Chem 431A-L20-F'07 (week 7) admin: Online quiz deadline Chapt 4 tonight -no exceptions. Chapt 5 deadline in next week, Monday. NO CLASS Monday, Nov12. Veterans Day	Last lecture: 1) Cooperativity and Allostery of Hb 2) structure of Hb (two αβ dimers) 3) MWC and KNF models of Hb. 4) Group Quiz

Today: group quiz
1) MWC and KNF models of Hb.
2) Bohr effect. CO_2 transport. 2.3-BPG
3) Brief intro to antibody structure

Last time, surprise group quiz Consider a hypothetical dimeric hemoglobin (Hb) with a P_{50} of 4.0 kPa. What % of the total O_2 that can be carried by Hb will be delivered to tissues which are at a pO_2 of 4.0 kPa? The lungs are at 12.0 kPa. You don't have to draw any graphs. Will the Mb be able to accept the O_2 if it's P_{50} is 0.25 kPa? Prove it with a calculation.	Answer: use Hill equation: (it's general enough: if Mb, then n=1, if tetramer, then n=4 (approximately) $\theta = (P_{O2})^n / \{(P_{50})^n +(P_{O2})^n \}$ get %saturation in lungs (that should be close to 100%) then get %sat'n in tissues (that should be quite low). Then subtract the 2 to see how much molecule loses to the tissue. If Mb has a higher %sat'n than Hb, then it means it is capable of getting the O_2 released by Hb.

Last time 2 models of allosteric binding: **MWC or concerted or symmetry model:** 1) an allosteric protein is an oligomer of symmetrically related subunits 2) Each subunit can exist in 2 conformational states designated R and T; these are in equilibrium 3) Ligand can bind to a subunit in either conformation. Only the conformational change alters the affinity for the ligand. 4) The molecular symmetry of the protein is conserved during the conformational change.	**Koshland et al model, sequential model** 1) ligand-binding induces a conformational change in the subunit to which it binds. 2) cooperative interactions arise through influence of those conformational changes on neighboring subunits 3) Conformational changes occur sequentially. (Reduces to the MWC model if the interactions between subunits is strong). Both features are observed in Hb – O_2 binding. ** look at the conformational changes.

Hb transports H^+ and CO_2 . both pH and $[CO_2]$ affect O_2 binding by Hb *Carbonic anhydrase*: $CO_2 + H_2O \rightarrow H^+ + HCO_3^-$. Raising $[H^+]$ and lowering pH. Go over *Bohr effect*. (Fig. 5-16) Hb binds O_2 and H^+ *inversely* to each other like: $\mathbf{H}\text{-}Hb^+ + O_2 \Leftrightarrow Hb\text{-}O_2 + H^+$	H^+ binds to the His^{146} HC3 residue (C-terminal aa) of the β subunit. CO_2 also binds as *carbamate* group to N-terminal's α amino group of all 4 subunits inversely to O_2 binding. $CO_2 + H_2N\text{-subunit} \rightarrow {}^-O_2C\text{-NH-subunit} + H^+$ (carbaminohemoglobin)

Another ligand:(*heterotropic* allosteric modulation): 1 CO_2^- 2 H C-PO_4^{2-} 3 H_2C-PO_4^{2-} **2,3 *bisphosphoglycerate*** (2,3-BPG) binds to the (+charge sidegroups of) central cavity between the β subunits of the T state. It's binding to Hb has inverse relation to O_2binding: Hb-BPG + O_2 ⇔ Hb-O_2+ BPG .BPG lowers affinity of Hb to O_2 (fig 5-17) Absence of BPG makes it behave like Mb	Fetal Hb contains: $\gamma_2\beta_2$ not $\alpha_2\beta_2$ as in adults. $\gamma_2\beta_2$ has a lower affinity for 2,3- BPG and therefore higher affinity for O_2. Fetus needs to extract O_2 from the moth2r's Hb and therefore must have a lower affinity for O_2 than the Hb of the mother **Sickle cell anemia:** Due to single aa substitution: a Val instead of a Glu in the position #6 of the β chain. Consider the hydrophobic vs ionic R group substitution: the "sticky Hϕ patch" causes the Hb to aggregate into strands when in Hb in the deoxy form. Need to avoid vigorous exercise.

Other examples of protein-ligand interaction: Hb is useful model but also different from other ligand protein binding. It uses prosthetic group (heme) and it has a small molecule ligand, O_2. Its best example of *complementary* binding is that of 2,3 BPG Antibodies (immunoglobulins) The immune system provides another example of *complementary* interactions between proteins and ligands. *Immunoglobulins* or *antibodies* bind to bacteria, viruses, foreign cells, or even large foreign molecules thus destroying them in some way.	Target molecules or cells of the antibodies are ***antigens***. Any particular molecular structure within antigen that antibody binds to is the ***antigenic determinant* or *epitope***. Small molecules ≤ 5000 daltons usually not antigens but if bound to larger molecules they can be, called ***haptens***. **Immunoglobulin G** (IgG) (one of 5 Ig's in verteb) -has 4 polypeptide chains: 2 ***heavy chains*** and 2 ***light chains***. Linkage is by noncovalent intxns and disulfide bonds. MW150,000, 3 domains: one Fc & two Fab fragments. Fc=easily **c**rystallized fragment Fab=antigen binding fragment (identical, sites of variability for different antibodies) Fab binds by induced fit. K_d as low as 10^{-10}! tight-fit

Other examples: Actin, Myosin & Molec. motors Basis for mechanical motion of cells and organisms. Muscle tissue: contain ***myosin*** and ***actin***. (≈ 540 kdaltons) **Myosin**= has 6 subunits: 2 heavy, 4 light chains. c-termini: 2 supercoiled α-helices n-termini: 2 domains with 2 light chains each myosin aggregates = *thick filaments*	**Actin** = found in most eukaryotic Cells Globular actin, *G-actin* are monomers that form long polymers: *F-actin* . Actin is associated with the *thin filament* which contains 3 proteins: F-actin, *tropomyosin* and *troponin* Look at the force generation of a cycle