

Biochemistry study guide/outline covering: Binding affinity, Allostery & cooperative binding, Hemoglobin (Hb), Enzyme fundamentals & mechanisms, Basic types of reaction types/enzyme classes (loosely based on EC classes), Enzyme kinetics, and Enzyme inhibitors

Here is a lightly-modified version of an outline/study guide I provide my students for their second Concept Check. Shared in case it can be helpful to others.

- Review your amino acids:
 - At this point in the course, I expect you to know the 1-letter and 3-letter abbreviations and be able to associate those with amino acid properties (e.g. if you see "E," you should think "glutamate, Glu, "acidic", often -)
 - I also expect you to be comfortable with mutation notation for amino acid substitutions (e.g. E6V indicates that there's normally a glutamate at position 6 of the amino acid chain, but now there's a valine)

Binding affinity

- The main way we talk about and compare binding affinities is through the **K_d**
 - K_d is the **equilibrium dissociation** constant
 - $K_d = [A][B]/[AB]$ (unbound over bound)
 - Has units of concentration -> convenient
 - K_d = the concentration of one binding partner at which half of the other is bound ($\frac{1}{2} B_{max}$)
 - At concentration $> K_d$, more than $\frac{1}{2}$ is bound
 - At concentration $< K_d$, less than $\frac{1}{2}$ is bound
 - Has an inverse relationship with affinity
 - **The lower the K_d the stronger the affinity**
 - You need fewer chances to bump into something in order for half of the copies of molecule to be bound at equilibrium
 - Could be because you bind tighter and/or release faster
 - It comes from the rate constants of binding (k_{on}) and unbinding (k_{off})
 - $K_d = k_{on}/k_{off}$
 - It does not tell us anything about rate - if you want k_{on} or k_{off} , you have to measure them directly (or at least measure one of them and the K_d to find the other)
 - K_a is the **equilibrium association** constant
 - Has units of 1/concentration -> *not* convenient
 - Has a direct relationship with affinity
 - **The higher the K_a the stronger the affinity**
- You should be able to interpret and sketch binding curves - both linear and semilog scale
 - All binding curves look sigmoidal on a semilog plot
 - Higher affinity things will be shifted to the left → takes a higher concentration of partner to reach $\frac{1}{2} B_{max}$
- Lower affinity things will be shifted to the right → takes less partner to reach $\frac{1}{2} B_{max}$
- Gas-binding affinity is often reported as **p50** values
 - The p50 is like a K_d (so an inverse measure of binding affinity), but in terms of partial pressures (which are often used to report gas concentrations)
 - The p50 for O_2 binding to hemoglobin is the partial pressure of O_2 needed for 50% hemoglobin saturation
 - A lower p50 indicates higher affinity

Allostery & cooperative binding

- **Allostery**: when something (such as ligand binding) at one place on a molecule and affects what's going on someplace else in the molecule
 - Typically relayed through conformational changes
 - Can be brought about by allosteric modulators that bind and/or by the addition of post-translational modifications like phosphorylation
- **Modulator**: something, often an allosteric binder, that impacts binding of a ligand
 - **Positive modulator**: increases binding affinity
 - e.g. O_2 with regards to hemoglobin binding more O_2
 - **Negative modulator**: decreases binding affinity
 - e.g. BPG towards hemoglobin binding more O_2
 - **Homotropic** if it influences binding of the same ligand
 - e.g. binding of one O_2 to hemoglobin affects the affinity for binding of more O_2 to the other Hb subunits
 - **Heterotropic** if it influences binding of a different ligand
 - e.g. binding of BPG to hemoglobin affects the affinity for binding of O_2 to Hb
- **Cooperative binding**: when binding of a ligand to one binding site influences the binding of a subsequent ligand
 - Need to have multiple ligand-binding sites (could be on single chain or on separate subunits) in order to have the possibility of cooperative binding
 - **Positive cooperativity**: binding of one ligand *increases affinity* towards subsequent ligands
 - **Negative cooperativity**: binding of one ligand *decreases affinity* towards subsequent ligands
 - You will not need to identify negative cooperativity from a graph
- Not on concept check but included for reference: the **Hill coefficient**, n_H , is a measure of the extent and direction of cooperativity:
 - $n_H > 1$: positive cooperativity
 - $n_H < 1$: negative cooperativity
 - $n_H = 1$: no cooperativity
- Positive cooperativity will show as a **sigmoidal curve** even on a linear scale of equilibrium binding
 - Caution: All binding curves (even non-cooperative) will look sigmoidal on a log scale
 - If you see a sigmoidal curve on a log scale, you can't tell if it's cooperative or not

Hemoglobin (Hb)

- 4 subunits: 2 α & 2 β in adult hemoglobin
- Each subunit binds 1 heme (which is bound to 1 iron) & can bind to one oxygen molecule (O_2)
 - The iron is coordinated by (bonding to) nitrogen lone pairs in the heme & histidines in the Hb
- Hemoglobin shows **positive cooperativity** with regards to O_2 binding
 - Binding of one O_2 increases affinity of other sites towards O_2 → leads to increased binding at other sites
 - This occurs due to structural transitions between a **low- O_2 -affinity taut (T) state**, and a **high- O_2 -affinity relaxed (R) state**
 - Remember R, red, oxygen-bound state
 - You're relaxed when you have oxygen - and so's your Hb!
 - Taut state gets its name from the tautness (tightness) between the subunits that occurs due to **salt bridges** that form between charged amino acid residues
 - The more oxygen there is around (the higher the pO_2 (concentration of oxygen in terms of partial pressure)) the more likely oxygen is to bind (just by chance alone)
 - Binding of oxygen to the iron in heme pulls on the iron → pulls on the Histidine coordinated to the iron → pulls on the rest of the protein chain the His is attached to → pulls on the other subunits → breaks salt bridges → stabilizes R state → more O_2 binds
 - Offloading O_2 of reverses things → brings charged residues close together allows salt bridges to form again → stabilizes T state → O_2 released
 - This allows Hb to efficiently pick up oxygen in the lungs (where pO_2 (concentration of oxygen in terms of partial pressure) is high and Hb is in the high-affinity R state) and hold onto it as it transports it to the tissues, where pO_2 is low, and thus the Hb transitions to the T state, offloading oxygen to the places in need & not picking much of it back up until it gets back to the lungs, where there's plenty of O_2
 - On a graph (even on a linear x-axis), this shows up as a sigmoidal curve
- If the subunits were isolated, each subunit would still be able to bind O_2 , but wouldn't show cooperativity
 - This is similar to myoglobin, which looks like a single subunit of hemoglobin
- The **Bohr effect** further fine-tunes Hb's affinity & cooperativity towards O_2 → **explains how low pH decreases O_2 affinity (shift rightwards in the binding curve)**
 - CO_2 dissolves in blood & leads to carbonic acid formation → decreases pH (increases $[H^+]$)
 - **CO_2 is higher in your tissues** than in the lungs because your tissues are burning lots of energy (thus producing CO_2 as a waste product) and can't just breathe it out like they could in the lungs
 - **pH is lower in tissues** than in lungs
 - Some of the salt bridges that form between the subunits to stabilize the T state are dependent on a Histidine being protonated
 - At lower pH there are more protons around so His more likely to protonate (remember His has a $pK_a \sim 6$, so it can readily go back & forth under physiological conditions, but is more likely to protonate if there are more protons around)
 - At a lower pH (like in the tissues), His protonates → helps form salt bridges that stabilize T state → Hb offloads O_2 and doesn't pick it back up readily
 - **Overall process: $\uparrow CO_2$ in tissues leads to $\downarrow pH$ → His protonates → induces inter-subunit salt bridges that stabilize low-affinity T state → oxygen offloads & doesn't bind readily → stays mostly unbound until more O_2 around & pH is $\uparrow$ (such as in lungs)**
- Some CO_2 also directly modifies the N-termini of the subunits, leading to additional inter-subunit salt bridges
- CO (carbon monoxide) competes with O_2 , binding for the same binding site, but CO binds more tightly
 - CO binding locks Hb into the R conformation, making it difficult to release oxygen
 - Since they're binding at the same location, CO also reduces B_{max} of O_2 binding to Hb

- BPG is a negative modulator of O₂ binding
 - Binds to central channel through salt bridges → stabilizes T form (not enough room in the channel in the R form) → decreases O₂ affinity
 - Fetal Hb has lower affinity for BPG → higher O₂ affinity
 - People at higher altitudes make more BPG so they can dump out more O₂ in their tissues
- Modulators of O₂ binding:
 - O₂: positive, homotropic
 - BPG: negative, heterotropic
 - CO₂: negative, heterotropic
 - CO: positive, heterotropic

Enzyme fundamentals & mechanisms

Note: You will not need to propose any mechanisms (draw your own arrow-pushing diagrams), but you should be able to interpret ones that are provided & describe the role of the different residues involved

- Key properties of enzymes:
 - Catalyze (speed up) a reaction in both directions
 - By reducing the activation energy required for the reaction to occur
 - DO NOT change overall thermodynamic favorability - cannot change equilibrium constants, can only increase the speed at which you can reach that equilibrium
 - Don't get used up in the process
 - Lower the activation energy required for the reaction to occur ($\Delta G^\ddagger$) by lowering the energy of the Transition State (TS) (more below)
- Enzymes use combinations of strategies including...
 - **Entropy reduction (proximity catalysis)**
 - Help molecules find one another & stay in the right orientation to react
 - Binding to the enzyme kinda pre-pays the entropic cost of bringing the molecules together, and the enthalpic gains from binding to the enzyme help offset the cost
 - **Acid/base catalysis**
 - General bases
 - Take proton to strengthen the nucleophile - make it more attack-y
 - This nucleophile is often water (e.g. they take a proton from H_2O to produce a hydroxide ion, OH^-)
 - Stabilize positive charge in the transition state to lower the activation barrier
 - General acids
 - Donate proton to strengthen the electrophile - make it more vulnerable to attack
 - Can make for a better leaving group
 - Stabilize negative charge in the transition state to lower the activation barrier
 - **Metal catalysis**
 - Can stabilize negative charge in the transition state to lower the activation barrier
 - Can give & take electrons to help with redox catalysis
 - Can coordinate with lone pairs on amino acid residues (e.g. His) and/or form ionic interactions with acidic amino residues and/or negatively-charged parts of substrate (e.g. phosphate groups in ATP) to serve as a sort of hub to hold things together in the right orientation
 - **Covalent catalysis**
 - Offer an alternative reaction route w/lower activation energy
- **Hints about types of catalysis taking place**
 - If protons are getting given or taken → acid/base
 - If an amino acid in the active site is donating a proton → general acid (in that step)
 - If an amino acid in the active site is taking a proton → general base (in that step)
 - If the enzyme is forming a covalent bond to a substrate → covalent
 - If a metal is present → metal
 - Always → proximity
- Enzymes often bind to helper molecules called **cofactors** that provide additional functional groups to work with
 - Can be metals or small organic molecules
- **Transition states vs reaction intermediates:**

- You will not have to draw any transition states but should know what they are & why they're important
- You also will not have to draw any energy coordinate diagrams but should be comfortable with what they represent
- **Transition states (TS)** are fleeting moments (shorter than length of bond vibration) in which bonds are being broken & formed
 - Involve **partial covalent bonds** & partial charges
 - **Can't be isolated** but we know they have to exist
 - **High energy points**
 - **Peaks** on reaction coordinate diagrams
 - Often represented with **brackets** & the symbol $\ddagger$
 - **Peaks** on reaction coordinate diagrams
 - The TS with the highest activation energy represents the slowest step in a reaction → will be the rate-determining step (RDS)
- **Reaction intermediates** are (at least theoretically) isolatable molecules with **full covalent bonds**
 - **Lower energy points**
 - **Valleys (troughs)** on reaction coordinate diagrams
- **Enzymes bind (and thus stabilize) the transition state (TS) the most tightly**
 - Interactions (noncovalent IMFs & sometimes covalent bonds) with the TS provide "**binding energy**" that lowers the free energy of the TS → decreases the activation barrier and thus allows the reaction to happen faster
- Amino acid residues that are protonatable/deprotonatable can act as **both general acids & general bases** (remember that the enzyme has to be regenerated each cycle so something that acts as an acid, donating a proton, must then act as a base, accepting a proton, in order to be able to act as an acid again)
 - Amino acids with hydroxyl or thiol groups: Ser, Thr, Tyr, Cys
 - Amino acids with amine groups: Lys, Arg, His
 - Amino acids with carboxyl groups: Asp, Glu

Basic types of reaction types/enzyme classes (loosely based on EC classes)

- **Reduction & oxidation**
 - **oxidoreductases:** catalyze redox reactions (electron transfers)
 - names like thingamabob- “dehydrogenase”, “reductase,” or “oxidase”
- **Group transfer**
 - **transferases:** catalyze group transfer
 - names like thingamabob-transferases (e.g. aminotransferases, methyltransferases), kinases (these are one of the main types of transferases we'll see)
- **Hydrolysis**
 - **hydrolases:** use water to break bonds
 - names like thingamabob-ase, where “thingamabob” is the substrate that's getting broken. Examples include nucleases, proteases
- **Elimination &/or bond forming or breaking other ways**
 - **lyases:** add or remove things to make (or break) bonds (often double bonds, but not always)
 - includes things with names like thingamabob- “decarboxylase,” “aldolase,” “dehydratase,” and “synthase”
- **Isomerization**
 - **isomerases:** modify a single molecule (like moving an oxygen 1 carbon over or flipping a bond that points up to one that points down) w/o changing its atomic composition
 - includes many thingamabob isomerases or mutases, epimerases, racemases, tautomerases...
- **Ligation**
 - **Ligases:** use ATP or a similar molecule to join together 2 things
 - Names like thingamabob ligase, as well as some “carboxylases,” “synthetases,” etc. sprinkled in there

Enzyme kinetics:

- Michaelis-Menten (M-M) kinetics describes kinetics of simple enzymes measured in the steady-state
 - MM requires many assumptions & is only reasonable early in the reaction, before product build-up, and when $[S] \gg [E]$
 - Steady-state assumption refers to the concentration of $[ES]$ remaining constant. The MM equation describes kinetics of simple enzymes measured in the steady-state:
$$V = \frac{V_{max} [S]}{K_M + [S]}$$
 - You do not need to memorize this - and will not have to do any calculations on the exam - but you do need to know what those terms represent
 - V : velocity of product formation at any point
 - V_{max} - maximum velocity, the fastest that a lot of copies of an enzyme can do work - depends on enzyme concentration
 - K_M - see below
- k_{cat} is aka the “turnover number” - it's a measure of how “good” an enzyme is at converting a substrate into product
 - Mathematically, it is $V_{max}/[E]$ - it basically tells you how fast one copy of an enzyme can work
 - For a simple reaction, it's the rate constant for the slowest step, which is typically k_2 in the general equation
 - **Higher k_{cat} is “better”**
 - E.g. a k_{cat} of 10 s^{-1} means a single enzyme can turn over (convert to product) 10 substrate molecules per second, whereas a k_{cat} of 0.1 s^{-1} means it would take 10s to turn over 1 substrate molecule
- K_M is an (imperfect) indicator of substrate **affinity** - it's similar to the K_d
 - Substrate concentration at which initial velocity, $v_0 = \frac{1}{2} V_{max}$
 - **Lower K_M is “better,”** higher affinity, (akin to how a lower K_d was higher affinity towards ligand when we were just talking about binding thermodynamics)
- k_{cat}/K_M is the “specificity constant” or “catalytic efficiency”
 - A measure of overall “how good” is an enzyme towards a specific substrate
 - Can be used to compare same enzyme towards different substrates, but shouldn't be used to compare different enzymes
 - Higher catalytic efficiency $\rightarrow$ converts substrate to product more quickly and/or without requiring as much substrate (work fast w/little waste)
- **Mutations to catalytic residues** - ones directly involved as general acids/bases, nucleophiles, electrophiles, TS-stabilizers, etc. **will likely impact k_{cat} more than K_M**
 - Such mutations will typically decrease k_{cat}
 - More conservative mutations (e.g. Glu to Asp) are likely to have a lesser effect than more drastic mutations (e.g. Glu to Ala) but still can have a big effect
- **Mutations to residues involved in substrate binding** but not directly involved in the catalytic mechanism **will likely impact K_M more than k_{cat}** (but may still have an effect on k_{cat} if they were helping position things for catalysis)
 - Such mutations will typically increase K_M
 - More conservative mutations (e.g. Glu to Asp) are likely to have a lesser effect than more drastic mutations (e.g. Glu to Ala) but still can have a big effect
- **General strategy for mutagenesis problems:**
 - Determine the role of the amino acid residue
 - Consider which properties are important for that role

- Compare the properties of the amino acids in question
- Determine whether the properties of the substituted amino acid would be conducive to the needed role and/or how the properties of the substituted amino acids might hinder it

Enzyme inhibitors:

- In the presence of an inhibitor, the inherent “goodness” of an enzyme doesn’t change - it still actually has the same constants, K_M , k_{cat} (and thus v_{max}), etc. BUT what we measure may change - so we call what we measure the “apparent” K_M (K_M^{app}) & apparent V_{max} (V_{max}^{app})
- **Irreversible covalent inhibitors**
 - Often “mechanism-based,” aka “suicide” inhibitors → take advantage of covalent catalysis mechanisms → enzyme attacks inhibitor just like it would attack a substrate, but the inhibitor has a poor leaving group, so gets stuck on, preventing the enzyme from regenerating
- There are 4 main types of **reversible inhibitors**:
 - **Competitive**
 - Binds E in active site
 - Competes with S for binding site
 - Resembles substrate/TS
 - ↑ apparent K_M (K_M^{app}) but doesn’t affect apparent V_{max} (V_{max}^{app})
 - **Noncompetitive (form of mixed)**
 - Binds E & ES at allosteric site with equal affinity & prevents conversion from S to P
 - Does not affect K_M^{app} , but ↓ V_{max}^{app}
 - **Uncompetitive**
 - Binds ES (but NOT E) at allosteric site & prevents conversion from S to P
 - ↓ K_M^{app} & ↓ V_{max}^{app}
 - **Mixed**
 - Binds E & ES at allosteric site with different affinities & prevents conversion from S to P
 - May ↑ or ↓ K_M^{app} , and will ↓ V_{max}^{app}
- **Competitive:**
 - Binds to **active site**
 - Competes with substrate for binding to active site
 - **Binds E only** (because its binding spot is already taken in ES!)
 - Can be competed out
 - Remember that each time the enzyme lets go of a substrate or an inhibitor, it then has a chance of picking up a substrate or an inhibitor
 - The relative concentrations of the 2 will determine which it is more likely to bump into first
 - If you have enough of the substrate it can “win”
 - The relative affinities of the 2 will determine how likely it is to stick & stay stuck to what it bumps into
 - You’ll need more substrate to compete out a stronger inhibitor
 - ↑ K_M^{app} but doesn’t affect V_{max}^{app} (you’ll just take more S to reach it)
 - Typically **look like substrate &/or TS**
 - Strongest inhibitors will look more like TS because that’s what the enzyme binds tightest
 - K_i is a measure of inhibitor affinity - it’s basically the dissociation constant, K_d , for the inhibitor
 - Lower K_i → stronger affinity
- **Noncompetitive:**
 - Binds to an **allosteric site** (not active site, or at least not in the same spot as the substrate) & prevents conversion of substrate to product

- Typically **will not** resemble substrate/TS because it's binding to a different spot
- Does not compete with substrate for binding, so cannot be competed out, but can be diluted out
 - If there's barely any around, the enzyme's unlikely to find another copy to pick up if it lets go, so if you dilute it a ton, then let it reach equilibrium, there'd basically be none bound
- **Binds E & ES with equal affinity**
- **Does not affect K_M^{app} , but $\downarrow V_{max}^{app}$**
 - substrate binding isn't affected, but the conversion to product is so the apparent affinity won't change, but the apparent rate of conversion will slow down since those bindings aren't productive
- Memory tip: it's noncompetitive because its binding is basically independent of S binding, so S binding isn't affected by its presence
- **Uncompetitive:**
 - Similarly to noncompetitive...
 - it **binds to an allosteric site** & prevents conversion of substrate to product
 - Typically **will not** resemble substrate/TS because it's binding to a different spot
 - Does not compete with substrate for binding, so cannot be competed out, but can be diluted out
 - But as opposed to noncompetitive, it **only binds ES (not E)**
 - **$\downarrow K_M^{app}$ & $\downarrow V_{max}^{app}$**
 - substrate binding is increased because binding to ES (forming ESI) draws away ES from the equilibrium binding equation $E + S \rightleftharpoons ES$, leading to a rightward shift (more S binding) thanks to the whole Le Chatlier's principle thing
 - Memory tip: it's uncompetitive because it's binding to basically the "opposite" thing as the substrate, and actually increasing the K_M^{app} , as opposed to a competitive inhibitor, which raises it
- **Mixed inhibition:**
 - Really similar to noncompetitive, except that it has different affinities for E & ES
 - Noncompetitive is really just an "idealized" form of mixed inhibition that isn't very realistic
 - **Binds E & ES, but with different affinities**
 - **Can $\downarrow$ or $\uparrow K_M^{app}$, but will always $\downarrow V_{max}^{app}$**
 - Will $\uparrow K_M^{app}$ if has higher affinity for E (more like competitive except it's binding to a different site)
 - Will $\downarrow K_M^{app}$ if it has higher affinity for ES (more like uncompetitive)

Type of inhibitor	Binds to	Effect on V_{max}^{app}	Effect on K_M^{app}	Overcome by:
Competitive	E	No change	$\uparrow$	Diluting out, competing out
Noncompetitive	E & ES - with equal affinity	$\downarrow$	No change	Diluting out
Uncompetitive	ES	$\downarrow$	$\downarrow$	Diluting out
Mixed	E & ES - with different affinities	$\downarrow$	May be $\uparrow$ or $\downarrow$	Diluting out