BCH622 - Using All-Atom Contact Analysis

Reading

Read the Word et al. (1999) J. Mol. Biol. **285**, 1711 paper (pdf is on the course web site) on the all-atom contact-dot method. In Procedures, you can skip from "scoring" to the start of Results.

Are the faces of aromatic rings considered to be H-bond acceptors? _____ Are CH groups considered to be possible H-bond donors? _____

Where are many clashes seen in large or low-resolution nucleic acid structures?
_____

Of the four successive steps of correction applied to the 100 data files, which one lowered the clashscore the most? _____

What is the explanation given for why the χ3 angle of Met prefers gauche (+/- 60°) over trans values?
_____

Graphics assignment

In this exercise, after a brief "opener", you will go through two all-atom contact example files for analyzing interfaces; view a demo explaining Reduce's correction of Asn/Gln/His "flips; run Reduce on ubiquitin to add H atoms (on the MolProbity web site) and then Probe to generate contact dots and look at them, and compare with a more recent, high-resolution structure; and lastly try a kinemage set up to interactively update contacts as you rotate sidechains, for evaluating a single-site mutation.

1. ☐ **3Dlit_tetrPlanar_CO.kin (400KB)**

As an initial step, to make sure you're up to speed with the 3D shapes of sidechains like Asp vs Leu, go through the same "3D lit" exercise you did as a timed test, but this time as a leisurely exercise to actually learn the distinction. Rotate each potential example, to see whether the whole group lines up as planar from some view or not, and to get a feel for what the non-planar, tetrahedral groups look like. Remember that the tetrahedral ones actually have a 4th atom attached (the H), not shown here or in most graphics you'll encounter.

2. 1dad (dethiobiotin synthase) protein/ligand dots

Now you will calculate all-atom contacts for the interface between two sets of atoms, in this case between a protein and its bound ADP. In MolProbity, fetch the PDB file 1dad; add H to it; accept 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.
Now temporarily turn off master buttons for the contacts and overlaps, to see just H-bonds (pillows of pale green dots). 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 -- blue and green favorable van der Waals, as well as H-bonds.
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 in your project, therefore, consult the "Tips" page or the "probe -h" help.

3. 1brs (barnase/barstar) interface contact dots

In MolProbity, fetch the PDB file 1brs. Notice in the thubnail kinemage that there are _____ copies of the complex in the repeating unit of the crystal; continue. On the main page, choose "Edit PDB file", choose edit option to delete all chains except A and D (which will give you one complex with a barnase enzyme and a barstar inhibitor). Then Add hydrogens with the defaults and continue.

Back on the main page, choose "Visualize interface contacts". For the source pattern, choose only Protein and chain A; for the target pattern, choose only Protein and chain D, at the top choose "kinemage only", and then "Run Probe". Choose "View in KiNG", to see the contact-dot interface for the Barnase/Barstar, probably the most-studied example of a protein/protein contact.

There are a good many waters in or near the contact (peachtint balls), but only 3 or 4 H-bond to both proteins. Turn off the buttons for waters and for H's, and zoom in on the contact region somewhat. Move the view back and forth gently, to study the size and shape of the interface as oultined by the contact dots. Are there any clashes ("bad overlaps)? _____ ; any small overlaps? _____ Turn off buttons for the vdw contacts and the small overlaps, to concentrate on H-bonds. Turning on just one master button at a time for contact type, count how many H-bonds (pale green dot pillows) are McMc (mainchain-mainchain): _____, how many are McSc: _____, and how many are ScSc: _____.

Close the window, continue, log out and destroy files.

4. ☐ 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 0.5A 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 NH2 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 NH2 interact non-equivalently with 2 of the 3 waters. Which end of the amide is more forgiving, the O or N? _____

5. 1ubq (ubiquitin) self dots

In MolProbity, fetch the PDB file 1ubq; move the thumbnail kinemage to make sure it is a small, monomeric β-sheet protein with one helix; check the tabulated info about the file; what is its resolution? _____. 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 in part 4). 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, poor bond angle geometry, and very poor sidechain rotamers. What is the MolProbity score? _____

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°, 0°, or +120°). For Ile 13, chi ___ is _____°; for Leu 15, chi1 is _____° and chi2 is _____°. 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 (at common resolutions), the position is uncertain and clashes are more probable. Find, center on, and zoom in on the gold-highlighted Ile sidechain (meaning that it has a poor rotamer): residue _____. Turn to look down the Cα-Cβ bond to judge the χ1 angle; is it staggered, eclipsed, or in between? _____ Turn the vdw contacts back on, center on an interior sidechain, zoom in, and look for some areas with especially nice packing. Close the KiNG window and return to the multi-criterion chart. What is the % score for your possibly-backwards Ile? _____ How far off from eclipsed (+/-60° or 180°) is its χ1 angle? _____

Close the kinemage window, and continue. Back on the main page, logout of MolProbity and delete files. Then, start another session to compare with a newer, 1.31Å structure of ubiquitin (done by Lindsay Deis). Browse to upload the file ubiquitin_1.31A_2012.pdb that you got from the course website; does it include H atoms? _____. Move the thumbnail image around - how many chains are there? _____ On the MolProbity main page, choose to analyze all-atom contacts and geometry. Admire the resulting summary chart. What is the MolProbity score? _____

In the multi-criterion chart, what is the chi1 angle of Ile 13 this time? _____ chi1 of Leu 15? _____ chi2? _____ Close the chart window, continue, and exit from MolProbity.

Open the ubiquitin_1.31A_2012-multi_map.kin file in KiNG; it's the same multi-kin you just made, but with part of the (large) electron map. [The gray balls are Zn ions and the pink hets are acetates, added to aid crystallization.] Edit/Find point "zn 1" to find the highest-occupancy Zn. Turn on the electron density map, sidechains, and waters. How many ligands does the Zn have? _____ Are they closer to tetrahedral or to square-planar? _____ Edit/Find point "cb 13" to center on Ile 13. Is there any doubt at all about its staggered conformation? _____ Move over to Leu 15; can you see the boomerang shape that sets the direction of tetrahedral bend at the cg? _____ Close the kinemage with out saving.

[Note: For part 6, if the remote update in KiNG doesn't work, check that the pdbH file name and location (same place as kin file) 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 line generated automatically in the remote update process. That command line is displayed in the appropriate dialog box when you are working in KiNG, and you will want to

cross-check file and program names if troubleshooting is needed.]

6. ☐ 1lmb6_85aH-KiNG.kin (340KB) (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.