

Lecture #5: Enzymes Characteristics and Properties

(1) Classification

(2) Catalytic power and specificity

- Nitrogenase
- Alcohol dehydrogenase
- Phenylalanine dehydrogenase

(3) Enzymes as catalysts

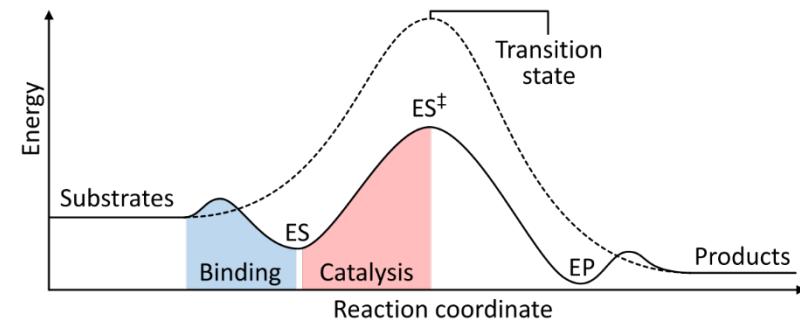
- Absolute rate theory

(4) Enzyme - substrate interactions

- (a) lock & key model
- (b) induced fit model
- (c) transition state model
- (d) quantum tunnelling model

(5) Enzymes as proteins

- ribozymes
- Protein folding



PROTEIN STRUCTURE

Scaffold to support and position active site

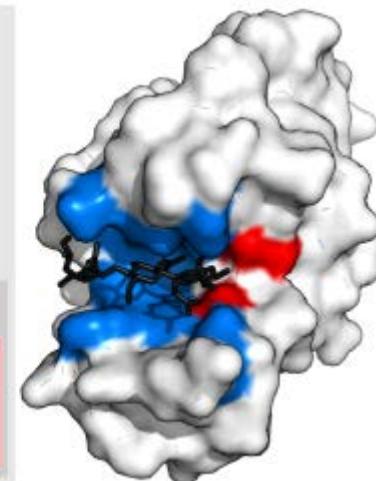
ACTIVE SITE

BINDING SITES

Bind and orient substrate(s)

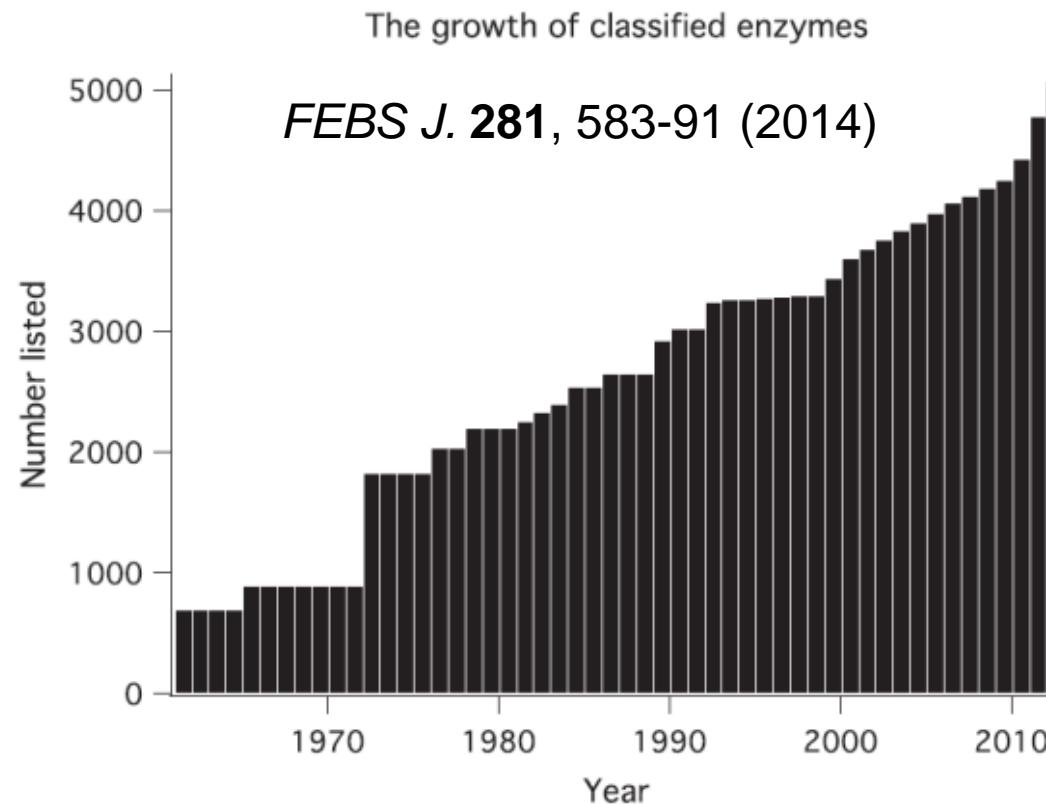
CATALYTIC SITE

Reduce chemical activation energy



1. Nomenclature/Classification

- Enzymes placed into **7 categories** based on reaction being catalyzed
 - many enzymes given trivial names, e.g., catalase
 - suffix "ase" added to the substrate name
- ExplorEnz-The Enzyme Database
 - <http://www.enzyme-database.org/search.php>
 - trivial name for proteases end with “in”
 - systematic naming: Enzyme Commission (1955)



ExplorEnz - The Enzyme Database



Home Search Enzymes by Class New/Amended Enzymes Statistics Forms News Information Downloads

Simple search

For advanced search option, click [here](#).

Search for in all fields Use regular expressions [\[what are these?\]](#) Full-text search
or
 select fields:

| | |
|--|-------------------------------------|
| <input type="checkbox"/> EC Number | <input type="checkbox"/> Comments |
| <input type="checkbox"/> Accepted name | <input type="checkbox"/> References |
| <input type="checkbox"/> Reaction | <input type="checkbox"/> PubMed ID |
| <input type="checkbox"/> Other name(s) | <input type="checkbox"/> Glossary |
| <input type="checkbox"/> Systematic name | |

and display (highlighting matches)
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| <input type="checkbox"/> Glossary | |

Sort results by displaying entries per page.

The E.C. Number: (a. b. c. d)

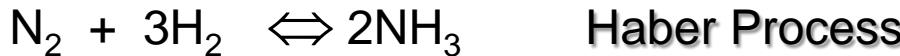
- #a - enzyme class or type of reaction that is catalyzed
- #b - often refers to the **subclass** or type of substrate
- #c - refers to the **sub subclass**: more precise determination of the reaction being catalysed
- #d - the **enzyme serial number** assigned by the Enzyme Commission.

TABLE 6–3 International Classification of Enzymes

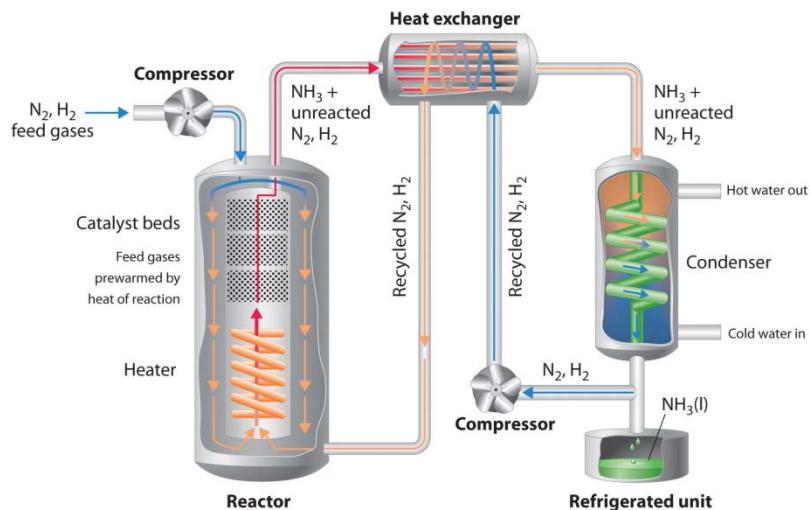
| Class no. | Class name | Type of reaction catalyzed |
|-----------|-----------------|--|
| 1 | Oxidoreductases | Transfer of electrons (hydride ions or H atoms) |
| 2 | Transferases | Group transfer reactions |
| 3 | Hydrolases | Hydrolysis reactions (transfer of functional groups to water) |
| 4 | Lyases | Cleavage of C—C, C—O, C—N, or other bonds by elimination, leaving double bonds or rings, or addition of groups to double bonds |
| 5 | Isomerases | Transfer of groups within molecules to yield isomeric forms |
| 6 | Ligases | Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to cleavage of ATP or similar cofactor |
| 7 | Translocases | Movement of ions or molecules across membranes from site 1 to site 2 |

2. Catalytic power and specificity

-catalytic power is illustrated by the ability of enzymes to catalyze reactions under conditions of 37°C, pH 7.2, 1 atmosphere



- Haber process for synthesis of ammonia
 - Important chemical synthetic process
 - explosives, fibers & plastics, pharmaceuticals, fertilizers, pulp & paper, metallurgy



Industry
800K
900 atm
 Fe, H^+

Nitrogenase
300K
1atm
 Fe, Mo, neutral

Broader Impact of Understanding the Nitrogen Fixation

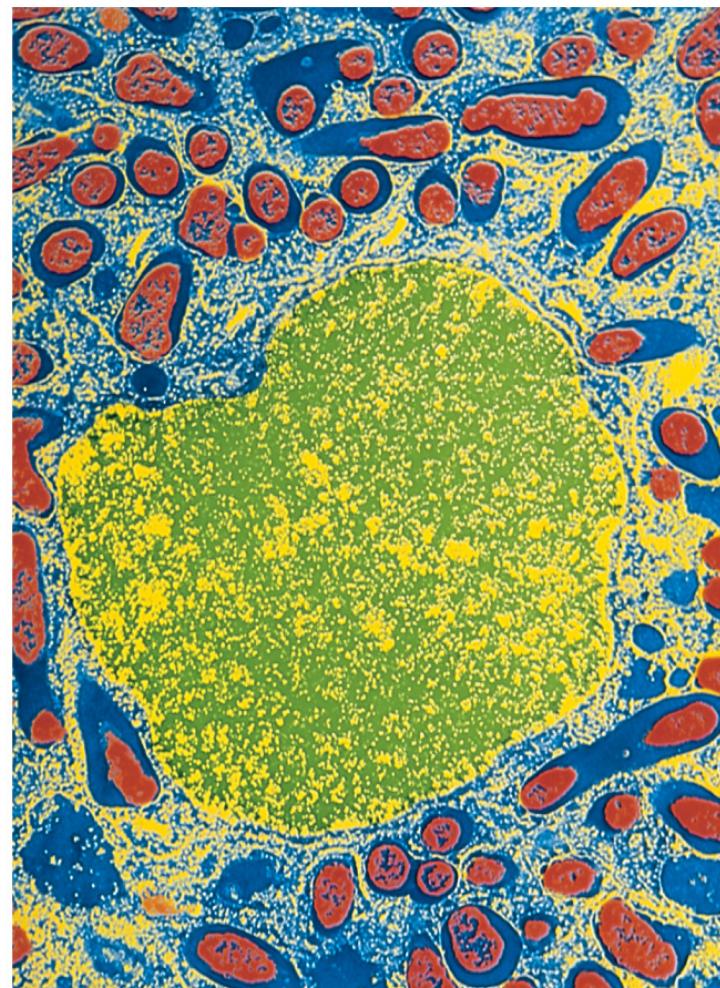
- Industrial synthesis of NH_3 via the Haber process is one of mankind's most significant chemical processes.
 - made chemical fertilizer possible!
 - yields over 100 million tons of fertilizer annually
 - sustains life of **over one-third** of human population on Earth
 - **consumes nonrenewable energy** (1–2% of total annual energy)
- Mimicking biological nitrogen fixation (biomimetic nitrogen fixation) may yield significant energy savings, or allow use of renewable energy sources.

Nitrogen-Fixing Nodules



(a)

Jeremy Burgess/Science Source



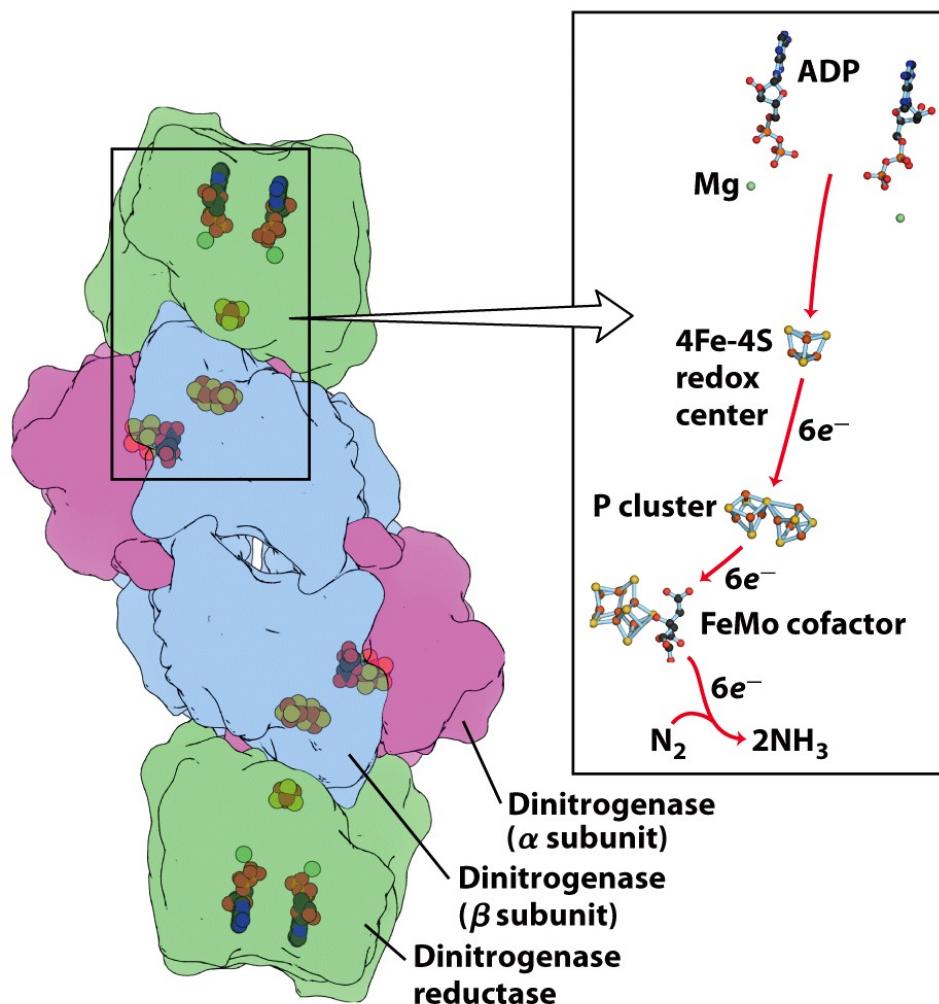
(b)

Jeremy Burgess/Science Source

Figure 22-6

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Nitrogenase Complex



- Nitrogenase complex consists of 2 identical dinitrogenase reductase molecules
 - Each has a $4\text{Fe}-4\text{S}$ redox center and 2 binding sites for ATP
- 2 identical dinitrogenase heterodimers (2α and 2β subunits)
- each dinitrogenase has two Fe-containing cofactors
 - a P-cluster (Fe-S center)
 - an FeMo cofactor
- ADP is bound at the ATP site

Figure 22-3a
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Nitrogenase: electron transfer cofactors

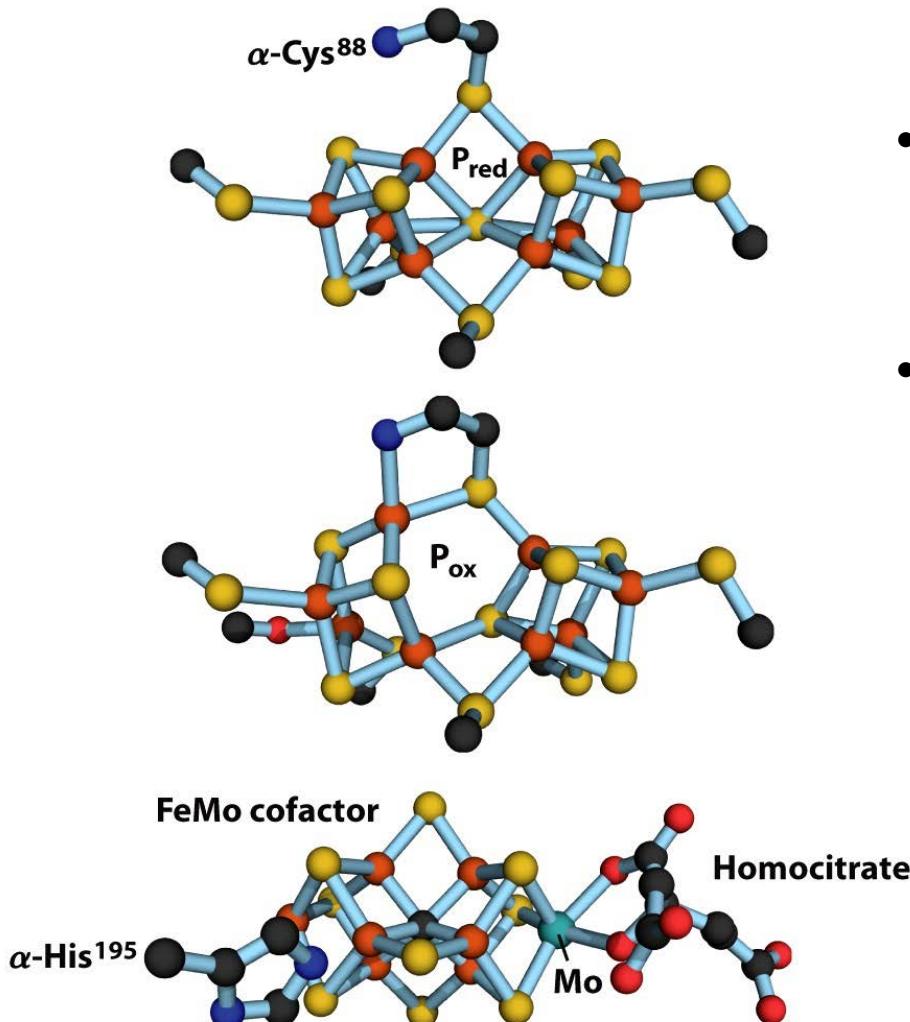
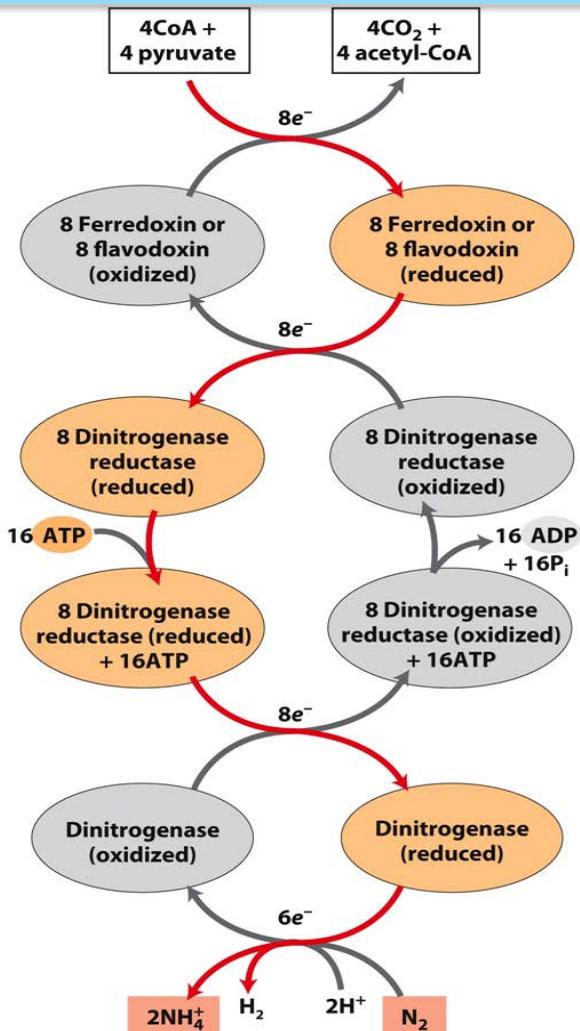


Figure 22-3b
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- P-cluster consists of a pair of 4Fe-4S centers that share a S atom making a 8Fe-7S center
- FeMo center consists of 7 Fe and 9 inorganic S atoms, Cys residue and a single C atom in the center of the FeS cluster
- A Mo atom with ligands to 3 inorganic S atoms, a His residue and 2 O atoms from homocitrate

Electron Path in Nitrogen fixation by the Nitrogenase Complex



- Electrons are transferred from pyruvate to dinitrogenase via ferredoxin and dinitrogenase reductase
- 6 electrons required to fix (directly) each molecule of N_2
- Additionally, 2 electrons required to reduce 2 molecules of H^+ to H_2
- Grand Total of 8 electrons required to fix each molecule of N_2 in pathway

Figure 22-4

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Catalytic Power is illustrated by the rate-fold enhancement of enzymes

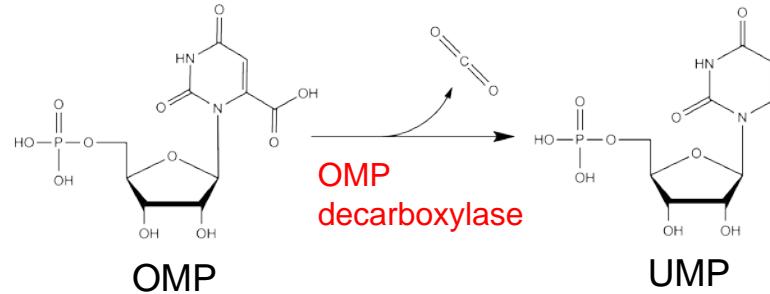
TABLE 8.1 Rate enhancement by selected enzymes

| Enzyme | Nonenzymatic half-life | Uncatalyzed rate ($k_{un} \text{ s}^{-1}$) | Catalyzed rate ($k_{cat} \text{ s}^{-1}$) | Rate enhancement (k_{cat}/k_{un}) |
|----------------------------|------------------------|--|---|---------------------------------------|
| OMP decarboxylase | 78,000,000 years | 2.8×10^{-16} | 39 | 1.4×10^{17} |
| Staphylococcal nuclease | 130,000 years | 1.7×10^{-13} | 95 | 5.6×10^{14} |
| AMP nucleosidase | 69,000 years | 1.0×10^{-11} | 60 | 6.0×10^{12} |
| Carboxypeptidase A | 7.3 years | 3.0×10^{-9} | 578 | 1.9×10^{11} |
| Ketosteroid isomerase | 7 weeks | 1.7×10^{-7} | 66,000 | 3.9×10^{11} |
| Triose phosphate isomerase | 1.9 days | 4.3×10^{-6} | 4,300 | 1.0×10^9 |
| Chorismate mutase | 7.4 hours | 2.6×10^{-5} | 50 | 1.9×10^6 |
| Carbonic anhydrase | 5 seconds | 1.3×10^{-1} | 1×10^6 | 7.7×10^6 |

Abbreviations: OMP, orotidine monophosphate; AMP, adenosine monophosphate.

Source: After A. Radzicka and R. Wofenden. *Science* 267 (1995):90–93.

S5:Table 8-1

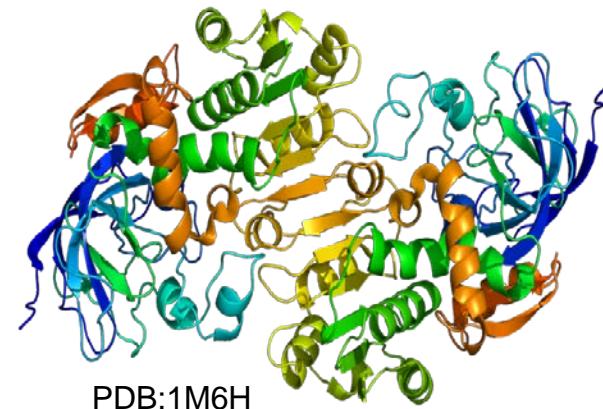


- cells and tissues of living organisms have to respond quickly to cellular demands
- Such activities as growth, maintenance and repair, and extracting energy from food have to be carried out efficiently and continuously
- Enzymes may accelerate reactions by factors up to 10^{17} or more

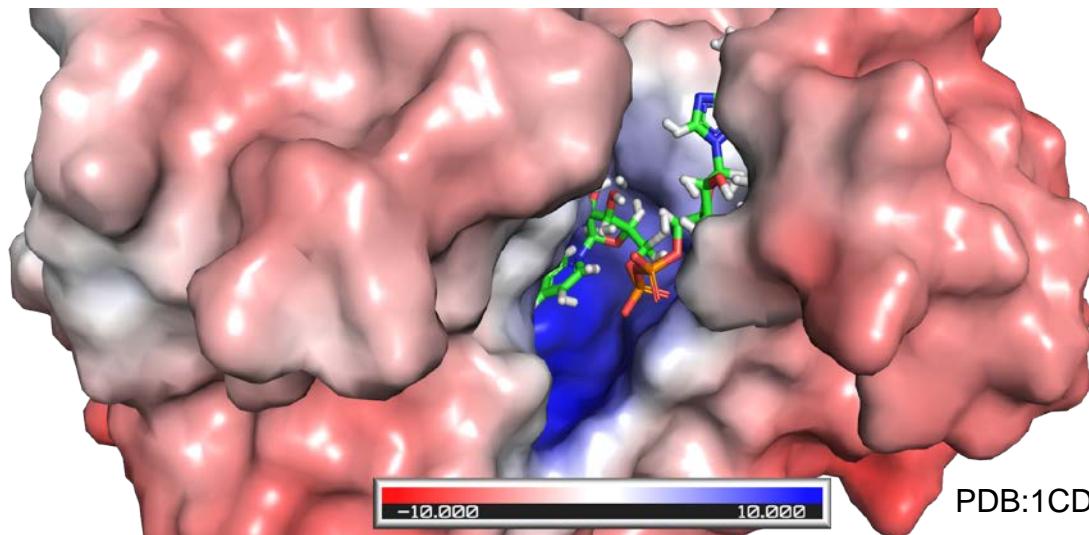
- Specificity- relates to the binding of the substrate to the enzyme

- Group specific enzymes: use a variety of substrates, each containing a certain functional group that is modified

Alcohol dehydrogenase



- Absolute specific enzymes: utilize one substrate only (or a specific pair) in one reaction

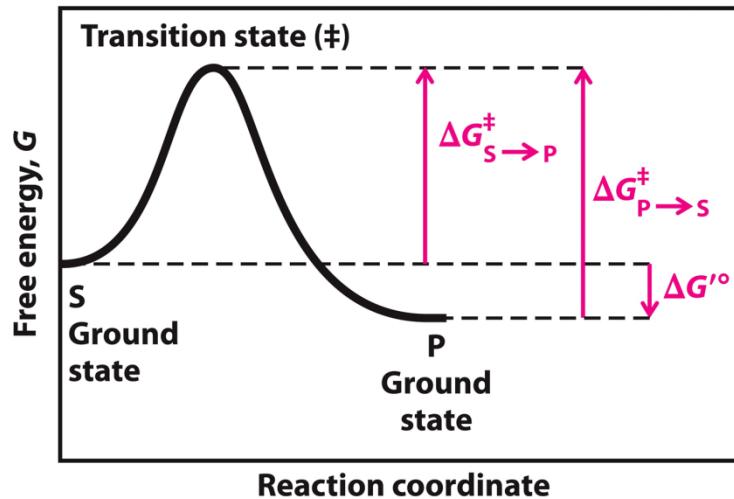


Phenylalanine dehydrogenase

Geometric and electrostatic complementarity between Phe dehydrogenase and its substrates

3. Enzymes as catalysts

- any molecule that increases the speed (or rate) of a chemical reaction without undergoing a permanent change in its structure



Absolute Rate Theory



Henry Eyring

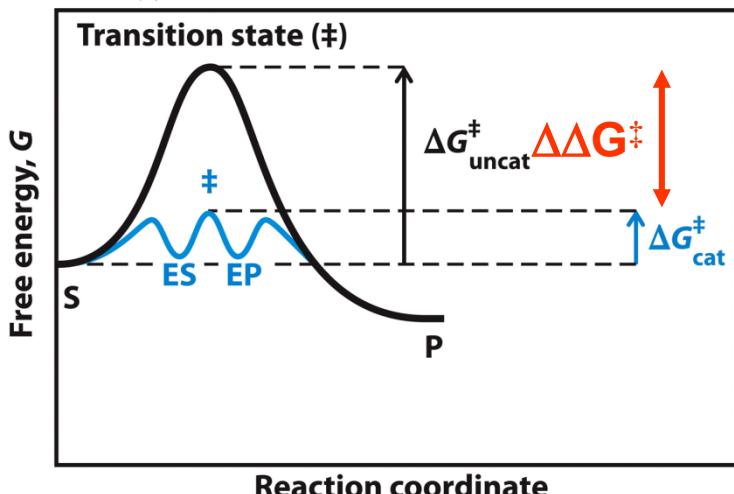
<https://www.youtube.com/watch?v=CQ1Ug4DKlrQ>

$$k = k_b T / h^* e^{(-\Delta G^\ddagger / RT)}$$

and

$$k = k_b T / h^* e^{(+\Delta \Delta G^\ddagger / RT)}$$

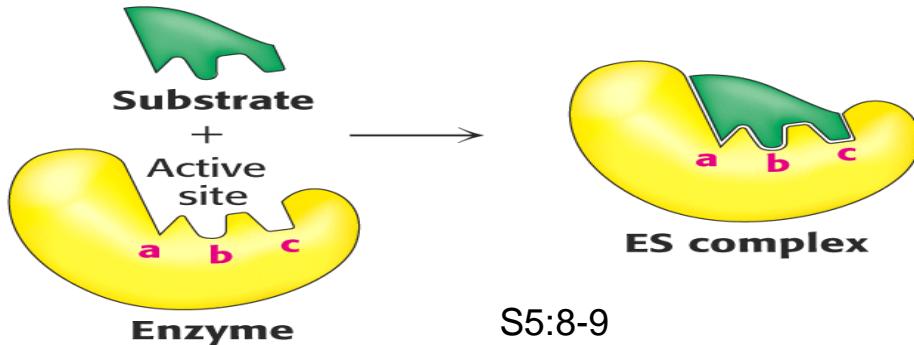
TABLE 11.1 The relationship between transition-state stabilization ($\Delta \Delta G^\ddagger$) and rate enhancement for reactions catalyzed at 37 °C.



| $\Delta \Delta G^\ddagger$ (kJ/mol) | Rate Enhancement |
|-------------------------------------|------------------|
| 24 | 10^4 |
| 36 | 10^6 |
| 47 | 10^8 |
| 59 | 10^{10} |
| 71 | 10^{12} |
| 83 | 10^{14} |
| 95 | 10^{16} |

4. Enzyme-substrate interactions

- (i) lock and key (Fischer 1894)



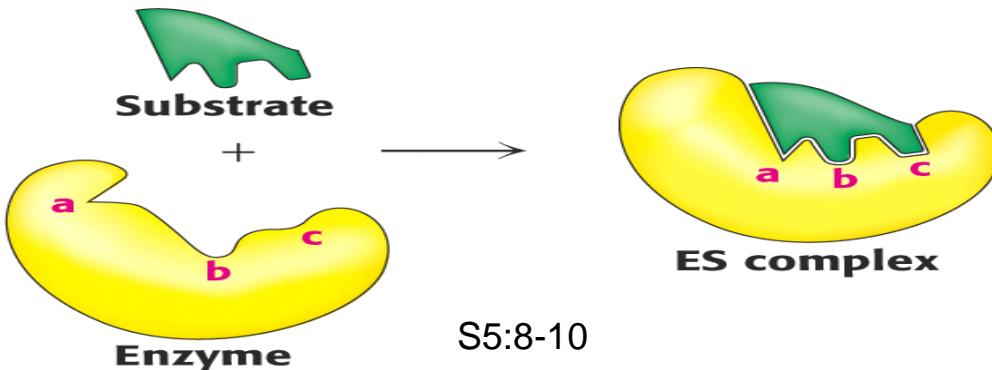
Emil Fischer
Nobel Prize in Chemistry in 1902

- Driven by
 - van der Waals contacts
 - Electrostatic interactions
 - Hydrophobic associations
 - Dipole-dipole stabilization
- Theory is correct at the first level but the situation is even more complex than Fischer imagined!

<https://www.youtube.com/watch?v=EiMBsgNZh-M>

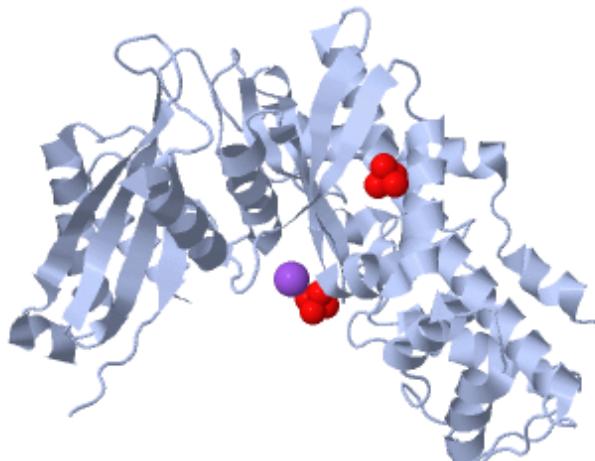
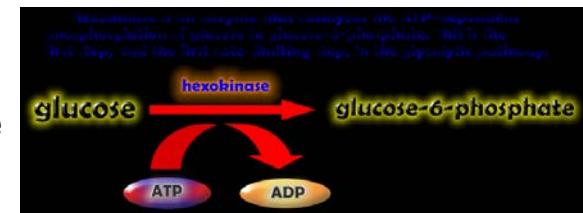
A short video (55 s) of the lock and key mechanism

- (ii) induced-fit (Koshland 1958)

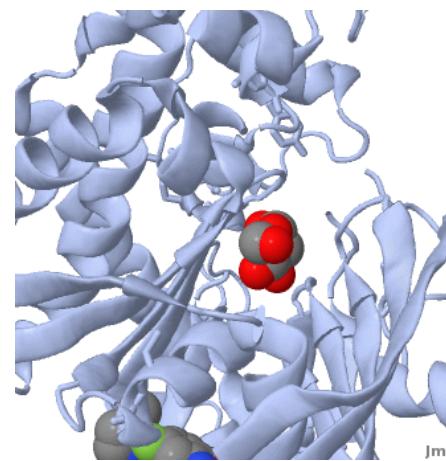


Daniel Koshland
U of California, Berkley

- The binding of a substrate or effector to the enzyme induces a conformational change in the enzyme



Human glucokinase
PDB: 1v4t



Jmol

•(iii) transition-state stabilization

https://www.youtube.com/watch?v=k5dGwr_LlzQ

- Enzymes work to force the substrates into a structural conformation that resembles the transition state

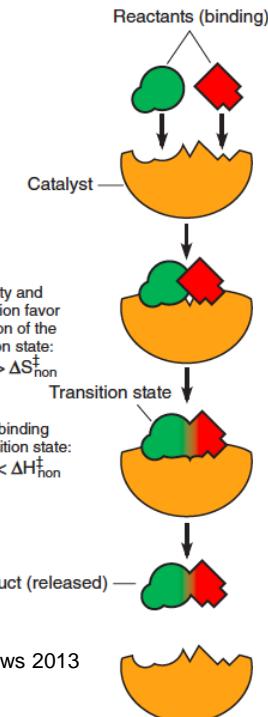
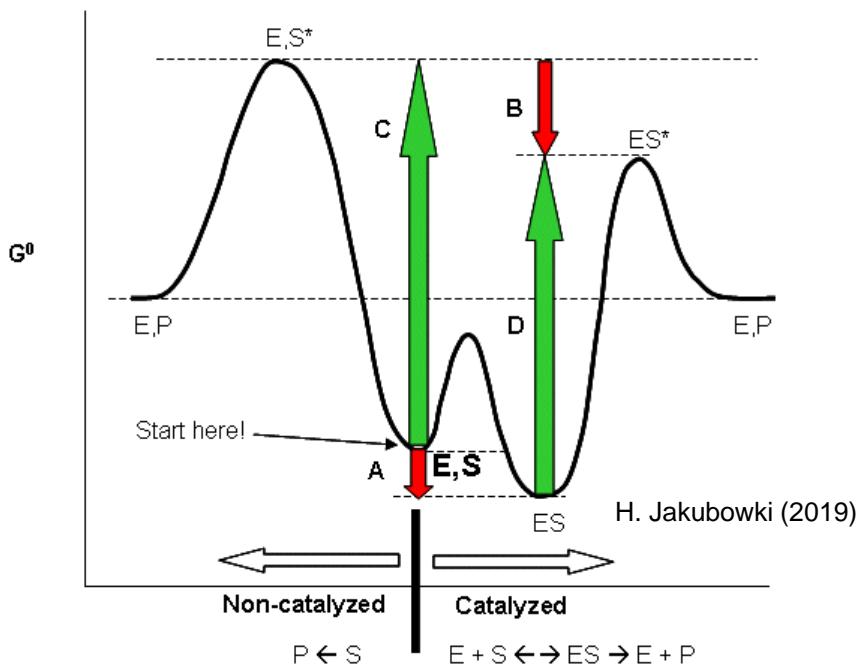


Fig. 11-16 Biochemistry Matthews 2013

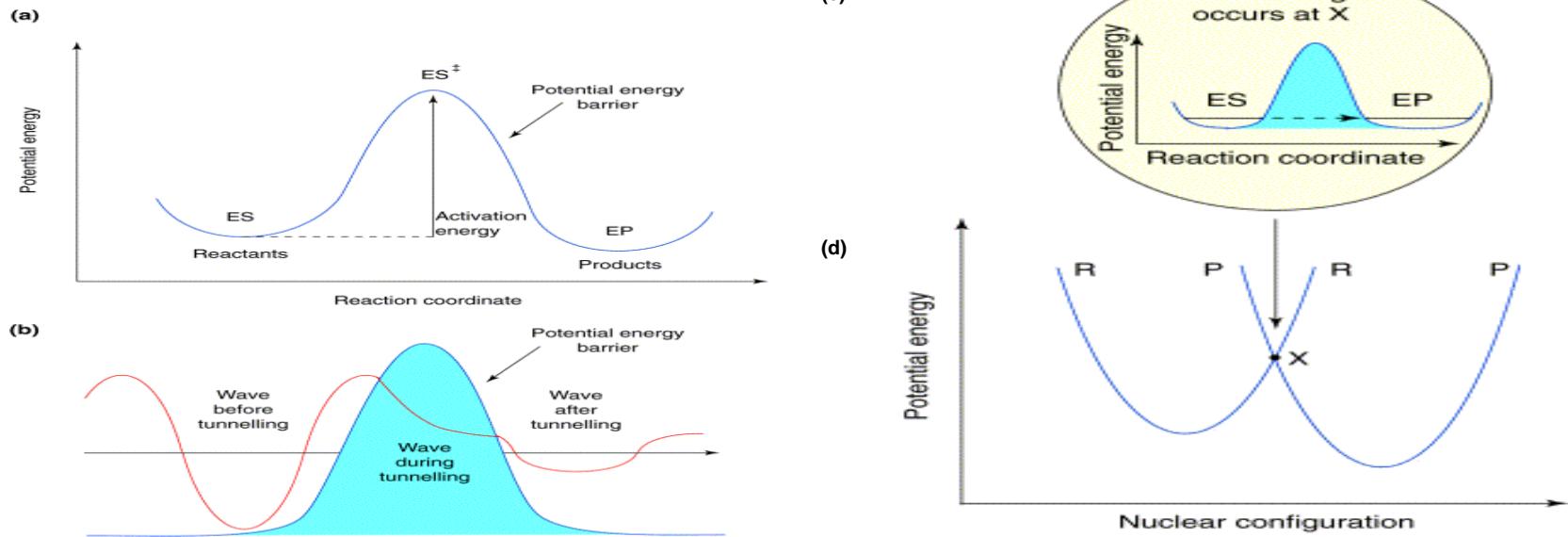
- Linus Pauling postulated that "the only thing that a catalyst must do is to bind the transition state more tightly than the substrate."



Linus Pauling

Nobel Prize (1954) in Chemistry ... for chemical bond theory...

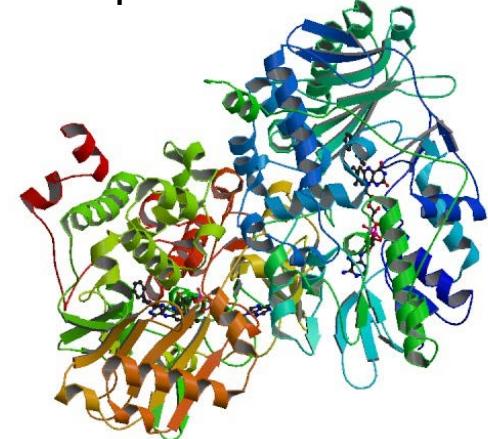
- (iv) quantum tunnelling



Sutcliffe & Scrutton, TIBS (2000) 25, 405-408.

TIBS

- If the reaction coordinate is treated like a wave-function, then the thinner the energy barrier, the greater is the probability that the wave can “quantum tunnel” or “short-circuit” the barrier
 - The tunnelling does not occur until the geometry of the protein (and product) is distorted (at X on (d) above)
 - Thermally-induced conformational change in the protein is a prerequisite for the tunnelling reaction
- Monoamine oxidase
- $k_{\text{cat}}/k_{\text{uncat}} = 10^{22}$ fold



Human monoamine oxidase PDB:1OJ9

5. Enzymes as proteins

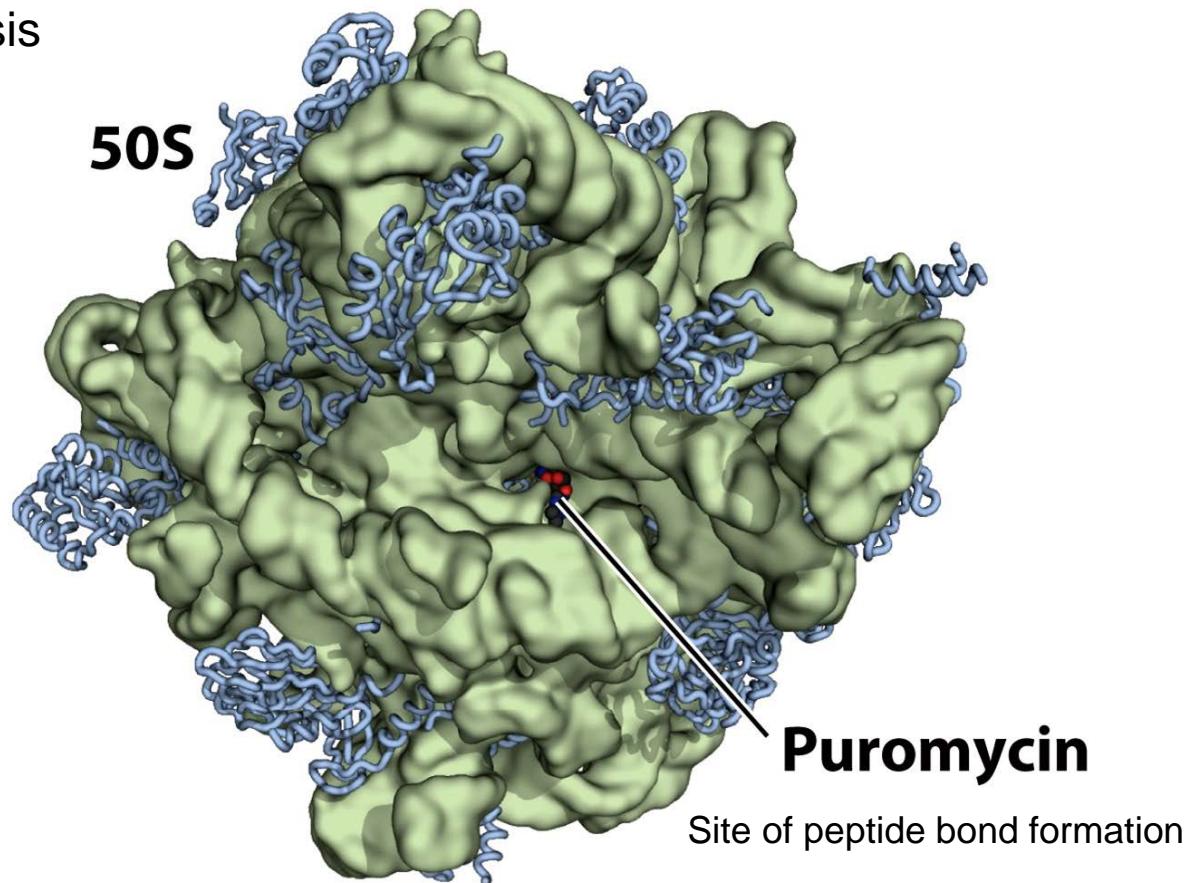
- Not all enzymes are proteins
 - Ribozymes (cleave RNA to carry out RNA splicing)
 - Ribosome is a ribozyme that catalyzes the formation of the peptide bond during protein synthesis



Thomas A. Steitz

Nobel Prize Chemistry 2009

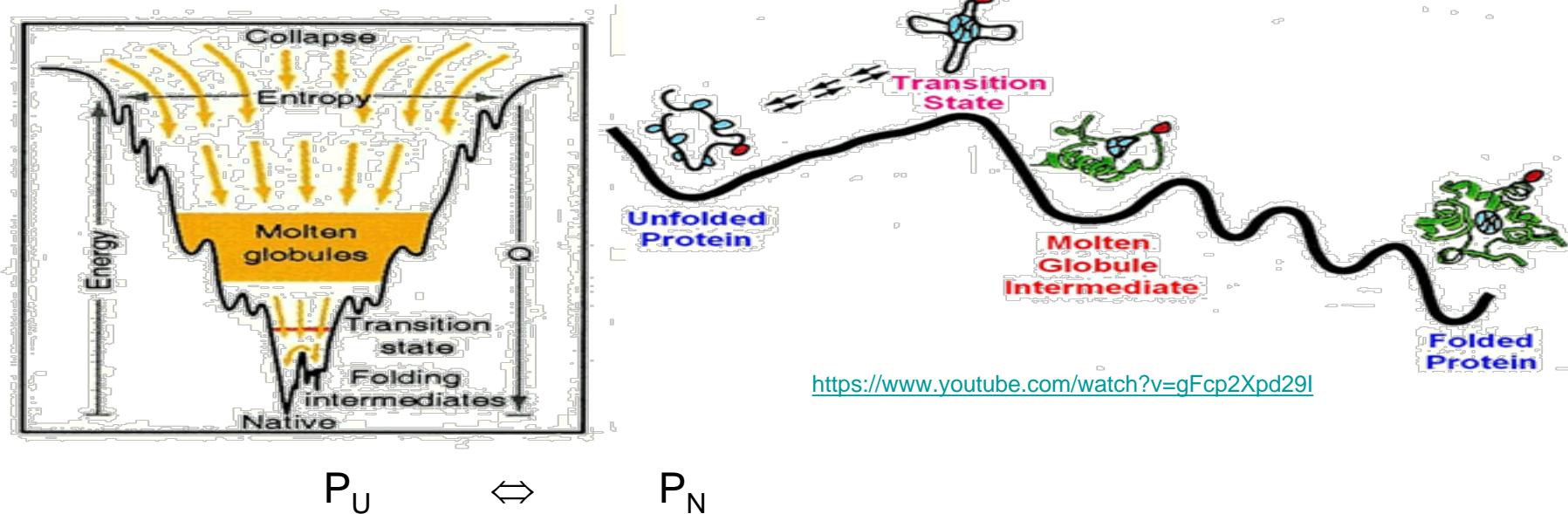
Structure of 50S bacterial ribosome



Box 27-2 Figure 1
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5'-AUUACAGG-3'

- proteins are composed of amino acids linked by peptide bonds
- important feature is 3-dimensional organization (2° , 3° , 4° structures)
- destroyed by heat/denaturants (guanidinium HCl, urea, organic solvents, detergents)
- mechanical disruption
- keep at low temperature (ice)
 - proteolysis
 - thermal denaturation



$$K_N = [P_N]/[P_U]$$

$$\Delta G_N = -RT\ln K_N$$

ΔG_N for most proteins range from -20 to -100 kJ/mol

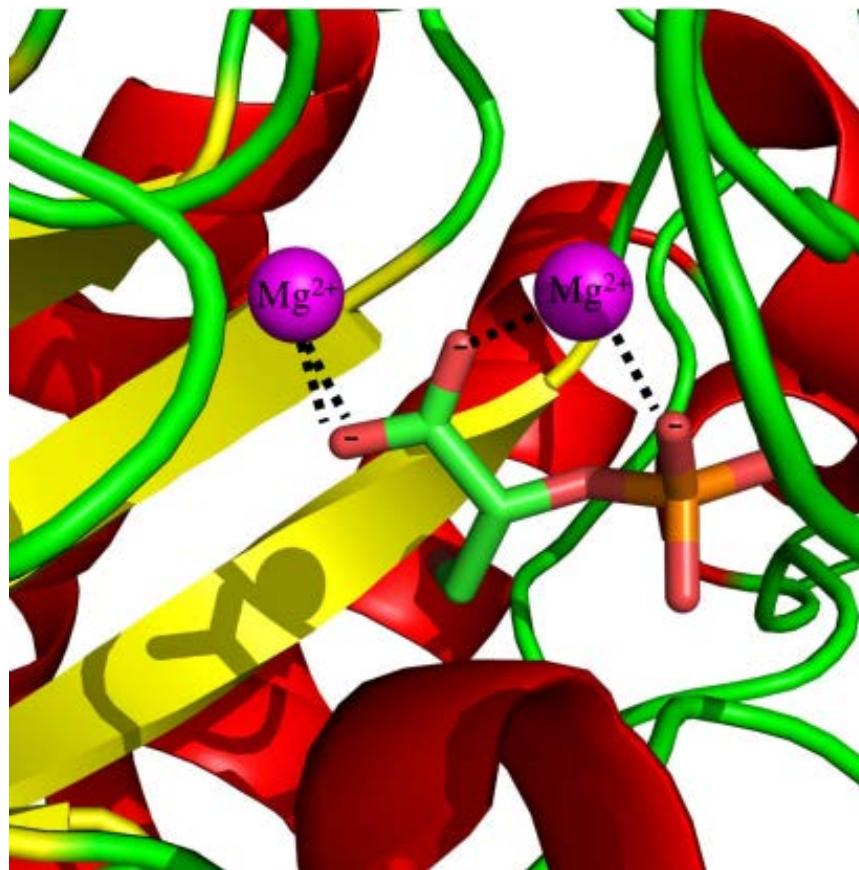


Enzyme cofactors

- **Perform certain tasks better than amino acids:**
 - Binding or **transfer of a chemical group** in the substrate
 - **Stabilizing** charged intermediates electrostatically
 - Transfer of electrons or protons to/from the substrate
 - Polarization of a chemical bond in the substrate or enzyme
 - **Substrate transport** between different enzyme parts
 - Channeling light energy to the substrate (activation)

Enzyme cofactors

- **Include two main types:**
 - Cationic metals (e.g. Mg^{2+} , Cu^{2+} , Zn^{2+} , Mn^{2+} , Co^{2+} , Fe^{2+})



Enzyme cofactors

- **Perform certain tasks better than amino acids**
 - Organic cofactors that **transfer chemical groups** between substrates or substrate and enzyme
- Lipoic acid [257] and CoA [258] – carry acyl groups.
- TPP – transfers aldehydes [98].
- Coenzyme B₁₂ (CoB₁₂) [259] and SAM [260] – transfer methyl (–CH₃) groups.
- Tetrahydrofolate (THF) – transfers single-carbon groups^{*1}.
- Biotin – transfers carboxyl (–CO₂) groups [253,254]

Kinetic Concepts-Appendix Material

(1) Kinetic Concepts

Reaction Rates

- Chemical kinetics deals with the rates of chemical reactions
- Law of Mass action "the rate of a chemical reaction is proportional to the active masses of the reacting substances"
- Thus the forward rate of EQ1: $A \rightleftharpoons B \propto [A]$
- For more complex rxns EQ2: $2A + B \rightleftharpoons 3C$ "The rate of a chemical rxn is proportional to the molar concentrations of the reacting substances, with each conc. round to the power equal to the stoichiometric coefficient of that substance in the balanced chemical equation"
 - Thus EQ:2 rate $\propto [A]^2 [B]$
- Kinetics addresses the following questions
 - (a) What is the rate (velocity) of the rxn
 - (b) How can the rate of the rxn be altered?
 - (c) What is the path by which the rxn proceeds?

Reaction Velocity

- speed with which a chemical reaction proceeds
- rate can be described by the appearance of product or the disappearance of a reactant

EQ:1 $A \rightleftharpoons B$

$$\text{Reaction rate } V = \frac{-d[A]}{dt} = \frac{d[B]}{dt}$$

- the derivative $d[]/dt$ refers to change in concn over time
- the derivative is the slope of a plot of concn versus time
 - may be straight line on a curve

Straight line

- slope is fixed and can be determined from any set of 2 points (see Fig., above)

Curved line

- slope at any point is given by the slope of the tangent to the curve at that point
 - slope is given by $dy/dx = \text{value of } \Delta y/\Delta x \text{ as } \Delta x \text{ approaches zero}$
 - slope of the tangent at the origin ($t = 0$) is the initial velocity
- velocity always has a positive value (initial - final, or final - initial)

- For a more complex rxn, the stoichiometric coefficients of the reactant and products must be considered

e.g. $2A + B \rightleftharpoons 3C$

$$v = \frac{-1}{2} \frac{d[A]}{dt} = \frac{-d[B]}{dt} = \frac{1}{3} \frac{d[C]}{dt}$$

Rate Constants

$$v = k[A] \quad \text{rate law } A \rightleftharpoons B$$

v = rate constant (rate coefficient, specific reaction rate)

- expression of the velocity as a function of reactant concentrations is known as the rate equation (rate law)

Order of reactions

- concn terms in the rate equation may be more complex and may be raised to specific powers. These powers can't be inferred from the stoichiometry of the rxn but rather have to be determined experimentally
- the exponent (powers) of the concn terms in a rate equation define the order of the rxn
 - usually range 0-3 (exponents) but may be fractional values
 - more than 1 substance in a rxn has a nonzero order called "mixed order rxn"
 - rxn that is independent of reactant concn is a "zero order" rxn
 - units of the rate constant are such that the units on the right side of the rate equation must always be identical to those on the left side

(1) Zero Order Rxns



Assume that: $\frac{-d[A]}{dt} = k$

rewritten: $d[A] = -kdt$

integrate: $[A] = -kt + C$ (C = integration constant)

evaluate integration constant: t = 0 then [A] = [A]_o and C = A_o so:

$$[A] = -kt + [A]_o$$

A plot of [A] as function of t will yield a straight line, slope = -k

(2) First order reactions

- follow an exponential time course

Assume rxn can be described:

$$\frac{-d[A]}{dt} = k[A]$$

rewritten as: $\frac{-d[A]}{[A]} = kdt$

integrate: $\ln[A] = -kt + C$ C = integration constant

evaluate C: $t = 0$ then $[A] = [A]_o$ then $C = \ln[A]_o$
 therefore: $\ln[A] = -kt + \ln[A]_o$
 or $A = [A]_o e^{-kt}$

Thus, a plot of $\ln[A]$ versus time gives slope = $-k$ and intercept of $\ln[A]_o$
 Alternatively, a plot of $[A]$ versus time will yield an exponential curve

Half Life ($t_{1/2}$)

- defined as the time required for the concn of a reactant to decrease to one half of its initial value
- so when $t = t_{1/2}$ then $[A] = \frac{1}{2}[A]_o$

$$\text{becomes } \ln \frac{[A]_o}{2} = -kt_{1/2} + \ln[A]_o$$

$$\ln 0.5 [A]_o - \ln[A]_o = -kt_{1/2}$$

$$\ln \left(\frac{0.5[A]_o}{[A]_o} \right) = -kt_{1/2}$$

$$-0.693 = -kt_{1/2}$$

$$t_{1/2} = \frac{0.693}{k}$$

2nd Order Rxn

Consider $A + A \rightleftharpoons B$

$$-\frac{1}{2} \frac{d[A]}{dt} = k[A]^2$$

$$\text{rearrange: } \frac{-d[A]}{[A]^2} = 2 k dt$$

$$\text{integrate: } \frac{1}{[A]} = 2 kt + C \quad C = \text{integration constant}$$

$$\text{evaluate: } [A] = [A]_o \text{ when } t = 0 \text{ so } C = \frac{1}{[A]_o}$$

$$\text{thus: } \frac{1}{[A]} = 2 kt + \frac{1}{[A]_o}$$

A plot of $\frac{1}{[A]}$ versus t gives a slope of $2k$, intercept = $\frac{1}{[A]_o}$

Determining Rxn order

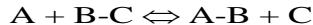
- (1) If a plot of $[A]$ versus t is linear then zero order rxn
- (2) If a plot of $\ln[A]$ versus t is linear then 1st order rxn
- (3) If a plot of $\frac{1}{[A]}$ versus t is linear then 2nd order rxn

(i) Transition State Theory (TS Theory)

-goal of kinetic theory is to describe the reaction rates in terms of physical properties of reacting molecules

-theory developed by Henry Eyring in 1930 known as "Transition State Theory" or "Absolute Rate Theory"

-consider a bimolecular reaction involving 3 atoms (A,B,C)

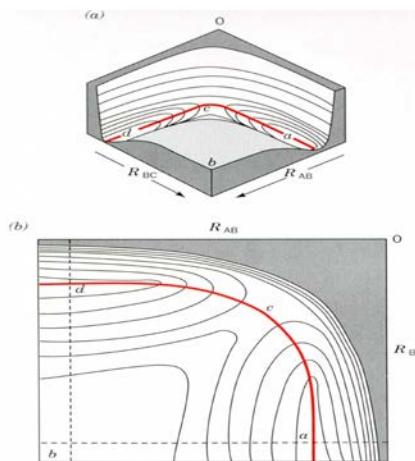


-atom A must approach the diatomic molecule B-C so that a high energy (unstable) complex A...B...C exists in which A-B covalent bond is in process of forming while the B-C bond is breaking

-consider the reaction of an H atom with H₂:



-potential energy of this triatomic system as a function of the relative positions of its component atoms is plotted in Fig (a) and (b) below (V&V_F13-3).



-shape is of 2 long valleys parallel to the coordinate axes with sheer walls rising towards the axes and steep ones rising towards a plateau where both coordinates are large (region of point b)

-2 valleys are joined by a pass or saddle near the origin of the diagram (point c)

-minimum energy configuration is an H₂ molecule and an isolated atom, i.e., with one coordinate large and the other at the H₂ covalent bond distance [near points a (the reactants and d (the products)]

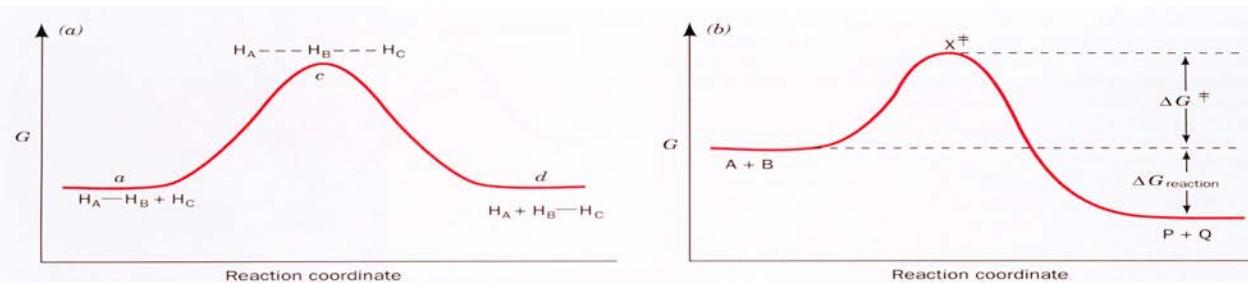
-during collision, reactants generally approach one another with little deviation from minimum energy rxn pathway (line a-c-d) because other trajectories would require much greater energy

-as the atom and molecule come together they increasingly repel one another (have increasing potential energy) and therefore usually fly apart

-if the system has sufficient kinetic energy to continue to coalesce, it will cause the covalent bond of the H₂ molecule to weaken until ultimately, if the system reaches the saddle point (point c) there is an equal probability that either reaction will occur or that the system will

decompose back to its reactants

- at this "saddle point" the system is at its "transition state" and hence to be an "activated complex"
- since the concentration of activated complex is small, the decomposition of the activated complex is postulated to be the rate-determining process of the reaction
- minimum energy pathway of a reaction is known as the "reaction coordinate"
- Fig (a) (V&V_F13-4) called a "Transition State Diagram" and shows the potential energy of the $\text{H} + \text{H}_2$ system along the reaction coordinate (line a-c-d in Fig. above)



- transition state is point of highest energy in the reaction coordinate
- if atoms in the triatomic system are of different types (Fig (b)) the transition state diagram is no longer symmetrical because there is an energy difference between reactants and products

Thermodynamics of the Transition State

Consider a bimolecular reaction:



where k is the ordinary rate constant and k' is the rate constant for the decomposition of X^\ddagger to products

-activated complex occurs at an energy maximum and is only metastable

-TS theory assumes that X^\ddagger is in rapid equilibrium with the reactant

$$[3] K^\ddagger = [X^\ddagger]/[A][B] \text{ where } K^\ddagger \text{ is an equilibrium constant}$$

This central assumption of TS theory permits a thermodynamic description of reaction mechanism and rates

- if K^\ddagger is an equilibrium constant then:

$$[4] \Delta G^\ddagger = -RT\ln K^\ddagger$$

-where ΔG^\ddagger is Gibbs Free Energy of the activated complex less that of the reactants

-T is absolute temperature

-R is the gas constant = $8.3145 \text{ J}\cdot\text{K}^{-1}\text{mole}^{-1}$

-combining equation [2], [3], and [4]:

$$[2] d[P]/dt = k'[X^\ddagger] \text{ substitute } [X^\ddagger] = K^\ddagger[A][B] \text{ [3]}$$

$$d[P]/dt = k'K^\ddagger[A][B] \text{ substitute } K^\ddagger + e^{-\Delta G^\ddagger/RT}[A][B]$$

-equation indicates that the rate of reaction depends not only on the concentration of reactants but also **decreases exponentially** with ΔG^\ddagger .

Thus, the **larger** the difference between the free energy of the transition state and that of the reactants (less stable is the TS) the **slower** the reaction

Evaluate k' :

- k' is the rate of passage of the activated complex over the maximum in the TS diagram (activation barrier of reaction)

Assumption: activated complex is held together by a bond that is associated with the reaction coordinate and assumed to be so weak that it flies apart during the first vibrational excursion

[6] $k' = \kappa v$ -where v is the vibrational freq of the bond that breaks as activated complex decomposes to products

- κ is the transmission coefficient, probability that the breakdown of the activated complex, X^\ddagger , will be in the direction of product formation rather than back to reactants

-for most spontaneous reactions in solution, κ is between 0.5 and 1.0

$$[7] v = \epsilon/h \text{ Planck's Law}$$

ϵ is the average energy of the vibration that leads to the decomposition of X^\ddagger

h is Planck's constant ($6.6261 \times 10^{-34} \text{ J}\cdot\text{s}$)

-statistical mechanics indicates that at temperature, T, the classical energy of an oscillator is: [8]
 $\epsilon = k_B T$ where k_B is Boltzmann constant ($1.3807 \times 10^{-23} \text{ J}\cdot\text{K}^{-1}$)

-combining equation [6] - [8]

[7] $v = \epsilon/h$ and $v = k_B T/h$ (substitute [8] into [7])

-substitute [7] into [6]:

$$[9] k' = \kappa k_B T/h$$

-assume that $\kappa = 1$ (for most reactions)

Now combine [2] and [5]

[2] $d[P]/dt = k[A][B]$ and [5] $d[P]/dt = k'e^{-\Delta G^\ddagger/RT}[A][B]$

[10] $k[A][B] = k'e^{-\Delta G^\ddagger/RT}[A][B]$ substitute [9] into [10]

$$[11] k = (k_B T/h)e^{-\Delta G^\ddagger/RT}$$

-indicates that the rate of reaction **decreases** as its free energy of activation, ΔG^\ddagger **increases** -as T increases so does k

-enzymes are usually proteins and so they are sensitive to high temperature and become denatured so there is a limit on T

-TS theory is an ideal system: real systems are much more complicated but quantitatively similar