Assembling Molecular Systems for NAMD

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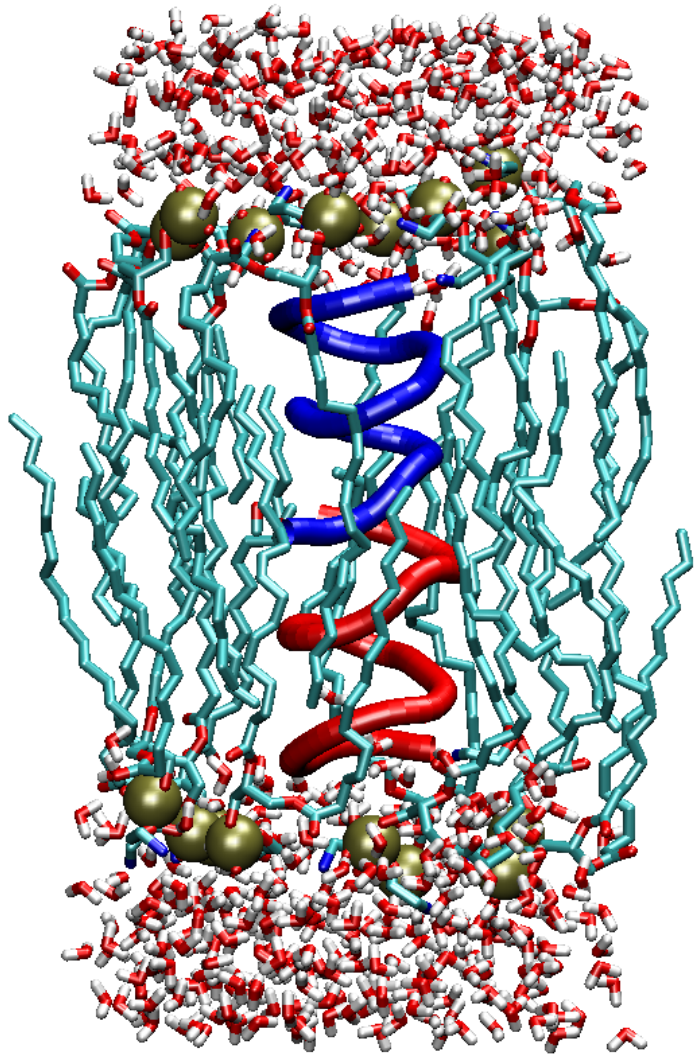
General Strategy

- Determine the components of the simulation (protein, dna, water, ions, lipids, etc.)
- Prepare individual components, if necessary.
  - Use psfgen or some other modeling program to add missing atoms, modify ionization states, graft functional groups onto particular residues, etc.
- Combine molecular components.
  - Overlay pre-equilibrated solvent
  - Generate solvent units on the fly
- Minimize

Example: Building Gramicidin A

- Obtain GA structure from the PDB databank (www.rcsb.org)
- Deal with non-standard N-terminal and C-terminal residues
- Build a lipid membrane around the peptide
- Add water
- Equilibrate



Building the Protein Structure

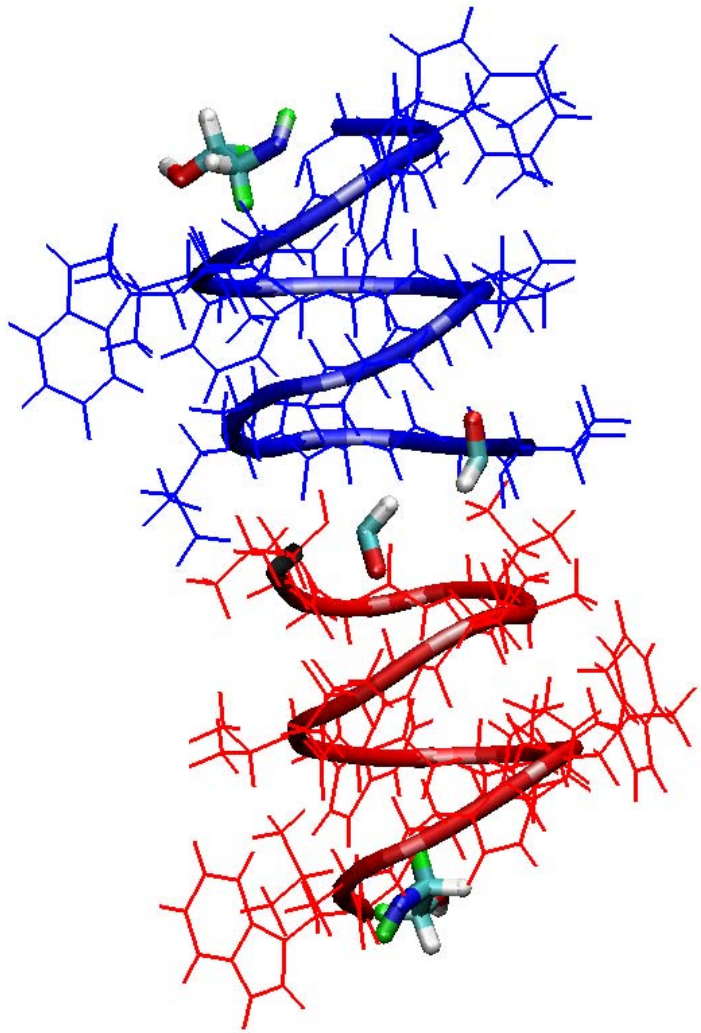
- Split the structure into connected segments
- Delete the hydrogens
  - Positions can be obtained from the topology file
  - Avoid naming problems
- Many atom names in the PDB file are different in the topology file - use psfgen's alias command to specify the mapping

Dealing with Unknown Residues

- Your system may contain residues that aren't in your topology file
- In many cases the residue can be built as a chimera out of existing topology groups
- Exotic new groups may require quantum chemistry to parameterize accurately

Example: GA Protein Structure

- D-Val and D-Leu residues
- Formyl group at N-terminus, ethanolamide group at C-terminus
- Created new topology, parameter entries by analogy with existing structures and terms.

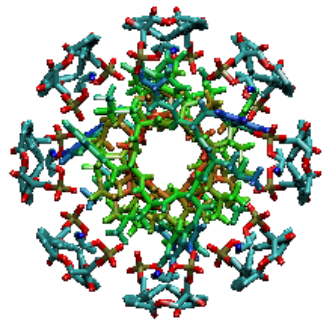


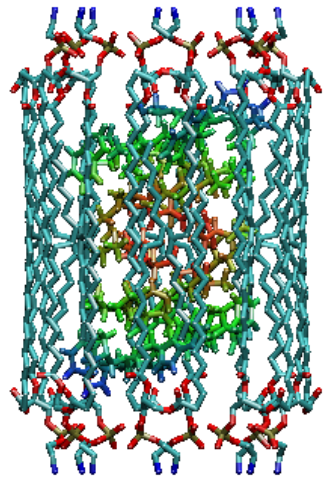
Adding a Lipid Bilayer

- *Ab initio*: surround the protein with lipids obtained from an ideal structure.
- *Lipid library*: Take pre-equilibrated lipid-water pieces and fit them around the protein.
- *Pre-existing membrane*: Cut a hole in an existing membrane (equilibrated or not) and place the protein inside.

Example: Building a lipid bilayer for Gramicidin A

- Start with idealized POPE structure, lipid tails straightened.
- Replicate the structure 16 times using psfgen.
- Position lipids geometrically using VMD.
- Position protein with the bilayer by eye.





Adding Water

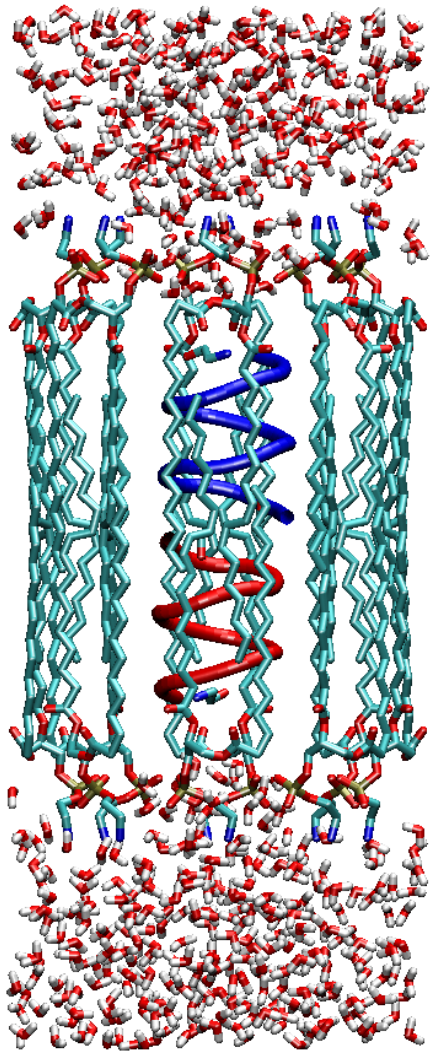
- Many modeling programs (e.g. MSI's *Quanta*) have a built-in solvate feature
- The program *solvate* from Grubmuller can add water as well as ions around a protein
- For membrane systems, take a pre-equilibrated block of water and add it to the system.
- The VMD solvate package has a flexible set of options for placing water around arbitrary structures.

Combining Simulation Components

- Once you have all the components (protein, water, membrane, etc.), combine them into one structure.
- Load the structure into VMD, and use atom selections to create PDB files containing the atoms you want to keep.
- Use *psfgen* to assemble the new PDB files into a reasonable starting configuration.

Example: Solvating Gramicidin

- Begin with a block of equilibrated water.
- Overlay the entire system with the water.
- Chop water outside the desired periodic cell, inside the membrane, and too close to protein or membrane.



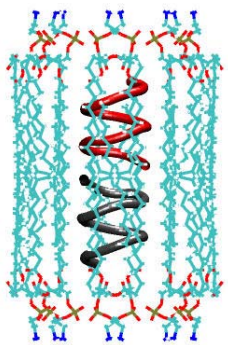
VMD's solvate package

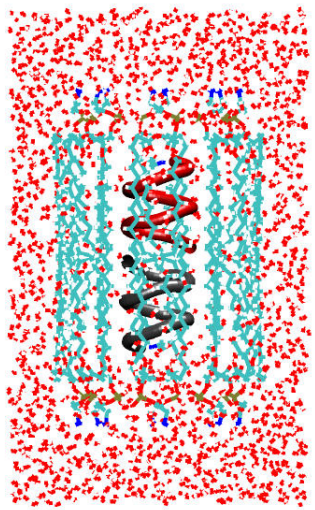
- The *solvate* package uses psfgen commands and VMD's atom selection capabilities.
- The basic building block is a cube of water equilibrated in an NpT ensemble.
- *Solvate* replicates the water box as many times as necessary, renaming segments and removing overlapping atoms.

Solvate: simple example

- For our Gramicidin A system, we can solvate the entire system in one step:

```
solvate pope_gram.psf pope_gram.pdb \
  -o pope_gram_wat -s WT -b 2.4 -t 5 \
  -z 10 +z 10
```

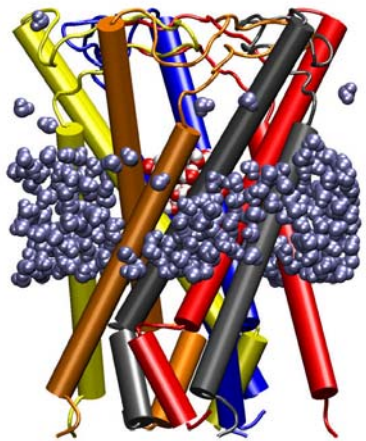


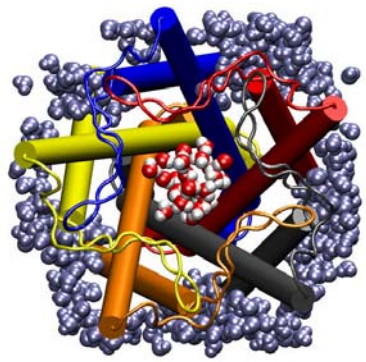


Solvate: complex example

- For a large membrane channel, one may need to solvate the pore, then remove waters outside the protein:

```
set badwat [atomselect top "segid WP1 and
  name OH2 and not same residue as
  ((sqr(x) + sqr(y) < 85) and z > -2 and
  within 8 of protein)"]
```





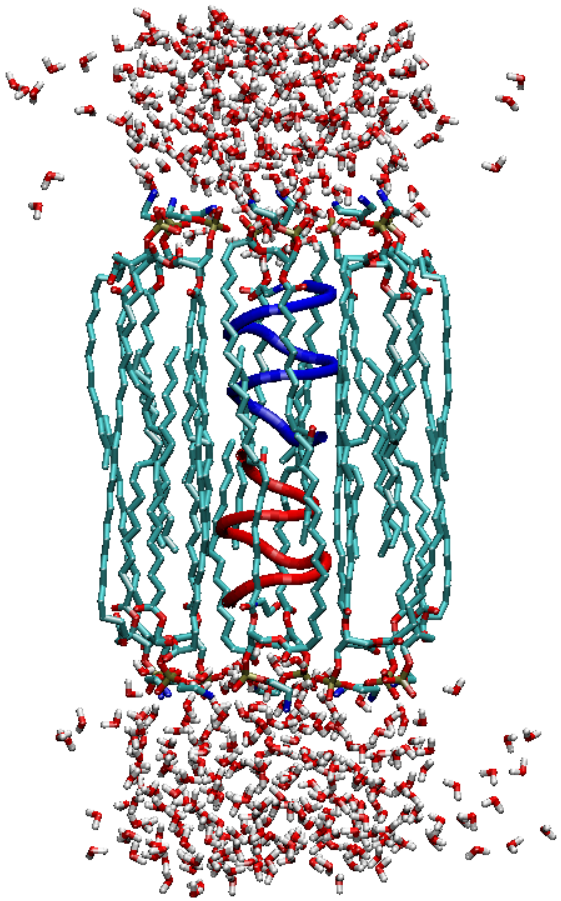
Minimization Issues

- After assembling the system, high-energy contacts usually remain.
- One wants to relieve these bad contacts without disturbing sensitive parts of the system.
- Minimize using the same force field parameters as will be used in the equilibration.
- Minimize until completion:
  - You want to start simulation from a well-defined starting point
  - No need to minimize down to the “bare metal” unless you’re doing normal mode analysis.

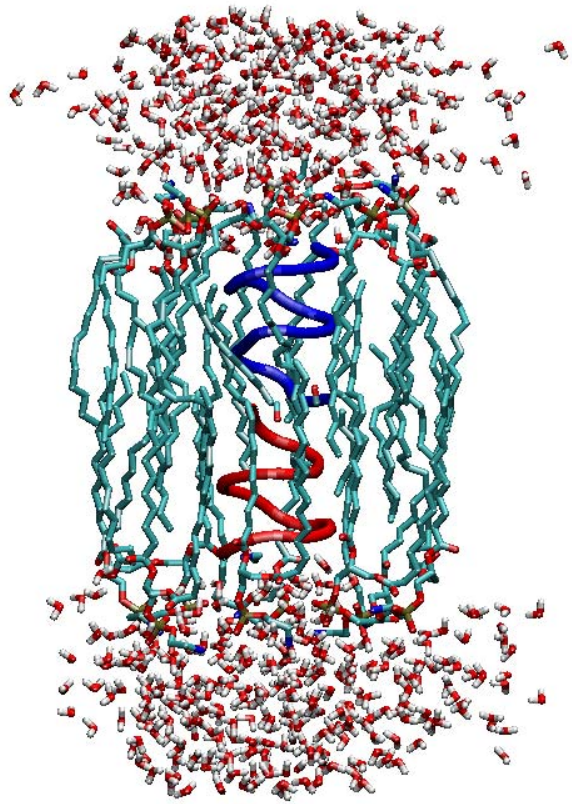
Keepin' it real with fixed atoms and restraints

- During minimization, fix protein backbone atoms until bad contacts have been removed.
- Put harmonic restraints on selected atoms during heating.
- Restraints and fixed atoms can be specified easily using VMD to mark the atoms; you can easily visualize which atoms are fixed.

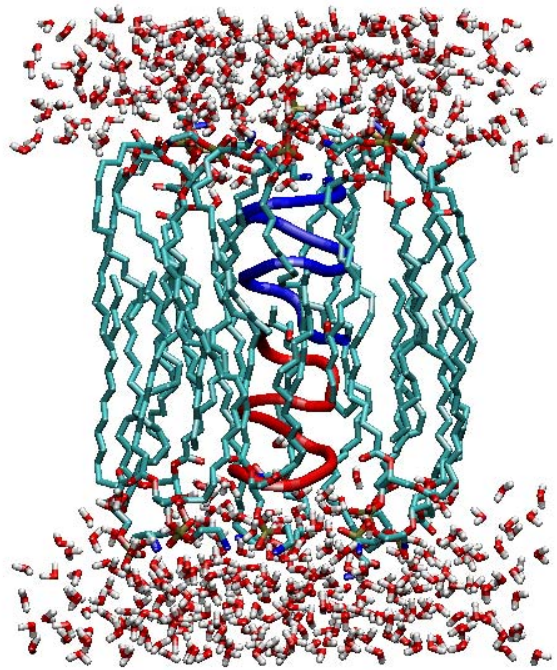
Example: Minimizing and Equilibrating Gramicidin A



Minimization



Restrained equilibration



Free equilibration

Minimization Setup

- First fix the protein backbone atoms and minimize everything else. Use VMD to specify the atoms to be fixed:

```
set all [atomselect top all]
set to_fix [atomselect top "protein and
  backbone"]
$all set beta 0
$to_fix set beta 1
$all writepdb fix_backbone.pdb
```

Minimization Protocol

- After minimizing non-backbone, minimize everything with no fixed atoms.
- Use VMD to examine results of minimization; look for disruption of side chains near system component boundaries or fixed atoms.
- When NAMD's reported gradient tolerance drops to below 1.0, you're doing well.

Heating Setup

- Put harmonic restraints on the CA atoms for heating and unit cell equilibration.
- Construct a PDB file using VMD to select CA atoms, just as for fixed atoms.
- NAMD input file contains:

```
bincoordinates          min_all.coor
binvelocities           min_all.vel
extendedSystem          min_all.xsc
constraints             on
consRef                 restrain_ca.pdb
consFile                restrain_ca.pdb
consKCol                B
```

Equilibration

- Turn on constant pressure to equilibrate the area of the membrane.
- Keep the restraints on the CA atoms until the membrane has reached a (meta)stable state.
- Release the CA atoms and continue equilibration until cell area, total energy, etc. have stabilized.

Are we done yet?

- Monitor RMSD of the protein; if it's a transmembrane protein, monitor loops and transmembrane parts separately.
- For membrane simulations, look at the surface area and the height of the unit cell.
- Total energy will appear to go down during equilibration in NAMD; don't be alarmed.