Welcome to BCII lesson 1

Chimie Biologique II

Biological Chemistry II

BIO-213

Teacher

Giovanni D’Angelo, IBI

About me…

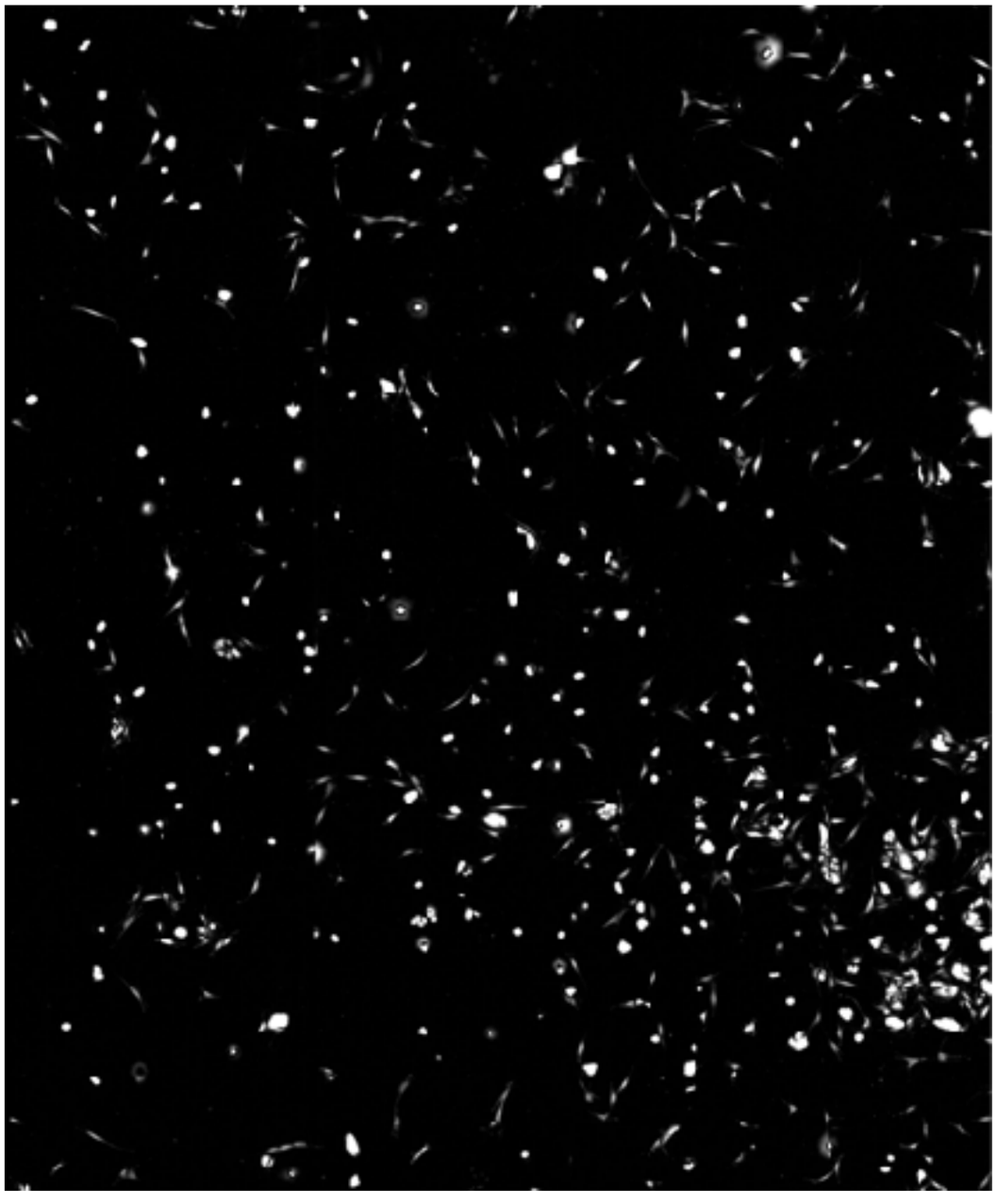
1998-2003 M.S. in Medical Biotechnology, University of Naples, School of Medicine, Italy

2004- 2008 PhD in Life Sciences, Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy

2009-2012 Postdoctoral fellow. Telethon Institute for Genetics and Medicine, Naples, Italy.

2013-2018 Researcher National Research Council of Italy, Naples, Italy

2018- Assistant Professor Institute of Bioengineering and Global Health Institute EPFL



Main Interests:

Lipid Metabolism , Membrane Cell Biology

General Overview

•Lecture material (slides, extended slides, exercises etc.) on Moodle

•Slides and exercises will be posted as PDFs

•Organization of exercises: 6 TAs

•Exam: 3h written exam, open book exam, questions will be provided in both English and French and you may answer in either language.

•Textbooks:

–Leningher - ‘Principles of Biochemistry ’

Vision and Rules

Vision for the course:

–Build a general understanding of cell metabolism
–Know the major metabolic pathways of the cell
–Know the tools and methods used to study cell metabolism

•Ask questions
•Take notes
•Read the books
•Attend lectures regularly
•Attend exercises regularly
•On March: Feedback questionnaire (too much, too fast/slow, boring/ exciting)

Teaching Model

Participative

Participation in the classroom
Questions
~~Emails~~
Moodle Forum

It pays back

Exercise Session Guidelines

- Please try/do the exercises ahead of time
- Pair up with colleagues and discuss
- Discuss with the TAs
- Constant participation will allow you to do better at the exam

Content of the course

The biochemical processes that occur within a living organism to:

1.convert **nutrients** into **energy** to be used in biological processes

2.**build up** the molecules that serve as **constituents** of cells

Metabolism

Structure of the course

13 lectures 4 chapters

1. Principles of Enzyme functioning - (lectures 1-2)

2. Energy Metabolism - (lectures 3-5)

3. Biosynthesis (lectures 5-9)

4. Visualising Metabolism (lectures 10-12)

5. Q/A (lecture 13)

Lecture1

General Principles of Bioenergetics

Enzymes

ENZYMES

What is an enzyme?

Enzymes are macromolecular biological catalysts that **accelerate** chemical reactions.

Enzymes possess extraordinary catalytic power and a high degree of specificity for their substrates, accelerating chemical reactions tremendously (10^6 to 10^15 folds). They operate in aqueous solutions under very mild conditions of temperature and pH, unlike many catalysts used in organic chemistry.

Enzymes catalyse the hundreds of stepwise reactions of metabolism, conserve and transform chemical energy, and synthesize biological macromolecules from simple precursors. Approximately 50% of drugs act by binding to enzymes..

ENZYMES ARE PROTEINS+COFACTORS

With the exception of catalytically active RNA molecules, all enzymes are composed of **proteins**.

Some enzymes require an additional chemical component called a **cofactor**, which can be an inorganic ion.

TABLE 6–1 Some Inorganic Ions That Serve as Cofactors for Enzymes

Ions	Enzymes
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

ENZYMES ARE PROTEINS+COFACTORS

Or complex organic or metalloorganic molecules known as **coenzymes**.

TABLE 6–2 Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

Note: The structures and modes of action of these coenzymes are described in Part II.

Vitamins are precursors of **coenzymes**

ENZYMES CLASSIFICATION

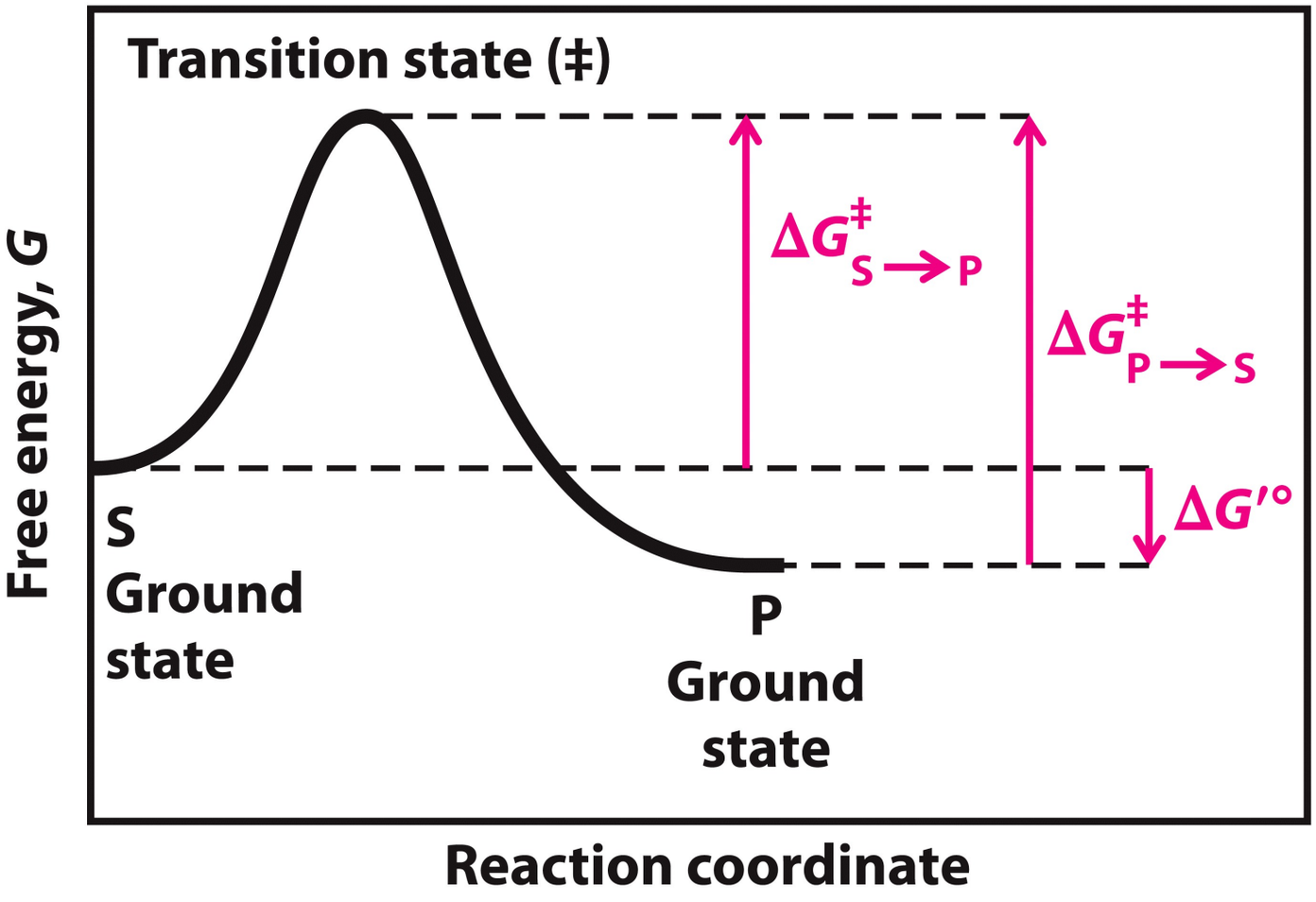
Enzymes are named by adding the suffix "**-ase**" to the name of their substrate or activity. Biochemists have adopted a system for naming and classifying enzymes based on the type of reaction they catalyze. Each enzyme is assigned a four-part classification number and a systematic name that identifies the reaction it catalyzes. For example, the enzyme commonly known as hexokinase is formally ATP:glucose phosphotransferase. Its Enzyme Commission number is 2.7.1.1, where the first number (2) denotes the class name (transferase), the second number (7) denotes the subclass (phosphotransferase), the third number (1) represents a phosphotransferase with a hydroxyl group as the acceptor, and the fourth number (1) designates D-glucose as the phosphoryl group acceptor.

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

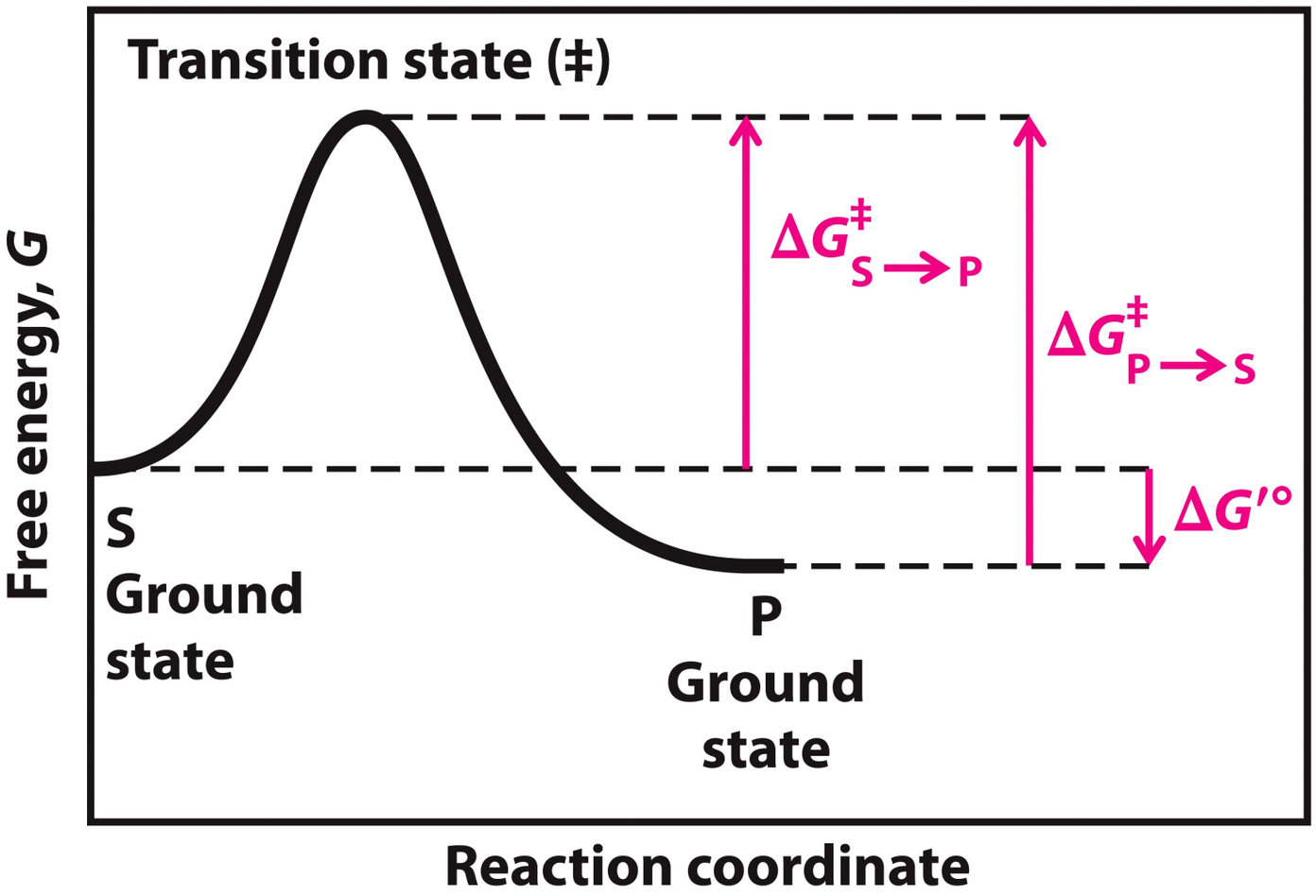
Transition State Theory (I)

Any reaction, such as S <-> P, can be described by a reaction coordinate diagram, where the free energy change is plotted as a function of the progress of the reaction. The free energy change (ΔG'0) and equilibrium position of the reaction are determined by the difference in ground state free energies of S and P. The rate of the reaction depends on the **height of the free energy barrier between S and P**, with the transition state occurring at the top of this barrier.



Transition State Theory (II)

The transition state **is not a chemical species** with any significant stability and should not be confused with a reaction intermediate. Instead, it is a fleeting molecular moment during which events such as bond breakage, bond formation, and charge development have progressed to the point where decay to either substrate or product is equally likely. The difference between the energy levels of the ground state and the transition state is the activation energy, ΔG‡. The rate of the reaction is inversely and exponentially proportional to the value of ΔG‡.

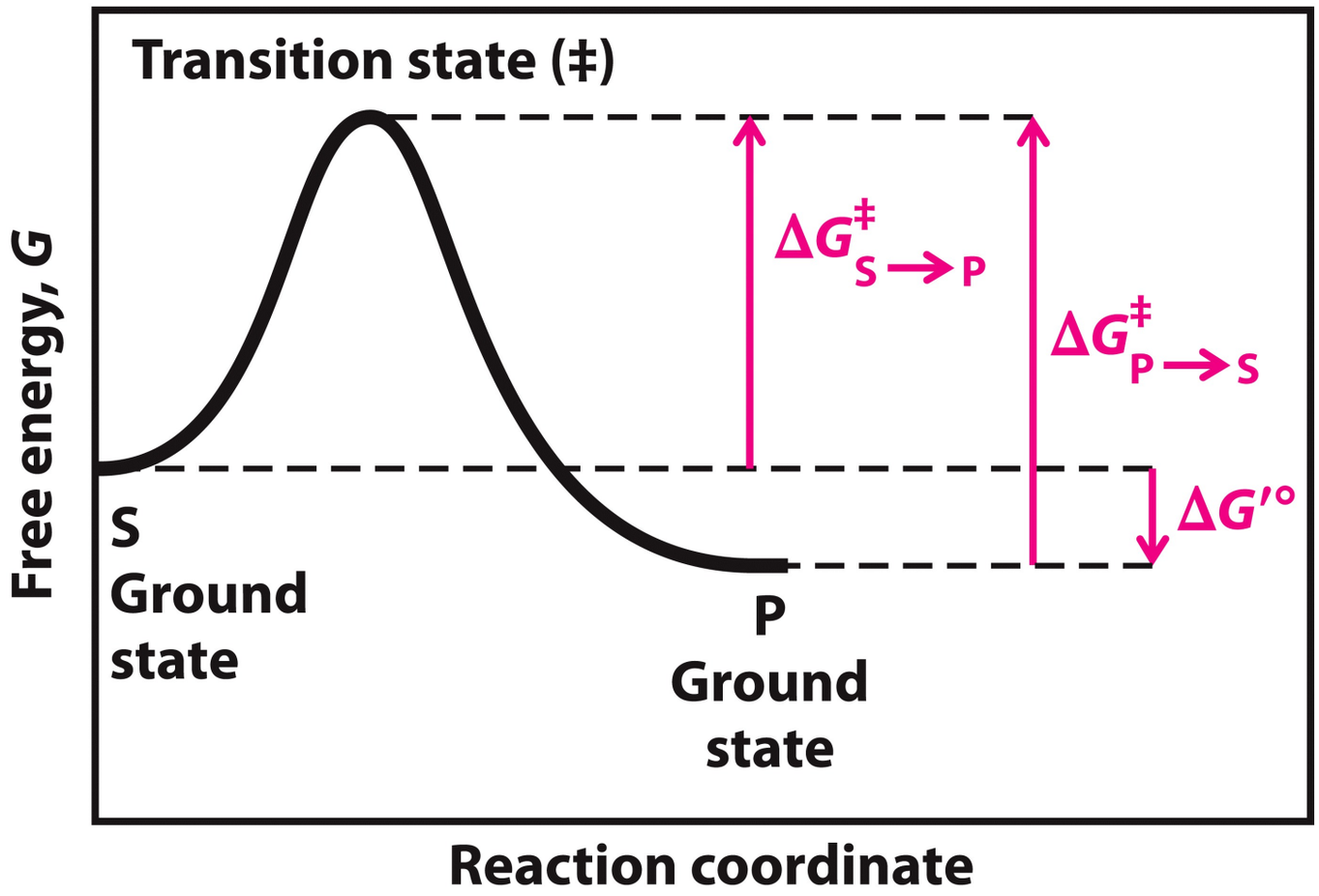


Relationship Between K'_{eq} and $\Delta G'^{0}$

The equilibrium constant (**Keq**) of a chemical reaction is the value of its reaction quotient at chemical equilibrium. In other words, Keq measures the extent to which reactants are converted to products in a reaction.

For the reaction S ⇄ P, Keq is calculated as [P]/[S] at equilibrium.

K'eq is the Keq under standard biochemical conditions (T 298 K, pH 7.0).



Relationship Between K'_{eq} and $\Delta G'^0$

The **standard free energy change** (ΔG^0) for reactions is defined under standard conditions (T = 298˚K; partial pressure of each gas = 1 atm; [] of each solute 1 M). Because biochemical systems commonly have H^+ concentrations far below 1 M, biochemists define a **biochemical standard free energy change**, **$\Delta G'^0$**, the standard free energy change at pH 7.0.

The equilibrium constant for a reaction (K'_{eq}) under standard biochemical conditions is mathematically linked to the $\Delta G'^0$

$$\mathbf{\Delta G'^0 = -RT \ln K'_{eq}.}$$

Where, **R** is the gas constant, 8.315 J/mol·K, and **T** is 298˚K (25˚C). Note that a large negative value of $\Delta G'^0$ reflects a favorable equilibrium in which the ratio of products to reactants is much greater than 1/1.

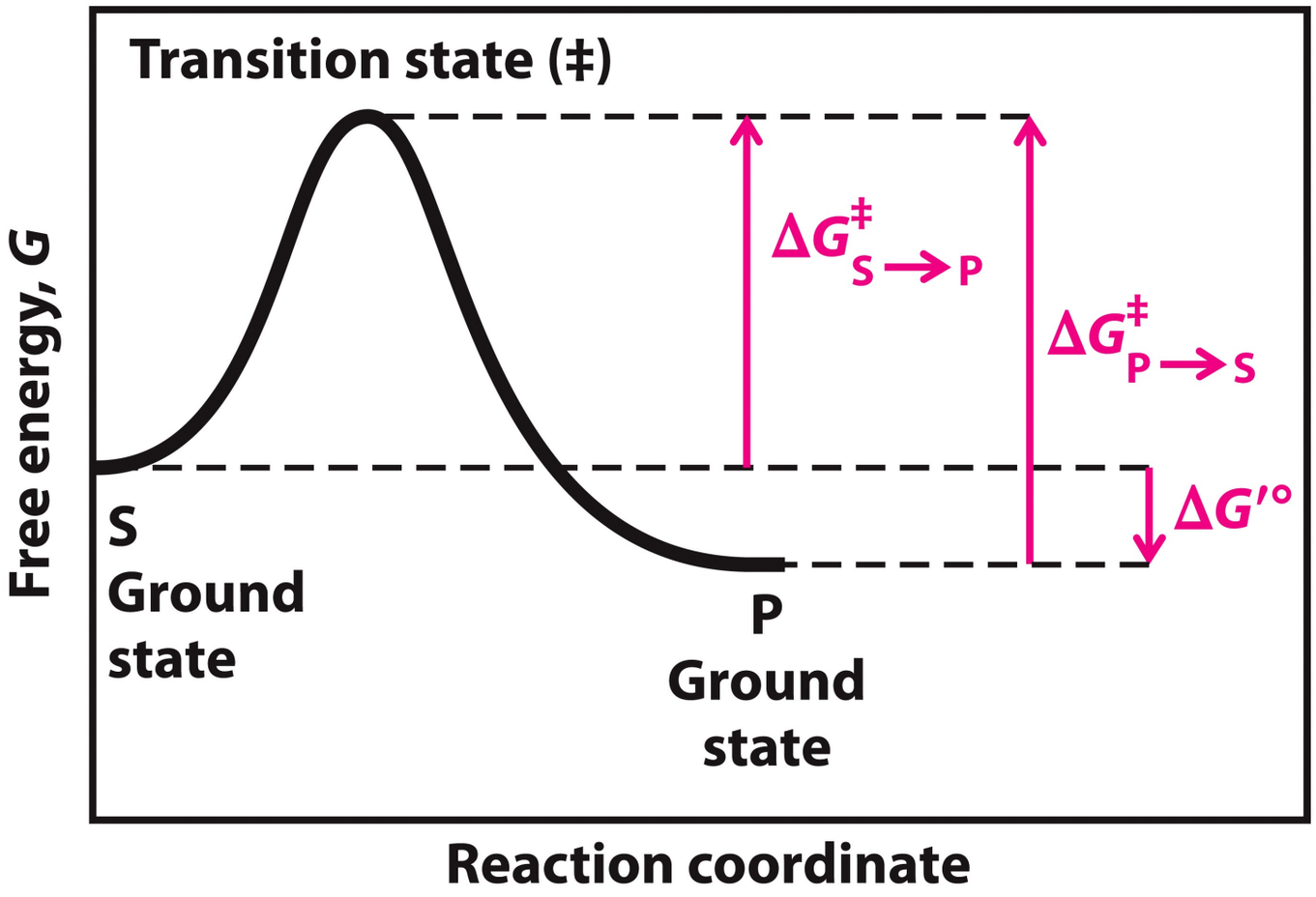
TABLE 6–4 Relationship between K'_{eq} and $\Delta G'^\circ$

K'_{eq}	$\Delta G'^\circ$ (kJ/mol)
10^{-6}	34.2
10^{-5}	28.5
10^{-4}	22.8
10^{-3}	17.1
10^{-2}	11.4
10^{-1}	5.7
1	0.0
10^{1}	−5.7
10^{2}	−11.4
10^{3}	−17.1

Note: The relationship is calculated from $\Delta G'^\circ = -RT \ln K'_{eq}$ (Eqn 6–3).

Relationship Between ΔG‡ and Rxn Rate

The rate of a chemical reaction is determined by the concentration of the reactant(s) and by a rate constant usually denoted by ***k***. For the unimolecular reaction S -> P, the rate (or velocity) of the reaction, represented by **V**, indicating the amount of S that reacts per unit time is expressed by a rate equation, **V** = ***k*** [S]. In this reaction, the rate depends only on the concentration of S. [In this case ***k*** is expressed as s^-1]



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

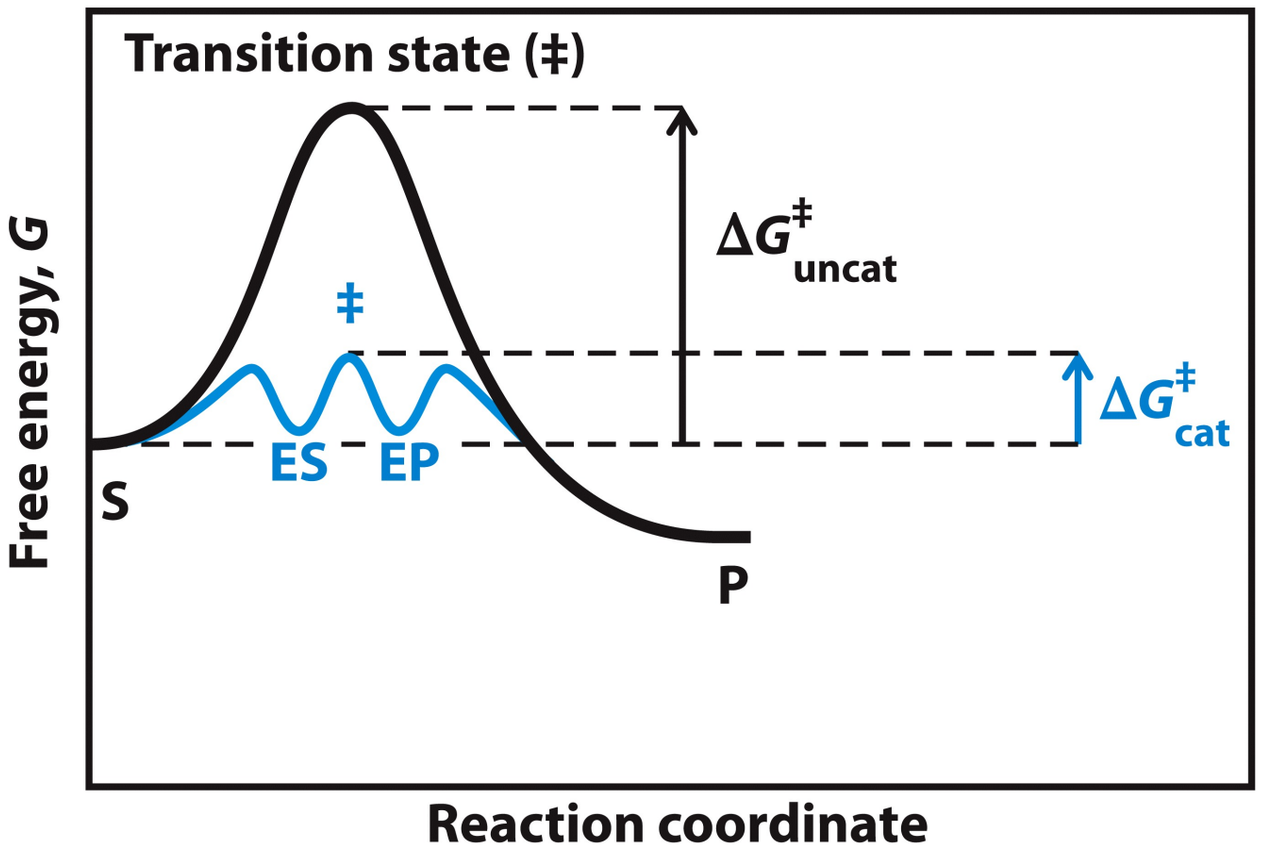
Where, k is the Boltzmann constant and *h* is the Planck constant. Note that the magnitude of a rate constant ***k*** is exponentially and inversely related to the **ΔG‡**. Thus, a lower activation energy means a faster reaction rate.

Enzymes Affect Rxn Rates

Like other catalysts, enzymes **enhance reaction rates** by lowering activation energies. They **have no effect on the position of reaction equilibria**. The example provided illustrates an enzyme which follows the simple enzymatic steps of

$$E + S \rightleftarrows ES \rightleftarrows EP \rightleftarrows E + P.$$

(E-enzyme; S-substrate; P-product; ES-transient complex between the enzyme and substrate; EP-transient complex between the enzyme and product). In the presence of the enzyme, three peaks appear in the reaction coordinate diagram. The highest peak indicates the rate-limiting step of the overall reaction. As discussed below, the binding energy resulting from the interaction of the enzyme with the transition state strongly contributes to lowering the activation energy of the reaction and accelerating its rate.



Catalytic Power and Specificity of Enzymes

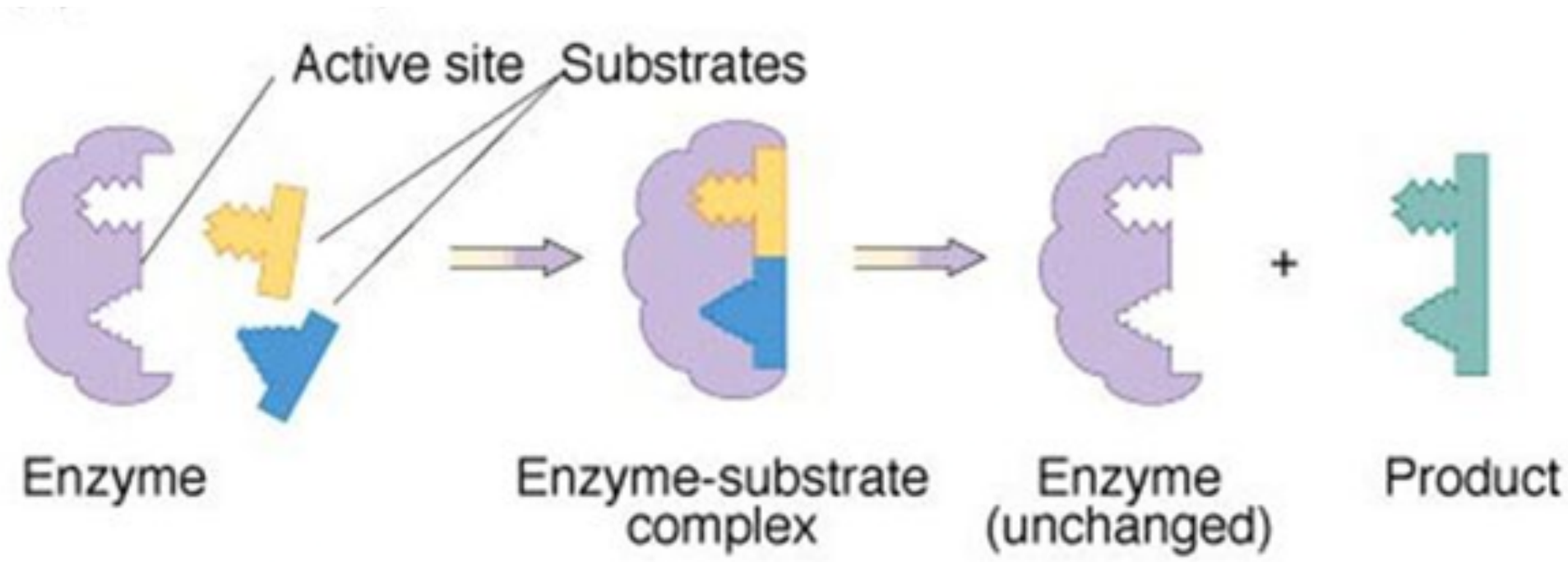
Enzymes commonly bring about enhancements in reaction rates in the range of 5 to 17 orders of magnitude. They are also highly specific, readily discriminating between substrates with quite similar structures.

TABLE 6–5 Some Rate Enhancements Produced by Enzymes

Cyclophilin	**10^5**
Carbonic anhydrase	**10^7**
Triose phosphate isomerase	**10^9**
Carboxypeptidase A	**10^{11}**
Phosphoglucomutase	**10^{12}**
Succinyl-CoA transferase	**10^{13}**
Urease	**10^{14}**
Orotidine monophosphate decarboxylase	**10^{17}**

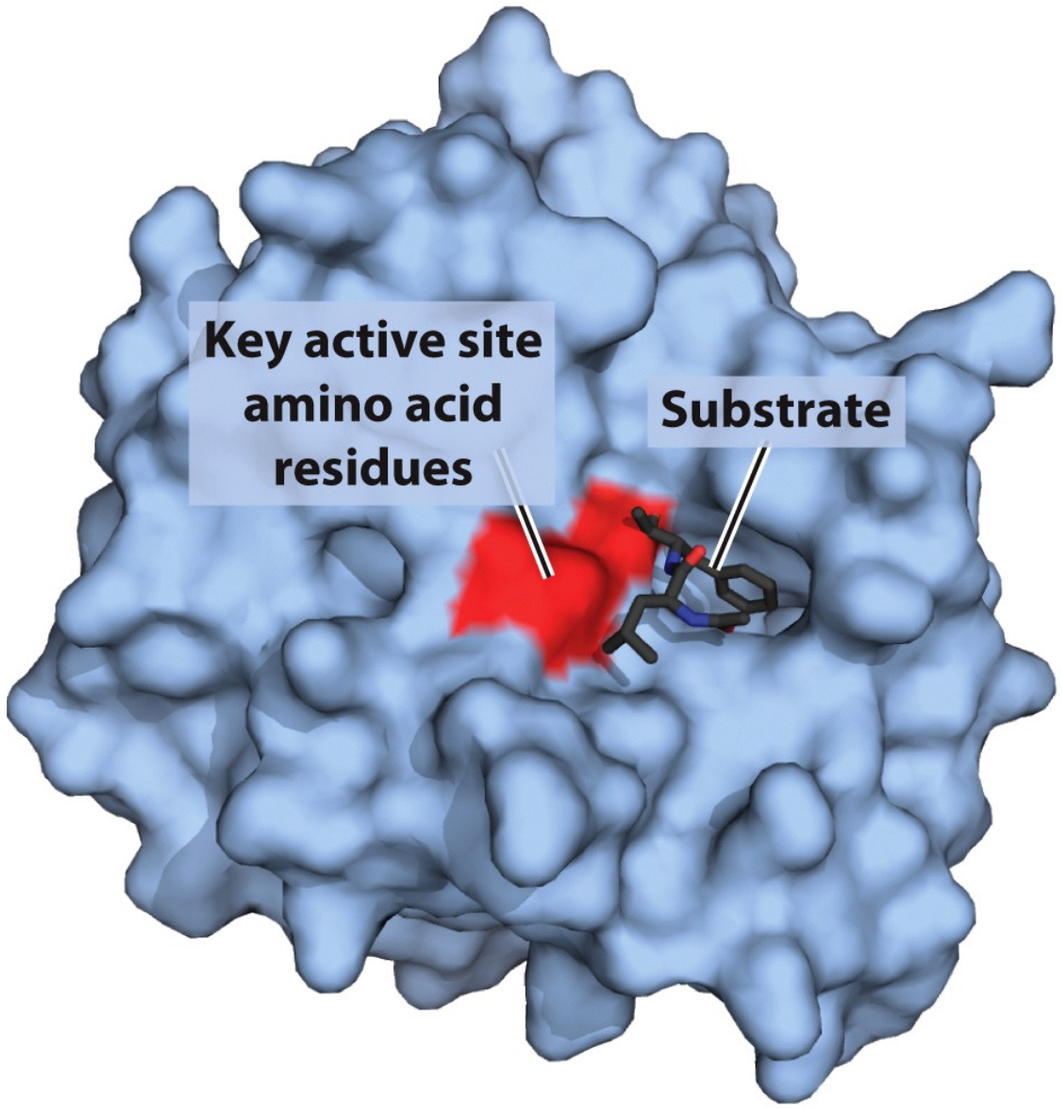
Catalytic Mechanism of Enzymes

The rate enhancements observed for enzymes result from two distinct but interwoven mechanisms. First, catalytic functional groups on an enzyme react with a substrate, lowering the activation energy barrier for reactions by providing an alternative, lower-energy reaction path. Second, non-covalent binding interactions between the substrate and enzyme release a small amount of free energy with each interaction, helping to lower the energy of the transition state. The energy derived from the enzyme-substrate interaction is referred to as the binding energy, ΔG_B.



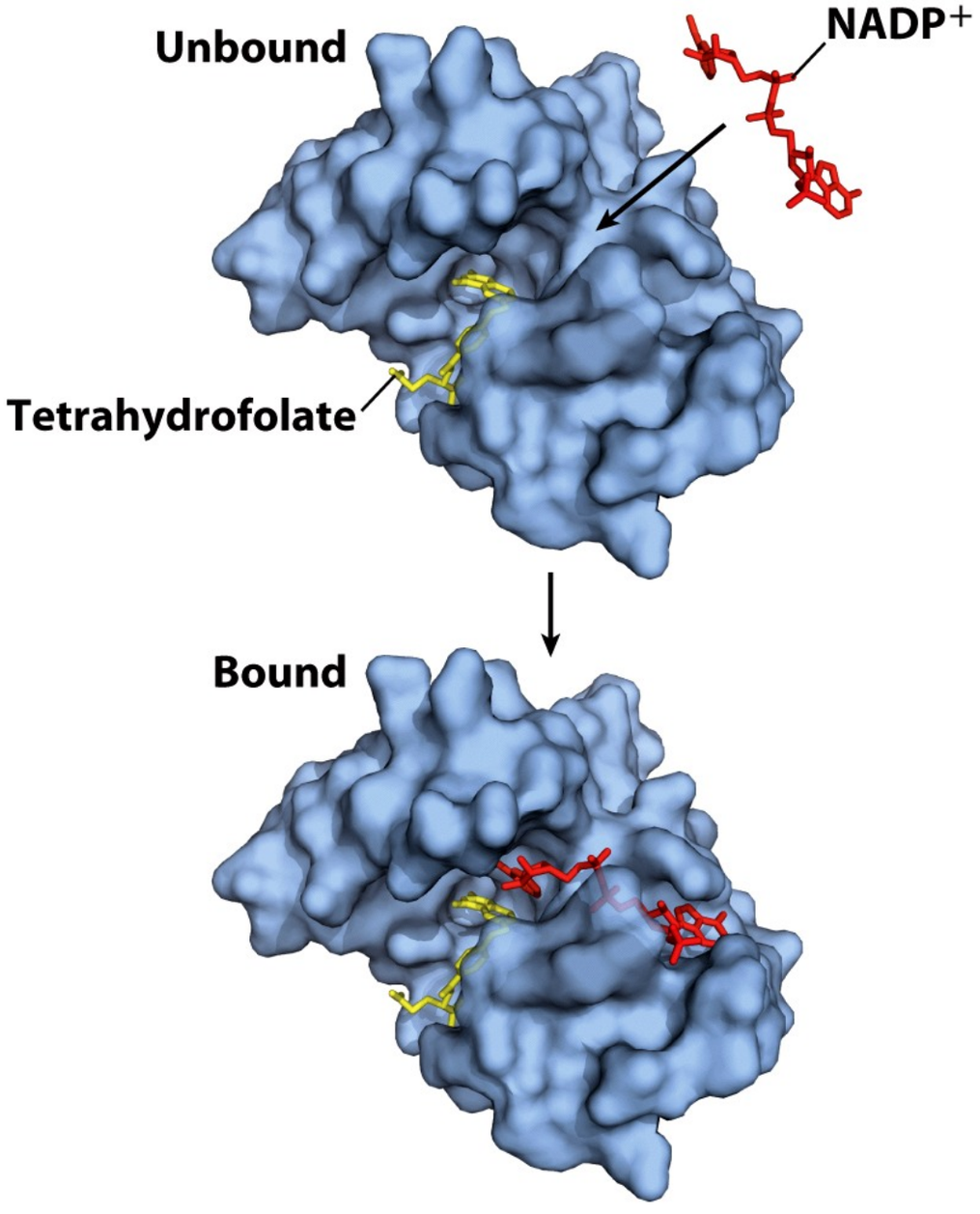
Active Site (I)

Uncatalyzed reactions tend to be slow because biological molecules are stable under physiological conditions. Enzymes increase the rates of biological reactions by providing a specific environment within which a reaction can occur more rapidly. Enzyme-catalyzed reactions take place within the confines of a **pocket on the enzyme called the active site**, where the reactant molecule is referred to as the substrate. The active site's surface is lined with amino acid residues, each possessing substituent groups that **bind to the substrate and catalyze its chemical transformation**. Often, the active site encloses the substrate, sequestering it from the solution. Here, we highlight the active site of the enzyme chymotrypsin.



Active Site (II)

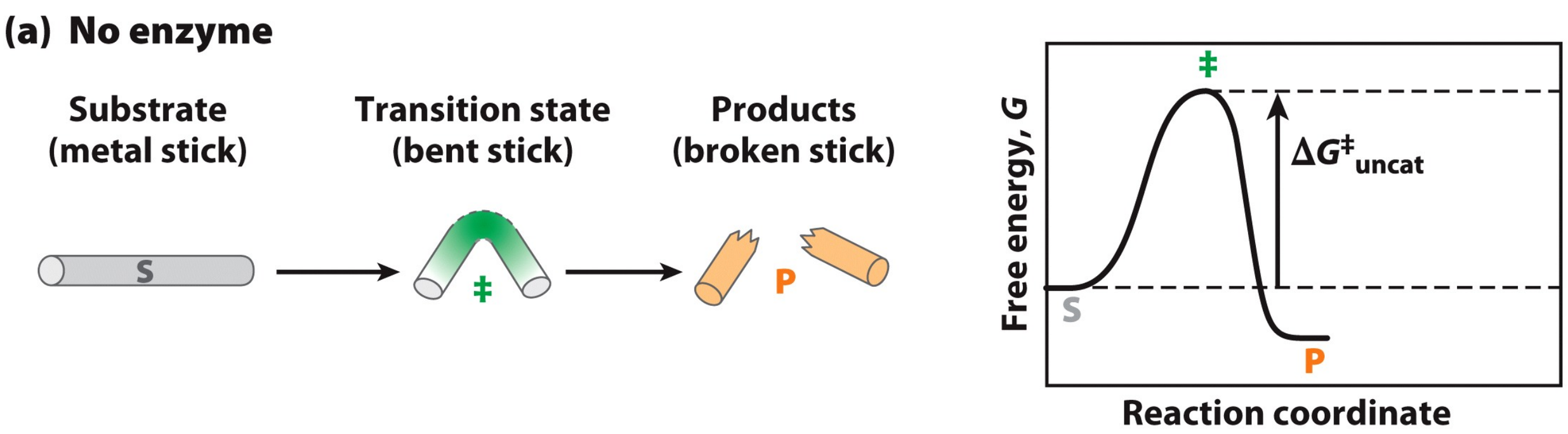
The active site of an enzyme has a surface that is complementary to its substrate (and products). This complementarity is **responsible for the specificity of enzyme reactions**. The idea that the enzyme and substrate are complementary to one another was first proposed by Emil Fischer in 1894. He suggested that the two components fit together like a **lock and key**, a proposal that greatly influenced the development of biochemistry. However, it is slightly misleading, as precise complementarity between an enzyme and its substrate would be counterproductive to efficient catalysis. Later, it was proposed that the enzyme must be more complementary to the reaction transition state than to the substrate per se for efficient catalysis to occur.



10’ Break

TS Complementarity and Rate Enhancement

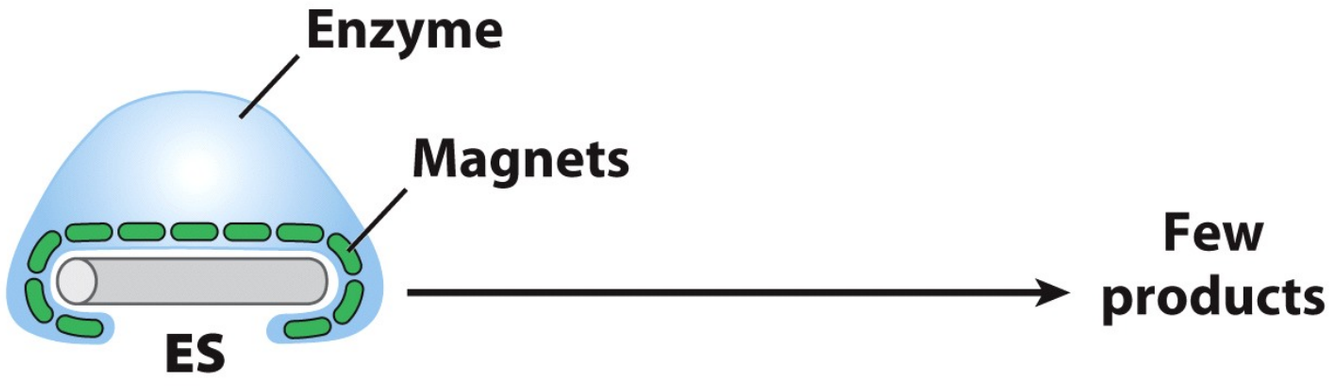
The importance of transition state complementarity to rate enhancement can be illustrated using an example of a hypothetical 'stickase' that catalyzes the breakage of a metal stick and binds to the stick via magnetic interactions. In the uncatalyzed reaction, the stick must first be bent to a transition state structure before breaking. Due to the high activation energy barrier of the bent stick transition state, the overall reaction (which has a negative free energy change) is slow.

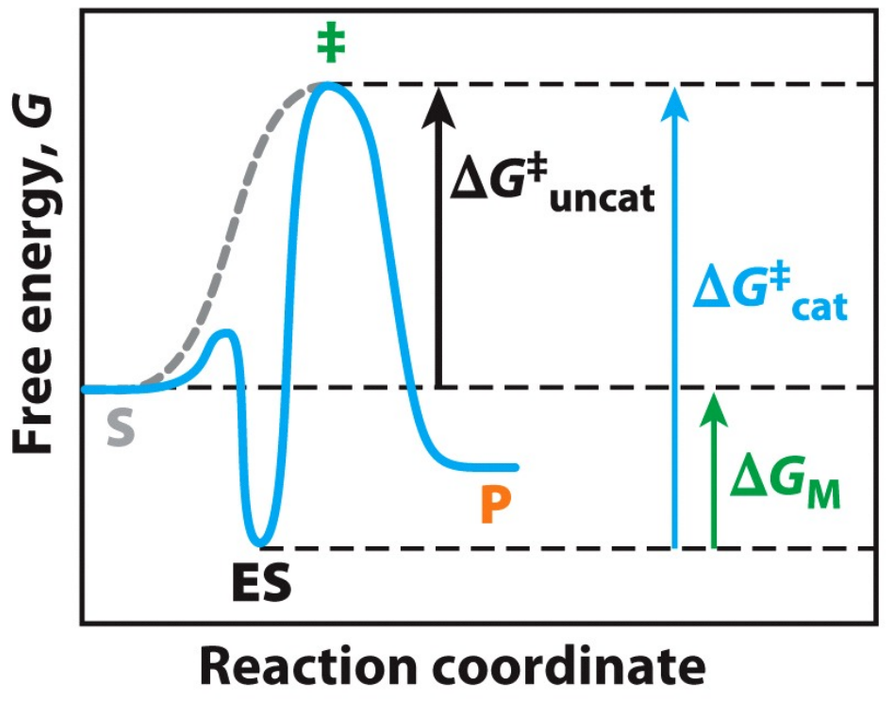


TS Complementarity and Rate Enhancement

If the stickase were precisely complementary to the metal bar, the rate of the reaction would not improve, as the enzyme would actually stabilize the structure of the stick. Under these conditions, the ES complex corresponds to a trough in the reaction coordinate diagram from which the substrate would have difficulty escaping.

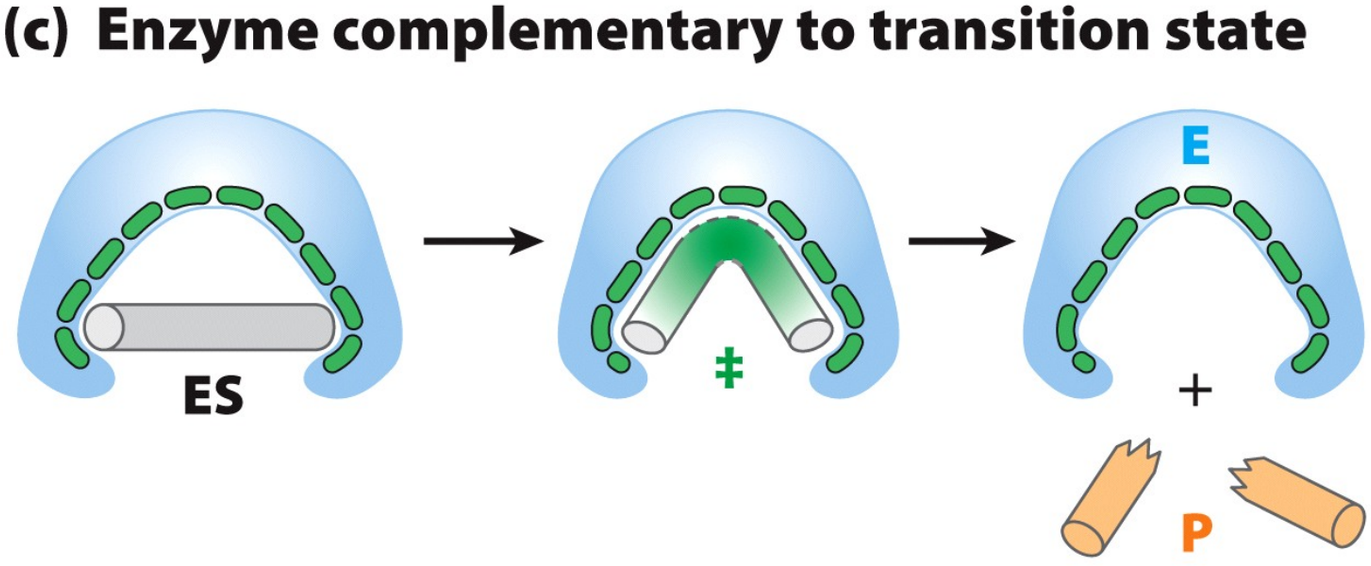
(b) Enzyme complementary to substrate

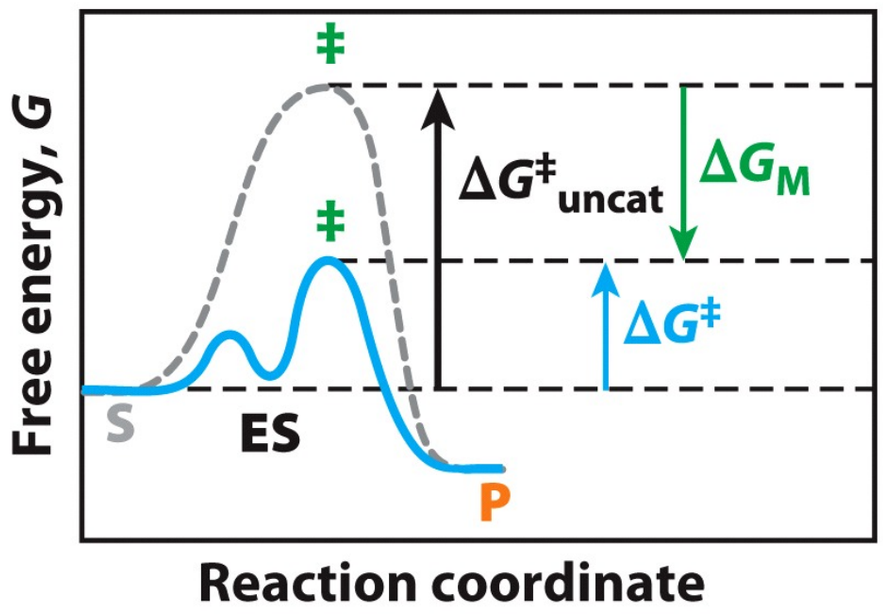




TS Complementarity and Rate Enhancement

However, if the stickase were more complementary to the transition state of the reaction, the increase in free energy required to draw the stick into a bent and partially broken conformation would be offset or paid for by the magnetic interactions (binding energy) between the enzyme and the substrate in its transition state. This energy payment translates into a lower net activation energy and a faster reaction rate.

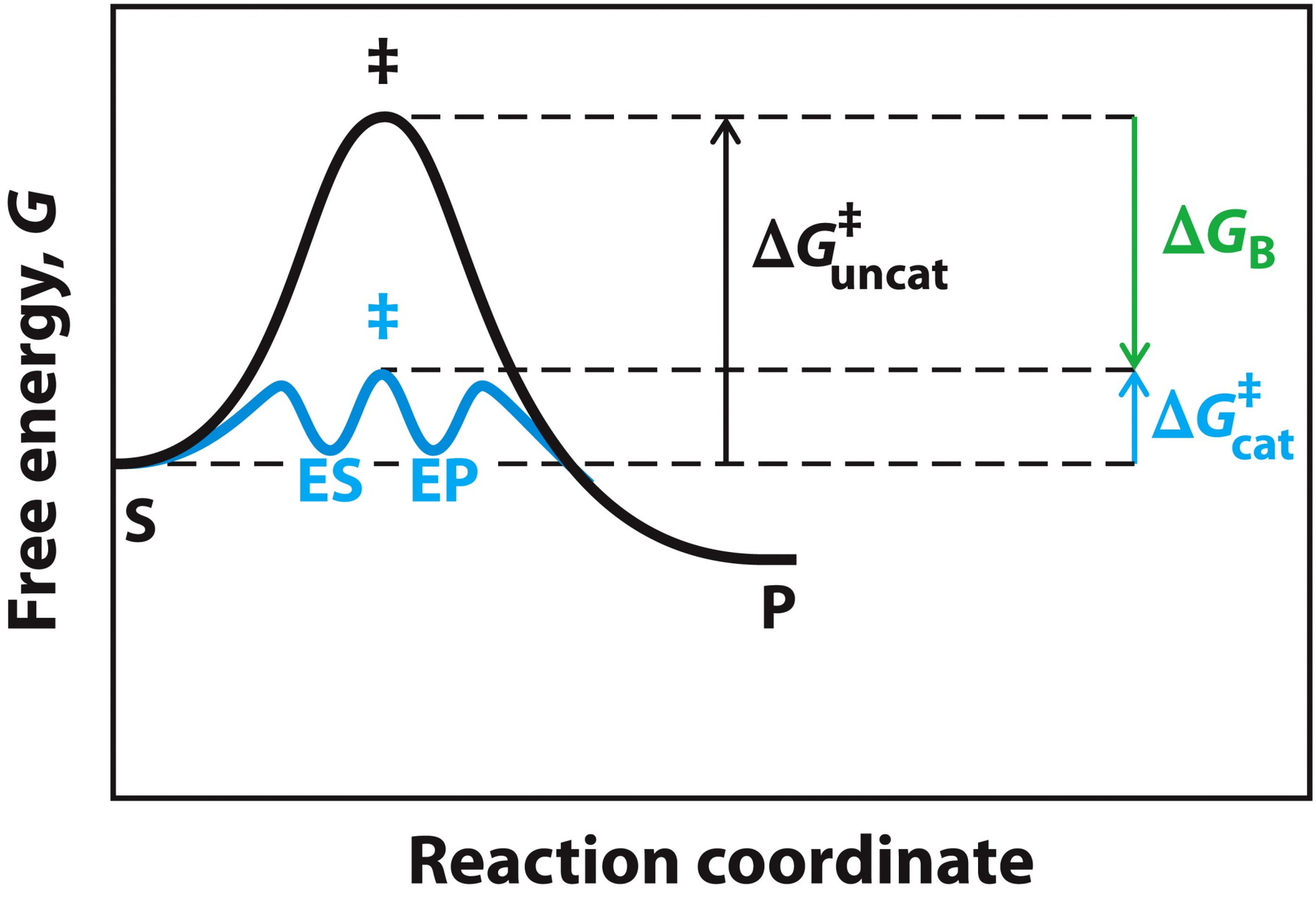




TS Complementarity and Rate Enhancement

Real enzymes work on an analogous principle. Some weak interactions are formed in the ES complex, but the full complement of such interactions between the substrate and enzyme is formed only when the substrate reaches the transition state. The free energy (binding energy) released by the formation of these interactions partially offsets the energy required to reach the top of the energy hill. The summation of the unfavorable (positive) activation energy $\Delta G^{\ddagger}$ and the favorable (negative) binding energy ΔG_B results in a lower net activation energy. Even on the enzyme, the transition state is not a stable species but rather a brief point in time when the substrate spends atop an energy hill. The enzyme-catalyzed reaction is much faster than the uncatalyzed process because the hill is much smaller. The important point is that weak binding interactions between the enzyme and the substrate provide a substantial driving force for enzymatic catalysis.

TS Complementarity and Rate Enhancement



Binding Energy and Specificity

For a reaction to take place, significant physical and thermodynamic factors contributing to $\Delta G^{\ddagger}$ must be overcome. These include

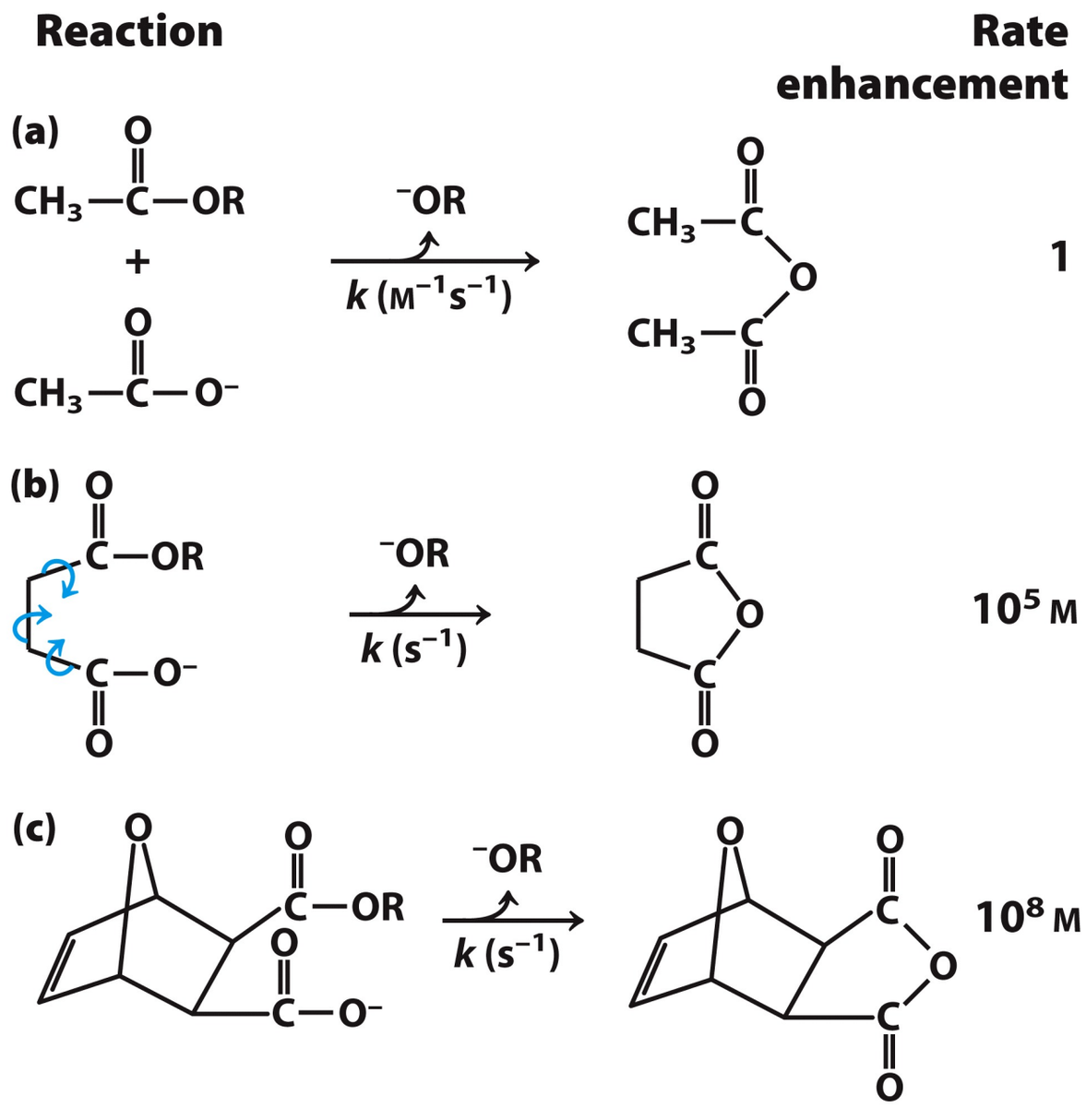
1) the entropy (freedom of motion) of molecules in solution, which reduces the possibility that they will react together,
2) the solvation shell of hydrogen-bonded water molecules that surrounds and helps to stabilize most biomolecules in solution,
3) the distortion of substrates that must occur in many reactions, and
4) the need for proper alignment of catalytic functional groups on the enzyme.

All of these factors can be overcome due to the binding energy released upon the interaction of the enzyme with the transition state. Binding energy also imparts specificity to an enzyme, enabling it to discriminate between its substrate and a competing molecule with a similar structure.

Binding Energy and Specificity

The mechanism by which binding energy compensates for physical and thermodynamic factors that impede reaction rates is as follows:

1. **Entropy reduction:** One benefit of binding two substrates to an enzyme is the restriction in their motions before reacting. Binding energy holds the substrates in the proper orientation for a reaction (a substantial contribution to catalysis), as productive collisions between molecules in solution can be exceedingly rare. Studies have shown that constraining the motion of two reactants can produce rate enhancements of many orders of magnitude.



Binding Energy and Specificity

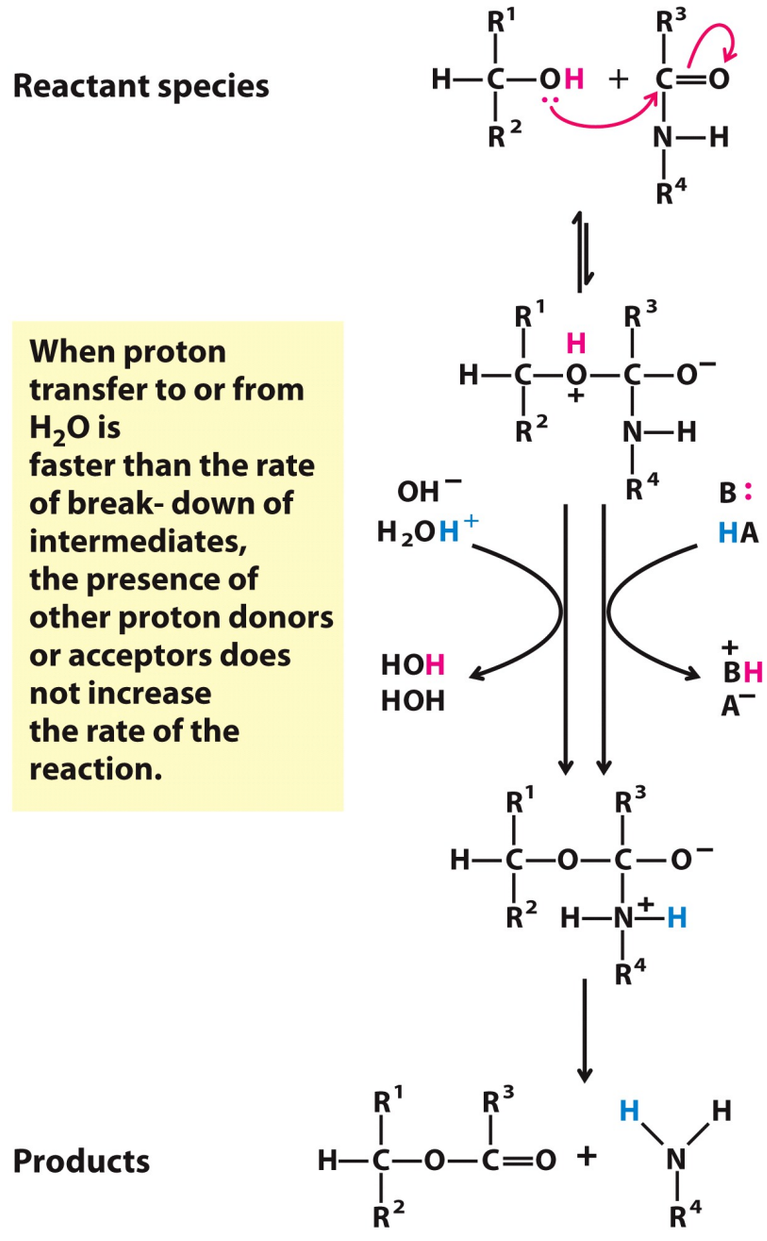
2. Desolvation: The formation of weak bonds between the enzyme and substrate leads to the desolvation of the substrate. Removing bound water molecules from the substrate eliminates potential impediments to the reaction.

3. **Substrate distortion**: Binding energy, involving weak interactions formed only in the reaction transition state, helps compensate thermodynamically for any distortion, primarily electronic redistribution, that the substrate must undergo to react.

4.Catalytic group alignment: Enzymes typically undergo conformational changes when the substrate binds, induced by multiple weak interactions with the substrate. The alignment of catalytic functional groups is referred to as induced fit, serving to position specific functional groups on the enzyme properly to catalyze the reaction.

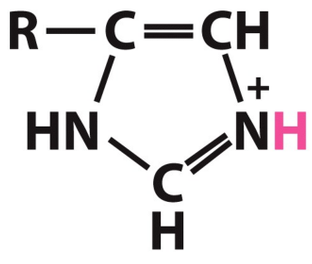
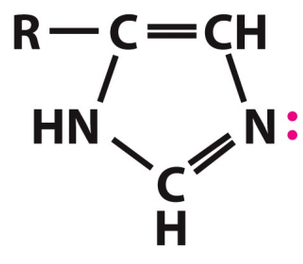
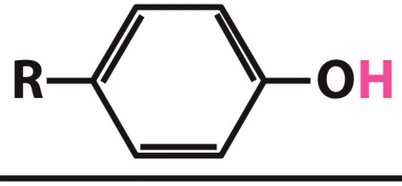
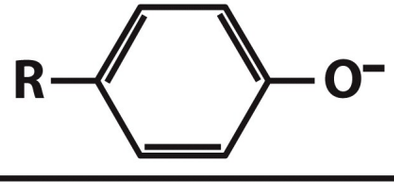
Other Contributions to Enzyme Catalysis: General Acid-base Catalysis

Many biochemical reactions involve the formation of unstable charged intermediates that tend to break down rapidly to their constituent reactant species, thus slowing the reaction. Charged intermediates can often be stabilized by the transfer of protons to or from the substrate or intermediate, forming a species that breaks down more readily to products. Proton transfers mediated by weak acids and bases other than water, such as the functional groups in the side-chains of amino acids, are referred to as general-acid-base catalysis.



Other Contributions to Enzyme Catalysis: General Acid-base Catalysis

Amino acid side-chains that are commonly involved in general acid-base catalysis

Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp		
Lys, Arg		
Cys		
His		
Ser		
Tyr		

Other Contributions to Enzyme Catalysis: Covalent Catalysis

In covalent catalysis, a transient covalent bond is formed between the enzyme and the substrate. Consider the hydrolysis of a bond between groups A and B:

$$A\text{-}B + H_2O \rightarrow A + B$$

In the presence of a covalent catalyst (an enzyme with the nucleophilic group X:), the reaction becomes

$$1)\ A\text{-}B + X: \rightarrow A\text{-}X + B$$
$$2)\ A\text{-}X + H_2O \rightarrow A + X:$$

This alteration in the pathway of the reaction results in catalysis if the new pathway has a lower activation energy than the uncatalyzed pathway. Both of the new steps must be faster than the uncatalyzed reaction. Several amino acid side-chains and the functional groups of some enzyme cofactors can serve as nucleophiles in the formation of covalent bonds with substrates. These covalent complexes always undergo further reactions to regenerate the free enzyme.

Other Contributions to Enzyme Catalysis: Metal Ion Catalysis

Metals, whether tightly bound to the enzyme or taken up from solution along with the substrate, can participate in catalysis in several ways. Ionic interactions between an enzyme-bound metal and a substrate can help orient the substrate for reaction or stabilize charged reaction transition states. This use of weak binding interactions between metal and substrate is similar to some of the uses of enzyme-substrate binding energy described earlier. Metals can also mediate oxidation-reduction reactions by reversible changes in the metal ion's oxidation state. Nearly a third of all enzymes require one or more metal ions for catalytic activity.

Enzymes - Take Home Messages

- Enzymes accelerate chemical reactions
- Enzymes are proteins often complexed with cofactors/ coenzymes
- Enzymes reduce the activation energy required for chemical reactions
- Enzymes have an active site that is complementary to the reaction transition state
- Enzymes are extremely specific (*in vivo*)
- Acid-base, covalent, and metal ions driven catalysis add to non covalent binding to the transition state to enhance reaction rates