Name:_____

CMB BCH MBP MCB UPC IWM 258

Structure Worksheet #1
Handedness & Amino Acid Roles

Kinemage file -- c1Basics.kin

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 box or press "a" on the keyboard. For a closer view, turn on "Pickcenter" , click an atom, and zoom in on the scale scrollbar. [Note: on older Mage versions, pickcenter is persistent and you should turn it off.] In kinemages 1 and 6, move the rotatable bonds with the horizontal sliders provided. (If you need additional help in using the Mage program, go through the tutorial in file **Demo5_4a.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 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 botton 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 rotation slider bars. Clicking in the bar to right or left of the "thumbmark" will change the

angle by 2.5° degrees at a time; clicking on the right or left arrows will change it by 0.25°. One angle value above the slider 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 0 and psi to 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. Choose "Next" on the KINEMAGES pulldown menu, to go to kinemage 2.

Kinemage 2

Kinemage 2 animates disulfide formation in an engineered mutant of T4 phage lysozyme. Click repeatedly on the onscreen button labeled "ANIMATE" (or press the 'a' keyboard key) to change between the reduced form, with SH Cys, and the oxidized form, in which the Cys sulfurs are covalently bonded into a disulfide. Sulfur atoms are shown as gold balls, and H atoms (only present in the reduced SH form) in brown. Often there is essentially no backbone movement when a disulfide forms, but in this case two separate domains of the T4 lysozyme structure hinge to come somewhat closer together when the SS bond forms; choose View2 to see this motion. View3 is a closeup of the SS bridge, which is in the righthand hook conformation, one of the two commonest conformations for disulfides; the other common one is the lefthand spiral. For the LH spiral the 3 dihedral angles across the SS are -, -, - while for the RH hook they are +, +, - (the handedness is named after the central dihedral around the S to S bond). We'll practice measuring dihedrals in kinemage 6, but to try this one choose "measures" on the TOOLS menu and click successively on the Cb, Sg, Sg', and Cb' of the SS; the dihedral angle is the last number shown: _____.
Try it in the opposite order, which should give the same number.

Kinemage 3

Kinemage 3 shows the Calpha backbone of dihydrofolate reductase (DHFR), with each type of side chain on a separate button. View1 shows the central beta sheet from the side. View2 is an edge view, showing the twist of the beta sheet. Click "Animate" to switch among the four groupings of side chains (use View2) and see how they differ in overall location. The strongly hydrophobic residues Cys, Phe, Ile, Leu, Met, Val, Trp (in gold) predominantly pack against each other to form the inside core

of the protein, while the permanent charges Asp, Glu, Lys, Arg (+ in skyblue and - in pink) are nearly always on the outside. The neutral and polar groups (in cyan) are more evenly distributed, with a more modest preference for the outside. Pro (green) is also usually outside, even though its side chain has no polar atoms, since it is so good at ending helices and turning corners. Gly (labeled with a "G" at the Calpha) has 3 different sorts of roles: it occurs a) at corners or at ends of pieces of secondary structure (often with otherwise disallowed conformational angles), b) at especially tight contacts inside a protein, and c) where flexibility of motion is needed. Many amino acids have roles at particular positions in specific secondary structures. Gly is by far the commonest amino acid at helix C-termini; to see Gly 86 ending an alpha helix, click in the hypertext jump command in the Mage text window; it looks like *{Kin3, view3 ... }*. At this typical Gly "C-cap" the backbone turns insideout relative to the normal pattern in helix; the peptides on either side of the Gly both make H-bonds to the previous helical turn, but in the wrong order. The N atom of Gly 86 is too far (3.62 Å) to H-bond with the usual n-4 CO; how far is it from the n-3 CO? _____ Å

Ser 77 is the "N-cap" residue at the start of this same alpha helix. The N-cap residue is defined as the one whose preceding peptide is out of the helix but whose following peptide is in the helix. Turn the image around, and perhaps zoom it smaller, to see that the backbone past the Ser N-cap follows the helical spiral, while the backbone before it goes in a completely different direction; the Calpha of the N-cap residue is where that abrupt change takes place. Ser, Thr, Asn, and Asp are all very common in N-cap positions, usually making an H-bond from the side-chain oxygen to a free backbone NH group in the first turn of the helix, as in this case (H-bond shown in purple). Interestingly, Gln is the least preferred residue in the N-cap position (the extra methylene group in its side chain, vs. Asn, does not allow the side-chain oxygen to get into the correct geometry for the H-bond), which emphasizes that residues whose properties look superficially similar do not always play equivalent roles in protein structure. Gln, on the other hand, is especially common in exposed positions in the middle of helices; to see one in such a place, choose "Find" on the "Tools" pulldown menu, enter "gln" and "102", make sure pickcenter is on, and accept. Zoom smaller and rotate the image, to see that the Gln is indeed exposed. Pro prefers corners in the backbone, many of which are at one end of a piece of secondary structure. To see Pro 25 at the beginning of a helix (at top) and 3 other Pro (bottom) at the ends of beta strands, click at the hypertext jump which begins: *{Kin 3 v=4 ...}*. Which position is Pro 25 (Ncap, Ncap+1, etc.)? _____

Arg and Lys are another pair of similar residues with often-divergent roles. Click on the hypertext in the Mage text window which looks like: *{Kin 3 v=5, alloff ... }* for a close-up of Arg 158: the aliphatic part of its side chain makes hydrophobic contacts to the Trp 133 ring, while the positively charged guanidinium group at its end reaches over to interact electrostatically with the negative helix dipole at the C-terminus of a helix and also H-bonds to one of the free backbone CO groups. Zoom out to see that helix; its Ncap is residue _____ _____ (for a double blank, please give the residue name and number). [Remember that the cap residue Cα, is the dividing point

between peptides clearly in the helix and peptides clearly not in the helix.] An Arg guanidinium can make as many as 5 planar H-bonds to oxygens and often does so, therefore tending to have a well-defined, tied-down position. Lys, in contrast, makes H-bonds with its very freely rotatable NH3 group, which is often quite mobile in proteins and interacts well with bulk solvent. Of the 7 Lys in DHFR, 2 of them were so mobile that only a few side-chain atoms could be located in the crystallographic structure: use the Find tool to center on Lys 76, which is only shown out to its Cbeta.

The main function of the large hydrophobic residues is to form hydrophobic cores; however, the different types pack together in distinctive geometries and have different preferences for backbone conformation. Click on the hypertext jump: *{Kin 3 v=6, ... }* to see 4 aromatic side chains in a row (Y151, F153, & Y111 on the beta sheet and W30 from a helix), some interacting with their rings perpendicular (the more common arrangement) and one pair with stacked rings: _____ _____ and _____ _____. Click in the hypertext jump, *{Kin 3 v=7, ... }*, to see a set of 8 Ile and Val on both sides of the beta sheet. Of the aliphatic side chains, the ones with branched Cbetas (Ile, Val) prefer beta sheet, while Leu and Met prefer alpha helices. Individual residue types can be toggled on one at a time in this kinemage - try that, back in View1 (note that buttons are labeled with both one-letter and 3-letter amino acid codes). If all amino acids were equally represented in DHFR, there would be approximately 159 residues/20 = 8 of each. Amino acids that occur much more often than that in DHFR include Asp (14), Ala (13), and Ile (12). What is the least common hydrophobic amino acid in DHFR? _____ with _____ examples

Kinemage 4

Kinemage 4 shows 27 superimposed examples of leucine side chains from 11 protein structures at 0.9 to 1.2 Å resolution (the button for each example gives its 4-character PDB code and residue number). Only a few combinations of side-chain "Chi" dihedral angles occur in these very accurate structures; such clusters of preferred conformations are known as "rotamers". All of them have one of the three possible staggered values for both Chi1 and Chi2 (near -60°, near 180° or trans, or near +60° degrees). Leu is surprisingly strongly constrained, with over 90% in one of just two rotamers: either Chi1=-60,Chi2=trans (examples shown in gold) or Chi1=trans,Chi2=+60 (in cyan). To understand better where the constraints might come from, turn off the minor rotamers (in pink), turn on "H", and move around.

Kinemage 5

Kinemage 5 shows in 3-D the di-iron center in ribonucleotide reductase, which produces a catalytically important free radical on the neighboring tyrosine (upper left). Each Fe has 6 ligands arranged octahedrally, including the bridging oxygen ion, a bridging Glu, waters, and other carboxyl O's and histidine N's (marked with color-coded balls). What is the shortest distance for one of the O-Fe bonds? _____ Å To see the local environment of these side chains within the mostly-helical protein subunit, choose View2 and turn on "Calphas".

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, click and hold down the mouse in the horizontal bond-rotation scrollbar (on the arrow for slow motion, and on the scrollbar just inside the arrow for rapid motion); then click and hold at the opposite end to rotate back. 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? _____°

Turn on "measures" 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 "dhdrl=" will give the dihedral angle defined by all 4 atoms); does it match the value above the scroll bar? _____ Turn the "measure" button on the righthand panel off and then on again (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 press the "m" key on the keyboard to get rid of the white measure lines, and click in the hypertext jump, *{Kin 6 v=3 ...}*, 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. [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. First, with measures on, pick the N, C, Calpha, and Cbeta atoms of Thr 9, in that zigzag order, to define normal Cbeta geometry. Now choose "draw new" under the "Edit" menu and turn on the "construct4" button. Click on the N, C, and then Calpha of the Gly you found with positive phi (Gly 10), and accept the "last measures" default in the resulting dialog box. The construct-line tool draws a Cbeta for the Gly with the same geometry as the reference one you measured for Thr 9. Turn Contstruct 4 back off. That hypothetical Cbeta is just 2.3 Å away from another atom (an impossibly close bump distance): which one? atom _____ of residue _____ _____. Click the "eraselast" button to get rid of the new Cbeta, and we will now make what would be the Cbeta of a D-amino acid. With Construct 4 on, click on the N, C, and Calpha of the Gly again, but this time in the construct dialog-box change the 125 dihedral value to -125 and then accept. You now have a D-Ala residue at position 10; turn the "construct" button back off, and press the 'm' key to get rid of the measure lines. 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.

In order to help us improve this kinemage lesson and worksheet, would you please tell us about any places where you had specific problems?

What part(s) did you find most interesting or enjoyable?