

Name: _____



Worksheet 3: Handedness & Amino Acid Roles

rev 090828

Kinimage file -- [c1Basics-2-5-QKiNG.kin](#)

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

Text field setup for Questions, marked Q: ... (with answer space) _____.

In KiNG: select check box at top left of text window "Allow text to be edited".

After you are finished, select menu item File:Save as...

In KiNG: Choose "Kinimage #2" in the scrollable upper-right panel.

{Kinimage 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).

Q: We'll practice measuring dihedrals in kinimage 6, but to try this one choose "Measure angle & dihedral" 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.

{Kinimage 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 (choose on the Views pulldown menu) 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. [Don't forget to often drag slowly back and forth with the mouse, to rotate and see 3D depth in the structure.]

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 here: *{Kin 3, view 3, master= {Gly,Pro} on, master= {other} on, master= {Hphobics} off, master= {charges} off, master= {ca only} off, master= {ca+mc} on}*.

Q: Turn to view from the helix end, to see that 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 this position, 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 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 point" on the KiNG "Edit" pulldown menu, enter "gln 102" (Mage "Find" is under "Tools" and put "gln" and "102" in the separate lines), turn on pickcenter, 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 here: *{Kin 3 v=4, alloff m={Gly,Pro} on, m={ca+mc} on}*.

Q: Which position is Pro 25 (Ncap, Ncap+1, etc.)? _____

Arg and Lys are another pair of similar residues with often-divergent roles. Click here: *{Kin 3 v=5, alloff m={charges} on, m={Hphobics} on, m={ca+mc} on}* 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 (see Chapter 2) and also H-bonds to one of the free backbone CO groups.

Q: 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 Calpha 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 "Find ..." to center on Lys 76, which is only shown out to its Cbeta since the rest was mobile and invisible.

Q: The main function of the large hydrophobic residues is to form hydrophobic cores; the different types pack together in distinctive geometries and have different preferences for backbone conformation. Click here: *{Kin 3 v=6, m={other} on, m={Hphobics} on, m={charges} off, m={ca only} on, m={ca+mc} off}* 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 here: *{Kin 3 v=7, m={Hphobics} on, m={Gly,Pro} on, m={other} off, m={ca only} on, m={ca+mc} off}* 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. If all amino acids were equally represented in DHFR, there would be approximately $159 \text{ residues} / 20 = 8$ of each. Amino acids that occur much more often than that in DHFR include Asp (14), Ala (13), and Ile (12). Amino acids with very few examples in DHFR are Cys (only 2), Gln (4), and Tyr (4).

Q: What is the least common hydrophobic amino acid in DHFR? _____ with _____ examples.

{Kinemage 4} has backbone in white, and shows 27 superimposed examples of leucine side chains from 11 protein structures at 0.9 to 1.2Å 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 -60,trans (examples shown in gold) or trans,+60 (in cyan). To understand better where the constraints might come from, turn off the minor rotamers (in pink) and turn on "H", and move around.

{Kinemage 5} shows in three dimensions 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).

Q: 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".