Wrksht-Hemoglobin is the hypertext/graphics of HbAllo-KiNGques.kin

Become familiar with hemoglobin structure by following the hypertext and graphics of HbAllo-KiNGques.kin, noting especially the joint influences of local and global changes on each other.

Worksheet questions are included in the text to focus your attention on specific features.
A separate Worksheet page with just the questions is attached below for handing in your answers.

Hypertext kinemages in KiNG: direct interaction between the "Text window" and the "KiNG graphics" window. Clicking in the *{… enclosed text …}* causes the coded choices to be made in the KiNG graphics display, for instance, changing which kinemage as well as view and on/off button states are active. Note that the reader has to click in the graphics window to allow graphics mouse and keyboard actions.

This exercise will provide you with the 3-D structure familiarity to more easily follow the in-class presentation and discussion of the structure-based view of the dynamic equilibrium that is the HB allostery, and of the unity behind the structure-based versus the equation-based approaches to understanding.

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.
FYI: The structures 1HCO.pdb and 3HHB.pdb used in this kinemage are discussed in:
Baldwin (1980) "The crystal structure of human carbonmonoxy haemoglobin at 2.7A resolution", J. Mol. Biol. 136: 103. (file 1HCO)
Fermi, Perutz, Shaanan, & Fourme (1984) "The crystal structure of human deoxy haemoglobin at 1.74A resolution", J. Mol. Biol. 175: 159. (file 3HHB)

Original structural presentation of Hb allostery: (optional background reading)
Perutz (1970) "Stereochemistry of cooperative effects in haemoglobin", Nature 228: 726-734.
Perutz (1970) "The Bohr Effect and Combination with Organic Phosphates", Nature 228: 734-739.

In these first papers Perutz mentions the aspects of his observations that are not explained by the MWC or the Koshland models of allostery. Although Perutz was explicitly looking for and finding direct linkage pathways by which O2 binding at one heme could influence affinity at another, he also describes the effect of DPG as just stabilizing the deoxy conformation.
Last paragraph of the second paper is:

"This study has perhaps conveyed a too mechanistic picture of the cooperative effects involved in respiratory transport. In fact we should imagine a dynamic system, in which both the tertiary structure of each subunit, and the quaternary structure of the haemoglobin molecule as a whole, oscillate rapidly and continuously between the oxy and the deoxy conformations. The concentrations of the different ligands alter the equilibrium between these two conformations of the various components rather than switching any of them completely to one or the other conformation. This also means that the transition between the two quaternary structures could occur at any stage of the reaction, depending on the concentration of H+, CO2, and DPG, and on other factors, and that the mechanism would work equally well regardless of the sequence of reaction of the subunits."

Wrksht-Hemoglobin-Questions Name:_____

Worksheet Hemoglobin: Structural Basis of Allostery
Just the Questions from HbAllo-KiNGques.kin Text Window: printed to go along with hypertext

{Kinemage 2, View 1}
For an initial glimpse of the different roles of alpha vs beta subunits, measure the distance changes between heme pairs: in deoxy (blue) the alpha 1 Fe to alpha 2 Fe distance is _____ Å; in oxy (pink) it is _____ Å; the alpha-alpha distance change is _____ Å. In deoxy, the beta 1 Fe to beta 2 Fe distance is _____ Å; in oxy it is _____ Å; the beta-beta distance change is _____ Å, longer in the _____ state.

{Kinemage 1, View 4} …. 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 Calpha 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.

{Kin 2, View 4 }… Identify the two charged side¬chains that make intersubunit salt links in the deoxy form but not in the oxy form: ____ ____ and ____ ____.

{Kin 2, View 5} 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, keeping 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 (left or right as seen in this view).

{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 ____ ____.

{Kin 2, View 8} 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.

{Kinemage 2, View 9}. 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.