Myoglobin and Hemoglobin

* Ligand- molecule that binds reversibly to a protein.
  + Hemoglobin is allosteric and myoglobin does not
* Allostery - communication of binding from one site
* Protein interacts with other protein
* Ligand- small (glucose / atp)
* ligand reversibly binds to protein’s binding site
  + They are tailor to bind to certain ligands, highly specific
* When ligand bind to protein, it will induce a conformational change to proteins
* Binding of ligand will have an effect on the protein
* Tertiary structure arrangement define the structure
  + Induce change in 3 structure
* Monomeric- one binding site
* Olligeric- multiple binding site, then it becomes complicated bc both can bind to ligand.
  + Interface-surface they share
  + Oligomeric binding of the ligand change in conformational change in blue subunit and because they share an interface.
  + The conformational change that happens to the blue subunit will also cause a conformational change to the pink subunit.
  + Conformational change in pink distorts the structure of binding site of the pink subunit so that it can no longer bind to ligands.
  + Communicate through the interface to the pink to switch the binding site
* Allostery - communication of binding site from one to other
  + Pink - negative allostery one turns off another
  + Or positive allostery where binding of one binding site improves the other site.
* New
* Quantification of ligand binding
* Interaction of ligand
* Equilibrium with P l
* Easier to consider the dissociation (Kd)
* More intuitive number
* Describe the nature of that ligand protein interaction.
  + Determine Kd - binding curve
  + **Y fraction that is bound to ligand**
    - How much protein is bound to ligand at different concentration of ligand.
* Characteristics of binding curve
  + First fill out very quickly as ligand increase then you run out of binding site then it plateaus off.
  + Kd is the ligand concentration that gives rise of 50 % of protein binding site filled with ligand
  + Ligand concentration that gives a fractional binding of 0.5
  + How much binding at different ligand concentration -- Kd
* You can measure the look at curve and the Kd to see which is the better binding ligand
* Shift to the right Kd -- fill 50 % of the ligand binding site of the blue at a higher concentration of ligand than the red.
  + You have to add more ligand of the blue then the red affinity of the blue is lower than the red. Kd is larger than you have to fill in a lot of ligand decrease affinity.
* Red has greater affinity than blue
* New
* Myoglobin and hemoglobin (allostery)
* myoglobin -- Storage protein
  + Well of oxygen with burse of oxygen
* hemoglobin - heavy
  + Oxygen transport protein move in red blood cell
  + Deliver oxygen to tissue
  + Oxygen binding protein
* Both bind to oxygen the same way
  + Uses Heme
* Non protonation group bind irreversibly to protein and pass function to the protein
* Heme can bind to oxygen add function
  + Different part
  + Iron ion fe 2+ center of protoporphrin ring is hexacoordinated. Coordinated by 4 nitrogen atom. 2 available coordination site for fe 2+. Planar
  + Z view
    - nitrogen donated by histidine to fill ½ available coordination site myo + His (proximal his)
* Monomeric- myoglobin donates His residue sidechain to fill one of the available coordination sites, proximal His residue in both myo/ hem
* 6th available coordination site -- bind to a molecule of oxygen gas. Ligand binding site
  + Oxygen molecule binding on the other side.
* Single molecule of heme is capable of binding single molecule of O2. Binding to more O2 --> incorporate more molecule of heme.
* the proximal his residue immediate adj to heme can sense where the oxygen is bind to heme or not
* Proximal His so important
* New
* Myoglobin monomeric almost all alpha helices
* Heme sit deep in the myoglobin structure
* Loops named by to helices they join together
* New
* Myoglobin
* Free heme bind to CO with much greater affinity than O2.
* Structure of myoglobin promote O2 than diatomic gases (CO)
  + The way it does it is by forming more interaction with oxygen not found when bound to CO.
  + Because of electronic structure when it bound to heme. Tilted geometry.
    - Distal His E7 above oxygen binding it position perfectly to have H bonding with O is tilted when it binds.
    - E7 that forms more binding energy → decrease Kd.
    - Using this structure promote binding of O2 than CO
    - 20 kJ of binding energy decreases Kd of binding of heme to oxygen than the binding of free heme to oxygen
* New
* Increasing concentration of oxygen → Myoglobin is fully bound
* Decrease oxygen remain the same
* Extremely low conc of oxygen is when myoglobin starts to release bound oxygen
* Myoglobin storage so you want high affinity and you want to hold on to the oxygen until the thing really need oxygen
* Binding of myoglobin perfect for storage bc so high affinity
  + Transport NAH
  + You want it to release the oxygen at not supper super low.
* New
* Tertiary structures are very similar myo +hg
* Hemoglobin
  + Key difference oligemic 4 subunit not all subunit have the same primary sequence.
  + 2 alpha and 2 beta subunit
  + Each contains a bound heme.
  + Capable of 4 oxygen binding sites.
* New
* Arrangement of alpha and beta create 4 distinct interface
  + Interface means of communication one subunit to another
  + Horizontal and vertical
* How many when the residues extent of the interface more residues buried more stable the allosteric structure
  + The more residues buried the within interface the more stable the allosteric structure.
* new
* What happen when hemoglobin binds to oxygen
* For allostery to occur, Ligand binding → conformational change of one subunit and it is transmitted and influence adj subunit → conformational change of the other subunit.
* Crystal of deoxy hemoglobin (absence of oxy) and took it to expose it to oxygen.
  + Crystals shattered
* Molecule of hemoglobin within crystal are changing shape in response to binding.
* New
* Compare two conformational structures
* Deoxy hemoglobin
  + T state- tense
  + Low affinity for O2
  + Predominates when O2 is not bound
* R state-oxy hemoglobin after exposure of o2 to hemoglobin structure difference
  + Relaxed state
  + High affinity for o2. O2 bind is promoting their conformational change
* New
* T state is called tense state because there are additional interaction present between subunit. There are not much in R.
* More constrained by additional interactions.
* Additional interactions between different subunits.
* Asp fg 1 first residue of that loop (fg loop)
  + In tense state, Asp fg 1 forms an **ionic interaction** with sidechain of His HC3. beta subunit. Both beta.
    - C terminus 3 residue helix H
* Because hc3 is the most C terminus so it has carboxyl end. His hc3 can forms ionic interaction with a charged sidechain lys C5 alpha subunit.
  + Network of ionic network from beta to alpha
* Heme group proximal his f8 will sense presence bound O is at f8
* when oxygen is bound to heme, it will induce change in proximal his residue in helix f
* Be transmitted to asp fg1 and it will be transmitted to ionic interaction
* Oxy binding to transmitted from beta to alpha because of chain of ionic interactions.
* Ligand binding affect alpha subunit bc of the ionic interaction
* new
* Tense state
* Asp 94
* Tyr hc 2 pac into H helix penultimate tyr but expelled in R
* Tyrosine moves so much from t-> r
* New
* When oxygen binds to fe 2+ ion, it will pull fe2+ ion downwards → puckered heme to planar heme in r state when oxy is here
* Change in electronic structure will pull fe2+ ion downwards into plane of heme rings.
* Proximal His pulled down with fe 2+ ion when fe 2+ is binds to ion
  + Displaced his sidechain 0.6 angstrom downwards (+tilted) but enough to trigger cascade of interaction
  + Come straight down then clash, prevent steric clashes 0.6 down and tilts because his is part of alpha helix, like a rod so when his tilt, whole helix will tilt.
  + Imparted on the position of helix f.
  + When his comes down it tilts to prevent steric clashes
  + His helix f not change whole helix tilts -> large tilting helix f tilts
* Helix f end (proximal his f8). Asp fg 1 will change.
* Change position of asp fg1
  + By change the position then it will break the ionic position of ionic interaction.
    - Collapsed the network of ionic interaction.
    - Cumulative effect sidechain, subunit slide to each other like a compacted structure.
* Break interaction you broken interaction on the other side. Change conformation from relaxed state adj alpha 1 to relaxed state.
  + Induced structural conformation.
* New
* If all are in R state then binding curve like myoglobin
* If locked in T state, then release oxygen in tissue but never fill with oxygen bc affinity is so low.

Lecture 12: Allosteric Models

* Tetrameric- hemoglobin
* Allosteric model- principle ligand binding of one binding communicated to an adjacent to
* There are no intermediate when some of the subunit are view using crystal microscopy.
* New
* **All these interactions are in equilibrium with each other**.
* Oxygen binding reposition is equilibrium with t state.
* Not really dominos effect/ cascade
* New

1) T state ionic bonds holding each other with each other tension between subunits

* + Tension will release if you could release bonds

2) Take T state -- expose to high conc oxygen at lungs

* + Affinity for oxygen is fairly low at that point
  + Oxygen binding in heme group in alpha 1 subunit will result in conformational change will break some of ionic bond that links alpha 1 and alpha 2 subunit; broken some of the bonds linking them together
  + Adjacent subunits b1 and b2 more relaxed
  + B1 and b2 more relaxed easier for to undergo conformational change or binding to oxygen

3) broken enough ionic bonds → release enough tension → everything can collapsed to the R state.

* + When all the subunits have release the tension. All the subunits will collapse.

4) Result of binding of two oxygen, 4 subunits are in R state, 2 not bound but in high affinity state because they have high affinity → eventually all R state

* + At first it is difficult to bind to oxygen but after each successive oxygen binding event, it gets easier. Convert others in high affinity state to bind quickly
  + Cumulative effect is hemoglobin goes low affinity to high affinity
* **The whole thing is an equilibrium between T and R state.**
* **Oxygen can bind to any of the subunit.**
* New
* Hemoglobin binding curve
  + **Sigmoidal shape**- indicative cooperative binding (allostery)
* Allosteric protein
  + Low conc-> t state/ low affinity state
* As we increase oxygen conc, we push some of the subunits to bind to oxygen then relax hemoglobin and generate t -> r transition that will result in high affinity r state
  + Affinity for R starts from low -> high
  + Then we fill up all the binding site and then plateau
* Cooperative binding → sigmoidal shape
* Difference in cooperative binding and allostery
  + Allostery - How one communicate to another subunit
  + Cooperative binding is what we observe when we measure binding curve
* Cooperative binding indicator for allostery.
* New
* Study allosteric
* Degree of cooperativity can be quantified using a modified version of Hill curve
* P+nL<->PLn n number of bound ligand number of ligand binding sites
* Fractional bonding
* Cooperative binding exist or not - Hill equation
* Slope=nH (Hill coefficient)
  + Can explain cooperative binding exist or not
  + N reflect the degree of interaction between binding site.
  + Myoglobin n=1 positive allostery n>1
    - Slope of curve never reach # of subunit.
  + Highly allosteric NH is significant.
    - Positive cooperativity.
      * Ligand binding in one site improve another.
  + Myoglobin NH is 1 no allostery
  + Negative cooperative nh <1 never zero
    - Impairing another by binding to another.
* New
* Model used to explain allostery
* MWC model
* Concerted model
* All or nothing model of hemoglobin transition
  + Five main points
    - Only two conformations exist all T or all R
      * Nobody see intermediate
    - All t and all r are in shifting equilibrium between each state.
    - All subunit make t ->r transition simultaneously
      * Switches to all t or r
    - Ligand can bind to both t and r but has higher affinity for R
    - Each successive ligand binding increase likelihood of T-> R transition.
  + Limitation: you don’t see protein conformation when oxygen is bound based on the model
* Sequential model
  + Four main points
    - Requires existence of many mixed state tetramer conformations
      * As soon as a heme binds to completely shape and changes the shape of polypeptide
      * Nearby shape change conformation slightly affinity also increase.
    - Ligand binding induces T to R in single subunit
    - Ligand binding induces T to R in single subunit.
    - Transition in one subunit increases likelihood of transition in adjacent unit
  + Have both t and r and intermediate states
  + Limitation: the only time we have r state is when all 4 subunits is filled with oxygen then it R state. But when three of them are filled then it should have R state.
* New
* Evidence on MWC model: cannot explain negative cooperativity
* To explain negative cooperativity: need 3 states
* Sequential requires intermediate state but these are never observed in experiments
* How do these models relate to hemoglobin
  + Both mwc and sequential both have the same curve for one thing so it doesn’t give us enough insight on how allostery works.
* New
* Hemoglobin also transports H+ and CO2 transport
  + Produced as respiration.
* How to deal with CO2? Not very water soluble.
  + Solution: carbonic anhydrase take CO2 reacts with water to form bicarbonate so it is more soluble in plasma to move back to the lungs
  + to reconverted to CO2.
  + CO2+H2O<-> H++HCO3-
  + New Problem: Will produce large amounts of H+.
    - pH will decrease significantly as we produce bicarbonate hemoglobin will manage the decrease the pH transporting protons back up to lungs. Hg bind to CO2 in a matter irreversibly with oxygen.
* Bohr effect.
  + Decrease pH → binding curve to the right
    - pH decrease as affinity of hg decreases
    - Because more protons less affinity for oxygen
* New
* Hg bind reversibly to proton
  + Need a negative sidechain proton transport
* His HC3 present in subunits
* Under circumstances of Lungs
  + Protonation of His HC3 promotes formation of ionic bond with Asp FG1
    - Addition of the proton to the His.
  + In order for his to form interaction with Asp ionic T state, His has to be protonated → will promote ionic interactions.
* Ionic bond→ T state so protonation of His will increase T state formation over r state
  + When T state forms, oxygen released.
  + Proton binding reversely binding to o2
* Why H and O2 reverse? bc H favors t states
* When hg is at the lung, it is fully at the R state. Ionic bonds not formed. They are at different conformation.
* 7.6 then His will be deprotonated. As his move lung to tissue pH will decrease and release in protonation R to T.
  + Result in protonation of His side chain favor ionic interaction r-> t exportation of oxygen from heme.
* CO2 same situation as proton.
* CO2 is capable reacting with n terminus; it creates carbamate ion covalently bound to the N terminus of each of 4 hg subunit.
* Carbamate ion is negatively charged
  + is capable of forming ionic interaction only in T state.
  + Just like proton in histidine, carbamate form interactions with N terminus
* When CO2 binds to the amino terminus, it promotes T state and release of oxygen in the tissue.
* Protonated/ carbomated hg free to move back to lung where high oxygen conc convert t state back to r state→ the deprotonation His sidechain decarbamation of amino group carbon and H free to be excreted out of lungs.
* New
* Oxygen acts allosteric not the only allosteric effect
  + BPG
    - Bpg binds to the center of cavity.
    - One bpg per tetrameric hg
* There’s a cavity in T state but not in R state.
  + Cavity is lined with positively sidechains from His
  + Only binds to T bc cavity is only in T state
  + When bound inhibits t->r
  + R cavity closes blocks binding of bpg
  + High bpg bind to cavity and resist t->r transition promotes t state
* Adaptation for living high attitude.
* New
* Concentration of oxygen the same at high altitude and sea level in **the tissue**
* When bpg stabilizes t state barrier to the high barrier r state
* 2,3,6, shift to the right allosteric effector of hemoglobin bind somewhere different than oxygen and it changes the affinity of oxygen. Positive charges that repel each other.
* T state very unstable so it would want to shift to R state.
  + Bpg many negative charges and small enough by going into that pocket, can interact with positively subunits.
  + Stabilizes the state equilibrium back to the T state
  + T state can exist because it is more stable than w/o bpg. Pure form is unstable because so many positive repelling charges.
* When you go up the conc of bpg increases
* Bpg stabilizes t state
* Much easy to go from r->t transformation
* Low affinity for oxygen at high conc of bpg than
* 100% lungs up alitude 60% bound to oxygen transported 40% of the total mount when lungs to tissues
* 90% of oxygen bound to hg at high altitude 60% bound to tissue transport capable 40-> 30%
* Same amount of oxygen just harder to breathe. BPG helps loosen the affinity of oxygen to hg
* **Same amount of oxygen in tissues regardless of altitude**
* Decrease the amount of r state oxygen
* Transport capacity 30% in sea level 30% of oxygen is transported out and in high altitude 37% of oxygen is transported out.
* BPG concentration to rise when in higher altitudes.
  + Bound heme slight decrease when more bpg
    - Bc bpg bind to t state resisting r state which means less affinity.
  + By stabilizing t state, bpg is allowing hg to recover its oxygen transport capacity.
    - Because bpg binds to t state oxygen transported