Lecture 13

- Rate enhancement is large doesnt' mean it occurs faster
  - Fold increase uncatalyzed vs catalyzed
  - High rate enhancement reaction time does not mean it will go faster
    - 1 every 10 billion year, 10^7 rate enhancement 10 times per sec

- active site Catalysis happens with key amino acid residues
- Substrate bind adjacent to active site
  - Enzyme tailor to substrate
  - What determines the shape of active site-> what is tertiary structure of enzyme-> rise of particular shape of active-> bind to substrate
- Interacting - weak interaction within protein molecule - h bonds / hydro/ionic use to bind to substrate molecule
  - Negative charge arginine charge change significant binding.

- Catalysis 20 amino acid - sometimes not enough not more catalysis power
  - Extra catalytic power is provided by cofactors.
- Cofactor - no protein group bind to enzyme extra chemistry to make reaction happen
  - Metal ions
    - Catalytic metal ion bind there coordinated by by amino acid sidechain
    - Exchanging electrons
    - Electron shuttle
  - Coenzyme
    - prosthetic - heme hemoglobin oxygen bind lots of enzymes to use heme
      - Bind irreversibly never leave
    - Cosubstrate - not reversibily bound come and go
      - Bind to enzyme the same time substrate binds
      - Co substrate different from the substrate is part of an enzyme complex
      - Regenerated by the end to bind again later
- Catalysis means that
  - Look the same reaction at the beginning and end
- Important NAD (cosubstrate)
  - Converted to nadh nad electron shuttle transition
  - Take an enzyme that require cofactor for activity
- Without cofactor
  - Apoenzyme w/o cofactor
- With cofactor
  - Holoenzyme active species of the enzyme

- Free energy is thermodynamics or what is delta g
- In between is kinetics beginning and end
  - Catalysis play role in kinetics don't play role in influencing thermodynamics
  - won't alter g product
  - Only the conversion between reactant and product
- High free energy intermediate or transition state
  - Species that exist in the lowest concentration- transition state
- Formation of mountain->energetic barrier
  - High free energy high energetic barrier
  - Lower free energy low energy barrier

        - Lower free energy in the transition state will increase concentration, allow it occur more quickly

- Catalyst rate
- Presence of enzyme transition state energy decreased significantly
- Concentration high reduce free energy
- They do some symmetrically
    - Catalyst accelerate the forward and reverse reactions equally both be same
    - Easier to go from reactant to product reverse will also be catalyze.

- clicker
- Transition-> lowest concentration
    - Enzyme's role is to increase concentration of transition state.
- B is correct lowest free energy stabilizing of high free energy
    - Positive oxygen energetic negative sidechain stabilize neutralize to lower free energy reaction proceed more quickly
- New
- Acid base catalysis
    - Group that can use as acid and base
    - Nuc attack by proteases
- Covalent catalysis
    - Always nuc attack by enzyme to substrate to generate a covalent bond between enzyme and substrate
    - Covalent is gonna be decompose to generate final products
    - In toy example
        - Transition state will have high free energy because it will have a charge
        - Lone pair nuc attack to generate intermediate enzyme and substrate liberate one of them
        - But enzyme is still there, decompose enzyme take intermediate go under hydrolysis to get a and b
    - Almost all of nuc are bases deprotonated serine very good nuc deprotonated-> things are good
    - Imidazole common nuc bc his is not a strong base.
    - If strong base, then the covalent bond immediate is gunna be so stable that it won't be easily decomposed.then its not complete
        - Needs a Strong enough base that can be easily decomposed
            - Enzyme covalent catalysis star: histidine
- Metal ion
    - Binding to substrate negative charge strong
    - Good at changing oxidation state
    - Electron changing
    - Useful for shield energetic unfavorable negative charge stabilize negative charge
- Entropy reduction- chance of collision
    - Form platform to hold two together
    - Reacting group immediately next to each order
        - Fix in fix reduce entropy collision reaction
    - Increase likeliness to collide with react increase proceeding
    - Different chemical scaffold different entropy value
    - Place them in the most reactive orientation

        - Rate enhancement go up when then you restrict rotational and translational motion of substrate

- Preferentially binding
    - Take comparing to binding to substrate and transition state when fit perfectly with substrate very few go to product
        - A lot of interactions with substrate -> Decrease in free energy in enzyme substrate complex no alteration in free energy alteration to transition
        - Transition state free energy gap is bigger when we bind to substrate
    - Enzyme form addition interaction with the transition state is better.
        - Turn off enzyme you can take small molecule to like transition state but not complete it once bound stuck.
    - Medicine that make inhibitors using strong affinity.
        - Out compete substrate and turn off the activity of the enzyme.

Lecture 14

- Enzyme mechanism- serine proteases
  - Not exclusive to one mechanism
  - Can use many acid base covalent etc
- Proteases cleave a scissile peptide bond with different specificities
  - Molecular basis of specificity

- Proteases catalyze the cleavage of peptide bond
  - Amide bond is the scissile peptide bond
- The breaking bond
  - Result carbon particle positive charge nuc attack at carbon covalent catalysis and then decompose the covalent bond
- Tertiary structure of serine protease why they have different specific molecular basis
  - Pocket surface of protease adjacent to active site in the pocket to accomodate rn-1
  - Trypsin large pocket involved with substrate rn-1 insert a pocket and positive charge tight interact with asp residue. Lysine and arg can fit and
  - Chymo also quite large
    - Can bc neutral so hydrophobic
  - Elastase shallower only accomodate small residue
- Why protease different specific based on pocket
- Binding of the substrate is immediate next door active site
- Specific pocket active site very similar
  - Feature center around catalytic triad
  - Asp his and serine all
  - serine protease have this combination
  - Catalytic triad bc
    - Reaction can be done more easily with more residue sidechain cumulative effect in difficult reaction
- How to determine the catalytic triad
  - Reactive analogues small molecule like substrate bind to sit in active site
  - It will initiate the catalysis will get struck and form covalent bond with substrate anayue and residue catalytic active
- 3 proteases exhibit both convergent and divergent evolution
- Typrin / elase/chmyo divergent evolution common ancestor diverge overtime but little difference in the
  - Convergent because when you look at tertiary structure, they look vastly different
  - Catalytic triad almost identical
  - Arrangement of crucial for function/ catalysis
  - 3department slightly
- Convergent evolution different starting point.
- Divergent can't take same ancestor scramble and same function
  - Serine help of the cell useful activity

    - Overall tertiary structure different but catalytic triad is the same

- mechanism
- Need a good nuc
- Serine right next to scissile bond is right under serine
- Turn serine into better nuc by deprotonation of serine-> they be good nuc
- To dep use his, act as base accept H and serine nuc attack

    - Base catalysis
- Problem His not a good base
    - Solution: Asp polarize his sidechain so it can dep serine (electrostatic catalysis)
- Tetrahedral intermediate planar (when double bond O) to tetrahedral change in geometry (transition state)
- Now to decompose
- H started out dep now get back give back substrate
    - Give H to nitrogen from protonated his
    - nitrogen break sisscle between C-N
    - Acid catalysis
- End of part a
    - ½ product completed amino terminus is done fragment
    - When break sissicle c from tetrahedral to planar (acyl enzyme intermediate)
- The other part is attached to enzyme
- When first product departs space, the gap is filled with water
- Water form another nuc attack to carbonyl C
    - Use His to make better nuc
        - Take h from water-> hydroxide now base again
            - Base catalysis
- Deprotonated water acyl enzyme intermediate 2nd intermediate hydroxyl group second tetrahedral intermediate
- All is left break serine from C fragment
    - Use his as acid protonated serine sidechain allow two product carboxyl terminus to bye bye
    - Left serine protonated his deprotonated
        - General acid catalysis
- Back to normal
- Key points
    - His goes thru many trials of p/d/p/ bc pka 6 so it can take in proton and give up. Moderate easy to manipulate environment
    - Proline twisted so it would work pull them away from catalytic triad

- Chief catalytic strategies used by serine proteases is preferential binding of the transition state
    - Bc it forms Additional interaction with transition
- Active site substrate is at its active planar geometry
    - When enzyme is bound to substrate, there is a space in active site-> oxyanion hole
- After nucleophile attack, covalent catalysis turns planar-> tetrahedral.
- Oxygen neutral to negative change in geometry push to another position.
- Shift in position pushes the negative of oxygen into oxyanion hole
    - surrounding it are amide nitrogen, capable of donating hydrogen bond to create amide bonds.
- Substrate-enzyme interaction- When its planar, substrate is too far away to form amide bonds with the nitrogen
- Transition state-enzyme interaction- amide formation of 2 more amide bond. Not in enzyme and substrate. 40 kJ of binding energy extra degree of catalytic power

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- Experiment to determine which residues are important for catalysis.
- Cleavage of a substrate subtilisin wildlife and uncatalyzed rate
    - Determination of important residues by mutating them to Alanine or glycine

        - If decrease in rate then they are involved in the catalysis.
    - Even tho you eliminated this important residues you still get something more than uncatalyzed.
        - This means mutating all three catalytic triad residues to alanine still gives ~10^3-fold faster rate than uncatalyzed.
            - Solved: bc of preferential binding state holding poplypetide in place-> addition interaction in transition is enough to promote the reaction to go faster than the absence of any enzyme.
- Clicker: You discover a protease that cleaves polypeptides only after glutamic acid residues. What are the most likely physical characteristics of the protease's specificity pocket.
    - A pocket that can accommodate a longer side chain and is lined with groups that bear a positive charge at physiological pH.
    - Specificity pocket is not active preferential binding is a characteristic of the active site.

- Regulation of serine proteases/ zymogens
    - Trypsin is digestive protease that break up food
        - Produced in the pancreas.
        - On the way you don't want them to be active when they are getting transport to site of function.
        - Active at the time and location to be active.
    - Zymogens are inactive of serine protease when they translate of a much longer polypeptide chain. Not present active form.
        - Extra residues located in N terminus
    - Once it is in lower intestine N terminus is chopped off
    - Proteolytic cleavage. trysinogen is cleaved by trysin. They will break this bond and create more of themselves.

- Comparing the 3rd structure of trysin and trysinogen
- When ILE in there in the active form, it blocks the oxyanion hole so it can't accommodate the tetrahedral intermediate.
    - -> making the oxyanion hole disorder.
    - After cleavage, the ile moves down creating room. Making it ordered now.
- Movement of ile from off to on through ordering of the oxyanion hole which allows the proteins to be degraded.

Lecture 15- Enzyme kinetic

- Rate of which substrate-> product
- Fundamental
    - How to measure enzyme kinetic
    - Rate equation enzymes catalyze reaction
- Rate order
    - Reaction order indicates the number of molecules participating in an elementary reaction
    - Order of the reaction tells us how many molecules that participate within chemical reaction.
    - Rate = change /change in time.
    - Equation
    - k=Likelihood of this reaction occuring
        - Likelihood increase as the reactant increase
    - Velocity proportional to concentration.
    - Second order when it is proportional to the sq of the velocity
    - Bimolecular velocity velocity is k (a)(B) first order in respect to a or b

- To describe progress first order reaction
- Negative slope. Slope =-k to see which is faster or slower calculate half life is independent of the con of reactant.
    - characteristic for 1st order.
- Second order half is
    - Half life proportional of A
    - Dependant of A
    - Rate slows down as concentration decreases.
- Enzyme catalyzed rate catalyzed beta fructo break down sugar for each sucrose.
- Hyperbola all enzyme catalyzed reactions.
- Low sucrose 2nd order as it continues to increase rate slows down looks more first order
- Increase initial concentration line plateaus then flat. Reaction rate at very high velocity independent of A zeroth order.
- At high substrate behavior
- Enzyme catalyzed formation

- 2 conponents E +S-> ES **ES -> Product turnover catalytic step to create product**
- Rate that identify each step. E+S reversible substrate can bind and dissociate equilibrium of E interacting with S.
- K_1 k_{-1} high affinity want to bind to substrate push to right
- Second step irreversibly forward more quickly
- Second order behavior: Rate k_2 low substrate rate limiting step formation of ES complex not much
    - How much ES complex is based on how substrate you have. K1 rate limiting.
    - Expects second order behavior when the [substrate] is low based on previous slide
    - Why is it second order because there are two things: [E]+[S] that gets to ES
- First order behavior: High substrate lots of ES so k2 is rate limiting.
    - Expects first order behavior when the [substrate] is high based on previous slide graph
    - Only [ES] is one rate determining
- Zeroth order
        - *behavior baseball player enzyme*

        - *Ball is substrate*
        - *Too busy hitting balls change of rate pitched ball faster and faster overwhelmed he can't do anything. Player completely saturated with substrate/ balls.*
    - Only so many active site when all are saturated you can't convert them any quicker when all are full.
    - Saturated so independent of the initial concentration of substrate.

- Want to describe all 3 behaviors in an equation
- Steady state kinetics
- Assumptions
    - Reaction is at equilibrium
        - Calculate overall velocity
            - Dependent on
                - ES complex
                - How quickly turn into product
    - Initial formation of ES set equation at equilibrium.
- Assumption: rate of formation and dissociate k1 and k-1 much more quickly than k2.
    - Come to equilibrium much quickly before taking measurement
    - Steady state: every time es turn to product enzyme can find another substrate to make product
    - Or **Conc of ES complex doesn't change much over the course of reaction**
        - Or formation of ES=loss of ES
    - Low conc of enzyme and significant excess of substrate.
- Rate don't have to be at an equal rate to be at equilibrium

- Rate equation take account 2 steps
    - Formation of es complex
    - Rate of formation of ES complex=k1
    - formation of ES decomposition- k_{-1}[ES]-K2[ES]
- Because k1+k2=k-1+k2 but k2 doesn't really exist bc k-2 is irreversible
- Form es complex product or turn back both will be different from ES.
- Equilibrium constant ratio of rate of removal of es complex /formation of
- kM tells me about first step
    - how much es complex in that reaction.
- Conversion of ES to product first order process (simple rate equation)
- V_{max} max velocity this enzyme achieve fraction of that max velocity based on how much substrate there.
- When reaction starts to plateau, that is V_{max} when zeroth order.
    - Under enzyme is completely filled
- $V_0=V_{max}[S]/ (K_M+[S])$
    - Rate equation for enzyme catalyzed reaction

- As initial substrate increase, rate increases, slope increase
    - Measure the initial velocity at different initial substrate concentration
    - Measure slopes at each point of that graph (take the derivative)
- Measure slope -> rate rate
- Hyperbola graph 2nd, 1st , zeroth
- Use michaelis plot to determine vmax and km
    - **Vmax -max at zeroth**

    - Extrapolate from the curve where the plateau is flat when V max. Use curve to estimate
    - **50% of Vmax(per unit of time) what gives rise to that--kM**
        - Km (molar) equivalent to substrate concentration that gives rise to 50% of vmax.
        - 50% vmax -- 50% of enzyme bound to substrate
    - V max all is bound to substrate
- Vmax low affinity for substrate
- Add a lot of substrate to fill 50% substrate
- Weak affinity km high add a lot of substrates to get to 50% of the active site
- High affinity low km don't need to a lot of substrate to get to 50% of the active
- Value km for a specific enzyme substrate complex
- Specific for each case

- You would be able to identify the active residue of km.
    - What the preferred substrate.
- Other measure using Km

- Kcat
    - Vomax is proportional to total enzyme concentration
    - Useful for us to come up of a parameter independent of the total concentration of enzyme
        - How quickly a single molecule is capable of converting substrate to product.
        - Turnover number
        - For most simple system kcat=k2
- Substrate concentration compare single rate equation how quickly/efficiently substrate -> enzyme kcat/kM
- Approx when substrate very low. Se
- Approx second order constant.
- Measure of catalytic efficiency.
    - Include both overall efficiency.
    - 2 enzyme one high vmax or kcat high km; max rate high but need to add a lot of substrates to get there → inefficient enzyme
    - High would be high
    - High vmax or kcat low km; Less substrate to get high enzymatic activity→ efficient

Lecture 16 -- Enzyme Inhibition

- Equilibrium km -position of this equation between ES and E and s
- Turnover step rate of this catalytic is Vmax
- When 100% is present in
- Pushed all the way to the right
- Saturated with substrate k cat because Vmax / Et
- Kcat is independent of enzyme concentration
- Max rate
- Approx second order rate constant to approx velocity when substrate conc is low
- kcat/Km = catalytic efficiency
- Low kM high affinity to the enzyme
- Much faster catalytic step cleaves
- High kcat/kM shows that it is efficient clear that it prefers trosine
- Enzyme kinetic about mechanism +important residues
- new
- Wild type enzyme measure mekalis menten
- Particular one that would be important for catalysis
- Make mutation (substituted with something inert
- Remeasure values of vmax or km
- Serine
- Km stay the same means the affinity is the same
    - Not a substrate binding substrate
    - Affecting vmax means serine involved in catalysis
- Vmax stay the same but Km increased
    - Isn't involved in catalysis but km increased means affinity decrease
    - Plays a role in enzyme binding.
- Using Km and Vmax can be used to find out the mechanism.
- New
- Displaying on micharelis-menten
- Easy to see what happen when enzyme is motified
    - Shifts kM increased or whatever
    - Limitation: error of approx
    - Error with extrapolation
- Lineweaver-Burk Plot
    - Gives accurate measure of Km
    - Double reciprocial of michael menten equation
    - Straight lines
    - intercept y :1/vmax
    - x- intercept: 1/km
    - Defined value for Km and Vmax
    - Less error on that
    - Downside: datapoint is clustered
- Inhibition
    - Bind to enzyme and prohibit it
    - What kind of inhibitor it is
    - Competitive inhibitor- competes with substrate
    - Prevent binding of the substrate
    - Unable to catalyze the reaction
    - Able to form the same interaction as substrate
    - Can't be able to turn to substrate

- Bind greater affinity than substrate you have succeeded.

- Look like the natural substrate
- Carboxylate group succinate binding to it the same way
- Competitive inhibitor
  - K_I is the inhibition constant- how much needed to be added to inhibit 50%
  - Ki is low bind with high affinity
  - Always bind to tamiflu over sialyic acid
  - High affinity over its natural substrate → effective inhibitor
    - Fits perfects in active site
    - So well all sorts of interactions

- Design transition state
  - Tailored to transition state
  - Wants tetrahedral transition state

- Effects on vmax and km
- Lineweaver burke plot more effective
- Competitive inhibitor compete to bind to free enzyme
- Alternative form E+I
- E+I or E+S exclusive
  - EI+ S no reaction
  - Addition of inhibitor influence position of [ES] affects Km
- Competitive Inhibition
  - In lineweaver plot, slope gets steeper as you increase the amount of inhibitors.
  - Intercept the value at same point **vmax no effect**
    - **Why?**
    - Equal affinity, equal concentration equal amount of substrate binding to enzyme
    - You can add in enough substrate to outcompete the inhibitor to get to product
    - **What is changing is how much substrate you need to add in to outcompete the inhibitor to full saturation**
      - Need to add a lot to full 50% of Vmax
  - Km is changing.
  - 1/km becomes more negative
  - **Km increasing**
    - **Bold is why km is increasing**
- Noncompetitive inhibition- Not gunna compete with substrate to bind to active site
  - Bind a position away from active site
  - When bind structural conformation
  - Move active site around to inhibits catalysis
  - Bind to susbtrate whether it is bound or not
  - Can bind to free enzyme and ES also substrate complex
  - Both EI and ESI non reactive
  - Only need to worry about inhibitor site
  - Pure noncompetitive inhibitor
  - Increasing concentration
  - Slope is increase
  - Y intercept increases as we increases

        - Increase conc of inhibitor vmax decreases
    - Same x intercept
        - Km no change
    - Bind to ES to form ESI
        - Removing ES from reaction
        - Increasing NCI more will form ESI decrease conc of enzyme substrate complex decrease vmax bc ES is drawn out
    - KM stays to the same
        - We have convert E+I to Ei and pulling ES +I to ESI
        - Because we are pulling on both sides of the pulley.
        - Equilibrium doesn't change
        - The Km doesn't change
        - Bound with equal affinity to free enzyme and ESI→ pure noncompetitive enzyme
        - Pulls on both sides equally
- Mixed inhibition
    - Bind with different affinity to free enzyme and ESI
    - Binds preferentially to free enzyme
        - Y intercept increases X
        - Increase in km
        - Pull on
    - Bind ESI
        - Decrease in km
        - **However, for both, you will see a decrease in Vmax**
- Uncompetitive inhibition exist only to ESI
    - **Only ESI** no free enzyme
    - Binds to substrate open binding site to enzyme
    - Distort shape to open bind
    - Bind when substrate
    - Parallel lines.
    - Increase conc inhibitor
    - Y int increasing vmax **decreasing with inhibitor**
    - X int more negative km is decreasing
    - Binding to ES pulling
        - Decrease in vmax
        - Bind to substrate more tightening then it is.
        - In the presence of un drawn more towards ES complex
        - Higher affinity when inhibitor is around

- Uncompetitive example occurs when two substrates bind in obligate order
- A competitive inhibitor of substrate 2 can act as an uncompetitive inhibitor of substrate 1
- But the inhibitor for substrate 2 will be competitive for 1.
- kM for substrate 1 for decrease