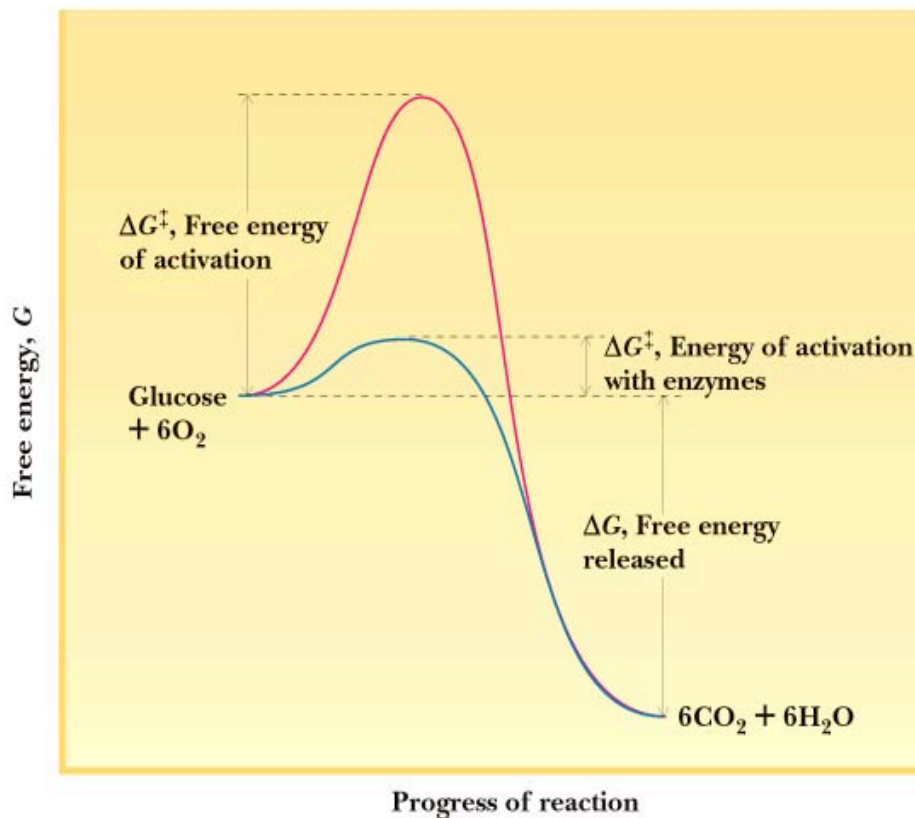


## Lecture 12: Introduction to Enzyme Kinetics

- Catalytic Power, Specificity, Regulation
- Enzyme Nomenclature
- Coenzymes and Cofactors
- First and Second Order Kinetics
- Catalysis and the Transition State

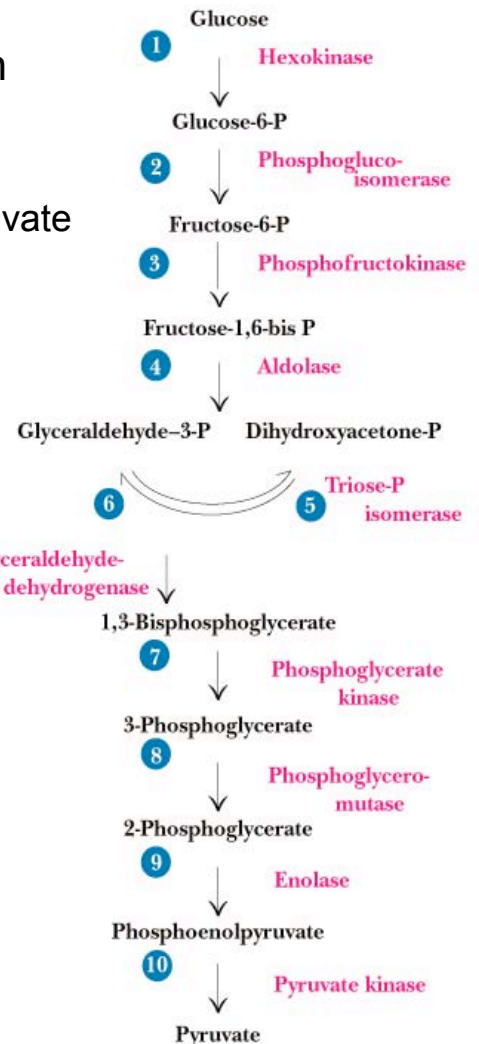
### Enzymes

Enzymes endow cells with the capacity to exert kinetic control over thermodynamic potentiality



## Enzymes are the Agents of Metabolic Function

- e.g. glycolysis - 10 enzymes catalyze 10 chemical reactions in the pathway that converts glucose to pyruvate
- There are **regulatory enzymes** at key junctions in the pathway



## Catalytic Power

- Catalytic power is defined as

$$\frac{\text{catalyzed rate}}{\text{uncatalyzed rate}}$$

- Enzymes can accelerate reactions as much as  $10^{16}$  over uncatalyzed rates
- Urease is a good example:
  - Catalyzed rate:  $3 \times 10^4/\text{sec}$
  - Uncatalyzed rate:  $3 \times 10^{-10}/\text{sec}$
  - Ratio is  $1 \times 10^{14}$

## Specificity

- Enzymes selectively recognize proper substrates over other molecules
- Enzymes produce products in very high yields - often much greater than 95%
- Specificity is controlled by structure - the unique fit of substrate with enzyme controls the selectivity for substrate and the product yield

## Regulation

- At the genetic level (transcription control)
- Activation by substrate, product, or other metabolites
- Inhibition by substrate, product, or other metabolites

# Enzyme Nomenclature

*classified according to function*

- 1. Oxidoreductases
  - 1.1 acting on C-OH as e<sup>-</sup> donor
    - 1.1.1 with NAD(P) as electron acceptor
    - 1.1.3 with O<sub>2</sub> as electron acceptor
  - 1.2 acting on C=O as e<sup>-</sup> donor
    - 1.2.3 with O<sub>2</sub> as electron acceptor
  - 1.3 acting on CH-CH as e<sup>-</sup> donor
    - 1.3.1 with NAD(P) as acceptor
  
- 2. Transferases
  - 2.1 transferring C<sub>1</sub> groups
    - 2.1.1 CH<sub>3</sub> (methyl transferases)
    - 2.1.2 C-O (hydroxymethyl and formyl transferases)
    - 2.1.3 C=O (carboxyl and carbamoyl transferases)
  - 2.2 transferring aldehydes or ketones
  - 2.3 acyl transferases
  - 2.4 glycosyl transferases
  - 2.6 transferring N groups
    - 2.6.1 amino transferases
  - 2.7 transferring P groups
    - 2.7.1 alcohol group as acceptor (ser, thr, tyr kinases)
  
- 3. Hydrolases
  - 3.1 hydrolyse ester linkages
    - 3.1.1 carboxylic ester hydrolases
    - 3.1.3 phosphoric monoester hydrolases
    - 3.1.4 phosphoric diester hydrolases
  
- 4. Lyases (addition to double bonds)
  - 4.1 C=C lyases
    - 4.1.1 carboxy lyases
    - 4.1.2 aldehyde lyases
  - 4.2 C=O lyases
    - 4.2.1 addition of water (hydrolases)
  - 4.3 C=N lyases
    - 4.3.1 ammonia lyases

- 5. Isomerases (isomerization reactions)
  - 5.1 racemases and epimerases
    - 5.1.3 acting on carbohydrates
  - 5.2 cis-trans isomerases
  
- 6. Ligases (ATP-dependent bond formation)
  - 6.1 C-O bond formation
    - 6.1.1 amino acid-RNA ligases
  - 6.2 C-S bond formation
  - 6.3 C-N bond formation
  - 6.4 C-C bond formation
    - 6.4.1 carboxylases

# Coenzymes and Cofactors

*nonprotein components essential for activity*

- Metal ions
  - be careful not to confuse  $\text{Na}^+$  or  $\text{Mg}^{2+}$  that might be present as counterions (these are not cofactors)
- Organic molecules
  - Including some vitamins, e.g. thiamine (vit.  $\text{B}_1$ )
- With the cofactor - holoenzyme
- Without the cofactor - apoenzyme

Enzyme Cofactors: Some Metal Ions and Coenzymes and the Enzymes with Which They Are Associated

Metal Ions and Some Enzymes That Require Them		Coenzymes Serving as Transient Carriers of Specific Atoms or Functional Groups		Representative Enzymes Using Coenzymes
Metal Ion	Enzyme	Coenzyme	Entity Transferred	
$\text{Fe}^{2+}$ or $\text{Fe}^{3+}$	Cytochrome oxidase	Thiamine pyrophosphate (TPP)	Aldehydes	Pyruvate dehydrogenase
	Catalase	Flavin adenine dinucleotide (FAD)	Hydrogen atoms	Succinate dehydrogenase
	Peroxidase	Nicotinamide adenine dinucleotide (NAD)	Hydride ion ( $\text{H}^-$ )	Alcohol dehydrogenase
$\text{Cu}^{2+}$	Cytochrome oxidase			
$\text{Zn}^{2+}$	DNA polymerase	Coenzyme A (CoA)	Acyl groups	Acetyl-CoA carboxylase
	Carbonic anhydrase	Pyridoxal phosphate (PLP)	Amino groups	Aspartate aminotransferase
	Alcohol dehydrogenase	5'-Deoxyadenosylcobalamin (vitamin $\text{B}_{12}$ )	H atoms and alkyl groups	Methylmalonyl-CoA mutase
$\text{Mg}^{2+}$	Hexokinase			
	Glucose-6-phosphatase	Biotin (biocytin)	$\text{CO}_2$	Propionyl-CoA carboxylase
$\text{Mn}^{2+}$	Arginase	Tetrahydrofolate (THF)	Other one-carbon groups	Thymidylate synthase
$\text{K}^+$	Pyruvate kinase (also requires $\text{Mg}^{2+}$ )			
$\text{Ni}^{2+}$	Urease			
Mo	Nitrate reductase			
Se	Glutathione peroxidase			

# Enzyme Kinetics

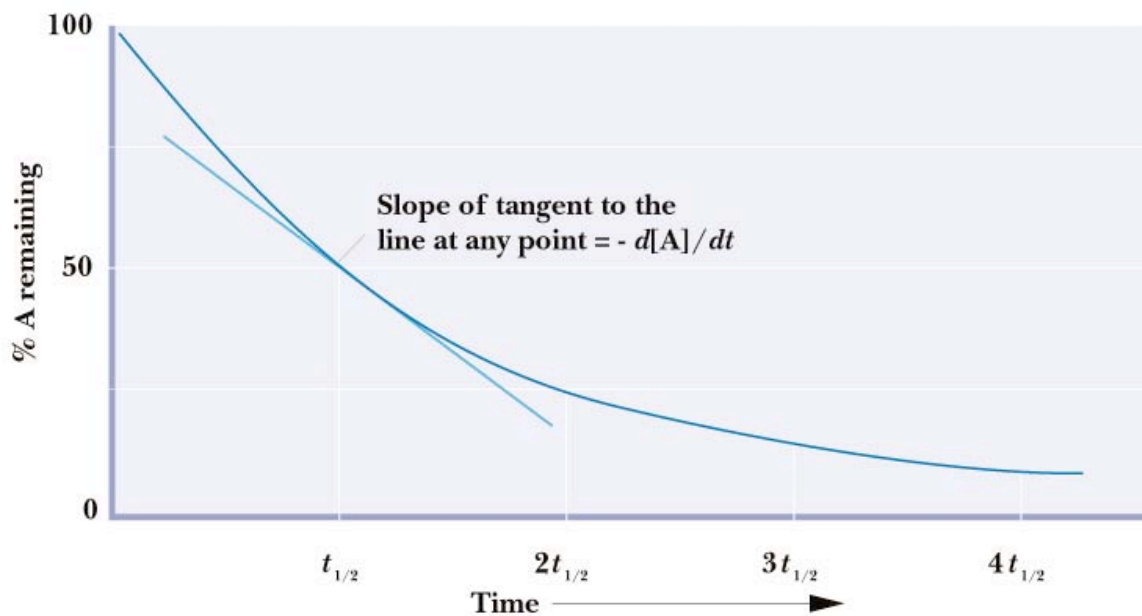
recap these terms from gen. chem.

- rate or velocity
- rate constant
- rate law
- order of a reaction
- molecularity of a reaction

## First Order Kinetics



$$\text{Rate } v = \frac{d[P]}{dt} = -\frac{d[A]}{dt} = k[A]$$



## Second Order Kinetics



$$\text{Rate } v = \frac{d[P]}{dt} = -\frac{d[A]}{dt} = -\frac{d[B]}{dt} = k[A][B]$$

second order, bimolecular

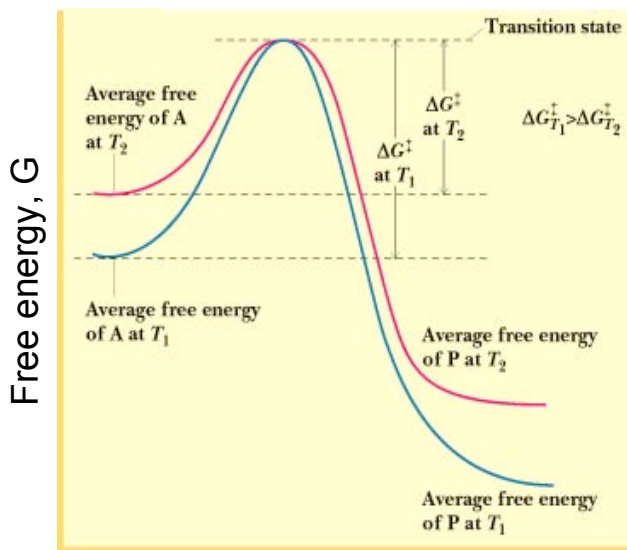
# The Transition State

*understand the difference between  $\Delta G$  and  $\Delta G^\ddagger$*

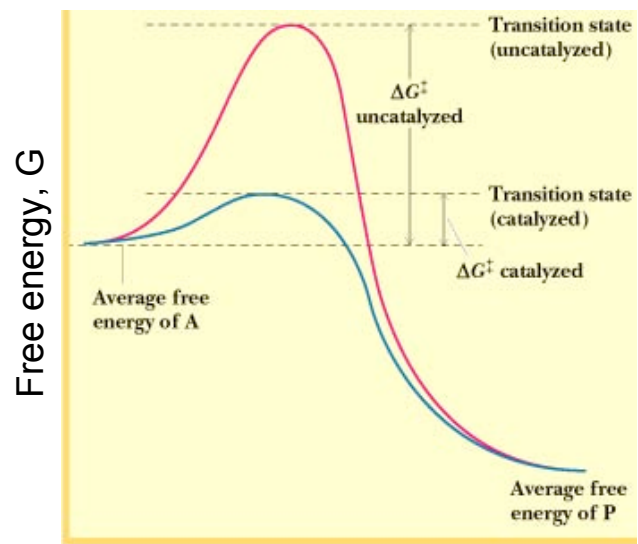
- The overall free energy change for a reaction is related to the equilibrium constant K
- The free energy of activation for a reaction is related to the rate constant k
- It is extremely important to appreciate this distinction

## What Enzymes Do....

- Enzymes accelerate reactions by lowering the free energy of activation
- Enzymes do this by binding the transition state of the reaction better than the substrate (or the product)



Progress of reaction



Progress of reaction