

Name: _____



Worksheet #7

Analysis of Mutants

Files: [1lmb6_85aH.kin](#) || [1lmb6_85aH.pdb](#) || [2simHdot.kin](#) || [2simH.pdb](#)

Mutations

In this exercise you will use the information from all-atom contacts and sidechain rotamer conformations to explore whether or not a specific mutation can be accommodated without moving the surrounding structure (especially the backbone). This is done in the interactive KiNG/Probe system. In this procedure, KiNG sends coordinates to Probe which actually runs as a remote job. Probe is not a Java program but a platform-specific compiled version is supplied as part of the KiNG bundle. However, the interaction is usually so fast that it appears to be an internal function in KiNG.

A. Putting a buried Trp into lambda repressor

Launch KiNG and open the 1lmb6_85aH-KiNG.kin file to see the starting structure, a small all-helix domain. Choose Tyr 22 on the Views menu to try a mostly buried aromatic as a candidate for mutation to Trp. [Remember to rotate the image back & forth often as you work.] You should probably enlarge the graphics window, but don't go completely full-screen because you'll be using other windows also.

Under the "Tools" menu select "Structural Biology" then "Sidechain mutator". A dialog box comes up in which you need to navigate to where you downloaded the coordinate file 1lmb6_85aH.pdb, choose this file. Ctrl-click, option-click, or middle-click on any atom in Tyr 22: select "Trp". Now there are 2 more little dialog boxes: a "Model manager" and a "Rotator" box labeled with this mutation which has a list of the 7 Trp rotamers; place this dialog box where you can find and click in it easily without covering the graphics too much. The rotamers are selected by clicking on their names and the dials allow adjustment from rotamer positions; try clicking on one to reset the "molten" (orange) sidechain to that conformation. Turn OFF the "frozen" (blue) model. Each rotamer is named by a string of characters, one for each chi angle (two, for Trp), with m p or t for minus (near -60), plus (near +60) or trans (near 180), or a number if not near those values. Examples are mt or p-90.

To evaluate which sidechain conformations are possible, you'll need to see the all-atom contacts. In the "Model manager" dialog box toggle on "Probe Dots". Now you should see contact dots around the Trp, probably with lots of red spikes because very few alternatives work for a buried sidechain. Try each rotamer in turn. All but one are really dreadful, but one fits quite well. What is the name of that good rotamer? _____ Is its conformation close to that of the original Tyr, or quite different? _____

The Trp is sandwiched between several other sidechain with blue, green, and yellow dots on its face indicating a pretty good fit. Try moving chi2 in each direction (click and drag near the outer edge of the dial for fine control, the + and - boxes make very fine, 0.1 deg changes) to see if you can make the fit even better. Turn so you can see the Ne1 at the back, inner end of the Trp sidechain, where there is also a small clash. Try moving chi1 a little to make that overlap smaller, without getting in trouble elsewhere. A good, final fit has chi1 = _____, chi2 = _____. (The active angle value is shown in black near the middle of the dial, the original value is gray.) Double-clicking on the dial face resets to the original values.

What sidechain does the Trp e1 NH touch? _____. It would prefer to form an H-bond, but that cannot be done here. All other factors are extremely favorable, however, and the mutation turned out to be just as stable and fast-folding as the original, and is used for fluorescence measurements.

[Optional: Replacing a buried Tyr or Phe with Trp does not usually work. To see a more typical case, try the same procedure on Phe 76.]

Quit from KiNG without saving.

B. Evaluating Thr to Val mutations in a neuraminidase

Open 2simHdot.kin in KiNG. The kinemage opens with a view for Thr 166 (or else choose that view), which has a largely exposed but H-bonded sidechain. Rotate the image, and identify the atom that makes an H-bond with the Thr 166 sidechain: _____ of _____. Now turn on wide and close contacts, and identify two more atoms (in different residues) that make van der Waals contacts with the sidechain of Thr 166: _____ of _____ and _____ of _____.

To test the importance of the Thr O_{g1} H-bond for stability or function, one might propose making a “conservative” mutation to Val. Under the “Tools” menu select “Structural Biology” then “Sidechain mutator”. A dialog box comes up in which you need to navigate to where you downloaded the coordinate file 2simH.pdb, choose this file. Ctrl-click, option-click, or middle-click the any atom in Thr 166; select “Val”. Now there are 2 more little dialog boxes: a “Model manager” and a “Rotator” box labeled with this mutation which has a list of the Val rotamers; place this dialog box where you can find and click in it easily without covering the graphics too much. Try each of the rotamers from the rotator dialog box. Can any one be made to overlay the original Thr atoms? _____ To avoid confusion turn off the “dots” group (just below “2simH” on the right-side button panel). Turn on “Probe dots” in the “Model manager” dialog box. Are any of the rotamers acceptable? (i.e. clash-free or nearly so) _____ Try moving chi1 near the best rotamer (by no more than 15-20 degrees). Can it be made acceptable? _____ If so, at what chi value? _____ Release the mobile “molten” group by clicking the “Finished” button in the rotator dialog box.

Go to the view for Thr 43, which is somewhat more buried and also H-bonded. Try the same procedure on it: middle click on any atom of the “frozen” (blue) model read in earlier, or of the original (cyan) model. Is one of the Val rotamers acceptable? _____ Which one is best? _____ (More usually, a Thr to Val change uses a rotamer different than the original Thr one, since the geometry at an H-bond is usually too tight to fit a methyl.)

[Optional: Misfit sidechains in crystal structures can be corrected using this same KiNG/Probe remote-update procedure (ask for Sidechain mutator, so it gets idealized, but pick the same residue name). Val 358 is fit wrong in 2sim: go to that view and turn on the contact dots (“dots” group) to see its bad clashes. Look down the C_{alpha}-C_{beta} bond to see that the chi1 angle is eclipsed. Use the remote update procedure to refit Val 358 with an idealized rotamer that puts atoms in the same region of space but has very much better contacts. Unfortunately, as is often the case, misfits in one place influence neighbors. This Val 358 when adjusted has a slightly too-close contact with Phe 330, which in turn has a too-close contact with Ile 312, but Ile 312 can be adjusted to relieve its contact with Phe 330. Refinement is poor at fixing rotamers, however, putting the model with corrected Val 358 back into refinement would probably result in these other minor corrections.]