

Homework 3 -- Homology modeling by hand.

due Feb 17

Save the final result in moe format. Upload the file to the website.

<http://www.bioinfo.rpi.edu/bystrc/courses/biol4550/homework.html>

You can upload again (overwrites) if you want to make changes.

Step 1 -- PDB search

- ✓ Open course web page (<http://www.bioinfo.rpi.edu/bystrc/courses/biol4550>) and download "Sequence 1" as file *strepto.seq*.
- ✓ Open MOE, go to Sequence Editor (ctrl-q), hereafter called "SEQ"
- ✓ SEQ: **File/Open** strepto.seq
- ✓ SEQ: **Annotate/2o structure/Predict**
- ✓ SEQ: **Compute/PDBsearch**. Load chain 1. Settings: set E-value cutoff to 0.001, Tuple size to 3. **Load** the alignment labeled 2IGH. Select **1EM7.A** and **Load Selected**
- ✓ Align the sequences: **SEQ: Alignment/Align..**
Sequence alignment only.

Step 2 -- Mutate aligned residues

- ✓ Color the residues: **SEQ: Residues/Color residues** (Function)
- ✓ Turn on sequence numbering. Write down residue numbers of indels and non-conservative mutations.
- ✓ Mutate-by-hand. Change 1EM7.A into *strepto* one residue at a time:
SEQ: Edit/Mutate
Select an amino acid in 1EM7.A, then click on a residue in 1EM7.A to mutate it. **Do not mutate the query!!** Watch the MOE window swap sidechains as you mutate.
- ✓ When done, look at it. Are there any bad contacts?
- ✓ Back in MOE window (ctrl-m):
- ✓ When done mutating (the sequence of 1EM7.A is now the same as the query), **energy minimize**:
MOE: Selection/Protein/backbone
Edit/Potential/Fix
Fixing prevents the backbone atoms from moving during energy minimization.

Selection/Invert

Edit/Potential/Unfix (this allows side chains to move)

- ✓ **Compute/Structure preparation** (click **Correct** and/or **Protonate3D**)
Compute/energy minimize, Forcefield..., Load/AMBER94
Click **Fix Hydrogens**, and/or **Fix charges** if either button is highlighted. **Apply, Close.**

Step 3 -- Make a deletion

- ✓ Delete the residue in the template that is not in the target. Select residue in SEQ window.
Edit/Delete/Residues
- ✓ Make a bond between the carbon and the nitrogen to create a new peptide bond. Select two atoms. **Builder**, bond button.
- ✓ Using **Window/Atom Manager**, find the two atoms you bonded. Make sure they are both *sp2 hybridized*, and that they have *zero* charge.
- ✓ Select four residues, two before and two after the deletion. **Edit/Potential/Unfix**. Invert selection. **Edit/Potential/Fix**.
- ✓ **Compute/Structure preparation**
- ✓ **GizMOE/Minimize**
- ✓ Push and pull to help atoms by hydrogen bonds.
- ✓ **Cancel/GizMOE_minimize** when converged. (Save)

Step 4 -- Make an insertion

- ✓ Create a new chain having the sequence of the insert. **Edit/Build/Protein**, **Clink AAs**.
- ✓ Split the template chain at the insertion point. Select (right-mouse) the residue after the insertion. **SEQ>Edit/Split chain**
- ✓ Middle-mouse drag chain label of new chain so that the three parts of the sequence are in order.

- ACDEF
- GH
- IKLMNP

where GH is the inserted sequence.

- ✓ Select all three chain tags. **Edit/Join chains**.
- ✓ Create new peptide bonds. Select appropriate N and C atoms. **Builder**, bond button.
- ✓ Using **Window/Atom Manager**, find the two atoms you bonded. Make sure they are both *sp² hybridized*, and that they have *zero* charge.
- ✓ Selected inserted atoms. **Shift-meta-middlemouse** drag so that the insert is close to the right place.
- ✓ In the SEQ window, select the insert and one residue before and after. **Edit/Potential/unfix**. Invert selection. **Edit/Potential/Fix**.
- ✓ **Compute/Structure preparation**
- ✓ **GizMOE/Minimize**
- ✓ Push and pull to help atoms by hydrogen bonds. (Select. **Shift-meta-middlemouse** drag. Unselect.)
- ✓ **Cancel/GizMOE_minimize** when converged. (Save)

Step 5 -- Submit

- ✓ *Save the molecular file in moe format. Upload the file to the homework website. Select Homework 3. You can upload again if you want to make changes.*