NAMD User's Guide

Version 2.14b1

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Description

The NAMD *User's Guide* describes how to run and use the various features of the molecular dynamics program NAMD. This guide includes the capabilities of the program, how to use these capabilities, the necessary input files and formats, and how to run the program both on uniprocessor machines and in parallel.

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1 Introduction

NAMD is a parallel molecular dynamics program for UNIX platforms designed for high-performance simulations in structural biology. This document describes how to use NAMD, its features, and the platforms on which it runs. The document is divided into several sections:

Section 1 gives an overview of NAMD.

Section 2 lists the basics for getting started.

Section 3 describes NAMD file formats.

Section 4 explains PSF file generation with psfgen.

Section 5 presents the potential functions, non-bonded interactions, and full electrostatics.

Section 6 explains Generalized Born implicit solvent simulations.

Section 7 lists standard minimization and dynamics parameters.

Section 16 lists performance tuning parameters.

Section 8 explains user defined forces. conformation change calculations.

Section 9 describes collective variable-based calculations.

Section 10 explains alchemical free energy calculations.

Section 11 presents accelerated sampling methods.

Section 15 lists runtime analysis options.

Section 17 provides hints for X-PLOR users.

Section 18 provides sample configuration files.

Section 19 gives details on running NAMD.

Section 20 gives details on installing NAMD.

1.1 NAMD and molecular dynamics simulations

Molecular dynamics (MD) simulations compute atomic trajectories by solving equations of motion numerically using empirical force fields, such as the CHARMM force field, that approximate the actual atomic force in biopolymer systems. Detailed information about MD simulations can be found in several books such as [1, 74]. In order to conduct MD simulations, various computer programs have been developed including X-PLOR [14] and CHARMM [13]. These programs were originally developed for serial machines. Simulation of large molecules, however, require enormous computing power. One way to achieve such simulations is to utilize parallel computers. In recent years, distributed memory parallel computers have been offering cost-effective computational power. NAMD was designed to run efficiently on such parallel machines for simulating large molecules. NAMD is particularly well suited to the increasingly popular Beowulf-class PC clusters, which are quite similar to the workstation clusters for which is was originally designed. Future versions of NAMD will also make efficient use of clusters of multi-processor workstations or PCs.

NAMD has several important features:

- **Force Field Compatibility**
  The force field used by NAMD is the same as that used by the programs CHARMM [13] and X-PLOR [14]. This force field includes local interaction terms consisting of bonded interactions between 2, 3, and 4 atoms and pairwise interactions including electrostatic and van der Waals forces. This commonality allows simulations to migrate between these three programs.

- **Efficient Full Electrostatics Algorithms**
  NAMD incorporates the Particle Mesh Ewald (PME) algorithm, which takes the full electrostatic interactions into account. This algorithm reduces the computational complexity of electrostatic force evaluation from $O(N^2)$ to $O(N \log N)$.

- **Multiple Time Stepping**
  The velocity Verlet integration method [1] is used to advance the positions and velocities of the atoms in time. To further reduce the cost of the evaluation of long-range electrostatic forces, a multiple time step scheme is employed. The local interactions (bonded, van der Waals and electrostatic interactions within a specified distance) are calculated at each time step. The longer range interactions (electrostatic interactions beyond the specified distance) are only computed less often. This amortizes the cost of computing the electrostatic forces over several timesteps. A smooth splitting function is used to separate a quickly varying short-range portion of the electrostatic interaction from a more slowly varying long-range component. It is also possible to employ an intermediate timestep for the short-range non-bonded interactions, performing only bonded interactions every timestep.

- **Input and Output Compatibility**
  The input and output file formats used by NAMD are identical to those used by CHARMM and X-PLOR. Input formats include coordinate files in PDB format [6], structure files in X-PLOR PSF format, and energy parameter files in either CHARMM or X-PLOR formats. Output formats include PDB coordinate files and binary DCD trajectory files. These similarities assure that the molecular dynamics trajectories from NAMD can be read by CHARMM or X-PLOR and that the user can exploit the many analysis algorithms of the latter packages.

- **Dynamics Simulation Options**
  MD simulations may be carried out using several options, including
  - Constant energy dynamics,
  - Constant temperature dynamics via
    - Velocity rescaling,
    - Velocity reassignment,
    - Langevin dynamics,
  - Periodic boundary conditions,
  - Constant pressure dynamics via
    - Berendsen pressure coupling,
    - Nosé-Hoover Langevin piston,
  - Energy minimization,
  - Fixed atoms,

- Rigid waters,
- Rigid bonds to hydrogen,
- Harmonic restraints,
- Spherical or cylindrical boundary restraints.

- **Easy to Modify and Extend**
  Another primary design objective for NAMD is extensibility and maintainability. In order to achieve this, it is designed in an object-oriented style with C++. Since molecular dynamics is a new field, new algorithms and techniques are continually being developed. NAMD's modular design allows one to integrate and test new algorithms easily. If you are contemplating a particular modification to NAMD you are encouraged to contact the developers for guidance.

- **Interactive MD simulations**
  A system undergoing simulation in NAMD may be viewed and altered with VMD; for instance, forces can be applied to a set of atoms to alter or rearrange part of the molecular structure. For more information on VMD, see http://www.ks.uiuc.edu/Research/vmd/.

- **Load Balancing**
  An important factor in parallel applications is the equal distribution of computational load among the processors. In parallel molecular simulation, a spatial decomposition that evenly distributes the computational load causes the region of space mapped to each processor to become very irregular, hard to compute and difficult to generalize to the evaluation of many different types of forces. NAMD addresses this problem by using a simple uniform spatial decomposition where the entire model is split into uniform cubes of space called *patches*. An initial load balancer assigns patches and the calculation of interactions among the atoms within them to processors such that the computational load is balanced as much as possible. During the simulation, an incremental load balancer monitors the load and performs necessary adjustments.

1.2 Acknowledgments

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2 Getting Started

2.1 What is needed

Before running NAMD, explained in section 19, the following are be needed:

- A CHARMM force field in either CHARMM or X-PLOR format.
- An X-PLOR format PSF file describing the molecular structure.
- The initial coordinates of the molecular system in the form of a PDB file.
- A NAMD configuration file.

NAMD provides the `psfgen` utility, documented in Section 4, which is capable of generating the required PSF and PDB files by merging PDB files and guessing coordinates for missing atoms. If `psfgen` is insufficient for your system, we recommend that you obtain access to either CHARMM or X-PLOR, both of which are capable of generating the required files.

2.2 NAMD configuration file

Besides these input and output files, NAMD also uses a file referred to as the *configuration file*. This file specifies what dynamics options and values that NAMD should use, such as the number of timesteps to perform, initial temperature, etc. The options and values in this file control how the system will be simulated. The NAMD configuration file is specified on the NAMD command line, either before or after the various parallel execution options described in section 19.

A NAMD configuration file contains a set of options and values. The options and values specified determine the exact behavior of NAMD, what features are active or inactive, how long the simulation should continue, etc. Section 2.2.1 describes how options are specified within a NAMD configuration file. Section 2.2.5 lists the parameters which are required to run a basic simulation. Section 17 describes the relation between specific NAMD and X-PLOR dynamics options. Several sample NAMD configuration files are shown in section 18.

During execution NAMD will change to the directory containing the configuration file so that all file paths in the configuration file are relative to the configuration file directory. Multiple configuration files may be specified on the command line and the will be read in order, but all file paths will be relative to the first configuration file to call a "run" (or "minimize" or "startup") command, or to the last configuration file if "run" is not called.

Commands or parameters may also be specified directly on the command line via `--keyword value` argument pairs, for example `--outputenergies 100 --run 100 -- checkpoint`. This may be used to include multiple configuration files without altering the working directory via `--source /path/to/second.conf`. Note that escaping or quoting of command line parameter values containing spaces may be difficult or impossible on some systems due to multiple levels of scripts called during the NAMD parallel launch process and because the keyword and value are simply merged into a single string that is passed to the Tcl interpreter.

If the argument `--tclmain` is present, all following arguments will be passed to the Tcl interpreter as a script file and arguments accessible via the standard argc and argv variables. Note that Charm++ arguments such as `+pemap` are processed during Charm++ startup and will not be passed to Tcl.

If the first argument is `+tclsh`, Charm++ argument parsing and startup are not performed, the Tcl interpreter is initialized without NAMD scripting features, and all following arguments are passed to Tcl. Statically linked packages such as psfgen are available via "package require ...".

2.2.1 Configuration parameter syntax

Each line in the configuration files consists of a *keyword* identifying the option being specified, and a *value* which is a parameter to be used for this option. The keyword and value can be separated by only white space:

```
keyword            value
```

or the keyword and value can be separated by an equal sign and white space:

```
keyword      =     value
```

Blank lines in the configuration file are ignored. Comments are prefaced by a `#` and may appear on the end of a line with actual values:

```
keyword            value          #  This is a comment
```

or may be at the beginning of a line:

```
#  This entire line is a comment . . .
```

Some keywords require several lines of data. These are generally implemented to either allow the data to be read from a file:

```
keyword            filename
```

or to be included inline using Tcl-style braces:

```
keyword {
  lots of data
}
```

The specification of the keywords is case insensitive so that any combination of upper and lower case letters will have the same meaning. Hence, `DCDfile` and `dcdfile` are equivalent. The capitalization in the values, however, may be important. Some values indicate file names, in which capitalization is critical. Other values such as `on` or `off` are case insensitive.

2.2.2 Tcl scripting interface and features

When compiled with Tcl (all released binaries) the config file is parsed by Tcl in a fully backwards compatible manner with the added bonus that any Tcl command may also be used. This alone allows:

- the "source" command to include other files (works w/o Tcl too!),
- the "print" command to display messages ("puts" to stdout fails on some platforms),
- environment variables through the env array ("$env(USER)"), and

- user-defined variables ("set base sim23", "dcdfile $base.dcd").

Additional features include:

- The "callback" command takes a 2-parameter Tcl procedure which is then called with a list of labels and a list of values during every timestep, allowing analysis, formatting, whatever.
- The "run" command takes a number of steps to run (overriding the now optional numsteps parameter, which defaults to 0) and can be called repeatedly. You can "run 0" just to get energies. Normally the preceeding timestep is repeated to account for any modifications to the energy function; this can be avoided with "run norepeat".
- The "minimize" command is similar to "run" and performs minimization for the specified number of force evaluations.
- The "startup" command will trigger simulation startup as would the first "run" or "minimize" command, but without any force/energy evaluation.
- Configuration file parameter introspection is supported by invoking a (case-insensitive) parameter keyword with no argument (e.g., "numsteps") and by the helper commands "isset" and "istrue". Note that keywords are not parsed until the first "run" command, and before this values are treated as unformatted strings, so for example "eFieldOn" and "eField" may return "yes" and "1 2 3" before the first "run" command, but "1" and "1.0 2.0 3.0" after parsing ("istrue eFieldOn" would return "1" in both cases). Similarly, "isset badparam" will return "0" before parsing but raise an "unknown parameter" error after.
- Between "run" commands the reassignTemp, rescaleTemp, and langevinTemp parameters can be changed to allow simulated annealing protocols within a single config file. The useGroupPressure, useFlexibleCell, useConstantArea, useConstantRatio, LangevinPiston, LangevinPistonTarget, LangevinPistonPeriod, LangevinPistonDecay, LangevinPistonTemp, SurfaceTensionTarget, BerendsenPressure, BerendsenPressureTarget, BerendsenPressureCompressibility, and BerendsenPressureRelaxationTime parameters may be changed to allow pressure equilibration. The fixedAtoms, constraintScaling, and nonbondedScaling parameters may be changed to preserve macromolecular conformation during minimization and equilibration (fixedAtoms may only be disabled, and requires that fixedAtomsForces is enabled to do this). The consForceScaling parameter may be changed to vary steering forces or to implement a time-varying electric field that affects specific atoms. The eField, eFieldFreq, and eFieldPhase parameters may be changed to implement at time-varying electric field that affects all atoms. The updateGridforceScale parameter may be called to change the scaling factor applied to gridforces potentials. The alchLambda and alchLambda2 parameters may be changed during alchemical free energy runs. The DCDfile may be changed to write binary coordinate trajectory output to separate files. The restartname may be changed to write restart output to separate files.
- The "checkpoint" and "revert" commands (no arguments) allow a scripted simulation to save and restore (in memory) to a single prior state. The "output" and "reinitatoms" commands support multiple saved states using files. Multiple saved states in memory are supported by the commands "checkpointStore", "checkpointLoad", "checkpointSwap", and "checkpointFree", all of which take a string key as an argument, plus an optional second argument that

is either replica index (the checkpoint is stored asynchronously on the target replica) or the keyword "global" (the target replica is computed as a hash of the key).

- The "output" command takes an output file basename and causes .coor, .vel, and .xsc files to be written with that name. Alternatively, "output withforces" and "output onlyforces" will write a .force file either in addition to or instead of the regular files.

- The "reinitatoms" command reinitializes coordinates, velocities, and periodic cell dimensions to those initially read in (random velocities are generated if they were not read from a file). An optional file basename argument (matching that passed to the output command) causes .coor, .vel, and .xsc files to be read, assuming the format indicated by the binaryoutput parameter.

- The "move" command repositions individual atoms, including fixed atoms. Arguments are a 1-based atom ID, "to" or "by", and a list of three numbers, e.g., "move 1 by {0.4 0.2 -0.1}". Atoms may not be moved by more than a single patch between "run" commands.

- The "exit" command writes output files and exits cleanly.

- The "abort" command concatenates its arguments into an error message and exits immediately without writing output files.

- The "numPes", "numNodes", and "numPhysicalNodes" commands allow performance-tuning parameters to be set based on the parallel execution environment.

- The "reinitvels" command reinitializes velocities to a random distribution based on the given temperature.

- The "rescalevels" command rescales velocities by the given factor.

- The "reloadCharges" command reads new atomic charges from the given file, which should contain one number for each atom, separated by spaces and/or line breaks.

- The "consForceConfig" command takes a list of 0-based atom indices and a list of forces which replace the existing set of constant forces (constantForce must be on).

- The "measure" command allows user-programmed calculations to be executed in order to facilitate automated methods. (For example, to revert or change a parameter.) A number of measure commands are included in the NAMD binary; the module has been designed to make it easy for users to add additional measure commands.

- The "coorfile" command allows NAMD to perform force and energy analysis on trajectory files. "coorfile open dcd `filename`" opens the specified DCD file for reading. "coorfile read" reads the next frame in the opened DCD file, replacing NAMD's atom coordinates with the coordinates in the frame, and returns 0 if successful or -1 if end-of-file was reached. "coorfile skip" skips past one frame in the DCD file; this is significantly faster than reading coordinates and throwing them away. "coorfile close" closes the file. The "coorfile" command is not available on the Cray T3E.

  Force and energy analysis are especially useful in the context of pair interaction calculations; see Sec. 15.1 for details, as well as the example scripts in Sec. 18.

Please note that while NAMD has traditionally allowed comments to be started by a # appearing anywhere on a line, Tcl only allows comments to appear where a new statement could begin. With Tcl config file parsing enabled (all shipped binaries) both NAMD and Tcl comments are allowed before the first "run" command. At this point only pure Tcl syntax is allowed. In addition, the ";#" idiom for Tcl comments will only work with Tcl enabled. NAMD has also traditionally allowed parameters to be specified as "param=value". This is supported, but only before the first "run" command. Some examples:

```
# this is my config file                              <- OK
reassignFreq 100 ; # how often to reset velocities  <- only w/ Tcl
reassignTemp 20 # temp to reset velocities to       <- OK before "run"
run 1000                                            <- now Tcl only
reassignTemp 40 ; # temp to reset velocities to     <- ";" is required
```

NAMD has also traditionally allowed parameters to be specified as "param=value" as well as "param value". This is supported, but only before the first "run" command. For an easy life, use "param value".

2.2.3 Multiple-copy/replica-exchange scripting interface

Multiple-copy (or replica-based) algorithms are supported by the following commands, which utilize two-sided semantics modeled on MPI:

- myReplica
- numReplicas
- replicaBarrier
- replicaSend *data dest*
- replicaRecv *source*
- replicaSendrecv *data dest source*
- replicaAtomSend *dest*
- replicaAtomRecv *source*
- replicaAtomSendrecv *dest source*

The replicaSend/Sendrecv *data* argument may be any string, and hence any Tcl object (e.g., a list) that can be represented as a string. Data received from the *source* replica is returned by replicaRecv/Sendrecv. In order to ensure message ordering, replicaSend/Sendrecv will block until the corresponding remote receive call (except when replicaSend is called from inside replicaEval, as discussed below).

The parameter replicaUniformPatchGrids must be true for atom exchange (replicaAtom...) or remote checkpointing (checkpoint... with a second argument, see below).

The following additional commands utilize one-sided semantics, and should provide a complete feature set for running a simulation with fewer NAMD replica partitions than logical replicas:

- checkpointStore *key* ?*replica* or global?
- checkpointLoad *key* ?*replica* or global?
- checkpointSwap *key* ?*replica* or global?
- checkpointFree *key* ?*replica* or global?
- replicaEval *replica script*
- replicaYield ?*seconds*?
- replicaDcdFile *index*—off ?*filename*?

The *key* can be any string. By default the checkpoint is stored in the memory of the replica the command is called on. If you specify a replica index the checkpoint is stored asynchronously in that replica's memory. If you specify "global" a hash is computed based on the key to select the replica on which to store the checkpoint. You can have checkpoints with the same key stored on multiple replicas at once if you really want to. The checkpoint... commands will not return until the checkpoint operation has completed.

Storing checkpoints is not atomic. If two replicas try to store a checkpoint with the same key on the same replica at the same time you may end up with a mix of the two (and probably duplicate/missing atoms). If one replica tries to load a checkpoint while another replica is storing it the same may happen. You cannot store a checkpoint on a replica until that replica has created its own patch data structures. This can be guaranteed by calling "startup" and "replicaBarrier" before any remote checkpoint calls.

The replicaEval command asynchronously executes its script in the top-level context of the target replica's Tcl interpreter and returns the result or error. This should be general enough to build any kind of work scheduler or shared data structure you need. If you want to call replicaEval repeatedly, e.g., to check if some value has been set, you should call "replicaYield *seconds*" in between, as this will introduce a delay but still enable processing of asynchronous calls from other replicas. Potentially blocking functions such as replicaRecv should not be called from within replicaEval, nor should functions such as run, checkpointLoad/Store, and replicaAtomSend/Recv that would require the simulation of the remote replica to be halted. It is allowed to call replicaSend (but not replicaSendrecv) from within replicaEval, since replicaSend is non-blocking and one-sided (but potentially overtaking) in this context. Rather than polling a remote replica (e.g., for work) via replicaEval, it is more efficient to register a request via replicaEval and then call replicaRecv to wait for notification.

The replicaDcdFile command is similar to the dcdFile command in that it changes the trajectory output file, but the file is actually opened by a different replica partition and may be written to by any other partition that calls replicaDcdFile with the same index but no filename argument. If a filename argument is given, any file currently associated with the index is closed and a new file created, even if the new and old filenames are the same. The new file is created only when the next trajectory frame is written, not during the replicaDcdFile command itself. The caller must ensure that an index is not used before it is associated with a filename, and that each index is in use by only one replica at a time. The keyword "off" will return to writing the local trajectory file set by the dcdFile command.

2.2.4 Python scripting interface and features

NAMD may be compiled with an embedded Python interpreter via the config script option --with-python. Both Python 2.x and 3.x versions are supported, with 3.x the default if found. The config script option --python-prefix can be used to specify the path to the python installation to be used. The default embedded Tcl interpreter is also required to enable Python scripting. Released NAMD binaries do not support Python scripting at this time due to portability issues with the extensive Python library.

Python scripting is accessed via the Tcl "python" command, which functions in either expression mode or script mode. When passed a single-line string, the Python interpreter will evaluate the expression in the string and return the result. Python containers (tuples, lists, etc.) are converted to Tcl lists and all other values are converted to their string representation, which is typically compatible with Tcl. For example, "[python (1 + 1, 'abc' + '123')]" evaluates to the Tcl list "2 abc123".

When the python command is passed a single multi-line string (typically enclosed in braces), the Python interpreter will execute the code in the string and return nothing. Because of Python's indentation-sensitive syntax the enclosed code can not be indented.

Calls to Tcl from Python code are supported by the tcl module functions tcl.call(), which takes the Tcl command name and its arguments as separate arguments and performs limited container and string conversions as described above, and tcl.eval(), which passes a single string unmodified to the Tcl interpreter. Both functions return the result as a string, so numerical results must be explicitly cast to float or int as appropriate.

NAMD simulation parameters and commands are wrapped for convenience by the "namd" object. Any NAMD simulation parameter may be set by assigning to the corresponding case-insensitive attribute of the namd object, e.g., "namd.timestep = 1.0", and similarly read (as a string) by access, e.g., "ts = float(namd.TimeStep)". Assignment corresponds exactly to normal config file parsing, i.e., "timestep 1.0", and hence multiple assignment will generate an error just as would repeated parameters. For convenience, multiple parameters may be set at once by passing them as keyword arguments, e.g., "namd(langevin=True, langevinDamping=5., langevinTemp=100.)". NAMD (and other) commands in the Tcl interpreter may be called as a method of the namd object, e.g., "namd.run(1000)" and "namd.output('myfile')".

The NAMD 1-4scaling parameter is incompatible with Python syntax, but may be accessed several other ways, e.g., "namd.param('1-4scaling',1.0)", "tcl.call('1-4scaling',1.0)", or "tcl.eval('1-4scaling 1.0')".

The namd object is available as the namd *module*, which can be accessed from user-written Python modules by the standard import statement (i.e, "import namd").

The following example illustrates various aspects of the Python scripting interface:

```
set a 1
cutoff 12.0
python {
# do not indent
namd.pairlistDist = float(namd.Cutoff) + float(tcl.eval("set a")) # cast strings to float
b = 2
namd(switching=True, switchdist = float(namd.cutoff) - b) # case insensitive
}
set c [python $a + b]
```

2.2.5 Required NAMD configuration parameters

The following parameters are *required* for every NAMD simulation:

- `numsteps` (page 83),
- `coordinates` (page 26),
- `structure` (page 26),
- `parameters` (page 26),
- `exclude` (page 56),
- `outputname` (page 27),
- one of the following three:
  - `temperature` (page 83),
  - `velocities` (page 27),
  - `binvelocities` (page 27).

These required parameters specify the most basic properties of the simulation. In addition, it is highly recommended that `pairlistdist` be specified with a value at least one greater than `cutoff`.

3 Input and Output Files

NAMD was developed to be compatible with existing molecular dynamics packages, especially the packages X-PLOR [14] and CHARMM [13]. To achieve this compatibility, the set of input files which NAMD uses to define a molecular system are identical to the input files used by X-PLOR and CHARMM. Thus it is trivial to move an existing simulation from X-PLOR or CHARMM to NAMD. A description of these molecular system definition files is given in Section 3.1.

In addition, the output file formats used by NAMD were chosen to be compatible with X-PLOR and CHARMM. In this way the output from NAMD can be analyzed using X-PLOR, CHARMM, or a variety of the other tools that have been developed for the existing output file formats. Descriptions of the output files formats are also given in Section 3.1.

3.1 File formats

3.1.1 PDB files

The PDB (Protein Data Bank) format is used for coordinate, velocity, force, or other data being read or written by NAMD. This is the standard format for coordinate data for most other molecular dynamics programs as well, including X-PLOR and CHARMM. A full description of this file format can be obtained from the PDB web site at `http://www.rcsb.org/pdb/`. Positions in PDB files are stored in Å. Velocities in PDB files are stored in Å/ps and may be divided by PDBVELFACTOR=20.45482706 to convert to the NAMD internal units used in DCD and NAMD binary files. Forces in PDB files are stored in kcal/mol/Å. NAMD binary files (below) should be preferred to PDB files in most cases due to their higher precision.

3.1.2 X-PLOR format PSF files

NAMD uses the same protein structure files that X-PLOR does. These files may be generated with psfgen, VMD, X-PLOR, or CHARMM. CHARMM can generate an X-PLOR format PSF file with the command "`write psf card xplor`".

3.1.3 CHARMM19, CHARMM22, and CHARMM27 parameter files

NAMD supports CHARMM19, CHARMM22, and CHARMM27 parameter files in both X-PLOR and CHARMM formats. (X-PLOR format is the default, CHARMM format parameter files may be used given the parameter "`paraTypeCharmm on`".) For a full description of the format of commands used in these files, see the X-PLOR and CHARMM User's Manual [14].

3.1.4 DCD trajectory files

NAMD produces DCD trajectory files in the same format as X-PLOR and CHARMM. The DCD files are single precision binary FORTRAN files, so are transportable between computer architectures. The file readers in NAMD and VMD can detect and adapt to the endianness of the machine on which the DCD file was written, and the utility program `flipdcd` is also provided to reformat these files if needed. The exact format of these files is very ugly but supported by a wide range of analysis and display programs. The timestep is stored in the DCD file in NAMD internal units and must be multiplied by TIMEFACTOR=48.88821 to convert to fs. Positions in DCD files are stored in Å. Velocities in DCD files are stored in NAMD internal units and must be multiplied by PDBVELFACTOR=20.45482706 to convert to Å/ps. Forces in DCD files are stored in kcal/mol/Å.

3.1.5 NAMD binary files

NAMD uses a trivial double-precision binary file format for coordinates, velocities, and forces. Due to its high precision this is the default output and restart format. VMD refers to these files as the "namdbin" format. The file consists of the atom count as a 32-bit integer followed by all three position or velocity components for each atom as 64-bit double-precision floating point, i.e., NXYZXYZXYZXYZ... where N is a 4-byte int and X, Y, and Z are 8-byte doubles. If the number of atoms the file contains is known then the atom count can be used to determine endianness. The file readers in NAMD and VMD can detect and adapt to the endianness of the machine on which the binary file was written, and the utility program `flipbinpdb` is also provided to reformat these files if needed. Positions in NAMD binary files are stored in Å. Velocities in NAMD binary files are stored in NAMD internal units and must be multiplied by PDBVELFACTOR=20.45482706 to convert to Å/ps. Forces in NAMD binary files are stored in kcal/mol/Å.

3.2 NAMD configuration parameters

3.2.1 Input files

- `coordinates` < coordinate PDB file >
  Acceptable Values: UNIX filename
  Description: The PDB file containing initial position coordinate data. Note that path names can be either absolute or relative. Only one value may be specified.

- `structure` < PSF file >
  Acceptable Values: UNIX filename
  Description: The X-PLOR format PSF file describing the molecular system to be simulated. Only one value may be specified.

- `parameters` < parameter file >
  Acceptable Values: UNIX filename
  Description: A CHARMM19, CHARMM22, or CHARMM27 parameter file that defines all or part of the parameters necessary for the molecular system to be simulated. At least one parameter file must be specified for each simulation. Multiple definitions (but only one file per definition) are allowed for systems that require more than one parameter file. The files will be read in the order that they appear in the configuration file. If duplicate parameters are read, a warning message is printed and the last parameter value read is used. Thus, the order that files are read can be important in cases where duplicate values appear in separate files.

- `paraTypeXplor` < Is the parameter file in X-PLOR format? >
  Acceptable Values: `on` or `off`
  Default Value: `on`
  Description: Specifies whether or not the parameter file(s) are in X-PLOR format. X-PLOR format is the default for parameter files! Caveat: The PSF file should be also constructed with X-PLOR in case of an X-PLOR parameter file because X-PLOR stores information about the multiplicity of dihedrals in the PSF file. See the X-PLOR manual for details.

- `paraTypeCharmm` < Is the parameter file in CHARMM format? >
  Acceptable Values: `on` or `off`

Default Value: `off`
Description: Specifies whether or not the parameter file(s) are in CHARMM format. X-PLOR format is the default for parameter files! Caveat: The information about multiplicity of dihedrals will be obtained directly from the parameter file, and the full multiplicity will be used (same behavior as in CHARMM). If the PSF file originates from X-PLOR, consecutive multiple entries for the same dihedral (indicating the dihedral multiplicity for X-PLOR) will be ignored.

- `velocities` < velocity PDB file >
Acceptable Values: UNIX filename
Description: The PDB file containing the initial velocities for all atoms in the simulation. This is typically a restart file or final velocity file written by NAMD during a previous simulation. Either the `temperature` or the `velocities`/`binvelocities` option must be defined to determine an initial set of velocities. Both options cannot be used together.

- `binvelocities` < binary velocity file >
Acceptable Values: UNIX filename
Description: The binary file containing initial velocities for all atoms in the simulation. A binary velocity file is created as output from NAMD by activating the `binaryrestart` or `binaryoutput` options. The `binvelocities` option should be used as an alternative to `velocities`. Either the `temperature` or the `velocities`/`binvelocities` option must be defined to determine an initial set of velocities. Both options cannot be used together.

- `bincoordinates` < binary coordinate restart file >
Acceptable Values: UNIX filename
Description: The binary restart file containing initial position coordinate data. A binary coordinate restart file is created as output from NAMD by activating the `binaryrestart` or `binaryoutput` options. Note that, in the current implementation at least, the `bincoordinates` option must be used in addition to the `coordinates` option, but the positions specified by `coordinates` will then be ignored.

- `cwd` < default directory >
Acceptable Values: UNIX directory name
Description: The default directory for input and output files. If a value is given, all filenames that do not begin with a / are assumed to be in this directory. For example, if `cwd` is set to `/scr`, then a filename of `outfile` would be modified to `/scr/outfile` while a filename of `/tmp/outfile` would remain unchanged. If no value for `cwd` is specified, than all filenames are left unchanged *but are assumed to be relative to the directory which contains the configuration file given on the command line.*

3.2.2 Output files

- `outputname` < output file prefix >
Acceptable Values: UNIX filename prefix
Description: At the end of every simulation, NAMD writes two files, one containing the final coordinates and another containing the final velocities of all atoms in the simulation. This option specifies the file prefix for these two files as well as the default prefix for trajectory and restart files. The position coordinates will be saved to a file named as this prefix with `.coor`

appended. The velocities will be saved to a file named as this prefix with `.vel` appended. For example, if the prefix specified using this option was `/tmp/output`, then the two files would be `/tmp/output.coor` and `/tmp/output.vel`.

- `binaryoutput` < use binary output files? >
  Acceptable Values: `yes` or `no`
  Default Value: `yes`
  Description: Enables the use of binary output files. If this option is not set to `no`, then the final output files will be written in binary rather than PDB format. Binary files preserve more accuracy between NAMD restarts than ASCII PDB files, but the binary files are not guaranteed to be transportable between computer architectures. (The atom count record is used to detect wrong-endian files, which works for most atom counts. The utility program `flipbinpdb` is provided to reformat these files if necessary.)

- `restartname` < restart files prefix >
  Acceptable Values: UNIX filename prefix
  Default Value: *outputname*`.restart`
  Description: The prefix to use for restart filenames. NAMD produces restart files that store the current positions and velocities of all atoms at some step of the simulation. This option specifies the prefix to use for restart files in the same way that `outputname` specifies a filename prefix for the final positions and velocities. If `restartname` is defined then the parameter `restartfreq` must also be defined.

- `restartfreq` < frequency of restart file generation >
  Acceptable Values: positive integer
  Description: The number of timesteps between the generation of restart files.

- `restartsave` < use timestep in restart filenames? >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: Appends the current timestep to the restart filename prefix, producing a sequence of restart files rather than only the last version written.

- `binaryrestart` < use binary restart files? >
  Acceptable Values: `yes` or `no`
  Default Value: `yes`
  Description: Enables the use of binary restart files. If this option is not set to `no`, then the restart files will be written in binary rather than PDB format. Binary files preserve more accuracy between NAMD restarts than ASCII PDB files, but the binary files are not guaranteed to be transportable between computer architectures. (The atom count record is used to detect wrong-endian files, which works for most atom counts. The utility program `flipbinpdb` is provided to reformat these files if necessary.)

- `DCDfile` < coordinate trajectory output file >
  Acceptable Values: UNIX filename
  Default Value: *outputname*`.dcd`
  Description: The binary DCD position coordinate trajectory filename. This file stores the trajectory of all atom position coordinates using the same format (binary DCD) as X-PLOR. If `DCDfile` is defined, then `DCDfreq` must also be defined.

- `DCDfreq` < timesteps between writing coordinates to trajectory file >
  Acceptable Values: positive integer
  Description: The number of timesteps between the writing of position coordinates to the trajectory file. The initial positions will not be included in the trajectory file. Positions in DCD files are stored in Å.

- `DCDUnitCell` < write unit cell data to dcd file? >
  Acceptable Values: `yes` or `no`
  Default Value: `yes` if periodic cell
  Description: If this option is set to `yes`, then DCD files will contain unit cell information in the style of Charmm DCD files. By default this option is enabled if the simulation cell is periodic in all three dimensions and disabled otherwise.

- `velDCDfile` < velocity trajectory output file >
  Acceptable Values: UNIX filename
  Default Value: *outputname*`.veldcd`
  Description: The binary DCD velocity trajectory filename. This file stores the trajectory of all atom velocities using the same format (binary DCD) as X-PLOR. If `velDCDfile` is defined, then `velDCDfreq` must also be defined.

- `velDCDfreq` < timesteps between writing velocities to trajectory file >
  Acceptable Values: positive integer
  Description: The number of timesteps between the writing of velocities to the trajectory file. The initial velocities will not be included in the trajectory file. Velocities in DCD files are stored in NAMD internal units and must be multiplied by PDBVELFACTOR=20.45482706 to convert to Å/ps.

- `forceDCDfile` < force trajectory output file >
  Acceptable Values: UNIX filename
  Default Value: *outputname*`.forcedcd`
  Description: The binary DCD force trajectory filename. This file stores the trajectory of all atom forces using the same format (binary DCD) as X-PLOR. If `forceDCDfile` is defined, then `forceDCDfreq` must also be defined.

- `forceDCDfreq` < timesteps between writing force to trajectory file >
  Acceptable Values: positive integer
  Description: The number of timesteps between the writing of forces to the trajectory file. The initial forces will not be included in the trajectory file. Forces in DCD files are stored in kcal/mol/Å. *In the current implementation only those forces that are evaluated during the timestep that a frame is written are included in that frame. This is different from the behavior of TclForces and is likely to change based on user feedback. For this reason it is strongly recommended that forceDCDfreq be a multiple of fullElectFrequency.*

3.2.3 Standard output

NAMD logs a variety of summary information to standard output. The standard units used by NAMD are Angstroms for length, kcal/mol for energy, Kelvin for temperature, and bar for pressure. Wallclock or CPU times are given in seconds unless otherwise noted.

BOUNDARY energy is from spherical boundary conditions and harmonic restraints, while MISC energy is from external electric fields and various steering forces. TOTAL is the sum of the various potential energies, and the KINETIC energy. TOTAL2 uses a slightly different kinetic energy that is better conserved during equilibration in a constant energy ensemble. TOTAL3 is another variation with much smaller short-time fluctuations that is also adjusted to have the same running average as TOTAL2. Defects in constant energy simulations are much easier to spot in TOTAL3 than in TOTAL or TOTAL2.

PRESSURE is the pressure calculated based on individual atoms, while GPRESSURE incorporates hydrogen atoms into the heavier atoms to which they are bonded, producing smaller fluctuations. The TEMPAVG, PRESSAVG, and GPRESSAVG are the average of temperature and pressure values since the previous ENERGY output; for the first step in the simulation they will be identical to TEMP, PRESSURE, and GPRESSURE.

- `outputEnergies` < timesteps between energy output >
  Acceptable Values: positive integer
  Default Value: 1
  Description: The number of timesteps between each energy output of NAMD. This value specifies how often NAMD should output the current energy values to **stdout** (which can be redirected to a file). By default, this is done every step. For long simulations, the amount of output generated by NAMD can be greatly reduced by outputting the energies only occasionally.

- `mergeCrossterms` < add crossterm energy to dihedral? >
  Acceptable Values: yes or no
  Default Value: yes
  Description: If crossterm (or CMAP) terms are present in the potential, the energy is added to the dihedral energy to avoid altering the energy output format. Disable this feature to add a separate "CROSS" field to the output.

- `outputMomenta` < timesteps between momentum output >
  Acceptable Values: nonnegative integer
  Default Value: 0
  Description: The number of timesteps between each momentum output of NAMD. If specified and nonzero, linear and angular momenta will be output to **stdout**.

- `outputPressure` < timesteps between pressure output >
  Acceptable Values: nonnegative integer
  Default Value: 0
  Description: The number of timesteps between each pressure output of NAMD. If specified and nonzero, atomic and group pressure tensors will be output to **stdout**.

- `outputTiming` < timesteps between timing output >
  Acceptable Values: nonnegative integer
  Default Value: the greater of `firstLdbStep` or 10× `outputEnergies`
  Description: The number of timesteps between each timing output of NAMD. If nonzero, CPU and wallclock times and memory usage will be output to **stdout**. These data are from node 0 only; CPU times and memory usage for other nodes may vary.

3.3 AMBER file and force field support

AMBER format PARM file and coordinate file can be read by NAMD, which allows one to use AMBER force field to carry out all types of simulations that NAMD has supported. NAMD can read PARM files in either the format used in AMBER 6 or the new format defined in AMBER 7. The output of the simulation (restart file, DCD file, etc.) will still be in traditional format that has been used in NAMD.

- `amber` < use AMBER format force field? >
  Acceptable Values: yes or no
  Default Value: no
  Description: If `amber` is set to on, then `parmfile` must be defined, and `structure` and `parameters` should not be defined.

- `parmfile` < AMBER format PARM file >
  Acceptable Values: UNIX filename
  Description: This file contains complete topology and parameter information of the system.

- `ambercoor` < AMBER format coordinate file >
  Acceptable Values: UNIX filename
  Description: This file contains the coordinates of all the atoms. Note that `coordinates` can also be used for PDB format coordinate file. When `amber` is set to on, either `ambercoor` or `coordinates` must be defined, but not both.

- `readexclusions` < Read exclusions from PARM file? >
  Acceptable Values: yes or no
  Default Value: yes
  Description: PARM file explicitly gives complete exclusion (including 1-4 exclusions) information. When `readexclusions` is set to on, NAMD will read all exclusions from PARM file and will not add any more; alternatively, if `readexclusions` is set to off, NAMD will ignore the exclusions in PARM file and will automatically generate them according to the exclusion policy specified by `exclude`.

- `scnb` < VDW 1-4 scaling factor >
  Acceptable Values: decimal ≥ 1.0
  Default Value: 2.0
  Description: Same meaning as SCNB in AMBER. Note that in NAMD, 1-4 vdw interactions are DIVIDED by `scnb`, whereas 1-4 electrostatic interactions are MULTIPLIED by `1-4scaling`. So `1-4scaling` should be set to the inverse of SCEE value used in AMBER.

Caveat:
1. Polarizable parameters in AMBER are not supported.
2. NAMD does not support the 10-12 potential terms in some old AMBER versions. When non-zero 10-12 parameter is encountered in PARM file, NAMD will terminate.
3. NAMD has several exclusion policy options, defined by `exclude`. The way AMBER dealing with exclusions corresponds to the "scaled1-4" in NAMD. So for simulations using AMBER force field, one would specify "exclude scaled1-4" in the configuration file, and set `1-4scaling` to the inverse value of SCEE as would be used in AMBER.

4. NAMD does not read periodic box lengths in PARM or coordinate file. They must be explicitly specified in NAMD configuration file.
5. By default, NAMD applies switching functions to the non-bond interactions within the cutoff distance, which helps to improve energy conservation, while AMBER does not use switching functions so it simply truncates the interactions at cutoff. However, if "authentic" AMBER cutoff simulations are desired, the switching functions could be turned off by specifying "switching off" in NAMD configuration file.
6. NAMD and AMBER may have different default values for some parameters (e.g., the tolerance of SHAKE). One should check other sections of this manual for accurate descriptions of the NAMD options.

Following are two examples of the NAMD configuration file to read AMBER force field and carry out simulation. They may help users to select proper NAMD options for AMBER force field. For the convenience of AMBER users, the AMBER 6 sander input files are given in the left for comparison, which would accomplish similar tasks in AMBER.

Example 1: Non-periodic boundary system, cutoff simulation

```
---AMBER----      ---NAMD---

 TITLE
 &cntrl
  ntb=0, igb=2,   # non-periodic, use cutoff for non-bond
  nstlim=1000,    numsteps       1000  # Num of total steps
  ntpr=50,        outputEnergies 50  # Energy output frequency
  ntwr=50,        restartfreq    50  # Restart file frequency
  ntwx=100,       DCDfreq        100  # Trajectory file frequency
  dt=0.001,       timestep       1  # in unit of fs (This is default)
  tempi=0.,       temperature    0  # Initial temp for velocity assignment
  cut=10.,        cutoff         10
                  switching      off  # Turn off the switching functions
  scee=1.2,       exclude        scaled1-4
                  1-4scaling     0.833333  # =1/1.2, default is 1.0
  scnb=2.0        scnb           2  # This is default
 &end
                  amber          on  # Specify this is AMBER force field
                  parmfile       prmtop  # Input PARM file
                  ambercoor      inpcrd  # Input coordinate file
                  outputname     md  # Prefix of output files
```

Example 2: Periodic boundary system, PME, NVE ensemble, using SHAKE algorithm

```
---AMBER----      ---NAMD---

 TITLE
 &cntrl
  ntc=2, ntf=2,   # SHAKE to the bond between each hydrogen and it mother atom
```

```
                rigidBonds     all
 tol=0.0005,    rigidTolerance 0.0005  # Default is  0.00001
 nstlim=500,    numsteps       500  # Num of total steps
 ntpr=50,       outputEnergies 50  # Energy output frequency
 ntwr=100,      restartfreq    100  # Restart file frequency
 ntwx=100,      DCDfreq        100  # Trajectory file frequency
 dt=0.001,      timestep       1  # in unit of fs (This is default)
 tempi=300.,    temperature    300  # Initial temp for velocity assignment
 cut=9.,        cutoff         9
                switching      off  # Turn off the switching functions
&end
&ewald          PME            on  # Use PME for electrostatic calculation
                # Orthogonal periodic box size
 a=62.23,       cellBasisVector1   62.23  0  0
 b=62.23,       cellBasisVector2   0  62.23  0
 c=62.23,       cellBasisVector3   0  0  62.23
 nfft1=64,      PMEGridSizeX   64
 nfft2=64,      PMEGridSizeY   64
 nfft3=64,      PMEGridSizeZ   64
 ischrgd=1,     # NAMD doesn't force neutralization of charge
&end
                amber          on  # Specify this is AMBER force field
                parmfile       FILENAME  # Input PARM file
                ambercoor      FILENAME  # Input coordinate file
                outputname     PREFIX  # Prefix of output files
                exclude        scaled1-4
                1-4scaling     0.833333  # =1/1.2, default is 1.0
```

3.4 GROMACS file support

NAMD has the ability to load GROMACS ASCII topology (.top) and coordinate (.gro) files, which allows you to run most GROMACS simulations in NAMD. All simulation output will still be in the traditional NAMD formats.

- `gromacs` < use GROMACS format force field? >
  Acceptable Values: on or off
  Default Value: off
  Description: If `gromacs` is set to on, then `grotopfile` must be defined, and `structure` and `parameters` should not be defined.

- `grotopfile` < GROMACS format topology/parameter file >
  Acceptable Values: UNIX filename
  Description: This file contains complete topology and parameter information of the system.

- `grocoorfile` < GROMACS format coordinate file >
  Acceptable Values: UNIX filename
  Description: This file contains the coordinates of all the atoms. Note that `coordinates`

can also be used for PDB format coordinate file. When `gromacs` is set to `on`, either `grocoorfile` or `coordinates` must be defined, but not both.

However, NAMD does not have support for many GROMACS-specific options:

- Dummies (fake atoms with positions generated from the positions of real atoms) are not supported.
- The GROMACS `pairs` section, where explicit 1–4 parameters are given between pairs of atoms, is not supported, since NAMD calculates its 1–4 interactions exclusively by type.
- Similarly, `exclusions` are not supported. The biggest problem here is that GROMACS RB dihedrals are supposed to imply exclusions, but NAMD does not support this.
- Constraints, restraints, and `settles` are not implemented in NAMD.
- In some cases, it may not work to override some but not all of the parameters for a bond, atom, etc. In this case, NAMD will generate an error and stop. The parser will sometimes not tolerate correct GROMACS files or fail to detect errors in badly formatted files.
- NAMD does not support all the types of bond potentials that exist in GROMACS, but approximates them with harmonic or sinusoidal potentials.
- NAMD does not read periodic box lengths in the coordinate file. They must be explicitly specified in the NAMD configuration file.

4 Creating PSF Structure Files

The `psfgen` structure building tool consists of a portable library of structure and file manipulation routines with a Tcl interface. Current capabilities include

- reading CHARMM topology files
- reading psf files in X-PLOR/NAMD format
- extracting sequence data from single segment PDB files
- generating a full molecular structure from sequence data
- applying patches to modify or link different segments
- writing NAMD and VMD compatible PSF structure files
- extracting coordinate data from PDB files
- constructing (guessing) missing atomic coordinates
- deleting selected atoms from the structure
- writing NAMD and VMD compatible PDB coordinate files

We are currently refining the interface of `psfgen` and adding features to create a complete molecular building solution. We welcome your feedback on this new tool.

4.1 New commands and Functionalities

The version 2.0 of `psfgen` was extensively modified and improved to meet the current standards in the size of the structures, and the modern versions of additive CHARMM force field, and polarizable DRUDE force field (`http://mackerell.umaryland.edu/charmm_ff.shtml`).

New functionalities include:

- hydrogen mass repartition
- structure preparation for Drude force field
- structure preparation containing colinear lone pairs (halogen atoms in the latest additive CHARMM force field version)
- psfgen log file to store all the information printed to the console

To use the Drude force field, one only needs to load the Drude topology files and prepare the structures as per usual. Most commands are available for both lone pairs in the additive and polarizable force fields, although some operations are not yet available for Drude particles. Atom modification operations, e.g., psfset, and queries with the segment command on the drude particles, are not implemented. We advise the user to use VMD to assign beta and occupancy values during the structure preparation. writemol and readmol commands are not compatible with structure preparation for Drude Force field.

The new `psfgen` log file allows the user to save all the information regularly printed out during a `psfgen` execution script to a file. It is possible to open and close multiple log files in a `psfgen` script, but only one file is active at any given moment. An example of an application of multiple log files is to save the information of the loading process of the topology files to one log file and the rest of information of structure preparation to another file, as demonstrated below:

```
psfgen_logfile "load_topoplogy.log"

topology top_all22_prot.rtf
topology top_all36_carb.rtf
topology top_all36_lipid.rtf
topology top_all36_prot.rtf
topology top_all36_cgenff.rtf
topology toppar_water_ions.str

psfgen_logfile close

psfgen_logfile "structure_preparation.log"

segment BPTI {
 pdb output/6PTI_protein.pdb
}

patch DISU BPTI:5 BPTI:55
patch DISU BPTI:14 BPTI:38
patch DISU BPTI:30 BPTI:51

pdbalias atom ILE CD1 CD
coordpdb output/6PTI_protein.pdb BPTI

pdbalias residue HOH TIP3
segment SOLV {
 auto none
 pdb output/6PTI_water.pdb
}

pdbalias atom HOH O OH2
coordpdb output/6PTI_water.pdb SOLV

guesscoord

writepsf output/bpti.psf
writepdb output/bpti.pdb

psfgen_logfile close
```

4.2 Ordinary Usage

`psfgen` is currently distributed in two forms. One form is as a standalone program implemented as a Tcl interpreter which reads commands from standard output. You may use loops, variables, etc. as you would in a VMD or NAMD script. You may use psfgen interactively, but we expect it to be run most often with a script file redirected to standard input. The second form is as a Tcl package which can be imported into any Tcl application, including VMD. All the commands available to the standalone version of psfgen are available to the Tcl package; using `psfgen` within VMD lets you harness VMD's powerful atom selection capability, as well as instantly view the result of your structure building scripts. Examples of using `psfgen` both with and without VMD are provided in this document.

Generating PSF and PDB files for use with NAMD will typically consist of the following steps:

1. Preparing separate PDB files containing individual segments of protein, solvent, etc. before running `psfgen`.
2. Reading in the appropriate topology definition files and aliasing residue and atom names found in the PDB file to those found in the topology files. This will generally include selecting a default protonation state for histidine residues.
3. Generating the default structure using segment and pdb commands.
4. Applying additional patches to the structure.
5. Reading coordinates from the PDB files.
6. Deleting unwanted atoms, such as overlapping water molecules.
7. Guessing missing coordinates of hydrogens and other atoms.
8. Writing PSF and PDB files for use in NAMD.

4.2.1 Preparing separate PDB files

Many PDB files in the PDB databank contain multiple chains, corresponding to protein subunits, water, and other miscellaneous groups. Protein subunits are often identified by their chain ID in the PDB file. In `psfgen`, each of these groups must be assigned to their own *segment*. This applies most strictly in the case of protein chains, each of which must be assigned to its own segment so that N-terminal and C-terminal patches can be applied. You are free to group water molecules into whatever segments you choose.

Chains can be split up into their own PDB files using your favorite text editor and/or Unix shell commands, as illustrated in the BPTI example below. If you are using VMD you can also use atom selections to write pieces of the structure to separate files:

```
# Split a file containing protein and water into separate segments.
# Creates files named myfile_water.pdb, myfile_frag0.pdb, myfile_frag1.pdb,...
# Requires VMD.
mol load pdb myfile.pdb
set water [atomselect top water]
$water writepdb myfile_water.pdb
```

```
set protein [atomselect top protein]
set chains [lsort -unique [$protein get pfrag]]
foreach chain $chains {
  set sel [atomselect top "pfrag $chain"]
  $sel writepdb myfile_frag${chain}.pdb
}
```

4.2.2 Deleting unwanted atoms

The `delatom` command described below allows you to delete selected atoms from the structure. It's fine to remove atoms from your structure before building the PSF and PDB files, but you should never edit the PSF and PDB files created by `psfgen` by hand as it will probably mess up the internal numbering in the PSF file.

Very often the atoms you want to delete are water molecules that are either too far from the solute, or else outside of the periodic box you are trying to prepare. In either case VMD atom selections can be used to select the waters you want to delete. For example:

```
# Load a pdb and psf file into both psfgen and VMD.
resetpsf
readpsf myfile.psf
coordpdb myfile.pdb
mol load psf myfile.psf pdb myfile.pdb
# Select waters that are more than 10 Angstroms from the protein.
set badwater1 [atomselect top "name OH2 and not within 10 of protein"]
# Alternatively, select waters that are outside our periodic cell.
set badwater2 [atomselect top "name OH2 and (x<-30 or x>30 or y<-30 or>30
                              or z<-30 or z>30)"]
# Delete the residues corresponding to the atoms we selected.
foreach segid [$badwater1 get segid] resid [$badwater1 get resid] {
  delatom $segid $resid
}
# Have psfgen write out the new psf and pdb file (VMD's structure and
# coordinates are unmodified!).
writepsf myfile_chopwater.psf
writepdb myfile_chopwater.pdb
```

4.3 BPTI Example

To actually run this demo requires

- the program `psfgen` from any NAMD distribution,
- the CHARMM topology and parameter files `top_all22_prot.inp` and `par_all22_prot.inp` from http://mackerell.umaryland.edu/charmm_ff.shtml, and
- the BPTI PDB file `6PTI.pdb` available from the Protein Data Bank at http://www.pdb.org/ by searching for `6PTI` and downloading the complete structure file in PDB format.

Building the BPTI structure

In this demo, we create the files `bpti.psf` and `bpti.pdb` in the output directory which can then be used for a simple NAMD simulation.

```
# File: bpti_example.tcl
# Requirements: topology file top_all22_prot.inp in directory toppar
#               PDB file 6PTI.pdb in current directory

# Create working directory; remove old output files
mkdir -p output
rm -f output/6PTI_protein.pdb output/6PTI_water.pdb

# (1) Split input PDB file into segments}
grep -v '^HETATM' 6PTI.pdb > output/6PTI_protein.pdb
grep 'HOH' 6PTI.pdb > output/6PTI_water.pdb

# (2) Embed the psfgen commands in this script
psfgen << ENDMOL

# (3) Read topology file
topology toppar/top_all22_prot.inp

# (4) Build protein segment
segment BPTI {
 pdb output/6PTI_protein.pdb
}

# (5) Patch protein segment
patch DISU BPTI:5 BPTI:55
patch DISU BPTI:14 BPTI:38
patch DISU BPTI:30 BPTI:51

# (6) Read protein coordinates from PDB file
pdbalias atom ILE CD1 CD    ; # formerly "alias atom ..."
coordpdb output/6PTI_protein.pdb BPTI

# (7) Build water segment
pdbalias residue HOH TIP3   ; # formerly "alias residue ..."
segment SOLV {
 auto none
 pdb output/6PTI_water.pdb
}

# (8) Read water coordinaes from PDB file
pdbalias atom HOH O OH2     ; # formerly "alias atom ..."
coordpdb output/6PTI_water.pdb SOLV
```

```
# (9) Guess missing coordinates
guesscoord

# (10) Write structure and coordinate files
writepsf output/bpti.psf
writepdb output/bpti.pdb

# End of psfgen commands
ENDMOL
```

Step-by-step explanation of the script:

(1) Split input PDB file into segments. 6PTI.pdb is the original file from the Protein Data Bank. It contains a single chain of protein and some PO4 and H2O HETATM records. Since each segment must have a separate input file, we remove all non-protein atom records using grep. If there were multiple chains we would have to split the file by hand. Create a second file containing only waters.

(2) Embed the psfgen commands in this script. Run the psfgen program, taking everything until "ENDMOL" as input. You may run psfgen interactively as well. Since psfgen is built on a Tcl interpreter, you may use loops, variables, etc., but you must use `$$` for variables when inside a shell script. If you want, run psfgen and enter the following commands manually.

(3) Read topology file. Read in the topology definitions for the residues we will create. This must match the parameter file used for the simulation as well. Multiple topology files may be read in since psfgen and NAMD use atom type names rather than numbers in psf files.

(4) Build protein segment. Actually build a segment, calling it BPTI and reading the sequence of residues from the stripped pdb file created above. In addition to the pdb command, we could specify residues explicitly. Both angles and dihedrals are generated automatically unless "auto none" is added (which is required to build residues of water). The commands "first" and "last" may be used to change the default patches for the ends of the chain. The structure is built when the closing } is encountered, and some errors regarding the first and last residue are normal.

(5) Patch protein segment. Some patch residues (those not used to begin or end a chain) are applied after the segment is built. These contain all angle and dihedral terms explicitly since they were already generated. In this case we apply the patch for a disulfide link three separate times.

(6) Read protein coordinates from PDB file. The same file used to generate the sequence is now read to extract coordinates. In the residue ILE, the atom CD is called CD1 in the pdb file, so we use "pdbalias atom" to define the correct name. If the segment names in the pdb file match the name we gave in the segment statement, then we don't need to specify it again; in this case we do specify the segment, so that all atoms in the pdb file must belong to the segment.

(7) Build water segment. Build a segment for the crystal waters. The residue type for water depends on the model, so here we alias HOH to TIP3. Because CHARMM uses an additional H-H bond we must disable generation of angles and dihedrals for segments containing water. Then read the pdb file.

(8) Read water coordinates from PDB file. Alias the atom type for water oxygen as well and read coordinates from the file to the segment SOLV. Hydrogen doesn't show up in crystal structures so it is missing from this pdb file.

(9) Guessing missing coordinates. The tolopogy file contains default internal coordinates which can be used to guess the locations of many atoms, hydrogens in particular. In the output pdb file, the occupancy field of guessed atoms will be set to 0, atoms which are known are set to 1, and atoms which could not be guessed are set to -1. Some atoms are "poorly guessed" if needed bond lengths and angles were missing from the topology file. Similarly, waters with missing hydrogen coordinates are given a default orientation.

Write structure and coordinate files. Now that all of the atoms and bonds have been created, we can write out the psf structure file for the system. We also create the matching coordinate pdb file. The psf and pdb files are a matched set with identical atom ordering as needed by NAMD.

Using generated files in NAMD.

The files bpti.pdb and bpti.psf can now be used with NAMD, but the initial coordinates require minimization first. The following is an example NAMD configuration file for the BPTI example.

```
# NAMD configuration file for BPTI

# molecular system
structure output/bpti.psf

# force field
paratypecharmm on
parameters toppar/par_all22_prot.inp
exclude scaled1-4
1-4scaling 1.0

# approximations
switching on
switchdist 8
cutoff 12
pairlistdist 13.5
margin 0
stepspercycle 20

#integrator
timestep 1.0
```

```
#output
outputenergies 10
outputtiming 100
binaryoutput no

# molecular system
coordinates output/bpti.pdb

#output
outputname output/bpti
dcdfreq 1000

#protocol
temperature 0
reassignFreq 1000
reassignTemp 25
reassignIncr 25
reassignHold 300

#script

minimize 1000

run 20000
```

4.4 Building solvent around a protein

The following script illustrates how `psfgen` and VMD can be used together to add water around a protein structure. It assumes you already have a psf and pdb file for your protein, as well as a box of water which is large enough to contain the protein. For more information on how atomselections can be used within VMD scripts, see the VMD User's Guide.

```
proc addwater { psffile pdbfile watpsf watpdb } {
# Create psf/pdb files that contain both our protein as well as
# a box of equilibrated water.  The water box should be large enough
# to easily contain our protein.
resetpsf
readpsf $psffile pdb $pdbfile
readpsf $watpsf pdb $watpdb

# Load the combined structure into VMD
writepsf combine.psf
writepdb combine.pdb
mol load psf combine.psf pdb combine.pdb

# Assume that the segid of the water in watpsf is QQQ
# We want to delete waters outside of a box ten Angstroms
```

```
# bigger than the extent of the protein.
set protein [atomselect top "not segid QQQ"]
set minmax [measure minmax $protein]
foreach {min max} $minmax { break }
foreach {xmin ymin zmin} $min { break }
foreach {xmax ymax zmax} $max { break }
    set xmin [expr $xmin - 10]
    set ymin [expr $ymin - 10]
    set zmin [expr $zmin - 10]
    set xmax [expr $xmax + 10]
    set ymax [expr $ymax + 10]
    set zmax [expr $zmax + 10]

# Center the water on the protein.  Also update the coordinates held
# by psfgen.
set wat [atomselect top "segid QQQ"]
$wat moveby [vecsub [measure center $protein] [measure center $wat]]
foreach atom [$wat get {segid resid name x y z}] {
foreach {segid resid name x y z} $atom { break }
coord $segid $resid $name [list $x $y $z]
}

# Select waters that we don't want in the final structure.
set outsidebox [atomselect top "segid QQQ and (x <= $xmin or y <= $ymin \
or z <= $zmin or x >= $xmax or y >= $ymax or z >= $xmax)"]
set overlap [atomselect top "segid QQQ and within 2.4 of (not segid QQQ)"]

# Get a list of all the residues that are in the two selections, and delete
# those residues from the structure.
set reslist [concat [$outsidebox get resid] [$overlap get resid]]
set reslist [lsort -unique -integer $reslist]

foreach resid $reslist {
delatom QQQ $resid
}

# That should do it - write out the new psf and pdb file.
writepsf solvate.psf
writepdb solvate.pdb

# Delete the combined water/protein molecule and load the system that
# has excess water removed.
mol delete top
mol load psf solvate.psf pdb solvate.pdb

# Return the size of the water box
```

```
return [list [list $xmin $ymin $zmin] [list $xmax $ymax $zmax]]
}
```

4.5 New Commands in the version 2.0

- `psfgen_logfile` <*file name*> [`close`]
  Purpose: Open or close a log file to store all information printed to the console.
  Arguments: <*file name*>: Valid file name in the current directory.
  `close`: Close the active log file. The file name should not be included in the closing command. Example above.
  Context: Any part of the script, context independent. May call multiple times.

- `hmassrepart` [*dowater <1 0>*] [*mass <target hydrogen mass>*]
  Purpose: Partition the mass of heavy atoms into the bonded hydrogen atoms.
  Arguments: `dowater`: 1 for true, 0 for false. Partition the water molecules. Default value 0.
  `mass`: Target for the hydrogen atoms' mass. Default value 3.024 amu.
  Context: After loading or preparing the structure.

- `vpbonds` [1 0]
  Purpose: Print the bonds between the virtual particles (drude particles and lone pairs) and their hosts.
  Arguments: 1 for true, 0 for false. Default value 1.
  Context: Before writing the psf file. May call multiple times. WARNING: To run simulations containing lone pairs or Drude particles on NAMD 2.13, set vpbonds to 0.

4.6 List of Commands

- `topology` [`list`] <*file name*>
  Purpose: Read in molecular topology definitions from file.
  Arguments: <*file name*>: CHARMM format topology file.
  `list`: Lists all currently specified topology files.
  `residues`: Return a list of the known residue topologies.
  `patches`: Return a list of the known residue patches.
  Context: Beginning of script, before segment. May call multiple times.

- `topology alias` <*alternate residue name*> <*existing residue name*>
  Purpose: Provide alternate names for residues found in topology file. An alternate name used to generate a residue will be used on output. Compare to "pdbalias residue" below, in which the real name is used on output.
  Arguments: <*alternate residue name*>: Desired residue name.
  <*existing residue name*>: Residue name found in topology file.
  Context: Before reading sequence with pdb. May call multiple times.

- `pdbalias residue` <*alternate name*> <*real name*>
  Purpose: Provide translations from residues found in PDB files to proper residue names read in from topology definition files. Proper names from topology files will be used in generated PSF and PDB files. Compare to "topology alias" above, in which the alias is is used as the residue name in generated files. This command also exists under the deprecated name `alias`.

Arguments: <*alternate name*>: Residue name found in PDB file.
<*real name*>: Residue name found in topology file or aliases.
Context: Before reading sequence with pdb. May call multiple times.

- `segment` [`segids`] [`resids`] [`residue`] [`first`] [`last`] <*segment ID*> [*resid*] [*atom name*] { <*commands*> }
  Purpose: Build a segment of the molecule. A segment is typically a single chain of protein or DNA, with default patches applied to the termini. Segments may also contain pure solvent or lipid. Options [`segids`] [`resids`] [`residue`] [`first`] [`last`] are used to query information about the specified segment.
  Arguments: `segids`: Return a list of segids for the molecule in the current context.
  `resids`: Return a list of resids for the given segment in the current context.
  `residue`: Return the residue name of the residue in the given segment with the given resid.
  `atoms`: Return a list of atoms for the given segment with the given resid.
  `coordinates`: Return x, y, z coordinates for the given atom.
  `velocities`: Return x, y, z velocities for the given atom.
  `mass`: Return the mass of the given atom.
  `charge`: Return the charge of the given atom.
  `atomid`: Return the one-based atomid of the given atom. These are only assigned/updated when writing a file. Therefore `writepsf`, `writepdb`, or `writemol` must be called to avoid returning old atomid values or zero.
  `first`: Returns the name of the patch that was applied to the beginning of the specified segment.
  `last`: Returns the name of the patch that was applied to the end of the specified segment.
  <*segment ID*>: Unique name for segment, 1–4 characters.
  <*commands*>: Sequence of commands in Tcl syntax to build the primary structure of the segment, including auto, first, last, residue, pdb, etc.
  Context: After topology definitions and residue aliases. May call multiple times. Structure information is generated at the end of every segment command.

- `auto` [`angles`] [`dihedrals`] [`none`]
  Purpose: Override default settings from topology file for automatic generation of angles and dihedrals for the current segment.
  Arguments: `angles`: Enable generation of angles from bonds.
  `dihedrals`: Enable generation of dihedrals from angles.
  `none`: Disable generation of angles and dihedrals.
  Context: Anywhere within segment, does not affect later segments.

- `first` <*patch name*>
  Purpose: Override default patch applied to first residue in segment. Default is read from topology file and may be residue-specific.
  Arguments: <*patch name*>: Single-target patch residue name or `none`.
  Context: Anywhere within segment, does not affect later segments.

- `last` <*patch name*>
  Purpose: Override default patch applied to last residue in segment. Default is read from topology file and may be residue-specific.

Arguments: <*patch name*>: Single-target patch residue name or `none`.
Context: Anywhere within segment, does not affect later segments.

- `residue` <*resid*> <*resname*> [*chain*]
  Purpose: Add a single residue to the end of the current segment.
  Arguments: <*resid*>: Unique name for residue, 1–5 characters, usually numeric. <*resname*>: Residue type name from topology file. <*chain*>: Single-character chain identifier.
  Context: Anywhere within segment.

- `pdb` <*file name*>
  Purpose: Extract sequence information from PDB file when building segment. Residue IDs will be preserved, residue names must match entries in the topology file or should be aliased before pdb is called.
  Arguments: <*file name*>: PDB file containing known or aliased residues.
  Context: Anywhere within segment.

- `mutate` <*resid*> <*resname*>
  Purpose: Change the type of a single residue in the current segment.
  Arguments: <*resid*>: Unique name for residue, 1–5 characters, usually numeric. <*resname*>: New residue type name from topology file.
  Context: Within segment, after target residue has been created.

- `patch` [`list`] <*patch residue name*> <*segid:resid*> [...]
  Purpose: Apply a patch to one or more residues. Patches make small modifications to the structure of residues such as converting one to a terminus, changing the protonation state, or creating disulphide bonds between a pair of residues.
  Arguments: `list`: Lists all patches applied explicitey using the command 'patch'.
  `listall`: Lists all currently applied patches including default patches.
  <*patch residue name*>: Name of patch residue from topology definition file.
  <*segid:resid*>: List of segment and residue pairs to which patch should be applied.
  Context: After one or more segments have been built.

- `regenerate` [`angles`] [`dihedrals`]
  Purpose: Remove all angles and/or dihedrals and completely regenerate them using the segment automatic generation algorithms. This is only needed if patches were applied that do not correct angles and bonds. Segment and file defaults are ignored, and angles/dihedrals for the entire molecule are regenerated from scratch.
  Arguments: `angles`: Enable generation of angles from bonds.
  `dihedrals`: Enable generation of dihedrals from angles.
  Context: After one or more segments have been built.

- `regenerate` [`resids`]
  Purpose: Remove insertion codes and minimally modify resids to retain uniqueness. No modifications will be made in segments that have monotonically increasing resids and do not contain insertion codes. Within a segment, no modifications will be made to residues preceeding the first non-increasing resid or residue with an insertion code.
  Arguments: `resids`: Enable regeneration of resids to remove insertion codes.
  Context: After one or more segments have been built.

- `multiply` <*factor*> <*segid*[*:resid*[*:atomname*]]> [...]
  Purpose: Create multiple images of a set of atoms for use in locally enhanced sampling. The beta column of the output pdb file is set to 1...<*factor*> for each image. Multiple copies of bonds, angles, etc. are created. Atom, residue or segment names are not altered; images are distinguished only by beta value. This is not a normal molecular structure and may confuse other tools.
  Arguments: <*factor*>:
  <*segid:resid:atomname*>: segment, residue, or atom to be multiplied. If :resid is omitted the entire segment is multiplied; if :atomname is omitted the entire residue is multiplied. May be repeated as many times as necessary to include all atoms.
  Context: After one or more segments have been built, all patches applied, and coordinates guessed. The effects of this command may confuse other commands.

- `delatom` <*segid*> [*resid*] [*atomname*]
  Purpose: Delete one or more atoms. If only `segid` is specified, all atoms from that segment will be removed from the structure. If both `segid` and `resid` are specified, all atoms from just that residue will be removed. If `segid`, `resid`, and `atomname` are all specified, just a single atom will be removed.
  Arguments: <*segid*>: Segment ID of target atom.
  <*resid*>: Residue ID of target atom (optional).
  <*atomname*>: Name of target atom (optional).
  Context: After one or more segments have been built.

- `resetpsf`
  Purpose: Delete all segments in the structure. The topology definitions and aliases are left intact. If you want to clear the topology and aliases as well, use `psfcontext reset` instead.
  Arguments:
  Context: After one or more segments have been built.

- `psfcontext` [*context*] [`new`] [`delete`]
  Purpose: Switches between complete contexts, including structure, topology definitions, and aliases. If no arguments are provided, the current context is returned. If <*context*> or `new` is specified, a new context is entered and the old context is returned. If `delete` is also specified, the old context is destroyed and "deleted <*old context*>" is returned. An error is returned if the specified context does not exist or if `delete` was specified and the current context would still be in use. *It may be possible to write robust, error-tolerant code with this interface, but it would not be easy. Please employ the following revised* `psfcontext` *usage instead.*
  Arguments: <*context*>: Context ID returned by psfcontext.
  Context: At any time.

- `psfcontext mixedcase`
  Purpose: Make context case sensitive by preserving case of all segment, residue, atom, and patch names on input.
  Arguments:
  Context: Before reading files requiring case sensitive behavior, normally as the first command.

- `psfcontext allcaps`
  Purpose: Make context case insensitive by converting all segment, residue, atom, and patch

names to upper case characters on input. This is the default behavior and should match the behavior of versions prior to 1.5.0.
Arguments:
Context: Before reading files requiring case insensitive behavior, not needed in normal use.

- `psfcontext reset`
  Purpose: Clears the structure, topology definitions, and aliases, creating clean environment just like a new context.
  Arguments:
  Context: At any time.

- `psfcontext create`
  Purpose: Creates a new context and returns its ID, but does not switch to it. This is different from `psfcontext new` above, which switches to the newly created context and returns the current context's ID.
  Arguments:
  Context: At any time.

- `psfcontext delete` <*context*>
  Purpose: Deletes the specified context. An error is returned if the specified context does not exist or would still be in use. This is different from `psfcontext` <*context*> `delete` above, which switches to the specified context and deletes the current one.
  Arguments: <*context*>: Context ID returned by psfcontext.
  Context: At any time.

- `psfcontext eval` <*context*> { <*commands*> }
  Purpose: Evaluates <*commands*> in the specified context, returning to the current context on exit. This should be totally robust, returning to the orignal context in case of errors and preventing its deletion when nested.
  Arguments: <*context*>: Context ID returned by `psfcontext create`.
  <*commands*>: Script to be executed in the specified context.
  Context: At any time.

- `psfcontext stats`
  Purpose: Returns the total numbers of contexts that have been created and destroyed. This is useful for checking if a script is leaking contexts.
  Arguments:
  Context: At any time.

- `writepsf` [`charmm`] [`x-plor`] [`cmap`] [`nocmap`] [`nopatches`] <*file name*>
  Purpose: Write out structure information as PSF file. A simplified session log is listed in the REMARKS section of the PSF file.
  Arguments: `charmm`: Use CHARMM format (numbers for atom types).
  `x-plor`: Use X-PLOR format (names for atom types), the default format required by NAMD.
  `cmap`: Write cross-term entries to PSF file if present, the default.
  `nocmap`: Do not write cross-term entries to PSF file, even if present.
  `nopatches`: Do not write list of applied patches to PSF file header.
  <*file name*>: PSF file to be generated.
  Context: After all segments have been built and patched.

- `readpsf` <*file name*> [`pdb`] [*pdb file name*] [`namdbin`] [*namdbin file name*] [`velnamdbin`] [*velocity file name*]
  Purpose: Read in structure information from PSF file and add it to the structure. Optionally also read coordinates and insertion codes from a PDB file, assuming that the atom order is the same in both files. Optionally also read coordinates a NAMD binary file, assuming that the atom order is the same as the psf file. It is an error if any segments in the PSF file already exist.
  Arguments: <*file name*>: PSF file in X-PLOR format (names for atom types).
  `pdb`: Read coordinates and insertion codes from PDB file.
  <*pdb file name*>: PDB file with atoms in same order as PSF file.
  `namdbin`: Read coordinates from NAMD binary file.
  <*namdbin file name*>: NAMD binary file with atoms in same order as PSF file.
  `velnamdbin`: Read velocities from NAMD binary file.
  <*velocity file name*>: NAMD binary velocity file with atoms in same order as PSF file.
  Context: Anywhere but within segment.

- `pdbalias atom` <*residue name*> <*alternate name*> <*real name*>
  Purpose: Provide translations from atom names found in PDB files to proper atom names read in from topology definition files. Proper names from topology files will be used in generated PSF and PDB files. This command also exists under the deprecated name `alias`.
  Arguments: <*residue name*>: Proper or aliased residue name.
  <*alternate name*>: Atom name found in PDB file.
  <*real name*>: Atom name found in topology file.
  Context: Before reading coordinates with coordpdb. May call multiple times.

- `coordpdb` <*file name*> [*segid*] [`namdbin`] [*namdbin file name*]
  Purpose: Read coordinates from PDB file, matching segment, residue and atom names.
  Arguments: <*file name*>: PDB file containing known or aliased residues and atoms.
  <*segid*>: If specified override segment IDs in PDB file.
  `namdbin`: Read coordinates from NAMD binary file.
  <*namdbin file name*>: NAMD binary file with atoms in same order as PDB file.
  Context: After segment has been generated and atom aliases defined.

- `guesscoord`
  Purpose: Guesses coordinates of atoms for which they were not explicitly set. Calculation is based on internal coordinate hints contained in toplogy definition files. When these are insufficient, wild guesses are attempted based on bond lengths of 1 Å and angles of 109°.
  Arguments: None.
  Context: After stucture has been generated and known coordinates read in.

- `coord` <*segid*> <*resid*> <*atomname*> <{ *x y z* }>
  Purpose: WILL BE DEPRECATED AFTER VERSON 1.6 (use `psfset coord` instead) Set coordinates for a single atom.
  Arguments: <*segid*>: Segment ID of target atom.
  <*resid*>: Residue ID of target atom.
  <*atomname*>: Name of target atom.
  <{ *x y z* }>: Coordinates to be assigned.
  Context: After structure has been generated.

- `psfset` <*attribute*> <*segid*> [*resid*] [*atomname*] <*value*>
  Purpose: Set an attribute for a given segment, residue, or atom.
  Arguments: <*attribute*>: Segment attributes: `segid`: the name of the segment Residue attributes: `resname`: the name of the residue Atom attributes: `name`: the name of the atom, `mass`: the mass of the atom, `charge`: the charge of the atom, `beta`: the PDB bfactor of the atom, `coord`: the coordinates of the atom as {x y z}, `vel`: the velocity of the atom as {vx vy vz}
  <*segid*>: Segment ID of target segment, residue, or atom.
  <*resid*>: Residue ID of target residue or atom.
  <*atomname*>: Name of target atom.
  <*value*>: Value to be assigned.
  Context: After structure has been generated or read from file.

- `writepdb` <*file name*>
  Purpose: Writes PDB file containing coordinates. Atom order is identical to PSF file generated by writepsf (unless structure has been changed). The O field is set to 1 for atoms with known coordinates, 0 for atoms with guessed coordinates, and -1 for atoms with no coordinate data available (coordinates are set to 0 for these atoms).
  Arguments: <*file name*>: PDB file to be written.
  Context: After structure and coordinates are complete.

- `writenamdbin` <*file name*> [`velnamdbin`] [*velocity file name*]
  Purpose: Writes NAMD binary file containing coordinates. Atom order is identical to PSF file generated by writepsf (unless structure has been changed). Coordinates are set to 0 for atoms with no coordinate data.
  Arguments: <*file name*>: NAMD binary file to be written.
  `velnamdbin`: Also write velocities to NAMD binary file.
  <*velocity file name*>: NAMD binary velocity file to be written.
  Context: After structure and coordinates are complete.

4.7 Example of a Session Log

The command "writepsf" prints a simple session log as "REMARKS" at the beginning of the PSF file. The log contains information about applied patches and used topology files which not stored in the standard records of PSF files. These informations are also available after a PSF file was read by command "readpsf". Here'a a simple axample:

```
PSF

       1 !NTITLE
 REMARKS original generated structure x-plor psf file
 REMARKS 4 patches were applied to the molecule.
 REMARKS topology 1LOV_autopsf-temp.top
 REMARKS segment P1 { first NTER; last CTER; auto angles dihedrals }
 REMARKS segment O1 { first NONE; last NONE; auto none  }
 REMARKS segment W1 { first NONE; last NONE; auto none  }
 REMARKS defaultpatch NTER P1:1
 REMARKS defaultpatch CTER P1:104
```

```
 REMARKS patch DISU P1:10  P1:2
 REMARKS patch DISU P1:103  P1:6

    1704 !NATOM
       1 P1   1    ALA  N    NH3   -0.300000       14.0070           0
...
```

All patches that were applied explicitely using the "patch" command are listed following the keyword "patch", but the patches that result from default patching like the first and last patches of a segment are marked as "defaultpatch". Further the segment based patching rules are listed along with the angle/dihedral autogeneration rules.

5 Force Field Parameters

5.1 Potential energy functions

Evaluating the force is the most computationally demanding part of molecular dynamics. The force is the negative gradient of a scalar potential energy function,

$$\vec{F}(\vec{r}) = -\nabla U(\vec{r}), \tag{1}$$

and, for systems of biomolecules, this potential function involves the summing,

$$U(\vec{r}) = \sum U_{\text{bonded}}(\vec{r}) + \sum U_{\text{nonbonded}}(\vec{r}), \tag{2}$$

over a large number of bonded and nonbonded terms. The bonded potential terms involve 2–, 3–, and 4–body interactions of covalently bonded atoms, with $O(N)$ terms in the summation. The nonbonded potential terms involve interactions between all pairs of atoms (usually excluding pairs of atoms already involved in a bonded term), with $O(N^2)$ terms in the summation, although fast evaluation techniques are used to compute good approximations to their contribution to the potential with $O(N)$ or $O(N \log N)$ computational cost.

5.1.1 Bonded potential energy terms

The bonded potential terms involve 2–, 3–, and 4–body interactions of covalently bonded atoms.

The 2–body spring bond potential describes the harmonic vibrational motion between an (i, j)–pair of covalently bonded atoms,

$$U_{\text{bond}} = k(r_{ij} - r_0)^2, \tag{3}$$

where $r_{ij} = \|\vec{r}_j - \vec{r}_i\|$ gives the distance between the atoms, r_0 is the equilibrium distance, and k is the spring constant.

The 3–body angular bond potential describes the angular vibrational motion occurring between an (i, j, k)–triple of covalently bonded atoms,

$$U_{\text{angle}} = k_\theta(\theta - \theta_0)^2 + k_{\text{ub}}(r_{ik} - r_{\text{ub}})^2, \tag{4}$$

where, in the first term, θ is the angle in radians between vectors $\vec{r}_{ij} = \vec{r}_j - \vec{r}_i$ and $\vec{r}_{kj} = \vec{r}_j - \vec{r}_k$, θ_0 is the equilibrium angle, and k_θ is the angle constant. The second term is the Urey–Bradley term used to describe a (noncovalent) spring between the outer i and k atoms, active when constant $k_{\text{ub}} \neq 0$, where, like the spring bond, $r_{ik} = \|\vec{r}_k - \vec{r}_i\|$ gives the distance between the pair of atoms and r_{ub} is the equilibrium distance.

The 4–body torsion angle (also known as dihedral angle) potential describes the angular spring between the planes formed by the first three and last three atoms of a consecutively bonded (i, j, k, l)–quadruple of atoms,

$$U_{\text{tors}} = \begin{cases} k(1 + \cos(n\psi + \phi)) & \text{if } n > 0, \\ k(\psi - \phi)^2 & \text{if } n = 0, \end{cases} \tag{5}$$

where ψ is the angle in radians between the (i, j, k)–plane and the (j, k, l)–plane. The integer constant n is nonnegative and indicates the periodicity. For $n > 0$, ϕ is the phase shift angle and k is the multiplicative constant. For $n = 0$, ϕ acts as an equilibrium angle and the units of k change to potential/rad^2. A given (i, j, k, l)–quadruple of atoms might contribute multiple terms to the potential, each with its own parameterization. The use of multiple terms for a torsion angle allows for complex angular variation of the potential, effectively a truncated Fourier series.

5.1.2 Nonbonded potential energy terms

The nonbonded potential terms involve interactions between all (i, j)–pairs of atoms, usually excluding pairs of atoms already involved in a bonded term. Even using a fast evaluation methods the cost of computing the nonbonded potentials dominates the work required for each time step of an MD simulation.

The Lennard–Jones potential accounts for the weak dipole attraction between distant atoms and the hard core repulsion as atoms become close,

$$U_{\mathrm{LJ}} = (-E_{\min}) \left[\left(\frac{R_{\min}}{r_{ij}} \right)^{12} - 2 \left(\frac{R_{\min}}{r_{ij}} \right)^{6} \right], \tag{6}$$

where $r_{ij} = \|\vec{r}_j - \vec{r}_i\|$ gives the distance between the pair of atoms. The parameter $E_{\min} = U_{\mathrm{LJ}}(R_{\min})$ is the minimum of the potential term ($E_{\min} < 0$, which means that $-E_{\min}$ is the well-depth). The Lennard–Jones potential approaches 0 rapidly as r_{ij} increases, so it is usually truncated (smoothly shifted) to 0 past a cutoff radius, requiring $O(N)$ computational cost.

The electrostatic potential is repulsive for atomic charges with the same sign and attractive for atomic charges with opposite signs,

$$U_{\mathrm{elec}} = \epsilon_{14} \frac{C q_i q_j}{\epsilon_0 r_{ij}}, \tag{7}$$

where $r_{ij} = \|\vec{r}_j - \vec{r}_i\|$ gives the distance between the pair of atoms, and q_i and q_j are the charges on the respective atoms. Coulomb's constant C and the dielectric constant ϵ_0 are fixed for all electrostatic interactions. The parameter ϵ_{14} is a unitless scaling factor whose value is 1, except for a modified 1–4 interaction, where the pair of atoms is separated by a sequence of three covalent bonds (so that the atoms might also be involved in a torsion angle interaction), in which case $\epsilon_{14} = \varepsilon$, for a fixed constant $0 \leq \varepsilon \leq 1$. Although the electrostatic potential may be computed with a cutoff like the Lennard–Jones potential, the $1/r$ potential approaches 0 much more slowly than the $1/r^6$ potential, so neglecting the long range electrostatic terms can degrade qualitative results, especially for highly charged systems. There are other fast evaluation methods that approximate the contribution to the long range electrostatic terms that require $O(N)$ or $O(N \log N)$ computational cost, depending on the method.

5.2 Non-bonded interactions

NAMD has a number of options that control the way that non-bonded interactions are calculated. These options are interrelated and can be quite confusing, so this section attempts to explain the behavior of the non-bonded interactions and how to use these parameters.

5.2.1 Van der Waals interactions

The simplest non-bonded interaction is the van der Waals interaction. In NAMD, van der Waals interactions are always truncated at the cutoff distance, specified by `cutoff`. The main option that effects van der Waals interactions is the `switching` parameter. With this option set to `on`, a smooth switching function will be used to truncate the van der Waals potential energy smoothly at the cutoff distance. A graph of the van der Waals potential with this switching function is shown in Figure 1. If `switching` is set to `off`, the van der Waals energy is just abruptly truncated at the cutoff distance, so that energy may not be conserved.

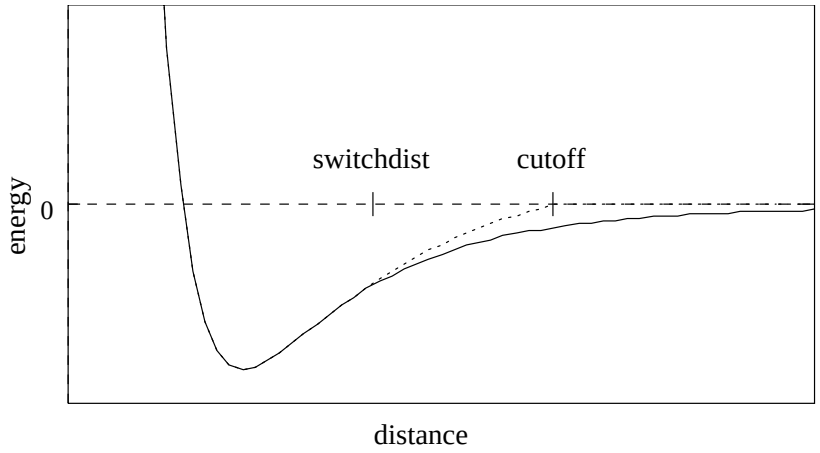

Figure 1: Graph of van der Waals potential with and without the application of the switching function. With the switching function active, the potential is smoothly reduced to 0 at the cutoff distance. Without the switching function, there is a discontinuity where the potential is truncated.

The switching function used is based on the X-PLOR switching function. The parameter `switchdist` specifies the distance at which the switching function should start taking effect to bring the van der Waals potential to 0 smoothly at the cutoff distance. Thus, the value of `switchdist` must always be less than that of `cutoff`.

5.2.2 Electrostatic interactions

The handling of electrostatics is slightly more complicated due to the incorporation of multiple timestepping for full electrostatic interactions. There are two cases to consider, one where full electrostatics is employed and the other where electrostatics are truncated at a given distance.

First let us consider the latter case, where electrostatics are truncated at the cutoff distance. Using this scheme, all electrostatic interactions beyond a specified distance are ignored, or assumed to be zero. If `switching` is set to `on`, rather than having a discontinuity in the potential at the cutoff distance, a shifting function is applied to the electrostatic potential as shown in Figure 2. As this figure shows, the shifting function shifts the entire potential curve so that the curve intersects the x-axis at the cutoff distance. This shifting function is based on the shifting function used by X-PLOR.

Next, consider the case where full electrostatics are calculated. In this case, the electrostatic interactions are not truncated at any distance. In this scheme, the `cutoff` parameter has a slightly different meaning for the electrostatic interactions — it represents the *local interaction distance*, or distance within which electrostatic pairs will be directly calculated every timestep. Outside of this distance, interactions will be calculated only periodically. These forces will be applied using a multiple timestep integration scheme as described in Section 7.3.4.

5.2.3 Non-bonded force field parameters

- `cutoff` < local interaction distance common to both electrostatic and van der Waals calculations (Å) >
  Acceptable Values: positive decimal
  Description: See Section 5.2 for more information.

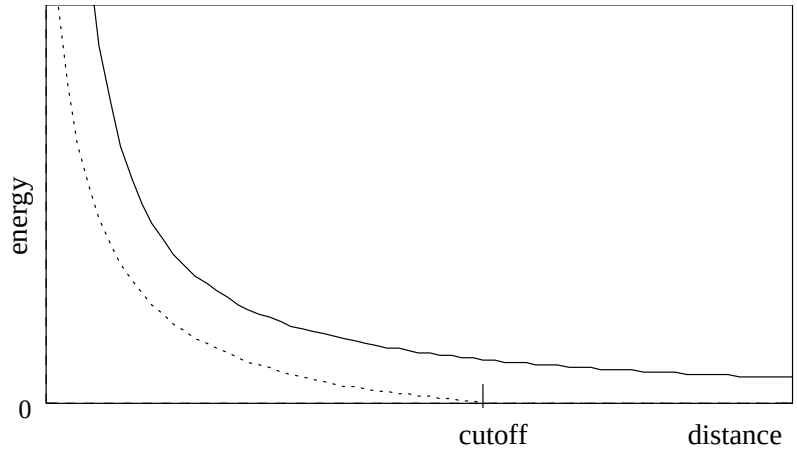

Figure 2: Graph showing an electrostatic potential with and without the application of the shifting function.

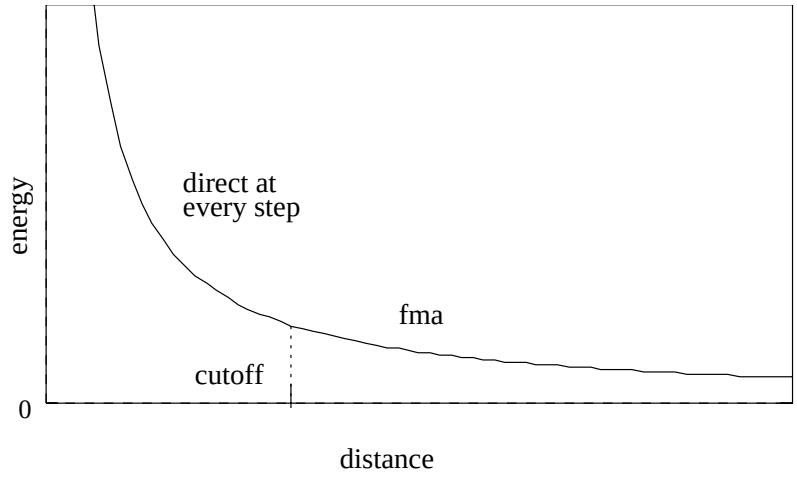

Figure 3: Graph showing an electrostatic potential when full electrostatics are used within NAMD, with one curve portion calculated directly and the other calculated using PME.

- `switching` < use switching function? >
  Acceptable Values: `on` or `off`
  Default Value: `on`
  Description: If `switching` is specified to be `off`, then a truncated cutoff is performed. If `switching` is turned `on`, then smoothing functions are applied to both the electrostatics and van der Waals forces. For a complete description of the non-bonded force parameters see Section 5.2. If `switching` is set to `on`, then `switchdist` must also be defined.

- `vdwForceSwitching` < use force switching for VDW? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: If both `switching` and `vdwForceSwitching` are set to `on`, then CHARMM force switching is used for van der Waals forces.

- `switchdist` < distance at which to activate switching/splitting function for electrostatic and van der Waals calculations (Å) >

Acceptable Values: positive decimal ≤ `cutoff`
Description: Distance at which the switching function should begin to take effect. This parameter only has meaning if `switching` is set to `on`. The value of `switchdist` must be less than or equal to the value of `cutoff`, since the switching function is only applied on the range from `switchdist` to `cutoff`. For a complete description of the non-bonded force parameters see Section 5.2.

- `exclude` < non-bonded exclusion policy to use >
Acceptable Values: `none`, `1-2`, `1-3`, `1-4`, or `scaled1-4`
Description: This parameter specifies which pairs of bonded atoms should be excluded from non-bonded interactions. With the value of `none`, no bonded pairs of atoms will be excluded. With the value of `1-2`, all atom pairs that are directly connected via a linear bond will be excluded. With the value of `1-3`, all `1-2` pairs will be excluded along with all pairs of atoms that are bonded to a common third atom (i.e., if atom A is bonded to atom B and atom B is bonded to atom C, then the atom pair A-C would be excluded). With the value of `1-4`, all `1-3` pairs will be excluded along with all pairs connected by a set of two bonds (i.e., if atom A is bonded to atom B, and atom B is bonded to atom C, and atom C is bonded to atom D, then the atom pair A-D would be excluded). With the value of `scaled1-4`, all `1-3` pairs are excluded and all pairs that match the `1-4` criteria are modified. The electrostatic interactions for such pairs are modified by the constant factor defined by `1-4scaling`. The van der Waals interactions are modified by using the special 1-4 parameters defined in the parameter files. The value of `scaled1-4` is necessary to enable the modified 1-4 VDW parameters present in the CHARMM parameter files.

- `1-4scaling` < scaling factor for 1-4 electrostatic interactions >
Acceptable Values: 0 ≤ decimal ≤ 1
Default Value: 1.0
Description: Scaling factor for 1-4 electrostatic interactions. This factor is only used when the `exclude` parameter is set to `scaled1-4`. In this case, this factor is used to modify the electrostatic interactions between 1-4 atom pairs. If the `exclude` parameter is set to anything but `scaled1-4`, this parameter has no effect regardless of its value.

- `dielectric` < dielectric constant for system >
Acceptable Values: decimal ≥ 1.0
Default Value: 1.0
Description: Dielectric constant for the system. A value of 1.0 implies no modification of the electrostatic interactions. Any larger value will lessen the electrostatic forces acting in the system.

- `nonbondedScaling` < scaling factor for nonbonded forces >
Acceptable Values: decimal ≥ 0.0
Default Value: 1.0
Description: Scaling factor for electrostatic and van der Waals forces. A value of 1.0 implies no modification of the interactions. Any smaller value will lessen the nonbonded forces acting in the system.

- `vdwGeometricSigma` < use geometric mean to combine L-J sigmas >
Acceptable Values: `yes` or `no`

Default Value: `no`
Description: Use geometric mean, as required by OPLS, rather than traditional arithmetic mean when combining Lennard-Jones sigma parameters for different atom types.

- `limitdist` < maximum distance between pairs for limiting interaction strength(Å) >
Acceptable Values: non-negative decimal
Default Value: `0.`
Description: The electrostatic and van der Waals potential functions diverge as the distance between two atoms approaches zero. The potential for atoms closer than `limitdist` is instead treated as $ar^2 + c$ with parameters chosen to match the force and potential at `limitdist`. This option should primarily be useful for alchemical free energy perturbation calculations, since it makes the process of creating and destroying atoms far less drastic energetically. The larger the value of `limitdist` the more the maximum force between atoms will be reduced. In order to not alter the other interactions in the simulation, `limitdist` should be less than the closest approach of any non-bonded pair of atoms; 1.3 Å appears to satisfy this for typical simulations but the user is encouraged to experiment. There should be no performance impact from enabling this feature.

- `LJcorrection` < Apply long-range corrections to the system energy and virial to account for neglected vdW forces? >
Acceptable Values: `yes` or `no`
Default Value: `no`
Description: Apply an analytical correction to the reported vdW energy and virial that is equal to the amount lost due to switching and cutoff of the LJ potential. The correction will use the average of vdW parameters for all particles in the system and assume a constant, homogeneous distribution of particles beyond the switching distance. See [99] for details (the equations used in the NAMD implementation are slightly different due to the use of a different switching function). Periodic boundary conditions are required to make use of tail corrections.

5.2.4 PME parameters

PME stands for Particle Mesh Ewald and is an efficient full electrostatics method for use with periodic boundary conditions. None of the parameters should affect energy conservation, although they may affect the accuracy of the results and momentum conservation.

- `PME` < Use particle mesh Ewald for electrostatics? >
Acceptable Values: `yes` or `no`
Default Value: `no`
Description: Turns on particle mesh Ewald.

- `PMETolerance` < PME direct space tolerance >
Acceptable Values: positive decimal
Default Value: 10^{-6}
Description: Affects the value of the Ewald coefficient and the overall accuracy of the results.

- `PMEInterpOrder` < PME interpolation order >
Acceptable Values: positive integer

Default Value: 4 (cubic)
Description: Charges are interpolated onto the grid and forces are interpolated off using this many points, equal to the order of the interpolation function plus one.

- `PMEGridSpacing` < maximum space between grid points >
Acceptable Values: positive real
Description: The grid spacing partially determines the accuracy and efficiency of PME. If any of the grid sizes below are not set, then PMEGridSpacing must be set (recommended value is 1.0 Å) and will be used to calculate them. If a grid size is set, then the grid spacing must be at least PMEGridSpacing (if set, or a very large default of 1.5).

- `PMEGridSizeX` < number of grid points in x dimension >
Acceptable Values: positive integer
Description: The grid size partially determines the accuracy and efficiency of PME. For speed, `PMEGridSizeX` should have only small integer factors (2, 3 and 5).

- `PMEGridSizeY` < number of grid points in y dimension >
Acceptable Values: positive integer
Description: The grid size partially determines the accuracy and efficiency of PME. For speed, `PMEGridSizeY` should have only small integer factors (2, 3 and 5).

- `PMEGridSizeZ` < number of grid points in z dimension >
Acceptable Values: positive integer
Description: The grid size partially determines the accuracy and efficiency of PME. For speed, `PMEGridSizeZ` should have only small integer factors (2, 3 and 5).

- `PMEProcessors` < processors for FFT and reciprocal sum >
Acceptable Values: positive integer
Default Value: larger of x and y grid sizes up to all available processors
Description: For best performance on some systems and machines, it may be necessary to restrict the amount of parallelism used. Experiment with this parameter if your parallel performance is poor when PME is used.

- `FFTWEstimate` < Use estimates to optimize FFT? >
Acceptable Values: `yes` or `no`
Default Value: `no`
Description: Do not optimize FFT based on measurements, but on FFTW rules of thumb. This reduces startup time, but may affect performance.

- `FFTWUseWisdom` < Use FFTW wisdom archive file? >
Acceptable Values: `yes` or `no`
Default Value: `yes`
Description: Try to reduce startup time when possible by reading FFTW "wisdom" from a file, and saving wisdom generated by performance measurements to the same file for future use. This will reduce startup time when running the same size PME grid on the same number of processors as a previous run using the same file.

- `FFTWWisdomFile` < name of file for FFTW wisdom archive >
Acceptable Values: file name
Default Value: FFTW_NAMD_*version_platform*.txt

Description: File where FFTW wisdom is read and saved. If you only run on one platform this may be useful to reduce startup times for all runs. The default is likely sufficient, as it is version and platform specific.

5.2.5 MSM parameters

The multilevel summation method (MSM) [44] is an alternative to PME for calculating full electrostatic interactions. The use of the FFT in PME has two drawbacks: (1) it generally requires the use of periodic boundary conditions, in which the simulation describes an infinite three-dimensional lattice, with each lattice cell containing a copy of the simulated system, and (2) calculation of the FFT becomes a considerable performance bottleneck to the parallel scalability of MD simulations, due to the many-to-many communication pattern employed. MSM avoids the use of the FFT in its calculation, instead employing the nested interpolation in real space of softened pair potentials, which permits in addition to periodic boundary conditions the use of semi-periodic boundaries, in which there is periodicity along just one or two basis vectors, or non-periodic boundaries, in which the simulation is performed in a vacuum. Also, better parallel scaling has been observed with MSM when scaling a sufficiently large system to a large number of processors. See the MSM research web page (`http://www.ks.uiuc.edu/Research/msm/`) for more information.

In order to use the MSM, one need only specify "MSM on" in the configuration file. For production use, we presently recommend using the default "MSMQuality 0" (C^1 cubic interpolation with C^2 Taylor splitting), which has been validated to correctly reproduce the PME results [44]. At this time, we discourage use of the higher order interpolation schemes (Hermite, quintic, etc.), as they are still under development. With cubic interpolation, MSM now gets roughly half the performance of PME. Comparable performance and better scaling for MSM have been observed with the optimizations described in Ref. [44], which will be available shortly.

For now, NAMD's implementation of the MSM does not calculate the long-range electrostatic contribution to the virial, so use with a barostat for constant pressure simulation is inappropriate. (Note that the experiments in Ref. [44] involving constant pressure simulation with MSM made use of a custom version that is incompatible with some other NAMD features, so is not yet available.) The performance of PME is generally still better for smaller systems with smaller processor counts. MSM is the only efficient method in NAMD for calculating full electrostatics for simulations with semi-periodic or non-periodic boundaries.

The periodicity is defined through setting the cell basis vectors appropriately, as discussed in Sec. 7. The cutoff distance, discussed earlier in this section, also determines the splitting distance between the MSM short-range part, calculated exactly, and long-range part, interpolated from the grid hierarchy; this splitting distance is the primary control for accuracy for a given interpolation and splitting, although most simulations will likely want to keep the cutoff set to the CHARMM-prescribed value of 12 Å.

The configuration options specific to MSM are listed below. A simulation employing non-periodic boundaries in one or more dimensions might have atoms that attempt to drift beyond the predetermined extent of the grid. In the case that an atom does drift beyond the grid, the simulation will be halted prematurely with an error message. Several options listed below deal with defining the extent of the grid along non-periodic dimensions beyond what can be automatically determined by the initial coordinates. It is also recommended for non-periodic simulation to configure boundary restraints to contain the atoms, for instance, through Tcl boundary forces in Sec. 8.11.

- `MSM` < Use multilevel summation method for electrostatics? >

Acceptable Values: `yes` or `no`
Default Value: `no`
Description: Turns on multilevel summation method.

- `MSMGridSpacing` < spacing between finest level grid points (Å) >
  Acceptable Values: positive real
  Default Value: 2.5
  Description: The grid spacing determines in part the accuracy and efficiency of MSM. An error versus cost analysis shows that the best tradeoff is setting the grid spacing to a value close to the inter-particle spacing. The default value works well in practice for atomic scale simulation. This value will be exact along non-periodic dimensions. For periodic dimensions, the grid spacing must evenly divide the basis vector length; the actual spacing for a desired grid spacing h is guaranteed to be within the interval $\left[\frac{4}{5}h, \frac{6}{5}h\right)$.

- `MSMQuality` < select the approximation quality >
  Acceptable Values: $0, 1, 2, 3, 4$
  Default Value: 0
  Description: This parameter offers a simplified way to select higher order interpolation and splitting for MSM. The available choices are:

  - 0 sets C^1 cubic ($p = 3$) interpolation with C^2 Taylor splitting,
  - 1 sets C^1 Hermite ($p = 4$) interpolation with C^3 Taylor splitting,
  - 2 sets C^1 quintic ($p = 5$) interpolation with C^3 Taylor splitting,
  - 3 sets C^1 septic ($p = 7$) interpolation with C^4 Taylor splitting,
  - 4 sets C^1 nonic ($p = 9$) interpolation with C^5 Taylor splitting.

  We presently recommend using the default selection, which has been validated to correctly reproduce the PME results [44], and discourage use of the higher order interpolation schemes, as they are still under development. With cubic interpolation, MSM now gets roughly half the performance of PME. Comparable performance and better scaling for MSM have been observed with the optimizations described in Ref. [44], which will be available shortly.

  There is generally a tradeoff between quality and performance. Empirical results show that the C^1 interpolation schemes offer a little better accuracy than the alternative interpolation schemes that have greater continuity. Also, better accuracy has been observed by using a splitting function with $C^{\lceil (p+1)/2 \rceil}$ continuity where p is the order of the interpolant.

- `MSMApprox` < select the interpolant >
  Acceptable Values: $0, 1, \ldots, 7$
  Default Value: 0
  Description: Select the interpolation scheme:

  - 0 sets C^1 cubic ($p = 3$) interpolation,
  - 1 sets C^1 quintic ($p = 5$) interpolation,
  - 2 sets C^2 quintic ($p = 5$) interpolation,
  - 3 sets C^1 septic ($p = 7$) interpolation,
  - 4 sets C^3 septic ($p = 7$) interpolation,

- 5 sets C^1 nonic ($p = 9$) interpolation,
- 6 sets C^4 nonic ($p = 9$) interpolation,
- 7 sets C^1 Hermite ($p = 4$) interpolation.

- `MSMSplit` < select the splitting >
  Acceptable Values: $0, 1, \ldots, 6$
  Default Value: 0
  Description: Select the splitting function:
  - 0 sets C^2 Taylor splitting,
  - 1 sets C^3 Taylor splitting,
  - 2 sets C^4 Taylor splitting,
  - 3 sets C^5 Taylor splitting,
  - 4 sets C^6 Taylor splitting,
  - 5 sets C^7 Taylor splitting,
  - 6 sets C^8 Taylor splitting.

- `MSMLevels` < maximum number of levels >
  Acceptable Values: non-negative integer
  Default Value: 0
  Description: Set the maximum number of levels to use in the grid hierarchy. Although setting slightly lower than the default might (or might not) improve performance and/or accuracy for non-periodic simulation, it is generally best to leave this at the default value "0" which will then automatically adjust the levels to the size of the given system.

- `MSMPadding` < grid padding (Å) >
  Acceptable Values: non-negative real
  Default Value: 2.5
  Description: The grid padding applies only to non-periodic dimensions, for which the extent of the grid is automatically determined by the maximum and minimum of the initial coordinates plus the padding value.

- `MSMxmin, MSMymin, MSMzmin` < minimum x-, y-, z-coordinate (Å) >
  Acceptable Values: real
  Description: Set independently the minimum x-, y-, or z-coordinates of the simulation. This parameter is applicable only to non-periodic dimensions. It is useful in conjunction with setting a boundary restraining force with Tcl boundary forces in Sec. 8.11.

- `MSMxmax, MSMymax, MSMzmax` < maximum x-, y-, z-coordinate (Å) >
  Acceptable Values: real
  Description: Set independently the maximum x-, y-, or z-coordinates of the simulation. This parameter is applicable only to non-periodic dimensions. It is useful in conjunction with setting a boundary restraining force with Tcl boundary forces in Sec. 8.11.

- `MSMBlockSizeX, MSMBlockSizeY, MSMBlockSizeZ` < block size for grid decomposition >
  Acceptable Values: positive integer
  Default Value: 8

Description: Tune parallel performance by adjusting the block size used for parallel domain decomposition of the grid. Recommended to keep the default.

- `MSMSerial` < Use serial long-range solver? >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: Enable instead the slow serial long-range solver. Intended to be used only for testing and diagnostic purposes.

5.2.6 Full direct parameters

The direct computation of electrostatics is not intended to be used during real calculations, but rather as a testing or comparison measure. Because of the $\mathcal{O}(N^2)$ computational complexity for performing direct calculations, this is *much* slower than using PME or MSM to compute full electrostatics for large systems. In the case of periodic boundary conditions, the nearest image convention is used rather than a full Ewald sum.

- `FullDirect` < calculate full electrostatics directly? >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: Specifies whether or not direct computation of full electrostatics should be performed.

5.2.7 Tabulated nonbonded interaction parameters

In order to support coarse grained models and semiconductor force fields, the tabulated energies feature replaces the normal van der Waals potential for specified pairs of atom types with one interpolated from user-supplied energy tables. The electrostatic potential is not altered.

Pairs of atom types to which the modified interactions apply are specified in a CHARMM parameter file by an `NBTABLE` section consisting of lines with two atom types and a corresponding interaction type name. For example, tabulated interactions for SI-O, O-O, and SI-SI pairs would be specified in a parameter file as:

```
NBTABLE
SI O SIO
O O OO
SI SI SISI
```

Each interaction type must correspond to an entry in the energy table file. The table file consists of a header formatted as:

```
# multiple comment lines
<number_of_tables> <table_spacing (A)> <maximum_distance (A)>
```

followed by *number_of_tables* energy tables formatted as:

```
TYPE <interaction type name>
0 <energy (kcal/mol)> <force (kcal/mol/A)>
<table_spacing> <energy (kcal/mol)> <force (kcal/mol/A)>
<2*table_spacing> <energy (kcal/mol)> <force (kcal/mol/A)>
```

```
<3*table_spacing> <energy (kcal/mol)> <force (kcal/mol/A)>
...
<maximum_distance - 3*table_spacing> <energy (kcal/mol)> <force (kcal/mol/A)>
<maximum_distance - 2*table_spacing> <energy (kcal/mol)> <force (kcal/mol/A)>
<maximum_distance - table_spacing> <energy (kcal/mol)> <force (kcal/mol/A)>
```

The table entry at *maximum_distance* will match the energy of the previous entry but have a force of zero. The maximum distance must be at least equal to the nonbonded cutoff distance and entries beyond the cutoff distance will be ignored. For the above example with a cutoff of 12 Å the table file could look like:

```
# parameters for silicon dioxide
3 0.01 14.0
TYPE SIO
0 5.092449e+26 3.055469e+31
0.01 5.092449e+14 3.055469e+17
0.02 7.956951e+12 2.387085e+15
0.03 6.985526e+11 1.397105e+14
...
13.98 0.000000e+00 -0.000000e+00
13.99 0.000000e+00 -0.000000e+00
TYPE OO
0 1.832907e+27 1.099744e+32
0.01 1.832907e+15 1.099744e+18
0.02 2.863917e+13 8.591751e+15
0.03 2.514276e+12 5.028551e+14
...
13.98 0.000000e+00 -0.000000e+00
13.99 0.000000e+00 -0.000000e+00
TYPE SISI
0 0.000000e+00 -0.000000e+00
0.01 0.000000e+00 -0.000000e+00
...
13.98 0.000000e+00 -0.000000e+00
13.99 0.000000e+00 -0.000000e+00
```

The following three parameters are required for tabulated energies.

- `tabulatedEnergies` < use tabulated energies >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: Specifies whether or not tabulated energies will be used for van der Waals interactions between specified pairs of atom types.

- `tabulatedEnergiesFile` < file containing energy table >
  Acceptable Values: file name
  Description: Provides one energy table for each interaction type in parameter file. See format above.

- `tableInterpType` < cubic or linear interpolation >
  Acceptable Values: `cubic` or `linear`
  Description: Specifies the order for interpolating between energy table entries.

5.3 Water Models

NAMD currently supports the 3-site TIP3P water model, the 4-site TIP4P water model, and the 5-site SWM4-NDP water model (from the Drude force field) [61]. TIP3P is the current default water model. Usage of alternative water models is described below.

- `waterModel` < using which water model? >
  Acceptable Values: `tip3`, `tip4`, `swm4`
  Default Value: `tip3`
  Description: Specifies the water model to be used. When using the TIP3P water model, the ordering of atoms within each TIP3P water molecule must be oxygen, hydrogen, hydrogen. When using the TIP4P water model, the ordering of atoms within each TIP4P water molecule must be oxygen, hydrogen, hydrogen, lone pair. When using the SWM4-NDP water model, the ordering of atoms within each SWM4-NDP water molecule must be oxygen, Drude particle, lone pair, hydrogen, hydrogen. Alternative orderings will fail.

5.4 Drude polarizable force field

The Drude oscillator model represents induced electronic polarization by introducing an auxiliary particle attached to each polarizable atom via a zero-length harmonic spring. The advantage with the Drude model is that it preserves the simple particle-particle Coulomb electrostatic interaction employed in nonpolarizable force fields, thus its implementation in NAMD is more straightforward than alternative models for polarization. NAMD performs the integration of Drude oscillators by employing a novel dual Langevin thermostat to "freeze" the Drude oscillators while maintaining the normal "warm" degrees of freedom at the desired temperature [51]. Use of the Langevin thermostat enables better parallel scalability than the earlier reported implementation which made use of a dual Nosé-Hoover thermostat acting on, and within, each nucleus-Drude pair [62]. Performance results show that the NAMD implementation of the Drude model maintains good parallel scalability with an increase in computational cost by not more than twice that of using a nonpolarizable force field [51].

Excessive "hyperpolarization" of Drude oscillators can be prevented by two different schemes. The default "hard wall" option reflects elongated springs back towards the nucleus using a simple collision model. Alternatively, the Drude oscillators can be supplemented by a flat-bottom quartic restraining potential (usually with a large force constant).

The Drude polarizable force field requires some extensions to the CHARMM force field. An anisotropic spring term is added to account for out-of-plane forces from a polarized atom and its covalently bonded neighbor with two more covalently bonded neighbors (similar in structure to an improper bond). The screened Coulomb correction of Thole is calculated between pairs of Drude oscillators that would otherwise be excluded from nonbonded interaction and optionally between non-excluded, nonbonded pairs of Drude oscillators that are within a prescribed cutoff distance [110, 111]. Also included in the Drude force field are the use of off-centered massless interaction sites, so called "lone pairs" (LPs), to avoid the limitations of centrosymmetric-based Coulomb interactions [43]. The coordinate of each LP site is constructed based on three "host"

atoms. The calculated forces on the massless LP must be transferred to the host atoms, preserving total force and torque. After an integration step of velocities and positions, the position of the LP is updated based on the three host atoms, along with additional geometry parameters that give displacement and in-plane and out-of-plane angles. See our research web page (http://www.ks.uiuc.edu/Research/Drude/) for additional details and parallel performance results.

5.4.1 Required input files

No additional files are required by NAMD to use the Drude polarizable force field. However, it is presently beyond the capability of the `psfgen` tool to generate the PSF file needed to perform a simulation using the Drude model. For now, CHARMM is needed to generate correct input files.

The CHARMM force field parameter files specific to the Drude model are required. The PDB file must also include the Drude particles (mass between 0.05 and 1.0) and the LPs (mass 0). The Drude particles always immediately follow their parent atom. The PSF file augments the "atom" section with additional columns that include the "Thole" and "alpha" parameters for the screened Coulomb interactions of Thole. The PSF file also requires additional sections that list the LPs, including their host atoms and geometry parameters, and list the anisotropic interaction terms, including their parameters. A Drude-compatible PSF file is denoted by the keyword "DRUDE" given along the top line.

5.4.2 Standard output

The NAMD logging to standard output is extended to provide additional temperature data on the cold and warm degrees of freedom. Four additional quantities are listed on the `ETITLE` and `ENERGY` lines:

`DRUDECOM` gives the temperature for the warm center-of-mass degrees of freedom,

`DRUDEBOND` gives the temperature for the cold Drude oscillator degrees of freedom,

`DRCOMAVG` gives the average temperature (averaged since the previously reported temperature) for the warm center-of-mass degrees of freedom,

`DRBONDAVG` gives the average temperature (averaged since the previously reported temperature) for the cold Drude oscillator degrees of freedom.

The energies resulting from the Drude oscillators and the anisotropic interactions are summed into the `BOND` energy. The energies resulting from the LPs and the screened Coulomb interactions of Thole are summed into the `ELECT` energy.

5.4.3 Drude force field parameters

The Drude model should be used with the Langevin thermostat enabled (`Langevin=on`). Doing so permits the use of normal sized time steps (e.g., 1 fs). The Drude model is also compatible with constant pressure simulation using the Langevin piston. Long-range electrostatics may be calculated using PME. The nonbonded exclusions should generally be set to use either the 1-3 exclusion policy (`exclude=1-3`) or the scaled 1-4 exclusion policy (`exclude=scaled1-4`).

The Drude water model (SWM4-NDP) is a 5-site model with four charge sites and a negatively charged Drude particle [61], with the particles ordered in the input files as oxygen, Drude particle, LP, hydrogen, hydrogen. The atoms in the water molecules should be constrained

(`rigidBonds=water`), with use of the SETTLE algorithm recommended (`useSettle=on`). Explicitly setting the water model (`waterModel=swm4`) is optional.

- `drude` < Perform integration of Drude oscillators? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: The integration uses a dual Langevin thermostat to freeze the Drude oscillators while maintaining the warm degrees of freedom at the desired temperature. Must also enable the Langevin thermostat. If `drude` is set to `on`, then `drudeTemp` must also be defined.

- `drudeTemp` < temperature for freezing the Drude oscillators (K) >
  Acceptable Values: non-negative decimal
  Description: For stability, the Drude oscillators must be kept at a very cold termpature. Using a Langevin thermostat, it is possible to set this temperature to 0 K.

- `drudeDamping` < damping coefficient for Drude oscillators (1/ps) >
  Acceptable Values: positive decimal
  Description: The Langevin coupling coefficient to be applied to the Drude oscillators. If not given, `drudeDamping` is set to the value of `langevinDamping`, but values of as much as an order of magnitude greater may be appropriate.

- `drudeNbTholeCut` < nonbonded Thole interaction radius (Å) >
  Acceptable Values: positive decimal
  Default Value: 5.0
  Description: If `drudeNbTholeCut` is non-zero, the screened Coulomb correction of Thole is also calculated for non-excluded, nonbonded pairs of Drude oscillators that are within this radius of interaction.

- `drudeHardWall` < use collisions to correct hyperpolarization? >
  Acceptable Values: on or off
  Default Value: on
  Description: Excessively elongated Drude oscillator bonds are avoided by reflective collisions induced at a fixed cutoff, `drudeBondLen`. A large number of such events is usually indicative of unstable/unphysical dynamics and a simulation will stop if double the cutoff is exceeded.

- `drudeBondLen` < hyperpolarization cutoff (Å) >
  Acceptable Values: positive decimal
  Default Value: 0.25
  Description: If using `drudeHardWall on`, this is the distance at which collisions occur. Otherwise, this is the distance at which an additional quartic restraining potential is applied to each Drude oscillator. In this latter case, a value of 0.2 Å (slightly smaller than default) is recommended.

- `drudeBondConst` < Drude oscillator restraining force constant >
  Acceptable Values: positive decimal
  Default Value: 40000.0
  Description: If `drudeHardWall off` and `drudeBondConst` is non-zero, an additional quartic restraining potential is applied to a Drude oscillator if its length exceeds `drudeBondLen`.

5.5 MARTINI Residue-Based Coarse-Grain Forcefield

The MARTINI forcefield for residue-based coarse-grain models allows simulation of several tens of atoms as only several large coarse-grained particles [72, 73, 78]. In the MARTINI model, each protein residue is represented by a backbone bead and usually one or more sidechain beads.

When preparing MARTINI simulations it is important to include only those dihedrals specified by the forcefield. Using the "auto dihedrals" or "regenerate dihedrals" feature of psfgen will create dihedrals for all possible sets of four bonded atoms. This is incorrect for MARTINI and will result in energy jumps because the dihedral potential function is degenerate for the angles of 180 degrees allowed by cosine-based angles.

When using MARTINI the following configuration parameters should be set as indicated:

```
cosAngles on
martiniSwitching on
dielectric 15.0
PME off
```

- `cosAngles` < enable the MARTINI cosine-based angle potential function >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not the MARTINI forcefield is being used, specifically cosine-based angle potential function. The cosine-based potential will only be used for angles in CHARMM parameter files that specify the cos keyword.

- `martiniSwitching` < enable the MARTINI Lennard-Jones switching function? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not the MARTINI forcefield is being used, specifically the Lennard-Jones switching function.

- `martiniDielAllow` < Allow dielectrics != 15.0 for use with MARTINI >
  Acceptable Values: `on` or `off`
  Description: `off` Allows user to specify a `dielectric` not equal to 15.0, ie a non-standard dielectric for MARTINI.

5.6 Constraints and Restraints

5.6.1 Bond constraint parameters

- `rigidBonds` < controls if and how ShakeH is used >
  Acceptable Values: `none`, `water`, `all`
  Default Value: `none`
  Description: When `water` is selected, the hydrogen-oxygen and hydrogen-hydrogen distances in waters are constrained to the nominal length or angle given in the parameter file, making the molecules completely rigid. When `rigidBonds` is `all`, waters are made rigid as described above and the bond between each hydrogen and the (one) atom to which it is bonded is similarly constrained. For the default case `none`, no lengths are constrained.

- `rigidTolerance` < allowable bond-length error for ShakeH (Å) >
  Acceptable Values: positive decimal

Default Value: 1.0e-8
Description: The ShakeH algorithm is assumed to have converged when all constrained bonds differ from the nominal bond length by less than this amount.

- `rigidIterations` < maximum ShakeH iterations >
  Acceptable Values: positive integer
  Default Value: 100
  Description: The maximum number of iterations ShakeH will perform before giving up on constraining the bond lengths. If the bond lengths do not converge, a warning message is printed, and the atoms are left at the final value achieved by ShakeH. Although the default value is 100, convergence is usually reached after fewer than 10 iterations.

- `rigidDieOnError` < maximum ShakeH iterations >
  Acceptable Values: `on` or `off`
  Default Value: `on`
  Description: Exit and report an error if rigidTolerance is not achieved after rigidIterations.

- `useSettle` < Use SETTLE for waters. >
  Acceptable Values: `on` or `off`
  Default Value: `on`
  Description: If rigidBonds are enabled then use the non-iterative SETTLE algorithm to keep waters rigid rather than the slower SHAKE algorithm.

5.6.2 Position restraint parameters

The following describes the parameters for the position restraints feature of NAMD. For historical reasons the term "constraints" has been carried over from X-PLOR. This feature allows a restraining potential to each atom of an arbitrary set during the simulation.

- `constraints` < are position restraints active? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not position restraints are active. If it is set to `off`, then no position restraints are computed. If it is set to `on`, the potential $k \times (\mathbf{x} - \mathbf{x}_0)^p$ is applied to each atom. Per-atom values for k can be defined by either `conskfile` or `conskcol`, for $\mathbf{x}_0$ by `consref`, and for p by `consexp`.

- `consexp` < exponent for position restraint energy function >
  Acceptable Values: positive, even integer
  Default Value: 2
  Description: Exponent to be use in the position restraint energy function. This value must be a positive integer, and only even values really make sense. This parameter is used only if `constraints` is set to `on`.

- `consref` < PDB file containing restraint reference positions >
  Acceptable Values: UNIX file name
  Description: PDB file to use for reference positions for position restraints. Each atom that has a positive force constant will be restrained about the position specified in this file.

- `conskfile` < PDB file containing force constant values >
  Acceptable Values: UNIX filename
  Description: PDB file to use for force constants for position restraints.

- `conskcol` < column of PDB file containing force constant >
  Acceptable Values: `X`, `Y`, `Z`, `O`, or `B`
  Description: Column of the PDB file to use for the position restraint force constant. This parameter may specify any of the floating point fields of the PDB file, either X, Y, Z, occupancy, or beta-coupling (temperature-coupling). Regardless of which column is used, a value of 0 indicates that the atom qshould not be restrained. Otherwise, the value specified is used as the force constant for that atom's restraining potential.

- `constraintScaling` < scaling factor for position restraint energy function >
  Acceptable Values: positive
  Default Value: 1.0
  Description: The position restraint energy function is multiplied by this parameter, making it possible to gradually turn off restraints during equilibration. This parameter is used only if `constraints` is set to `on`.

- `selectConstraints` < Restrain only selected Cartesian components of the coordinates? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: This option is useful to restrain the positions of atoms to a plane or a line in space. If active, this option will ensure that only selected Cartesian components of the coordinates are restrained. E.g.: Restraining the positions of atoms to their current z values with no restraints in x and y will allow the atoms to move in the x-y plane while retaining their original z-coordinate. Restraining the x and y values will lead to free motion only along the z coordinate.

- `selectConstrX` < Restrain X components of coordinates >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Restrain the Cartesian x components of the positions.

- `selectConstrY` < Restrain Y components of coordinates >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Restrain the Cartesian y components of the positions.

- `selectConstrZ` < Restrain Z components of coordinates >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Restrain the Cartesian z components of the positions.

5.6.3 Fixed atoms parameters

Atoms may be held fixed during a simulation. NAMD avoids calculating most interactions in which all affected atoms are fixed unless `fixedAtomsForces` is specified.

- `fixedAtoms` < are there fixed atoms? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not fixed atoms are present.

- `fixedAtomsForces` < are forces between fixed atoms calculated? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not forces between fixed atoms are calculated. This option is required to turn fixed atoms off in the middle of a simulation. These forces will affect the pressure calculation, and you should leave this option off when using constant pressure if the coordinates of the fixed atoms have not been minimized. The use of constant pressure with significant numbers of fixed atoms is not recommended.

- `fixedAtomsFile` < PDB file containing fixed atom parameters >
  Acceptable Values: UNIX filename
  Default Value: `coordinates`
  Description: PDB file to use for the fixed atom flags for each atom. If this parameter is not specified, then the PDB file specified by `coordinates` is used.

- `fixedAtomsCol` < column of PDB containing fixed atom parameters >
  Acceptable Values: `X`, `Y`, `Z`, `O`, or `B`
  Default Value: `O`
  Description: Column of the PDB file to use for the containing fixed atom parameters for each atom. The coefficients can be read from any floating point column of the PDB file. A value of 0 indicates that the atom is not fixed.

5.6.4 Extra bond, angle, and dihedral restraints

Additional bond, angle, and dihedral energy terms may be applied to system, allowing secondary or tertiary structure to be restrained, for example. Extra bonded terms are not considered part of the molecular structure and hence do not alter nonbonded exclusions. The energies from extra bonded terms are included with the normal bond, angle, and dihedral energies in NAMD output.

All extra bonded terms are harmonic potentials of the form $U(x) = k(x-x_{ref})^2$ except dihedrals and impropers with a non-zero periodicity specified, which use $U(x) = k(1 + cos(nx - x_{ref}))$. The only difference between dihedrals and impropers is the output field that their potential energy is added to.

Due to a very old bug all NAMD releases prior to 2.13 have used the MARTINI cosine-based angle potential function for all extra angles. Since workflows may unknowingly depend on this undocumented behavior, cosine-based angles remain the default, but a warning is printed unless the desired behavior is specified via the new option extraBondsCosAngles (defaults to "on", set to "off" to use the normal harmonic angle potential function for all extra angles).

The extra bonded term implementation shares the parallel implementation of regular bonded terms in NAMD, allowing large numbers of extra terms to be specified with minimal impact on parallel scalability. Extra bonded terms do not have to duplicate normal bonds/angles/dihedrals, but each extra bond/angle/dihedral should only involve nearby atoms. If the atoms involved are too far apart a bad global bond count will be reported in parallel runs.

Extra bonded terms are enabled via the following options:

- `extraBonds` < enable extra bonded terms? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not extra bonded terms are present.

- `extraBondsCosAngles` < are extra angles cosine-based? >
  Acceptable Values: `on` or `off`
  Default Value: `on`
  Description: Specifies whether or not all extra angles are cosine-based for consistency with previous versions. Set to `off` to use normal harmonic angle potential for all extra angles.

- `extraBondsFile` < file containing extra bonded terms >
  Acceptable Values: file
  Description: File containing extra bonded terms. May be repeated for multiple files.

The extra bonds file(s) should contain lines of the following formats:

- `bond <atom> <atom> <k> <ref>`
- `angle <atom> <atom> <atom> <k> <ref>`
- `dihedral <atom> <atom> <atom> <atom> <k> <ref>`
- `dihedral <atom> <atom> <atom> <atom> <k> <n> <ref>`
- `improper <atom> <atom> <atom> <atom> <k> <ref>`
- `improper <atom> <atom> <atom> <atom> <k> <n> <ref>`
- `wall <atom> <atom> <k> <lower> <upper>`
- `# <comment ...>`

In all cases `<atom>` is a **zero-based** atom index (the first atom has index 0), `<ref>` is a reference distance in Å (bond) or angle in degrees (others), and `<k>` is a spring constant in the potential energy function $U(x) = k(x - x_{ref})^2$ or, for dihedrals and impropers with periodicity `<n>` specified and not 0, $U(x) = k(1 + cos(nx - x_{ref}))$. Note that x_{ref} is only a minimum for the harmonic potential; the sinusoidal potential has minima at $(x_{ref} + 180)/n + i \times 360/n$.

Use of `wall` implements a harmonic wall potential similar to the Colvars harmonic wall restraint. The potential function is

$$U(x) = \begin{cases} k(x - x_{\text{upper}})^2, & \text{if } x > x_{\text{upper}} \\ 0, & \text{if } x_{\text{lower}} \leq x \leq x_{\text{upper}} \\ k(x - x_{\text{lower}})^2, & \text{if } x < x_{\text{lower}} \end{cases} .$$

6 Generalized Born Implicit Solvent

Generalized Born implicit solvent (GBIS) is a fast but approximate method for calculating molecular electrostatics in solvent as described by the Poisson Boltzmann equation which models water as a dielectric continuum. GBIS enables the simulation of atomic structures without including explicit solvent water. The elimination of explicit solvent greatly accelerates simulations; this speedup is lessed by the increased computational complexity of the implicit solvent electrostatic calculation and a longer interaction cutoff. These are discussed in greater detail below.

6.1 Theoretical Background

Water has many biologically necessary properties, one of which is as a dielectric. As a dielectric, water screens (lessens) electrostatic interactions between charged particles. Water can therefore be crudely modeled as a dielectric continuum. In this manner, the electrostatic forces of a biological system can be expressed as a system of differential equations which can be solved for the electric field caused by a collection of charges.

6.1.1 Poisson Boltzmann Equation

The Poisson Boltzmann equation (PBE),

$$\vec{\nabla} \cdot \left[\epsilon(\vec{r})\vec{\nabla}\Psi(\vec{r})\right] = -4\pi\rho^f(\vec{r}) - 4\pi \sum_i c_i^\infty q_i \lambda(\vec{r}) \cdot \exp\left[\frac{-q_i\Psi(\vec{r})}{k_B T}\right]$$

is a nonlinear equation which solves for the electrostatic field, $\Psi(\vec{r})$, based on the position dependent dielectric, $\epsilon(\vec{r})$, the position-dependent accessibility of position $\vec{r}$ to the ions in solution, $\lambda(\vec{r})$, the solute charge distribution, $\rho^f(\vec{r})$, and the bulk charge density, c_i^∞, of ion q_i. While this equation does exactly solve for the electrostic field of a charge distribution in a dielectric, it is very expensive to solve, and therefore not suitable for molecular dynamics.

6.1.2 Generalized Born

The Generalized Born (GB) equation is an approximation of the PBE. It models atoms as charged spheres whose internal dielectric is lower than that of the environment. The screening which each atom, i, experiences is determined by the local environment; the more atom i is surrounded by other atoms, the less it's electrostatics will be screened since it is more surrounded by low dielectric; this property is called one atom descreening another. Different GB models calculate atomic descreening differently. Descreening is used to calculate the Born radius, α_i, of each atom. The Born radius of an atom measures the degree of descreening. A large Born radius represents small screening (strong electric field) as if the atom were in vacuum. A small Born radius represents large screening (weak electric field) as if the atom were in bulk water. The next section describes how the Born radius is calculated and how it is used to calculate electrostatics.

6.1.3 Generalized Born Equations

In a GB simulation, the total electrostatic force on an atom, i, is the net Coulomb force on atom i (from nearby atoms) minus the GB force on atom i (also caused by nearby atoms):

$$\vec{F}_i = \vec{F}_i^{Coulomb} - \vec{F}_i^{GB}.$$

Forces are contributed by other nearby atoms within a cutoff. The GB force on atom i is the derivative of the total GB energy with respect to relative atom distances r_{ij},

$$\vec{F}_i^{GB} = -\sum_j \left[\frac{dE_T^{GB}}{dr_{ij}}\right] \hat{r}_{ji} \tag{8}$$

$$= -\sum_j \left[\sum_k \frac{\partial E_T^{GB}}{\partial \alpha_k}\frac{d\alpha_k}{dr_{ij}} + \frac{\partial E_{ij}^{GB}}{\partial r_{ij}}\right] \hat{r}_{ji} \tag{9}$$

$$= -\sum_j \left[\frac{\partial E_T^{GB}}{\partial \alpha_i}\frac{d\alpha_i}{dr_{ij}} + \frac{\partial E_T^{GB}}{\partial \alpha_j}\frac{d\alpha_j}{dr_{ij}} + \frac{\partial E_{ij}^{GB}}{\partial r_{ij}}\right] \hat{r}_{ji} \, . \tag{10}$$

where the partial derivatives are included since the Born radius, α, is a function of all relative atom distances. The total GB energy of the system is

$$E_T^{GB} = \sum_i \sum_{j>i} E_{ij}^{GB} + \sum_i E_{ii}^{GB} \, , \tag{11}$$

where E_{ii}^{GB} is the Born radius dependent self energy of atom i, and the GB energy between atoms i and j is given by

$$E_{ij}^{GB} = -k_e D_{ij} \frac{q_i q_j}{f_{ij}} \, . \tag{12}$$

The dielectric term [104] is

$$D_{ij} = \left(\frac{1}{\epsilon_p} - \frac{\exp\left(-\kappa f_{ij}\right)}{\epsilon_s}\right) \, , \tag{13}$$

and the GB function [107] is

$$f_{ij} = \sqrt{r_{ij}^2 + \alpha_i \alpha_j \exp\left(\frac{-r_{ij}^2}{4\alpha_i \alpha_j}\right)} \, . \tag{14}$$

As the Born radii of atoms i and j decrease (increasing screening), the effective distance between the atoms (f_{ij}) increases. The implicit solvent implemented in NAMD is the model of Onufriev, Bashford and Case [83, 84] which calculates the Born radius as

$$\alpha_k = \left[\frac{1}{\rho_{k0}} - \frac{1}{\rho_k}\tanh\left(\delta\psi_k - \beta\psi_k^2 + \gamma\psi_k^3\right)\right]^{-1} \tag{15}$$

where

$$\psi_k = \rho_{k0} \sum_l H_{kl} \, . \tag{16}$$

H_{ij} is the piecewise descreening function [84, 45, 95]; the seven piecewise regimes are

$$\text{Regimes} = \begin{cases} 0 & r_{ij} > r_c + \rho_{js} & \text{(sphere } j \text{ beyond cutoff)} \\ \text{I} & r_{ij} > r_c - \rho_{js} & \text{(sphere } j \text{ partially within cutoff)} \\ \text{II} & r_{ij} > 4\rho_{js} & \text{(artificial regime for smoothing)} \\ \text{III} & r_{ij} > \rho_{i0} + \rho_{js} & \text{(spheres not overlapping)} \\ \text{IV} & r_{ij} > |\rho_{i0} - \rho_{js}| & \text{(spheres overlapping)} \\ \text{V} & \rho_{i0} < \rho_{js} & \text{(sphere } i \text{ inside sphere } j\text{)} \\ \text{VI} & \text{otherwise} & \text{(sphere } j \text{ inside sphere } j\text{)} \end{cases} \tag{17}$$

and the values of H_{ij} are

$$H_{ij} = \begin{cases} 0 & 0 \\ \text{I} & \frac{1}{8r_{ij}}\left[1 + \frac{2r_{ij}}{r_{ij}-\rho_{js}} + \frac{1}{r_c^2}\left(r_{ij}^2 - 4r_c r_{ij} - \rho_{js}^2\right) + 2\ln\frac{r_{ij}-\rho_{js}}{r_c}\right] \\ \text{II} & \frac{\rho_{js}^2}{r_{ij}^2}\frac{\rho_{js}}{r_{ij}^2}\left[a + \frac{\rho_{js}^2}{r_{ij}^2}\left(b + \frac{\rho_{js}^2}{r_{ij}^2}\left(c + \frac{\rho_{js}^2}{r_{ij}^2}\left(d + \frac{\rho_{js}^2}{r_{ij}^2}e\right)\right)\right)\right] \\ \text{III} & \frac{1}{2}\left[\frac{\rho_{js}}{r_{ij}^2-\rho_{js}^2} + \frac{1}{2r_{ij}}\ln\frac{r_{ij}-\rho_{js}}{r_{ij}+\rho_{js}}\right] \\ \text{IV} & \frac{1}{4}\left[\frac{1}{\rho_{i0}}\left(2 - \frac{1}{2r_{ij}\rho_{i0}}\left(r_{ij}^2 + \rho_{i0}^2 - \rho_{js}^2\right)\right) - \frac{1}{r_{ij}+\rho_{js}} + \frac{1}{r_{ij}}\ln\frac{\rho_{i0}}{r_{ij}+\rho_{js}}\right] \\ \text{V} & \frac{1}{2}\left[\frac{\rho_{js}}{r_{ij}^2-\rho_{js}^2} + \frac{2}{\rho_{i0}} + \frac{1}{2r_{ij}}\ln\frac{\rho_{js}-r_{ij}}{r_{ij}+\rho_{js}}\right] \\ \text{VI} & 0 \end{cases} \tag{18}$$

Below are defined the derivatives of the above functions which are required for force calculations.

$$\frac{\partial E_{ij}}{\partial r_{ij}} = -k_e\left[\frac{q_i q_j}{f_{ij}}\frac{\partial D_{ij}}{\partial r_{ij}} - \frac{q_i q_j D_{ij}}{f_{ij}^2}\frac{\partial f_{ij}}{\partial r_{ij}}\right] \tag{19}$$

$$\frac{\partial D_{ij}}{\partial r_{ij}} = \frac{\kappa}{\epsilon_s}\exp\left(-\kappa f_{ij}\right)\frac{\partial f_{ij}}{\partial r_{ij}} \tag{20}$$

$$\frac{\partial f_{ij}}{\partial r_{ij}} = \frac{r_{ij}}{f_{ij}}\left[1 - \frac{1}{4}\exp\left(\frac{-r_{ij}^2}{4\alpha_i\alpha_j}\right)\right] \tag{21}$$

$$\frac{d\alpha_k}{dr_{ij}} = \frac{\alpha_k^2}{\rho_k}\left(1 - \tanh^2\left(\delta\psi_k - \beta\psi_k^2 + \gamma\psi_k^3\right)\right)\left(\delta - 2\beta\psi_k + 3\beta\psi_k^2\right)\frac{d\psi_k}{dr_{ij}} \tag{22}$$

$$\begin{aligned} \frac{d\psi_k}{dr_{ij}} &= \rho_{k0}\sum_l \frac{dH_{kl}}{dr_{ij}} && (23) \\ &= \rho_{k0}\sum_l \frac{\partial H_{kl}}{\partial r_{kl}}\frac{dr_{kl}}{dr_{ij}} && (24) \\ &= \rho_{k0}\left[\frac{\partial H_{kj}}{\partial r_{kj}}\delta_{ki} + \frac{\partial H_{ki}}{\partial r_{ki}}\delta_{kj}\right] && (25) \end{aligned}$$

$$\begin{aligned} \frac{d\alpha_k}{dr_{ij}} = &\quad \frac{\alpha_i^2\rho_{i0}}{\rho_i}\left(1 - \tanh^2\left(\delta\psi_i - \beta\psi_i^2 + \gamma\psi_i^3\right)\right)\left(\delta - 2\beta\psi_i + 3\beta\psi_i^2\right)\frac{\partial H_{ij}}{\partial r_{ij}}\delta_{ki} \\ + &\quad \frac{\alpha_j^2\rho_{j0}}{\rho_j}\left(1 - \tanh^2\left(\delta\psi_j - \beta\psi_j^2 + \gamma\psi_j^3\right)\right)\left(\delta - 2\beta\psi_j + 3\beta\psi_j^2\right)\frac{\partial H_{ji}}{\partial r_{ij}}\delta_{kj} \end{aligned} \tag{26}$$

$$\frac{\partial E_{ij}}{\partial \alpha_i} = -\frac{1}{\alpha_i}\frac{k_e q_i q_j}{2f_{ij}^2}\left(\frac{\kappa}{\epsilon_s}\exp\left(-\kappa f_{ij}\right) - \frac{D_{ij}}{f_{ij}}\right)\left(\alpha_i\alpha_j + \frac{r_{ij}^2}{4}\right)\exp\left(\frac{-r_{ij}^2}{4\alpha_i\alpha_j}\right) \tag{27}$$

$$\frac{\partial E_{ij}}{\partial \alpha_j} = -\frac{1}{\alpha_j}\frac{k_e q_i q_j}{2f_{ij}^2}\left(\frac{\kappa}{\epsilon_s}\exp\left(-\kappa f_{ij}\right) - \frac{D_{ij}}{f_{ij}}\right)\left(\alpha_i\alpha_j + \frac{r_{ij}^2}{4}\right)\exp\left(\frac{-r_{ij}^2}{4\alpha_i\alpha_j}\right) \tag{28}$$

$$\frac{\partial H_{ij}}{\partial r_{ij}} = \begin{cases} 0 & 0 \\ \text{I} & \left[-\frac{(r_c+\rho_{js}-r_{ij})(r_c-\rho_{js}+r_{ij})\left(\rho_{js}^2+r_{ij}^2\right)}{8r_c^2 r_{ij}^2(\rho_{js}-r_{ij})^2} - \frac{1}{4r_{ij}^2}\ln\frac{r_{ij}-\rho_{js}}{r_c} \right] \\ \text{II} & \left[-4a\frac{\rho_{js}^3}{r_{ij}^5} - 6b\frac{\rho_{js}^5}{r_{ij}^7} - 8c\frac{\rho_{js}^7}{r_{ij}^9} - 10d\frac{\rho_{js}^9}{r_{ij}^{11}} - 12e\frac{\rho_{js}^{11}}{r_{ij}^{13}} \right] \\ \text{III} & \frac{1}{2}\left[-\frac{\rho_{js}\left(r_{ij}^2+\rho_{js}^2\right)}{r_{ij}\left(r_{ij}^2-\rho_{js}^2\right)^2} - \frac{1}{2r_{ij}^2}\ln\frac{r_{ij}-\rho_{js}}{r_{ij}+\rho_{js}} \right] \\ \text{IV} & \frac{1}{4}\left[-\frac{1}{2\rho_{i0}^2} + \frac{r_{ij}^2\left(\rho_{i0}^2-\rho_{js}^2\right)-2r_{ij}\rho_{js}^3+\rho_{js}^2\left(\rho_{i0}^2-\rho_{js}^2\right)}{2r_{ij}^2\rho_{i0}^2(r_{ij}+\rho_{js})^2} - \frac{1}{r_{ij}^2}\ln\frac{\rho_{i0}}{r_{ij}+\rho_{js}} \right] \\ \text{V} & \frac{1}{2}\left[-\frac{\rho_{js}\left(r_{ij}^2+\rho_{js}^2\right)}{r_{ij}\left(r_{ij}^2-\rho_{js}^2\right)^2} - \frac{1}{2r_{ij}^2}\ln\frac{\rho_{js}-r_{ij}}{r_{ij}+\rho_{js}} \right] \\ \text{VI} & 0 \end{cases} \tag{29}$$

Other variables referenced in the above GB equations are

- r_{ij} - distance between atoms i and j; calculated from atom coordinates.
- κ - debye screening length; calculated from ion concentration, $\kappa^{-1} = \sqrt{\frac{\epsilon_0\epsilon_p kT}{2N_A e^2 I}}$; $\kappa^{-1} = 10$ Å for 0.1 M monovalent salt.
- ϵ_s - dielectric constant of solvent.
- ϵ_p - dielectric constant of protein.
- α_i - Born radius of atom i.
- ρ_i - intrinsic radius of atom i taken from Bondi [10].
- ρ_0 - intrinsic radius offset; $\rho_0 = 0.09$ Å by default [84].
- $\rho_{i0} = \rho_i - \rho_0$
- $\rho_{is} = \rho_{i0} S_{ij}$
- S_{ij} - atom radius scaling factor [45, 104].
- k_e - Coulomb's constant, $\frac{1}{4\pi\epsilon_0}$, 332.063711 kcal Å / e^2.
- $\{\delta, \beta, \gamma\} = \{0.8, 0, 2.91\}$ or $\{1.0, 0.8, 4.85\}$ [84]

6.2 3-Phase Calculation

The GBIS algorithm requires three phases of calculation, with each phase containing an iteration over all atom pairs with the cutoff. In phase 1, the screening of atom pairs is summed; at the conclusion of phase 1, the Born radii are calculated. In phase 2, the $\frac{\partial E_{ij}^{GB}}{\partial r_{ij}}$ force contribution (hereafter called the dEdr force) is calculated as well as the partial derivative of the Born radii with respect to the atom positions, $\frac{d\alpha_i}{dr_{ij}}$. In phase 3, the $\frac{\partial E_T^{GB}}{\partial \alpha_i}\frac{d\alpha_i}{dr_{ij}}$ force contribution (hereafter called the dEda force) is calculated.

6.3 Configuration Parameters

When using GBIS, user's should not use `PME` (because it is not compatible with GBIS). Periodic boundary conditions are supported but are optional. User's will need to increase `cutoff`; 16-18 Å is a good place to start but user's will have to check their system's behavior and increase `cutoff` accordingly. GBIS interactions are never excluded regardless of the type of force field used, thus user's can choose any value for `exclude` without affecting GBIS; user's should still choose `exclude` based on the force field as if using explicit solvent. When using GBIS, multiple timestepping behaves as follows: the dEdr force is calculated every `nonbondedFreq` steps (as with explicit solvent, 2 is a reasonable frequency) and the dEda force is calculated every `fullElectFrequency` steps (because dEda varies more slowly than dEdr, 4 is a reasonable frequency).

- `GBIS` < Use Generalized Born Implicit Solvent? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Turns on GBIS method in NAMD.

- `solventDielectric` < dielectric of water >
  Acceptable Values: positive decimal
  Default Value: 78.5
  Description: Defines the dielectric of the solvent, usually 78.5 or 80.

- `intrinsicRadiusOffset` < shrink the intrinsic radius of atoms (Å) >
  Acceptable Values: positive decimal
  Default Value: 0.09
  Description: This offset shrinks the intrinsic radius of atoms (used only for calculating Born radius) to give better agreement with Poisson Boltzmann calculations. Most users should not change this parameter.

- `ionConcentration` < concentration of ions in solvent (Molar) >
  Acceptable Values: positive decimal
  Default Value: 0.2
  Description: An ion concentration of 0 M represents distilled water. Increasing the ion concentration increases the electrostatic screening.

- `GBISDelta` < GB^{OBC} parameter for calculating Born radii >
  Acceptable Values: decimal
  Default Value: 1.0
  Description: Use $\{\texttt{GBISDelta}, \texttt{GBISBeta}, \texttt{GBISGamma}\} = \{1.0, 0.8, 4.85\}$ for GB^{OBC}II and $\{0.8, 0.0, 2.90912\}$ for GB^{OBC}I. See $\{\alpha, \beta, \gamma\}$ in [84] for more information.

- `GBISBeta` < GB^{OBC} parameter for calculating Born radii >
  Acceptable Values: decimal
  Default Value: 0.8
  Description: See `GBISDelta`.

- `GBISGamma` < GB^{OBC} parameter for calculating Born radii >
  Acceptable Values: decimal
  Default Value: 4.85
  Description: See `GBISDelta`.

- `alphaCutoff` < cutoff used in calculating Born radius and derivatives (phases 1 and 3) (Å) >
  Acceptable Values: positive decimal
  Default Value: 15
  Description: Cutoff used for calculating Born radius. Only atoms within this cutoff descreen an atom. Though `alphaCutoff` can bet set to be larger or shorter than `cutoff`, since atom forces are more sensitive to changes in position than changes in Born radius, user's should generally set `alphaCutoff` to be shorter than `cutoff`.

- `SASA` < whether or not to calculate SASA >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: The nonpolar / hydrophobic energy contribution from implicit solvent is calculated; it is proportional to the solvent-accessible surface area (SASA) which is calculated by the Linear Combination of Pairwise Overlap (LCPO) method [116]. It evaluated every `nonbondedFreq` steps and its energy is added into the reported `ELECT` energy.

- `surfaceTension` < surface tension of SASA energy >
  Acceptable Values: positive decimal
  Default Value: 0.005 kcal/mol/Å^2
  Description: Surface tension used when calculating hydrophobic `SASA` energy; $E_{\text{nonpolar}} =$ surfaceTension $\times$ surfaceArea.

Below is a sample excerpt from a NAMD config file for nonbonded and multistep parameters when using `GBIS` and `SASA`:

```
#GBIS parameters
GBIS on
ionConcentration 0.3
alphaCutoff 14
#nonbonded parameters
switching on
switchdist 15
cutoff 16
pairlistdist 18
#hydrophobic energy
sasa on
surfaceTension 0.006
#multistep parameters
timestep 1
nonbondedFreq 2
fullElectFrequency 4
```

7 Standard Minimization and Dynamics Parameters

7.1 Boundary Conditions

In addition to periodic boundary conditions, NAMD provides spherical and cylindrical boundary potentials to contain atoms in a given volume. To apply more general boundary potentials written in Tcl, use `tclBC` as described in Sec. 8.11.

7.1.1 Periodic boundary conditions

NAMD provides periodic boundary conditions in 1, 2 or 3 dimensions. The following parameters are used to define these boundary conditions.

- `cellBasisVector1` < basis vector for periodic boundaries (Å) >
  Acceptable Values: vector
  Default Value: 0 0 0
  Description: Specifies a basis vector for periodic boundary conditions.

- `cellBasisVector2` < basis vector for periodic boundaries (Å) >
  Acceptable Values: vector
  Default Value: 0 0 0
  Description: Specifies a basis vector for periodic boundary conditions.

- `cellBasisVector3` < basis vector for periodic boundaries (Å) >
  Acceptable Values: vector
  Default Value: 0 0 0
  Description: Specifies a basis vector for periodic boundary conditions.

- `cellOrigin` < center of periodic cell (Å) >
  Acceptable Values: position
  Default Value: 0 0 0
  Description: When position rescaling is used to control pressure, this location will remain constant. Also used as the center of the cell for wrapped output coordinates.

- `extendedSystem` < XSC file to read cell parameters from >
  Acceptable Values: file name
  Description: In addition to .coor and .vel output files, NAMD generates a .xsc (eXtended System Configuration) file which contains the periodic cell parameters and extended system variables, such as the strain rate in constant pressure simulations. Periodic cell parameters will be read from this file if this option is present, ignoring the above parameters.

- `XSTfile` < XST file to write cell trajectory to >
  Acceptable Values: file name
  Default Value: *outputname*`.xst`
  Description: NAMD can also generate a .xst (eXtended System Trajectory) file which contains a record of the periodic cell parameters and extended system variables during the simulation. If `XSTfile` is defined, then `XSTfreq` must also be defined.

- `XSTfreq` < how often to append state to XST file >
  Acceptable Values: positive integer

Description: Like the `DCDfreq` option, controls how often the extended system configuration will be appended to the XST file.

- `wrapAll` < wrap all coordinates around periodic boundaries? >
  Acceptable Values: on or off
  Default Value: off
  Description: Coordinates are normally output relative to the way they were read in. Hence, if part of a molecule crosses a periodic boundary it is not translated to the other side of the cell on output. This option applies a translation to the center-of-mass of each molecule or contiguous cluster of bonded atoms to keep it within the periodic unit cell. The translation has usually *no effect on the physical trajectory*, because the force field potentials used in NAMD follow the minimum-image convention for interatomic distances. However, some complex quantities, for example the center of mass of a multimeric protein, will be undefined as a result of this option. If you plan on applying external forces (`SMD`, `tclForces` or `Colvars`) to such quantities, it is recommended to keep this option off, and to possibly replace it with a custom restraint.

- `wrapWater` < wrap water coordinates around periodic boundaries? >
  Acceptable Values: on or off
  Default Value: off
  Description: This option is similar to the `wrapAll` option, but its effect is restricted to water molecules only.

- `wrapNearest` < use nearest image to cell origin when wrapping coordinates? >
  Acceptable Values: on or off
  Default Value: off
  Description: Coordinates are normally wrapped to the diagonal unit cell centered on the origin. This option, combined with `wrapWater` or `wrapAll`, wraps coordinates to the nearest image to the origin, providing hexagonal or other cell shapes.

7.1.2 Spherical harmonic boundary conditions

NAMD provides spherical harmonic boundary conditions. These boundary conditions can consist of a single potential or a combination of two potentials. The following parameters are used to define these boundary conditions.

- `sphericalBC` < use spherical boundary conditions? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not spherical boundary conditions are to be applied to the system. If set to `on`, then `sphericalBCCenter`, `sphericalBCr1` and `sphericalBCk1` must be defined, and `sphericalBCexp1`, `sphericalBCr2`, `sphericalBCk2`, and `sphericalBCexp2` can optionally be defined.

- `sphericalBCCenter` < center of sphere (Å) >
  Acceptable Values: position
  Description: Location around which sphere is centered.

- `sphericalBCr1` < radius for first boundary condition (Å) >
  Acceptable Values: positive decimal
  Description: Distance at which the first potential of the boundary conditions takes effect. This distance is a radius from the center.

- `sphericalBCk1` < force constant for first potential >
  Acceptable Values: non-zero decimal
  Description: Force constant for the first harmonic potential. A positive value will push atoms toward the center, and a negative value will pull atoms away from the center.

- `sphericalBCexp1` < exponent for first potential >
  Acceptable Values: positive, even integer
  Default Value: 2
  Description: Exponent for first boundary potential. The only likely values to use are 2 and 4.

- `sphericalBCr2` < radius for second boundary condition (Å) >
  Acceptable Values: positive decimal
  Description: Distance at which the second potential of the boundary conditions takes effect. This distance is a radius from the center. If this parameter is defined, then `sperical BCk2` must also be defined.

- `sphericalBCk2` < force constant for second potential >
  Acceptable Values: non-zero decimal
  Description: Force constant for the second harmonic potential. A positive value will push atoms toward the center, and a negative value will pull atoms away from the center.

- `sphericalBCexp2` < exponent for second potential >
  Acceptable Values: positive, even integer
  Default Value: 2
  Description: Exponent for second boundary potential. The only likely values to use are 2 and 4.

7.1.3 Cylindrical harmonic boundary conditions

NAMD provides cylindrical harmonic boundary conditions. These boundary conditions can consist of a single potential or a combination of two potentials. The following parameters are used to define these boundary conditions.

- `cylindricalBC` < use cylindrical boundary conditions? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not cylindrical boundary conditions are to be applied to the system. If set to `on`, then `cylindricalBCCenter`, `cylindricalBCr1`, `cylindricalBCl1` and `cylindricalBCk1` must be defined, and `cylindricalBCAxis`, `cylindricalBCexp1`, `cylindricalBCr2`, `cylindricalBCl2`, `cylindricalBCk2`, and `cylindricalBCexp2` can optionally be defined.

- `cylindricalBCCenter` < center of cylinder (Å) >
  Acceptable Values: position
  Description: Location around which cylinder is centered.

- `cylindricalBCAxis` < axis of cylinder (Å) >
  Acceptable Values: `x`, `y`, or `z`
  Description: Axis along which cylinder is aligned.

- `cylindricalBCr1` < radius for first boundary condition (Å) >
  Acceptable Values: positive decimal
  Description: Distance at which the first potential of the boundary conditions takes effect along the non-axis plane of the cylinder.

- `cylindricalBCl1` < distance along cylinder axis for first boundary condition (Å) >
  Acceptable Values: positive decimal
  Description: Distance at which the first potential of the boundary conditions takes effect along the cylinder axis.

- `cylindricalBCk1` < force constant for first potential >
  Acceptable Values: non-zero decimal
  Description: Force constant for the first harmonic potential. A positive value will push atoms toward the center, and a negative value will pull atoms away from the center.

- `cylindricalBCexp1` < exponent for first potential >
  Acceptable Values: positive, even integer
  Default Value: 2
  Description: Exponent for first boundary potential. The only likely values to use are 2 and 4.

- `cylindricalBCr2` < radius for second boundary condition (Å) >
  Acceptable Values: positive decimal
  Description: Distance at which the second potential of the boundary conditions takes effect along the non-axis plane of the cylinder. If this parameter is defined, then `cylindricalBCl2` and `spericalBCk2` must also be defined.

- `cylindricalBCl2` < radius for second boundary condition (Å) >
  Acceptable Values: positive decimal
  Description: Distance at which the second potential of the boundary conditions takes effect along the cylinder axis. If this parameter is defined, then `cylindricalBCr2` and `spericalBCk2` must also be defined.

- `cylindricalBCk2` < force constant for second potential >
  Acceptable Values: non-zero decimal
  Description: Force constant for the second harmonic potential. A positive value will push atoms toward the center, and a negative value will pull atoms away from the center.

- `cylindricalBCexp2` < exponent for second potential >
  Acceptable Values: positive, even integer
  Default Value: 2
  Description: Exponent for second boundary potential. The only likely values to use are 2 and 4.

7.2 Energy Minimization

7.2.1 Conjugate gradient parameters

The default minimizer uses a sophisticated conjugate gradient and line search algorithm with much better performance than the older velocity quenching method. The method of conjugate gradients is used to select successive search directions (starting with the initial gradient) which eliminate repeated minimization along the same directions. Along each direction, a minimum is first bracketed (rigorously bounded) and then converged upon by either a golden section search, or, when possible, a quadratically convergent method using gradient information.

For most systems, it just works.

- `minimization` < Perform conjugate gradient energy minimization? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Turns efficient energy minimization `on` or `off`.

- `minTinyStep` < first initial step for line minimizer >
  Acceptable Values: positive decimal
  Default Value: 1.0e-6
  Description: If your minimization is immediately unstable, make this smaller.

- `minBabyStep` < max initial step for line minimizer >
  Acceptable Values: positive decimal
  Default Value: 1.0e-2
  Description: If your minimization becomes unstable later, make this smaller.

- `minLineGoal` < gradient reduction factor for line minimizer >
  Acceptable Values: positive decimal
  Default Value: 1.0e-4
  Description: Varying this might improve conjugate gradient performance.

7.2.2 Velocity quenching parameters

You can perform energy minimization using a simple quenching scheme. While this algorithm is not the most rapidly convergent, it is sufficient for most applications. There are only two parameters for minimization: one to activate minimization and another to specify the maximum movement of any atom.

- `velocityQuenching` < Perform old-style energy minimization? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Turns slow energy minimization `on` or `off`.

- `maximumMove` < maximum distance an atom can move during each step (Å) >
  Acceptable Values: positive decimal
  Default Value: 0.75 × `cutoff/stepsPerCycle`
  Description: Maximum distance that an atom can move during any single timestep of minimization. This is to insure that atoms do not go flying off into space during the first few timesteps when the largest energy conflicts are resolved.

7.3 Dynamics

7.3.1 Timestep parameters

- `numsteps` < number of timesteps >
  Acceptable Values: positive integer
  Description: The number of simulation timesteps to be performed. An integer greater than 0 is acceptable. The total amount of simulation time is `numsteps` × `timestep`.

- `timestep` < timestep size (fs) >
  Acceptable Values: non-negative decimal
  Default Value: 1.0
  Description: The timestep size to use when integrating each step of the simulation. The value is specified in femtoseconds.

- `firsttimestep` < starting timestep value >
  Acceptable Values: non-negative integer
  Default Value: 0
  Description: The number of the first timestep. This value is typically used only when a simulation is a continuation of a previous simulation. In this case, rather than having the timestep restart at 0, a specific timestep number can be specified.

7.3.2 Initialization

- `temperature` < initial temperature (K) >
  Acceptable Values: positive decimal
  Description: Initial temperature value for the system. Using this option will generate a random velocity distribution for the initial velocities for all the atoms such that the system is at the desired temperature. Either the `temperature` or the `velocities/binvelocities` option must be defined to determine an initial set of velocities. Both options cannot be used together.

- `COMmotion` < allow initial center of mass motion? >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: Specifies whether or not motion of the center of mass of the entire system is allowed. If this option is set to `no`, the initial velocities of the system will be adjusted to remove center of mass motion of the system. Note that this does not preclude later center-of-mass motion due to external forces such as random noise in Langevin dynamics, boundary potentials, and harmonic restraints.

- `seed` < random number seed >
  Acceptable Values: positive integer
  Default Value: pseudo-random value based on current UNIX clock time
  Description: Number used to seed the random number generator if `temperature` or `langevin` is selected. This can be used so that consecutive simulations produce the same results. If no value is specified, NAMD will choose a pseudo-random value based on the current UNIX clock time. The random number seed will be output during the simulation startup so that its value is known and can be reused for subsequent simulations. Note that if

Langevin dynamics are used in a parallel simulation (i.e., a simulation using more than one processor) even using the same seed will *not* guarantee reproducible results.

7.3.3 Conserving momentum

- `zeroMomentum` < remove center of mass drift due to PME >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: If enabled, the net momentum of the simulation and any resultant drift is removed before every full electrostatics step. This correction should conserve energy and have minimal impact on parallel scaling. This feature should only be used for simulations that would conserve momentum except for the slight errors in PME. (Features such as fixed atoms, harmonic restraints, steering forces, and Langevin dynamics do not conserve momentum; use in combination with these features should be considered experimental.) Since the momentum correction is delayed, enabling outputMomenta will show a slight nonzero linear momentum but there should be no center of mass drift.

7.3.4 Multiple timestep parameters

To further reduce the cost of computing full electrostatics, NAMD uses a multiple timestepping integration scheme. In this scheme, the total force acting on each atom is broken into two pieces, a quickly varying local component and a slower long range component. The local force component is defined in terms of a *splitting function*. The local force component consists of all bonded and van der Waals interactions as well as that portion of electrostatic interactions for pairs that are separated by less than the local interaction distance determined by the splitting function. The long range component consists only of electrostatic interactions outside of the local interaction distance. Since the long range forces are slowly varying, they are not evaluated every timestep. Instead, they are evaluated every k timesteps, specified by the NAMD parameter `fullElectFrequency`. An impulse of k times the long range force is applied to the system every k timesteps (i.e., the r-RESPA integrator is used). For appropriate values of k, it is believed that the error introduced by this infrequent evaluation is modest compared to the error already incurred by the use of the numerical (Verlet) integrator. Improved methods for incorporating these long range forces are currently being investigated, with the intention of improving accuracy as well as reducing the frequency of long range force evaluations.

In the scheme described above, the van der Waals forces are still truncated at the local interaction distance. Thus, the van der Waals cutoff distance forms a lower limit to the local interaction distance. While this is believed to be sufficient, there are investigations underway to remove this limitation and provide full van der Waals calculations in $\mathcal{O}(N)$ time as well.

One of the areas of current research being studied using NAMD is the exploration of better methods for performing multiple timestep integration. Currently the only available method is the impulse-based Verlet-I or r-RESPA method which is stable for timesteps up to 4 fs for long-range electrostatic forces, 2 fs for short-range nonbonded forces, and 1 fs for bonded forces Setting `rigid all` (i.e., using SHAKE) increases these timesteps to 6 fs, 2 fs, and 2 fs respectively but eliminates bond motion for hydrogen. The mollified impulse method (MOLLY) reduces the resonance which limits the timesteps and thus increases these timesteps to 6 fs, 2 fs, and 1 fs while retaining all bond motion.

- `fullElectFrequency` < number of timesteps between full electrostatic evaluations >
  Acceptable Values: positive integer factor of `stepspercycle`
  Default Value: `nonbondedFreq`
  Description: This parameter specifies the number of timesteps between each full electrostatics evaluation. It is recommended that `fullElectFrequency` be chosen so that the product of `fullElectFrequency` and `timestep` does not exceed 4.0 unless `rigidBonds all` or `molly on` is specified, in which case the upper limit is perhaps doubled.

- `nonbondedFreq` < timesteps between nonbonded evaluation >
  Acceptable Values: positive integer factor of `fullElectFrequency`
  Default Value: 1
  Description: This parameter specifies how often short-range nonbonded interactions should be calculated. Setting `nonbondedFreq` between 1 and `fullElectFrequency` allows triple timestepping where, for example, one could evaluate bonded forces every 1 fs, short-range nonbonded forces every 2 fs, and long-range electrostatics every 4 fs.

- `MTSAlgorithm` < MTS algorithm to be used >
  Acceptable Values: `impulse/verletI` or `constant/naive`
  Default Value: `impulse`
  Description: Specifies the multiple timestep algorithm used to integrate the long and short range forces. `impulse/verletI` is the same as r-RESPA. `constant/naive` is the stale force extrapolation method.

- `longSplitting` < how should long and short range forces be split? >
  Acceptable Values: `c1`, `c2`
  Default Value: `c1`
  Description: Specifies the method used to split electrostatic forces between long and short range potentials. The `c1` option uses a cubic polynomial splitting function,

$$S_3(r) = 1 - \frac{3}{2}\left(\frac{r}{r_{\text{cut}}}\right) + \frac{1}{2}\left(\frac{r}{r_{\text{cut}}}\right)^3,$$

  to affect C^1 continuity in the splitting of the electrostatic potential [103]. The `c2` option uses a quintic polynomial splitting function,

$$S_5(r) = 1 - 10\left(\frac{r}{r_{\text{cut}}}\right)^3 + 15\left(\frac{r}{r_{\text{cut}}}\right)^4 - 6\left(\frac{r}{r_{\text{cut}}}\right)^5,$$

  to affect C^2 continuity in the splitting of the electrostatic potential. The S_5 splitting function, contributed by Bruce Berne, Ruhong Zhou, and Joe Morrone, produces demonstrably better long time stability than S_3 without requiring any additional computational cost during simulation, since the nonbonded forces are calculated via a lookup table. Note that earlier options `xplor` and `sharp` are no longer supported.

- `molly` < use mollified impulse method (MOLLY)? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: This method eliminates the components of the long range electrostatic forces which contribute to resonance along bonds to hydrogen atoms, allowing a fullElectFrequency

of 6 (vs. 4) with a 1 fs timestep without using `rigidBonds all`. You may use `rigidBonds water` but using `rigidBonds all` with MOLLY makes no sense since the degrees of freedom which MOLLY protects from resonance are already frozen.

- `mollyTolerance` < allowable error for MOLLY >
  Acceptable Values: positive decimal
  Default Value: 0.00001
  Description: Convergence criterion for MOLLY algorithm.

- `mollyIterations` < maximum MOLLY iterations >
  Acceptable Values: positive integer
  Default Value: 100
  Description: Maximum number of iterations for MOLLY algorithm.

7.4 Temperature Control and Equilibration

7.4.1 Langevin dynamics parameters

NAMD is capable of performing Langevin dynamics, where additional damping and random forces are introduced to the system. This capability is based on that implemented in X-PLOR which is detailed in the X-PLOR *User's Manual* [14], although a different integrator is used.

- `langevin` < use Langevin dynamics? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not Langevin dynamics active. If set to `on`, then the parameter `langevinTemp` must be set and the parameters `langevinFile` and `langevinCol` can optionally be set to control the behavior of this feature.

- `langevinTemp` < temperature for Langevin calculations (K) >
  Acceptable Values: positive decimal
  Description: Temperature to which atoms affected by Langevin dynamics will be adjusted. This temperature will be roughly maintained across the affected atoms through the addition of friction and random forces.

- `langevinDamping` < damping coefficient for Langevin dynamics (1/ps) >
  Acceptable Values: positive decimal
  Default Value: per-atom values from PDB file
  Description: Langevin coupling coefficient to be applied to all atoms (unless `langevinHydrogen` is `off`, in which case only non-hydrogen atoms are affected). If not given, a PDB file is used to obtain coefficients for each atom (see `langevinFile` and `langevinCol` below).

- `langevinHydrogen` < Apply Langevin dynamics to hydrogen atoms? >
  Acceptable Values: `on` or `off`
  Default Value: `on`
  Description: If `langevinDamping` is set then setting `langevinHydrogen` to `off` will turn off Langevin dynamics for hydrogen atoms. This parameter has no effect if Langevin coupling coefficients are read from a PDB file.

- `langevinFile` < PDB file containing Langevin parameters >
  Acceptable Values: UNIX filename
  Default Value: `coordinates`
  Description: PDB file to use for the Langevin coupling coefficients for each atom. If this parameter is not specified, then the PDB file specified by `coordinates` is used.

- `langevinCol` < column of PDB from which to read coefficients >
  Acceptable Values: `X`, `Y`, `Z`, `O`, or `B`
  Default Value: `O`
  Description: Column of the PDB file to use for the Langevin coupling coefficients for each atom. The coefficients can be read from any floating point column of the PDB file. A value of 0 indicates that the atom will remain unaffected.

7.4.2 Temperature coupling parameters

NAMD is capable of performing temperature coupling, in which forces are added or reduced to simulate the coupling of the system to a heat bath of a specified temperature. This capability is based on that implemented in X-PLOR which is detailed in the X-PLOR *User's Manual* [14].

- `tCouple` < perform temperature coupling? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not temperature coupling is active. If set to `on`, then the parameter `tCoupleTemp` must be set and the parameters `tCoupleFile` and `tCoupleCol` can optionally be set to control the behavior of this feature.

- `tCoupleTemp` < temperature for heat bath (K) >
  Acceptable Values: positive decimal
  Description: Temperature to which atoms affected by temperature coupling will be adjusted. This temperature will be roughly maintained across the affected atoms through the addition of forces.

- `tCoupleFile` < PDB file with tCouple parameters >
  Acceptable Values: UNIX filename
  Default Value: `coordinates`
  Description: PDB file to use for the temperature coupling coefficient for each atom. If this parameter is not specified, then the PDB file specified by `coordinates` is used.

- `tCoupleCol` < column of PDB from which to read coefficients >
  Acceptable Values: `X`, `Y`, `Z`, `O`, or `B`
  Default Value: `O`
  Description: Column of the PDB file to use for the temperature coupling coefficient for each atom. This value can be read from any floating point column of the PDB file. A value of 0 indicates that the atom will remain unaffected.

7.4.3 Stochastic velocity rescaling parameters

The stochastic velocity rescaling method originated by [15] can be viewed as an extension (and correction) of the Berendsen method. The implementation in NAMD is based on that from GROMACS, with some slight performance modifications during random number generation.

- `stochRescale` < perform stochastic rescaling? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not stochastic rescaling is active. If set to `on`, then the parameters `stochRescaleTemp` and `stochRescalePeriod` must be set.

- `stochRescaleTemp` < temperature for heat bath (K) >
  Acceptable Values: positive decimal
  Description: Temperature to which all atoms will be periodically readjusted toward. This temperature will be correctly maintained (in the canonical sense) over all atoms by rescaling the velocities with both deterministic (using the instantaneous temperature) and stochastic components.

- `stochRescalePeriod` < time parameter (ps) for temperature coupling >
  Acceptable Values: positive decimal
  Description: The stochastic rescaling algorithm holds for an arbitrary time parameter introduced when solving the Fokker-Planck equation. For systems predominantly composed of liquid water a value near 2 ps is appropriate and values between 0.5 and 2 ps are common in the literature for simulations of biomolecules. Larger values will generally result in weaker coupling and thus more NVE-like dynamics, but may also lead to slow (i.e. incorrect) convergence to the correct ensemble.

- `stochRescaleFreq` < number of timesteps between rescalings >
  Acceptable Values: positive integer
  Default Value: `stepsPerCycle`
  Description: The stochastic rescaling algorithm is invoked at fixed intervals. The effective time parameter is technically the ratio `stochRescaleFreq`/`stochRescalePeriod` (after converting into proper units using the value of `timestep`). The default should be adequate for most applications, but a smaller value closer to the frequency at which the nonbonded list is rebuilt would also be appropriate. When using multiple time stepping, it is important that rescaling occurs at timesteps that are integer multiples of the slowest interaction type (usually `fullElectFrequency`).

- `stochRescaleHeat` < Should heat transfer and work be computed? >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: When active, the *cumulative* heat transfer with the thermostat will be reported as `HEAT`. The work due to the thermostat and integrator can then be computed as the change in total energy less the heat transfer and is reported as `WORK`. Note that the work includes all sources, including non-conservative elements of the Hamiltonian, but should otherwise approach zero for simulations at or near equilibrium. The accumulation starts at `firstTimestep` and can be reset from `Tcl` by re-setting this to zero. **This is an experimental option and not yet guaranteed for any specific purpose.**

7.4.4 Temperature rescaling parameters

NAMD allows equilibration of a system by means of temperature rescaling. Using this method, all of the velocities in the system are periodically rescaled so that the entire system is set to the

desired temperature. The following parameters specify how often and to what temperature this rescaling is performed.

- `rescaleFreq` < number of timesteps between temperature rescaling >
  Acceptable Values: positive integer
  Description: The equilibration feature of NAMD is activated by specifying the number of timesteps between each temperature rescaling. If this value is given, then the `rescaleTemp` parameter must also be given to specify the target temperature.

- `rescaleTemp` < temperature for equilibration (K) >
  Acceptable Values: positive decimal
  Description: The temperature to which all velocities will be rescaled every `rescaleFreq` timesteps. This parameter is valid only if `rescaleFreq` has been set.

7.4.5 Temperature reassignment parameters

NAMD allows equilibration of a system by means of temperature reassignment. Using this method, all of the velocities in the system are periodically reassigned so that the entire system is set to the desired temperature. The following parameters specify how often and to what temperature this reassignment is performed.

- `reassignFreq` < number of timesteps between temperature reassignment >
  Acceptable Values: positive integer
  Description: The equilibration feature of NAMD is activated by specifying the number of timesteps between each temperature reassignment. If this value is given, then the `reassignTemp` parameter must also be given to specify the target temperature.

- `reassignTemp` < temperature for equilibration (K) >
  Acceptable Values: positive decimal
  Default Value: `temperature` if set, otherwise none
  Description: The temperature to which all velocities will be reassigned every `reassignFreq` timesteps. This parameter is valid only if `reassignFreq` has been set.

- `reassignIncr` < temperature increment for equilibration (K) >
  Acceptable Values: decimal
  Default Value: 0
  Description: In order to allow simulated annealing or other slow heating/cooling protocols, `reassignIncr` will be added to `reassignTemp` after each reassignment. (Reassignment is carried out at the first timestep.) The `reassignHold` parameter may be set to limit the final temperature. This parameter is valid only if `reassignFreq` has been set.

- `reassignHold` < holding temperature for equilibration (K) >
  Acceptable Values: positive decimal
  Description: The final temperature for reassignment when `reassignIncr` is set; `reassignTemp` will be held at this value once it has been reached. This parameter is valid only if `reassignIncr` has been set.

7.4.6 Lowe-Andersen dynamics parameters

NAMD can perform Lowe-Andersen dynamics, a variation of Andersen dynamics whereby the radial relative velocities of atom pairs are randomly modified based on a thermal distribution. The Lowe-Andersen thermostat is Galilean invariant, therefore conserving momentum, and is thus independent of absolute atom velocities. Forces are applied only between non-bonded, non-hydrogen pairs of atoms. When using rigid bonds, forces are applied to the center of mass of hydrogen groups. The implementation is based on Koopman and Lowe [58].

- `loweAndersen` < use Lowe-Andersen dynamics? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not Lowe-Andersen dynamics are active. If set to `on`, then the parameter `loweAndersenTemp` must be set and the parameters `loweAndersenCutoff` and `loweAndersenRate` can optionally be set.

- `loweAndersenTemp` < temperature for Lowe-Andersen calculations (K) >
  Acceptable Values: positive decimal
  Description: Temperature of the distribution used to set radial relative velocities. This determines the target temperature of the system.

- `loweAndersenCutoff` < cutoff radius for Lowe-Andersen collisions (Å) >
  Acceptable Values: positive decimal
  Default Value: 2.7
  Description: Forces are only applied to atoms within this distance of one another.

- `loweAndersenRate` < rate for Lowe-Andersen collisions (1/ps) >
  Acceptable Values: positive decimal
  Default Value: 50
  Description: Determines the probability of a collision between atoms within the cutoff radius. The probability is the rate specified by this keyword times the non-bonded timestep.

7.5 Pressure Control

Constant pressure simulation (and pressure calculation) require periodic boundary conditions. Pressure is controlled by dynamically adjusting the size of the unit cell and rescaling all atomic coordinates (other than those of fixed atoms) during the simulation.

Pressure values in NAMD output are in bar. PRESSURE is the pressure calculated based on individual atoms, while GPRESSURE incorporates hydrogen atoms into the heavier atoms to which they are bonded, producing smaller fluctuations. The TEMPAVG, PRESSAVG, and GPRESSAVG are the average of temperature and pressure values since the previous ENERGY output; for the first step in the simulation they will be identical to TEMP, PRESSURE, and GPRESSURE.

The phenomenological pressure of bulk matter reflects averaging in both space and time of the sum of a large positive term (the kinetic pressure, nRT/V), and a large cancelling negative term (the static pressure). The instantaneous pressure of a simulation cell as simulated by NAMD will have mean square fluctuations (according to David Case quoting Section 114 of *Statistical Physics* by Landau and Lifshitz) of $kT/(V\beta)$, where β is the compressibility, which is RMS of roughly 100 bar for a 10,000 atom biomolecular system. Much larger fluctuations are regularly observed in practice.

The instantaneous pressure for a biomolecular system is well defined for "internal" forces that are based on particular periodic images of the interacting atoms, conserve momentum, and are translationally invariant. When dealing with externally applied forces such as harmonic constraints, fixed atoms, and various steering forces, NAMD bases its pressure calculation on the relative positions of the affected atoms in the input coordinates and assumes that the net force will average to zero over time. For time periods during with the net force is non-zero, the calculated pressure fluctuations will include a term proportional to the distance to the affected from the user-defined cell origin. A good way to observe these effects and to confirm that pressure for external forces is handled reasonably is to run a constant volume cutoff simulation in a cell that is larger than the molecular system by at least the cutoff distance; the pressure for this isolated system should average to zero over time.

Because NAMD's impluse-basd multiple timestepping system alters the balance between bonded and non-bonded forces from every timestep to an average balance over two steps, the calculated pressure on even and odd steps will be different. The PRESSAVG and GPRESSAVG fields provide the average over the non-printed intermediate steps. If you print energies on every timestep you will see the effect clearly in the PRESSURE field.

The following options affect all pressure control methods.

- `useGroupPressure` < group or atomic quantities >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: Pressure can be calculated using either the atomic virial and kinetic energy (the default) or a hydrogen-group based pseudo-molecular virial and kinetic energy. The latter fluctuates less and is required in conjunction with rigidBonds (SHAKE).

- `useFlexibleCell` < anisotropic cell fluctuations >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: NAMD allows the three orthogonal dimensions of the periodic cell to fluctuate independently when this option is enabled.

- `useConstantRatio` < constant shape in first two cell dimensions >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: When enabled, NAMD keeps the ratio of the unit cell in the x-y plane constant while allowing fluctuations along all axes. The `useFlexibleCell` option is required for this option.

- `useConstantArea` < constant area and normal pressure conditions >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: When enabled, NAMD keeps the dimension of the unit cell in the x-y plane constant while allowing fluctuations along the z axis. This is not currently implemented in Berendsen's method.

7.5.1 Berendsen pressure bath coupling

NAMD provides constant pressure simulation using Berendsen's method. The following parameters are used to define the algorithm.

- `BerendsenPressure` < use Berendsen pressure bath coupling? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not Berendsen pressure bath coupling is active. If set to `on`, then the parameters `BerendsenPressureTarget`, `BerendsenPressureCompressibility` and `BerendsenPressureRelaxationTime` must be set and the parameter `BerendsenPressureFreq` can optionally be set to control the behavior of this feature.

- `BerendsenPressureTarget` < target pressure (bar) >
  Acceptable Values: positive decimal
  Description: Specifies target pressure for Berendsen's method. A typical value would be 1.01325 bar, atmospheric pressure at sea level.

- `BerendsenPressureCompressibility` < compressibility (bar^{-1}) >
  Acceptable Values: positive decimal
  Description: Specifies compressibility for Berendsen's method. A typical value would be 4.57E-5 bar^{-1}, corresponding to liquid water. The higher the compressibility, the more volume will be adjusted for a given pressure difference. The compressibility and the relaxation time appear only as a ratio in the dynamics, so a larger compressibility is equivalent to a smaller relaxation time.

- `BerendsenPressureRelaxationTime` < relaxation time (fs) >
  Acceptable Values: positive decimal
  Description: Specifies relaxation time for Berendsen's method. If the instantaneous pressure did not fluctuate randomly during a simulation and the compressibility estimate was exact then the inital pressure would decay exponentially to the target pressure with this time constant. Having a longer relaxation time results in more averaging over pressure measurements and hence smaller fluctuations in the cell volume. A reasonable choice for relaxation time would be 100 fs. The compressibility and the relaxation time appear only as a ratio in the dynamics, so a larger compressibility is equivalent to a smaller relaxation time.

- `BerendsenPressureFreq` < how often to rescale positions >
  Acceptable Values: positive multiple of `nonbondedFrequency` and `fullElectFrequency`
  Default Value: `nonbondedFrequency` or `fullElectFrequency` if used
  Description: Specifies number of timesteps between position rescalings for Berendsen's method. Primarily to deal with multiple timestepping integrators, but also to reduce cell volume fluctuations, cell rescalings can occur on a longer interval. This could reasonably be between 1 and 20 timesteps, but the relaxation time should be at least ten times larger.

7.5.2 Nosé-Hoover Langevin piston pressure control

NAMD provides constant pressure simulation using a modified Nosé-Hoover method in which Langevin dynamics is used to control fluctuations in the barostat. This method should be combined with a method of temperature control, such as Langevin dynamics, in order to simulate the NPT ensemble.

The Langevin piston Nose-Hoover method in NAMD is a combination of the Nose-Hoover constant pressure method as described in GJ Martyna, DJ Tobias and ML Klein, "Constant pressure

molecular dynamics algorithms", J. Chem. Phys 101(5), 1994, with piston fluctuation control implemented using Langevin dynamics as in SE Feller, Y Zhang, RW Pastor and BR Brooks, "Constant pressure molecular dynamics simulation: The Langevin piston method", J. Chem. Phys. 103(11), 1995.

The equations of motion are:

$$\begin{aligned} r' &= p/m + e'r \\ p' &= F - e'p - gp + R \\ V' &= 3Ve' \\ e'' &= 3V/W(P - P_0) - g_e e' + R_e/W \\ W &= 3N\tau^2 kT \\ < R^2 > &= 2mgkT/h \\ \tau &= \text{oscillationperiod} \\ < R_e^2 > &= 2Wg_e kT/h \end{aligned}$$

Here, W is the mass of piston, R is noise on atoms, and R_e is the noise on the piston.

The user specifies the desired pressure, oscillation and decay times of the piston, and temperature of the piston. The compressibility of the system is not required. In addition, the user specifies the damping coefficients and temperature of the atoms for Langevin dynamics.

The following parameters are used to define the algorithm.

- `LangevinPiston` < use Langevin piston pressure control? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not Langevin piston pressure control is active. If set to `on`, then the parameters `LangevinPistonTarget`, `LangevinPistonPeriod`, `LangevinPistonDecay` and `LangevinPistonTemp` must be set.

- `LangevinPistonTarget` < target pressure (bar) >
  Acceptable Values: positive decimal
  Description: Specifies target pressure for Langevin piston method. A typical value would be 1.01325 bar, atmospheric pressure at sea level.

- `LangevinPistonPeriod` < oscillation period (fs) >
  Acceptable Values: positive decimal
  Description: Specifies barostat oscillation time scale for Langevin piston method. If the instantaneous pressure did not fluctuate randomly during a simulation and the decay time was infinite (no friction) then the cell volume would oscillate with this angular period. Having a longer period results in more averaging over pressure measurements and hence slower fluctuations in the cell volume. A reasonable choice for the piston period would be 200 fs.

- `LangevinPistonDecay` < damping time scale (fs) >
  Acceptable Values: positive decimal
  Description: Specifies barostat damping time scale for Langevin piston method. A value larger than the piston period would result in underdamped dynamics (decaying ringing in the cell volume) while a smaller value approaches exponential decay as in Berendsen's method above. A smaller value also corresponds to larger random forces with increased coupling to

the Langevin temperature bath. Typically this would be chosen equal to or smaller than the piston period, such as 100 fs.

- `LangevinPistonTemp` < noise temperature (K) >
  Acceptable Values: positive decimal
  Description: Specifies barostat noise temperature for Langevin piston method. This should be set equal to the target temperature for the chosen method of temperature control.

- `SurfaceTensionTarget` < Surface tension target (dyn/cm) >
  Acceptable Values: decimal
  Default Value: 0.0
  Description: Specifies surface tension target. Must be used with `useFlexibleCell` and periodic boundary conditions. The pressure specified in `LangevinPistonTarget` becomes the pressure along the z axis, and surface tension is applied in the x-y plane.

- `StrainRate` < initial strain rate >
  Acceptable Values: decimal triple (x y z)
  Default Value: 0. 0. 0.
  Description: Optionally specifies the initial strain rate for pressure control. Is overridden by value read from file specified with `extendedSystem`. There is typically no reason to set this parameter.

- `ExcludeFromPressure` < Should some atoms be excluded from pressure rescaling? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not to exclude some atoms from pressure rescaling. The coordinates and velocites of such atoms are not rescaled during constant pressure simulations, though they do contribute to the virial calculation. May be useful for membrane protein simulation. EXPERIMENTAL.

- `ExcludeFromPressureFile` < File specifying excluded atoms >
  Acceptable Values: PDB file
  Default Value: coordinates file
  Description: PDB file with one column specifying which atoms to exclude from pressure rescaling. Specify 1 for excluded and 0 for not excluded.

- `ExcludeFromPressureCol` < Column in PDB file for specifying excluded atoms >
  Acceptable Values: O, B, X, Y, or Z
  Default Value: O
  Description: Specifies which column of the pdb file to check for excluded atoms.

8 User Defined Forces

There are several ways to apply external forces to simulations with NAMD. These are described below.

8.1 Constant Forces

NAMD provides the ability to apply constant forces to some atoms. There are three parameters that control this feature.

- `constantForce` < Apply constant forces? >
  Acceptable Values: yes or no
  Default Value: no
  Description: Specifies whether or not constant forces are applied.

- `consForceFile` < PDB file containing forces to be applied >
  Acceptable Values: UNIX filename
  Description: The X, Y, Z and occupancy (O) fields of this file are read to determine the constant force vector of each atom, which is (X,Y,Z)*O, in unit of kcal/(mol*Å). The occupancy (O) serves as a scaling factor, which could expand the range of the force applied. (One may be unable to record very large or very small numbers in the data fields of a PDB file due to limited space). Zero forces are ignored.

  Specifying `consforcefile` is optional; constant forces may be specified or updated between runs by using the consForceConfig command.

- `consForceScaling` < Scaling factor for constant forces >
  Acceptable Values: decimal
  Default Value: 1.0
  Description: Scaling factor by which constant forces are multiplied. May be changed between run commands.

8.2 External Electric Field

NAMD provides the ability to apply a constant electric field to the molecular system being simulated. Energy due to the external field will be reported in the MISC column and will be continuous even in simulations using periodic boundary conditions as unwrapped coordinates are used to calculate energy and pressure, resulting in linearly increasing pressure over time for systems with free ions. To avoid this effect, for periodic simulations the new `eFieldNormalized` option should be used with the electric field vector multiplied by the cell dimension. There are three parameters that control this feature.

- `eFieldOn` < apply electric field? >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: Specifies whether or not an electric field is applied.

- `eField` < electric field vector >
  Acceptable Values: vector of decimals (x y z)
  Description: Vector which describes the electric field to be applied. Units are

kcal/(mol Å e), which is natural for simulations. This parameter may be changed between `run` commands, allowing a square wave or other approximate wave form to be applied.

- `eFieldNormalized` < electric field vector scaled by cell basis vectors? >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: Specifies whether or not that eField vector has been scaled by the cell basis vectors, thus indicating the voltage drop across the cell in units of kcal/(mol e). The eField vector is then scaled by the reciprocal lattice vectors at each timestep. When eFieldNormalized is true the eField forces do not contribute to the pressure calculation.

8.3 Grid Forces

NAMD provides the ability to specify grids describing a potential in the simulation space. Each atom is affected by the potential based on its charge and its position, using the potential function interpolated from the specified grid(s). Energy due to the grid-defined field will be reported in the MISC column of the output, unless a scaling factor not proportional to (1,1,1) is used.

NAMD allows the definition of multiple grids, each with a separate set of defining parameters. This is specified using a tag field in each of the mgridforceXXX commands. The tag is an alphanumeric string without spaces which identifies to which grid the specified field applies.

The grid file format is a subset of the DataExplorer DX file format, as shown below:

```
# Lines at the beginning of the file starting with a # symbol
# are ignored as comments
# Variables (replaced by numbers in an actual file):
#   xn, yn, and zn are the number of data points along each dimension;
#   xorg, yorg, and zorg is the origin of the grid, in angstroms;
#   x[1-3]del, y[1-3]del, and z[1-3]del are the basis vectors which transform
#   grid indices to coordinates in angstroms:
#      x(i,j,k) = xorg + i * x1del + j * y1del + k * z1del
#      y(i,j,k) = yorg + i * x2del + j * y2del + k * z2del
#      z(i,j,k) = zorg + i * x3del + j * y3del + k * z3del
#
#   Grid data follows, with three values per line, ordered z fast, y medium,
#   and x slow. Exactly xn*yn*zn values should be given. Grid data is then
#   terminated with a field object.
#
#  Note: Other features of the DX file format are not handled by this code
#
object 1 class gridpositions counts xn yn zn
origin xorg yorg zorg
delta x1del y1del z1del
delta x2del y2del z2del
delta x3del y3del z3del
object 2 class gridconnections counts xn yn zn
object 3 class array type double rank 0 items [ xn*yn*zn ] data follows
f1 f2 f3
f4 f5 f6
```

```
.
.
.
object 4 class field
component "positions" value 1
component "connections" value 2
component "data" value 3
```

Each dimension of the grid may be specified as continuous or not. If the grid is not continuous in a particular dimension, the potential grid is padded with one border slices on each non-continuous face of the grid, and border grid values are computed so that the force felt by an atom outside the grid goes to zero. If the grid is continuous along a particular dimension, atoms outside the grid are affected by a potential that is interpolated from the grid and its corresponding periodic image along that dimension.

To calculate the force on an atom due to the grid, the atom's coordinates are transformed according to the current basis vectors of the simulation box to a coordinate frame that is centered at the center of the specified grid. Note that the size and spatial coordinates of the grid remain fixed, and are not scaled as the size of the simulation box fluctuates. For atoms within the grid, the force is computed by analytically determining the gradient of the tricubic polynomial used to interpolate the potential from surrounding grid values. For atoms outside the grid, the state of the `mgridforcecont[1,2,3]` determine whether the force is zero, or computed from the images of the grid as described above. Note that if the grid is ever larger than the periodic box, it is truncated at the edge of that box. The consequence of this is that the computed potential will not vary smoothly at the edges, introducing numerical instability.

NAMD also supports non-uniform grids, allowing regions of a grid to be defined at higher resolution. Non-uniform grids are structured hierarchically, with a single *maingrid* which has one or more *subgrid*s. Each subgrid spans a number of maingrid cells in each of the three dimensions, and effectively redefines the data in that region. The subgrids are usually defined at higher resolution, with the restriction that the number of cells along each dimension is an integral number of the original number in the maingrid. Note that the maingrid still has data points in regions where subgrids are defined, and that, on the boundary of a subgrid, *they must agree with the values in the subgrid*. Subgrids, in turn, may have subgrids of their own, which may have subgrids of their own, etc.

A non-uniform grid file takes the form of a special comment block followed by multiple normal grid definitions. The special comment block defines the grid hierarchy, and consists of comments beginning with `# namdnugrid`. An example follows:

```
# namdnugrid version 1.0
# namdnugrid maingrid subgrids count 2
# namdnugrid subgrid 1 generation 1 min x1 y1 z1 max x2 y2 z2 subgrids count 2
# namdnugrid subgrid 2 generation 2 min x3 y3 z3 max x4 y4 z4 subgrids count 0
# namdnugrid subgrid 3 generation 2 min x5 y5 z5 max x6 y6 z6 subgrids count 0
# namdnugrid subgrid 4 generation 1 min x7 y7 z7 max x8 y8 z8 subgrids count 0
```

The maingrid is described by the number of subgrids. Subgrids are additionally described by a subgrid number; a generation number, which should be one higher than the generation of its supergrid; and `min` and `max` attributes, which describe the location of the subgrid within its supergrid. In this example, the maingrid has two subgrids, subgrid 1 and subgrid 4, labeled `generation 1`.

The first of these subgrids has two subgrids of its own (`generation 2`). Notice that subgrids are described immediately after their supergrid. The `min` and `max` attributes are given in units of grid *cells* of the supergrid. For example, a subgrid with `min 0 0 0 max 1 1 1` would redefine 8 cells of its supergrid, the space between gridpoints (0, 0, 0) and (2, 2, 2) in grid coordinates. Following the comment block, the maingrid and subgrids are defined in the format described above, in the same order as the comment block.

The following parameters describe the grid-based potentials.

- `mgridforce` < apply grid forces? >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: Specifies whether or not any grid forces are being applied.

- `mgridforcefile` < tag > < PDB file specifying force multipliers and charges for each atomd >
  Acceptable Values: UNIX file name
  Description: The force on each atom is scaled by the corresponding value in this PDB file. By setting the force multiplier to zero for an atom, it will not be affected by the grid force.

- `mgridforcecol` < tag > < column of PDB from which to read force multipliers >
  Acceptable Values: X, Y, Z, O, or B
  Default Value: B
  Description: Which column in the PDB file specified by `mgridforcefile` contains the scaling factor

- `mgridforcechargecol` < tag > < column of PDB from which to read atom charges >
  Acceptable Values: X, Y, Z, O, or B
  Default Value: Atom charge used for electrostatics.
  Description: Which column in the PDB file specified by `mgridforcefile` contains the atom charge. By default, the charge value specified for the short-range Columb interactions are also used for the grid force. Both `mgridforcecol` and `mgridforceqcol` can be specified, in which case the apparent charge of the atom will be the product of the two values.

- `mgridforcepotfile` < tag > < grid potential file name >
  Acceptable Values: UNIX file name
  Description: File specifying the grid size, coordinates, and potential values.

- `mgridforcevolts` < tag > < grid potential units in eV/charge >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: If set, the grid potential values are expressed in eV. Otherwise, values are in kcal/(mol *charge*)

- `mgridforcescale` < tag > < scale factor for grid potential >
  Acceptable Values: Vector of decimals $scale_x$ $scale_y$ $scale_z$
  Default Value: 1 1 1
  Description: Defines the scale factors that modulate the amplitude of the grid potential forces in each dimension. When the three values are the same number, the grid potential's value is also included in the MISC column of the energy output. After initialization, the

three scale factors may be updated between "run" commands by the `updategridforcescale` command. In the special case when "0 0 0" is given for this option, the corresponding grid potential can be used a collective variable in the Colvars module (Sec. 9), allowing the use of restraint potentials and fully time-dependent forces.

- `updategridforcescale` < tag > < scale factor for grid potential >
  Acceptable Values: Vector of decimals $scale_x$ $scale_y$ $scale_z$
  Default Value: 1 1 1
  Description: Provides new scale factors to be applied to the grid potential values. This comand can be issued between "run" commands to modify the amplitude of the grid potential. The values provided remain constant for the duration of each "run" command.

- `mgridforcecont1` < tag > < Is grid continuous in the direction of the first basis vector >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: By specifying that the grid is continuous in a direction, atoms outside of the grid will be affected by a force determined by interpolating based on the values at the edge of the grid with the values of the corresponding edge of the periodic image of the grid. The current size of the simulation box is taken into account, so that as the simulation box size fluctuates, the force on an atom outside of the grid varies continuously until it re-enters the opposite edge of the grid. If the grid is not continuous in this direction, the interpolated force on atoms near the edge of the grid is calculated so that it continuously approaches zero as an atom approaches the edge of the grid.

- `mgridforcecont2` < tag > < Is grid continuous in the direction of the second basis vector >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: Operates the same as `mgridforcecont1` except applies in the direction of the second basis vector

- `mgridforcecont3` < tag > < Is grid continuous in the direction of the third basis vector >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: Operates the same as `mgridforcecont1` except applies in the direction of the third basis vector

- `mgridforcevoff` < tag > < Offset periodic images of the grid by specified amounts >
  Acceptable Values: vector of decimals (x y z)
  Default Value: (0 0 0)
  Description: If a continuous grid is used along a particular basis vector, it may be desirable to shift the potentials in the image to manipulate the potential outside the grid. For example, consider the case where the potential is a ramp in the x direction and the grid is defined for points $[0, N)$, with a potential $f(i, j, k)$ given by $f(i, j, k) = f_0 + i(f_1 - f_0)/N$. By shifting the images of the grid, the potential can be transformed as illustrated in Fig. 4.

- `mgridforcelite` < tag > < Is grid to use Gridforce Lite interpolation? >
  Acceptable Values: `yes` or `no`
  Default Value: `no`

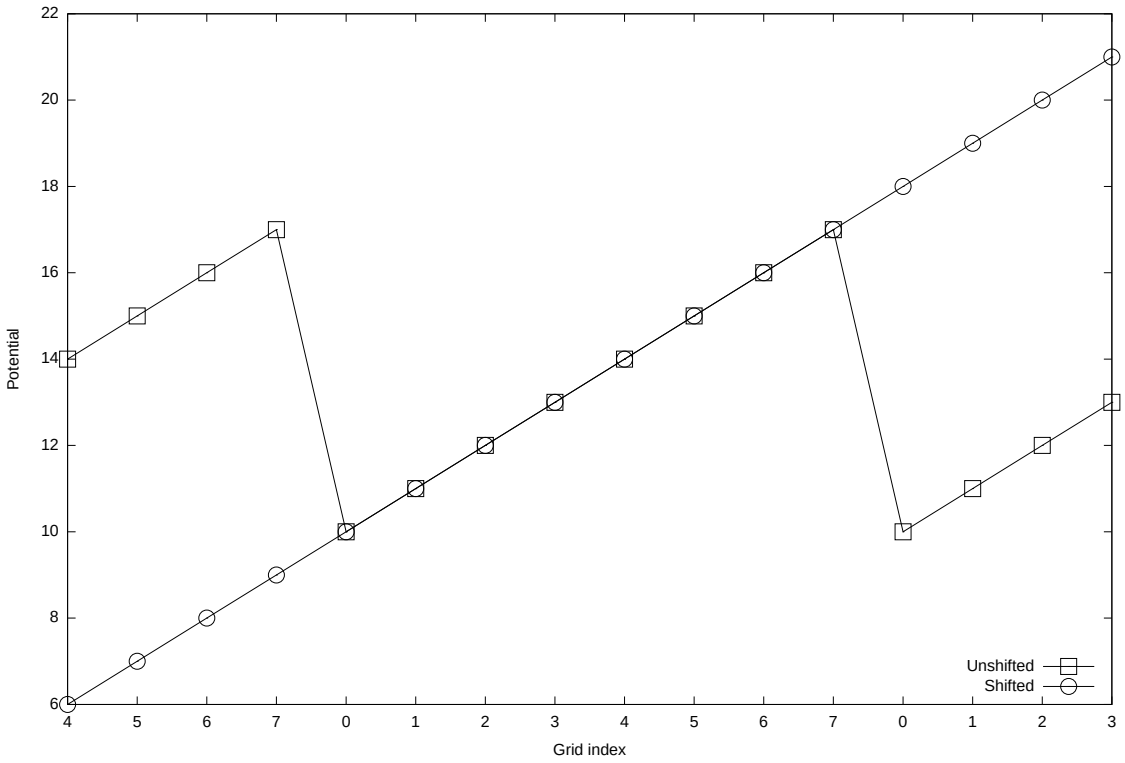

Figure 4: Graph showing a slice of a ramp potential, with eight grid points along the axis, and a periodic cell size which just contains the grid. The Unshifted case shows how the pontential is not smooth when `mgridforcevoff` is not specified, or set to zero. The Shifted potential shows the grid that results when `mgridfocevoff` is set so that the wrapped potential is offset so that the potential has constant slope at the periodic boundaries.

Description: When Gridforce Lite is enabled, a faster but less accurate interpolation method is used to compute forces. Specifically, rather than computing a tri-cubic interpolation of the potential, from which the force is then computed analytically, Gridforce Lite computes force as a linear interpolation. This method also increases the memory required by Gridforce. Note that Gridforce Lite is incompatible with use of the `mgridforcecont[123]` keywords and with non-uniform grids.

8.4 Moving Constraints

Moving constraints feature works in conjunction with the Harmonic Constraints (see an appropriate section of the User's guide). The reference positions of all constraints will move according to

$$\vec{r}(t) \;=\; \vec{r}_0 \;+\; \vec{v}t\,. \tag{30}$$

A velocity vector $\vec{v}$ (`movingConsVel`) needs to be specified.

The way the moving constraints work is that the moving reference position is calculated every integration time step using Eq. 30, where $\vec{v}$ is in Å/timestep, and t is the current timestep (i.e., `firstTimestep` plus however many timesteps have passed since the beginning of NAMD run). Therefore, one should be careful when restarting simulations to appropriately update the

`firstTimestep` parameter in the NAMD configuration file or the reference position specified in the reference PDB file.
NOTE: NAMD actually calculates the constraints potential with $U = k(x - x_0)^d$ and the force with $F = dk(x - x_0)$, where d is the exponent `consexp`. The result is that if one specifies some value for the force constant k in the PDB file, effectively, the force constant is $2k$ in calculations. This caveat was removed in SMD feature.

The following parameters describe the parameters for the moving harmonic constraint feature of NAMD.

- `movingConstraints` < Are moving constraints active >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Should moving restraints be applied to the system. If set to `on`, then `movingConsVel` must be defined. May not be used with `rotConstraints`.

- `movingConsVel` < Velocity of the reference position movement >
  Acceptable Values: vector in Å/timestep
  Description: The velocity of the reference position movement. Gives both absolute value and direction

8.5 Rotating Constraints

The constraints parameters are specified in the same manner as for usual (static) harmonic constraints. The reference positions of all constrained atoms are then rotated with a given angular velocity about a given axis. If the force constant of the constraints is sufficiently large, the constrained atoms will follow their reference positions.

A rotation matrix M about the axis unit vector v is calculated every timestep for the angle of rotation corresponding to the current timestep. angle $= \Omega t$, where Ω is the angular velocity of rotation.

From now on, all quantities are 3D vectors, except the matrix M and the force constant K.

The current reference position R is calculated from the initial reference position R_0 (at $t = 0$), $R = M(R_0 - P) + P$, where P is the pivot point.

Coordinates of point N can be found as $N = P + ((R - P) \cdot v)v$. Normal from the atom pos to the axis is, similarly, normal $= (P + ((X - P) \cdot v)v) - X$ The force is, as usual, $F = K(R - X)$; This is the force applied to the atom in NAMD (see below). NAMD does not know anything about the torque applied. However, the torque applied to the atom can be calculated as a vector product torque $= F \times normal$ Finally, the torque applied to the atom with respect to the axis is the projection of the torque on the axis, i.e., $torque_{proj} = torque \cdot v$

If there are atoms that have to be constrained, but not moved, this implementation is not suitable, because it will move *all* reference positions.

Only one of the moving and rotating constraints can be used at a time.

Using very soft springs for rotating constraints leads to the system lagging behind the reference positions, and then the force is applied along a direction different from the "ideal" direction along the circular path.

Pulling on N atoms at the same time with a spring of stiffness K amounts to pulling on the whole system by a spring of stiffness NK, so the overall behavior of the system is as if you are pulling with a very stiff spring if N is large.

In both moving and rotating constraints the force constant that you specify in the constraints pdb file is multiplied by 2 for the force calculation, i.e., if you specified $K = 0.5$ kcal/mol/Å^2 in the pdb file, the force actually calculated is $F = 2K(R - X) = 1$ kcal/mol/Å^2 $(R - X)$. SMD feature of namd2 does the calculation without multiplication of the force constant specified in the config file by 2.

- `rotConstraints` < Are rotating constraints active >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Should rotating restraints be applied to the system. If set to `on`, then `rotConsAxis`, `rotConsPivot` and `rotConsVel` must be defined. May not be used with `movingConstraints`.

- `rotConsAxis` < Axis of rotation >
  Acceptable Values: vector (may be unnormalized)
  Description: Axis of rotation. Can be any vector. It gets normalized before use. If the vector is 0, no rotation will be performed, but the calculations will still be done.

- `rotConsPivot` < Pivot point of rotation >
  Acceptable Values: position in Å
  Description: Pivot point of rotation. The rotation axis vector only gives the direction of the axis. Pivot point places the axis in space, so that the axis goes through the pivot point.

- `rotConsVel` < Angular velocity of rotation >
  Acceptable Values: rate in degrees per timestep
  Description: Angular velocity of rotation, degrees/timestep.

8.6 Symmetry Restraints

Symmetry restraints are based on symmetrical relationships between monomers. Defined monomers are transformed to overlap and an average position for each atom is calculated. After the average structure is transformed back, a harmonic force is calculated which drives each monomer to the average.

- `symmetryRestraints` < Are symmetry restraints active? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Should Symmetry constraining forces be applied to the system. If symmetry restraints are enabled, `symmetryk`* and `symmetryFile` must be defined in the input file as well. *See `symmetryk` entry for details.

- `symmetryFirstFullStep` < First step to apply full harmonic force >
  Acceptable Values: Non-negative integer
  Default Value: `symmetryFirstStep`
  Description: Force constant `symmetryk` linearly increased from `symmetryFirstStep` to `symmetryFirstFullStep`

- `symmetryLastFullStep` < Last step to apply full harmonic force >
  Acceptable Values: Non-negative integer

Default Value: `symmetryLastStep`
Description: Force constant `symmetryk` linearly decreased from `symmetryLastFullStep` to `symmetryLastStep`

- `symmetryk` < Constant for harmonic restraining forces >
Acceptable Values: Positive value
Description: Harmonic force constant. Scaled down by number of atoms in the monomer. If this setting is omitted, the value in the occupancy column of the pdb file specified by `symmetrykFile` will be used as the constant for that atom. This allows the user to specify the constant on a per-atom basis.

- `symmetrykFile` < pdb containing per atom force constants >
Acceptable Values: Path to pdb file
Description: pdb where the occupancy column specifies the per atom force constants. If using overlapping symmetry groups, you must include one additional `symmetrykfile` per `symmetryFile`

- `symmetryScaleForces` < Scale symmetry restraints over time >
Acceptable Values: `on` or `off`
Default Value: `off`
Description: If turned on, the harmonic force applied by the symmetry restraints will linearly evolve with each time step based on `symmetryFirstFullStep` and `symmetryLastFullStep`.

- `symmetryFile` < File for symmetry information >
Acceptable Values: Path to PDB file
Description: Restrained atoms are those whose occupancy (O) is nonzero in the symmetry pdb file. The file must contain no more atoms than the structure file and those atoms present must have the exact same index as the structure file (i.e., the file may contain a truncated atom selection "index $< N$" but not an arbitrary selection). The value in the occupancy column represent the "symmetry group" the atom belongs to. These symmetry groups are used for denoting monomers of the same type. These groups will be transformed by the matrices in their own `symmetryMatrixFile` and averaged separetely from other groups. The designation in the occupancy column should be an integer value starting at 1 and proceeding in ascending order, mirroring the order of the corresponding matrix file within the configuration file (e.g. the first symmetryMatrixFile contains the matrices for symmetry group 1). The value in the atom's beta column represents its monomer designation. This should be an integer value starting at 1 and proceeding in ascending order, relative to the order of the corresponding transformation matrix found in the symmetry group's `symmetryMatrixFile`. If an atom is contained in more than one symmetry group, additional pdb files can be listed. These pdb files should follow the same rules as the first one (unique group and monomer identifiers in increasing order).

- `symmetryMatrixFile` < File for transformation matrices >
Acceptable Values: Path to matrix file
Description: The `symmetryMatrixFile` is a path to a file that contains a list of transformation matrices to make the monomers overlap. The file should contain one (and only one) matrix for each monomer in the order of monomer ID designated in the symmetryFile.

Each symmetry group should have its own symmetryMatrixFile file containing only the matrices used by the monomers in that group. These should be formatted with spaces between columns and NO spaces between rows as follows:

1 0 0 0
0 1 0 0
0 0 1 0
0 0 0 1

with different matrices separated by a single blank line (and no line before the first or after the last matrix). This file is OPTIONAL. Leave this line out to have namd generate the transformations for you.

- `symmetryFirstStep` < first symmetry restraint timestep >
  Acceptable Values: Non-negative integer
  Default Value: 0
  Description:

- `symmetryLastStep` < last symmetry restraint timestep >
  Acceptable Values: Positive integer
  Default Value: infinity
  Description: Symmetry restraints are applied only between `symmetryFirstStep` and `symmetryLastStep`. Use these settings with caution and ensure restraints are only being applied when necessary (e.g. not during equilibration).

8.7 Targeted Molecular Dynamics (TMD)

In TMD, subset of atoms in the simulation is guided towards a final 'target' structure by means of steering forces. At each timestep, the RMS distance between the current coordinates and the target structure is computed (after first aligning the target structure to the current coordinates). The force on each atom is given by the gradient of the potential

$$U_{TMD} = \frac{1}{2}\frac{k}{N}\left[RMS(t) - RMS^*(t)\right]^2 \tag{31}$$

where $RMS(t)$ is the instantaneous best-fit RMS distance of the current coordinates from the target coordinates, and $RMS^*(t)$ evolves linearly from the initial RMSD at the first TMD step to the final RMSD at the last TMD step. The spring constant k is scaled down by the number N of targeted atoms.

Atoms can be separated into non-overlapping constraint domains by assigning integer values in the beta column of the `TMDFile`. Forces on the atoms will be calculated for each domain independently of the other domains.

Within each domain, the set of atoms used to fit the target structure can be different from the set of atoms that are biased towards the target structure. If the altloc field in the `TMDFile` is not ' ' or '0' then the atom is fitted. If the occupancy is non-zero then the atom is biased. If none of the atoms in a domain have altloc set then all biased atoms are fitted.

Note that using different atoms for fitting and biasing or not using the same spring constant for all target atoms within a domain will result in forces conserving neither energy nor momentum. In this case harmonic restraints and Langevin dynamics are likely needed.

- `TMD` < Is TMD active >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Should TMD steering forces be applied to the system. If TMD is enabled, `TMDk`, `TMDFile`, and `TMDLastStep` must be defined in the input file as well.

- `TMDk` < Elastic constant for TMD forces >
  Acceptable Values: Positive value in $kcal/mol/\text{Å}^2$.
  Description: The value of k in Eq. 31. A value of 200 seems to work well in many cases. If this setting is omitted, the value in the occupancy column of the pdb file specified by `TMDFile` will be used as the constant for that atom. This allows the user to specify the constant on a per-atom basis.

- `TMDOutputFreq` < How often to print TMD output >
  Acceptable Values: Positive integer
  Default Value: 1
  Description: TMD output consists of lines of the form `TMD ts targetRMS currentRMS` where `ts` is the timestep, `targetRMS` is the target RMSD at that timestep, and `currentRMS` is the actual RMSD.

- `TMDFile` < File for TMD information >
  Acceptable Values: Path to PDB file
  Description: Biased atoms are those whose occupancy (O) is nonzero in the TMD PDB file. Fitted atoms are those whose altloc field is not ' ' or '0', if present, otherwise all biased atoms are fitted. The file must contain no more atoms than the structure file and those atoms present must have the exact same index as the structure file (i.e., the file may contain a truncated atom selection "index < N" but not an arbitrary selection). The coordinates for the target structure are also taken from the targeted atoms in this file. Non-targeted atoms are ignored. The beta column of targetted atoms is used to designate non-overlapping constraint domains. Forces will be calculated for atoms within a domain separately from atoms of other domains.

- `TMDFirstStep` < first TMD timestep >
  Acceptable Values: Positive integer
  Default Value: 0
  Description:

- `TMDLastStep` < last TMD timestep >
  Acceptable Values: Positive integer
  Description: TMD forces are applied only between `TMDFirstStep` and `TMDLastStep`. The target RMSD evolves linearly in time from the initial to the final target value.

- `TMDInitialRMSD` < target RMSD at first TMD step >
  Acceptable Values: Non-negative value in Å
  Default Value: from coordinates
  Description: In order to perform TMD calculations that involve restarting a previous NAMD run, be sure to specify `TMDInitialRMSD` with the same value in each NAMD input file, and use the NAMD parameter `firstTimestep` in the continuation runs so that the target RMSD continues from where the last run left off.

- `TMDFinalRMSD` < target RMSD at last TMD step >
  Acceptable Values: Non-negative value in Å
  Default Value: 0
  Description: If no `TMDInitialRMSD` is given, the initial RMSD will be calculated at the first TMD step. `TMDFinalRMSD` may be less than or greater than `TMDInitialRMSD`, depending on whether the system is to be steered towards or away from a target structure, respectively. Forces are applied only if $RMS(t)$ is betwween `TMDInitialRMSD` and $RMS*(t)$; in other words, only if the current RMSD fails to keep pace with the target value.

- `TMDDiffRMSD` < Is double-sided TMD active? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Turns on the double-sided TMD feature which targets the transition between two structures. This is accomplished by modifying the TMD force such that the potential is based on the difference of RMSD's from the two structures:

$$U_{TMD} = \frac{1}{2}\frac{k}{N}\left[DRMS(t) - DRMS^*(t)\right]^2 \tag{32}$$

  where $DRMS(t)$ is RMS1(t) - RMS2(2) (RMS1 being the RMSD from structure 1 and RMS2 the RMSD from structure 2). The first structure is specified as normal in `TMDFile` and the second structure should be specified in `TMDFile2`, preserving any domain designations found in `TMDFile`.

- `TMDFile2` < Second structure file for double-sided TMD >
  Acceptable Values: Path to PDB file
  Description: PDB file defining the second structure of a double sided TMD. This file should contain the same number of atoms as `TMDFile` along with the same domain designations if any are specified.

8.8 Steered Molecular Dynamics (SMD)

The SMD feature is independent from the harmonic constraints, although it follows the same ideas. In both SMD and harmonic constraints, one specifies a PDB file which indicates which atoms are 'tagged' as constrained. The PDB file also gives initial coordinates for the constraint positions. One also specifies such parameters as the force constant(s) for the constraints, and the velocity with which the constraints move.

There are two major differences between SMD and harmonic constraints:

- In harmonic constraints, each tagged atom is harmonically constrained to a reference point which moves with constant velocity. In SMD, it is the *center of mass* of the tagged atoms which is constrained to move with constant velocity.

- In harmonic constraints, each tagged atom is constrained in all three spatial dimensions. In SMD, tagged atoms are constrained *only along the constraint direction* (unless the optional `SMDk2` keyword is used.)

The center of mass of the SMD atoms will be harmonically constrained with force constant k (`SMDk`) to move with velocity v (`SMDVel`) in the direction $\vec{n}$ (`SMDDir`). SMD thus results in the

following potential being applied to the system:

$$U(\vec{r}_1, \vec{r}_2, ..., t) \;=\; \frac{1}{2}k\left[vt - (\vec{R}(t) - \vec{R}_0)\cdot\vec{n}\right]^2. \tag{33}$$

Here, $t \equiv N_{ts}dt$ where N_{ts} is the number of elapsed timesteps in the simulation and dt is the size of the timestep in femtoseconds. Also, $\vec{R}(t)$ is the current center of mass of the SMD atoms and R_0 is the initial center of mass as defined by the coordinates in `SMDFile`. Vector $\vec{n}$ is normalized by NAMD before being used.

Optionally, one may also specify a transverse force constant k_2 (`SMDk2`). The potential then becomes

$$U(\vec{r}_1, \vec{r}_2, ..., t) \;=\; \frac{1}{2}k\left[vt - (\vec{R}(t) - \vec{R}_0)\cdot\vec{n}\right]^2 + \frac{1}{2}k_2\left[\left(\vec{R}(t) - \vec{R}_0\right)^2 - \left((\vec{R}(t) - \vec{R}_0)\cdot\vec{n}\right)^2\right]. \tag{34}$$

In this case, the force constant k controls the potential *parallel* to the pulling direction $\vec{n}$, while the transverse force constant k_2 controls the potential *perpendicular* to $\vec{n}$.

Output NAMD provides output of the current SMD data. The frequency of output is specified by the `SMDOutputFreq` parameter in the configuration file. Every `SMDOutputFreq` timesteps NAMD will print the current timestep, current position of the center of mass of the restrained atoms, and the current force applied to the center of mass (in piconewtons, pN). The output line starts with word `SMD`

Parameters The following parameters describe the parameters for the SMD feature of NAMD.

- `SMD` < Are SMD features active >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Should SMD harmonic constraint be applied to the system. If set to `on`, then `SMDk`, `SMDFile`, `SMDVel`, and `SMDDir` must be defined. Specifying `SMDOutputFreq` is optional.

- `SMDFile` < SMD constraint reference position >
  Acceptable Values: UNIX filename
  Description: File to use for the initial reference position for the SMD harmonic constraints. All atoms in this PDB file with a nonzero value in the *occupancy* column will be tagged as SMD atoms. The coordinates of the tagged SMD atoms will be used to calculate the initial center of mass. During the simulation, this center of mass will move with velocity `SMDVel` in the direction `SMDDir`. The actual atom order in this PDB file must match that in the structure or coordinate file, since the atom number field in this PDB file will be ignored.

- `SMDk` < force constant to use in SMD simulation >
  Acceptable Values: positive real
  Description: SMD harmonic constraint force constant. Must be specified in kcal/mol/Å^2. The conversion factor is 1 kcal/mol = 69.479 pN Å.

- `SMDk2` < force constant for transverse direction to use in SMD simulation >
  Acceptable Values: positive real
  Default Value: 0
  Description: SMD transverse harmonic constraint force constant. Must be specified in kcal/mol/Å^2. The conversion factor is 1 kcal/mol = 69.479 pN Å.

- `SMDVel` < Velocity of the SMD reference position movement >
  Acceptable Values: nonzero real, Å/timestep
  Description: The velocity of the SMD center of mass movement. Gives the absolute value.

- `SMDDir` < Direction of the SMD center of mass movement >
  Acceptable Values: non-zero vector
  Description: The direction of the SMD reference position movement. The vector does not have to be normalized, it is normalized by NAMD before being used.

- `SMDOutputFreq` < frequency of SMD output >
  Acceptable Values: positive integer
  Default Value: 1
  Description: The frequency in timesteps with which the current SMD data values are printed out.

8.9 Interactive Molecular Dynamics (IMD)

NAMD now works directly with VMD to allow you to view and interactively steer your simulation. With IMD enabled, you can connect to NAMD at any time during the simulation to view the current state of the system or perform interactive steering.

- `IMDon` < is IMD active? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not to listen for an IMD connection.

- `IMDport` < port number to expect a connection on >
  Acceptable Values: positive integer
  Description: This is a free port number on the machine that node 0 is running on. This number will have to be entered into VMD.

- `IMDfreq` < timesteps between sending coordinates >
  Acceptable Values: positive integer
  Description: This allows coordinates to be sent less often, which may increase NAMD performance or be necessary due to a slow network.

- `IMDwait` < wait for an IMD connection? >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: If `no`, NAMD will proceed with calculations whether a connection is present or not. If `yes`, NAMD will pause at startup until a connection is made, and pause when the connection is lost.

- `IMDignore` < ignore interactive steering forces >
  Acceptable Values: `yes` or `no`
  Default Value: `no`
  Description: If `yes`, NAMD will ignore any steering forces generated by VMD to allow a simulation to be monitored without the possibility of perturbing it.

8.10 Tcl Forces and Analysis

NAMD provides a limited Tcl scripting interface designed for applying forces and performing on-the-fly analysis. This interface is efficient if only a few coordinates, either of individual atoms or centers of mass of groups of atoms, are needed. In addition, information must be requested one timestep in advance. To apply forces individually to a potentially large number of atoms, use `tclBC` instead as described in Sec. 8.11. The following configuration parameters are used to enable the Tcl interface:

- `tclForces` < is Tcl interface active? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not Tcl interface is active. If it is set to `off`, then no Tcl code is executed. If it is set to `on`, then Tcl code specified in `tclForcesScript` parameters is executed.

- `tclForcesScript` < input for Tcl interface >
  Acceptable Values: file or {script}
  Description: Must contain either the name of a Tcl script file or the script itself between { and } (may include multiple lines). This parameter may occur multiple times and scripts will be executed in order of appearance. The script(s) should perform any required initialization on the Tcl interpreter, including requesting data needed during the first timestep, and define a procedure `calcforces { }` to be called every timestep.

At this point only low-level commands are defined. In the future this list will be expanded. Current commands are:

- `print <anything>`
  This command should be used instead of `puts` to display output. For example, "`print Hello World`".

- `atomid <segname> <resid> <atomname>`
  Determines atomid of an atom from its segment, residue, and name. For example, "`atomid br 2 N`".

- `addatom <atomid>`
  Request coordinates of this atom for next force evaluation, and the calculated total force on this atom for current force evaluation. Request remains in effect until `clearconfig` is called. For example, "`addatom 4`" or "`addatom [atomid br 2 N]`".

- `addgroup <atomid list>`
  Request center of mass coordinates of this group for next force evaluation. Returns a group ID which is of the form `gN` where `N` is a small integer. This group ID may then be used to find coordinates and apply forces just like a regular atom ID. Aggregate forces may then be applied to the group as whole. Request remains in effect until `clearconfig` is called. For example, "`set groupid [addgroup { 14 10 12 }]`".

- `clearconfig`
  Clears the current list of requested atoms. After `clearconfig`, calls to `addatom` and `addgroup` can be used to build a new configuration.

- `getstep`
  Returns the current step number.

- `loadcoords <varname>`
  Loads requested atom and group coordinates (in Å) into a local array. `loadcoords` should only be called from within the `calcforces` procedure. For example, "`loadcoords p`" and "`print $p(4)`".

- `loadforces <varname>`
  Loads the forces applied in the previous timestep (in kcal mol^{-1} $Å^{-1}$) into a local array. `loadforces` should only be called from within the `calcforces` procedure. For example, "`loadforces f`" and "`print $f(4)`".

- `enabletotalforces/disabletotalforces`
  Enables/disables the "`loadtotalforces`" command, described below, which is disabled by default to avoid unneeded work and communication.

- `loadtotalforces <varname>`
  Loads the total forces on each requested atom and group in the previous time step (in kcal $mol^{-1}Å^{-1}$) into a local array. The total force also includes external forces. Note that the "`loadforces`" command returns external forces applied by the user. Therefore, one can subtract the external force on an atom from the total force on this atom to get the pure force arising from the simulation system. Note that "`enabletotalforces`" must be called first.

- `loadmasses <varname>`
  Loads requested atom and group masses (in amu) into a local array. `loadmasses` should only be called from within the `calcforces` procedure. For example, "`loadcoords m`" and "`print $m(4)`".

- `addforce <atomid|groupid> <force vector>`
  Applies force (in kcal mol^{-1} $Å^{-1}$) to atom or group. `addforce` should only be called from within the `calcforces` procedure. For example, "`addforce $groupid { 1. 0. 2. }`".

- `addenergy <energy (kcal/mol)>`
  This command adds the specified energy to the MISC column (and hence the total energy) in the energy output. For normal runs, the command does not affect the simulation trajectory at all, and only has an artificial effect on its energy output. However, it can indeed affect minimizations.

With the commands above and the functionality of the Tcl language, one should be able to perform any on-the-fly analysis and manipulation. To make it easier to perform certain tasks, some Tcl routines are provided below.

Several vector routines (`vecadd`, `vecsub`, `vecscale`) from the VMD Tcl interface are defined. Please refer to VMD manual for their usage.

The following routines take atom coordinates as input, and return some geometry parameters (bond, angle, dihedral).

- `getbond <coor1> <coor2>`
  Returns the length of the bond between the two atoms. Actually the return value is simply the distance between the two coordinates. "coor1" and "coor2" are coordinates of the atoms.

- `getangle <coor1> <coor2> <coor3>`
  Returns the angle (from 0 to 180) defined by the three atoms. "coor1", "coor2" and "coor3" are coordinates of the atoms.

- `getdihedral <coor1> <coor2> <coor3> <coor4>`
  Returns the dihedral (from -180 to 180) defined by the four atoms. "coor1", "coor2", "coor3" and "coor4" are coordinates of the atoms.

The following routines calculate the derivatives (gradients) of some geometry parameters (angle, dihedral).

- `anglegrad <coor1> <coor2> <coor3>`
  An angle defined by three atoms is a function of their coordinates: $\theta\left(\vec{r_1}, \vec{r_2}, \vec{r_3}\right)$ (in radian). This command takes the coordinates of the three atoms as input, and returns a list of $\{\frac{\partial\theta}{\partial\vec{r_1}}\ \frac{\partial\theta}{\partial\vec{r_2}}\ \frac{\partial\theta}{\partial\vec{r_3}}\}$. Each element of the list is a 3-D vector in the form of a Tcl list.

- `dihedralgrad <coor1> <coor2> <coor3> <coor4>`
  A dihedral defined by four atoms is a function of their coordinates: $\phi\left(\vec{r_1}, \vec{r_2}, \vec{r_3}, \vec{r_4}\right)$ (in radian). This command takes the coordinates of the four atoms as input, and returns a list of $\{\frac{\partial\phi}{\partial\vec{r_1}}\ \frac{\partial\phi}{\partial\vec{r_2}}\ \frac{\partial\phi}{\partial\vec{r_3}}\ \frac{\partial\phi}{\partial\vec{r_4}}\}$. Each element of the list is a 3-D vector in the form of a Tcl list.

As an example, here's a script which applies a harmonic constraint (reference position being 0) to a dihedral. Note that the "addenergy" line is not really necessary – it simply adds the calculated constraining energy to the MISC column, which is displayed in the energy output.

```
tclForcesScript {
  # The IDs of the four atoms defining the dihedral
  set aid1 112
  set aid2 123
  set aid3 117
  set aid4 115

  # The "spring constant" for the harmonic constraint
  set k 3.0

  addatom $aid1
  addatom $aid2
  addatom $aid3
  addatom $aid4

  set PI 3.1416

  proc calcforces {} {

    global aid1 aid2 aid3 aid4 k PI

    loadcoords p
```

```
    # Calculate the current dihedral
    set phi [getdihedral $p($aid1) $p($aid2) $p($aid3) $p($aid4)]
    # Change to radian
    set phi [expr $phi*$PI/180]

    # (optional) Add this constraining energy to "MISC" in the energy output
    addenergy [expr $k*$phi*$phi/2.0]

    # Calculate the "force" along the dihedral according to the harmonic constraint
    set force [expr -$k*$phi]

    # Calculate the gradients
    foreach {g1 g2 g3 g4} [dihedralgrad $p($aid1) $p($aid2) $p($aid3) $p($aid4)] {}

    # The force to be applied on each atom is proportional to its
    # corresponding gradient
    addforce $aid1 [vecscale $g1 $force]
    addforce $aid2 [vecscale $g2 $force]
    addforce $aid3 [vecscale $g3 $force]
    addforce $aid4 [vecscale $g4 $force]
  }
}
```

8.11 Tcl Boundary Forces

While the `tclForces` interface described above is very flexible, it is only efficient for applying forces to a small number of pre-selected atoms. Applying forces individually to a potentially large number of atoms, such as applying boundary conditions, is much more efficient with the `tclBC` facility described below.

- `tclBC` < are Tcl boundary forces active? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not Tcl interface is active. If it is set to `off`, then no Tcl code is executed. If it is set to `on`, then Tcl code specified in the `tclBCScript` parameter is executed.

- `tclBCScript` < input for Tcl interface >
  Acceptable Values: {script}
  Description: Must contain the script itself between { and } (may include multiple lines). This parameter may occur only once. The script(s) should perform any required initialization on the Tcl interpreter and define a procedure `calcforces <step> <unique> [args...]` to be called every timestep.

- `tclBCArgs` < extra args for tclBC calcforces command >
  Acceptable Values: {args...}
  Description: The string (or Tcl list) provided by this option is appended to the tclBC

calcforces command arguments. This parameter may appear multiple times during a run in order to alter the parameters of the boundary potential function.

The script provided in `tclBCScript` and the `calcforces` procedure it defines are executed in multiple Tcl interpreters, one for every processor that owns patches. These `tclBC` interpreters do not share state with the Tcl interpreter used for `tclForces` or config file parsing. The `calcforces` procedure is passed as arguments the current timestep, a "unique" flag which is non-zero for exactly one Tcl interpreter in the simulation (that on the processor of patch zero), and any arguments provided to the most recent `tclBCArgs` option. The "unique" flag is useful to limit printing of messages, since the command is invoked on multiple processors.

The `print`, `vecadd`, `vecsub`, `vecscale`, `getbond`, `getangle`, `getdihedral`, `anglegrad`, and `dihedralgrad` commands described under `tclForces` are available at all times.

The `wrapmode <mode>` command, available in the `tclBCScript` or the `calcforces` procedure, determines how coordinates obtained in the calcforces procedure are wrapped around periodic boundaries. The options are:

- `patch`, (default) the position in NAMD's internal patch data structure, requires no extra calculation and is almost the same as `cell`
- `input`, the position corresponding to the input files of the simulation
- `cell`, the equivalent position in the unit cell centered on the `cellOrigin`
- `nearest`, the equivalent position nearest to the `cellOrigin`

The following commands are available from within the `calcforces` procedure:

- `nextatom`
  Sets the internal counter to a new atom and return 1, or return 0 if all atoms have been processed (this may even happen the first call). This should be called as the condition of a while loop, i.e., `while {[nextatom]} { ... }` to iterate over all atoms. One one atom may be accessed at a time.
- `dropatom`
  Excludes the current atom from future iterations on this processor until `cleardrops` is called. Use this to eliminate extra work when an atom will not be needed for future force calculations. If the atom migrates to another processor it may reappear, so this call should be used only as an optimization.
- `cleardrops`
  All available atoms will be iterated over by `nextatom` as if `dropatom` had never been called.
- `getcoord`
  Returns a list {x y z} of the position of the current atom wrapped in the periodic cell (if there is one) in the current wrapping mode as specified by `wrapmode`.
- `getcell`
  Returns a list of 1–4 vectors containing the cell origin (center) and as many basis vectors as exist, i.e., `{{ox oy oz} {ax ay az} {bx by bz} {cx cy cz}}`. It is more efficient to set the wrapping mode than to do periodic image calculations in Tcl.

- `getmass`
  Returns the mass of the current atom.
- `getcharge`
  Returns the charge of the current atom.
- `getid`
  Returns the 1-based ID of the current atom.
- `addforce {<fx> <fy> <fz>}`
  Adds the specified force to the current atom for this step.
- `addenergy <energy>`
  Adds potential energy to the BOUNDARY column of NAMD output.

As an example, these spherical boundary condition forces:

```
sphericalBC        on
sphericalBCcenter  0.0,0.0,0.0
sphericalBCr1      48
sphericalBCk1      10
sphericalBCexp1    2
```

Are replicated in the following script:

```
tclBC on
tclBCScript {
  proc veclen2 {v1} {
    foreach {x1 y1 z1} $v1 { break }
    return [expr $x1*$x1 + $y1*$y1 + $z1*$z1]
  }

  # wrapmode input
  # wrapmode cell
  # wrapmode nearest
  # wrapmode patch ;# the default

  proc calcforces {step unique R K} {
    if { $step % 20 == 0 } {
      cleardrops
      # if $unique { print "clearing dropped atom list at step $step" }
    }
    set R [expr 1.*$R]
    set R2 [expr $R*$R]
    set tol 2.0
    set cut2 [expr ($R-$tol)*($R-$tol)]

    while {[nextatom]} {
      # addenergy 1 ; # monitor how many atoms are checked
```

```
      set rvec [getcoord]
      set r2 [veclen2 $rvec]
      if { $r2 < $cut2 } {
        dropatom
        continue
      }
      if { $r2 > $R2 } {
        # addenergy 1 ; # monitor how many atoms are affected
        set r [expr sqrt($r2)]
        addenergy [expr $K*($r - $R)*($r - $R)]
        addforce [vecscale $rvec [expr -2.*$K*($r-$R)/$r]]
      }
    }
  }
}

tclBCArgs {48.0 10.0}
```

8.12 External Program Forces

This feature allows an external program to be called to calculate forces at every force evaluation, taking all atom coordinates as input.

- `extForces` < Apply external program forces? >
  Acceptable Values: yes or no
  Default Value: no
  Description: Specifies whether or not external program forces are applied.

- `extForcesCommand` < Force calculation command >
  Acceptable Values: UNIX shell command
  Description: This string is the argument to the system() function at every forces evaluation and should read coordinates from the file specified by extCoordFilename and write forces to the file specified by extForceFilename.

- `extCoordFilename` < Temporary coordinate file >
  Acceptable Values: UNIX filename
  Description: Atom coordinates are written to this file, which should be read by the extForcesCommand. The format is one line of "atomid charge x y z" for every atom followed by three lines with the periodic cell basis vectors "a.x a.y a.z", "b.x b.y b.z", and "c.x c.y c.z". The atomid starts at 1 (not 0). For best performance the file should be in /tmp and not on a network-mounted filesystem.

- `extForceFilename` < Temporary force file >
  Acceptable Values: UNIX filename
  Description: Atom forces are read from this file after extForcesCommand in run. The format is one line of "atomid replace fx fy fz" for every atom followed by the energy on a line by itself and then, optionally, three lines of the virial "v.xx v.xy v.xz", "v.yx v.yy v.yz", "v.zx v.zy v.zz" where, e.g., v.xy = - fx * y for a non-periodic force. The atomid starts at 1 (not 0)

and all atoms must be present and in order. The energy is added to the MISC output field. The replace flag should be 1 if the external program force should replace the forces calculated by NAMD for that atom and 0 if the forces should be added. For best performance the file should be in /tmp and not on a network-mounted filesystem.

9 Collective Variable-based Calculations (Colvars)

The features described in this section were originally contributed to NAMD by Giacomo Fiorin (NIH) and Jérôme Hénin (CNRS, France) and are currently developed at this external repository: https://github.com/Colvars/colvars
An updated version of this section can also be downloaded as a separate manual:
HTML: https://colvars.github.io/colvars-refman-namd/colvars-refman-namd.html
PDF: https://colvars.github.io/pdf/colvars-refman-namd.pdf

See section 9.7 for specific changes that affect compatibility between versions. Please ask any usage questions through the NAMD mailing list, and development questions through GitHub.

Overview

In molecular dynamics simulations, it is often useful to reduce the large number of degrees of freedom of a physical system into few parameters whose statistical distributions can be analyzed individually, or used to define biasing potentials to alter the dynamics of the system in a controlled manner. These have been called 'order parameters', 'collective variables', '(surrogate) reaction coordinates', and many other terms.

Here we use primarily the term 'collective variable', often shortened to *colvar*, to indicate any differentiable function of atomic Cartesian coordinates, $\boldsymbol{x}_i$, with i between 1 and N, the total number of atoms:

$$\xi(t) \ = \xi(\boldsymbol{X}(t)) \ = \xi\left(\boldsymbol{x}_i(t), \boldsymbol{x}_j(t), \boldsymbol{x}_k(t), \ldots\right) \ , \quad 1 \leq i, j, k \ldots \leq N \tag{35}$$

The module is designed to perform multiple tasks concurrently during or after a simulation, the most common of which are:

- apply restraints or biasing potentials to multiple variables, tailored on the system by choosing from a wide set of basis functions, without limitations on their number or on the number of atoms involved; while this can in principle be done through a TclForces script, using the Colvars module is both easier and computationally more efficient;
- calculate potentials of mean force (PMFs) along any set of variables, using different enhanced sampling methods, such as Adaptive Biasing Force (ABF), metadynamics, steered MD and umbrella sampling; variants of these methods that make use of an ensemble of replicas are supported as well;
- calculate statistical properties of the variables, such as running averages and standard deviations, correlation functions of pairs of variables, and multidimensional histograms: this can be done either at run-time without the need to save very large trajectory files, or after a simulation has been completed using VMD and the `cv` command or NAMD and the `coorfile read` command as illustrated in 18.

Detailed explanations of the design of the Colvars module are provided in reference [33]. Please cite this reference whenever publishing work that makes use of this module.

9.1 Writing a Colvars configuration: a crash course

The Colvars configuration is a plain text file or string that defines collective variables, biases, and general parameters of the Colvars module. It is passed to the module using back-end-specific commands documented in section 9.2.

Now let us look at a complete, non-trivial configuration. Suppose that we want to run a steered MD experiment where a small molecule is pulled away from a protein binding site. In Colvars terms, this is done by applying a moving restraint to the distance between the two objects. The configuration will contain two blocks, one defining the distance variable (see section 9.3 and 9.3.2), and the other the moving harmonic restraint (9.5.5).

```
colvar {
  name dist
  distance {
    group1 { atomNumbersRange 42-55 }
    group2 {
      psfSegID PR
      atomNameResidueRange CA 15-30
    }
  }
}

harmonic {
  colvars dist
  forceConstant 20.0
  centers 4.0         # initial distance
  targetCenters 15.0  # final distance
  targetNumSteps 500000
}
```

Reading this input in plain English: the variable here named *dist* consists in a distance function between the centers of two groups: the ligand (atoms 42 to 55) and the α-carbon atoms of residues 15 to 30 in the protein (segment name PR). To the "*dist*" variable, we apply a `harmonic` potential of force constant 20 kcal/mol/Å^2, initially centered around a value of 4 Å, which will increase to 15 Å over 500,000 simulation steps.

The atom selection keywords are detailed in section 9.4.

9.2 Enabling and controlling the Colvars module in NAMD

Here, we document the syntax of the commands and parameters used to set up and use the Colvars module in NAMD. One of these parameters is the configuration file or the configuration text for the module itself, whose syntax is described in 9.2.4 and in the following sections.

9.2.1 Units in the Colvars module

The "internal units" of the Colvars module are the units in which values are expected to be in the configuration file, and in which collective variable values, energies, etc. are expressed in the output and colvars trajectory files. Generally **the Colvars module uses internally the same**

units as its back-end MD engine, with the exception of VMD, where different unit sets are supported to allow for easy setup, visualization and analysis of Colvars simulations performed with any simulation engine.

Note that **angles** are expressed in degrees, and derived quantites such as force constants are based on degrees as well. Atomic coordinates read from **XYZ files** (and PDB files where applicable) are expected to be expressed in Ångström, no matter what unit system is in use by the back-end or the Colvars Module.

To avoid errors due to reading configuration files written in a different unit system, it can be specified within the input:

- `units` < Unit system to be used >
  Context: global
  Acceptable Values: string
  Description: A string defining the units to be used internally by Colvars. In NAMD the only allowed value is NAMD's native units: *real* (Å, kcal/mol).

9.2.2 NAMD parameters

To enable a Colvars-based calculation, the `colvars on` command must be added to the NAMD script. Two optional commands, `colvarsConfig` and `colvarsInput` can be used to define the module's configuration or continue a previous simulation. Because these are static parameters, it is typically more convenient to use the `cv` command in the rest of the NAMD script.

- `colvars` < Enable the Colvars module >
  Context: NAMD configuration file
  Acceptable Values: boolean
  Default Value: `off`
  Description: If this flag is on, the Colvars module within NAMD is enabled.

- `colvarsConfig` < Configuration file for the collective variables >
  Context: NAMD configuration file
  Acceptable Values: UNIX filename
  Description: Name of the Colvars configuration file (9.2.4, 9.2.5 and following sections). This file can also be provided by the Tcl command `cv configfile`. Alternatively, the contents of the file (as opposed to the file itself) can be given as a string argument to the command `cv config`.

- `colvarsInput` < Input state file for the collective variables >
  Context: NAMD configuration file
  Acceptable Values: UNIX filename
  Description: Keyword used to specify the input state file's name (9.2.6). If the input file is meant to be loaded within a Tcl script section, the `cv load` command may be used instead.

9.2.3 Using the `cv` command to control the Colvars module

At any moment after the first initialization of the Colvars module, several options can be read or modified by the Tcl command `cv`, with the following syntax:
`cv <subcommand> [args ...]`

The most frequent uses of the `cv` command are discussed here. For a complete list of all subcommands of `cv`, see section 9.6.

Setting up the Colvars module If the NAMD configuration parameter `colvars` is on, the `cv` command can be used anywhere in the NAMD script, and will be invoked as soon as NAMD begins processing Tcl commands.

To define collective variables and biases, configuration can be loaded using either:

```
cv configfile colvars-file.in
```

to load configuration from a file, or:

```
cv config "keyword { ...  }"
```

to load configuration as a string argument.

The latter version is particularly useful to dynamically define the Colvars configuration. For example, when running an ensemble of umbrella sampling simulations in NAMD, it may be convenient to use an identical NAMD script, and let the queuing system assist in defining the window. In this example, in a Slurm array job an environment variable is used to define the window's numeric index (starting at zero), and the umbrella restraint center (starting at 2 for the first window, and increasing in increments of 0.25 for all other windows):

```
cv configfile colvars-definition.in
set window $env(SLURM_ARRAY_TASK_ID)
cv config "harmonic {
  name us_${window}
  colvars xi
  centers [expr 2.0 + 0.25 * ${window}]
  ...
}"
```

Using the Colvars version in scripts The vast majority of the syntax in Colvars is backward-compatible, adding keywords when new features are introduced. However, when using multiple versions simultaneously it may be useful to test within the script whether the version is recent enough to support the desired feature. `cv version` can be used to get the Colvars version for this use:

```
if { [cv version] >= "2020-02-25" } {
  cv config "(use a recent feature)"
}
```

Loading and saving the Colvars state and other information After a configuration is fully defined, `cv load` may be used to load a state file from a previous simulation that contains e.g. data from history-dependent biases), to either continue that simulation or analyze its results:

```
cv load <oldjob>.colvars.state
```

or more simply using the prefix of the state file itself:

```
cv load <oldjob>
```

The latter is much more convenient in combination with the NAMD `reinitatoms` command, for example:

```
reinitatoms <oldjob>
cv load <oldjob>
```
The step number contained by the loaded file will be used internally by Colvars to control time-dependent biases, unless `firstTimestep` is issued, in which case that value will be used.

When the system's topology is changed during simulation via the `structure` command (e.g. in constant-pH simulations), it is generally best to reset and re-initalize the module from scratch before loading the corresponding snapshot:
```
structure newsystem.psf
reinitatoms <snapshot>
cv reset
cv configfile ...
cv load <snapshot>
```

`cv save`, analogous to `cv load`, saves all restart information to a state file. This is normally not required during a simulation if `colvarsRestartFrequency` (see 9.2.5) is defined (either directly or indirectly by the NAMD restart frequency), but it is necessary in post-processing e.g. with VMD. Because not only a state file (used to continue simulations) but also other data files (used to analyze the trajectory) are written, it is generally clearer to use `cv save` with a prefix rather than a file name:
```
cv save <job>
```
See 9.6.1 for a complete list of scripting commands used to manage the Colvars module.

Managing collective variables After one or more collective variables are defined, they can be accessed via `cv colvar [args ...]`. For example, to recompute the collective variable `xi` the following command can be used:
```
cv colvar xi update
```
This ordinarily is not needed during a simulation run, where all variables are recomputed at every step (along with biasing forces acting on them). However, when analyzing an existing trajectory a call to `update` is generally required.

While in all typical cases all configuration of the variables is done with `cv config` or `cv configfile`, a limited set of changes can be enacted at runtime using `cv colvar <name> modifycvcs [args ...]`. Each argument is a string passed to the function or functions that are used to compute the variable, and are called colvar components, or CVCs (9.3.1). For example, a variable `DeltaZ` made of a single `distanceZ` CVC can be made periodic with a period equal to the unit cell dimension along the Z-axis:
```
cv colvar DeltaZ modifycvcs "period $Lz"
```
where `$Lz` is obtained outside Colvars.
This option is currently limited to changing the values of `componentCoeff` (see 9.3.15) and `componentExp` (see 9.3.15) (e.g. to update the polynomial superposition parameters on the fly), of `period` (see 9.3.13) and `wrapAround` (see 9.3.13), and of the `forceNoPBC` option for all components that support it.

If the variable is computed using more than one CVC, it is possible to selectively turn some of them on or off:
```
cv colvar xi cvcflags <flags>
```
where `<flags>` is a list of 0/1 values, one per component. This is useful for example when Tcl script-based path collective variables in Cartesian coordinates (9.3.10) are used, to minimize

computational cost by disabling the computation of terms that are very close to zero.

Important: None of the changes enacted by `modifycvcs` or `cvcflags` will be saved to state files, and will be lost when restarting a simulation, deleting the corresponding collective variable, or resetting the module with `cv reset`.

Applying and analyzing forces on collective variables As soon as a collective variable is up to date (during a MD run or after its `update` method has been called), forces can be applied to it, e.g. as part of a custom restraint implemented by `scriptedColvarForces` (see 9.5.12):
`cv colvar xi addforce $force`
where `$force` is a scalar or a vector (depending on the type of variable `xi`) and is defined by the user's function. The force will be physically applied to the corresponding atoms during the simulation after Colvars communicates all forces to the rest of NAMD. Until then, the total force applied to `xi` from all biases can be retrieved by:
`cv colvar xi getappliedforce`
(see also the use of the `outputAppliedForce` (see 9.3.19) option).

To obtain the total force projected on the variable `xi`:
`cv colvar xi gettotalforce`
Note that not all types of variable support this option, and the value of the total force may not be available immediately: see `outputTotalForce` (see 9.3.19) for more details.

See 9.6.2 for a complete list of scripting commands used to manage collective variables.

Managing collective variable biases Because biases depend only upon data internal to the Colvars module (i.e. they do not need atomic coordinates from NAMD), it is generally easy to create them or update their configuration at any time. For example, given the most current value of the variable `xi`, an already-defined restraint on it named `harmonic_xi` can be updated as:
`cv bias harmonic_xi update`
Again, this is not generally needed during a running simulation, when an automat ic update of each bias is already carried out.

Calling `update` for a bias is most useful for just-defined biases or when changing their configuration. When `update` is called e.g. as part of the function invoked by `scriptedColvarForces` (see 9.5.12), it is executed before any biasing forces are applied to the variables, thus allowing to modify them. This use of `update` is often used e.g. in the definition of custom bias-exchange algorithms as part of the NAMD script. Because a bias is a relatively light-weight object, the easiest way to change the configuration of an existing bias is deleting it and re-creating it:

```
# Delete the restraint "harmonic_xi"
cv bias harmonic_xi delete
# Re-define it, but using an updated restraint center
cv config "harmonic {
  name harmonic_xi
  centers ${new_center}]
  ...
}"
# Now update it (based on the current value of "xi")
cv bias harmonic_xi update
```

It is also possible to make the change subject to a condition on the energy of the new bias:

```
...
cv bias harmonic_xi update
if { [cv bias harmonic_xi energy] < ${E_accept} } {
    ...
}
```

Loading and saving the state of individual biases Some types of bias are history-dependent, and the magnitude of their forces depends not only on the values of their corresponding variables, but also on previous simulation history. It is thus useful to load information from a state file that contains information specifically for one bias only, for example:
`cv bias metadynamics1 load old.colvars.state`
or alternatively, using the prefix of the file instead of its full name:
`cv bias metadynamics1 load old`
A corresponding `save` function is also available:
`cv bias metadynamics1 save new`
This pair of functions is also used internally by Colvars to implement e.g. multiple-walker metadynamics (9.5.4), but they can be called from a scripted function to implement alternative coupling schemes.

See 9.6.3 for a complete list of scripting commands used to manage biases.

9.2.4 Configuration syntax used by the Colvars module

All the parameters defining variables and their biasing or analysis algorithms are read from the file specified by the configuration option `colvarsConfig`, or by the Tcl commands `cv config` and `cv configfile`. *None of the keywords described in the remainder of this manual are recognized directly in the NAMD configuration file, unless as arguments of* `cv config`. Each configuration line follows the format "`keyword value`", where the keyword and its value are separated by any white space. The following rules apply:

- keywords are case-insensitive (`upperBoundary` is the same as `upperboundary` and `UPPERBOUNDARY`): their string values are however case-sensitive (e.g. file names);
- a long value, or a list of multiple values, can be distributed across multiple lines by using curly braces, "{" and "}": the opening brace "{" must occur on the same line as the keyword, following a space character or other white space; the closing brace "}" can be at any position after that; any keywords following the closing brace on the same line are not valid (they should appear instead on a different line);
- many keywords are nested, and are only meaningful within a specific context: for every keyword documented in the following, the "parent" keyword that defines such context is also indicated in parentheses;
- the '`=`' sign between a keyword and its value, deprecated in the NAMD main configuration file, is not allowed;
- Tcl syntax is generally not available, but it is possible to use Tcl variables or bracket expansion of commands within a configuration string, when this is passed via the command `cv config`

`...`; this is particularly useful when combined with parameter introspection (see 2.2.2), e.g. `cv config "colvarsTrajFrequency [DCDFreq]"`;

- if a keyword requiring a boolean value (`yes|on|true` or `no|off|false`) is provided without an explicit value, it defaults to '`yes|on|true`'; for example, '`outputAppliedForce`' may be used as shorthand for '`outputAppliedForce on`';
- the hash character `#` indicates a comment: all text in the same line following this character will be ignored.

9.2.5 Global keywords

The following keywords are available in the global context of the Colvars configuration, i.e. they are not nested inside other keywords:

- `colvarsTrajFrequency` < Colvar value trajectory frequency >
  Context: global
  Acceptable Values: positive integer
  Default Value: 100
  Description: The values of each colvar (and of other related quantities, if requested) are written to the file *outputName*`.colvars.traj` every these many steps throughout the simulation. If the value is 0, such trajectory file is not written. For optimization the output is buffered, and synchronized with the disk only when the restart file is being written.

- `colvarsRestartFrequency` < Colvar module restart frequency >
  Context: global
  Acceptable Values: positive integer
  Default Value: NAMD parameter `restartFreq`
  Description: The state file and any other output files produced by Colvars are written every these many steps (the trajectory file is still written every `colvarsTrajFrequency` (see 9.2.5) steps). It is generally a good idea to leave this parameter at its default value, unless needed for special cases or to disable automatic writing of output files altogether. Writing can still be invoked at any time via the command `cv save`.

- `indexFile` < Index file for atom selection (GROMACS "ndx" format) >
  Context: global
  Acceptable Values: UNIX filename
  Description: This option reads an index file (usually with a `.ndx` extension) as produced by the `make_ndx` tool of GROMACS. This keyword may be repeated to load multiple index files. A group with the same name may appear multiple times, as long as it contains the same indices in identical order each time: an error is raised otherwise. The names of index groups contained in this file can then be used to define atom groups with the `indexGroup` keyword. Other supported methods to select atoms are described in 9.4.

- `smp` < Whether SMP parallelism should be used >
  Context: global
  Acceptable Values: boolean
  Default Value: `on`
  Description: If this flag is enabled (default), SMP parallelism over threads will be used to compute variables and biases, provided that this is supported by the NAMD build in use.

To illustrate the flexibility of the Colvars module, a non-trivial setup is represented in Figure 5. The corresponding configuration is given below. The options within the `colvar` blocks are described in 9.3, those within the `harmonic` and `histogram` blocks in 9.5. **Note:** *except* `colvar`, *none of the keywords shown is mandatory.*

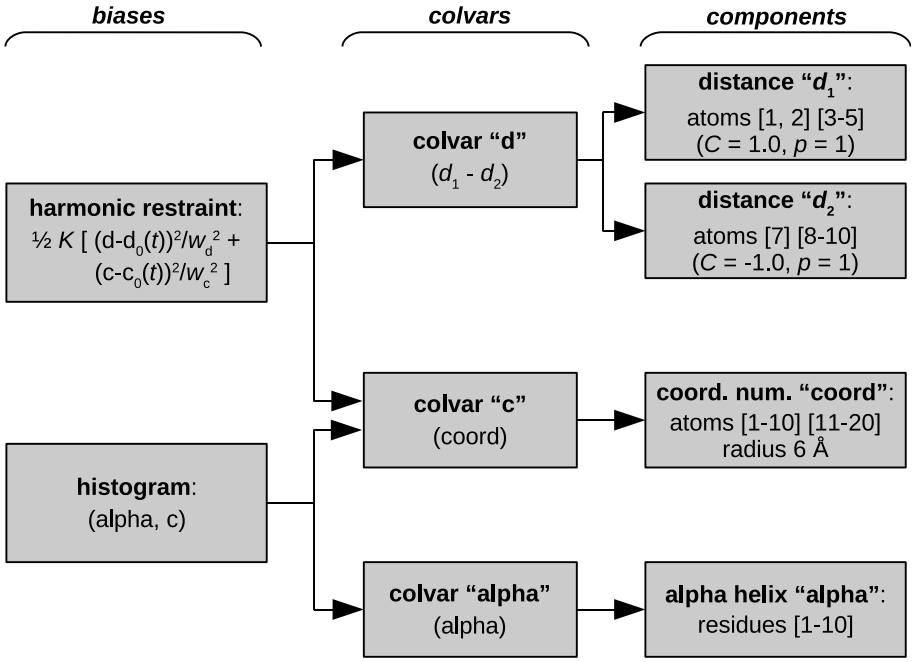

Figure 5: Graphical representation of a Colvars configuration. The colvar called "d" is defined as the difference between two distances: the first distance (d_1) is taken between the center of mass of atoms 1 and 2 and that of atoms 3 to 5, the second (d_2) between atom 7 and the center of mass of atoms 8 to 10. The difference $d = d_1 - d_2$ is obtained by multiplying the two by a coefficient $C = +1$ or $C = -1$, respectively. The colvar called "c" is the coordination number calculated between atoms 1 to 10 and atoms 11 to 20. A harmonic restraint is applied to both d and c: to allow using the same force constant K, both d and c are scaled by their respective fluctuation widths w_d and w_c. A third colvar "alpha" is defined as the α-helical content of residues 1 to 10. The values of "c" and "alpha" are also recorded throughout the simulation as a joint 2-dimensional histogram.

```
colvar {
  # difference of two distances
  name d
  width 0.2 # 0.2 Å of estimated fluctuation width
  distance {
    componentCoeff 1.0
    group1 { atomNumbers 1 2 }
    group2 { atomNumbers 3 4 5 }
  }
  distance {
    componentCoeff -1.0
```

```
    group1 { atomNumbers 7 }
    group2 { atomNumbers 8 9 10 }
  }
}

colvar {
  name c
  coordNum {
    cutoff 6.0
    group1 { atomNumbersRange 1-10 }
    group2 { atomNumbersRange 11-20 }
  }
}

colvar {
  name alpha
  alpha {
    psfSegID PROT
    residueRange 1-10
  }
}

harmonic {
  colvars d c
  centers 3.0 4.0
  forceConstant 5.0
}

histogram {
  colvars c alpha
}
```

Section 9.3 explains how to define a colvar and its behavior, regardless of its specific functional form. To define colvars that are appropriate to a specific physical system, Section 9.4 documents how to select atoms, and section 9.3 lists all of the available functional forms, which we call "colvar components". Finally, section 9.5 lists the available methods and algorithms to perform biased simulations and multidimensional analysis of colvars.

9.2.6 Input state file

Because many of the methods implemented in Colvars are history-dependent, a *state file* is often needed to continue a long simulation over consecutive runs. Such state file is written automatically at the end of any simulation with Colvars, and contains data accumulated during that simulation along with the step number at the end of it. The step number read from the state file is then used to control such time-dependent biases: because of this essential role, the step number internal to Colvars may not always match the step number reported by the MD program that carried during the simulation (which may instead restart from zero each time). If a state file is not given, the

NAMD command `firstTimestep` may be used to control the Colvars step number.

Depending on the configuration, a state file may need to be loaded issued at the beginning of a new simulation when time-dependent biasing methods are applied (moving restraints, metadynamics, ABF, ...). When the Colvars module is initialized in NAMD, the `colvarsInput` keyword can be used to give the name of the state file. After initialization, a state file may be loaded at any time with the Tcl command `cv load`.

It is possible to load a state file even if the configuration has changed: for example, new variables may be defined or restraints be added in between consecutive runs. For each newly defined variable or bias, no information will be read from the state file if this is unavailable: such new objects will remain uninitialized until the first compute step. Conversely, any information that the state file has about variables or biases that are not defined any longer is silently ignored. *Because these checks are done by the names of variables or biases, it is the user's responsibility to ensure that these are consistent between runs.*

9.2.7 Output files

During a simulation with collective variables defined, the following three output files are written:

- A *state file*, named *outputName*`.colvars.state`; this file is in ASCII (plain text) format, regardless of the value of `binaryOutput` in the NAMD configuration. This file is written at the end of the specified run, but can also be written at any time with the command `cv save` (9.2.3).
  This is the only Colvars output file needed to continue a simulation.

- If the parameter `colvarsRestartFrequency` (see 9.2.5) is larger than zero, a *restart file* is written every that many steps: this file is fully equivalent to the final state file. The name of this file is *restartName*`.colvars.state`.

- If the parameter `colvarsTrajFrequency` (see 9.2.5) is greater than 0 (default: 100), a *trajectory file* is written during the simulation: its name is *outputName*`.colvars.traj`; unlike the state file, it is not needed to restart a simulation, but can be used later for post-processing and analysis.

Other output files may also be written by specific methods, e.g. the ABF or metadynamics methods (9.5.2, 9.5.4). Like the trajectory file, they are needed only for analyzing, not continuing a simulation. All such files' names also begin with the prefix *outputName*.

Lastly, the total energy of all biases or restraints applied to the colvars appears under the NAMD standard output, under the MISC column.

9.3 Defining collective variables

A collective variable is defined by the keyword `colvar` followed by its configuration options contained within curly braces:

```
colvar {
  name xi
  <other options>
  function_name {
    <parameters>
```

```
    <atom selection>
  }
}
```

There are multiple ways of defining a variable:

- The *simplest and most common way* way is using one of the precompiled functions (here called "components"), which are listed in section 9.3.1. For example, using the keyword `rmsd` (section 9.3.5) defines the variable as the root mean squared deviation (RMSD) of the selected atoms.
- A new variable may also be constructed as a linear or polynomial combination of the components listed in section 9.3.1 (see 9.3.15 for details).
- A user-defined mathematical function of the existing components (see list in section 9.3.1), or of the atomic coordinates directly (see the `cartesian` keyword in 9.3.8). The function is defined through the keyword `customFunction` (see 9.3.16) (see 9.3.16 for details).
- A user-defined Tcl function of the existing components (see list in section 9.3.1), or of the atomic coordinates directly (see the `cartesian` keyword in 9.3.8). The function is provided by a separate Tcl script, and referenced through the keyword `scriptedFunction` (see 9.3.17) (see 9.3.17 for details).

Choosing a component (function) is the only parameter strictly required to define a collective variable. It is also highly recommended to specify a name for the variable:

- `name` < Name of this colvar >
  Context: `colvar`
  Acceptable Values: string
  Default Value: "`colvar`" + numeric id
  Description: The name is an unique case-sensitive string which allows the Colvars module to identify this colvar unambiguously; it is also used in the trajectory file to label to the columns corresponding to this colvar.

9.3.1 Choosing a function

In this context, the function that computes a colvar is called a *component*. A component's choice and definition consists of including in the variable's configuration a keyword indicating the type of function (e.g. `rmsd`), followed by a definition block specifying the atoms involved (see 9.4) and any additional parameters (cutoffs, "reference" values, ...). *At least one component must be chosen to define a variable:* if none of the keywords listed below is found, an error is raised.

The following components implement functions with a scalar value (i.e. a real number):

- `distance` (see 9.3.2): distance between two groups;
- `distanceZ` (see 9.3.2): projection of a distance vector on an axis;
- `distanceXY` (see 9.3.2): projection of a distance vector on a plane;
- `distanceInv` (see 9.3.2): mean distance between two groups of atoms (e.g. NOE-based distance);

- `angle` (see 9.3.3): angle between three groups;
- `dihedral` (see 9.3.3): torsional (dihedral) angle between four groups;
- `dipoleAngle` (see 9.3.3): angle between two groups and dipole of a third group;
- `dipoleMagnitude` (see **??**) magnitude of the dipole of a group of atoms;
- `polarTheta` (see 9.3.3): polar angle of a group in spherical coordinates;
- `polarPhi` (see 9.3.3): azimuthal angle of a group in spherical coordinates;
- `coordNum` (see 9.3.4): coordination number between two groups;
- `selfCoordNum` (see 9.3.4): coordination number of atoms within a group;
- `hBond` (see 9.3.4): hydrogen bond between two atoms;
- `rmsd` (see 9.3.5): root mean square deviation (RMSD) from a set of reference coordinates;
- `eigenvector` (see 9.3.5): projection of the atomic coordinates on a vector;
- `mapTotal` (see **??**): total value of a volumetric map;
- `orientationAngle` (see 9.3.6): angle of the best-fit rotation from a set of reference coordinates;
- `orientationProj` (see 9.3.6): cosine of `orientationProj` (see 9.3.6);
- `spinAngle` (see 9.3.6): projection orthogonal to an axis of the best-fit rotation from a set of reference coordinates;
- `tilt` (see 9.3.6): projection on an axis of the best-fit rotation from a set of reference coordinates;
- `gyration` (see 9.3.5): radius of gyration of a group of atoms;
- `inertia` (see 9.3.5): moment of inertia of a group of atoms;
- `inertiaZ` (see 9.3.5): moment of inertia of a group of atoms around a chosen axis;
- `alpha` (see 9.3.7): α-helix content of a protein segment.
- `dihedralPC` (see 9.3.7): projection of protein backbone dihedrals onto a dihedral principal component.

Some components do not return scalar, but vector values:

- `distanceVec` (see 9.3.2): distance vector between two groups (length: 3);
- `distanceDir` (see 9.3.2): unit vector parallel to distanceVec (length: 3);
- `cartesian` (see 9.3.8): vector of atomic Cartesian coordinates (length: N times the number of Cartesian components requested, X, Y or Z);
- `distancePairs` (see 9.3.8): vector of mutual distances (length: $N_1 \times N_2$);

- `orientation` (see 9.3.6): best-fit rotation, expressed as a unit quaternion (length: 4).

The types of components used in a colvar (scalar or not) determine the properties of that colvar, and particularly which biasing or analysis methods can be applied.

What if "X" is not listed? If a function type is not available on this list, it may be possible to define it as a polynomial superposition of existing ones (see 9.3.15), a custom function (see 9.3.16), or a scripted function (see 9.3.17).

In the rest of this section, all available component types are listed, along with their physical units and the ranges of values, if limited. Such limiting values can be used to define `lowerBoundary` (see 9.3.18) and `upperBoundary` (see 9.3.18) in the parent colvar.

For each type of component, the available configurations keywords are listed: when two components share certain keywords, the second component references to the documentation of the first one that uses that keyword. The very few keywords that are available for all types of components are listed in a separate section 9.3.12.

9.3.2 Distances

`distance`: **center-of-mass distance between two groups.** The `distance` {...} block defines a distance component between the two atom groups, `group1` and `group2`.
List of keywords (see also 9.3.15 for additional options):

- `group1` < First group of atoms >
  Context: `distance`
  Acceptable Values: Block `group1` {...}
  Description: First group of atoms.

- `group2`: analogous to `group1`

- `forceNoPBC` < Calculate absolute rather than minimum-image distance? >
  Context: `distance`
  Acceptable Values: boolean
  Default Value: `no`
  Description: By default, in calculations with periodic boundary conditions, the `distance` component returns the distance according to the minimum-image convention. If this parameter is set to `yes`, PBC will be ignored and the distance between the coordinates as maintained internally will be used. This is only useful in a limited number of special cases, e.g. to describe the distance between remote points of a single macromolecule, which cannot be split across periodic cell boundaries, and for which the minimum-image distance might give the wrong result because of a relatively small periodic cell.

- `oneSiteTotalForce` < Measure total force on group 1 only? >
  Context: `angle`, `dipoleAngle`, `dihedral`
  Acceptable Values: boolean
  Default Value: `no`
  Description: If this is set to `yes`, the total force is measured along a vector field (see equation (61) in section 9.5.2) that only involves atoms of `group1`. This option is only useful for ABF, or custom biases that compute total forces. See section 9.5.2 for details.

The value returned is a positive number (in Å), ranging from 0 to the largest possible interatomic distance within the chosen boundary conditions (with PBCs, the minimum image convention is used unless the `forceNoPBC` option is set).

`distanceZ`: projection of a distance vector on an axis. The `distanceZ` {...} block defines a distance projection component, which can be seen as measuring the distance between two groups projected onto an axis, or the position of a group along such an axis. The axis can be defined using either one reference group and a constant vector, or dynamically based on two reference groups. One of the groups can be set to a dummy atom to allow the use of an absolute Cartesian coordinate.
List of keywords (see also 9.3.15 for additional options):

- `main` < Main group of atoms >
  Context: `distanceZ`
  Acceptable Values: Block `main` {...}
  Description: Group of atoms whose position $\boldsymbol{r}$ is measured.

- `ref` < Reference group of atoms >
  Context: `distanceZ`
  Acceptable Values: Block `ref` {...}
  Description: Reference group of atoms. The position of its center of mass is noted $\boldsymbol{r}_1$ below.

- `ref2` < Secondary reference group >
  Context: `distanceZ`
  Acceptable Values: Block `ref2` {...}
  Default Value: none
  Description: Optional group of reference atoms, whose position $\boldsymbol{r}_2$ can be used to define a dynamic projection axis: $\boldsymbol{e} = (\|\boldsymbol{r}_2 - \boldsymbol{r}_1\|)^{-1} \times (\boldsymbol{r}_2 - \boldsymbol{r}_1)$. In this case, the origin is $\boldsymbol{r}_m = 1/2(\boldsymbol{r}_1 + \boldsymbol{r}_2)$, and the value of the component is $\boldsymbol{e} \cdot (\boldsymbol{r} - \boldsymbol{r}_m)$.

- `axis` < Projection axis (Å) >
  Context: `distanceZ`
  Acceptable Values: `(x, y, z)` triplet
  Default Value: `(0.0, 0.0, 1.0)`
  Description: The three components of this vector define a projection axis $\boldsymbol{e}$ for the distance vector $\boldsymbol{r} - \boldsymbol{r}_1$ joining the centers of groups `ref` and `main`. The value of the component is then $\boldsymbol{e} \cdot (\boldsymbol{r} - \boldsymbol{r}_1)$. The vector should be written as three components separated by commas and enclosed in parentheses.

- `forceNoPBC`: see definition of `forceNoPBC` in sec. 9.3.2 (`distance` component)

- `oneSiteTotalForce`: see definition of `oneSiteTotalForce` in sec. 9.3.2 (`distance` component)

This component returns a number (in Å) whose range is determined by the chosen boundary conditions. For instance, if the z axis is used in a simulation with periodic boundaries, the returned value ranges between $-b_z/2$ and $b_z/2$, where b_z is the box length along z (this behavior is disabled if `forceNoPBC` is set).

`distanceXY`**: modulus of the projection of a distance vector on a plane.** The `distanceXY` {...} block defines a distance projected on a plane, and accepts the same keywords as the component `distanceZ`, i.e. `main`, `ref`, either `ref2` or `axis`, and `oneSiteTotalForce`. It returns the norm of the projection of the distance vector between `main` and `ref` onto the plane orthogonal to the axis. The axis is defined using the `axis` parameter or as the vector joining `ref` and `ref2` (see `distanceZ` above).
List of keywords (see also 9.3.15 for additional options):

- `main`: see definition of `main` in sec. 9.3.2 (`distanceZ` component)
- `ref`: see definition of `ref` in sec. 9.3.2 (`distanceZ` component)
- `ref2`: see definition of `ref2` in sec. 9.3.2 (`distanceZ` component)
- `axis`: see definition of `axis` in sec. 9.3.2 (`distanceZ` component)
- `forceNoPBC`: see definition of `forceNoPBC` in sec. 9.3.2 (`distance` component)
- `oneSiteTotalForce`: see definition of `oneSiteTotalForce` in sec. 9.3.2 (`distance` component)

`distanceVec`**: distance vector between two groups.** The `distanceVec` {...} block defines a distance vector component, which accepts the same keywords as the component `distance`: `group1`, `group2`, and `forceNoPBC`. Its value is the 3-vector joining the centers of mass of `group1` and `group2`.
List of keywords (see also 9.3.15 for additional options):

- `group1`: see definition of `group1` in sec. 9.3.2 (`distance` component)
- `group2`: analogous to `group1`
- `forceNoPBC`: see definition of `forceNoPBC` in sec. 9.3.2 (`distance` component)
- `oneSiteTotalForce`: see definition of `oneSiteTotalForce` in sec. 9.3.2 (`distance` component)

`distanceDir`**: distance unit vector between two groups.** The `distanceDir` {...} block defines a distance unit vector component, which accepts the same keywords as the component `distance`: `group1`, `group2`, and `forceNoPBC`. It returns a 3-dimensional unit vector $\mathbf{d} = (d_x, d_y, d_z)$, with $|\mathbf{d}| = 1$.
List of keywords (see also 9.3.15 for additional options):

- `group1`: see definition of `group1` in sec. 9.3.2 (`distance` component)
- `group2`: analogous to `group1`
- `forceNoPBC`: see definition of `forceNoPBC` in sec. 9.3.2 (`distance` component)
- `oneSiteTotalForce`: see definition of `oneSiteTotalForce` in sec. 9.3.2 (`distance` component)

`distanceInv`**: mean distance between two groups of atoms.** The `distanceInv` {...} block defines a generalized mean distance between two groups of atoms 1 and 2, weighted with exponent $1/n$:

$$d_{1,2}^{[n]} = \left(\frac{1}{N_1 N_2} \sum_{i,j} \left(\frac{1}{\|\mathbf{d}^{ij}\|} \right)^n \right)^{-1/n} \tag{36}$$

where $\|\mathbf{d}^{ij}\|$ is the distance between atoms i and j in groups 1 and 2 respectively, and n is an even integer.
List of keywords (see also 9.3.15 for additional options):

- `group1`: see definition of `group1` in sec. 9.3.2 (`distance` component)
- `group2`: analogous to `group1`
- `oneSiteTotalForce`: see definition of `oneSiteTotalForce` in sec. 9.3.2 (`distance` component)
- `exponent` < Exponent n in equation 36 >
  Context: `distanceInv`
  Acceptable Values: positive even integer
  Default Value: 6
  Description: Defines the exponent to which the individual distances are elevated before averaging. The default value of 6 is useful for example to applying restraints based on NOE-measured distances.

This component returns a number in Å, ranging from 0 to the largest possible distance within the chosen boundary conditions.

9.3.3 Angles

`angle`**: angle between three groups.** The `angle` {...} block defines an angle, and contains the three blocks `group1`, `group2` and `group3`, defining the three groups. It returns an angle (in degrees) within the interval $[0 : 180]$.
List of keywords (see also 9.3.15 for additional options):

- `group1`: see definition of `group1` in sec. 9.3.2 (`distance` component)
- `group2`: analogous to `group1`
- `group3`: analogous to `group1`
- `forceNoPBC`: see definition of `forceNoPBC` in sec. 9.3.2 (`distance` component)
- `oneSiteTotalForce`: see definition of `oneSiteTotalForce` in sec. 9.3.2 (`distance` component)

`dipoleAngle`: **angle between two groups and dipole of a third group.** The `dipoleAngle` {...} block defines an angle, and contains the three blocks `group1`, `group2` and `group3`, defining the three groups, being `group1` the group where dipole is calculated. It returns an angle (in degrees) within the interval $[0 : 180]$.
List of keywords (see also 9.3.15 for additional options):

- `group1`: see definition of `group1` in sec. 9.3.2 (`distance` component)
- `group2`: analogous to `group1`
- `group3`: analogous to `group1`
- `forceNoPBC`: see definition of `forceNoPBC` in sec. 9.3.2 (`distance` component)
- `oneSiteTotalForce`: see definition of `oneSiteTotalForce` in sec. 9.3.2 (`distance` component)

`dihedral`: **torsional angle between four groups.** The `dihedral` {...} block defines a torsional angle, and contains the blocks `group1`, `group2`, `group3` and `group4`, defining the four groups. It returns an angle (in degrees) within the interval $[-180 : 180]$. The Colvars module calculates all the distances between two angles taking into account periodicity. For instance, reference values for restraints or range boundaries can be defined by using any real number of choice.
List of keywords (see also 9.3.15 for additional options):

- `group1`: see definition of `group1` in sec. 9.3.2 (`distance` component)
- `group2`: analogous to `group1`
- `group3`: analogous to `group1`
- `group4`: analogous to `group1`
- `forceNoPBC`: see definition of `forceNoPBC` in sec. 9.3.2 (`distance` component)
- `oneSiteTotalForce`: see definition of `oneSiteTotalForce` in sec. 9.3.2 (`distance` component)

`polarTheta`: **polar angle in spherical coordinates.** The `polarTheta` {...} block defines the polar angle in spherical coordinates, for the center of mass of a group of atoms described by the block `atoms`. It returns an angle (in degrees) within the interval $[0 : 180]$. To obtain spherical coordinates in a frame of reference tied to another group of atoms, use the `fittingGroup` (9.4.2) option within the `atoms` block. An example is provided in file `examples/11_polar_angles.in` of the Colvars public repository.
List of keywords (see also 9.3.15 for additional options):

- `atoms` < Atom group >
  Context: `polarPhi`
  Acceptable Values: `atoms` {...} block
  Description: Defines the group of atoms for the COM of which the angle should be calculated.

`polarPhi`: azimuthal angle in spherical coordinates. The `polarPhi` {...} block defines the azimuthal angle in spherical coordinates, for the center of mass of a group of atoms described by the block `atoms`. It returns an angle (in degrees) within the interval $[-180 : 180]$. The Colvars module calculates all the distances between two angles taking into account periodicity. For instance, reference values for restraints or range boundaries can be defined by using any real number of choice. To obtain spherical coordinates in a frame of reference tied to another group of atoms, use the `fittingGroup` (9.4.2) option within the `atoms` block. An example is provided in file `examples/11_polar_angles.in` of the Colvars public repository.
List of keywords (see also 9.3.15 for additional options):

- `atoms` < Atom group >
  Context: `polarPhi`
  Acceptable Values: `atoms` {...} block
  Description: Defines the group of atoms for the COM of which the angle should be calculated.

9.3.4 Contacts

`coordNum`: coordination number between two groups. The `coordNum` {...} block defines a coordination number (or number of contacts), which calculates the function $(1-(d/d_0)^n)/(1-(d/d_0)^m)$, where d_0 is the "cutoff" distance, and n and m are exponents that can control its long range behavior and stiffness [49]. This function is summed over all pairs of atoms in `group1` and `group2`:

$$C(\texttt{group1},\texttt{group2}) \;=\; \sum_{i\in\texttt{group1}} \sum_{j\in\texttt{group2}} \frac{1-(|\mathbf{x}_i-\mathbf{x}_j|/d_0)^n}{1-(|\mathbf{x}_i-\mathbf{x}_j|/d_0)^m} \tag{37}$$

List of keywords (see also 9.3.15 for additional options):

- `group1`: see definition of `group1` in sec. 9.3.2 (`distance` component)
- `group2`: analogous to `group1`
- `cutoff` < "Interaction" distance (Å) >
  Context: `coordNum`
  Acceptable Values: positive decimal
  Default Value: 4.0
  Description: This number defines the switching distance to define an interatomic contact: for $d \ll d_0$, the switching function $(1-(d/d_0)^n)/(1-(d/d_0)^m)$ is close to 1, at $d = d_0$ it has a value of n/m (1/2 with the default n and m), and at $d \gg d_0$ it goes to zero approximately like d^{m-n}. Hence, for a proper behavior, m must be larger than n.
- `cutoff3` < Reference distance vector (Å) >
  Context: `coordNum`
  Acceptable Values: "`(x, y, z)`" triplet of positive decimals
  Default Value: `(4.0, 4.0, 4.0)`
  Description: The three components of this vector define three different cutoffs d_0 for each direction. This option is mutually exclusive with `cutoff`.
- `expNumer` < Numerator exponent >
  Context: `coordNum`

Acceptable Values: positive even integer
Default Value: 6
Description: This number defines the n exponent for the switching function.

- `expDenom` < Denominator exponent >
  Context: `coordNum`
  Acceptable Values: positive even integer
  Default Value: 12
  Description: This number defines the m exponent for the switching function.

- `group2CenterOnly` < Use only `group2`'s center of mass >
  Context: `coordNum`
  Acceptable Values: boolean
  Default Value: `off`
  Description: If this option is `on`, only contacts between each atoms in `group1` and the center of mass of `group2` are calculated (by default, the sum extends over all pairs of atoms in `group1` and `group2`). If `group2` is a `dummyAtom`, this option is set to `yes` by default.

- `tolerance` < Pairlist control >
  Context: `coordNum`
  Acceptable Values: decimal
  Default Value: 0.0
  Description: This controls the pairlist feature, dictating the minimum value for each summation element in Eq. 37 such that the pair that contributed the summation element is included in subsequent simulation timesteps until the next pairlist recalculation. For most applications, this value should be small (eg. 0.001) to avoid missing important contributions to the overall sum. Higher values will improve performance by reducing the number of pairs that contribute to the sum. Values above 1 will exclude all possible pair interactions. Similarly, values below 0 will never exclude a pair from consideration. To ensure continuous forces, Eq. 37 is further modified by subtracting the tolerance and then rescaling so that each pair covers the range $[0, 1]$.

- `pairListFrequency` < Pairlist regeneration frequency >
  Context: `coordNum`
  Acceptable Values: positive integer
  Default Value: 100
  Description: This controls the pairlist feature, dictating how many steps are taken between regenerating pairlists if the tolerance is greater than 0.

This component returns a dimensionless number, which ranges from approximately 0 (all interatomic distances are much larger than the cutoff) to $N_{\texttt{group1}} \times N_{\texttt{group2}}$ (all distances are less than the cutoff), or $N_{\texttt{group1}}$ if `group2CenterOnly` is used. For performance reasons, at least one of `group1` and `group2` should be of limited size or `group2CenterOnly` should be used: the cost of the loop over all pairs grows as $N_{\texttt{group1}} \times N_{\texttt{group2}}$. Setting `tolerance` > 0 ameliorates this to some degree, although every pair is still checked to regenerate the pairlist.

`selfCoordNum`: coordination number between atoms within a group. The `selfCoordNum` {...} block defines a coordination number similarly to the component `coordNum`, but the function

is summed over atom pairs within `group1`:

$$C(\texttt{group1}) = \sum_{i \in \texttt{group1}} \sum_{j>i} \frac{1-(|\mathbf{x}_i - \mathbf{x}_j|/d_0)^n}{1-(|\mathbf{x}_i - \mathbf{x}_j|/d_0)^m} \tag{38}$$

The keywords accepted by `selfCoordNum` are a subset of those accepted by `coordNum`, namely `group1` (here defining *all* of the atoms to be considered), `cutoff`, `expNumer`, and `expDenom`.
List of keywords (see also 9.3.15 for additional options):

- `group1`: see definition of `group1` in sec. 9.3.4 (`coordNum` component)
- `cutoff`: see definition of `cutoff` in sec. 9.3.4 (`coordNum` component)
- `cutoff3`: see definition of `cutoff3` in sec. 9.3.4 (`coordNum` component)
- `expNumer`: see definition of `expNumer` in sec. 9.3.4 (`coordNum` component)
- `expDenom`: see definition of `expDenom` in sec. 9.3.4 (`coordNum` component)
- `tolerance`: see definition of `tolerance` in sec. 9.3.4 (`coordNum` component)
- `pairListFrequency`: see definition of `pairListFrequency` in sec. 9.3.4 (`coordNum` component)

This component returns a dimensionless number, which ranges from approximately 0 (all interatomic distances much larger than the cutoff) to $N_{\texttt{group1}} \times (N_{\texttt{group1}} - 1)/2$ (all distances within the cutoff). For performance reasons, `group1` should be of limited size, because the cost of the loop over all pairs grows as $N^2_{\texttt{group1}}$.

`hBond`: **hydrogen bond between two atoms.** The `hBond` {...} block defines a hydrogen bond, implemented as a coordination number (eq. 37) between the donor and the acceptor atoms. Therefore, it accepts the same options `cutoff` (with a different default value of 3.3 Å), `expNumer` (with a default value of 6) and `expDenom` (with a default value of 8). Unlike `coordNum`, it requires two atom numbers, `acceptor` and `donor`, to be defined. It returns an adimensional number, with values between 0 (acceptor and donor far outside the cutoff distance) and 1 (acceptor and donor much closer than the cutoff).
List of keywords (see also 9.3.15 for additional options):

- `acceptor` < Number of the acceptor atom >
  Context: `hBond`
  Acceptable Values: positive integer
  Description: Number that uses the same convention as `atomNumbers`.
- `donor`: analogous to `acceptor`
- `cutoff`: see definition of `cutoff` in sec. 9.3.4 (`coordNum` component)
  Note: default value is 3.3 Å.
- `expNumer`: see definition of `expNumer` in sec. 9.3.4 (`coordNum` component)
  Note: default value is 6.
- `expDenom`: see definition of `expDenom` in sec. 9.3.4 (`coordNum` component)
  Note: default value is 8.

9.3.5 Collective metrics

`rmsd`: root mean square displacement (RMSD) from reference positions. The block `rmsd` {...} defines the root mean square replacement (RMSD) of a group of atoms with respect to a reference structure. For each set of coordinates $\{\mathbf{x}_1(t), \mathbf{x}_2(t), \ldots \mathbf{x}_N(t)\}$, the colvar component `rmsd` calculates the optimal rotation $U^{\{\mathbf{x}_i(t)\}\rightarrow\{\mathbf{x}_i^{(\mathrm{ref})}\}}$ that best superimposes the coordinates $\{\mathbf{x}_i(t)\}$ onto a set of reference coordinates $\{\mathbf{x}_i^{(\mathrm{ref})}\}$. Both the current and the reference coordinates are centered on their centers of geometry, $\mathbf{x}_{\mathrm{cog}}(t)$ and $\mathbf{x}_{\mathrm{cog}}^{(\mathrm{ref})}$. The root mean square displacement is then defined as:

$$\mathrm{RMSD}(\{\mathbf{x}_i(t)\}, \{\mathbf{x}_i^{(\mathrm{ref})}\}) \;=\; \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left|U\left(\mathbf{x}_i(t) - \mathbf{x}_{\mathrm{cog}}(t)\right) - \left(\mathbf{x}_i^{(\mathrm{ref})} - \mathbf{x}_{\mathrm{cog}}^{(\mathrm{ref})}\right)\right|^2} \qquad (39)$$

The optimal rotation $U^{\{\mathbf{x}_i(t)\}\rightarrow\{\mathbf{x}_i^{(\mathrm{ref})}\}}$ is calculated within the formalism developed in reference [26], which guarantees a continuous dependence of $U^{\{\mathbf{x}_i(t)\}\rightarrow\{\mathbf{x}_i^{(\mathrm{ref})}\}}$ with respect to $\{\mathbf{x}_i(t)\}$.
List of keywords (see also 9.3.15 for additional options):

- `atoms` < Atom group >
  Context: `rmsd`
  Acceptable Values: `atoms` {...} block
  Description: Defines the group of atoms of which the RMSD should be calculated. Optimal fit options (such as `refPositions` and `rotateReference`) should typically NOT be set within this block. Exceptions to this rule are the special cases discussed in the *Advanced usage* paragraph below.

- `refPositions` < Reference coordinates >
  Context: `rmsd`
  Acceptable Values: space-separated list of `(x, y, z)` triplets
  Description: This option (mutually exclusive with `refPositionsFile`) sets the reference coordinates for RMSD calculation, and uses these to compute the roto-translational fit. It is functionally equivalent to the option `refPositions` (see 9.4.2) in the atom group definition, which also supports more advanced fitting options.

- `refPositionsFile` < Reference coordinates file >
  Context: `rmsd`
  Acceptable Values: UNIX filename
  Description: This option (mutually exclusive with `refPositions`) sets the reference coordinates for RMSD calculation, and uses these to compute the roto-translational fit. It is functionally equivalent to the option `refPositionsFile` (see 9.4.2) in the atom group definition, which also supports more advanced fitting options.

- `refPositionsCol` < PDB column containing atom flags >
  Context: `rmsd`
  Acceptable Values: `O`, `B`, `X`, `Y`, or `Z`
  Description: If `refPositionsFile` is a PDB file that contains all the atoms in the topology, this option may be provided to set which PDB field is used to flag the reference coordinates for `atoms`.

- `refPositionsColValue` < Atom selection flag in the PDB column >
  Context: `rmsd`
  Acceptable Values: positive decimal
  Description: If defined, this value identifies in the PDB column `refPositionsCol` of the file `refPositionsFile` which atom positions are to be read. Otherwise, all positions with a non-zero value are read.

- `atomPermutation` < Alternate ordering of atoms for RMSD computation >
  Context: `rmsd`
  Acceptable Values: List of atom numbers
  Description: If defined, this parameter defines a re-ordering (permutation) of the 1-based atom numbers that can be used to compute the RMSD, typically due to molecular symmetry. This parameter can be specified multiple times, each one defining a new permutation: the returned RMSD value is the minimum over the set of permutations. For example, if the atoms making up the group are 6, 7, 8, 9, and atoms 7, 8, and 9 are invariant by circular permutation (as the hydrogens in a CH3 group), a symmetry-adapted RMSD would be obtained by adding:
  `atomPermutation 6 8 9 7`
  `atomPermutation 6 9 7 8`
  Note that *this does not affect the least-squares roto-translational fit*, which is done using the topology ordering of atoms, and the reference positions in the order provided. Therefore, this feature is mostly useful when using custom fitting parameters within the atom group, such as `fittingGroup` (see 9.4.2), or when fitting is disabled altogether.

This component returns a positive real number (in Å).

Advanced usage of the `rmsd` component. In the standard usage as described above, the `rmsd` component calculates a minimum RMSD, that is, current coordinates are optimally fitted onto the same reference coordinates that are used to compute the RMSD value. The fit itself is handled by the atom group object, whose parameters are automatically set by the `rmsd` component. For very specific applications, however, it may be useful to control the fitting process separately from the definition of the reference coordinates, to evaluate various types of non-minimal RMSD values. This can be achieved by setting the related options (`refPositions`, etc.) explicitly in the atom group block. This allows for the following non-standard cases:

1. applying the optimal translation, but no rotation (`rotateReference off`), to bias or restrain the shape and orientation, but not the position of the atom group;

2. applying the optimal rotation, but no translation (`centerReference off`), to bias or restrain the shape and position, but not the orientation of the atom group;

3. disabling the application of optimal roto-translations, which lets the RMSD component describe the deviation of atoms from fixed positions in the laboratory frame: this allows for custom positional restraints within the Colvars module;

4. fitting the atomic positions to different reference coordinates than those used in the RMSD calculation itself (by specifying `refPositions` (see 9.4.2) or `refPositionsFile` (see 9.4.2) within the atom group as well as within the `rmsd` block);

5. applying the optimal rotation and/or translation from a separate atom group, defined through `fittingGroup`: the RMSD then reflects the deviation from reference coordinates in a separate, moving reference frame (see example in the section on `fittingGroup` (see 9.4.2)).

`eigenvector`: projection of the atomic coordinates on a vector. The block `eigenvector {...}` defines the projection of the coordinates of a group of atoms (or more precisely, their deviations from the reference coordinates) onto a vector in $\mathbb{R}^{3n}$, where n is the number of atoms in the group. The computed quantity is the total projection:

$$p(\{\mathbf{x}_i(t)\},\{\mathbf{x}_i^{(\mathrm{ref})}\}) \;=\; \sum_{i=1}^{n} \mathbf{v}_i \cdot \left(U(\mathbf{x}_i(t) - \mathbf{x}_{\mathrm{cog}}(t)) - (\mathbf{x}_i^{(\mathrm{ref})} - \mathbf{x}_{\mathrm{cog}}^{(\mathrm{ref})}) \right), \tag{40}$$

where, as in the `rmsd` component, U is the optimal rotation matrix, $\mathbf{x}_{\mathrm{cog}}(t)$ and $\mathbf{x}_{\mathrm{cog}}^{(\mathrm{ref})}$ are the centers of geometry of the current and reference positions respectively, and $\mathbf{v}_i$ are the components of the vector for each atom. Example choices for $(\mathbf{v}_i)$ are an eigenvector of the covariance matrix (essential mode), or a normal mode of the system. It is assumed that $\sum_i \mathbf{v}_i = 0$: otherwise, the Colvars module centers the $\mathbf{v}_i$ automatically when reading them from the configuration.
List of keywords (see also 9.3.15 for additional options):

- `atoms`: see definition of `atoms` in sec. 9.3.5 (`rmsd` component)
- `refPositions`: see definition of `refPositions` in sec. 9.3.5 (`rmsd` component)
- `refPositionsFile`: see definition of `refPositionsFile` in sec. 9.3.5 (`rmsd` component)
- `refPositionsCol`: see definition of `refPositionsCol` in sec. 9.3.5 (`rmsd` component)
- `refPositionsColValue`: see definition of `refPositionsColValue` in sec. 9.3.5 (`rmsd` component)
- `vector` < Vector components >
  Context: `eigenvector`
  Acceptable Values: space-separated list of `(x, y, z)` triplets
  Description: This option (mutually exclusive with `vectorFile`) sets the values of the vector components.
- `vectorFile` < file containing vector components >
  Context: `eigenvector`
  Acceptable Values: UNIX filename
  Description: This option (mutually exclusive with `vector`) sets the name of a coordinate file containing the vector components; the file is read according to the same format used for `refPositionsFile`. For a PDB file specifically, the components are read from the X, Y and Z fields. **Note:** *The PDB file has limited precision and fixed-point numbers: in some cases, the vector components may not be accurately represented; a XYZ file should be used instead, containing floating-point numbers.*
- `vectorCol` < PDB column used to flag participating atoms >
  Context: `eigenvector`
  Acceptable Values: `O` or `B`
  Description: Analogous to `atomsCol`.

- `vectorColValue` < Value used to flag participating atoms in the PDB file >
  Context: `eigenvector`
  Acceptable Values: positive decimal
  Description: Analogous to `atomsColValue`.

- `differenceVector` < The $3n$-dimensional vector is the difference between `vector` and `refPositions` >
  Context: `eigenvector`
  Acceptable Values: boolean
  Default Value: `off`
  Description: If this option is `on`, the numbers provided by `vector` or `vectorFile` are interpreted as another set of positions, $\mathbf{x}'_i$: the vector $\mathbf{v}_i$ is then defined as $\mathbf{v}_i = \left(\mathbf{x}'_i - \mathbf{x}_i^{(\mathrm{ref})}\right)$. This allows to conveniently define a colvar ξ as a projection on the linear transformation between two sets of positions, "A" and "B". For convenience, the vector is also normalized so that $\xi = 0$ when the atoms are at the set of positions "A" and $\xi = 1$ at the set of positions "B".

This component returns a number (in Å), whose value ranges between the smallest and largest absolute positions in the unit cell during the simulations (see also `distanceZ`). Due to the normalization in eq. 40, this range does not depend on the number of atoms involved.

`gyration`: radius of gyration of a group of atoms. The block `gyration` {...} defines the parameters for calculating the radius of gyration of a group of atomic positions $\{\mathbf{x}_1(t), \mathbf{x}_2(t), \dots \mathbf{x}_N(t)\}$ with respect to their center of geometry, $\mathbf{x}_{\mathrm{cog}}(t)$:

$$R_{\mathrm{gyr}} = \sqrt{\frac{1}{N}\sum_{i=1}^{N} |\mathbf{x}_i(t) - \mathbf{x}_{\mathrm{cog}}(t)|^2} \tag{41}$$

This component must contain one `atoms` {...} block to define the atom group, and returns a positive number, expressed in Å.
List of keywords (see also 9.3.15 for additional options):

- `atoms`: see definition of `atoms` in sec. 9.3.5 (`rmsd` component)

`inertia`: total moment of inertia of a group of atoms. The block `inertia` {...} defines the parameters for calculating the total moment of inertia of a group of atomic positions $\{\mathbf{x}_1(t), \mathbf{x}_2(t), \dots \mathbf{x}_N(t)\}$ with respect to their center of geometry, $\mathbf{x}_{\mathrm{cog}}(t)$:

$$I = \sum_{i=1}^{N} |\mathbf{x}_i(t) - \mathbf{x}_{\mathrm{cog}}(t)|^2 \tag{42}$$

Note that all atomic masses are set to 1 for simplicity. This component must contain one `atoms` {...} block to define the atom group, and returns a positive number, expressed in Å^2.
List of keywords (see also 9.3.15 for additional options):

- `atoms`: see definition of `atoms` in sec. 9.3.5 (`rmsd` component)

`dipoleMagnitude`: **dipole magnitude of a group of atoms.** The `dipoleMagnitude` {...} block defines the dipole magnitude of a group of atoms (norm of the dipole moment's vector), being `atoms` the group where dipole magnitude is calculated. It returns the magnitude in elementary charge e times Å.
List of keywords (see also 9.3.15 for additional options):

- `atoms`: see definition of `atoms` in sec. 9.3.5 (`rmsd` component)

`inertiaZ`: **total moment of inertia of a group of atoms around a chosen axis.** The block `inertiaZ` {...} defines the parameters for calculating the component along the axis $\mathbf{e}$ of the moment of inertia of a group of atomic positions $\{\mathbf{x}_1(t), \mathbf{x}_2(t), \dots \mathbf{x}_N(t)\}$ with respect to their center of geometry, $\mathbf{x}_{\mathrm{cog}}(t)$:

$$I_{\mathbf{e}} = \sum_{i=1}^{N} \left((\mathbf{x}_i(t) - \mathbf{x}_{\mathrm{cog}}(t)) \cdot \mathbf{e} \right)^2 \tag{43}$$

Note that all atomic masses are set to 1 for simplicity. This component must contain one `atoms` {...} block to define the atom group, and returns a positive number, expressed in Å^2.
List of keywords (see also 9.3.15 for additional options):

- `atoms`: see definition of `atoms` in sec. 9.3.5 (`rmsd` component)
- `axis` < Projection axis (Å) >
  Context: `inertiaZ`
  Acceptable Values: `(x, y, z)` triplet
  Default Value: `(0.0, 0.0, 1.0)`
  Description: The three components of this vector define (when normalized) the projection axis $\mathbf{e}$.

9.3.6 Rotations

`orientation`: **orientation from reference coordinates.** The block `orientation` {...} returns the same optimal rotation used in the `rmsd` component to superimpose the coordinates $\{\mathbf{x}_i(t)\}$ onto a set of reference coordinates $\{\mathbf{x}_i^{(\mathrm{ref})}\}$. Such component returns a four dimensional vector $\mathbf{q} = (q_0, q_1, q_2, q_3)$, with $\sum_i q_i^2 = 1$; this *quaternion* expresses the optimal rotation $\{\mathbf{x}_i(t)\} \rightarrow \{\mathbf{x}_i^{(\mathrm{ref})}\}$ according to the formalism in reference [26]. The quaternion (q_0, q_1, q_2, q_3) can also be written as $(\cos(\theta/2), \sin(\theta/2)\mathbf{u})$, where θ is the angle and $\mathbf{u}$ the normalized axis of rotation; for example, a rotation of 90° around the z axis is expressed as "`(0.707, 0.0, 0.0, 0.707)`". The script `quaternion2rmatrix.tcl` provides Tcl functions for converting to and from a 4×4 rotation matrix in a format suitable for usage in VMD.

As for the component `rmsd`, the available options are `atoms`, `refPositionsFile`, `refPositionsCol` and `refPositionsColValue`, and `refPositions`.

Note: `refPositions`and `refPositionsFile` define the set of positions *from which* the optimal rotation is calculated, but this rotation is not applied to the coordinates of the atoms involved: it is used instead to define the variable itself.
List of keywords (see also 9.3.15 for additional options):

- `atoms`: see definition of `atoms` in sec. 9.3.5 (`rmsd` component)
- `refPositions`: see definition of `refPositions` in sec. 9.3.5 (`rmsd` component)

- `refPositionsFile`: see definition of `refPositionsFile` in sec. 9.3.5 (`rmsd` component)
- `refPositionsCol`: see definition of `refPositionsCol` in sec. 9.3.5 (`rmsd` component)
- `refPositionsColValue`: see definition of `refPositionsColValue` in sec. 9.3.5 (`rmsd` component)
- `closestToQuaternion` < Reference rotation >
  Context: `orientation`
  Acceptable Values: "`(q0, q1, q2, q3)`" quadruplet
  Default Value: `(1.0, 0.0, 0.0, 0.0)` ("null" rotation)
  Description: Between the two equivalent quaternions (q_0, q_1, q_2, q_3) and $(-q_0, -q_1, -q_2, -q_3)$, the closer to `(1.0, 0.0, 0.0, 0.0)` is chosen. This simplifies the visualization of the colvar trajectory when sampled values are a smaller subset of all possible rotations. **Note:** *this only affects the output, never the dynamics.*

Tip: stopping the rotation of a protein. To stop the rotation of an elongated macromolecule in solution (and use an anisotropic box to save water molecules), it is possible to define a colvar with an `orientation` component, and restrain it through the `harmonic` bias around the identity rotation, `(1.0, 0.0, 0.0, 0.0)`. Only the overall orientation of the macromolecule is affected, and *not* its internal degrees of freedom. The user should also take care that the macromolecule is composed by a single chain, or disable `wrapAll` otherwise.

`orientationAngle`: **angle of rotation from reference coordinates.** The block `orientationAngle {...}` accepts the same base options as the component `orientation`: `atoms`, `refPositions`, `refPositionsFile`, `refPositionsCol` and `refPositionsColValue`. The returned value is the angle of rotation θ between the current and the reference positions. This angle is expressed in degrees within the range [0°:180°].
List of keywords (see also 9.3.15 for additional options):

- `atoms`: see definition of `atoms` in sec. 9.3.5 (`rmsd` component)
- `refPositions`: see definition of `refPositions` in sec. 9.3.5 (`rmsd` component)
- `refPositionsFile`: see definition of `refPositionsFile` in sec. 9.3.5 (`rmsd` component)
- `refPositionsCol`: see definition of `refPositionsCol` in sec. 9.3.5 (`rmsd` component)
- `refPositionsColValue`: see definition of `refPositionsColValue` in sec. 9.3.5 (`rmsd` component)

`orientationProj`: **cosine of the angle of rotation from reference coordinates.** The block `orientationProj {...}` accepts the same base options as the component `orientation`: `atoms`, `refPositions`, `refPositionsFile`, `refPositionsCol` and `refPositionsColValue`. The returned value is the cosine of the angle of rotation θ between the current and the reference positions. The range of values is [-1:1].
List of keywords (see also 9.3.15 for additional options):

- `atoms`: see definition of `atoms` in sec. 9.3.5 (`rmsd` component)
- `refPositions`: see definition of `refPositions` in sec. 9.3.5 (`rmsd` component)

- `refPositionsFile`: see definition of `refPositionsFile` in sec. 9.3.5 (`rmsd` component)
- `refPositionsCol`: see definition of `refPositionsCol` in sec. 9.3.5 (`rmsd` component)
- `refPositionsColValue`: see definition of `refPositionsColValue` in sec. 9.3.5 (`rmsd` component)

`spinAngle`: **angle of rotation around a given axis.** The complete rotation described by `orientation` can optionally be decomposed into two sub-rotations: one is a "*spin*" rotation around **e**, and the other a "*tilt*" rotation around an axis orthogonal to **e**. The component `spinAngle` measures the angle of the "spin" sub-rotation around **e**.
List of keywords (see also 9.3.15 for additional options):

- `atoms`: see definition of `atoms` in sec. 9.3.5 (`rmsd` component)
- `refPositions`: see definition of `refPositions` in sec. 9.3.5 (`rmsd` component)
- `refPositionsFile`: see definition of `refPositionsFile` in sec. 9.3.5 (`rmsd` component)
- `refPositionsCol`: see definition of `refPositionsCol` in sec. 9.3.5 (`rmsd` component)
- `refPositionsColValue`: see definition of `refPositionsColValue` in sec. 9.3.5 (`rmsd` component)
- `axis` < Special rotation axis (Å) >
  Context: `tilt`
  Acceptable Values: `(x, y, z)` triplet
  Default Value: `(0.0, 0.0, 1.0)`
  Description: The three components of this vector define (when normalized) the special rotation axis used to calculate the `tilt` and `spinAngle` components.

The component `spinAngle` returns an angle (in degrees) within the periodic interval $[-180 : 180]$.

Note: the value of `spinAngle` is a continuous function almost everywhere, with the exception of configurations with the corresponding "tilt" angle equal to 180° (i.e. the `tilt` component is equal to -1): in those cases, `spinAngle` is undefined. If such configurations are expected, consider defining a `tilt` colvar using the same axis **e**, and restraining it with a lower wall away from -1.

`tilt`: **cosine of the rotation orthogonal to a given axis.** The component `tilt` measures the cosine of the angle of the "tilt" sub-rotation, which combined with the "spin" sub-rotation provides the complete rotation of a group of atoms. The cosine of the tilt angle rather than the tilt angle itself is implemented, because the latter is unevenly distributed even for an isotropic system: consider as an analogy the angle θ in the spherical coordinate system. The component `tilt` relies on the same options as `spinAngle`, including the definition of the axis **e**. The values of `tilt` are real numbers in the interval $[-1 : 1]$: the value 1 represents an orientation fully parallel to **e** (tilt angle = 0°), and the value -1 represents an anti-parallel orientation.
List of keywords (see also 9.3.15 for additional options):

- `atoms`: see definition of `atoms` in sec. 9.3.5 (`rmsd` component)
- `refPositions`: see definition of `refPositions` in sec. 9.3.5 (`rmsd` component)

- `refPositionsFile`: see definition of `refPositionsFile` in sec. 9.3.5 (`rmsd` component)
- `refPositionsCol`: see definition of `refPositionsCol` in sec. 9.3.5 (`rmsd` component)
- `refPositionsColValue`: see definition of `refPositionsColValue` in sec. 9.3.5 (`rmsd` component)
- `axis`: see definition of `axis` in sec. 9.3.6 (`spinAngle` component)

9.3.7 Protein structure descriptors

`alpha`: α-helix content of a protein segment. The block `alpha` {...} defines the parameters to calculate the helical content of a segment of protein residues. The α-helical content across the $N+1$ residues N_0 to N_0+N is calculated by the formula:

$$\alpha\left(\mathrm{C}_\alpha^{(N_0)},\mathrm{O}^{(N_0)},\mathrm{C}_\alpha^{(N_0+1)},\mathrm{O}^{(N_0+1)},\ldots\mathrm{N}^{(N_0+5)},\mathrm{C}_\alpha^{(N_0+5)},\mathrm{O}^{(N_0+5)},\ldots\mathrm{N}^{(N_0+N)},\mathrm{C}_\alpha^{(N_0+N)}\right) = \tag{44}$$

$$\frac{1}{2(N-2)}\sum_{n=N_0}^{N_0+N-2}\mathrm{angf}\left(\mathrm{C}_\alpha^{(n)},\mathrm{C}_\alpha^{(n+1)},\mathrm{C}_\alpha^{(n+2)}\right) + \frac{1}{2(N-4)}\sum_{n=N_0}^{N_0+N-4}\mathrm{hbf}\left(\mathrm{O}^{(n)},\mathrm{N}^{(n+4)}\right), \tag{45}$$

where the score function for the $\mathrm{C}_\alpha-\mathrm{C}_\alpha-\mathrm{C}_\alpha$ angle is defined as:

$$\mathrm{angf}\left(\mathrm{C}_\alpha^{(n)},\mathrm{C}_\alpha^{(n+1)},\mathrm{C}_\alpha^{(n+2)}\right) = \frac{1-\left(\theta(\mathrm{C}_\alpha^{(n)},\mathrm{C}_\alpha^{(n+1)},\mathrm{C}_\alpha^{(n+2)})-\theta_0\right)^2/\left(\Delta\theta_{\mathrm{tol}}\right)^2}{1-\left(\theta(\mathrm{C}_\alpha^{(n)},\mathrm{C}_\alpha^{(n+1)},\mathrm{C}_\alpha^{(n+2)})-\theta_0\right)^4/\left(\Delta\theta_{\mathrm{tol}}\right)^4}, \tag{46}$$

and the score function for the $\mathrm{O}^{(n)} \leftrightarrow \mathrm{N}^{(n+4)}$ hydrogen bond is defined through a `hBond` colvar component on the same atoms.

List of keywords (see also 9.3.15 for additional options):

- `residueRange` < Potential α-helical residues >
  Context: `alpha`
  Acceptable Values: "<Initial residue number>-<Final residue number>"
  Description: This option specifies the range of residues on which this component should be defined. The Colvars module looks for the atoms within these residues named "`CA`", "`N`" and "`O`", and raises an error if any of those atoms is not found.

- `psfSegID` < PSF segment identifier >
  Context: `alpha`
  Acceptable Values: string (max 4 characters)
  Description: This option sets the PSF segment identifier for the residues specified in `residueRange`. This option is only required when PSF topologies are used.

- `hBondCoeff` < Coefficient for the hydrogen bond term >
  Context: `alpha`
  Acceptable Values: positive between 0 and 1
  Default Value: 0.5
  Description: This number specifies the contribution to the total value from the hydrogen bond terms. 0 disables the hydrogen bond terms, 1 disables the angle terms.

- `angleRef` < Reference $C_\alpha - C_\alpha - C_\alpha$ angle >
  Context: `alpha`
  Acceptable Values: positive decimal
  Default Value: 88°
  Description: This option sets the reference angle used in the score function (46).

- `angleTol` < Tolerance in the $C_\alpha - C_\alpha - C_\alpha$ angle >
  Context: `alpha`
  Acceptable Values: positive decimal
  Default Value: 15°
  Description: This option sets the angle tolerance used in the score function (46).

- `hBondCutoff` < Hydrogen bond cutoff >
  Context: `alpha`
  Acceptable Values: positive decimal
  Default Value: 3.3 Å
  Description: Equivalent to the `cutoff` option in the `hBond` component.

- `hBondExpNumer` < Hydrogen bond numerator exponent >
  Context: `alpha`
  Acceptable Values: positive integer
  Default Value: 6
  Description: Equivalent to the `expNumer` option in the `hBond` component.

- `hBondExpDenom` < Hydrogen bond denominator exponent >
  Context: `alpha`
  Acceptable Values: positive integer
  Default Value: 8
  Description: Equivalent to the `expDenom` option in the `hBond` component.

This component returns positive values, always comprised between 0 (lowest α-helical score) and 1 (highest α-helical score).

`dihedralPC`: protein dihedral principal component The block `dihedralPC {...}` defines the parameters to calculate the projection of backbone dihedral angles within a protein segment onto a *dihedral principal component*, following the formalism of dihedral principal component analysis (dPCA) proposed by Mu et al.[79] and documented in detail by Altis et al.[2]. Given a peptide or protein segment of N residues, each with Ramachandran angles ϕ_i and ψ_i, dPCA rests on a variance/covariance analysis of the $4(N-1)$ variables $\cos(\psi_1), \sin(\psi_1), \cos(\phi_2), \sin(\phi_2) \cdots \cos(\phi_N), \sin(\phi_N)$. Note that angles ϕ_1 and ψ_N have little impact on chain conformation, and are therefore discarded, following the implementation of dPCA in the analysis software Carma.[39]

For a given principal component (eigenvector) of coefficients $(k_i)_{1 \leq i \leq 4(N-1)}$, the projection of the current backbone conformation is:

$$\xi = \sum_{n=1}^{N-1} k_{4n-3} \cos(\psi_n) + k_{4n-2} \sin(\psi_n) + k_{4n-1} \cos(\phi_{n+1}) + k_{4n} \sin(\phi_{n+1}) \tag{47}$$

`dihedralPC` expects the same parameters as the `alpha` component for defining the relevant residues (`residueRange` and `psfSegID`) in addition to the following:
List of keywords (see also 9.3.15 for additional options):

- `residueRange`: see definition of `residueRange` in sec. 9.3.7 (`alpha` component)
- `psfSegID`: see definition of `psfSegID` in sec. 9.3.7 (`alpha` component)
- `vectorFile` < File containing dihedral PCA eigenvector(s) >
  Context: `dihedralPC`
  Acceptable Values: file name
  Description: A text file containing the coefficients of dihedral PCA eigenvectors on the cosine and sine coordinates. The vectors should be arranged in columns, as in the files output by Carma.[39]
- `vectorNumber` < File containing dihedralPCA eigenvector(s) >
  Context: `dihedralPC`
  Acceptable Values: positive integer
  Description: Number of the eigenvector to be used for this component.

9.3.8 Raw data: building blocks for custom functions

`cartesian`: vector of atomic Cartesian coordinates. The `cartesian` {...} block defines a component returning a flat vector containing the Cartesian coordinates of all participating atoms, in the order $(x_1, y_1, z_1, \cdots, x_n, y_n, z_n)$.
List of keywords (see also 9.3.15 for additional options):

- `atoms` < Group of atoms >
  Context: `cartesian`
  Acceptable Values: Block `atoms` {...}
  Description: Defines the atoms whose coordinates make up the value of the component. If `rotateReference` or `centerReference` are defined, coordinates are evaluated within the moving frame of reference.

`distancePairs`: set of pairwise distances between two groups. The `distancePairs` {...} block defines a $N_1 \times N_2$-dimensional variable that includes all mutual distances between the atoms of two groups. This can be useful, for example, to develop a new variable defined over two groups, by using the `scriptedFunction` feature.
List of keywords (see also 9.3.15 for additional options):

- `group1`: see definition of `group1` in sec. 9.3.2 (`distance` component)
- `group2`: analogous to `group1`
- `forceNoPBC`: see definition of `forceNoPBC` in sec. 9.3.2 (`distance` component)

This component returns a $N_1 \times N_2$-dimensional vector of numbers, each ranging from 0 to the largest possible distance within the chosen boundary conditions.

9.3.9 Geometric path collective variables

The geometric path collective variables define the progress along a path, s, and the distance from the path, z. These CVs are proposed by Leines and Ensing[63] , which differ from that[12] proposed by Branduardi et al., and utilize a set of geometric algorithms. The path is defined as a series of frames in the atomic Cartesian coordinate space or the CV space. s and z are computed as

$$s = \frac{m}{M} \pm \frac{1}{2M}\left(\frac{\sqrt{(\mathbf{v}_1\cdot\mathbf{v}_3)^2 - |\mathbf{v}_3|^2(|\mathbf{v}_1|^2 - |\mathbf{v}_2|^2)} - (\mathbf{v}_1\cdot\mathbf{v}_3)}{|\mathbf{v}_3|^2} - 1\right) \tag{48}$$

$$z = \sqrt{\left(\mathbf{v}_1 + \frac{1}{2}\left(\frac{\sqrt{(\mathbf{v}_1\cdot\mathbf{v}_3)^2 - |\mathbf{v}_3|^2(|\mathbf{v}_1|^2 - |\mathbf{v}_2|^2)} - (\mathbf{v}_1\cdot\mathbf{v}_3)}{|\mathbf{v}_3|^2} - 1\right)\mathbf{v}_4\right)^2} \tag{49}$$

where $\mathbf{v}_1 = \mathbf{s}_m - \mathbf{z}$ is the vector connecting the current position to the closest frame, $\mathbf{v}_2 = \mathbf{z} - \mathbf{s}_{m-1}$ is the vector connecting the second closest frame to the current position, $\mathbf{v}_3 = \mathbf{s}_{m+1} - \mathbf{s}_m$ is the vector connecting the closest frame to the third closest frame, and $\mathbf{v}_4 = \mathbf{s}_m - \mathbf{s}_{m-1}$ is the vector connecting the second closest frame to the closest frame. m and M are the current index of the closest frame and the total number of frames, respectively. If the current position is on the left of the closest reference frame, the $\pm$ in s turns to the positive sign. Otherwise it turns to the negative sign.

The equations above assume: (i) the frames are equidistant and (ii) the second and the third closest frames are neighbouring to the closest frame. When these assumptions are not satisfied, this set of path CV should be used carefully.

`gspath`: progress along a path defined in atomic Cartesian coordinate space. In the `gspath` {...} and the `gzpath` {...} block all vectors, namely $\mathbf{z}$ and $\mathbf{s}_k$ are defined in atomic Cartesian coordinate space. More specifically, $\mathbf{z} = [\mathbf{r}_1, \cdots, \mathbf{r}_n]$, where $\mathbf{r}_i$ is the i-th atom specified in the `atoms` block. $\mathbf{s}_k = [\mathbf{r}_{k,1}, \cdots, \mathbf{r}_{k,n}]$, where $\mathbf{r}_{k,i}$ means the i-th atom of the k-th reference frame.
List of keywords (see also 9.3.15 for additional options):

- `atoms` < Group of atoms >
  Context: `gspath` and `gzpath`
  Acceptable Values: Block `atoms` {...}
  Description: Defines the atoms whose coordinates make up the value of the component.

- `refPositionsCol` < PDB column containing atom flags >
  Context: `gspath` and `gzpath`
  Acceptable Values: `O`, `B`, `X`, `Y`, or `Z`
  Description: If `refPositionsFileN` is a PDB file that contains all the atoms in the topology, this option may be provided to set which PDB field is used to flag the reference coordinates for `atoms`.

- `refPositionsFileN` < File containing the reference positions for fitting >
  Context: `gspath` and `gzpath`
  Acceptable Values: UNIX filename
  Description: The path is defined by multiple `refPositionsFile`s which are similiar to `refPositionsFile` in the `rmsd` CV. If your path consists of 10 nodes, you can list the coordinate file (in PDB or XYZ format) from `refPositionsFile1` to `refPositionsFile10`.

- `useSecondClosestFrame` < Define $\mathbf{s}_{m-1}$ as the second closest frame? >
  Context: `gspath` and `gzpath`
  Acceptable Values: boolean
  Default Value: `on`
  Description: The definition assumes the second closest frame is neighbouring to the closest frame. This is not always true especially when the path is crooked. If this option is set to `on` (default), $\mathbf{s}_{m-1}$ is defined as the second closest frame. If this option is set to `off`, $\mathbf{s}_{m-1}$ is defined as the left or right neighbouring frame of the closest frame.

- `useThirdClosestFrame` < Define $\mathbf{s}_{m+1}$ as the third closest frame? >
  Context: `gspath` and `gzpath`
  Acceptable Values: boolean
  Default Value: `off`
  Description: The definition assumes the third closest frame is neighbouring to the closest frame. This is not always true especially when the path is crooked. If this option is set to `on`, $\mathbf{s}_{m+1}$ is defined as the third closest frame. If this option is set to `off` (default), $\mathbf{s}_{m+1}$ is defined as the left or right neighbouring frame of the closest frame.

- `fittingAtoms` < The atoms that are used for alignment >
  Context: `gspath` and `gspath`
  Acceptable Values: Group of atoms
  Description: Before calculating $\mathbf{v}_1$, $\mathbf{v}_2$, $\mathbf{v}_3$ and $\mathbf{v}_4$, the current frame need to be aligned to the corresponding reference frames. This option specifies which atoms are used to do alignment.

`gzpath`**: distance from a path defined in atomic Cartesian coordinate space. List of keywords** (see also 9.3.15 for additional options):

- `useZsquare` < Compute z^2 instead of z >
  Context: `gzpath`
  Acceptable Values: boolean
  Default Value: `off`
  Description: z is not differentiable when it is zero. This implementation workarounds it by setting the derivative of z to zero when $z = 0$. Another workaround is set this option to `on`, which computes z^2 instead of z, and then z^2 is differentiable when it is zero.

The usage of `gzpath` and `gspath` is illustrated below:

```
colvar {
  # Progress along the path
  name gs
  # The path is defined by 5 reference frames (from string-00.pdb to
string-04.pdb)
  # Use atomic coordinate from atoms 1, 2 and 3 to compute the path
  gspath {
    atoms {atomnumbers { 1 2 3 }}
    refPositionsFile1 string-00.pdb
    refPositionsFile2 string-01.pdb
```

```
    refPositionsFile3 string-02.pdb
    refPositionsFile4 string-03.pdb
    refPositionsFile5 string-04.pdb
  }
}
colvar {
  # Distance from the path
  name gz
  # The path is defined by 5 reference frames (from string-00.pdb to
string-04.pdb)
  # Use atomic coordinate from atoms 1, 2 and 3 to compute the path
  gzpath {
    atoms {atomnumbers { 1 2 3 }}
    refPositionsFile1 string-00.pdb
    refPositionsFile2 string-01.pdb
    refPositionsFile3 string-02.pdb
    refPositionsFile4 string-03.pdb
    refPositionsFile5 string-04.pdb
  }
}
```

`linearCombination`: **Helper CV to define a linear combination of other CVs** This is a helper CV which can be defined as a linear combination of other CVs. It maybe useful when you want to define the `gspathCV {...}` and the `gzpathCV {...}` as combinations of other CVs.

`gspathCV`: **progress along a path defined in CV space.** In the `gspathCV {...}` and the `gzpathCV {...}` block all vectors, namely $\mathbf{z}$ and $\mathbf{s}_k$ are defined in CV space. More specifically, $\mathbf{z} = [\xi_1, \cdots, \xi_n]$, where ξ_i is the i-th CV. $\mathbf{s}_k = [\xi_{k,1}, \cdots, \xi_{k,n}]$, where $\xi_{k,i}$ means the i-th CV of the k-th reference frame. It should be note that these two CVs requires the `pathFile` option, which specifies a path file. Each line in the path file contains a set of space-seperated CV value of the reference frame. The sequence of reference frames matches the sequence of the lines.
List of keywords (see also 9.3.15 for additional options):

- `useSecondClosestFrame` < Define $\mathbf{s}_{m-1}$ as the second closest frame? >
  Context: `gspathCV` and `gzpathCV`
  Acceptable Values: boolean
  Default Value: `on`
  Description: The definition assumes the second closest frame is neighbouring to the closest frame. This is not always true especially when the path is crooked. If this option is set to `on` (default), $\mathbf{s}_{m-1}$ is defined as the second closest frame. If this option is set to `off`, $\mathbf{s}_{m-1}$ is defined as the left or right neighbouring frame of the closest frame.

- `useThirdClosestFrame` < Define $\mathbf{s}_{m+1}$ as the third closest frame? >
  Context: `gspathCV` and `gzpathCV`
  Acceptable Values: boolean
  Default Value: `off`
  Description: The definition assumes the third closest frame is neighbouring to the closest

frame. This is not always true especially when the path is crooked. If this option is set to `on`, $\mathbf{s}_{m+1}$ is defined as the third closest frame. If this option is set to `off` (default), $\mathbf{s}_{m+1}$ is defined as the left or right neighbouring frame of the closest frame.

- `pathFile` < The file name of the path file. >
  Context: `gspathCV` and `gzpathCV`
  Acceptable Values: UNIX filename
  Description: Defines the nodes or images that constitutes the path in CV space. The CVs of an image are listed in a line of `pathFile` using space-seperated format. Lines from top to button in `pathFile` corresponds images from initial to last.

`gzpathCV`**: distance from a path defined in CV space. List of keywords** (see also 9.3.15 for additional options):

- `useZsquare` < Compute z^2 instead of z >
  Context: `gzpathCV`
  Acceptable Values: boolean
  Default Value: `off`
  Description: z is not differentiable when it is zero. This implementation workarounds it by setting the derivative of z to zero when $z = 0$. Another workaround is set this option to `on`, which computes z^2 instead of z, and then z^2 is differentiable when it is zero.

The usage of `gzpathCV` and `gspathCV` is illustrated below:

```
colvar {
  # Progress along the path
  name gs
  # Path defined by the CV space of two dihedral angles
  gspathCV {
    pathFile ./path.txt
    dihedral {
      name 001
      group1 {atomNumbers {5}}
      group2 {atomNumbers {7}}
      group3 {atomNumbers {9}}
      group4 {atomNumbers {15}}
    }
    dihedral {
      name 002
      group1 {atomNumbers {7}}
      group2 {atomNumbers {9}}
      group3 {atomNumbers {15}}
      group4 {atomNumbers {17}}
    }
  }
}
colvar {
  # Distance from the path
```

```
  name gz
  gzpathCV {
    pathFile ./path.txt
    dihedral {
      name 001
      group1 {atomNumbers {5}}
      group2 {atomNumbers {7}}
      group3 {atomNumbers {9}}
      group4 {atomNumbers {15}}
    }
    dihedral {
      name 002
      group1 {atomNumbers {7}}
      group2 {atomNumbers {9}}
      group3 {atomNumbers {15}}
      group4 {atomNumbers {17}}
    }
  }
}
```

9.3.10 Arithmetic path collective variables

The arithmetic path collective variable in CV space uses the same formula as the one proposed by Branduardi[12] et al., except that it computes s and z in CV space instead of RMSDs in Cartesian space. Moreover, this implementation allows different coefficients for each CV components as described in [59]. Assuming a path is composed of N reference frames and defined in an M-dimensional CV space, then the equations of s and z of the path are

$$s = \frac{\sum_{i=1}^{N} i \exp\left(-\lambda \sum_{j=1}^{M} c_j^2 \left(x_j - x_{i,j}\right)^2\right)}{\sum_{i=1}^{N} \exp\left(-\lambda \sum_{j=1}^{M} c_j^2 \left(x_j - x_{i,j}\right)^2\right)} \tag{50}$$

$$z = -\frac{1}{\lambda} \ln\left(\sum_{i=1}^{N} \exp\left(-\lambda \sum_{j=1}^{M} c_j^2 \left(x_j - x_{i,j}\right)\right)\right) \tag{51}$$

where c_j is the coefficient(weight) of the j-th CV, $x_{i,j}$ is the value of j-th CV of i-th reference frame and x_j is the value of j-th CV of current frame. λ is a parameter to smooth the variation of s and z.

`aspathCV`: progress along a path defined in CV space. This colvar component computes the s variable.
List of keywords (see also 9.3.15 for additional options):

- `weights` < Coefficients of the collective variables >
  Context: `aspathCV` and `azpathCV`
  Acceptable Values: space-separated numbers in a {...} block
  Default Value: {`1.0` ...}

Description: Define the coefficients. The j-th value in the $\{\ldots\}$ block corresponds the c_j in the equations.

- `lambda` < Smoothness of the variation of s and z >
  Context: `aspathCV` and `azpathCV`
  Acceptable Values: decimal
  Default Value: inverse of the mean square displacements of successive reference frames
  Description: The value of λ in the equations.

- `pathFile` < The file name of the path file. >
  Context: `aspathCV` and `azpathCV`
  Acceptable Values: UNIX filename
  Description: Defines the nodes or images that constitutes the path in CV space. The CVs of an image are listed in a line of `pathFile` using space-seperated format. Lines from top to button in `pathFile` corresponds images from initial to last.

`azpathCV`: distance from a path defined in CV space. This colvar component computes the z variable. Options are the same as in 9.3.10.

The usage of `azpathCV` and `aspathCV` is illustrated below:

```
colvar {
  # Progress along the path
  name as
  # Path defined by the CV space of two dihedral angles
  aspathCV {
    pathFile ./path.txt
    weights {1.0 1.0}
    lambda 0.005
    dihedral {
      name 001
      group1 {atomNumbers {5}}
      group2 {atomNumbers {7}}
      group3 {atomNumbers {9}}
      group4 {atomNumbers {15}}
    }
    dihedral {
      name 002
      group1 {atomNumbers {7}}
      group2 {atomNumbers {9}}
      group3 {atomNumbers {15}}
      group4 {atomNumbers {17}}
    }
  }
}
colvar {
  # Distance from the path
  name az
```

```
  azpathCV {
    pathFile ./path.txt
    weights {1.0 1.0}
    lambda 0.005
    dihedral {
      name 001
      group1 {atomNumbers {5}}
      group2 {atomNumbers {7}}
      group3 {atomNumbers {9}}
      group4 {atomNumbers {15}}
    }
    dihedral {
      name 002
      group1 {atomNumbers {7}}
      group2 {atomNumbers {9}}
      group3 {atomNumbers {15}}
      group4 {atomNumbers {17}}
    }
  }
}
```

Path collective variables in Cartesian coordinates The path collective variables defined by Branduardi et al. [12] are based on RMSDs in Cartesian coordinates. Noting d_i the RMSD between the current set of Cartesian coordinates and those of image number i of the path:

$$s = \frac{1}{N-1} \frac{\sum_{i=1}^{N} (i-1) \exp\left(-\lambda d_i^2\right)}{\sum_{i=1}^{N} \exp\left(-\lambda d_i^2\right)} \tag{52}$$

$$z = -\frac{1}{\lambda} \ln \left(\sum_{i=1}^{N} \exp(-\lambda d_i^2) \right) \tag{53}$$

where λ is the smoothing parameter.

These coordinates are implemented as Tcl-scripted combinations of `rmsd` components. The implementation is available as file `colvartools/pathCV.tcl`, and an example is provided in file `examples/10_pathCV.namd` of the Colvars public repository. It implements an optimization procedure, whereby the distance to a given image is only calculated if its contribution to the sum is larger than a user-defined `tolerance` parameter. All distances are calculated every `freq` timesteps to update the list of nearby images.

9.3.11 Volumetric map-based variables

Volumetric maps of the Cartesian coordinates, typically defined as mesh grid along the three Cartesian axes, may be used to define collective variables. This feature is currently available in NAMD, implemented as an interface between Colvars and GridForces (see section 8). Please cite [34] when using this implementation of collective variables based on volumetric maps.

`mapTotal`: total value of a volumetric map Given a function of the Cartesian coordinates $\phi(\mathbf{x}) = \phi(x, y, z)$, a `mapTotal` collective variable component $\Phi(\mathbf{X})$ is defined as the sum of the values of the function $\phi(\mathbf{x})$ evaluated at the coordinates of each atom, $\mathbf{x}_i$:

$$\Phi(\mathbf{X}) = \sum_{i=1}^{N} \phi(\mathbf{x}_i) \tag{54}$$

This formulation allows, for example, to "count" the number of atoms within a region of space by using a positive-valued function $\phi(\mathbf{x})$, such as for example the number of water molecules in a hydrophobic cavity [34].

Because the volumetric map itself and the atoms affected by it are defined externally to Colvars, this component has a very limited number of keywords. **List of keywords** (see also 9.3.15 for additional options):

- `mapName` < Specify the name of the volumetric map to use as a colvar >
  Context: `mapTotal`
  Acceptable Values: string
  Description: The value of this option specifies the label of the volumetric map to use for this collective variable component. This label must identify a map already loaded in NAMD via `mGridForcePotFile`, and its value of `mGridForceScale` needs to be set to (0, 0, 0), so that its collective force can be computed dynamically.

Example: biasing the number of molecules inside a cavity using a volumetric map.
Firstly, a volumetric map that has a value of 1 inside the cavity and 0 outside should be prepared. A reasonable starting point may be obtained, for example, with VMD: using an existing trajectory where the cavity is occupied by solvent and a spatial selection that identifies all the molecules within the cavity, `volmap occupancy -allframes -combine max` computes the occupancy map as a step function (values 1 or 0), and `volutil -smooth ...` makes it a continuous map, suitable for use as a MD simulation bias. A PDB file defining the selection (for example, where all water oxygens and ions have an occupancy of 1 and other atoms 0) is also prepared using VMD. Both the map file and the atom selection file are then loaded via the `mGridForcePotFile` and related NAMD commands:

```
mGridForce yes
mGridForcePotFile Cavity cavity.dx   # OpenDX map file
mGridForceFile Cavity water-sel.pdb  # PDB file used for atom selection
mGridForceCol Cavity O               # Use the occupancy column of the PDB file
mGridForceChargeCol Cavity O         # Use occ.  again (default:  electrostatic
charge)
mGridForceScale Cavity 0.0 0.0 0.0   # Do not use GridForces for this map
```

The value of `mGridForceScale` is particularly important, because it determines the GridForces force constant for the "Cavity" map. A non-zero value enables a conventional GridForces calculation, where the force constant remains fixed within each `run` command and the forces on the atoms depend only on their positions in space. However, setting `mGridForceScale` to zero signals to NAMD that the force acting through the volumetric map may be computed dynamically, as part of a collective-variable biasing scheme. To do so, the map labeled "Cavity" needs to be

referred to in the Colvars configuration:

```
cv config "
colvar {
  name n_waters
  mapTotal {
    mapName Cavity # Same label as the GridForce map
  }
}"
```

The variable "`n_waters`" may then be used with any of the enhanced sampling methods available (9.5): new forces applied to it at each simulation step will be transmitted to the corresponding atoms within the same step.

Multiple volumetric maps collective variables To study processes that involve changes in shape of a macromolecular aggregate (for example, deformations of lipid membranes) it is useful to define collective variables based on more than one volumetric map at a time, measuring the relative similarity with each map while still achieving correct thermodynamic sampling of each state.

This is achieved by combining multiple `mapTotal` components, each based on a differently-shaped volumetric map, into a single collective variable ξ. To track transitions between states, the contribution of each map to ξ should be discriminated from the others, for example by assigning to it a different weight. The "Multi-Map" progress variable [34] uses a weight sum of these components, using linearly-increasing weights:

$$\xi(\mathbf{X}) = \sum_{k=1}^{K} \Phi_k(\mathbf{X}) = \sum_{k=1}^{K} k \sum_{i=1}^{N} \phi_k(\mathbf{x}_i) \tag{55}$$

where K is the number of maps employed and each Φ_k is a `mapTotal` component.

Example: transitions between macromolecular shapes using volumetric maps.
A series of map files, each representing a different shape, is loaded into NAMD:

```
mGridForce yes
for { set k 1 } { $k <= $K } { incr k } {
  mGridForcePotFile Shape_$k map_$k.dx  # Density map of the k-th state
  ...
}
```

and these are then used to define a multiple-map collective variable:

```
# Collect the definition of all components into one string
set components ""
for { set k 1 } { $k <= $K } { incr k } {
  set components "${components}
  mapTotal {
    mapName Shape_$k
    componentCoeff $k
  }
"
}
```

```
# Use this string to define the variable
cv config "
colvar {
  name shapes
  ${components}
}"
```

The above example illustrates a use case where a weighted sum (i.e. a linear combination) is used to define a single variable from multiple components. Depending on the problem under study, non-linear functions may be more appropriate. These may be defined a custom functions if implemented (see 9.3.16), or scripted functions (see 9.3.17).

9.3.12 Shared keywords for all components

The following options can be used for any of the above colvar components in order to obtain a polynomial combination or any user-supplied function provided by `scriptedFunction` (see 9.3.15).

- `name` < Name of this component >
  Context: any component
  Acceptable Values: string
  Default Value: type of component + numeric id
  Description: The name is an unique case-sensitive string which allows the Colvars module to identify this component. This is useful, for example, when combining multiple components via a `scriptedFunction`. It also defines the variable name representing the component's value in a `customFunction` (see 9.3.16) expression.

- `scalable` < Attempt to calculate this component in parallel? >
  Context: any component
  Acceptable Values: boolean
  Default Value: `on`, if available
  Description: If set to `on` (default), the Colvars module will attempt to calculate this component in parallel to reduce overhead. Whether this option is available depends on the type of component: currently supported are `distance`, `distanceZ`, `distanceXY`, `distanceVec`, `distanceDir`, `angle` and `dihedral`. This flag influences computational cost, but does not affect numerical results: therefore, it should only be turned off for debugging or testing purposes.

9.3.13 Periodic components

The following components returns real numbers that lie in a periodic interval:

- `dihedral`: torsional angle between four groups;

- `spinAngle`: angle of rotation around a predefined axis in the best-fit from a set of reference coordinates.

In certain conditions, `distanceZ` can also be periodic, namely when periodic boundary conditions (PBCs) are defined in the simulation and `distanceZ`'s axis is parallel to a unit cell vector.

In addition, a custom or scripted scalar colvar may be periodic depending on its user-defined expression. It will only be treated as such by the Colvars module if the period is specified using the `period` keyword, while `wrapAround` is optional.

The following keywords can be used within periodic components, or within custom variables (9.3.16), or wthin scripted variables 9.3.17).

- `period` < Period of the component >
  Context: `distanceZ`, custom colvars
  Acceptable Values: positive decimal
  Default Value: 0.0
  Description: Setting this number enables the treatment of `distanceZ` as a periodic component: by default, `distanceZ` is not considered periodic. The keyword is supported, but irrelevant within `dihedral` or `spinAngle`, because their period is always 360 degrees.

- `wrapAround` < Center of the wrapping interval for periodic variables >
  Context: `distanceZ`, `dihedral`, `spinAngle`, custom colvars
  Acceptable Values: decimal
  Default Value: 0.0
  Description: By default, values of the periodic components are centered around zero, ranging from $-P/2$ to $P/2$, where P is the period. Setting this number centers the interval around this value. This can be useful for convenience of output, or to set the walls for a `harmonicWalls` in an order that would not otherwise be allowed.

Internally, all differences between two values of a periodic colvar follow the minimum image convention: they are calculated based on the two periodic images that are closest to each other.

Note: linear or polynomial combinations of periodic components (see 9.3.15) may become meaningless when components cross the periodic boundary. Use such combinations carefully: estimate the range of possible values of each component in a given simulation, and make use of ***`wrapAround`*** *to limit this problem whenever possible.*

9.3.14 Non-scalar components

When one of the following components are used, the defined colvar returns a value that is not a scalar number:

- `distanceVec`: 3-dimensional vector of the distance between two groups;
- `distanceDir`: 3-dimensional unit vector of the distance between two groups;
- `orientation`: 4-dimensional unit quaternion representing the best-fit rotation from a set of reference coordinates.

The distance between two 3-dimensional unit vectors is computed as the angle between them. The distance between two quaternions is computed as the angle between the two 4-dimensional unit vectors: because the orientation represented by `q` is the same as the one represented by $-$`q`, distances between two quaternions are computed considering the closest of the two symmetric images.

Non-scalar components carry the following restrictions:

- Calculation of total forces (`outputTotalForce` option) is currently not implemented.

- Each colvar can only contain one non-scalar component.
- Binning on a grid (`abf`, `histogram` and `metadynamics` with `useGrids` enabled) is currently not implemented for colvars based on such components.

Note: while these restrictions apply to individual colvars based on non-scalar components, no limit is set to the number of scalar colvars. To compute multi-dimensional histograms and PMFs, use sets of scalar colvars of arbitrary size.

Calculating total forces In addition to the restrictions due to the type of value computed (scalar or non-scalar), a final restriction can arise when calculating total force (`outputTotalForce` option or application of a `abf` bias). total forces are available currently only for the following components: `distance`, `distanceZ`, `distanceXY`, `angle`, `dihedral`, `rmsd`, `eigenvector` and `gyration`.

9.3.15 Linear and polynomial combinations of components

To extend the set of possible definitions of colvars $\xi(\mathbf{r})$, multiple components $q_i(\mathbf{r})$ can be summed with the formula:

$$\xi(\mathbf{r}) = \sum_i c_i [q_i(\mathbf{r})]^{n_i} \tag{56}$$

where each component appears with a unique coefficient c_i (1.0 by default) the positive integer exponent n_i (1 by default).

Any set of components can be combined within a colvar, provided that they return the same type of values (scalar, unit vector, vector, or quaternion). By default, the colvar is the sum of its components. Linear or polynomial combinations (following equation (56)) can be obtained by setting the following parameters, which are common to all components:

- `componentCoeff` < Coefficient of this component in the colvar >
  Context: any component
  Acceptable Values: decimal
  Default Value: `1.0`
  Description: Defines the coefficient by which this component is multiplied (after being raised to `componentExp`) before being added to the sum.

- `componentExp` < Exponent of this component in the colvar >
  Context: any component
  Acceptable Values: integer
  Default Value: `1`
  Description: Defines the power at which the value of this component is raised before being added to the sum. When this exponent is different than 1 (non-linear sum), total forces and the Jacobian force are not available, making the colvar unsuitable for ABF calculations.

Example: To define the *average* of a colvar across different parts of the system, simply define within the same colvar block a series of components of the same type (applied to different atom groups), and assign to each component a `componentCoeff` of $1/N$.

9.3.16 Custom functions

Collective variables may be defined by specifying a custom function as an analytical expression. The expression is parsed by Lepton, the lightweight expression parser written by Peter Eastman (https://simtk.org/projects/lepton). Lepton produces efficient evaluation routines for the function and its derivatives.

- `customFunction` < Compute colvar as a custom function of its components >
  Context: `colvar`
  Acceptable Values: string
  Description: Mathematical expression to define the colvar as a closed-form function of its colvar components. See below for the detailed syntax of Lepton expressions. Multiple mentions of this keyword can be used to define a vector variable (as in the example below).

- `customFunctionType` < Type of value returned by the scripted colvar >
  Context: `colvar`
  Acceptable Values: string
  Default Value: `scalar`
  Description: With this flag, the user may specify whether the colvar is a scalar or one of the following vector types: `vector3` (a 3D vector), `unit_vector3` (a normalized 3D vector), or `unit_quaternion` (a normalized quaternion), or `vector`. Note that the scalar and vector cases are not necessary, as they are detected automatically.

The expression may use the collective variable components as variables, referred to by their user-defined `name`. Scalar elements of vector components may be accessed by appending a 1-based index to their `name`, as in the example below. When implementing generic functions of Cartesian coordinates rather than functions of existing components, the `cartesian` component may be particularly useful. A scalar-valued custom variable may be manually defined as periodic by providing the keyword `period`, and the optional keyword `wrapAround`, with the same meaning as in periodic components (see 9.3.13 for details). A vector variable may be defined by specifying the `customFunction` parameter several times: each expression defines one scalar element of the vector colvar, as in this example:

```
colvar {
  name custom

  # A 2-dimensional vector function of a scalar x and a 3-vector r
  customFunction cos(x) * (r1 + r2 + r3)
  customFunction sqrt(r1 * r2)

  distance {
    name x
    group1 { atomNumbers 1 }
    group2 { atomNumbers 50 }
  }
  distanceVec {
    name r
    group1 { atomNumbers 10 11 12 }
```

```
    group2 { atomNumbers 20 21 22 }
  }
}
```

Numeric constants may be given in either decimal or exponential form (e.g. 3.12e-2). An expression may be followed by definitions for intermediate values that appear in the expression, separated by semicolons. For example, the expression:
`a^2 + a*b + b^2; a = a1 + a2; b = b1 + b2`
is exactly equivalent to:
`(a1 + a2)^2 + (a1 + a2) * (b1 + b2) + (b1 + b2)^2`.
The definition of an intermediate value may itself involve other intermediate values. All uses of a value must appear *before* that value's definition.

Lepton supports the usual arithmetic operators +, -, *, /, and ^ (power), as well as the following functions:

sqrt	Square root
exp	Exponential
log	Natural logarithm
erf	Error function
erfc	Complementary error function
sin	Sine (angle in radians)
cos	Cosine (angle in radians)
sec	Secant (angle in radians)
csc	Cosecant (angle in radians)
tan	Tangent (angle in radians)
cot	Cotangent (angle in radians)
asin	Inverse sine (in radians)
acos	Inverse cosine (in radians)
atan	Inverse tangent (in radians)
atan2	Two-argument inverse tangent (in radians)
sinh	Hyperbolic sine
cosh	Hyperbolic cosine
tanh	Hyperbolic tangent
abs	Absolute value
floor	Floor
ceil	Ceiling
min	Minimum of two values
max	Maximum of two values
delta	delta(x) = 1 if $x = 0$, 0 otherwise
step	step(x) = 0 if $x < 0$, 1 if $x >= 0$
select	select(x, y, z) = z if $x = 0$, y otherwise

9.3.17 Scripted functions

When scripting is supported (default in NAMD), a colvar may be defined as a scripted function of its components, rather than a linear or polynomial combination. When implementing generic functions of Cartesian coordinates rather than functions of existing components, the `cartesian` component may be particularly useful. A scalar-valued scripted variable may be manually defined

as periodic by providing the keyword `period`, and the optional keyword `wrapAround`, with the same meaning as in periodic components (see 9.3.13 for details).

An example of elaborate scripted colvar is given in example 10, in the form of path-based collective variables as defined by Branduardi et al[12] (Section 9.3.10).

- `scriptedFunction` < Compute colvar as a scripted function of its components >
  Context: `colvar`
  Acceptable Values: string
  Description: If this option is specified, the colvar will be computed as a scripted function of the values of its components. To that effect, the user should define two Tcl procedures: `calc_<scriptedFunction>` and `calc_<scriptedFunction>_gradient`, both accepting as many parameters as the colvar has components. Values of the components will be passed to those procedures in the order defined by their sorted `name` strings. Note that if all components are of the same type, their default names are sorted in the order in which they are defined, so that names need only be specified for combinations of components of different types. `calc_<scriptedFunction>` should return one value of type <scriptedFunctionType>, corresponding to the colvar value. `calc_<scriptedFunction>_gradient` should return a Tcl list containing the derivatives of the function with respect to each component. If both the function and some of the components are vectors, the gradient is really a Jacobian matrix that should be passed as a linear vector in row-major order, i.e. for a function $f_i(x_j)$: $\nabla_x f_1 \nabla_x f_2 \cdots$.

- `scriptedFunctionType` < Type of value returned by the scripted colvar >
  Context: `colvar`
  Acceptable Values: string
  Default Value: `scalar`
  Description: If a colvar is defined as a scripted function, its type is not constrained by the types of its components. With this flag, the user may specify whether the colvar is a scalar or one of the following vector types: `vector3` (a 3D vector), `unit_vector3` (a normalized 3D vector), or `unit_quaternion` (a normalized quaternion), or `vector` (a vector whose size is specified by `scriptedFunctionVectorSize`). Non-scalar values should be passed as space-separated lists.

- `scriptedFunctionVectorSize` < Dimension of the vector value of a scripted colvar >
  Context: `colvar`
  Acceptable Values: positive integer
  Description: This parameter is only valid when `scriptedFunctionType` is set to `vector`. It defines the vector length of the colvar value returned by the function.

9.3.18 Defining grid parameters

Many algorithms require the definition of boundaries and/or characteristic spacings that can be used to define discrete "states" in the collective variable, or to combine variables with very different units. The parameters described below offer a way to specify these parameters only once for each variable, while using them multiple times in restraints, time-dependent biases or analysis methods.

- `width` < Unit of the variable, or grid spacing >
  Context: `colvar`

Acceptable Values: positive decimal
Default Value: 1.0
Description: This number defines the effective unit of measurement for the collective variable, and is used by the biasing methods for the following purposes. Harmonic (9.5.5), harmonic walls (9.5.7) and linear restraints (9.5.8) use it to set the physical unit of the force constant, which is useful for multidimensional restraints involving multiple variables with very different units (for examples, $\AA$ or degrees °) with a single, scaled force constant. The values of the scaled force constant in the units of each variable are printed at initialization time. Histograms (9.5.10), ABF (9.5.2) and metadynamics (9.5.4) all use this number as the initial choice for the grid spacing along this variable: for this reason, `width` should generally be no larger than the standard deviation of the colvar in an unbiased simulation. Unless it is required to control the spacing, it is usually simplest to keep the default value of 1, so that restraint force constants are provided with their full physical unit.

- `lowerBoundary` < Lower boundary of the colvar >
Context: `colvar`
Acceptable Values: decimal
Default Value: natural boundary of the function
Description: Defines the lowest end of the interval of "relevant" values for the variable. This number can be, for example, a true physical boundary imposed by the choice of function (e.g. the `distance` function is always larger than zero): if this is the case, and only one function is used to define the variable, the default value of this number is set to the lowest end of the range of values of that function, if available (see Section 9.3.1). Alternatively, this value may be provided by the user, to represent for example the left-most point of a PMF calculation along this variable. In the latter case, it is the user's responsibility to either *(a)* ensure the variable does not go significantly beyond the boundary (for example by adding a `harmonicWalls` restraint, 9.5.7), or *(b)* instruct the code that this is a true physical boundary by setting `hardLowerBoundary` (see 9.3.18).

- `upperBoundary` < Upper boundary of the colvar >
Context: `colvar`
Acceptable Values: decimal
Default Value: natural boundary of the function
Description: Similarly to `lowerBoundary`, defines the highest of the "relevant" values of the variable.

- `hardLowerBoundary` < Whether the lower boundary is the physical lower limit >
Context: `colvar`
Acceptable Values: boolean
Default Value: provided by the component
Description: When the colvar has a "natural" boundary (for example, a `distance` colvar cannot go below 0) this flag is automatically enabled. For more complex variable definitions, or when `lowerBoundary` (see 9.3.18) is provided directly by the user, it may be useful to set this flag explicitly. This option does not affect simulation results, but enables some internal optimizations by letting the code know that the variable is unable to cross the lower boundary, regardless of whether restraints are applied to it.

- `hardUpperBoundary` < Whether the upper boundary is the physical upper limit of the colvar's values >
  Context: `colvar`
  Acceptable Values: boolean
  Default Value: provided by the component
  Description: Analogous to `hardLowerBoundary`.

- `expandBoundaries` < Allow to expand the two boundaries if needed >
  Context: `colvar`
  Acceptable Values: boolean
  Default Value: `off`
  Description: If defined, `lowerBoundary` and `upperBoundary` may be automatically expanded to accommodate colvar values that do not fit in the initial range. Currently, this option is used by the metadynamics bias (9.5.4) to keep all of its hills fully within the grid. This option cannot be used when the initial boundaries already span the full period of a periodic colvar.

Grid files: multicolumn text format Many simulation methods and analysis tools write files that contain functions of the collective variables tabulated on a grid (e.g. potentials of mean force or multidimentional histograms) for the purpose of analyzing results. Such files are produced by ABF (9.5.2), metadynamics (9.5.4), multidimensional histograms (9.5.10), as well as any restraint with optional thermodynamic integration support (9.5.1).

In some cases, these files may also be read as input of a new simulation. Suitable input files for this purpose are typically generated as output files of previous simulations, or directly by the user in the specific case of ensemble-biased metadynamics (9.5.4). This section explains the "multicolumn" format used by these files. For a multidimensional function $f(\xi_1, \xi_2, \dots)$ the multicolumn grid format is defined as follows:

```
#  N_cv
#  min(ξ1)        width(ξ1)        npoints(ξ1)        periodic(ξ1)
#  min(ξ2)        width(ξ2)        npoints(ξ2)        periodic(ξ2)
#  ...            ...              ...                ...
#  min(ξ_Ncv)     width(ξ_Ncv)     npoints(ξ_Ncv)     periodic(ξ_Ncv)

   ξ1^1           ξ2^1             ...                ξ_Ncv^1            f(ξ1^1, ξ2^1, ..., ξ_Ncv^1)
   ξ1^1           ξ2^1             ...                ξ_Ncv^2            f(ξ1^1, ξ2^1, ..., ξ_Ncv^2)
   ...            ...              ...                ...                ...
```

Lines beginning with the character "`#`" are the header of the file. $N_{\rm cv}$ is the number of collective variables sampled by the grid. For each variable ξ_i, `min`(ξ_i) is the lowest value sampled by the grid (i.e. the left-most boundary of the grid along ξ_i), `width`(ξ_i) is the width of each grid step along ξ_i, `npoints`(ξ_i) is the number of points and `periodic`(ξ_i) is a flag whose value is 1 or 0 depending on whether the grid is periodic along ξ_i. In most situations:

- `min`(ξ_i) is given by the `lowerBoundary` (see 9.3.18) keyword of the variable ξ_i;

- `width`(ξ_i) is given by the `width` (see 9.3.18) keyword;

- `npoints`(ξ_i) is calculated from the two above numbers and the `upperBoundary` (see 9.3.18) keyword;
- `periodic`(ξ_i) is set to 1 if and only if ξ_i is periodic and the grids' boundaries cover its period.

Exception: there is a slightly different header in PMF files computed by ABF (9.5.2) or by other biases with an optional thermodynamic integration (TI) estimator (9.5.1). In this case, free-energy gradients are accumulated on an `(npoints)`-long grid along each variable ξ: after these gradients are integrated, the resulting PMF is discretized on a grid with `(npoints+1)` points along ξ. Therefore, the edges of the PMF's grid extend `width`/2 above and below the original boundaries (unless these are periodic). The format of the file's header is otherwise unchanged.

After the header, the rest of the file contains values of the tabulated function $f(\xi_1, \xi_2, \ldots \xi_{N_{\mathrm{cv}}})$, one for each line. The first N_{cv} columns contain values of ξ_1, ξ_2, $\ldots \xi_{N_{\mathrm{cv}}}$ and the last column contains the value of the function f. Points are sorted in ascending order with the fastest-changing values at the right ("C-style" order). Each sweep of the right-most variable $\xi_{N_{\mathrm{cv}}}$ is terminated by an empty line. For two dimensional grid files, this allows quick visualization by programs such as GNUplot.

Example 1: multicolumn text file for a one-dimensional histogram with `lowerBoundary` = 15, `upperBoundary` = 48 and `width` = 0.1.

```
#  1
#  15      0.1           330   0

   15.05   6.14012e-07
   15.15   7.47644e-07
   ...     ...
   47.85   1.65944e-06
   47.95   1.46712e-06
```

Example 2: multicolumn text file for a two-dimensional histogram of two dihedral angles (periodic interval with 6° bins):

```
#  2
#  -180.0  6.0     30           1
#  -180.0  6.0     30           1

   -177.0  -177.0  8.97117e-06
   -177.0  -171.0  1.53525e-06
   ...     ...     ...
   -177.0   177.0  2.442956-06

   -171.0  -177.0  2.04702e-05
   ...     ...     ...
```

9.3.19 Trajectory output

- `outputValue` < Output a trajectory for this colvar >

Context: `colvar`
Acceptable Values: boolean
Default Value: `on`
Description: If `colvarsTrajFrequency` is non-zero, the value of this colvar is written to the trajectory file every `colvarsTrajFrequency` steps in the column labeled "<`name`>".

- `outputVelocity` < Output a velocity trajectory for this colvar >
  Context: `colvar`
  Acceptable Values: boolean
  Default Value: `off`
  Description: If `colvarsTrajFrequency` is defined, the finite-difference calculated velocity of this colvar are written to the trajectory file under the label "`v_`<`name`>".

- `outputEnergy` < Output an energy trajectory for this colvar >
  Context: `colvar`
  Acceptable Values: boolean
  Default Value: `off`
  Description: This option applies only to extended Lagrangian colvars. If `colvarsTrajFrequency` is defined, the kinetic energy of the extended degree and freedom and the potential energy of the restraining spring are are written to the trajectory file under the labels "`Ek_`<`name`>" and "`Ep_`<`name`>".

- `outputTotalForce` < Output a total force trajectory for this colvar >
  Context: `colvar`
  Acceptable Values: boolean
  Default Value: `off`
  Description: If `colvarsTrajFrequency` is defined, the total force on this colvar (i.e. the projection of all atomic total forces onto this colvar — see equation (61) in section 9.5.2) are written to the trajectory file under the label "`fs_`<`name`>". For extended Lagrangian colvars, the "total force" felt by the extended degree of freedom is simply the force from the harmonic spring. Due to design constraints, the total force reported by NAMD to Colvars was computed at the previous simulation step. **Note:** not all components support this option. The physical unit for this force is kcal/mol, divided by the colvar unit U.

- `outputAppliedForce` < Output an applied force trajectory for this colvar >
  Context: `colvar`
  Acceptable Values: boolean
  Default Value: `off`
  Description: If `colvarsTrajFrequency` is defined, the total force applied on this colvar by Colvars biases are written to the trajectory under the label "`fa_`<`name`>". For extended Lagrangian colvars, this force is actually applied to the extended degree of freedom rather than the geometric colvar itself. The physical unit for this force is kcal/mol divided by the colvar unit.

9.3.20 Extended Lagrangian

The following options enable extended-system dynamics, where a colvar is coupled to an additional degree of freedom (fictitious particle) by a harmonic spring. This extended coordinate masks the

colvar and replaces it transparently from the perspective of biasing and analysis methods. Biasing forces are then applied to the extended degree of freedom, and the actual geometric colvar (function of Cartesian coordinates) only feels the force from the harmonic spring. This is particularly useful when combined with an `abf` (see 9.5.2) bias to perform eABF simulations (9.5.3).

Note that for some biases (`harmonicWalls` (see 9.5.7), `histogram` (see 9.5.10)), this masking behavior is controlled by the keyword `bypassExtendedLagrangian` (see 9.5). Specifically for `harmonicWalls`, the default behavior is to bypass extended Lagrangian coordinates and act directly on the actual colvars.

- `extendedLagrangian` < Add extended degree of freedom >
  Context: `colvar`
  Acceptable Values: boolean
  Default Value: `off`
  Description: Adds a fictitious particle to be coupled to the colvar by a harmonic spring. The fictitious mass and the force constant of the coupling potential are derived from the parameters `extendedTimeConstant` and `extendedFluctuation`, described below. Biasing forces on the colvar are applied to this fictitious particle, rather than to the atoms directly. This implements the extended Lagrangian formalism used in some metadynamics simulations [49]. The energy associated with the extended degree of freedom is reported along with bias energies under the MISC title in NAMD's energy output.

- `extendedFluctuation` < Standard deviation between the colvar and the fictitious particle (colvar unit) >
  Context: `colvar`
  Acceptable Values: positive decimal
  Description: Defines the spring stiffness for the `extendedLagrangian` mode, by setting the typical deviation between the colvar and the extended degree of freedom due to thermal fluctuation. The spring force constant is calculated internally as k_BT/σ^2, where σ is the value of `extendedFluctuation`.

- `extendedTimeConstant` < Oscillation period of the fictitious particle (fs) >
  Context: `colvar`
  Acceptable Values: positive decimal
  Default Value: `200`
  Description: Defines the inertial mass of the fictitious particle, by setting the oscillation period of the harmonic oscillator formed by the fictitious particle and the spring. The period should be much larger than the MD time step to ensure accurate integration of the extended particle's equation of motion. The fictitious mass is calculated internally as $k_BT(\tau/2\pi\sigma)^2$, where τ is the period and σ is the typical fluctuation (see above).

- `extendedTemp` < Temperature for the extended degree of freedom (K) >
  Context: `colvar`
  Acceptable Values: positive decimal
  Default Value: thermostat temperature
  Description: Temperature used for calculating the coupling force constant of the extended variable (see `extendedFluctuation`) and, if needed, as a target temperature for extended Langevin dynamics (see `extendedLangevinDamping`). This should normally be left at its default value.

- `extendedLangevinDamping` < Damping factor for extended Langevin dynamics (ps^{-1}) >
  Context: `colvar`
  Acceptable Values: positive decimal
  Default Value: `1.0`
  Description: If this is non-zero, the extended degree of freedom undergoes Langevin dynamics at temperature `extendedTemp`. The friction force is minus `extendedLangevinDamping` times the velocity. This is useful because the extended dynamics coordinate may heat up in the transient non-equilibrium regime of ABF. Use moderate damping values, to limit viscous friction (potentially slowing down diffusive sampling) and stochastic noise (increasing the variance of statistical measurements). In doubt, use the default value.

9.3.21 Multiple time-step variables

- `timeStepFactor` < Compute this colvar once in a certain number of timesteps >
  Context: `colvar`
  Acceptable Values: positive integer
  Default Value: `1`
  Description: Instructs this colvar to activate at a time interval equal to the base (MD) timestep times `timeStepFactor`.[32] At other time steps, the value of the variable is not updated, and no biasing forces are applied. Any forces exerted by biases are accumulated over the given time interval, then applied as an impulse at the next update.

9.3.22 Backward-compatibility

- `subtractAppliedForce` < Do not include biasing forces in the total force for this colvar >
  Context: `colvar`
  Acceptable Values: boolean
  Default Value: `off`
  Description: If the colvar supports total force calculation (see 9.3.14), all forces applied to this colvar by biases will be removed from the total force. This keyword allows to recover some of the "system force" calculation available in the Colvars module before version 2016-08-10. Please note that removal of *all* other external forces (including biasing forces applied to a different colvar) is *no longer supported*, due to changes in the underlying simulation engines (primarily NAMD). This option may be useful when continuing a previous simulation where the removal of external/applied forces is essential. *For all new simulations, the use of this option is not recommended.*

9.3.23 Statistical analysis

Run-time calculations of statistical properties that depend explicitly on time can be performed for individual collective variables. Currently, several types of time correlation functions, running averages and running standard deviations are implemented. For run-time computation of histograms, please see the histogram bias (9.5.10).

- `corrFunc` < Calculate a time correlation function? >
  Context: `colvar`
  Acceptable Values: boolean
  Default Value: `off`

Description: Whether or not a time correlaction function should be calculated for this colvar.

- `corrFuncWithColvar` < Colvar name for the correlation function >
  Context: `colvar`
  Acceptable Values: string
  Description: By default, the auto-correlation function (ACF) of this colvar, ξ_i, is calculated. When this option is specified, the correlation function is calculated instead with another colvar, ξ_j, which must be of the same type (scalar, vector, or quaternion) as ξ_i.

- `corrFuncType` < Type of the correlation function >
  Context: `colvar`
  Acceptable Values: `velocity`, `coordinate` or `coordinate_p2`
  Default Value: `velocity`
  Description: With `coordinate` or `velocity`, the correlation function $C_{i,j}(t) = \langle \Pi\left(\xi_i(t_0), \xi_j(t_0+t)\right)\rangle$ is calculated between the variables ξ_i and ξ_j, or their velocities. $\Pi(\xi_i, \xi_j)$ is the scalar product when calculated between scalar or vector values, whereas for quaternions it is the cosine between the two corresponding rotation axes. With `coordinate_p2`, the second order Legendre polynomial, $(3\cos(\theta)^2 - 1)/2$, is used instead of the cosine.

- `corrFuncNormalize` < Normalize the time correlation function? >
  Context: `colvar`
  Acceptable Values: boolean
  Default Value: `on`
  Description: If enabled, the value of the correlation function at $t = 0$ is normalized to 1; otherwise, it equals to $\langle O\left(\xi_i, \xi_j\right)\rangle$.

- `corrFuncLength` < Length of the time correlation function >
  Context: `colvar`
  Acceptable Values: positive integer
  Default Value: `1000`
  Description: Length (in number of points) of the time correlation function.

- `corrFuncStride` < Stride of the time correlation function >
  Context: `colvar`
  Acceptable Values: positive integer
  Default Value: `1`
  Description: Number of steps between two values of the time correlation function.

- `corrFuncOffset` < Offset of the time correlation function >
  Context: `colvar`
  Acceptable Values: positive integer
  Default Value: `0`
  Description: The starting time (in number of steps) of the time correlation function (default: $t = 0$). **Note:** *the value at $t = 0$ is always used for the normalization.*

- `corrFuncOutputFile` < Output file for the time correlation function >
  Context: `colvar`
  Acceptable Values: UNIX filename

Default Value: *outputName*.<name>.corrfunc.dat
Description: The time correlation function is saved in this file.

- runAve < Calculate the running average and standard deviation >
  Context: colvar
  Acceptable Values: boolean
  Default Value: off
  Description: Whether or not the running average and standard deviation should be calculated for this colvar.

- runAveLength < Length of the running average window >
  Context: colvar
  Acceptable Values: positive integer
  Default Value: 1000
  Description: Length (in number of points) of the running average window.

- runAveStride < Stride of the running average window values >
  Context: colvar
  Acceptable Values: positive integer
  Default Value: 1
  Description: Number of steps between two values within the running average window.

- runAveOutputFile < Output file for the running average and standard deviation >
  Context: colvar
  Acceptable Values: UNIX filename
  Default Value: *outputName*.<name>.runave.traj
  Description: The running average and standard deviation are saved in this file.

9.4 Selecting atoms

To define collective variables, atoms are usually selected as groups. Each group is defined using an identifier that is unique in the context of the specific colvar component (e.g. for a distance component, the two groups are group1 and group2). The identifier is followed by a brace-delimited block containing selection keywords and other parameters, including an optional name:

- name < Unique name for the atom group >
  Context: atom group
  Acceptable Values: string
  Description: This parameter defines a unique name for this atom group, which can be referred to in the definition of other atom groups (including in other colvars) by invoking atomsOfGroup as a selection keyword.

9.4.1 Atom selection keywords

Selection keywords may be used individually or in combination with each other, and each can be repeated any number of times. Selection is incremental: each keyword adds the corresponding atoms to the selection, so that different sets of atoms can be combined. However, atoms included by multiple keywords are only counted once. Below is an example configuration for an atom group called "atoms". **Note:** *this is an unusually varied combination of selection*

keywords, demonstrating how they can be combined together: most simulations only use one of them.

```
atoms {

  # add atoms 1 and 3 to this group (note:  the first atom in the system is 1)
  atomNumbers {
    1 3
  }

  # add atoms starting from 20 up to and including 50
  atomNumbersRange 20-50

  # add all the atoms with occupancy 2 in the file atoms.pdb
  atomsFile atoms.pdb
  atomsCol O
  atomsColValue 2.0

  # add all the C-alphas within residues 11 to 20 of segments "PR1" and "PR2"
  psfSegID PR1 PR2
  atomNameResidueRange CA 11-20
  atomNameResidueRange CA 11-20

  # add index group (requires a .ndx file to be provided globally)
  indexGroup Water
}
```

The resulting selection includes atoms 1 and 3, those between 20 and 50, the C_α atoms between residues 11 and 20 of the two segments `PR1` and `PR2`, and those in the index group called "Water". The indices of this group are read from the file provided by the global keyword `indexFile` (see 9.2.5).

The complete list of selection keywords available in NAMD is:

- `atomNumbers` < List of atom numbers >
  Context: atom group
  Acceptable Values: space-separated list of positive integers
  Description: This option adds to the group all the atoms whose numbers are in the list. *The number of the first atom in the system is 1: to convert from a VMD selection, use "atomselect get serial".*

- `indexGroup` < Name of index group to be used (GROMACS format) >
  Context: atom group
  Acceptable Values: string
  Description: If the name of an index file has been provided by `indexFile`, this option allows to select one index group from that file: the atoms from that index group will be used to define the current group.

- `atomsOfGroup` < Name of group defined previously >
  Context: atom group

Acceptable Values: string
Description: Refers to a group defined previously using its user-defined `name`. This adds all atoms of that named group to the current group.

- `atomNumbersRange` < Atoms within a number range >
  Context: atom group
  Acceptable Values: <Starting number>-<Ending number>
  Description: This option includes in the group all atoms whose numbers are within the range specified. *The number of the first atom in the system is 1.*

- `atomNameResidueRange` < Named atoms within a range of residue numbers >
  Context: atom group
  Acceptable Values: <Atom name> <Starting residue>-<Ending residue>
  Description: This option adds to the group all the atoms with the provided name, within residues in the given range.

- `psfSegID` < PSF segment identifier >
  Context: atom group
  Acceptable Values: space-separated list of strings (max 4 characters)
  Description: This option sets the PSF segment identifier for `atomNameResidueRange`. Multiple values may be provided, which correspond to multiple instances of `atomNameResidueRange`, in order of their occurrence. This option is only necessary if a PSF topology file is used.

- `atomsFile` < PDB file name for atom selection >
  Context: atom group
  Acceptable Values: UNIX filename
  Description: This option selects atoms from the PDB file provided and adds them to the group according to numerical flags in the column `atomsCol`. **Note:** *the sequence of atoms in the PDB file provided must match that in the system's topology.*

- `atomsCol` < PDB column to use for atom selection flags >
  Context: atom group
  Acceptable Values: `O`, `B`, `X`, `Y`, or `Z`
  Description: This option specifies which PDB column in `atomsFile` is used to determine which atoms are to be included in the group.

- `atomsColValue` < Atom selection flag in the PDB column >
  Context: atom group
  Acceptable Values: positive decimal
  Description: If defined, this value in `atomsCol` identifies atoms in `atomsFile` that are included in the group. If undefined, all atoms with a non-zero value in `atomsCol` are included.

- `dummyAtom` < Dummy atom position (Å) >
  Context: atom group
  Acceptable Values: `(x, y, z)` triplet
  Description: Instead of selecting any atom, this option makes the group a virtual particle at a fixed position in space. This is useful e.g. to replace a group's center of geometry with a user-defined position.

9.4.2 Moving frame of reference.

The following options define an automatic calculation of an optimal translation (`centerReference`) or optimal rotation (`rotateReference`), that superimposes the positions of this group to a provided set of reference coordinates. This can allow, for example, to effectively remove from certain colvars the effects of molecular tumbling and of diffusion. Given the set of atomic positions $\mathbf{x}_i$, the colvar ξ can be defined on a set of roto-translated positions $\mathbf{x}'_i = R(\mathbf{x}_i - \mathbf{x}^{\mathrm{C}}) + \mathbf{x}^{\mathrm{ref}}$. $\mathbf{x}^{\mathrm{C}}$ is the geometric center of the $\mathbf{x}_i$, R is the optimal rotation matrix to the reference positions and $\mathbf{x}^{\mathrm{ref}}$ is the geometric center of the reference positions.

Components that are defined based on pairwise distances are naturally invariant under global roto-translations. Other components are instead affected by global rotations or translations: however, they can be made invariant if they are expressed in the frame of reference of a chosen group of atoms, using the `centerReference` and `rotateReference` options. Finally, a few components are defined by convention using a roto-translated frame (e.g. the minimal RMSD): for these components, `centerReference` and `rotateReference` are enabled by default. In typical applications, the default settings result in the expected behavior.

Warning on rotating frames of reference and periodic boundary conditions. `rotateReference` affects coordinates that depend on minimum-image distances in periodic boundary conditions (PBC). After rotation of the coordinates, the periodic cell vectors become irrelevant: the rotated system is effectively non-periodic. A safe way to handle this is to ensure that the relevant inter-group distance vectors remain smaller than the half-size of the periodic cell. If this is not desirable, one should avoid the rotating frame of reference, and apply orientational restraints to the reference group instead, in order to keep the orientation of the reference group consistent with the orientation of the periodic cell.

Warning on rotating frames of reference and ABF. Note that `centerReference` and `rotateReference` may affect the Jacobian derivative of colvar components in a way that is not taken into account by default. Be careful when using these options in ABF simulations or when using total force values.

- `centerReference` < Implicitly remove translations for this group >
  Context: atom group
  Acceptable Values: boolean
  Default Value: `off`
  Description: If this option is `on`, the center of geometry of the group will be aligned with that of the reference positions provided by either `refPositions` or `refPositionsFile`. Colvar components will only have access to the aligned positions. **Note**: unless otherwise specified, `rmsd` and `eigenvector` set this option to `on` *by default.*

- `rotateReference` < Implicitly remove rotations for this group >
  Context: atom group
  Acceptable Values: boolean
  Default Value: `off`
  Description: If this option is `on`, the coordinates of this group will be optimally superimposed to the reference positions provided by either `refPositions` or `refPositionsFile`. The rotation will be performed around the center of geometry if `centerReference` is `on`, or

around the origin otherwise. The algorithm used is the same employed by the `orientation` colvar component [26]. Forces applied to the atoms of this group will also be implicitly rotated back to the original frame. **Note**: unless otherwise specified, `rmsd` and `eigenvector` set this option to `on` *by default*.

- `refPositions` < Reference positions for fitting (Å) >
  Context: atom group
  Acceptable Values: space-separated list of `(x, y, z)` triplets
  Description: This option provides a list of reference coordinates for `centerReference` and/or `rotateReference`, and is mutually exclusive with `refPositionsFile`. If only `centerReference` is `on`, the list may contain a single (x, y, z) triplet; if also `rotateReference` is `on`, the list should be as long as the atom group, and *its order must match the order in which atoms were defined*.

- `refPositionsFile` < File containing the reference positions for fitting >
  Context: atom group
  Acceptable Values: UNIX filename
  Description: This option provides a list of reference coordinates for `centerReference` and/or `rotateReference`, and is mutually exclusive with `refPositions`. The acceptable file format is XYZ, which is read in double precision, or PDB; *the latter is discouraged if the precision of the reference coordinates is a concern*. Atomic positions are read differently depending on the following scenarios: **(i)** the file contains exactly as many records as the atoms in the group: all positions are read in sequence; **(ii)** (most common case) the file contains coordinates for the entire system: only the positions corresponding to the numeric indices of the atom group are read; **(iii)** if the file is a PDB file and `refPositionsCol` is specified, positions are read according to the value of the column `refPositionsCol` (which may be the same as `atomsCol`). In each case, atoms are read from the file *in order of increasing number*.

- `refPositionsCol` < PDB column containing atom flags >
  Context: atom group
  Acceptable Values: `O`, `B`, `X`, `Y`, or `Z`
  Description: Like `atomsCol` for `atomsFile`, indicates which column to use to identify the atoms in `refPositionsFile` (if this is a PDB file).

- `refPositionsColValue` < Atom selection flag in the PDB column >
  Context: atom group
  Acceptable Values: positive decimal
  Description: Analogous to `atomsColValue`, but applied to `refPositionsCol`.

- `fittingGroup` < Use an alternate set of atoms to define the roto-translation >
  Context: atom group
  Acceptable Values: Block `fittingGroup { ... }`
  Default Value: This atom group itself
  Description: If either `centerReference` or `rotateReference` is defined, this keyword defines an alternate atom group to calculate the optimal roto-translation. Use this option to define a continuous rotation if the structure of the group involved changes significantly (a typical symptom would be the message "Warning: discontinuous rotation!").

The following example illustrates the use of `fittingGroup` as part of a Distance to Bound Configuration (DBC) coordinate for use in ligand restraints for binding affinity calculations.[94] The group called "`atoms`" describes coordinates of a ligand's atoms, expressed in a moving frame of reference tied to a binding site (here within a protein). An optimal roto-translation is calculated automatically by fitting the C_α trace of the rest of the protein onto the coordinates provided by a PDB file. To define a DBC coordinate, this atom group would be used within an `rmsd` (see 9.3.5) function.

```
# Example:  defining a group "atoms" (the ligand) whose coordinates are
expressed
# in a roto-translated frame of reference defined by a second group (the
receptor)
atoms {

  atomNumbers 1 2 3 4 5 6 7 # atoms of the ligand (1-based)

  centerReference yes
  rotateReference yes
  fittingGroup {
    # define the frame by fitting alpha carbon atoms
    # in 2 protein segments close to the site
    psfSegID PROT PROT
    atomNameResidueRange CA 1-40
    atomNameResidueRange CA 59-100
  }
  refPositionsFile all.pdb # can be the entire system
}
```

The following two options have default values appropriate for the vast majority of applications, and are only provided to support rare, special cases.

- `enableFitGradients` < Include the roto-translational contribution to colvar gradients >
  Context: atom group
  Acceptable Values: boolean
  Default Value: `on`
  Description: When either `centerReference` or `rotateReference` is on, the gradients of some colvars include terms proportional to $\partial R/\partial \mathbf{x}_i$ (rotational gradients) and $\partial \mathbf{x}^{\mathrm{C}}/\partial \mathbf{x}_i$ (translational gradients). By default, these terms are calculated and included in the total gradients; if this option is set to `off`, they are neglected. In the case of a minimum RMSD component, this flag is automatically disabled because the contributions of those derivatives to the gradients cancel out.

- `enableForces` < Apply forces from this colvar to this group >
  Context: atom group
  Acceptable Values: boolean
  Default Value: `on`
  Description: If this option is `off`, no forces are applied the atoms in the group. Other

forces are not affected (i.e. those from the MD engine, from other colvars, and other external forces). For dummy atoms, this option is `off` by default.

9.4.3 Treatment of periodic boundary conditions.

In simulations with periodic boundary conditions, NAMD maintains the coordinates of all the atoms within a molecule contiguous to each other (i.e. there are no spurious "jumps" in the molecular bonds). The Colvars module relies on this when calculating a group's center of geometry, but this condition may fail if the group spans different molecules. In that case, writing the NAMD output and restart files using `wrapAll` or `wrapWater` could produce wrong results when a simulation run is continued from a previous one. The user should then determine, according to which type of colvars are being calculated, whether `wrapAll` or `wrapWater` can be enabled.

In general, internal coordinate wrapping by NAMD does not affect the calculation of colvars if each atom group satisfies one or more of the following:

i) it is composed by only one atom;

ii) it is used by a colvar component which does not make use of its center of geometry, but only of pairwise distances (`distanceInv`, `coordNum`, `hBond`, `alpha`, `dihedralPC`);

iii) it is used by a colvar component that ignores the ill-defined Cartesian components of its center of mass (such as the x and y components of a membrane's center of mass modeled with `distanceZ`);

iv) it has all of its atoms within the same molecular fragment.

9.4.4 Performance of a Colvars calculation based on group size.

In simulations performed with message-passing programs (such as NAMD or LAMMPS), the calculation of energy and forces is distributed (i.e., parallelized) across multiple nodes, as well as over the processor cores of each node. When Colvars is enabled, certain atomic coordinates are collected on a single node, where the calculation of collective variables and of their biases is executed. This means that for simulations over large numbers of nodes, a Colvars calculation may produce a significant overhead, coming from the costs of transmitting atomic coordinates to one node and of processing them. The latency-tolerant design and dynamic load balancing of NAMD may alleviate both factors, but a noticeable performance impact may be observed.

Performance can be improved in multiple ways:

- The calculation of variables, components and biases can be distributed over the processor cores of the node where the Colvars module is executed. Currently, an equal weight is assigned to each colvar, or to each component of those colvars that include more than one component. The performance of simulations that use many colvars or components is improved automatically. For simulations that use a single large colvar, it may be advisable to partition it in multiple components, which will be then distributed across the available cores. In NAMD, this feature is enabled in all binaries compiled using SMP builds of Charm++ with the CkLoop extension. If printed, the message "SMP parallelism is available." indicates the availability of the option. If available, the option is turned on by default, but may be disabled using the keyword `smp` (see 9.2.5) if required for debugging.

- NAMD also offers a parallelized calculation of the centers of mass of groups of atoms. This option is on by default for all components that are simple functions of centers of mass, and is controlled by the keyword `scalable` (see 9.3.12). When supported, the message "Will enable scalable calculation for group ..." is printed for each group.

- As a general rule, the size of atom groups should be kept relatively small (up to a few thousands of atoms, depending on the size of the entire system in comparison). To gain an estimate of the computational cost of a large colvar, one can use a test calculation of the same colvar in VMD (hint: use the `time` Tcl command to measure the cost of running `cv update`).

9.5 Biasing and analysis methods

A biasing or analysis method can be applied to existing collective variables by using the following configuration:

```
<biastype> {
  name <name>
  colvars <xi1> <xi2> ...
  <parameters>
}
```

The keyword <biastype> indicates the method of choice. There can be multiple instances of the same method, e.g. using multiple `harmonic` blocks allows defining multiple restraints.

All biasing and analysis methods implemented recognize the following options:

- `name` < Identifier for the bias >
  Context: colvar bias
  Acceptable Values: string
  Default Value: <type of bias><bias index>
  Description: This string is used to identify the bias or analysis method in the output, and to name some output files. **Tip:** because the default name depends on the order of definition, but the outcome of the simulation does not, it may be convenient to assign consistent names for certain biases; for example, you may want to name a moving harmonic restraint `smd`, so that it can always be identified regardless of the presence of other restraints.

- `colvars` < Collective variables involved >
  Context: colvar bias
  Acceptable Values: space-separated list of colvar names
  Description: This option selects by name all the variables to which this bias or analysis will be applied.

- `outputEnergy` < Write the current bias energy to the trajectory file >
  Context: colvar bias
  Acceptable Values: boolean
  Default Value: `off`
  Description: If this option is chosen and `colvarsTrajFrequency` is not zero, the current value of the biasing energy will be written to the trajectory file during the simulation. The total energy of all Colvars biases is also reported by NAMD, as part of the MISC title.

- `outputFreq` < Frequency (number of steps) at which output files are written >
  Context: colvar bias
  Acceptable Values: positive integer
  Default Value: `colvarsRestartFrequency` (see 9.2.5)
  Description: If this bias produces aggregated data that needs to be written to disk (for example, a PMF), this number specifies the number of steps after which these data are written to files. A value of zero disables writing files for this bias during the simulation (except for `outputEnergy` (see 9.5), which is controlled by `colvarsTrajFrequency` (see 9.2.5)). All output files are also written at the end of a simulation run, regardless of the value of this number.

- `bypassExtendedLagrangian` < Apply bias to actual colvars, bypassing extended coordinates >
  Context: colvar bias
  Acceptable Values: boolean
  Default Value: `off`
  Description: This option is implemented by the `harmonicWalls` (see 9.5.7) and `histogram` (see 9.5.10) biases. It is only relevant if the bias is applied to one or several extended-Lagrangian colvars (9.3.20), for example within an eABF (9.5.3) simulation. Usually, biases use the value of the extended coordinate as a proxy for the actual colvar, and their biasing forces are applied to the extended coordinates as well. If `bypassExtendedLagrangian` is enabled, the bias behaves as if there were no extended coordinates, and accesses the value of the underlying colvars, applying any biasing forces along the gradients of those variables.

- `stepZeroData` < Accumulate data starting at step 0 of a simulation run >
  Context: colvar bias
  Acceptable Values: boolean
  Default Value: `off`
  Description: This option is meaningful for biases that record and accumulate data during a simulation, such as ABF (9.5.2), metadynamics (9.5.4), histograms (9.5.10) and in general any bias that accumulates free-energy samples with thermodynamic integration, or TI (9.5.1). When this option is disabled (default), data will only be recorded into the bias after the first coordinate update: this is generally the correct choice in simulation runs. Biasing energy and forces will always be computed for all active biases, regardless of this option. Note that in some cases the bias may require data from previous simulation steps: for example, TI requires total atomic forces (see `outputTotalForce` (see 9.3.19)) which are only available at the following step in NAMD; turning on this flag in those cases will raise an error.

9.5.1 Thermodynamic integration

The methods implemented here provide a variety of estimators of conformational free-energies. These are carried out at run-time, or with the use of post-processing tools over the generated output files. The specifics of each estimator are discussed in the documentation of each biasing or analysis method.

A special case is the traditional thermodynamic integration (TI) method, used for example to compute potentials of mean force (PMFs). Most types of restraints (9.5.5, 9.5.7, 9.5.8, ...) as well as metadynamics (9.5.4) can optionally use TI alongside their own estimator, based on the keywords documented below.

- `writeTIPMF` < Write the PMF computed by thermodynamic integration >
  Context: colvar bias
  Acceptable Values: boolean
  Default Value: `off`
  Description: If the bias is applied to a variable that supports the calculation of total forces (see `outputtotalForce` (see 9.3) and 9.3.14), this option allows calculating the corresponding PMF by thermodynamic integration, and writing it to the file *outputName*.<`name`>`.ti.pmf`, where <`name`> is the name of the bias and the contents of the file are in multicolumn text format (9.3.18). The total force includes the forces applied to the variable by all bias, except those from this bias itself. If any bias applies time-dependent forces besides the one using this option, an error is raised.

- `writeTISamples` < Write the free-energy gradient samples >
  Context: colvar bias
  Acceptable Values: boolean
  Default Value: `off`
  Description: This option allows to compute total forces for use with thermodynamic integration as done by the keyword `writeTIPMF` (see 9.5). The names of the files containing the variables' histogram and mean thermodynamic forces are *outputName*.<`name`>`.ti.count` and *outputName*.<`name`>`.ti.force`, respectively: these can be used by `abf_integrate` (see 9.5.2) or similar utility. Note that because the `.force` file contains mean forces instead of free-energy gradients, `abf_integrate` <`filename`> `-s -1.0` should be used. This option is on by default when `writeTIPMF` is on, but can be enabled separately if the bias is applied to more than one variable, making not possible the direct integration of the PMF at runtime. If any bias applies time-dependent forces besides the one using this option, an error is raised.

In adaptive biasing force (ABF) (9.5.2) the above keywords are not recognized, because their functionality is either included already (conventional ABF) or not available (extended-system ABF).

9.5.2 Adaptive Biasing Force

For a full description of the Adaptive Biasing Force method, see reference [28]. For details about this implementation, see references [46] and [47]. **When publishing research that makes use of this functionality, please cite references [28] and [47].**

An alternate usage of this feature is the application of custom tabulated biasing potentials to one or more colvars. See `inputPrefix` and `updateBias` below.

Combining ABF with the extended Lagrangian feature (9.3.20) of the variables produces the extended-system ABF variant of the method (9.5.3).

ABF is based on the thermodynamic integration (TI) scheme for computing free energy profiles. The free energy as a function of a set of collective variables $\boldsymbol{\xi} = (\xi_i)_{i\in[1,n]}$ is defined from the canonical distribution of $\boldsymbol{\xi}$, $\mathcal{P}(\boldsymbol{\xi})$:

$$A(\boldsymbol{\xi}) = -\frac{1}{\beta}\ln\mathcal{P}(\boldsymbol{\xi}) + A_0 \tag{57}$$

In the TI formalism, the free energy is obtained from its gradient, which is generally calculated in the form of the average of a force $\boldsymbol{F}_\xi$ exerted on $\boldsymbol{\xi}$, taken over an iso-$\boldsymbol{\xi}$ surface:

$$\boldsymbol{\nabla}_\xi A(\boldsymbol{\xi}) = \langle -\boldsymbol{F}_\xi \rangle_{\boldsymbol{\xi}} \tag{58}$$

Several formulae that take the form of (58) have been proposed. This implementation relies partly on the classic formulation [18], and partly on a more versatile scheme originating in a work by Ruiz-Montero et al. [93], generalized by den Otter [29] and extended to multiple variables by Ciccotti et al. [23]. Consider a system subject to constraints of the form $\sigma_k(\boldsymbol{x}) = 0$. Let $(\boldsymbol{v}_i)_{i\in[1,n]}$ be arbitrarily chosen vector fields ($\mathbb{R}^{3N} \to \mathbb{R}^{3N}$) verifying, for all i, j, and k:

$$\boldsymbol{v}_i \cdot \boldsymbol{\nabla}_{\boldsymbol{x}} \xi_j = \delta_{ij} \tag{59}$$

$$\boldsymbol{v}_i \cdot \boldsymbol{\nabla}_{\boldsymbol{x}} \sigma_k = 0 \tag{60}$$

then the following holds [23]:

$$\frac{\partial A}{\partial \xi_i} = \langle \boldsymbol{v}_i \cdot \boldsymbol{\nabla}_{\boldsymbol{x}} V - k_B T \boldsymbol{\nabla}_{\boldsymbol{x}} \cdot \boldsymbol{v}_i \rangle_{\boldsymbol{\xi}} \tag{61}$$

where V is the potential energy function. $\boldsymbol{v}_i$ can be interpreted as the direction along which the force acting on variable ξ_i is measured, whereas the second term in the average corresponds to the geometric entropy contribution that appears as a Jacobian correction in the classic formalism [18]. Condition (59) states that the direction along which the total force on ξ_i is measured is orthogonal to the gradient of ξ_j, which means that the force measured on ξ_i does not act on ξ_j.

Equation (60) implies that constraint forces are orthogonal to the directions along which the free energy gradient is measured, so that the measurement is effectively performed on unconstrained degrees of freedom. In NAMD, constraints are typically applied to the lengths of bonds involving hydrogen atoms, for example in TIP3P water molecules (parameter `rigidBonds`, section 5.6.1).

In the framework of ABF, $\mathbf{F}_\xi$ is accumulated in bins of finite size $\delta\xi$, thereby providing an estimate of the free energy gradient according to equation (58). The biasing force applied along the collective variables to overcome free energy barriers is calculated as:

$$\mathbf{F}^{\mathrm{ABF}} = \alpha(N_\xi) \times \boldsymbol{\nabla}_{\boldsymbol{x}} \widetilde{A}(\boldsymbol{\xi}) \tag{62}$$

where $\boldsymbol{\nabla}_{\boldsymbol{x}} \widetilde{A}$ denotes the current estimate of the free energy gradient at the current point $\boldsymbol{\xi}$ in the collective variable subspace, and $\alpha(N_\xi)$ is a scaling factor that is ramped from 0 to 1 as the local number of samples N_ξ increases to prevent nonequilibrium effects in the early phase of the simulation, when the gradient estimate has a large variance. See the `fullSamples` parameter below for details.

As sampling of the phase space proceeds, the estimate $\boldsymbol{\nabla}_{\boldsymbol{x}} \widetilde{A}$ is progressively refined. The biasing force introduced in the equations of motion guarantees that in the bin centered around $\boldsymbol{\xi}$, the forces acting along the selected collective variables average to zero over time. Eventually, as the undelying free energy surface is canceled by the adaptive bias, evolution of the system along $\boldsymbol{\xi}$ is governed mainly by diffusion. Although this implementation of ABF can in principle be used in arbitrary dimension, a higher-dimension collective variable space is likely to be difficult to sample and visualize. Most commonly, the number of variables is one or two, sometimes three.

ABF requirements on collective variables The following conditions must be met for an ABF simulation to be possible and to produce an accurate estimate of the free energy profile. Note that these requirements do not apply when using the extended-system ABF method (9.5.3).

1. *Only linear combinations* of colvar components can be used in ABF calculations.

2. *Availability of total forces* is necessary. The following colvar components can be used in ABF calculations: `distance`, `distance_xy`, `distance_z`, `angle`, `dihedral`, `gyration`, `rmsd` and `eigenvector`. Atom groups may not be replaced by dummy atoms, unless they are excluded from the force measurement by specifying `oneSiteTotalForce`, if available.

3. *Mutual orthogonality of colvars.* In a multidimensional ABF calculation, equation (59) must be satisfied for any two colvars ξ_i and ξ_j. Various cases fulfill this orthogonality condition:

   - ξ_i and ξ_j are based on non-overlapping sets of atoms.
   - atoms involved in the force measurement on ξ_i do not participate in the definition of ξ_j. This can be obtained using the option `oneSiteTotalForce` of the `distance`, `angle`, and `dihedral` components (example: Ramachandran angles ϕ, ψ).
   - ξ_i and ξ_j are orthogonal by construction. Useful cases are the sum and difference of two components, or `distance_z` and `distance_xy` using the same axis.

4. *Mutual orthogonality of components*: when several components are combined into a colvar, it is assumed that their vectors $\boldsymbol{v}_i$ (equation (61)) are mutually orthogonal. The cases described for colvars in the previous paragraph apply.

5. *Orthogonality of colvars and constraints*: equation 60 can be satisfied in two simple ways, if either no constrained atoms are involved in the force measurement (see point 3 above) or pairs of atoms joined by a constrained bond are part of an *atom group* which only intervenes through its center (center of mass or geometric center) in the force measurement. In the latter case, the contributions of the two atoms to the left-hand side of equation 60 cancel out. For example, all atoms of a rigid TIP3P water molecule can safely be included in an atom group used in a `distance` component.

Parameters for ABF ABF depends on parameters from collective variables to define the grid on which free energy gradients are computed. In the direction of each colvar, the grid ranges from `lowerBoundary` to `upperBoundary`, and the bin width (grid spacing) is set by the `width` (see 9.3.18) parameter. The following specific parameters can be set in the ABF configuration block:

- `name`: see definition of `name` in sec. 9.5 (biasing and analysis methods)
- `colvars`: see definition of `colvars` in sec. 9.5 (biasing and analysis methods)
- `outputEnergy`: see definition of `outputEnergy` in sec. 9.5 (biasing and analysis methods)
- `outputFreq`: see definition of `outputFreq` in sec. 9.5 (biasing and analysis methods)
- `stepZeroData`: see definition of `stepZeroData` in sec. 9.5 (biasing and analysis methods)
- `fullSamples` < Number of samples in a bin prior to application of the ABF >
  Context: `abf`
  Acceptable Values: positive integer
  Default Value: 200
  Description: To avoid nonequilibrium effects due to large fluctuations of the force exerted along the colvars, it is recommended to apply a biasing force only after a the estimate has started converging. If `fullSamples` is non-zero, the applied biasing force is scaled by a factor

$\alpha(N_\xi)$ between 0 and 1. If the number of samples N_ξ in the current bin is higher than `fullSamples`, the factor is one. If it is less than half of `fullSamples`, the factor is zero and no bias is applied. Between those two thresholds, the factor follows a linear ramp from 0 to 1: $\alpha(N_\xi) = (2N_\xi/\text{fullSamples}) - 1$.

- `maxForce` < Maximum magnitude of the ABF force >
  Context: `abf`
  Acceptable Values: positive decimals (one per colvar)
  Default Value: disabled
  Description: This option enforces a cap on the magnitude of the biasing force effectively applied by this ABF bias on each colvar. This can be useful in the presence of singularities in the PMF such as hard walls, where the discretization of the average force becomes very inaccurate, causing the colvar's diffusion to get "stuck" at the singularity. To enable this cap, provide one non-negative value for each colvar. The unit of force is kcal/mol divided by the colvar unit.

- `hideJacobian` < Remove geometric entropy term from calculated free energy gradient? >
  Context: `abf`
  Acceptable Values: boolean
  Default Value: `no`
  Description: In a few special cases, most notably distance-based variables, an alternate definition of the potential of mean force is traditionally used, which excludes the Jacobian term describing the effect of geometric entropy on the distribution of the variable. This results, for example, in particle-particle potentials of mean force being flat at large separations. Setting this parameter to `yes` causes the output data to follow that convention, by removing this contribution from the output gradients while applying internally the corresponding correction to ensure uniform sampling. It is not allowed for colvars with multiple components.

- `historyFreq` < Frequency (in timesteps) at which ABF history files are accumulated >
  Context: `abf`
  Acceptable Values: positive integer
  Default Value: 0
  Description: If this number is non-zero, the free energy gradient estimate and sampling histogram (and the PMF in one-dimensional calculations) are written to files on disk at the given time interval. History file names use the same prefix as output files, with "`.hist`" appended (*outputName*`.hist.pmf`). `historyFreq` must be a multiple of `outputFreq` (see 9.5).

- `inputPrefix` < Filename prefix for reading ABF data >
  Context: `abf`
  Acceptable Values: list of strings
  Description: If this parameter is set, for each item in the list, ABF tries to read a gradient and a sampling files named <`inputPrefix`>`.grad` and <`inputPrefix`>`.count`. This is done at startup and sets the initial state of the ABF algorithm. The data from all provided files is combined appropriately. Also, the grid definition (min and max values, width) need not be the same that for the current run. This command is useful to piece together data from simulations in different regions of collective variable space, or change the colvar boundary values and widths. Note that it is not recommended to use it to switch to a smaller width, as

that will leave some bins empty in the finer data grid. This option is NOT compatible with reading the data from a restart file (`colvarsInput` option of the NAMD config file).

- `applyBias` < Apply the ABF bias? >
  Context: `abf`
  Acceptable Values: boolean
  Default Value: `yes`
  Description: If this is set to no, the calculation proceeds normally but the adaptive biasing force is not applied. Data is still collected to compute the free energy gradient. This is mostly intended for testing purposes, and should not be used in routine simulations.

- `updateBias` < Update the ABF bias? >
  Context: `abf`
  Acceptable Values: boolean
  Default Value: `yes`
  Description: If this is set to no, the initial biasing force (e.g. read from a restart file or through `inputPrefix`) is not updated during the simulation. As a result, a constant bias is applied. This can be used to apply a custom, tabulated biasing potential to any combination of colvars. To that effect, one should prepare a gradient file containing the gradient of the potential to be applied (negative of the bias force), and a count file containing only values greater than `fullSamples`. These files must match the grid parameters of the colvars.

Multiple-replica ABF

- `shared` < Apply multiple-replica ABF, sharing force samples among the replicas? >
  Context: `abf`
  Acceptable Values: boolean
  Default Value: `no`
  Description: This is command requires that NAMD be compiled and executed with multiple-replica support. If `shared` is set to yes, the total force samples will be synchronized among all replicas at intervals defined by `sharedFreq`. This implements the multiple-walker ABF scheme described in [77]; this implementation is documented in [25]. Thus, it is as if total force samples among all replicas are gathered in a single shared buffer, which why the algorithm is referred to as shared ABF. Shared ABF allows all replicas to benefit from the sampling done by other replicas and can lead to faster convergence of the biasing force.

- `sharedFreq` < Frequency (in timesteps) at which force samples are synchronized among the replicas >
  Context: `abf`
  Acceptable Values: positive integer
  Default Value: `outputFreq` (see 9.5)
  Description: In the current implementation of shared ABF, each replica maintains a separate buffer of total force samples that determine the biasing force. Every `sharedFreq` steps, the replicas communicate the samples that have been gathered since the last synchronization time, ensuring all replicas apply a similar biasing force.

Output files The ABF bias produces the following files, all in multicolumn text format (9.3.18):

- *outputName*`.grad`: current estimate of the free energy gradient (grid), in multicolumn;

- *outputName*`.count`: histogram of samples collected, on the same grid;
- *outputName*`.pmf`: integrated free energy profile or PMF (for dimensions 1, 2 or 3).

Also in the case of one-dimensional calculations, the ABF bias can report its current energy via `outputEnergy`; in higher dimensions, such computation is not implemented and the energy reported is zero.

If several ABF biases are defined concurrently, their name is inserted to produce unique filenames for output, as in *outputName*`.abf1.grad`. This should not be done routinely and could lead to meaningless results: only do it if you know what you are doing!

If the colvar space has been partitioned into sections (*windows*) in which independent ABF simulations have been run, the resulting data can be merged using the `inputPrefix` option described above (a run of 0 steps is enough).

Multidimensional free energy surfaces If a one-dimensional calculation is performed, the estimated free energy gradient is integrated using a simple rectangle rule. In dimension 2 or 3, it is calculated as the solution of a Poisson equation:

$$\Delta A(\xi) = -\nabla \cdot \langle F_\xi \rangle \tag{63}$$

wehere ΔA is the Laplacian of the free energy. The potential of mean force is written under the file name `<outputName>.pmf`, in a plain text format (see 9.3.18) that can be read by most data plotting and analysis programs (e.g. Gnuplot). This applies periodic boundary conditions to periodic coordinates, and Neumann boundary conditions otherwise (imposed free energy gradient at the boundary of the domain). Note that the grid used for free energy discretization is extended by one point along non-periodic coordinates, but not along periodic coordinates.

In dimension 4 or greater, integrating the discretized gradient becomes non-trivial. The standalone utility `abf_integrate` is provided to perform that task. Because 4D ABF calculations are uncommon, **this tool is practically deprecated** by the Poisson integration described above.

`abf_integrate` reads the gradient data and uses it to perform a Monte-Carlo (M-C) simulation in discretized collective variable space (specifically, on the same grid used by ABF to discretize the free energy gradient). By default, a history-dependent bias (similar in spirit to metadynamics) is used: at each M-C step, the bias at the current position is incremented by a preset amount (the *hill height*). Upon convergence, this bias counteracts optimally the underlying gradient; it is negated to obtain the estimate of the free energy surface.

`abf_integrate` is invoked using the command-line:

```
abf_integrate <gradient_file> [-n <nsteps>] [-t <temp>] [-m (0|1)] [-h <hill_height>] [-f
<factor>]
```

The gradient file name is provided first, followed by other parameters in any order. They are described below, with their default value in square brackets:

- `-n`: number of M-C steps to be performed; by default, a minimal number of steps is chosen based on the size of the grid, and the integration runs until a convergence criterion is satisfied (based on the RMSD between the target gradient and the real PMF gradient)
- `-t`: temperature for M-C sampling (unrelated to the simulation temperature) [500 K]
- `-s`: scaling factor for the gradients; when using a histogram of total forces obtained from `outputTotalForce` (see 9.3.19) or the `.force` file written by `writeTISamples` (see 9.5.1), a scaling factor of -1 should be used [1.0]

- `-m`: use metadynamics-like biased sampling? (0 = false) [1]
- `-h`: increment for the history-dependent bias ("hill height") [0.01 kcal/mol]
- `-f`: if non-zero, this factor is used to scale the increment stepwise in the second half of the M-C sampling to refine the free energy estimate [0.5]

Using the default values of all parameters should give reasonable results in most cases.

`abf_integrate` produces the following output files:

- `<gradient_file>.pmf`: computed free energy surface
- `<gradient_file>.histo`: histogram of M-C sampling (not usable in a straightforward way if the history-dependent bias has been applied)
- `<gradient_file>.est`: estimated gradient of the calculated free energy surface (from finite differences)
- `<gradient_file>.dev`: deviation between the user-provided numerical gradient and the actual gradient of the calculated free energy surface. The RMS norm of this vector field is used as a convergence criteria and displayed periodically during the integration.

Note: Typically, the "deviation" vector field does not vanish as the integration converges. This happens because the numerical estimate of the gradient does not exactly derive from a potential, due to numerical approximations used to obtain it (finite sampling and discretization on a grid).

9.5.3 Extended-system Adaptive Biasing Force (eABF)

Extended-system ABF (eABF) is a variant of ABF (9.5.2) where the bias is not applied directly to the collective variable, but to an extended coordinate ("fictitious variable") λ that evolves dynamically according to Newtonian or Langevin dynamics. Such an extended coordinate is enabled for a given colvar using the `extendedLagrangian` and associated keywords (9.3.20). The theory of eABF and the present implementation are documented in detail in reference [64].

Defining an ABF bias on a colvar wherein the `extendedLagrangian` option is active will perform eABF automatically; there is no dedicated option.

The extended variable λ is coupled to the colvar $z = \xi(q)$ by the harmonic potential $(k/2)(z-\lambda)^2$. Under eABF dynamics, the adaptive bias on λ is the running estimate of the average spring force:

$$F^{\text{bias}}(\lambda^*) = \langle k(\lambda - z) \rangle_{\lambda^*} \tag{64}$$

where the angle brackets indicate a canonical average conditioned by $\lambda = \lambda^*$. At long simulation times, eABF produces a flat histogram of the extended variable λ, and a flattened histogram of ξ, whose exact shape depends on the strength of the coupling as defined by `extendedFluctuation` in the colvar. Coupling should be somewhat loose for faster exploration and convergence, but strong enough that the bias does help overcome barriers along the colvar ξ.[64] Distribution of the colvar may be assessed by plotting its histogram, which is written to the *outputName*`.zcount` file in every eABF simulation. Note that a `histogram` bias (9.5.10) applied to an extended-Lagrangian colvar will access the extended degree of freedom λ, not the original colvar ξ; however, the joint histogram may be explicitly requested by listing the name of the colvar twice in a row within the `colvars` parameter of the `histogram` block.

The eABF PMF is that of the coordinate λ, it is not exactly the free energy profile of ξ. That quantity can be calculated based on either the CZAR estimator or the Zheng/Yang estimator.

CZAR estimator of the free energy The *corrected z-averaged restraint* (CZAR) estimator is described in detail in reference [64]. It is computed automatically in eABF simulations, regardless of the number of colvars involved. Note that ABF may also be applied on a combination of extended and non-extended colvars; in that case, CZAR still provides an unbiased estimate of the free energy gradient.

CZAR estimates the free energy gradient as:

$$A'(z) = -\frac{1}{\beta}\frac{d\ln\tilde{\rho}(z)}{dz} + k(\langle\lambda\rangle_z - z). \tag{65}$$

where $z = \xi(q)$ is the colvar, λ is the extended variable harmonically coupled to z with a force constant k, and $\tilde{\rho}(z)$ is the observed distribution (histogram) of z, affected by the eABF bias.

Parameters for the CZAR estimator are:

- `CZARestimator` < Calculate CZAR estimator of the free energy? >
  Context: `abf`
  Acceptable Values: boolean
  Default Value: `yes`
  Description: This option is only available when ABF is performed on extended-Lagrangian colvars. When enabled, it triggers calculation of the free energy following the CZAR estimator.

- `writeCZARwindowFile` < Write internal data from CZAR to a separate file? >
  Context: `abf`
  Acceptable Values: boolean
  Default Value: `no`
  Description: When this option is enabled, eABF simulations will write a file containing the z-averaged restraint force under the name *outputName*`.zgrad`. The same information is always included in the colvars state file, which is sufficient for restarting an eABF simulation. These separate file is only useful when joining adjacent windows from a stratified eABF simulation, either to continue the simulation in a broader window or to compute a CZAR estimate of the PMF over the full range of the coordinate(s). **Important warning.** Unbiased free-energy estimators from eABF dynamics rely on some form of sampling histogram. When running stratified (windowed) calculations this histogram becomes discontinuous, and as a result the free energy gradient estimated by CZAR is inaccurate at the window boundary, resulting in visible "blips" in the PMF. As a workaround, we recommend manually replacing the two free energy gradient values at the boundary, either with the ABF values from .grad files (accurate in the limit of tight coupling), or with values interpolated for the neighboring values of the CZAR gradient.

Similar to ABF, the CZAR estimator produces two output files in multicolumn text format (9.3.18):

- *outputName*`.czar.grad`: current estimate of the free energy gradient (grid), in multicolumn;
- *outputName*`.czar.pmf`: only for one-dimensional calculations, integrated free energy profile or PMF.

The sampling histogram associated with the CZAR estimator is the z-histogram, which is written in the file *outputName*`.zcount`.

Zheng/Yang estimator of the free energy This feature has been contributed to NAMD by the following authors:

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This implementation is fully documented in [36]. The Zheng and Yang estimator [121] is based on Umbrella Integration [53]. The free energy gradient is estimated as :

$$A'(\xi^*) = \frac{\sum_{\lambda} N(\xi^*, \lambda) \left[\frac{(\xi^* - \langle \xi \rangle_\lambda)}{\beta \sigma_\lambda^2} - k(\xi^* - \lambda) \right]}{\sum_{\lambda} N(\xi^*, \lambda)} \tag{66}$$

where ξ is the colvar, λ is the extended variable harmonically coupled to ξ with a force constant k, $N(\xi, \lambda)$ is the number of samples collected in a (ξ, λ) bin, which is assumed to be a Gaussian function of ξ with mean $\langle \xi \rangle_\lambda$ and standard deviation σ_λ.

The estimator is enabled through the following option:

- `UIestimator` < Calculate UI estimator of the free energy? >
  Context: `abf`
  Acceptable Values: boolean
  Default Value: `no`
  Description: This option is only available when ABF is performed on extended-Lagrangian colvars. When enabled, it triggers calculation of the free energy following the UI estimator.

Usage for multiple–replica eABF. The eABF algorithm can be associated with a multiple–walker strategy [77, 25] (9.5.2). To run a multiple–replica eABF simulation, start a multiple-replica NAMD run (option `+replicas`) and set `shared on` in the Colvars config file to enable the multiple–walker ABF algorithm. It should be noted that in contrast with classical MW–ABF simulations, the output files of an MW–eABF simulation only show the free energy estimate of the corresponding replica.

One can merge the results, using `./eabf.tcl -mergemwabf [merged_filename] [eabf_output1] [eabf_output2] ...`, e.g., `./eabf.tcl -mergemwabf merge.eabf eabf.0.UI eabf.1.UI eabf.2.UI eabf.3.UI`.

If one runs an ABF–based calculation, breaking the reaction pathway into several non–overlapping windows, one can use `./eabf.tcl -mergesplitwindow [merged_fileprefix] [eabf_output] [eabf_output2] ...` to merge the data accrued in these non–overlapping windows. This option can be utilized in both eABF and classical ABF simulations, e.g., `./eabf.tcl -mergesplitwindow merge window0.czar window1.czar window2.czar window3.czar`, `./eabf.tcl -mergesplitwindow merge window0.UI window1.UI window2.UI window3.UI` or `./eabf.tcl -mergesplitwindow merge abf0 abf1 abf2 abf3`.

9.5.4 Metadynamics

The metadynamics method uses a history-dependent potential [60] that generalizes to any type of colvars the conformational flooding [40] and local elevation [48] methods, originally formulated to use as colvars the principal components of a covariance matrix or a set of dihedral angles, respectively. The metadynamics potential on the colvars $\boldsymbol{\xi} = (\xi_1, \xi_2, \ldots, \xi_{N_{\mathrm{cv}}})$ is defined as:

$$V_{\mathrm{meta}}(\boldsymbol{\xi}(t)) \;=\; \sum_{t'=\delta t, 2\delta t, \ldots}^{t'<t} W \prod_{i=1}^{N_{\mathrm{cv}}} \exp\left(-\frac{(\xi_i(t) - \xi_i(t'))^2}{2\sigma_{\xi_i}^2}\right), \tag{67}$$

where V_{meta} is the history-dependent potential acting on the *current* values of the colvars $\boldsymbol{\xi}$, and depends only parametrically on the *previous* values of the colvars. V_{meta} is constructed as a sum of N_{cv}-dimensional repulsive Gaussian "hills", whose height is a chosen energy constant W, and whose centers are the previously explored configurations $(\boldsymbol{\xi}(\delta t), \boldsymbol{\xi}(2\delta t), \ldots)$.

During the simulation, the system evolves towards the nearest minimum of the "effective" potential of mean force $\tilde{A}(\boldsymbol{\xi})$, which is the sum of the "real" underlying potential of mean force $A(\boldsymbol{\xi})$ and the the metadynamics potential, $V_{\mathrm{meta}}(\boldsymbol{\xi})$. Therefore, at any given time the probability of observing the configuration $\boldsymbol{\xi}^*$ is proportional to $\exp\left(-\tilde{A}(\boldsymbol{\xi}^*)/\kappa_{\mathrm{B}}T\right)$: this is also the probability that a new Gaussian "hill" is added at that configuration. If the simulation is run for a sufficiently long time, each local minimum is canceled out by the sum of the Gaussian "hills". At that stage the "effective" potential of mean force $\tilde{A}(\boldsymbol{\xi})$ is constant, and $-V_{\mathrm{meta}}(\boldsymbol{\xi})$ is an estimator of the "real" potential of mean force $A(\boldsymbol{\xi})$, save for an additive constant:

$$A(\boldsymbol{\xi}) \;\simeq\; -V_{\mathrm{meta}}(\boldsymbol{\xi}) + K \tag{68}$$

Such estimate of the free energy can be provided by enabling `writeFreeEnergyFile`. Assuming that the set of collective variables includes all relevant degrees of freedom, the predicted error of the estimate is a simple function of the correlation times of the colvars τ_{ξ_i}, and of the user-defined parameters W, σ_{ξ_i} and δt [16]. In typical applications, a good rule of thumb can be to choose the ratio $W/\delta t$ much smaller than $\kappa_{\mathrm{B}}T/\tau_{\boldsymbol{\xi}}$, where $\tau_{\boldsymbol{\xi}}$ is the longest among $\boldsymbol{\xi}$'s correlation times: σ_{ξ_i} then dictates the resolution of the calculated PMF.

If the metadynamics parameters are chosen correctly, after an equilibration time, t_e, the estimator provided by eq. 68 oscillates on time around the "real" free energy, thereby a better estimate of the latter can be obtained as the time average of the bias potential after t_e [70, 27]:

$$A(\boldsymbol{\xi}) \;=\; -\frac{1}{t_{tot} - t_e} \int_{t_e}^{t_{tot}} V_{\mathrm{meta}}(\boldsymbol{\xi}, t) dt \tag{69}$$

where t_e is the time after which the bias potential grows (approximately) evenly during the simulation and t_{tot} is the total simulation time. The free energy calculated according to eq. 69 can thus be obtained averaging on time mutiple time-dependent free energy estimates, that can be printed out through the keyword `keepFreeEnergyFiles`. An alternative is to obtain the free energy profiles by summing the hills added during the simulation; the hills trajectory can be printed out by enabling the option `writeHillsTrajectory`.

Treatment of the PMF boundaries In typical scenarios the Gaussian hills of a metadynamics potential are interpolated and summed together onto a grid, which is much more efficient than computing each hill independently at every step (the keyword `useGrids` (see 9.5.4) is `on` by default).

This numerical approximation typically yields neglibile errors in the resulting PMF [33]. However, due to the finite thickness of the Gaussian function, the metadynamics potential would suddenly vanish each time a variable exceeds its grid boundaries.

To avoid such discontinuity the Colvars metadynamics code will keep an explicit copy of each hill that straddles a grid's boundary, and will use it to compute metadynamics forces outside the grid. This measure is taken to protect the accuracy and stability of a metadynamics simulation, except in cases of "natural" boundaries (for example, the [0 : 180] interval of an `angle` colvar) or when the flags `hardLowerBoundary` (see 9.3.18) and `hardUpperBoundary` (see 9.3.18) are explicitly set by the user. Unfortunately, processing explicit hills alongside the potential and force grids could easily become inefficient, slowing down the simulation and increasing the state file's size.

In general, it is a good idea to *define a repulsive potential to avoid hills from coming too close to the grid's boundaries*, for example as a `harmonicWalls` restraint (see 9.5.7).

Example: Using harmonic walls to protect the grid's boundaries.

```
colvar {
  name r
  distance { ...  }
  upperBoundary 15.0
  width 0.2
}

metadynamics {
  name meta_r
  colvars r
  hillWeight 0.001
  hillWidth 2.0
}

harmonicWalls {
  name wall_r
  colvars r
  upperWall 13.0
  upperWallConstant 2.0
}
```

In the colvar `r`, the `distance` function used has a `lowerBoundary` automatically set to 0 Å by default, thus the keyword `lowerBoundary` itself is not mandatory and `hardLowerBoundary` is set to `yes` internally. However, `upperBoundary` does not have such a "natural" choice of value. The metadynamics potential `meta_r` will individually process any hill whose center is too close to the `upperBoundary`, more precisely within fewer grid points than 6 times the Gaussian σ parameter plus one. It goes without saying that if the colvar `r` represents a distance between two freely-moving molecules, it will cross this "threshold" rather frequently.

In this example, where the value of `hillWidth` (2σ) amounts to 2 grid points, the threshold is 6+1 = 7 grid points away from `upperBoundary`. In explicit units, the `width` of r is $w_r = 0.2$ Å, and the threshold is 15.0 - 7×0.2 = 13.6 Å.

The `wall_r` restraint included in the example prevents this: the position of its `upperWall` is 13 Å,

i.e. 3 grid points below the buffer's threshold (13.6 Å). For the chosen value of `upperWallConstant`, the energy of the `wall_r` bias at `r` $= r_{\text{upper}} = 13.6$ Å is:

$$E^* = \frac{1}{2}k\left(\frac{r - r_{\text{upper}}}{w_r}\right)^2 = \frac{1}{2}2.0\,(-3)^2 = 9 \text{ kcal/mol}$$

which results in a relative probability $\exp(-E^*/\kappa_{\text{B}}T) \simeq 3 \times 10^{-7}$ that `r` crosses the threshold. The probability that `r` exceeds `upperBoundary`, which is further away, has also become vanishingly small. At that point, you may want to set `hardUpperBoundary` to `yes` for `r`, and let `meta_r` know that no special treatment near the grid's boundaries will be needed.

What is the impact of the wall restraint onto the PMF? Not a very complicated one: the PMF reconstructed by metadynamics will simply show a sharp increase in free-energy where the wall potential kicks in (`r` $>$ 13 Å). You may then choose between using the PMF only up until that point and discard the rest, or subtracting the energy of the `harmonicWalls` restraint from the PMF itself. Keep in mind, however, that the statistical convergence of metadynamics may be less accurate where the wall potential is strong.

In summary, although it would be simpler to set the wall's position `upperWall` and the grid's boundary `upperBoundary` to the same number, the finite width of the Gaussian hills calls for setting the former strictly within the latter.

Basic configuration keywords To enable a metadynamics calculation, a `metadynamics {...}` block must be defined in the Colvars configuration file. Its mandatory keywords are `colvars` (see 9.5), the variables involved, `hillWeight` (see 9.5.4), the weight parameter W, and the widths 2σ of the Gaussian hills in each dimension given by the single dimensionless parameter `hillWidth` (see 9.5.4), or more explicitly by the `gaussianSigmas` (see 9.5.4).

- `name`: see definition of `name` in sec. 9.5 (biasing and analysis methods)
- `colvars`: see definition of `colvars` in sec. 9.5 (biasing and analysis methods)
- `outputEnergy`: see definition of `outputEnergy` in sec. 9.5 (biasing and analysis methods)
- `outputFreq`: see definition of `outputFreq` in sec. 9.5 (biasing and analysis methods)
- `writeTIPMF`: see definition of `writeTIPMF` in sec. 9.5 (biasing and analysis methods)
- `writeTISamples`: see definition of `writeTISamples` in sec. 9.5 (biasing and analysis methods)
- `stepZeroData`: see definition of `stepZeroData` in sec. 9.5 (biasing and analysis methods)
- `hillWeight` < Height of each hill (kcal/mol) >
  Context: `metadynamics`
  Acceptable Values: positive decimal
  Description: This option sets the height W of the Gaussian hills that are added during this run. Lower values provide more accurate sampling of the system's degrees of freedom at the price of longer simulation times to complete a PMF calculation based on metadynamics.

- `hillWidth` < Width 2σ of a Gaussian hill, measured in number of grid points >
  Context: `metadynamics`
  Acceptable Values: positive decimal
  Description: This keyword sets the Gaussian width $2\sigma_{\xi_i}$ for all colvars, expressed in *number of grid points*, with the grid spacing along each colvar ξ determined by the respective value of `width` (see 9.3.18). Values between 1 and 3 are recommended for this option: smaller numbers will fail to adequately interpolate each Gaussian function [33], while larger values may be unable to account for steep free-energy gradients. The values of each half-width σ_{ξ_i} in the physical units of ξ_i are also printed by NAMD at initialization time; alternatively, they may be set explicitly via `gaussianSigmas` (see 9.5.4).

- `gaussianSigmas` < Half-widths σ of the Gaussian hill (one for each colvar) >
  Context: `metadynamics`
  Acceptable Values: space-separated list of decimals
  Description: This option sets the parameters σ_{ξ_i} of the Gaussian hills along each colvar ξ_i, expressed in *the same unit of* ξ_i. No restrictions are placed on each value, but a warning will be printed if `useGrids` (see 9.5.4) is `on` and the Gaussian width $2\sigma_{\xi_i}$ is smaller than the corresponding grid spacing, `width`(ξ_i). If not given, default values will be computed from the dimensionless number `hillWidth` (see 9.5.4).

- `newHillFrequency` < Frequency of hill creation >
  Context: `metadynamics`
  Acceptable Values: positive integer
  Default Value: `1000`
  Description: This option sets the number of steps after which a new Gaussian hill is added to the metadynamics potential. The product of this number and the integration time-step defines the parameter δt in eq. 67. Higher values provide more accurate statistical sampling, at the price of longer simulation times to complete a PMF calculation.

Output files When interpolating grids are enabled (default behavior), the PMF is written by default every `colvarsRestartFrequency` steps to the file *outputName*`.pmf` in multicolumn text format (9.3.18). The following two options allow to disable or control this behavior and to track statistical convergence:

- `writeFreeEnergyFile` < Periodically write the PMF for visualization >
  Context: `metadynamics`
  Acceptable Values: boolean
  Default Value: `on`
  Description: When `useGrids` and this option are `on`, the PMF is written every `outputFreq` (see 9.5) steps.

- `keepFreeEnergyFiles` < Keep all the PMF files >
  Context: `metadynamics`
  Acceptable Values: boolean
  Default Value: `off`
  Description: When `writeFreeEnergyFile` and this option are `on`, the step number is included in the file name, thus generating a series of PMF files. Activating this option can be

useful to follow more closely the convergence of the simulation, by comparing PMFs separated by short times.

- `writeHillsTrajectory` < Write a log of new hills >
  Context: `metadynamics`
  Acceptable Values: boolean
  Default Value: `off`
  Description: If this option is `on`, a file containing the Gaussian hills written by the `metadynamics` bias, with the name:
  "*outputName*`.colvars.<name>.hills.traj`",
  which can be useful to post-process the time series of the Gassian hills. Each line is written every `newHillFrequency`, regardless of the value of `outputFreq` (see 9.5). When `multipleReplicas` is `on`, its name is changed to:
  "*outputName*`.colvars.<name>.<replicaID>.hills.traj`".
  The columns of this file are the centers of the hills, $\xi_i(t')$, followed by the half-widths, σ_{ξ_i}, and the weight, W. **Note:** prior to version 2020-02-24, the full-width 2σ of the Gaussian was reported in lieu of σ.

Performance optimization The following options control the computational cost of metadynamics calculations, but do not affect results. Default values are chosen to minimize such cost with no loss of accuracy.

- `useGrids` < Interpolate the hills with grids >
  Context: `metadynamics`
  Acceptable Values: boolean
  Default Value: `on`
  Description: This option discretizes all hills for improved performance, accumulating their energy and their gradients on two separate grids of equal spacing. Grids are defined by the values of `lowerBoundary`, `upperBoundary` and `width` for each colvar. Currently, this option is implemented for all types of variables except the non-scalar types (`distanceDir` or `orientation`). If `expandBoundaries` is defined in one of the colvars, grids are automatically expanded along the direction of that colvar.

- `rebinGrids` < Recompute the grids when reading a state file >
  Context: `metadynamics`
  Acceptable Values: boolean
  Default Value: `off`
  Description: When restarting from a state file, the grid's parameters (boundaries and widths) saved in the state file override those in the configuration file. Enabling this option forces the grids to match those in the current configuration file.

- `keepHills` < Write each individual hill to the state file >
  Context: `metadynamics`
  Acceptable Values: boolean
  Default Value: `off`
  Description: When `useGrids` and this option are `on`, all hills are saved to the state file in their analytic form, alongside their grids. This makes it possible to later use exact

analytic Gaussians for `rebinGrids`. To only keep track of the history of the added hills, `writeHillsTrajectory` is preferable.

Ensemble-Biased Metadynamics The ensemble-biased metadynamics (EBMetaD) approach [69] is designed to reproduce a target probability distribution along selected collective variables. Standard metadynamics can be seen as a special case of EBMetaD with a flat distribution as target. This is achieved by weighing the Gaussian functions used in the metadynamics approach by the inverse of the target probability distribution:

$$V_{\mathrm{EBmetaD}}(\boldsymbol{\xi}(t)) \;=\; \sum_{t'=\delta t,2\delta t,\ldots}^{t'<t} \frac{W}{\exp\left(S_\rho\right)\rho_{exp}(\boldsymbol{\xi}(t'))} \prod_{i=1}^{N_{\mathrm{cv}}} \exp\left(-\frac{(\xi_i(t)-\xi_i(t'))^2}{2\sigma_{\xi_i}^2}\right), \tag{70}$$

where $\rho_{exp}(\boldsymbol{\xi})$ is the target probability distribution and $S_\rho = -\int \rho_{exp}(\boldsymbol{\xi}) \log \rho_{exp}(\boldsymbol{\xi})\, \mathrm{d}\boldsymbol{\xi}$ its corresponding differential entropy. The method is designed so that during the simulation the resulting distribution of the collective variable $\boldsymbol{\xi}$ converges to $\rho_{exp}(\boldsymbol{\xi})$. A practical application of EBMetaD is to reproduce an "experimental" probability distribution, for example the distance distribution between spectroscopic labels inferred from Förster resonance energy transfer (FRET) or double electron-electron resonance (DEER) experiments [69].

The PMF along ξ can be estimated from the bias potential and the target ditribution [69]:

$$A(\boldsymbol{\xi}) \;\simeq\; -V_{\mathrm{EBmetaD}}(\boldsymbol{\xi}) - \kappa_{\mathrm{B}} T \log \rho_{exp}(\boldsymbol{\xi}) \tag{71}$$

and obtained by enabling `writeFreeEnergyFile`. Similarly to eq. 69, a more accurate estimate of the free energy can be obtained by averaging (after an equilibration time) multiple time-dependent free energy estimates (see `keepFreeEnergyFiles`).

The following additional options define the configuration for the ensemble-biased metadynamics approach:

- `ebMeta` < Perform ensemble-biased metadynamics >
  Context: `metadynamics`
  Acceptable Values: boolean
  Default Value: `off`
  Description: If enabled, this flag activates the ensemble-biased metadynamics as described by Marinelli et al.[69]. The target distribution file, `targetdistfile`, is then required. The keywords `lowerBoundary`, `upperBoundary` and `width` for the respective variables are also needed to set the binning (grid) of the target distribution file.

- `targetDistFile` < Target probability distribution file for ensemble-biased metadynamics >
  Context: `metadynamics`
  Acceptable Values: multicolumn text file
  Description: This file provides the target probability distribution, $\rho_{exp}(\boldsymbol{\xi})$, reported in eq. 70. The latter distribution must be a tabulated function provided in a multicolumn text format (see 9.3.18). The provided distribution is then normalized.

- `ebMetaEquilSteps` < Number of equilibration steps for ensemble-biased metadynamics >
  Context: `metadynamics`
  Acceptable Values: positive integer

Description: The EBMetaD approach may introduce large hills in regions with small values of the target probability distribution (eq. 70). This happens, for example, if the probability distribution sampled by a conventional molecular dynamics simulation is significantly different from the target distribution. This may lead to instabilities at the beginning of the simulation related to large biasing forces. In this case, it is useful to introduce an equilibration stage in which the bias potential gradually switches from standard metadynamics (eq. 67) to EBmetaD (eq. 70) as $\lambda V_{\text{meta}}(\boldsymbol{\xi}) + (1-\lambda)V_{\text{EBmetaD}}(\boldsymbol{\xi})$, where $\lambda = (\texttt{ebMetaEquilSteps} - \texttt{step})/\texttt{ebMetaEquilSteps}$ and `step` is the current simulation step number.

- `targetDistMinVal` < Minimum value of the target distribution in reference to its `maximum value` >
  Context: `metadynamics`
  Acceptable Values: positive decimal
  Description: It is useful to set a minimum value of the target probability distribution to avoid values of the latter that are nearly zero, leading to very large hills. This parameter sets the minimum value of the target probability distribution that is expressed as a fraction of its `maximum value`: `minimum value = maximum value X targetDistMinVal`. This implies that 0 < `targetDistMinVal` < 1 and its default value is set to 1/1000000. To avoid divisions by zero (see eq. 70), if `targetDistMinVal` is set as zero, values of ρ_{exp} equal to zero are replaced by the smallest positive value read in the same file.

As with standard metadynamics, multidimensional probability distributions can be targeted using a single `metadynamics` block using multiple `colvars` and a multidimensional target distribution file (see 9.3.18). Instead, multiple probability distributions on different variables can be targeted separately in the same simulation by introducing multiple `metadynamics` blocks with the `ebMeta` option.

Example: EBmetaD configuration for a single variable.

```
colvar {
  name r
  distance {
    group1 { atomNumbers 991 992 }
    group2 { atomNumbers 1762 1763 }
  }
  upperBoundary 100.0
  width 0.1
}

metadynamics {
  name ebmeta
  colvars r
  hillWeight 0.01
  hillWidth 3.0
  ebMeta on
  targetDistFile targetdist1.dat
  ebMetaEquilSteps 500000
}
```

where `targetdist1.dat` is a text file in "multicolumn" format (9.3.18) with the same `width` as the variable `r` (0.1 in this case):

```
#  1
#  0.0     0.1      1000   0

     0.05   0.0012
     0.15   0.0014
    ...     ...
    99.95   0.0010
```

Tip: Besides setting a meaninful value for `targetDistMinVal`, the exploration of unphysically low values of the target distribution (which would lead to very large hills and possibly numerical instabilities) can be also prevented by restricting sampling to a given interval, using e.g. `harmonicWalls` restraint (9.5.7).

Well-tempered metadynamics The following options define the configuration for the "well-tempered" metadynamics approach [4]:

- `wellTempered` < Perform well-tempered metadynamics >
  Context: `metadynamics`
  Acceptable Values: boolean
  Default Value: `off`
  Description: If enabled, this flag causes well-tempered metadynamics as described by Barducci et al.[4] to be performed, rather than standard metadynamics. The parameter `biasTemperature` is then required. This feature was contributed by Li Li (Luthey-Schulten group, Department of Chemistry, UIUC).

- `biasTemperature` < Temperature bias for well-tempered metadynamics >
  Context: `metadynamics`
  Acceptable Values: positive decimal
  Description: When running metadynamics in the long time limit, collective variable space is sampled to a modified temperature $T+\Delta T$. In conventional metadynamics, the temperature "boost" ΔT would constantly increases with time. Instead, in well-tempered metadynamics ΔT must be defined by the user via `biasTemperature`. The written PMF includes the scaling factor $(T + \Delta T)/\Delta T$ [4]. A careful choice of ΔT determines the sampling and convergence rate, and is hence crucial to the success of a well-tempered metadynamics simulation.

Multiple-walker metadynamics Metadynamics calculations can be performed concurrently by multiple replicas that share a common history. This variant of the method is called multiple-walker metadynamics [90]: the Gaussian hills of all replicas are periodically combined into a single biasing potential, intended to converge to a single PMF.

In the implementation here described [33], replicas communicate through files. This arrangement allows launching the replicas either (1) as a bundle (i.e. a single job in a cluster's queueing system) or (2) as fully independent runs (i.e. as separate jobs for the queueing system). One advantage of the use case (1) is that an identical Colvars configuration can be used for all replicas (otherwise, `replicaID` needs to be manually set to a different string for each replica). However,

the use case (2) is less demanding in terms of high-performance computing resources: a typical scenario would be a computer cluster (including virtual servers from a cloud provider) where not all nodes are connected to each other at high speed, and thus each replica runs on a small group of nodes or a single node.

Whichever way the replicas are started (coupled or not), a shared filesystem is needed so that each replica can read the files created by the others: paths to these files are stored in the shared file `replicasRegistry`. This file, and those listed in it, are read every `replicaUpdateFrequency` steps. Each time the Colvars state file is written (for example, `colvarsRestartFrequency` steps), the file named:
outputName`.colvars.`*name*`.`*replicaID*`.state`
is written as well; this file contains only the state of the metadynamics bias, which the other replicas will read in turn. In between the times when this file is modified/replaced, new hills are also temporarily written to the file named:
outputName`.colvars.`*name*`.`*replicaID*`.hills`
Both files are only used for communication, and may be deleted after the replica begins writing files with a new *outputName*.

Example: Multiple-walker metadynamics with file-based communication.

```
metadynamics {
  name mymtd
  colvars x
  hillWeight 0.001
  newHillFrequency 1000
  hillWidth 3.0

  multipleReplicas on
  replicasRegistry /shared-folder/mymtd-replicas.txt
  replicaUpdateFrequency 50000 # Best if larger than newHillFrequency
}
```

The following are the multiple-walkers related options:

- `multipleReplicas` < Enable multiple-walker metadynamics >
  Context: `metadynamics`
  Acceptable Values: boolean
  Default Value: `off`
  Description: This option turns on multiple-walker communication between replicas.

- `replicasRegistry` < Multiple replicas database file >
  Context: `metadynamics`
  Acceptable Values: UNIX filename
  Description: If `multipleReplicas` is `on`, this option sets the path to the replicas' shared database file. It is best to use an absolute path (especially when running individual replicas in separate folders).

- `replicaUpdateFrequency` < How often hills are shared between replicas >
  Context: `metadynamics`
  Acceptable Values: positive integer

Description: If `multipleReplicas` is `on`, this option sets the number of steps after which each replica tries to read the other replicas' files. On a networked file system, it is best to use a number of steps that corresponds to at least a minute of wall time.

- `replicaID` < Set the identifier for this replica >
  Context: `metadynamics`
  Acceptable Values: string
  Default Value: replica index (only if a shared communicator is used)
  Description: If `multipleReplicas` is `on`, this option sets a unique identifier for this replicas. When the replicas are launched in a single command (i.e. they share a parallel communicator and are tightly synchronized) this value is optional, and defaults to the replica's numeric index (starting at zero). However, when the replicas are launched as independent runs this option is required.

- `writePartialFreeEnergyFile` < Periodically write the contribution to the PMF from this replica >
  Context: `metadynamics`
  Acceptable Values: boolean
  Default Value: `off`
  Description: If `multipleReplicas` is `on`, enabling this option produces an additional file *outputName*`.partial.pmf`, which can be useful to monitor the contribution of each replica to the total PMF (which is written to the file *outputName*`.pmf`). **Note:** the name of this file is chosen for consistency and convenience, *but its content is not a PMF* and it is not expected to converge, even if the total PMF does.

9.5.5 Harmonic restraints

The harmonic biasing method may be used to enforce fixed or moving restraints, including variants of Steered and Targeted MD. Within energy minimization runs, it allows for restrained minimization, e.g. to calculate relaxed potential energy surfaces. In the context of the Colvars module, harmonic potentials are meant according to their textbook definition:

$$V(\xi) = \frac{1}{2}k\left(\frac{\xi - \xi_0}{w_\xi}\right)^2 \tag{72}$$

There are two noteworthy aspects of this expression:

1. Because the *standard coefficient of* $1/2$ of the harmonic potential is included, this expression differs from harmonic bond and angle potentials historically used in common force fields, where the factor was typically omitted resulting in a non-standard definition of the force constant.

2. The variable ξ is not only centered at ξ_0, but is also *scaled by its characteristic length scale* w_ξ (keyword `width` (see 9.3.18)). The resulting dimensionless variable $z = (\xi - \xi_0)/w_\xi$ is typically easier to treat numerically: for example, when the forces typically experienced by ξ are much smaller than k/w_ξ and k is chosen equal to $\kappa_\mathrm{B}T$ (thermal energy), the resulting probability distribution of z is approximately a Gaussian with mean equal to 0 and standard deviation equal to 1.

This property can be used for setting the force constant in umbrella-sampling ensemble runs: if the restraint centers are chosen in increments of w_ξ, the resulting distributions of ξ are most often optimally overlapped. In regions where the underlying free-energy landscape induces highly skewed distributions of ξ, additional windows may be added as needed, with spacings finer than w_ξ.

Beyond one dimension, the use of a scaled harmonic potential also allows a standard definition of a multi-dimensional restraint with a unified force constant:

$$V(\xi_1, \ldots, \xi_M) = \frac{1}{2} k \sum_{i=1}^{M} \left(\frac{\xi_i - \xi_0}{w_\xi} \right)^2 \tag{73}$$

If one-dimensional or homogeneous multi-dimensional restraints are defined, and there are no other uses for the parameter w_ξ, `width` *can be left at its default value of* 1.

A harmonic restraint is defined by a `harmonic {...}` block, which may contain the following keywords:

- `name`: see definition of `name` in sec. 9.5 (biasing and analysis methods)
- `colvars`: see definition of `colvars` in sec. 9.5 (biasing and analysis methods)
- `outputEnergy`: see definition of `outputEnergy` in sec. 9.5 (biasing and analysis methods)
- `writeTIPMF`: see definition of `writeTIPMF` in sec. 9.5 (biasing and analysis methods)
- `writeTISamples`: see definition of `writeTISamples` in sec. 9.5 (biasing and analysis methods)
- `stepZeroData`: see definition of `stepZeroData` in sec. 9.5 (biasing and analysis methods)
- `forceConstant` < Scaled force constant (kcal/mol) >
  Context: `harmonic`
  Acceptable Values: positive decimal
  Default Value: `1.0`
  Description: This option defines a *scaled* force constant k for the harmonic potential (eq. 73). To ensure consistency for multidimensional restraints, it is divided internally by the square of the specific `width` of each variable (which is 1 by default). This makes all values effectively dimensionless and of commensurate size. For instance, if this force constant is set to the thermal energy $\kappa_B T$ (equal to RT if molar units are used), then the amplitude of the thermal fluctuations of each variable ξ will be on the order of its `width`, w_ξ. This can be used to estimate the optimal spacing of umbrella-sampling windows (under the assumption that the force constant is larger than the curvature of the underlying free energy). *The values of the actual force constants k/w_ξ^2 are always printed when the restraint is defined.*
- `centers` < Initial harmonic restraint centers >
  Context: `harmonic`
  Acceptable Values: space-separated list of colvar values
  Description: The centers (equilibrium values) of the restraint, ξ_0, are entered here. The number of values must be the number of requested colvars. Each value is a decimal number if the corresponding colvar returns a scalar, a "`(x, y, z)`" triplet if it returns a unit vector

or a vector, and a "`(q0, q1, q2, q3)`" quadruplet if it returns a rotational quaternion. If a colvar has periodicities or symmetries, its closest image to the restraint center is considered when calculating the harmonic potential.

Tip: A complex set of restraints can be applied to a system, by defining several colvars, and applying one or more harmonic restraints to different groups of colvars. In some cases, dozens of colvars can be defined, but their value may not be relevant: to limit the size of the colvars trajectory file, it may be wise to disable `outputValue` for such "ancillary" variables, and leave it enabled only for "relevant" ones.

Moving restraints: steered molecular dynamics The following options allow to change gradually the centers of the harmonic restraints during a simulations. When the centers are changed continuously, a steered MD in a collective variable space is carried out.

- `targetCenters` < Steer the restraint centers towards these targets >
  Context: `harmonic`
  Acceptable Values: space-separated list of colvar values
  Description: When defined, the current `centers` will be moved towards these values during the simulation. By default, the centers are moved over a total of `targetNumSteps` steps by a linear interpolation, in the spirit of Steered MD. If `targetNumStages` is set to a nonzero value, the change is performed in discrete stages, lasting `targetNumSteps` steps *each*. This second mode may be used to sample successive windows in the context of an Umbrella Sampling simulation. When continuing a simulation run, the `centers` specified in the configuration file <`colvarsConfig`> are overridden by those saved in the restart file <`colvarsInput`>. To perform Steered MD in an arbitrary space of colvars, it is sufficient to use this option and enable `outputAccumulatedWork` and/or `outputAppliedForce` within each of the colvars involved.

- `targetNumSteps` < Number of steps for steering >
  Context: `harmonic`
  Acceptable Values: positive integer
  Description: In single-stage (continuous) transformations, defines the number of MD steps required to move the restraint centers (or force constant) towards the values specified with `targetCenters` or `targetForceConstant`. After the target values have been reached, the centers (resp. force constant) are kept fixed. In multi-stage transformations, this sets the number of MD steps *per stage*.

- `outputCenters` < Write the current centers to the trajectory file >
  Context: `harmonic`
  Acceptable Values: boolean
  Default Value: `off`
  Description: If this option is chosen and `colvarsTrajFrequency` is not zero, the positions of the restraint centers will be written to the trajectory file during the simulation. This option allows to conveniently extract the PMF from the colvars trajectory files in a steered MD calculation.

Note on restarting moving restraint simulations: Information about the current step and stage of a simulation with moving restraints is stored in the restart file (state file). Thus, such

simulations can be run in several chunks, and restarted directly using the same colvars configuration file. In case of a restart, the values of parameters such as `targetCenters`, `targetNumSteps`, etc. should not be changed manually.

Moving restraints: umbrella sampling The centers of the harmonic restraints can also be changed in discrete stages: in this cases a one-dimensional umbrella sampling simulation is performed. The sampling windows in simulation are calculated in sequence. The colvars trajectory file may then be used both to evaluate the correlation times between consecutive windows, and to calculate the frequency distribution of the colvar of interest in each window. Furthermore, frequency distributions on a predefined grid can be automatically obtained by using the `histogram` bias (see 9.5.10).

To activate an umbrella sampling simulation, the same keywords as in the previous section can be used, with the addition of the following:

- `targetNumStages` < Number of stages for steering >
  Context: `harmonic`
  Acceptable Values: non-negative integer
  Default Value: `0`
  Description: If non-zero, sets the number of stages in which the restraint centers or force constant are changed to their target values. If zero, the change is continuous. Each stage lasts `targetNumSteps` MD steps. To sample both ends of the transformation, the simulation should be run for `targetNumSteps` × (`targetNumStages` + 1).

Changing force constant The force constant of the harmonic restraint may also be changed to equilibrate [31].

- `targetForceConstant` < Change the force constant towards this value >
  Context: `harmonic`
  Acceptable Values: positive decimal
  Description: When defined, the current `forceConstant` will be moved towards this value during the simulation. Time evolution of the force constant is dictated by the `targetForceExponent` parameter (see below). By default, the force constant is changed smoothly over a total of `targetNumSteps` steps. This is useful to introduce or remove restraints in a progressive manner. If `targetNumStages` is set to a nonzero value, the change is performed in discrete stages, lasting `targetNumSteps` steps *each*. This second mode may be used to compute the conformational free energy change associated with the restraint, within the FEP or TI formalisms. For convenience, the code provides an estimate of the free energy derivative for use in TI. A more complete free energy calculation (particularly with regard to convergence analysis), while not handled by the Colvars module, can be performed by post-processing the colvars trajectory, if `colvarsTrajFrequency` is set to a suitably small value. It should be noted, however, that restraint free energy calculations may be handled more efficiently by an indirect route, through the determination of a PMF for the restrained coordinate.[31]

- `targetForceExponent` < Exponent in the time-dependence of the force constant >
  Context: `harmonic`
  Acceptable Values: decimal equal to or greater than 1.0

Default Value: `1.0`
Description: Sets the exponent, α, in the function used to vary the force constant as a function of time. The force is varied according to a coupling parameter λ, raised to the power α: $k_\lambda = k_0 + \lambda^\alpha(k_1 - k_0)$, where k_0, k_λ, and k_1 are the initial, current, and final values of the force constant. The parameter λ evolves linearly from 0 to 1, either smoothly, or in `targetNumStages` equally spaced discrete stages, or according to an arbitrary schedule set with `lambdaSchedule`. When the initial value of the force constant is zero, an exponent greater than 1.0 distributes the effects of introducing the restraint more smoothly over time than a linear dependence, and ensures that there is no singularity in the derivative of the restraint free energy with respect to lambda. A value of 4 has been found to give good results in some tests.

- `targetEquilSteps` < Number of steps discarded from TI estimate >
Context: `harmonic`
Acceptable Values: positive integer
Description: Defines the number of steps within each stage that are considered equilibration and discarded from the restraint free energy derivative estimate reported reported in the output.

- `lambdaSchedule` < Schedule of lambda-points for changing force constant >
Context: `harmonic`
Acceptable Values: list of real numbers between 0 and 1
Description: If specified together with targetForceConstant, sets the sequence of discrete λ values that will be used for different stages.

9.5.6 Computing the work of a changing restraint

If the restraint centers or force constant are changed continuosly (`targetNumStages` undefined) it is possible to record the net work performed by the changing restraint:

- `outputAccumulatedWork` < Write the accumulated work of the changing restraint to the Colvars trajectory file >
Context: `harmonic`
Acceptable Values: boolean
Default Value: `off`
Description: If `targetCenters` or `targetForceConstant` are defined and this option is enabled, the accumulated work from the beginning of the simulation will be written to the trajectory file (`colvarsTrajFrequency` must be non-zero). When the simulation is continued from a state file, the previously accumulated work is included in the integral. This option allows to conveniently extract the estimated PMF of a steered MD calculation (when `targetCenters` is used), or of other simulation protocols.

9.5.7 Harmonic wall restraints

The `harmonicWalls` {...} bias is closely related to the harmonic bias (see 9.5.5), with the following two differences: *(i)* instead of a center a *lower wall* and/or an *upper wall* are defined, outside of

which the bias implements a half-harmonic potential;

$$V(\xi) = \begin{cases} \frac{1}{2}k\left(\frac{\xi-\xi_{\text{upper}}}{w_\xi}\right)^2 & \text{if } \xi > \xi_{\text{upper}} \\ 0 & \text{if } \xi_{\text{lower}} \leq \xi \geq \xi_{\text{upper}} \\ \frac{1}{2}k\left(\frac{\xi-\xi_{\text{lower}}}{w_\xi}\right)^2 & \text{if } \xi < \xi_{\text{lower}} \end{cases} \tag{74}$$

where ξ_{lower} and ξ_{upper} are the lower and upper wall thresholds, respectively; *(ii)* because an interval between two walls is defined, only scalar variables can be used (but any number of variables can be defined, and the wall bias is intrinsically multi-dimensional).

Note: this bias replaces the keywords `lowerWall`, `lowerWallConstant`, `upperWall` and `upperWallConstant` defined in the `colvar` context. Those keywords are deprecated.

The `harmonicWalls` bias implements the following options:

- `name`: see definition of `name` in sec. 9.5 (biasing and analysis methods)
- `colvars`: see definition of `colvars` in sec. 9.5 (biasing and analysis methods)
- `outputEnergy`: see definition of `outputEnergy` in sec. 9.5 (biasing and analysis methods)
- `writeTIPMF`: see definition of `writeTIPMF` in sec. 9.5 (biasing and analysis methods)
- `writeTISamples`: see definition of `writeTISamples` in sec. 9.5 (biasing and analysis methods)
- `stepZeroData`: see definition of `stepZeroData` in sec. 9.5 (biasing and analysis methods)
- `lowerWalls` < Position of the lower wall >
  Context: `colvar`
  Acceptable Values: Space-separated list of decimals
  Description: Defines the values ξ_{lower} below which a confining restraint on the colvar is applied to each colvar ξ.
- `upperWalls` < Position of the lower wall >
  Context: `colvar`
  Acceptable Values: Space-separated list of decimals
  Description: Defines the values ξ_{upper} above which a confining restraint on the colvar is applied to each colvar ξ.
- `forceConstant`: see definition of `forceConstant` in sec. 9.5.5 (Harmonic restraints)
- `lowerWallConstant` < Force constant for the lower wall >
  Context: `harmonicWalls`
  Acceptable Values: positive decimal
  Default Value: `forceConstant`
  Description: When both sets of walls are defined (lower and upper), this keyword allows setting different force constants for them. As with `forceConstant`, the specified constant is divided internally by the square of the specific `width` of each variable (see also the equivalent keyword for the harmonic restraint, `forceConstant` (see 9.5.5)). The force constant reported in the output as "k", and used in the change of force constant scheme, is the geometric mean of `upperWallConstant` and `upperWallConstant`.

- `upperWallConstant`: analogous to `lowerWallConstant`
- `targetForceConstant`: see definition of `targetForceConstant` in sec. 9.5.5 (harmonic restraints)
- `targetForceConstant` < Change the force constant(s) towards this value >
  Context: `harmonicWalls`
  Acceptable Values: positive decimal
  Description: This keyword allows changing either one or both of the wall force constants over time. In the case that `lowerWallConstant` and `upperWallConstant` have the same value, the behavior of this keyword is identical to the corresponding keyword in the `harmonic` restraint; otherwise, the change schedule is applied to the geometric mean of the two constant. When only one set of walls is defined (`lowerWall` or `upperWalls`), only the respective force constant is changed. **Note:** if only one of the two force constants is meant to change over time, it is possible to use two instances of `harmonicWalls`, and apply the changing schedule only to one of them.
- `targetNumSteps`: see definition of `targetNumSteps` in sec. 9.5.5 (harmonic restraints)
- `targetForceExponent`: see definition of `targetForceExponent` in sec. 9.5.5 (harmonic restraints)
- `targetEquilSteps`: see definition of `targetEquilSteps` in sec. 9.5.5 (harmonic restraints)
- `targetNumStages`: see definition of `targetNumStages` in sec. 9.5.5 (harmonic restraints)
- `lambdaSchedule`: see definition of `lambdaSchedule` in sec. 9.5.5 (harmonic restraints)
- `outputAccumulatedWork`: see definition of `outputAccumulatedWork` in sec. 9.5.5 (harmonic restraints)
- `bypassExtendedLagrangian` < Apply bias to actual colvars, bypassing extended coordinates >
  Context: `harmonicWalls`
  Acceptable Values: boolean
  Default Value: `on`
  Description: This option behaves as `bypassExtendedLagrangian` (see 9.5) for other biases, but **it defaults to `on`, unlike in the general case**. Thus, by default, the `harmonicWalls` bias applies to the actual colvars, so that the distribution of the colvar between the walls is unaffected by the bias, which then applies a flat-bottom potential *as a function of the colvar value*. This bias will affect the extended coordinate distribution near the walls. If `bypassExtendedLagrangian` is disabled, `harmonicWalls` applies a flat-bottom potential *as a function of the extended coordinate*. Conversely, this bias will then modify the distribution of the actual colvar value near the walls.

Example 1: harmonic walls for one variable with two different force constants.

```
harmonicWalls {
  name mywalls
  colvars dist
  lowerWall 22.0
```

```
  upperWall 38.0
  lowerWallConstant 2.0
  upperWallConstant 10.0
}
```

Example 2: harmonic walls for two variables with a single force constant.

```
harmonicWalls {
  name mywalls
  colvars phi psi
  lowerWall -180.0 0.0
  upperWall 0.0 180.0
  forceConstant 5.0
}
```

9.5.8 Linear restraints

The linear restraint biasing method is used to minimally bias a simulation. There is generally a unique strength of bias for each CV center, which means you must know the bias force constant specifically for the center of the CV. This force constant may be found by using experiment directed simulation described in section 9.5.9. Please cite Pitera and Chodera when using [87].

- `name`: see definition of `name` in sec. 9.5 (biasing and analysis methods)
- `colvars`: see definition of `colvars` in sec. 9.5 (biasing and analysis methods)
- `outputEnergy`: see definition of `outputEnergy` in sec. 9.5 (biasing and analysis methods)
- `forceConstant` < Scaled force constant (kcal/mol) >
  Context: `linear`
  Acceptable Values: positive decimal
  Default Value: `1.0`
  Description: This option defines a *scaled* force constant for the linear bias. To ensure consistency for multidimensional restraints, it is divided internally by the specific `width` of each variable (which is 1 by default), so that all variables are effectively dimensionless and of commensurate size. See also the equivalent keyword for the harmonic restraint, `forceConstant` (see 9.5.5). *The values of the actual force constants k/w_ξ are always printed when the restraint is defined.*
- `centers` < Initial linear restraint centers >
  Context: `linear`
  Acceptable Values: space-separated list of colvar values
  Description: These are analogous to the `centers` (see 9.5.5) keyword of the harmonic restraint. Although they do not affect dynamics, they are here necessary to ensure a well-defined energy for the linear bias.
- `writeTIPMF`: see definition of `writeTIPMF` in sec. 9.5 (biasing and analysis methods)
- `writeTISamples`: see definition of `writeTISamples` in sec. 9.5 (biasing and analysis methods)

- `targetForceConstant`: see definition of `targetForceConstant` in sec. 9.5.5 (Harmonic restraints)
- `targetNumSteps`: see definition of `targetNumSteps` in sec. 9.5.5 (Harmonic restraints)
- `targetForceExponent`: see definition of `targetForceExponent` in sec. 9.5.5 (Harmonic restraints)
- `targetEquilSteps`: see definition of `targetEquilSteps` in sec. 9.5.5 (Harmonic restraints)
- `targetNumStages`: see definition of `targetNumStages` in sec. 9.5.5 (Harmonic restraints)
- `lambdaSchedule`: see definition of `lambdaSchedule` in sec. 9.5.5 (Harmonic restraints)
- `outputAccumulatedWork`: see definition of `outputAccumulatedWork` in sec. 9.5.5 (Harmonic restraints)

9.5.9 Adaptive Linear Bias/Experiment Directed Simulation

Experiment directed simulation applies a linear bias with a changing force constant. Please cite White and Voth [117] when using this feature. As opposed to that reference, the force constant here is scaled by the `width` corresponding to the biased colvar. In White and Voth, each force constant is scaled by the colvars set center. The bias converges to a linear bias, after which it will be the minimal possible bias. You may also stop the simulation, take the median of the force constants (ForceConst) found in the colvars trajectory file, and then apply a linear bias with that constant. All the notes about units described in sections 9.5.8 and 9.5.5 apply here as well. **This is not a valid simulation of any particular statistical ensemble and is only an optimization algorithm until the bias has converged**.

- `name`: see definition of `name` in sec. 9.5 (biasing and analysis methods)
- `colvars`: see definition of `colvars` in sec. 9.5 (biasing and analysis methods)
- `centers` < Collective variable centers >
  Context: `alb`
  Acceptable Values: space-separated list of colvar values
  Description: The desired center (equilibrium values) which will be sought during the adaptive linear biasing. The number of values must be the number of requested colvars. Each value is a decimal number if the corresponding colvar returns a scalar, a "`(x, y, z)`" triplet if it returns a unit vector or a vector, and a "`q0, q1, q2, q3)`" quadruplet if it returns a rotational quaternion. If a colvar has periodicities or symmetries, its closest image to the restraint center is considered when calculating the linear potential.
- `updateFrequency` < The duration of updates >
  Context: `alb`
  Acceptable Values: An integer
  Description: This is, N, the number of simulation steps to use for each update to the bias. This determines how long the system requires to equilibrate after a change in force constant ($N/2$), how long statistics are collected for an iteration ($N/2$), and how quickly energy is added to the system (at most, $A/2N$, where A is the `forceRange`). Until the force constant

has converged, the method as described is an optimization procedure and not an integration of a particular statistical ensemble. It is important that each step should be uncorrelated from the last so that iterations are independent. Therefore, N should be at least twice the autocorrelation time of the collective variable. The system should also be able to dissipate energy as fast as $N/2$, which can be done by adjusting thermostat parameters. Practically, N has been tested successfully at significantly shorter than the autocorrelation time of the collective variables being biased and still converge correctly.

- `forceRange` < The expected range of the force constant in units of energy >
  Context: `alb`
  Acceptable Values: A space-separated list of decimal numbers
  Default Value: 3 k_bT
  Description: This is largest magnitude of the force constant which one expects. If this parameter is too low, the simulation will not converge. If it is too high the simulation will waste time exploring values that are too large. A value of 3 k_bT has worked well in the systems presented as a first choice. This parameter is dynamically adjusted over the course of a simulation. The benefit is that a bad guess for the forceRange can be corrected. However, this can lead to large amounts of energy being added over time to the system. To prevent this dynamic update, add `hardForceRange yes` as a parameter

- `rateMax` < The maximum rate of change of force constant >
  Context: `alb`
  Acceptable Values: A list of space-separated real numbers
  Description: This optional parameter controls how much energy is added to the system from this bias. Tuning this separately from the `updateFrequency` and `forceRange` can allow for large bias changes but with a low `rateMax` prevents large energy changes that can lead to instability in the simulation.

9.5.10 Multidimensional histograms

The `histogram` feature is used to record the distribution of a set of collective variables in the form of a N-dimensional histogram. A `histogram` block may define the following parameters:

- `name`: see definition of `name` in sec. 9.5 (biasing and analysis methods)
- `colvars`: see definition of `colvars` in sec. 9.5 (biasing and analysis methods)
- `outputFreq`: see definition of `outputFreq` in sec. 9.5 (biasing and analysis methods)
- `stepZeroData`: see definition of `stepZeroData` in sec. 9.5 (biasing and analysis methods)
- `outputFile` < Write the histogram to a file >
  Context: `histogram`
  Acceptable Values: UNIX filename
  Default Value: *outputName*.<`name`>`.dat`
  Description: Name of the file containing histogram data (multicolumn format), which is written every `outputFreq` (see 9.5) steps. For the special case of 2 variables, Gnuplot may be used to visualize this file. If `outputFile` is set to `none`, the file is not written.

- `outputFileDX` < Write the histogram to a file >
  Context: `histogram`
  Acceptable Values: UNIX filename
  Default Value: *outputName*.<`name`>.`dx`
  Description: Name of the file containing histogram data (OpenDX format), which is written every `outputFreq` (see 9.5) steps. For the special case of 3 variables, VMD may be used to visualize this file. This file is written by default if the dimension is 3 or more. If `outputFileDX` is set to `none`, the file is not written.

- `gatherVectorColvars` < Treat vector variables as multiple observations of a scalar variable? >
  Context: `histogram`
  Acceptable Values: UNIX filename
  Default Value: `off`
  Description: When this is set to `on`, the components of a multi-dimensional colvar (e.g. one based on `cartesian`, `distancePairs`, or a vector of scalar numbers given by `scriptedFunction`) are treated as multiple observations of a scalar variable. This results in the histogram being accumulated multiple times for each simulation step). When multiple vector variables are included in `histogram`, these must have the same length because their components are accumulated together. For example, if ξ, λ and τ are three variables of dimensions 5, 5 and 1, respectively, for each iteration 5 triplets (ξ_i, λ_i, τ) $(i = 1, \ldots 5)$ are accumulated into a 3-dimensional histogram.

- `weights` < Treat vector variables as multiple observations of a scalar variable? >
  Context: `histogram`
  Acceptable Values: list of space-separated decimals
  Default Value: all weights equal to 1
  Description: When `gatherVectorColvars` is `on`, the components of each multi-dimensional colvar are accumulated with a different weight. For example, if x and y are two distinct `cartesian` variables defined on the same group of atoms, the corresponding 2D histogram can be weighted on a per-atom basis in the definition of `histogram`.

As with any other biasing and analysis method, when a histogram is applied to an extended-system colvar (9.3.20), it accesses the value of the extended coordinate rather than that of the actual colvar. This can be overridden by enabling the `bypassExtendedLagrangian` (see 9.5) option. A *joint histogram* of the actual colvar and the extended coordinate may be collected by specifying the colvar name twice in a row in the `colvars` parameter (e.g. `colvars myColvar myColvar`): the first instance will be understood as the actual colvar, and the second, as the extended coordinate.

- `bypassExtendedLagrangian`: see definition of `bypassExtendedLagrangian` in sec. 9.5 (biasing and analysis methods)

Grid definition for multidimensional histograms Like the ABF and metadynamics biases, `histogram` uses the parameters `lowerBoundary`, `upperBoundary`, and `width` to define its grid. These values can be overridden if a configuration block `histogramGrid { ...}` is provided inside the configuration of `histogram`. The options supported inside this configuration block are:

- `lowerBoundaries` < Lower boundaries of the grid >
  Context: `histogramGrid`

Acceptable Values: list of space-separated decimals
Description: This option defines the lower boundaries of the grid, overriding any values defined by the `lowerBoundary` keyword of each colvar. Note that when `gatherVectorColvars` is `on`, each vector variable is automatically treated as a scalar, and a single value should be provided for it.

- `upperBoundaries`: analogous to `lowerBoundaries`
- `widths`: analogous to `lowerBoundaries`

9.5.11 Probability distribution-restraints

The `histogramRestraint` bias implements a continuous potential of many variables (or of a single high-dimensional variable) aimed at reproducing a one-dimensional statistical distribution that is provided by the user. The M variables $(\xi_1, \dots, \xi_M)$ are interpreted as multiple observations of a random variable ξ with unknown probability distribution. The potential is minimized when the histogram $h(\xi)$, estimated as a sum of Gaussian functions centered at $(\xi_1, \dots, \xi_M)$, is equal to the reference histogram $h_0(\xi)$:

$$V(\xi_1, \dots, \xi_M) = \frac{1}{2} k \int (h(\xi) - h_0(\xi))^2 \, \mathrm{d}\xi \tag{75}$$

$$h(\xi) = \frac{1}{M\sqrt{2\pi\sigma^2}} \sum_{i=1}^{M} \exp\left(-\frac{(\xi - \xi_i)^2}{2\sigma^2} \right) \tag{76}$$

When used in combination with a `distancePairs` multi-dimensional variable, this bias implements the refinement algorithm against ESR/DEER experiments published by Shen *et al* [98].

This bias behaves similarly to the `histogram` bias with the `gatherVectorColvars` option, with the important difference that *all* variables are gathered, resulting in a one-dimensional histogram. Future versions will include support for multi-dimensional histograms.

The list of options is as follows:

- `name`: see definition of `name` in sec. 9.5 (biasing and analysis methods)
- `colvars`: see definition of `colvars` in sec. 9.5 (biasing and analysis methods)
- `outputEnergy`: see definition of `outputEnergy` in sec. 9.5 (biasing and analysis methods)
- `lowerBoundary` < Lower boundary of the colvar grid >
  Context: `histogramRestraint`
  Acceptable Values: decimal
  Description: Defines the lowest end of the interval where the reference distribution $h_0(\xi)$ is defined. Exactly one value must be provided, because only one-dimensional histograms are supported by the current version.
- `upperBoundary`: analogous to `lowerBoundary`
- `width` < Width of the colvar grid >
  Context: `histogramRestraint`
  Acceptable Values: positive decimal
  Description: Defines the spacing of the grid where the reference distribution $h_0(\xi)$ is defined.

- `gaussianSigma` < Standard deviation of the approximating Gaussian >
  Context: `histogramRestraint`
  Acceptable Values: positive decimal
  Default Value: $2 \times$ `width`
  Description: Defines the parameter σ in eq. 76.

- `forceConstant` < Force constant (kcal/mol) >
  Context: `histogramRestraint`
  Acceptable Values: positive decimal
  Default Value: `1.0`
  Description: Defines the parameter k in eq. 75.

- `refHistogram` < Reference histogram $h_0(\xi)$ >
  Context: `histogramRestraint`
  Acceptable Values: space-separated list of M positive decimals
  Description: Provides the values of $h_0(\xi)$ consecutively. The mid-point convention is used, i.e. the first point that should be included is for $\xi =$ `lowerBoundary`+`width`/2. If the integral of $h_0(\xi)$ is not normalized to 1, $h_0(\xi)$ is rescaled automatically before use.

- `refHistogramFile` < Reference histogram $h_0(\xi)$ >
  Context: `histogramRestraint`
  Acceptable Values: UNIX file name
  Description: Provides the values of $h_0(\xi)$ as contents of the corresponding file (mutually exclusive with `refHistogram`). The format is that of a text file, with each line containing the space-separated values of ξ and $h_0(\xi)$. The same numerical conventions as `refHistogram` are used.

- `writeHistogram` < Periodically write the instantaneous histogram $h(\xi)$ >
  Context: `metadynamics`
  Acceptable Values: boolean
  Default Value: `off`
  Description: If `on`, the histogram $h(\xi)$ is written every `colvarsRestartFrequency` steps to a file with the name *outputName*`.<name>.hist.dat`This is useful to diagnose the convergence of $h(\xi)$ against $h_0(\xi)$.

9.5.12 Defining scripted biases

Rather than using the biasing methods described above, it is possible to apply biases provided at run time as a Tcl script, in the spirit of `TclForces`.

- `scriptedColvarForces` < Enable custom, scripted forces on colvars >
  Context: global
  Acceptable Values: boolean
  Default Value: `off`
  Description: If this flag is enabled, a Tcl procedure named `calc_colvar_forces` accepting one parameter should be defined by the user. It is executed at each timestep, with the current step number as parameter, between the calculation of colvars and the application of bias forces. This procedure may use the `cv` command to access the values of colvars (e.g. `cv colvar xi value`), apply forces on them (`cv colvar xi addforce $F`) or add energy

to the simulation system (`cv addenergy $E`), effectively defining custom collective variable biases.

9.5.13 Performance of scripted biases

If concurrent computation over multiple threads is available (this is indicated by the message "SMP parallelism is available." printed at initialization time), it is useful to take advantage of the scripting interface to combine many components, all computed in parallel, into a single variable.

The default SMP schedule is the following:

1. distribute the computation of all components across available threads;
2. on a single thread, collect the results of multi-component variables using polynomial combinations (see 9.3.15), or custom functions (see 9.3.16), or scripted functions (see 9.3.17);
3. distribute the computation of all biases across available threads;
4. compute on a single thread any scripted biases implemented via the keyword `scriptedColvarForces` (see 9.5.12).
5. communicate on a single thread forces to NAMD.

The following options allow to fine-tune this schedule:

- `scriptingAfterBiases` < Scripted colvar forces need updated biases? >
  Context: global
  Acceptable Values: boolean
  Default Value: `on`
  Description: This flag specifies that the `calc_colvar_forces` procedure (last step in the list above) is executed only after all biases have been updated (next-to-last step) For example, this allows using the energy of a restraint bias, or the force applied on a colvar, to calculate additional scripted forces, such as boundary constraints. When this flag is set to `off`, it is assumed that only the values of the variables (but not the energy of the biases or applied forces) will be used by `calc_colvar_forces`: this can be used to schedule the calculation of scripted forces and biases concurrently to increase performance.

9.6 Scripting interface (Tcl): list of commands

This section lists all the commands used in NAMD to control the behavior of the Colvars module from within a run script.

9.6.1 Commands to manage the Colvars module

- `cv addenergy <E>`
  `Add an energy to the MD engine (no effect in VMD)`
  `Parameters`
  `E : float - Amount of energy to add`
- `cv config <conf>`
  `Read configuration from the given string`
  `Parameters`
  `conf : string - Configuration string`

- cv configfile <conf_file>
  Read configuration from a file
  Parameters
  conf_file : string - Path to configuration file

- cv delete
  Delete this Colvars module instance (VMD only)

- cv frame [frame]
  Get or set current frame number (VMD only)
  Parameters
  frame : integer - Frame number (optional)

- cv getconfig
  Get the module's configuration string read so far

- cv getenergy
  Get the current Colvars energy

- cv help [command]
  Get the help string of the Colvars scripting interface
  Parameters
  command : string - Get the help string of this specific command (optional)

- cv list [param]
  Return a list of all variables or biases
  Parameters
  param : string - "colvars" or "biases"; default is "colvars" (optional)

- cv listcommands
  Get the list of script functions, prefixed with "cv_", "colvar_" or "bias_"

- cv load <prefix>
  Load data from a state file into all matching colvars and biases
  Parameters
  prefix : string - Path to existing state file or input prefix

- cv loadfromstring <buffer>
  Load state data from a string into all matching colvars and biases
  Parameters
  buffer : string - String buffer containing the state information

- cv molid [molid]
  Get or set the molecule ID on which Colvars is defined (VMD only)
  Parameters
  molid : integer - Molecule ID; -1 means undefined (optional)

- cv printframe
  Return the values that would be written to colvars.traj

- cv printframelabels
  Return the labels that would be written to colvars.traj
- cv reset
  Delete all internal configuration
- cv resetindexgroups
  Clear the index groups loaded so far, allowing to replace them
- cv save <prefix>
  Change the prefix of all output files and save them
  Parameters
  prefix : string - Output prefix with trailing ".colvars.state" gets removed)
- cv savetostring
  Write the Colvars state to a string and return it
- cv units [units]
  Get or set the current Colvars unit system
  Parameters
  units : string - The new unit system (optional)
- cv update
  Recalculate colvars and biases
- cv version
  Get the Colvars Module version number

9.6.2 Commands to manage individual collective variables

- cv colvar name addforce <force>
  Apply the given force onto this colvar and return the same
  Parameters
  force : float or array - Applied force; must match colvar dimensionality
- cv colvar name cvcflags <flags>
  Enable or disable individual components by setting their active flags
  Parameters
  flags : integer array - Zero/nonzero value disables/enables the CVC
- cv colvar name delete
  Delete this colvar, along with all biases that depend on it
- cv colvar name get <feature>
  Get the value of the given feature for this colvar
  Parameters
  feature : string - Name of the feature
- cv colvar name getappliedforce
  Return the total of the forces applied to this colvar

- cv colvar name getatomgroups
  Return the atom indices used by this colvar as a list of lists

- cv colvar name getatomids
  Return the list of atom indices used by this colvar

- cv colvar name getconfig
  Return the configuration string of this colvar

- cv colvar name getgradients
  Return the atomic gradients of this colvar

- cv colvar name gettotalforce
  Return the sum of internal and external forces to this colvar

- cv colvar name help [command]
  Get a help summary or the help string of one colvar subcommand
  Parameters
  command : string - Get the help string of this specific command (optional)

- cv colvar name modifycvcs <confs>
  Modify configuration of individual components by passing string arguments
  Parameters
  confs : sequence of strings - New configurations; empty strings are skipped

- cv colvar name run_ave
  Get the current running average of the value of this colvar

- cv colvar name set <feature> <value>
  Set the given feature of this colvar to a new value
  Parameters
  feature : string - Name of the feature
  value : string - String representation of the new feature value

- cv colvar name state
  Print a string representation of the feature state of this colvar

- cv colvar name type
  Get the type description of this colvar

- cv colvar name update
  Recompute this colvar and return its up-to-date value

- cv colvar name value
  Get the current value of this colvar

- cv colvar name width
  Get the width of this colvar

9.6.3 Commands to manage individual biases

- cv bias name bin
  Get the current grid bin index (1D ABF only for now)

- cv bias name bincount [index]
  Get the number of samples at the given grid bin (1D ABF only for now)
  Parameters
  index : integer - Grid index; defaults to current bin (optional)

- cv bias name binnum
  Get the total number of grid points of this bias (1D ABF only for now)

- cv bias name delete
  Delete this bias

- cv bias name energy
  Get the current energy of this bias

- cv bias name get <feature>
  Get the value of the given feature for this bias
  Parameters
  feature : string - Name of the feature

- cv bias name getconfig
  Return the configuration string of this bias

- cv bias name help [command]
  Get a help summary or the help string of one bias subcommand
  Parameters
  command : string - Get the help string of this specific command (optional)

- cv bias name load <prefix>
  Load data into this bias
  Parameters
  prefix : string - Read from a file with this name or prefix

- cv bias name loadfromstring <buffer>
  Load state data into this bias from a string
  Parameters
  buffer : string - String buffer containing the state information

- cv bias name save <prefix>
  Save data from this bias into a file with the given prefix
  Parameters
  prefix : string - Prefix for the state file of this bias

- cv bias name savetostring
  Save data from this bias into a string and return it

- `cv bias name set <feature> <value>`
  `Set the given feature of this bias to a new value`
  `Parameters`
  `feature : string - Name of the feature`
  `value : string - String representation of the new feature value`

- `cv bias name share`
  `Share bias information with other replicas (multiple-walker scheme)`

- `cv bias name state`
  `Print a string representation of the feature state of this bias`

- `cv bias name update`
  `Recompute this bias and return its up-to-date energy`

9.7 Syntax changes from older versions

The following is a list of syntax changes in Colvars since its first release. Many of the older keywords are still recognized by the current code, thanks to specific compatibility code. *This is not a list of new features:* its primary purpose is to make you aware of those improvements that affect the use of old configuration files with new versions of the code.
Note: if you are using any of the NAMD and VMD tutorials:
https://www.ks.uiuc.edu/Training/Tutorials/
please be aware that *several of these tutorials are not actively maintained*: for those cases, this list will help you reconcile any inconsistencies.

- **Colvars version 2016-06-09 or later (NAMD version 2.12b1 or later).**
  The legacy keyword `refPositionsGroup` has been renamed `fittingGroup` (see 9.4.2) for clarity (the legacy version is still supported).

- **Colvars version 2016-08-10 or later (NAMD version 2.12b1 or later).**
  "System forces" have been replaced by "total forces" (see for example `outputTotalForce` (see 9.3.19)). See the following page for more information:
  https://colvars.github.io/README-totalforce.html

- **Colvars version 2017-01-09 or later (NAMD version 2.13b1 or later).**
  A new type of restraint, `harmonicWalls` (see 9.5.7), replaces and improves upon the legacy keywords `lowerWall` and `upperWall`: these are still supported as short-hands.

- **Colvars version 2018-11-15 or later (NAMD version 2.14b1 or later).**
  The global `analysis` keyword has been discontinued: specific analysis tasks are controlled directly by the keywords `corrFunc` (see 9.3.23) and `runAve` (see 9.3.23), which continue to remain `off` by default.

- **Colvars version 2020-02-25 or later (NAMD version 2.14b1 or later).**
  The parameter `hillWidth` (see 9.5.4), expressing the Gaussian width 2σ in relative units (number of grid points), does not have a default value any more. A new alternative parameter `gaussianSigmas` (see 9.5.4) allows setting the σ parameters explicitly for each variable if needed.
  Furthermore, to facilitate the use of other analysis tools such as for example `sum_hills`:

https://www.plumed.org/doc-v2.6/user-doc/html/sum_hills.html
the format of the file written by `writeHillsTrajectory` (see 9.5.4) has also been changed to use σ instead of 2σ. This change does not affect how the biasing potential is written in the state file, or the simulated trajectory.

- **Colvars version 2020-02-25 or later (NAMD version 2.14b1 or later).**
  The legacy keywords `lowerWall` and `upperWall` of a `colvar` definition block do not have default values any longer, and need to be set explicitly, preferably as part of the `harmonicWalls` restraint. When using an ABF bias, it is recommended to set the two walls equal to `lowerBoundary` (see 9.3.18) and `upperBoundary` (see 9.3.18), respectively. When using a metadynamics bias, it is recommended to set the two walls strictly *within* `lowerBoundary` (see 9.3.18) and `upperBoundary` (see 9.3.18); see 9.5.4 for details.

Up-to-date documentation can always be accessed at:
https://colvars.github.io/colvars-refman-namd/colvars-refman-namd.html

10 Alchemical Free Energy Methods[1]

Alchemical free energy calculations model the physically impossible but computationally realizable process of gradually mutating a subset of atoms of a system from one state to another, through a series of intermediate steps. Two alternative methods for alchemical calculation of free energies from molecular dynamics simulation are available in NAMD: Free energy perturbation (FEP) and thermodynamic integration (TI).

10.1 Theoretical Background

Free energy differences can be obtained through four different routes: (i) probability densities, (ii) free energy perturbation, (iii) thermodynamic integration, or (iv) nonequilibrium work approaches [22]. Within NAMD, alchemical transformations are modeled following the second and the third routes, both of which rely upon the use of a general extent parameter often referred to as the coupling parameter [8, 71, 54, 55] for the description of chemical changes in the molecular systems between the reference and the target states.

10.1.1 The dual–topology paradigm

In a typical alchemical transformation setup involving the alteration of one chemical species into an alternate one in the course of the simulation, the atoms in the molecular topology can be classified into three groups, (i) a group of atoms that do not change during the simulation — *e.g.* the environment, (ii) the atoms describing the reference state, a, of the system, and (iii) the atoms that correspond to the target state, b, at the end of the alchemical transformation. The atoms representative of state a should *never* interact with those of state b throughout the MD simulation. Such a setup, in which atoms of both the initial and the final states of the system are present in the molecular topology file — *i.e.* the `psf` file — is characteristic of the so–called "dual topology" paradigm [37, 86, 3]. The hybrid Hamiltonian of the system is a function of the general extent parameter, λ, which connects smoothly state a to state b. In the simplest case, such a connection may be achieved by linear combination of the corresponding Hamiltonians:

$$\mathcal{H}(\mathbf{x}, \mathbf{p}_x; \lambda) = \mathcal{H}_0(\mathbf{x}, \mathbf{p}_x) + \lambda \mathcal{H}_b(\mathbf{x}, \mathbf{p}_x) + (1 - \lambda)\mathcal{H}_a(\mathbf{x}, \mathbf{p}_x) \tag{77}$$

where $\mathcal{H}_a(\mathbf{x}, \mathbf{p}_x)$ describes the interaction of the group of atoms representative of the reference state, a, with the rest of the system. $\mathcal{H}_b(\mathbf{x}, \mathbf{p}_x)$ characterizes the interaction of the target topology, b, with the rest of the system. $\mathcal{H}_0(\mathbf{x}, \mathbf{p}_x)$ is the Hamiltonian describing those atoms that do not undergo any transformation during the MD simulation.

For instance, in the point mutation of an alanine side chain into that of glycine, by means of a free energy calculation — either free energy perturbation or thermodynamic integration, the topology of both the methyl group of alanine and the hydrogen borne by the C_α in glycine co–exist throughout the simulation (see Figure 6), yet without actually seeing each other.

The energy and forces are defined as a function of λ, in such a fashion that the interaction of the methyl group of alanine with the rest of the protein is effective at the beginning of the simulation, *i.e.* $\lambda = 0$, while the glycine C_α hydrogen atom does not interact with the rest of the protein, and *vice versa* at the end of the simulation, *i.e.* $\lambda = 1$. For intermediate values of λ, both the alanine

[1] The features described in this section were contributed by Surjit B. Dixit, Christophe Chipot (Nancy Université, Université Henri Poincaré, France), Floris Buelens (Institute of Structural and Molecular Biology, University of London, Birkbeck, UK), and Christopher Harrison (University of Illinois, Urbana, IL USA).

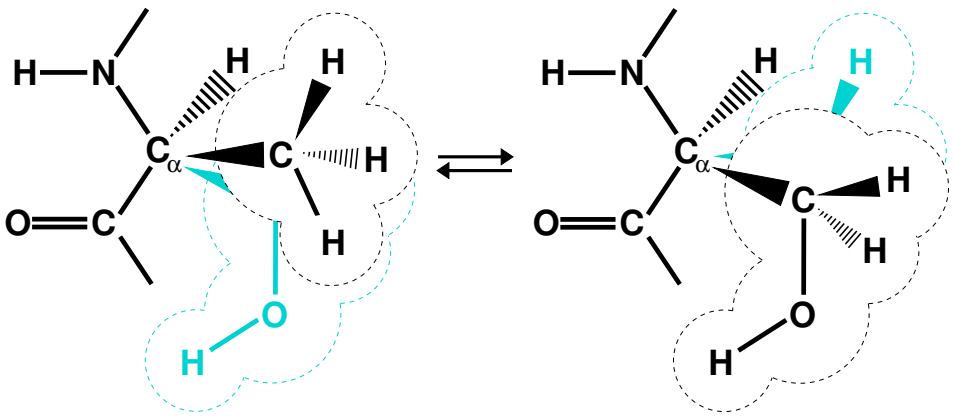


Figure 6: Dual topology description for an alchemical simulation. Case example of the mutation of alanine into serine. The lighter color denotes the non–interacting, alternate state.

and the glycine side chains participate in nonbonded interactions with the rest of the protein, scaled on the basis of the current value of λ. It should be clearly understood that these side chains never interact with each other.

It is noteworthy that end points of alchemical transformations carried out in the framework of the dual–topology paradigm have been shown to be conducive to numerical instabilities from molecular dynamics simulations, often coined as "end–point catastrophes". These scenarios are prone to occur when λ becomes close to 0 or 1, and incoming atoms instantly appear where other particles are already present, which results in a virtually infinite potential as the interatomic distance tends towards 0. Such "end–point catastrophes" can be profitably circumvented by introducing a so–called soft–core potential [7, 67], aimed at a gradual scaling of the short–range nonbonded interactions of incoming atoms with their environment, as shown in Equation 78. What is really being modified is the value of a coupling parameter (λ_{LJ} or λ_{elec}) that scales the interactions — *i.e.*, if set to 0, the latter are off; if set to 1, they are on — in lieu of the actual value of λ provided by the user.

$$V_{\mathrm{NB}}(r_{ij}) = \lambda_{\mathrm{LJ}}\varepsilon_{ij}\left[\left(\frac{R_{ij}^{\min\,2}}{r_{ij}^2+\delta(1-\lambda_{\mathrm{LJ}})}\right)^6 - \left(\frac{R_{ij}^{\min\,2}}{r_{ij}^2+\delta(1-\lambda_{\mathrm{LJ}})}\right)^3\right] + \lambda_{\mathrm{elec}}\frac{q_i q_j}{\varepsilon_1 r_{ij}} \qquad (78)$$

It is also worth noting that the free energy calculation does not alter intermolecular bonded potentials, *e.g.* bond stretch, valence angle deformation and torsions, in the course of the simulation. In calculations targeted at the estimation of free energy differences between two states characterized by distinct environments — *e.g.* a ligand, bound to a protein in the first simulation, and solvated in water, in the second — as is the case for most free energy calculations that make use of a thermodynamic cycle, perturbation of intramolecular terms may, by and large, be safely avoided [11]. This property is controlled by the `alchDecouple` keyword described in

10.1.2 Free Energy Perturbation

Within the FEP framework [8, 21, 22, 38, 57, 71, 109, 112, 122], the free energy difference between two alternate states, a and b, is expressed by:

$$\Delta A_{a\to b} = -\frac{1}{\beta}\ \ln\left\langle \exp\left\{-\beta\left[\mathcal{H}_b(\mathbf{x},\mathbf{p}_x) - \mathcal{H}_a(\mathbf{x},\mathbf{p}_x)\right]\right\}\right\rangle_a \tag{79}$$

Here, $\beta^{-1} \equiv k_B T$, where k_B is the Boltzmann constant, T is the temperature. $\mathcal{H}_a(\mathbf{x},\mathbf{p}_x)$ and $\mathcal{H}_b(\mathbf{x},\mathbf{p}_x)$ are the Hamiltonians describing states a and b, respectively. $\langle\cdots\rangle_a$ denotes an ensemble average over configurations representative of the initial, reference state, a.

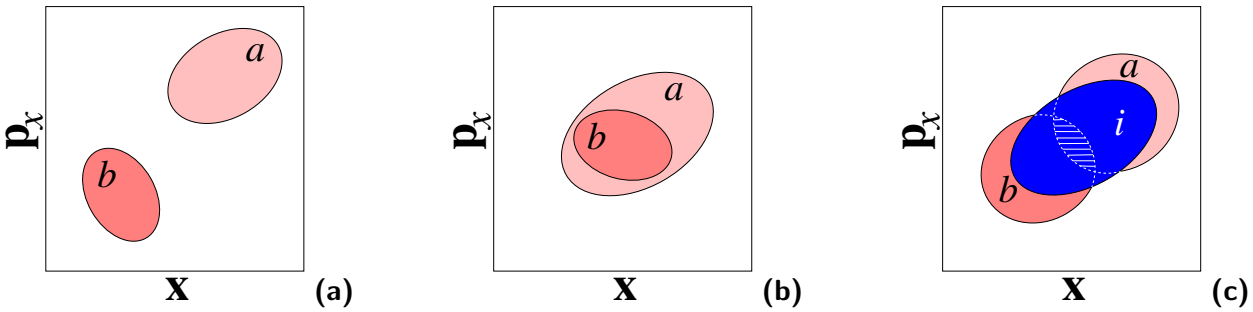


Figure 7: Convergence of an FEP calculation. If the ensembles representative of states a and b are too disparate, equation (79) will not converge **(a)**. If, in sharp contrast, the configurations of state b form a subset of the ensemble of configurations characteristic of state a, the simulation is expected to converge **(b)**. The difficulties reflected in case **(a)** may be alleviated by the introduction of mutually overlapping intermediate states that connect a to b **(c)**. It should be mentioned that in practice, the kinetic contribution, $\mathcal{T}(\mathbf{p}_x)$, is assumed to be identical for state a and state b.

Convergence of equation (79) implies that low–energy configurations of the target state, b, are also configurations of the reference state, a, thus resulting in an appropriate overlap of the corresponding ensembles — see Figure 7. Transformation between the two thermodynamic states is replaced by a series of transformations between non–physical, intermediate states along a well–delineated pathway that connects a to b. This pathway is characterized by the general extent parameter [8, 54, 55, 71], λ, that makes the Hamiltonian and, hence, the free energy, a continuous function of this parameter between a and b:

$$\Delta A_{a\to b} = -\frac{1}{\beta}\ \sum_{i=1}^{N} \ln\left\langle \exp\left\{-\beta\left[\mathcal{H}(\mathbf{x},\mathbf{p}_x;\lambda_{i+1}) - \mathcal{H}(\mathbf{x},\mathbf{p}_x;\lambda_i)\right]\right\}\right\rangle_i \tag{80}$$

Here, N stands for the number of intermediate stages, or "windows" between the initial and the final states — see Figure 7.

10.1.3 Thermodynamic Integration

An alternative to the perturbation formula for free energy calculation is Thermodynamic Integration (TI). With the TI method, the free energy is given as [55, 108, 35]:

$$\Delta A = \int_0^1 \left\langle \frac{\partial \mathcal{H}(\mathbf{x},\mathbf{p}_x;\lambda)}{\partial \lambda} \right\rangle_\lambda d\lambda \tag{81}$$

In the multi-configuration thermodynamic integration approach [108] implemented in NAMD, $\langle \partial \mathcal{H}(\mathbf{x}, \mathbf{p}_x; \lambda)/\partial\lambda \rangle_\lambda$, the ensemble average of the derivative of the internal energy with respect to λ, is collected for a series of discrete λ values and written to `tiOutFile`. These values are analyzed by the separately distributed script `NAMD_ti.pl`, which performs the integration of individual energy components and reports back the total ΔA values for the transformation.

10.2 Implementation of the free energy methods in NAMD

The procedures implemented in NAMD are particularly adapted for performing free energy calculations that split the λ reaction path into a number of non–physical, intermediate states, or "windows". Separate simulations can be started for each window. Alternatively, the Tcl scripting ability of NAMD can be employed advantageously to perform the complete simulation in a single run. An example, making use of such a script, is supplied at the end of this section. However, **the setup of sequential alchemical trsnaformations can be simplified** by calling the script library `fep.tcl`, found in the `lib/alch` directory of the NAMD distribution. This library provides two helper procedures, `runFEP` to run a series of evenly spaced windows, and `runFEPlist` to specify a list of λ values to be sampled.

The following keywords can be used to run alchemical free energy calculations, whether FEP or TI.

- `alch` < Is an alchemical transformation to be performed? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Turns on alchemical transformation methods in NAMD.

- `alchType` < Which method is to be employed for the alchemical transformation? >
  Acceptable Values: `fep` or `ti`
  Default Value: `ti`
  Description: Turns on Hamiltonian scaling and ensemble averaging for alchemical FEP or TI.

- `alchWCA` < Turn on/off Weeks-Chandler-Andersen (WCA) decomposition. >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: When active, WCA decomposition changes the lambda dependence of the van der Waals perturbation following the repulsion/dispersion scheme proposed by Deng and Roux [30]. For example, for appearing atoms, all interactions are still fully coupled at `alchLambda` = `alchVdwLambdaEnd`, but repulsive components are instead fully coupled according to the new `alchRepLambdaEnd` keyword. No dispersive interactions (including terms from `LJcorrection`) are coupled until the repulsive interactions are fully coupled. By virtue of the formulation, `alchVdwShiftCoeff` does not have any effect in this scheme and any non-zero values are ignored. Note that this scheduling is completely separate from electrostatic coupling and the two may overlap in any way desired (this may not be stable!). In order to achieve the exact decoupling scheme proposed by Deng and Roux, one ought to set `alchRepLambdaEnd` < `alchVdwLambdaEnd` = `alchElecLambdaStart` < 1. **This scheme has only been widely tested when a single alchemical group is being used.** Due to current limitations, this scheme is not available when `alchType` is set to `ti`.

- `alchLambda` < Current value of the coupling parameter >
  Acceptable Values: positive decimal between 0.0 and 1.0
  Description: The coupling parameter value determining the progress of the perturbation for FEP or TI. This parameter is unnecessary when using the `runFEP` procedure of `fep.tcl`.

- `alchLambda2` < Forward projected value of the coupling parameter >
  Acceptable Values: positive decimal between 0.0 and 1.0
  Description: The `lambda2` value corresponds to the coupling parameter to be used for sampling in the next window. The free energy difference between `alchLambda2` and `alchLambda` is calculated. Through simulations at progressive values of `alchLambda` and `alchLambda2` the total free energy difference may be determined. This parameter is unnecessary when using the `runFEP` procedure of `fep.tcl`.

- `alchLambdaIDWS` < Backward value of the coupling parameter for Interleaved Double-Wide Sampling >
  Acceptable Values: decimal between 0.0 and 1.0, negative to disable
  Description: Setting this parameter between 0 and 1 activates Interleaved Double-Wide Sampling (IDWS), whereby the target lambda value alternates between `alchLambda2` and `alchLambdaIDWS`. The switch occurs every `fullElectFrequency` steps if defined, or `nonbondedFrequency` otherwise. Setting this parameter to a negative value (including between run statements) disables IDWS. When IDWS is active, the alchemy output file contains `FepEnergy` line headers for the forward energy differences, and `FepE_Back` for backward energy differences. FEP free energy estimates given in output are based on forward data only. The fepout file can be postprocessed with the python script `deinterleave_idws.py`, found in the `lib/alch` directory of the NAMD distribution. This tool produces separate fepout files for the forward and backwards samples, suitable for computing e.g. a Bennett's Acceptance Ratio (BAR) estimate of the free energy difference. When using the `runFEP` or `runFEPlist` procedure of `fep.tcl`, IDWS can be enabled simply by adding a `true` flag to the argument list.

- `alchEquilSteps` < Number of equilibration steps in a window, prior to data collection >
  Acceptable Values: positive integer less than `numSteps` or `run`
  Default Value: 0
  Description: In each window `alchEquilSteps` steps of equilibration can be performed before ensemble averaging is initiated. The output also contains the data gathered during equilibration and is meant for analysis of convergence properties of the alchemical free energy calculation.

- `alchFile` < `pdb` file with perturbation flags >
  Acceptable Values: filename
  Default Value: coordinates
  Description: `pdb` file to be used for indicating the status of all atoms pertaining to the system, with respect to the alchemical transformation. If this parameter is not declared specifically, then the `pdb` file specified by `coordinates` is utilized for this information.

- `alchCol` < Column in the `alchFile` that carries the perturbation flag >
  Acceptable Values: X, Y, Z, O or B
  Default Value: B

Description: Column of the `pdb` file to use for retrieving the status of each atom, *i.e.* a flag that indicates which atom will be perturbed in the course of the alchemical transformation. A value of `-1` in the specified column indicates that the atom will vanish as λ moves from 0 to 1, whereas a value of `1` indicates that it will grow.

- `alchOutFreq` < Frequency of free energy output in time–steps >
  Acceptable Values: positive integer
  Default Value: 5
  Description: Every `alchOutFreq` number of MD steps, the output file `alchOutFile` is updated by dumping energies that are used for ensemble averaging. This variable could be set to `1` to include all the configurations for ensemble averaging. Yet, it is recommended to update `alchOutFile` energies at longer intervals to avoid large files containing highly correlated data, unless a post–treatment, *e.g.* Bennett's acceptance ratio (BAR) [5] or simple overlap sampling (SOS) [65], is to be performed.

- `alchOutFile` < Alchemical free energy output filename >
  Acceptable Values: filename
  Default Value: `outfilename`
  Description: An output file named `alchOutFile`, containing the FEP energies, or `tiOutFile`, containing the TI derivatives, dumped every `alchOutFreq` steps.

- `alchVdwShiftCoeff` < Soft-core van der Waals radius-shifting coefficient >
  Acceptable Values: positive decimal
  Default Value: 5
  Description: This is a radius-shifting coefficient of λ that is used to construct the modified vdW interactions during alchemical free energy calculations. Providing a positive value for `alchVdWShiftCoeff` ensures that the vdW potential is finite everywhere for small values of λ, which significantly improves the accuracy and convergence of FEP and TI calculations, and also prevents overlapping particles from making the simulation unstable. During FEP and TI, assuming $\lambda = 0$ denotes an absence of interaction, the interatomic distances used in the Lennard-Jones potential are shifted according to [7, 67]: $r^2 \rightarrow r^2 + \texttt{alchVdWShiftCoeff} \times (1 - \lambda)$

- `alchElecLambdaStart` < Value of λ to introduce electrostatic interactions >
  Acceptable Values: positive decimal
  Default Value: 0.5
  Description: In order to avoid the so–called "end-point catastrophes", it is crucial to avoid situations where growing particles overlap with existing particles with an unbounded interaction potential, which would approach infinity as the interaction distance approaches zero [7, 22]. One possible route for avoiding overlap of unbounded electrostatic potentials consists of allowing a bounded (soft-core) vdW potential, using a positive value of `alchVdWShiftCoeff`, to repel first all overlapping particles at low values of λ. As λ increases, once the particles are repelled, it becomes safe to turn on FEP or TI electrostatics.

  In the current implementation, the electrostatic interactions of an exnihilated, or appearing, particle are linearly coupled to the simulation over the λ value range of `alchElecLambdaStart` – 1.0. At λ values less than or equal to the user-defined value of `alchElecLambdaStart`, electrostatic interactions of the exnihilated particle are fully decoupled from the simulation. Coupling of electrostatic interactions then increases linearly for increasing values of λ until

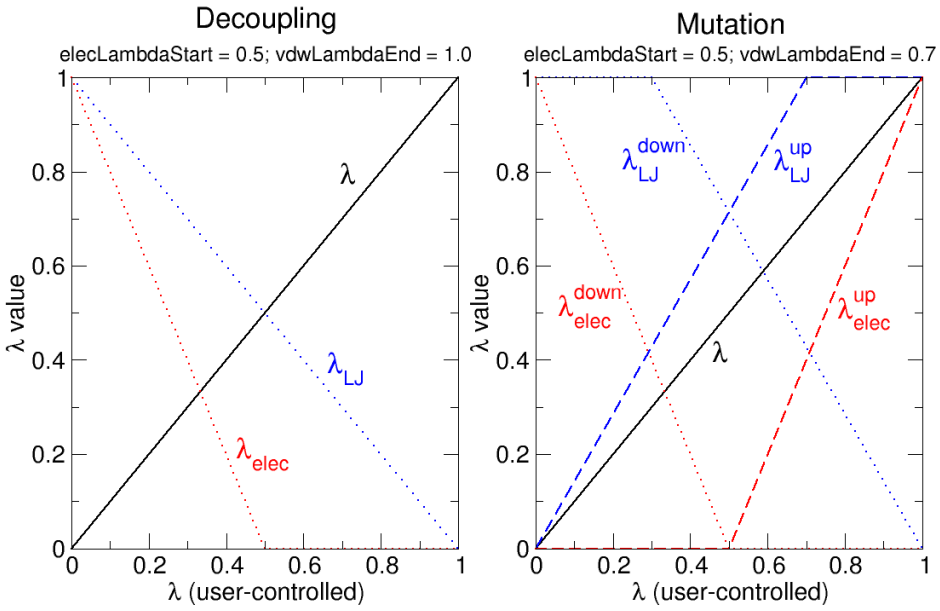


Figure 8: Relationship of user-defined λ to coupling of electrostatic or vdW interactions to a simulation, given specific values of `alchElecLambdaStart` or `alchVdwLambdaEnd`.

λ=1.0, at which point electrostatic interactions of the exnihilated particle are fully coupled to the simulation.

For annihilated, or vanishing, particles the electrostatic interactions are linearly decoupled from the simulation over the λ value range of 0 – (1.0 - `alchElecLambdaStart`). At λ=0 electrostatic interactions are fully coupled to the simulation, and then linearly decreased with increasing λ such that at λ values greater than or equal to (1.0 - `alchElecLambdaStart`) electrostatic interactions are completely decoupled from the simulation. Two examples, shown in Figure 8, describe the relationship between the user-defined value of λ and the coupling of electrostatic or vdW interactions to the simulation.

- `alchVdwLambdaEnd` < Value of λ to cancel van der Waals interactions >
  Acceptable Values: positive decimal
  Default Value: 1.0
  Description: If the `alchElecLambdaStart` option is used, it may also be desirable to separate the scaling of van der Waals and electrostatic interactions. `alchVdwLambdaEnd` sets the value of λ above which all vdW interactions are fully enabled for exnihilated particles.

  For an exnihilated particle, vdW interactions are fully decoupled at λ=0. The coupling of vdW interactions to the simulation is then increased with increasing values of λ such that at values of λ greater than or equal to `alchVdwLambdaEnd` the vdW interactions of the exnihilated particle are fully coupled to the simulation.

  For an annihilated particle, vdW interactions are completely coupled to the simulation for λ values between 0 and (1 - `alchVdwLambdaEnd`). Then, vdW interactions of the annihilated particle are linearly decoupled over the range of λ values between (1 - `alchVdwLambdaEnd`) and 1.0. VdW interactions are only fully decoupled when λ reaches 1.0.

New as of version 2.12: The energy and virial terms added by `LJcorrection on` are now also controlled by the vdW λ schedule. The average Lennard-Jones A and B coefficients are computed separately at both endpoints and then coupled linearly. In most practical situations the energy difference is extremely negligible, but this is more theoretically sound than the old behavior of averaging both endpoints together. However, the kinetic energy component of the virial *does* still count the endpoints together, as if annihilated alchemical atoms were an ideal gas. Again, this is likely quite negligible, nor is it clear that this should be treated specially.

- `alchRepLambdaEnd` < Value of λ to cancel van der Waals repulsive interactions >
  Acceptable Values: positive decimal
  Default Value: 0.5
  Description: This parameter is only used when `alchWCA` is `on`, in which case it MUST be less than or equal to `alchVdwLambdaEnd`. For appearing atoms, this marks both the value at which repulsive interactions are completely coupled and at which dispersive interactions beging to become coupled (but are still zero).

- `alchBondLambdaEnd` < Value of λ to cancel bonded interactions >
  Acceptable Values: positive decimal
  Default Value: 0.0
  Description: **New as of version 2.12** Bonded terms involving alchemical atoms may now also be scaled on a schedule similar to vdW interactions. Although this is more theoretically sound in many situations, this behavior is off by default.

- `alchBondDecouple` < Enable scaling of bonded terms within alchemical groups >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: This is essentially a bonded term analogue of the `alchDecouple` keyword. Setting `alchBondDecouple on`, causes bonded terms between alchemical atoms *in the same group* to also be scaled. This means that alchemical atoms are annihilated into ideal gas atoms instead of ideal gas molecules. In this case it is recommended to use the approach of Axelsen and Li [3] by way of the `extraBonds` keyword. Using `alchBondDecouple on` is strictly necessary if it is desired to have the endpoint (potential) energies of a dual-topology PSF match those of a non-alchemical PSF.

- `alchDecouple` < Disable scaling of nonbonded interactions within alchemical partitions >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: With `alchDecouple` set to `on`, only nonbonded interactions of perturbed, incoming and outgoing atoms with their environment are scaled, while interactions within the subset of perturbed atoms are preserved. On the contrary, if `alchDecouple` is set to `off`, interactions within the perturbed subset of atoms are also scaled and contribute to the cumulative free energy. In most calculations, intramolecular annihilation free energies are not particularly informative, and decoupling ought to be preferred. Under certain circumstances, it may, however, be desirable to scale intramolecular interactions, provided that the latter are appropriately accounted for in the thermodynamic cycle [22].

10.3 Examples of input files for running alchemical free energy calculations

Note: In this section the lambda values are specified manually. For sequential sampling of lambda values, it is simpler to call the `runFEP` or `runFEPlist` procedure of `fep.tcl`. See the comments in that file for instructions.

The first example illustrates the use of TCL scripting for running an alchemical transformation with the FEP feature of NAMD. In this calculation, λ is changed continuously from 0 to 1 by increments of $\delta\lambda = 0.1$.

```
alch            On
alchType        fep
alchFile        ion.fep
alchCol         X
alchOutfile     ion.fepout
alchOutFreq     5
alchEquilSteps 5000

set Lambda0     0.0
set dLambda     0.1

while {$Lambda0 < 1.0} {
 alchLambda $Lambda0
 set Lambda0 [expr $Lambda0 + $dLambda]
 alchLambda2 $Lambda0
 run  10000
}
```

Enable alchemical simulation module
Set alchemical method to FEP
File containing the information about growing/shrinking atoms described in column `X`.
Output file containing the free energy.
Frequency at which `fepOutFreq` is updated.
Number of equilibration steps per λ–state.

Starting value of λ.
Increment of λ, *i.e.* $\delta\lambda$.

TCL script to increment λ:
(1) set `lambda` value;
(2) increment λ;
(3) set `lambda2` value;
(4) run 10,000 MD steps.

The user should be reminded that by setting `run 10000`, 10,000 MD steps will be performed, which includes the preliminary `fepEquilSteps` equilibration steps. This means that here, the ensemble average of equation (80) will be computed over 5,000 MD steps.

Alternatively, λ–states may be declared explicitly, avoiding the use of TCL scripting:

```
alchLambda      0.0
alchLambda2     0.1
run             10000
```

(1) set `alchLambda` value;
(2) set `alchLambda2` value;
(3) run 10,000 MD steps.

This option is generally preferred to set up windows of diminishing widths as $\lambda \to 0$ or 1 — a way to circumvent end–point singularities caused by appearing atoms that may clash with their surroundings.

The following second input is proposed for the measuring via TI the free energy of a particle insertion.

```
alch            On              ;# Enable alchemical simulation module
alchType        ti              ;# Set method to thermodynamic integration
alchFile        ion.alch.pdb    ;# PDB file with perturbation flags
alchCol         B               ;# Perturbation flags in Beta column
alchOutfile     ion.ti.out
alchOutFreq     5
alchEquilSteps 5000

alchVdWShiftCoeff    1          ;# Enable soft-core vdW potential
alchElecLambdaStart  0.1        ;# Introduce electrostatics for lambda > 0.1
```

```
alchLambda 0
run 10000
alchLambda 0.00001
run 10000
alchLambda 0.0001
run 10000
alchLambda 0.001
run 10000
alchLambda 0.01
run 10000

set Lambda           0.1

while {$Lambda <= 0.9} {
  alchLambda $Lambda
  run 10000
  set Lambda [expr $Lambda + 0.1]
}

alchLambda 0.99
run 10000
alchLambda 0.999
run 10000
alchLambda 0.9999
run 10000
alchLambda 0.99999
run 10000
alchLambda 1
run 10000
```

Robust sampling of the free energy of particle insertion is enabled by the use of soft-core van der Waals scaling with the `alchVdWShiftCoeff` parameter, delayed introduction of electrostatics with a non-zero `alchElecLambdaStart` value, and very gradual scaling of λ towards its end points.

10.4 Description of a free energy calculation output

10.4.1 Free Energy Perturbation

When running FEP, the `alchOutFile` contains electrostatic and van der Waals energy data calculated for `alchLambda` and `alchLambda2`, written every `alchOutFreq` steps. The column `dE` is the energy difference of the single configuration, `dE_avg` and `dG` are the instantaneous ensemble average of the energy and the calculated free energy at the time step specified in column 2, respectively. The temperature is specified in the penultimate column. Upon completion of `alchEquilSteps` steps, the calculation of `dE_avg` and `dG` is restarted. The accumulated net free energy change is written at each lambda value and at the end of the simulation.

Whereas the FEP module of NAMD supplies free energy differences determined from equation (79), the wealth of information available in `alchOutFile` may be utilized profitably to explore

different routes towards the estimation of ΔA. Both BAR and SOS methods, which combine advantageously *direct* and *reverse* transformations to improve convergence and accuracy of the calculation, represent relevant alternatives to brute–force application of the FEP formula [65].

Within the SOS framework, the free energy difference between states λ_i and λ_{i+1} is expressed as:

$$\exp(-\beta\Delta A_{i\rightarrow i+1}) = \frac{\left\langle \exp\left\{-\frac{\beta}{2}\left[\mathcal{H}(\mathbf{x},\mathbf{p}_x;\lambda_{i+1}) - \mathcal{H}(\mathbf{x},\mathbf{p}_x;\lambda_i)\right]\right\}\right\rangle_i}{\left\langle \exp\left\{-\frac{\beta}{2}\left[\mathcal{H}(\mathbf{x},\mathbf{p}_x;\lambda_i) - \mathcal{H}(\mathbf{x},\mathbf{p}_x;\lambda_{i+1})\right]\right\}\right\rangle_{i+1}} \tag{82}$$

and can be readily used with the statistical information provided by the forward and the backward runs.

10.4.2 Thermodynamic Integration

When running TI free energy calculations, the `elec_dU/dl`, `vdW_dU/dl`, and `bond_dU/dl` values reported in `alchOutFile` are the derivatives of the internal energy with respect to the scaling factors for each interaction type (*i.e.* electrostatics, etc.). `dU/dl` values are locally averaged over the last `alchOutFreq` steps. Cumulative averages for each component are reported alongside in the `_avg` columns.

The electrostatic, vdW, and bond values are separated following a partition scheme — that is, the "appearing" and the "disappearing" atoms are accounted for separately. "Partition 1" contains those atoms whose interactions are switched up as λ increases — *i.e.* flagged with `1` in the `alchFile`. "Partition 2" represents those atoms whose interactions are switched down as λ increases — *i.e.* flagged with `-1`. ΔA values for each component are obtained by integrating from $\lambda = 0$ to 1 using the respective `ELEC / VDW / BOND LAMBDA` listed for each partition after the title.

New as of version 2.12: The output in `alchOutFile` has been extensively revised and now more closely matches the NAMD standard output. Additional accounting for bonded term scaling is now also included.

The choice of λ values will depend on the application, but in general it is important to examine the shape of the curve to ensure that sampling is adequate to give a good estimate of the integral. In particular, it will be necessary to sample more finely towards the end points in order to accurately account for the strong repulsive van der Waals forces encountered when inserting particles into a system (see Figure 9).

10.5 Hybrid single–dual topology approach for relative binding free energy calculation of ligand to receptor

An effective hybrid single–dual topology protocol is designed for the calculation of relative binding affinities of small ligands to a receptor. The protocol was developed as an expansion of the existing dual-topology relative alchemical free energy calculations [50], for either free energy perturbation or thermodynamic integration. In this protocol, the alchemical end states are represented as two separate molecules sharing a common substructure identified through maximum structural mapping. Within the substructure, an atom-to-atom correspondence is established, and each pair of corresponding atoms are holonomically constrained to share identical coordinates at all time throughout the simulation, as shown in Figure 10. The forces are projected and combined at each step for propagation.

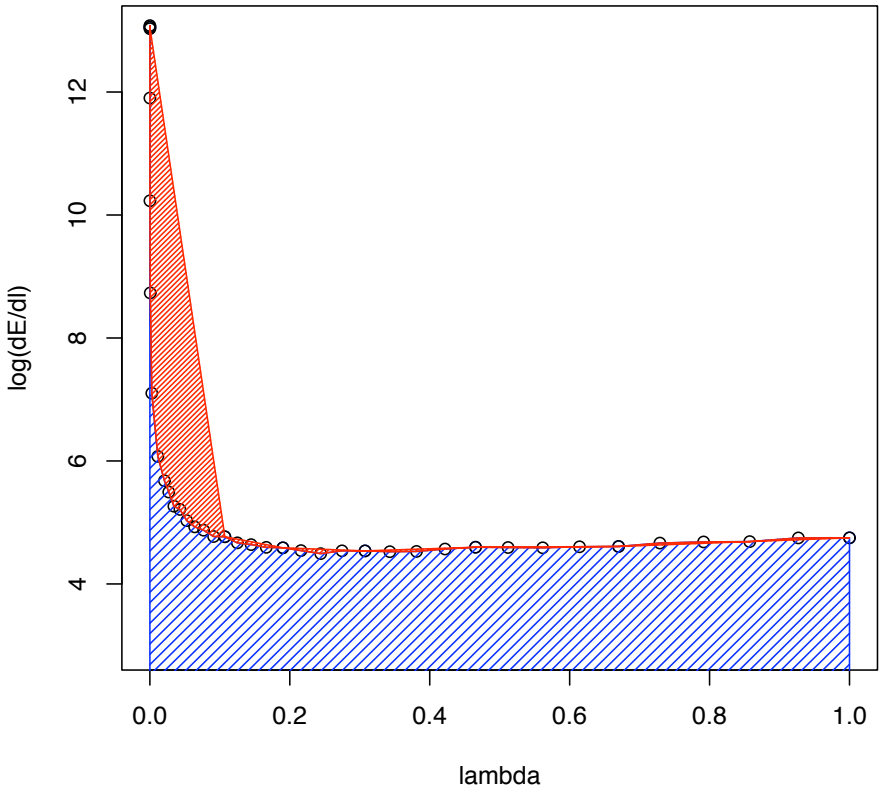


Figure 9: Sample TI data ($log(\langle \frac{\partial U}{\partial \lambda} \rangle)$ against λ). The blue shaded area shows the integral with fine sampling close to the end point. The red area shows the difference when λ values are more sparse. In this example, insufficient sampling before $\lambda \simeq 0.1$ can result in a large overestimation of the integral. Beyond $\simeq 0.2$, sparser sampling is justified as $dE/d\lambda$ is not changing quickly.

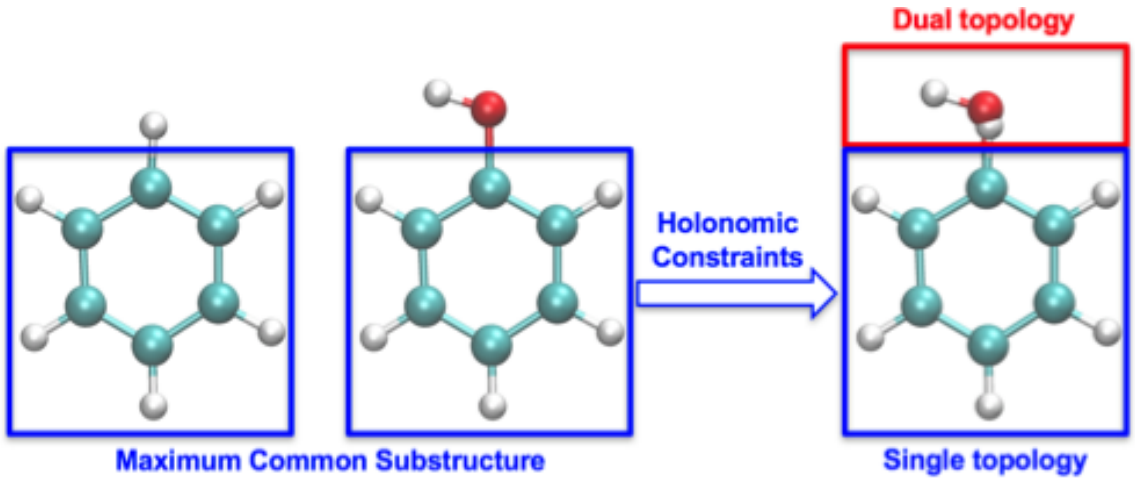


Figure 10: Hybrid single–dual topology setup generated by applying holonomic constraints on the maximum common substructure.

As it is based on the existing dual topology setup, the major input files including PDB, PSF and alchemical flag files adopt the same format as before, with two more partitions accommodating the initial/end states of the single topology region. Determining the common substructure generally requires a special setup tool to determine the maximum structural mapping that generate the partitions present in the PDB and PSF files. The dual-topology setup also implements Shobana bonded terms to support the ring topology change problem [100], for which a separate input file lists all unperturbed bonded terms on a ring. The current implementation supports both relative solvation free energies of small molecules and relative binding affinities of drug compounds to proteins. To enhance sampling of the dual-topology region, the alchemical calculations can be carried out within a replica-exchange MD scheme supported by the multiple-copy algorithm module of NAMD, with periodic attempted swapping of the thermodynamic coupling parameter λ betwen neighboring states.

It needs to be noted that the protocol is currently implemented only on CPU, with a GPU implementation in development. VMD does not yet provide a hybrid topology setup tool, and CHARMM-GUI is testing a beta version (that is not yet available online) to automatically generate all input files for NAMD. For the time being, users can utilize an alternative hybrid structure preparation tool, such as FESetup or AmberTools, and then manually convert the generated CHARMM-formatted input files into a format that can be read by NAMD.

The following keywords enable hybrid single–dual topology simulation.

- `singleTopology` < Enable hybrid single–dual topology? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Enable the use of hybrid single–dual topology for alchemical transformation, which extends the default dual topology setup.

- `sdBondScaling` < Are Shobana terms enabled? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Enable the use of selected Shobana terms, the unperturbed bond, angle, and dihedral terms on a transformed ring, that remove the possible artificial effects of dummy atoms. For a more detailed elucidation, please see reference [100].

- `unperturbedBondFile` < file listing unperturbed bonded terms >
  Acceptable Values: filename
  Description: This must be defined if `sdBondScaling` is `on`. The file lists the selected unperturbed bond, angle, and dihedral terms that remove the possible artificial effects of dummy atoms. When `sdBondScaling` is `off`, the file will be skipped.

11 Accelerated Sampling Methods

11.1 Accelerated Molecular Dynamics

Accelerated molecular dynamics (aMD) [42] is an enhanced-sampling method that improves the conformational space sampling by reducing energy barriers separating different states of a system. The method modifies the potential energy landscape by raising energy wells that are below a certain threshold level, while leaving those above this level unaffected. As a result, barriers separating adjacent energy basins are reduced, allowing the system to sample conformational space that cannot be easily accessed in a classical MD simulation.

Please include the following two references in your work using the NAMD implementation of aMD:

- Accelerated Molecular Dynamics: A Promising and Efficient Simulation Method for Biomolecules, D. Hamelberg, J. Mongan, and J. A. McCammon. *J. Chem. Phys.*, 120:11919-11929, 2004.
- Implementation of Accelerated Molecular Dynamics in NAMD, Y. Wang, C. Harrison, K. Schulten, and J. A. McCammon, *Comp. Sci. Discov.*, 4:015002, 2011.

11.1.1 Theoretical background

In the original form of aMD [42], when the system's potential energy falls below a threshold energy, E, a boost potential is added, such that the modified potential, $V^*(\mathbf{r})$, is related to the original potential, $V(\mathbf{r})$, via

$$V^*(\mathbf{r}) = V(\mathbf{r}) + \Delta V(\mathbf{r}), \tag{83}$$

where $\Delta V(\mathbf{r})$ is the boost potential,

$$\Delta V(\mathbf{r}) = \begin{cases} 0 & V(\mathbf{r}) \geq E \\ \frac{(E-V(\mathbf{r}))^2}{\alpha+E-V(\mathbf{r})} & V(\mathbf{r}) < E. \end{cases} \tag{84}$$

As shown in the following figure, the threshold energy E controls the portion of the potential surface affected by the boost, while the acceleration factor α determines the shape of the modified potential. Note that α cannot be set to zero, otherwise the derivative of the modified potential is discontinuous.

From an aMD simulation, the ensemble average, $\langle A \rangle$, of an observable, $A(\mathbf{r})$, can be calculated using the following reweighting procedure:

$$\langle A \rangle = \frac{\langle A(\mathbf{r}) \exp(\beta \Delta V(\mathbf{r})) \rangle^*}{\langle \exp(\beta \Delta V(\mathbf{r})) \rangle^*}, \tag{85}$$

in which β=$1/k_BT$, and $\langle ... \rangle$ and $\langle ... \rangle^*$ represent the ensemble average in the original and the aMD ensembles, respectively.

Currently, aMD can be applied in three modes in NAMD: aMDd, aMDT, and aMDdual [115]. The boost energy is applied to the dihedral potential in the aMDd mode (the default mode), and to the total potential in the aMDT mode. In the dual boost mode (aMDdual) [41], two independent boost energies are applied, one on the dihedral potential and the other on the (Total - Dihedral) potential.

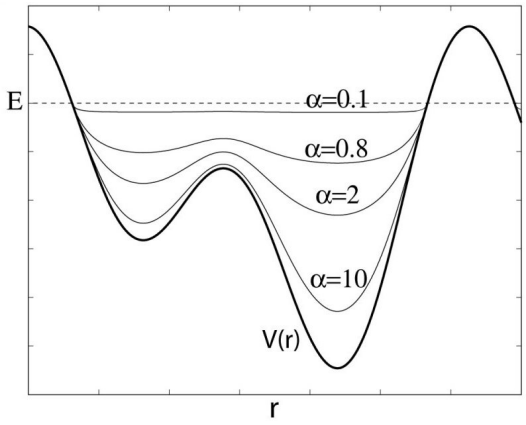


Figure 11: Schematics of the aMD method. When the original potential (thick line) falls below a threshold energy E (dashed line), a boost potential is added. The modified energy profiles (thin lines) have smaller barriers separating adjacent energy basins.

11.1.2 NAMD parameters

The following parameters are used to enable accelerated MD:

- `accelMD` < Is accelerated molecular dynamics active? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies if accelerated MD is active.

- `accelMDdihe` < Apply boost to dihedrals? >
  Acceptable Values: `on` or `off`
  Default Value: `on`
  Description: Only applies boost to the dihedral potential. By default, `accelMDdihe` is turned on and the boost energy is applied to the dihedral potential of the simulated system. When `accelMDdihe` is turned off, aMD switches to the `accelMDT` mode, and the boost is applied to the total potential.

- `accelMDE` < Threshold energy E >
  Acceptable Values: Real number
  Description: Specifies the threshold energy E in the aMD equations.

- `accelMDalpha` < Acceleration factor α >
  Acceptable Values: Positive real number
  Description: Specifies the acceleration factor α in the aMD equations.

- `accelMDdual` < Use dual boost mode? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: When `accelMDdual` is on, aMD switches to the dual boost mode. Two independent boost potentials will be applied: one to the dihedral potential that is controlled by

the parameters `accelMDE` and `accelMDalpha`, and a second to the (Total - Dihedral) potential that is controlled by the `accelMDTE` and `accelMDTalpha` parameters described below.

- `accelMDTE` < Threshold energy E in the dual boost mode >
  Acceptable Values: Real number
  Description: Specifies the threshold energy E used in the calculation of boost energy for the (Total - Dihedral) potential. This option is only available when `accelMDdual` is turned on.

- `accelMDTalpha` < Acceleration factor α in the dual boost mode >
  Acceptable Values: Positive real number
  Description: Specifies the acceleration factor α used in the calculation of boost energy for the (Total - Dihedral) potential. This option is only available when `accelMDdual` is turned on.

- `accelMDFirstStep` < First accelerated MD step >
  Acceptable Values: Zero or positive integer
  Default Value: 0
  Description: Accelerated MD will only be performed when the current step is equal to or higher than `accelMDFirstStep`, and equal to or lower than `accelMDLastStep`. Otherwise regular MD will be performed.

- `accelMDLastStep` < Last accelerated MD step >
  Acceptable Values: Zero or positive integer
  Default Value: 0
  Description: Accelerated MD will only be performed when the current step is equal to or higher than `accelMDFirstStep`, and equal to or lower than `accelMDLastStep`. Otherwise regular MD will be performed. Note that the accelMDLastStep parameter only has an effect when it is positive. When accelMDLastStep is set to zero (the default), aMD is 'open-ended' and will be performed till the end of the simulation.

- `accelMDOutFreq` < Frequency in steps of aMD output >
  Acceptable Values: Positive integer
  Default Value: 1
  Description: An aMD output line will be printed to the log file at the frequency specified by `accelMDOutFreq`. The aMD output will contain the boost potential (dV) at the current timestep, the average boost potential ($dVAVG$) since the last aMD output, and various potential energy values at the current timestep. The boost potential dV can be used to reconstruct the ensemble average described earlier.

11.2 Gaussian Accelerated Molecular Dynamics

Gaussian accelerated molecular dynamics (GaMD) [76] is a type of accelerated molecular dynamics (aMD) calculation. It is an enhanced sampling method that works by adding a harmonic boost potential to smoothen the system's potential energy surface. By constructing a boost potential that follows Gaussian distribution, accurate reweighting of the GaMD simulations is achieved using cumulant expansion to the second order.

Please include the following two references in your work using the NAMD implementation of GaMD:

- Gaussian Accelerated Molecular Dynamics: Unconstrained Enhanced Sampling and Free Energy Calculation, Y. Miao, V. Feher, and J. A. McCammon. *J. Chem. Theory Comput.*, 11:3584-3595, 2015.
- Gaussian Accelerated Molecular Dynamics in NAMD, Y.T. Pang, Y. Miao, Y. Wang, and J. A. McCammon, *J. Chem. Theory Comput.*, 13:9-19, 2017.

11.2.1 Theoretical background

GaMD enhances conformational sampling of biomolecules by adding a harmonic boost potential to smoothen the system's potential energy surface [76], as illustrated below:

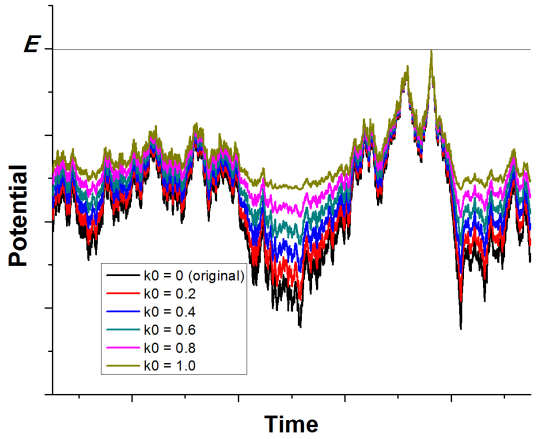


Figure 12: Schematic illustration of GaMD. When the threshold energy E is set to the maximum potential ($iE = 1$ mode), the system's potential energy surface is smoothened by adding a harmonic boost potential that follows a Gaussian distribution. The coefficient k_0, which falls in the range of $0 - 1.0$, determines the magnitude of the applied boost potential.

Consider a system with N atoms at positions $\mathbf{r} = \{\mathbf{r}_1, \cdots, \mathbf{r}_N\}$. When the system's potential energy $V(\mathbf{r})$ is lower than a threshold energy E, the following boost potential is added:

$$V^*(\mathbf{r}) = V(\mathbf{r}) + \Delta V(\mathbf{r}), \tag{86}$$

where $\Delta V(\mathbf{r})$ is the boost potential,

$$\Delta V(\mathbf{r}) = \begin{cases} \frac{1}{2}k\left(E - V(\mathbf{r})\right)^2, & V(\mathbf{r}) < E \\ 0, & V(\mathbf{r}) \geq E. \end{cases} \tag{87}$$

where k is the harmonic force constant.

As explained in reference [76], the two adjustable parameters E and k are automatically determined by the following three criteria. First, ΔV should not change the relative order of the biased potential values, i.e., for any two arbitrary potential values $V_1(\mathbf{r})$ and $V_2(\mathbf{r})$ found on the original energy surface, if $V_1(\mathbf{r}) < V_2(\mathbf{r})$, then one should have $V_1^*(\mathbf{r}) < V_2^*(\mathbf{r})$. Second, the difference between potential energy values on the smoothened energy surface should be smaller than that of the original, i.e., if $V_1(\mathbf{r}) < V_2(\mathbf{r})$, then one should have $V_2^*(\mathbf{r}) - V_1^*(\mathbf{r}) < V_2(\mathbf{r}) - V_1(\mathbf{r})$. By combining the above two criteria and plugging in the formula of $V^*(\mathbf{r})$ and ΔV, one obtains

$$V_{\max} \leq E \leq V_{\min} + \frac{1}{k} \tag{88}$$

where $V_{\min}$ and $V_{\max}$ are the system's minimum and maximum potential energies. To ensure that Eqn. (88) is valid, k needs to satisfy: $k \leq \frac{1}{V_{\max}-V_{\min}}$. Define $k \equiv k_0 \cdot \frac{1}{V_{\max}-V_{\min}}$, then $0 < k_0 \leq 1$. Third, the standard deviation of ΔV needs to be small enough (i.e., narrow distribution) to ensure accurate reweighting using cumulant expansion to the second order: $\sigma_{\Delta V} = k\,(E - V_{\text{avg}})\,\sigma_V \leq \sigma_0$, where V_{avg} and σ_V are the average and standard deviation of the system's potential energies, $\sigma_{\Delta V}$ is the standard deviation of ΔV, while σ_0 is a user-specified upper limit (e.g., $10k_BT$) in order to achieve accurate reweighting.

iE = 1 mode: When E is set to $E = V_{\max}$ according to Eqn. (88), k_0 is calculated as:

$$k_0 = \min(1.0, k_0') = \min\left(1.0, \frac{\sigma_0}{\sigma_V} \cdot \frac{V_{\max} - V_{\min}}{V_{\max} - V_{\text{avg}}}\right) \tag{89}$$

iE = 2 mode: Alternatively, when E is set to $E = V_{\min} + \frac{1}{k}$, k_0 is calculated as:

$$k_0 = k_0'' \equiv \left(1 - \frac{\sigma_0}{\sigma_V} \cdot \frac{V_{\max} - V_{\min}}{V_{\text{avg}} - V_{\min}}\right) \tag{90}$$

If k_0'' obtained from the above equation is smaller than 0 or greater than 1, then k_0 will be calculated using Eqn. (89).

For more details on GaMD and the corresponding reweighting using cumulant expansion, see reference [76][85].

11.2.2 NAMD parameters

Same as aMD, three modes are available for applying boost potential in GaMD: (1) boosting the dihedral energy only, (2) boosting the total potential energy, and (3) boosting both the dihedral and total potential energy (i.e., "dual-boost").

Some parameters from aMD, including: `accelMD`, `accelMDdihe`, `accelMDdual`, `accelMDFirstStep`, `accelMDLastStep` and `accelMDOutFreq` are shared by GaMD (see Section 11.1 for details). The following is a list of input parameters unique to a GaMD run:

- `accelMDG` < Is Gaussian accelerated MD on? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether Gaussian accelerated MD (GaMD) is on. Only available when `accelMD` is on.

- `accelMDGiE` < Flag to set the threshold energy for adding boost potential >
  Acceptable Values: 1 or 2
  Default Value: 1
  Description: Specifies how the threshold energy E is set in GaMD. A value of 1 indicates that the threshold energy E is set to its lower bound $E = V_{\max}$. A value of 2 indicates that the threshold energy is set to its upper bound $E = V_{\min} + (V_{\max} - V_{\min})/k_0$.

- `accelMDGcMDPrepSteps` < Number of preparatory cMD steps >
  Acceptable Values: Zero or Positive integer
  Default Value: 200,000
  Description: The number of preparatory conventional MD (cMD) steps in GaMD. This value should be smaller than `accelMDGcMDSteps` (see below). Potential energies

are not collected for calculating the values of $V_{\max}$, $V_{\min}$, V_{avg}, σ_V during the first `accelMDGcMDPrepSteps`.

- `accelMDGcMDSteps` < Number of total cMD steps >
  Acceptable Values: Zero or Positive integer
  Default Value: 1,000,000
  Description: The number of total cMD steps in GaMD. With `accelMDGcMDPrepSteps` < t < `accelMDGcMDSteps`, $V_{\max}$, $V_{\min}$, V_{avg}, σ_V are collected and at $t =$ `accelMDGcMDSteps`, E and k_0 are computed.

- `accelMDGEquiPrepSteps` < Number of preparatory equilibration steps in GaMD >
  Acceptable Values: Zero or Positive integer
  Default Value: 200,000
  Description: The number of preparatory equilibration steps in GaMD. This value should be smaller than `accelMDGEquiSteps` (see below). With `accelMDGcMDSteps` < t < `accelMDGEquiPrepSteps` + `accelMDGcMDSteps`, GaMD boost potential is applied according to E and k_0 obtained at $t =$ `accelMDGcMDSteps`.

- `accelMDGEquiSteps` < Number of total equilibration steps in GaMD >
  Acceptable Values: Zero or Positive integer
  Default Value: 1,000,000
  Description: The number of total equilibration steps in GaMD. With `accelMDGEquiPrepSteps` + `accelMDGcMDSteps` < t < `accelMDGEquiSteps` + `accelMDGcMDSteps`, GaMD boost potential is applied, and E and k_0 are updated every step.

- `accelMDGStatWindow` < Number of steps to calculate average and standard deviation in GaMD >
  Acceptable Values: Integer
  Default Value: -1
  Description: The number of simulation steps used to calculate the average and standard deviation of potential energies, as well as the frequency of recalculating the boost potential during equilibration steps. When it is set to a negative number, all the steps throughout the cMD and equilibration stage (except the preparatory steps) will be used to calculate the average and standard deviation without resetting, and the boost potential will be updated every step during equilibration steps. When used, it is recommended to be set to about 4 times the total number of atoms in the system. Note that `accelMDGcMDPrepSteps`, `accelMDGcMDSteps`, `accelMDGEquiPrepSteps` and `accelMDGEquiSteps` need to be multiples of `accelMDGStatWindow`.

- `accelMDGSigma0P` < Upper limit of the standard deviation of the total boost potential in GaMD >
  Acceptable Values: Positive real number
  Default Value: 6.0 (kcal/mol)
  Description: Specifies the upper limit of the standard deviation of the total boost potential. This option is only available when `accelMDdihe` is off or when `accelMDdual` is on.

- `accelMDGSigma0D` < Upper limit of the standard deviation of the dihedral boost potential in GaMD >

Acceptable Values: Positive real number
Default Value: 6.0 (kcal/mol)
Description: Specifies the upper limit of the standard deviation of the dihedral boost potential. This option is only available when `accelMDdihe` or `accelMDdual` is on.

- `accelMDGRestart` < Flag to restart GaMD simulation >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether the current GaMD simulation is the continuation of a previous run. If this option is turned on, the GaMD restart file specified by `accelMDGRestartFile` (see below) will be read.

- `accelMDGRestartFile` < Name of GaMD restart file >
  Acceptable Values: UNIX filename
  Description: A GaMD restart file that stores the current number of steps, maximum, minimum, average and standard deviation of the dihedral and/or total potential energies (depending on the `accelMDdihe` and `accelMDdual` parameters). This file is saved automatically every `restartfreq` steps. If `accelMDGRestart` is turned on, this file will be read and the simulation will restart from the point where the file was written.

11.3 Solute Scaling and REST2

Solute scaling improves sampling efficiency by scaling the intramolecular potential energy of a protein to lower barriers separating different confirmations [114]. The potential is scaled based on a parameter β,

$$U^{\mathrm{SS}}(\vec{r}) = \beta U_{\mathrm{pp}}(\vec{r}) + \sqrt{\beta} U_{\mathrm{pw}}(\vec{r}) + U_{\mathrm{ww}}(\vec{r}), \tag{91}$$

with U_{pp} denoting protein–protein interactions, U_{pw} denoting protein–water interactions, and U_{ww} denoting water–water interactions, effectively "heating" the protein's interatomic interactions whenever $\beta < 1$. The NAMD implementation is made efficient by rescaling the force field parameters for the affected atoms [52]. In particular, this parameter scaling approach makes the calculation compatible with existing CUDA force kernels.

The NAMD implementation provides additional flexibility to solute scaling by allowing different scaling factors for electrostatics, van der Waals, and bonded interactions, as described in the following section. Solute scaling can be combined with replica exchange to produce a powerful sampling enhancement method that is highly transferable and provides higher efficiency than traditional temperature exchange methods. In the literature, this replica exchange solute scaling method is known as REST2, due to its improvement of the earlier REST (replica exchange solute tempering) method that directly scaled the temperature of the solute. Sample files are available in directory `lib/replica/REST2`, with script file `lib/replica/REST2/rest2_remd.namd` demonstrating use of solute scaling with multiple replicas.

11.3.1 NAMD parameters

The following parameters are used to control solute scaling:

- `soluteScaling` < Is replica exchange solute tempering enabled? >
  Acceptable Values: `on` or `off`
  Default Value: `off`

Description: Specifies whether or not REST2 is enabled. If set on, then `soluteScaling` must also be set.

- `soluteScalingFactor` < Solute scaling factor >
  Acceptable Values: non-negative
  Description: This options sets the scaling factor β, and is typically set lower than 1 to reduce potential energy barriers for the solute.

- `soluteScalingFactorCharge` < Solute scaling factor for electrostatics >
  Acceptable Values: non-negative
  Default Value: `soluteScalingFactor`
  Description: Scaling factor applied to just the electrostatics interactions. If not specified, this is set to `soluteScalingFactor`.

- `soluteScalingFactorVdw` < Solute scaling factor for van der Waals >
  Acceptable Values: non-negative
  Default Value: `soluteScalingFactor`
  Description: Scaling factor applied to just the van der Waals interactions. If not specified, this is set to `soluteScalingFactor`.

- `soluteScalingFile` < PDB file with scaling flags >
  Acceptable Values: UNIX filename
  Default Value: `coordinates`
  Description: PDB file used to flag solute atoms for scaling. If undefined, this defaults to the coordinate PDB file.

- `soluteScalingCol` < Column of PDB file >
  Acceptable Values: `X`, `Y`, `Z`, `O`, or `B`
  Default Value: `O`
  Description: Column of the PDB file used to flag solute atoms for scaling. If undefined, this defaults to the `O` (occupancy) column. A value of 1.0 marks the atom for scaling.

- `soluteScalingAll` < Apply scaling also to bond and angle interactions? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: If set on, `scalingFactor` is applied also to bond and angle interactions. Otherwise, `scalingFactor` is applied only to dihedral, improper, and crossterm interactions.

11.4 Adaptive Tempering

Adaptive tempering is akin to a single-copy replica exchange method for dynamically updating the simulation temperature. The temperature T is a new random variable in the range $[Tmin, Tmax]$ that is governed by the equation $dE/dT = E - E(T) - 1/T + sqrt(2)T\xi$, where ξ is Gaussian white noise. The effect is that when the potential energy for a given structure is lower than the (so far calculated) average energy, the temperature is lowered. Conversely when the current energy is higher than the average energy, the temperature is raised. The effect is faster conformational sampling to find minimum energy structures. The method is implemented exactly as described by Zhang and Ma in J. Chem. Phys. 132, 244101 (2010) (using Equation 18 of their paper to calculate the average energy at a given temperature from the histogram of energies).

The dynamic temperature is realized either by changing the temperature of the Langevin thermostat or by velocity rescaling.

11.4.1 NAMD parameters

The following parameters are used to adaptive tempering:

- `adaptTempMD` < Is adaptive tempering active? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not adaptive tempering is used. If set to on then the following parameters are required to be set: either all of (`adaptTempTmin`, `adaptTempTmax`, `adaptTempBins`, `adaptTempDt`) or `adaptTempInFile` (but not both).

- `adaptTempFreq` < steps between temperature updates >
  Acceptable Values: Positive integers
  Default Value: 10
  Description: The number of steps between temperature updates. Note that the potential energy at the current is calculated and added to the temperature-energy histogram at every step.

- `adaptTempTmin` < minimum temperature (K) >
  Acceptable Values: Positive real number
  Description: Sets the minimum temperature to be used in the simulation.

- `adaptTempTmax` < maximum temperature (K) >
  Acceptable Values: Positive real number
  Description: Sets the maximum temperature to be used in the simulation.

- `adaptTempBins` < number of temperature bins >
  Acceptable Values: Positive integer
  Default Value: 1000
  Description: Sets the number of bins to subdivide the temperature range. Each bin stores the average energy for the given temperature

- `adaptTempDt` < stepsize for temperature updates >
  Acceptable Values: Positive real numbers
  Default Value: 10^{-4}
  Description: Integration timestep for temperature updates. This is unrelated to the simulation timestep and only scales the size of the step taken in temperature space every `adaptTempFreq` steps.

- `adaptTempInFile` < adaptive tempering input filename >
  Acceptable Values: UNIX filename
  Description: The input file containing restart information for adaptive tempering (written out by `adaptTempRestartFile`).

- `adaptTempRestartFile` < adaptive tempering restart filename >
  Acceptable Values: UNIX filename
  Description: The file to write out restart information for adaptive tempering.

- `adaptTempRestartFreq` < steps between writing restart file >
  Acceptable Values: Positive integer
  Description: Frequency of writing restart file.

- `adaptTempLangevin` < send temperature updates to langevin thermostat? >
  Acceptable Values: `on` or `off`
  Default Value: `on`
  Description: Setting this to on will cause the langevin thermostat to use the updated temperatures from adaptive tempering. Note that either one of adaptTempLangevin or adaptTempRescaling have to be on.

- `adaptTempRescaling` < send temperature to velocity rescaling thermostat? >
  Acceptable Values: `on` or `off`
  Default Value: `on`
  Description: Setting this to on will cause the veloctiy rescaling thermostat to use the updated temperatures from adaptive tempering. Note that either one of adaptTempLangevin or adaptTempRescaling have to be on.

- `adaptTempOutFreq` < steps between printing adaptive tempering output >
  Acceptable Values: Positive integers
  Default Value: 10
  Description: The number of timesteps between printing adaptive tempering output to the log file.

- `adaptTempFirstStep` < step to start adaptive tempering >
  Acceptable Values: Non-negative integers
  Default Value: 0
  Description: The first timestep from which adaptive tempering will be run.

- `adaptTempLastStep` < step to stop adaptive tempering >
  Acceptable Values: Positive integers
  Description: The last timestep to apply adaptive tempering.

- `adaptTempCgamma` < dynamic bin averaging constant >
  Acceptable Values: Non-negative real number
  Default Value: 0.1
  Description: The calculation of the mean energy for a given bin is weighted by a factor of 1 - Cgamma / samples to damp out old statistics. Setting Cgamma to zero restores the use of a standard arithmetic mean to calculate the mean energy for each bin.

- `adaptTempRandom` < assign random temperature if we step out of range? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: If set to on and the temperature steps out of [`adaptTempTmin`, `adaptTempTmax`], a random temperature in that range is assigned. Otherwise the previous temperature is kept.

11.5 Locally enhanced sampling

Locally enhanced sampling (LES) [92, 101, 102] increases sampling and transition rates for a portion of a molecule by the use of multiple non-interacting copies of the enhanced atoms. These enhanced atoms experience an interaction (electrostatics, van der Waals, and covalent) potential that is divided by the number of copies present. In this way the enhanced atoms can occupy the same space, while the multiple instances and reduces barriers increase transition rates.

11.5.1 Structure generation

To use LES, the structure and coordinate input files must be modified to contain multiple copies of the enhanced atoms. `psfgen` provides the `multiply` command for this purpose. NAMD supports a maximum of 255 copies, which should be sufficient.

Begin by generating the complete molecular structure and guessing coordinates as described in Sec. 4. As the last operation in your script, prior to writing the psf and pdb files, add the `multiply` command, specifying the number of copies desired and listing segments, residues, or atoms to be multiplied. For example, `multiply 4 BPTI:56 BPTI:57` will create four copies of the last two residues of segment BPTI. You must include all atoms to be enhanced in a single `multiply` command in order for the bonded terms in the psf file to be duplicated correctly. Calling `multiply` on connected sets of atoms multiple times will produce unpredictable results, as may running other commands after `multiply`.

The enhanced atoms are duplicated exactly in the structure—they have the same segment, residue, and atom names. They are distinguished only by the value of the B (beta) column in the pdb file, which is 0 for normal atoms and varies from 1 to the number of copies created for enhanced atoms. The enhanced atoms may be easily observed in VMD with the atom selection `beta != 0`.

11.5.2 Simulation

In practice, LES is a simple method used to increase sampling; no special output is generated. The following parameters are used to enable LES:

- `les` < is locally enhanced sampling active? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not LES is active.

  `lesFactor` < number of LES images to use >
  Acceptable Values: positive integer equal to the number of images present
  Description: This should be equal to the factor used in `multiply` when creating the structure. The interaction potentials for images is divided by `lesFactor`.

- `lesReduceTemp` < reduce enhanced atom temperature? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Enhanced atoms experience interaction potentials divided by `lesFactor`. This allows them to enter regions that would not normally be thermally accessible. If this is not desired, then the temperature of these atoms may be reduced to correspond with the reduced potential. This option affects velocity initialization, reinititialization, reassignment, and the target temperature for langevin dynamics. Langevin dynamics is recommended with

this option, since in a constant energy simulation energy will flow into the enhanced degrees of freedom until they reach thermal equilibrium with the rest of the system. The reduced temperature atoms will have reduced velocities as well, unless `lesReduceMass` is also enabled.

- `lesReduceMass` < reduce enhanced atom mass? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Used with `lesReduceTemp` to restore velocity distribution to enhanced atoms. If used alone, enhanced atoms would move faster than normal atoms, and hence a smaller timestep would be required.

- `lesFile` < PDB file containing LES flags >
  Acceptable Values: UNIX filename
  Default Value: `coordinates`
  Description: PDB file to specify the LES image number of each atom. If this parameter is not specified, then the PDB file containing initial coordinates specified by `coordinates` is used.

- `lesCol` < column of PDB file containing LES flags >
  Acceptable Values: `X`, `Y`, `Z`, `O`, or `B`
  Default Value: `B`
  Description: Column of the PDB file to specify the LES image number of each atom. This parameter may specify any of the floating point fields of the PDB file, either X, Y, Z, occupancy, or beta-coupling (temperature-coupling). A value of 0 in this column indicates that the atom is not enhanced. Any other value should be a positive integer less than `lesFactor`.

11.6 Replica exchange simulations

The `lib/replica/` directory contains Tcl scripts that implement replica exchange both for parallel tempering (temperature exchange) and umbrella sampling (exchanging collective variable biases). This replaces the old Tcl server and socket connections driving a separate NAMD process for every replica used in the simulation.

A NAMD build based on Charm++ 6.5.0 or later using one of the "LRTS" (low-level runtime system) machine layers is required! Current LRTS machine layers include mpi, netlrts, verbs (for InfiniBand), gemini_gni-crayxe, gni-crayxc, and pamilrts-bluegeneq.

Only temperature-exchange simulations are described below. To employ replicas for umbrella sampling you will need to understand this material, collective variable-based calculations (Sec. 9), and basic Tcl programming to adapt the examples in `lib/replica/umbrella/` and `lib/replica/umbrella2d/` until further documentation and a tutorial are available.

This implementation is designed to be modified to implement exchanges of parameters other than temperature or via other temperature exchange methods. The scripts should provide a good starting point for any simulation method requiring a number of loosely interacting systems.

Replica exchanges and energies are recorded in the .history files written in the output directories. These can be viewed with, e.g., "`xmgrace output/*/*.history`" and processed via awk or other tools. There is also a script to load the output into VMD and color each frame according to replica index. An example simulation folds a 66-atom model of a deca-alanine helix in about 10 ns.

`replica.namd` is the master script for replica temperature-exchange simulations. To run:

```
cd example
mkdir output
(cd output; mkdir 0 1 2 3 4 5 6 7)
mpirun namd2 +replicas 8 job0.conf +stdout output/%d/job0.%d.log
mpirun namd2 +replicas 8 job1.conf +stdout output/%d/job1.%d.log
```

The number of MPI ranks must be a multiple of the number of replicas (+replicas). Be sure to increment jobX for +stdout option on command line.

`show_replicas.vmd` is a script for loading replicas into VMD; first source the replica exchange conf file and then this script, then repeat for each restart conf file or for example just do "`vmd -e load_all.vmd`". This script will likely destroy anything else you are doing in VMD at the time, so it is best to start with a fresh VMD. `clone_reps.vmd` provides the `clone_reps` commmand to copy graphical representation from the top molecule to all other molecules.

`sortreplicas`, found in the namd2 binary directory, is a program to un-shuffle replica trajectories to place same-temperature frames in the same file. Usage:

```
sortreplicas <job_output_root> <num_replicas> <runs_per_frame> [final_step]
```

where job_output_root is the job specific output base path, including %s or %d for separate directories as in output/%s/fold_alanin.job1 This will be extended with .%d.dcd .%d.history for input files and .%d.sort.dcd .%d.sort.history for output files. The optional final_step parameter will truncate all output files after the specified step, which is useful in dealing with restarts from runs that did not complete. Colvars trajectory files are similarly processed if they are found.

A replica exchange config file should define the following Tcl variables:

- `num_replicas`, the number of replica simulations to use,
- `min_temp`, the lowest replica target temperature,
- `max_temp`, the highest replica target temperature,
- `steps_per_run`, the number of steps between exchange attempts,
- `num_runs`, the number of runs before stopping (should be divisible by `runs_per_frame` × `frames_per_restart`).
- `runs_per_frame`, the number of runs between trajectory outputs,
- `frames_per_restart`, the number of frames between restart outputs,
- `namd_config_file`, the NAMD config file containing all parameters, needed for the simulation except `seed`, `langevin`, `langevinTemp`, `outputEnergies`, `outputname`, `dcdFreq`, `temperature`, `bincoordinates`, `binvelocities`, or `extendedSystem`, which are provided by `replica.namd`,
- `output_root`, the directory/fileroot for output files, optionally including a "%s" that is replaced with the replica index to use multiple output directories,
- `psf_file`, the psf file for `show_replicas.vmd`,
- `initial_pdb_file`, the initial coordinate pdb file for `show_replicas.vmd`,

- `fit_pdb_file`, the coodinates that frames are fit to by `show_replicas.vmd` (e.g., a folded structure),

The `lib/replica/example/` directory contains all files needed to fold a 66-atom model of a deca-alanine helix:

- `alanin_base.namd`, basic config options for NAMD,
- `alanin.params`, parameters,
- `alanin.psf`, structure,
- `unfolded.pdb`, initial coordinates,
- `alanin.pdb`, folded structure for fitting in `show_replicas.vmd`,
- `fold_alanin.conf`, config file for `replica_exchange.tcl` script,
- `job0.conf`, config file to start alanin folding for 10 ns,
- `job1.conf`, config file to continue alanin folding another 10 ns, and
- `load_all.vmd`, load all output into VMD and color by replica index.

The `fold_alanin.conf` config file contains the following settings:

```
set num_replicas 8
set min_temp 300
set max_temp 600
set steps_per_run 1000
set num_runs 10000
# num_runs should be divisible by runs_per_frame * frames_per_restart
set runs_per_frame 10
set frames_per_restart 10
set namd_config_file "alanin_base.namd"
set output_root "output/%s/fold_alanin" ; # directories must exist

# the following used only by show_replicas.vmd
set psf_file "alanin.psf"
set initial_pdb_file "unfolded.pdb"
set fit_pdb_file "alanin.pdb"
```

11.7 Random acceleration molecular dynamics simulations

The "lib/ramd" directory stores the tcl scripts and the example files for the implementation of the Random Acceleration Molecular Dynamics (RAMD) simulation method in NAMD. The RAMD method can be used to carry out molecular dynamics (MD) simulations with an additional randomly oriented acceleration applied to the center of mass of one group of atoms (referred to below as "ligand") in the system. It can, for example, be used to identify egress routes for a ligand from a buried protein binding site. Since its original implementation in the ARGOS [66, 120] program, the method has been implemented in AMBER 8 [96], and CHARMM [17]. The first implementation

of RAMD in NAMD using a tcl script (available as supplementary material in [113]) provided only limited functionality compared to the AMBER 8 implementation and was followed with an implementation of RAMD and RAMD–MD in NAMD [24, 9]. Recently the RAMD method was improved in speed by using NAMD vector implementations and streamlining the code. The current implementation is now focused on the RAMD simulation and was used in the τRAMD procedure for the estimation of relative drug-target residence times [56].

Additional information is found in the README file in the "lib/ramd" directory. The user is encouraged to carefully read this information before starting production runs.

The two required scripts are stored in "lib/ramd/scripts": (i) ramd–5.tcl defines the simulation parameters and passes them from the NAMD configuration file to the main script, (ii) "ramd–5_script.tcl" adds the randomly oriented force and performs all related computations.

Two examples for running RAMD are included in the directory "lib/ramd/example/". The examples can be started using the RAMD-force.sh shell scripts.

The specific RAMD simulation parameters to be provided in the NAMD configuration file (listed below) should be preceded by the keyword "ramd". The default values for these parameters are only given as guidance. They may not to be suitable for other systems.

Mandatory parameter settings:

- `ramd lastProtAtom` < Last index of protein atom >
  Acceptable Values: `positive integer`
  Description: Specifies the index of the last protein atom.

- `ramd firstRamdAtom` < First index of ligand atom >
  Acceptable Values: `positive integer`
  Description: Specifies the index of the first ligand atom.

- `ramd lastRamdAtom` < Last index of ligand atom >
  Acceptable Values: `positive integer`
  Description: Specifies the index of the last ligand atom.

- `ramd ramdfilename` < Name of ramd output file >
  Acceptable Values: `Valid file name`
  Description: Specified the name of the file where the ramd logs are written.

Optional parameter settings with a default. Depending on your simulation system, you might want to change these settings:

- `ramd firstProtAtom` < First index of protein atom >
  Acceptable Values: `positive integer`
  Default Value: `1`
  Description: Specifies the index of the first protein atom.

- `ramd ramdSteps` < Set number of steps in RAMD block >
  Acceptable Values: `positive integer`
  Default Value: `50`
  Description: Specifies the number of steps in 1 RAMD block; the simulations are evaluated every 'ramdSteps' steps.

- `ramd forceRAMD` < Set acceleration force >
  Acceptable Values: `positive decimal`
  Default Value: `16.0`
  Description: Specifies the force to be applied. Replaces the acceleration (accel) specified in previous releases. Defaults to 16 kcal/mol/Angstrom

- `ramd rMinRamd` < Set threshold for distance travelled RAMD >
  Acceptable Values: `positive decimal`
  Default Value: `0.01`
  Description: Specifies a threshold value for the distance in Angstroms travelled by the ligand in 1 RAMD block. In RAMD simulations the direction of the acceleration is changed if the ligand has travelled less than 'rMinRamd' Å in the evaluated block.

- `ramd forceOutFreq` < Set frequency of RAMD forces output >
  Acceptable Values: `positive integer`, Must be divisor of `ramdSteps`
  Default Value: `10`
  Description: Every 'forceOutFreq' steps, detailed output of forces will be written.

- `ramd maxDist` < Set center of mass separation >
  Acceptable Values: `positive decimal`
  Default Value: `50`
  Description: Specifies the distance in Angstroms between the the centers of mass of the ligand and the protein when the simulation is stopped.

- `ramd ramdSeed` < Set RAMD seed >
  Acceptable Values: `positive integer`
  Default Value: `14253`
  Description: Specifies seed for the random number generator for generation of RAMD force directions. Change this parameter if you wish to run different trajectories with identical parameters.

- `ramd debugLevel` < Set debug level of RAMD >
  Acceptable Values: `integer value`
  Default Value: 0
  Description: Activates verbose output if set to an integer greater than 0. Should be used only for testing purposes because the very dense output is full of information only relevant for debugging.

- `ramd namdVersion` < Set the NAMD version >
  Acceptable Values: `float value`
  Default Value: 2.13
  Description: After NAMD version 2.10 a call to *enabletotalforces* is done to enable tcl processing in NAMD

Note: In the current RAMD implementation, combined RAMD-MD simulations, where RAMD blocks alternate with standard MD blocks are not available. In case you are are interested in this feature, please contact the RAMD developers at mcmsoft@h-its.org

Scripts for using RAMD in the τRAMD procedure for computing residence times are available at: https://www.h-its.org/downloads/ramd/.

12 Structure based simulations

12.1 Hybrid MD-Go Simulation

12.1.1 Hybrid MD-Go model

NAMD incorporates a hybrid MD-Go model (hereby referred to as Go) to study the conformation changes in biomolecular systems. The method replaces the physical-based nonbonded interactions with a smoother knowledge-based potential energy surface. Bonded interactions are taken from the classical force fields. By removing energetic traps along a MD trajectory, the system will be able to sample states not normally accessible to classical MD simulations.

12.1.2 Hybrid MD-Go considerations

Typically, Go simulations are conducted in the **absence of solvent** and with **electrostatic and van der Waals forces** in the system **turned off** to improve conformational space exploration. Due to the current implementation of Go, the partial charges and van der Waals radii need to be set to zero in the psf and parameter file to remove the physical nonbonded interactions. Additionally, NAMD uses a **reference PDB structure** to construct the Go pairwise potential between atoms.

Finally, the Go model in NAMD introduces the idea of chain types. Consider modeling a protein-nucleic acid complex. Using classical all-atom MD, a single force field describes all possible nonbonded interactions. With Go, however, one can create separate nonbonded force fields to describe the protein and nucleic acid interactions. In order to create separate force fields, atoms are grouped together using chain types where the chain types are taken from the occupancy field of the reference PDB file. For argument sake, assume that the protein atoms have an occupancy value of 1.0 and that the nucleic acid atoms have an occupancy value of 2.0. One now must define three separate Go potentials for intra-protein, intra-nucleic acid, and inter-protein-nucleic acid interactions. In terms of chain types, this corresponds to (1) between atom pairs fully in chain 1, (2) between atom pairs fully in chain 2, (3) between atom pairs where one atom is in chain 1 and the other atom is in chain 2 respectively. To run Go, a minimum of one chain type must be defined.

12.1.3 Configuration file modifications

The following configuration parameters are used to setup and run a Go simulation:

- `GoForcesOn` < Are Go forces turned on? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether or not Go forces should be calculated. If turned `'off'`, Go forces will not be calculated. If turned `'on'`, Go forces will be calculated. By default, the Go forces will be calculated in addition to the electrostatic and van der Waals forces. To simulate a system using only Go forces, the partial charges and Lennard-Jones parameters can be set to zero in the force field files.

- `GoParameters` < Parameter file defining Go potential >
  Acceptable Values: file

Description: File contains parameters to define the Go pairwise forces between different chain types. All possible chain type pairing combinations must be enumerated. Chain types are defined in the GoCoordinates file. The format for the GoParameters file is described in the next section.

- `GoCoordinates` < Reference structure for Go simulation >
  Acceptable Values: PDB file
  Description: PDB file contains the reference structure used to define the Go potential. The file need not be the same file used to initialize the coordinates of the MD simulation; however, it must contain the same number of atoms in the same order as given in the structure (.psf) and coordinates (.coor) file. Additionally, the occupancy fields of the PDB file will be read to determine which chain type an individual atom belongs to, and, thus, which pairwise Go potential to use to calculate forces. By default, the occupancy value of 0.0 turns off the Go potential for that particular atom.

- `GoMethod` < controls method for storing Go contact information >
  Acceptable Values: `lowmem` or `matrix`
  Description: Specifies whether the Go contacts should be calculated on the fly or stored in a matrix respectively. In most cases, `'lowmem'` will be sufficient. However, for smaller systems, the `'matrix'` does offer a slight performance speedup in terms of wall time. Variable is only used if GoForcesOn is `'on'`

The following sections describe the format of the GoParameter file.

12.1.4 GoParameter format

When running a Go simulation, the atoms are partitioned into chains according to the occupancy value given in the GoCoordinates file. For every possible pairwise combination between chains, a Go potential is defined by the following equations:

Let $r_{i,j}^{ref}$ be the pairwise distance between atoms i and j in the reference structure. If $r_{i,j}^{ref}$ is less than the Go cutoff distance, the pairwise potential between atoms i and j is given by:

$$V_{Go}(r_{i,j}, \epsilon, \sigma_{i,j}^{ref}, a, b) = 4\epsilon\left[\left(\frac{\sigma_{i,j}^{ref}}{r_{i,j}}\right)^{a} - \left(\frac{\sigma_{i,j}^{ref}}{r_{i,j}}\right)^{b}\right]$$

where $\sigma_{i,j}^{ref}$ is given as $\left(\frac{b}{a}\right)^{\frac{1}{b-a}} r_{i,j}^{ref}$. If $r_{i,j}^{ref}$ is greater than the Go cutoff distance, the pairwise potential between atoms i and j is given by:

$$V_{Go}(r_{i,j}, \epsilon^{rep}, \sigma^{rep}, expRep) = 4\epsilon^{rep}(\frac{\sigma_{i,j}^{rep}}{r_{i,j}})^{expRep}$$

For each pairwise chain combination, the following parameters are needed to define the Go potential:

- **chaintypes (2 floats):** (*first_chain second_chain*) Defines the pairwise chain interaction

- **epsilon (1 float):** (ϵ) Determines the ϵ constant of the Go potential in units of $kcal \cdot mol^{-1} \cdot \mathring{A}^{-2}$
- **exp_a (1 integer):** (a) Determines the 'a' constant for the Go potential
- **exp_b (1 integer):** (b) Determines the 'b' constant for the Go potential
- **expRep (1 integer):** (*expRep*) Determines the 'expRep' constant for the Go potential
- **sigmaRep (1 float):** (σ^{rep}) Determines the σ^{rep} constant for the Go potential in units of $\mathring{A}$
- **epsilonRep (1 float):** (ϵ^{rep}) Determines the ϵ^{rep} constant for the Go potential in units of $kcal \cdot mol^{-1} \cdot \mathring{A}^{-2}$
- **cutoff (1 float):** (*cutoff*) Defines the Go cutoff distance for this particular pairwise chain in units of $\mathring{A}$
- **[Optional] restriction (1 integer):** Determines if interactions between the i^{th} and i^{th} + *integer* adjacent residue should be excluded. Multiple restriction between adjacent residues can be defined within a chaintype. Each additional new restriction is given on its own line.

Each pairwise chaintype should be written in its own block of text with each entry given its own line. It is recommended that individual pairwise potential be separated by a blank line.

12.2 Running SMOG simulations

12.2.1 SMOG model considerations

NAMD supports the SMOG model from published from Onuchic's lab [118, 119]. The input files for SMOG can be generated from the SMOG website (`http://smog-server.org`) [82]. It is recommended to run these simulations with 1-4 exclusions (as opposed to scaled 1-4), a 0.5fs timestep, and with a 0.5fs timestep (as described in [118, 119]).

12.2.2 Configuration file modifications

As the SMOG model uses GROMACS topology and coordinate files, the GROMACS configuration parameters—`gromacs`, `grotopfile`, grocoorfile—must be defined. The description for the GROMACS configuration parameters are reproduced below:

- `gromacs` < use GROMACS format force field? >
  Acceptable Values: on or off
  Default Value: off
  Description: If `gromacs` is set to on, then `grotopfile` must be defined, and `structure` and `parameters` should not be defined.
- `grotopfile` < GROMACS format topology/parameter file >
  Acceptable Values: UNIX filename
  Description: This file contains complete topology and parameter information of the system.

- `grocoorfile` < GROMACS format coordinate file >
  Acceptable Values: UNIX filename
  Description: This file contains the coordinates of all the atoms. Note that `coordinates` can also be used for PDB format coordinate file. When `gromacs` is set to `on`, either `grocoorfile` or `coordinates` must be defined, but not both.

To run a SMOG simulation, the following extra parameters must be defined.

- `GromacsPair` < Are GROMACS pair forces turned on? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: This variable determines if the pair section of the GROMACS topology file grotopfile is evaluated. Currently, only Lennard-Jones type pairs are supported. Variable is only used if gromacs variable is `'on'`.

- `staticAtomAssignment` < Optimization to fix atoms onto a specific node >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies if atoms should be statically fixed to a node. This will change the internode communication and will give a significant speed-up to MD simulations if the atoms are moving rapidly. It is suggested that SMOG simulations use the staticAtomAssignment flag.

13 Constant-pH Simulations [1]

Constant-pH MD in NAMD is based on the scheme first proposed by Stern [105] and later revised and extended by Chen and Roux [19]. A detailed description of the modifications and improvements made in the NAMD implementation has been presented elsewhere by Radak, *et al.* [88] and this is likely the best comprehensive description of the method, its uses, and its limitations/pitfalls. Herein the goal is to provide a working understanding of how the implementation works and what kinds of data it produces.

13.1 Overview and Theoretical Background

Constant-pH MD is a simulation methodology specially formulated for the treatment of variable protonation states. This is to be contrasted with conventional force-field based MD simulations, which generally treat protonation states by assuming they are fixed. Consider, for example, a protein with two titratable residues which may both be either protonated or deprotonated (Figure 13); the system has four possible protonation states. In the conventional route, the user must enumerate these possibilities, construct distinct topologies, and then simulate the cases individually. The simulations for each state must then be connected by either asserting knowledge about the system (*e.g.*, by assuming that only certain states are of biological importance) or by performing additional simulations to probe transitions between states directly (*e.g.*, by performing free energy calculations). In a constant-pH MD simulation, knowledge of the transformations is not assumed and is instead actively explored by interconverting between the various protonation states. This is especially useful when the number of protonation states is extremely large and/or prior information on the importance of particular states is not available.

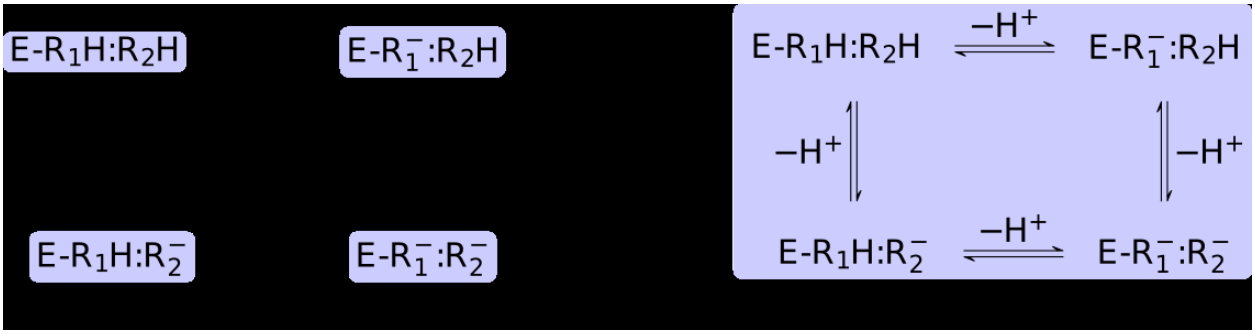


Figure 13: The core difference between conventional and constant-pH MD can be illustrated by a simple enzyme E with four protonation states describing the occupancy of two titratable residues, R_1 and R_2. A conventional MD simulation handles the states *separately* (left panel). The relative importance of the states must be known beforehand or computed by other means. Conversely, a constant-pH MD simulation handles the states *collectively* and actively simulates interconversion (right panel). Determining the relative importance of the states is a direct result of the simulation.

[1] The features described in this section were implemented by Brian K. Radak (Argonne National Laboratory, Argonne, IL USA) with considerable technical support from James C. Phillips (University of Illinois, Urbana, IL USA) and Wei Jiang (Argonne National Laboratory). The algorithm draws heavily from earlier work by Yunjie Chen and Benoît Roux and later by Donghyuk Suh (University of Chicago, Chicago, IL USA), as well as time spent as a postdoctoral scholar at University of Chicago. Testing and validation were also aided by Christophe Chipot (Université de Lorraine, Vandœuvre-lès-Nancy cedex France and University of Illinois).

In formal terms, conventional MD samples from a canonical ensemble, whereas constant-pH MD samples from a semi-grand canonical ensemble. The new partition function,

$$\Xi(\mathrm{pH}) = \sum_{\boldsymbol{\lambda}\in\mathcal{S}} Q_{\boldsymbol{\lambda}} 10^{-n_{\boldsymbol{\lambda}}\mathrm{pH}}, \tag{92}$$

is essentially a weighted summation of canonical partition functions, $Q_{\boldsymbol{\lambda}}$, each of which are defined by an occupancy vector, $\boldsymbol{\lambda}$. The elements of $\boldsymbol{\lambda}$ are either one or zero depending on whether a given protonation site is or is not occupied, respectively. For a vector of length m, the set of all protonation states, $\mathcal{S}$, has at most 2^m members. In order to sample from the corresponding semi-grand canonical distribution function, a simulation must explore *both* the phase space defined by the canonical paritition functions and the state space defined by the different occupancy vectors. The fraction of simulation time spent in each state is dictated by the weights in the summation and these depend on the pH and the number of protons, $n_{\boldsymbol{\lambda}}$, in the system (*i.e.*, the sum of the elements in $\boldsymbol{\lambda}$).

Although a constant-pH MD system may contain any number of titratable protons, the base transformation is always the movement of *one* proton from a molecule into a bath of non-interacting protons "in solution." For a generic chemical species A, this corresponds to the usual deprotonation reaction definition, except with fixed pH:

$$\mathrm{HA} \underset{\text{pH fixed}}{\overset{-\mathrm{H}^+}{\rightleftharpoons}} \mathrm{A}^-.$$

In the language of statistical mechanics the species HA and A^- refer to all terms in Eq. (92) which do and do not, respectively, contain the specific proton in question (*i.e.*, the particular element of $\boldsymbol{\lambda}$ is one or zero). By taking out a factor of $10^{-\mathrm{pH}}$, this can be re-written as

$$\Xi(\mathrm{pH}) = \Xi_{\mathrm{A}^-}(\mathrm{pH}) + \Xi_{\mathrm{HA}}(\mathrm{pH})10^{-\mathrm{pH}}$$

and then recast as a statistical mechanical analog of the Henderson-Hasselbalch equation by recognizing that $\Xi_{\mathrm{A}^-}(\mathrm{pH})/\Xi_{\mathrm{HA}}(\mathrm{pH})$ is just the ratio of deprotonated / protonated fractions of species A. The *protonated* fraction is then

$$P_{\mathrm{HA}}(\mathrm{pH}) = \frac{1}{1+10^{\mathrm{pH}-\mathrm{p}K_{\mathrm{a}}(\mathrm{pH})}}; \qquad \mathrm{p}K_{\mathrm{a}}(\mathrm{pH}) \equiv -\log\frac{\Xi_{\mathrm{A}^-}(\mathrm{pH})}{\Xi_{\mathrm{HA}}(\mathrm{pH})}. \tag{93}$$

In practice, $P_{\mathrm{HA}}(\mathrm{pH})$ can be calculated from a simulation by simply counting the fraction of time spent in state HA (*e.g.*, the fraction of time a specific element of $\boldsymbol{\lambda}$ is one). Note also that $\mathrm{p}K_{\mathrm{a}}(\mathrm{pH})$ is formally a pH dependent function unless the system only contains one proton (or type of proton).

In most experimental contexts, a different form of Eq. (93) is used which is often referred to as a "generalized" Hill equation. This corresponds to a specific choice of pH dependence such that

$$\mathrm{p}K_{\mathrm{a}}(\mathrm{pH}) \approx \mathrm{p}K_{\mathrm{a}}^{(\mathrm{a})} + (1-n)\left(\mathrm{pH} - \mathrm{p}K_{\mathrm{a}}^{(\mathrm{a})}\right).$$

The constant n is then known as the Hill coefficient and the so-called apparent $\mathrm{p}K_{\mathrm{a}}$, $\mathrm{p}K_{\mathrm{a}}^{(\mathrm{a})}$, generally corresponds to the inflection point of a plot of $P_{\mathrm{HA}}(\mathrm{pH})$. Both quantities are usually determined by non-linear regression after P_{HA} has been determined at different pH values.

13.2 Implementation Details

In NAMD, each canonical partition function is represented by a specific force field description embodied in a PSF – in order to change the protonation state the underlying PSF must also be modified. This is accomplished by a close coupling to `psfgen`. The models that can be used with constant-pH MD are thus limited to only those which can be completely implemented within `psfgen`. This also means that NAMD requires access to residue topology files (RTFs) during the course of a simulation. These must be specified with the `psfgen topology` command.

For consistency between topological descriptions, NAMD uses "dummy" atoms to represent non-interacting protons. These atoms have the same mass as protons but only interact with the system via a minimal number of force field bonded terms. This formalism guarantees that: 1) the number of atoms/coordinates during the simulation remains fixed and 2) the thermodynamics of the model is unchanged. The latter point is subtle and warrants comment. As implemented in NAMD, constant-pH MD only captures the thermodynamics of the semi-grand canonical ensemble. There is no active description of proton dissociation events. However, this is more of a limitation of classical MD than a particular shortcoming of NAMD. A useful analogy may be the use of Langevin dynamics as a thermostat as opposed to a phenomonological model for Brownian motion.

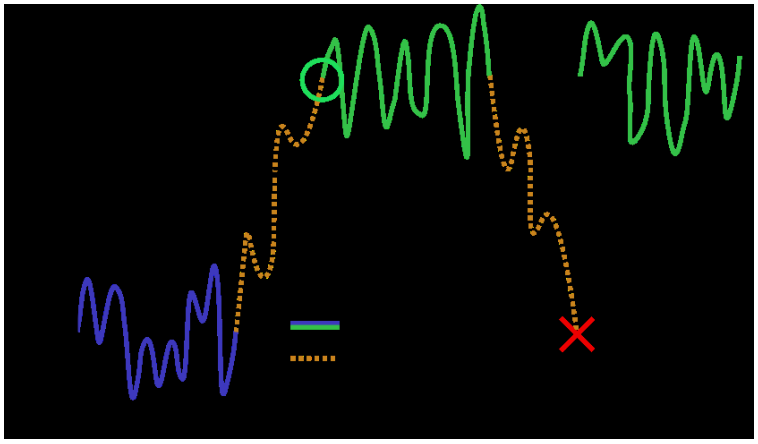


Figure 14: The basic constant-pH MD scheme in NAMD is to alternate equilibrium sampling in a fixed protonation state followed by a nonequilibrium MD Monte Carlo move to sample other protonation states. The latter move can be accepted or rejected. If accepted, the simulation continues in the new protonation state. If the move is rejected, sampling continues as if the move were never attempted at all.

The basic scheme in NAMD is to alternately sample the protonation state and then the configuration space within that state. Protonation state sampling is accomplished by an alchemical coupling scheme that forcibly turns off interactions with the current protonation state and turns on interactions with a candidate protonation state. This nonequilibrium "switching" is accomplished with the alchemy code (specifically the thermodynamic integration code branch) and necessarily has lower performance (by about 30%) than regular MD due to the added electrostatic calculations in the reciprocal space (*i.e.*, when using PME). However, the configuration space sampling should still have normal performance. The switching process exerts work on the system and thus drives the system out of equilibrium. However, an appropriately designed Monte Carlo (MC) move using an accept/reject criterion can recover the correct semi-grand canonical equilibrium distribution in both the state and configuration spaces [81, 20]. The resulting scheme is a hybrid nonequilibrium

MD/MC (neMD/MC) algorithm. The most important conceptual change from conventional MD is that, rather than being a continuous trajectory, the simulation now becomes a series of cycles composed of an MD and neMD/MC step. This means that the length of the simulation is no longer simply determined by the number of steps (`numsteps`) but rather the number of cycles. The length of a cycle is also determined by two parts – the amount of time on equilibrium sampling and the amount of time executing the switch.

It may be profitable/necessary to vary the switch time depending on the type of protonation change that is being effected. Indeed, this is a critical factor in the efficiency of the method. That is, if the switch is too short, then moves are unlikely to be accepted and effort will be wasted when the move is rejected. However, if the switch is too long, then an inordinate amount of effort will be spent sampling the state space and there will be fewer resources left for exploring the configuration space. Some basic qualities of the system that affect sampling have been determined using nonequilibrium linear response theory [89]. In short, there are intrinsic limits based on: 1) the extent that differing interactions between each state fluctuate (according to some variance, σ_0^2) and 2) the "molecular" time scale, τ_m, on which these fluctuations change. These effects are roughly captured by the expression [89, 88]:

$$\tau_\mathrm{opt} \leq \frac{\sigma_0^2 \tau_\mathrm{m}}{2.83475},$$

where τ_opt is some optimal switching time, in the sense of maximizing the rate at which protonation states interconvert. Overall, switching times on the order of tens of picoseconds tend to be optimal in that they balance the high cost of switching versus the high acceptance rate at longer switching times (in the infinite time limit the perturbation is adiabatic and exerts zero work). For titratable groups exposed primarily to aqueous solvent, a switch on the order of 10-20 ps appears to give near optimal results [89, 88]. An equivalent formulation of the above expression is that mean acceptance rates around 20-25% are likely near optimal.

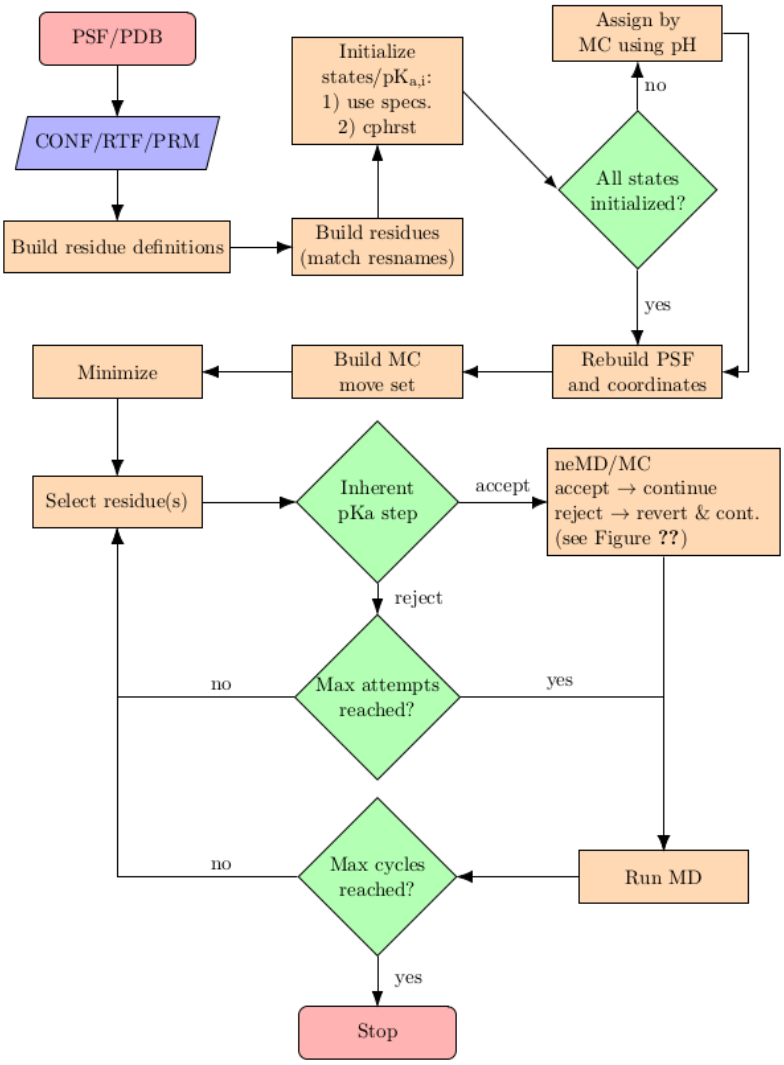
Important Limitations:

For various reasons concerning the implementation, constant-pH simulations are currently *incompatible* with the following NAMD functionalities in all or most situations:

- Any system using GPUs/CUDA
- Generalized Born implict solvent (`GBIS`)
- Alchemical free energy calculations, *e.g.*, ligand binding (`alch`)
- Drude polarizable force fields
- Hybrid quantum mechanical/molecular mechanical simulations
- Collective variables (`colvars`)
- `extraBonds`

This list is neither exhaustive nor definitive. In many instances the problem may be overcome by modest additional developments.

`namdcph` Execution Flow Chart



13.3 New Commands and Keywords

The constant-pH implementation is largely implemented in Tcl and can be found in `/lib/namdcph/namdcph.tcl`, where the base directory is the NAMD source home directory. When that file has been loaded with a suitable `source` command, the following commands and keywords are available and appear to the user in a way similar to NAMD syntax. The most significant change from normal NAMD usage is that there is generally no need to use the `run` command. One should instead use the new `cphRun` command; this can only be used *once* per script for now. *NB*, all commands and keywords are currently case sensitive!

`cphRun` < Run constant-pH MD >
Arguments: <*numsteps*> [*numcycles*]
Defaults: `numcycles` = 1
Description: Execute `numcycles` cycles of constant-pH MD with the current settings. Each cycle consists of 1) a neMD/MC move in both configuration and protonation space and 2) MD based sampling in configuration space. By default, configuration space sampling simply consists of `numsteps` dynamics, as in conventional MD. The nature of the neMD/MC moves, however, is more elaborate and controlled by other keywords, *many of which are required* (see below).

13.3.1 Required Keywords

- `pH` < pH value that the system is in contact with >
  Acceptable Values: decimal (usually between 0 and 14)
  Description: The `pH` is effectively a chemical potential applied to protons *only*. This value affects the details of neMD/MC moves but otherwise has no effect on the system dynamics.

- `cphConfigFile` < File defining titratable residues >
  Acceptable Values: filename
  Description: The `cphConfigFile` contains definitions for the available titratable residues. This is essentially meta information regarding the RTF contents, but also includes experimental references and additional force field parameterization.

- `cphNumstepsPerSwitch` < Number of steps during nonequilibrium switching >
  Acceptable Values: [*integer* [<*move label*> <*integer*>] ...]
  Description: Each move must have an associated number of steps per switch. If an odd number number of arguments is specified, then the first such argument is assumed to be a default number for all such moves. After this (or if an even number of arguments is specified) all remaining arguments are assumed to be specific assignments for a given move label of the form <*segid*>:<*resid*>:<*resname*>/<*segid*>:<*resid*>:<*resname*>/....

13.3.2 Commonly Used Options

- `cphSetResidueState` < Set the initial state of one or more titratable residues. >
  Acceptable Values: <*segid*>:<*resid*>:<*resname*> <*state*> [...]
  Description: Initial residue states can be assigned in three ways (in descending order of precedence): 1) via this command, 2) from a `cphRestartFile`, and 3) randomly from the assigned `pH` and the current inherent pKa of each residue.

- `cphSetResiduepKai` < Set the inherent pKa of one or more titratable residues. >
  Acceptable Values: <*segid*>:<*resid*>:<*resname*> <*pKai*> [...]
  Description: The two step inherent pKa algorithm implemented here permits on-the-fly update of an estimate for the pKa(s) of each residue. These can either be guessed at the outset (the default is to use the reference pKa) or updated as the simulation progresses. A more accurate estimate of the inherent pKa increases the statistical efficiency of the method, but the long time result is formally unbiased regardless of the value. If an extremely large or extremely small value is assigned, then the residue will be assigned the most probable protonation state at the given pH and likely remain fixed in that state.

- `cphExcludeResidue` < Exclude one or more residues from being titratable >
  Acceptable Values: <*segid*>:<*resid*>:<*resname*> [...]
  Description: By default, any residue that matches a titratable residue type will be allowed to change protonation state. This command permits specific residues to be excluded from consideration in a manner that is similar to assigning an extreme inherent pKa (see `cphSetResiduepKai`). The main differences are that 1) the protonation state will not be modified and remain as it is in the original PSF and 2) the protons in the residue will *not* be tracked in the `cphlog` file. This command is not always recommended, but is currently necessary for handling disulfide linkages.

- `cphRestartFile` < Restart file for constant-pH >
  Acceptable Values: filename
  Description: Constant pH requires additional checkpoint information regarding the state of the titratable residues and the nature of the neMD/MC moves. This (optional) information is read from the file specified here. After/during a simulation, this information is written to `[outputname]`.cphrst.

- `cphRestartFreq` < Frequency at which constant-pH checkpoint files are written >
  Acceptable Values: Non-negative integer
  Default Value: 0
  Description: Checkpoint information is written to `[outputname]`.cphrst every `cphRestartFreq` cycles (*not* MD steps). A checkpoint file is *always* written at the end of the last cycle.

- `cphOutFile` < Log file for constant-pH >
  Acceptable Values: filename
  Default Value: `[outputname]`.cphlog
  Description: Titratable residue state information is logged here after every cycle.

- `cphProposalWeight` < MC move label and weight specifications >
  Acceptable Values: <*move label*> <*weight*> [[<*move label*> <*weight*>] ...]
  Description: During each cycle, MC moves are selected from the move set and then accepted/rejected according to a Metropolis criterion based on the combined inherent pKa information and pH. The move weight affects the probability that such a move is selected. Note that *this does not affect the probability that any given proposal is accepted*, it merely increases the number of attempts at the given proposal. This may be useful in a system where one desires specific attention on a given process, such as proton transfer or the exchange of a given residue, but one does not want to assume that all other residue protonation states are

nominally fixed. By default all moves are assigned equal weights of 1.0. During the simulation these are automatically normalized to a discrete probability mass function.

- `cphMaxProposalAttempts` < Maximum number of switch proposal attempts per cycle >
  Acceptable Values: integer
  Default Value: 0
  Description: During each cycle, MC moves are selected from the move set and then accepted/rejected according to a Metropolis criterion based on the combined inherent pKa information and pH. This process stops when either a switch move is accepted or a maximum limit is reached. Any value less than one defaults to the number of titratable residues in the system.

- `cphNumMinSteps` < Number of steps of minimization before dynamics >
  Acceptable Values: integer
  Default Value: 0
  Description: This is a replacement for the normal minimize command, which is not compatible with constant-pH due to PSF modifications during initialization. Setting this option to a modest number (100–200, say) might be necessary when randomizing protonation states based on pH, since in that case it cannot be assumed that the starting structure is representative of the initial protonation state.

13.3.3 Specialized Options

- `cphForceConstant` < force constant for alchemical switches (in kcal/mol-Å^2) >
  Acceptable Values: Non-negative decimal
  Default Value: 100.0
  Description: During "dual-topology" alchemical switches, a harmonic bond is formed between analogous atoms in each alchemical region. This rigorously leaves all static thermodynamic quantities intact and is generally expected to improve the stability of dynamic quantities.

- `cphMDBasename` < basename of intermediate files for equilibrium MD >
  Acceptable Values: string
  Default Value: namdcph.md
  Description: PSF/coordinate modifications are currently done via the file system and utilize intermediate files. It may be advantageous to direct this I/O to a fast temporary directory.

- `cphSWBasename` < basename of intermediate files for nonequilibrium (switch) MD >
  Acceptable Values: string
  Default Value: namdcph.sw
  Description: PSF/coordinate modifications are currently done via the file system and utilize intermediate files. It may be advantageous to direct this I/O to a fast temporary directory.

Undocumented Features:
The constant-pH code is actively under development, although future work will almost exclusively be in adding new features and capabilities as well as improving performance. Because the code is fairly lightweight and available in `Tcl`, the intrepid user may discover "easter egg" features which are not listed in the documentation. **USE UNDOCUMENTED FEATURES AT YOUR OWN RISK.** Such undocumented features may work (and even be advisable) for specific problems, but have not undergone as rigorous of testing and may be prone to unintended consequences.

13.4 Minimal Examples

Constant-pH simulations can be carried out with largely the same options as conventional MD simulations (with some exceptions, see previous sections). The follwing examples assume that: 1) PSF and PDB files for the system of interest have already been constructed and 2) appropriate simulation keywords have already been chosen (*e.g.*, for PME, Langevin dynamics, *etc.*).

```
# End conventional settings...
source .../namd/lib/namdcph/namdcph.tcl
# Constant-pH MD requires additional force field files _during_ the simulation.
# In general, all RTFs used to construct the system need to be included with
# the ‘‘topology’’ command (just as in psfgen). Additional constant-pH specific
# RTF and PRM files are also necessary, as well as an accompanying
# configuration file in JSON format.
#
cphConfigFile <path to JSON config file>
topology <path to RTF>
topology <path to another RTF>
pH 7.0
# The following defaults all nonequilibrium switches to 5000 steps and then
# increases the time for residue 5 of segid PROA to 7500 steps -- multiple
# residues can be specified
#
cphNumStepsPerSwitch 5000 PROA:5:ASP 7500
# Run 100 minimization cycles before starting dynamics.
cphNumMinSteps 100
# Run 2500 steps of MD between attempted protonation state changes. Run 10
# cycles of MD and neMD/MC. The _upper_ bound of the simulation is thus:
#
# 10*(2500 + 7500) = 100000 steps
#
# but the actual simulation may be shorter in length.
#
cphRun 2500 10
```

Restarting a simulation
The following assumes that a simulation has already been run (as in the example above). For clarity we shall assume that `outputname` was set to "foo" such that restart files have been written to foo.coor and foo.vel (normal output) as well as foo.psf, foo.pdb, and foo.cphrst (constant-pH specific output).

```
# End conventional settings...
source .../namd/lib/namdcph/namdcph.tcl
# Constant-pH MD requires additional force field files _during_ the simulation.
# In general, all RTFs used to construct the system need to be included with
# the ‘‘topology’’ command (just as in psfgen). Additional constant-pH specific
# RTF and PRM files are also necessary, as well as an accompanying
# configuration file in JSON format.
#
cphConfigFile <path to JSON config file>
topology <path to RTF>
topology <path to another RTF>
pH 7.0

structure foo.psf
coordinates foo.pdb
binCoordinates foo.coor
binVelocities foo.vel
cphRestartFile foo.cphrst
# NB: switch times and inherent pKa values are read here and no longer need to
# be specified as during initialization

cphRun 2500 10
```

14 Hybrid QM/MM Simulations

Even though molecular mechanics (MM) force-fields are based on quantum mechanical calculations and experimental observations, only quantum mechanics (QM) can give a complete and accurate understanding of many biochemical processes, particularly those involving chemical reactions or charge redistribution. Nevertheless, even with the advanced hardware technology available today, the computational cost of studying nanosecond-long dynamics of entire systems relying solely on QM methodologies is usually prohibitive. A common route to circumvent this cost barrier is to confine the QM formalism to a sub-region of a system and to include the effects of the surrounding system through MM simulations, leading to hybrid QM/MM simulations [97].

NAMD's comprehensive QM/MM suite [75] was developed to provide easy setup, visualization and analysis of QM/MM simulations through the graphical user interface VMD/QwikMD [91], and a broad range of QM methods through NAMD's new "QMForces" module. The QM/MM interface in NAMD supports the simulation of many independent QM regions, and smooth integration with a vast collection of enhanced sampling methods. In hybrid QM/MM simulations, NAMD offloads part of its standard force and energy calculations to a QM program, either through native interfaces to MOPAC [106, 68] or ORCA [80], or through a flexible generic interface requiring a wrapper script, where exemplary Python wrappers are provided for Gaussian, TeraChem and Q-CHEM. Multiple QM-MM coupling schemes are implemented, allowing for both mechanically and electrostatically embedded QM regions to be used (see description in Nature Methods [75]). QM/MM simulations require the same input files used for classical MD, with additional options in the configuration file. QM and MM atoms covalently bound are usually treated by redistributing the MM atom's charge over its nearest MM neighbors and by capping the QM atom with a hydrogen atom, as shown in Figure 15 for a solvated tri-alanine QM/MM calculation using the NAMD/ORCA interface. Tests of the QM/MM interface for accuracy, stability and performance, are provided as supporting information in Nature Methods [75].

If employing NAMD QM/MM please cite:

> NAMD goes quantum: An integrative suite for hybrid simulations. Melo*, M. C. R.; Bernardi*, R. C.; Rudack T.; Scheurer, M.; Riplinger, C.; Phillips, J. C.; Maia, J. D. C.; Rocha, G. D.; Ribeiro, J. V.; Stone, J. E.; Neese, F.; Schulten, K.; Luthey-Schulten, Z.; Nature Methods, 2018 (doi:10.1038/nmeth.4638)

14.1 Division of Labor

The basic idea behind a hybrid QM/MM simulation in NAMD is to use a classical force field to treat the classical atoms in the system (or "MM atoms"), and pass the information that describes the quantum atoms in the system (or "QM atoms") to a Quantum Chemistry (QC) software, which is expected to produce gradients for all QM atoms, as well as the total energy of the QM region (and optionally partial charges). All bonded and non-bonded interactions among MM atoms are handled by NAMD's force field. Similarly, all bonded and non-bonded interactions among QM atoms are handled by the QC software in its chosen theory level. Treatment of covalent bonds between QM and MM atoms will be described in a following section.

The non-bonded interactions between QM and MM atoms are handled differently, and can be modified and regulated by the user. Van der Waals interactions are always calculated, and can be done using either the default force field parameters, or specific (user-defined) parameters for QM atoms. Parameter modifications for QM atoms have been proposed in order to compensate

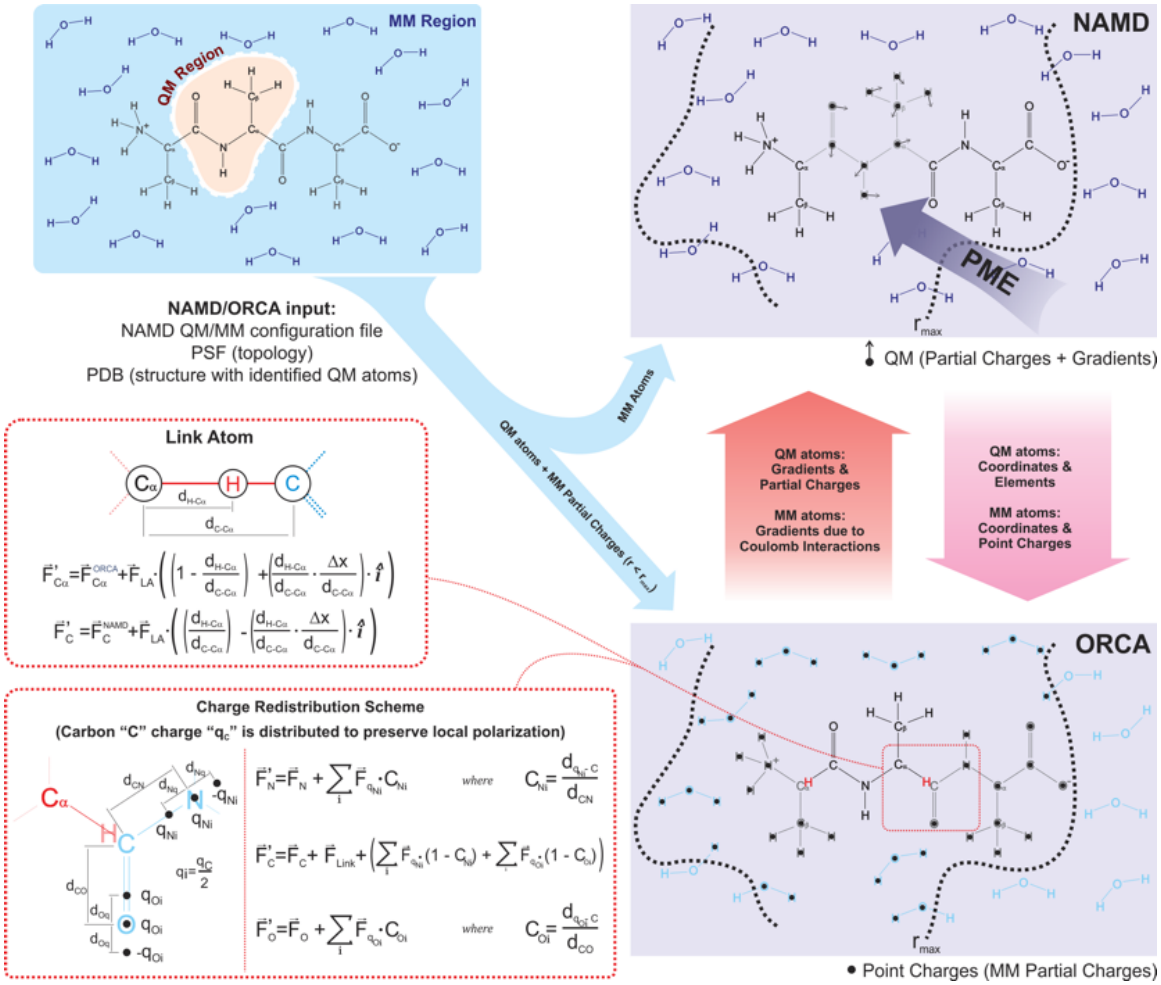


Figure 15: Graphical representation of NAMD-ORCA interconnection. Only the contribution of MM charges beyond rmax are calculated by NAMD (via PME), with the direct electrostatic calculation performed by ORCA. The image assumes the charge shift redistribution scheme, where the partial charge of the linking MM atom is shifted to its nearest MM neighbors.

for over-polarization that these atoms may exhibit in hybrid QM/MM simulations. Larger van der Waals radii and/or shallower well depths should then be provided for all element types that occur among QM atoms (see the "qmVdwParams" keyword).

14.2 Mechanical and Electrostatic Embedding

Electrostatic interactions between QM and MM atoms deserve a more detailed discussion due to the abundance and diversity of available alternatives. The first decision to be made is whether there will be electrostatic interactions between the two portions of a system, QM and MM. In the "mechanical embedding" scheme, only positions and elements of atoms in the QM region are passed on to the chosen QC software for energy and force calculations. This way, QM and MM atoms share only van der Waals interactions.

In the "electrostatic embedding" scheme, on the other hand, the partial charges of MM atoms surrounding all QM atoms are used to approximate the electrostatic environment where QM atoms are found (the scheme is selected with the "qmElecEmbed" keyword). See Figure 16. This process can be customized in a variety of ways, the first of which is deciding if a smoothing function will be used to avoid an abrupt decay in electrostatic force due to the cutoff used in the selection of surrounding point charges (this option is activated with the "qmSwitching" keyword).

Classical point charge utilization can be further customized by choosing which smoothing function will be used, and if the total charge of selected partial charges should be modified to (A) have a whole charge or (B) have a complementary charge to that of the QM region, so that the sum of charges from QM atoms and classical partial charges add to zero (see Figure 16).

With electrostatic embedding, QM atoms are influenced by the charges in the classical region. In order to balance the forces acting on the system, NAMD uses partial charges for the QM atoms to calculate the electrostatic interaction with classical point charges. There are two possibilities for the origin of the QM partial charges: the original partial charges found in the force field parameter files can be used, or updated partial charges can be gathered at each step from the QC software output (controllable through the "qmChargeMode" keyword). The continuous update in charge distribution allows for a partial re-parameterization of the targeted molecule at each time step, which can lead to an improved description of the interactions of a ligand as it repositions over the surface of a protein, for example, or as it moves through a membrane.

In case PME is activated by the user, NAMD will automatically apply the necessary corrections to handle the QM region, allowing it to be influenced by long range interactions from the entire system.

14.3 Covalent Bonds Divided by the QM/MM Barrier

Hybrid QM/MM simulations of biomolecular systems often present situations where only a portion of a molecule should be treated quantum mechanically, usually to save computational resources since the cost of simulating QM regions rises rapidly with the number of simulated toms. In order to deal with chemical bonds that are split by the QM/MM division of the biomolecular system, that is, bonds that have one atom in the quantum (QM) region and another in the classical (MM) region (we will call these "QM/MM bonds"), NAMD makes approximations to the molecular system in order to bridge differences in simulation type (QM vs. MM), and minimize errors involved in the QM/MM division of the system (Figure 17 A and B).

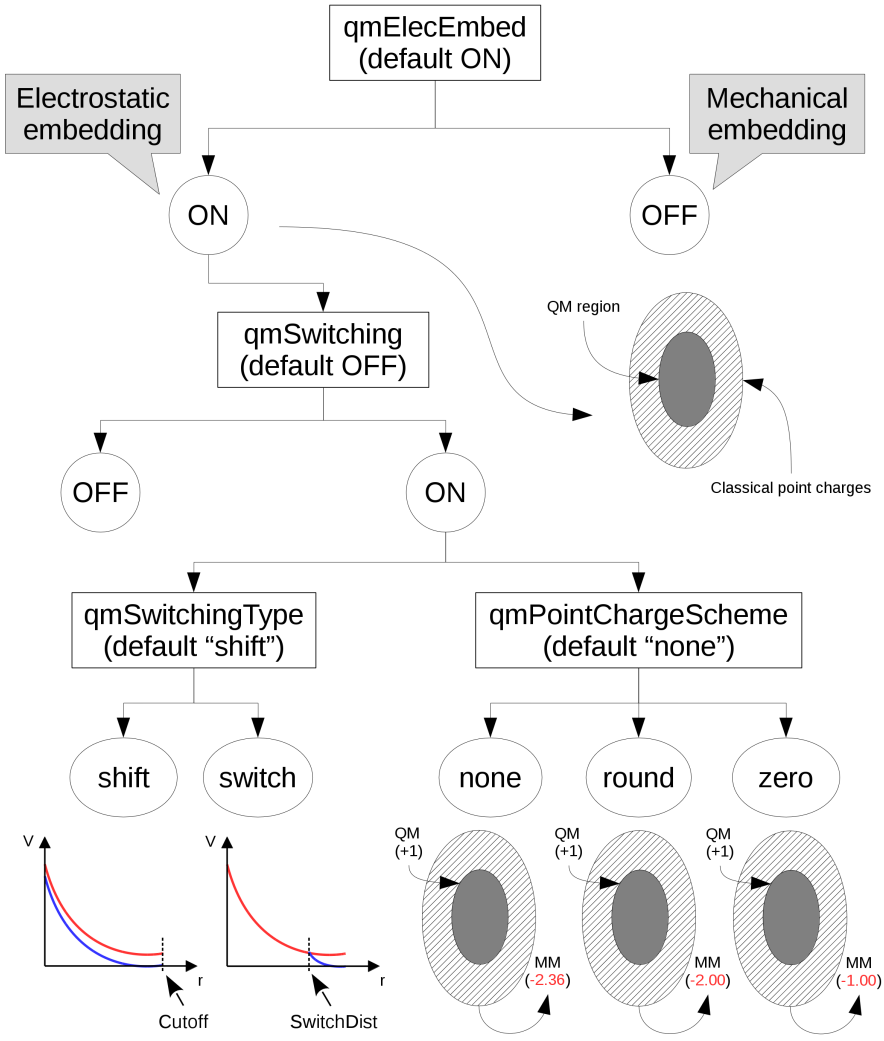


Figure 16: Diagram of options that control the use and manipulation of classical point charges. Default values are indicated below their respective keyword. “Cutoff” and “SwitchDist” are keywords used in NAMD to configure the calculations of electrostatic and van der Waals interactions.

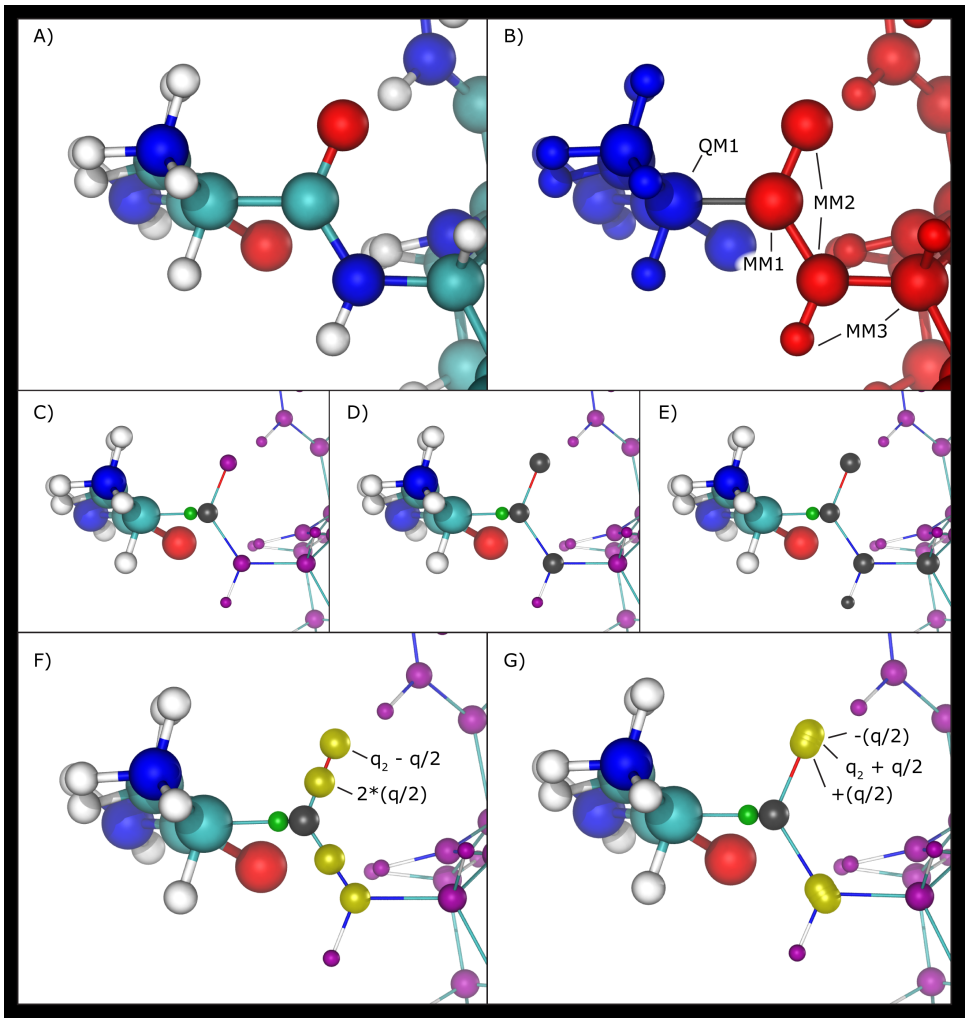


Figure 17: Treatment of QM/MM bonds. A) Illustration of all atoms in the vicinity of the QM/MM bond, colored by element: cyan for carbon, white for hydrogen, blue for nitrogen and red for oxygen. B) To the left, in blue, is the region that will be treated with the chosen QC software. To the right, in red, the region treated classically by NAMD. The bond in gray is the one crossing the QM/MM division. The atom marked as **QM1** is the quantum atom directly connected to the classical atom on the other side of the QM/MM division. Analogously, the atom marked as **MM1** is the classical atom directly connected to the quantum atom on the other side of the QM/MM division. Atoms marked as **MM2** are directly bonded to the **MM1** atom, and atoms marked **MM3** are directly bonded to **MM2** atoms. C) **Z1** method. Ignored partial charges are indicated in the image with a gray sphere taking the place of its respective classical atom. Directly adjacent to MM1 is a green sphere representing the link atom that is placed along the QM1-MM1 covalent bond. All remaining partial charges representing classical atoms that are passed on to the QC software are indicated in purple spheres. D) **Z2** method. E) **Z3** method. F) RCD method. Virtual point charges, are represented in yellow spheres. The text indicates the total charge placed at each position, where **q** indicates the charge of the MM1 atom and **q2** represents the partial charge of the MM2 atom at that position. The yellow spheres at MM2 atom positions indicate their partial charge has been changed from its original value. G) CS method. Since in this case the virtual point charges are placed very close to the MM2 atom position, the yellow spheres representing them show significant overlapping.

14.3.1 Link Atoms

As previously mentioned, the information regarding atoms in the QM region is passed on to the chosen QC software, that is, their respective positions and element types, but in order to maintain (or approximate) the effect of the chemical bond between the QM atom and the MM atom, NAMD creates and places a link atom (usually a hydrogen) along the "broken" QM/MM bond. The user can fine-tune this process by choosing the method of placement of the link atom and even the element of such atom (keywords "qmBondDist" and "qmLinkElement").

The introduction of the link atom will invariably place it very near the classical atom involved in the QM/MM bond, therefore the use and placement of partial charges from classical atoms becomes highly relevant. Under the mechanical embedding scheme, the QC software only receives the atoms in the QM region and the link atoms created to approximate QM/MM bonds, so no manipulation of partial charges is required. On the other hand, usual QM/MM simulations are done under the electrostatic embedding scheme, in which case the partial charges of classical atoms involved in the QM/MM bonds and classical atoms directly connected to them require special treatment.

14.3.2 Point Charge Alterations

Several methods have been proposed to handle this situation, and the QM/MM interface developed here implements the most widely accepted ones. One can be chosen using the "qmBondScheme" keyword (Figure 17 C to G). In all implemented methods, the classical atom participating in the QM/MM bond (MM1 atom) does not have its partial charge passed on to the QC software, since this would create excessive repulsion (or attraction) on the link atom. This is, in fact, the entirety of the "Z1" method: ignoring the partial charge of the MM1 atom. Analogously, "Z2" and "Z3" ignore all partial charges up to MM2 and MM3 atoms, respectively (Figure 17 C to E).

The Redistributed Charge and Dipole (RCD) method (Figure 17 F) is more elaborate, as it rearranges the partial charge of the MM1 atom (indicated as q) so that the total charge of the region is maintained as well as the dipole moments of the bonds between MM1 and MM2 atoms. This is done by creating "virtual" point charges, which are passed on to the QC software as if they represented partial charges of classical atoms. More specifically, the RCD method creates a virtual point charge in the middle of all MM1-MM2 bonds with a charge of $2q/n$, where n is the number of MM2 atoms connected to MM1, and also subtracts a charge q/n from each of the MM2 atoms, so that the total charge of the region remains constant while approximating the dipole moment of the MM1-MM2 bonds. This way there will be no point charge placed at the position of the MM1 atom, but its partial charge is not simply removed, it is redistributed.

A similar approach is taken by the Charge Shifting (CS) method (Figure 17 G). In this case, the MM1 partial charge is equally distributed across the MM2 atoms, and two virtual point charges are placed along the direction of the MM1-MM2 bond, one before the MM2 atom and one after, each one with a charge of $+q/n$ and $-q/n$ respectively. This method will also keep the total charge of the region constant while trying to preserve the local dipoles formed by all MM1-MM2 bonds.

14.3.3 Link Atom Charge and Charge Groups

Along with the gradient over all QM atoms, NAMD can also use the partial charge derived from the QC calculation to update the charge distribution of atoms in the QM region. When a QM/MM bond exists, however, part of the charge of the region will be placed on the link atom, and in order to keep the charge of the QM region constant, the link atom charge is re-distributed on the QM

region. This seemingly simple mechanism can cause problems unless special care is be taken when deciding which bond will mark the division of QM and MM regions.

Many force fields divide the topologies of biomolecules in "charge groups" (Figure 18 A and B). What this means is that not only will the partial charges of all atoms of a molecule add up to the whole number that represents the charge of the molecule, they will also add up to whole numbers in sub groups of atoms (look for the "GROUP" statements in http://www.ks.uiuc.edu/Training/Tutorials/namd/namd-tutorial-unix-html/node24.html to see an example). Therefore, one needs to make sure that the chosen QM/MM bond(s) sits in between "charge groups", so the total sum of partial charges of atoms defining a QM region is a whole number. This is especially important in order to keep the total charge of the system constant. Since the QC calculation will always distribute a whole charge over all atoms of a system (QM atoms plus a link atom), if the partial charge of QM atoms is not initially a whole number, it will be forced into a whole number after the first QC step, where the charge of the link atom is distributed over the QM region. This will create a mismatch between QM and MM charges, changing the total charge of the entire system (QM plus MM regions).

An example can be seen in Figure 18, bonds 1 and 3 are chosen as the QM/MM bonds, the charge distribution seen in Figure 18 C shows a whole charge for the QM region (and consequently for the MM region). Therefore, any charge placed on link atoms can be redistributed to the QM atoms with no change in total system charge. However, if bonds 2 and 3 are chosen for the QM/MM bond (Figure 18 D), the charge of the MM region would be +1.16, while the charge of the QM region would be −1.16. Since the QC calculation would place a pre-determined whole charge on the region (−1, in this case), the updated charge of the QM region after the first simulation step would change the total charge of the system to +0.16, in this example.

14.4 Custom Quantum Chemistry Software

In order to offer the broad range of tools and technologies present in NAMD to all researchers who develop and/or employ specialized Quantum Chemistry tools, the QM/MM interface is prepared to utilize any QC tool that can be wrapped in a script that converts input and output files to specified formats. This flexible interface will improve development and testing of new tools, as well as their quick integration utilization in hybrid dynamics.

We currently provide in the `libs/qmmm/` directory (and mirrored at http://www.ks.uiuc.edu/Research/qmmm/Scripts/) Python wrapper scripts for GAUSSIAN, TeraChem, and Q-Chem. Other wrapper scripts can be generated, based on these templates, in any other language, as long as they are provided to NAMD in an executable form. Although natively supported, we also provide a python wrapper script for ORCA, with extended comments explaining the format in which NAMD will write data for the QC software and the format in which NAMD expects to find the results.

14.5 Independent QM Regions

Aiming at large macromolecular simulations that could take advantage of localized QM resolution, NAMD allows the user to set up multiple independent QM regions in the same molecular system. For example, one could study a multimeric complex that contains several active sites and have all active sites be calculated with a chosen QC software simultaneously (Figure 19). Each active site would be calculated independently of all others, by its own execution of the QC software, keeping the calculation cost low and without impacting the overall efficiency of the simulation, since all QM regions would be calculated in parallel.

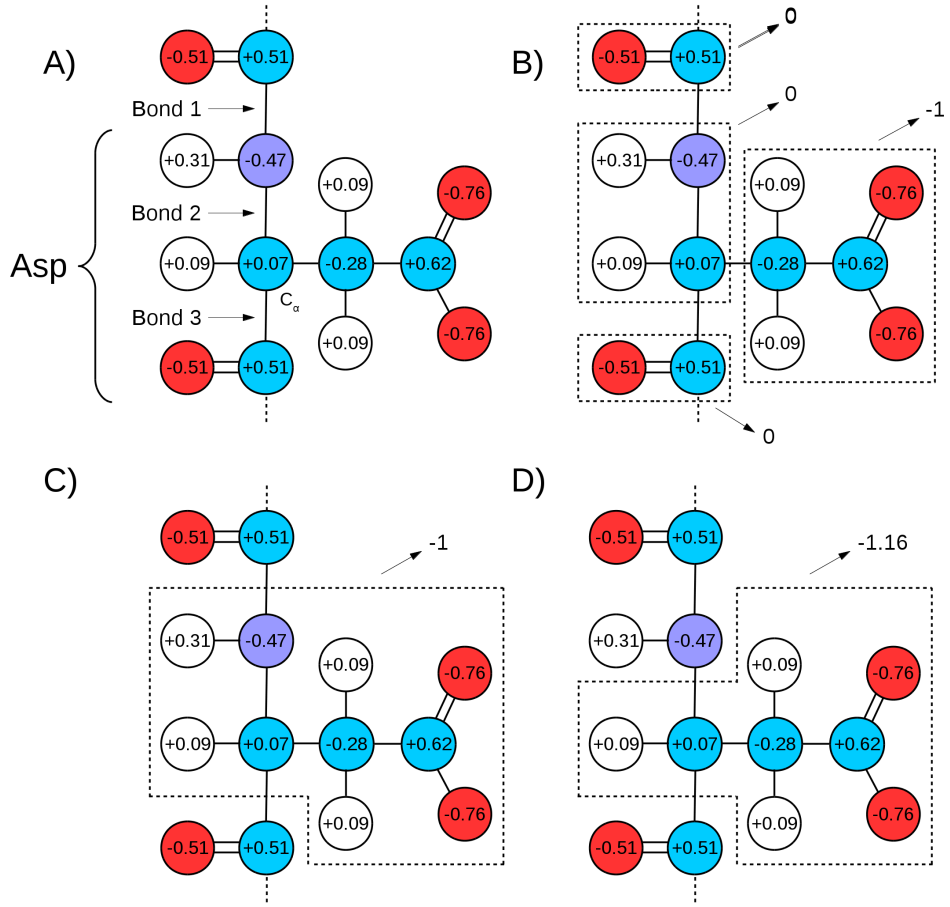


Figure 18: Charge Groups and QM/MM Bonds. A) Illustration of aspartate and the distribution of charge over its atoms as in CHARMM36 force field parameters. Circles in red indicate oxygen atoms, blue indicate nitrogen atoms, cyan for carbon atoms, and white for hydrogen atoms. "Bond 1" indicates the peptide bond, "Bond 2" indicates the one between the alpha carbon and the peptide bond nitrogen, and "Bond 3" the bond between the alpha carbon and the peptide bond carbon. B) Charge groups are indicated with dashed squares, along with their total charges. C) Depiction of the atoms in the QM region if Bonds 1 and 3 are used to separate it from the MM region. The total charge of QM region is -1. D) Depiction of QM region if the same is defined by Bonds 2 and 3. In this case, the total charge of QM region is -1.16.

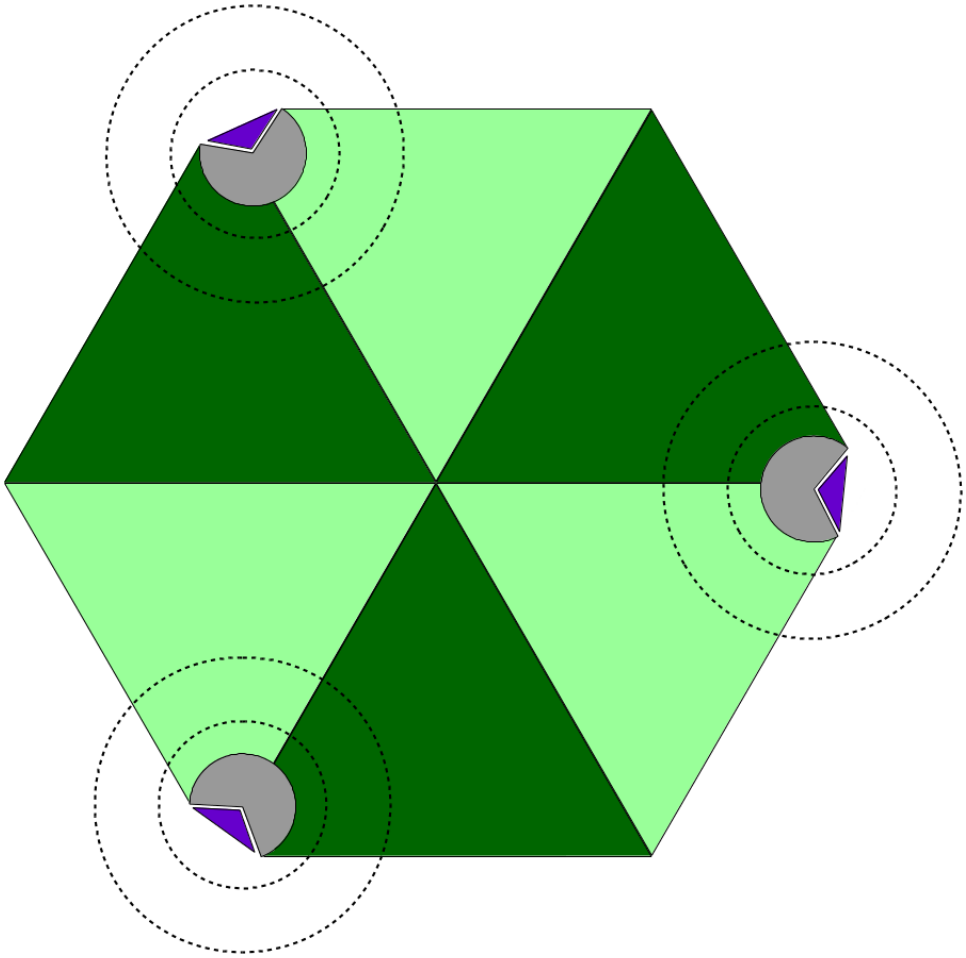


Figure 19: Diagram of Multiple QM Regions. The illustration depicts a hetero-hexameric complex (light and dark green triangles) that combine to create three active sites (in gray). Active sites are bound to a target molecule (purple triangle). The inner and outer dashed circles represent, respectively, the boundary of a QM region and the limits of the classical point charge shell around that region.

Identifying the different QM regions and which atoms belong to each one of them can be simply accomplished in the input PDB file, or in a dedicated PDB file (keyword "qmParamPDB"). Since each region can contain different sets of molecules, their charges and multiplicities are indicated separately (see keywords "qmCharge" and "qmMult").

For simulations of large systems that are distributed across several computer nodes, one can control how many independent QM regions are calculated in each node. This would prevent large simulations from running out of memory if two or more large QM regions are placed in the same node (see keyword "qmSimsPerNode").

14.6 Keywords

- `qmForces` < Calculate QM? >
  Acceptable Values: yes or no
  Default Value: no
  Description: Turns on or off the QM calculations.

- `qmParamPDB` < Set QM atoms >
  Acceptable Values: PDB file
  Description: Name of an optional secondary PDB file where the OCCupancy or BETA column has the indications for QM or MM atoms. QM atoms should have an integer bigger than zero (0) and MM atoms should have zero as the beta or occupancy field. The same file may have indications for bonds between a QM atom and an MM atom. This should be provided in case the PDB file passed to the "coordinates" keyword already has data on its beta or occupancy columns, such as when a SMD simulations is being performed.

- `qmColumn` < Which column? >
  Acceptable Values: "beta" or "occ"
  Description: Indicates which column has the QM/MM field. Required.

- `qmSimsPerNode` < Sims per node >
  Acceptable Values: postive integer
  Default Value: 1
  Description: Number of independent simultaneous QM simulations per node.

- `qmBondColumn` < Which bond column? >
  Acceptable Values: "beta" or "occ"
  Description: Indicates which column has the QM/MM bond information. This will tell NAMD which atoms are at the ends of a covalent bond split by the QM/MM barrier, with one atom being quantum and one being classical. There is no default value. If this parameter is provided, NAMD will parse options for dealing with QM/MM bonds.

- `qmBondDist` < Use qmBondColumn value for distance? >
  Acceptable Values: "on" or "off"
  Default Value: off
  Description: Indicates whether the value in the BondColumn will be used to define the

distance between the QM atom and the Link Atom that will replace the MM atom in the QM system.

- `qmBondValueType` < Does qmBondColumn value give length or ratio? >
  Acceptable Values: "len" or "ratio"
  Default Value: len
  Description: Indicates if the values in the BondColumn represent either the length ("len") between the QM and link atoms or the ratio ("ratio") between the QM-MM distance and the one which will be used as the QM-Link distance.

- `qmLinkElement` < Set link atom element >
  Acceptable Values: string, for example "4 9 Cl"
  Default Value: H
  Description: User defined link atom element. Two syntaxes are allowed: if there is only one QM-MM bond, a string with the element symbol is allowed (such as "H" or "Cl"). If there are two or more bonds, the string needs to have the two atoms that compose the bond, and then the element (such as "4 9 Cl"). The default element for all link atoms is hydrogen.

- `qmBondScheme` < Select QM-MM bond scheme >
  Acceptable Values: "CS" or "RCD" or "Z1" or "Z2" or "Z3"
  Default Value: CS
  Description: Indicates what will be the treatment given to QM-MM bonds in terms of charge distribution and link atom creation and placement. CS: Charge Shift Scheme; RCD: Redistributed Charge and Dipole method; Z1: Only ignored MM1 partial charge, with no charge redistribution; Z2: Ignores MM1 and all MM2 partial charges, with no charge redistribution; Z3: Ignores MM1 and all MM2 and MM3 partial charges, with no charge redistribution.

- `qmElecEmbed` < Should point charges be used in QM? >
  Acceptable Values: "on" or "off"
  Default Value: on
  Description: Indicates if classical point charges should be used in QM calculations.

- `qmSwitching` < Use switching on point charges? >
  Acceptable Values: "on" or "off"
  Default Value: off
  Description: This will scale down the point charges representing the classical system as to replicate the switching procedure that NAMD applies to all charged interaction (see "switching").

- `qmSwitchingType` < Set functional form of switching >
  Acceptable Values: "switch" or "shift"
  Default Value: shift
  Description: This option is used to decide which kind of function will be used to scale down point charges sent to QM calculations. SHIFT: This will "shift down" the entire shell of point charges so that electrostactic interactions reach zero at the cutoff distance. SWITCH: This

will only change point charges in the sub-volume between the switchdist and cutoff distance, so that electrostactic interactions reach zero at the cutoff distance.

- `qmPointChargeScheme` < Set point charge scheme >
  Acceptable Values: "none" or "round" or "zero"
  Default Value: none
  Description: This option allows the user to decide if and how the point charges presented to the QM system will be altered. NONE: Nothing will be done. ROUND: This will change the most distant point charges so that the total sum of point charges is a whole number. ZERO: This will adjust the most distant point charges so that the total sum of point charges is 0.

- `qmBaseDir` < Set directory for file I/O >
  Acceptable Values: directory path
  Description: This should be a fast read/write location, such as a RAM drive (/dev/shm on most linux distros). The user needs to make sure this directory exists in the node(s) running the QM calculation(s).

- `qmReplaceAll` < Use only QM gradients for forces? >
  Acceptable Values: "on" or "off"
  Default Value: off
  Description: Indicates to NAMD that ALL forces form NAMD will be ignored and only the gradients from the QM software will be applied on the atoms. This IS NOT NECESSARY in any regular QM/MM simulation, and will prevent the use of any other feature from NAMD such as SMD.

- `qmVdwParams` < Modify type names for QM atoms? >
  Acceptable Values: "on" or "off"
  Default Value: off
  Description: The QM code will change all QM atoms' van der Waals types to "q"+element (e.g., all carbons will be qC and all hydrogens will be qH) for VdW interactions. This means that a parameter file with epsilon and sigma values for these atom types must be provided along with the regular parameter files. For example, if using CHARMM force field, the new file should be in CHARMM format.

- `qmPCStride` < Set stride for point charge >
  Acceptable Values: integer
  Default Value: 1
  Description: Sets a stride for new point charge determination. The same set of classical atoms will be sent to QM calculations as point charges, but with updated positions.

- `qmCustomPCSelection` < Provide custom point charge selection? >
  Acceptable Values: "on" or "off"
  Default Value: off
  Description: Indicates that one or more file(s) will be provided with a custom selection of

point charges. Each file will have a selection for a single QM group. This selection will be kept during the entire simulation.

- `qmCustomPCFile` < File for custom point charge selection >
  Acceptable Values: PDB file
  Description: The file will have, in the "qmColumn", the same QM ID provided for a single QM group. All other groups will have zero (0) in this column. In the second column (beta or occupancy), the classical or quantum atoms (from other QM regions) that need to be passed as point charges will be identified by a non-zero number.
  Example/Format:
```
qmCustomPCFile system/system.customPC.1.pdb
qmCustomPCFile system/system.customPC.2.pdb
qmCustomPCFile system/system.customPC.3.pdb
qmCustomPCFile system/system.customPC.4.pdb
```

- `qmLiveSolventSel` < Keep track of solvent? >
  Acceptable Values: "on" or "off"
  Default Value: off
  Description: With Live Solvent Selection (LSS), NAMD will automatically keep track of the solvent molecules for all QM Groups, and will exchange classical solvent molecules with QM solvent molecules every "QMLSSFreq" steps.

- `qmLSSResname` < Set residue name for LSS >
  Acceptable Values: residue name
  Default Value: TIP3
  Description: Indicates which residue name will be used in LSS.

- `qmLSSFreq` < Set frequency of LSS >
  Acceptable Values: integer, multiple of stepspercycle
  Default Value: 100
  Description: Frequency of LSS. Must be a multiple of stepspercycle.

- `qmLSSMode` < How solvent molecules are selected >
  Acceptable Values: "dist" or "COM"
  Default Value: dist
  Description: For LSS, this indicates how solvent molecules are selected. In all cases, the closest solvent molecules are selected, and if a classical solvent molecule is closer than a QM one, they are swaped. DIST: This mode will use the smallest distance between a solvent atom and a non-solvent QM atom to sort solvent molecules. This is best used when the non-solvent QM atoms form irregular volumes (when the COM is not very representatve), and/or volumes with high solvent accessibility (such as a drug, or a small peptide, in solution). COM: This mode will sort solvent molecules based on Center Of Mass distance between the solvent COM and the COM of a selection for each QM group (see "qmLSSRef" keyword). Best used with small QM regions that have limited solvent accessibility, such as an active site.

- `qmLSSRef` < Which residues for COM of QM atoms? >
  Acceptable Values: string

Description: This will indicate which residues are to be used in the determination of the COM of non-solvent QM atoms. Only these atoms will be used to determine the closest set of solvent molecules. The keyword takes a string composed of the QM group ID, the segment name and the residue ID.
Example/Format:

```
qmLSSRef "1 RP1 9"
qmLSSRef "2 RP1 3"
qmLSSRef "2 RP1 2"
qmLSSRef "3 AP1 9"
qmLSSRef "3 AP1 3"
qmLSSRef "4 AP1 9"
```

- `qmConfigLine` < Pass string to QM configuration >
  Acceptable Values: string
  Description: The string passed to "qmConfigLine" will be copied and pasted at the very begining of the configuration file for the chosen QM software if either ORCA or MOPAC are selected.
  Example/Format (QM/MM NAMD-ORCA):

```
qmConfigLine "!  PM3 ENGRAD"
qmConfigLine "%%output PrintLevel Mini Print\[P_Mulliken\] 1 Print\[P_AtCharges_M\] 1 end"
```

  Example/Format (QM/MM NAMD-MOPAC):

```
qmConfigLine "PM7 XYZ T=2M 1SCF MOZYME CUTOFF=9.0 AUX LET GRAD QMMM GEO-OK"
qmConfigLine "Test System"
```

- `qmMult` < Set multiplicity of QM region >
  Acceptable Values: string
  Description: Multiplicity of the QM region. This is needed for proper construction of ORCA's input file. Each string must be composed of the QM region ID and its multiplicity.
  Example/Format:

```
qmMult "1 1"
qmMult "2 1"
qmMult "3 1"
qmMult "4 1"
```

- `qmCharge` < Set charge of each QM region >
  Acceptable Values: string
  Description: Indicates the charge of each QM region. If no charge is provided for a QM region, NAMD calculates the total charge automatically based on the given parameter set. Each string must be composed of the QM region ID and its total charge.
  Example/Format:

```
qmCharge "1 1"
qmCharge "2 -1"
qmCharge "3 1"
qmCharge "4 -1"
```

- `qmSoftware` < Which QM software? >
  Acceptable Values: "mopac" or "orca" or "custom"
  Description: Required for QM/MM, this indicates which QM software should be used.

In case the user wants to apply another QM code, this can be done by using the "custom" qmSoftware. In this case, NAMD will call the executable defined in the qmExecPath variable and will give it one argument: the full path to the input file.

INPUT: This input file will contain on the first line the number of QM atoms (X) and the number of point charges in the file (Y, which may be 0 or more), separated by a space. The following X+Y lines will have four (4) fields: X, Y and Z coordinates, and a fourth field which will depend on the type of entry. For QM atoms, the field will contain the element of the QM atom. For point charge lines, the field will contain the charge of the point charge.

OUTPUT: The expected output file whould be placed in the same directory as the input file, and should be named `"*inputfile*.result"` (meaning it will have the same path and name of the input file, plus the suffix `".result"`). This file should have, on its first line, the energy of the system and the number of point charges that were passed to ORCA, and that ORCA calculated forces on (zero, if using mechanical embedding). The two numbers should be separated by a single white space. Following the standard for the INPUT file, there will be another X+Y lines in the OUTPUT file. On the following X lines (where X is the number of QM atoms passed in the input file), there must be four (4) fields: the x, y and z components of the TOTAL FORCE applied on that atom, and on the fourth field, the charge of the atom. If the user indicates that charges from the QM software should not be used (see "qmChargeMode"), the fourth field should have zeroes, but should not be empty. On the following Y lines (where Y is the number of point charges), there must be only three (3) fields: the x, y and z components of the electrostatic force applied on that point charge. Energy should be in Kcal/mol and forces in Kcal/mol/Angstrom.

- `qmExecPath` < Set path to QM executable >
  Acceptable Values: path
  Description: Required for QM/MM, this indicates the path to the QM code executable.

- `qmSecProc` < Set path to secondary executable >
  Acceptable Values: path
  Description: Indicates a secondary executable that NAMD will call AFTER each QM software execution for each QM group. The executable is called with two arguments: the complete path and name of the input file used for each QM software execution; and the simulation step. This option can be used for an extra-processing at every step, e.g., for saving all QM outputs every step.

- `qmPrepProc` < Set path to initial executable >
  Acceptable Values: path
  Description: Indicates an executable that must be called BEFORE the FIRST QM software execution of each QM group. The executable is called with one argument: the complete path and name of the input file used for each QM software execution. This can be used to setup a charge distribution for a molecule of interest, for example.

- `qmChargeMode` < Set charge calculation mode >
  Acceptable Values: "none" or "mulliken" or "chelpg"
  Default Value: mulliken

Description: Charge calculation mode expected from the QM software. This indicates if charges should be read from the QM software and updated at every step, or if the original force field atom charges should be used. In case you are using ORCA, two charge options are allowed, Mulliken or CHELPG. We must know the kind of charge requested by the user so that the proper format is expected, read and processed. NONE: No charges are read from the QM software output and the original force field charges are preserved. MULLIKEN: This is the only other option for MOPAC and one possibility for ORCA. In case you are using the custom QM software interface, choose this option in order to use the charges calculated in the custom QM software, irrespective of the actual theory used for that calculation. CHELPG: This is a second possibility for ORCA.

- `qmOutStride` < Set frequency of QM charge output >
  Acceptable Values: integer
  Default Value: 0 (not saving)
  Description: Frequency of QM charge output. A dedicated DCD file will be created to store the charge data for all QM atoms in the system. This independent frequency allows the user to store whole-system data at a larger stride to save time and space.

- `qmPositionOutStride` < Set frequency of QM-only position output >
  Acceptable Values: integer
  Default Value: 0 (not saving)
  Description: Frequency of QM-only position output. A dedicated DCD file will be created to store the position data for all QM atoms in the system. This independent frequency allows the user to store whole-system data at a larger stride to save time and space.

- `qmEnergyStride` < Set frequency of QM specific energy output >
  Acceptable Values: integer
  Default Value: 1
  Description: Frequency of QM-only energy output. A dedicated energy output line will be created to indicate the energy calculated by the QM code. This independent frequency allows the user to store QM-specific energy data at a larger stride to save time and space.

- `qmChargeFromPSF` < Set charge of QM region from PSF file >
  Acceptable Values: "on" or "off"
  Default Value: off
  Description: Automatically determine charge of QM regions by adding the charges of atoms in each region.

- `qmCSMD` < Apply conditional-SMD to QM atoms? >
  Acceptable Values: "on" or "off"
  Default Value: off
  Description: Apply conditional SMD to QM atoms in order to steer the simulation within the QM region, while avoiding bringing atoms too close together and destabilizing the molecule. C-SMD works like regular SMD, but with pairs of atoms. The first atom is pulled by a string connected to a virtual particle, and the direction of motion of the virtual particle is updated to follow a second atom. The force on the first atom will stop being

applied when they come closer than a cutoff value.

- `qmCSMDFile` < Set cSMD information >
  Acceptable Values: cSMD file
  Description: Name of a text file indicating pairs of atoms that will be brought closer in space. In the file, each line defines a cSMD bias, with the following syntax:
  Atom1 Atom2 Force(Kcal/Mol/A) Speed(A/step) Cutoff(A)

15 Runtime Analysis

15.1 Pair interaction calculations

NAMD supportes the calculation of interaction energy calculations between two groups of atoms. When enabled, pair interaction information will be calculated and printed in the standard output file on its own line at the same frequency as energy output. The format of the line is `PAIR INTERACTION: STEP:` *step* `VDW_FORCE:` *fx fy fz* `ELECT_FORCE:` *fx fy fz*. The displayed force is the force on atoms in group 1 and is units of kcal/mol/Å.

For trajectory analysis the recommended way to use this set of options is to use the NAMD Tcl scripting interface as described in Sec. 2.2.2 to run for 0 steps, so that NAMD prints the energy without performing any dynamics.

- `pairInteraction` < is pair interaction calculation active? >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: Specifies whether pair interaction calculation is active.

- `pairInteractionFile` < PDB file containing pair interaction flags >
  Acceptable Values: UNIX filename
  Default Value: `coordinates`
  Description: PDB file to specify atoms to use for pair interaction calculations. If this parameter is not specified, then the PDB file containing initial coordinates specified by `coordinates` is used.

- `pairInteractionCol` < column of PDB file containing pair interaction flags >
  Acceptable Values: `X`, `Y`, `Z`, `O`, or `B`
  Default Value: `B`
  Description: Column of the PDB file to specify which atoms to use for pair interaction calculations. This parameter may specify any of the floating point fields of the PDB file, either X, Y, Z, occupancy, or beta-coupling (temperature-coupling).

- `pairInteractionSelf` < compute within-group interactions instead of bewteen groups >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: When active, NAMD will compute bonded and nonbonded interactions only for atoms within group 1.

- `pairInteractionGroup1` < Flag to indicate atoms in group 1? >
  Acceptable Values: integer
  Description:

- `pairInteractionGroup2` < Flag to indicate atoms in group 2? >
  Acceptable Values: integer
  Description: These options are used to indicate which atoms belong to each interaction group. Atoms with a value in the column specified by `pairInteractionCol` equal to `pairInteractionGroup1` will be assigned to group 1; likewise for group 2.

15.2 Pressure profile calculations

NAMD supports the calculation of lateral pressure profiles as a function of the z-coordinate in the system. The algorithm is based on that of Lindahl and Edholm (JCP 2000), with modifications to enable Ewald sums based on Sonne et al (JCP 122, 2005).

The simulation space is partitioned into slabs, and half the virial due to the interaction between two particles is assigned to each of the slabs containing the particles. This amounts to employing the Harasima contour, rather than the Irving-Kirkwood contour, as was done in NAMD 2.5. The diagonal components of the pressure tensor for each slab, averaged over all timesteps since the previous output, are recorded in the NAMD output file. The units of pressure are the same as in the regular NAMD pressure output; i.e., bar.

The total virial contains contributions from up to four components: kinetic energy, bonded interactions, nonbonded interactions, and an Ewald sum. All but the Ewald sums are computed online during a normal simulation run (this is a change from NAMD 2.5, when nonbonded contributions to the Ewald sum were always computed offline). If the simulations are performed using PME, the Ewald contribution should be estimated using a separate, offline calculation based on the saved trajectory files. The nonbonded contribution using a cutoff different from the one used in the simulation may also be computed offline in the same fashion as for Ewald, if desired.

Pressure profile calculations may be performed in either constant volume or constant pressure conditions. If constant pressure is enabled, the slabs thickness will be rescaled along with the unit cell; the dcdUnitCell option will also be switched on so that unit cell information is stored in the trajectory file.

NAMD 2.6 now reports the lateral pressure partitioned by interaction type. Three groups are reported: kinetic + rigid bond restraints (referred to as "internal", bonded, and nonbonded. If Ewald pressure profile calculations are active, the Ewald contribution is reported in the nonbonded section, and no other contributions are reported.

NAMD 2.6 also permits the pressure profile to be partitioned by atom type. Up to 15 atom groups may be assigned, and individual contribution of each group (for the "internal" pressures) and the pairwise contributions of interactions within and between groups (for the nonbonded and bonded pressures) are reported in the output file.

- `pressureProfile` < compute pressure profile >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: When active, NAMD will compute kinetic, bonded and nonbonded (but not reciprocal space) contributions to the pressure profile. Results will be recorded in the NAMD output file in lines with the format `PRESSUREPROFILE: ts Axx Ayy Azz Bxx Byy Bzz ...` , where `ts` is the timestep, followed by the three diagonal components of the pressure tensor in the first slab (the slab with lowest z), then the next lowest slab, and so forth. The output will reflect the pressure profile averaged over all the steps since the last output.

  NAMD also reports kinetic, bonded and nonbonded contributions separately, using the same format as the total pressure, but on lines beginning with `PPROFILEINTERNAL`, `PPROFILEBONDED`, and `PPROFILENONBONDED`.

- `pressureProfileSlabs` < Number of slabs in the spatial partition >
  Acceptable Values: Positive integer
  Default Value: 10

Description: NAMD divides the entire periodic cell into horizontal slabs of equal thickness; `pressureProfileSlabs` specifies the number of such slabs.

- `pressureProfileFreq` < How often to output pressure profile data >
  Acceptable Values: Positive integer
  Default Value: 1
  Description: Specifies the number of timesteps between output of pressure profile data.

- `pressureProfileEwald` < Enable pressure profile Ewald sums >
  Acceptable Values: `on` or `off`
  Default Value: `off`
  Description: When enabled, only the Ewald contribution to the pressure profile will be computed. For trajectory analysis the recommended way to use this option is to use the NAMD Tcl scripting interface as described in Sec. 2.2.2 to run for 0 steps, so that NAMD prints the pressure profile without performing any dynamics.

  The Ewald sum method is as described in Sonne et al. (JCP 122, 2005). The number of k vectors to use along each periodic cell dimension is specified by the `pressureProfileEwald`n parameters described below.

- `pressureProfileEwaldX` < Ewald grid size along X >
  Acceptable Values: Positive integer
  Default Value: 10
  Description:

- `pressureProfileEwaldY` < Ewald grid size along Y >
  Acceptable Values: Positive integer
  Default Value: 10
  Description:

- `pressureProfileEwaldZ` < Ewald grid size along Z >
  Acceptable Values: Positive integer
  Default Value: 10
  Description:

- `pressureProfileAtomTypes` < Number of atom type partitions >
  Acceptable Values: Positive integer
  Default Value: 1
  Description: If `pressureProfileAtomTypes` is greater than 1, NAMD will calculate the separate contributions of each type of atom to the internal, bonded, nonbonded, and total pressure. In the case of the internal contribution, there will be n pressure profile data sets reported on each `PPROFILEINTERNAL` line, where n is the number of atom types. All the partial pressures for atom type 1 will be followed by those for atom type 2, and so forth. The other three pressure profile reports will contain $n(n+1)/2$ data sets. For example, if there are $n = 3$ atom types, the six data sets arising from the three inter-partition and the three intra-partition interactions will be reported in the following order: 1–1, 1–2, 1–3, 2–2, 2–3, 3–3. The total pressure profile, reported on the `PRESSUREPROFILE` line, will contain the internal contributions in the data sets corresponding to 1–1, 2–2, etc.

- `pressureProfileAtomTypesFile` < Atom type partition assignments >
  Acceptable Values: PDB file
  Default Value: coordinate file
  Description: If `pressureProfileAtomTypes` is greater than 1, NAMD will assign atoms to types based on the corresponding value in `pressureProfileAtomTypesCol`. The type for each atom must be strictly less than `pressureProfileAtomTypes`!

- `pressureProfileAtomTypesCol` < `pressureProfileAtomTypesFile` PDB column >
  Acceptable Values: PDB file
  Default Value: B
  Description:

Here is an example snippet from a NAMD input that can be used to compute the Ewald component of the pressure profile. It assumes that the coordinates were saved in the dcd file `pp03.dcd`) every 500 timesteps.

```
Pme             on
PmeGridSizeX    64
PmeGridSizeY    64
PmeGridSizeZ    64

exclude         scaled1-4
1-4scaling      1.0

switching on
switchdist      9
cutoff          10
pairlistdist    11

pressureProfile        on
pressureProfileSlabs   30
pressureProfileFreq    100
pressureProfileAtomTypes 6
pressureProfileAtomTypesFile atomtypes.pdb
pressureProfileEwald  on
pressureProfileEwaldX  16
pressureProfileEwaldY  16
pressureProfileEwaldZ  16

set ts 0
firstTimestep $ts

coorfile open dcd pp03.dcd
while { [coorfile read] != -1 } {
  incr ts 500
  firstTimestep $ts
  run 0
```

```
}
coorfile close
```

16 Performance Tuning

16.1 NAMD performance tuning concepts

The simulation performance obtained from NAMD depends on many factors. The particular simulation protocol being run is one of the largest single factors associated with NAMD performance, as different simulation methods invoke different code that can have substantially different performance costs, potentially with a different degree of parallel scalability, message passing activity, hardware acceleration through the use of GPUs or CPU vectorization, and other attributes that also contribute to overall NAMD performance.

Measuring performance. When NAMD first starts running, it does significant I/O, FFT tuning, GPU context setup, and other work that is unrelated to normal simulation activity, so it is important to measure performance only when NAMD has completed startup all of the processing units are running at full speed. The best way to measure NAMD performance accurately requires running NAMD for 500 to 1,000 steps of normal dynamics (not minimization), so that load balancing has a chance to take place several times, and all of the CPUs and GPUs have ramped up to 100% clock rate. NAMD provides "Benchmark time:" and "TIMING:" measurements in its output, which can be used for this purpose. Here, we are only interested in the so-called wall clock time.

NAMD configuration and I/O performance. Aside from the choice of major simulation protocol and associated methods in use, it is also important to consider the performance impacts associated with routine NAMD configuration parameters such as those that control the frequency of simulation informational outputs and various types of I/O. Simulation outputs such as energy information may require NAMD to do additional computations above and beyond standard force evaluation calculations. We advise that NAMD simulation configuration parameters be selected such that output of energies (via the `outputEnergies` parameter) be performed only as much as is strictly necessary, since they otherwise serve to slow down the simulation due to the extra calculations they require. NAMD writes "restart" files to enable simulations that were terminated unexpectedly (for any reason) to be conveniently restarted from the most recently written restart file available. While it is desirable to have a relatively recent restart point to continue from, writing restart information costs NAMD extra network communication and disk I/O. If restart files are written too frequently, this extra activity and I/O will slow down the simulation. A reasonable estimate for restart frequency is to choose the value such that NAMD writes restart files about once every ten minutes of wall clock time. At such a rate, the extra work and I/O associated with writing the restart files should remain an insignificant factor in NAMD performance.

Computational (arithmetic) performance. NAMD is provided in a variety of builds that support platform-specific techniques such as CPU vectorization and GPU acceleration to achieve higher arithmetic performance, thereby increasing NAMD simulation throughput. Whenever possible NAMD builds should be compiled such that CPU vector instructions are enabled, and highly tuned platform-specific NAMD code is employed for performance-critical force computations. The so-called "SMP" builds of NAMD benefit from reduced memory use and can in many cases perform better overall, but one trade-off is that the communication thread is unavailable for simulation work. NAMD performance can be improved by explicitly setting CPU affinity using the appropriate Charm++ command line flags, e.g., `++ppn 7 +commap 0,8 +pemap 1-7,9-15` as an example.

It is often beneficial to reserve one CPU core for the operating system, to prevent harmful operating system noise or "jitter", particularly when running NAMD on large scale clusters or supercomputers. The Cray `aprun -r 1` command reserves and forces the operating system to run on the last CPU core.

State-of-the-art compute-optimized GPU accelerators, can provide NAMD with simulation performance equivalent to several CPU sockets (on the order of 100 CPU cores) when used to greatest effect, e.g., when GPUs have sufficient work per GPU. In general, effective GPU acceleration currently requires on the order of 10,000 atoms per GPU assuming a fast network interconnect. NAMD currently requires several CPU cores to drive each GPU effectively, ensuring that there is always work ready and available for the GPU. For contemporary CPU and GPU hardware, the most productive ratios of CPU core counts per GPU tend to range from 8:1 to 25:1 depending on the details of the hardware involved.

Networking performance. When running NAMD on more than a single node, it is important to use a NAMD version that is optimal for the underlying network hardware and software you intend to run on. The Charm++ runtime system on which NAMD is based supports a variety of underlying networks, so be sure to select a NAMD/Charm++ build that is most directly suited for your hardware platform. In general, we advise users to avoid the use of an MPI-based NAMD build as it will underperform compared with a native network layer such as InfiniBand IB verbs (often referred to as "verbs"), the Cray-specific "gni-crayxc" or "gni-crayxe" layer, or the IBM PAMI message passing layer, as practical examples.

16.2 Non-bonded interaction distance-testing

The last critical parameter for non-bonded interaction calculations is the parameter `pairlistdist`. To reduce the cost of performing the non-bonded interactions, NAMD uses a *non-bonded pair list* which contained all pairs of atoms for which non-bonded interactions should be calculated. Performing the search for pairs of atoms that should have their interactions calculated is an expensive operation. Thus, the pair list is only calculated periodically, at least once per cycle. Unfortunately, pairs of atoms move relative to each other during the steps between preparation of the pair list. Because of this, if the pair list were built to include only those pairs of atoms that are within the cutoff distance when the list is generated, it would be possible for atoms to drift closer together than the cutoff distance during subsequent timesteps and yet not have their non-bonded interactions calculated.

Let us consider a concrete example to better understand this. Assume that the pairlist is built once every ten timesteps and that the cutoff distance is 8.0 Å. Consider a pair of atoms A and B that are 8.1 Å apart when the pairlist is built. If the pair list includes only those atoms within the cutoff distance, this pair would not be included in the list. Now assume that after five timesteps, atoms A and B have moved to only 7.9 Å apart. A and B are now within the cutoff distance of each other, and should have their non-bonded interactions calculated. However, because the non-bonded interactions are based solely on the pair list and the pair list will not be rebuilt for another five timesteps, this pair will be ignored for five timesteps causing energy not to be conserved within the system.

To avoid this problem, the parameter `pairlistdist` allows the user to specify a distance greater than the `cutoff` distance for pairs to be included in the pair list, as shown in Figure 20. Pairs that are included in the pair list but are outside the cutoff distance are simply ignored. So in the above

example, if the `pairlistdist` were set to 10.0 Å, then the atom pair A and B would be included in the pair list, even though the pair would initially be ignored because they are further apart than the cutoff distance. As the pair moved closer and entered the cutoff distance, because the pair was already in the pair list, the non-bonded interactions would immediately be calculated and energy conservation would be preserved. The value of `pairlistdist` should be chosen such that no atom pair moves more than `pairlistdist` − `cutoff` in one cycle. This will insure energy conservation and efficiency.

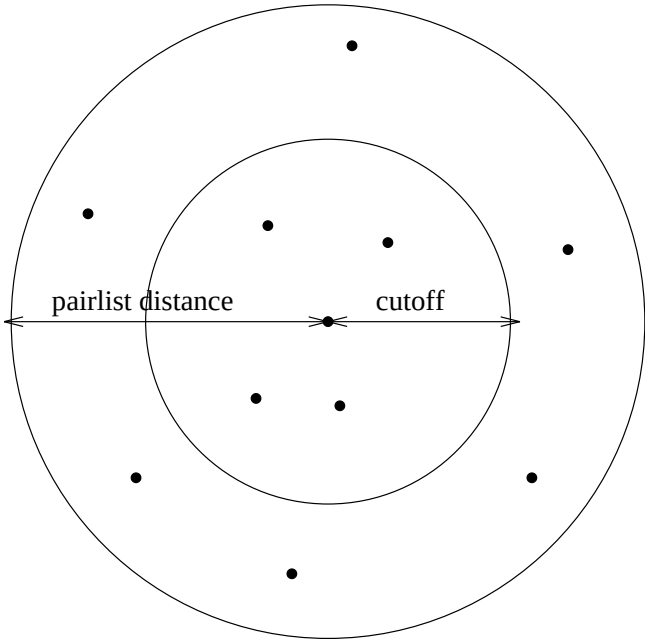


Figure 20: Depiction of the difference between the cutoff distance and the pair list distance. The pair list distance specifies a sphere that is slightly larger than that of the cutoff so that pairs are allowed to move in and out of the cutoff distance without causing energy conservation to be disturbed.

The `pairlistdist` parameter is also used to determine the minimum patch size. Unless the `splitPatch` parameter is explicitly set to `position`, hydrogen atoms will be placed on the same patch as the "mother atom" to which they are bonded. These *hydrogen groups* are then distance tested against each other using only a cutoff increased by the the value of the `hgroupCutoff` parameter. The size of the patches is also increased by this amount. NAMD functions correctly even if a hydrogen atom and its mother atom are separated by more than half of `hgroupCutoff` by breaking that group into its individual atoms for distance testing. Margin violation warning messages are printed if an atom moves outside of a safe zone surrounding the patch to which it is assigned, indicating that `pairlistdist` should be increased in order for forces to be calculated correctly and energy to be conserved.

Margin violations mean that atoms that are in non-neighboring patches may be closer than the cutoff distance apart. This may sometimes happen in constant pressure simulations when the cell shrinks (since the patch grid remains the same size). The workaround is to increase the margin parameter so that the simulation starts with fewer, larger patches. Restarting the simulation will also regenerate the patch grid.

In rare special circumstances atoms that are involved in bonded terms (bonds, angles, dihedrals,

or impropers) or nonbonded exclusions (especially implicit exclusions due to bonds) will be placed on non-neighboring patches because they are more than the cutoff distance apart. This can result in the simulation dying with a message of "bad global exclusion count". If an "atoms moving too fast; simulation has become unstable", "bad global exclusion count", or similar error happens on the first timestep then there is likely something very wrong with the input coordinates, such as the atoms with uninitialized coordinates or different atom orders in the PSF and PDB file. Looking at the system in VMD will often reveal an abnormal structure. Be aware that the atom IDs in the "Atoms moving too fast" error message are 1-based, while VMD's atom indices are 0-based. If an "atoms moving too fast; simulation has become unstable", "bad global exclusion count", or similar error happens later in the simulation then the dynamics have probably become unstable, resulting in the system "exploding" apart. Energies printed at every timestep should show an exponential increase. This may be due to a timestep that is too long, or some other strange feature. Saving a trajectory of every step and working backwards in can also sometimes reveal the origin of the instability.

- `pairlistdist` < distance between pairs for inclusion in pair lists (Å) >
  Acceptable Values: positive decimal ≥ `cutoff`
  Default Value: `cutoff`
  Description: A pair list is generated `pairlistsPerCycle` times each cycle, containing pairs of atoms for which electrostatics and van der Waals interactions will be calculated. This parameter is used when `switching` is set to `on` to specify the allowable distance between atoms for inclusion in the pair list. This parameter is equivalent to the X-PLOR parameter `CUTNb`. If no atom moves more than `pairlistdist`−`cutoff` during one cycle, then there will be no jump in electrostatic or van der Waals energies when the next pair list is built. Since such a jump is unavoidable when truncation is used, this parameter may only be specified when `switching` is set to `on`. If this parameter is not specified and `switching` is set to `on`, the value of `cutoff` is used. A value of at least one greater than `cutoff` is recommended.

- `stepspercycle` < timesteps per cycle >
  Acceptable Values: positive integer
  Default Value: 20
  Description: Number of timesteps in each cycle. Each cycle represents the number of timesteps between atom reassignments. For more details on non-bonded force evaluation, see Section 5.2.

- `splitPatch` < how to assign atoms to patches >
  Acceptable Values: `position` or `hydrogen`
  Default Value: `hydrogen`
  Description: When set to `hydrogen`, hydrogen atoms are kept on the same patch as their parents, allowing faster distance checking and rigid bonds.

- `hgroupCutoff (Å)` < used for group-based distance testing >
  Acceptable Values: positive decimal
  Default Value: 2.5
  Description: This should be set to twice the largest distance which will ever occur between a hydrogen atom and its mother. Warnings will be printed if this is not the case. This value is also added to the margin.

- `margin` < extra length in patch dimension (Å) >
  Acceptable Values: positive decimal
  Default Value: 0.0
  Description: An internal tuning parameter used in determining the size of the cubes of space with which NAMD uses to partition the system. The value of this parameter will not change the physical results of the simulation. Unless you are very motivated to get the *very* best possible performance, just leave this value at the default.

- `pairlistMinProcs` < min procs for pairlists >
  Acceptable Values: positive integer
  Default Value: 1
  Description: Pairlists may consume a large amount of memory as atom counts, densities, and cutoff distances increase. Since this data is distributed across processors it is normally only problematic for small processor counts. Set pairlistMinProcs to the smallest number of processors on which the simulation can fit into memory when pairlists are used.

- `pairlistsPerCycle` < regenerate x times per cycle >
  Acceptable Values: positive integer
  Default Value: 2
  Description: Rather than only regenerating the pairlist at the beginning of a cycle, regenerate multiple times in order to better balance the costs of atom migration, pairlist generation, and larger pairlists.

- `outputPairlists` < how often to print warnings >
  Acceptable Values: non-negative integer
  Default Value: 0
  Description: If an atom moves further than the pairlist tolerance during a simulation (initially (pairlistdist - cutoff)/2 but refined during the run) any pairlists covering that atom are invalidated and temporary pairlists are used until the next full pairlist regeneration. All interactions are calculated correctly, but efficiency may be degraded. Enabling outputPairlists will summarize these pairlist violation warnings periodically during the run.

- `pairlistShrink` < tol *= (1 - x) on regeneration >
  Acceptable Values: non-negative decimal
  Default Value: 0.01
  Description: In order to maintain validity for the pairlist for an entire cycle, the pairlist tolerance (the distance an atom can move without causing the pairlist to be invalidated) is adjusted during the simulation. Every time pairlists are regenerated the tolerance is reduced by this fraction.

- `pairlistGrow` < tol *= (1 + x) on trigger >
  Acceptable Values: non-negative decimal
  Default Value: 0.01
  Description: In order to maintain validity for the pairlist for an entire cycle, the pairlist tolerance (the distance an atom can move without causing the pairlist to be invalidated) is adjusted during the simulation. Every time an atom exceeds a trigger criterion that is some fraction of the tolerance distance, the tolerance is increased by this fraction.

- `pairlistTrigger` < trigger is atom beyond (1 - x) * tol >
  Acceptable Values: non-negative decimal
  Default Value: 0.3
  Description: The goal of pairlist tolerance adjustment is to make pairlist invalidations rare while keeping the tolerance as small as possible for best performance. Rather than monitoring the (very rare) case where atoms actually move more than the tolerance distance, we reduce the trigger tolerance by this fraction. The tolerance is increased whenever the trigger tolerance is exceeded, as specified by pairlistGrow.

17 Translation between NAMD and X-PLOR configuration parameters

NAMD was designed to provide many of the same molecular dynamics functions that X-PLOR provides. As such, there are many similarities between the types of parameters that must be passed to both X-PLOR and NAMD. This section describes relations between similar NAMD and X-PLOR parameters.

- **NAMD Parameter:** `cutoff`
  X-PLOR Parameter: `CTOFNB`
  When full electrostatics are not in use within NAMD, these parameters have exactly the same meaning — the distance at which electrostatic and van der Waals forces are truncated. When full electrostatics are in use within NAMD, the meaning is still very similar. The van der Waals force is still truncated at the specified distance, and the electrostatic force is still computed at every timestep for interactions within the specified distance. However, the NAMD integration uses multiple time stepping to compute electrostatic force interactions beyond this distance every `stepspercycle` timesteps.

- **NAMD Parameter:** `vdwswitchdist`
  X-PLOR Parameter: `CTONNB`
  Distance at which the van der Waals switching function becomes active.

- **NAMD Parameter:** `pairlistdist`
  X-PLOR Parameter: `CUTNb`
  Distance within which interaction pairs will be included in pairlist.

- **NAMD Parameter:** `1-4scaling`
  X-PLOR Parameter: `E14Fac`
  Scaling factor for 1-4 pair electrostatic interactions.

- **NAMD Parameter:** `dielectric`
  X-PLOR Parameter: `EPS`
  Dielectric constant.

- **NAMD Parameter:** `exclude`
  X-PLOR Parameter: `NBXMod`
  Both parameters specify which atom pairs to exclude from non-bonded interactions. The ability to ignore explicit exclusions is *not* present within NAMD, thus only positive values of `NBXMod` have NAMD equivalents. These equivalences are

  - `NBXMod=1` is equivalent to `exclude=none` — no atom pairs excluded,
  - `NBXMod=2` is equivalent to `exclude=1-2` — only 1-2 pairs excluded,
  - `NBXMod=3` is equivalent to `exclude=1-3` — 1-2 and 1-3 pairs excluded,
  - `NBXMod=4` is equivalent to `exclude=1-4` — 1-2, 1-3, and 1-4 pairs excluded,
  - `NBXMod`=5 is equivalent to `exclude=scaled1-4` — 1-2 and 1-3 pairs excluded, 1-4 pairs modified.

- **NAMD Parameter:** `switching`
  X-PLOR Parameter: `SHIFt, VSWItch, and TRUNcation`
  Activating the NAMD option `switching` is equivalent to using the X-PLOR options `SHIFt` and `VSWItch`. Deactivating `switching` is equivalent to using the X-PLOR option `TRUNcation`.

- **NAMD Parameter:** `temperature`
  X-PLOR Parameter: `FIRSttemp`
  Initial temperature for the system.

- **NAMD Parameter:** `rescaleFreq`
  X-PLOR Parameter: `IEQFrq`
  Number of timesteps between velocity rescaling.

- **NAMD Parameter:** `rescaleTemp`
  X-PLOR Parameter: `FINAltemp`
  Temperature to which velocities are rescaled.

- **NAMD Parameter:** `restartname`
  X-PLOR Parameter: `SAVE`
  Filename prefix for the restart files.

- **NAMD Parameter:** `restartfreq`
  X-PLOR Parameter: `ISVFrq`
  Number of timesteps between the generation of restart files.

- **NAMD Parameter:** `DCDfile`
  X-PLOR Parameter: `TRAJectory`
  Filename for the position trajectory file.

- **NAMD Parameter:** `DCDfreq`
  X-PLOR Parameter: `NSAVC`
  Number of timesteps between writing coordinates to the trajectory file.

- **NAMD Parameter:** `velDCDfile`
  X-PLOR Parameter: `VELOcity`
  Filename for the velocity trajectory file.

- **NAMD Parameter:** `velDCDfreq`
  X-PLOR Parameter: `NSAVV`
  Number of timesteps between writing velocities to the trajectory file.

- **NAMD Parameter:** `numsteps`
  X-PLOR Parameter: `NSTEp`
  Number of simulation timesteps to perform.

18 Sample configuration files

This section contains some simple example NAMD configuration files to serve as templates.

This file shows a simple configuration file for alanin. It performs basic dynamics with no output files or special features.

```
# protocol params
numsteps        1000

# initial config
coordinates     alanin.pdb
temperature     300K
seed            12345

# output params
outputname      /tmp/alanin
binaryoutput    no

# integrator params
timestep        1.0

# force field params
structure       alanin.psf
parameters      alanin.params
exclude         scaled1-4
1-4scaling      1.0
switching       on
switchdist      8.0
cutoff          12.0
pairlistdist    13.5
stepspercycle   20
```

This file is again for alanin, but shows a slightly more complicated configuration. The system is periodic, a coordinate trajectory file and a set of restart files are produced.

```
# protocol params
numsteps        1000

# initial config
coordinates     alanin.pdb
temperature     300K
seed            12345

# periodic cell
cellBasisVector1   33.0 0 0
cellBasisVector2   0 32.0 0
cellBasisVector3   0 0 32.5

# output params
outputname      /tmp/alanin
binaryoutput    no
DCDfreq         10
restartfreq     100

# integrator params
timestep        1.0

# force field params
structure       alanin.psf
parameters      alanin.params
exclude         scaled1-4
1-4scaling      1.0
switching       on
switchdist      8.0
cutoff          12.0
pairlistdist    13.5
stepspercycle   20
```

This file shows another simple configuration file for alanin, but this time with full electrostatics using PME and multiple timestepping.

```
# protocol params
numsteps        1000

# initial config
coordinates     alanin.pdb
temperature     300K
seed            12345

# periodic cell
cellBasisVector1   33.0 0 0
cellBasisVector2   0 32.0 0
cellBasisVector3   0 0 32.5

# output params
outputname      /tmp/alanin
binaryoutput    no
DCDfreq         10
restartfreq     100

# integrator params
timestep        1.0
fullElectFrequency  4

# force field params
structure       alanin.psf
parameters      alanin.params
exclude         scaled1-4
1-4scaling      1.0
switching       on
switchdist      8.0
cutoff          12.0
pairlistdist    13.5
stepspercycle   20

# full electrostatics
PME             on
PMEGridSizeX    32
PMEGridSizeY    32
PMEGridSizeZ    32
```

This file demonstrates the analysis of a DCD trajectory file using NAMD. The file `pair.pdb` contains the definition of pair interaction groups; NAMD will compute the interaction energy and force between these groups for each frame in the DCD file. It is assumed that coordinate frames were written every 1000 timesteps. See Sec. 15.1 for more about pair interaction calculations.

```
# initial config
coordinates     alanin.pdb
temperature     0

# output params
outputname      /tmp/alanin-analyze
binaryoutput    no

# integrator params
timestep        1.0

# force field params
structure       alanin.psf
parameters      alanin.params
exclude         scaled1-4
1-4scaling      1.0
switching       on
switchdist      8.0
cutoff          12.0
pairlistdist    13.5
stepspercycle   20

# Atoms in group 1 have a 1 in the B column; group 2 has a 2.
pairInteraction on
pairInteractionFile pair.pdb
pairInteractionCol B
pairInteractionGroup1 1
pairInteractionGroup2 2

# First frame saved was frame 1000.
set ts 1000

coorfile open dcd /tmp/alanin.dcd

# Read all frames until nonzero is returned.
while { ![coorfile read] } {
  # Set firstTimestep so our energy output has the correct TS.
  firstTimestep $ts
  # Compute energies and forces, but don't try to move the atoms.
  run 0
```

```
  incr ts 1000
}
coorfile close
```

19 Running NAMD

NAMD runs on a variety of serial and parallel platforms. While it is trivial to launch a serial program, a parallel program depends on a platform-specific library such as MPI to launch copies of itself on other nodes and to provide access to a high performance network such as Myrinet or InfiniBand if one is available.

For typical workstations (Windows, Linux, Mac OS X, or other Unix) with only ethernet networking (hopefully gigabit), NAMD uses the Charm++ native communications layer and the program charmrun to launch namd2 processes for parallel runs (either exclusively on the local machine with the ++local option or on other hosts as specified by a nodelist file). The namd2 binaries for these platforms can also be run directly (known as standalone mode) for single process runs.

19.1 Individual Windows, Linux, Mac OS X, or Other Unix Workstations

Individual workstations use the same version of NAMD as workstation networks, but running NAMD is much easier. If your machine has only one processor core you can run the any non-MPI namd2 binary directly:

```
namd2 <configfile>
```

Windows, Mac OX X (Intel), and Linux-x86_64-multicore released binaries are based on "multicore" builds of Charm++ that can run multiple threads. These multicore builds lack a network layer, so they can only be used on a single machine. For best performance use one thread per processor with the +p option:

```
namd2 +p<procs> <configfile>
```

For other multiprocessor workstations the included charmrun program is needed to run multiple namd2 processes. The ++local option is also required to specify that only the local machine is being used:

```
charmrun namd2 ++local +p<procs> <configfile>
```

You may need to specify the full path to the namd2 binary.

19.2 Windows Clusters and Workstation Networks

The Win64-MPI version of NAMD runs on Windows HPC Server and should be launched as you would any other MPI program.

19.3 Linux Clusters with InfiniBand or Other High-Performance Networks

Charm++ provides a special ibverbs network layer that uses InfiniBand networks directly through the OpenFabrics OFED ibverbs library. This avoids efficiency and portability issues associated with MPI. Look for pre-built ibverbs NAMD binaries or specify ibverbs when building Charm++. The newer verbs network layer should offer equivalent performance to the ibverbs layer, plus support for multi-copy algorithms (replicas).

Intel Omni-Path networks are incompatible with the pre-built ibverbs NAMD binaries. Charm++ for verbs can be built with –with-qlogic to support Omni-Path, but the Charm++

MPI network layer performs better than the verbs layer. Hangs have been observed with Intel MPI but not with OpenMPI, so OpenMPI is preferred. See "Compiling NAMD" below for MPI build instructions. NAMD MPI binaries may be launched directly with mpiexec rather than via the provided charmrun script.

Writing batch job scripts to run charmrun in a queueing system can be challenging. Since most clusters provide directions for using mpiexec to launch MPI jobs, charmrun provides a ++mpiexec option to use mpiexec to launch non-MPI binaries. If "mpiexec -n *procs* ..." is not sufficient to launch jobs on your cluster you will need to write an executable mympiexec script like the following from TACC:

```
#!/bin/csh
shift; shift; exec ibrun $*
```

The job is then launched (with full paths where needed) as:

```
charmrun +p<procs> ++mpiexec ++remote-shell mympiexec namd2 <configfile>
```

Charm++ now provides the option ++mpiexec-no-n for the common case where mpiexec does not accept "-n *procs*" and instead derives the number of processes to launch directly from the queueing system:

```
charmrun +p<procs> ++mpiexec-no-n ++remote-shell ibrun namd2 <configfile>
```

For workstation clusters and other massively parallel machines with special high-performance networking, NAMD uses the system-provided MPI library (with a few exceptions) and standard system tools such as mpirun are used to launch jobs. Since MPI libraries are very often incompatible between versions, you will likely need to recompile NAMD and its underlying Charm++ libraries to use these machines in parallel (the provided non-MPI binaries should still work for serial runs.) The provided charmrun program for these platforms is only a script that attempts to translate charmrun options into mpirun options, but due to the diversity of MPI libraries it often fails to work.

19.4 Linux or Other Unix Workstation Networks

The same binaries used for individual workstations as described above (other than pure "multicore" builds and MPI builds) can be used with charmrun to run in parallel on a workstation network. The only difference is that you must provide a "nodelist" file listing the machines where namd2 processes should run, for example:

```
group main
host brutus
host romeo
```

The "group main" line defines the default machine list. Hosts brutus and romeo are the two machines on which to run the simulation. Note that charmrun may run on one of those machines, or charmrun may run on a third machine. All machines used for a simulation must be of the same type and have access to the same namd2 binary.

By default, the "rsh" command is used to start namd2 on each node specified in the nodelist file. You can change this via the CONV_RSH environment variable, i.e., to use ssh instead of

rsh run "setenv CONV_RSH ssh" or add it to your login or batch script. You must be able to connect to each node via rsh/ssh without typing your password; this can be accomplished via a .rhosts files in your home directory, by an /etc/hosts.equiv file installed by your sysadmin, or by a .ssh/authorized_keys file in your home directory. You should confirm that you can run "ssh hostname pwd" (or "rsh hostname pwd") without typing a password before running NAMD. Contact your local sysadmin if you have difficulty setting this up. If you are unable to use rsh or ssh, then add "setenv CONV_DAEMON" to your script and run charmd (or charmd_faceless, which produces a log file) on every node.

You should now be able to try running NAMD as:

```
charmrun namd2 +p<procs> <configfile>
```

If this fails or just hangs, try adding the ++verbose option to see more details of the startup process. You may need to specify the full path to the namd2 binary. Charmrun will start the number of processes specified by the +p option, cycling through the hosts in the nodelist file as many times as necessary. You may list multiprocessor machines multiple times in the nodelist file, once for each processor.

You may specify the nodelist file with the "++nodelist" option and the group (which defaults to "main") with the "++nodegroup" option. If you do not use "++nodelist" charmrun will first look for "nodelist" in your current directory and then ".nodelist" in your home directory.

Some automounters use a temporary mount directory which is prepended to the path returned by the pwd command. To run on multiple machines you must add a "++pathfix" option to your nodelist file. For example:

```
group main ++pathfix /tmp\_mnt /
host alpha1
host alpha2
```

There are many other options to charmrun and for the nodelist file. These are documented at in the Charm++ Installation and Usage Manual available at http://charm.cs.uiuc.edu/manuals/ and a list of available charmrun options is available by running charmrun without arguments.

If your workstation cluster is controlled by a queueing system you will need build a nodelist file in your job script. For example, if your queueing system provides a HOST_FILE environment variable:

```
set NODES = `cat $HOST_FILE`
set NODELIST = $TMPDIR/namd2.nodelist
echo group main >! $NODELIST
foreach node ( $nodes )
  echo host $node >> $NODELIST
end
@ NUMPROCS = 2 * $#NODES
charmrun namd2 +p$NUMPROCS ++nodelist $NODELIST <configfile>
```

Note that NUMPROCS is twice the number of nodes in this example. This is the case for dual-processor machines. For single-processor machines you would not multiply $#NODES by two.

Note that these example scripts and the setenv command are for the csh or tcsh shells. They must be translated to work with sh or bash.

19.5 Shared-Memory and Network-Based Parallelism (SMP Builds)

The Linux-x86_64-ibverbs-smp and Solaris-x86_64-smp released binaries are based on "smp" builds of Charm++ that can be used with multiple threads on either a single machine like a multicore build, or across a network. SMP builds combine multiple worker threads and an extra communication thread into a single process. Since one core per process is used for the communication thread SMP builds are typically slower than non-SMP builds. The advantage of SMP builds is that many data structures are shared among the threads, reducing the per-core memory footprint when scaling large simulations to large numbers of cores.

SMP builds launched with charmrun use +p to specify the total number of PEs (worker threads) and ++ppn to specify the number of PEs per process. Thus, to run one process with one communication and three worker threads on each of four quad-core nodes one would specify:

```
charmrun namd2 +p12 ++ppn 3 <configfile>
```

For MPI-based SMP builds one would specify any mpiexec options needed for the required number of processes and pass +ppn to the NAMD binary as:

```
mpiexec -n 4 namd2 +ppn 3 <configfile>
```

See the Cray XE/XK/XC directions below for a more complex example.

19.6 Cray XE/XK/XC

First load modules for the GNU compilers (XE/XK only, XC should use Intel), topology information, huge page sizes, and the system FFTW 3 library:

```
module swap PrgEnv-cray PrgEnv-gnu
module load rca
module load craype-hugepages8M
module load fftw
```

The CUDA Toolkit module enables dynamic linking, so it should only be loaded when building CUDA binaries and never for non-CUDA binaries:

```
module load cudatoolkit
```

For CUDA or large simulations on XE/XK use gemini_gni-crayxe-persistent-smp and for smaller XE simulations use gemini_gni-crayxe-persistent. For XC similarly use gni-crayxc-persistent-smp or gni-crayxc-persistent.

For XE/XK use CRAY-XE-gnu and (for CUDA) the "–with-cuda" config option, the appropriate "–charm-arch" parameter, and –with-fftw3. For XC use instead CRAY-XC-intel but all other options the same.

Your batch job will need to load modules and set environment variables:

```
module swap PrgEnv-cray PrgEnv-gnu
module load rca
module load craype-hugepages8M
setenv HUGETLB_DEFAULT_PAGE_SIZE 8M
setenv HUGETLB_MORECORE no
```

To run an SMP build with one process per node on 16 32-core nodes:

```
aprun -n 16 -r 1 -N 1 -d 31 /path/to/namd2 +ppn 30 +pemap 1-30 +commap 0 <configfile>
```

or the same with 4 processes per node:

```
aprun -n 64 -N 4 -d 8 /path/to/namd2 +ppn 7 \
          +pemap 1-7,9-15,17-23,25-31 +commap 0,8,16,24 <configfile>
```

or non-SMP, leaving one core free for the operating system:

```
aprun -n 496 -r 1 -N 31 -d 1 /path/to/namd2 +pemap 0-30 <configfile>
```

The explicit +pemap and +commap settings are necessary to avoid having multiple threads assigned to the same core (or potentially all threads assigned to the same core). If the performance of NAMD running on a single compute node is much worse than comparable non-Cray host then it is very likely that your CPU affinity settings need to be fixed.

All Cray XE/XK/XC network layers support multi-copy algorithms (replicas).

19.7 Xeon Phi Processors (KNL)

Special Linux-KNL-icc and CRAY-XC-KNL-intel builds enable vectorizable mixed-precision kernels while preserving full alchemical and other functionality. Multi-host runs require multiple smp processes per host (as many as 13 for Intel Omni-Path, 6 for Cray Aries) in order to drive the network. Careful attention to CPU affinity settings (see below) is required, as is 1 or 2 (but not 3 or 4) hyperthreads per PE core (but only 1 per communication thread core).

There appears to be a bug in the Intel 17.0 compiler that breaks the non-KNL-optimized NAMD kernels (used for alchemical free energy, etc.) on KNL. Therefore the Intel 16.0 compilers are recommended on KNL.

19.8 SGI Altix UV

Use Linux-x86_64-multicore and the following script to set CPU affinity:

```
namd2 +setcpuaffinity `numactl --show | awk '/^physcpubind/ {printf \
   "+p%d +pemap %d",(NF-1),$2; for(i=3;i<=NF;++i){printf ",%d",$i}}'` ...
```

19.9 IBM POWER Clusters

Run the verbs or ibverbs version of NAMD as on any other cluster, using poe in place of mpiexec as the process launcher, for example:

```
charmrun +p<procs> ++mpiexec-no-n ++remote-shell poe namd2 <configfile>
```

The details of job submission will vary between sites. For example, two nodes with two tasks per node on LSF are -n 4 -R "span[ptile=2]" with charmrun options +p36 ++ppn 9 ++mpiexec-no-n ++remote-shell poe

19.10 CPU Affinity

NAMD may run faster on some machines if threads or processes are set to run on (or not run on) specific processor cores (or hardware threads). On Linux this can be done at the process level with the numactl utility, but NAMD provides its own options for assigning threads to cores. This feature is enabled by adding +setcpuaffinity to the namd2 command line, which by itself will cause NAMD (really the underlying Charm++ library) to assign threads/processes round-robin to available cores in the order they are numbered by the operating system. This may not be the fastest configuration if NAMD is running fewer threads than there are cores available and consecutively numbered cores share resources such as memory bandwidth or are hardware threads on the same physical core.

If needed, specific cores for the Charm++ PEs (processing elements) and communication threads (on SMP builds) can be set by adding the +pemap and (if needed) +commap options with lists of core sets in the form "lower[-upper[:stride[.run]]][,...]". A single number identifies a particular core. Two numbers separated by a dash identify an inclusive range (lower bound and upper bound). If they are followed by a colon and another number (a stride), that range will be stepped through in increments of the additional number. Within each stride, a dot followed by a run will indicate how many cores to use from that starting point. For example, the sequence 0-8:2,16,20-24 includes cores 0, 2, 4, 6, 8, 16, 20, 21, 22, 23, 24. On a 4-way quad-core system three cores from each socket would be 0-15:4.3 if cores on the same chip are numbered consecutively. There is no need to repeat cores for each node in a run as they are reused in order.

For example, the IBM POWER7 has four hardware threads per core and the first thread can use all of the core's resources if the other threads are idle; threads 0 and 1 split the core if threads 2 and 3 are idle, but if either of threads 2 or 3 are active the core is split four ways. The fastest configuration of 32 threads or processes on a 128-thread 32-core is therefore "+setcpuaffinity +pemap 0-127:4". For 64 threads we need cores 0,1,4,5,8,9,... or 0-127:4.2. Running 4 processes with +ppn 31 would be "+setcpuaffinity +pemap 0-127:32.31 +commap 31-127:32"

For Intel processors, including KNL, where hyperthreads on the same core are not numbered consecutively, hyperthreads may be mapped to consecutive PEs by appending [+span] to a core set, e.g., "+pemap 0-63+64+128+192" to use all threads on a 64-core, 256-thread KNL with threads mapped to PEs as 0,64,128,192,1,65,129,193,...

For an Altix UV or other machines where the queueing system assigns cores to jobs this information must be obtained with numactl –show and passed to NAMD in order to set thread affinity (which will improve performance):

```
namd2 +setcpuaffinity ‘numactl --show | awk ’/^physcpubind/ {printf \
   "+p%d +pemap %d",(NF-1),$2; for(i=3;i<=NF;++i){printf ",%d",$i}}’‘ ...
```

19.11 CUDA GPU Acceleration

NAMD does not offload the entire calculation to the GPU, and performance may therefore be limited by the CPU. In general all available CPU cores should be used, with CPU affinity set as described above.

Energy evaluation is slower than calculating forces alone, and the loss is much greater in CUDA-accelerated builds. Therefore you should set outputEnergies to 100 or higher in the simulation config file. Forces evaluated on the GPU differ slightly from a CPU-only calculation, an effect more visible in reported scalar pressure values than in energies.

NAMD now has the entire force calculation offloaded to GPU for conventional MD simulation options. However, not all advanced features are compatible with CUDA-accelerated NAMD builds,

in particular, any simulation option that requires modification to the functional form of the non-bonded forces. Note that QM/MM simulation is also disabled for CUDA-accelerated NAMD, because the calculation is bottlenecked by the QM calculation rather than the MM force calculation, so can benefit from CUDA acceleration of the QM part when available. Table 1 lists the parts of NAMD that are accelerated with CUDA-capable GPUs, and Table 2 lists the advanced simulation options that are disabled within a CUDA-accelerated NAMD build.

Table 1: NAMD GPU: What is accelerated?

Accelerated	**Not Accelerated**
short-range non-bonded	integration
PME reciprocal sum	rigid bonds
bonded terms	grid forces
implicit solvent	collective variables
NVIDIA GPUs only	

Table 2: NAMD GPU: What features are disabled?

Disabled	**Not Disabled**
Alchemical (FEP and TI)	Memory optimized builds
Locally enhanced sampling	Conformational free energy
Tabulated energies	Collective variables
Drude (nonbonded Thole)	Grid forces
Go forces	Steering forces
Pairwaise interaction	Almost everything else
Pressure profile	
QM/MM	

To benefit from GPU acceleration you will need a CUDA build of NAMD and a recent NVIDIA video card. CUDA builds will not function without a CUDA-capable GPU and a driver that supports CUDA 8.0. If the installed driver is too old NAMD will exit on startup with the error "CUDA driver version is insufficient for CUDA runtime version."

Finally, if NAMD was not statically linked against the CUDA runtime then the libcudart.so file included with the binary (copied from the version of CUDA it was built with) must be in a directory in your LD_LIBRARY_PATH before any other libcudart.so libraries. For example, when running a multicore binary (recommended for a single machine):

```
setenv LD_LIBRARY_PATH ".:$LD_LIBRARY_PATH"
(or LD_LIBRARY_PATH=".:$LD_LIBRARY_PATH"; export LD_LIBRARY_PATH)
./namd2 +p8 +setcpuaffinity <configfile>
```

Each namd2 thread can use only one GPU. Therefore you will need to run at least one thread for each GPU you want to use. Multiple threads in an SMP build of NAMD can share a single GPU, usually with an increase in performance. NAMD will automatically distribute threads equally

among the GPUs on a node. Specific GPU device IDs can be requested via the +devices argument on the namd2 command line, for example:

```
./namd2 +p8 +setcpuaffinity +devices 0,2 <configfile>
```

Devices are shared by consecutive threads in a process, so in the above example threads 0–3 will share device 0 and threads 4–7 will share device 2. Repeating a device will cause it to be assigned to multiple master threads, which is allowed only for different processes and is advised against in general but may be faster in certain cases. When running on multiple nodes the +devices specification is applied to each physical node separately and there is no way to provide a unique list for each node.

When running a multi-node parallel job it is recommended to have one process per device to maximize the number of communication threads. If the job launch system enforces device segregation such that not all devices are visible to each process then the +ignoresharing argument must be used to disable the shared-device error message.

When running a multi-copy simulation with both multiple replicas and multiple devices per physical node, the +devicesperreplica <n> argument must be used to prevent each replica from binding all of the devices. For example, for 2 replicas per 6-device host use +devicesperreplica 3.

GPUs of compute capability < 3.0 are no longer supported and are ignored. GPUs with two or fewer multiprocessors are ignored unless specifically requested with +devices.

While charmrun with ++local will preserve LD_LIBRARY_PATH, normal charmrun does not. You can use charmrun ++runscript to add the namd2 directory to LD_LIBRARY_PATH with the following executable runscript:

```
#!/bin/csh
setenv LD_LIBRARY_PATH "${1:h}:$LD_LIBRARY_PATH"
$*
```

For example:

```
./charmrun ++runscript ./runscript +p60 ./namd2 ++ppn 15 <configfile>
```

An InfiniBand network is highly recommended when running CUDA-accelerated NAMD across multiple nodes. You will need either an ibverbs NAMD binary (available for download) or an MPI NAMD binary (must build Charm++ and NAMD as described above) to make use of the InfiniBand network. The use of SMP binaries is also recommended when running on multiple nodes, with one process per GPU and as many threads as available cores, reserving one core per process for the communication thread.

The CUDA (NVIDIA's graphics processor programming platform) code in NAMD is completely self-contained and does not use any of the CUDA support features in Charm++. When building NAMD with CUDA support you should use the same Charm++ you would use for a non-CUDA build. Do NOT add the cuda option to the Charm++ build command line. The only changes to the build process needed are to add –with-cuda and possibly –cuda-prefix ... to the NAMD config command line.

Right now, NAMD does not support all features available on GPUs. Thus, some keywords were introduced to help the user have a better control of the calculation. These keywords are relevant only for CUDA builds, and are ignored if the user is running a CPU build.

19.11.1 Keywords

- `bondedCUDA` < 0 to 255 >
  Acceptable Values: Integer value between 0 and 255
  Default Value: 255
  Description: NAMD provides CUDA kernels for calculating six different bonded force terms. The bondedCUDA parameter acts as a bit mask that can disable particular kernels. Any partial sum of the following values can be used to enable only the specified bonded terms:
  - bonds = 1
  - angles = 2
  - dihedrals = 4
  - impropers = 8
  - exclusions = 16
  - crossterms = 32

- `usePMECUDA` < Offload entire PME reciprocal sum to GPU? >
  Acceptable Values: "on" or "off"
  Default Value: on
  Description: The entire PME reciprocal sum is offloaded to GPUs, when using no more than four nodes. Otherwise usePMECUDA is disabled by default.

- `PMEoffload` < Offload PME gridding/ungridding procedures to GPU? >
  Acceptable Values: "on" or "off"
  Default Value: off
  Description: The gridding and ungridding procedures for calculating the PME reciprocal sum is offloaded to GPUs, with the FFT calculation still performed by CPUs. PMEoffload is enabled by default only for PMEinterpOrder > 4.

  For huge systems (10 million atoms and above) where the parallel FFT limits performance, it is desirable to use PMEoffload in conjunction with increased order interpolation and increased grid spacing, in order to decrease the overall communication latency by decreasing the overall grid size by a factor of 8 while maintaining the same accuracy for the calculation.
  Exemplary use:
  ```
  PME on
  PMEinterpOrder 8
  PMEgridSpacing 2.0
  PMEoffload on ;# enabled by default for these PME settings
  ```

19.12 Xeon Phi Acceleration

NAMD supports offloading calculations to Intel Xeon Phi coprocessors. This feature is new and should be considered experimental. Observed speedups are around a factor of two, but parallel scaling is degraded.

The Xeon Phi coprocessor is supported in NAMD similar to CUDA GPUs. Binaries are not provided, so you will need to build from source code (see "Compiling NAMD" below) specifying –with-mic to the config script. As with CUDA, multicore or ibverbs-smp builds are strongly recommended. A recent Intel compiler is obviously required to compile for Xeon Phi.

19.13 Memory Usage

NAMD has traditionally used less than 100MB of memory even for systems of 100,000 atoms. With the reintroduction of pairlists in NAMD 2.5, however, memory usage for a 100,000 atom system with a 12A cutoff can approach 300MB, and will grow with the cube of the cutoff. This extra memory is distributed across processors during a parallel run, but a single workstation may run out of physical memory with a large system.

To avoid this, NAMD now provides a pairlistMinProcs config file option that specifies the minimum number of processors that a run must use before pairlists will be enabled (on fewer processors small local pairlists are generated and recycled rather than being saved, the default is "pairlistMinProcs 1"). This is a per-simulation rather than a compile time option because memory usage is molecule-dependent.

Additional information on reducing memory usage may be found at http://www.ks.uiuc.edu/Research/namd/wiki/index.cgi?NamdMemoryReduction

19.14 Improving Parallel Scaling

While NAMD is designed to be a scalable program, particularly for simulations of 100,000 atoms or more, at some point adding additional processors to a simulation will provide little or no extra performance. If you are lucky enough to have access to a parallel machine you should measure NAMD's parallel speedup for a variety of processor counts when running your particular simulation. The easiest and most accurate way to do this is to look at the "Benchmark time:" lines that are printed after 20 and 25 cycles (usually less than 500 steps). You can monitor performance during the entire simulation by adding "outputTiming *steps*" to your configuration file, but be careful to look at the "wall time" rather than "CPU time" fields on the "TIMING:" output lines produced. For an external measure of performance, you should run simulations of both 25 and 50 cycles (see the stepspercycle parameter) and base your estimate on the additional time needed for the longer simulation in order to exclude startup costs and allow for initial load balancing.

Multicore builds scale well within a single node, but may benefit from setting CPU affinity using the +setcpuaffinity +pemap <map> +commap <map> options described in CPU Affinity above. Experimentation is needed.

We provide standard (UDP), TCP, and ibverbs (InfiniBand) precompiled binaries for Linux clusters. The TCP version may be faster on some networks but the UDP version now performs well on gigabit ethernet. The ibverbs version should be used on any cluster with InfiniBand, and for any other high-speed network you should compile an MPI version.

SMP builds generally do not scale as well across nodes as single-threaded non-SMP builds because the communication thread is both a bottleneck and occupies a core that could otherwise be used for computation. As such they should only be used to reduce memory consumption or if for scaling reasons you are not using all of the cores on a node anyway, and you should run benchmarks to determine the optimal configuration.

Extremely short cycle lengths (less than 10 steps) will limit parallel scaling, since the atom migration at the end of each cycle sends many more messages than a normal force evaluation. Increasing margin from 0 to 1 while doubling stepspercycle and pairlistspercycle may help, but it is important to benchmark. The pairlist distance will adjust automatically, and one pairlist per ten steps is a good ratio.

NAMD should scale very well when the number of patches (multiply the dimensions of the patch grid) is larger or rougly the same as the number of processors. If this is not the case, it may

be possible to improve scaling by adding "twoAwayX yes" to the config file, which roughly doubles the number of patches. (Similar options twoAwayY and twoAwayZ also exist, and may be used in combination, but this greatly increases the number of compute objects. twoAwayX has the unique advantage of also improving the scalability of PME.)

Additional performance tuning suggestions and options are described at http://www.ks.uiuc.edu/Research/namd/wiki/?NamdPerformanceTuning

20 NAMD Availability and Installation

NAMD is distributed freely for non-profit use. NAMD 2.14b1 is based on the Charm++ messaging system and the Converse communication layer (http://charm.cs.uiuc.edu/) which have been ported to a wide variety of parallel platforms. This section describes how to obtain and install NAMD 2.14b1.

20.1 How to obtain NAMD

NAMD may be downloaded from http://www.ks.uiuc.edu/Research/namd/. You will be required to provide minimal registration information and agree to a license before receiving access to the software. Both source and binary distributions are available.

20.2 Platforms on which NAMD will currently run

NAMD should be portable to any parallel platform with a modern C++ compiler to which Charm and Converse have been ported. Precompiled NAMD 2.14b1 binaries are available for download for the following platforms:

- Windows (7, 8, 10, etc.) on x86-64 processors
- Mac OS X on Intel processors
- Linux on x86-64 processors
- Windows, Mac OS X, or Linux with NVIDIA GPUs (CUDA)

NAMD may be compiled for the following additional platforms:

- Cray XT/XE/XK/XC
- IBM Blue Gene L/P/Q
- Linux or AIX on POWER processors
- Linux on ARM processors
- Linux on ARM or POWER processors with NVIDIA GPUs (CUDA)
- Linux on x86-64 processors with Intel Xeon Phi coprocessors (MIC)

20.3 Installing NAMD

A NAMD binary distribution need only be untarred or unzipped and can be run directly in the resulting directory. When building from source code, "make release" will generate a self-contained directory and .tar.gz or .zip archive that can be moved to the desired installation location. Windows and CUDA builds include Tcl .dll and CUDA .so files that must be in the dynamic library path.

20.4 Compiling NAMD

We provide complete and optimized binaries for all common platforms to which NAMD has been ported. It should not be necessary for you to compile NAMD unless you wish to add or modify features or to improve performance by using an MPI library that takes advantage of special networking hardware.

Directions for compiling NAMD are contained in the release notes, which are available from the NAMD web site http://www.ks.uiuc.edu/Research/namd/ and are included in all distributions.

20.5 Documentation

All available NAMD documentation is available for download without registration via the NAMD web site http://www.ks.uiuc.edu/Research/namd/.

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