Exercises 4, 5

Synopsis

- In these two exercises we will build a homology model for a target that actually has a **known structure** (1BBZ.C). Why would we do that? The structure is known! Answer: To evaluate the accuracy of the loop modeling and to correct errors. We will use a **distant homolog** as the template (2IIM.A).

- After Exercise 4 (next two pages) look carefully at your modeled loop. *Are there H-bonds that are missing?*

- In Exercise 5, we add in some H-bonds that may be missing in the automatically generated loop.

Exercise 4

- Download and open **Exercise4.moe** from the website
- Contained in the file are two homologous structures. 1BBZ.C will be the target (we will use only the sequence), and 2IIM.A will be the template.
- Align the sequences/structures using the defaults. Write down the RMSD.
- Locate the two loops where there are indels. Find the best anchor points and **unalign all of the residues between the anchor points**. The alignment should look approximately like the one below.
- Make the target sequence (mouseover chain 1/meta key/residues/to clipboard, Edit/Paste, Force: raw_seq, OK. Now chain 3 is identical to 1BBZ.C (but chain 3 has no coordinates)
- Align chain 3 to chain 1 by pushing chain 3 until it is identical to chain 1. Delete chain 1 (1BBZ true target coords)
- Run Homology->Homology model.. Create 10 models.

1	1BBZ.C ABL TYROSINE KINASE
	NLFVALYDFVASGDNTLSITKGEKLRVLGYNHNG···EWCEAQ·····TKNGQGWVPSNYITPVNS
2	2IIM.A PROTO-ONCOGENE TYROSINE-PROTEIN KINASE LCK
	GSPLQDNLVIALHSYEPSHDGDLGFEKGEQLRILE·····QSGEWWKAQSLTTG···QEGFIPFNFVAKA

Exercise 4, part 2

- Close all.
- Retrieve 1BBZ.C from File/Protein database. (Or use 1bbzC.pdb on the website. Remember 1BBZ is the "true target", the correct structure of the model. We are using it to see how good our predicted model is.)
- Open the database file that was created by Homology model (promodel.mdb, or whatever you named it.) in Database Viewer (DV)
- In DV, Right-click on each mode and Send to MOE. (Do not clear system)
- Superimpose. (all chains, all residues, use current alignment)
- Remove the 5 models that have the worst RMSDs.
- Select residues 28-35. Superimpose. (all chains, selected residues, use current alignment)
- Select the chain with the best RMSD to the true target. Delete the other chains.
- Hide all. Display as Ribbon. <u>Save MOE file, upload as Exercise 4.</u>
- Keep the MOE file for Exercise 5.

Exercise 5

- Open your model from Ex. 4.
- Does your model look like this in the 29-38 region? Notice the gap between the VL (29-35) and the SCR (36-38).
- Lines indicate *H-bonds that are possible but not made*.
- Make them using restraints as shown on the next page. It should cause the VL to fill the gap.
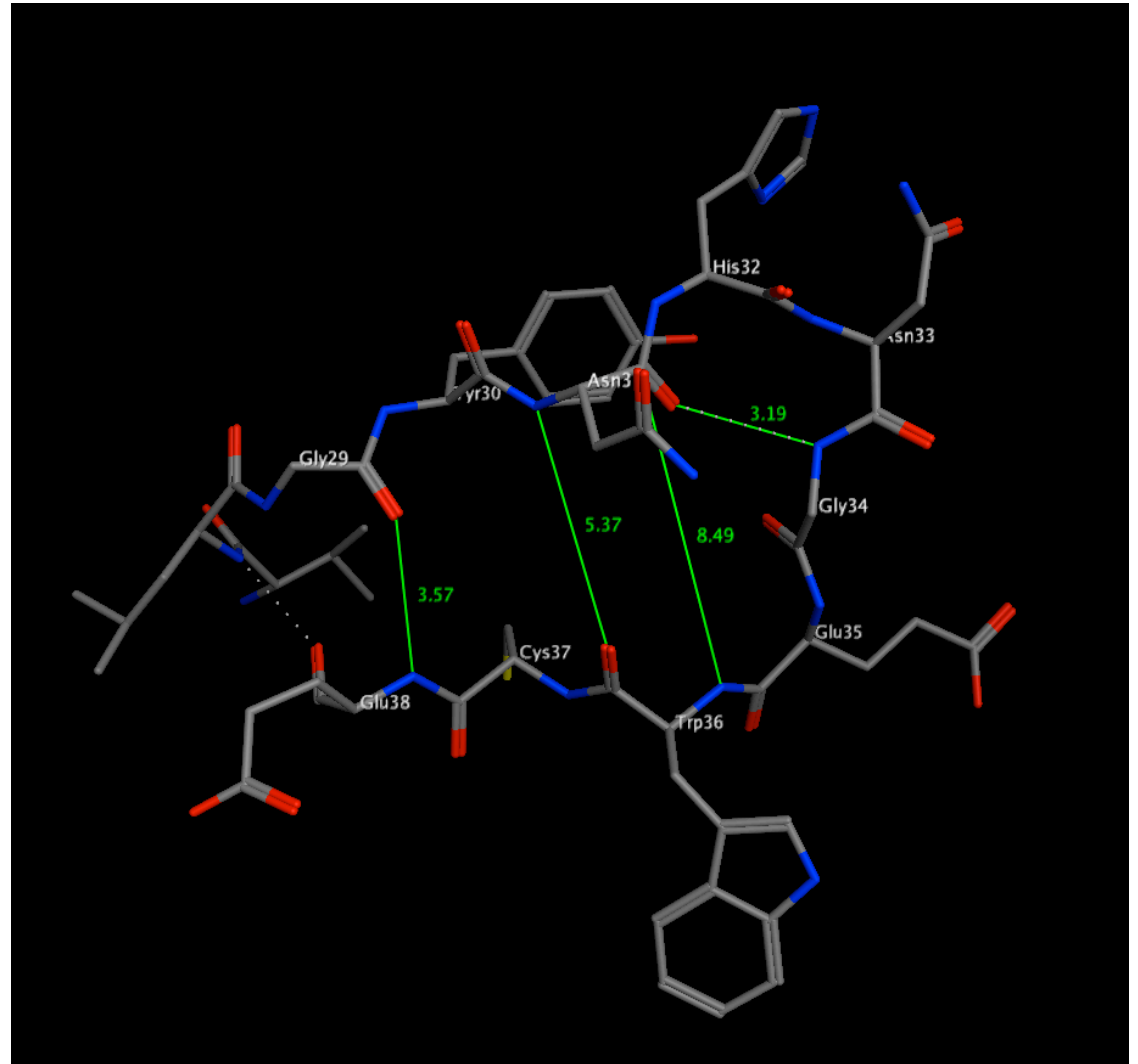
Ex 5. Filling the VL gap

- Restrain the following distances to 2.8 using a weight = 50
  - G29 O --> E
  - N31 N --> W36 O
  - N31 OD2 --> W36 N
  - N31 O --> G34 N
- Select residues 29-35. Unfix them. Invert selection. Fix everything else.
- Energy minimize using GizMOE->Minimize. Help by grabbing and pulling if necessary.
- Retrieve the true target (1BBZ) from File/Protein database again. (or from the course website 1bbzC.pdb)
- Superimpose model and true target. Use "Report RMSD".
- Is the model closer to the true target than before filling the VL gap?
- Hide everything except residues 25-40 of both structure.
- Color carbons by chain.
- **Save and upload** the MOE file as **Exercise 5.**