**Part 1: Building Structure using Chimera**

1. Open the Build Structure dialog box by clicking **Tools > Structure Editing > Build Structure**
2. In the **Start Structure** tab, choose Add peptide sequence, input the peptide sequence GGGGG in the Peptide Sequence text box, then click **Apply** (for hexapeptides type GGGGGG, for heptapeptides GGGGGGG, etc). At this stage glycines will be used to avoid any inversion of chirality as a result of cyclization. Step 13 involves swapping out the glycines with the desired residues.
3. Set ***ϕ***/***ψ*** angles in the pop-up dialog, Click Ok after setting the angles. In most purposes, you should choose random angles for each ***ϕ***/***ψ*.**
4. Show the **Command Line** with **Tools > General Controls > Command Line.**
5. Delete the OXT atom of the C-terminus by using the command:

delete :5@OXT

1. Add a bond between the N-terminal N-atom and the C-terminal C-atom:

bond :5@C :1@N

1. Open the **Minimize Structure** dialog using T**ools > Structure Editing > Minimize Structure**.
2. Click **Minimize** after setting minimization parameters in the **Minimize Structure** dialog box. Tinker with these parameters such that the optimization runs long enough for the structure to stop moving.
3. In the pop-up **Add Hydrogens for Dock Prep** dialog box, set the hydrogen adding method and protonation states for the different residues. Click **Ok**. Normally, you want to simulate your system at normal pH so the given positive and negative amino acids should be protonated.
4. In the pop-up **Assign Charges for Minimize** dialog box, set the charge assignment method and click **Ok**.
5. After this minimization procedure, some oddities may occur. Because Chimera is not set up for cyclic peptides, it is very possible (and fairly common) for *cis* peptide bonds to form, or for D-amino acids to appear. If this does happen, start the process over again with re-randomized dihedral angles
6. Now that the peptide has been cyclized, it is time to mutate some of the Gly residues to the residues we wish to simulate.
7. Now, to get the desired amino acids, write on the command

swapaa arg #:1.a

Now repeat this for all of the given residues that you want, editing the index of the residue and the name of the residue for each additional amino acid.

1. If you wish to insert a D-amino acid, (convert the chirality of F), in the command line type:

invert :#@ca (# being the residue number)

1. At this point you should have a cyclic peptide with your desired sequence!

**Alignment of the 2 configurations:**

Often, you would like to do the above procedure at least twice in order to get two or more starting configurations for your simulation. After creating the two (or more) initial confirmations (s1, s2), you can make sure they are substantially different calculating RMSD using the VMD. To check the RMSD of your initial confirmations type in the command line:

$vmd -m s1.pdb s2.pdb (for windows, just load s1.pdb and s2.pdb by going **File-> New Molecule**)

Then, in vmd, go to **Extension > Analysis > RMSD Trajectory tool**

Check the **Backbone** box

Click on **ALIGN**

Click on **RMSD**

**Removal of all hydrogen atoms except one:**

Save a version of your .pdb called, for example, s1\_GNSRV\_hydro.pdb and open it with Chimera. Remove hydrogen atoms from the initial structures using chimera with command: “delete element.H”. Then, after making a .pdb of your structure without hydrogens, s1\_GNSRV\_finished.pdb, add a hydrogen atom back to the N-terminal nitrogen pasting the line containing the hydrogen atom from N-terminal nitrogen of s1\_GNSRV\_hydro.pdb to s1\_GNSRV\_finished.pdb.

(Another way of doing this that may be more convenient is by deleting all of the other hydrogens except for the hydrogen connected to the N-terminal nitrogen. You can do this by typing in the command line

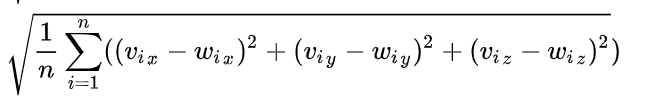
select element.H

~select :1.A@H #unselect hydrogen connected to N-terminal

delete sel

The significance of this step will be clarified later (in Part 2, 2.c). In short, deletion of all of the hydrogens except one will facilitate Gromacs understanding that your peptide is cyclic; not linear.

**Quiz Answers!**

1. Build a linear peptide with all glycines and random dihedrals, bond the C and N terminals, complete an energy minimization, check for cis bonds and throw away seqs if necessary. Swap in other amino acids, remove all hydrogens except one.
2. Because glycine is achiral, if you cyclize with all glycines, Chimera doesn’t have a chance to make D-amino acids.
3. Finding a cis bond, having structures that are too similar.
4. Backbone
5. The backbone RMSD is a metric indicating the difference between two protein structures. It is calculated as

Where you have two backbone structures, v and w, each with n atoms.

**Quiz Answers Pt. 2!**

1. aminoacids.rtp, aminoacids.hdb, specbond.dat
2. In aminoacids.rtp, you want to create new residues for the 1st and last residues in your sequence. These residues will be identical to their linear counterparts except that they will lack the bond and dihedrals that connect them together. In aminoacids.hdb, you need to create entries for your new residues that are identical to their linear counterparts. However, the 1st residue in your sequence should not have a hydrogen connected to its N-terminal nitrogen. Specbond.dat should be altered to connect your custom residues together
3. fudgeQQ goes from 0.8333 to 1.0. There are also 1–5 and 1–6 interactions added. Residue-specific torsional parameters are added.
4. Your topology file, your .gro file, your posre.itp.