

Name: _____



Worksheet 2: Dihedrals & Handedness

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Kinemage file -- [c1Basics-1n6-KiNG.kin](#)
PDB file -- [4FXN.pdb](#)

Study the kinemages in this file, following along on this worksheet and answering its questions. Use chapter 1 of the Branden & Tooze textbook for background. Practice dragging back & forth slowly with the mouse to rotate the molecule and see it as 3-dimensional, and clicking on an atom to identify it. Turn things on & off with the button boxes. To animate, either click the “Animate” button or press “a” on the keyboard. For a closer view, turn on “Pickcenter”, click an atom, and zoom in on the scale scrollbar (alternatively, center with a right-click). In kinemages 1 and 6, move the rotatable bonds with the horizontal sliders provided in Mage or in KiNG select Tools/Specialty/Suite Rotation.

(If you need additional help in using the KiNG program, review the tutorial file [Demo5_4a-KiNG.kin](#).)

Kinemage 1

Kinemage 1 illustrates a 5-residue fragment of polypeptide chain, to show its geometry and atom names and to allow rotation of one residue’s worth of the variable dihedral angles Phi, Psi, and Chi. Hydrogen atoms (in brown) are included, so this illustrates the full chemical geometry of a protein. Turn them off (with the “H” button box) to see the heavy atoms alone, more typical of what is directly known for most crystal structures since hydrogen atoms diffract x-rays very weakly. Atoms are depicted in this type of “skeletal” or “stick” representation as the intersections or ends of lines which show the chemical connectivity of the molecule. In this case, non-carbon atoms are shown as colored balls (red for O and blue for N), giving a partial “ball&stick” representation. For a skeletal model, one aspect of the basic physical nature of the molecule - namely the chemical bonding arrangement - is explicitly clear, and the model does not hide parts of itself; on the other hand, another important physical aspect - the bulk and the steric constraints - can only be inferred by the relative position of the atoms. Choose View3 (on the VIEWS pulldown menu) and turn on the “spheres” button temporarily to see a rough space-filling representation, which has the opposite properties of showing how big the atoms are but of hiding things that are inside. Note that in Mage and KiNG you can click on a sphere and identify it.

Can you turn the H atoms on & off in this representation? _____

Now turn off the spheres and go back to View1. Selected atom labels can be turned on, or the angle labels turned off, with the button box controls on the right side of the screen. They identify the atom names along the backbone, or they label the variable dihedral angles that determine conformation.

(Of course, an atom can also be identified just by clicking on it, but then the residues will have specific numbers, such as 45 for the Phe, rather than being called n, n+1, etc.) The dihedral angles Phi (around the N-Calpha bond) and Psi (around the Calpha-C bond) of the central Phe residue can be changed using the Tools: Specialty: Suite Rotation. The Suite Rotation box has one dial and a set of axis choices. Dragging the dial “hand” changes the angle freely, clicking in the + - boxes to right or left of the dial will change the angle by 0.1° degrees at a time for fine control. A double-click on the dial resets it to its original angle. One angle value below the middle changes as you change the angle, while the other stays at the original value. As you change one of the angles, look for positions at which atoms on either side are too close together (that is what determines the allowed conformations). For instance, try setting phi to about 0 and psi to about 180. Does that look possible? _____
What bumps? _____

The central Phe (residue n) is shown in cyan with a full side chain and a green ball at its Calpha. The Calpha-Cbeta bonds are colored seagreen, both to better illustrate the tetrahedral geometry of the Calpha atom and also to point out that the Cbeta position is fixed with respect to the polypeptide backbone. Were it not for the unique amino acid glycine which lacks a Cbeta, one would probably consider the Cbeta as part of the main chain rather than the side chain. View2 looks down the Cbeta-Calpha bond, showing that the starting conformation is close to one of the three staggered positions of Chi1 (the first side-chain dihedral angle) expected from simple steric considerations around a carbon-carbon single bond: +60°, -60°, or 180°. What is the actual Chi1 value? _____
(One can return to the startup view by choosing View1.) Try rotating Chi1 and Chi2.

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.

Sidechain Mutator: 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. We'll keep using this new "mutation" dialog box, so do NOT "finish" it.

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.) (The "el" key - lower case l - toggles between clashes and dots colored by atom type and colored by contact severity.)

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

In the new mutation dialog box, click the check box "Use D-amino acid".

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.