# Notes 10/31

Heather Graehl

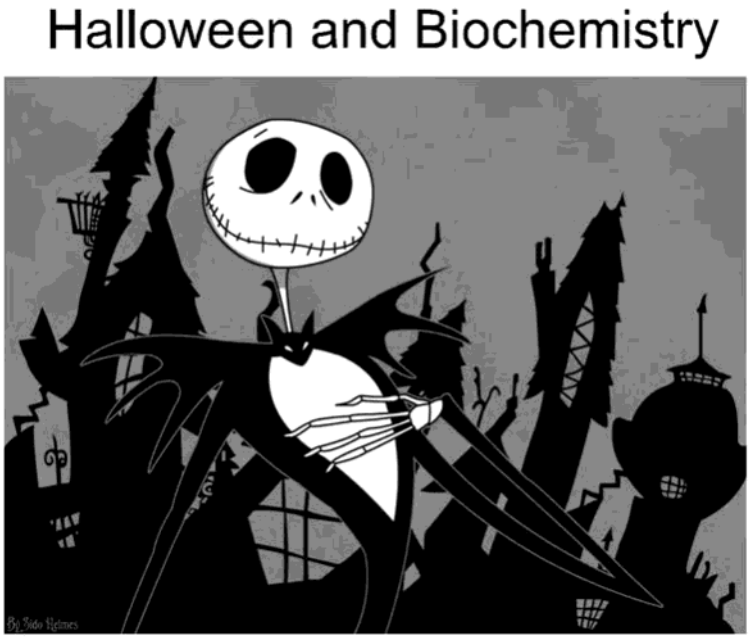
Wednesday, October 31, 2007
8:59 AM

Notes 1031

Audio recording started: 9:01 AM Wednesday, October 31, 2007

Slides posted after 11am

- **Slide 1: Halloween and Biochemistry**
  - Skeleton made of collagen
    - 3 left handed helices twisted about each other in right handed
    - 3.3 residues per turn (not alpha helix)
    - High amount of glycine
    - Proleohydroxylase - enzyme involved in collagen
      - Need vitamin C (asorbic acid)




- **Slide 2: Recall Catalytic Mechanisms**
  - Acid-base catalysis
  - Covalent catalysis
  - Metal ion catalysis
  - Electrostatic catalysis
  - Catalysis through proximity and orientation effects
  - Catalysis by preferential transition state binding

## Recall Catalytic Mechanisms

- Acid-base cat.
- Covalent cat.
- Metal ion cat.
- Electrostatic cat.
- Catalysis through proximity and orientation effects
- Catalysis by preferential transition state binding

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- **Slide 3: Catalytic Teamwork**
  - Enzymes are catalytically efficient because they employ many catalytic mechanisms at the same time

## Catalytic Teamwork

- Enzymes are catalytically efficient because they employ many catalytic mechanisms at the same time

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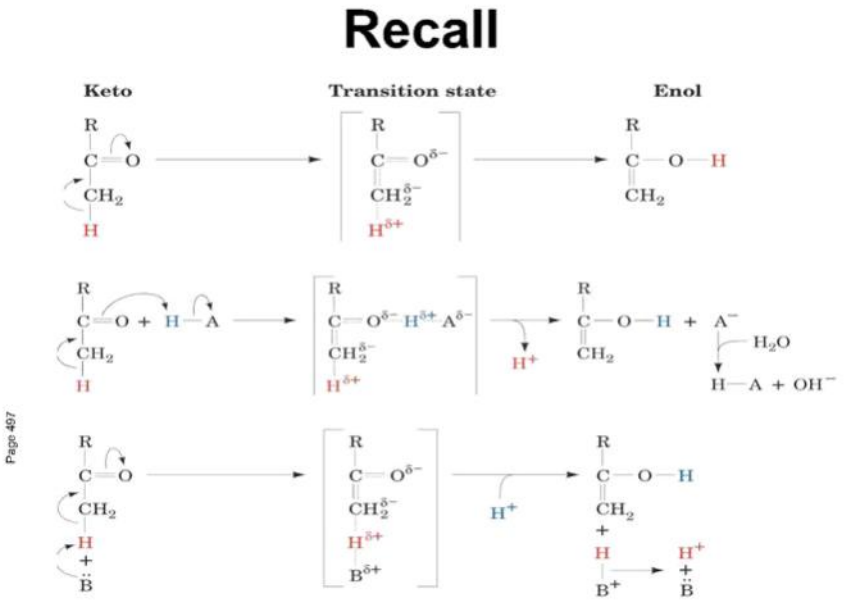
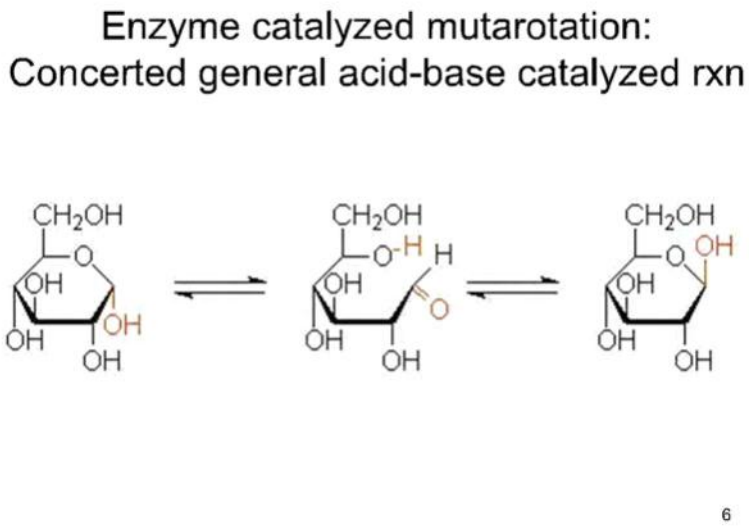
- **Slide 4: Recall (keto enol tautonomerization)**
  - Keto Enol Tautonomerization is either acid or base catalyized



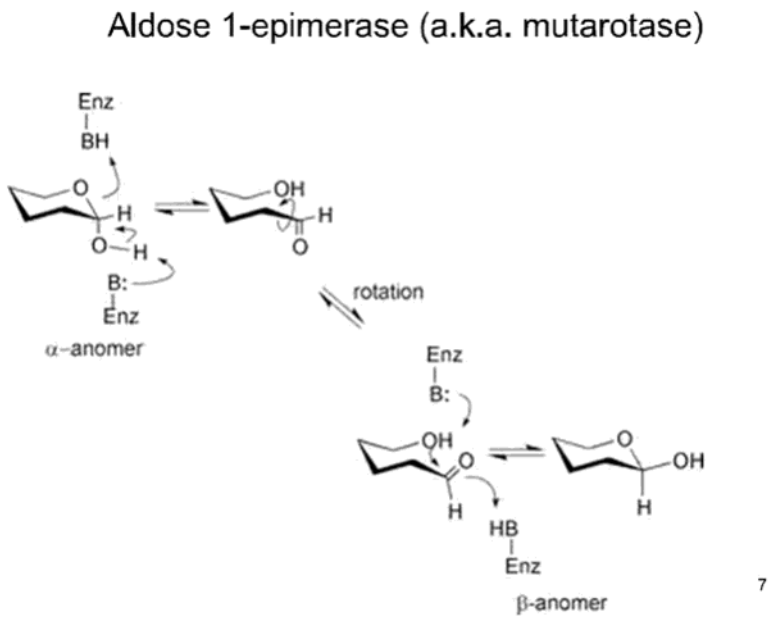

- **Slide 5: concerted general acid-base catalyzed reaction**


- **Slide 6: Enzyme catalyzed mutarotation. Concerted general acid-base catalyzed reaction**
  - Mutarotation - changing anomeric carbon between alpha and beta.

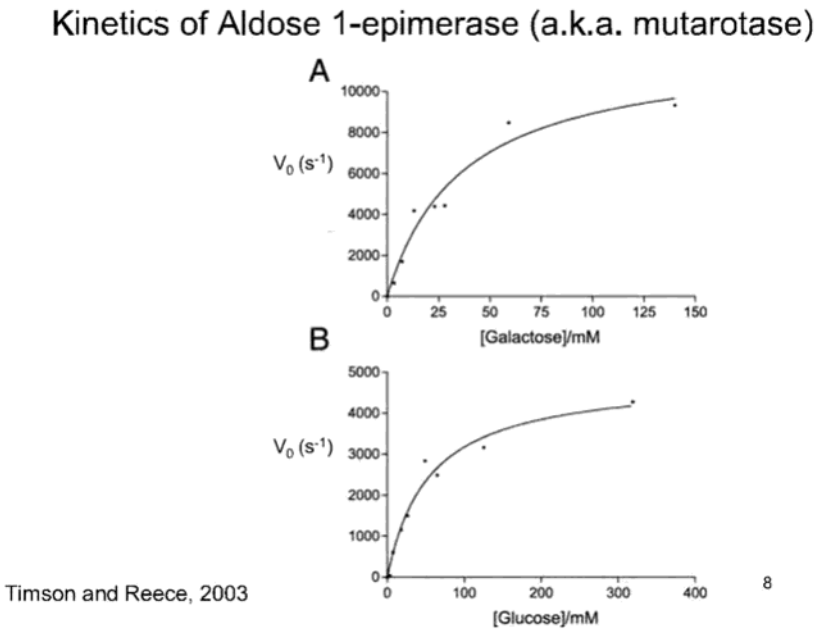
        - Happens in aqueous solution
    - Left side - alpha D glucose
    - Right side - beta D glucose
    - Beta is more prevalent at equilibrium
    - Mutarotation occurs slowly in aqueous solution but enzymes speed up
        - What type of enzyme expected to catalyze mutarotation: isomerase
        - Mutarotase (found in kidney) - important in carbohydrate catalysis




- **Slide 7: Aldose 1-epimerase (aka mutarotase)**
    - Named because sugar is aldose, occurs at carbon 1
    - Steps:
        - Enzyme has base in active site that can abstract proton off hydroxyl of glucose
        - Glucose will abstract proton off of hydrogen donor
        - Ring opens up
        - $180^0$ degree rotation
        - Enzyme abstracts proton back, and glucose (carbonyl group) abstracts proton back
    - Specific for 6 ring sugars… can work with some other than glucose




- **Slide 8: Kinetics of Aldose 1-epimerase (aka mutarotase)**
  - Expect something like this on exam
  - Two graphs of kinetic data
    - Vary concentration of substrate and keep enzyme concentration constant. Measured velocity of reaction
    - Graph A
      - Substrate: galactose
      - $V_{max}$ approx 10,000$s^{-1}$
      - $K_m$ approx 25mM
    - Graph B
      - Substrate: glucose
      - $V_{max}$ approx 4,500$s^{-1}$
      - $K_m$ approx 50mM
  - Which is better substrate… which is mutarotase more specific for?
    - Galactose (graph A)… lower $K_m$
    - Lower $K_m$ means enzyme does not require as much substrate to hit 1/2 $V_{max}$ (very general statement… enzymes are more complicated)
  - Which is more catalytically efficient?
    - Catalytic efficiency = $K_{cat}/K_m$
    - We don't have $K_{cat}$




- **Slide 9: Kinetics of Aldose 1-epimerase (aka mutarotase)**
  - Computer values form graphs A and B
  - Which is more catalyitcally efficient?
    - $K_{cat}/K_m$
    - A
      - $K_m$/mM=37
      - $K_{cat}/s^{-1}$ = 12000
      - $K_{cat}/K_m$ = 340000
    - B
      - $K_m$/mM= 54
      - $K_{cat}/s^{-1}$ = 4900
      - $K_{cat}/K_m$ = 90000
    - Catalytically perfect: $10^8$ or $10^9$
      - Lactose is closest

## Kinetics of Aldose 1-epimerase (a.k.a. mutarotase)

| | Sugar | |
|---|---|---|
| | Galactose | Glucose |
| $K_m$/mM | 37 ±10 | 54 ±11 |
| $k_{cat}/s^{-1}$ | 12 000 ±1 400 | 4 900 ±370 |
| $(k_{cat}/K_m)/l\ mol^{-1}\ s^{-1}$ | 340 000±56 000 | 90 000±12 000 |

Timson and Reece, 2003

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- **Slide 10: Concerted acid-base catalysis**
  - Certain residues are best for acid base catalysis
    - D,E,H,C,Y,K
      - Because they are acidic or basic amino acids

## Concerted acid-base catalysis

- Common enzymatic mech.
- D, E, H, C, Y, K

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- **Slide 11: Covalent Catalysis**

# Covalent Catalysis

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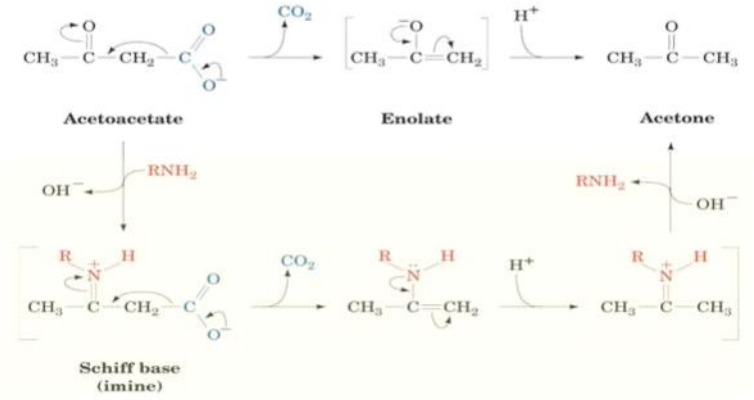
- **Slide 12: Fig. 15-4 Covalent Catalysis**
  - Top is uncatalyzed reaction
  - Bottom is reaction in presence of enzyme
    - Nucleophilic and electrophilic stages

## Fig. 15-4. Covalent Catalysis

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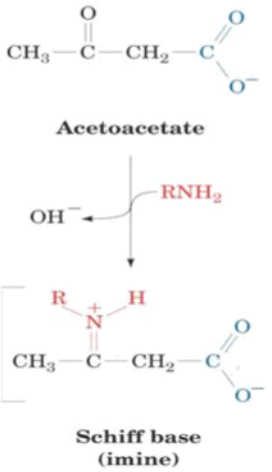
- **Slide 13: Nucleophilic reaction between catalyst and the substrate to form covalent bond**
  - Primary amine within active site

Nucleophilic reaction between catalyst and the substrate to form covalent bond

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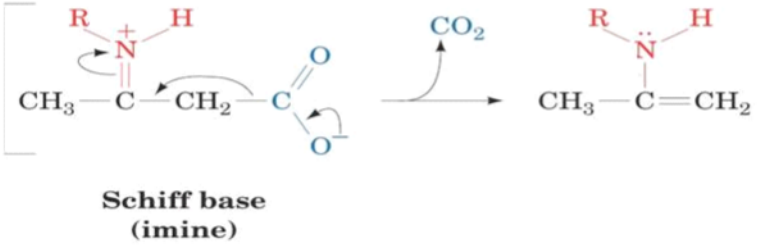
- **Slide 14: Withdrawn of electrons from the reaction center by the now electrophilic catalyst**

Withdrawl of electrons from the reaction center by the now electrophilic catalyst

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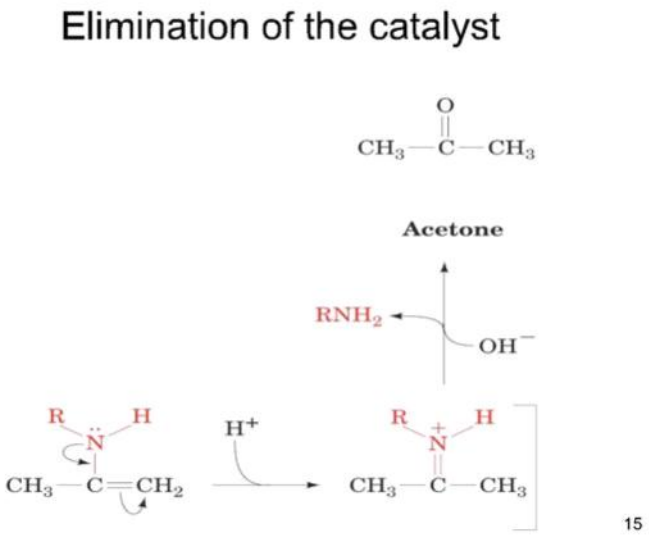
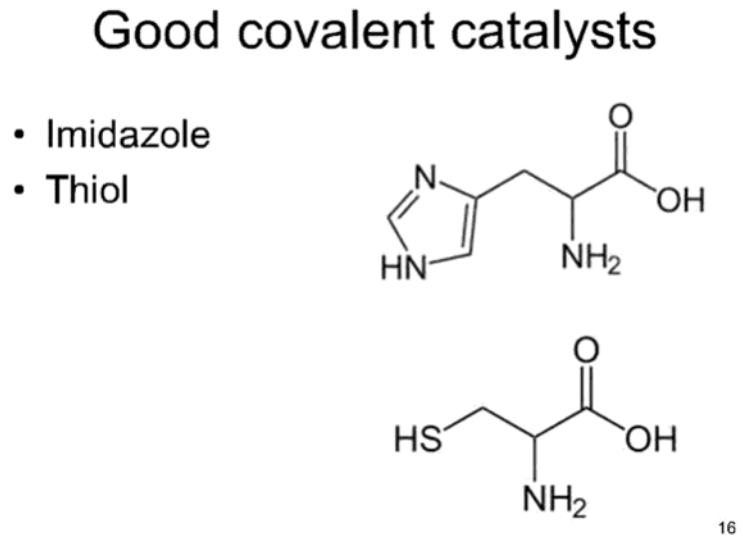
- **Slide 15: Elimination of catalyst**
  - Catalyst is recycled.
  - Reverse of 1st reaction
  - Base eliminates amine

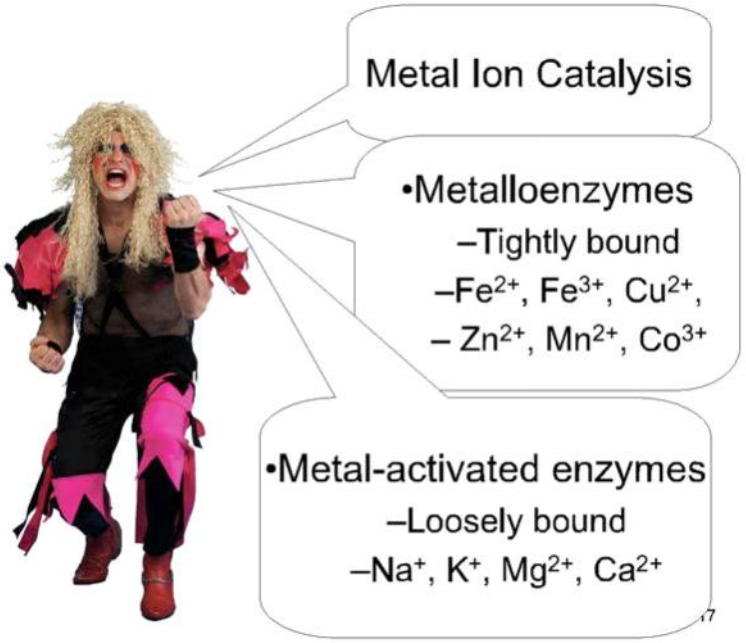



- **Slide 16: Good covalent catalysts**
  - Imidazole
  - Thiol
  - Good because it has nucleophilic and electrophilic parts. Have high polarizability

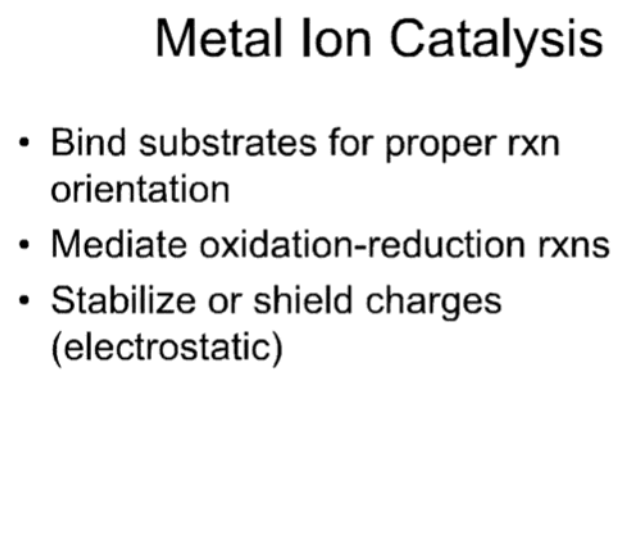



- **Slide 17: Metal Ion Catalysis**
  - Metalloenzymes

        - Tightly bound
        - $Fe^{2+}$, $Fe^{3+}$, $Cu^{2+}$
        - $Zn^{2+}$, $Mn^{2+}$, $Co^{3+}$
    - Metal-activated enzymes
        - Loosely bound
        - $Na^{+}$, $K^{+}$, $Mg^{2+}$, $Ca^{2+}$




- **Slide 18: Metal Ion Catalysis**
    - Bind substrates for proper reaction orientation
    - Mediate oxidation-reduction reactions
    - Stabilize or shield charges (electrostatic interaction)
    - Advantages of metal ion (say as opposed to proton)
        - Metal ions are not sensitive to pH
        - Sometimes has more than +1 charge
        - Referred to as super acids




- **Slide 19: Figure 15-5A**
  - Looking at active site in picture
  - 3 His for metal ion catalysis
  - $Zn^{2+}$
    - Liganded to 3 His and $HCO_3^-$
    - Makes bound water more acidic thus a source of OH
    - Works below neutral pH
  - $HCO_3^0$ interacts with protein
    - Van der Waals
    - H-bonding (Thr, Glu)

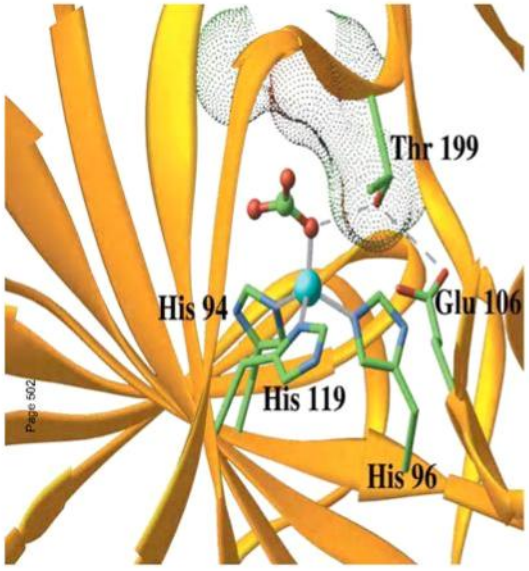



**Figure 15-5a**

- $Zn^{+2}$
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  - Makes bound water more acidic thus a source of OH-
  - Works below neutral pH
- $HCO_3^-$ interacts with protein
  - Van der Waals
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- **Slide 20: Charge stabilization**
  - Metal ions are charged to stabilize charges
  - Hexokinase
    - Uses ATP to transfer phosphoryl group
  - $Mg^{2+}$ stabilizes charges
    - Without $Mg^{2+}$ the phosphate groups might repel each other
  - E.C. Commision #
    - Hexokinase is of the transferase class, (not technically ligase)

## More metal ion catalysis…

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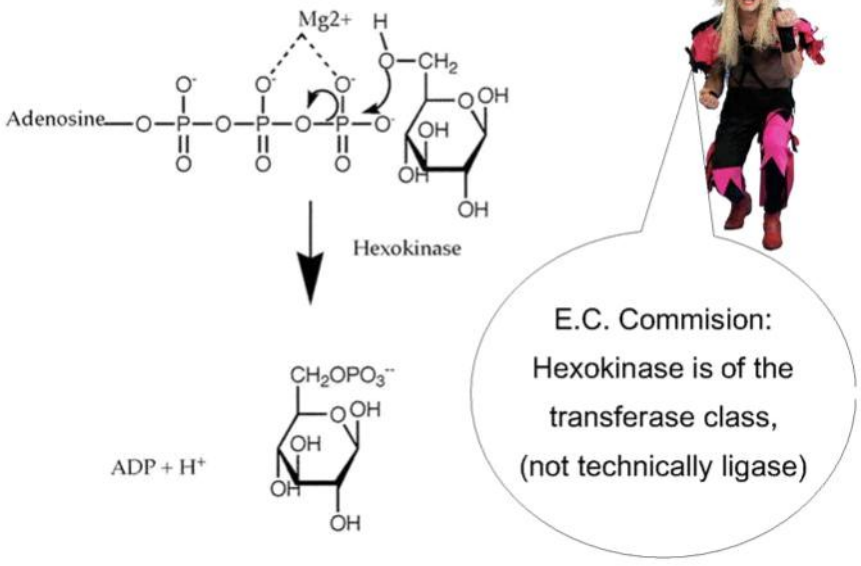
## Charge stabilization




- **Slide 22: Hexokinase classification**
  - Clas: transferase
  - Subclass: kinase
  - Kinase = enzyme that transfers phosphoryl group between ATP and…

## Hexokinase classification

- Class: transferase
- Subclass: kinase
- Kinase = enzyme that transfers phosphoryl groups between ATP and metabolite

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- **Slide 23: Remaining Catalytic Mechanisms**
  - Electrostatic catalysis
  - Proximity and orientation effects
  - Preferential transition state binding

## Remaining Catalytic Mechanisms

- Electrostatic cat.
- Proximity and orientation effects
- Preferential transition state binding

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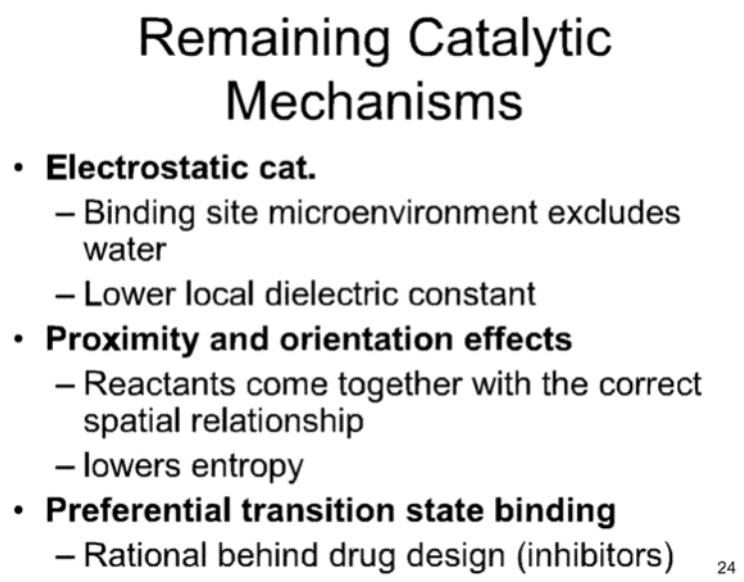
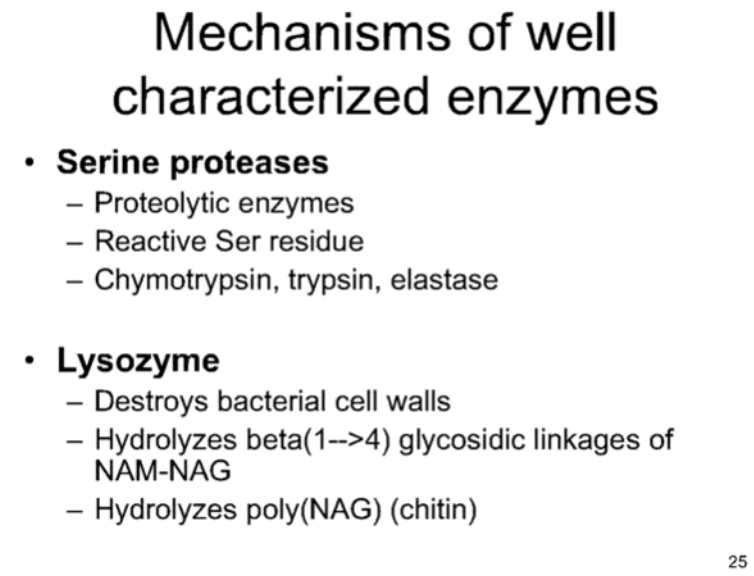
- **Slide 24: Remaining Catalytic Mechanisms**
  - Electrostatic catalsysis

        - Binding site microenvironment excludes
        - Results in lower local dielectric constant
            - Coulomb's Law: $F = kq_1q_2/Dr^2$
                - Dielectic constant is higher than say for example hexane
                - Tells us how well it will dissolve substrate
    - Proximity and orientation effects
        - Reactants come together with the correct spatial relationship
        - Lowers entropy
    - Preferential transition state binding
        - Rational behind drug design (inhibitors)
        - In general prefer transition state

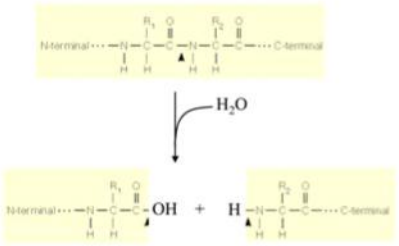



- **Slide 25: Mechanisms of well characterized enzymes**
    - Serine proteases
        - Proteolytic enzymes
            - Function: cleaves peptide bonds
        - Reactive Ser residue
        - Chymotrypsin, trypsin, elestase
    - Lysozyme
        - Destroys bacterial cell walls
            - Cell walls made of peptodoglycan
        - Hydroylzes beta (10>4) glycosidic linkages of NAM-NAG
        - Hydrolyzes poly(NAG) (chiten)




- **Slide 26: Serine Proteases**
  - Diverse group
  - Digestive team
    - Chymotrypsin
      - Prefers bulky hydrophobic residues
        - Ex Phenolalinine, Tryptophan, Tryrosine
    - Trypsin
      - Positively charged residues
        - Ex. Arginine, Lysine, Histidine
    - Elastase
      - Small neutral residues
        - Ex. Glycine, alanine, valine

# Serine Proteases

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