

Name: _____



Graphics Worksheet #8

Protein Motions (==2007)

Kinemage files: c6FldFlx-KiNG.kin | | HbAllo-KiNG.kin

Go through the kinemages of c6FldFlx.kin for yourself.

In kinemage 5, is it the closed or open form of GroEL that contains ATP? _____

In kinemage 10, what is so unusual about the conformational change of the antitrypsin?

Kinemages 12 and 13 of file c6FldFlx.kin each animate a representative example of protein motion on binding ligands. In each case, look to see which parts move relative to each other in what way, and think about what the functional need or advantage is for this motion.

Kinemage 12 shows a lactate dehydrogenase (LDH) subunit, with its doubly-wound nucleotide-binding domain in yellowtint and catalytic domain in bluetint. Animate to see how the movements on binding relate to the overall LDH subunit structure. What part moves most? _____

What is the largest movement you can measure for a Calpha? _____ Å

Which residue? _____

Would the NAD be significantly less buried without the catalytic domain? _____

Is there any hinge motion or shift between the two domains? _____

Choose View3 and turn on “side ch” for a closeup of the substrate-binding site, and animate again.

Could the oxamate get in or out in the ternary-complex conformation? _____

Kinemage 13 shows the 2-domain, alpha/beta structure of maltodextrin-binding protein. Animate to see the conformational change on binding maltose. What parts move relative to each other?

What is this type of motion usually called? _____

What is the movement for a residue near the binding site? _____ Å

What happens on the surface behind where the maltose binds (at right in View1)?

Which part of the protein was superimposed to make this kinemage? _____

If the other domain were superimposed, do you think it would show much internal motion? _____

There are 3 chains that run from one domain to the other;

for each, can you locate a “hinge” residue? _____, _____, _____ (Try turning on both forms at once.)

Now study the HbAllo.kin kinemage, working along in the explanations and exercises on the following pages... file HbAllo-KiNG.kin hypertext has been reworked for use in KiNG: use the hypertext commands in the kinemage text window to navigate through the exercise:

{Kin 1} An exercise in allostery - a hemoglobin subunit binding O₂

{Kin 2} An exercise in allostery - the Hb tetramer T -> R transition

For hemoglobin, its function as an oxygen-carrier in the blood is fundamentally linked to the equilibrium between the two main states of its quaternary structure, the unliganded “deoxy” or “T state” versus the liganded “oxy” or “R state”. The T-state is shown in shades of blue (bluetint alpha-chains, cyan betas, and skyblue hemes) and the R-state is in shades of pink (pinkint alphas, pink betas, and hotpink hemes), suggestive of the change in color between deoxygenated and oxygenated blood. The unliganded (deoxy) form is called the “T” (for “tense”) state because it contains extra stabilizing interactions between the subunits. In the high-affinity R-state conformation the interactions which oppose oxygen binding and stabilize the tetramer are somewhat weaker or “relaxed”. In some organisms this difference is so pronounced that their Hb molecules dissociate into dimers in the oxygenated form. Animation of the structural changes that occur during this transition can illuminate how such changes result in important functional properties, such as cooperativity of oxygen binding and allosteric control by pH and anions. Hemoglobin is definitely not a pure two-state system, but the T to R transition provides the major, first-level explanation of its function. The hemoglobin molecule (or “Hb”) is a tetramer of two alpha and two beta chains, of 141 and 146 residues in human. They are different but homologous, with a “globin fold” structure similar to myoglobin. The two crystal structures used here are human deoxy hemoglobin (PDB file 3HHB), which is in the T-state quaternary structure with no ligands at the O₂-binding site, and human carbonmonoxy hemoglobin (PDB file 1HCO), which is in the R-state quaternary structure and has ligands at all 4 sites.

Kinemage 1 shows a single alpha chain of hemoglobin, starting with an overview of the subunit. The 6 major and 2 short alpha-helices that make up the structure of a Hb subunit (the “globin fold”) are labeled A through H, which is the traditional naming scheme. For example, the proximal histidine (the tightest protein Fe ligand) is often called His F9, since it is residue 9 on helix F (it is residue 87 in the human alpha chain). The helices form an approximately-cylindrical bundle, with the heme and its central Fe atom bound in a hydrophobic pocket between the E and F helices. Turn on “highlights” and click the “animate” button, or press the “a” key on the keyboard, to cycle between the deoxy and oxy forms in this overview. Click here: [*{Kinemage 1, View 2, m= {highlights} on}*](#) for a closeup around the heme O₂-binding site. Animate back & forth between the deoxy and oxy forms. For this kinemage the two alpha1 heme groups were superimposed on each other, to give a local comparison at this site. The heme is quite domed in the blue T-state (deoxy) form, with the 5-coordinate, high-spin Fe (yellow ball) out of the plane. In the pink R-state form a CO molecule is bound at the left, the Fe, now 6-coordinate low-

spin, has moved into the heme plane, which has flattened. The proximal His (at right) connects the Fe to helices on the proximal side, making the Fe position sensitive to changes in the globin structure and vice versa. Remember that this kinemage shows a subunit in the all-unliganded versus the all-liganded states of Hb; when oxygen binds to just one subunit, then its internal structure undergoes some but not all of these changes, depending on conditions. O₂ binds in the same place as CO, with similar effects on the structure; however, for O₂ the outer atom is angled rather than straight. The equilibrium between free and bound O₂ is very rapid, with on and off rates that are sensitive to protein conformation. Both CO and NO dissociate from the Fe atom very slowly, so that these gases act as respiratory poisons. The alpha and beta chains differ somewhat in their rates and relative affinities for O₂ and other ligands, by virtue of heme-pocket differences, but the differences between affinities in the R vs T quaternary states are much larger. Both alpha and beta chains of Hb resemble myoglobin (the single-chain O₂-binder in muscle), both in overall tertiary structure and in using an Fe atom centered in a heme group as the site where oxygen is reversibly bound. The heme is surrounded by a hydrophobic pocket, which is necessary in order for it to bind oxygen reversibly without undergoing oxidation or other undesirable reactions. Click here: [*{Kin 1, View 3, m= {Hphobics} on}*](#) to see some of the hydrophobic sidechains that form the heme pocket. They actually surround the binding site so thoroughly that O₂ cannot get in or out without parts of the protein moving out of the way a bit, so that its dynamic properties are essential to have any O₂ binding at all; this restrictive process also increases the specificity of ligand binding. The shift between R and T state requires subunit interactions and does not occur in myoglobin, or in isolated alpha or beta chain monomers. These monomers bind O₂ quite tightly, which would work well for loading O₂ in the lungs but would not allow unloading it for delivery to the tissues. Therefore, the central critical feature of hemoglobin function is how it achieves, uses, and allosterically controls cooperativity between the 4 binding sites in the tetramer to tune O₂ binding for satisfying physiological needs. Click here: [*{Kin 1, View 4, m= {Hphobics} off}*](#) and animate, to see the linkage between changes at the O₂-binding site and changes in protein conformation, which shows ligand-dependent shifts in the region from the heme out to the subunit interface. Linkage of the heme Fe through the proximal His results in tertiary-structure changes that can then transmit their effects to other subunits in the tetrameric assemblage. This allows O₂ binding in one subunit to indirectly affect the affinity of other subunits. Briefly, inside the alpha chains the R/T equilibrium is reflected in changes in Fe spin state and position as it moves in or out of the heme plane. Click on the ball of the heme Fe in one form, then animate and pick the Fe again: the Fe atom moves _____ Å when the heme changes conformation. The proximal His changes distance and angle relative to the heme, and the F helix shifts; measure how far the C α of the proximal His moves between the two states: _____ Å. Tyr 140 moves and its H-bond to backbone weakens; measure the length of the H-bond between Tyr 140 OH and the backbone CO in the deoxy state: _____ Å vs its length in the oxy state: _____ Å. Both the C-terminus of the chain and Arg 141 move significantly at the interface, measure how far the central C atom of the guanidinium group at the end of Arg 141 moves: _____ Å. The small motion at the heme is thus amplified into a much larger change at the subunit interface. Changes at the subunit interface (coupled with changes at the Fe,

as we have seen) alter the equilibrium between the deoxy and oxy quaternary structures, and conversely a change of quaternary structure alters the balance between the two states inside a given subunit. Each O₂ that binds increases the likelihood of switching the tetramer into the oxy state, and once it switches, the O₂ affinity at all sites increases because the local structure changes have either already occurred or are easier to make. Click here: [*{Kin 1, View 5, m= {highlights} off, m= {axes} on}*](#) to show the alpha1 subunit, but centered for the whole tetramer (deoxy form), as it will be seen in View1 of Kinemage 13, including the 2-fold axes of symmetry of the tetramer.

Kinemage 2 shows multiple views of the quaternary-structure change for a tetramer of human hemoglobin. Animate to change between the T-state (deoxy) and R-state (oxy) forms. The starting view looks down one of the approximate 2-fold axes, with alpha subunits at the top and beta subunits at the bottom. Notice that the hemes are quite far apart, so that their interactions must be mediated by the protein. For a view down the exact crystallographic 2-fold axis from the Beta1-Beta2 end, click here: [*{Kinemage 2, View 2}*](#). The yellowtint crosses are phosphate sites present in deoxy but not oxy Hb. In oxy Hb, the beta subunits move closer together, squeezing out phosphates (such as 2,3 DPG), and allowing the N- and C-termini to interact. Measure the distance between the heme Fe atoms of the two beta subunits in the oxy state (pink): ____ Å vs in the deoxy state (blue) ____ Å. DPG and other phosphates bind very much more strongly to the deoxy quaternary structure; therefore they necessarily push the equilibrium toward deoxy Hb, and because of that they decrease O₂ affinity. Such regulatory phosphate molecules are useful in the blood, because their concentrations can be controlled to shift the Hb O₂-binding curve so that it is working across the steepest and most efficient part under conditions in the lungs and tissues. For instance, at high altitude the body makes more DPG, to unload O₂ more effectively in the muscles. To the first approximation the hemoglobin molecule consists of two “dimers” (Alpha1-Beta1 and Alpha2-Beta2), which rotate relative to each other as rigid bodies in the R-T transition. To see that rotation for just one dimer, click here: [*{Kin 2, View 3, m= {alpha1} off, m= {beta1} off}*](#). The Alpha1-Beta1 unit undergoes relatively little internal rearrangement, but its overall rotation is considerable. The net rotation of the two dimers alters their interactions with one another, most notably at the allosteric effector site between Beta1 and Beta2 (PO₄ binding) and at the important Alpha1-Beta2 interface, where mutations have the largest effect on Hb allosteric properties. For an overview of relative shifts at the critical Alpha1-Beta2 interface, click here: [*{Kin 2, View 4, m= {alpha1} on, m= {beta2} on, m= {alpha2} off, m= {beta1} off, m= {salt links} on, m= {hinge&ratchet} on}*](#). Identify the two long, charged side-chains that make intersubunit salt links in the deoxy form but not in the oxy form: ____ ____ and ____ _____. Although the symmetry is not exact, similar parts of the subunits contact each other: the C helix, and the “FG corner” between helices F and G. Animate, to see the relative motion of these two subunits, with a fairly stationary “hinge” on one side and a larger “ratchet” motion on the other. Click here: [*{Kin 2, View 5, m= {hinge&ratchet} on, m= {salt links} off, m= {axes} off}*](#) for a closeup that emphasizes the ratchet contact between the C helix of Alpha1 and the FG corner of Beta2; His 97 of the Beta2 FG corner makes a large jump against Thr 38 and Thr 41 of the Alpha1 C helix. Animate repeatedly, keep-

ing your eye on His 97. The His 97 ring is to the _____ of Thr 41 in deoxy and to the _____ of Thr 41 in oxy. Click here: [*{Kin 2, View 6, m= {hinge&ratchet} on, m= {salt links} off}*](#) for a closeup of the hinge contact, where the motions are mainly rotations without much shift, between the Alpha1 FG corner and the Beta2 C helix. Labels help identify these parts. The two long sidechains that rotate but keep the same contacts are _____ and _____. Since this is a complex motion orchestrated between the fit of two quite different sets of contacts in the two states, this interface is critical to making Hb allosteric work, and mutations of residues in this interface have been found to be especially likely to influence cooperativity and allostery.

There are salt links between Alpha1 and Alpha2, which stabilize the deoxy form. Click here: [*{Kin 2, View 7, m= {salt links} on, master= {a1a2 salt2} on, m= {hinge&ratchet} off, m= {alpha2} on, master= {beta1} off, master= {beta2} off}*](#) for an overview down the exact 2-fold axis between the subunits, showing that there are two equivalent sets of interactions, on either side of the twofold. Animate, to see the making and breaking of these interactions. Salt links at the C-terminus of Beta2 stabilize the deoxy T form and make a large contribution to the pH dependence of Hb oxygen binding, known as the Bohr Effect. Click here: [*{Kin 2, View 8, m= {salt links} on, m= {a1a2 salt2} off, m= {beta2} on, m= {axes} off}*](#) for a closeup to see the making and breaking of these interactions. His b 146 moves a great deal, disrupting the salt link (charged H-bond) to Asp b 94 that is formed in the T state: the cg atom of His b 146 moves _____ Å. Since His titrates near physiological pH, this interaction is quite pH sensitive. At low pH, when more protons are present, the His ring N is more likely to be protonated and positive; this strengthens its H-bond with Asp 94, thus favoring the T state and decreasing O₂ affinity. The pH effect, or Bohr Effect, can be considered as allosteric regulation by the binding of protons. It is important biologically, because it promotes oxygen unloading in the tissues where proton concentrations are elevated, for instance by the production of lactic acid in muscle.

To summarize some of these critical subunit interactions in the context of the whole hemoglobin tetramer, click here: [*{Kinemage 2, View 9, m= {salt links} on, m= {hinge&ratchet} on, m= {beta1} on, m= {axes} on}*](#). Animate, to follow components of the T-R changes in the contacts at the allosteric interface between the two dimers. Hemoglobin is a good example of how conformational changes are an equilibrium system - anything that strengthens, or binds selectively to, one of the two states shifts the equilibrium somewhat toward favoring that state. Oxygen itself, of course, shifts the equilibrium to favor the _____ state.

Kinemages 1 and 2 were adapted from "THE PROTEIN TOURIST #8 - THE T-R, DEOXY-OXY TRANSITION IN HUMAN HEMOGLOBIN", David Richardson, Celia Bonaventura, and Jane Richardson, Protein Science vol. 3, electronic supplement, Oct. 1994.

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