Lecture 15: Introduction to Enzymes

1. Transition state theory and relationship to the rate of catalysis.
2. Entropic and enthalpic stabilization of transition state.
3. Maximum rate of enzyme catalyzed reactions (diffusion controlled).

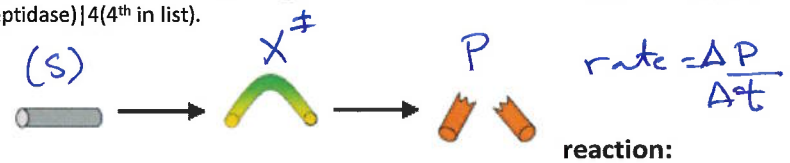
Most enzymes are proteins although some RNA enzymes (ribozymes) are also known. Enzymes catalyze a wide range of reactions, e.g.:

1) Oxidoreductases (redox)
2) Transferases
3) Hydrolases
4) Lyases
5) Isomerases
6) Ligases

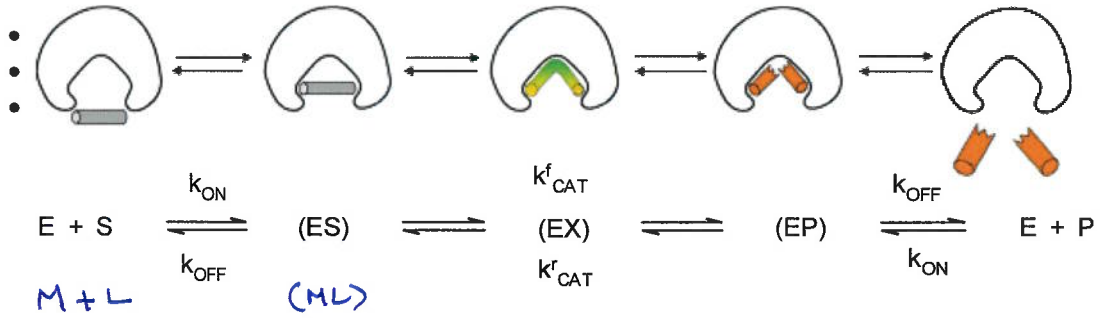
Nomenclature:

1. Many "historic" enzymes have names based on what the biochemists who discovered called them, e.g. Trypsin, Lysozyme.
2. Enzymes are commonly named by adding "-ase" to the name of their substrate or to a word describing their activity, e.g. Urease, Protease, DNA polymerase
3. A more standard system of nomenclature (based on an international agreement) exists now based on enzyme activities, e.g. Trypsin = EC 3.4.21.4. 3(hydrolase)|4(peptide bonds)|21(Serine endopeptidase)|4(4th in list).

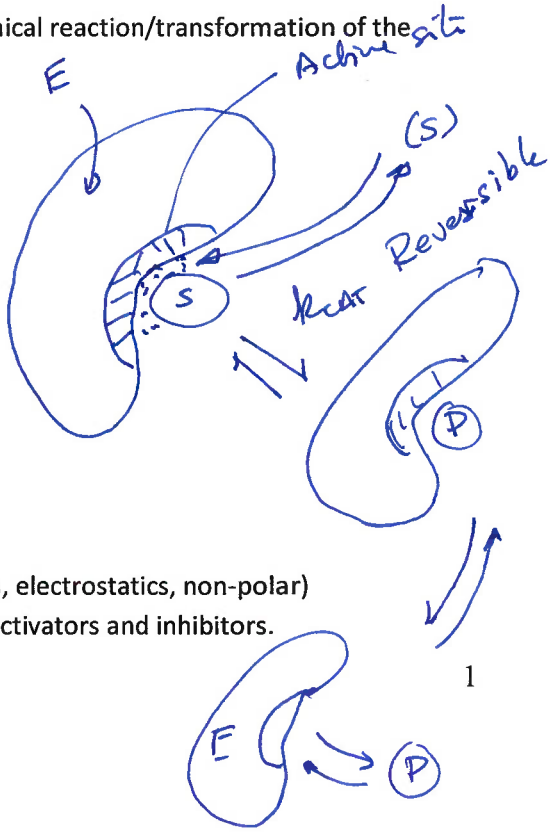
Uncatalyzed Reaction:



A simple enzyme-catalyzed reaction:



- The enzyme forms a complex with the **substrate** (S), often undergoing some degree of conformational change (*induced fit*).
- The interaction between the enzyme and the substrate are similar to protein-ligand interactions.
- Catalytic groups on the enzyme perform some chemical reaction/transformation of the bound substrate.
- The resultant **product** (P) is released.



Important Features of Enzyme Catalysis.

1. Enzymes increase the rate of reactions, in ***both*** directions
2. Enzymes do not change the equilibrium point of reactions. The equilibrium point depends on the relative energy of (S) and (P).
3. Enzymes are not changed by the overall reaction (They may be modified *during* the reaction).
4. Catalysis occurs at the "active site", which is usually **specific for certain substrates**. The active site has the following with respect to its substrate:
    - Geometric complementarity (Van der Waals)
    - Complementary functional groups (H-bonds, electrostatics, non-polar)
5. Enzymatic reactions are often regulated by allosteric activators and inhibitors.

Transition State Theory – Accounting for Rate Enhancements: *rate ∝ [Transition State]*

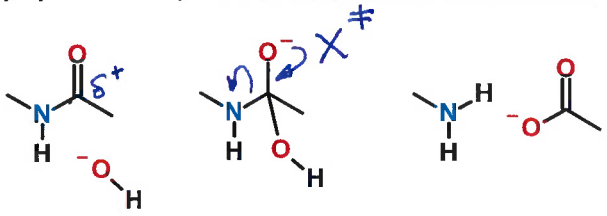
All reactions proceed through a high energy transition state ($X^{\ddagger}$).

- In the case of the uncatalyzed reaction, the substrate (S) is converted directly to the transition state which then changes to product (P). The energy barrier, or **activation energy,** between the (S) and the transition state is $\Delta G^{\ddagger}$.
- In the case of the catalyzed reaction, the substrate first binds to the enzyme to form the (ES) complex, this then goes to the enzyme bound transition state ($EX^{\ddagger}$). The activation barrier in this case is $\Delta G^{\ddagger}_{E}$.
- The energy difference between (S) and (P) must be the same (ΔG^{o}).

The energy of the transition state is high because of:

- Strained bonds in the transition state.
- Unfavorable entropy required to order groups when the transition state is formed.

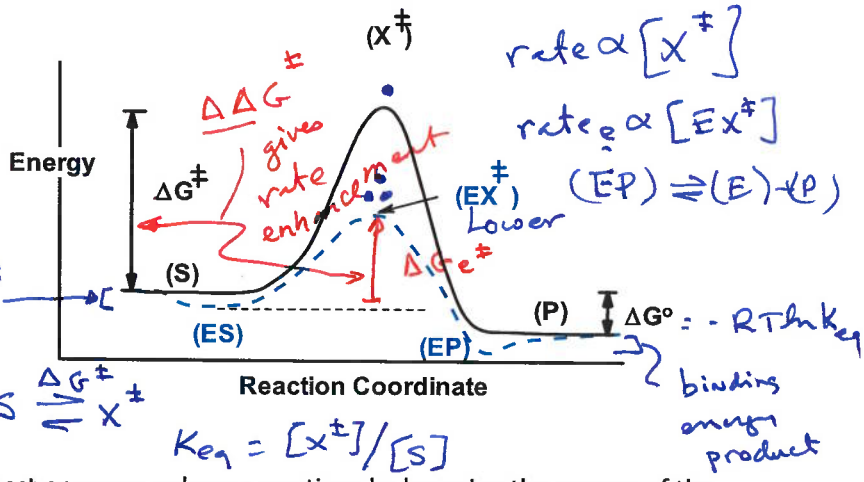
***Example**: In the hydrolysis of a peptide bond by hydroxide ion, the transition state is the unstable tetrahedral oxyanion.*



- The rate (v) of the reaction depends on the concentration of the transition state:

$$v \propto [X^{\ddagger}]$$
$$v_e \propto [EX^{\ddagger}]$$

- The concentration of $X^{\ddagger}$ is quantitatively related to the activation energy assuming the (S) is in equilibrium with the transition state (S)↔($X^{\ddagger}$):



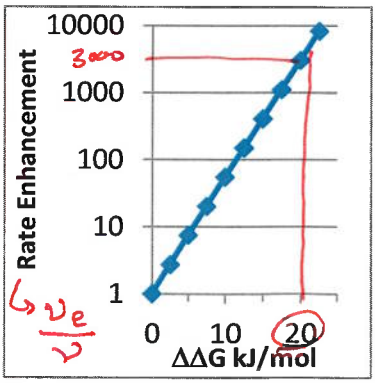
$$\frac{[X^{\pm}]}{[S]} = K_{EQ} = e^{-\Delta G^{\pm}/RT}$$
$$\frac{[EX^{\pm}]}{[ES]} = e^{-\Delta G^{\pm}_{E}/RT}$$

Most enzymes enhance reactions by lowering the energy of the transition state, thus increasing the concentration of the transition state, and thus the overall rate of the reaction.

Rate Enhancements:

- Small decreases in the energy of the transition state can lead to large rate enhancements.

$$v \propto \frac{[X^{\neq}]}{[S]} = K_{EQ} = e^{-\frac{\Delta G^{\neq}}{RT}}$$
$$v_e \propto \frac{[EX^{\neq}]}{[ES]} = K_{EQ} = e^{-\frac{\Delta G^{\neq}_{e}}{RT}}$$
$$\frac{v_e}{v} = e^{-\frac{\Delta G^{\neq}_{e} - \Delta G^{\neq}}{RT}} = e^{-\Delta\Delta G/RT}$$



Reflection:

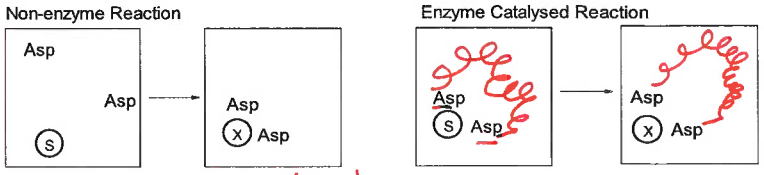
1. *Assuming a **new** hydrogen bond was made to the transition state during the reaction, by how much would the transition state be stabilized (i.e. how much energy is released when a hydrogen bond forms?)*
2. *How much would the rate increase?*

The transition state of the enzyme substrate complex is stabilized in two ways:

All enzymes!

1. **Entropy**: The enzyme may provide a number of functional groups to aid in catalysis. Since these groups are positioned in well-defined locations due to the folding of the protein, the entropy of bringing these groups into the catalytic site is substantially less than having these functional groups freely diffuse in solution. This entropy change contributes to $\Delta G^{\ddagger}$ via the relationship $\Delta G^{o} = -T\Delta S^{o}$.

In this example, two amino acids (Asp, Asp) are involved in cleaving a peptide bond, converting the substrate (a peptide) to product via the transition state "X".



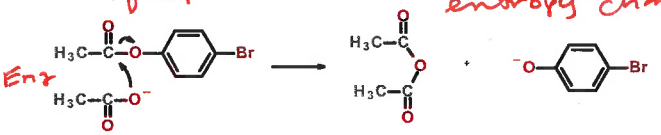
high entropy of Asp

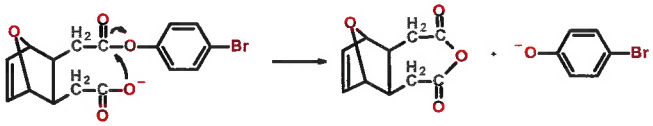
low (high order) entropy of Asp

No significant entropy change

Don't underestimate the effect of entropy in rate enhancement:

No Enz



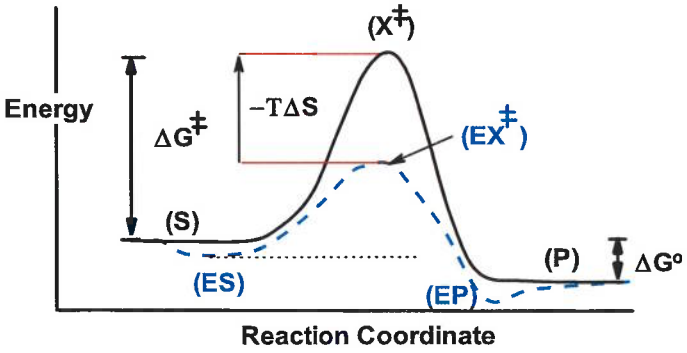


Top reaction: Model for uncatalyzed reaction, two "substrates" must become ordered with respect to each other.

Rate= 1

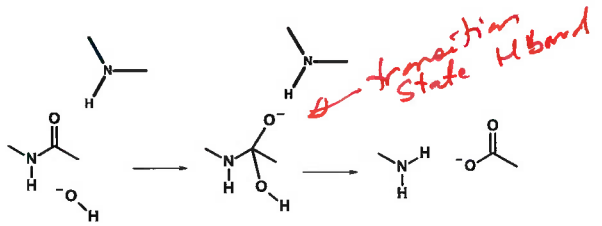
Bottom reaction: Model for enzyme catalyzed reaction, the two substrates are held in the right orientation (pre-ordered) by the cyclic bridge:

Rate = 10^6

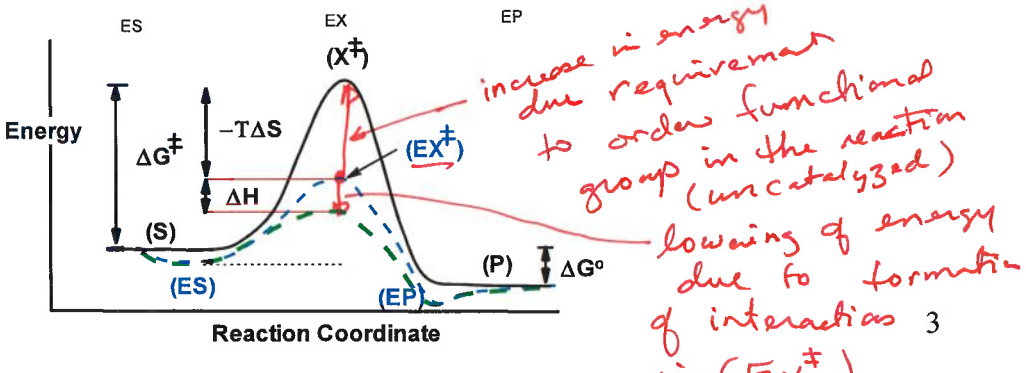


Some enzymes

2. **Enthalpy**: The enzyme transition-state complex is stabilized by direct interactions between the enzyme and the transition state. This reduces the free energy of the transition state due to ΔH^{o} (enthalpy). This is used by serine proteases, such as trypsin and chymotrypsin.



transition state H bond



increase in energy due requirement to order functional group in the reaction (uncatalyzed)

lowering of energy due to formation of interactions in (EX‡)