

# BCH622 :: 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 BCH622 course web site at <http://kinemage.biochem.duke.edu/teaching/BCH622/>.

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 c1Basics.kin

📄 [c1Basics.kin](#) (132KB)

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. 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** Open the provided kinemage file in KiNG

📄 [1a6m.kin.zip](#) (464KB)

- a. This kinemage shows the mainchain from a crystal structure of oxy-myoglobin refined at 1 Å 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 \(PDB\)](#) and retrieve the pdb file for this protein (search for the pdbid 1a6m, open the download menu on the resulting page, and download the full pdb text file). To open this file in KiNG go to File/Import then select 1a6m.pdb from the directory where you saved it (or drag-n-drop on the Mac). A Quickin window should open; choose "Open in Molikin". By default, Ball and Stick model is selected, but you should turn off Calphas and turn on backbone and sidechains in the bottom-right checklist. You may like balls on N,O,P,etc as well. Click "As New Kinemage" 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.
- f. Actually cannot just Go to the [Electron Density Server \(EDS\)](#) and retrieve the electron density map file for this protein (enter PDB code 1a6m and click on "submit"). There is a mismatch of calculated overall "R" value using this route to get a map from the EDS and the EDS will not serve it to you.
- g. So instead do an end run past the quality guardian at the EDS by Going to the [MolProbity Server](#) and retrieve the electron density map file for this protein (enter PDB code 1a6m and DECLARE THE "type" to be 2Fo-Fc map (EDS), the click on "fetch"). This gets the map calculated at the EDS by a direct download. When retrieved hit "continue", click on "electron density", and hit "Download" at the far right side of the page. Store this in your working directory where you have the PDB coordinate file.

With the 1a6m kinemage loaded in KiNG, Hit menu item "Tools"/ Structural biology/Electron density maps/ and select the 1a6m.omap.gz -- King can process from this zipped file. Now explore what good high resolution electron density shows you about the structure.

## 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

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

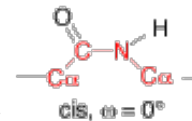
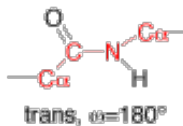
### 4. Cis peptides

- 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 (erythrocrucorin). 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":

- 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.