

Homework 2. Make a small protein

The sequence of our small protein will be

AAAAAAAAAAAAAAAAAAAAAAAA

- 24 alanines.

We will fold it into a classic right-handed beta-alpha-beta unit (see TOPS drawing), using MOE.

- Step by step instruction follow. However, *your results may vary*.

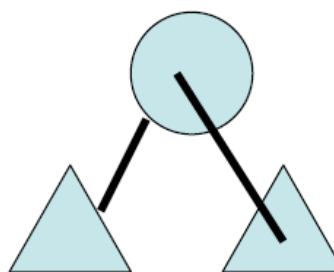


Figure 1

Step 1 -- Synthesize

• **Edit | Build | Protein**

- select “**extended**” and click ALA 8 times.
- You now have a 8-residue beta strand.
- select “**helix**”. Click ALA 8 more times.
- select “**extended**” again and hit ALA 8 more times.
- Now you have a beta-alpha-beta unit, but it is not “folded”.

Step 2 -- Fold

- Freeze the helix for the duration of this exercise. (Select helix. **Edit | Potential | Fix**, Invert selection, **Edit | Potential | Unfix**)

- Fold the two strands around the helix. Right-handed.

- Select residues 1-8

Use meta-key to rotate the strand parallel to the helix axis. (see figure 2)

- Select residues 17-24

• Use **meta-middlemouse** to rotate the strand parallel to the helix axis.

- Make it a **right-handed** $\beta\alpha\beta$ unit. (see Fig. 1)

• Try to rotate the strands so the the sidechains point in/out, and the oxygens/nitrogens point to each other, as they will when they make H-bonds. (see figure 3)

* In this view, strand 1 is on the right, connected to the helix (behind) at the top. This is right-handed.

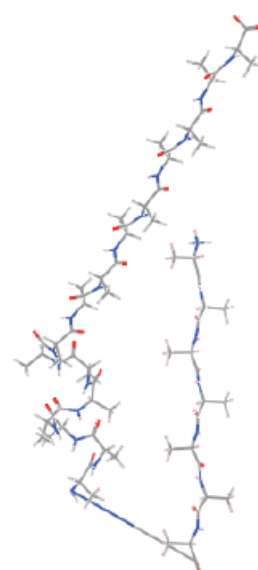


Figure 2

Step 3 -- H-bonds

Use restraints to make H-bonds:

Edit | Potential | Restrain

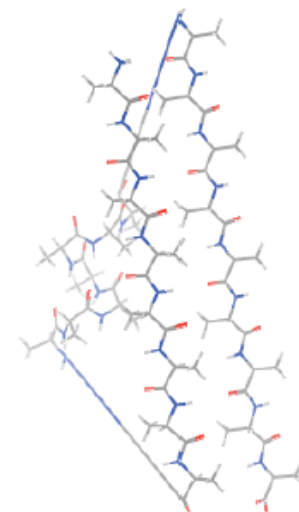


Figure 3

- Select “distance”. Set min, max target distances to 1.8 and 1.8.
- Set weight to 100
- Select O. Select H. Press Create.
- Do the same for O to N, but set the distance to 2.8, 2.8

O	N or H
1	19
19	3
3	21
21	5

Step 4 -- Energy minimize

Energy minimize.

• **Compute | Structure preparation...**

- Hit Minimize (side panel)

– Watch the stretched bonds relax to their ideal lengths.

• Check for bad geometry (this takes practice!)

- Examples of bad geometry are D-amino acids (usually created in the process of minimizing, since L and D amino acids both have the same low energy.)

- Cis-peptide bonds. Are the CAs both on the same side of the CO-N peptide bond? Is the peptide bond planar?

– To correct for bad geometry, push and pull atoms by selecting and dragging with **meta-shift-middlemouse**. (meta is Alt or Command, depending on your system.) Then hit Minimize.

[NOTE: I recommend using GizMOE to continuously minimize, instead of repeatedly hitting Minimize. To do this type "run 'gizmin.svl' " in the SVL mindow.]

To remove or modify a restraint

• **Window | Potential setup. Restraints**

– Find the one you want to delete or modify by looking at the atom names.

Correct errors in the beta sheet.

Inevitably, energy minimization is not enough to find the ideal structure. **Fix it by hand.**

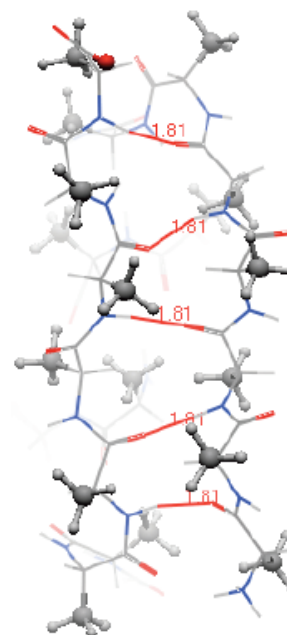


Figure 4

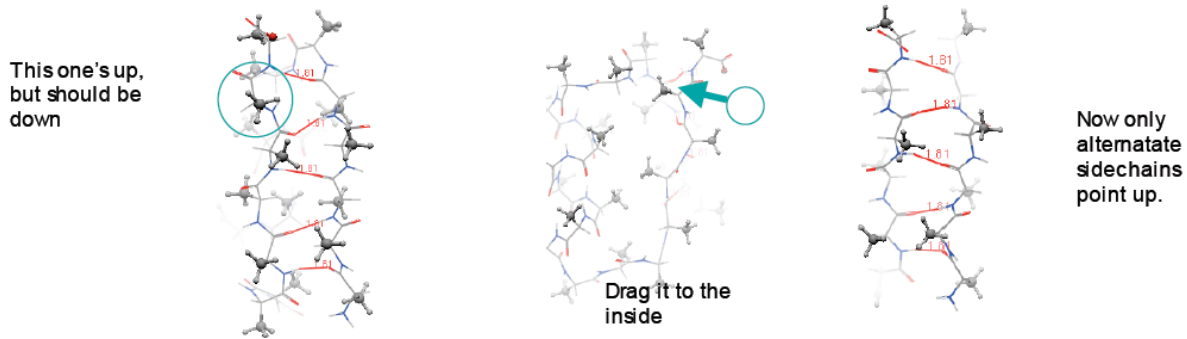


Figure 5

The beta-sheet should have its sidechains alternating sides, up-down-up-down, when viewed from the side. To make a *canonical parallel beta sheet*:

- Drag the sidechains of 2, 4, 19, and 21 to the outside of the sheet, if they are not there already.
- Drag the sidechains of 1,3,5,18,20 and 22 to the inside of the sheet (the side that packs against the helix).
- **Minimize**. Or **SVL: run 'gizmin.svl'**
- Let the Minimizer relax atoms into place.

After pushing and pulling, make sure all residues are still L-amino acids!

When dragging side chains, it is possible to convert L-amino acids to D-amino acids. There is no energy difference. Check chirality by eye (see **Lecture 1** for how to do this.) If you find a D-amino acid, make it L by selecting the CB atom and swiftly dragging it directly through the CA atom to the other side. Then let the energy minimize.

Knobs in holes packing

Select the side chains – **Selection | Protein | sidechain**

- Render them as spheres – (**Atoms** button. Pick spheres button.)
- *Push* and *pull* atoms so that beta sheet sidechains (knobs) fill the spaces between alpha helix sidechains

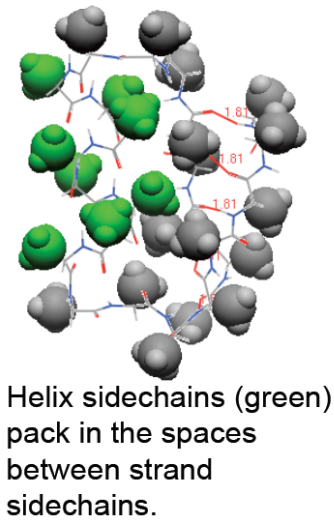


Figure 6

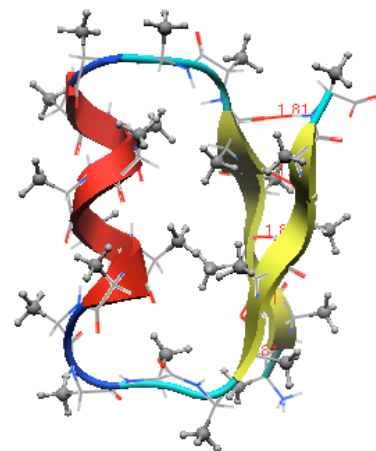


Figure 7

(holes).

Step 5 -- Save and submit

Make side chains ball-and-stick.
Add a cartoon backbone.
Save file as a MOE file (.moe)

Upload your MOE file to
<http://www.bioinfo.rpi.edu/bystrc/courses/biol4550/homework.html>
Be sure to check Homework 2.