

Map Fit // Model Building

Reading

C.I. Branden and J. Tooze (1999) "Chapter 18: Determination of Protein Structures" in Introduction to Protein Structure, Second Edition.

J.S. Richardson & D.C. Richardson (1985) "Interpretation of Electron Density Maps" in Methods in Enzymology, **115**, pp. 189-206. PMID: 3841180

In-class exercise (following week)

helix chain-trace in mini-maps

[Work with the stacked-sheets electron density contours from part of the Staph. nuclease 2 Å electron density map. This region is one of the α -helices; the object of the exercise is to build/envision an approximate model and (by identifying sidechain shapes in the map) to locate this region in the amino-acid sequence. A copy of the sequence will be provided, including a secondary-structure prediction to help you locate potential helical candidates in the sequence.]

Graphics assignment: Map-fitting into electron density, using KiNG.


The two aims are:

1. to learn where the coordinate data come from and judge their probable reliability vs resolution and solvent exposure, and
2. to learn shapes and appearances of sidechains in preparation for the chain-tracing class exercise.

There are 4 parts, for different fitting tasks at varying resolutions.

Part 1: 1mjf putative spermidine synthase at 1.8Å, structural genomics (SECSG): repositioning His ring.

The 1mjf map file is available from the class web site if needed, but you should try downloading it from a very useful web site called the Uppsala Electron Density Server (<http://eds.bmc.uu.se/eds/>). Enter the 1mjf code, go to "Maps" on the left-hand menu, and ask to generate the 2Fo-Fc map as O format; with the right mouse button, ask to save link target, to download the map file. Get the kin and PDB files from the class website.

Launch KiNG with file  [1mjfHa.kin](#) (380KB). With "Find point" on the Edit menu, type in "cg 263"; zoom in (drag down with the right mouse button), and clip to remove most of the obscuring things in front of the His ring - or, just choose view2. Under the Tools menu, choose Structural biology/ electron density maps and browse to find file 1mjf.omap.gz. You should see gray contours appear, and a small control window that should be moved off to the side. From this view above the ring, the H atoms stick out of density (which they should), but the heavier-atom fit looks very good, meaning that the χ^2 angle is fit well.

Choose view3, or rotate to view the ring in-plane from the sidechain end; the density is rounded, but about twice as wide as thick - the model is not correctly aligned to the plane of the density. Under the Tools menu, choose Structural biology/Sidechain rotator and then select 1mjfH.pdb as the model file; move the Model manager dialog off to one side, and turn on Probe dots. Click an atom in His 263 with the middle mouse button (with a one-button mouse, add the Control key) to activate fitting of His 263 - you should get an orange, idealized sidechain and a rotation-control window, which can be moved to a convenient position that doesn't block the graphics. Drag across the bottom of the chi2 circle to refit the angle to match the density. (Note that the left mouse button makes a rapid angle change and the right button a slow one.)

What value of χ^2 fits best? _____

What percentile score is given for this conformation in the rotamer window?

_____ Are the contacts and H-bonds for the His ring better or worse after your change? _____ Reset with "original" in the rotamer list. Note that χ_1 is near 180°, so try rotamers starting with "t". Which one fits? _____ Is it essentially the same as your fit? _____

Choose "Finished" and "Yes", to accept your refitting.

Explore the neighborhood in this map at 1.8Å resolution. (Zoom out a bit and recenter on what looks interesting, or use the Edit/Find point function.) For the nearby Phe 267, look for a dimple in the center of the ring; look end-on, to see that the cross-section of its 6-membered ring is more flattened than for the 5-membered His 263 ring.

**For Pro 63, look edge-on to the ring:
does the density clearly support the direction of Cy pucker? _____**

Quit out of KiNG without saving anything.

Part 2: 1bkr actin-binding domain (calponin homology) at 1.1Å resolution: refitting an Arg sidechain.

Launch KiNG with file [1BKRH-contact.kin](#) (496KB) (which you could have made in [MolProbity](#) and downloaded). Move it around and locate the biggest clump of red clash spikes. Center on it with a right-button click (or shift-click, for a one-button mouse), zoom in to view the Arg 32 guanidinium, and narrow the zclip. Under Tools/Structural biology/Electron density maps, browse to find file [1bkr_2FoFc.omap.gz](#) (1.3MB); move the map dialog to one side. There are bad clashes with waters, and the sidechain occupies only part of the density. It looks as though one of the waters was fit where the sidechain should be. To set up for refitting, choose Structural biology/Sidechain rotator" under the Tools menu, select the [1BKRH.pdb](#) (256KB) model file, turn off "dots" and enable Probe dots in the Model manager box. Then right-click on an atom in Arg 32 to enable rotations. Look at Arg C γ to confirm that χ_1 should be plus (opposite to the alpha H). Try out all the p rotamers, to find the one positioned closest to the sidechain density.

Shift the 4 dihedral angles to come as close as you can to matching the density; the

values are: chi1 _____, chi2 _____, chi3 _____, chi4 _____. Are there any bad clashes now? _____ What is the percentile score of your Arg conformation? _____ (Note that H-bonds to waters are not shown, since they are often changed when refitting.) Which water should be deleted? hoh # _____

Release and accept.

A refit Arg similar to yours was put back into crystallographic refinement. Append file [1bkr_later.kin](#) (80KB) to see the position it ended up in.

Does it fit the electron density convincingly better than the original model?

Quit out of KiNG

Part 3: Cabenol Ca⁺⁺ binding protein at 2.2Å resolution, structural genomics (SECSG): refitting a Trp sidechain.

Launch KiNG with file [cabenolH.kin](#) (244KB). Find trp 179, zoom way in, center on the ring, and clip down around the ring flat in the screen plane.

Measure (under Tools) the chi1 angle: _____.

That is too close to eclipsed; we initially found this problem on MolProbity's list of bad rotamers. Open the electron density map called [cabenol_2FoFc.omap.gz](#) (404KB).

Does the Trp ring fit the density fairly well? _____

Now open the difference density map ([cabenol_FoFc.omap.gz](#) (400KB)); click on "Presets" and choose Fo-Fc; move the dialog out of the way. Orange contours mean there is too much model in that region, and blue ones that there is not enough - in this case they strongly suggest that the ring is elongated in the wrong direction. Choose the Sidechain rotator tool with [cabenolH.pdb](#) (240KB) as the model, turn on Probe dots, and move the box out of the way.

Center-click an atom in the Trp, try rotamers (you know χ_1 should be m) to find the nearest, which is _____. Adjust the angles to get a good fit in the density and with good Probe contacts (but the other way around - it should have more atoms in the blue difference density than before). X1: _____, X2: _____, % _____. Find the ne1 atom; is there something that might provide an H-bond partner for it?

Finished, yes, to keep your change.

Discard the difference map, and zoom out. Center on a nearby helix and study the appearance of its density (perhaps adjust the map scope a bit larger with the top slider in its dialog box).

Look end-on down the helix axis; is there a hole down the density? _____ Try turning the model on & off to get a feeling for the density shape of helical backbone; do the CO groups stick outward or inward from the general backbone

worm shape? _____

Do they point N-terminal or C-terminal along the helix direction? _____

Find Tyr 180; compare the planarity of the ring, OH, and C β atoms with the pronounced "elbow" at the _____ atom.

Find a Met, to see what the sidechain sulfur looks like; turn on the 3sigma purple density, and slide it to higher values until it almost disappears: at what value? _____ for which Met? _____

Do a find on cg for lysines 23, 53, and 81, and evaluate their density; which one has strong density all the way to nz? _____; which has density that gradually tapers out? _____;

which disappears abruptly _____, last enclosed atom _____?

For this last case, adjust the density contour lower, to see why they fitted the sidechain where they did; at what value can you see to the end? _____

Sometimes the density for an exposed Lys is invisible beyond C β , because it is free to adopt so many conformations.

Part 4: 301d hammerhead ribozyme at 3.1Å resolution, to see lower-resolution density and a nucleic acid map.

Launch KiNG with file [301dH_mcCon.kin](#) (156KB), noting that there are some bad clashes along the backbone. Zoom in on the tetraloop at the top (or choose View2), and open electron density file [301d.omap.gz](#) (116KB). Move around in it to see the shapes of bases and backbone; try changing both contour levels, to see if you can find a gray contour that encloses the bases and a purple one that is just the phosphates; then try it in the crowded 151-152 region. Fill in the table below with your estimates of yes, no, or maybe for whether the listed feature can reliably be placed in density at this resolution.

	yes	maybe	no
overall fold or trace			
base pairing			
position of base			
orientation of base ring			
phosphate position			
sugar pucker			
backbone dihedral angles			