Lecture 13 (10/9/24)

I. **Protein Structure**
- A. Primary
  - 1. Peptide Bond
    - a. Planar, strong, ϕ/ψ angles
  - 2. Determination
    - a. Sequence determination; CHEMICAL
      - i. aa composition; Divide & conquer; Edman degradation
    - b. Sequence determination; PHYSICAL
      - i. Tandem Mass Spectrometry for proteins
    - c. Sequence determination; BIOLOGICAL
      - i. Genome sequenced; need partial sequence
    - d. Determination of Disulfide bonds
- B. Secondary
  - 1. Conformational structure; Levinthal paradox
  - 2. Pauling & Corey's predictions
    - a. α-Helix
    - b. β-sheets/strands
    - c. Connections between β-strands
    - d. Connections between α-helices; angle not important
  - 3. Super secondary structure
- C. Tertiary
  - 1. Picturing and classifications
  - 2. Topology
  - 3. Domains
  - 4. Intrinsically disordered
  - 5. Stability
- D. Quaternary
  - 1. Nomenclature
  - 2. Stability

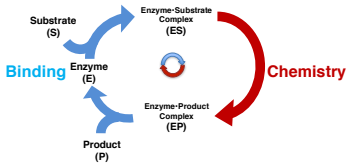
TODAY
- Reading: Ch6, 178-179, 194-195, 179-181, 184-185

II. **Protein Structure Determination**
- A. Quaternary structure
  - 1. How determined; Gel filtration & SDS-PAGE, Ultracentrifugation
- B. Tertiary structure
  - 1. X-ray diffraction/crystallography
  - 2. NMR spectroscopy
  - 3. Comparison: NMR *versus* X-ray crystallography
- C. Secondary structure
  - 1. Circular dichroism (CD)

III. **Collagen**
- A. Special Fibrous Protein:
- B. Clues to structure
- C. 4-S's
- D. Biosynthesis
- E. Disorders

IV. **ENZYMES: Binding & Catalysis**
1. General
2. Catalytic cycle; turnover number = k_{cat}
   - A. Binding
     - a. Models
     - b. Active site; complementarity
     - c. How? (lock & key; induced fit)
     - d. How tight? – Binding curves; K_d
       - i. Hyperbolic –saturation
       - ii. Sigmoidal –cooperativity in saturation
   - B. Catalysis
     - a. Catalytic power
       - i. Proficiency (rate enhancement)
       - ii. Assay of rate
       - iii. Rate *versus* [E]
3. Nomenclature
   - A. Reaction
   - B. Cofactors
   - C. Names
     - a. Trivial
     - b. Systematic
4. What do enzymes do
   - A. Transition-state theory
   - B. Catalytic strategies
     - a. Position
     - b. Polarization
     - c. Strain
     - d. desolvation
5. How do enzymes do it
   - A. Mechanistic strategies
     - a. Acid-base catalysis
     - b. Covalent
     - c. Metal ion

Enzymes



Now that we have some concept of binding, the first important part of the catalytic cycle, lets discuss the second part of the cycle:

Catalysis

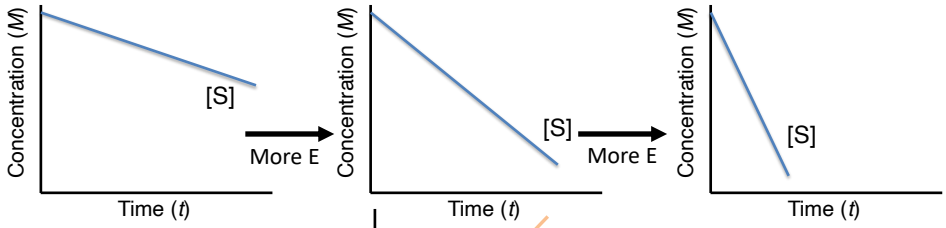
Four introductory aspects to Enzyme Catalysis:

1) Rate enhancement (proficiency)
2) How do you measure catalysis (enzyme activity)
3) What is the relationship between reaction rate and [E]?
4) Enzyme nomenclature
   a. reaction
   b. helpers
   c. enzymes

Enzymes

Catalysis

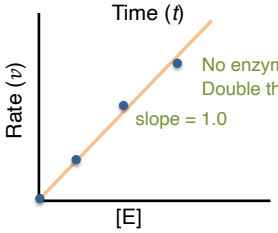
3) What is the relationship between reaction rate and [E]?



Rate (v) = k_{cat} Δ[S]

Rate (v) = k_{cat}[E]

(μM•sec^{-1}) (sec^{-1}) (μM)



No enzyme, "no rate" (really slow)

Double the [enzyme], double the rate

- The **turnover number** (k_{cat}) is the number of catalytic cycles/unit time per enzyme active site.
- Range of values for turnover number: from <1 up to 10^7 sec^{-1} → Said to be "Diffusion Controlled"

Enzyme	Catalyzed rate (k_{cat} s^{-1})
OMP decarboxylase	39
Staphylococcal nuclease	95
AMP nucleosidase	60
Carboxypeptidase A	578
Ketosteroid isomerase	66,000
Triose phosphate isomerase	4,300
Chorismate mutase	50
Carbonic anhydrase	1×10^6

Enzymes

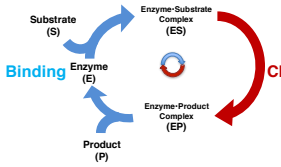
Enzyme nomenclature

a. Reaction Nomenclature

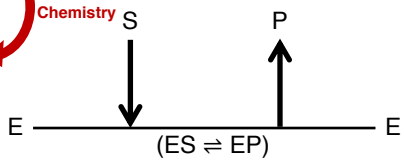
b. Enzyme helpers (cofactors)

c. Naming enzymes

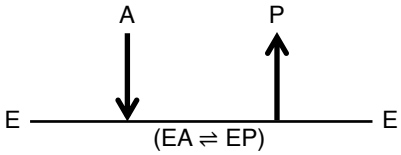
Reaction Nomenclature Enzymes



$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$



- Reaction coordinate written as a horizontal line
- Substrates binding with arrow down; substrates are denoted in order A, B, C
- Products released with arrow up; products are denoted in order P, Q, R
- Enzyme forms are denoted in order as E, F, G
- Define the number of substrates and/or products separately as *uni*=1, *bi*=2, *ter*=3
- For multi-substrate enzymes:
- Define the order of binding and release separately as *ordered* or *random*
- Define the relationship of substrates-to-products as *sequential* or *ping-pong*



This is a uni-uni reaction.

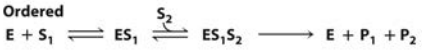
What about more complicated reactions?

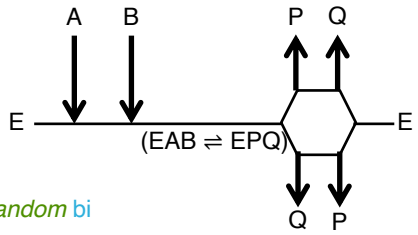
(No need to designate binding order or substrates-product relationship when there is only one substrate and one product)

Reaction Nomenclature Enzymes

(a) Enzyme reaction involving a ternary complex

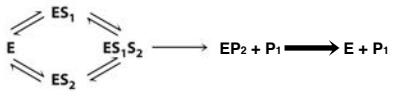
Ordered

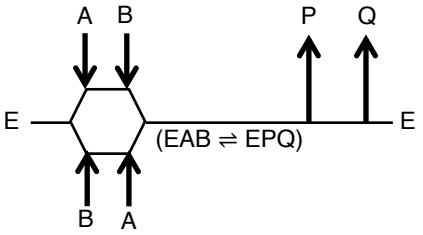




Sequential ordered bi *random* bi

Random order





Sequential random bi *ordered* bi

Reaction Nomenclature

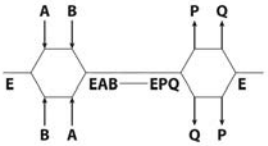
Enzymes

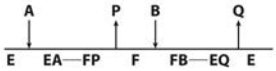
Cleland nomenclature

Two-substrate/Two-product reactions: bi bi

Ordered bi bi

Random bi bi





Sequential ordered bi *ordered* bi
Sequential ordered bi bi
Ordered bi bi

Sequential random bi *random* bi
Sequential random bi bi
Random bi bi

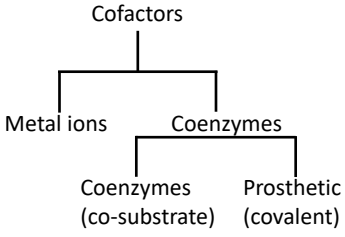
Ping-pong ordered bi *ordered* bi
Ping-pong bi bi

Which is the bi bi reaction catalyzed by LDH?

Enzyme Helpers

Enzymes

Cofactors are small molecules that some enzymes require for activity. The two main classes of cofactors are coenzymes (organic molecules derived from vitamins) and metals. Covalently bound coenzymes are called prosthetic groups.



An enzyme with its cofactor is a **holoenzyme**. Without the cofactor, the enzyme is called an **apoenzyme**.

Cofactors	Enzyme
Coenzyme†	
Thiamine pyrophosphate (TPP)	Pyruvate dehydrogenase
Flavin adenine dinucleotide (FAD)	Monoamine oxidase
Nicotinamide adenine dinucleotide (NAD^+)	Lactate dehydrogenase
Pyridoxal phosphate (PLP)	Glycogen phosphorylase
Coenzyme A (CoA)	Acetyl CoA carboxylase
Biotin	Pyruvate carboxylase
6′-Deoxyadenosyl cobalamin	Methylmalonyl mutase
Tetrahydrofolate	Thymidylate synthase
Metal	
Zn^{2+}	Carbonic anhydrase
Mg^{2+}	*Eco*RV
Ni^{2+}	Urease
Mo	Nitrogenase
Se	Glutathione peroxidase
$Mn^{2+\leftrightarrow 3+}$	Superoxide dismutase
K^+	Acetoacetyl CoA thiolase

*The enzymes listed are examples of enzymes that employ the indicated cofactor.
†Often derived from vitamins, coenzymes can be either tightly or loosely bound to the enzyme.

Naming Enzymes

Enzymes

*Trivial:

- Nearly all enzymes end with the suffix of "-ase."
- Generally, the names are of the form "substrate or product – reaction catalyzed." For example, lactate dehydrogenase is for an enzyme that removes a hydrogen (plus $2e^-$, i.e., a hydride) from lactate, yielding the carbonyl in pyruvate.
- There are two ways of naming enzymes; 1) Trivial and 2) Systematic

* Not all possible types listed

• Bullets are those also given as systematic

Name	Reaction Catalyzed
-dehydrogenase	redox/hydride transfer
• *lactate dehydrogenase*	
• *glyceraldehyde-3-phosphate dehydrogenase*	
-oxidase	redox/O_2 as oxidizer
cytochrome oxidase	
• *glucose oxidase*	
-oxygenase	redox/O_2 incorporated
cyclooxygenase	
Ribulose Bisphosphate Carboxylase Oxygenase	
-hydroxylase	redox/-OH incorporated
tyrosine hydroxylase	
• *phenylalanine hydroxylase*	
-kinase	transfer/P_i into substrate from ATP
• *hexose kinase*	
protein kinase A	
-hydrolase	hydrolysis with H_2O
• (esterase, deacylase)	*trypsin*
• *phospholipase C*	
-phosphorylase	hydrolysis with P_i instead of H_2O
glycogen phosphorylase b	
Thymidine phosphorylase	
-mutase	move P_i from one part of molecule to another
phosphoglycerate mutase	
• *phosphoglucose mutase*	
-isomerase	configuration change
• *triosephosphate isomerase*	
• *phosphogluco isomerase*	
-synthase	synthesis
• *fatty acid synthase*	
nitric oxide synthase	
-synthetase	synthesis that requires ATP
• *aminoacyl-tRNA synthetases*	
acyl-CoA synthetase	

Systematic

Great website: EC numbers (https://www.enzyme-database.org/class.php)

1.1.1.1 — Type, Sub-type, Sub-class, enzyme specific*

Types	Reaction	Examples		
		EC#	Name	Trivial Name
	At alcohol → Uses NAD			
-oxidoreductase	redox	1.1.1.28	L-lactate:NADH oxidoreductase	lactate dehydrogenase
		1.2.1.59	D-glyceraldehyde-3-phosphate:NAD+ oxidoreductase	glyceraldehyde-3-phosphate dehydrogenase
		1.1.3.4	β-D-glucose:oxygen 1-oxidoreductase	glucose oxidase
		1.14.16.1	L-phenylalanine,tetrahydrobiopterin:oxygen oxidoreductase (4-hydroxylating)	phenylalanine hydroxylase
	Nitrogen-group transfer → Primary amine			
-transferase	transfer	2.6.1.1	L-aspartate:2-oxoglutarate aminotransferase	Aspartate aminotransferase
		2.3.1.85	Acyl-CoA:malonyl-CoA C-acyltransferase	fatty acid synthase
		2.7.1.1	ATP:D-hexose 6-phosphotransferase	hexose kinase
	Peptide bonds → Serine mechanism			
-hydrolase	hydrolysis	*3.4.21.4*	*Serine endopeptidylamino acid hydrolase*	*trypsin*
		3.1.4.3	*Phosphatidylcholine:cholinephosphohydrolase*	*phospholipase C*
	C-C bond cleavage → Aldehyde product			
-lyase	bond cleavage	*4.1.2.13*	*Fructose 1,6-bisphosphate:triosephosphate lyase*	*aldolase*
		4.2.1.2	*(S)-malate hydro-lyase*	*fumarase*
		4.1.1.1	*2-oxo-acid carboxy-lyase (aldehyde-forming)*	*pyruvate decarboxylase*
	Intramolecular configuration change → Aldose:ketose			
-isomerase	configuration change	*5.3.1.9*	*Glucose-6-phophate isomerase*	*phosphoglucoisomerase*
		5.3.1.1	*D-glyceraldehyde-3-phosphate:aldose-ketose-isomerase*	*triosephosphate isomerase*
		5.1.1.4	*Proline racemase*	*proline racemase*
		5.4.2.11	*D-phosphoglycerate 2,3-phosphomutase (2,3-diphosphoglycerate-dependent)*	*phosphoglycerate mutase*
	Ester linkage → Only 1 subclass			
-ligase	synthesis	*6.1.1.1*	*Tyrosine aminoacyl-tRNA ligase*	*Tyr-aminoacyl-tRNA synthetases*
		6.4.1.1	*Pyruvate:carbon-dioxide ligase (ADP-forming)*	*pyruvate carboxylase*

*this is specific for alcohol dehydrogenase!

Naming Enzymes

Enzymes

Correlation of trivial and systematic:

OTHLIL

What to know:
1) Trivial: substrate-reaction-ase
   a) Dehydrogenase, hydrolase*, isomerase, kinase, synthase, synthetase
   b) *substrate-ase
2) Systematic: OTHLIL (1–6)
   a) Oxidoreductase, transferase, hydrolase, lyase, isomerase, ligase

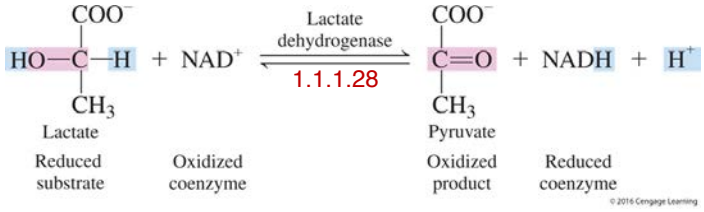
Main Classes	Selected Trivial Names	Type of Reaction Catalyzed
oxidoreductases	oxidases reductases dehydrogenases	oxidation of a substrate reduction of a substrate introduction of double bond (oxidation) by formal removal of two H atoms from a substrate, with one H being accepted by a coenzyme
transferases	transaminases kinases	transfer of an amino group between substrates transfer of a phosphate group between substrates
hydrolases	lipases proteases nucleases carbohydrases phosphatases	hydrolysis of ester linkages in lipids hydrolysis of amide linkages in proteins hydrolysis of sugar–phosphate ester bonds in nucleic acids hydrolysis of glycosidic bonds in carbohydrates hydrolysis of phosphate–ester bonds
lyases	dehydratases decarboxylases deaminases hydratases	removal of H_2O from a substrate removal of CO_2 from a substrate removal of NH_3 from a substrate addition of H_2O to a substrate
isomerases	racemases mutases	conversion of D isomer to L isomer, or vice versa transfer of a functional group from one position to another in the same molecule
ligases	synthetases carboxylases	formation of a new bond between two substrates, with participation of ATP formation of a new bond between a substrate and CO_2, with participation of ATP

Naming Enzymes

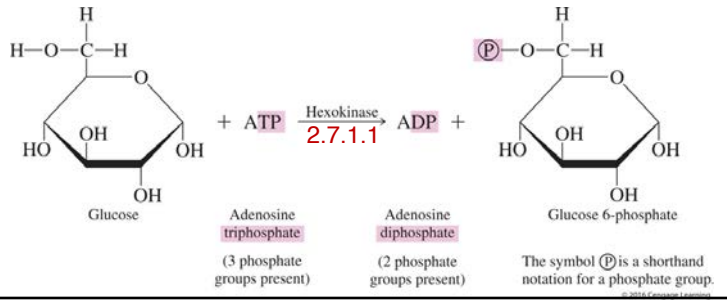
Enzymes

OTHLIL

Oxidoreductase: oxidation-reduction reaction



Transferase: transfer of functional group between molecules

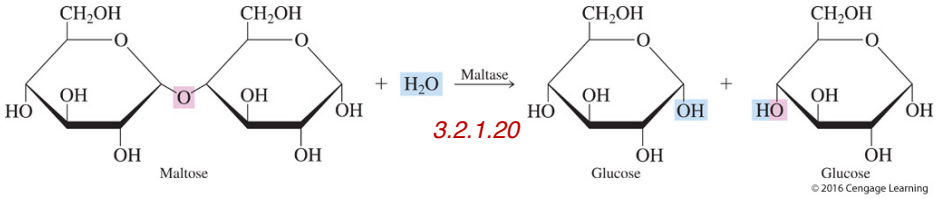


Naming Enzymes

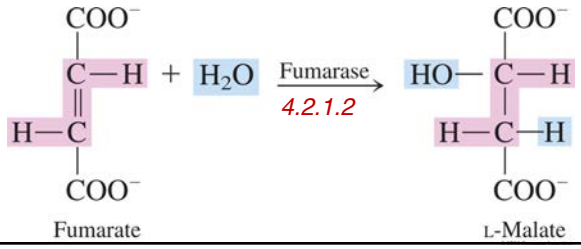
Enzymes

OTHLIL

Hydrolase: hydrolysis (addition of water breaks bond)



Lyase: addition of group to a double bond or
removal of group to form double bond without hydrolysis or oxidation

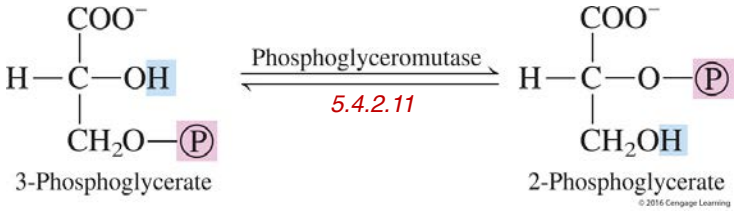


Naming Enzymes

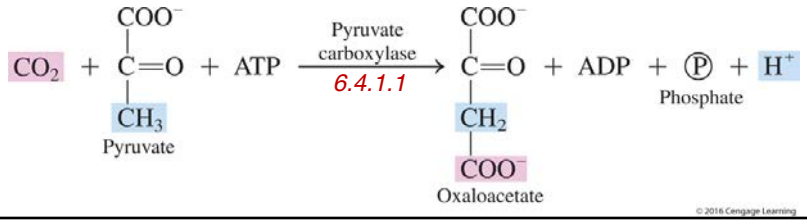
Enzymes

OTHLIL

Isomerase: transfer of functional group *within* a molecule (rearrangement)



Ligase: joining of two molecules (bond formation) coupled with ATP hydrolysis



ENZYMES

(The WHAT and the How)

What must ALL enzymes to to achieve these amazing rate enhancements?

How do enzymes do what they do…….. Mechanistically?

Enzymes

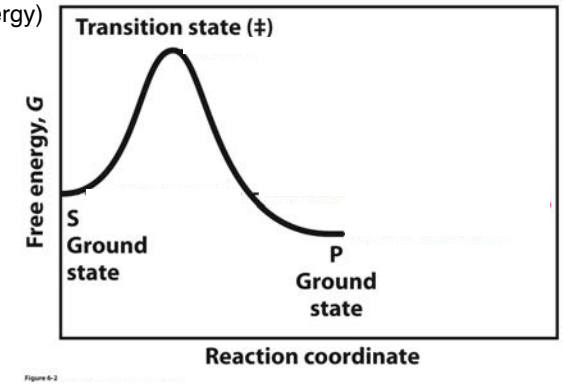
How do enzymes achieve these HUGE rate enhancements?

Let's review what governs the rates of chemical reactions: the transition state energy

OK, what does a similar plot look like using an enzyme?

Reaction Coordinate Diagram

Reaction rate ∝ $\Delta G^{\ddagger}$ (activation energy)



⊕ $\Delta G^{\ddagger}$ – Enzymes CAN change this!

⊖ ΔG – Enzymes CANNOT change this!

The Equilibrium Constant will NEVER be changed by an enzyme

Enzymes

How do enzymes achieve these HUGE rate enhancements?

Let's review what governs the rates of chemical reactions: the transition state energy

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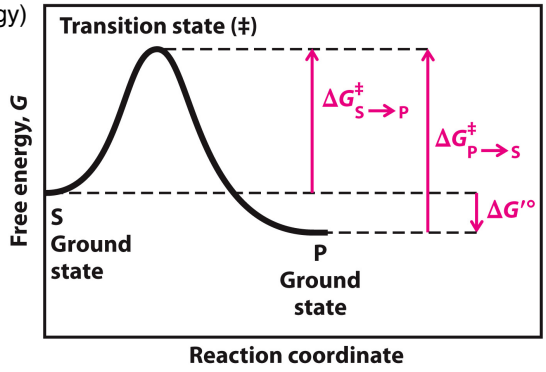
Reaction rate ∝ $\Delta G^{\ddagger}$ (activation energy)



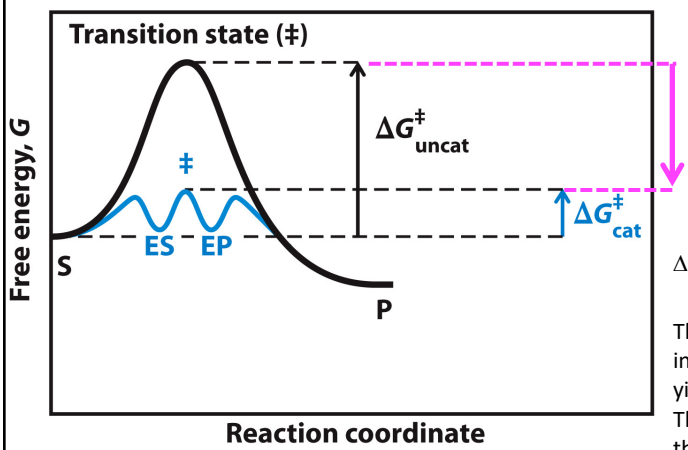
⊕ $\Delta G^{\ddagger}$ – Enzymes CAN change this!

⊖ ΔG – Enzymes CANNOT change this!

The Equilibrium Constant will NEVER be changed by an enzyme

Enzymes

Enzymes Can Decrease $\Delta G^{\ddagger}$



$\Delta\Delta G^{\ddagger}$ ⊖

$\Delta\Delta G^{\ddagger} = \Delta G^{\ddagger}_{cat} - \Delta G^{\ddagger}_{uncat}$

There is always a difference in activation energies that yields a negative $\Delta\Delta G^{\ddagger}$. This is the amount of energy that must be supplied somehow to the reaction by the enzyme

Enzymes

*Catalytic Strategies

versus

Mechanistic Strategies

WHAT must Enzymes do to lower Activation Energies?
-nearly all enzymes do these

HOW do Enzymes lower Activation Energies?
- enzymes may use none, one, or more of these

*Textbook uses this term a bit incorrectly. What they term Catalytic strategies are really those that answer HOW enzymes decrease the activation energy. The HOW-to strategies are really "Mechanistic" strategies.

Enzymes

Catalytic Strategies

- Position Effects: bind substrates where they need to be for reaction (rather than depending on random collisions)
- Polarization of bonds: make substrates more reactive by polarizing bonds (make better nucleophiles, electrophile, or leaving groups) (Electrostatics)
- Strain of bonds: bind substrates in such a way that they "look" like products (put strain on bonds that are to be broken (sessile)) (Geometry)
- De-solvation: assist in removal of water shell around substrates or adding to products upon release (S & P are usually in direct contact with residues at the active site (no water))

Enzymes

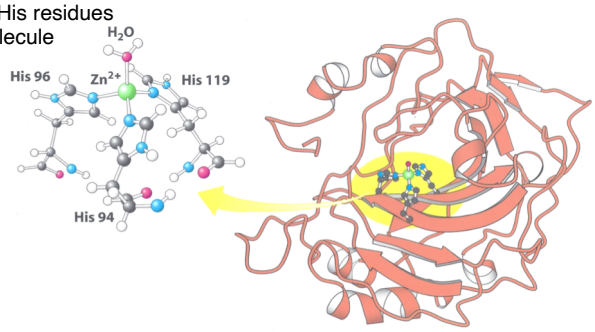
Catalytic Strategies

EXAMPLE: Carbonic Anhydrase

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

Carbonic Acid — Bicarbonate

- Named for the reverse reaction, the dehydration of Carbonic Acid to get water and carbon dioxide
- Mostly β-structure
- It needs a Zn^{2+} metal cofactor
- Held in the active site by 3 His residues
- Fourth ligand is a water molecule



Enzymes

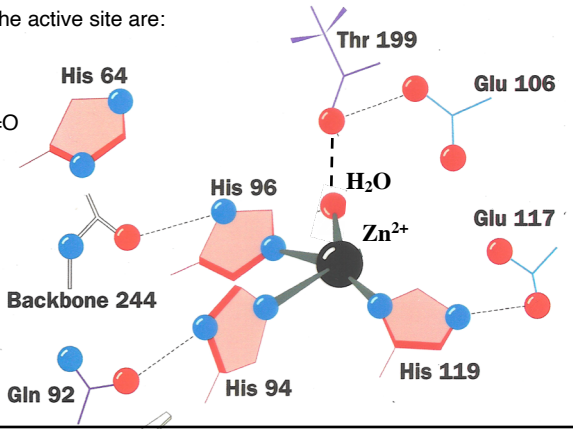
Catalytic Strategies

EXAMPLE: Carbonic Anhydrase

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

Carbonic Acid — Bicarbonate

- Other important residues in the active site are:
  - His-64
  - Thr-199
  - Glu-106
  - Glu-117, Gln-92, 244C=O

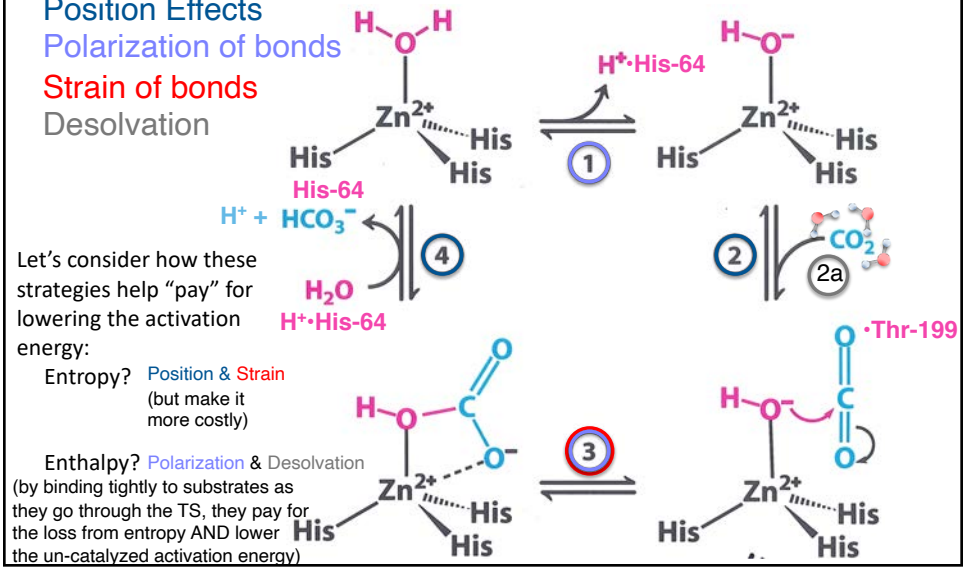


Enzymes

Catalytic Strategies

$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$

- Position Effects
- Polarization of bonds
- Strain of bonds
- Desolvation



Let's consider how these strategies help "pay" for lowering the activation energy:

Entropy? Position & Strain (but make it more costly)

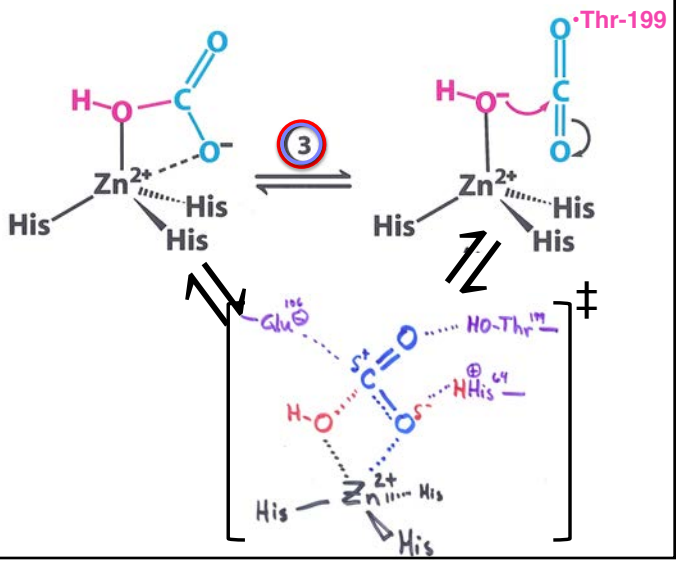
Enthalpy? Polarization & Desolvation (by binding tightly to substrates as they go through the TS, they pay for the loss from entropy AND lower the un-catalyzed activation energy)

Enzymes

Catalytic Strategies

$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$

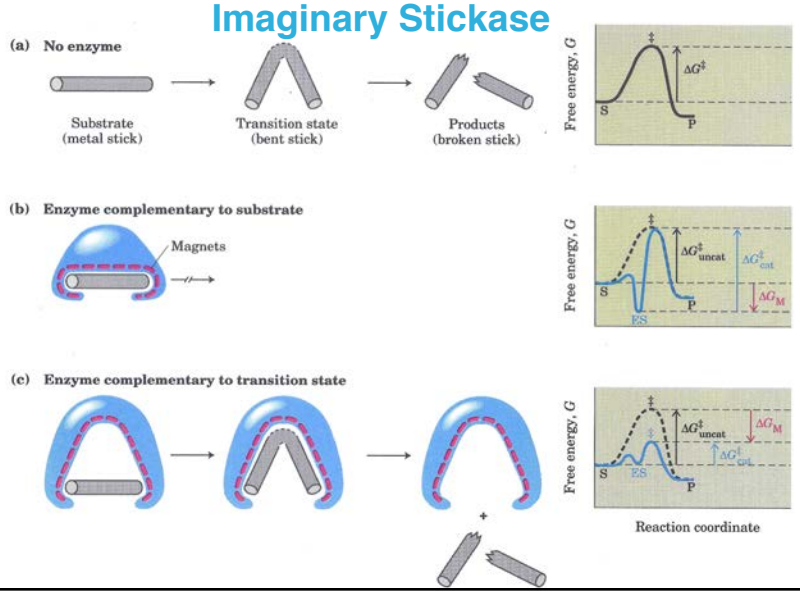
- Transition State Binding



What is another example of binding the TS?

Enzymes

Illustration of TS Stabilization Idea: Imaginary Stickase



Enzymes

*Catalytic Strategies

versus

Mechanistic Strategies

WHAT must Enzymes do to lower Activation Energies?
-nearly all enzymes do these

HOW do Enzymes lower Activation Energies?
- enzymes may use none, one, or more of these

*Textbook uses this term a bit incorrectly. What they term Catalytic strategies are really those that answer HOW enzymes decrease the activation energy. The HOW-to strategies are really "Mechanistic" strategies.

Enzymes

Mechanistic Strategies

HOW do Enzymes lower Activation Energies?
- enzymes use may use none, one, or more of these

There are THREE major strategies used by enzymes:

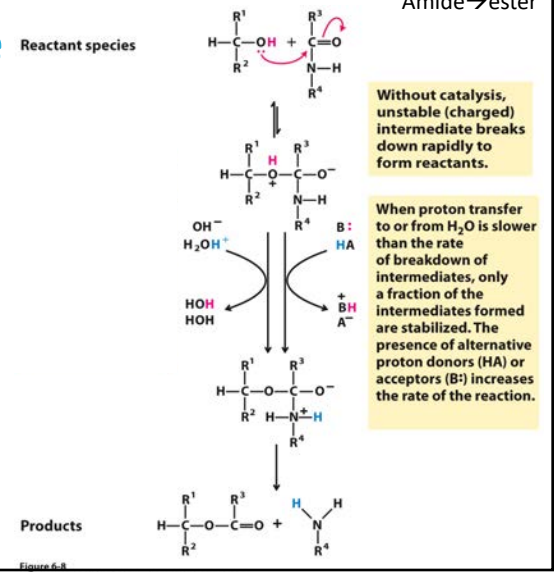
– acid-base catalysis: give and take protons
– covalent catalysis: change reaction paths
– metal ion catalysis: use redox cofactors, pK_a shifters

Enzymes

Mechanistic Strategies

Amide→ester

General Acid-Base Catalysis



EXAMPLES:
Carbonic Anhydrase
Proteases
Phosphoglyceromutase
Proline Racemase
….. and many many more

Enzymes

Mechanistic Strategies

General Acid-Base Catalysis

Amino Acids

Recall, pK_a values can shift by orders of magnitude in the micro-environment of the active site

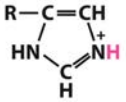
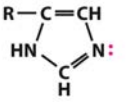
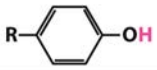
Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp	R—COOH	R—COO⁻
Lys, Arg		
Cys	R—SH	R—S⁻
His		
Ser	R—OH	R—O⁻
Tyr		

Figure 6-9

Enzymes

Mechanistic Strategies

Covalent Catalysis

- A transient covalent bond between the enzyme and the substrate
- Changes the reaction pathway
  - un-catalyzed:

$$A—B \xrightarrow{H_2O} A + B$$

  - catalyzed (X = catalyst):

$$A—B + X: \rightarrow A—X + B \xrightarrow{H_2O} A + X: + B$$

- Requires a nucleophile on the enzyme

Which Amino Acids?

Ser, Thr, Cys, Lys, Asp, Glu

EXAMPLES:
Carbonic Anhydrase
Proteases
Amino transferase
Aldolase

  - can be a reactive hydroxyl, thiolate, amine, or carboxylate
- May also be with metals (dative-covalent bond/coordinate)

Enzymes

Mechanistic Strategies

Metal Ion Catalysis

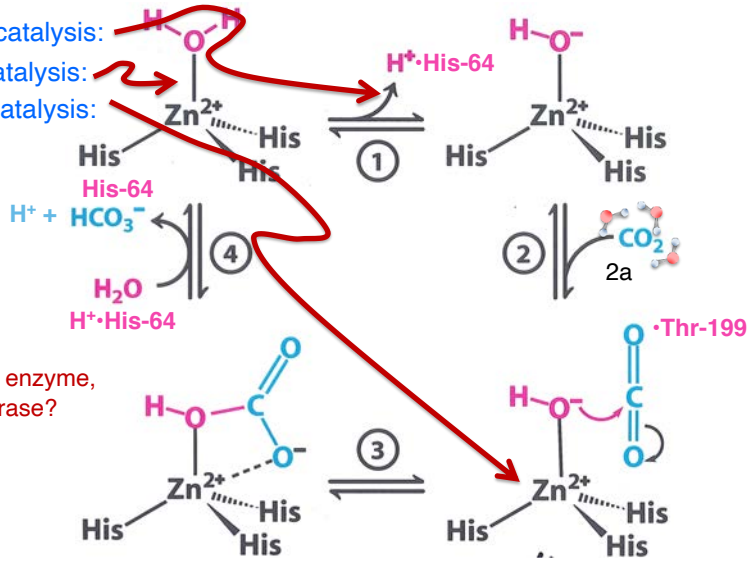
- Involves a metal ion bound to the enzyme
- Interacts with substrate to facilitate binding
  – stabilizes negative charges
- Participates in oxidation reactions

EXAMPLES:
Carbonic Anhydrase
Carboxylpeptidase A
Cytochrome oxidase

Enzymes

Mechanistic Strategies

– acid-base catalysis:
– covalent catalysis:
– metal ion catalysis:



What about our enzyme, carbonic anhydrase?