

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 hydrogens, 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 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.

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 compare 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 guanidinium 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).