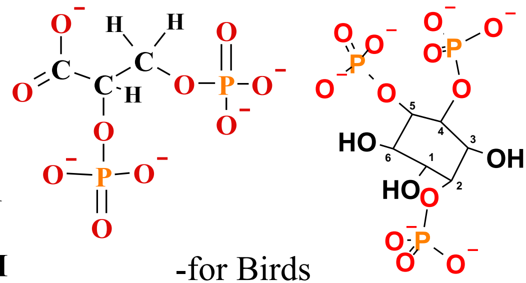
Āris Kaksis RSU 2023.

THE PROTEIN TOURIST#8-THE T-R, DEOXY-OXY TRANSITION IN HUMAN HEMOGLOBIN

2,3BisPhosphoGlycerate^{5-}=[**BPG^{5-}**]=5mM at see level **human homeostasis** and inositolphosphate in birds



David Richardson, Celia Bonaventura, and Jane Richardson
Protein Science vol. 3. Electronic supplement, Oct.1994
Kin.1- Hb tetramer: deoxy vs oxy transition animated
Kin.2- Hb T-R transition: alpha chain and heme close up
Kin.3- The alpha1-beta2 allosteric interface
Kin.4- Alpha1-alpha2 salt bridges;
Kin.5-Beta2 salt bridges.

Human **homeostasis** oxygen is arterial blood plasma concentration **[O_2]=6•10^{-5} M** -for Birds

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, known as the unliganded "**deoxy**" or "**T state**" versus the liganded "**oxy**" or "**R state**". We will use animated kinemages to illustrate the structural changes that occur during this transition and how such changes result in important functional properties, such as cooperativity of oxygen binding and allosteric control by pH and anions. Hemoglobin is not a pure two-state system, but the **T** to **R** transition provides the major, first-level explanation of its function and is the focus of this ProTour.

The hemoglobin molecule (which we will sometimes abbreviate as "Hb") is a tetramer of two alpha and two beta chains; in human, they contain 141 and 146 residues respectively. They are different but homologous, and they share an all-helical tertiary structure known as the "globin fold". In all of these kinemages, the **deoxy T state** is shown in shades of blue and the liganded **R state** in shades of pink, with the alpha chains slightly paler than the beta chains.

The two crystal structures illustrated here are human **deoxy** hemoglobin, which is in the **T-state** quaternary structure with no ligands at the **O_2**-binding site, and human **carbon mono oxy** hemoglobin, which is in the **R-state** quaternary structure and has ligands at all 4 sites. These two were the first matched pair of Hb structures solved at fairly high resolution, and were the subject of detailed comparisons (Baldwin & Chothia). Protein Data Bank (PDB) files 3HHB and 1HCO do not need to be realigned for most comparisons, although they include only a non-redundant half-dimmer; the full tetramer coordinates are available on-line from Brookhaven PDB (130.199.144.1) in directory /user_group/biological_units, as files pdb3hhb.bio and pdb1hco.bio.

Kinemage 1 shows multiple views of the quaternary-structure change for a tetramer of human hemoglobin. Click on the "ANIMATE" button to change between the **T-state (deoxy)** and **R-state (oxy)** forms. The unliganded **T-state** is shown in shades of blue (blue tint alpha-chains, cyan betas, and sky-blue hems) and the liganded **R-state** is in shades of pink (pink tint alphas, pink betas, and hot pink hems), suggestive of the change in color between **deoxygenated** and **oxygenated** blood. The structure is simplified by showing only the C alpha trace **backbones** (Amino Acid Cα trace). **View1** 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. The unliganded (**deoxy**) form is called the "**T**" (for "**tense**") state because it contains extra stabilizing interactions between the subunits , which we will see in detail in Kin. 4 and 5. 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 dimmers in the **oxygenated** form. Liganded human Hb will also dissociate when diluted, which poses problems for cell-free Hb-based blood substitutes because the dissociated protein is rapidly excreted.

$H_2COPO_3^{2-}$-$HCOPO_3^{2-}$-COO^->**BPG^{5-}** is glycerate dihydro oxy acid salt **G$^-$** of two **phosphate 2,3-esters** with **homeostasis** concentration [**BPG^{5-}**]= 5 mM and is glycolysis metabolite in erythrocytes which stabilizes **[O_2]** concentration with sensitive equilibrium shift to turn transition **oxyR → deoxyT** at lowered **[O_2]** concentration **BPG^{5-}** squeeze in to cavity desorbs oxygen 4 **O_2** , adsorbing 4 **H^+** on distal histidines 2*His63,58 keeping blood pH=7,36 constant.

Carbonic anhydrase CA protolysis reactivity create functional active bicarbonate buffer. [9,14]

Carbonic anhydrase CA drive high rate protolysis **CO_{2aqua}** with two water molecules:

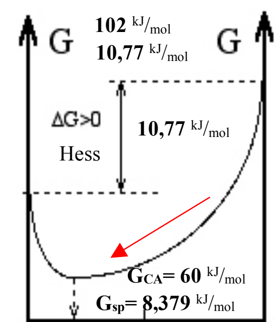
CO_{2aqua}+2H_2O+ΔG+Q=v1$\stackrel{CA}{\rightarrow}$$H_3O^+$+$HCO_3^-$, which velocity constant is: $k_{1CO2aqua}$=1,5×10^8 $M^{-1}s^{-1}$. [9]

endothermic................ ΔH_{Hess}=9.7576 $^{kJ}/_{mol}$; **endoergic**............ ΔG_{Hess}=**102** $^{kJ}/_{mol}$;. [9];

$\Delta H_{Hess}=\Delta H°_{H3O}+\Delta H°_{HCO3}-2\Delta H°_{H2O}-\Delta H°_{CO2}$=-285.81-689.93-(2*-285.85-413.7976) =9.7576.............. $^{kJ}/_{mol}$;

$\Delta G_{protolysisHess}=\Delta G°_{H3O}+\Delta G°_{HCO3}-2\Delta G°_{H2O}-\Delta G°_{CO2}$=-213,2746-**544,9688**-(2*-237,191-385,98)=**102**.........$^{kJ}/_{mol}$;

CA weak acid equilibrium $\mathbf{K_{eqCA}}=\frac{[HCO_3^-]_{aqua}\cdot[H_3O^+]}{[CO_2]_{aqua}\cdot[H_2O]^2}$ $=\mathbf{K_a}/[\mathbf{H_2O}]^2=10$^(-7,0512)/55,3457339^2=2,906*10^{-11}



Exothermic ΔH_{spHess}=-20,3....... $^{kJ}/_{mol}$ and endoergic **solubility** [$\mathbf{CO_{2aqua}}$]=0,000754 M for dissolution is ΔG_{spHess}=10,77.......$^{kJ}/_{mol}$ and protolysis constant is $\mathbf{K_{eqCA}}$=2,906*10^{-11} <1: therefore positive endoergic free energy change minimum:

$\mathbf{G_{CA}}$=-**R•T•ln**($\mathbf{K_{eqCA}}$) =-8,3144*298,15***ln**(2,906*10^(-11))=60 .... $^{kJ}/_{mol}$.

Endoergic $\mathbf{CO_{2gas}}$ solubility and $\mathbf{CO_{2aq}}$ protolysis Hess free energy change positive $\Delta\mathbf{G}_{spHess}$ 10.77 $^{kJ}/_{mol}$ and $\Delta\mathbf{G}_{protolysisHess}$ 102 $^{kJ}/_{mol}$, but minimizes reaching equilibrium mixture of solubility $\mathbf{G_{sp}}$=8.38 $^{kJ}/_{mol}$ and of protolysis $\Delta\mathbf{G_{min}}=\mathbf{G_{CA}}$ 60 $^{kJ}/_{mol}$: $\mathbf{CO_2}$+2$\mathbf{H_2O}$ protolysis generate indispensable concentrations $\mathbf{H_3O^+}$+$\mathbf{HCO_3^-}$ gradients of free energy accumulation $G_{spCO_2}+G_{CA}$=8,38 $^{kJ}/_{mol}$+60 $^{kJ}/_{mol}$. Using the gradients energy Brownian molecular engines drive irreversible homeostasis of $\mathbf{H_3O^+}$+$\mathbf{HCO_3}$ for transport down the gradient through membrane cannels exhaling $\mathbf{CO_{2gas}}$+$\mathbf{H_2O}$ and of $\mathbf{O_{2aqua}}$+$\mathbf{H_2O}$ for osmosis against the gradients through aquaporins inhaling $\mathbf{O_2}$. Photosynthesis with CA inhale $\mathbf{CO_{2gas}}$+$\mathbf{H_2O}$ through proton $\mathbf{H^+}$+$\mathbf{HCO_3}$ bicarbonate cannels and exhale $\mathbf{O_{2aqua}}$+$\mathbf{H_2O}$ through aquaporins cannels in osmosis manner.

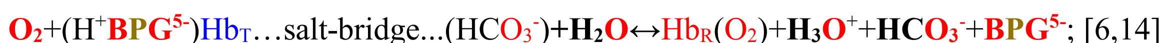
A+2B 50% C+D
$CO_{2aq}+2H_2O$ reactants
products $HCO_3^-+H_3O^+$
A 50% B
$\mathbf{CO_2}\uparrow_{gas}$ reactant
product $\mathbf{CO_{2aqua}}$

Prigogine attractor free energy change minimum ΔG_{min} reaching is Le Chatelier principle of equilibrium mixture. High rate protolysis attractor stay at equilibrium, while homeostasis continues, because is non-equilibrium state. Prigogine: "This equilibrium state is an “attractor” for non-equilibrium states." 1977. [4]

CA Carbonic Anhydrase drive irreversible dissolute carbon dioxide protolysis with two water molecules cooling Earth biosphere in photosynthesis : $\mathbf{CO_{2aqua}}$+2$\mathbf{H_2O}$+Δ**G**+**Q** $\xrightarrow{CA}$ $\mathbf{H_3O^+}$+$\mathbf{HCO_3^-}$ high solubility ratio $K_{CO2aqua+HCO3-}$=[CO_{2aqua}+HCO_3^-]/[$CO_2\uparrow_{air}$]=0.023 M/0,000754 M=30,6...... times for inhale . $\mathbf{CO_{2gas}}$+$\mathbf{H_2O}$. [14]

$\mathbf{O_{2aqua}}$ Hemoglobin shuttle exchange with protolysis generate $\mathbf{HCO_3^-}$ and $\mathbf{H^+}$ gradients across membranes.

Hemoglobin in tissue desorbs oxidant oxygen $\mathbf{O_{2aqua}}$ for exchange to generate HCO_3^-, H^+. In lungs release HCO_3^- and H^+ due to adsorption of oxygen $\mathbf{O_{2aqua}}$. [6] Exchange equilibrium affinity of hemoglobin to oxygen increases reaching mole fraction [$Hb_R(O_2)$]=0.96 concentration [$\mathbf{O_{2aqua}}$]=6*10^{-5} M in arterial blood and deoxy mole fraction lefts [($H^+$$\mathbf{BPG^{5-}}$)$Hb_T$...salt bridge...($HCO_3^-$)]=0.04. In tissue venous blood Bisphospho glycerate $\mathbf{BPG^{5-}}$ squeezed in hemoglobin creates 0.37=[(H^+ $\mathbf{BPG^{5-}}$)Hb_T...salt bridge...(HCO_3^-)] deoxy mole fraction and decreases oxy to [$Hb_R(O_2)$]=0.63 mole fraction. In circulation organism consume 0.37-0.04=0.33 oxygen mole fraction sustaining venous [$\mathbf{O_{2aqua}}$]=0.426·10^{-5} M concentration. Ratio [HCO_3^-]/[CO_{2aqua}]=0.0154 M/0.0076 M stabilizing at pH=7.36 level as multi functional Attractor of Self-Organization. Actual seven components concentrations at saturated oxygen arterial blood and venous state:



$\mathbf{O_2}$+($H^+$$\mathbf{BPG^{5-}}$)$Hb_T$…salt-bridge...($HCO_3^-$)+$\mathbf{H_2O}$↔$Hb_R(O_2)$+$\mathbf{H_3O^+}$+$\mathbf{HCO_3^-}$+$\mathbf{BPG^{5-}}$; [6,14]

K=[$Hb_R(O_2)$]*[BPG^{5-}]*[H_3O^+]*[HCO_3^-]/[($H^+$$BPG^{5-}$)$Hb_T$..salt bridge...($HCO_3^-$)]/[$H_2O$]/[$\mathbf{O_{2aqua}}$]=2.43*$10^{-8}$;

	[$Hb_R(O_2)$]*	[BPG^{5-}]*	[H_3O^+]*	[HCO_3^-]/	[Hb_T]/	[H_2O]/	[$\mathbf{O_{2aqua}}$]	
arterial state K=	0.96*	**0.005***	$10^{-7.36}$*	0.0154/	0.04/	55.3/	6/10^{-5}	=2.43*10^{-8};
venous state K=	0.63*	**0.005***	$10^{-7.36}$*	0.0154/	0.37/	55.3/	0.426/10^{-5}	=2.43*10^{-8};
high land venous state K=	0.48*	**0.008***	$10^{-7.36}$*	0.0154/	0.52/	55.3/	0.3692/10^{-5}	=2.43*10^{-8};

See level air Attractor [$\mathbf{O_2}$]=20.95% make in erythrocytes [$\mathbf{BPG^{5-}}$]=**5 mM**, but high land (see Oxygen in blood [6]) low air [$\mathbf{O_2}$] in erythrocytes have content of [$\mathbf{BPG^{5-}}$]=**8 mM** and keep equilibrium at K=2.43*10^{-8}.

Stabilized multi functional Attractor pH=7.36 keep [HCO_3^-]=0.0154 M, [CO_{2aqua}]=0.0076 M despite blood circulation cycle generate amounts of [$\mathbf{H^+}$]=459*6•10^{-5} M and 0.0275 M=[$\mathbf{HCO_3^-}$]. Arterial concentrations [$\mathbf{O_2}$]=6·10^{-5} M, [$Hb_R(O_2)$]=0.96, [(H^+)Hb_T..salt bridge..(HCO_3^-)]=0.04 and venous Homeostasis concentrations are [$\mathbf{O_2}$]=0.426·10^{-5} M, [$Hb_R(O_2)$]=0.63, [(H^+)Hb_T...salt bridge...(HCO_3^-)]=0.37.[6,14]

Try animating in **View₂** (choose from the "**Views**" pull down menu), which looks down the exact crystallographic 2-fold axis from the Beta1-Beta2 end. The **yellow** tint crosses **x** are **phosphates** -$\mathbf{OPO_3^{2-}}$sites present in **deoxy** but not **oxy** Hb. In **oxy** Hb, the beta subunits move closer together, squeezing out **phosphates** -$\mathbf{OPO_3^{2-}}$ (such as **2,3-BPG**$^{5-}$), and allowing the $\mathbf{N^-}$ and **C**-termini to interact. **BPG** and other **inositol 4,5-phosphates (birds** erythrocytes**)** bind very much more strongly to the **deoxy** quaternary structure; therefore

they necessarily push the equilibrium toward **deoxy** Hb, and they decrease O_2 affinity. Such regulatory **phosphate** -OPO_3^{2-} groups let maintain [O_2] concentration in **blood** controlled to shift the HbO_2 binding curve, which is working across the steepest and most efficient part in the **lungs**, to **deoxy venous blood** Hb in **tissues** when oxygen is desorbed to minimal [O_2]=**1.85•10^{-5}M**.

The interactions between alpha and beta subunits are critical for cooperativity in **oxygen** binding. To the first approximation, the Hb molecule consists of two "dimmers" (Alpha1-Beta1 and Alpha2-Beta2), which rotate relative to each other as rigid bodies in the **R-T** transition. **View3** looks down the approximate axis around which those two Alpha-Beta dimmers rotate. Animate with all 4 subunits present, and then turn off Alpha and Beta in each of two forms to see the rotation for just one dimmer. The Alpha-Beta unit undergoes relatively little internal rearrangement, but its overall rotation is considerable. The net rotation of the two dimmers alters their interactions with one another, most notably at the allosteric effector site between Beta1 and Beta2 (which we saw in **View2**) and at the important Alpha1-Beta2 interface (see Kin. 3) where mutations have the largest effect on Hb allosteric properties.

After looking at all the other kinemages, come back to this one to see some of those details in the context of the overall tetramer movements.

Kinemage 2 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 α 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** (His**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 the "highlights" button and choose **View2**, which is a close-up around the heme O_2-binding site. Click the "animate" button to cycle 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.

View3 is from an angle that shows the binding site more clearly. O_2 binds in the same place as **C=O**, with similar effects on the structure; however, for **O=O** the outer atom is angled rather than straight. The equilibrium between free and bound O_2 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 O_2 and other ligands, by virtue of heme-pocket differences, but the differences between affinities in the **R** vs **T quaternary states** are much larger.

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 O_2 quite tightly, which would work well for loading O_2 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 O_2 binding for satisfying physiological needs controlled oxygen [O_2]=**6•10^{-5}M** concentration in **blood** plasma.

Both alpha and beta chains of Hb resemble myoglobin (the single-chain O_2-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. Choose and turn on "Hb **hydrophobic**" temporarily to see some of the **hydrophobic** side chains that form the heme pocket. They actually surround the binding site so thoroughly that O_2 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 O_2 binding at all; this restrictive process also increases the specificity of ligand binding.

The binding-site linkage to changes in protein conformation are illustrated in **View5** (centered near the **OH** of Tyr 140), which shows ligand-dependent changes 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 O_2 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;
the proximal His changes distance and angle relative to the heme; the **F** helix shifts; Tyr140 moves and its H-

bond to **backbone** weakens; and both the **C**-terminus of the chain and Arg141 move significantly at the 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 O_2 that binds increases the likelihood of switching the tetramer into the **oxy** state, and once it switches, the O_2 affinity at all sites increases because the local structure changes have either, already occurred or are easier to make.

View6 backs off to show the entire alpha1 subunit, but centered for the whole tetramer (**deoxy form**), as it was seen in **View1** of Kinemage 1. Turn on "axes" to see the 2-fold axes of symmetry of the tetramer.

Kinemage 3 shows the critical Alpha1-Beta2 interface, where the subunits shift against each other between **deoxy T** and **oxy R states**. The startup view is an overview. 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 repeatedly, to see the relative motion of these two subunits, with a fairly stationary "hinge" near the top and a larger "ratchet" motion near the bottom. **View2** emphasizes the ratchet contact between the C helix of Alpha1 and the **FG** corner of Beta2; His97 of the Beta2 **FG** corner makes a large jump against Thr38 and Thr41 of the Alpha1 **C** helix. **View3** emphasizes 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. 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 allostery work, and mutations of residues in this interface have been found to be especially likely to influence cooperativity and allostery.

Kinemage 4 shows the salt links between Alpha1 and Alpha2, which stabilize the **deoxy** form. **View1** is 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. **View2** is a close up to see the making and breaking of these interactions. Note that Tyr140 -**OH** stays close to the carbonyl oxygen of Val93 in the **FG** corner, and that Lys127 from Alpha2 has a strong salt-link to the carboxy terminus of Alpha1 in the **deoxy** form and in the **oxy** form has a weaker H-bond to a main chain carbonyl oxygen.

Kinemage 5 shows the salt links at the **C**-terminus of Beta2, which stabilize the **deoxy T** form and make a large contribution to the **pH** dependence of **oxygen** binding, known as the Bohr Effect. **View1** is an overview, from a similar view as in Kin.3, but this time emphasizing the charged interactions nears the **C**-terminus of the beta chain. **View2** is a close-up to see the making and breaking of these interactions. Note that **Tyr145** -**OH** stays close to the carbonyl **oxygen** of **Val93** in the **FG** corner, while **β His146** moves a great deal, disrupting the salt link (charged **H-bond**) to **β Asp94** that is formed in the **T state**. 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 **Asp94**, thus favoring the **T state** and decreasing O_2 affinity. There is also a contribution to the Bohr Effect by charged side chains in the central cavity of the tetramer, where four hydrogen ions $4H^+$ bind and the **BPG^{5-} anion bind** favor the shift back equilibrium **R state lungs<=>**
$4O_2+(H^+His63,58)_4\text{betaVal1}(NH_4^+PO_4^{2-})+_2Hb_TG^-\leftrightarrow(His63,58)\text{betaVal1}(NH_4^+)_2Hb_R(O_2)_4+4H^++BPG^{5-}$ **tissues** to **T state** is **pH** dependent by the binding of protons to His(63,58). It is important biologically, because it promotes **oxygen** unloading in the **tissues** where proton $4H^+$ concentrations are elevated, for instance by the production of lactic acid in muscle.

To see some of these critical subunit interactions in the context of the whole hemoglobin tetramer, click here: *{Kinemage 1, **View 5**, master= {details} on}*. **Animate**, to see details of the **T-R** changes in the contacts at the allosteric interface between alpha1 and beta2.
Choose **View6** to see the formation and breakage of the **β His146** salt link, as described in Kin. 5.
Choose **View7** to look down the central cavity of the tetramer, this time from the alpha1-alpha2 end.

References.

[1] David R. Lide. CRC Handbook of Chemistry and Physics .90th ed. Taylor and Francis Group LLC; 2010 .

[2] Prigogine I., Defey R. Chemical Thermodynamics. Longmans Green & co ©; 1954.

[3] Prigogine I., Nicolis G. Self-Organization in Non-Equilibrium Systems. Wiley, 1977.

[4] Prigogine I. Time, Structure and Fluctuations. Lecture, The Nobel Praise in Chemistry; 1977.

[5] Kuman M. New light on the attractors creating order out of the chaos. Int J Complement Alt Med.; 2018; 11(6); 337.;

[6] Nelson DL, Cox MM. Lehninger Principles of Biochemistry. 5th ed. New York: W.H. Freman and company; 2008.

[7] Xing W, Yin G, Zhang J. Rotating Electrode Method and Oxygen Reduction Electrocatalysts. *Elsevier*; 6 (2014) .

[8] Alberty RA. Biochemical Thermodynamic's : Applications of Mathematics. John Wiley & Sons, Inc. 1-463, (2006).

[9] Pinard MA, Mahon B, McKenna R. Probing the Surface of Human Carbonic Anhydrase for Clues towards the Design of Isoform Specific Inhibitors. *BioMed Research International*; **2015**, 3 (2015).

[10] Kotz JC, Purcell KF. Chemistry and chemical reactivity. Saunders College Publishing; 1991.

[11] White VM. THE CARBON CYCLE, ISOTOPES, AND CLIMATE I and II. Lectures 37, 38; 2003 .

[12] Hanania J, Pomerantz C, Stenhouse K, Toor J, Donev J. Carbon cycle. University of Calgary's 2020 .

[13] Der wohltemperierte Planet. *Der Spiegel*. 2007 Nr.19:148-154. German .

[14] Kaksis A. The Biosphere Self-Organization Attractors drive perfect order homeostasis reactions to link bioenergetic with functionally activate oxygen and carbon dioxide molecules. 7th International Conference on New Trends in Chemistry September 25-26, 2021.27-32.

http://www.ebi.ac.uk/pdbe/entry/search/index?text:3HHB
http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=9606
http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606&lvl=3&lin=f&keep=1&srchmode=1&unlock

```
     1         10         20         30         40         50         60
    61         70         80         90        100        110        120
   121        130        140        150        160        170        180

  1 VHLTPEEKSA VTALWGKVNV DEVGGEALGR LLVVYPWTQR FFESFGDLST PDAVMGNPKV
 61 KAHGKKVLGA FSDGLAHLDN LKGTFATLSE LHCDKLHVDP ENFRLLGNVL VCVLAHHFGK
121 EFTPPVQAAY QKVVAGVANA LAHKYH       146 D HHB
```

http://www.ncbi.nlm.nih.gov/protein/3HHB_D

```
  1 VLSPADKTNV KAAWGKVGAH AGEYGAEALE RMFLSFPTTK TYFPHFDLSH GSAQVKGHGK
 61 KVADALTNAV AHVDDMPNAL SALSDLHAHK LRVDPVNFKL LSHCLLVTLA AHLPAEFTPA
121 VHASLDKFLA SVSTVLTSKY R            141 C HHB
```

http://www.ncbi.nlm.nih.gov/protein/3HHB_C

```
  1     10      20      30      40      50      60
  1 VHLTPEEKSA VTALWGKVNV DEVGGEALGR LLVVYPWTQR FFESFGDLST PDAVMGNPKV
 61 KAHGKKVLGA FSDGLAHLDN LKGTFATLSE LHCDKLHVDP ENFRLLGNVL VCVLAHHFGK
121 EFTPPVQAAY QKVVAGVANA LAHKYH       146 B HHB
```

http://www.ncbi.nlm.nih.gov/protein/3HHB_B

```
  1 VLSPADKTNV KAAWGKVGAH AGEYGAEALE RMFLSFPTTK TYFPHFDLSH GSAQVKGHGK
 61 KVADALTNAV AHVDDMPNAL SALSDLHAHK LRVDPVNFKL LSHCLLVTLA AHLPAEFTPA
121 VHASLDKFLA SVSTVLTSKY R           141 A HHB
```

http://www.ncbi.nlm.nih.gov/protein/3HHB_A

Coordinates from Brookhaven Data Bank files: 3HHB & 1HCO (human deoxy hemoglobin vs human CO "oxy")

References, for further information: To the structures used here:

1. Baldwin (1980) "The crystal structure of human carbon mono oxy hemoglobin at 2.7A resolution", J. Mol. Biol. 136: 103. (file 1HCO)
2. Fermi, Perutz, Shaanan, & Fourme (1984) "The crystal structure of human deoxy hemoglobin at 1.74A resolution", J. Mol. Biol. 175: 159. (file 3HHB)

General treatments of Hb allostery:

1. Perutz (1970) "Stereochemistry of cooperative effects in hemoglobin", Nature 228: 726
2. Baldwin & Chothia (1979) "Hemoglobin. The structural changes related to ligand binding and its allosteric mechanism", J. Mol. Biol. 129: 175.
3. Dickerson & Geis (1983) "Hemoglobin: Structure, Function, and Pathology", Benjamin/Cummings Publ., Menlo Park, CA
4. Perutz (1989) "Mechanisms of cooperativity and allosteric regulation in proteins", Quarterly Rev. of Biophys. 22: 139-236
5. Ackers, Doyle, Myers, & Daugherty (1992) "Molecular code for cooperativity in hemoglobin", Science 255: 54
6. Perutz, Fermi, Poyart, Pagnier, & Kister (1993) "A novel allosteric mechanism in hemoglobin: Structure of bovine deoxy hemoglobin, absence of specific chloride binding sites, and origin of the chloride-linked Bohr Effect in bovine and human hemoglobin", J. Mol. Biol. 233: 536

Recent Hb structures in other quaternary states or intermediates:

1. Silva, Rogers, & Arnone (1992) "A third quaternary structure of human hemoglobin A at 1.7A resolution", J. Biol. Chem. 267: 17248
2. Smith, Lattman, & Carter (1991) "The mutation beta99 Asp-Tyr stabilizes Y - A new, composite quaternary state of human hemoglobin", Proteins: Struct., Funct., Genet. 10: 81
3. Liddington, Derewenda, Dodson, Hubbard, & Dodson (1992) "High resolution crystal structures and comparisons of T state deoxy hemoglobin and two liganded T-state hemoglobins: T(alpha-oxy)hemoglobin and T(met)Hemoglobin", J. Mol. Biol. 228: 551

@kinemage 1
Hemoglobin tetramer - deoxy (blue shades) vs oxy (pink shades) animation. **View2** looks down the central cavity, which is wider in the deoxy state, forming phosphate sites. **View3** shows the quaternary structure change as rigid rotations of Alpha-Beta dimmers (turn off alpha2 and beta2 in both forms). After looking at all the other kinemages, come back to this one and animate in **Views 5-7** with "details" turned on.

@kinemage 2
Human hemoglobin alpha1 subunit, deoxy (blue shades) vs oxy (pink shades) structures. Click on "animate" to switch between the two structures, and turn on "highlights" for any of the close ups.
View1 shows the overall alpha subunit, **Views 2** and **3** are close ups of the heme site, shows the hydrophobic heme pocket, **View5** moves out from the heme toward the interface with alpha 2 (alpha 2 not shown), and **View6** goes to our standard orientation centered as if in the tetramer: see **View1** of Kin.1.