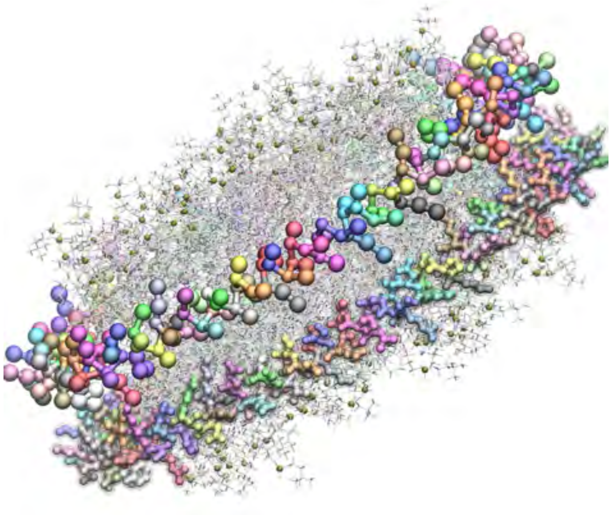
University of Illinois at Urbana-Champaign
Beckman Institute for Advanced Science and Technology
Theoretical and Computational Biophysics Group
Computational Biophysics Workshop

Residue-Based Coarse Graining using MARTINI Force Field in NAMD



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A current version of this tutorial is available at
http://www.ks.uiuc.edu/Training/Tutorials/

Contents

Introduction

In this session, we will learn about coarse-grained (CG) molecular dynamics (MD) simulations. Atomistic simulations are useful computational tools for investigating biological systems such as proteins, lipids and nucleic acids over timescales of nanoseconds. However, many interesting phenomena, including vesicle fusion, membrane deformation, protein-protein assembly etc., occur at longer time scales that fall outside the capabilities of atomic scale simulations. In order to reach the relevant timescales, simplification of the model is required. The term "coarse graining" (CG) can be used to refer to any simulation technique that simplifies the system by grouping several atoms of it into one component, thus to consist of fewer, larger components. CG thereby represents an attractive alternative to atomistic scale simulations since the reduction in interaction particles and number of degrees of freedom allow for simulations to be run over relatively long periods of time and length scales at a reduced level of detail.

This tutorial presents one CG method, termed residue-based coarse-grained (RBCG). In a residue-based coarse-grained (RBCG) model for biological systems comprising proteins and or lipids, several atoms are grouped together in a "virtual" bead that interacts through an effective potential. For example, each amino acid residue and 4 water molecules are represented by 2-5 beads and 1 bead respectively (Figure 1).The reduction of the number of degrees of freedom and the use of shorter-range potential functions makes the model computationally very efficient, allowing a increase in the base time-step and thus a reduction of the simulation time by 2 - 3 orders of magnitude compared to the traditional atomistic models. RBCG MD simulations were performed in NAMDusing the MARTINI CG force field developed and parametrized by the group of Siewert Marrink for use with GROMACS. For the implemented CG force field in NAMD to be functional, in order to reproduce the results of GROMACS, we adapted the GROMACS switching function for LJ potential and a shifting function for Colomb potential only for use of CG simulations.

The tutorial introduces tools for RBCG modeling that are provided in VMD as plugins (http://www.ks.uiuc.edu/Research/vmd/plugins/cgtools/).

For exercises, we will model protein-lipid assemblies called high-density lipoproteins (HDL) (Shih et al., *J. Str. Biol.*, **157**:579, 2007). HDL are known to function as cholesterol transporters, facilitating the removal of excess cholesterol from the body. Due to the heterogenity of native HDL particles, the details of how these protein-lipid particles form and the structure they assume in their lipid associated states are not well characterized. Coarse-grained (CG) molecular dynamics allows for long-time scale simulations needed to reveal the stable conformations and also self-assembly of discoidal HDL particles from disordered protein-lipid complexes. In this tutorial we focus on modeling a RBCG structure of discoidal HDL starting from all-atom for performing simulations to reveal the stablity of the widely accepted double-belt model.

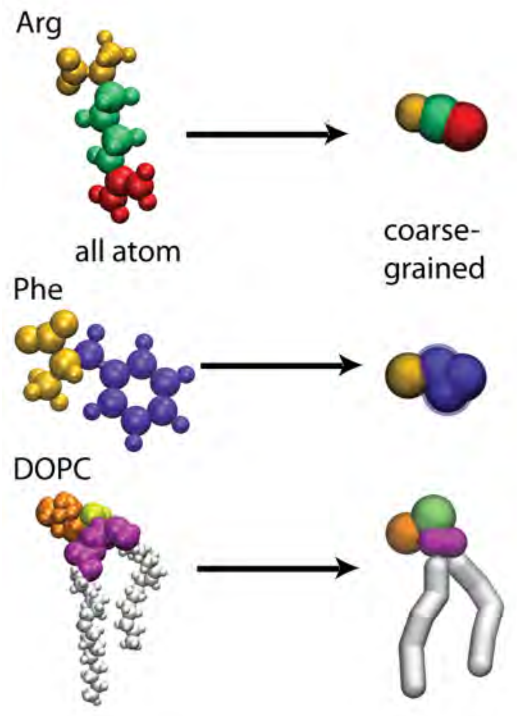


Figure 1: Mapping all-atom to coarse-grained structure . Left, amino acid residues and lipid shown in all-atom representation. Right, a coarse-grained representation of the same.

To perform simulations using the RBCG representation, one uses VMD and NAMD without any changes in comparison with the all-atom case, and work with the same file types as for all-atom modeling, such as PSF and PDB for structures, and topology, parameter, and configuration files for running simulations (see VMD and NAMD tutorials, http://www.ks.uiuc.edu/Training/Tutorials/). However, the RBCG PSF, PDB files first need to be created according to the all-atom model that one desires to coarse-grain. In this tutorial, we will learn how to use the RBCG plugins of VMD to build such files for simulations.

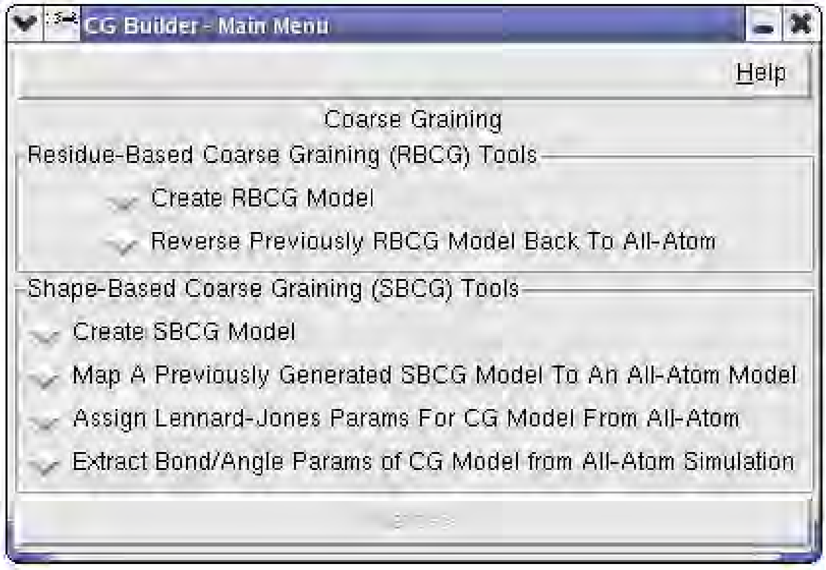
Required Programs

The following programs are required for this tutorial:

- **VMD:** The tutorial assumes that you already have a working knowledge of VMD, which is available at http://www.ks.uiuc.edu/Research/vmd/ (for all platforms). The VMD tutorial is available at http://www.ks.uiuc.edu/Training/Tutorials/vmd/tutorial-html/
- **NAMD ”Nightly build May 31, 2012 or later (Linux only)” or NAMD version 2.10 (for all platforms when available):** In order to perform simulations with the CG model in this tutorial, NAMD should be correctly installed on your computer. For installation instructions, please refer to the NAMD Users' Guide. The NAMD tutorial is available in both Unix/MacOSX and Windows versions:
  http://www.ks.uiuc.edu/Training/Tutorials/namd/namd-tutorial-unix-html/
  http://www.ks.uiuc.edu/Training/Tutorials/namd/namd-tutorial-win-html/

Most of the exercises in the tutorial are performed using Residue-Based Coarse-Graining (RBCG) Tools in VMD. The Tools are implemented as a set of plugins available with their Graphical User Interfaces (GUIs) through VMD menu:

`Extensions` → `Modeling` → `CG Builder`



CG Builder - Main Menu

Help

Coarse Graining

Residue-Based Coarse Graining (RBCG) Tools

- Create RBCG Model
- Reverse Previously RBCG Model Back To All-Atom

Shape-Based Coarse Graining (SBCG) Tools

- Create SBCG Model
- Map A Previously Generated SBCG Model To An All-Atom Model
- Assign Lennard-Jones Params For CG Model From All-Atom
- Extract Bond/Angle Params of CG Model from All-Atom Simulation

Figure 2: Main Graphical User Interface for the CG Builder Tools in VMD. Available are several tools for two CG models, one of which is the RBCG model addressed in this tutorial.

Getting Started

If you downloaded the tutorial from the web you will also need to download the appropriate files, unzip them, and place them in a directory of your choosing. You should then navigate to that directory as described below. The files for this tutorial are available at
http://www.ks.uiuc.edu/Training/Tutorials/

- **Unix/Mac OS X Users:** In a Terminal window type:

  ```
  cd <path to the directory rbcg-martini-tutorial/files/>
  ```

  You can list the content of this directory by using the command `ls`.

- **Windows Users:** Navigate to the `rbcg-martini-tutorial` → `files` directory using Windows Explorer.

You can find the files for this tutorial in the `rbcg-martini-tutorial/files`. Below you can see in Fig. 3, the organization of files and directories of `rbcg-martini-tutorial/files/` .

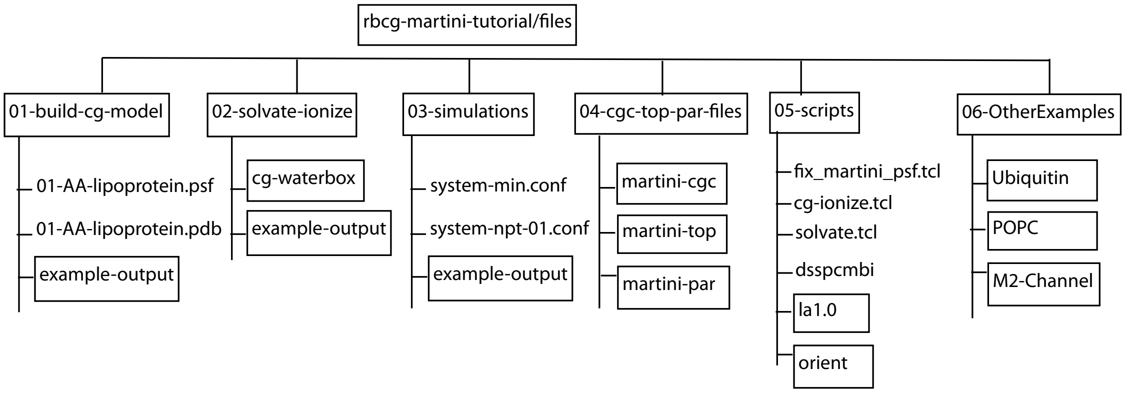


Figure 3: Directory Structure for tutorial exercises. Sample output for each exercise is provided in an "example-output" subdirectory within each folder.

To start VMD type `vmd` in a Unix terminal window. Double-click on the VMD application icon likely located in the `Applications` folder in Mac OS X, or click on the `Start` → `Programs` → `VMD` menu item in Windows.

1 Coarse-graining an atomic structure

In this unit you will build the PDB and PSF required for simulation of the lipoprotein assembly, learning how to take a raw all-atom structure and build a RBCG system out of it.

1.1 Structural model of lipoprotein.

High-density lipoproteins (HDL) are protein-lipid particles, which circulate in the blood collecting cholesterol. Apolipoprotein A-I (apo A-I), the primary protein component of HDL, is a 243 residue amphipathic protein containing an N-terminal globular domain and a C-terminal lipid binding domain. The lipid binding domain comprises 200 residues, however, the first 11 to 22 residues of the domain are known not to be involved in binding of lipids in the discoidal shaped HDL particles. Due to heterogenity of HDL particles, high resolution structures have been difficult to obtain. Nanodiscs are nanometer-sized discoidal HDL that are being developed as a platform for studying membrane proteins. The scaffold protein that were used to surround nanodiscs (MSP1) were engineered to contain the lipid binding domain of 200 residues. In this tutorial, we model a truncated discoidal HDL comprising a truncated lipid binding domain of apo A-I (MSP1 Δ(1-11) consisting of 189 residues by deleting the first 11 residues surrounding a lipid core consisting of 160 DPPC lipids. We employ RBCG VMD plugin to model this lipoprotein system.

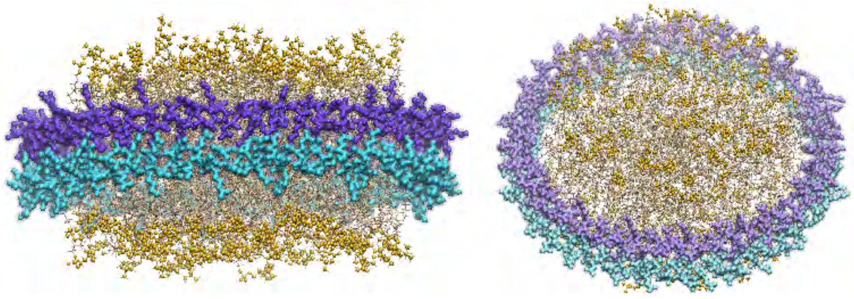


Figure 4: The discoidal HDL nanodisc shown in side (left) and top (right) view. The two monomers of the apo A-I lipid binding domain are shown in violet and cyan. DPPC lipids are shown tan with lipid head groups in yellow.

Provided for you is the all-atom PDB/PSF nanodisc structure with truncated apo A-I (MSP1 Δ(1-11)) (see Shih et al., *J. Str. Biol.*, **157**:579, 2007). To begin, you will build an all-atom PDB/PSF pair for the PDB structure of interest. This can be done using a PSFgen script or employing AutoPSF plugin in VMD. We assume that the reader is familiar with constructing a PSF from PDB. Such PDB and PSF are already created: see `01-AA-lipoprotien`, `01-AA-lipoprotein.psf` in the directory `01-build-cg-model/`.

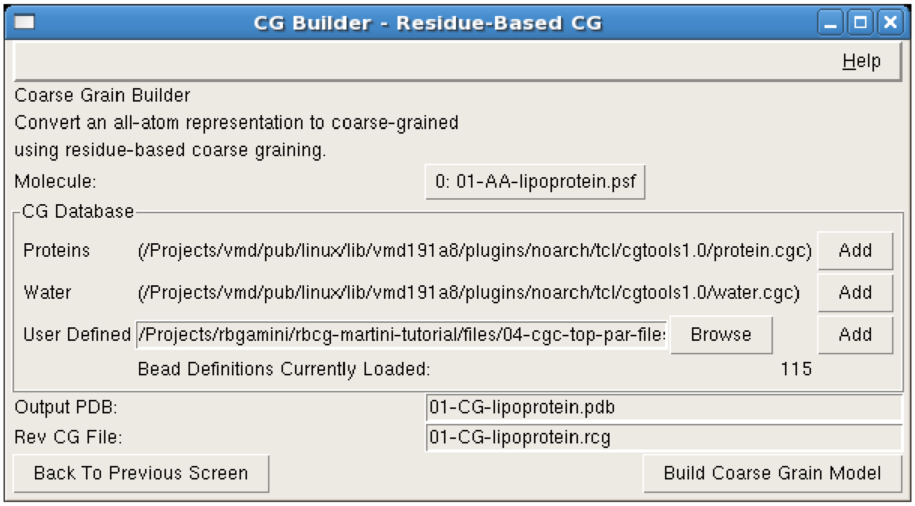
Navigate to the directory `01-build-cg-model/`. You can examine the segments of the truncated lipid binding domain of the apo A-I (MSP1 Δ(1-11) in VMD (files `01-AA-lipoprotein.pdb` and `01-AA-lipoprotein.psf` in `1-build-cg-model/`). One monomer is designated as `segname P1`, and the other as `segname P2`. The DPPC lipid patch is designated as `resname DPPC`.

1.2 Coarse-graining of a lipoprotein structure.

Let us now coarse-grain the all-atom lipoprotein structure .

1. Start VMD and load the all-atom lipoprotein structure (load `01-AA-lipoprotein.psf` and `01-AA-lipoprotein.pdb` into the same molecule).

2. Open the CG Builder in VMD (`Extensions` → `Modeling` → `CG Builder`), and choose the option "`Create RBCG Model`"and hit the button `Next->`. This will bring you to the RBCG Builder GUI (Fig. 5).



CG Builder - Residue-Based CG

Help

Coarse Grain Builder
Convert an all-atom representation to coarse-grained
using residue-based coarse graining.

Molecule: 0: 01-AA-lipoprotein.psf

CG Database

Proteins (/Projects/vmd/pub/linux/lib/vmd191a8/plugins/noarch/tcl/cgtools1.0/protein.cgc) Add

Water (/Projects/vmd/pub/linux/lib/vmd191a8/plugins/noarch/tcl/cgtools1.0/water.cgc) Add

User Defined /Projects/rbgamini/rbcg-martini-tutorial/files/04-cgc-top-par-file: Browse Add

Bead Definitions Currently Loaded: 115

Output PDB: 01-CG-lipoprotein.pdb

Rev CG File: 01-CG-lipoprotein.rcg

Back To Previous Screen

Build Coarse Grain Model

Figure 5: RBCG Builder GUI.

3. The first step in creating a coarse-grained model is to split the system into appropriate atom clusters and assign the correct bead types to them. The definitions of the atom clusters are provided in the .cgc files in the folder `04-cgc-top-par-files/martini-cgc/`. To model our lipoprotein system, choose the `User Defined` option to browse and add `martini-lipids.cgc` and `martini-protein.cgc`. Note: The options `Proteins` and `Water` are set to use old RBCG parameters.

CGC entries. Open one of the .cgc files (i.e. `martini-protein.cgc`), you will see entries of the form

```
CGBEGIN
(RESNAME) (BEADNAME) 0
(RESNAME) (ATOMNAME) 0 (the first atom should be what you consider the "central" atom of the cluster)
(RESNAME) (ATOMNAME) 0 (beyond that, the ordering is unimportant)
...
CGEND
```

each of which defines one bead assignment.

An annotated section of the standard CHARMM .top file is provided for illustrative purposes in Appendix A, showing the cgc bead assignments for all protein residues and for POPE lipids.

4. The main result of running the algorithm is the production of output files that are written on the hard drive, namely the RBCG PDB and RCG files. If you want to have specific names for those files, they can be changed in the RBCG Builder GUI before hitting "`Build Coarse Grain Model`" button. Here, the default filename for PDB/RCG `cg-01-AA-lipoprotein` is changed to `01-CG-lipoprotein`.

5. Hit the "`Build Coarse Grain Model`" button. Completion of the RBCG algorithm will take a few moments.

6. The output PDB file containing the newly constructed RBCG model is automatically loaded in VMD as a new molecule, overlapped with the original all-atom model. In case something fails, we have provided the output files generated in this step `01-CG-lipoprotein.pdb` and `01-CG-lipoprotein.rcg` in the `01-build-cg-model/example-output/` folder.

7. The RBCG output PDB file determines the structure of the coarse-grained lipoprotein model. To obtain the complete structure for display in VMD, or for subsequent simulations, we need to make a PSF file for the PDB. This can be done the same way as commonly achieved for all-atom files, namely, using a PSFgen script or by employing the AutoPSF VMD plugin. Start VMD and load the cg-lipoprotein structure (load `01-CG-lipoprotein.pdb`). To employ the AutoPSF plugin (`Extensions` → `Modeling` → `Automatic PSF Builder`), remember to delete the default topology file from the list of topologies in the plugin, and add the CG topology files (`martini-protein.top` and `martini-lipids.top`) located in the `04-cgc-top-par-files/martini-top/` directory. One caveat to keep in mind is to ensure you do not generate angles and dihedrals which are not defined in MARTINI. If you are using AutoPSF plugin, uncheck "Regenerate angles/dihedrals" under Options. Click

"Guess and split chains using current selections". Note that in the `Segments Identified` the NTER, CTER patches listed are not defined for RBCG in AutoPSF VMD plugin, therefore, we select each chain and hit "`Edit Chain`" to change the N terminal patch and C terminal patch to "`NONE`". Once the patches are changed, hit "`Create chains`". This will create a preliminary PSF file `01-CG-lipoprotein_autopsf.psf` and the corresponding PDB file `01-CG-lipoprotein_autopsf.psf` for your coarse-grained system.

MARTINI mapping for protein and lipids . In the MARTINI model, each protein residue is represented by a "backbone bead" and one or more "sidechain beads" (with the exception of ALA and GLY, which are modeled solely by a backbone bead). Ring-shaped sidechains (TRP, PHE, TYR, HIS) are treated in more detail, and contain 3-4 lighter sidechain beads. Fig. 1 shows three examples of coarse-grained residues. The type of bead to be assigned to each cluster of atoms is defined by the non-bonded interactions of that bead. There are four classes of beads: Q (charged), P (polar), N (nonpolar) and C (apolar), each with several subtypes. Q and N types have four subtypes, Qda, Qd, Qa, Q0 and Nda, Nd, Na, N0 which distinguish between the hydrogen-bonding capabilities of the atom cluster (da = donor or acceptor, d = donor, a = acceptor, 0 = no hydrogen bonding). P and C types have five subtypes, P1, P2, P3, P4, P5 and C1, C2, C3, C4, C5, wherein the subscripts 1-5 indicate increasing polar affinity. By convention, each bead has a mass of 72 amu. In addition to these "heavy" beads, there is also a class of light beads with mass 45 amu, used to describe ring shaped residues. These beads are indicated with a prepended "S", e.g. SP1, SC3, SQda, etc. In addition to their lighter mass, they are assigned a smaller vdW radius and the vdW interaction strength is scaled by 75%. To be clear, the mass of a bead in MARTINI is 72 (or 45) amu regardless of whether the masses of the atoms comprising that bead actually sum to 72. The modeling of lipid molecules is similar to that of proteins, except that the "small" bead types and secondary structure assignments are not needed. See again Fig. 1 for a depiction of a lipid in coarse-grained representation.

8. Correction for protein segments. The next step is to correct the coarse-grained PSF file so that the bead types reflect the secondary structure of the protein. A PSFgen script is provided: `fix_martini_psf.tcl` in the folder `05-scripts` for this purpose. Open the script to see how this is done. The script also allows you to choose for charged/uncharged N and C terminal. Copy the script to current working directory `01-build-cg-model`. Also copy `martini-protein.top`. Start VMD and load the "all-atom-lipoprotein structure" (`01-AA-lipoprotein.psf 01-AA-lipoprotein.pdb`). Make sure this is the top molecule. And run the following command in the VMD Tk Console:

```
   source fix_martini_psf.tcl
fix_martini_psf 0 martini-protein.top 01-CG-lipoprotein_autopsf.psf
```

```
01-CG-lipoprotein_autopsf.pdb CG-fix_martini_psf 1 ../05-scripts 1 -1
```

Note that the script uses the all-atom PSF PDB and RBCG PSF PDB files you have just created. The first argument here "0" refers to molid of the top molecule `01-AA-lipoprotein.psf` and `01-AA-lipoprotein.psf`. The second argument is the topology file `martini-protein.top` placed in the current directory; if you did not place these files in the directory where `fix_martini_psf.tcl` is located, you will need to specify the correct paths for all the input files while calling the proc `fix_martini_psf`. The fifth argument "`CG-fix_martini_psf`" refers to name of the corrected "output PSF/PDB" files. In the sixth argument we provide the "path" for DSSP secondary structure assignment plugin "`../05-script`"-the default is STRIDE. The last two arguments is for having a positive N terminus and negative C terminus.
Now open the original 01-CG-lipoprotein_autopsf.psf and the corrected `CG-fix_martini_psf.psf` PSF files with a text editor. Note that in the original PSF file, the bacbone beads (BAS) have generic types (such as P4, P5), whereas in the corrected PSF file, they have been give bead types corresponding to the secondary structure assignment for each residue.

MARTINI mapping for protein secondary structure . MARTINI is sensitive to the secondary structure of the protein it is being used to represent. Beads representing protein backbone are classified not only by their non-bonded interactions, but on their secondary structure and the secondary structures of their immediate neighbors. Backbone beads may be classified as Helix (H), Coil (C), Extended (E), Turn (T), Bend (B) or Free (F), and the secondary structure assignment determines the bonded interactions with neighboring backbone beads.

RBCG Builder output files. Sample RBCG Builder output files are provided in the folder `1-build-cg-model/example-output`, including also the output files from running the AutoPSF and PSFgen script `fix_martini_psf.tcl`. Note that all these output files are generally going to be somewhat different from those you create, due to the probabilistic nature of the RBCG algorithm.

We are almost there! We only have to solvate and ionize the system, then we can start using the NAMD to actually perform a simulation of the RBCG lipoprotein system.

1.3 Solvation and Ionization.

We will now solvate and ionize the system. We will use the VMD's Solvate Plugin, just as in the all-atom case, except that you will use the non-standard solvent option and place the system in a MARTINI water box in place of the default.

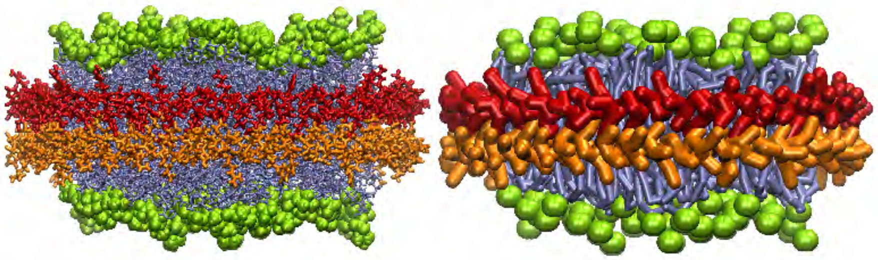


Figure 6: High-density lipoprotein (HDL) nanodisc structure. The two monomers of the apo A-I lipid binding domain are shown in orange and red and the DPPC lipids are shown in blue with lipid head groups in green. The all-atom structure is shown on the left, and an example of a RBCG structure is shown on the right. Both all-atom and RBCG structures are shown from the sideview.

1. First, load the files `01-CG-fix_martini_psf.psf` and `01-CG-fix_martini_psf.pdb` into VMD.

MARTINI water. Water molecules in MARTINI are represented by a single bead (of mass 72 amu). To protect against the possibility of the water box "freezing" on very large timescales, a second type of water molecule is defined with a slighly larger VdW radius. These are referred to as "antifreeze particles", and it is recommended that 10% of the waterbox be antifreeze particles.

2. To use the Solvate plugin, select `Extensions` → `Modeling` → `Add Solvation Box` in the main window.

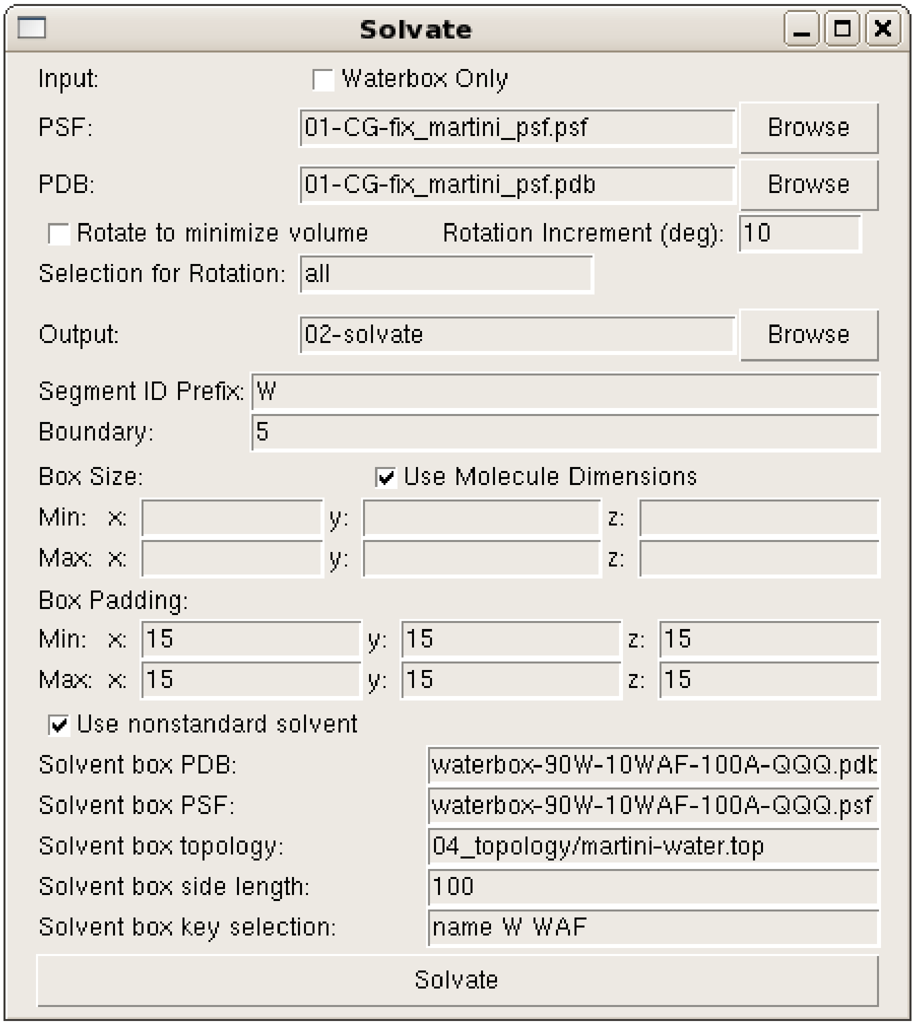
3. Set "Boundary", the minimum distance between water and solute to 5 instead of the default 2.4.

4. Set "Box Padding" to 15 angstrom padding in x, y and z directions.

5. Check the "nonstandard solvent" to use the equilibrated CG waterbox. The PDB/PSF files are provided in the folder `02-solvate-ionize/cg-waterbox/` and the TOP file is located in the folder `/04-cgc-top-par-files/martini-top`.

6. Set "Solvent box side length" to 100. This corresponds to the box side length of the provided equilibrated CG waterbox.

7. Set "Solvent box key selection" to "name W WAF". This corresponds to the water and anti-freeze CG beads.



Input: □ Waterbox Only

PSF: 01-CG-fix_martini_psf.psf

PDB: 01-CG-fix_martini_psf.pdb

□ Rotate to minimize volume Rotation Increment (deg): 10

Selection for Rotation: all

Output: 02-solvate

Segment ID Prefix: W

Boundary: 5

Box Size: ☑ Use Molecule Dimensions

Min: x: y: z:

Max: x: y: z:

Box Padding:

Min: x: 15 y: 15 z: 15

Max: x: 15 y: 15 z: 15

☑ Use nonstandard solvent

Solvent box PDB: waterbox-90W-10WAF-100A-QQQ.pdb

Solvent box PSF: waterbox-90W-10WAF-100A-QQQ.psf

Solvent box topology: 04_topology/martini-water.top

Solvent box side length: 100

Solvent box key selection: name W WAF

Figure 7: `Solvate` plugin in VMD.

8. Then click `Solvate` and wait for a minute or two. The `Solvate` should generate the `02-solvate` PSF PDB files.

9. To ionize and neutralize the system, we will use the modified version of autoionize PSFgen script `cg-ionize.tcl` provided in the folder `05-scripts`. Make sure the the output files of `Solvate` (`02-solvate.psf` and `02-solvate.pdb`) are currently loaded into VMD.

MARTINI ions. Ions are also represented as single (charged) particles of mass 72 amu.

10. Type in the Tk Console window:

```
source cg-ionize.tcl
autoionize -psf 02-solvate.psf -pdb 02-solvate.pdb -sc 0.1 -o 02-ionize
```

The `-sc 0.1` option tells `autoionize` to neutralize and set salt concentration to 0.1 (mol/L). The default `cation` is `CL` and default `anion` is `NA`. The option `-cation -anion` can be used to specify the cation and anion other than default ions.

11. Load the output files of autoionize (`02-ionize.psf` and `02-ionize.pdb`) into VMD and check the ions are really there and the system is neutral.

Type in the Tk Console window:

```
set all [atomselect top all]
measure sumweights $all weight charge
You should get:
```

0.0

Finally, after all this work, we are ready for minimization and equilibration with NAMD. This is described in the next unit.

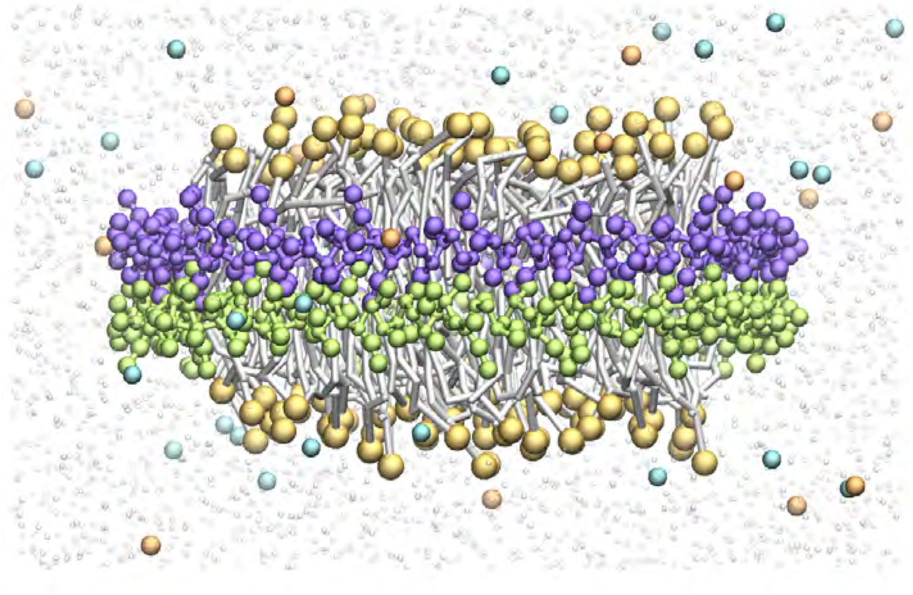


Figure 8: Final simulation system including the truncated apo A-I protein, DPPC, water, and ions.

2 Running a coarse-grained simulation

We are now almost ready to simulate the system of RBCG lipoprotien assembly. In this section we will discuss first how to write a NAMD configuration file for an RBCG system. We will then perform the simulation, and discuss the file outputs and simulation result.

To perform exercises, navigate to the folder `03-simulation/`.

2.1 Preparing a configuration file.

Since RBCG was designed to be compatible with NAMD, an RBCG configuration file looks similar to a normal, all-atom, NAMD configuration file that you might have used before.

1. A sample configuration file `system-npt-01.conf` has been prepared for you in the directory `example-output/`. Copy it to the folder where you want to run the simulation, and open it with a text editor. Remember to create in the same folder subfolder `output` and `input`, and add your CG files to the folder `input` analogously to how it is done in `example-output/`. Note that you will need here RBCG parameter files for CG lipids and proteins, which we have not used before. The paramter files are located in `04-cgc-top-par-files/martini-par/` folder.

2. The configuration file contains many options (entries in the first column), followed by their parameters (entries in the second column) specifically chosen for the simulated system. Assuming readers already have experience with NAMD simulations, here we will only go through those options that require special adjustments for an RBCG system. New NAMD users are encouraged to consult the NAMD Tutorial and NAMD User's Guide.

3. We introduce a new parameter `cosAngles`. This is required to allow for the cosine-based angle potential energy term conventionally used in GROMACS, as opposed to the harmonic angle term used in NAMD. `cosAngles` is turned `on`.

4. In the text editor displaying the content of `system-npt-01.conf`, scroll down to the section `# Force-Field Parameters`. Note all lines beginning with `#` are comments ignored by NAMD.

Under `# Force-Field Parameters`, you will find simulation options that might need different parameters than those of an all-atom simulation. These options define how you want the interactions between beads to be computed. `exclude` parameter is set `1-2`, `martiniSwitching`, a new key parameter, is turned `on`,`PME` is turned `off` and `dielectric` is set `15.0`.

It should be noted that for the use of MARTINI force field in NAMD, a GROMACS switching function is adapted for LJ potential with `switchdist 9.0` and `cutoff 12.0`, and a shifting function for Colomb potential with `switchdist`

`0.0 hardcoded in the NAMD source code` and `cutoff 12.0` .

5. Scroll down to the section `# Integrator Parameters`.

The parameter `timestep` has a value of `20.0`, implying that the integration timestep of the simulation is 20 fs/step. A typical all-atom simulation uses 1 or 2 fs/step, hence the RBCG gives a speedup of 20 from the choice of integration timestep alone. The choice of the timestep depends on how fast beads are moving in the simulation, and, thus, the maximal timestep possible (so that the simulation does not crash) is determined by the strength of interactions, e.g., stiffness of bonds, as mentioned above. If your simulation crashes with a timestep of 20 fs/step, starting the simulation with a shorter timestep might fix the problem. Then timestep can be increased when the system becomes stable later in the simulation. For minimization, one can do a larger value for timestep of 40 fs/step.

6. Constant temperature is maintained in this RBCG simulation using Langevin dynamics, as usually done in all-atom simulations. You can take a look at these parameters under `# Constant Temperature Control`. The Langevin dynamics introduces viscous drag and random forces acting on each CG bead, which can be used to mimic the viscosity of the solvent and the Brownian motion due to random hits from the molecules of the solvent. A single parameter, `langevinDamping`, is used to account for these effects. Here, `langevinDamping` is set to $1\,\mathrm{ps}^{-1}$.

7. We will first perform minimization before running the simulation in NPT. You can see that the simulation is designed to be minimized by 5000 steps (see `system-min.conf`) to eliminate the possible steric clashes, and subsequently run for 50,000,000 steps. This corresponds to 50,000,000 steps × 20 fs/step = 100 ns simulation time.

8. Close the text editor displaying configuration file. Run the minimization by typing `namd2 system-min.conf > system-min.log` in a terminal window. This is a short run of minimization for 5000 steps that takes about 5 minutes to complete. Once this step is complete, run a short simulation for 10,000,000 steps in NPT by typing `namd2 system-npt-01.conf > system-npt-01.log`

2.2 Simulation outputs.

On a one-processor machine, a 100 ns simulation will take about two days to complete, but actually we do not need to run the full simulation. The general trend is obvious already after about the first 10 ns, which can be achieved within an hour or two. If you do not wish to run the simulation yourself, you can use the files provided in `example-output/` for the following discussion on file outputs and results.

1. Open the logfile of the simulation, `system-npt-01.log`, with a text editor. If you did not run the simulation, use `example-output/system-npt-01.log`.

The logfile of a simulation contains useful information. When your simulation crashes, checking the logfile for the error message is the first step of fixing the problem. The logfile can also give you an estimate on how long a simulation will run. Find the words "`Benchmark time`" in `system-npt-01.log`, here you can find the speed of the simulation. Now let's examine the system via VMD.

2. Close the text editor. Open VMD, and load the psf file of the system, `02-ionize.psf`. If you did not run the simulation, make sure you use the provided `example-output/input/02-ionize.psf`.

3. Load the output dcd files, `system-npt-01.dcd`. If you did not run the simulation, use the one provided `example-output/output/system-npt-01.dcd`. In this case, you will have 1000 frames loaded in VMD, one frame for 20 picosecond of the simulation.

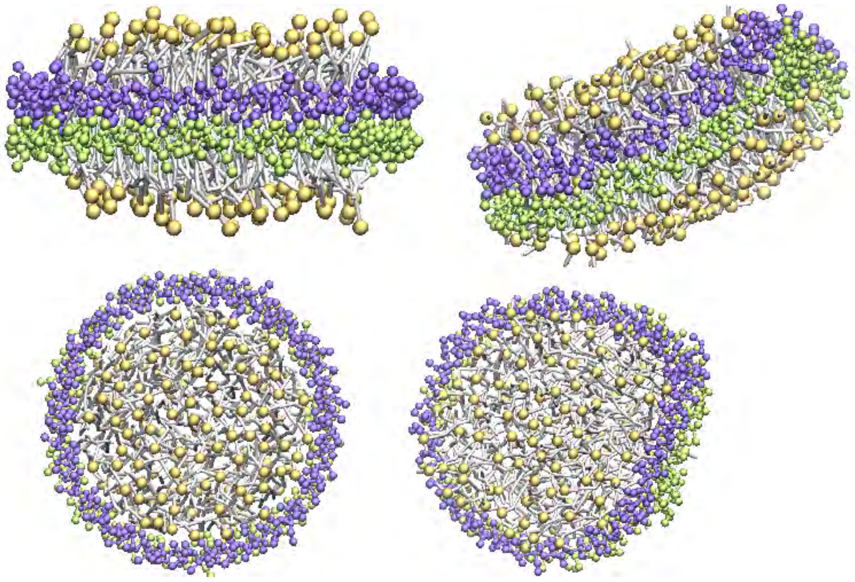


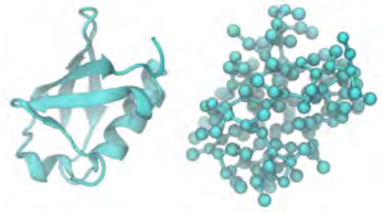
Figure 9: Simulation result of the lipoprotein system. Left: beginning of the simulation. Right: system after 100 ns.

Use VMD to take a look at the simulation result. Throughout the trajectory, the initial discoidal shape of the MSP1 (1-11) nanodisc is maintained well. After 100 ns of simulation time, the overall "double-belt" like configuration of the two apo A-I strands remained intact and stable such that the protein helices remained perpendicular to the lipid tail groups. A full trajectory of 100 ns is also provided for you `example-output/output/system-full5000.dcd`. The protein strands in this configuration had neither a gap nor an overlap with the protein ends just touching each other. This observation correlates well with previous all-atom MD simulations and experimental results suggesting that the first 11 N-terminal residues are not needed for the formation of nanodiscs of this size and composition. A simple RBCG simulation used here demonstrates very well stability of the double-belt confguration suggesting this an equilibrium structure of the nanodisc model of HDL. For more information on assembly of the lipoprotein system, please see Shih et al., *J. Str. Biol.*, **157**:579, 2007.

To familarize yourself with the process, you may want to work through several other examples. Below, we provide three examples which follows the same procedure as explained for the lipoprotein case. However, you can simply use the ready-to-use tcl scripts instead of the GUIs.

3 Other examples

3.1 Ubiquitin



Directory: `06-OtherExamples/Ubiquitin`

In this section, you will coarse-grain and set up simulation files for ubiquitin in a water box. Provided for you is `1UBQ.pdb`, downloaded from `www.rcsb.org`, and several build scripts.

Building the system (step 0)
The first step is to build an all-atom PDB/PSF pair for `1UBQ.pdb`. Do this by typing

```
vmd -dispdev text -e 00-make-AA-psf.tcl > 00-make-AA-psf.log
```

on the command line. This will create `AA-ubiquitin.pdb` and `AA-ubiquitin.psf`

Coarse-grain your system (step 1)
The next step is to coarse-grain the protein. This is done by `01-coarse-grain.tcl`.

To run this script, type

```
vmd -dispdev text -e 01-coarse-grain.tcl > 01-coarse-grain.log.
```

Open the script file with your text editor of choice and inspect its contents. This and all other scripts provided are commented so that you can understand what each part of the script does.

Create a PSF file (step 2)
Now you will create a preliminary psf file for your coarse-grained system. Do this with

```
vmd -dispdev text -e 02a-make-initial-CG-psf.tcl > 02a-make-initial-CG-psf.log
```

You should now have the files `cg-ubiquitin.pdb`, `cg-ubiquitin.rcg`, `cg-ubiquitin-init.pdb`, and `cg-ubiquitin-init.psf`.
The next step is to correct the coarse-grained psf file so that the bead types reflect the secondary structure of the protein. To do this, run

```
vmd -dispdev text -e 02b-correct-CG-psf.tcl > 02b-correct-CG-psf.log
```

Solvate (step 3)
Now that we have constructed the PDB/PSF pair for the protein, we can construct a water box around it. To do this, we will use the solvate procedure, using the MARTINI cg-waterbox. Open `03-solvate.tcl` with a text editor for an example of how to do this. Run solvate with

```
vmd -dispdev text -e 03a-solvate.tcl > 03a-solvate.log.
```

Next we will run a script to remove any waters too close to the protein that solvate might have missed, just as we would do for an all-atom system.

```
vmd -dispdev text -e 03b-remove-waters.tcl > 03b-remove-waters.log.
```

Ionize (step 4)
Last, we will neutralize the system using a modified version of the autoionize procedure (provided in `cgionize.tcl`). Copy the `martini-ions.top` file to the current directory. Run

```
vmd -dispdev text -e 04-ionize.tcl > 04-ionize.log.
```

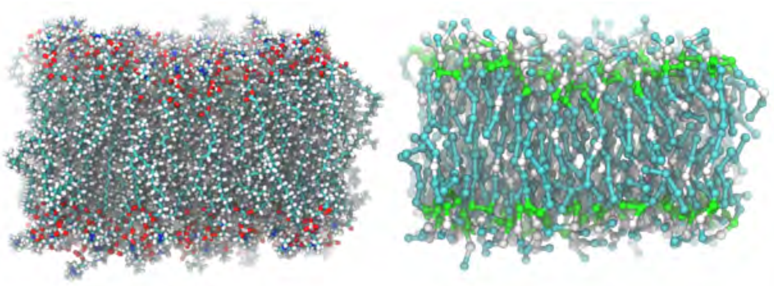
You should now have the files `ionized.pdb` and `ionized.psf` in your directory.

Set up the simulation files (step 5)
Some configuration files have been provided for you: `ubiquitin-min.conf`, `ubiquitin-01.conf`, and `ubiquitin-02.conf`. A short minimization is done in `ubiquitin-min.conf`. Equilibration is started in `ubiquitin-01.conf`, and

continued in `ubiquitin-02.conf`. It will often be necessary to run with a shorter timestep in the first stages of a simulation until the system stablilizes. This is why the timestep is set to 10 fs in `ubiquitin-01.conf` and then increased to 40 fs in `ubiquitin-02.conf`. As a general point of advice, if you find that your simulation crashes frequently with the “atoms moving too fast” error, temporarily decreasing the timestep may solve the problem.

Run the simulation (step 6)
This system is small enough to run on your desktop/laptop, so try running the simulations and open the trajectory in VMD.

3.2 Lipid Bilayer



Directory: `06-OtherExamples/POPC`

In this section, you will coarse-grain and set up simulation files for a system containing a membrane patch.

You are provided with a small all-atom POPC membrane patch, and several build scripts.

Coarse-grain your system (step 1)
The first step is to coarse-grain the lipid patch and build a coarse-grained PDB/PSF pair. Do this by running

```
vmd -dispdev text -e 01-coarse-grain.tcl > 01-coarse-grain.log,
```

and open the script in a text file to see how this is being done.

Create a PSF file (step 2)
Create a coarse-grained PDB/PDF pair for your system using

```
vmd -dispdev text -e 02-make-CG-psf.tcl > 02-make-CG-psf.log,
```

Unlike in the ubiquitin example, the psf file requires no further correction because this system contains no protein segments.

Solvate (step 3)
Now we will add a water box, again using solvate with the provided MARTINI files. Do this by running

```
vmd -dispdev text -e 03a-solvate.tcl > 03a-solvate.log.
```

Next we will run a script to remove any waters that may have ended up in the hydrophobic region on the outside of the membrane patch, again just as we would do for an all-atom system.

```
vmd -dispdev text -e 03b-remove-waters.tcl > 03b-remove-waters.log.
```

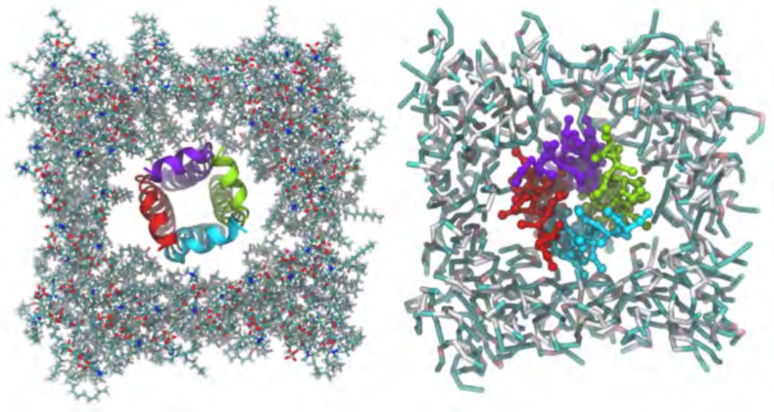
Ionize (step 4)
Finally, we will neutralize the system, again using the modified version of autoionize. Make sure, you copy the `martini-ions.top` to the current directory. And, run

```
vmd -dispdev text -e 04-ionize.tcl > 04-ionize.log.
```

Set up the simulation files (step 5)
Again, several configuration files (`popcpatch-min.conf`, `popcpatch-01.conf`, `popcpatch-02.conf`) have been provided for you. Note that since this is a membrane simulation `useFlexibleCell` should be turned on.

Run the simulation (step 6)
Again, the system should be small enough to run on one processor!

3.3 Membrane Protein



Directory: `06-OtherExamples/M2-channel`

In this section, you will coarse-grain and set up simulation files for a membrane protein system, containing both protein and lipid segments. The protein used in this section is the M2 proton channel (`2RLF.pdb`), which is composed of several non-covalently-bound homo-oliomers. Therefore, this example will demonstrate not only how to combine lipids and protein, but how to handle modular proteins in MARTINI. Please note that M2 was chosen as a proof-of-concept example merely because it is small and well known; whether it can be adequately modeled in RBCG representation is another matter. In all-atom simulations, the oligomers are held together in part by hydrogen-bonding interactions, which are lost in the coarse-graining process. To compensate, you must use the `extraBonds` feature of NAMD to add extra constraints to keep the oligomers together.

Create all-atom PDB and PSF files (step 0)
As in the ubiquitin case, we must first construct an all-atom PDB/PSF file pair for the protein using a PDB file downloaded from `www.rcsb.org` databse. The first script (`00-make-AA-psf.tcl`) replicates the protein from 2RLF, assembles the oligomer, and aligns it along the z-axis. To do this, simply run

```
vmd -dispdev text -e 00a-make-AA-psf.tcl > 00a-make-AA-psf.log.
```

Open the script file if you are interested in seeing how this is done.

Now, since this is a membrane simulation, the next thing to do is to combine it with the membrane. First run

```
vmd -dispdev text -e 00b-combine-with-lipids.tcl > 00b-combine-with-lipids.log.
```

Coarse-grain your system (step 1)
Now we coarse-grain the system:

```
vmd -dispdev text -e 01-coarse-grain.tcl > 01-coarse-grain.log,
```

Create a PSF file (step 2)
Create a preliminary PSF file with:

```
vmd -dispdev text -e 02a-make-initial-CG-psf.tcl > 02a-make-initial-CG-psf.log
```

Since this system contains both lipid and protein segments, we will need to correct the psf file to account for secondary structure:

```
vmd -dispdev text -e 02b-correct-CG-psf.tcl > 02b-correct-CG-psf.log
```

Solvate and Ionize (steps 3 and 4)
Now we solvate, remove unwanted waters, and ionize the system, just as before

(Note: Copy the martini-ions.top file to the current directory):

```
vmd -dispdev text -e 03a-solvate.tcl > 03a-solvate.log
vmd -dispdev text -e 03b-remove-waters.tcl > 03b-remove-waters.log
vmd -dispdev text -e 04-ionize.tcl > 04-ionize.log.
```

Set up the simulation files (step 5)
Usually for a membrane protein (protein embedded in membrane), one begins the simulation with the protein held in place, to let the lipids relax around the protein. For more information, refer to the Membrane Protein tutorial. We do this with NAMD's constraints option, and create the necessary constraint file here:

```
vmd -dispdev text -e 05a-make-constraints.tcl > 05a-make-constraints.log.
```

Last, since our protein contains several non-covalently-bonded segments, we must use NAMD's extrabonds feature to keep the protein from falling apart. This script shows an example of how to construct such a file:

```
vmd -dispdev text -e 05b-make-extrabonds.tcl > 05b-make-extrabonds.log.
```

If you are aware of particular interactions between monomers, it makes sense to represent those as extraBonds. Beyond that, the choice of extraBonds is a matter of trial-and-error. You must use your own judgement as to whether or not the extraBonded structure adequately represents your oligomeric protein.

Run the simulation (step 6)
This system is slightly larger than the first two, but should still be manageable on your desktop/laptop. So try running the simulation and open the trajectory in VMD.

This ends the RBCG tutorial. You are now ready to use RBCG!

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Appendix

This appendix is essentially a visual representation of the information encoded in the coarse-grain .cgc files, showing how each of the amino acids as well as a POPC lipid is divided into coarse-grained beads. Excerpts from the standard CHARMM topology file are shown with the Martini bead assignments overlaid.

```
RESI ALA          0.00
GROUP
ATOM N    NH1    -0.47  !     |
ATOM HN   H       0.31  !  HN-N
ATOM CA   CT1     0.07  !     |     HB1
ATOM HA   HB      0.09  !     |    /
GROUP                   !  HA-CA--CB-HB2
ATOM CB   CT3    -0.27  !     |    \
ATOM HB1  HA      0.09  !     |     HB3
ATOM HB2  HA      0.09  !   O=C
ATOM HB3  HA      0.09  !     |
GROUP                   !
ATOM C    C       0.51      BAS = P4
ATOM O    O      -0.51
BOND CB CA  N  HN  N  CA
BOND C  CA  C  +N  CA HA  CB HB1  CB HB2  CB HB3
DOUBLE O  C
```

```
RESI ARG          1.00
GROUP
ATOM N    NH1    -0.47  !     |                    HH11
ATOM HN   H       0.31  !  HN-N        SI1 = N0r    |
ATOM CA   CT1     0.07  !     |   HB1 HG1 HD1 HE    NH1-HH12
ATOM HA   HB      0.09  !     |   |   |   |   |   //(+)
GROUP                   !  HA-CA--CB--CG--CD--NE--CZ
ATOM CB   CT2    -0.18  !     |   |   |   |         \
ATOM HB1  HA      0.09  !     |   HB2 HG2 HD2        NH2-HH22
ATOM HB2  HA      0.09  !   O=C                       |
GROUP                   !     |                      HH21
ATOM CG   CT2    -0.18    BAS = P5              SI2 = Qdr
ATOM HG1  HA      0.09
ATOM HG2  HA      0.09
GROUP
ATOM CD   CT2     0.20
ATOM HD1  HA      0.09
ATOM HD2  HA      0.09
ATOM NE   NC2    -0.70
ATOM HE   HC      0.44
ATOM CZ   C       0.64
ATOM NH1  NC2    -0.80
ATOM HH11 HC      0.46
ATOM HH12 HC      0.46
ATOM NH2  NC2    -0.80
ATOM HH21 HC      0.46
ATOM HH22 HC      0.46
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB   CA   CG   CB   CD CG   NE CD   CZ NE
BOND NH2 CZ   N   HN   N   CA
BOND C    CA   C   +N   CA HA   CB HB1
BOND CB   HB2 CG   HG1 CG HG2 CD HD1 CD HD2
BOND NE   HE   NH1 HH11   NH1 HH12   NH2 HH21   NH2 HH22
DOUBLE O  C     CZ   NH1
```

```
RESI ASN          0.00
GROUP
ATOM N    NH1    -0.47  !     |
ATOM HN   H       0.31  !  HN-N
ATOM CA   CT1     0.07  !     |   HB1 OD1    HD21 (cis to OD1)
ATOM HA   HB      0.09  !     |   |   ||    /
GROUP                   !  HA-CA--CB--CG--ND2
ATOM CB   CT2    -0.18  !     |   |         \
ATOM HB1  HA      0.09  !     |   HB2        HD22 (trans to OD1)
ATOM HB2  HA      0.09  !   O=C
GROUP                   !     |            SID = P5n
ATOM CG   CC      0.55    BAS = P5
ATOM OD1  O      -0.55
GROUP
ATOM ND2  NH2    -0.62
ATOM HD21 H       0.32
ATOM HD22 H       0.30
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB CA  CG CB   ND2 CG
BOND N  HN  N  CA   C   CA    C +N
BOND CA HA  CB HB1  CB  HB2  ND2 HD21  ND2 HD22
DOUBLE C  O   CG  OD1
```

```
RESI ASP         -1.00
GROUP
ATOM N    NH1    -0.47  !     |
ATOM HN   H       0.31  !  HN-N     SID = Qad
ATOM CA   CT1     0.07  !     |   HB1   OD1
ATOM HA   HB      0.09  !     |   |    //
GROUP                   !  HA-CA--CB--CG
ATOM CB   CT2    -0.28  !     |   |    \
ATOM HB1  HA      0.09  !     |   HB2   OD2(-)
ATOM HB2  HA      0.09  !   O=C
ATOM CG   CC      0.62  !     |
ATOM OD1  OC     -0.76
ATOM OD2  OC     -0.76    BAS = P5
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB CA  CG CB  OD2 CG
BOND N  HN  N  CA   C   CA  C +N
BOND CA HA  CB HB1  CB HB2
DOUBLE  O   C   CG  OD1
```

```
RESI CYS          0.00
GROUP
ATOM N    NH1    -0.47  !     |
ATOM HN   H       0.31  !  HN-N   SID = C5c
ATOM CA   CT1     0.07  !     |   HB1
ATOM HA   HB      0.09  !     |   |
GROUP                   !  HA-CA--CB--SG
ATOM CB   CT2    -0.11  !     |   |     \
ATOM HB1  HA      0.09  !     |   HB2    HG1
ATOM HB2  HA      0.09  !   O=C
ATOM SG   S      -0.23  !     |
ATOM HG1  HS      0.16    BAS = P5
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB CA   SG CB   N HN  N  CA
BOND C  CA   C +N  CA HA  CB HB1
BOND CB HB2  SG HG1
DOUBLE O  C
```

```
RESI GLN          0.00
GROUP                     BAS = P5
ATOM N    NH1    -0.47  !     |
ATOM HN   H       0.31  !  HN-N
ATOM CA   CT1     0.07  !     |   HB1 HG1 OE1   HE21 (cis to OE1)
ATOM HA   HB      0.09  !     |   |   |   ||    /
GROUP                   !  HA-CA--CB--CG--CD--NE2
ATOM CB   CT2    -0.18  !     |   |   |          \
ATOM HB1  HA      0.09  !     |   HB2 HG2        HE22 (trans to OE1)
ATOM HB2  HA      0.09  !   O=C             SID = P4q
GROUP                   !     |
ATOM CG   CT2    -0.18
ATOM HG1  HA      0.09
ATOM HG2  HA      0.09
GROUP
ATOM CD   CC      0.55
ATOM OE1  O      -0.55
GROUP
ATOM NE2  NH2    -0.62
ATOM HE21 H       0.32
ATOM HE22 H       0.30
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB CA  CG  CB   CD  CG   NE2 CD
BOND N  HN  N   CA   C   CA
BOND C  +N  CA  HA   CB  HB1  CB  HB2  CG HG1
BOND CG HG2 NE2 HE21 NE2 HE22
DOUBLE O  C    CD  OE1
```

```
RESI GLU          -1.00
                          BAS = P5
GROUP
ATOM N    NH1    -0.47  !     |
ATOM HN   H       0.31  !  HN-N
ATOM CA   CT1     0.07  !     |   HB1 HG1   OE1
ATOM HA   HB      0.09  !     |   |   |    //
GROUP                   !  HA-CA--CB--CG--CD
ATOM CB   CT2    -0.18  !     |   |   |    \
ATOM HB1  HA      0.09  !     |   HB2 HG2   OE2(-)
ATOM HB2  HA      0.09  !   O=C
GROUP                   !     |       SID = Qae
ATOM CG   CT2    -0.28
ATOM HG1  HA      0.09
ATOM HG2  HA      0.09
ATOM CD   CC      0.62
ATOM OE1  OC     -0.76
ATOM OE2  OC     -0.76
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB CA  CG CB  CD CG  OE2 CD
BOND N  HN  N  CA C   CA
BOND C  +N  CA HA  CB HB1 CB  HB2 CG  HG1
BOND CG HG2
DOUBLE O  C   CD  OE1
```

```
RESI GLY          0.00
GROUP
ATOM N    NH1    -0.47  !     |
ATOM HN   H       0.31  !     N-H
ATOM CA   CT2    -0.02  !     |
ATOM HA1  HB      0.09  !     |
ATOM HA2  HB      0.09  ! HA1-CA-HA2
GROUP                   !     |
ATOM C    C       0.51  !     |
ATOM O    O      -0.51  !     C=O
                        !     |
BOND N HN  N  CA  C CA     BAS = P5
BOND C +N  CA HA1 CA HA2
DOUBLE O  C
```

```
RESI HSD          0.00  ! neutral HIS, proton on ND1
GROUP                                      SI3 = SP1h
ATOM N    NH1    -0.47  !     |            HD1    HE1
ATOM HN   H       0.31  !  HN-N             |     /
ATOM CA   CT1     0.07  !     |   HB1      ND1--CE1
ATOM HA   HB      0.09  !     |   |      /      ||
                                     SI1 = SC4h
GROUP                   !  HA-CA--CB--CG        ||
ATOM CB   CT2    -0.09  !     |   |      \\     ||
ATOM HB1  HA      0.09  !     |   HB2      CD2--NE2
ATOM HB2  HA      0.09  !   O=C             |
ATOM ND1  NR1    -0.36  !     |            HD2
ATOM HD1  H       0.32
                          BAS = P5       SI2 = SP1h
ATOM CG   CPH1   -0.05
GROUP
ATOM CE1  CPH2    0.25
ATOM HE1  HR1     0.13
ATOM NE2  NR2    -0.70
ATOM CD2  CPH1    0.22
ATOM HD2  HR3     0.10
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB   CA    CG   CB    ND1 CG    CE1 ND1
BOND NE2  CD2   N    HN    N   CA
BOND C    CA    C    +N    CA  HA    CB  HB1
BOND CB   HB2   ND1  HD1   CD2 HD2   CE1 HE1
DOUBLE O  C     CG   CD2    CE1  NE2
```

```
RESI HSE          0.00  ! neutral His, proton on NE2
GROUP                                      SI3 = SP1h
ATOM N    NH1    -0.47  !     |                 HE1
ATOM HN   H       0.31  !  HN-N               __  /
ATOM CA   CT1     0.07  !     |   HB1      ND1--CE1
ATOM HA   HB      0.09  !     |   |      /      |
                                     SI1 = SC4h
GROUP                   !  HA-CA--CB--CG        |
ATOM CB   CT2    -0.08  !     |   |      \\     |
ATOM HB1  HA      0.09  !     |   HB2      CD2--NE2
ATOM HB2  HA      0.09  !   O=C             |     \
ATOM ND1  NR2    -0.70  !     |            HD2    HE2
ATOM CG   CPH1    0.22
                          BAS = P5       SI2 = SP1h
ATOM CE1  CPH2    0.25
ATOM HE1  HR1     0.13
GROUP
ATOM NE2  NR1    -0.36
ATOM HE2  H       0.32
ATOM CD2  CPH1   -0.05
ATOM HD2  HR3     0.09
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB   CA    CG   CB    ND1 CG
BOND NE2  CD2   N    HN    N   CA
BOND C    CA    C    +N    NE2 CE1   CA   HA    CB HB1
BOND CB   HB2   NE2  HE2   CD2 HD2   CE1 HE1
DOUBLE    O     C    CD2 CG    CE1 ND1
```

```
RESI HSP          1.00  ! Protonated His  SI3 = SP1h
GROUP
ATOM N    NH1    -0.47  !     |            HD1    HE1
ATOM HN   H       0.31  !  HN-N             |     /
ATOM CA   CT1     0.07  !     |   HB1      ND1--CE1
ATOM HA   HB      0.09  !     |   |      /      ||
                                     SI1 = SC4h
GROUP                   !  HA-CA--CB--CG        ||
ATOM CB   CT2    -0.05  !     |   |      \\     ||
ATOM HB1  HA      0.09  !     |   HB2      CD2--NE2(+)
ATOM HB2  HA      0.09  !   O=C             |     \
ATOM CD2  CPH1    0.19  !     |            HD2    HE2
ATOM HD2  HR1     0.13
                          BAS = P5       SI2 = SQdh
ATOM CG   CPH1    0.19
GROUP
ATOM NE2  NR3    -0.51
ATOM HE2  H       0.44
ATOM ND1  NR3    -0.51
ATOM HD1  H       0.44
ATOM CE1  CPH2    0.32
ATOM HE1  HR2     0.18
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB   CA    CG   CB    ND1 CG    CE1 ND1
BOND NE2  CD2   N    HN    N   CA
BOND C    CA    C    +N   CA  HA   CB HB1
BOND CB   HB2   ND1 HD1   NE2 HE2   CD2 HD2 CE1 HE1
DOUBLE  O   C   CD2 CG      NE2 CE1
```

```
RESI ILE          0.00
                          BAS = P5
GROUP
ATOM N    NH1    -0.47  !     |   HG21 HG22
ATOM HN   H       0.31  !  HN-N      | /
ATOM CA   CT1     0.07  !     |      CG2--HG23
ATOM HA   HB      0.09  !     |     /
GROUP                   !  HA-CA--CB-HB    HD1
ATOM CB   CT1    -0.09  !     |     \       /
ATOM HB   HA      0.09  !     |      CG1--CD--HD2
GROUP                   !   O=C     / \     \
ATOM CG2  CT3    -0.27  !     | HG11 HG12  HD3
                                 SID = AC1i
ATOM HG21 HA      0.09
ATOM HG22 HA      0.09
ATOM HG23 HA      0.09
GROUP
ATOM CG1  CT2    -0.18
ATOM HG11 HA      0.09
ATOM HG12 HA      0.09
GROUP
ATOM CD   CT3    -0.27
ATOM HD1  HA      0.09
ATOM HD2  HA      0.09
ATOM HD3  HA      0.09
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB   CA    CG1 CB    CG2 CB    CD   CG1
BOND N    HN    N   CA     C   CA    C    +N
BOND CA   HA    CB  HB    CG1 HG11 CG1 HG12 CG2 HG21
BOND CG2 HG22 CG2 HG23 CD   HD1   CD   HD2   CD   HD3
DOUBLE  O   C
```

```
RESI LEU          0.00
GROUP
ATOM N    NH1    -0.47  !     |        HD11 HD12
ATOM HN   H       0.31  ! HN-N             | /
ATOM CA   CT1     0.07  !     |   HB1   CD1--HD13
ATOM HA   HB      0.09  !     |   |     /
GROUP                   ! HA-CA--CB--CG-HG
ATOM CB   CT2    -0.18  !     |   |     \
ATOM HB1  HA      0.09  !     |   HB2   CD2--HD23
ATOM HB2  HA      0.09  !   O=C            | \
GROUP                   !     |        HD21 HD22
ATOM CG   CT1    -0.09
ATOM HG   HA      0.09
GROUP
ATOM CD1  CT3    -0.27
ATOM HD11 HA      0.09
ATOM HD12 HA      0.09
ATOM HD13 HA      0.09
GROUP
ATOM CD2  CT3    -0.27
ATOM HD21 HA      0.09
ATOM HD22 HA      0.09
ATOM HD23 HA      0.09
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB  CA   CG  CB   CD1 CG   CD2 CG
BOND N   HN   N   CA    C   CA   C +N
BOND CA  HA   CB  HB1  CB  HB2  CG  HG   CD1 HD11
BOND CD1 HD12 CD1 HD13 CD2 HD21 CD2 HD22 CD2 HD23
DOUBLE   O   C
```

BAS = P5   SID = AC1l

```
RESI LYS          1.00
GROUP
ATOM N    NH1    -0.47  !     |
ATOM HN   H       0.31  ! HN-N
ATOM CA   CT1     0.07  !     |   HB1 HG1 HD1 HE1    HZ1
ATOM HA   HB      0.09  !     |   |   |   |   |     /
GROUP                   ! HA-CA--CB--CG--CD--CE--NZ--HZ2
ATOM CB   CT2    -0.18  !     |   |   |   |   |     \
ATOM HB1  HA      0.09  !     |   HB2 HG2 HD2 HE2    HZ3
ATOM HB2  HA      0.09  !   O=C
GROUP                   !     |
ATOM CG   CT2    -0.18
ATOM HG1  HA      0.09
ATOM HG2  HA      0.09
GROUP
ATOM CD   CT2    -0.18
ATOM HD1  HA      0.09
ATOM HD2  HA      0.09
GROUP
ATOM CE   CT2     0.21
ATOM HE1  HA      0.05
ATOM HE2  HA      0.05
ATOM NZ   NH3    -0.30
ATOM HZ1  HC      0.33
ATOM HZ2  HC      0.33
ATOM HZ3  HC      0.33
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB CA   CG CB   CD CG   CE CD   NZ CE
BOND N  HN   N  CA    C  CA
BOND C  +N   CA HA   CB HB1  CB HB2  CG HG1
BOND CG HG2  CD HD1  CD HD2  CE HE1  CE HE2
DOUBLE   O  C
BOND NZ HZ1  NZ HZ2  NZ HZ3
```

SI1 = C3k   SI2 = Qdk   BAS = P5

```
RESI MET          0.00
GROUP
ATOM N    NH1    -0.47  !     |
ATOM HN   H       0.31  ! HN-N
ATOM CA   CT1     0.07  !     |   HB1 HG1     HE1
ATOM HA   HB      0.09  !     |   |   |       |
GROUP                   ! HA-CA--CB--CG--SD--CE--HE3
ATOM CB   CT2    -0.18  !     |   |   |       |
ATOM HB1  HA      0.09  !     |   HB2 HG2     HE2
ATOM HB2  HA      0.09  !   O=C
GROUP                   !     |
ATOM CG   CT2    -0.14
ATOM HG1  HA      0.09
ATOM HG2  HA      0.09
ATOM SD   S      -0.09
ATOM CE   CT3    -0.22
ATOM HE1  HA      0.09
ATOM HE2  HA      0.09
ATOM HE3  HA      0.09
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB CA   CG CB   SD CG   CE SD
BOND N  HN   N  CA    C  CA   C  +N
BOND CA HA   CB HB1  CB HB2  CG HG1  CG HG2
BOND CE HE1  CE HE2  CE HE3
DOUBLE   O  C
```

SID = C5m   BAS = P5

SID = AC2p

```
RESI PRO          0.00
GROUP                   !        HD1 HD2
ATOM N    N      -0.29  !     |   \ /
ATOM CD   CP3     0.00  !     N---CD   HG1
ATOM HD1  HA      0.09  !     |     \  /
ATOM HD2  HA      0.09  !     |      CG
ATOM CA   CP1     0.02  !     |     /  \
ATOM HA   HB      0.09  ! HA-CA--CB   HG2
GROUP                   !     |   / \
ATOM CB   CP2    -0.18  !     | HB1 HB2
ATOM HB1  HA      0.09  !   O=C
ATOM HB2  HA      0.09  !     |
GROUP
ATOM CG   CP2    -0.18
ATOM HG1  HA      0.09
ATOM HG2  HA      0.09
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND C  CA  C   +N
BOND N  CA  CA  CB  CB  CG  CG  CD  N   CD
BOND HA CA  HG1 CG  HG2 CG  HD1 CD  HD2 CD  HB1 CB  HB2 CB
DOUBLE   O  C
```

BAS = P5

```
RESI PHE          0.00
GROUP
ATOM N    NH1    -0.47  !     |        HD1  HE1
ATOM HN   H       0.31  ! HN-N          |    |
ATOM CA   CT1     0.07  !     |   HB1  CD1--CE1
ATOM HA   HB      0.09  !     |   |    //      \\
GROUP                   ! HA-CA--CB--CG         CZ--HZ
ATOM CB   CT2    -0.18  !     |   |    \  __  /
ATOM HB1  HA      0.09  !     |   HB2  CD2--CE2
ATOM HB2  HA      0.09  !   O=C         |    |
GROUP                   !     |        HD2  HE2
ATOM CG   CA      0.00
GROUP
ATOM CD1  CA     -0.115
ATOM HD1  HP      0.115
GROUP
ATOM CE1  CA     -0.115
ATOM HE1  HP      0.115
GROUP
ATOM CZ   CA     -0.115
ATOM HZ   HP      0.115
GROUP
ATOM CD2  CA     -0.115
ATOM HD2  HP      0.115
GROUP
ATOM CE2  CA     -0.115
ATOM HE2  HP      0.115
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB  CA   CG CB   CD2 CG   CE1 CD1
BOND CZ  CE2  N   HN
BOND N   CA    C   CA   C   +N   CA  HA
BOND CB  HB1  CB HB2  CD1 HD1  CD2 HD2  CE1 HE1
DOUBLE   O  C    CD1 CG  CZ CE1   CE2 CD2
BOND CE2 HE2  CZ HZ
```

SI1 = SC4f   SI3 = SC4f   BAS = P5   SI2 = SC4f

```
RESI SER          0.00
GROUP
ATOM N    NH1    -0.47  !     |
ATOM HN   H       0.31  !  HN-N
ATOM CA   CT1     0.07  !     |   HB1
ATOM HA   HB      0.09  !     |   |
GROUP                   !  HA-CA--CB--OG
ATOM CB   CT2     0.05  !     |   |     \
ATOM HB1  HA      0.09  !     |   HB2    HG1
ATOM HB2  HA      0.09  !   O=C
ATOM OG   OH1    -0.66  !     |
ATOM HG1  H       0.43
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB CA   OG CB  N HN  N  CA
BOND C  CA  C +N  CA HA  CB HB1
BOND CB HB2  OG HG1
DOUBLE   O  C
```

BAS = P5

SID = P1s

```
RESI THR          0.00
GROUP
ATOM N    NH1    -0.47  !     |
ATOM HN   H       0.31  !  HN-N
ATOM CA   CT1     0.07  !     |     OG1--HG1
ATOM HA   HB      0.09  !     |    /
GROUP                   !  HA-CA--CB-HB
ATOM CB   CT1     0.14  !     |    \
ATOM HB   HA      0.09  !     |     CG2--HG21
ATOM OG1  OH1    -0.66  !   O=C    / \
ATOM HG1  H       0.43  !     | HG21 HG22
GROUP
ATOM CG2  CT3    -0.27
ATOM HG21 HA      0.09
ATOM HG22 HA      0.09
ATOM HG23 HA      0.09
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB CA  OG1 CB   CG2 CB    N   HN
BOND N  CA    C   CA    C   +N    CA  HA
BOND CB HB  OG1 HG1  CG2 HG21  CG2 HG22  CG2 HG23
DOUBLE  O   C
```

BAS = P5

SID = P1t

```
RESI TRP          0.00
GROUP
ATOM N    NH1    -0.47  !     |                  HE3
ATOM HN   H       0.31  !  HN-N                   |
ATOM CA   CT1     0.07  !     |   HB1            CE3
ATOM HA   HB      0.09  !     |   |             /  \\
GROUP                   !  HA-CA--CB---CG-----CD2   CZ3-HZ3
ATOM CB   CT2    -0.18  !     |   |     ||     ||      |
ATOM HB1  HA      0.09  !     |   HB2  CD1     CE2    CH2-HH2
ATOM HB2  HA      0.09  !   O=C        /  \   / \  //
GROUP                   !     |      HD1   NE1     CZ2
ATOM CG   CY     -0.03  !                   |       |
ATOM CD1  CA      0.035 !                  HE1     HZ2
ATOM HD1  HP      0.115
ATOM NE1  NY     -0.61
ATOM HE1  H       0.38
ATOM CE2  CPT     0.13
ATOM CD2  CPT    -0.02
GROUP
ATOM CE3  CA     -0.115
ATOM HE3  HP      0.115
GROUP
ATOM CZ3  CA     -0.115
ATOM HZ3  HP      0.115
GROUP
ATOM CZ2  CA     -0.115
ATOM HZ2  HP      0.115
GROUP
ATOM CH2  CA     -0.115
ATOM HH2  HP      0.115
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB  CA   CG  CB   CD2 CG   NE1 CD1
BOND CZ2 CE2
BOND N   HN   N   CA    C   CA   C   +N
BOND CZ3 CH2  CD2 CE3  NE1 CE2  CA  HA   CB  HB1
BOND CB  HB2  CD1 HD1  NE1 HE1  CE3 HE3  CZ2 HZ2
BOND CZ3 HZ3  CH2 HH2
DOUBLE  O   C   CD1 CG   CE2 CD2  CZ3 CE3  CH2 CZ2
```

SI1 = SC4w

SI3 = SC4w

BAS = P5

SI2 = SP1w

SI4 = SC4w

```
RESI TYR          0.00
GROUP
ATOM N    NH1    -0.47  !     |        HD1  HE1
ATOM HN   H       0.31  !  HN-N         |    |
ATOM CA   CT1     0.07  !     |   HB1  CD1--CE1
ATOM HA   HB      0.09  !     |   |   //      \\
GROUP                   !  HA-CA--CB--CG        CZ--OH
ATOM CB   CT2    -0.18  !     |   |     \  __  /      \
ATOM HB1  HA      0.09  !     |   HB2   CD2--CE2      HH
ATOM HB2  HA      0.09  !   O=C          |    |
GROUP                   !     |         HD2  HE2
ATOM CG   CA      0.00
GROUP
ATOM CD1  CA     -0.115
ATOM HD1  HP      0.115
GROUP
ATOM CE1  CA     -0.115
ATOM HE1  HP      0.115
GROUP
ATOM CZ   CA      0.11
ATOM OH   OH1    -0.54
ATOM HH   H       0.43
GROUP
ATOM CD2  CA     -0.115
ATOM HD2  HP      0.115
GROUP
ATOM CE2  CA     -0.115
ATOM HE2  HP      0.115
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB  CA   CG  CB   CD2 CG   CE1 CD1
BOND CZ  CE2  OH  CZ
BOND N   HN   N   CA    C   CA   C   +N
BOND CA  HA   CB  HB1  CB  HB2  CD1 HD1  CD2 HD2
BOND CE1 HE1  CE2 HE2  OH  HH
DOUBLE   O   C   CD1 CG  CE1  CZ  CE2 CD2
```

SI1 = SC4y

SI3 = SP1y

BAS = P5

SI2 = SC4y

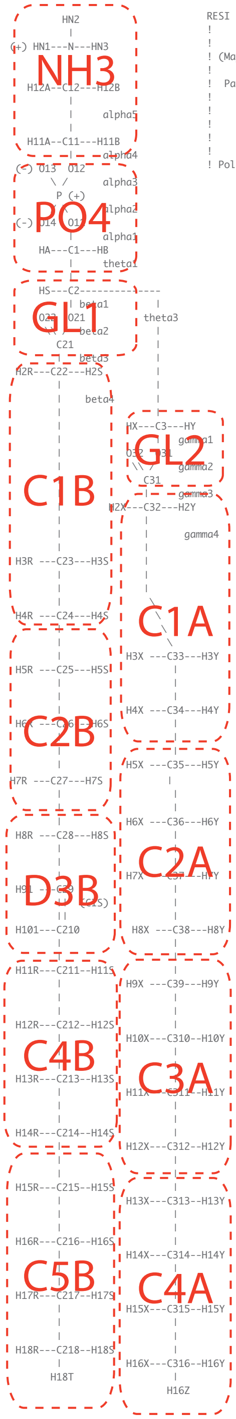
```
RESI VAL          0.00
GROUP
ATOM N    NH1    -0.47  !     |    HG11 HG12
ATOM HN   H       0.31  !  HN-N       | /
ATOM CA   CT1     0.07  !     |       CG1--HG13
ATOM HA   HB      0.09  !     |      /
GROUP                   !  HA-CA--CB-HB
ATOM CB   CT1    -0.09  !     |      \
ATOM HB   HA      0.09  !     |       CG2--HG21
GROUP                   !   O=C      / \
ATOM CG1  CT3    -0.27  !     |  HG21 HG22
ATOM HG11 HA      0.09
ATOM HG12 HA      0.09
ATOM HG13 HA      0.09
GROUP
ATOM CG2  CT3    -0.27
ATOM HG21 HA      0.09
ATOM HG22 HA      0.09
ATOM HG23 HA      0.09
GROUP
ATOM C    C       0.51
ATOM O    O      -0.51
BOND CB  CA    CG1 CB    CG2 CB    N   HN
BOND N   CA     C   CA     C   +N    CA HA
BOND CB  HB    CG1 HG11  CG1 HG12  CG1 HG13  CG2 HG21
BOND CG2 HG22  CG2 HG23
DOUBLE    O   C
```

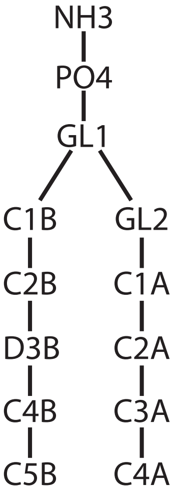
BAS = P5

SID = AC3v

```
RESI POPE         0.00 ! 1-palmytoil-2-oleoyl-sn-glycero-
!                        3-Phosphatidylethanolamine
!
! (May 14, 1999 Carlos F. Lopez)
!
!  Palmytoil - CH2
!              |
!      Oleyl - CH
!              |
!              CH2 - PO4 - CH2 - CH2 - NH3
!
! Polar Head and glycerol backbone
```

```
GROUP                   !
ATOM N     NH3L  -0.30 !
ATOM HN1   HCL    0.33 !
ATOM HN2   HCL    0.33 !
ATOM HN3   HCL    0.33 !
ATOM C12   CTL2   0.13 !
ATOM H12A  HAL2   0.09 !
ATOM H12B  HAL2   0.09 !
GROUP                   !
ATOM C11  CTL2   -0.08 !
ATOM H11A HAL2    0.09 !
ATOM H11B HAL2    0.09 !
ATOM P    PL      1.50 !
ATOM O13  O2L    -0.78 !
ATOM O14  O2L    -0.78 !
ATOM O11  OSL    -0.57 !
ATOM O12  OSL    -0.57 !
ATOM C1   CTL2   -0.08 !
ATOM HA   HAL2    0.09 !
ATOM HB   HAL2    0.09 !
GROUP                   !
ATOM C2   CTL1    0.04 !
ATOM HS   HAL1    0.09 !
ATOM O21  OSL    -0.34 !
ATOM C21  CL      0.63 !
ATOM O22  OBL    -0.52 !
ATOM C22  CTL2   -0.08 !
ATOM H2R  HAL2    0.09 !
ATOM H2S  HAL2    0.09 !
GROUP                   !
ATOM C3   CTL2   -0.05 !
ATOM HX   HAL2    0.09 !
ATOM HY   HAL2    0.09 !
ATOM O31  OSL    -0.34 !
ATOM C31  CL      0.63 !
ATOM O32  OBL    -0.52 !
ATOM C32  CTL2   -0.08 !
ATOM H2X  HAL2    0.09 !
ATOM H2Y  HAL2    0.09 !
GROUP                   !
ATOM C23  CTL2   -0.18 !
ATOM H3R  HAL2    0.09 !
ATOM H3S  HAL2    0.09 !
GROUP                   !
ATOM C24  CTL2   -0.18 !
ATOM H4R  HAL2    0.09 !
ATOM H4S  HAL2    0.09 !
GROUP                   !
ATOM C25  CTL2   -0.18 !
ATOM H5R  HAL2    0.09 !
ATOM H5S  HAL2    0.09 !
GROUP                   !
ATOM C26  CTL2   -0.18 !
ATOM H6R  HAL2    0.09 !
ATOM H6S  HAL2    0.09 !
GROUP                   !
ATOM C27  CTL2   -0.18 !
ATOM H7R  HAL2    0.09 !
ATOM H7S  HAL2    0.09 !
GROUP                   !
ATOM C28  CTL2   -0.18 !
ATOM H8R  HAL2    0.09 !
ATOM H8S  HAL2    0.09 !
GROUP                   !
ATOM C29  CEL1   -0.15 !
ATOM H91  HEL1    0.15 !
GROUP                   !
ATOM C210 CEL1   -0.15 !
ATOM H101 HEL1    0.15 !
GROUP                   !
ATOM C211 CTL2   -0.18 !
ATOM H11R HAL2    0.09 !
ATOM H11S HAL2    0.09 !
GROUP                   !
ATOM C212 CTL2   -0.18 !
ATOM H12R HAL2    0.09 !
ATOM H12S HAL2    0.09 !
GROUP                   !
ATOM C213 CTL2   -0.18 !
ATOM H13R HAL2    0.09 !
ATOM H13S HAL2    0.09 !
GROUP                   !
ATOM C214 CTL2   -0.18 !
ATOM H14R HAL2    0.09 !
ATOM H14S HAL2    0.09 !
GROUP                   !
ATOM C215 CTL2   -0.18 !
ATOM H15R HAL2    0.09 !
ATOM H15S HAL2    0.09 !
GROUP                   !
ATOM C216 CTL2   -0.18 !
ATOM H16R HAL2    0.09 !
ATOM H16S HAL2    0.09 !
GROUP                   !
ATOM C217 CTL2   -0.18 !
ATOM H17R HAL2    0.09 !
ATOM H17S HAL2    0.09 !
GROUP                   !
ATOM C218 CTL3   -0.27 !
ATOM H18R HAL3    0.09 !
ATOM H18S HAL3    0.09 !
ATOM H18T HAL3    0.09 !
GROUP                   !
ATOM C33  CTL2   -0.18 !
ATOM H3X  HAL2    0.09 !
ATOM H3Y  HAL2    0.09 !
GROUP                   !
ATOM C34  CTL2   -0.18 !
ATOM H4X  HAL2    0.09 !
ATOM H4Y  HAL2    0.09 !
```





```
GROUP                   !
ATOM C35  CTL2   -0.18 !
ATOM H5X  HAL2    0.09 !
ATOM H5Y  HAL2    0.09 !
GROUP                   !
ATOM C36  CTL2   -0.18 !
ATOM H6X  HAL2    0.09 !
ATOM H6Y  HAL2    0.09 !
GROUP                   !
ATOM C37  CTL2   -0.18 !
ATOM H7X  HAL2    0.09 !
ATOM H7Y  HAL2    0.09 !
GROUP                   !
ATOM C38  CTL2   -0.18 !
ATOM H8X  HAL2    0.09 !
ATOM H8Y  HAL2    0.09 !
GROUP                   !
ATOM C39  CTL2   -0.18 !
ATOM H9X  HAL2    0.09 !
ATOM H9Y  HAL2    0.09 !
GROUP                   !
ATOM C310 CTL2   -0.18 !
ATOM H10X HAL2    0.09 !
ATOM H10Y HAL2    0.09 !
GROUP                   !
ATOM C311 CTL2   -0.18 !
ATOM H11X HAL2    0.09 !
ATOM H11Y HAL2    0.09 !
GROUP                   !
ATOM C312 CTL2   -0.18 !
ATOM H12X HAL2    0.09 !
ATOM H12Y HAL2    0.09 !
GROUP                   !
ATOM C313 CTL2   -0.18 !
ATOM H13X HAL2    0.09 !
ATOM H13Y HAL2    0.09 !
GROUP                   !
ATOM C314 CTL2   -0.18 !
ATOM H14X HAL2    0.09 !
ATOM H14Y HAL2    0.09 !
GROUP                   !
ATOM C315 CTL2   -0.18 !
ATOM H15X HAL2    0.09 !
ATOM H15Y HAL2    0.09 !
GROUP                   !
ATOM C316 CTL3   -0.27 !
ATOM H16X HAL3    0.09 !
ATOM H16Y HAL3    0.09 !
ATOM H16Z HAL3    0.09 !
```