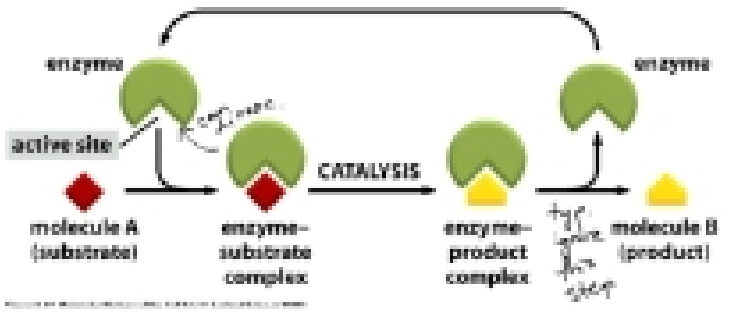
Main Ideas

- Enzymes are catalysts that **lower the activation energy** for a rxn and speed up the rxn rate
- Binding of a protein to a ligand (1st step of catalysis) can be measured by the **binding (equilibrium) constant**
- The Michaelis-Menten (MM) equation relates the **rate of an enzyme catalyzed rxn to the substrate concentration** and two constants, Km and kcat
- Based on the MM equation, the **best enzymes have a small Km and a large Vmax** (w/ Km= [S] at Vmax/2)
- Ex. of enzymatic amplification thru regulated proteolysis= **blood coagulation cascade**

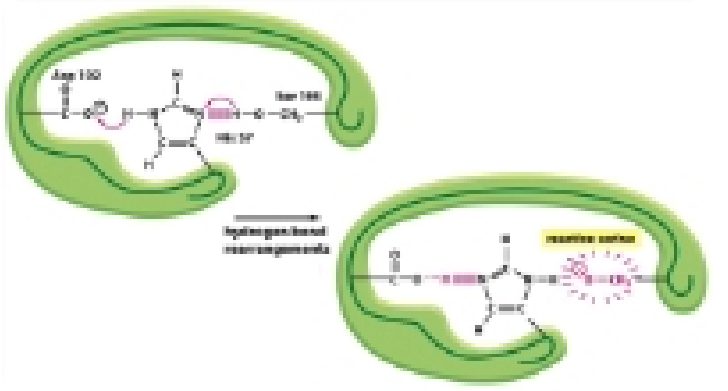
Learning Objectives

1) Understand the basic principles and steps of enzyme catalyzed rxns

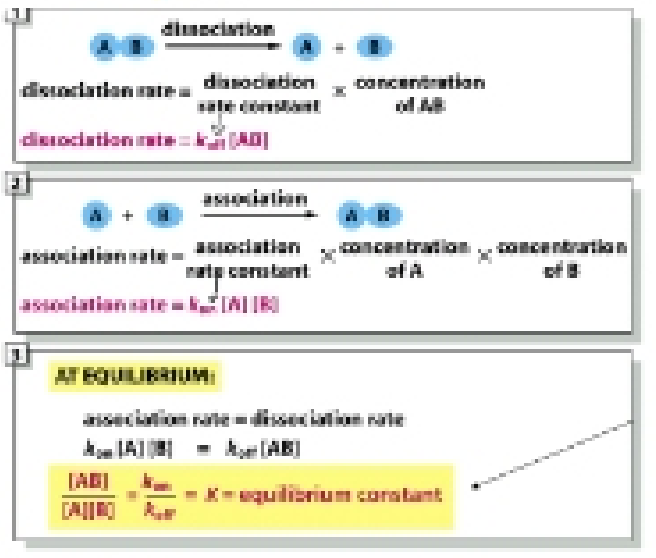
- **Enzymes** are catalysts and are everywhere
- Catalyzed rxns are typically connected in series
- Enzymes lower the **activation energy** that blocks chemical rxns --> hugely increase the rate/speed of chemical rxns, but don't affect/change Keq (the state where products and reactants are in equilibrium)



- They are **highly selective**= 1 enzyme: 1 rxn
  - Due to **active site**: pocket only accepts 1 substrate
- **Substrate/ligand**= molecule that binds to the enzyme
  - Binds thru non-covalent interactions and complementary binding surfaces
  - Hypothesis: 1) lock and key= fit each other perfectly 2) induced fit= don't fit perfectly initially, enzyme has to undergo a little change to fit perfectly



- Ex. **serine protease**= attacks peptide bonds on proteins, has a basic microenvi, so due to the interactions between its side chains via H-bonds, a nonreactive group-->reactive group
  - Active site: catalytic triad
- First step: **substrate binding**
  - Dynamic process: the substrate binds to other molecules, but their interactions are too weak, so they fall apart. Substrate only stays w/enzyme when it's supposed to be bound to it.
- **Equilibrium constant** measures binding strength
  - at equilbrium the association rate = the dissocation rate
  - k1= kon
- k2= kcat (catalysis)= ES--> E + P
  - this is the limiting step
  - ES is a transient intermediate



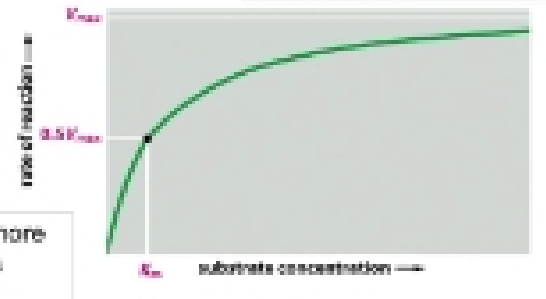
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

- understand steady state approximation: ES concentration stays the same, so d[ES]/dt= 0, helps us get to MM

2) Understand that the MM eqn describes how rxn velocity depends on substrate concentration

Michaelis-Menten Equation

$$v = \frac{d[P]}{dt} = \frac{k_2 [E_t][S]}{K_m + [S]}$$



When [S] >> K_m, then:

$$v = v_{max} = k_2[E_t]$$

- So the velocity of your rxn is the increase in product made over time and this depends on your substrate concentration

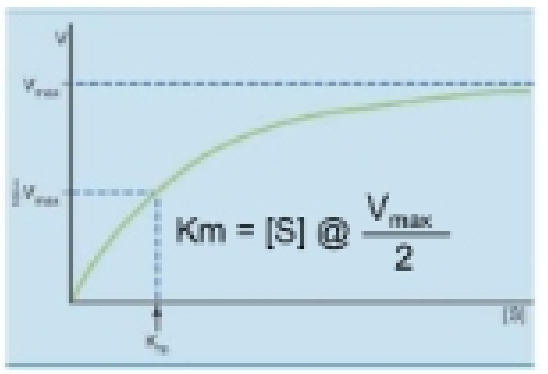
Using the Michaelis constant: $K_m = \frac{k_{-1} + k_2}{k_1}$

- **vmax**
  - once all the enzyme is bound by substrate, the max rxn rate is reached

$$v = \frac{k_2 [E_t][S]}{K_m + [S]} = \frac{v_{max} [S]}{K_m + [S]}$$

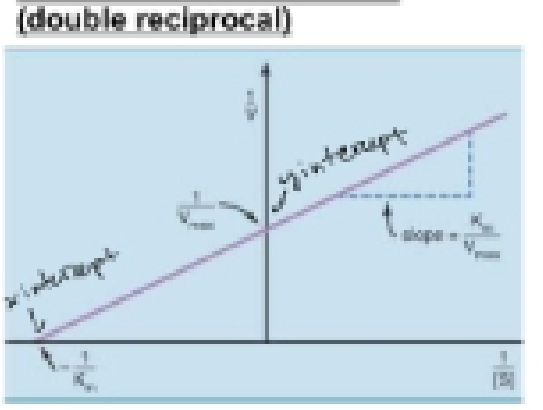
Note: $k_2 = k_{cat}$ (turnover number)

Direct Plot



$$v = \frac{v_{max} [S]}{K_m + [S]}$$

Lineweaver-Burk Plot (double reciprocal)



$$\frac{1}{v} = \frac{1}{v_{max}} + \frac{K_m}{v_{max}} \times \frac{1}{[S]}$$

Direct Plot:
- When your rxn has reached half of its max v, Km= S

If $k_{-1} >>> k_{cat} \Rightarrow K_m \approx K_d$ (the dissoc. Const)

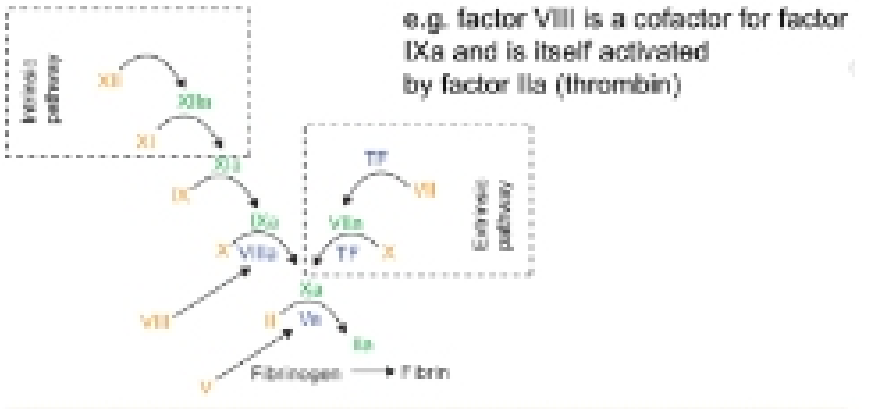
Ergo: K_m indicates affinity between substrate and enzyme

- **The best enzymes have the smallest Km** (least tendency to dissociate) **and largest Vmax!**

3) Be able to interpret a direct plot or a double reciprocal plot for an enzyme that follows MM kinetics
- See above

4) Be familiar w/the basic principle of an enzyme amplification cascade (ex. blood coagulation)
- **Enzymes and rate acceleration**
  - Range: 10^9-10^23 increase in rate
  - Factors that can influence rate:
    - Concentration of enzyme and substrate, affinity between E and S, ability of E to stabilize transition state (ES), bond strain, T of rxn and pH of rxn
- **Enzyme Cascade: blood coagulation**
  - Hemostatis: arrest of blood loss from ruptured blood vessels
    - Primary: vascular restriction + platelets adhere to injury site
    - Secondary: **coagulation**= the casese of rxns that produces a fibrin mesh (clot) that entraps the soft plug from above
  - Triggering coagulation
    - Membrane proteins exposed by injury serve as receptors for zymogens and protein co-factors that are either: already present in the blood or released by platelets following their activation

- Both intrinsic and extrinsinc cascade using **proteolysis** to **amplify the signal**
  - Multimembered cascade
  - One member gets activated and then activates the next zymogen --> etc.
  - One enzyme at the start of the cascade can activate many molecules of a given substrate



- **Factors XII, XI, IX, VII, X, and II: zymogens** (=precursor) **of serine proteases**
  - Cleavage of 1 zymogen-->active protease that activates another zymogen
  - Each zymogen is also reg. by **protein cofactors** that themselves are also reg. by proteases (+ feedback looping-->more amplification)
  - zymogens are cleaved (all these lose their N terminal parts to free up the serine protease part) to become active