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 ~103-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.
* K1 k-1 high affinity want to bind to substrate push to right
* Second step irreversibly forward more quickly
* Second order behavior: Rate k2 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)
* Vmax max velocity this enzyme achieve fraction of that max velocity based on how much substrate there.
* When reaction starts to plateau, that is Vmax when zeroth order.
  + Under enzyme is completely filled
* V0=Vmax[S]/ (KM+[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
  + KI 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