

# BCH222 - Using All-Atom Contact Analysis

## Reading

Word et al. (1999) J. Mol. Biol. **285**, 1711 on the contact-dot method (Read the parts marked in margins, but keep the paper for later reference.)

## Graphics assignment

To go through an all-atom contact example file; run Reduce to add H atoms (on the MolProbity web site), run Probe in 2 different modes to generate contact dots and look at them; and try a kinemage set up to interactively update contacts as you rotate side chains.

[ Note: If the remote update doesn't work, check that the pdb file name and location are right and that your computer's Probe program has been renamed or aliased to the name "probe" (rather than with a name that includes version number or date). This is because of the command lines generated "automagically" in the remote update process. The command lines are displayed in the appropriate dialog boxes when you are working in KiNG or Mage, and you will want to cross-check file and program names if troubleshooting is needed. ]

### 1. Demo5\_4b.kin (400KB)

Look at kinemage 5 and follow the text below, animating in each of these views.

*\*{KINEMAGE 5}\* All-atom Contacts to Choose Asn/Gln Flips*

Kinemage 5 shows the use of contact dots to illustrate detailed molecular contacts in the structure of ribonuclease F1. In this case, they show correct vs incorrect sidechain amide orientations for Asn/Gln residues. [H atoms added and optimized by Reduce and dots calculated by Probe, as explained in Word et al. 1999, J.Mol.Biol. **285**: 1735.] Contact dots occur on both van der Waals surfaces when two atoms are 0.5Å or less apart, and are color-coded by how small the gap is, with H-bonds in greentint and unfavorable overlaps as spikes in shades of orange and red. [To help understand which dots come from which atom, you can press the 'l' (el) key to toggle between gap coloring and atom-type coloring: O red, N blue, and C white, with H matching their bonded atoms. In the kinemage file, the atom colors are assigned by list and the gap colors separately for each dot.]

View 1 is a close-up of Asn10, which H-bonds to a Thr Og on the neighboring beta strand, with good van der Waals contacts as well as the H-bond. Animate to flip the Asn amide by 180 degrees, to see why it must be assigned in the original (green) orientation, since the NH<sub>2</sub> group would have bad clashes and no H-bond in the other (pink) orientation.

Views 2-4 show Asn/Gln sidechains on an alpha-helix. Gln15 is a "cap box" Gln which helps specify the N-terminus by H-bonding to the backbone NH of the helix N-cap residue: \_\_\_\_\_; it also has numerous favorable contacts with its surroundings, but is disastrous if flipped. Asn21 and Gln25, in successive helical turns, make one H-bond whose geometry is better with both sidechains in the original orientation than with both flipped (flipping just one would be very bad). Asn 29 just precedes the helix C-terminus and makes an Hd1 to carbonyl O H-bond with residue n-4 (the magenta spike means that the H-bond is just slightly too short).

In View 5, Asn44 reinforces a tight turn by making a double H-bond with the backbone of Phe 48. Rotate it around to appreciate the shell of good contacts around Od1, including an H-bond to water # \_\_\_\_\_. Animate to see that the flipped state would be sterically as well as electrostatically impossible. For a simpler contrast, try

turning off the "contacts" button temporarily and animate again.

In View 6, Gln57 lies nicely on a neighboring peptide and against the edge of a Pro ring, while the flipped state has approximately equivalent water H-bonds and peptide contacts, but clashes with the Pro. This illustrates that amide orientation can often be unambiguously determined by H atom contacts even when H-bonding is either equivalent or absent.

Asn 81 (View 7) makes a "pseudo-turn" where Od1 H-bonds to the NH of \_\_\_\_\_, plus 3 other sidechain H-bonds and good van der Waals contacts (everything is very slightly too tight, but not enough to worry about). The flipped orientation is blatantly wrong, with impossible clashes and no H-bonds; try turning off "contacts" temporarily for a simpler contrast.

Asn 83 (View8) is one of the cases (about 15% of Asn/Gln) that is so exposed it has almost no interactions. The score would be very slightly better in the pink orientation, but not enough that we would feel justified in flipping it. Indeed, in the molecule this sidechain probably samples both conformations. [Note that the dots visible at lower left come from Asn 81 and are not relevant to the 83 sidechain.]

Choosing the correct orientation for Asn 107 (View9) depends on the geometry of amide-water H-bonds. Turn off "contacts" and rotate to see how the O and the NH<sub>2</sub> interact non-equivalently with 2 of the 3 waters. Which end of the amide is more forgiving, the O or N? \_\_\_\_\_

View 10 is an overview, to see how this kinemage was pruned down to include only the parts needed for the set of close-up views. Also, the "contact" dots and the H atoms are given a "lens" parameter, which means they will only be shown within a specified radius (default=7.0) of the current center. [To see what was suppressed by the lens, toggle the 'e' key.] This improves rotation and comprehensibility of overviews while allowing more detail in close-ups.

## 2. 1ubq (ubiquitin) self dots

In MolProbity, fetch the PDB file 1ubq; move the thumbnail kinemage to make sure it is a small, monomeric  $\beta$ -sheet protein with one helix; check the tabulated info about the file. Continue. On the main page, click on "Add hydrogens" and run with the defaults. In the resulting page, click to regenerate H, applying only selected flips, then continue (you've already studied a flipkin). Back on the main page, choose "Analyze all-atom contacts and geometry", and read the options but just run with the defaults. On the summary page you will see that this file has excellent Ramachandran values, so-so all-atom clashes, and poor bond angle geometry, and very poor sidechain rotamers.

Choose to view the multi-criterion chart. Click on the heading of the "Rotamer" column to sort by that criterion. The first 6 residues each have at least one nearly-eclipsed sidechain chi angle (that is, near  $-120^\circ$ ,  $0^\circ$ , or  $+120^\circ$ ). For residue \_\_\_\_\_, chi \_\_\_ is \_\_\_\_\_ $^\circ$ ; for residue \_\_\_\_\_, chi \_\_\_ is \_\_\_\_\_ $^\circ$ . Note that 3 of the 6 also have bad clashes. Sort on the Clash column; which residue has the worst clash? \_\_\_\_\_. Close that window, and choose to view the multi-criterion kinemage on-line in KiNG.

Look at how the various types of outliers are flagged. Then turn on mainchain and sidechain. In the overview, try turning off master buttons for the different classes of dots, to see just the H-bonds (pale green pillows of dots) or just the bad clashes (red and pink spikes). Note that most clashes are on the outside, involving either high-B sidechains or waters. Pickcenter on one or two examples and zoom in to see what atoms are clashing; click on an atom to see both its identity and its B-factor. If B is greater than about 30 or 40, the position is uncertain and clashes are more probable. Find one of the gold-highlighted Leu or Ile sidechains that you think may have been fit backwards: \_\_\_\_\_. What is the value of its most-nearly eclipsed Chi angle? \_\_\_\_\_ Turn the vdW contacts back on, center on an interior sidechain, zoom in, and look for some areas with especially nice packing. Close the Java viewer (in the window behind it), return to the main page, and run the rotamer check function on 1ubq. Is your possibly-backwards Leu or Ile on the list of bad rotamers? \_\_\_\_\_

Close the kinemage window, and continue. Back on the main page, logout of MolProbity and delete files. Then,

start a new session for section 3.

### 3. 1dad (dethiobiotin synthase) protein/ligand dots

Now you will calculate all-atom contacts just for the interface between two sets of atoms, in this case between the protein and the bound ADP. Bring in the PDB file 1dad; add H to it, unchecking the option to make a Flipkin. Apply the flips, and continue.

Back on the main page, choose "Visualize interface contacts". For the source pattern, leave Protein and both chains checked, but uncheck the rest (DNA/RNA, hets, and waters); for the target pattern, leave hets and both chains, but uncheck the rest. Examine the various options offered, but run with the default settings. Choose to "View in KiNG".

You should see contact dots just around the pink ADP group. Pickcenter there, zoom, and perhaps adjust the clipping planes, to look at the interface.

Are there any bad clashes (hotpink spikes that don't go away when you turn off "small overlap")? \_\_\_\_\_ If so, between what atoms? \_\_\_\_\_ on ADP, \_\_\_\_\_ on protein.

[Temporarily turn off master buttons for the contacts and overlaps, to see just H-bonds.] How many H-bonds are there? \_\_\_\_\_ and to what parts of the ADP? \_\_\_\_\_

With contacts back on, admire the many good contacts that make the binding both favorable and specific. Close the window, continue, log out and destroy files.

As you saw, the interface feature in MolProbity can do many sorts of contact analyses. The Probe program itself has even wider options and more specific control over atom selections, when run off-line. If you want to do something more complex, therefore, look at the "Tips" page and the "probe -h" help.

### 4. 1iftH.kin (340KB) (ricin A chain)

For ricin, Reduce and Prekin have already been run for you and the active-site Glu177 highlighted. Open 1iftH.kin in KiNG and go to the closeup in View 2. Glu177 was known to be the catalytic residue for ricin's toxic cleavage of ribosomal RNA, and mutating it to Asp greatly reduces that activity. However, to everyone's consternation, the "control" mutation to Ala had nearly normal activity. In an attempt to explain that behavior, we noticed that Glu208 is perhaps close enough to move and substitute for the Glu177 carboxyl - you can test that idea.

Choose Tools -> Structural Biology -> Sidechain mutator, browse to locate the 1IFT.pdb file, middle-click on an atom in Glu 177, and choose to mutate it to Ala. You should get a new Ala sidechain in wide orange, but with no rotatable angles. Now choose Tools -> Structural Biology -> Sidechain rotator, and middle-click on Glu208 (behind in this view) to get a dialog for adjusting the sidechain; move the box off to one side. In the Model manager box, check "Probe dots"; you should see dots around the Ala177 and Glu208, including some good H-bonds.

Click in turn on each of the rotamers for Glu208 and identify the two quite different ones that put the 208 carboxyl near where the original 177 carboxyl had been:

those 2 rotamers are called \_\_\_\_\_ and \_\_\_\_\_

. Pick one of them to try optimizing by bond rotations; see if you can minimize the clashing and form an H-bond with the Tyr OH, while still being fairly near rotamer values and near at least one of the original 177 carboxyl O's. Would this conformation be possible in the Glu 177 to Asp mutant? \_\_\_\_\_

Quit from kinemage without saving.