BCH 622 - Exercise on crystallographic resolution, in trypsin electron density maps

Files needed for this exercise: 1HJ8_1.0A.kin, 1.0_1hj8.omap.gz, 1C9P_2.8A.kin, and 2.8_1c9p.omap.gz -- downloaded from the BCH 622 web page

***1HJ8 TRYPSIN AT 1.0A RESOLUTION ***

Open the kinemage file 1HJ8_1.0A.kin in KiNG.

Choose "beta" on the Views menu. You should see several vertical beta strands, with Val 52 at the center (click on an atom, to see its identity on the info line at the bottom of the graphics window). Drag right from "Structural biology" on the Tools menu, and release on "electron density map". Choose your 1hj8 omap.gz map, and OK its format. Be patient for it to load - it's very big. Move the contour window off to the side, and turn on both 1.2 (gray) and 3.0 sigma (purple) contour levels. You should be able to see clear density for all the non-H atoms. Note that the Val 52 sidechain is in a staggered orientation relative to its backbone.

Turn off the gray contours and move or click the slider for the purple contours up to 8.0 sigma, where the difference in x-ray scattering power between different atom types becomes evident. Click on some of the atoms with the largest peaks: what element type are they? _____ Most but not all of the C atoms have disappeared at this contour level. What element type here has intermediate size peaks (and thus intermediate scattering power)? _____

Not all atoms of a given type show up equally strongly, because they are not all equally well ordered. Click on several Calpha atoms (where the sidechain joins the backbone) that do show small purple density peaks; what are their B-factors (given on the info line, along with their identity)? _____ , _____ , _____ . Pick several Calphas without peaks; what are their B-factors? _____ , _____, _____.

Choose the "helix" view. Almost no peaks are visible at 8 sigma, but if you shift to 5 sigma most backbone atoms show, and at 4 sigma most sidechain atoms. This helix is at the C-terminal chain end and has somewhat higher B's. Right-click on the double ring of the Trp sidechain to center there, and turn on the gray 1.2 sigma contour. Is there a hole thru both 5-membered and 6-membered rings? _____ Zoom out (right-drag up), center near the chain end, and notice that the end is disordered enough for even the gray contour to disappear.

Look at the views for the Ser-His-Asp catalytic triad, benzamidine inhibitor, and Arg 66, which are all extremely clear and well-ordered. In the SS 42-59 view, note that the S atoms have even bigger peaks than oxygens. Radiation damage has oxidized and opened the disulfide bond in some fraction of the molecules. Click on the S atom of the open form; what is its occupancy? _____
The 2 conformations of that S atom are quite distinct, not just a smear.

Choose the "Gln 192" view. The backbone CO has two widely separated alternate conformations, one H-bonded to an SO4 (pink). The Gln Calpha and Cbeta densities are smeared between two close alternates, and beyond that the density essentially disappears (lower contour level doesn't help very much). What is the occupancy and B-factor for one of the Cg atoms? occ:_____ , B:_____ ; for one of the terminal N or O atoms? occ:_____ , B:_____ When an atom is not visible at all in the electron density, some crystallographers omit it from the model, some set its occupancy to zero, and some let its B refine very high. Note that the information content is much worse for this sidechain with high B (or low occupancy in each of probably many conformations) at atomic resolution than for the well-ordered parts of a much lower-resolution structure like the one below.

Choose the "neighbor e.d." view, which is part of a neighboring molecule in the crystal with no model shown in this kinemage. What is the amino-acid type of the residue at center? _____ Are the 5 atoms in its ring planar or puckered? _____

Keep in mind that initial maps seldom look this good; the phases and the density quality both improve during refinement.

Close KiNG.

*** 1C9P TRYPSIN AT 2.8A RESOLUTION ***

Open the 1C9P kin file in KiNG, and read in the 1c9p map. Go to the "beta" view. Click on some backbone atoms; what is the best (lowest) B-factor you find for one? _____ How does that compare with the B-values you found for similar atoms in the high-resolution structure? _____ Open the 2Fo-Fc map, and drag slowly back&forth to judge the density shape of the Val 52 sidechain in 3D. Is it concave left, concave right, or symmetrical? _____

Center on the Ile sidechain to the right of the Val, and drag left to view it from the side. Does the density show which branch is the longer one with the extra Cdelta atom? _____

Choose the "helix" view. Drag back&forth gently to see the spiral shape of the backbone density and the small bumps for the backbone O atoms. Center on the Trp sidechain. Is there a hole in either ring? _____ Would you know from the density that this was a Trp? _____

Choose the "His end" view to see the ring cross section. Could it be plausibly turned 90° to sit crosswise in the density? _____ ; turned by 15°? _____ Note the nearby Asp; the His orientation is fine-tuned by its hydrogen bonds, in the absence of higher resolution.

Look at the other views. Note that the disulfide S atoms are not resolved into separate peaks, altho their positions are clear. Click on one, then the other, S atom: what is their distance? _____Å. For Gln 192, note that it has quite reasonable density in this structure, presumably because it interacts with the BPTI inhibitor molecule.

Close KiNG.

Model quality, validation exercise.

You will need a web link to MolProbity (with Java), and the file 1JIRon1S83_Arg66_supr.kin downloaded from the kinemage.biochem.duke.edu BCH291 web site.

Part 1: MolProbity

Go to the MolProbity web service (at http://kinemage.biochem.duke.edu, click MolProbity on the navigation bar) and fetch PDB file 1JIR (not case sensitive). Check that you got a trypsin at 2.0Å resolution. What is the R value? _____%; the Rfree? _____% That is very good for 2Å, presumably because of information from previous structures at higher resolution. Continue to the main page, ask to add hyudrogens, and run with the default settings.
The resulting chart shows no His flips but 10 amide flips; the largest score differences are for Asn _____ and Gln _____ .

Pick "View in KiNG" for 1jirH-flipnq.kin, and animate between the two orientations for some of the views marked * for flips. Gln 30 has no clashes in the unfavored (pink) position, but in the clearly better flipped version (green) it makes _____ H-bonds.
Asn 48 makes a pseudo-turn H-bond to the backbone _____ atom of residue _____, but in the incorrect original position the NH2 has really dire clashes (not evident, of course, if the crystallographer had not added those H atoms).
Gln 64 is similar, but the clashes or H-bond are to the sidechain of _____ _____.
Close the KiNG window, and "regenerate H", accepting the flips; continue.

On the main page, chose "Analyze all-atom contacts and geometry", and run with the defaults. While waiting, you can preview the Ramachandran kin or pdf, seeing that this structure has excellent phi,psi values with no outliers. The summary statistics are also good, almost all evaluated as green; the clashscore of 7.94 is at the _____ percentile for this resolution. But good average scores do not protect against local errors. Click on "Multi-criterion chart" for per-residue scores. Click on "Rotamer" to sort by increasing rotamer quality. The worst rotamer is for Arg 66 (0% of the high-quality data is this bad, giving a a score of 0%); note that it also has a serious clash, with an overlap of _____Å. Sort on "clashes", to see that no other sidechain has both a bad rotamer and a bad clash.

Close the chart window, and view the multi-criterion kinemage in KiNG. On a backdrop of the Calpha trace and the non-water "het" groups (in pink, or gray balls for metals), this kinemage shows bad sidechain rotamers in gold and serious clashes as clumps of hotpink spikes. Find the gold sidechain for Arg 66; how many clash clumps does it have? _____ [Before flipping Gln 64, there would have been more.] Center on the Arg, zoom in, and turn on sidechains. The planar Arg guanidinium is stacked between the sidechains of residues _____ _____ and _____ _____. We will study Arg 66 further in the next part.

Close the KiNG window and continue to the main page. In the file list, click on the triangles to expand the outline, to see all the viewable or downloadable file you have accumulated. This time you will look at a further modified version of the multi-kin, so logout of MolProbity now: "logout" on left side panel, then click "Destroy all my files and log out" to clear your workspace on the server.

Model quality, validation exercise (cont'd).

Part 2: local comparison of 2Å and 1.25Å structures.

One of the few problems with the 1JIR bovine trypsin structure at 2Å resolution is Arg 66, with serious clashes and a very bad rotamer although it fits quite acceptably in the electron density. Rotamer and all-atom contact criteria were used to refit Arg 66, with the Asn 64 flip corrected and the Arg guanidinium group flipped over in its density to make two good H-bonds. To test the validity of that correction, we will coompare with a more recent porcine trypsin structure at 1.25Å resolution.

Open the 1JIRon1S83_Arg66_supr.kin kinemage in either Mage or KiNG, and note the green Ser-His-Asp sidechains of the trypsin active site. Go to the "Arg66" view, which shows the original 2Å 1JIR model (gold) in its 2.0Å density, and its all-atom contacts, with several bad clashes (red spikes) [with the starting button selection with "*1JIRa"].
Animate to "*refit Arg 66", the model refit by adding additional steric and dihedral constraints (orange bonds). It is an excellent rotamer; are all the clashes gone? _____
Now animate to "*1S83Ha", the actual 1S83 model refined at 1.25Å (cyan). Are the 1S83 atoms cleanly centered in their atomic-resolution density peaks? _____
All 5 guanidium NH's make H-bonds, 2 to Gln 64 Oe1, one to a water, and the other two to _____.

Turn off the "Arg dots" and the "1jir map" buttons;
turn on the "*refit Arg 66" button as well as the "*1S83Ha" button.
Click on pairs of equivalent atoms in these two sidechain models to find their separation (reported on the info line at the bottom of the graphics window); what is the largest difference? _____Å
Turn off the "*refit Arg 66" and turn on the original "*1JIRa". Is the original model clearly wrong? _____
What is the distance between its cd atom and the 1S83 cd? _____Å.
What is the largest distance between two equivalent (same name) atoms? _____Å for the _____ atom.
Protein structures always need to use extra information in the form of bond lengths and bond angles (known from high-resolution small-molecule crystal structures and from quantum calculations); at medium to low resolution we have seen that accuracy can be improved by also adding in knowledge about dihedral-angle preferences and all-atom sterics.

Go to the "Gln 64" view with only the original 1JIR model on; is the N or the O near Arg 66? _____
For 1JIR, Gln 64 was flagged by MolProbity as needing an amide flip for steric and H-bonding reasons.
Switch to the 1S83 model; is the N or the O near Arg 66? _____.
At this resolution, does one branch have clearly higher electron density? _____; which? _____.
The 2Å model seemed well centered in the 2Å map, but that model was displaced slightly from the position of the well-fit 1.25Å model. Note that the phases for the maps come from the model and this model bias tends to make the map fit whatever is the model!

Arg 66 and Gln 64 were incorrectly fit and refined into the wrong local-minimum conformation. Look at the next 3 views with the 1S83 map and both 1JIR and 1S83 models on, to compare the basic accuracy of correctly-fit sidechains at 2Å resolution. Which of the 3 sidechains matches the atomic-resolution map and model almost perfectly? _____ Which one deviates the most? _____, by what maximum atom separation? _____Å for the _____ atom. So, at 2Å resolution would you judge that a typical atom is known to an accuracy of about 2Å, 1Å, 0.2Å, or 0.1Å? _____Å However, you have seen that a few atoms may be displaced by very large amounts: 2-3Å if a group is flipped over (and even 5-10Å occasionally, if the local density is very poor).