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=n_H (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 CO_2 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.
    - $CO2+H2O <-> H^+ + HCO_3^-$
    - 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