

Name: _____



Worksheet 3: Dihedrals & Handedness

Kinemage file -- [c1Basics-B-KiNG.kin](#)
PDB file -- [4FXN.pdb](#)

Study the kinemage in this file, following along on this worksheet and answering its questions. Use chapter 1 of the Branden & Tooze textbook for background.

Kinemage 6

Kinemage 6 uses a short segment from flavodoxin, for practice with dihedral angles, the “measure” tool, and amino-acid handedness. The startup view shows just 4 backbone atoms and the bonds between them - this is the minimum for defining a single dihedral angle. Imagine it as a mechanical linkage with stiff bonds and rigid angles of about 120° connecting each pair of bonds, but with something like a rotating sleeve that allows rotation around the central bond. To see this rotation, select menu item “Tools: Specialty: Suite Rotation to get its dial box on screen. Select the ro1 dihedral, and drag in the dial region to change it. Choose View2 to look down the central bond (move the image back and forth a bit to see both ends of that bond) and rotate the dihedral angle again. This is a “phi” conformational angle, since it is rotation around the N-Calpha bond. Watch the numerical value of phi change as you rotate, and see what the geometry looks like near 180 degrees and near 0 degrees; which one is most extended (i.e., has the end atoms farthest apart)?

Turn on “Measure angles & dihedrals” under the “Tools” pulldown menu; the measure function reports the geometry of 4 successive atoms picked, including angles and dihedrals. Choose View1 again. Click on the bottom C atom, then on the ‘N’ atom (the “dist:” part will give the distance between those last 2 atoms picked), then on the ‘Calpha’ atom (now “angle:” will give the in-plane angle defined by the last 3 atoms picked), and finally on the ‘C’ atom (now “dihd:” will give the dihedral angle defined by all 4 atoms); does it match the value on the dial? _____ Type the “m” keyboard key twice (to restart the white lines), and then click on the 4 atoms in the opposite order to verify that the dihedral angle is the same when measured from either direction. Now type the “m” key on the keyboard to get rid of the white measure lines, and choose View3, turn off “dihedral” and turn on “flavodoxin” on the right-side button panel, to put up a short portion of flavodoxin, including some alpha helix, some extended strand, and the connection between them. Practice identifying backbone atom types N, Calpha, C and O by their geometry and relationships (first with the “side ch” button turned on, and then with it turned off). The biggest clues are that the CO (or “carbonyl”) sticks out, and that each entire peptide (the group of 5 atoms from one Calpha to the next) lies in one plane; in contrast, the tetrahedral Calpha lies at the 3D intersection of two peptides in DIFFERENT planes. Practice telling N-to-C-terminal polypeptide chain direction from the fact that the peptide N atom

precedes the Calpha, while the CO follows it. Check yourself by clicking on an atom to get its atom name (“ca” for the Calpha), residue name, and residue number on the information line at the bottom of the screen. What atom type has the “Gly” label? _____ [Don’t forget to often drag slowly back & forth with the mouse, to see 3-D depth in the structure.]

Click on atoms to find the residue numbers for the start (_____) and end (_____) of this entire segment of structure. Now click on successive Calphas along the chain, and notice the distance between each Calpha pair: to within about 0.1 Angstrom, it is always _____ Å.

Choose View4 for a closeup of the extended, or beta-strand, part of the structure. Turn “measures” back on, and starting at the first N (at the very beginning of the chain), click on the first 4 atoms in order along the backbone: N, Calpha, C, and N (but not the O, which sticks out from the continuous line); the dihedral angle displayed after that 4th atom-click is a psi angle (rotation around the Calpha-C bond) for Trp 6, and should read 132.9°. Then click on the next atom in order (the next Calpha) to get the near-180 omega dihedral angle around the 6-7 peptide bond: what is its actual value? _____ ° Then click on the next atom in order (a C) to get the phi angle for Ser 7: _____ °. [Notice that at each step the white lines produced by the measures function show you which 4 atoms define the currently-displayed dihedral angle. To measure the dihedral around a given bond, you must start one atom BEFORE that bond and finish one atom AFTER the bond.]

Choose View5 for a closeup of the helical part. By clicking your way along the backbone (starting at the C atom of residue Gly 10), measure the phi and psi angles of the fully-helical residues 11 and 12. They should be near -60, -40, and in between each phi,psi pair you should see a near -180 omega angle.

Asn 11: phi _____, psi _____
Thr 12: phi _____, psi _____

Choose View6, to concentrate on the connection between the strand and helix (residues Gly 8, Thr 9, and Gly 10). Because they are not fully in any piece of secondary-structure, their conformations are more variable. Measure their phi,psi angles, looking for one with a positive phi value; which residue is it? _____ what is that phi value? _____ Gly is uniquely able to adopt such conformations, because it has only an H in place of a Cbeta atom; to see why that is true in this particular case, let’s construct a hypothetical Cbeta onto this Gly. Do this in KiNG by mutating the Gly to an Ala. First, type “m” to turn off the measure highlights. Then under the “Tools” menu select “Structural Biology” then “Sidechain mutator”. A dialog box comes up in which you need to navigate to where you downloaded the flavodoxin coordinate file 4fxn.pdb, choose this file. Now a full model of flavodoxin overlays the original fragment. Ctrl-click, option-click, or middle-click the Gly Calpha atom: select “Ala”. Now there are 2 more little dialog boxes: a “Model manager” and a box labeled with this mutation. Turn off “refit H’s” in order to see just the heavy atoms like the Cbeta.

That hypothetical Cbeta is just 2.3 Å away from another atom (an impossibly close bump distance): which one? atom _____ of residue _____. (Select “Probe dots” in the “Model manager” dialog box to visualize this collision.)

We will now make what would be the Cbeta of a D-amino acid.

Under “Tools” menu select “Kin editing”: “Fudge Kins” which produces a “Fudge Kins” dialog box. Select “Adjust Dihedral” and “Move One Point”. Turn on markers so you can see the atom picking steps. Click in succession 4 particular points of Gly 10: n, c, ca, cb. This last atom picked will be the moved one point.

You now have a D-Ala residue at position 10. Rotate to look at the D-Ala from its Calpha H direction (the 4th, now-empty, tetrahedral direction from the Calpha); the Calpha should hump slightly toward you. If you have trouble identifying that direction, choose View7. From there, turn on the “corncrib, D” button for labels, and try the “corn crib” test for amino-acid handedness: the 3 branches leaving the Calpha atom should read CO, then R (“r group” of the side chain, in this case your new green Cbeta), and then N around in a clockwise direction for a normal biological L-amino acid, but counter-clockwise for a D-amino acid such as this one you just made. For comparison, try the same thing for the normal L-Thr at position 9: center on its Calpha (turn “pickcenter” on and click that Calpha), rotate to look from its H direction, and read off the CO, R, N branches, this time clockwise. If it doesn’t seem obvious at first, choose View8 and turn on the “corncrib, L” button for labels. Practice identifying both L and D forms, until you can do it without the help of preset views and labels. Remember that amino-acid handedness has strong effects on larger-scale structures: if we were made of D-amino acids, our alpha helices would be lefthanded, our beta sheets would twist the other way, and our enzymes would be specific for molecules of the opposite chirality.