**CHARMMGUI Tutorial: Preparing A Simple Globular Protein - Ubiquitin**

Visit the CHARMM-GUI website at https://charmm-gui.org/. Login to your account.

Click “Input Generator” in the menu to the left.
Click “Solution Builder” in the menu to the left.

Beside “Upload PDB File”, click “Browse...”. Select the file 1ubq.pdb

Move to the next page by clicking “Next Step: Select Model/Chain”.

Under Model/Chain Selection Option, it’s generally a good idea to keep crystallographic waters. So select the checkbox next to “Water” to include it in the model.

Click “Next Step: Manipulate PDB”.

On this page, one may make a number of possible modifications to the structure, adding missing atoms and such.

We will assign the correct protonation states of histidine manually. Select “Mutation”. Select the RESID for your histidine(s) (the residue number should be 68 for this structure). Change “Mutant” into “HSD” (or other correct protonation state).

When doing your own protein, you should pay special attention that “Disulfide bonds” are correctly added. This protein contains no cysteines, so we don’t need to worry about disulfide bonds at this time.

Click “Next Step: Generate PDB”.

Download the CHARMM PDB file, save it as “step1_pdbreader.pdb”. Check the protonation state of histidine in VMD. Also check your protein termini in VMD to ensure that they are correctly built and chemically valid.

Next, we need to add a water box. “Fit waterbox size to protein size” should be selected. Next, “Enter Edge Distance” should be 10.0.

“Include Ions” should be checked. Change “Basic Ion Types” to NaCl. Click “Add Simple Ion Type”. A concentration of 0.15 M should be good for most physiological situations. If unsure, consult other publications - perhaps you’re trying to recreate some experimental conditions. Click the “-” next to KCl to remove KCl ions. Make sure “Neutralizing” is selected beside the NaCl ions.

Click “Calculate Solvent Composition”. It will show you how many ions of each type are being added. There should only be Na+ and Cl- ions listed there.

Click “Next Step: Solvate Molecule”.

Once complete, you can download “step2_solvator.pdb” to ensure that the waterbox looks reasonable. Alternatively, you could use “view structure” in the CHARMM-GUI to take a quick view.

Take note of the System size A, B, and C values. Write these down for later.

Click “Next Step: Setup Periodic Boundary Condition”.

When that is finished, you may leave the forcefield as CHARMM36m.

Under “Input Generation Options”, select “OpenMM”.

Click “Next Step: Generate Equilibration and Dynamics Inputs”.

On the final page, you can download the files as a .tgz file.