

Files needed for this exercise: 1HJ8_1.0A.kin, 1C9P_2.8A.kin, downloaded from the BCH 291 web page

***1HJ8 TRYPsin AT 1.0A RESOLUTION ***

Take your browser to <http://kinemage.biochem.duke.edu> and click MolProbity in the navigation bar.
[If you don't have Java, follow the directions to get it.]

Set the "browse" file type to "kinemage", browse to find the 1HJ8_1.0A.kin, and upload it.
Continue. Set the "fetch" file type to "2Fo-Fc (EDS)" and the pdbID to 1HJ8, and fetch it (takes ~15 sec).
Back on the MolProbity main page, expand the kinemage entry in the file list and ask to view your 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 2Fo-Fc 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 C γ 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 the KiNG window.

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

Repeat the above procedure to upload the 1C9P kin file and fetch its 2Fo-Fc map from the EDS.

Open the kin file from the kin list on the MolProbity main page, for viewing in KiNG, and 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 degrees to sit crosswise in the density? _____ ; turned by 15 degrees? _____ 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: what is their distance? _____ A.

For Gln 192, note that it has quite reasonable density in this structure, presumably because it interacts with the BPTI inhibitor molecule.

Close the KiNG window and log out of MolProbity.