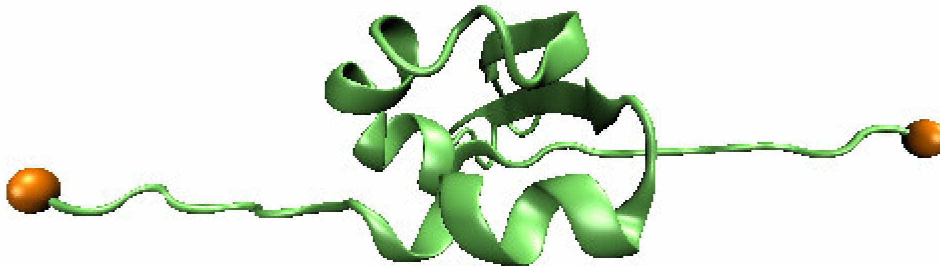
Laboratory 3:
Steered molecular dynamics

Nathan Hammond
nhammond@mit.edu

Issued: Friday, February 20, 2009
Due: Friday, February 27, 2009



In this lab you will:

1. Do some basic analysis of the molecular dynamics trajectories you generated in Lab 2 and determine whether your equilibration run was sufficiently long

2. Use Steered Molecular Dynamics to apply an external force to unfold a protein

Step 1: Analyzing an MD trajectory

Evaluate whether our equilibration run was sufficiently long

You'll find the files you need for this lab in the folder "/share/lab3".

First, we'll use the file "energy-rmsd.inp" to analyze the equilibration trajectory that we created in Lab 2. This file will measure a couple of things and write the results to an output file.

First, it computes the potential energy of the structure for each frame (each snapshot in time that we have in our trajectory file). Energy should reach a stable value fairly quickly during equilibration, probably within a few picoseconds.

Second, it computes the root mean square deviation of the alpha carbons at each frame. In other words, it computes the distance of each atom from its starting position, squares it, takes the mean of these square distances from all the alpha carbons, and takes the square root. We'll use this as a measure of how much the molecule is changing shape.

(Note, if our molecule did not change shape but drifted a few angstroms to the side, the distance between the atoms and their starting positions would be a bad measure of structural change, right? That's why we use the "coor orient" command to make CHARMM position the structure as closely as it can over the original coordinates before computing the RMSD.)

Create a folder to work in. You'll need "energy-rmsd.inp", your PSF file, the equilibration trajectory, and the minimized (not equilibrated) coordinates—you want to use the coordinates that correspond to the *start* of the trajectory you're analyzing.

Later, if you want to look at RMSD of the production run, you'll use the equilibrated coordinates with the "run" trajectory, because those are the coordinates that the "run" simulation started with.

Make whatever edits are needed to filenames, etc., in "energy-rmsd.inp". Run the script in CHARMM. It should take a few minutes to run. Write the output to something like "energy-rmsd.out"

This should generate a data file called "energy-rmsd.dat". Download this file and open it in Excel or another spreadsheet program. (If you need to separate your data into distinct columns in Excel, highlight the data, then select "Data→Text to Columns". Select "Delimited" and click "Next". Check "Comma" and click "Finish".)

Plot the energy against time. Is it drifting, or is it stable by the end of the equilibration run? Plot RMSD against time. Is it stable?

If either of these variables is drifting, you should run a longer equilibration before you move on to a real production run. My first run was 10 ps but since I still saw considerable drift I'm bumping it up to 100 ps. A thorough equilibration doesn't matter for these labs, but keep it in mind for your final projects. If you want to extend the equilibration time over what you've already done, you can modify the "min-equil.inp" script or the "run.inp" script you used for lab2. Set it to read the right restart (rst) file generated after your first equilibration run, and write the trajectory to a new file name so you don't overwrite your first equilibration trajectory. You don't need to repeat the minimization, so you can chop that part of the script.

We always expect the structure to drift a little bit from the PDB coordinates that we downloaded, but if it drifts very much then something is wrong. Most people consider a drift of 2 angstroms or less in the RMSD of the alpha carbons to be acceptable. Higher than this might indicate that our implicit solvent is not doing a good job of mimicking real solvent, that our cutoff distance is too short, that we goofed something up, or simply that the CHARMM parameter sets aren't accurate enough to do a good job of simulating this molecule.

Monitor the production run

Ordinarily you would do similar analysis of your production run to ensure that the structure was not drifting and that there were no unanticipated abrupt changes in energy. You don't need to do this for this lab, but you should do it wherever appropriate for your projects.

Step 2: Steered Molecular Dynamics

Running SMD

Now we'll see one of the most powerful applications of Molecular Dynamics. We are going to apply an external force to a few selected atoms in a way that would be difficult or impossible to do experimentally.

You'll need the script "pull.inp" from the "/share/lab3" folder. Go through the file, see what it does, and make the necessary edits.

One of the important features of this script is the "select" command. We use it in a number of places, but it's especially noteworthy here where we define the atoms "cterm" and "nterm" and then apply constraints or forces to these atoms selections. In the sample script I've selected just one atom. There's no reason you can't select more. Feel free to modify the script to apply forces to different atoms. If you want to pull on the termini, as I've done, just check the residue numbers of your

first (n-terminus) and last (c-terminus) residues in your PSF file, and update the selection terms to match.

Some ways to use "select" are listed below:

```
select all end                  !Select all atoms
select atom A 283 CA end        !Select alpha carbon in residue 282 of chain A
select type CA .and. ( resname ALA .or. resname GLY ) end
                                !Select the alpha carbons from any Alanine or
                                !   Glycine residue
select resid 283 .and. .not. type H* end        !Select all atoms in residue 283
                                !   whose type doesn't start with H.  This
                                !   will exclude all Hydrogen atoms.
select atom A 283 CA end .around. 5.0 end   !Select all atoms within 5 Angstroms
                                !   of atom A 283 CA
```

For more on using select, go to www.charmm.org. Under "Documentation" choose "Version c35b1" and open "select.doc".

Note where the force is applied using the "pull" command. Currently it's set to 1000 pN, but this can be changed. If you select more than one atom, 1000 pN will be applied to *each* atom. It may be appropriate to divide 1000 by the number of atoms to which you're applying force. These forces can be removed using "pull off". More info in "cons.doc" on the CHARMM website.

Also note that I have increased the value of "nsavc" under the "dynamics" command to 500. This means we are saving each 500th step in the trajectory, where in the previous lab we saved every 100th step. I've changed this simply to keep the size of our trajectory file reasonable.

I also increased the duration of the trajectory. Where before I set the "time" variable to 10 ps (and "nstep" in the dynamics command to 10000), in "pull.inp" I have increased this value to 200 ps (and "nstep" to 200000). Of course this is going to take about 20x longer to run.

To run the script, you'll need the restart file from equilibration (mine is 1by9_equil.rst), your PSF file, and the equilibrated coordinates.

Run the script in CHARMM and write the output to a file.

Examine the trajectory

This script might require a day or more to fully run. However, you may not have to wait this long to see some results. Download your trajectory to your computer and look at it in VMD. Is the molecule unfolding?

Does the unfolding occur smoothly, or are there points where it snags? Can you see the atoms that offer resistance? Maybe there are some hydrogen bonds that have to be ruptured, or some hydrophobic faces that have to be pulled apart and exposed to solvent. Using VMD, you can select individual atoms (see Lab 1) to know which residues are important to prevent unfolding and confer stability to the molecule. Think about what mutations you might want to carry out to make the molecule unfold more easily.

More info on CHARMM

There is a big community of CHARMM users, and a very active discussion forum on www.charmm.org. You can usually get an answer to any question within a day or two, sometimes from other novices and sometimes from the experts who moderate the forums. The knowledgeable but curmudgeonly Rick Venable will occasionally excoriate you for asking what he considers to be a dumb question, but that's just part of the initiation process. If you're lucky you might get Rick and Lennart Nilsson, two CHARMM gurus, arguing back and forth about your question and offering you conflicting advice. Or maybe you'll hear from another user saying, "Hey, I'm having the same problem. When you figure it out, let me know."

Welcome to the CHARMM community!

And beyond . . .

That's all for now. Great job. You've done a lot with molecular dynamics in a very short time.

Was it kind of fun? Did you find it just a teensy weensy bit addictive? Do you kind of have an urge to explore what else you can do with MD, even when it's not for a course assignment? I hope so.

Now go to work on your projects! Feel free to use the scripts from these labs and modify them as needed. And of course Alisha, Zhenya and I are available for help.