

BCH222 :: Structure of Biological Macromolecules

1st Graphics Assignment

Read Section I Background, parts A–C of the annotated, on–line "Anatomy & Taxonomy of Protein Structure", which include explanation of the web version, Intro, Amino Acid & Backbone, and Levels of Error. Links to the Anatax, software, and kinemage files can all be found on the BCH222 course web site at <http://kinemage.biochem.duke.edu/teaching/bch222/>.

If you are not already somewhat familiar with polypeptide geometry, please study Fig. 5 in the Anatax. You may also want to explore kinemage 1 in the [c1Basics.kin](#) (132KB) file, which shows the same information in 3D. Open it in KiNG, left–drag to move the viewpoint around, and turn on & off the angle and atom labels and the H atoms with buttons on the right–hand button panel. Turn on the "spheres", to get a feeling for the bulk of the atoms and their bonded interpenetration. With spheres off, enable bond rotations under the Tools menu by choosing Specialty → Suite rotation, which gives you a rotation widget with a choice of possible angles to rotate. Try PHI and PSI, then CHI1 and CHI2. [Don't use the "end" angles, which are a temporary artifact of the current process of updating KiNG to work with these lessons.] Left–button drag around the circle edge changes the angle quickly, right–button drag changes more slowly, and the + and – boxes very slowly. Quit out of KiNG when you're done.

Worksheet: Getting Comfortable with Protein Geometry

1. Finding direction and chain termini

- a. Open the provided kinemage file [1a6m.kin.zip](#) (464KB) in KiNG, which shows the mainchain from a crystal structure of oxy–myoglobin refined at 1Å resolution, with color–coded balls for the non–C atoms (O red, N blue). In the overview, drag to rotate the molecule slowly back & forth, to see in 3D the arrangement of helices that enclose the heme group (in pink). Turn on sidechains and sc atoms. The central Fe atom (orange) of the heme has an O₂ ligand bound on one side; what is the amino–acid type and the residue number of the sidechain ligand on the other side? [You can click on an atom in the sidechain and read its pointID at bottom left of the graphics window, or figure it out by the shape and atom types of the sidechain.]
- b. With just mainchain and mc atoms on, choose "helix side" from the Views pulldown menu. Given that the backbone carbonyl (CO) groups in a helix point toward the C–terminus and splay outward slightly, determine the N to C direction for the helix. In this view, is the beginning of the helix at the top or the bottom of the screen? _____
Then turn off mc atoms, and get used to identifying the carbonyl oxygens and their directionality without the red balls.
- c. Remembering that N precedes and CO follows the C–alpha in each residue, practice determining the local chain direction in loops and ends as well as helices. Move around in the molecule by a right–click to center on an atom, and zoom in or out as needed. [if you get confused about which is the C–alpha, briefly turn on either the sidechains, or the 'mc atoms' button for O and N atom markers.]

- d. Go to the [Protein Data Bank \(PDB\)](http://www.rcsb.org/pdb) and retrieve the pdb file for this protein (search for the pdbid 1a6m, then click the download icon next to the pdbid on the resulting page). To open this file in KiNG go to File/Import then select 1a6m.pdb from the directory where you saved it. A Molikin window should open. By default, Ball and Stick model is selected, but you will have to turn on backbone and sidechains in the bottom-right checklist. You may like balls on N,O,P,etc as well. Hit As New Kinimage at the bottom when done. This will be your kin.
- e. On your kin, find the helix from the "helix side" view in the provided kin. An easy way to do this is to select an atom in the provided kin's helix, note its residue number, then go to your kin and use Edit/Find Point. Type in the residue number and you will be taken to an atom in that residue. Determine the directionality of the helix and orient it in frame with the N to C direction going left to right. Go to Views/Save Current View and save this view with a descriptive name.

2. Backbone geometry

- a. On the provided kin, choose the "helix end" view, and observe how each peptide forms a straight line seen down the helix axis, while the Calphas are at the corners between peptides. For several examples, turn to look along the peptide plane to see which atoms are planar and how accurately. In a given peptide, which 5 atoms are co-planar?
 _____ , _____ , _____ , _____ , _____
- b. KiNG and Mage have tools to help study geometrical features. Pull down the "Tools" menu and turn on the "Measure" function; the information line at the bottom of the screen will now give you successive identity, distance, angle, and dihedral for the last 1, 2, 3, and 4 atoms you picked.
 Try it out on the provided kin to get dihedral angles phi, psi, and omega along the backbone (if you start with a carbonyl carbon and go forward along the main polypeptide chain, the first dihedral you get will be phi). In a helical region you should get phi, psi, and omega values near -60, -40, 180.
- c. On your kin, go to "Tools" and open the "Edit/Draw/Delete" window. Keep this on "Do nothing(navigate)" unless you plan to alter something. It is recommended to save your kin between edits since some edits are not readily reversed.
 On your kin, find a phi angle in a helix and highlight it using the Edit/Draw/Delete tools. "Paint points" works, but can be hard to control over such a small area (hints: turn off sidechains, you can repaint the backbone "white" if you color outside the lines). "Draw line segments" creates a new object which you can turn on and off, but is easier to control. Once you have highlighted the phi angle, position it nicely in frame and save that view. Name the view "Phi dihedral" followed by the measure of that angle as determined with the Measures function.
 Measure, highlight, and save a view of a nearby psi angle (don't overlap the highlights). Do the same for an omega angle.

3. Sidechains

- a. In the provided kin, turn on sidechains and sc atoms, choose the Pro face or Pro side view, and rotate between them. Note that the Pro ring is not planar. Two atoms are in both the mainchain and the sidechain of the Pro ring; identify which is Calpha and which is N.
 Now find a Pro residue in your kin (you can search for "pro" in place of a residue number in Edit/Find Point). Decide which of the 5 ring atoms (Cbeta, Cgamma, etc.) is most out of the plane defined by the other 4. Highlight this atom with a ball using "Draw balls" from Edit/Draw/Delete and save a view with a descriptive name.
 [NB: the consensus is that Pro should pucker at the Cgamma atom.]

- b. On the provided kin, turn on sidechain and turn off sc atoms. Practice identifying amino-acid residues by looking just at the sidechain shapes (e.g., Asp vs. Leu, Met vs Lys, even Glu vs Gln). Note especially the branch points, and whether those branches are tetrahedral or planar.

What are two pairs of residues that cannot be told apart this way?

_____ vs _____ and _____ vs _____ .

- c. Check yourself either by clicking on an atom to get its ID, or by turning on 'sc atoms' to mark the non-carbons.

4. Levels of error: the good parts at high resolution

- a. The original myoglobin map shown in the introduction to "Anatomy and Taxonomy of Protein Structures" was at 6Å resolution, where only the molecular shape and the position of helices can be seen. This 1a6m structure is at 1Å resolution, where almost every individual atom can be seen. In the provided kin, with both mainchain, sidechains, and their atoms on, as well as the "het groups", choose the heme view and turn on "heme" under the "ED map" group. Notice how thoroughly the map shape outlines all the atoms of the heme group. The gray contours are at 1.2 sigma (or about 0.6 electrons per cubic Å), while the purple contours are at 3 sigma (or about 1.6 electrons per cubic Å). Turn off the gray contours ("1.2 sigma" master button) temporarily to see that each atom has a separate density peak ("atomic resolution", where neighboring atoms appear distinct, is about 1.2Å or better). Which atom has the biggest peak? _____
- b. Choose the Phe 123 view, and turn on just the "Pro and helix" electron density, with both contour levels. Note the hole in the ring and the clear peaks for individual atoms. Turn sidewise to see the flatness of the ring and the tetrahedral "elbow bend" between backbone and sidechain. At what atom does the structure make the elbow bend? _____
- c. Choose the "Pro face" view. Note that the peaks for N atoms are a bit larger than for C, and O peaks a bit larger still; that's because C has 6 electrons, N has 7, and O has 8. This difference is clear in the good parts at atomic resolution, but cannot be distinguished at lower resolutions. Choose the Pro side view, or rotate around, to see the ring pucker. Which atom is out of the plane of the other 4? _____ is there any doubt about the location or direction of pucker? _____
- d. Choose the Arg 139 view, and turn on just the "Arg" electron density. Note that the Arg sidechain is very non-planar but still clear and well-ordered -- it has a favorable rotamer conformation and strong interactions with its surroundings. The 3 N atoms of the planar "guanidinium" end-group have 5 H-bond-donating hydrogen atoms attached, and in this case (not unusually), all 5 H-bonds are satisfied by H-bond acceptor atoms. One of those is the Ogamma of a nearby Ser sidechain; of what residue number? _____ Two others are isolated density peaks; turn on the "waters" button to see that they are water O atoms. The last two are in the density to the right of the Arg, with no model inside it; those are part of a neighboring myoglobin molecule in the crystal. Drag to rotate around, to see that the peaks are elongated for those atoms - they are somewhat disordered. In solution, H-bonds to them would be replaced by H-bonds to solvent; however, the Arg would still be well-ordered, because the Ser O and the 2 waters would stay fixed, since the waters also H-bond to another sidechain O - can you identify the sidechain of that other O? _____

Next week you will learn how to reliably identify H-bonds.

5. Levels of error: the bad parts with disorder

- a. Even at very high resolution, it is usual to have partly-disordered regions at the chain ends and for some of the surface sidechains; at lower resolution loop ends are often disordered, and occasionally entire domains. In the provided kin, choose the "high-B ends" view and turn on just the "ends & alts" density under the ED map group. Lys 140 is at the upper left in this view; its density is well-ordered out to the _____ atom, very weak at _____, and absent at _____. The chain N-terminus is at the upper right. Note that the density is weak or absent for the Val 1 Cgamma atoms, and the shape of the density that includes N, Ca, Cb is strange (drag to see it in 3D). In both cases, the problem is almost certainly due to the sidechain or chain-terminus adopting multiple conformations, but it was not possible to see those alternates and fit them.
- b. However, sometimes 2 or more conformations can be identified confidently. Turn on the "altb" master button, to see two versions for the sidechains of Lys _____ and Glu _____. The Glu is moving locally within one rotamer, while the Lys occupies two quite distinct rotamers. At lower resolution the density would appear smeared-out or would disappear altogether, although a single conformation is often fit. The key to identifying these uncertain regions is either to look at the electron density, or to pay attention to the B-factors of the atoms, which are larger the more the density is smeared out.

Return to the Molik window if it's still open or import 1a6m.pdb again. This time make sure sidechains are turned on and change "Color by" to B factor. Open as a new kinimage. In kinimages, both occupancy (when there are alternates) and B-factor are given in the pointID of an atom. Click on each atom in the sidechain of Lys 140; the B-factor for Ca is _____, for Cb is _____, for Cg is _____, for Cd is _____, for Ce is _____, and for Nz (you may need to turn off "sc atoms") the B is _____. For Lys 133, the occupancy of the "a" alternate is _____ and for the "b" alternate is _____.

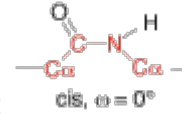
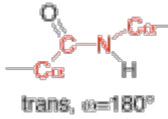
Often the conformation fit by the crystallographer is misleading only because it is not actually unique. However, multiple, smeared, or missing density can often lead to an erroneous fit that does not correspond to any possible local conformation. Later in the course you will learn more about how to identify and even correct such local errors.

- c. Zoom out and look at your new colored-by-B factor kin with and without sidechains. Find a sidechain for which all the member atoms have relatively low B factor (you can base this on the color). Get a nice view of this sidechain and save a view of it. Since the sidechain is probably in the interior of the protein, you should use the "Clipping" slider to clear away obscuring atoms on front of and behind your target. Using "Pick center" or a right click on the sidechain will help ensure that the clipping plane falls in the correct place.
- d. Save your modified kins (go to each of the two you made in the list of kins, then File/Save As and save just the current kinimage). You should end up with a file for each; email these to the TA when you turn in this assignment. Quit out of KiNG when you're done (otherwise you accumulate too much verbiage in the text window!).

6. Cis peptides

- a. Launch KiNG and open the file [c6FldFlx.kin](#) (796KB), and go to Kinemage 3 (use either the kinemage list at top of the button panel, or the *{ }* hypertext link in the text-window table of contents). Click the "Animate" button or hit the "a" keyboard key to switch back and forth between a trans peptide in myoglobin and a cis peptide in a related protein (erythrocyruorin). Scroll down in the text window to read about this

kinemage, and drag the image to view the cis/trans difference from many angles. Measure the distance from Calpha to Calpha across the trans peptide: _____ Å, and then across the cis peptide: _____ Å. Successive Calphas for most peptides are "trans" = "across" the CO-NH amide bond:



but some (almost always preceding a Pro) are "cis" = "same-side":

- b. Go to kinemage 4, which shows a proline isomerase enzyme, and read the text about it. Look especially at the closeup of the cis Ala-Pro substrate (view 5), and turn on "contacts" to see how the enzyme binds it tightly in order to catalyze the isomerization of the peptide bond between cis and trans forms. Identify the Calphas of the Ala and the Pro, and verify that the bond between them is indeed cis.

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