

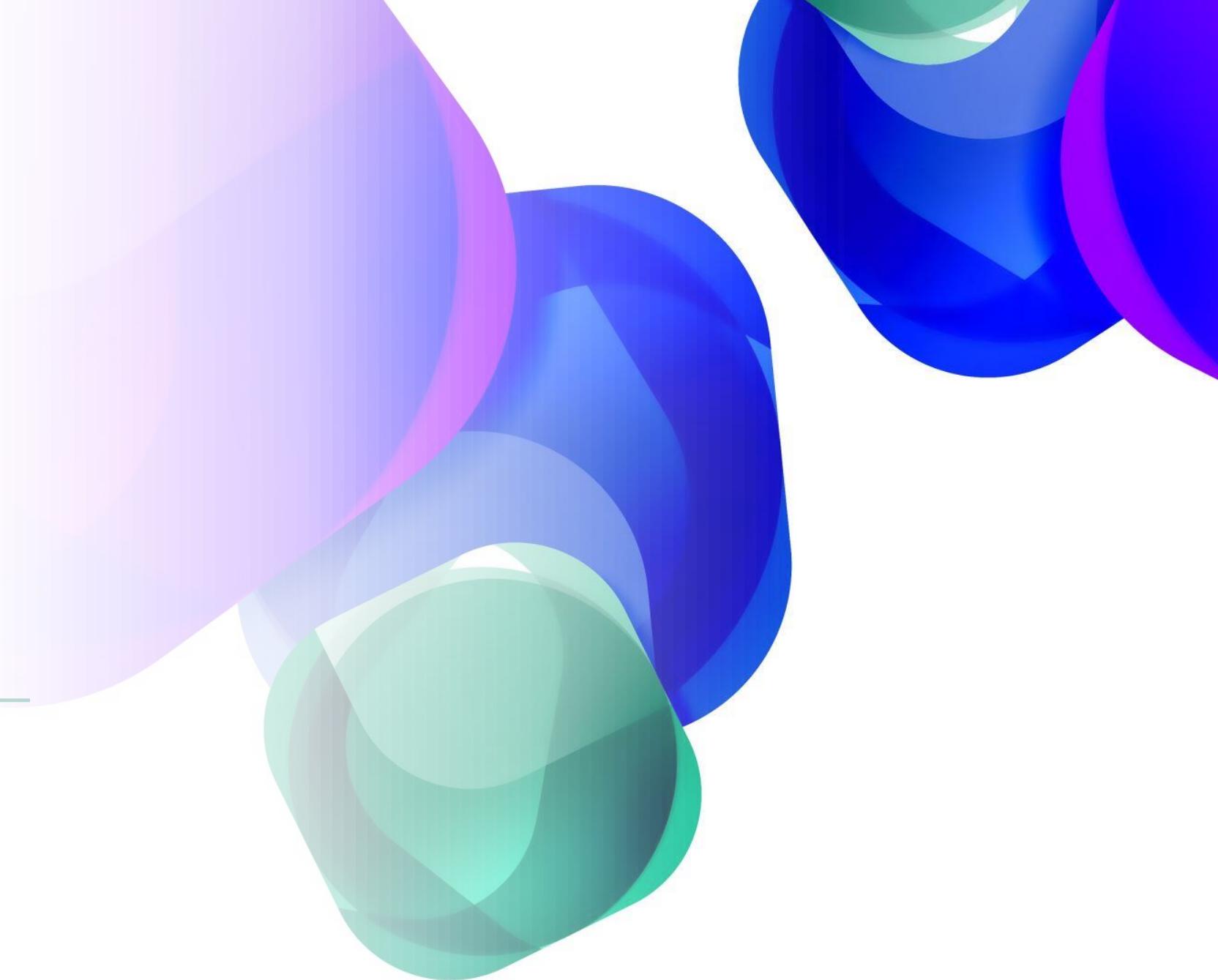


Enzyme Science and Engineering

Ravikrishnan Elangovan,

Department of Biochemical Engg and
Biotechnology,

Indian Institute of Technology, Delhi



Evaluation model

Minor 1	- 15 marks	Class ethics:
Minor 2	- 15 marks	1. Come ON time and participate! 2. Do not disturb 3. No mal practice
Major	- 30 marks	
Lab component	- 20 marks	
Quiz	- 20 marks	

Less than 70% attendance is one grade down!

Schedule for this semester

Class timing: Tue, Wed, Friday 9.00-10.00 AM

Lab timing: Mon, Friday 2.00-4.00 PM

Minor 1: 6-9th Feb 2023

Minor 2: 23-26th March 2023

Major: 1-10th May 2023

Every two weeks one quiz!

All lab practical's report will be submitted in MS teams

Topics to be covered!

Minor 1:

- Enzyme chemical and functional nature
- Enzyme source
- Enzyme purification
- Enzyme kinetics, inhibition and inactivation

Minor 2:

- Enzyme immobilization
- Enzyme reactors
- Diffusion restriction
- Enzyme improvement
- High throughput screening

Major:

- Enzyme for therapeutic applications
- Enzyme for biosensing
- Enzyme in bioethanol & biodiesel
- Non aqueous enzymology
- Biocatalysis

Why Enzymes?

- Chemical reactions at mild condition
- Highly specific reaction
- High catalytic rate
- 10000 and more enzymes

Some misconception:

Unstable:

Half life Aspartase – 24 months
Fumarase – 180 months
Protease – 2 months

Expensive:

Bacterial α amylase cost 2.4\$/Kg
Glucose isomerase cost 11 \$/Kg

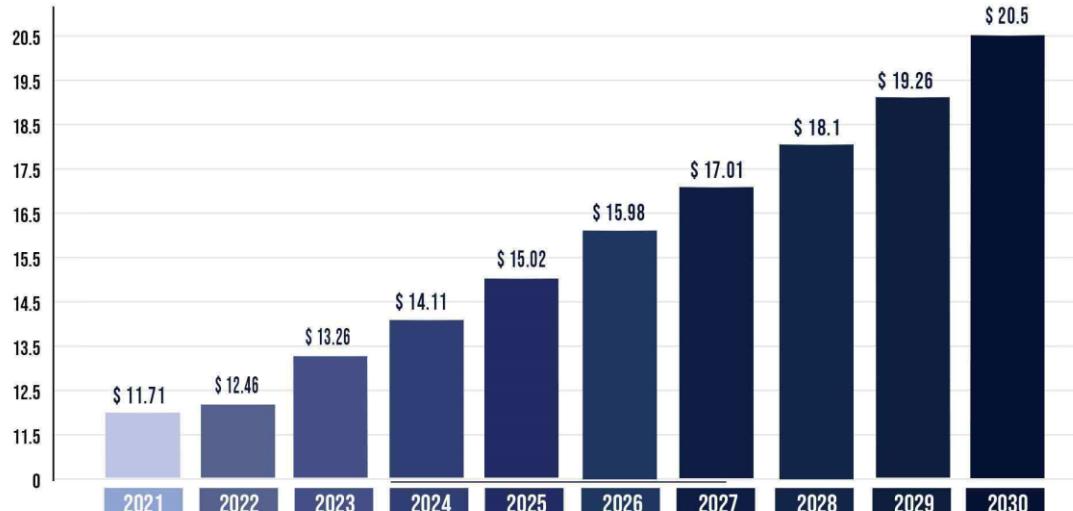
Low productivity:

e.g., acrylonitrile to acrylamide is produced at 400 g/L/hr

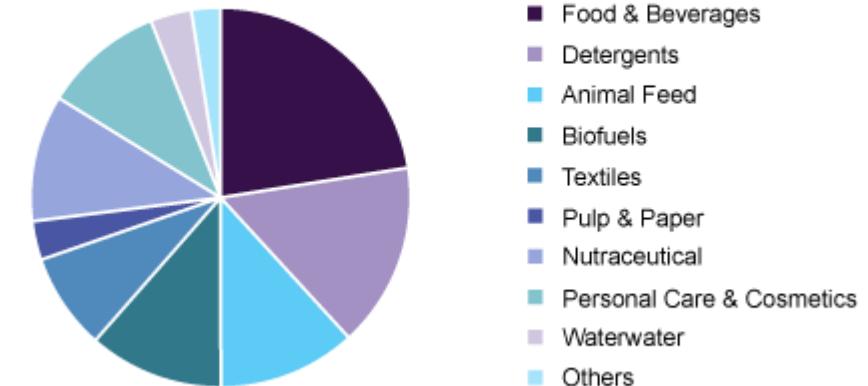
Motivation!

PRECEDENCE
RESEARCH

ENZYMES MARKET SIZE, 2021 TO 2030 [USD BILLION]



Global industrial enzymes market share, by application, 2019 (%)

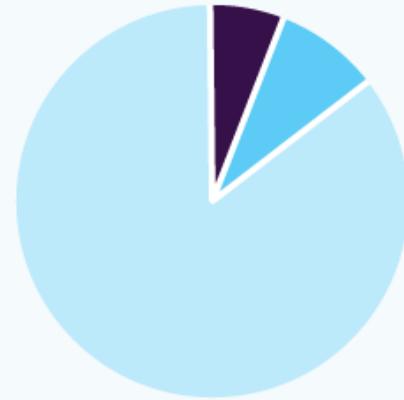


Source: www.grandviewresearch.com

Motivation!

Global Enzymes Market

share, by source, 2021 (%)



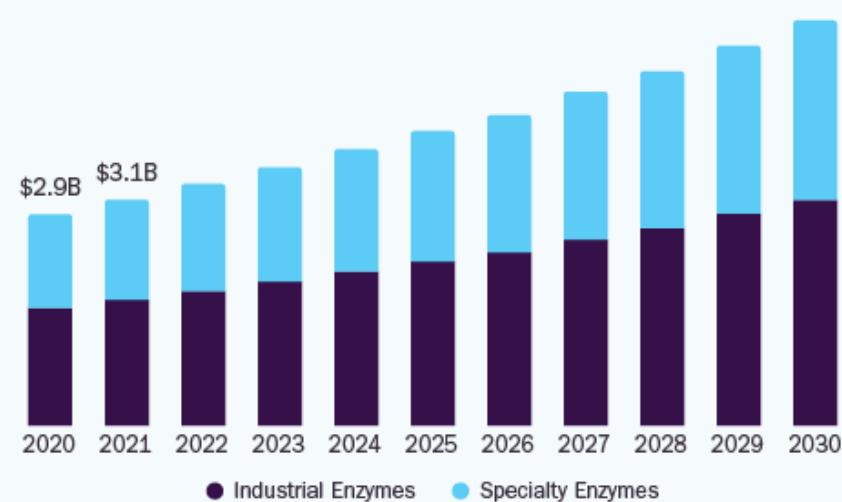
\$11.5B

Global Market Size,
2021

Source:
www.grandviewresearch.com

U.S. Enzymes Market

size, by type, 2020 - 2030 (USD Billion)



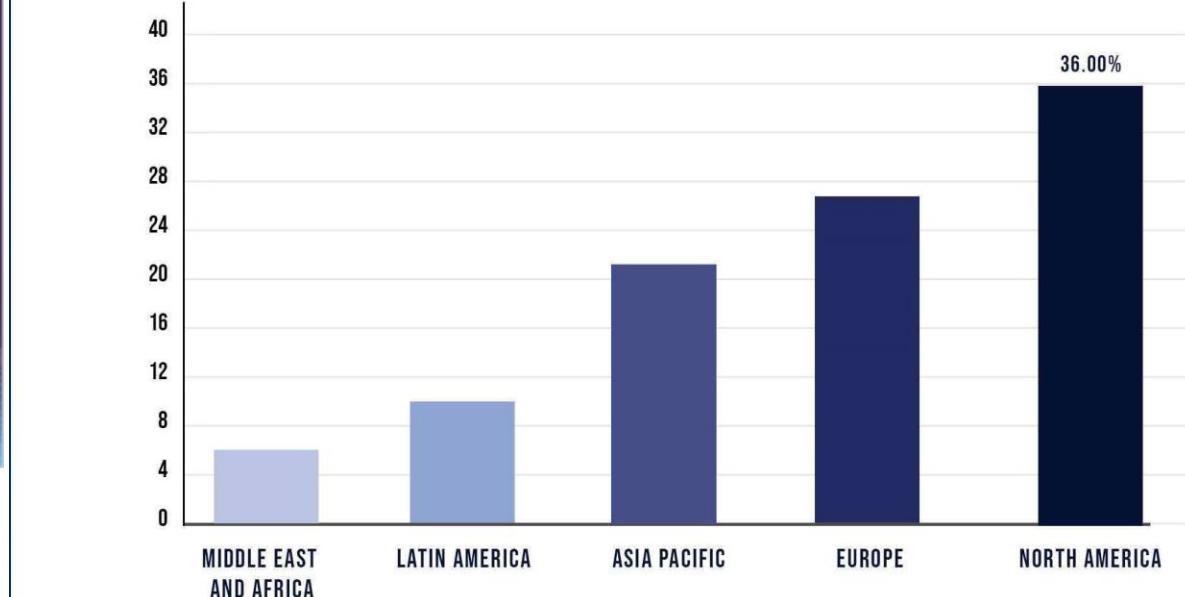
GRAND VIEW RESEARCH

6.8%

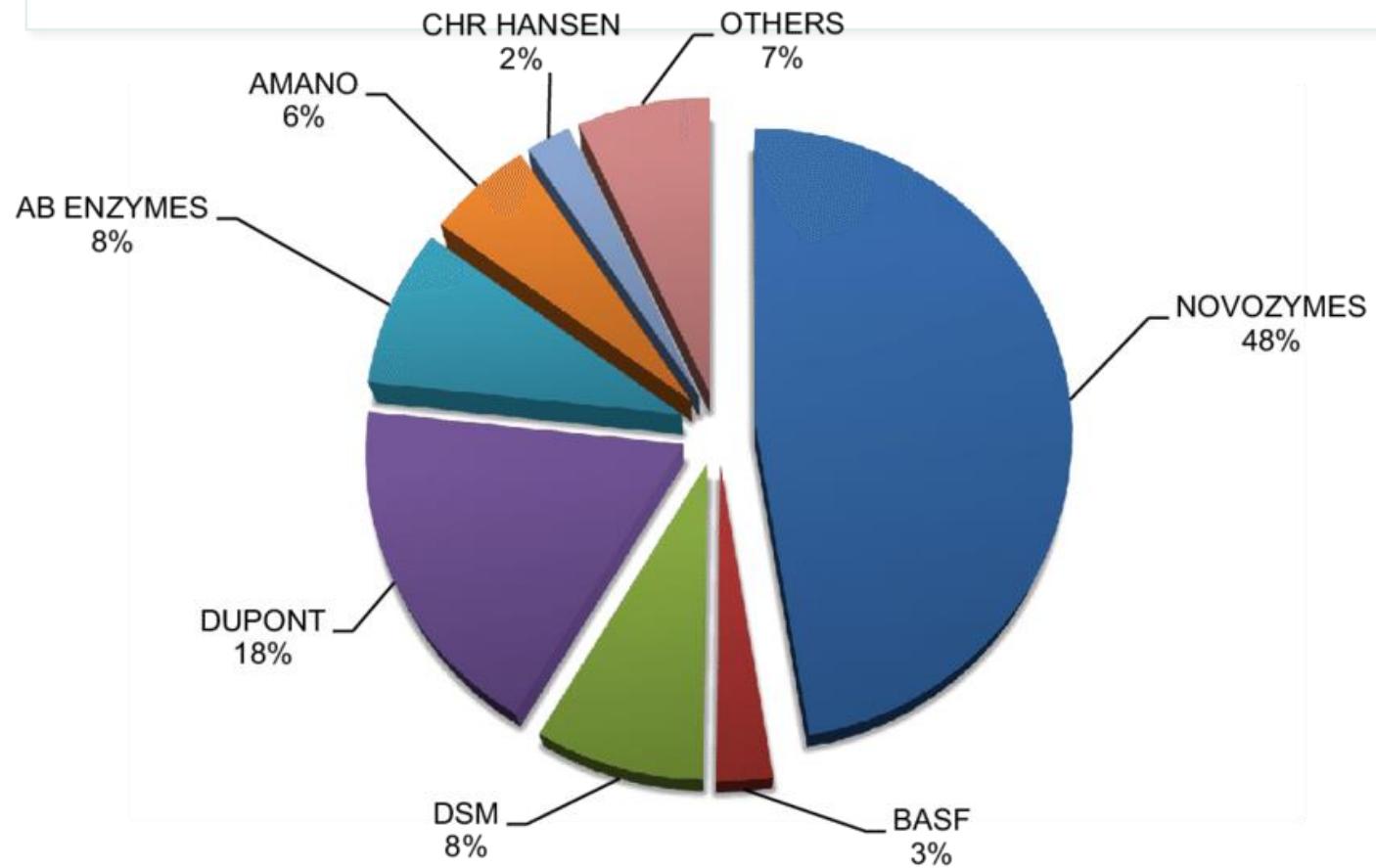
U.S. Market CAGR,
2022 - 2030

Source:
www.grandviewresearch.com

ENZYME MARKET SHARE, BY REGION, 2021 (%)



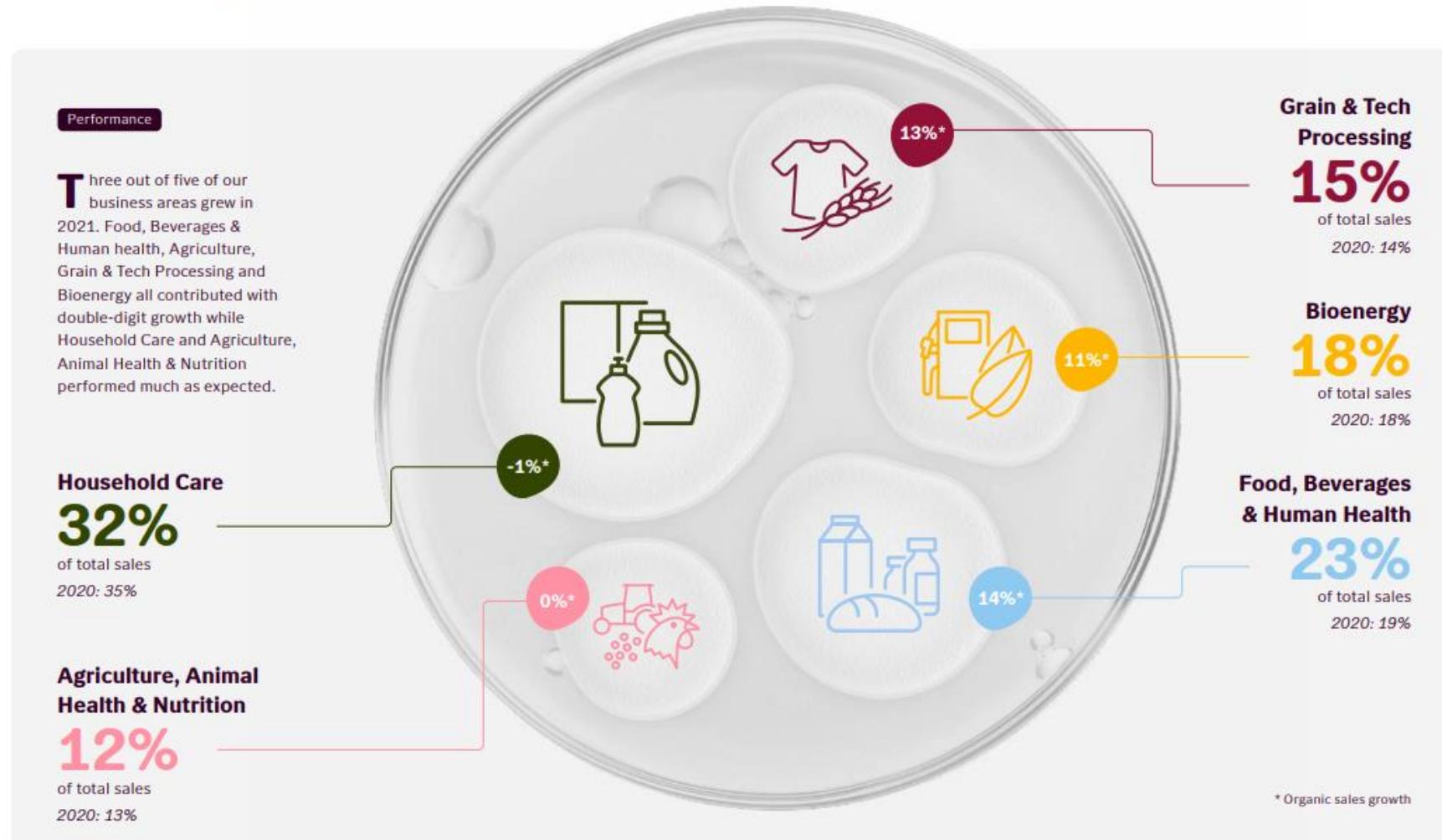
Motivation!



Novozymes

Sales by business area

<https://report2021.novozymes.com/#Facts-and-figures>



Enzyme applications

1. Starch conversions

- Production of glucose syrup
- Production of high fructose corn syrup
- Production of high maltose conversion syrups
- Production of cyclodextrins
- Production of ethanol

2. Lignocellulosic Biomass conversions

- Cellulose conversion
- Hemicellulose conversion
- Lignin conversion

- 3. Enzymes in the Production of Functional Oligosaccharides and Other Neutraceuticals
- 4. Enzymes in the Modification of Fats and Oils
- 5. Enzymes in the Animal Feed Industry
- 6. Enzymes in the Pulp and Paper Industry
- 7. Enzymes in the Fruit Juice Processing Industry
- 8. Enzymes in the Meat and Fish Processing Industry
- 9. Enzymes in the Dairy Industry
- 10. Enzymes in Detergents
- 11. Enzymes in the Leather Industry
- 12. Enzymes in the Production of Bulk and Fine Chemicals
- 13. Analytical Applications of Enzymes
- 14. Enzyme-Replacement Therapy



TEXTILE ENZYMES

For Cotton and Hosiery
Biopolishing

For Cotton and Denim Biofading

For Textile and Denim Desizing

For Textile Fabric Bioscouring

Concentrated Thermostable Alpha
Amylase

Concentrated Low Temperature
Alpha Amylase

Concentrated Cellulase for
Formulation (Biopolishing)

Concentrated Cellulase for
Formulation (Biofading)

Concentrated Pectinase for
Formulation (Bioscouring)

Concentrated Catalase for
Formulation (Peroxide Killer)

LEATHER ENZYMES

For Beamhouse Soaking

For Fat and Oil Degreasing

For Acid Bathing and Softening

For Alkaline Bathing and Dewrinkling

For Enzymatic Dehairing

Concentrated Alkaline protease for
Formulation

Concentrated Acid Protease for
Formulation

Concentrated Alkaline Lipase for
Formulation

Concentrated Acid Lipase for
Formulation

STARCH ENZYMES

For Starch Liquefaction

For Starch Saccharification

For Viscosity Reduction

For Dextran Hydrolysis

ANIMAL FEED ENZYMES

Phytase Enzyme for Animal Feed

B-Glucanase Enzyme for Animal
Feed

Cellulase Enzyme for Animal Feed

Xylanase Enzyme for Animal Feed

Amylase Enzyme for Animal Feed

Bacterial Protease Enzyme for
Animal Feed

Fungal Protease Enzyme for Animal
Feed

Lipase Enzyme for Animal Feed

DIETARY SUPPLEMENT ENZYMES

Fungal Amylase Enzyme for Dietary
Supplements

Fungal Xylanase Enzyme for Dietary
Supplements

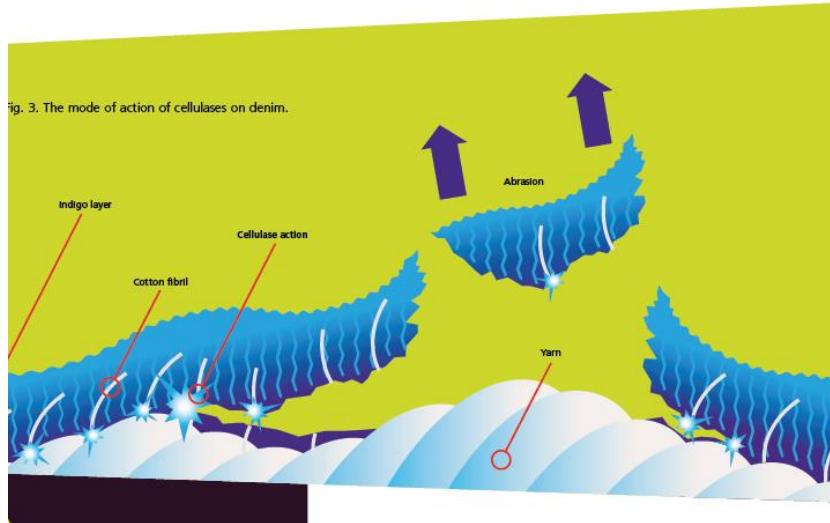
Fungal Lipase Enzyme for Dietary
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Fungal Cellulase Enzyme for Dietary
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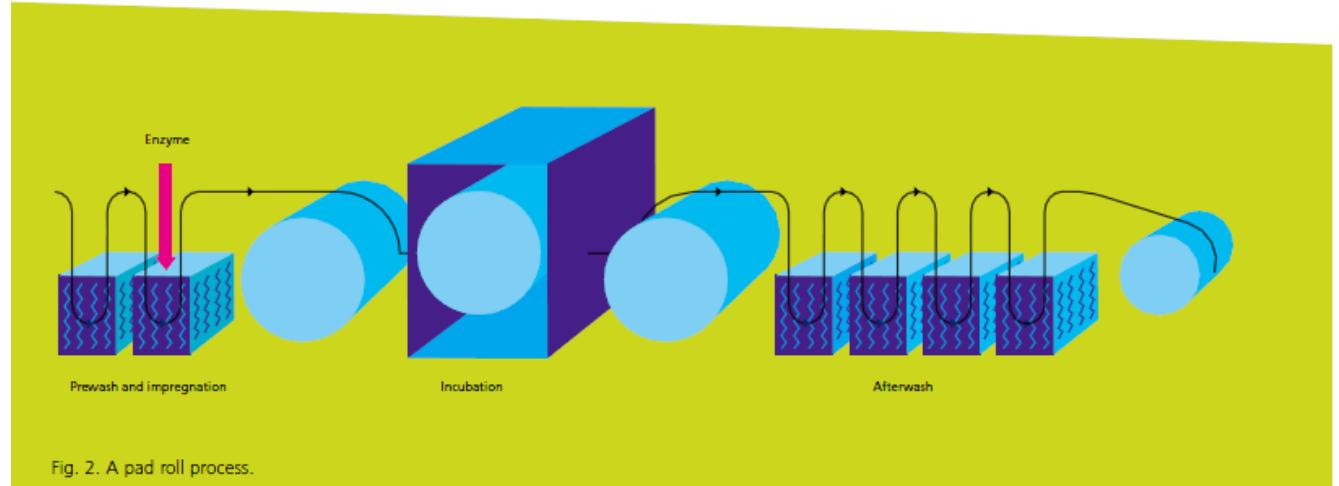
Cloth Industry

Faded Jeans



cellulase

Enzymatic desizing of cotton fabric



Otto Rohm first patented the use of pancreatic enzymes in 1913, since then use of enzymes in detergents have come a long way



- Proteases
- Lactases
- Maltases
- Lipases
- Alpha-amylase
- Cellulases

Lower temperature
Efficient
No environmental hazard
Softer fabric



- Papaya contains an enzyme, papain, which is a mild whitening agent and is used in toothpastes to remove enamel stains.



















L2 – Enzyme applications

Enzyme Science and Engineering

- Ravikrishnan Elangovan,
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- 13. Analytical Applications of Enzymes
- 14. Enzyme-Replacement Therapy

What are enzymes?

- Enzymes are proteins
- Catalysts are those that accelerate reaction without getting altered permanently
- Sometimes RNA's have enzymatic property
- 3D structure of proteins allows them to have this unique Functional property
- Enzyme are purified from
 - Animals, e.g., Rennet
 - Plants, e.g., Papain
 - Microbial sources, e.g., Cellulases

Why enzymes are useful?

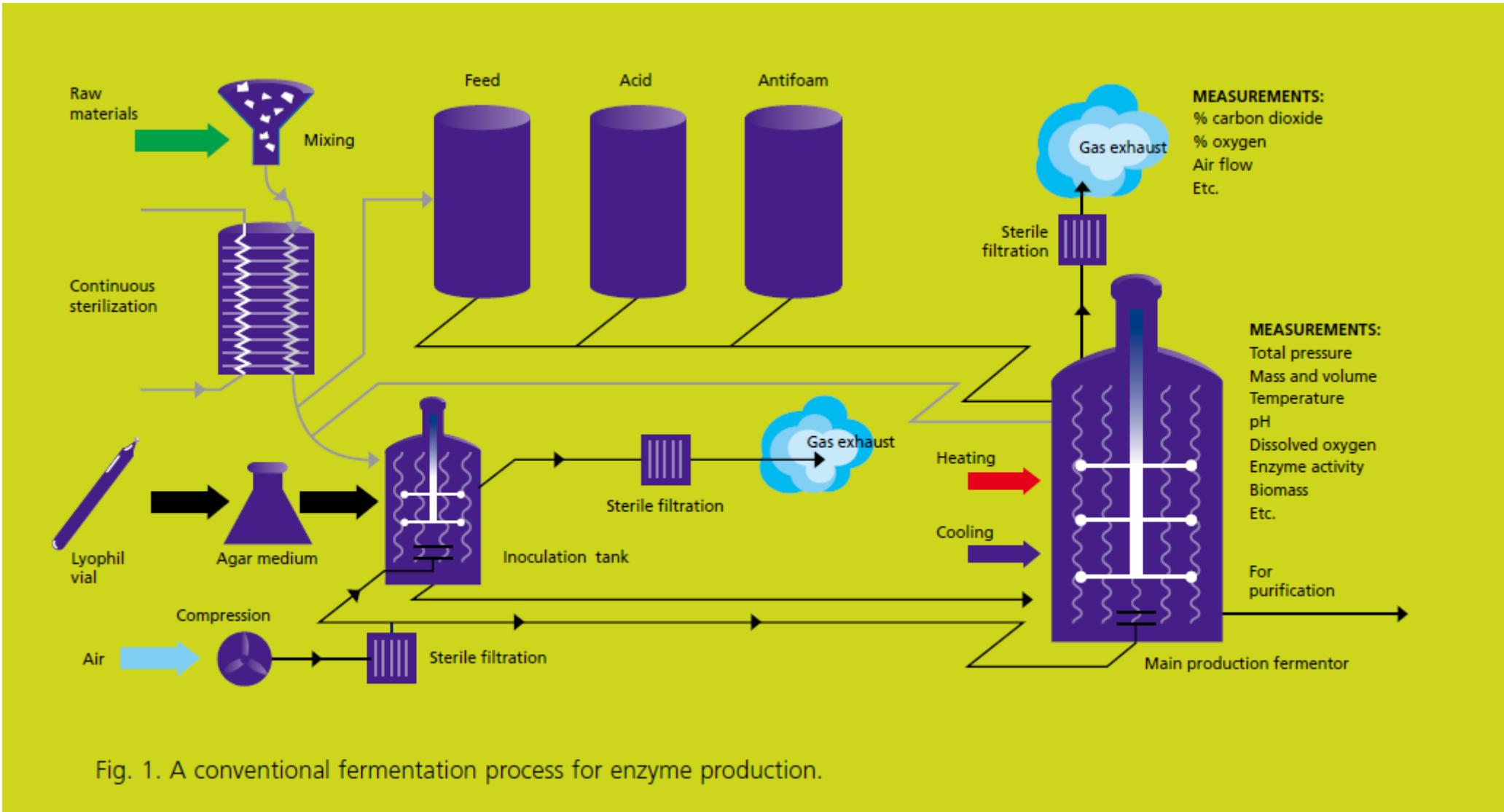
There are several characteristics of enzymes that make them ideal for industrial applications:

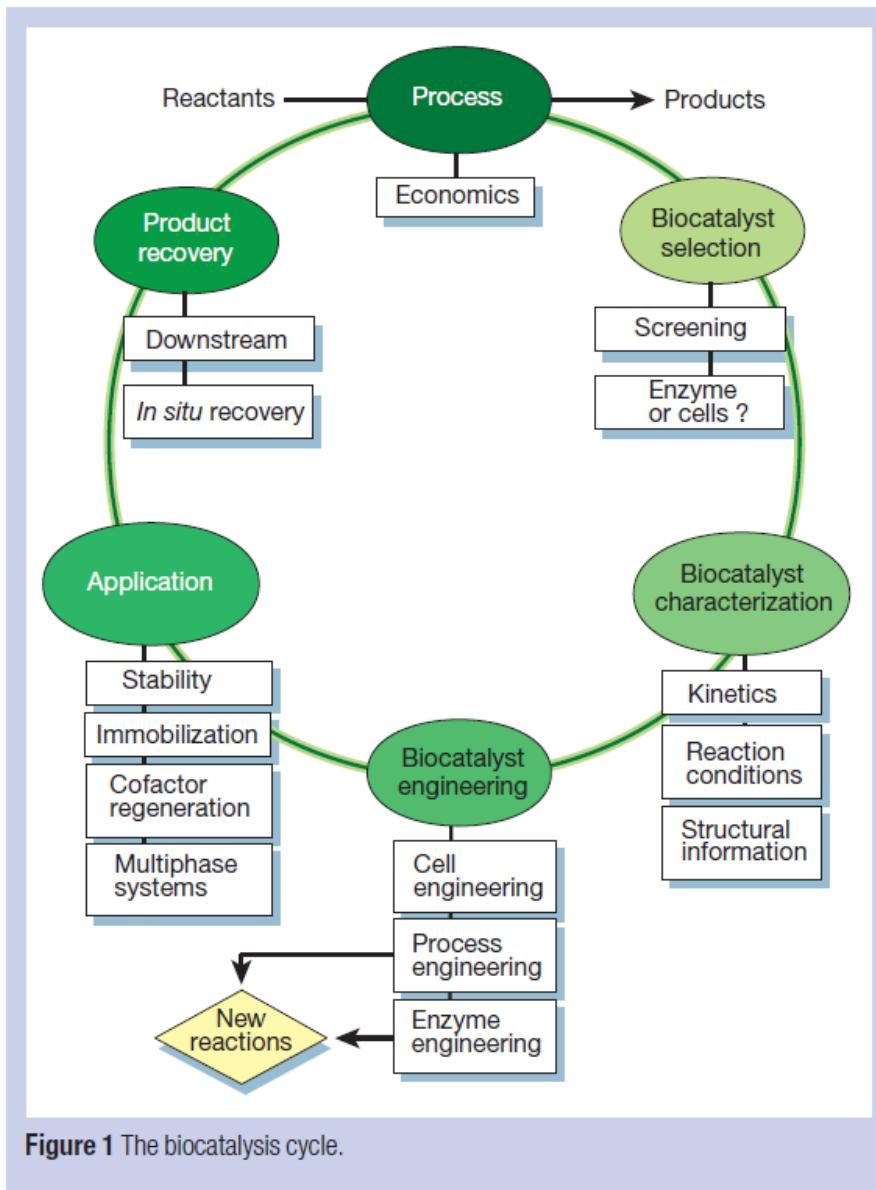
- Specificity: Enzymes are highly specific in their catalytic activity, meaning that they can catalyze a specific chemical reaction without affecting other reactions. This allows them to be used in targeted and precise ways in industrial processes.
- Efficiency: Enzymes can catalyze reactions at much lower temperatures and pressures than traditional chemical catalysts, making them more energy-efficient and cost-effective.
- Stability: Enzymes are relatively stable and can be used over a wide range of pH and temperature conditions. This makes them suitable for use in a variety of industrial processes.
- Renewability: Enzymes are biodegradable and can be produced sustainably, making them an environmentally friendly alternative to traditional chemical catalysts.
- Versatility: Enzymes can be used in a wide range of industries, including food processing, animal feed, textiles, paper and pulp production, and detergent manufacturing. This makes them a valuable tool in many different industrial applications.

Enzyme classification

ENZYME CLASS	INDUSTRIAL ENZYMES
EC 1: Oxidoreductases	catalase glucose oxidase laccase
EC 2: Transferases	glucosyltransferase
EC 3: Hydrolases	amylase cellulase lipase mannanase pectinase phytase protease pullulanase xylanase
EC 4: Lyases	pectate lyase alpha-acetolactate decarboxylase
EC 5: Isomerases	glucose isomerase
EC 6: Ligases	not used at present

Availability of enzyme!







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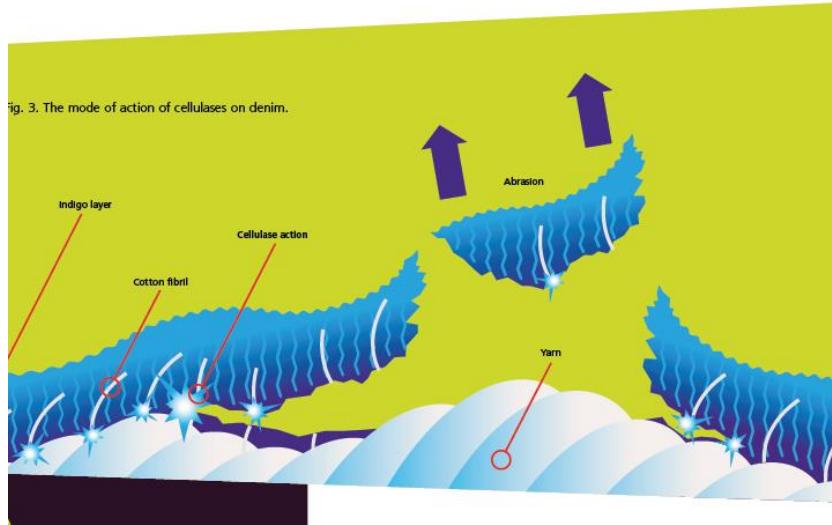
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Cloth Industry

Faded Jeans

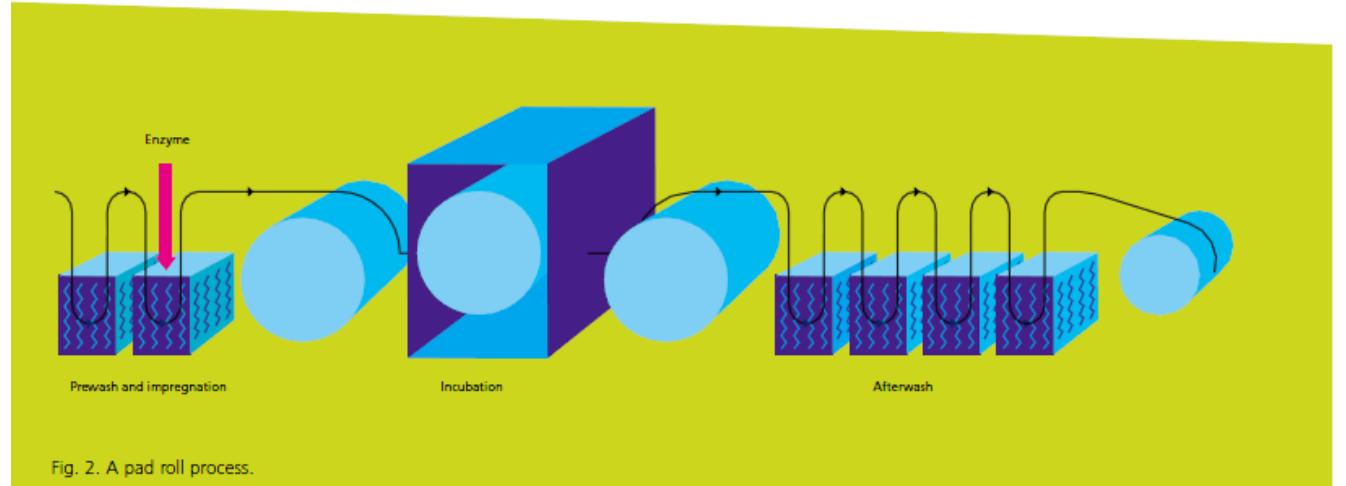


cellulase

Enzymatic desizing of cotton fabric



Enzymatic desizing is a method for removing the size, or starch, from cotton fabric.



Enzymes as detergents!

Otto Rohm first patented the use of pancreatic enzymes in 1913, since then use of enzymes in detergents have come a long way

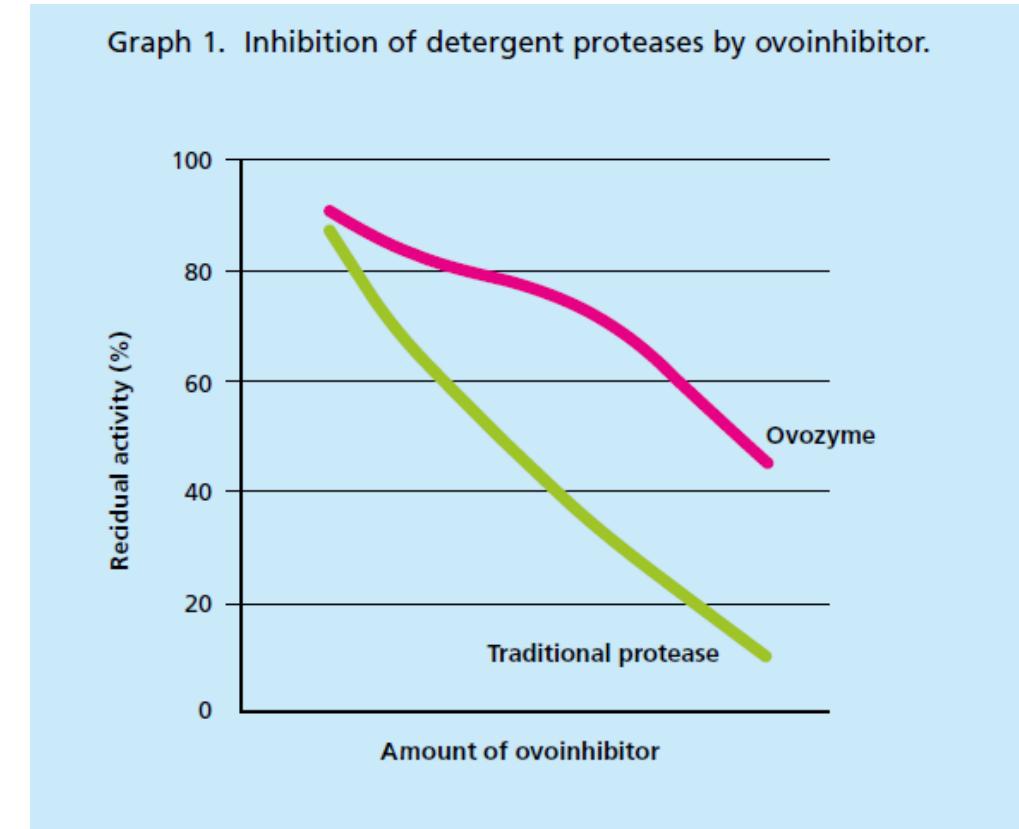


- Proteases
- Lactases
- Maltases
- Lipases
- Alpha-amylase
- Cellulases

- A better cleaning performance in general
- Rejuvenation of cotton fabric through the action of cellulases on fibers
- Reduced energy consumption by enabling lower washing temperatures
- Reduced water consumption through more effective soil release
- Minimal environmental impact since they are readily biodegradable
- Environmentally friendlier wash water effluents (in particular, phosphate-free and less alkaline)

The most widely used detergent enzymes are hydrolases, which remove soils consisting of proteins, lipids, and polysaccharides.

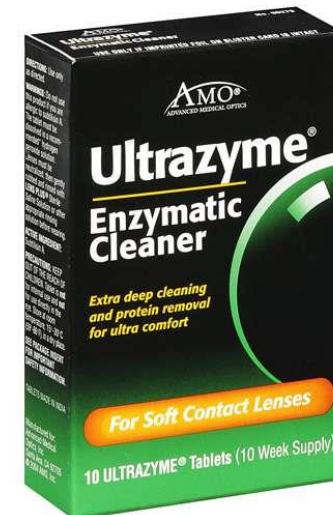
Ingredients	Example	Approximate Composition (%)
Builders	Sodium tripolyphosphate, nitrilotriacetic acid, sodium citrate, EDTA, Polycarboxylates	38
Surfactants	Sodium alkane sulphonate, alkyl sulphate	25
Bleaching agents	Sodium percarbonate, hydrogen peroxide, sodium perborate tetrahydrate, and chlorine	25
Antiredeposition agents	Sodium carboxymethyl cellulose/sodium polyacrylate/ polyethylene glycol	2
Foam regulators/soap	Soap (sodium alkane carboxylates)	3
Water softeners	Sodium sulphate	2.5
Binder /loosens dirt	Sodium metasilicate	1
Enzyme	Protease, lipase, amylase, cellulase	1
Optical brighteners	Triazine-stilbenes, bi-phenyl-stilbenes, coumarins, imidazolines, diazoles, triazoles	0.5
Solvents	Alcohol, acetone	Trace
Alkalies	Sodium hydroxide, sodium carbonate	Trace
Perfumes	Citronella, lavender oil, vanilla	Trace
Fabric softeners	Imidazolinium salts	Trace



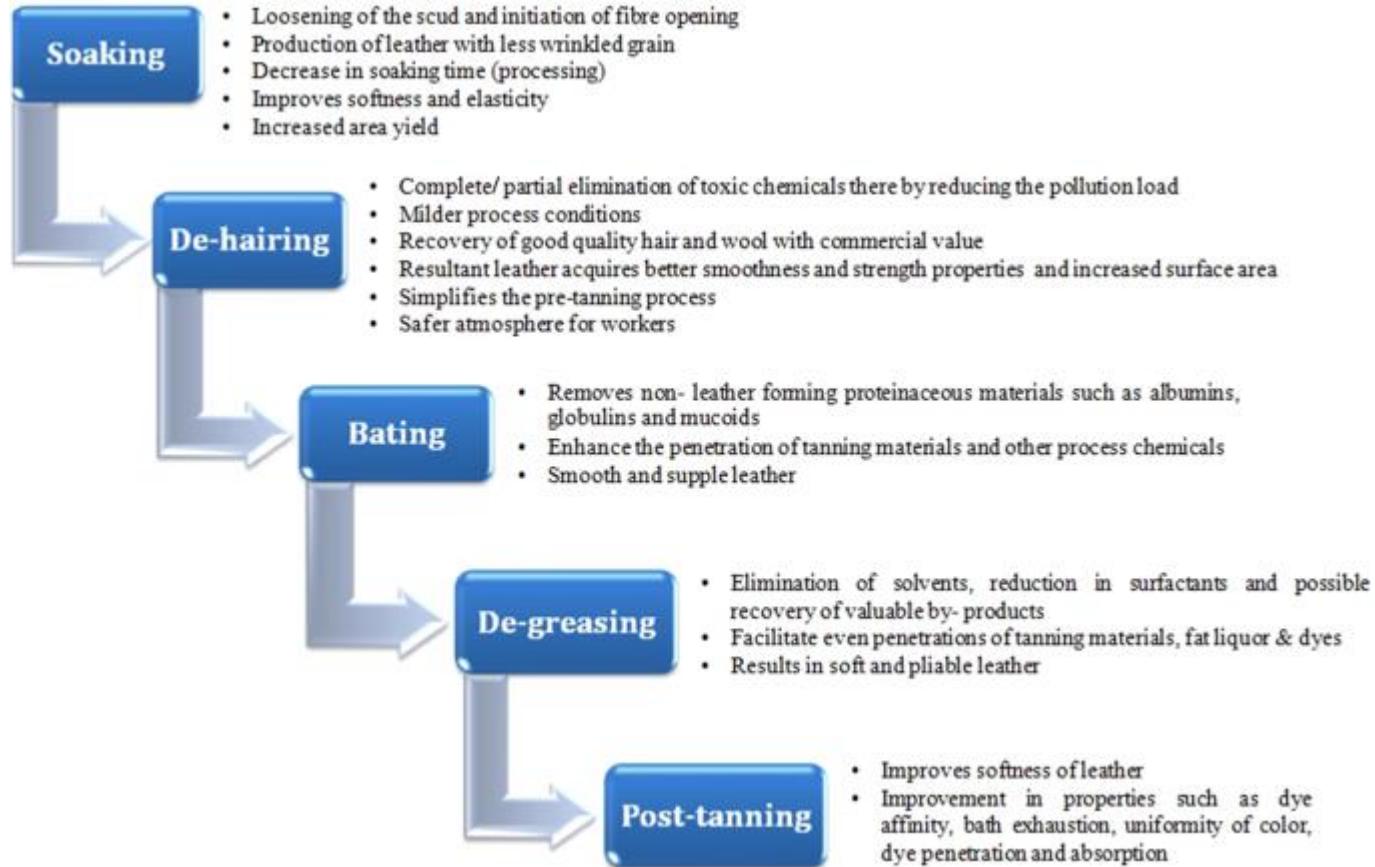
Microbial Enzymes in Detergents: A Review Sumeyra GÜRKÖK - Scientific Figure on ResearchGate. Available from:
https://www.researchgate.net/figure/COMPOSITION-OF-AN-ENZYME-DETERGENT_tbl1_337796251 [accessed 10 Jan, 2023]

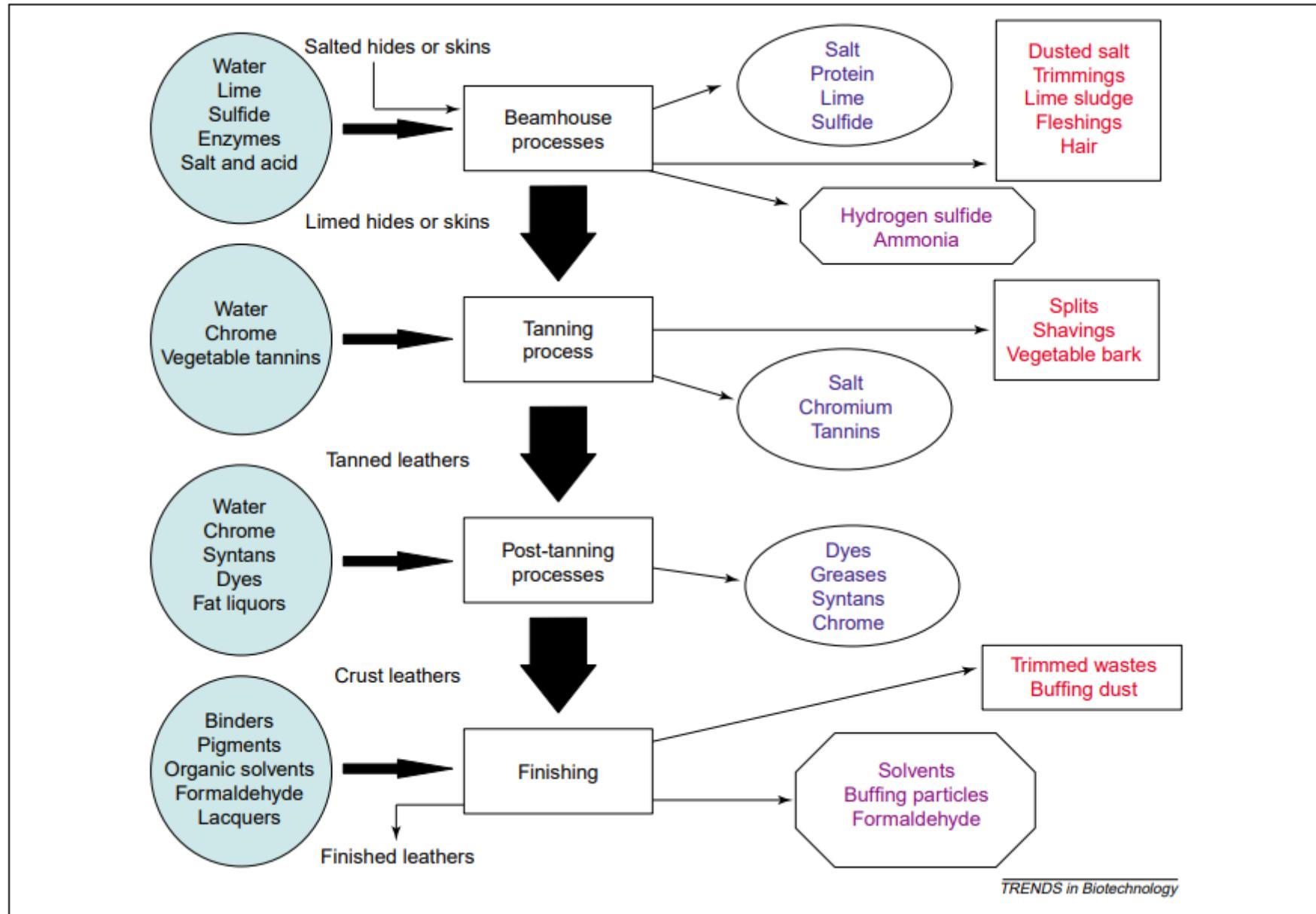


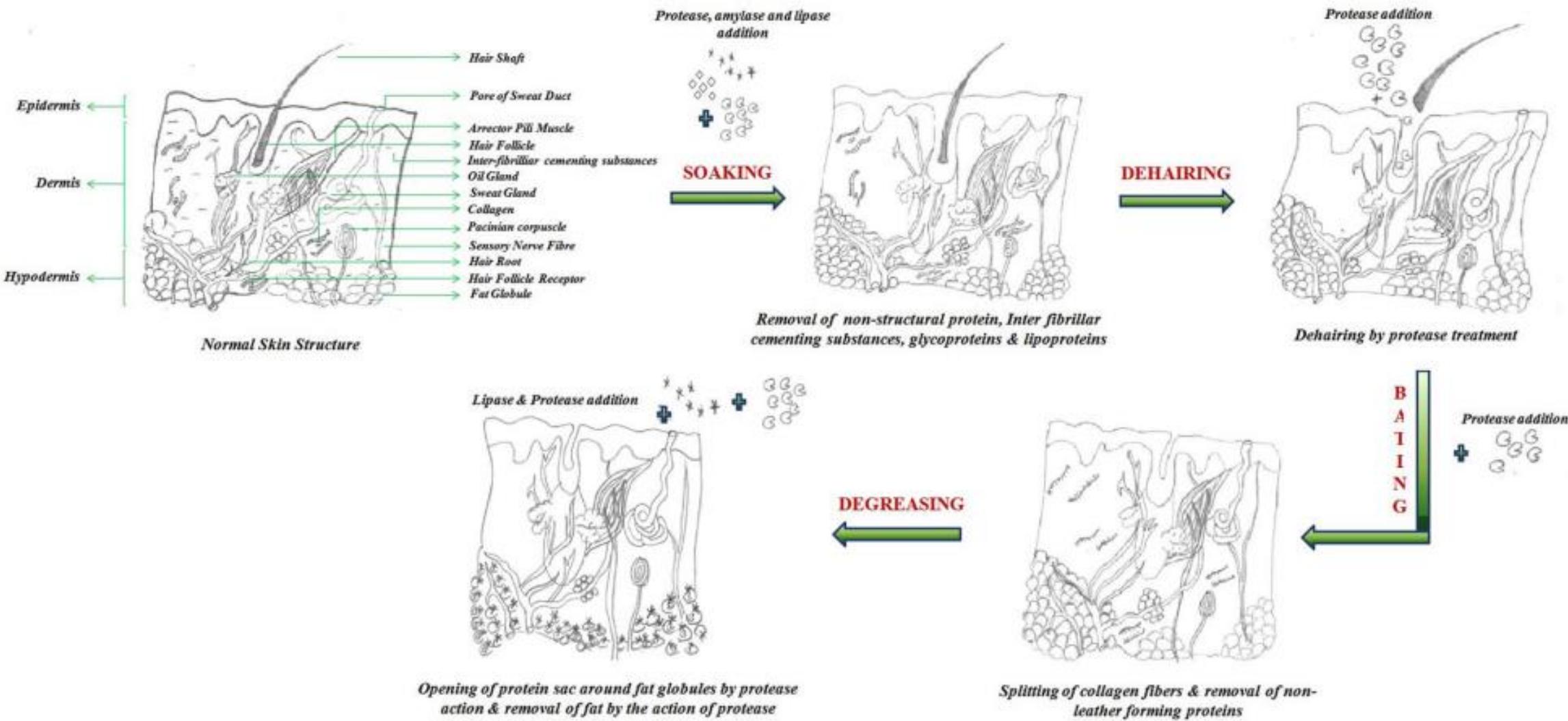
- Papaya contains an enzyme, papain, which is a mild whitening agent and is used in toothpastes to remove enamel stains.



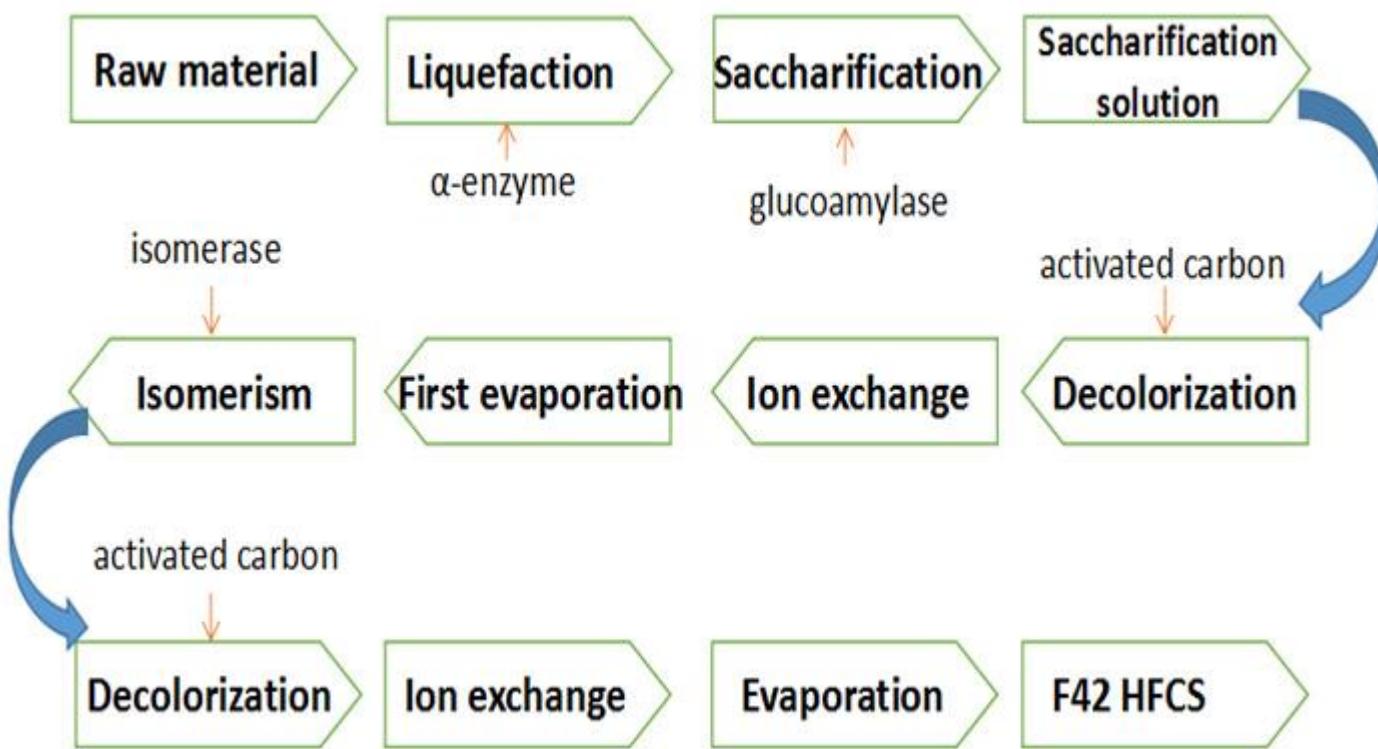
Enzymes in leather industry







Enzymes in food processing!

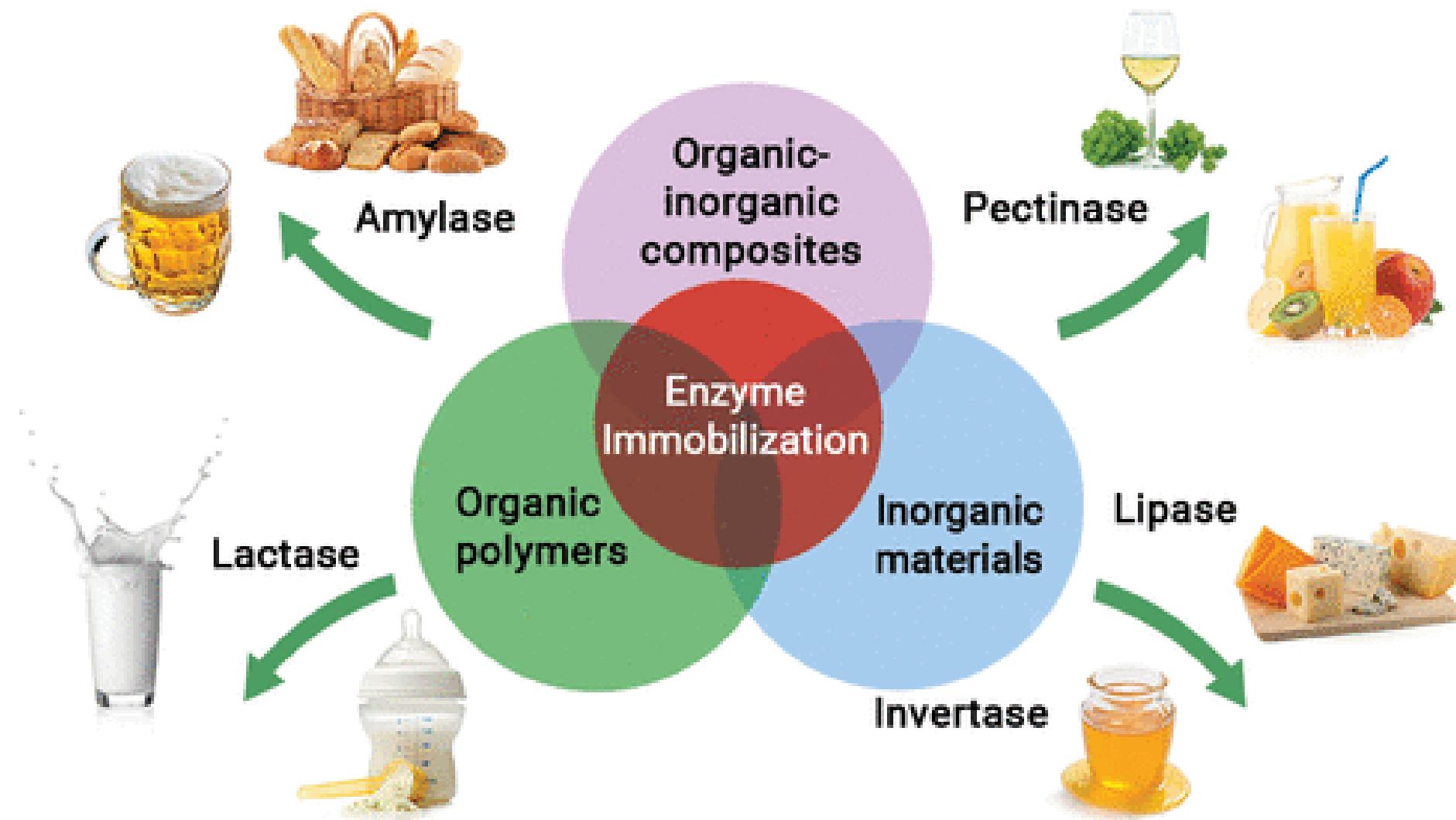


Thank you!

L3 Enzyme application continued

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Indian Institute of Technology - Delhi

Enzyme application in food industry



Enzyme application in food industry

Application Fields of Food rocessing	Enzymes	Technical Benefits
Dairy Industry	Chymosin, lipases, lysozymes	Cheese manufacturing.
	β -galactosidase, lactases	Breaking down lactose to glucose and galactose in milk processing to avoid lactose intolerance.
	lactoperoxidase	Cold sterilisation of milk: milk replacers for calves
	Acid proteinases	Milk coagulation
	Neutral proteinases and peptidases	Accelerated cheese ripening; de-bittering; enzyme modified cheese; production of hypoallergenic milk-based foods
Baking Industry	α -amylases	Degrading starch in flours and controlling the volume and crumb structure of bread.
	β -xylanases	Improving dough handling and dough stability.
	Oxidoreductase	Giving increased gluten strength.
	Lipases	Improving stability of the gas cells in dough.
	Proteases	Reducing the protein in flour

Enzyme application in starch modification

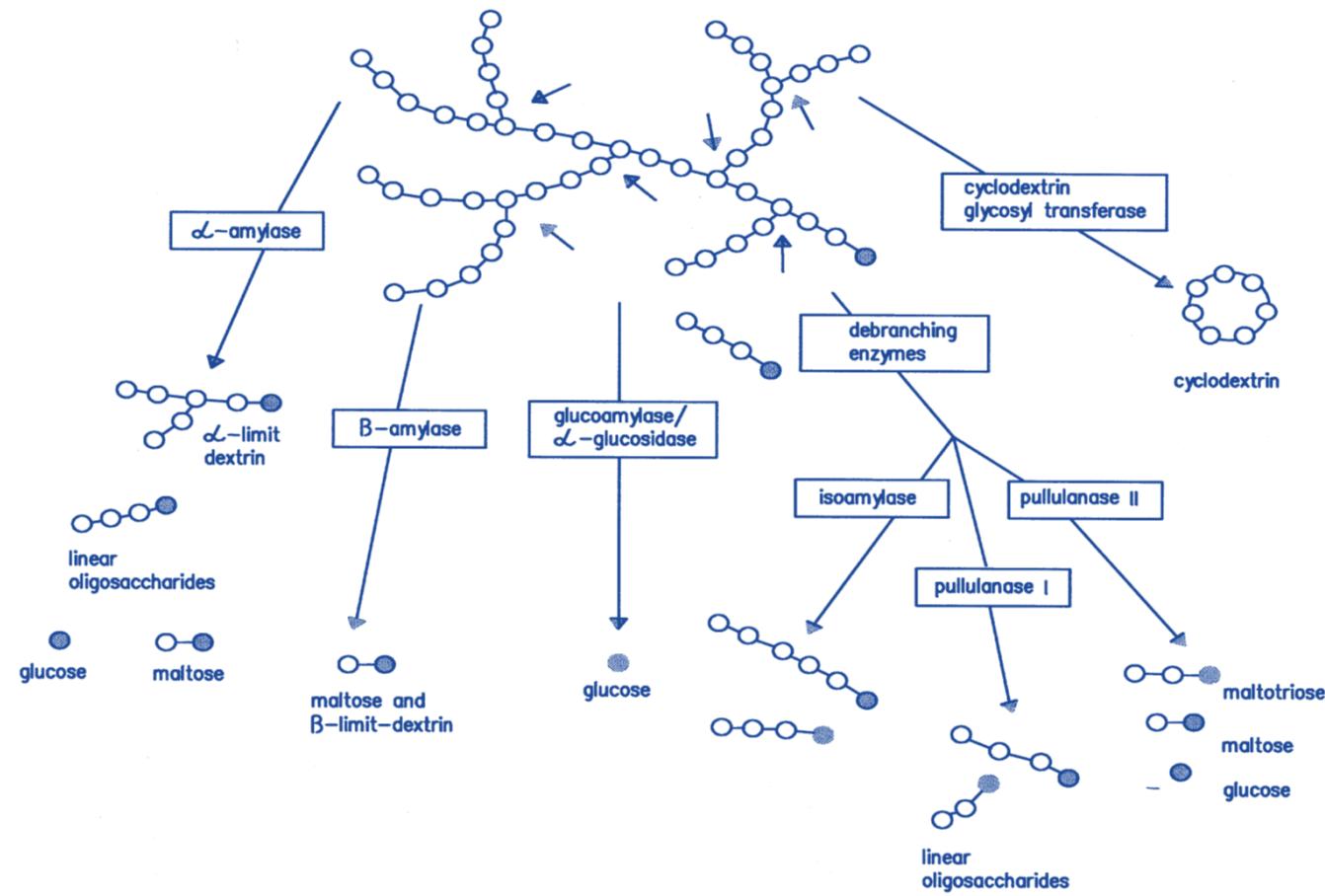
1. Alpha-amylase: This enzyme breaks down the large, complex starch molecules into smaller, simpler sugars like glucose and maltose.

2. Glucoamylase: This enzyme specifically hydrolyzes the alpha-1,4-glucosidic linkages of starch, which convert the glucose and dextrans into glucose. Glucoamylase can be used to produce a high fructose syrup, glucose syrup and corn syrup.

3. Pullulanase: This enzyme breaks down the branches in amylopectin, a component of starch, and thus increases the availability of the glucose units.

4. Beta-amylase: This enzyme breaks down the starch by attacking the alpha-1,4 linkages, releasing maltose and dextrin as the main products. Beta-amylase is used in production of beer, malt syrups, and malted milk.

5. Debranching enzymes: these enzymes are a group of enzymes which include alpha-1,6-glucosidase and limit dextrinase.

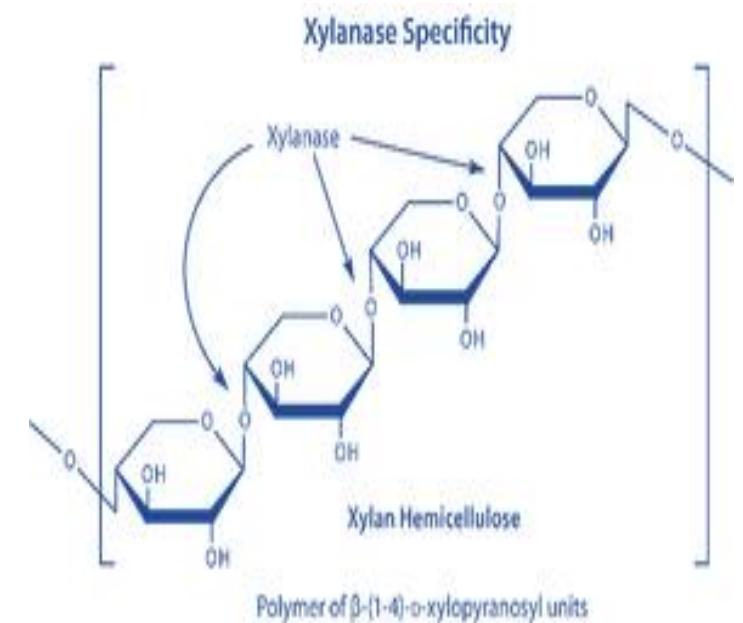


Enzyme application of Xylanase

Xylanase – preferred are those that act on the non-water soluble arabinoxylan fraction. It interferes with the formation of gluten network. Removal of not-extractable with water arabinoxylan fraction results in increase of high molecular weight solubilized in water arabinoxylans that in turns increase viscosity and dough stability; provide better crumb texture and increased loaf volume.

Increasing the efficiency of animal feed: Xylanase can also be added to animal feed to improve its nutritional value and increase the efficiency of animal growth. The enzyme breaks down the xylan in the feed, which makes the nutrients in the feed more accessible to the animals. This leads to faster growth rates and improved feed conversion efficiency.

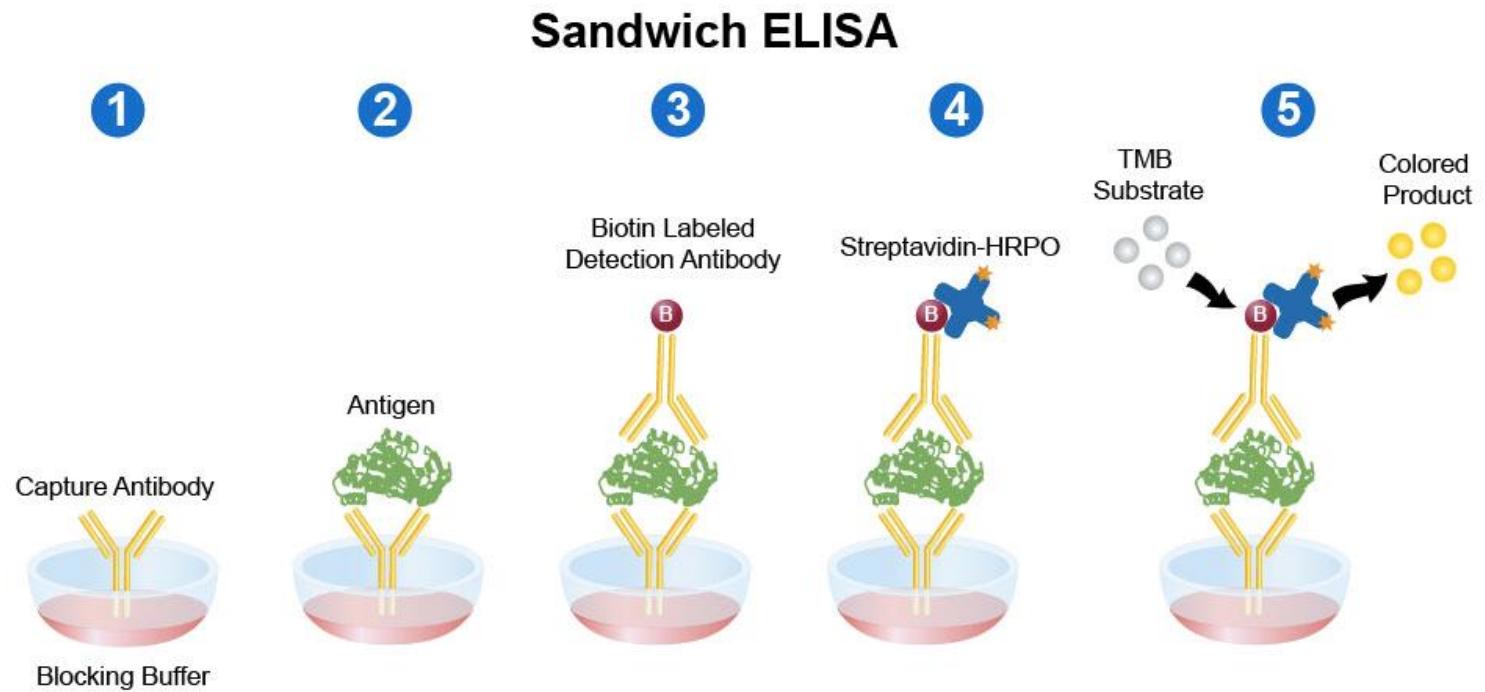
Xylanase can be used to enhance the nutritional value of food products by breaking down the xylan and releasing the trapped nutrients.



Xylanase can be used to break down the xylan in plant-based materials, such as corn stalks, corn cobs, and wood chips, which are used to make bioethanol. This allows more of the starch in the material to be converted into ethanol, which results in more efficient and cost-effective bioethanol production.

Enzyme application in analytical industry

test strips w/ Long shelf-life of upto 5 years
Lifetime Warranty



Enzymes as therapeutic agents

- Therapeutic enzymes have a broad variety of specific uses
 - Oncolytics
 - Anticoagulants
 - Thrombolytics
 - Replacements for metabolic deficiencies
 - Digestive aids
 - Metabolic storage disorders, etc
 - Miscellaneous enzymes of diverse function

Enzyme	Reaction	Use
Asparaginase	L-Asparagine H ₂ O L-aspartate + NH ₃	Leukaemia
Collagenase	Collagen hydrolysis	Skin ulcers
Glutaminase	L-Glutamine H ₂ O L-glutamate + NH ₃	Leukaemia
Hyaluronidase a	Hyaluronate hydrolysis	Heart attack
Lysozyme	Bacterial cell wall hydrolysis	Antibiotic
Rhodanase b	S ₂ O ₃ ²⁻ + CN ⁻ SO ₃ ²⁻ + SCN ⁻	Cyanide poisoning
Ribonuclease	RNA hydrolysis	Antiviral
β-Lactamase	Penicillin penicilloate	Penicillin allergy
Streptokinase c	Plasminogen plasmin	Blood clots
Trypsin	Protein hydrolysis	Inflammation
Uricase d	Urate + O ₂ allantoin	Gout
Urokinase e	Plasminogen plasmin	Blood clots

Enzyme application in medical treatments

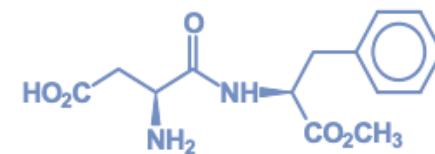
Challenges of Using Enzymes in Medical Treatments

- Enzymes are too large to enter many of the body's cells.
- Antigenic proteins elicit immune responses.
- Effective half-life in the circulatory system may be only a few minutes.
- Must be very pure and administered in very small concentrations.
- Must exhibit low K_m and high V_{max} to be maximally efficient at low concentrations.

Uricase is an enzyme that converts uric acid, which is a waste product of purine metabolism, into a more soluble form called allantoin. Uric acid can build up in the body and form crystals, which can deposit in joints and cause inflammation and pain, a condition known as gout.

Advantages of Biocatalysis

- Enzymes have a very good selectivity :
Stereoselectivity – enantioselectivity in most cases are > 99 %
Chemo- and regioselectivity – react on one location over another similar group without the need for protection groups.
- Mild reaction conditions:
aqueous solvent, room temperature, normal pressure, neutral pH
- Environmentally friendly: enzymes are biodegradable
- Fewer side reactions
- High efficiency
- Cheap and simple starting material can be used.
- Overall lower cost of production.



Aspartame (200)

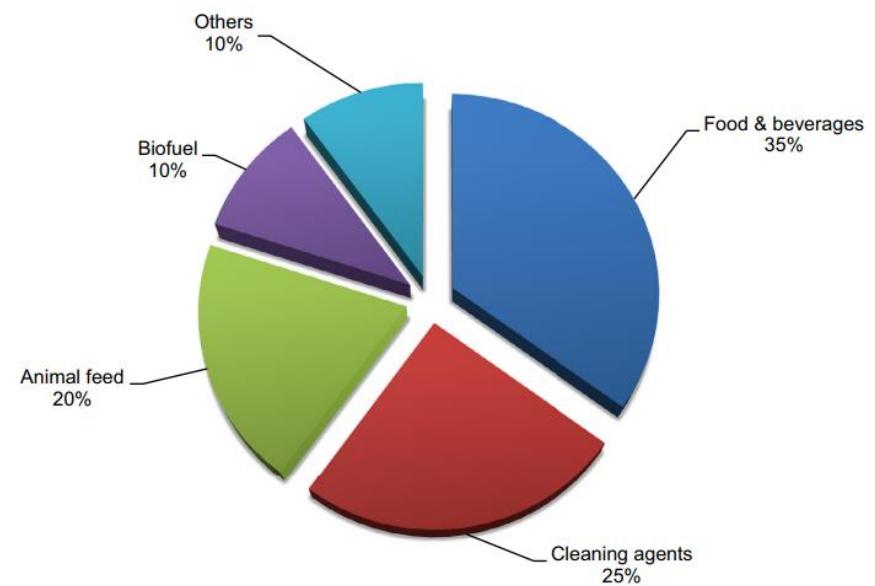
Table 1 Enzymes commonly used in organic synthesis

Enzymes	Reactions
Esterase, lipases	Ester hydrolysis, formation
Amidases (proteases, acylases)	Amide hydrolysis, formation
Dehydrogenases	Oxidoreduction of alcohols and ketones
Oxidases (mono- and dioxygenases)	Oxidation
Peroxidases	Oxidation, epoxidation, halohydration
Kinases	Phosphorylation (ATP-dependent)
Aldolases, transketolases	Aldol reaction (C–C bond)
Glycosidases, glycosyltransferases	Glycosidic bond formation
Phosphorylases, phosphatases	Formation and hydrolysis of phosphate
Sulphotransferases	Formation of sulphate esters
Transaminases	Amino acid synthesis (C–N bond)
Hydrolases	Hydrolysis
Isomerase, lyases, hydratases	Isomerization, addition, elimination, replacement

Enzyme application of Lipase enzyme

1. Food industry: Lipases are used in the production of various types of food, such as cheeses, chocolate, and flavors.
2. Biofuel production: Lipase enzymes can be used to break down vegetable oils and animal fats into fatty acids, which can then be converted into biodiesel.
3. Detergent industry: Lipases are used in laundry detergents to help remove stains from clothes by breaking down the fats and oils that make up the stains.
4. Pharmaceutical industry: Lipases are used in the production of certain drugs, such as oral lipid-based formulations, which are designed to improve the solubility and bioavailability of drugs.
5. Biotechnology: Lipases are used in various biotechnology applications such as the synthesis of natural and non-natural fatty acids and in the production of biodegradable plastics.
6. Cosmetic Industry: Lipases are used in the production of certain cosmetics, particularly in moisturizers and emollients that are used to hydrate and soften the skin.
7. Waste water treatment: Lipases are also used in the treatment of oily waste-water by breaking down oils and fats present in the waste-water and converting them into smaller molecules that can be more easily removed.

D. Guerrand: OCL 2017, 24(4), D403

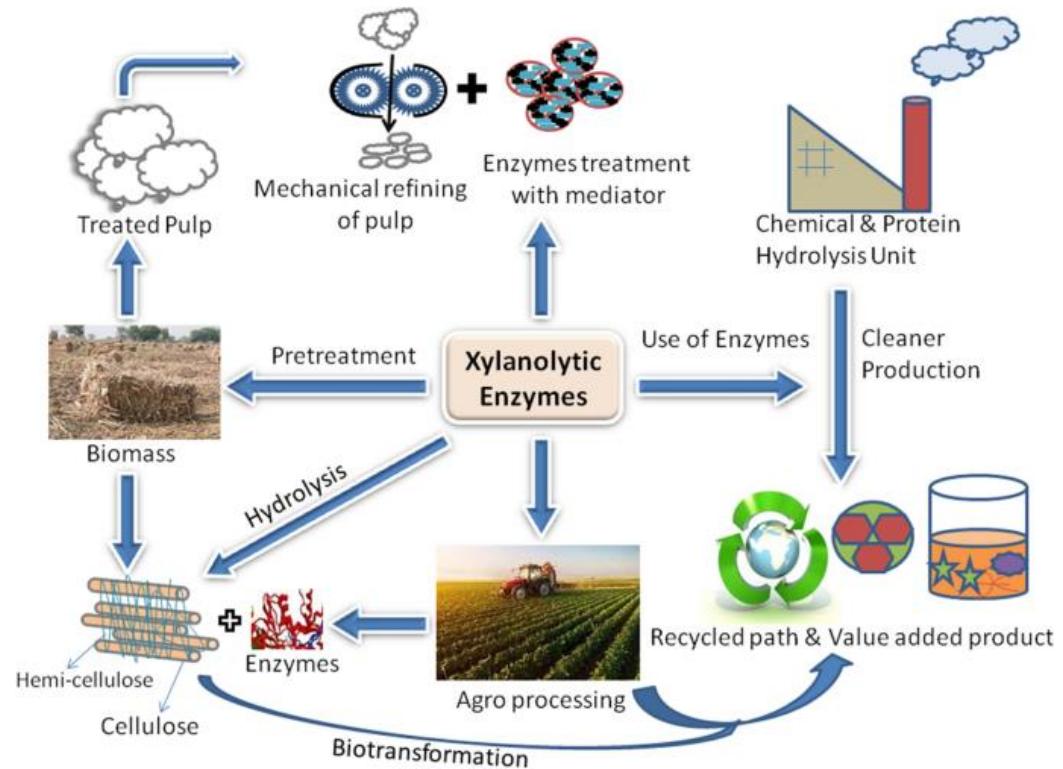


Enzyme application in paper industry

One major application of enzymes in the pulp and paper industry is in the production of paper from wood fibers. The fibers in wood are bound together by lignin, a complex polymer that is difficult to break down. Enzymes known as **laccases** and **manganese peroxidase** can be used to break down lignin, making the fibers easier to process and resulting in a stronger, whiter, and brighter paper.

Another application of enzymes in the pulp and paper industry is in the recycling of paper. Enzymes like **cellulases** and **xylanases** are used to break down the fibers in recycled paper, making it possible to recycle paper more efficiently and reduce the need for virgin fibers.

Enzymes are also used to improve the efficiency of the bleaching process, which is used to remove lignin and other impurities from the fibers. Enzymes like **peroxidases**, **laccases** and **lignin peroxidases** can be used to reduce the amount of chlorine or chlorine compounds needed in the bleaching process, reducing the environmental impact and production costs.

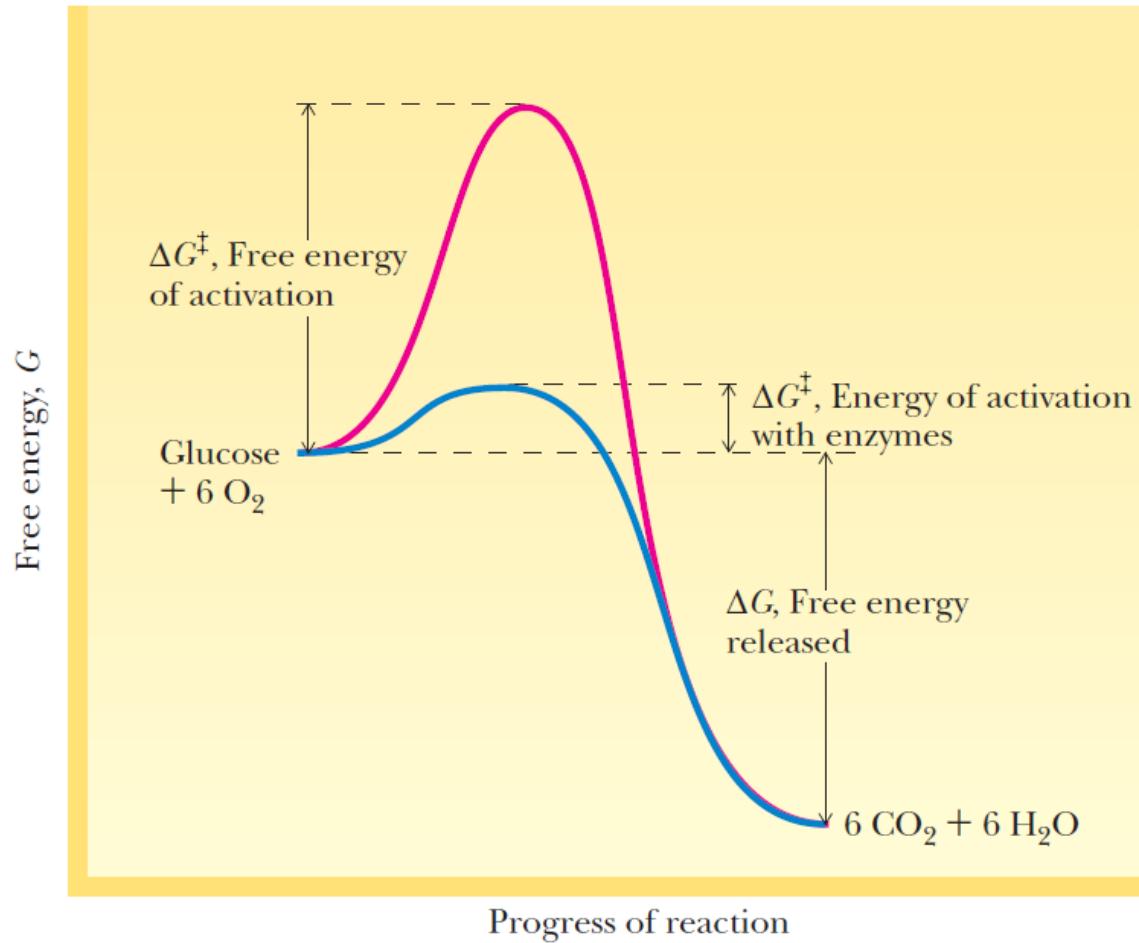


L4 Enzyme functional nature

Ravikrishnan Elangovan,
Department of Biochemical Engg and Biotechnology
Indian Institute of Technology - Delhi

Enzymes are catalyst,
That accelerate chemical reactions

Mostly enzymes are proteins,
some RNA enzymes also exist



Enzyme catalytic power



TABLE 14.1

A Comparison of Enzyme-Catalyzed Reactions and Their Uncatalyzed Counterparts

Reaction	Enzyme	Uncatalyzed Rate, v_u (sec $^{-1}$)	Catalyzed Rate, v_e (sec $^{-1}$)	v_e/v_u
Fructose-1,6-bisP \longrightarrow fructose-6-P + P _i	Fructose-1,6-bisphosphatase	2×10^{-20}	21	1.05×10^{21}
(Glucose) _n + H ₂ O \longrightarrow (glucose) _{n-2} + maltose	β -amylase	1.9×10^{-15}	1.4×10^3	7.2×10^{17}
DNA, RNA cleavage	Staphylococcal nuclease	7×10^{-16}	95	1.4×10^{17}
CH ₃ —O—PO ₃ ²⁻ + H ₂ O \longrightarrow CH ₃ OH + HPO ₄ ²⁻	Alkaline phosphatase	1×10^{-15}	14	1.4×10^{16}
$\text{H}_2\text{N}-\overset{\text{O}}{\underset{\parallel}{\text{C}}}-\text{NH}_2 + 2 \text{H}_2\text{O} + \text{H}^+ \longrightarrow 2 \text{NH}_4^+ + \text{HCO}_3^-$	Urease	3×10^{-10}	3×10^4	1×10^{14}
$\text{R}-\overset{\text{O}}{\underset{\parallel}{\text{C}}}-\text{O}-\text{CH}_2\text{CH}_3 + \text{H}_2\text{O} \longrightarrow \text{RCOOH} + \text{HOCH}_2\text{CH}_3$	Chymotrypsin	1×10^{-10}	1×10^2	1×10^{12}
Glucose + ATP \longrightarrow Glucose-6-P + ADP	Hexokinase	$<1 \times 10^{-13}$	1.3×10^{-3}	$>1.3 \times 10^{10}$
CH ₃ CH ₂ OH + NAD ⁺ \longrightarrow $\text{CH}_3\overset{\text{O}}{\underset{\parallel}{\text{C}}}\text{H} + \text{NADH} + \text{H}^+$	Alcohol dehydrogenase	$<6 \times 10^{-12}$	2.7×10^{-5}	$>4.5 \times 10^6$
CO ₂ + H ₂ O \longrightarrow HCO ₃ ⁻ + H ⁺	Carbonic anhydrase	10^{-2}	10^5	1×10^7
Creatine + ATP \longrightarrow Cr-P + ADP	Creatine kinase	$<3 \times 10^{-9}$	4×10^{-5}	$>1.33 \times 10^4$

Adapted from Koshland, D., 1956. Molecular geometry in enzyme action. *Journal of Cellular Comparative Physiology*, Supp. 1, 47:217; and Wolfenden, R., 2006. Degrees of difficulty of water-consuming reactions in the absence of enzymes. *Chemical Reviews* 106:3379–3396.

Enzyme specificity

Enzyme specificity:

- Molecular recognition based on shape and charge of the molecule at atomic level
- Even chiral groups will be distinguished

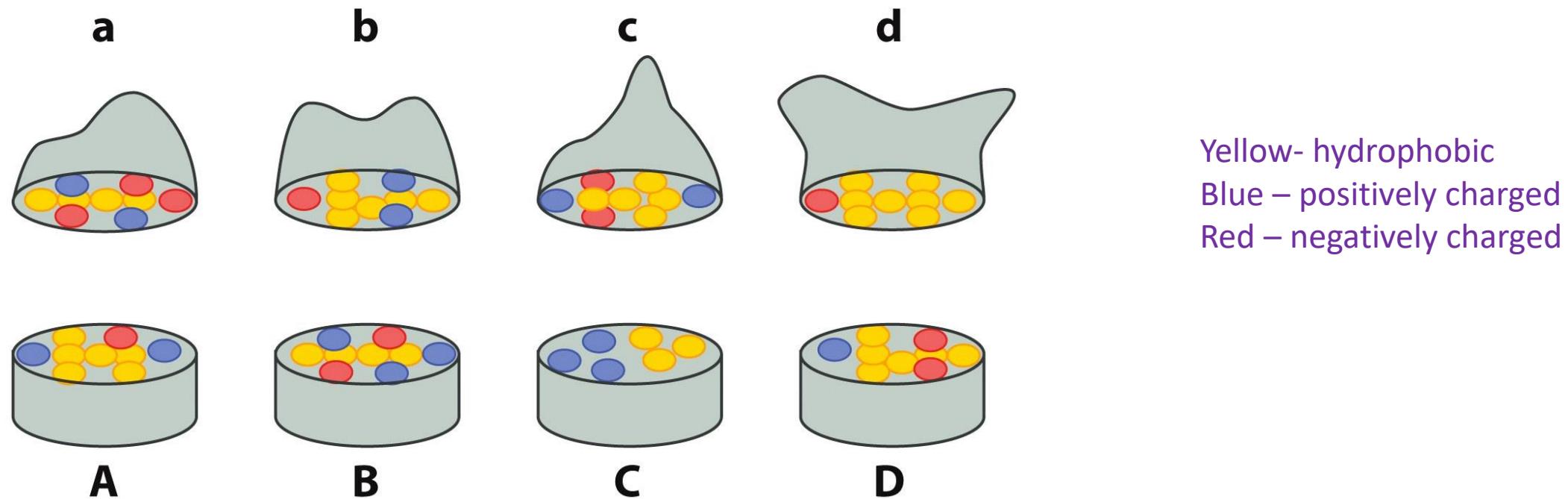


Figure 13.8 The Molecules of Life (© Garland Science 2013)

Enzyme cofactors and coenzymes

Cofactors: Are non protein components that are required for function of an Enzyme

e.g., Ca²⁺ ions

Prosthetic group: Organic molecule cofactor, tightly bound to enzyme. Eg., Haem group

Coenzyme: Organic molecule cofactor. They are chemically changed in course of enzymatic reaction Eg., NADH

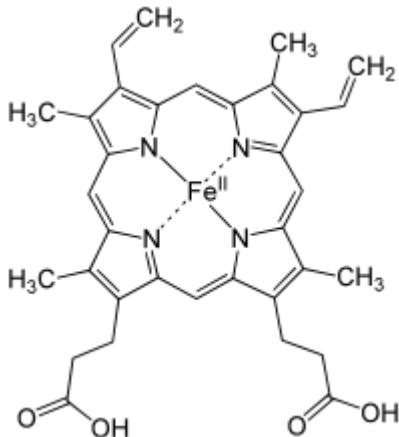
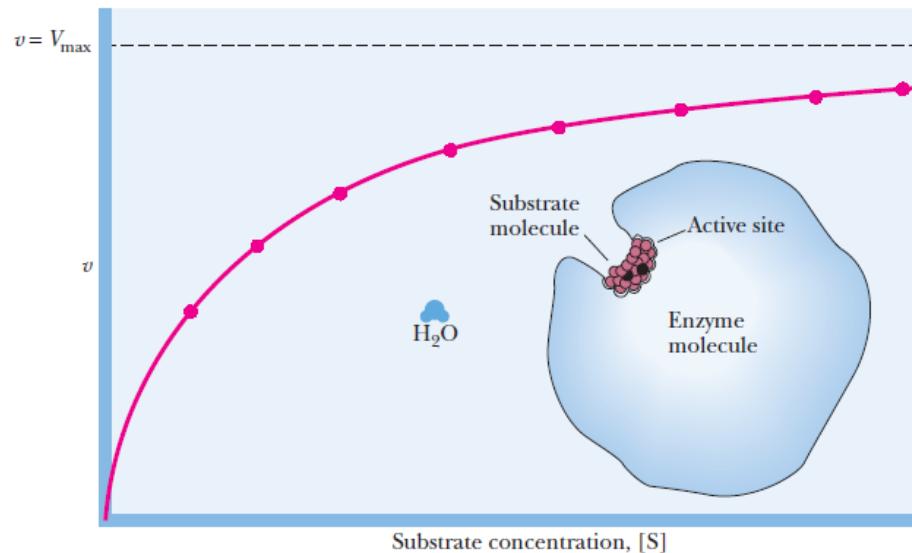


TABLE 13.2 Enzyme Cofactors: Some Metal Ions and Coenzymes and the Enzymes with Which They Are Associated

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

Enzyme kinetics



Michaelis–Menten equation

$$v = \frac{V_{\max}[S]}{K_m + [S]}$$

$$k_2 = \frac{V_{\max}}{[E_T]} = k_{\text{cat}}$$

TABLE 13.3 K_m Values for Some Enzymes

Enzyme	Substrate	K_m (mM)
Carbonic anhydrase	CO_2	12
Chymotrypsin	<i>N</i> -Benzoyltyrosinamide	2.5
	Acetyl-L-tryptophanamide	5
	<i>N</i> -Formyltyrosinamide	12
	<i>N</i> -Acetyltyrosinamide	32
	Glycyltyrosinamide	122
Hexokinase	Glucose	0.15
	Fructose	1.5
β -Galactosidase	Galactose	0.01

TABLE 13.4 Values of k_{cat} (Turnover Number) for Some Enzymes

Enzyme	k_{cat} (sec ⁻¹)
Catalase	40,000,000
Carbonic anhydrase	1,000,000
Acetylcholinesterase	14,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase I	15
Lysozyme	0.5

Catalytic efficiency of an Enzyme

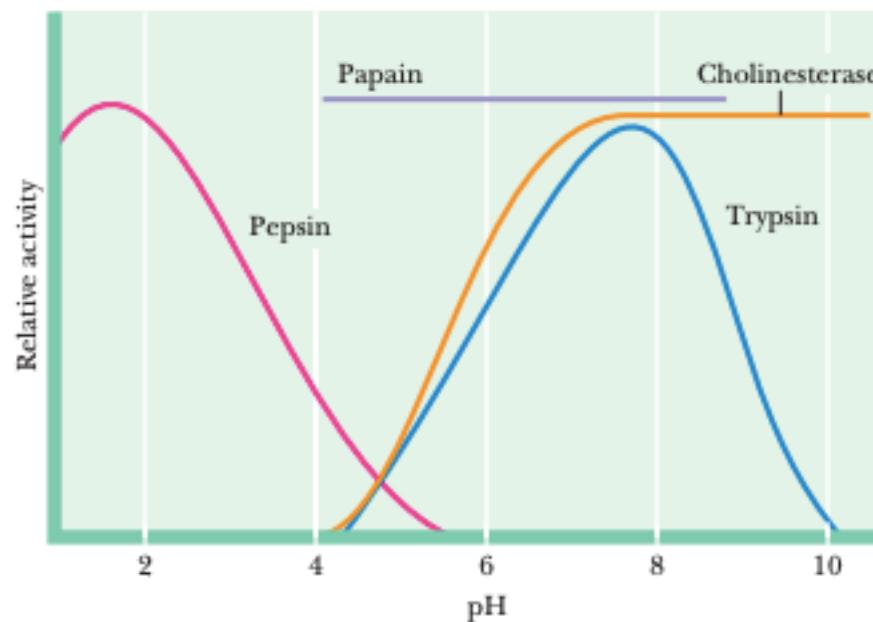
$$k_2 = \frac{V_{\max}}{[E_T]} = k_{\text{cat}}$$

Enzyme	Substrate	k_{cat} (sec $^{-1}$)	K_m (M)	k_{cat}/K_m (M $^{-1}$ sec $^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO ₂	1×10^6	0.012	8.3×10^7
	HCO ₃ ⁻	4×10^5	0.026	1.5×10^7
Catalase	H ₂ O ₂	4×10^7	1.1	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	800	5×10^{-6}	1.6×10^8
	Malate	900	2.5×10^{-5}	3.6×10^7
Triosephosphate isomerase	Glyceraldehyde-3-phosphate*	4.3×10^3	1.8×10^{-5}	2.4×10^8
β -Lactamase	Benzylpenicillin	2×10^3	2×10^{-5}	1×10^8

* K_m for glyceraldehyde-3-phosphate is calculated on the basis that only 3.8% of the substrate in solution is unhydrated and therefore reactive with the enzyme.

Adapted from Fersht, A., 1985. *Enzyme Structure and Mechanism*, 2nd ed. New York: W. H. Freeman.

Enzyme activity as function of pH



Optimum pH of Some Enzymes	
Enzyme	Optimum pH
Pepsin	1.5
Catalase	7.6
Trypsin	7.7
Fumarase	7.8
Ribonuclease	7.8
Arginase	9.7

A decrease in pH (more acidic) causes an increase in the concentration of hydrogen ions (H^+) in solution, which can cause the ionization of acidic functional groups such as carboxyl groups (-COOH) resulting in the formation of carboxylate groups (-COO-). This can cause the enzyme to lose its activity by altering its shape and function. Similarly, an increase in pH (more basic) can cause the ionization of basic functional groups such as amino groups (-NH₃⁺) and this can also affect the enzyme's activity.

Enzyme activity as function of temperature

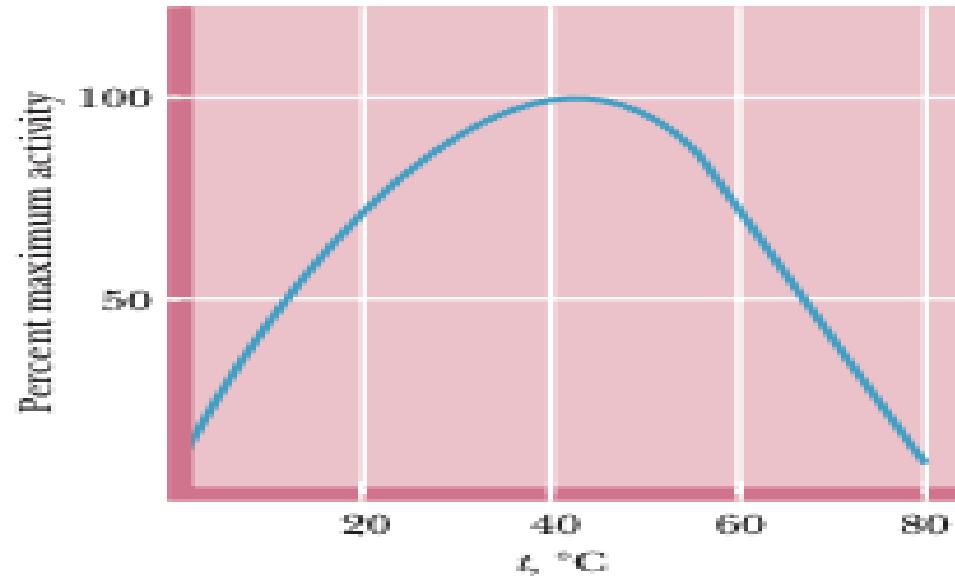
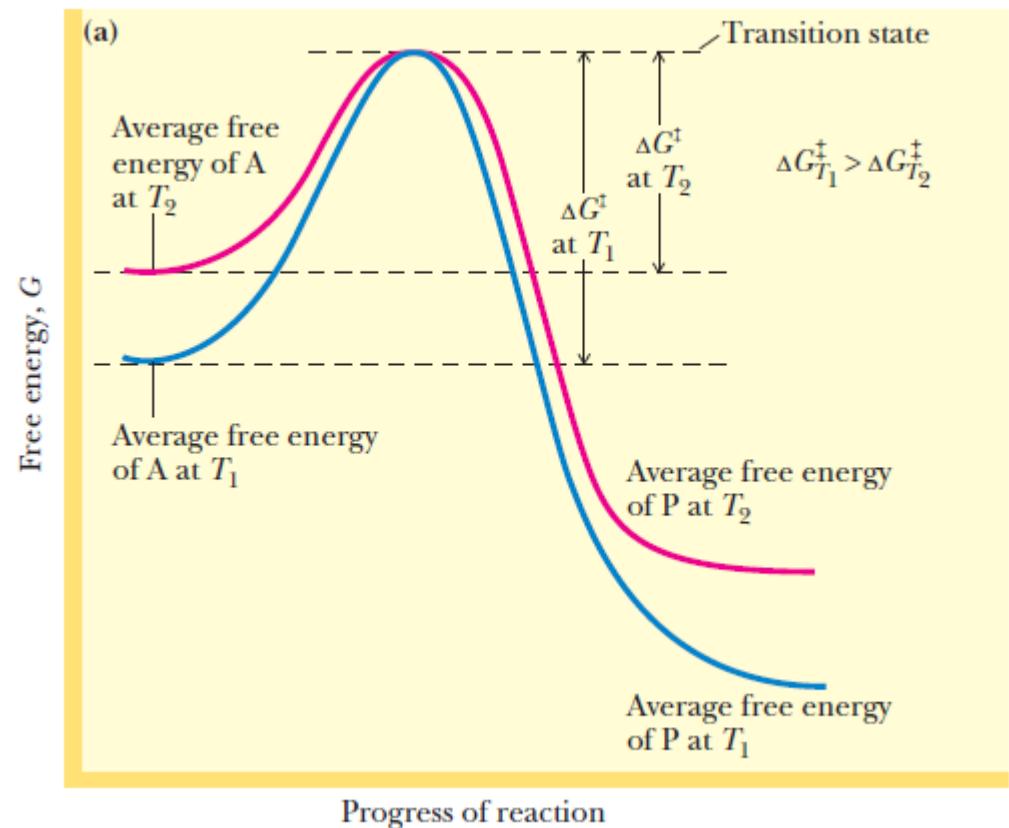
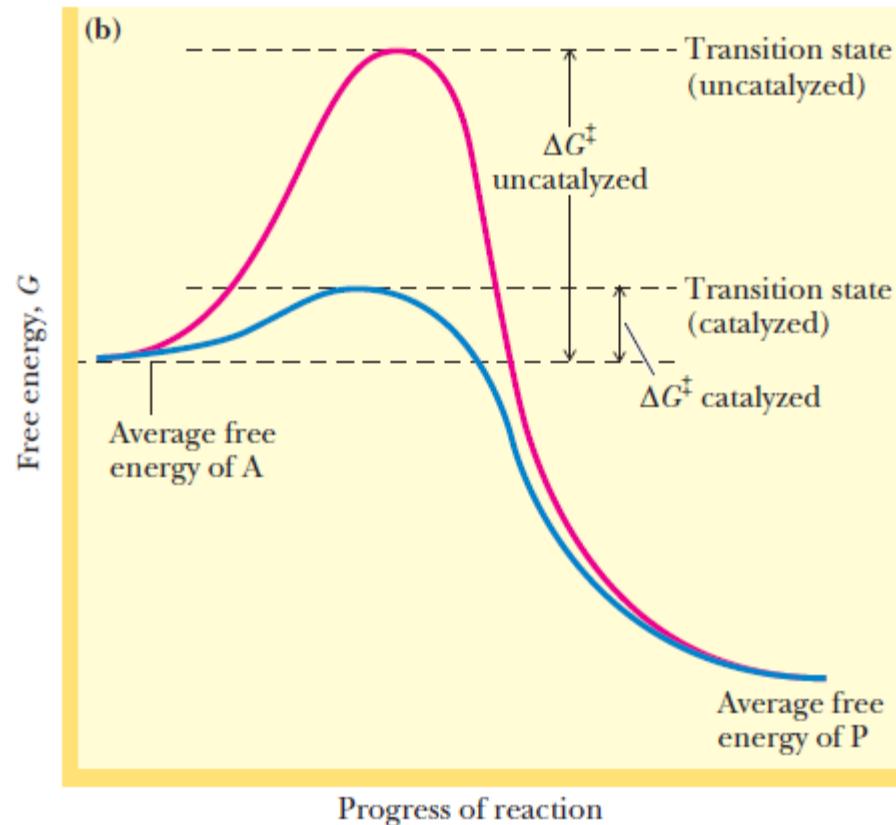


FIGURE 13.12 The effect of temperature on enzyme activity.

Enzyme thermodynamics

Mechanism :Catalysts Lower the Free Energy of Activation for a Reaction



Gibbs free energy

- Thermodynamics: changes in free energy, entropy, ...

$$\Delta G = \Delta H - T \cdot \Delta S$$

$$\Delta G = (\Delta U + P \cdot \Delta V) - T \cdot \Delta S$$

- For nearly all biochemical reactions ΔV is small and ΔH is almost equal to ΔU
- Hence, we can write:

$$\boxed{\Delta G = \Delta U - T \cdot \Delta S}$$

If ΔG is negative

Energy was released, products are simpler, greater entropy (2nd Law of Thermodynamics)
Exergonic / exothermic reaction (spontaneous)

If ΔG is positive

Energy input, product more complex, energy needed to go against 2nd Law
Endergonic / endothermic (non-spontaneous)

The Enthalpic term

- Changes in bonding
- van der Waals
- Hydrogen bonding
- Charge interactions

The Entropic term

- Changes the arrangement of the solvent or counterions
- Reflects the degrees of freedom
- Rotational & Translational changes

Protein folding

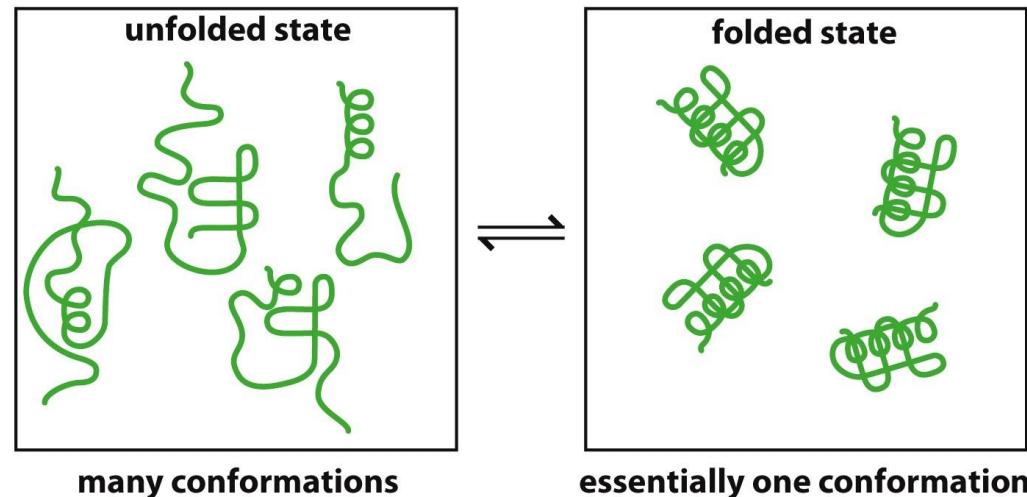


Figure 10.13 The Molecules of Life (© Garland Science 2013)

Protein folding is a spontaneous process?

$\Delta G < 0 ???$

Protein folding



$$K_{\text{folding}} = \frac{(F)}{(U)} = \frac{1}{K_{\text{unfolding}}}$$

$$\Delta G^\circ_{\text{unfolding}} = \Delta H^\circ - T \Delta S^\circ$$

Enthalpy change in protein folding

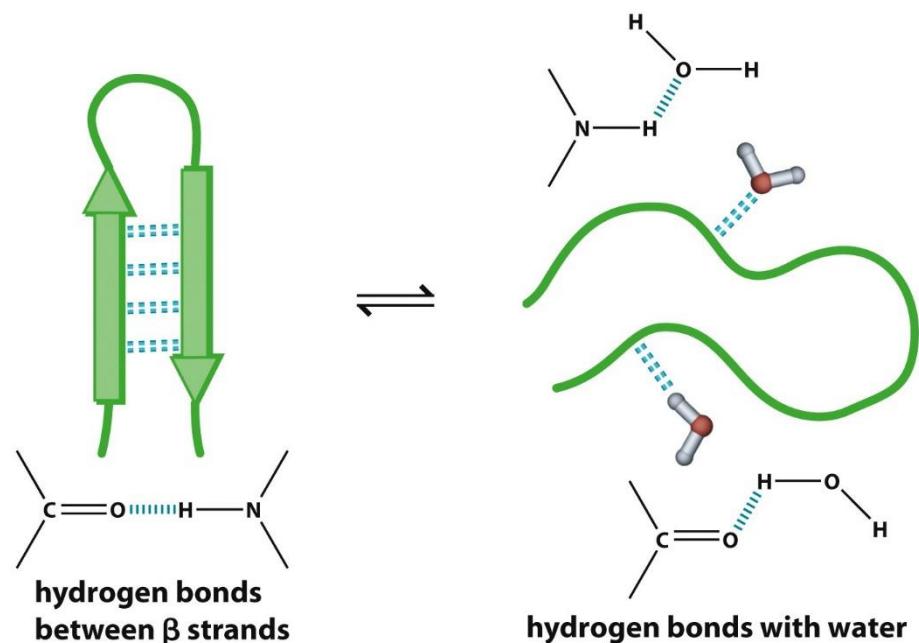


Figure 10.14 The Molecules of Life (© Garland Science 2013)

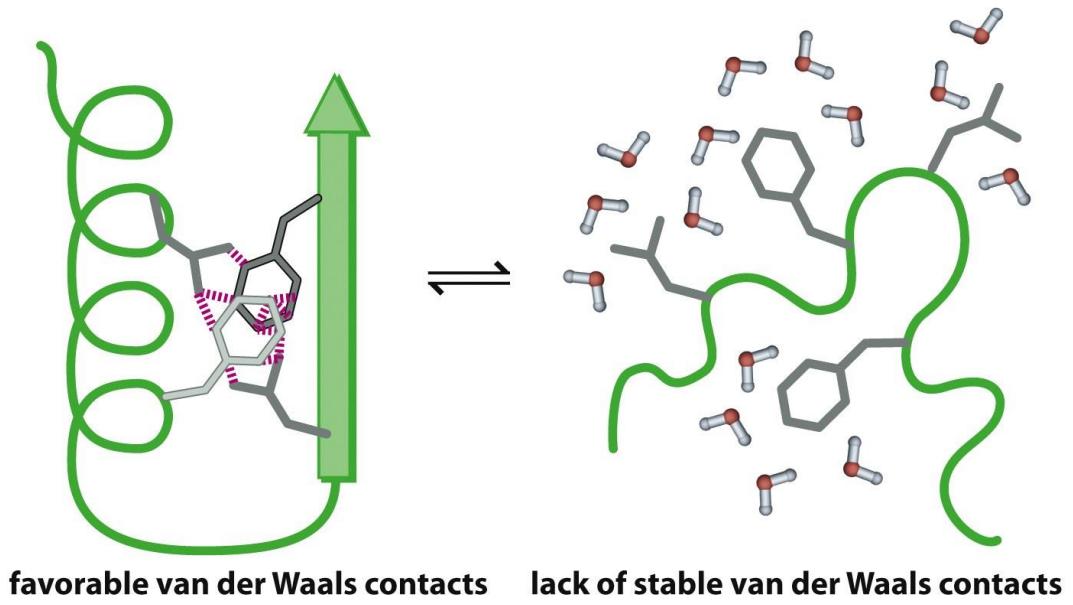


Figure 10.15 The Molecules of Life (© Garland Science 2013)

Entropy change in protein folding

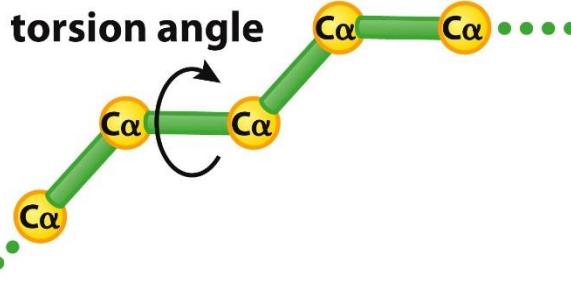


Figure 10.18 The Molecules of Life (© Garland Science 2013)

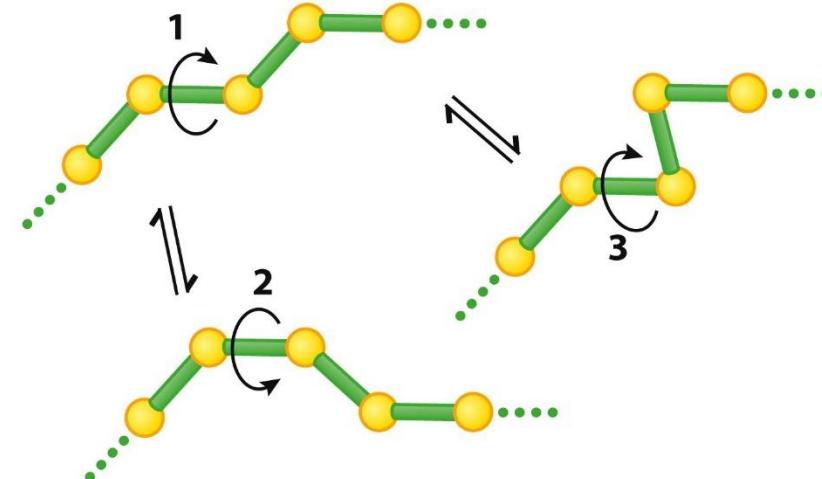


Figure 10.19 The Molecules of Life (© Garland Science 2013)

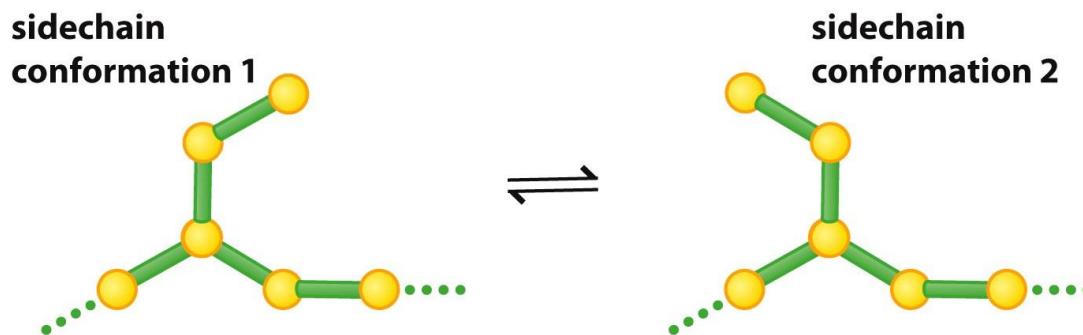


Figure 10.20 The Molecules of Life (© Garland Science 2013)

Entropy contribution from water

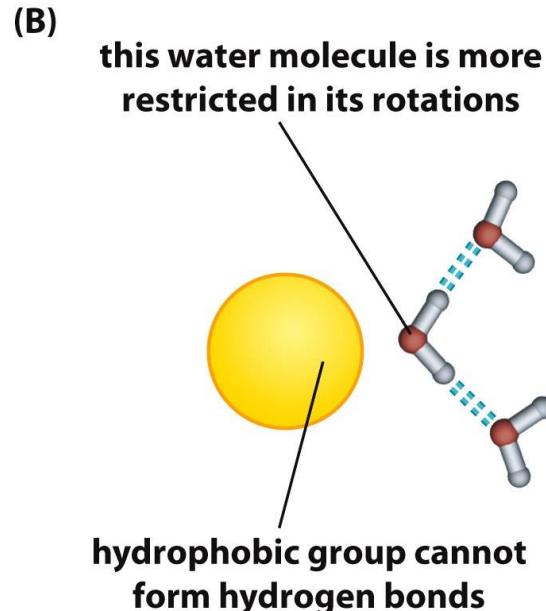
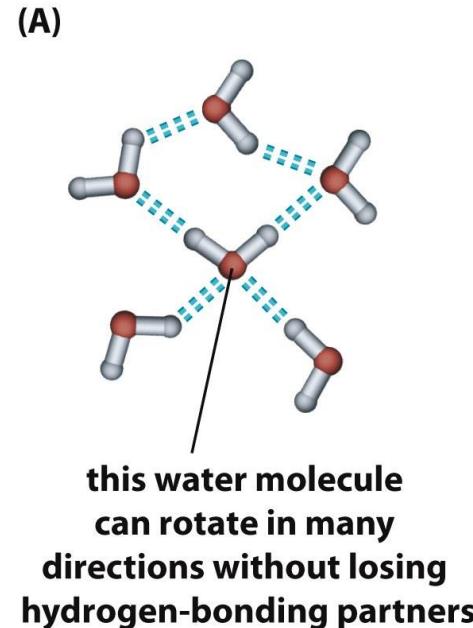


Figure 10.21 The Molecules of Life (© Garland Science 2013)

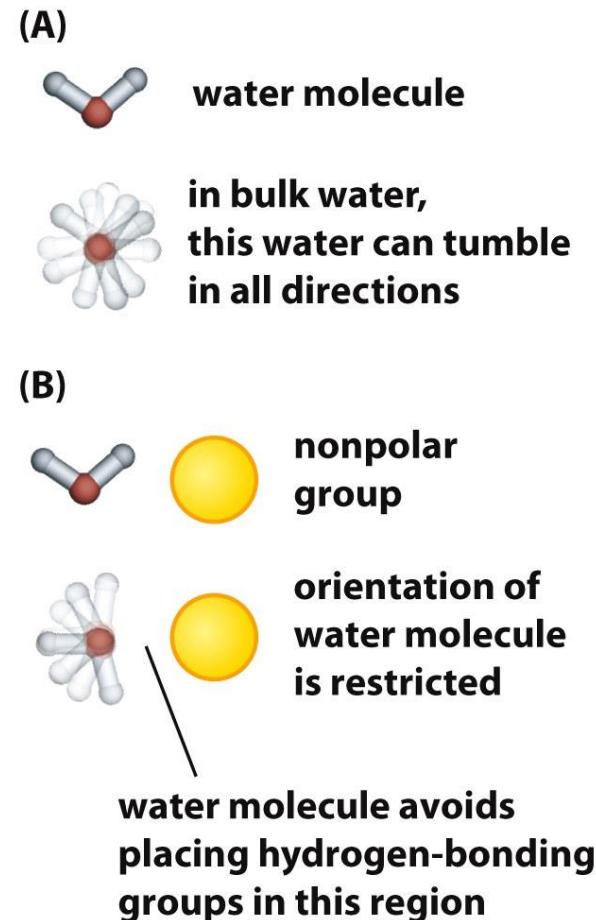


Figure 10.22 The Molecules of Life (© Garland Science 2013)

Protein folding

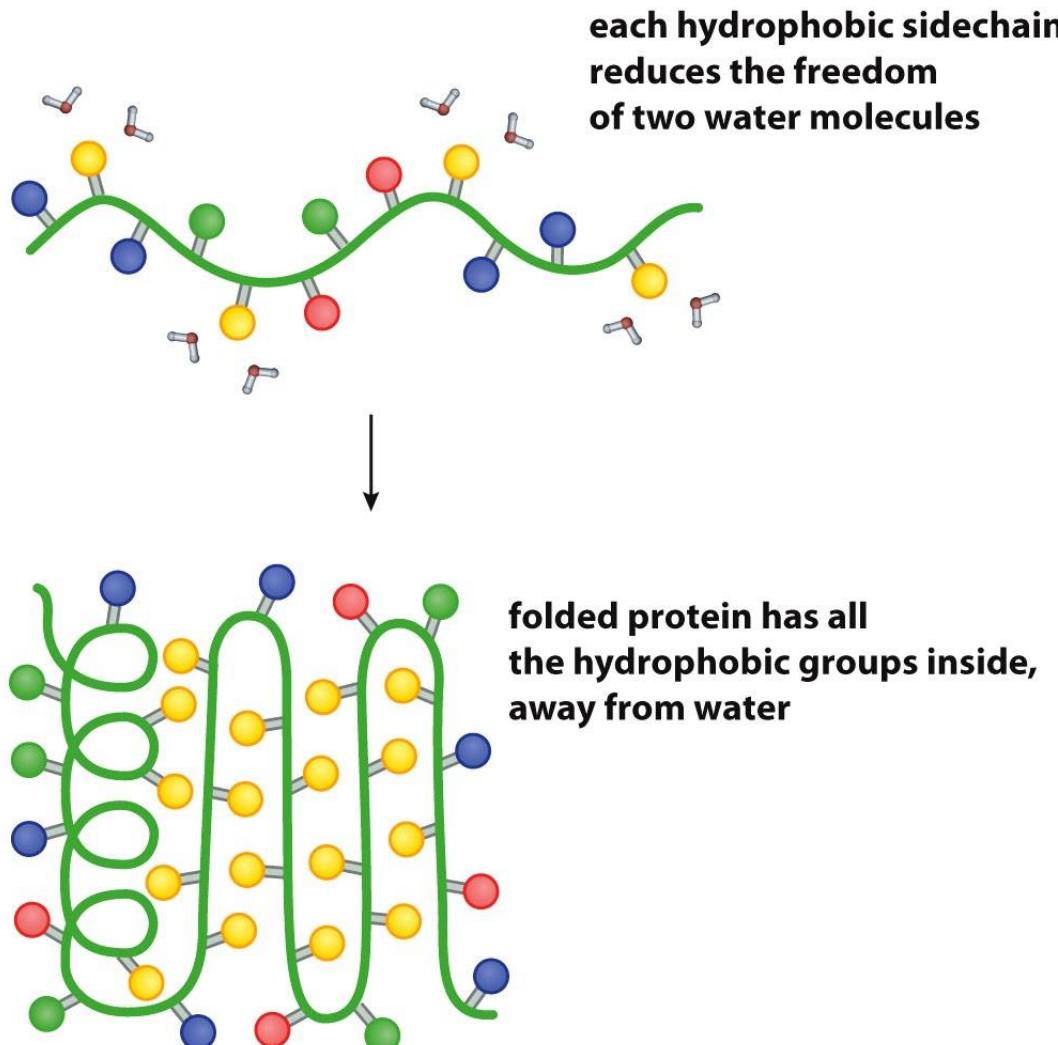
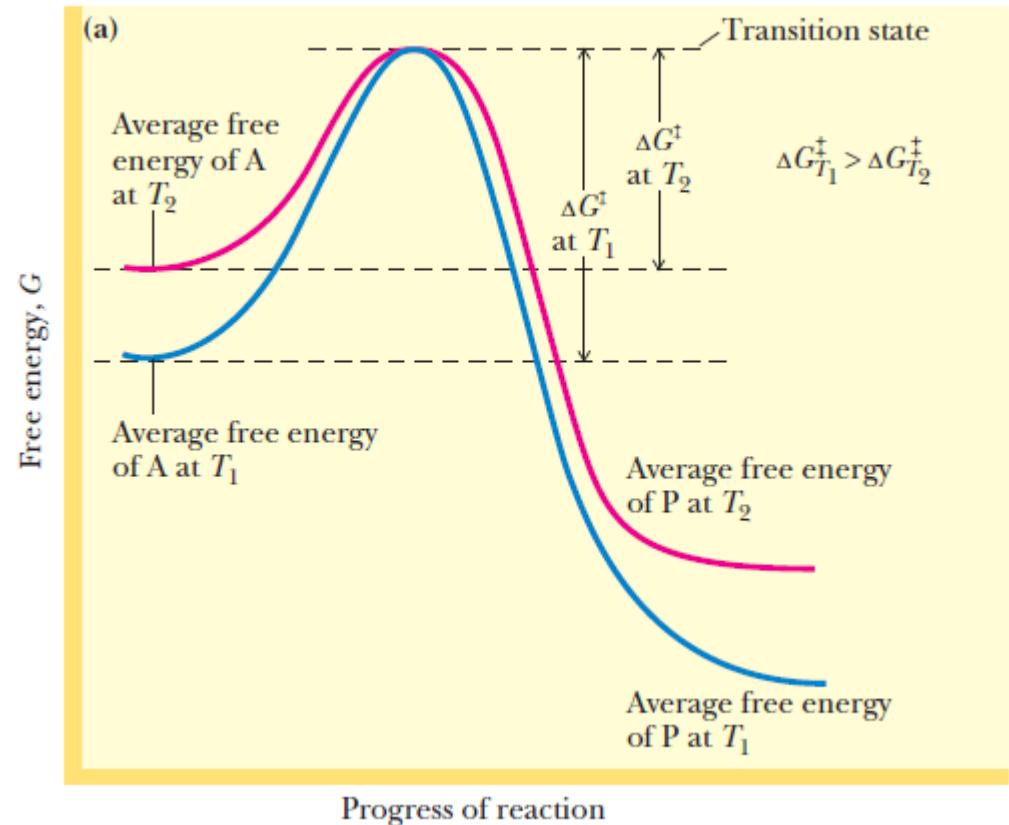
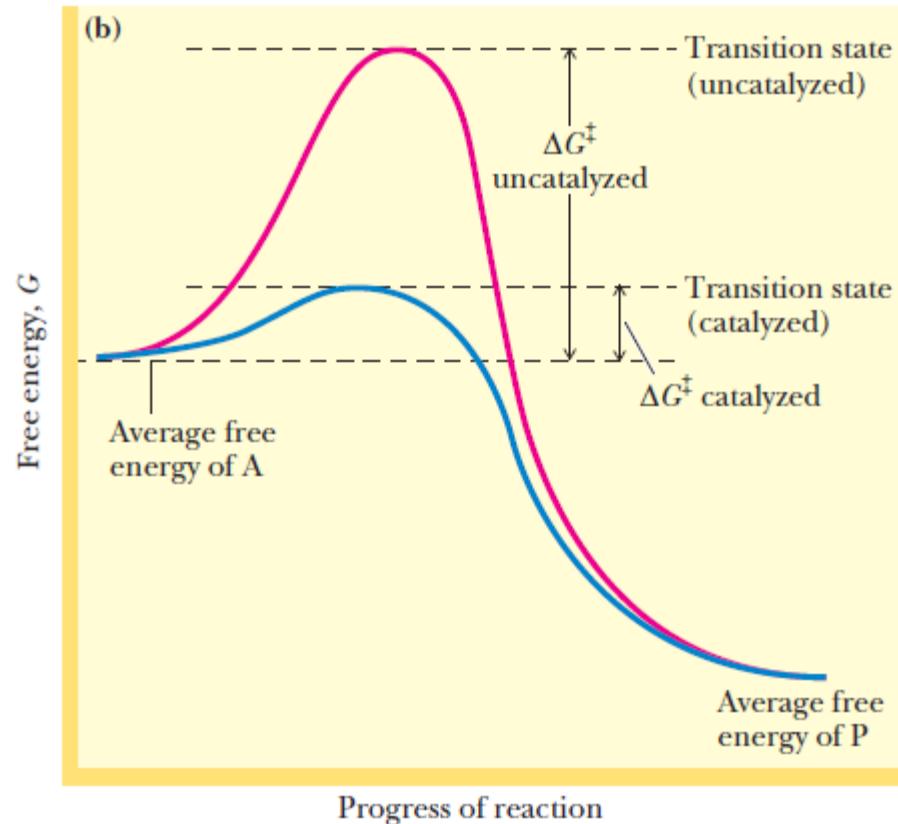


Figure 10.23 The Molecules of Life (© Garland Science 2013)

Enzyme mechanism

Mechanism :Catalysts Lower the Free Energy of Activation for a Reaction

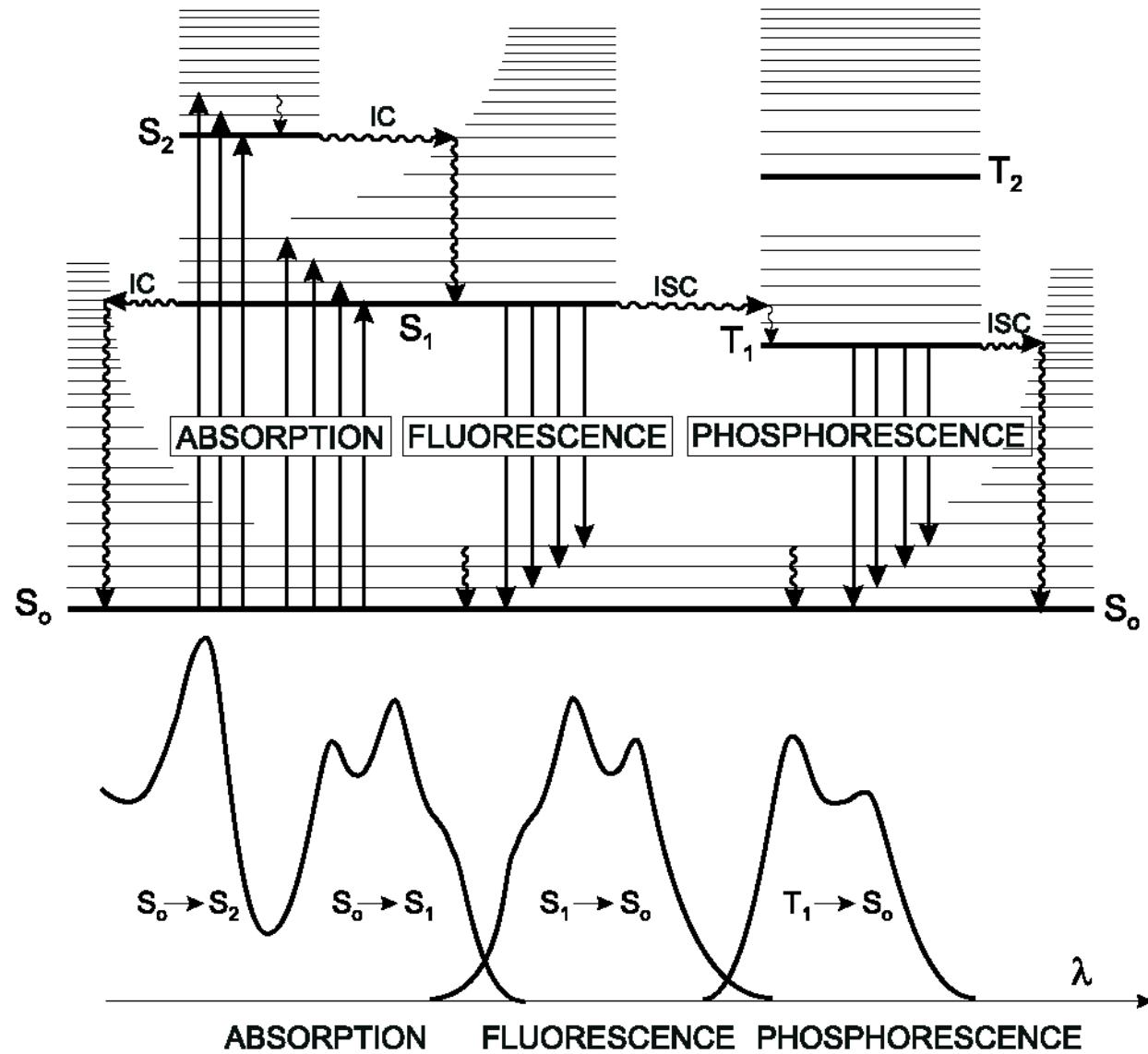


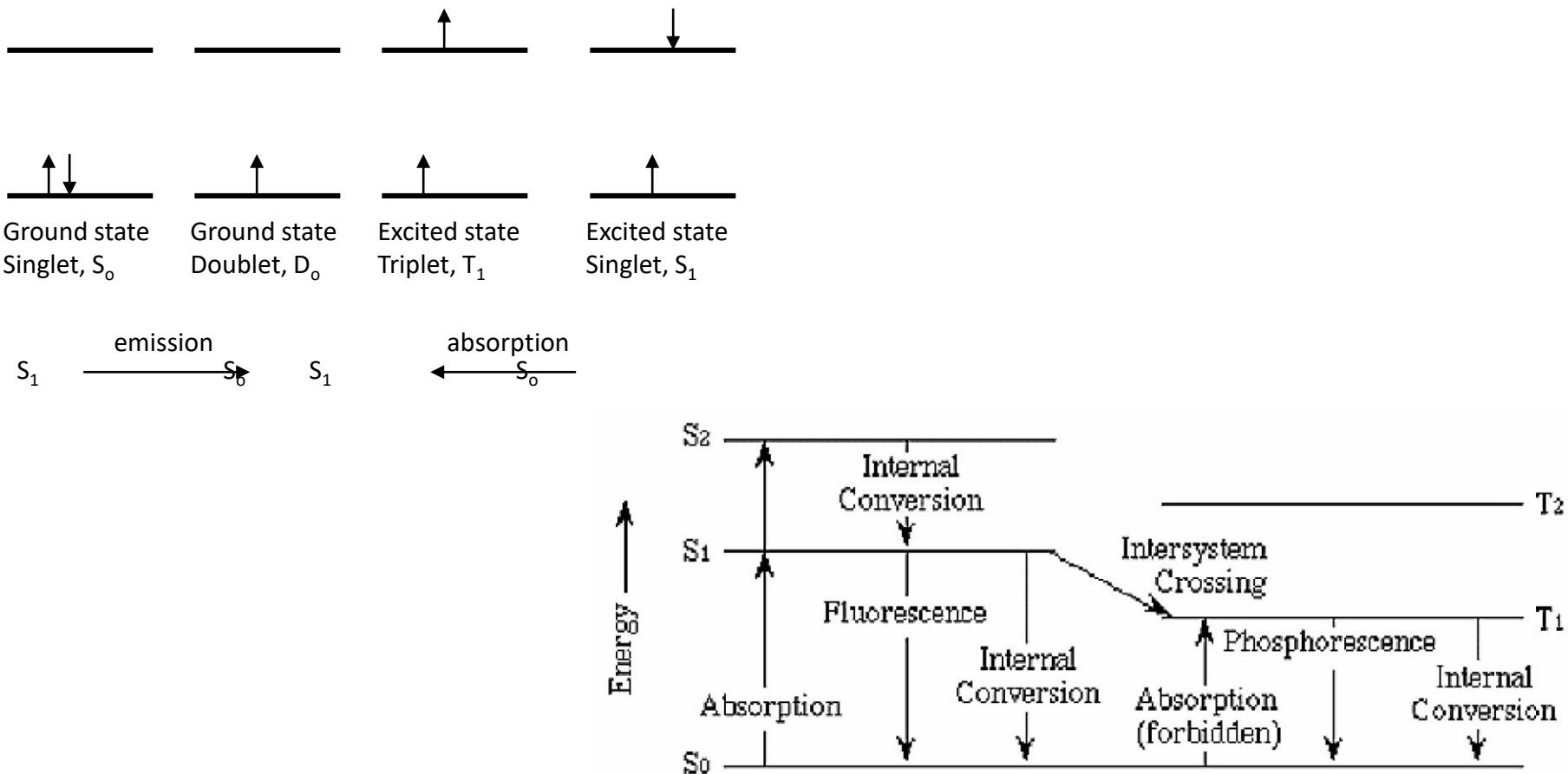
Thank you

L5 Enzyme functional nature: Activity measurement

Ravikrishnan Elangovan,
Department of Biochemical Engg and Biotechnology
Indian Institute of Technology - Delhi

Perrin-Jablonski diagram

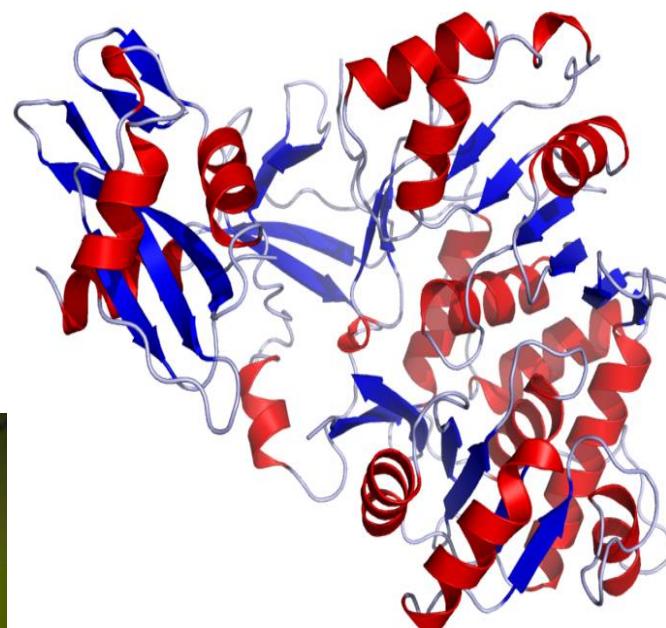
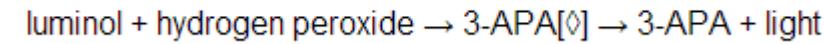
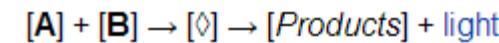
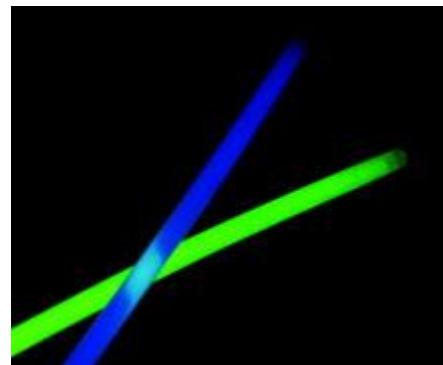




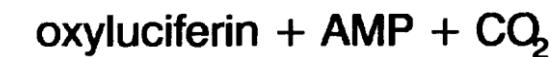
S is singlet and **T** is triplet.

The **S_0** state is the ground state and the subscript numbers identify individual states.

Chemi-luminenscence

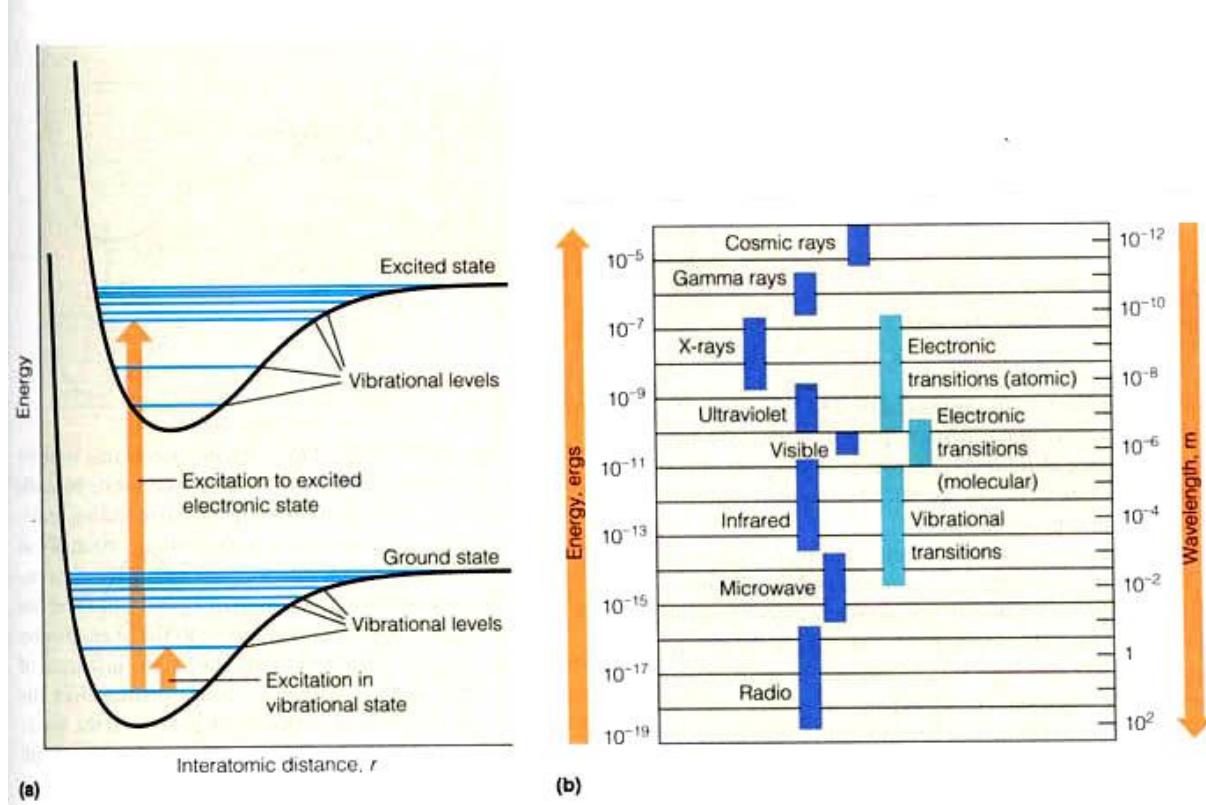


↓ **luciferase**



+

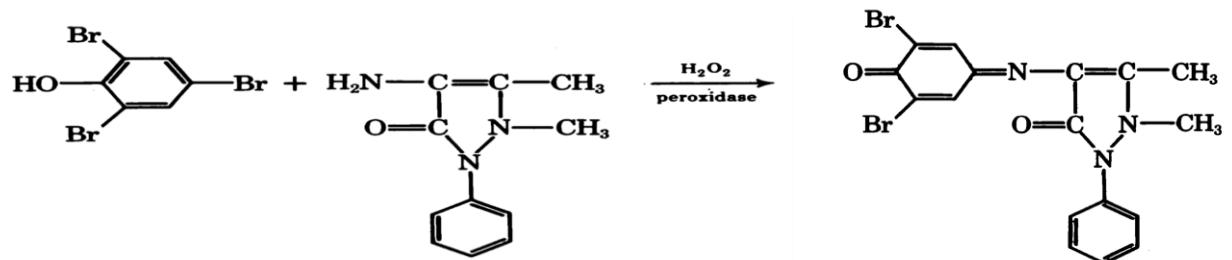
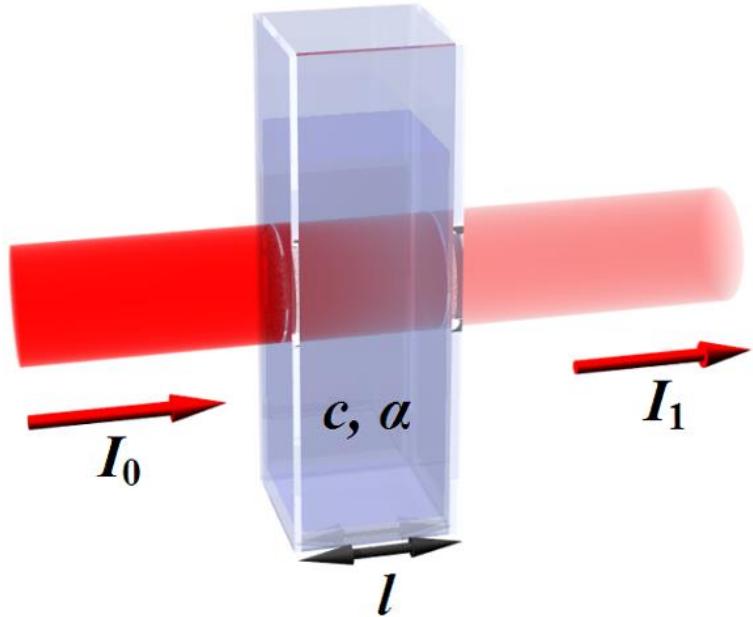




Frank-Condon Principle

- The nuclear motion (10^{-13} s) is negligible during the time required for an electronic excitation (10^{-16} s).
- Since the nuclei do not move during the excitation, the internuclear distances remain constant and “the most probable component of an electronic transition involves only the vertical transitions”.

Absorption



Color reaction

$$I_1/I_0 = e^{-\alpha l c}$$

l is the pass length

C is the concentration of absorbing material

α is the absorption coefficient

Absorption: The Beer-Lambert Law

- ✿ The Beer-Lambert law sortof has the wrong name...



Pierre Bouguer
(1698-1758)

Astronomer: Light is diminished as it passes through the atmosphere.

August Beer (1825-1863): Added absorption coefficient and related to conc. in solution.



Johan Lambert
(1728-1777)

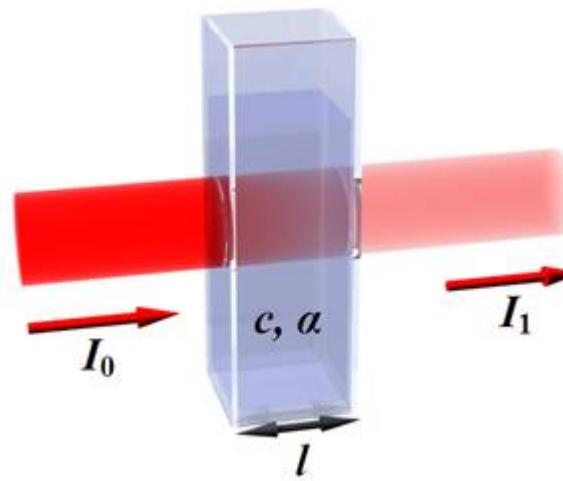
Mathematician, first to prove that π is irrational. No absorption coefficient.

$$A = -\log(I_1 / I_0) = \epsilon cl$$

Extinction coefficient

Concentration

Path length



Assumptions

TABLE 3-1
Assumptions of the absorption law

1. The incident radiation is monochromatic.
2. The absorbers (molecules, atoms, ions, etc.) act independently of each other.
3. The incident radiation consists of parallel rays, perpendicular to the surface of the absorbing medium.
4. The pathlength traversed is uniform over the cross section of the beam. (All rays traverse an equal distance of the absorbing medium.)
5. The absorbing medium is homogeneous and does not scatter the radiation.
6. The incident flux is not large enough to cause saturation effects. (Lasers can cause such effects, as discussed in Chapters 11 and 15.)

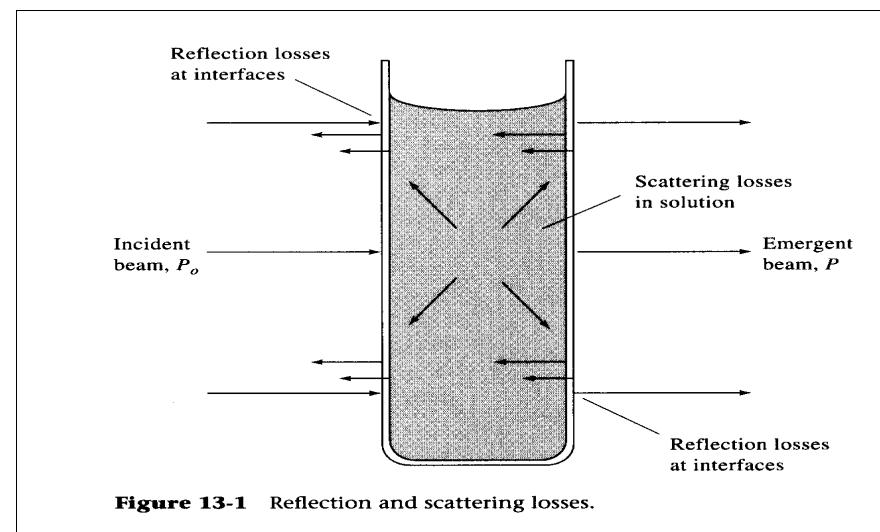
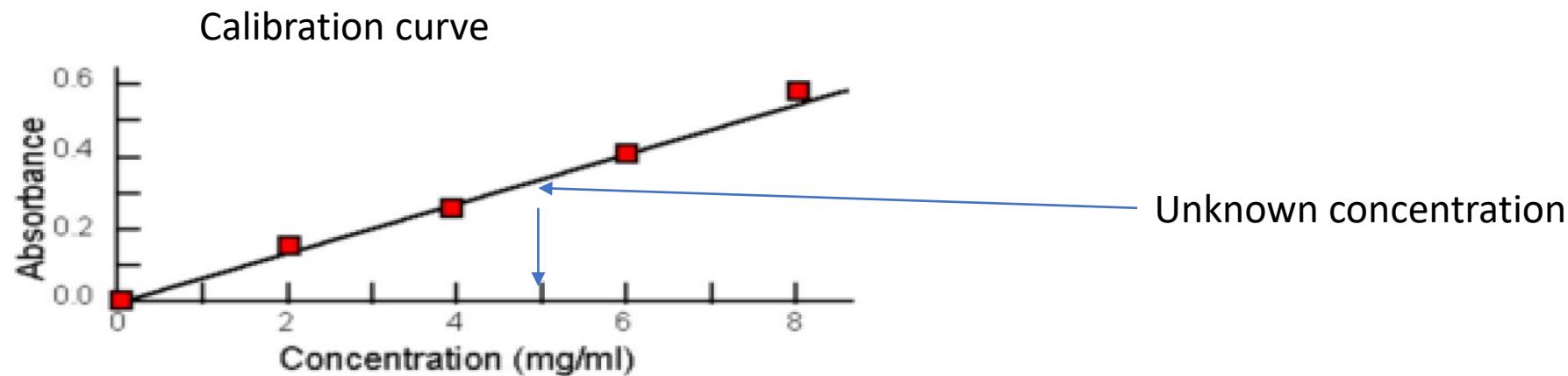


Figure 13-1 Reflection and scattering losses.

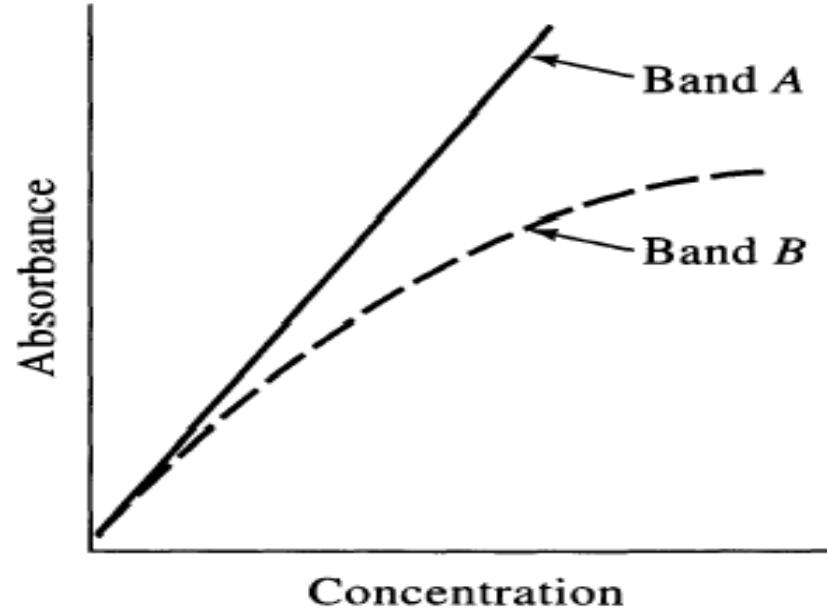
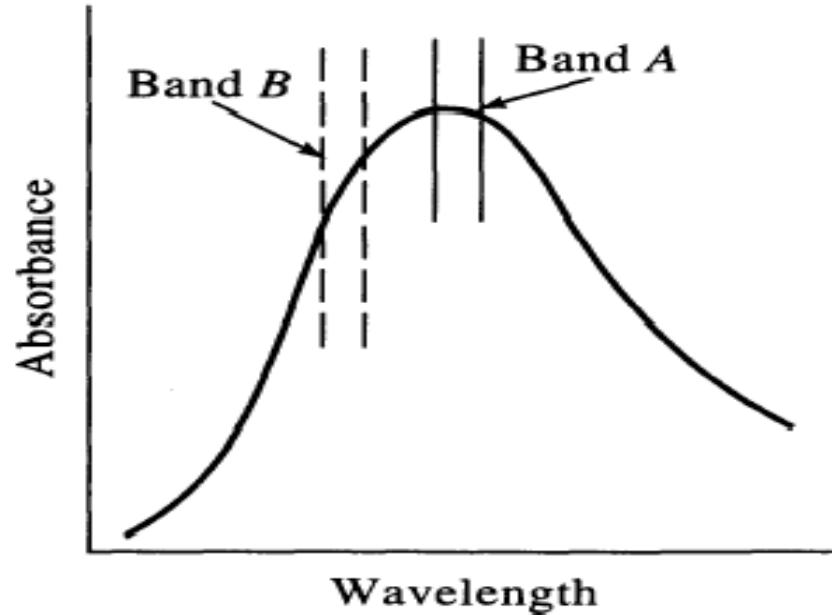
Beer's law and mixtures

- Each analyte present in the solution absorbs light!
- The magnitude of the absorption depends on its ϵ
- $A_{\text{total}} = A_1 + A_2 + \dots + A_n$
- $A_{\text{total}} = \epsilon_1 bc_1 + \epsilon_2 bc_2 + \dots + \epsilon_n bc_n$
- If $\epsilon_1 = \epsilon_2 = \epsilon_n$ then simultaneous determination is impossible
- Need $n\lambda$'s where ϵ 's are different to solve the mixture



Beer's Law Limitation

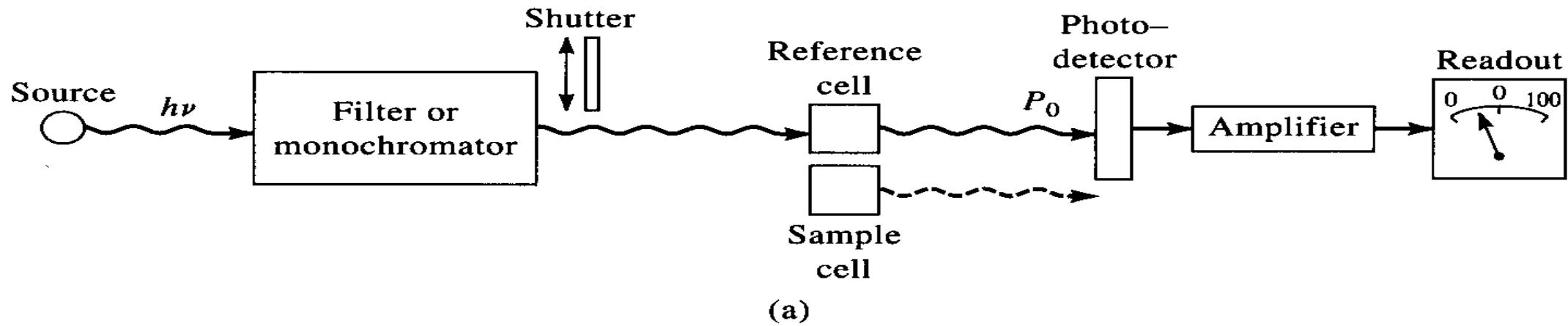
- Polychromatic Light
 - More than one wavelength cannot be used



Ideally, a monochromator will pass radiation of a single wavelength, but in reality the monochromator passes a band of radiation. The bandwidth of the spectrometer will affect the linearity of Beer's Law.

General Instrument Designs

Single beam



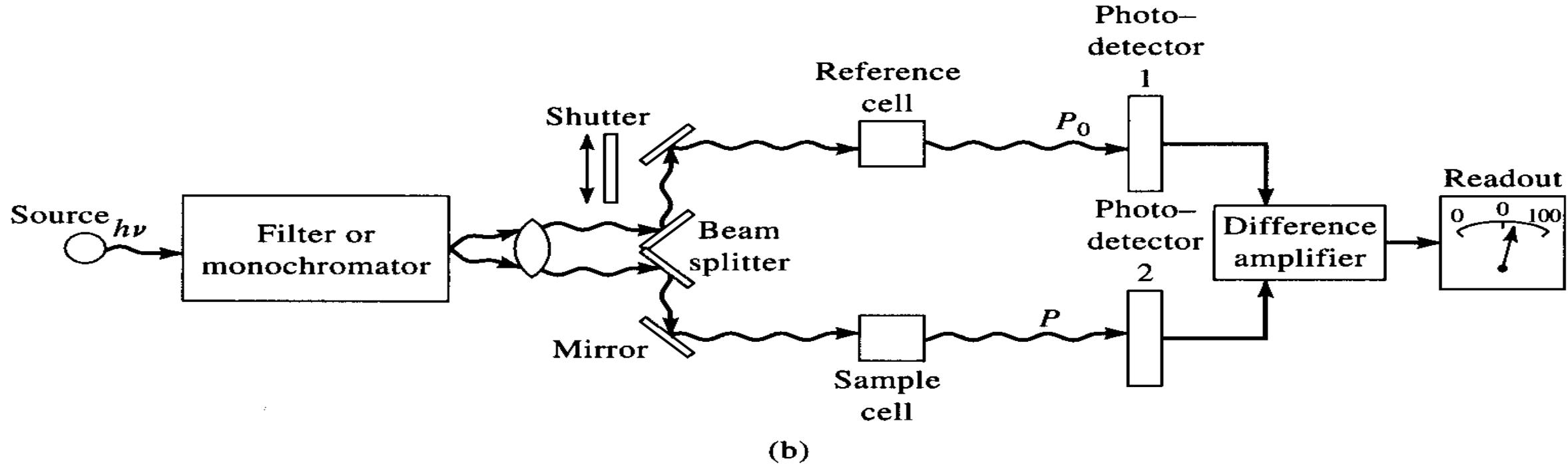
(a)

Photo

- 0% T is set with shutter in the beam path.
- 100% T is set with a reference in the beam path.
- Measurement is then made with the sample in the beam path.

General Instrument Designs

Double Beam: Space resolved

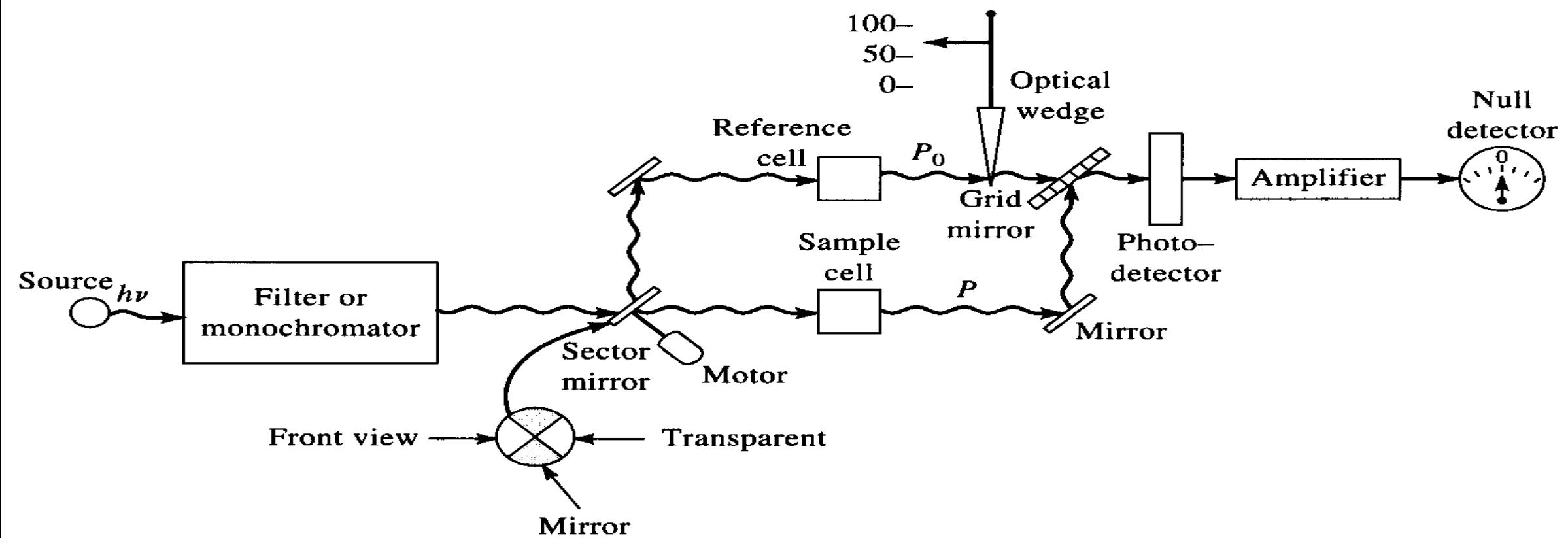


Sample and reference are measured simultaneously and the signal from the reference is subtracted from the sample signal.

A major drawback of this type of instrument is the requirement of two detectors, which makes the instrument more expensive.

General Instrument Designs

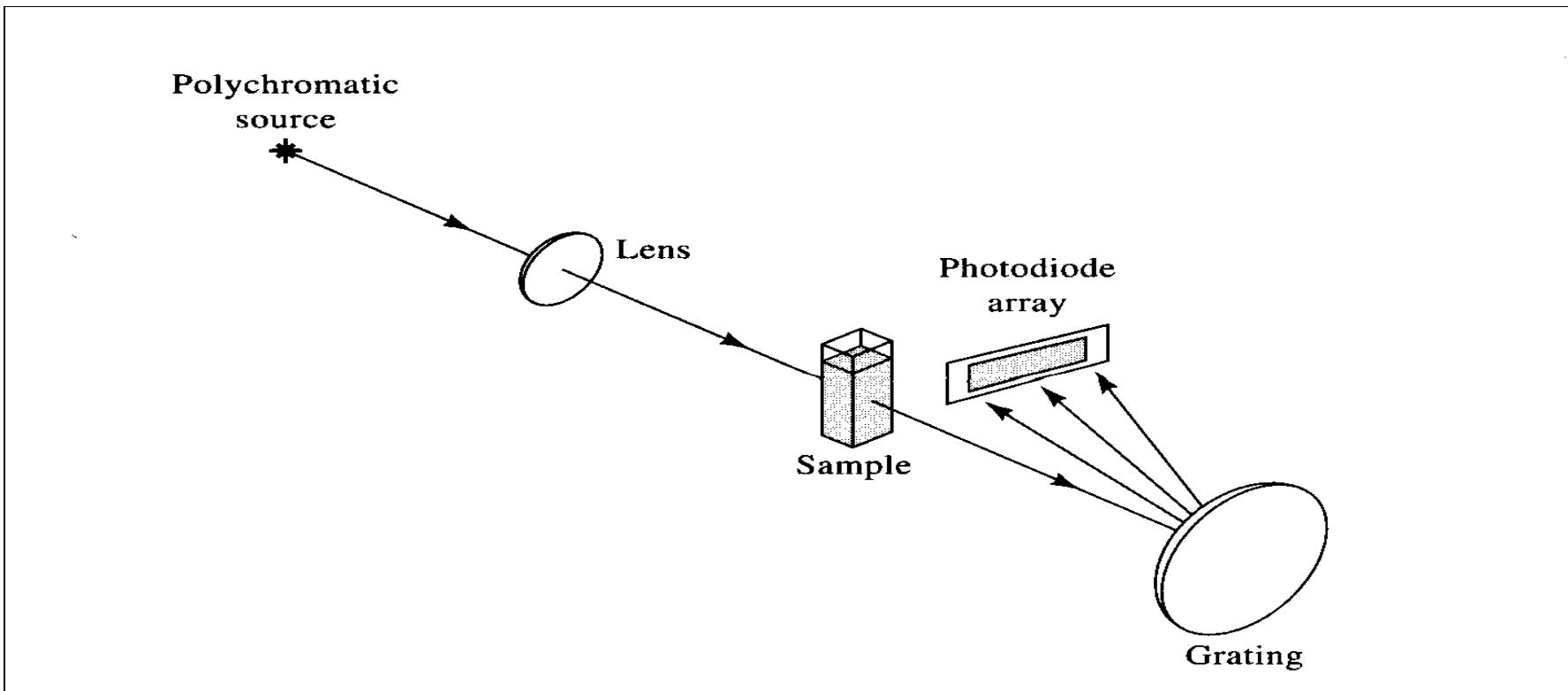
Double Beam: Time resolved



Advantages of a double-beam over a single-beam instrument:

- Compensate for variations in the source intensity.
- Compensate for drift in the detector and amplifier.
- Compensate for variation in intensity as a function of wavelength.

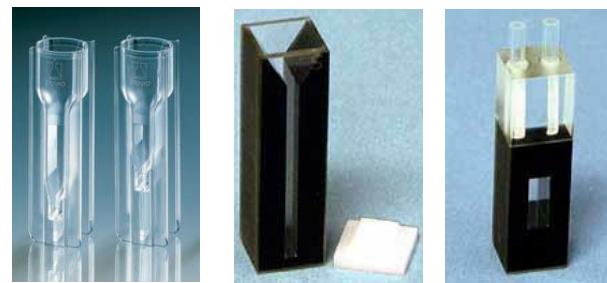
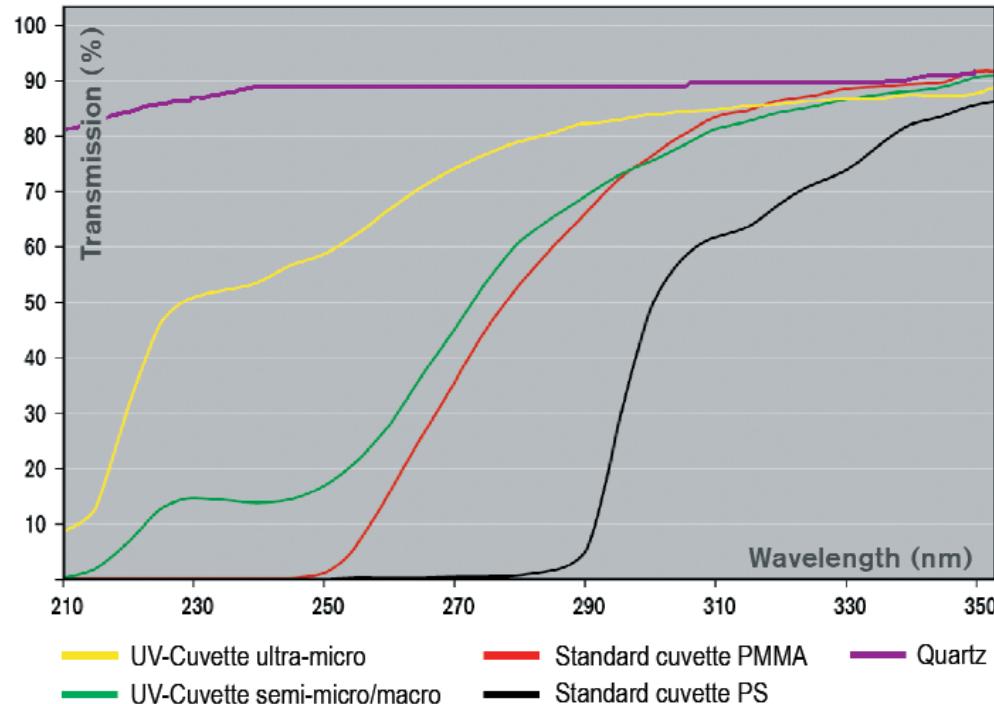
Multi-channel Design



- Able to “scan” (collect) an entire spectrum in ~ 0.1 sec.
- Uses signal averaging over a period of 1 sec or more to enhance signal-to noise ratio.
- Have high throughput of radiant energy due to the minimal optics.
- Typically use a deuterium lamp source for a spectral range of 200nm → 820 nm and have a spectral bandwidth (resolution) of 2 nm.

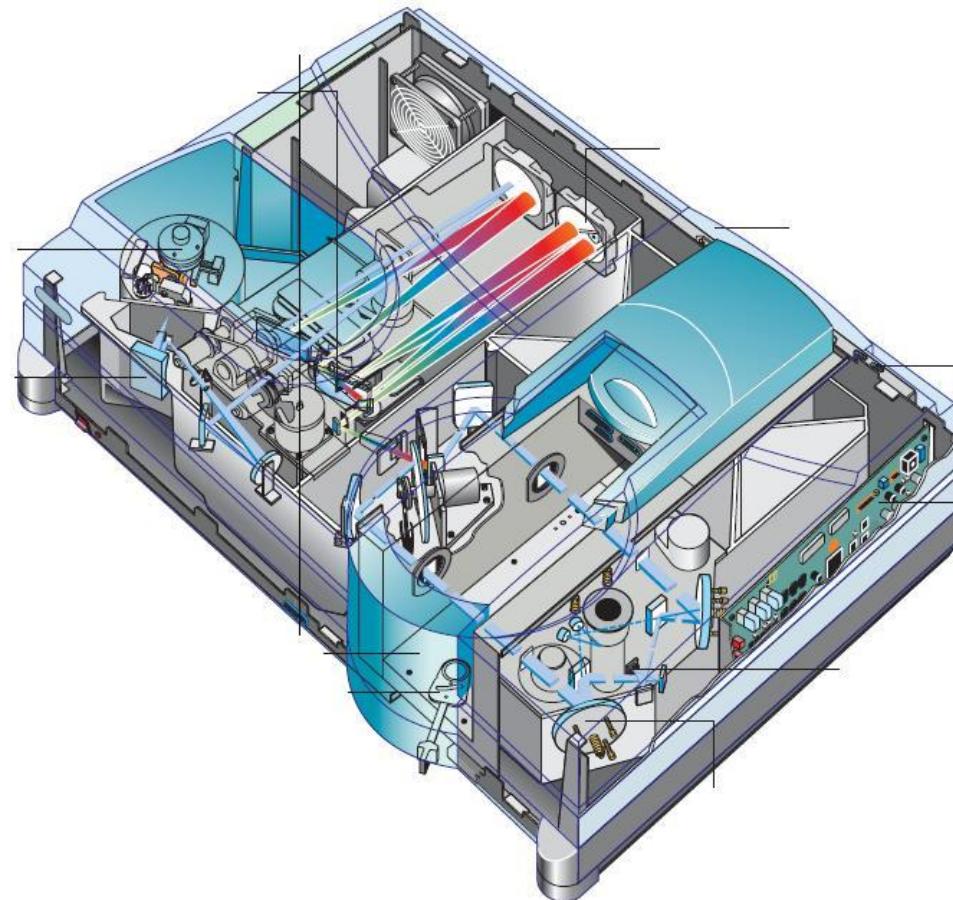
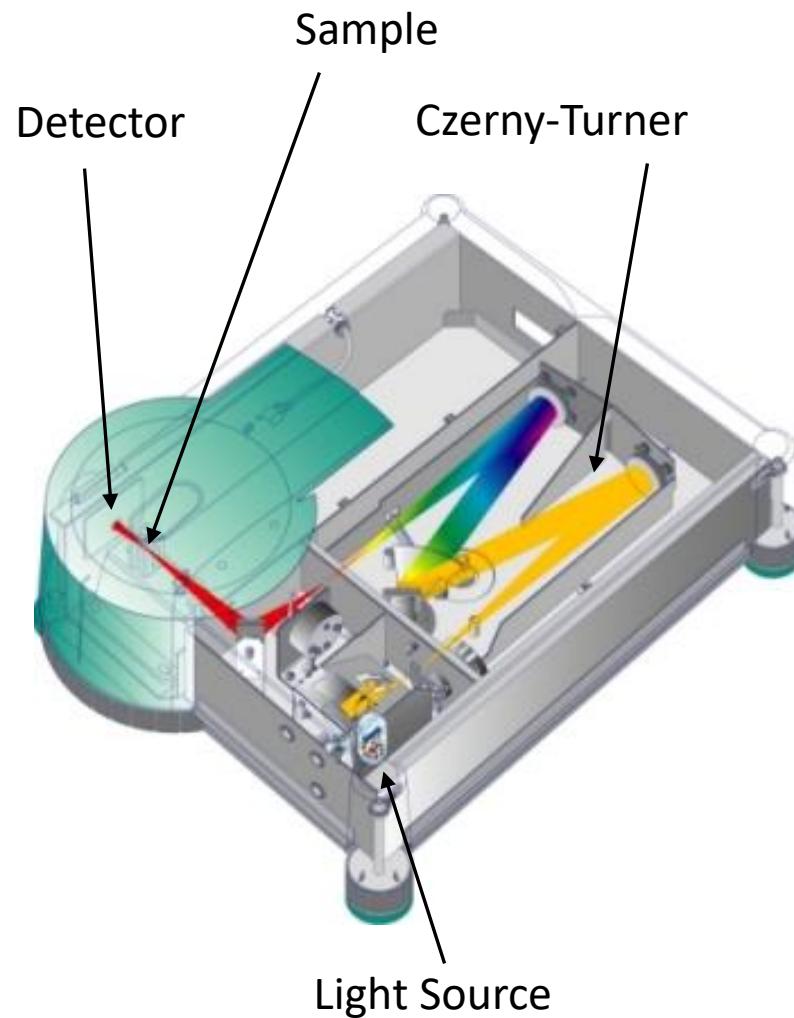
Sample Compartments/Holders

- ✿ The sample itself is held in a cuvette, usually plastic or quartz:





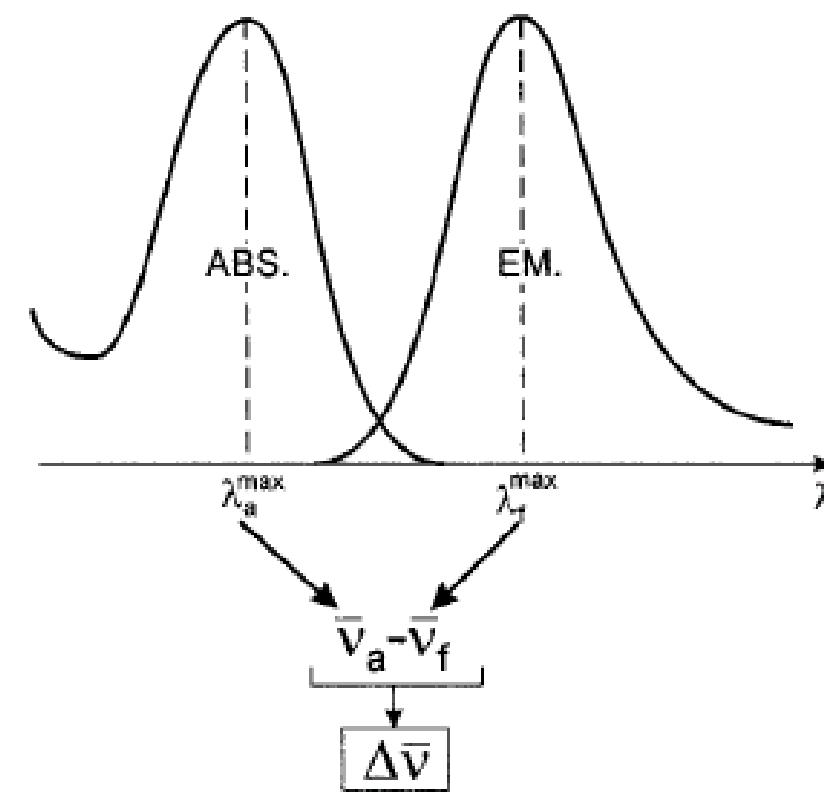
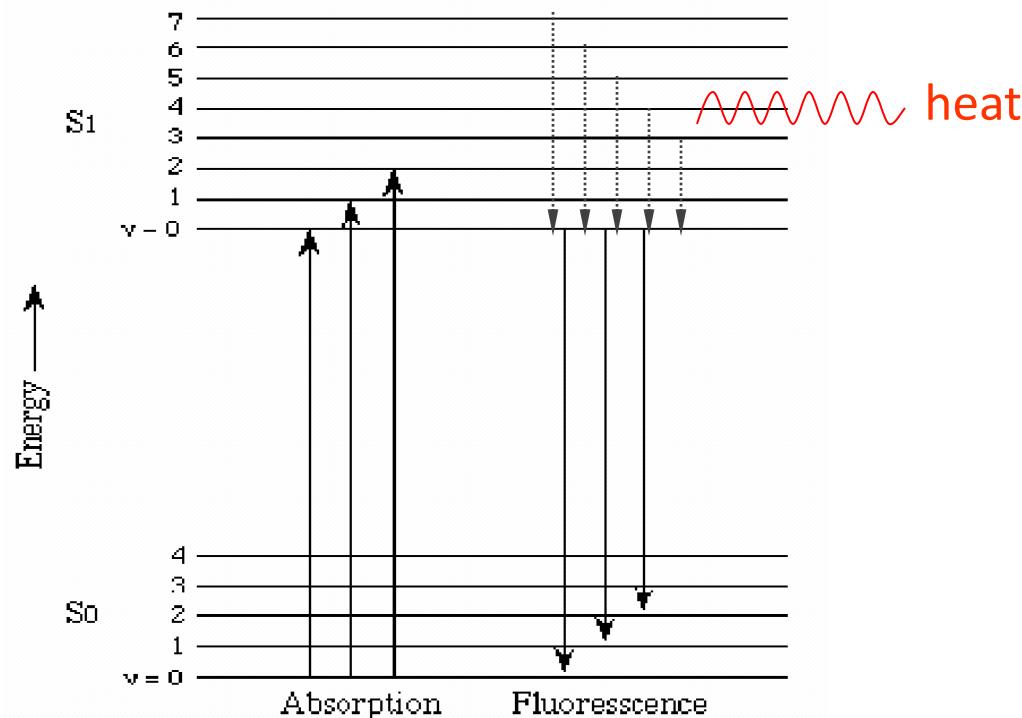
The Whole Instrument



Stokes shift

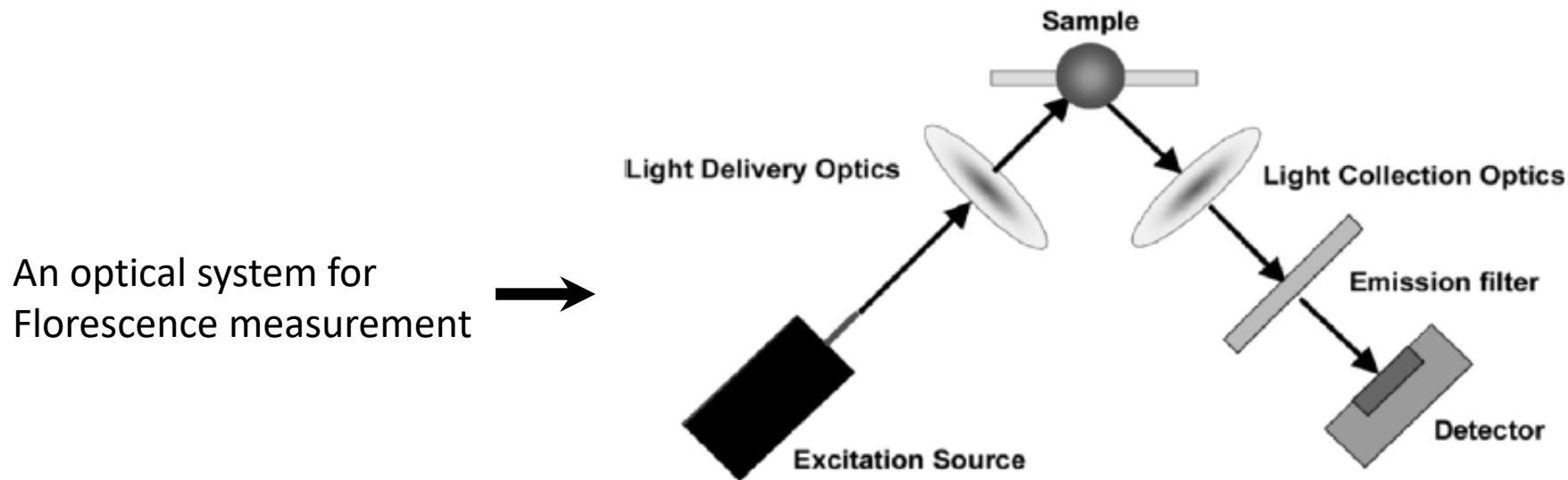
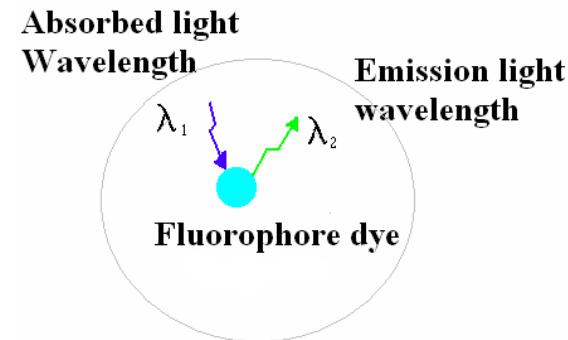
The Stokes shift is the gap between the maximum of the first absorption band and the maximum of the fluorescence spectrum

loss of vibrational energy in the excited state as heat by collision with solvent



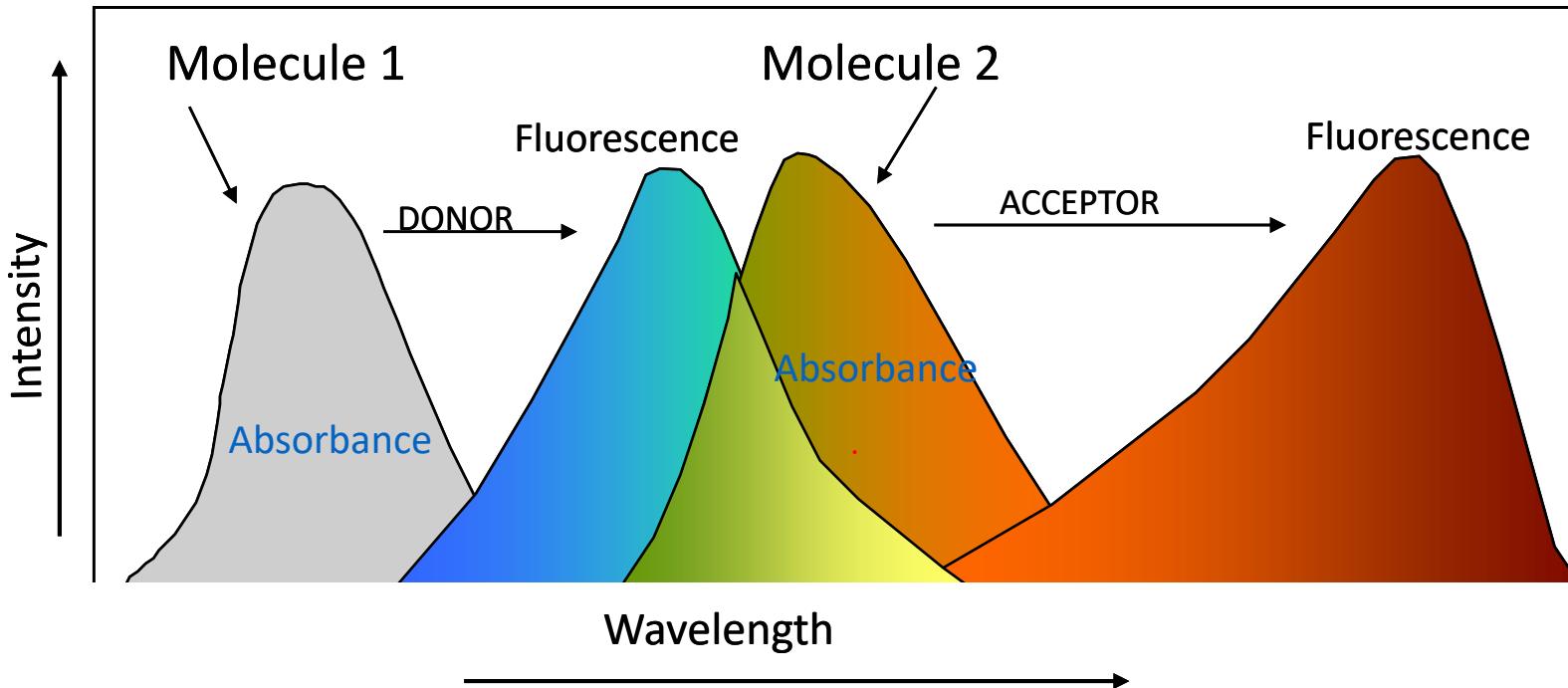
Transducers-Optical methods- Fluorescence

Fluorescence is a molecular absorption of light at one wavelength and its instantaneous emission of at longer wavelengths. Some molecules fluoresce naturally and others such as DNA can be modified for fluorescence detection by attachment of special fluorescent dyes



I. Principles of fluorescence

- Fluorescence energy transfer (FRET)



Non radiative energy transfer – a quantum mechanical process of resonance between transition dipoles

Effective between 10-100 Å only

Emission and excitation spectrum must significantly overlap

Donor transfers **non-radiatively** to the acceptor

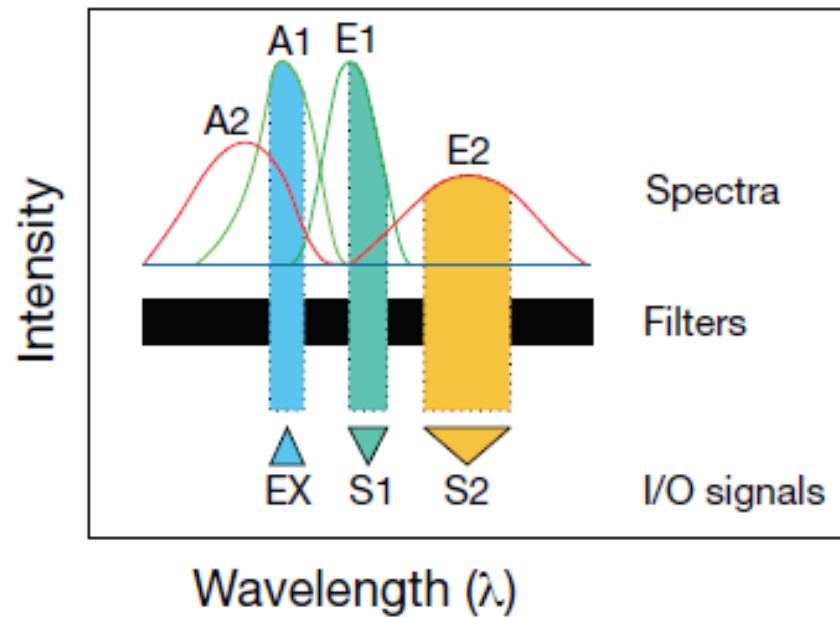
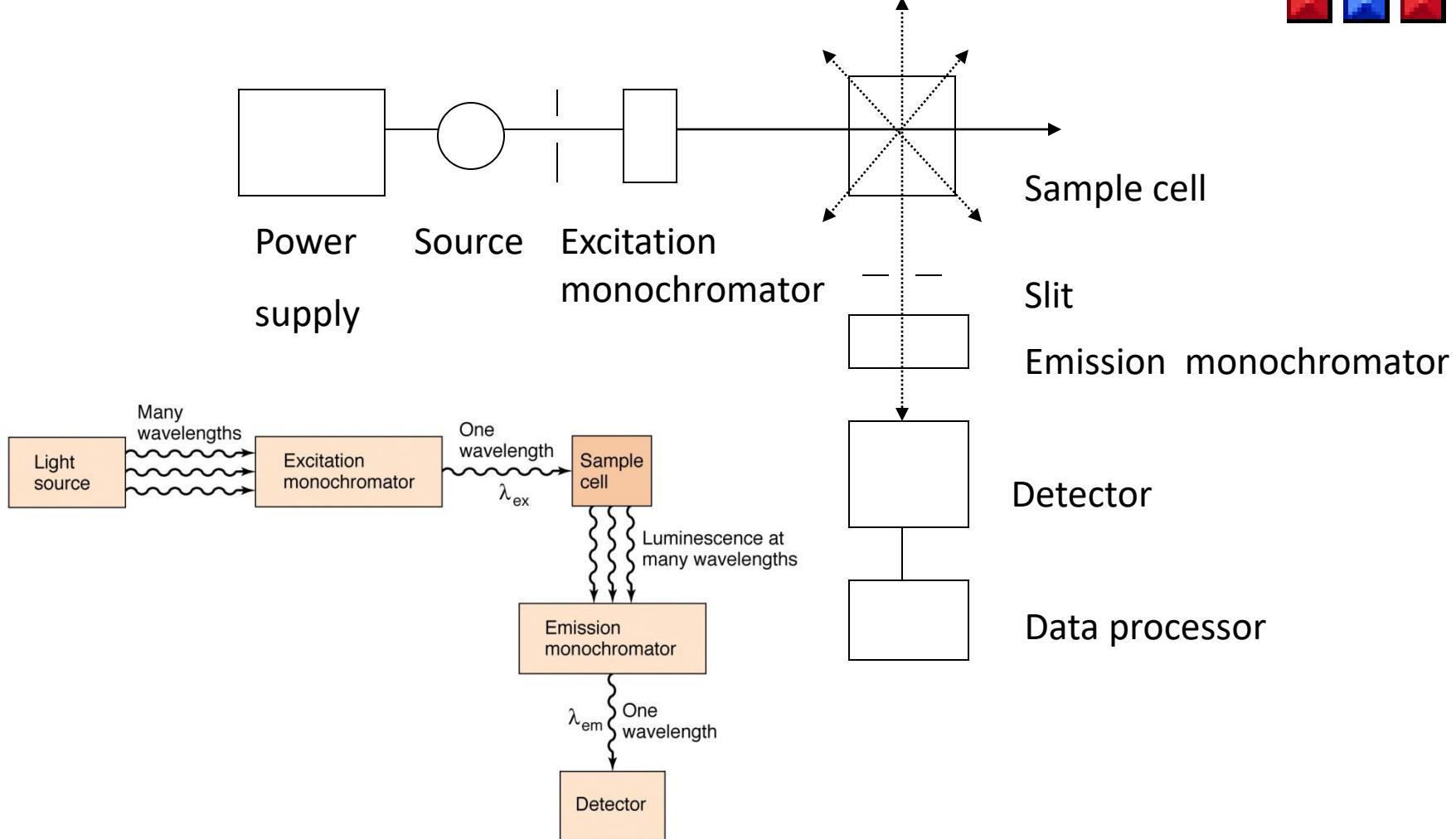
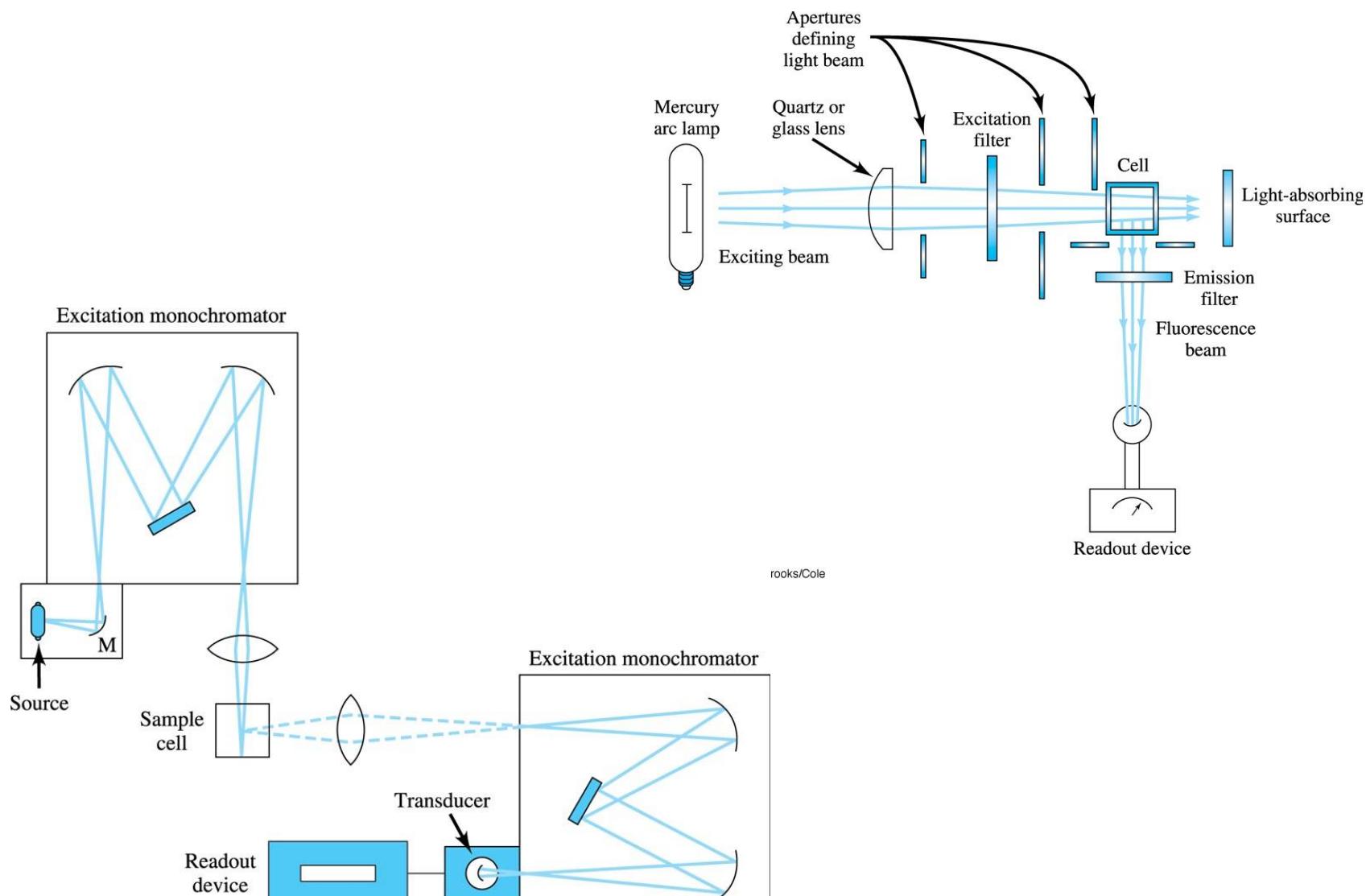


Figure 3 Fluorescence detection of mixed species. Excitation (EX) in overlapping absorption bands A1 and A2 produces two fluorescent species with spectra E1 and E2. Optical filters isolate quantitative emission signals S1 and S2.

Instrumentation for fluorescence spectroscopy

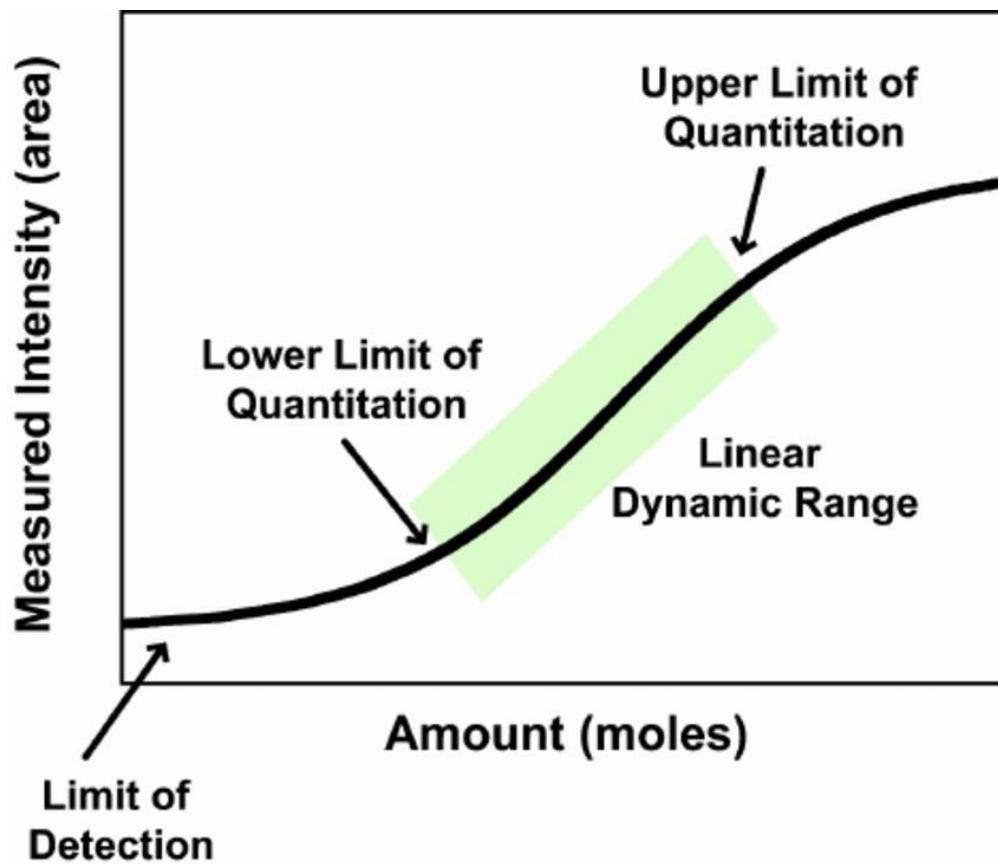


General layout of fluorescence spectrophotometer



© 2004 Thomson - Brooks/Cole

Schematic diagram of a typical spectrofluorometer.



Background noise ??

Sensitivity ?

Specificity ?

Figure 4 from Strategies and Challenges in Measuring Protein Abundance Using Stable Isotope Labeling and Tandem Mass Spectrometry; DOI: 10.5772/33421

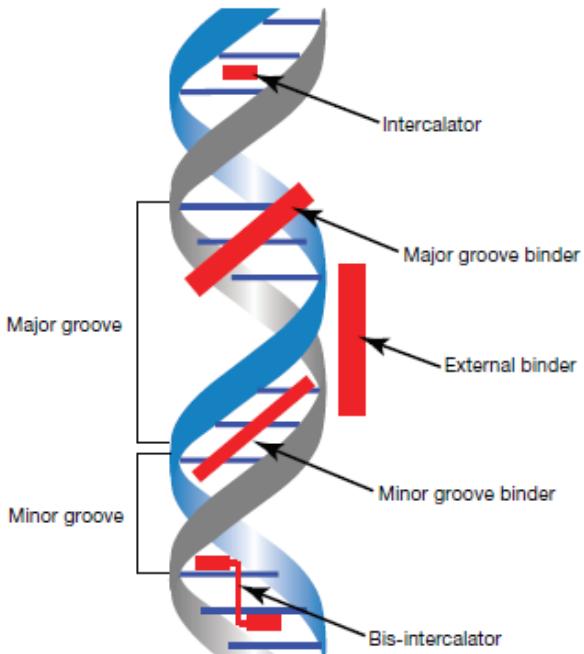


Figure 8.1.1 Schematic diagram showing the different binding modes of dyes (and other ligands) to DNA.

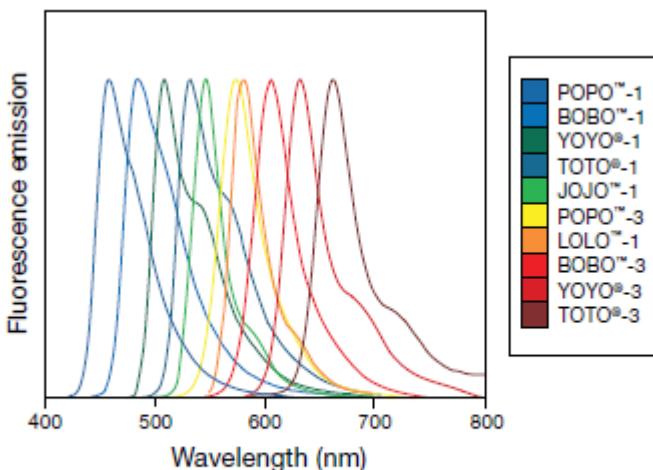


Figure 8.1.2 Normalized fluorescence emission spectra of DNA-bound cyanine dimers, identified by the color key on the sidebar.

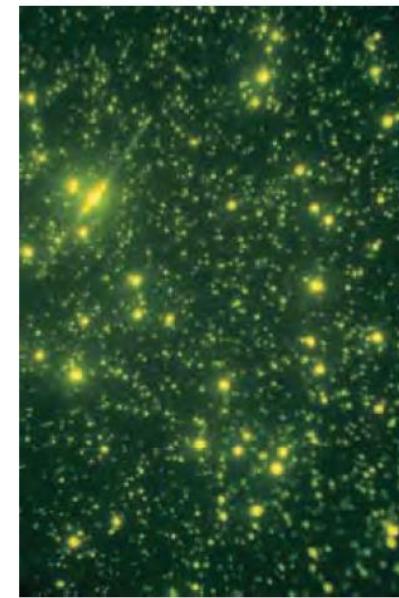


Figure 8.3.12 An environmental sample containing marine viruses (smallest dots), bacteria (larger, brighter dots) and a diatom (long thin cell with prominent nucleus) stained with SYBR® Green I nucleic acid stain (S7563, S7567, S7585). Image contributed by Jed Fuhrman, University of Southern California.

Enzyme fluorogenic substrates

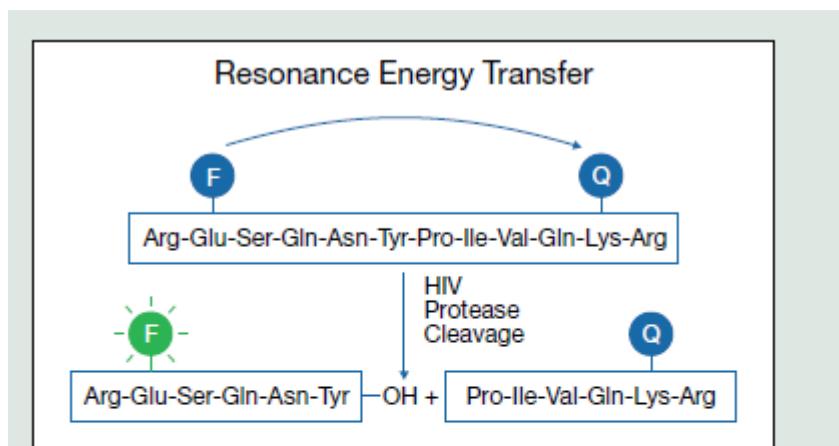
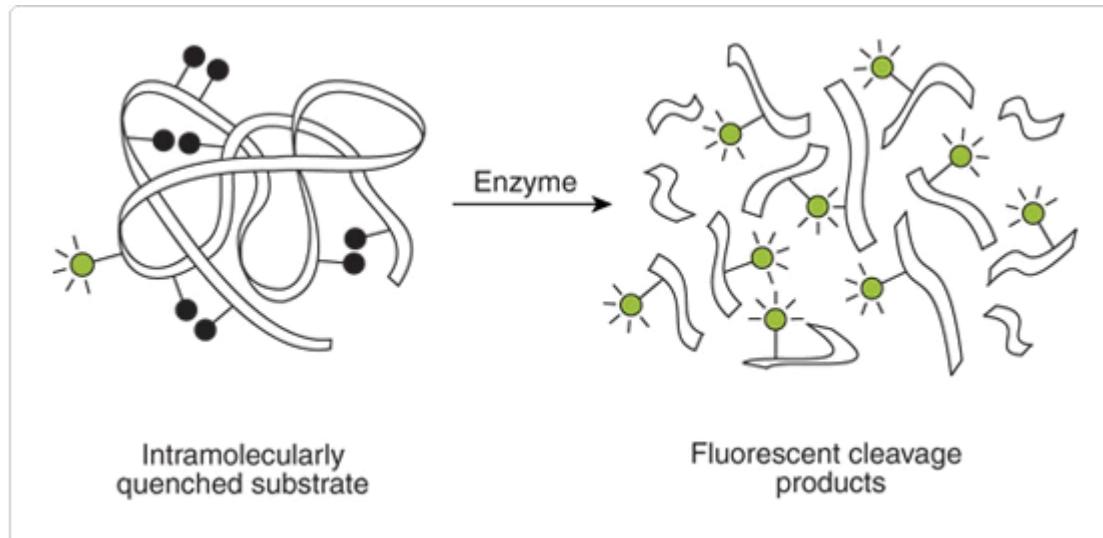


Figure 2 Principle of the fluorogenic response to protease cleavage exhibited by HIV protease substrate 1 (H2930). Quenching of the EDANS fluorophore (F) by distance-dependent resonance energy transfer to the dabcyl quencher (Q) is eliminated upon cleavage of the intervening peptide linker.

Some more applications

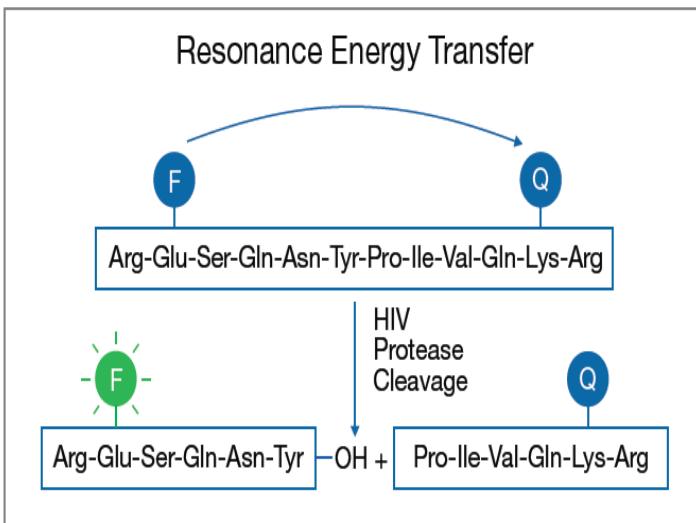


Figure 10.1.16 Principle of the fluorogenic response to protease cleavage exhibited by HIV protease substrate 1 (H2930). Quenching of the EDANS fluorophore (F) by distance-dependent resonance energy transfer to the dabcyl quencher (Q) is eliminated upon cleavage of the intervening peptide linker.

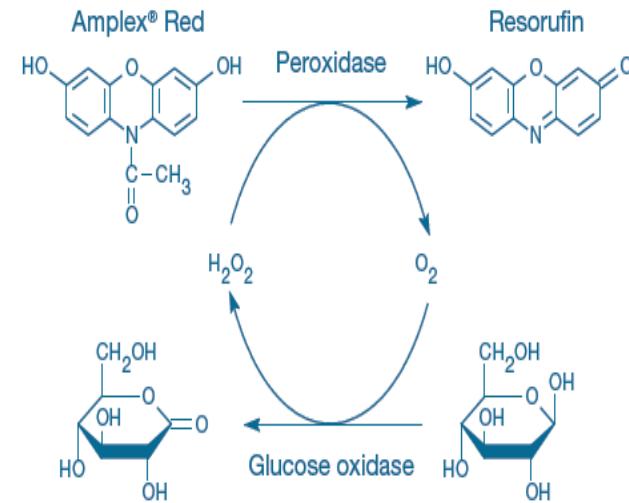
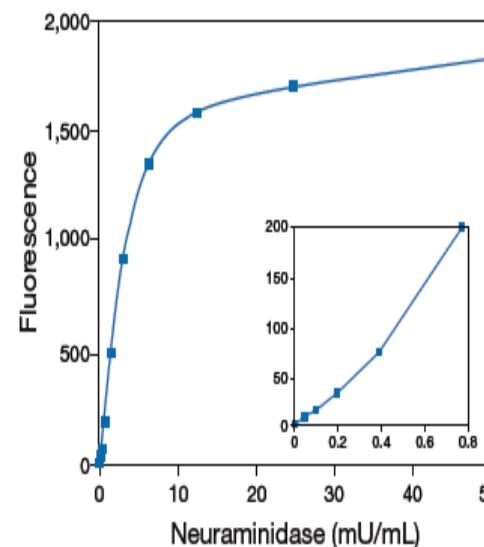
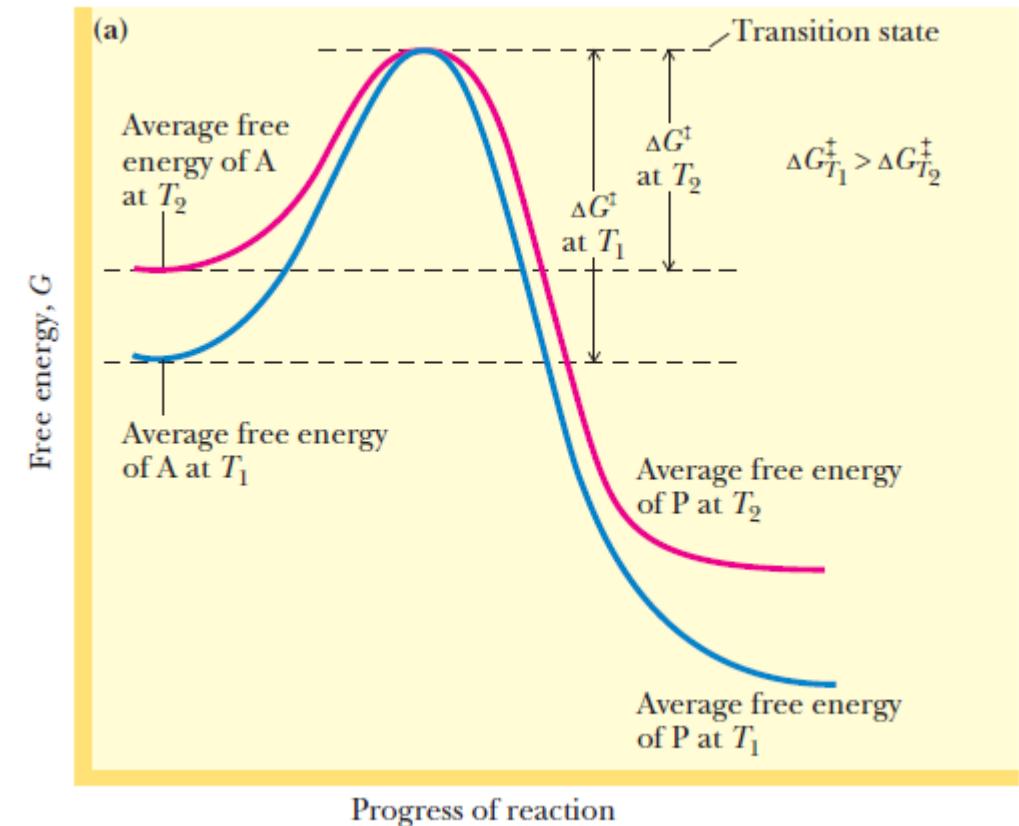
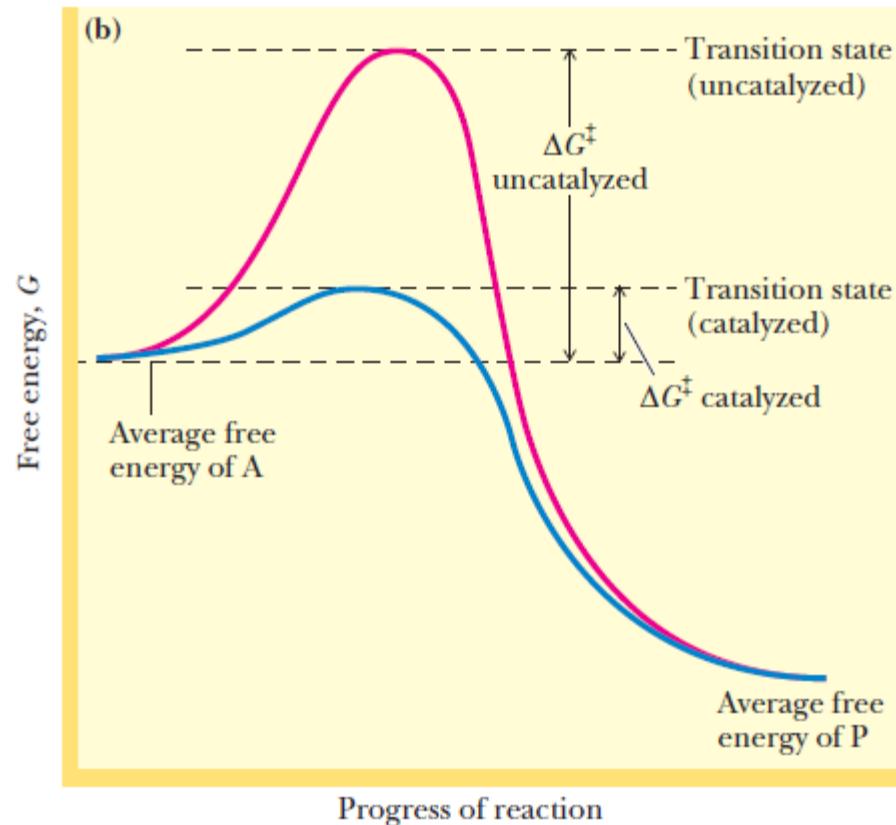


Figure 10.2.15 Principle of coupled enzymatic assays using our Amplex® Red reagent. Oxidation of glucose by glucose oxidase results in generation of H_2O_2 , which is coupled to conversion of the Amplex® Red reagent to fluorescent resorufin by HRP. The detection scheme shown here is used in our Amplex® Red Glucose/Glucose Oxidase Assay Kit (A22189).



Enzyme thermodynamics

Mechanism :Catalysts Lower the Free Energy of Activation for a Reaction



Gibbs free energy

- Thermodynamics: changes in free energy, entropy, ...

$$\Delta G = \Delta H - T \cdot \Delta S$$

$$\Delta G = (\Delta U + P \cdot \Delta V) - T \cdot \Delta S$$

- For nearly all biochemical reactions ΔV is small and ΔH is almost equal to ΔU
- Hence, we can write:

$$\boxed{\Delta G = \Delta U - T \cdot \Delta S}$$

If ΔG is negative

Energy was released, products are simpler, greater entropy (2nd Law of Thermodynamics)
Exergonic / exothermic reaction (spontaneous)

If ΔG is positive

Energy input, product more complex, energy needed to go against 2nd Law
Endergonic / endothermic (non-spontaneous)

The Enthalpic term

- Changes in bonding
- van der Waals
- Hydrogen bonding
- Charge interactions

The Entropic term

- Changes the arrangement of the solvent or counterions
- Reflects the degrees of freedom
- Rotational & Translational changes

Protein folding

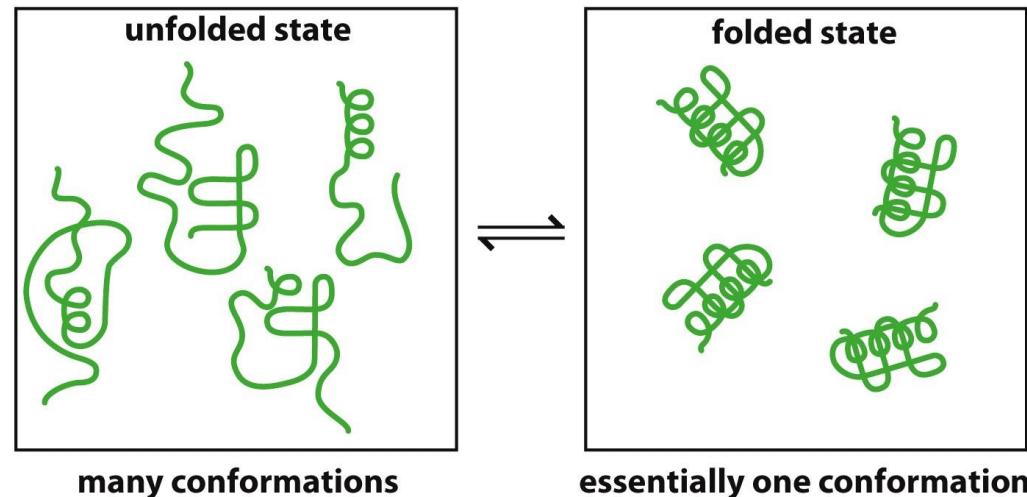


Figure 10.13 The Molecules of Life (© Garland Science 2013)

Protein folding is a spontaneous process?

$\Delta G < 0 ???$

Protein folding



$$K_{\text{folding}} = \frac{(F)}{(U)} = \frac{1}{K_{\text{unfolding}}}$$

$$\Delta G^\circ_{\text{unfolding}} = \Delta H^\circ - T \Delta S^\circ$$

Enthalpy change in protein folding

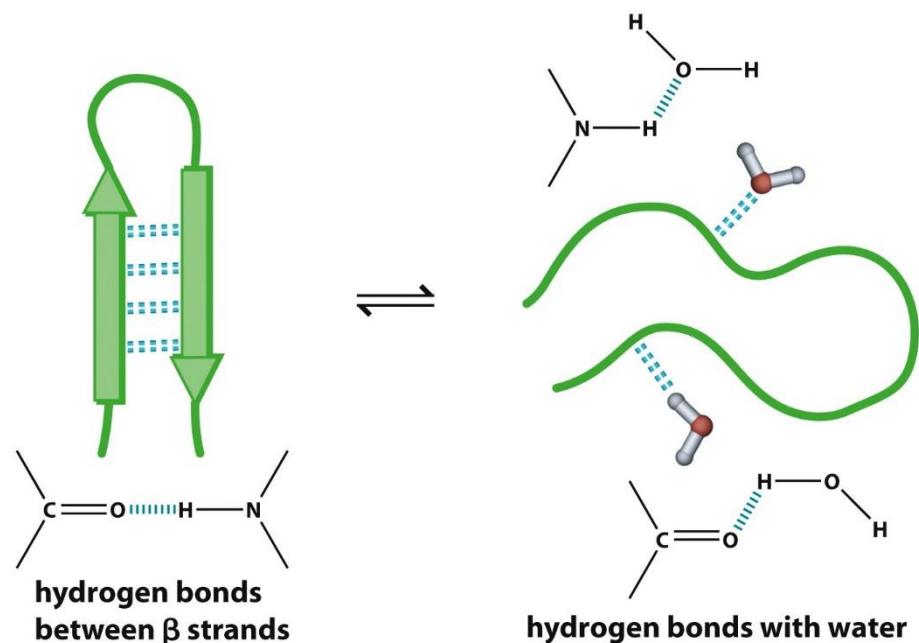


Figure 10.14 The Molecules of Life (© Garland Science 2013)

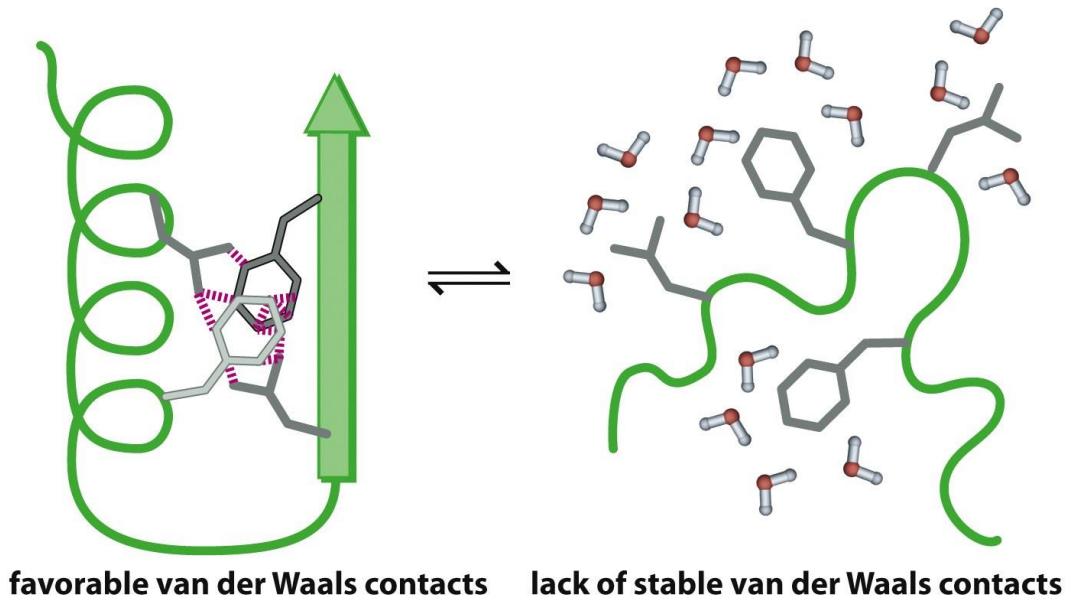


Figure 10.15 The Molecules of Life (© Garland Science 2013)

Entropy change in protein folding

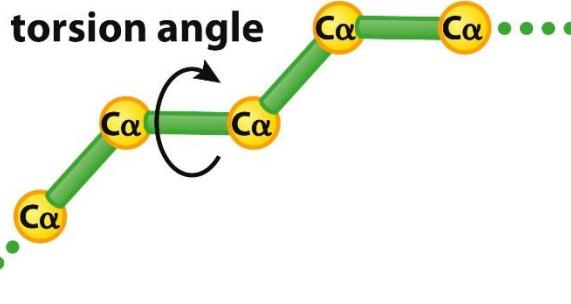


Figure 10.18 The Molecules of Life (© Garland Science 2013)

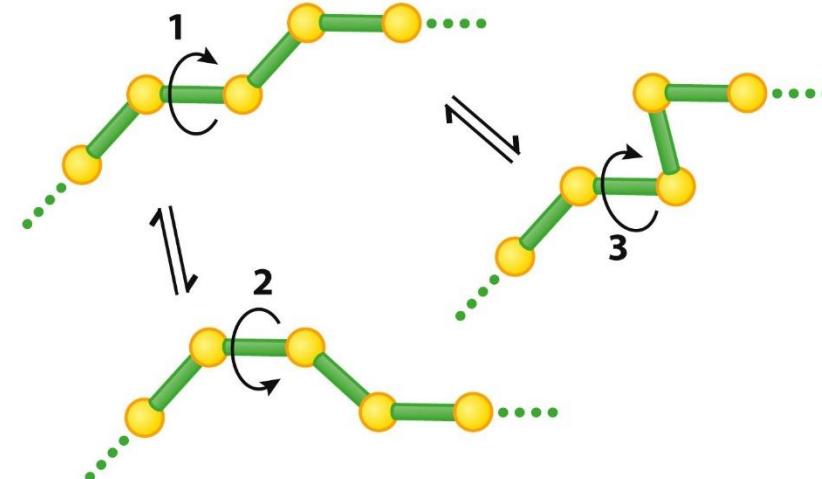


Figure 10.19 The Molecules of Life (© Garland Science 2013)

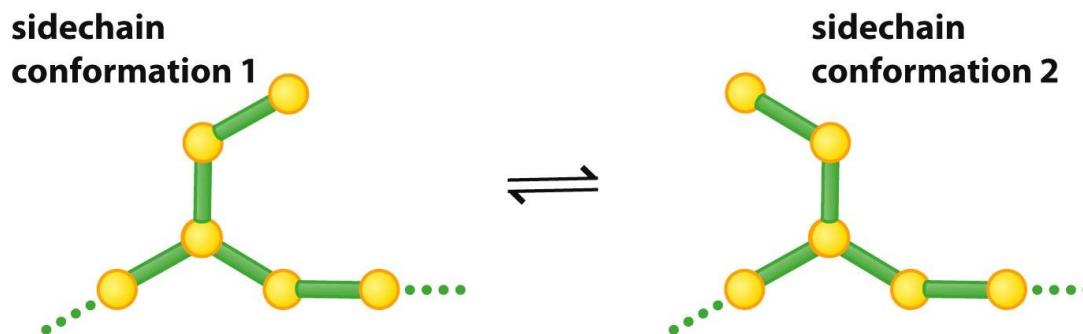


Figure 10.20 The Molecules of Life (© Garland Science 2013)

Entropy contribution from water

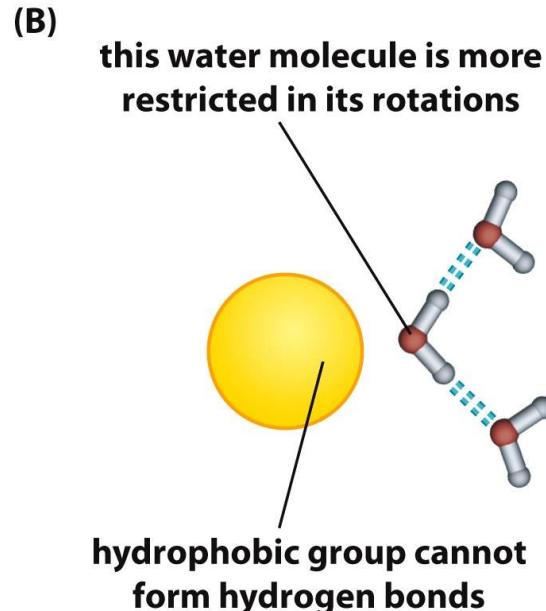
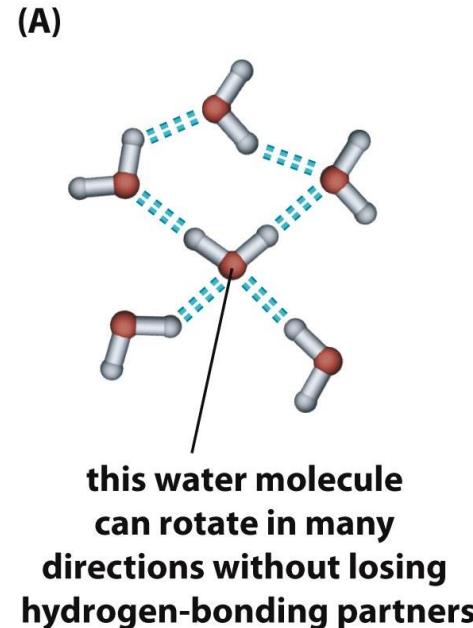


Figure 10.21 The Molecules of Life (© Garland Science 2013)

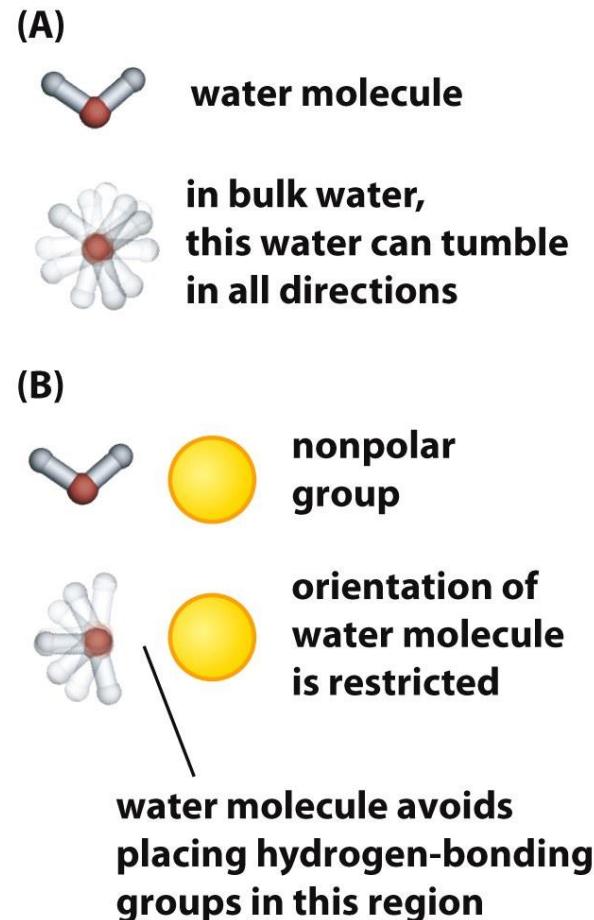


Figure 10.22 The Molecules of Life (© Garland Science 2013)

Protein folding

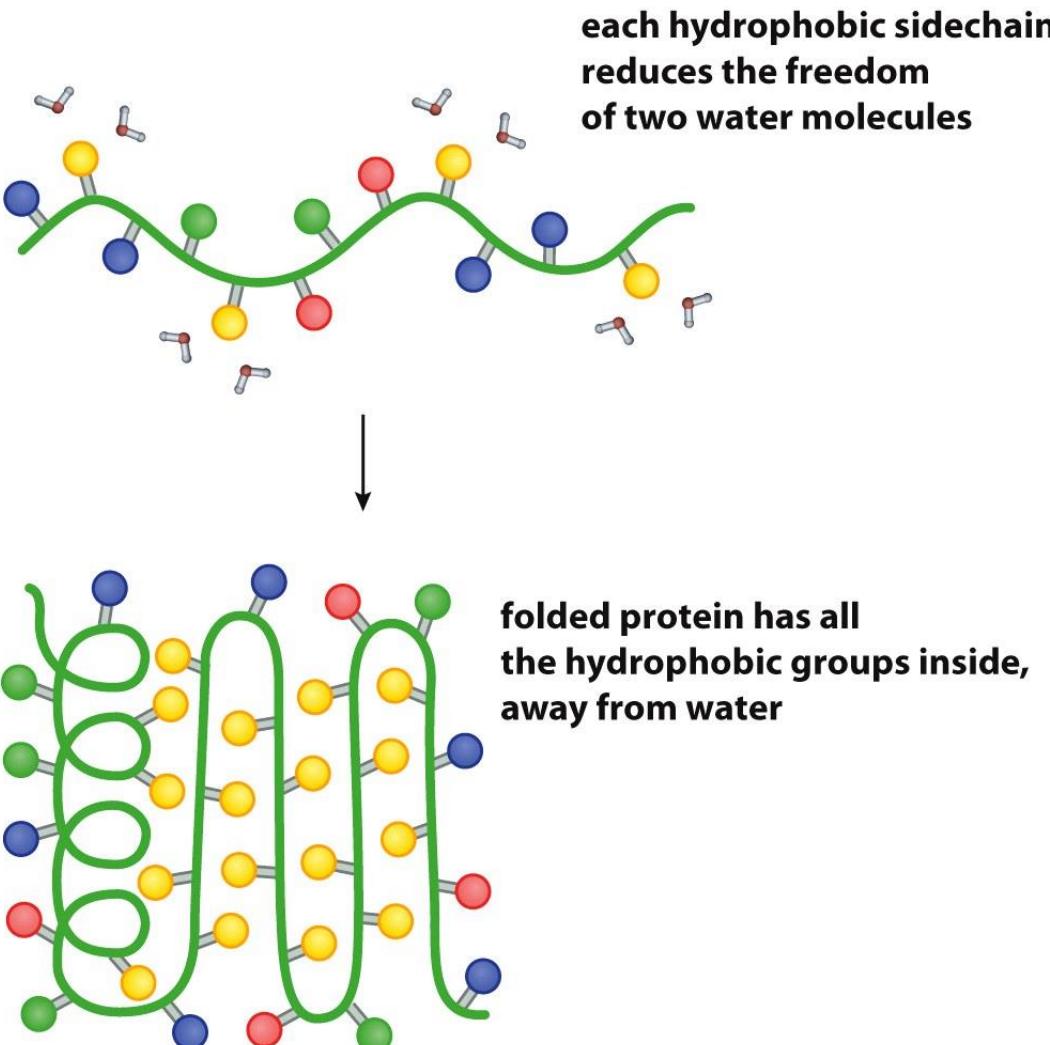


Figure 10.23 The Molecules of Life (© Garland Science 2013)

Enzyme mechanism

Mechanism :Catalysts Lower the Free Energy of Activation for a Reaction

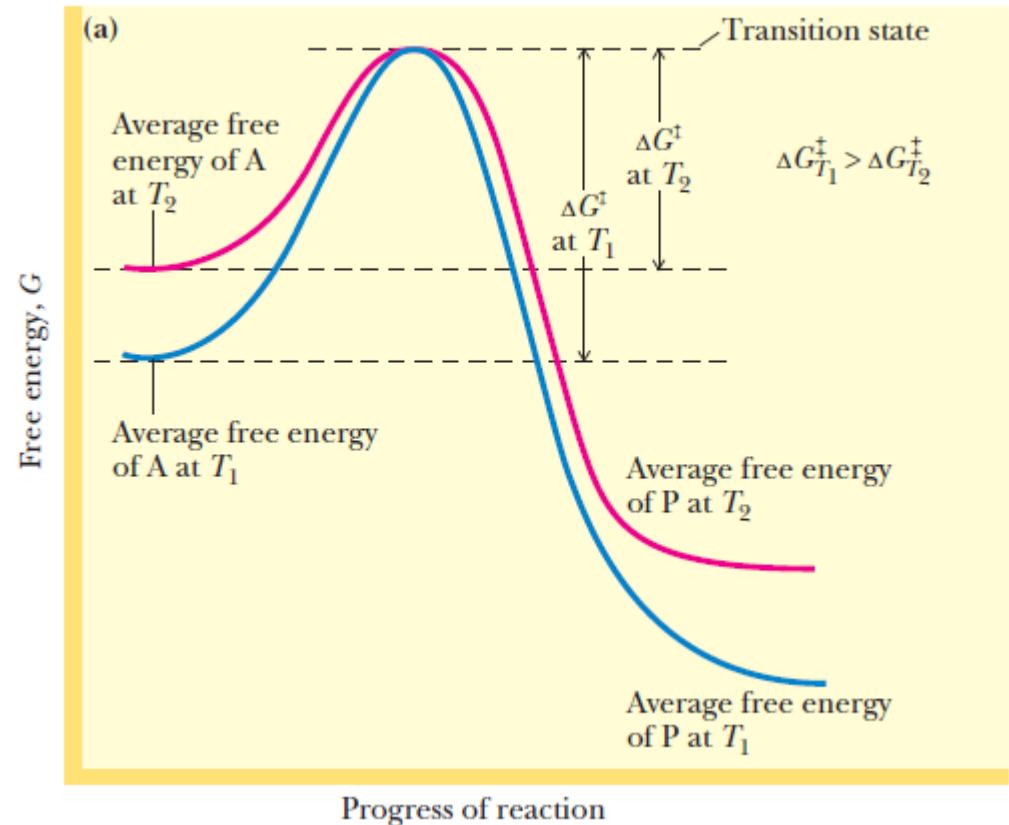
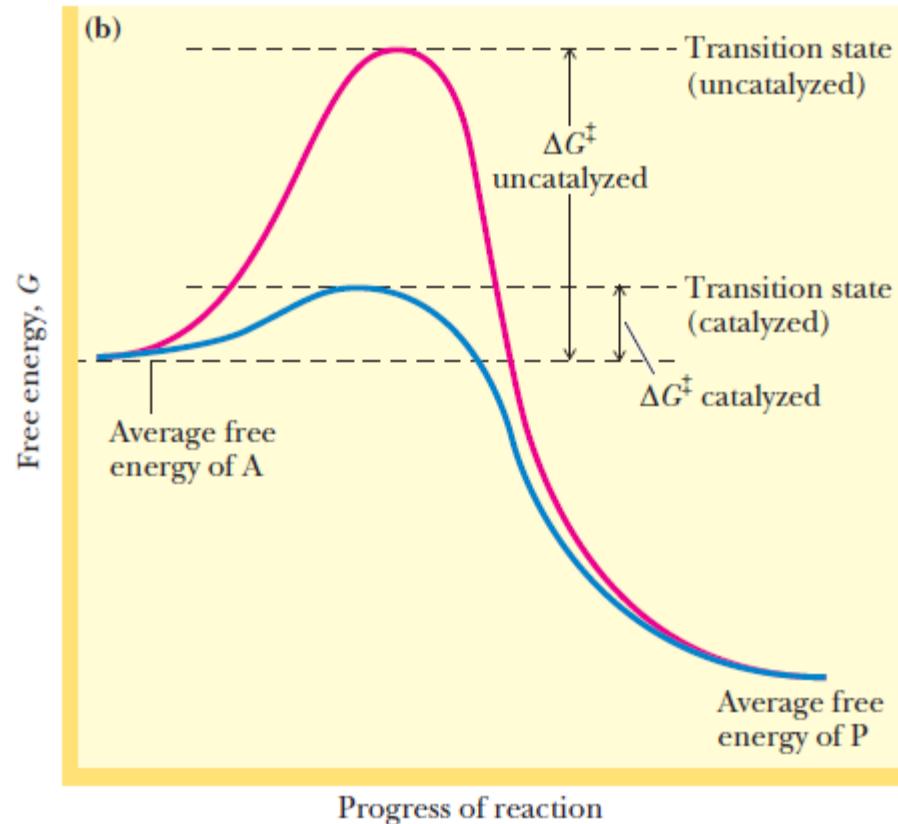


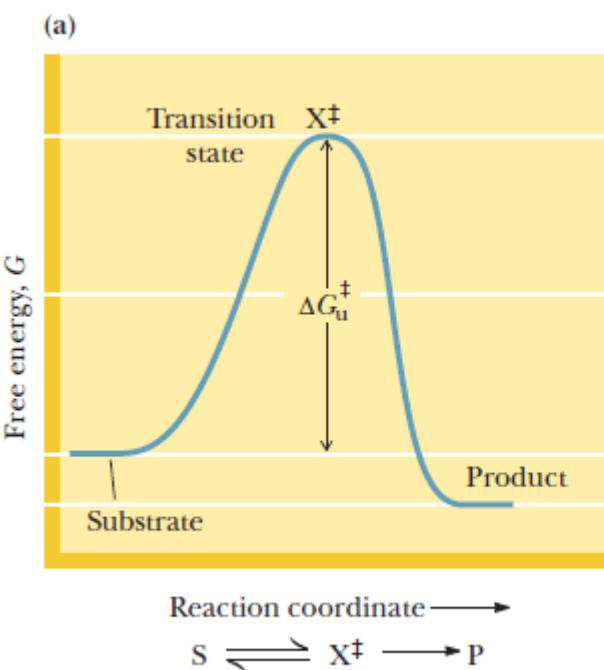
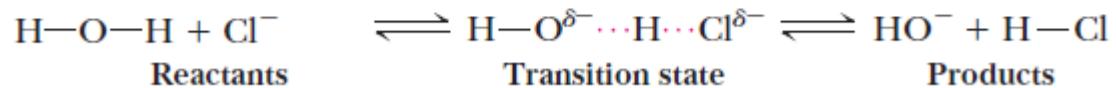
TABLE 14.1

A Comparison of Enzyme-Catalyzed Reactions and Their Uncatalyzed Counterparts

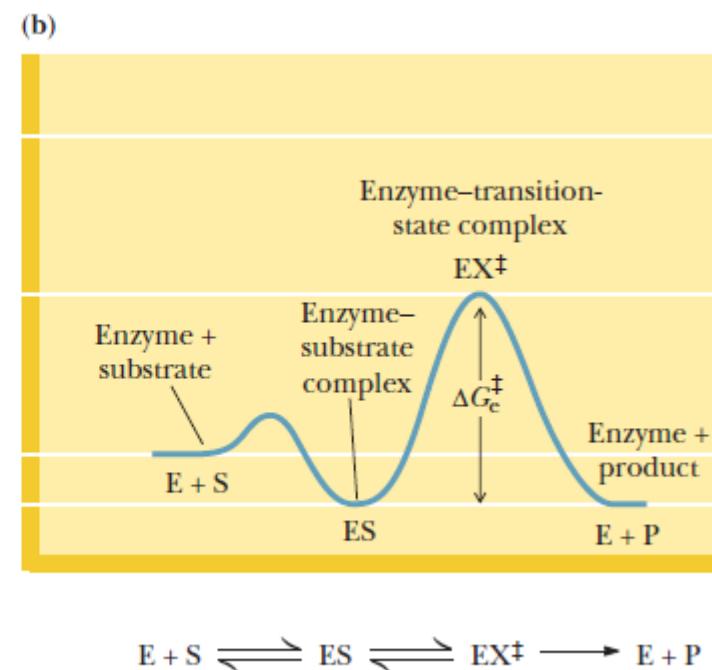
Reaction	Enzyme	Uncatalyzed Rate, v_u (sec $^{-1}$)	Catalyzed Rate, v_e (sec $^{-1}$)	v_e/v_u
Fructose-1,6-bisP \longrightarrow fructose-6-P + P _i	Fructose-1,6-bisphosphatase	2×10^{-20}	21	1.05×10^{21}
(Glucose) _n + H ₂ O \longrightarrow (glucose) _{n-2} + maltose	β -amylase	1.9×10^{-15}	1.4×10^3	7.2×10^{17}
DNA, RNA cleavage	Staphylococcal nuclease	7×10^{-16}	95	1.4×10^{17}
CH ₃ —O—PO ₃ ²⁻ + H ₂ O \longrightarrow CH ₃ OH + HPO ₄ ²⁻	Alkaline phosphatase	1×10^{-15}	14	1.4×10^{16}
$\text{H}_2\text{N}-\overset{\text{O}}{\underset{\parallel}{\text{C}}}-\text{NH}_2 + 2 \text{H}_2\text{O} + \text{H}^+ \longrightarrow 2 \text{NH}_4^+ + \text{HCO}_3^-$	Urease	3×10^{-10}	3×10^4	1×10^{14}
R—C(=O)—O—CH ₂ CH ₃ + H ₂ O \longrightarrow RCOOH + HOCH ₂ CH ₃	Chymotrypsin	1×10^{-10}	1×10^2	1×10^{12}
Glucose + ATP \longrightarrow Glucose-6-P + ADP	Hexokinase	$<1 \times 10^{-13}$	1.3×10^{-3}	$>1.3 \times 10^{10}$
CH ₃ CH ₂ OH + NAD ⁺ \longrightarrow CH ₃ CH(OH) + NADH + H ⁺	Alcohol dehydrogenase	$<6 \times 10^{-12}$	2.7×10^{-5}	$>4.5 \times 10^6$
CO ₂ + H ₂ O \longrightarrow HCO ₃ ⁻ + H ⁺	Carbonic anhydrase	10^{-2}	10^5	1×10^7
Creatine + ATP \longrightarrow Cr-P + ADP	Creatine kinase	$<3 \times 10^{-9}$	4×10^{-5}	$>1.33 \times 10^4$

Adapted from Koshland, D., 1956. Molecular geometry in enzyme action. *Journal of Cellular Comparative Physiology*, Supp. 1, 47:217; and Wolfenden, R., 2006. Degrees of difficulty of water-consuming reactions in the absence of enzymes. *Chemical Reviews* 106:3379–3396.

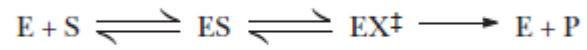
In all chemical reactions, the reacting atoms or molecules pass through a state that is intermediate in structure between the reactant(s) and the product(s). Consider the transfer of a proton from a water molecule to a chloride anion:



Without enzyme



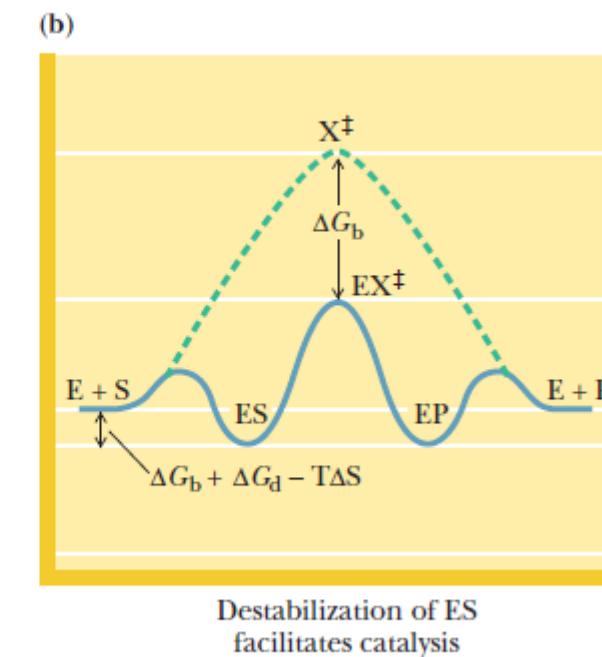
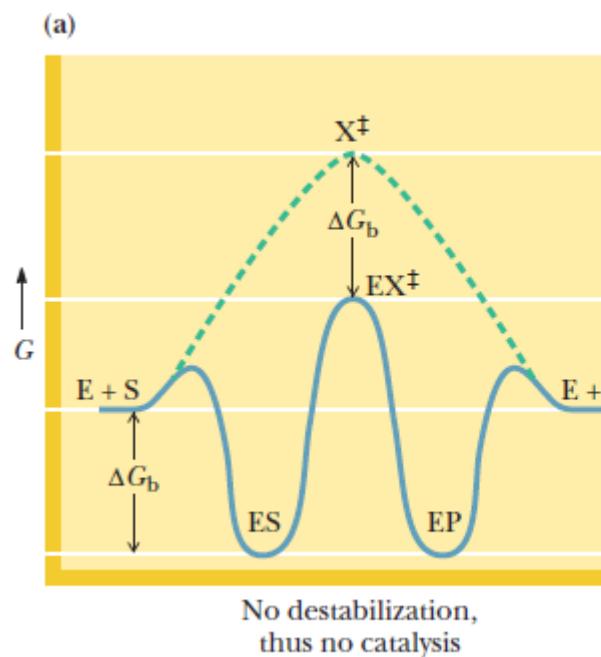
With enzyme



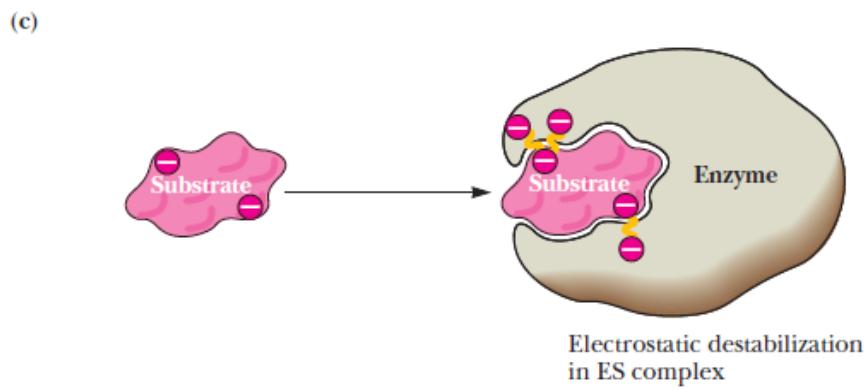
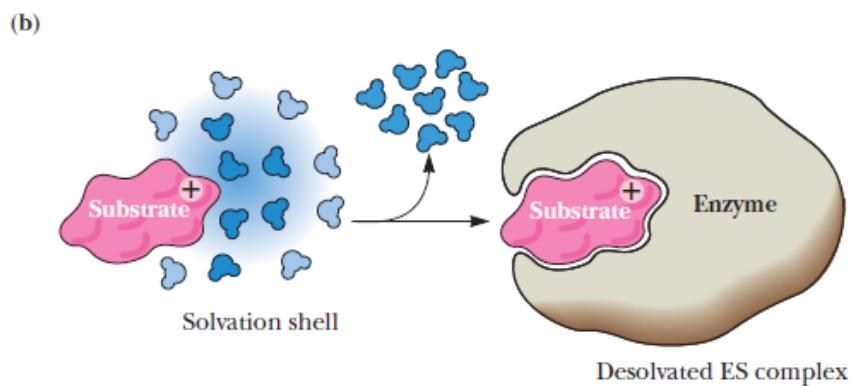
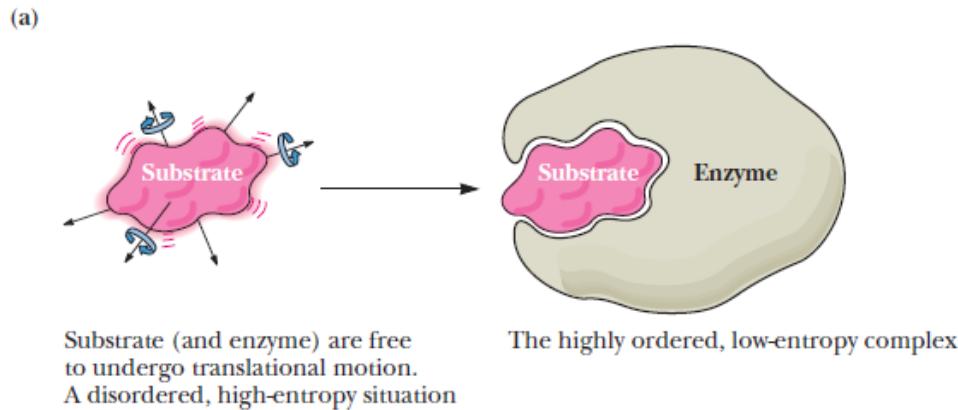
$$K_S = \frac{[E][S]}{[ES]}$$

$$K_T = \frac{[E][X^\ddagger]}{[EX^\ddagger]}$$

$$k_e/k_u \approx K_S/K_T$$



Partially de-stabilize ES complex



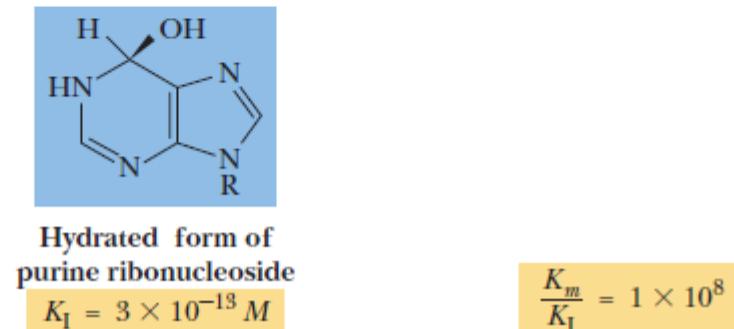
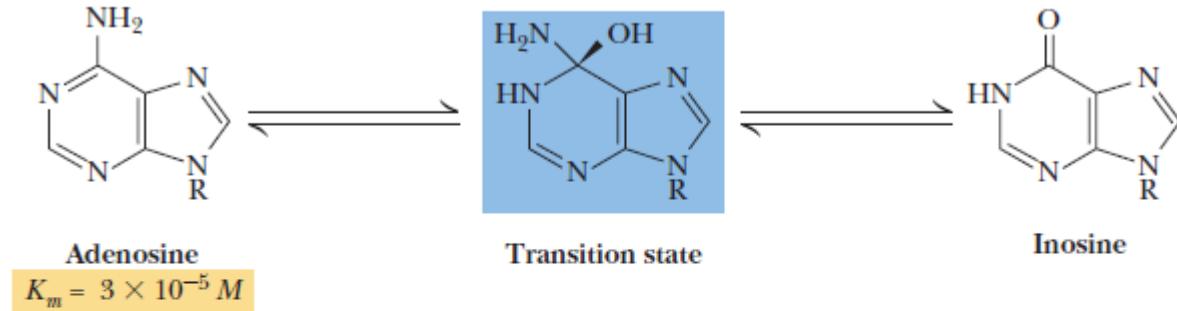
How analogs of transition state exploited?

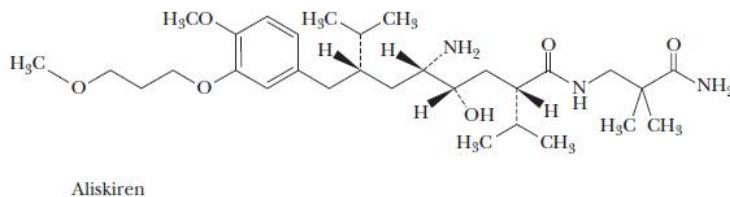
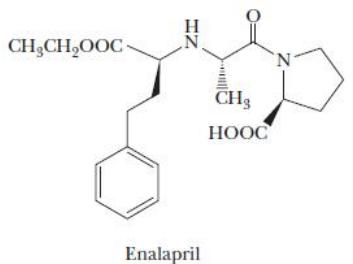
The value of K_T for fructose-1,6-bisphosphatase is an astounding $7 \times 10^{-26} M$!

This low value for binding constant means very tight binding between enzyme and transition complex

If you can mimic the transition state with similar compound, it will bind the enzyme complex very tightly.
This idea is exploited very well and many known drug today have designed on this principle

(b) Calf intestinal adenosine deaminase reaction

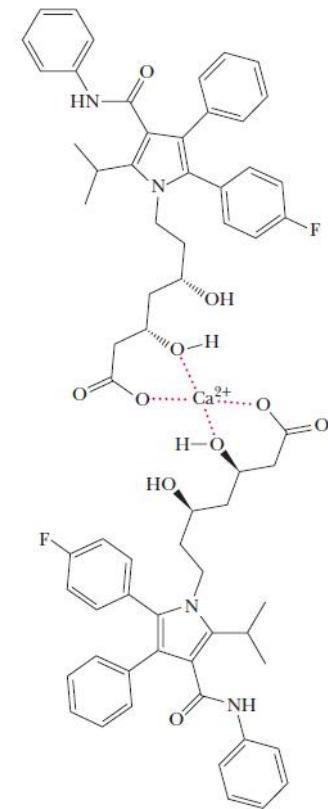
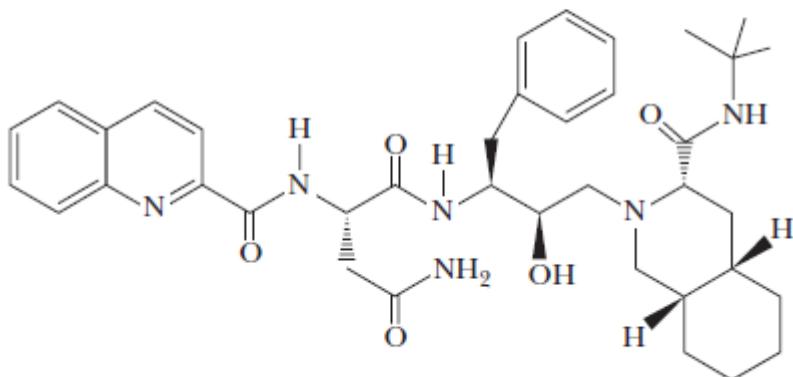




Angiotensin Converting Enzyme (ACE)

Protease Inhibitors Are AIDS Drugs

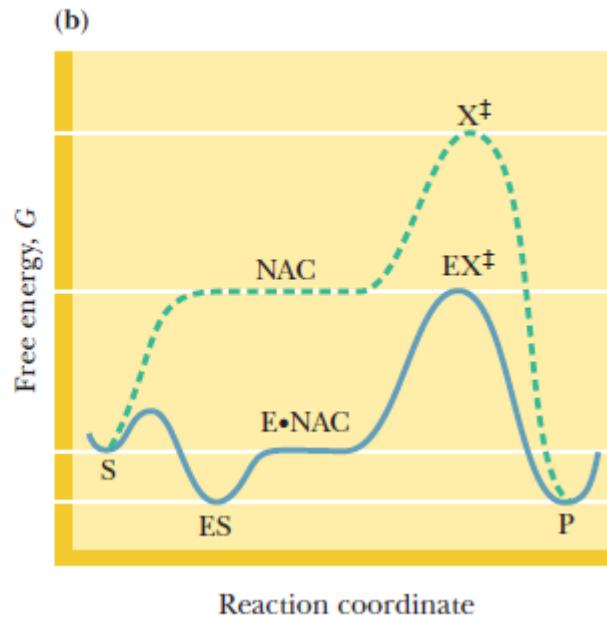
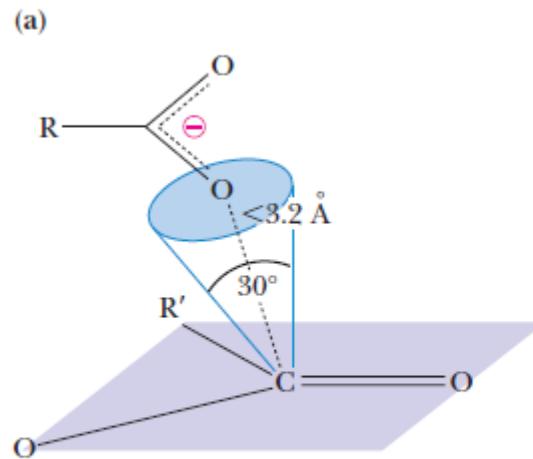
Crixivan (indinavir) by Merck, Invirase (saquinavir) by Roche, and similar “protease inhibitor” drugs are transition-state analogs for the HIV-1 protease, discussed on pages 470–471.



HMG-CoA reductase

Near attack conformations

Reacting atoms are in *Van der waals* contact and at an angle resembling the bond to be formed



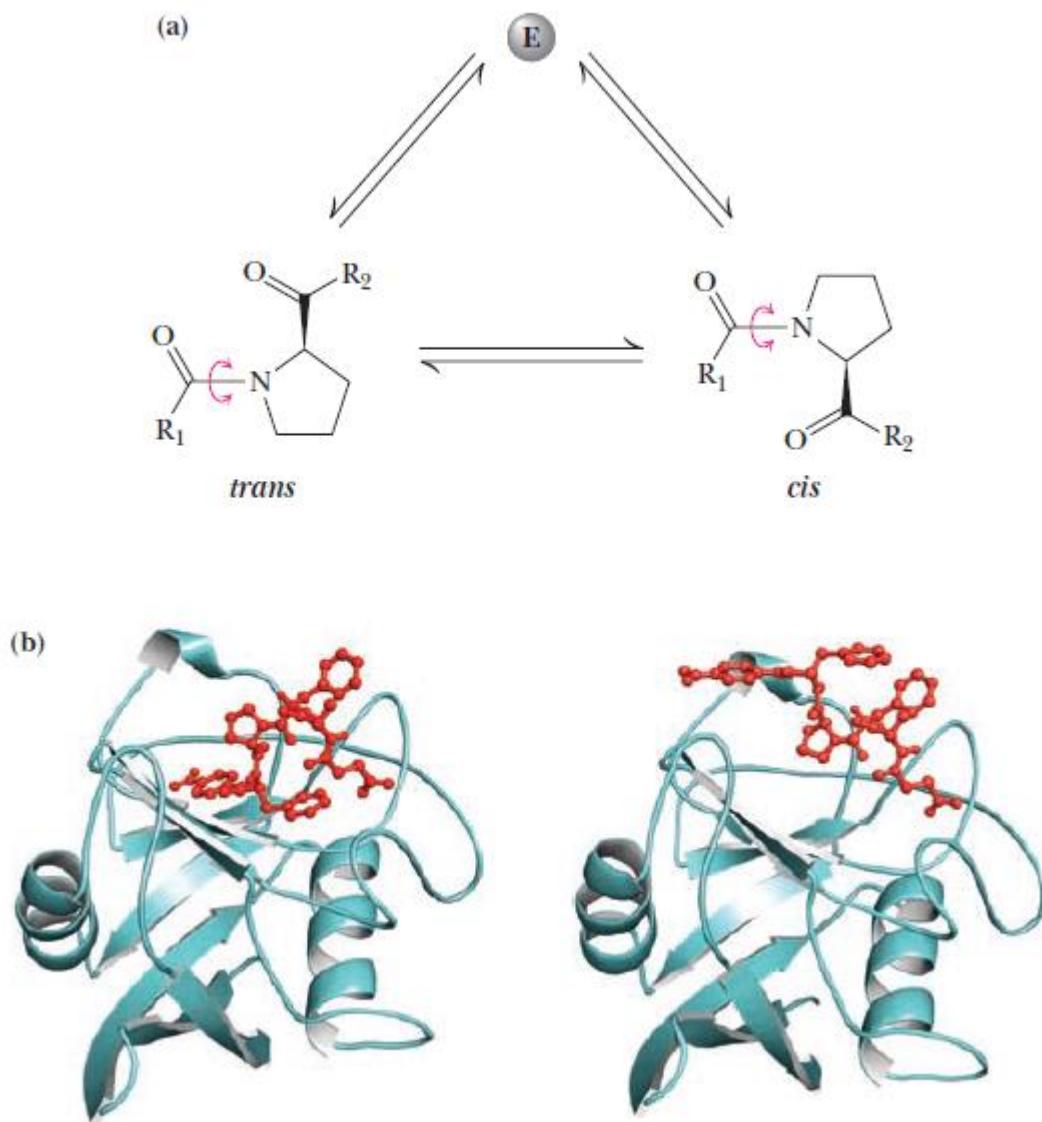
For reactions involving bonding between O, N, C & S atoms: NAC are characterized as Distance within 3.2 Å and ± 15 angle

What exactly happens at protein active site??

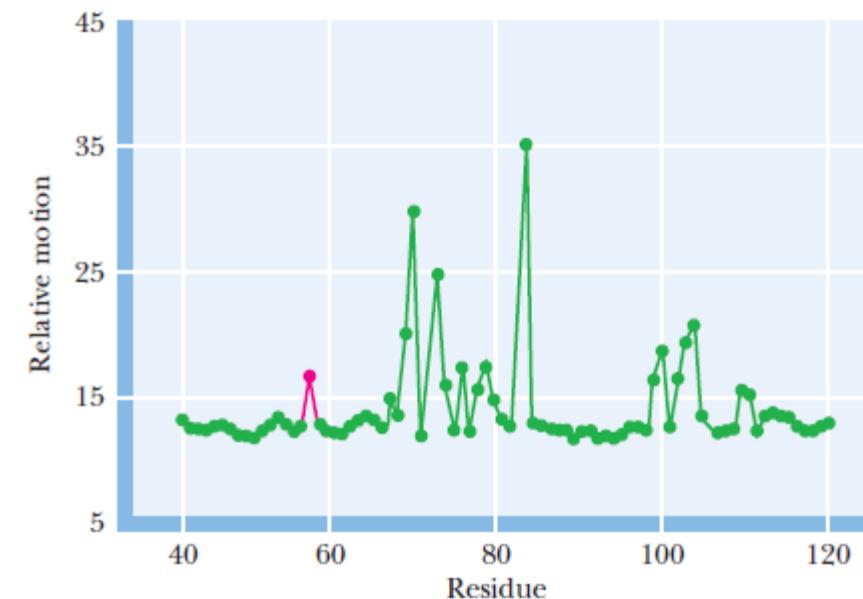
Different amino-acids in protein active site are in constant motions and they assist in

1. Substrate binding
2. Bring catalytic group into position around a substrate
3. Induce formation of NAC
4. Assist in bond making and bond breaking
5. Facilitate conversion of substrate to product

example



Human cyclophilin A is a prolyl isomerase, which catalyzes the interconversion between *trans* and *cis* conformations of proline in peptides.



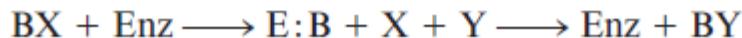
This network extends from the active site to the surface of the protein, and the motions in this network span time scales of femtoseconds to milliseconds. Such extensive networks of motion make it likely that the entire folded structure of the protein may be involved in catalysis at the active site.

Covalent Catalysis

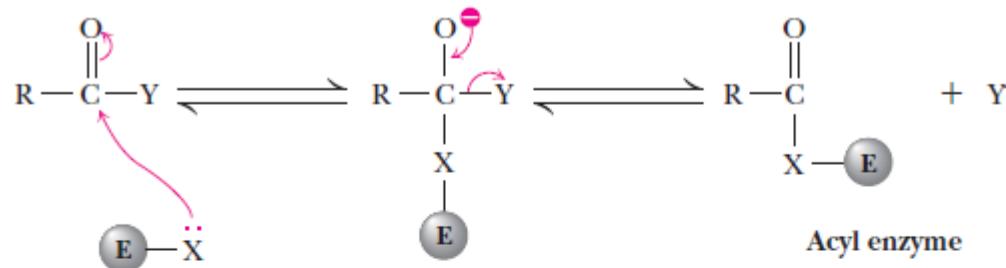
Some enzyme reactions derive much of their rate acceleration from the formation of covalent bonds between enzyme and substrate. Consider the reaction:



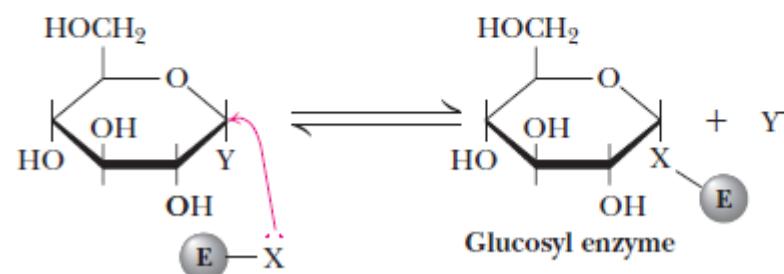
and an enzymatic version of this reaction involving formation of a covalent intermediate:



examples



Acyl enzyme



Glucosyl enzyme

Digestive serine protease

Ex., trypsin, chymotrypsin, thrombin, tissue plasminogen activator

Catalytic mechanism based on active site serine

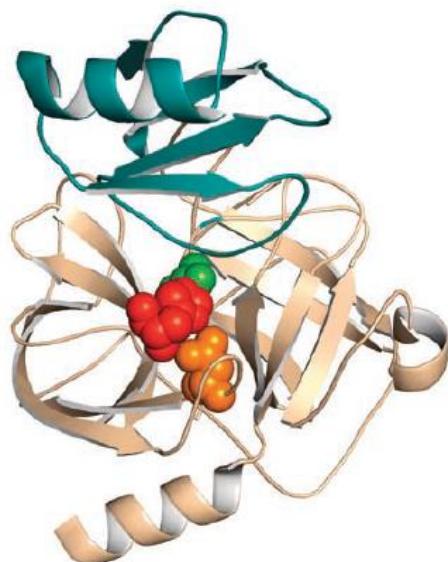


FIGURE 14.16 Structure of chymotrypsin (white) in a complex with eglin C (blue ribbon structure), a target protein. The residues of the catalytic triad (His⁵⁷, Asp¹⁰², and Ser¹⁹⁵) are highlighted. His⁵⁷ (red) is flanked by Asp¹⁰² (gold) and by Ser¹⁹⁵ (green). The catalytic site is filled by a peptide segment of eglin. Note how close Ser¹⁹⁵ is to the peptide that would be cleaved in the chymotrypsin reaction (pdb id = 1ACB).

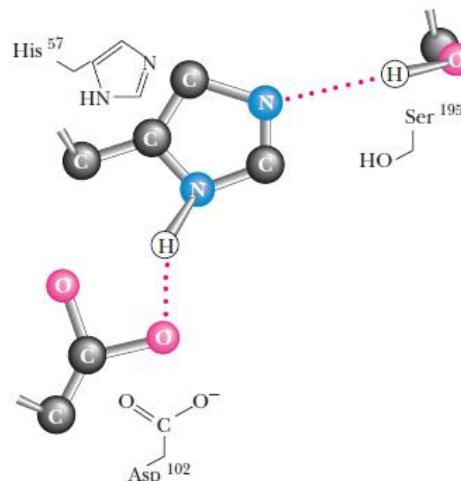
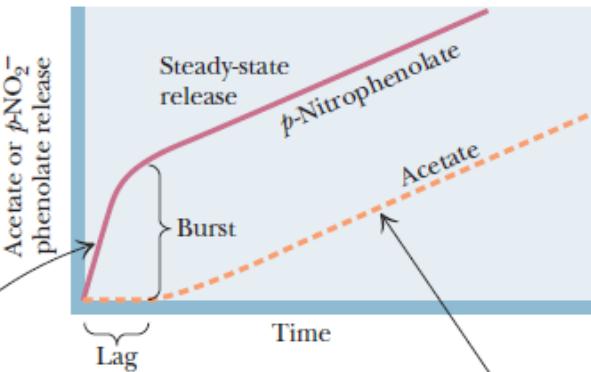
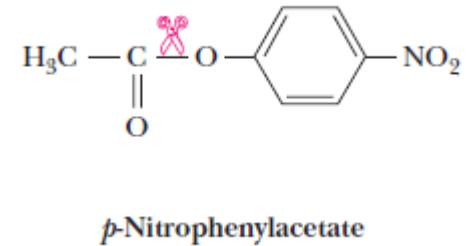
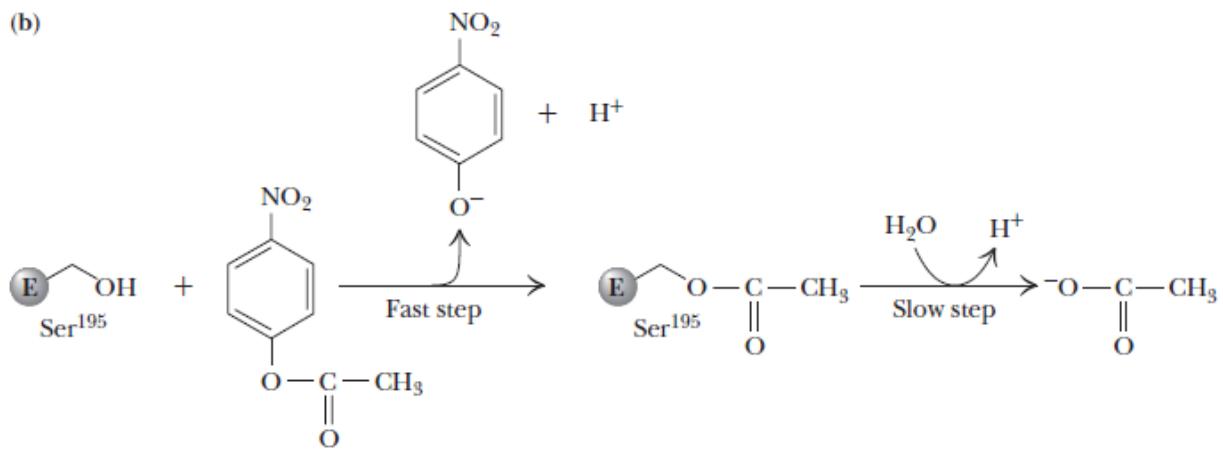


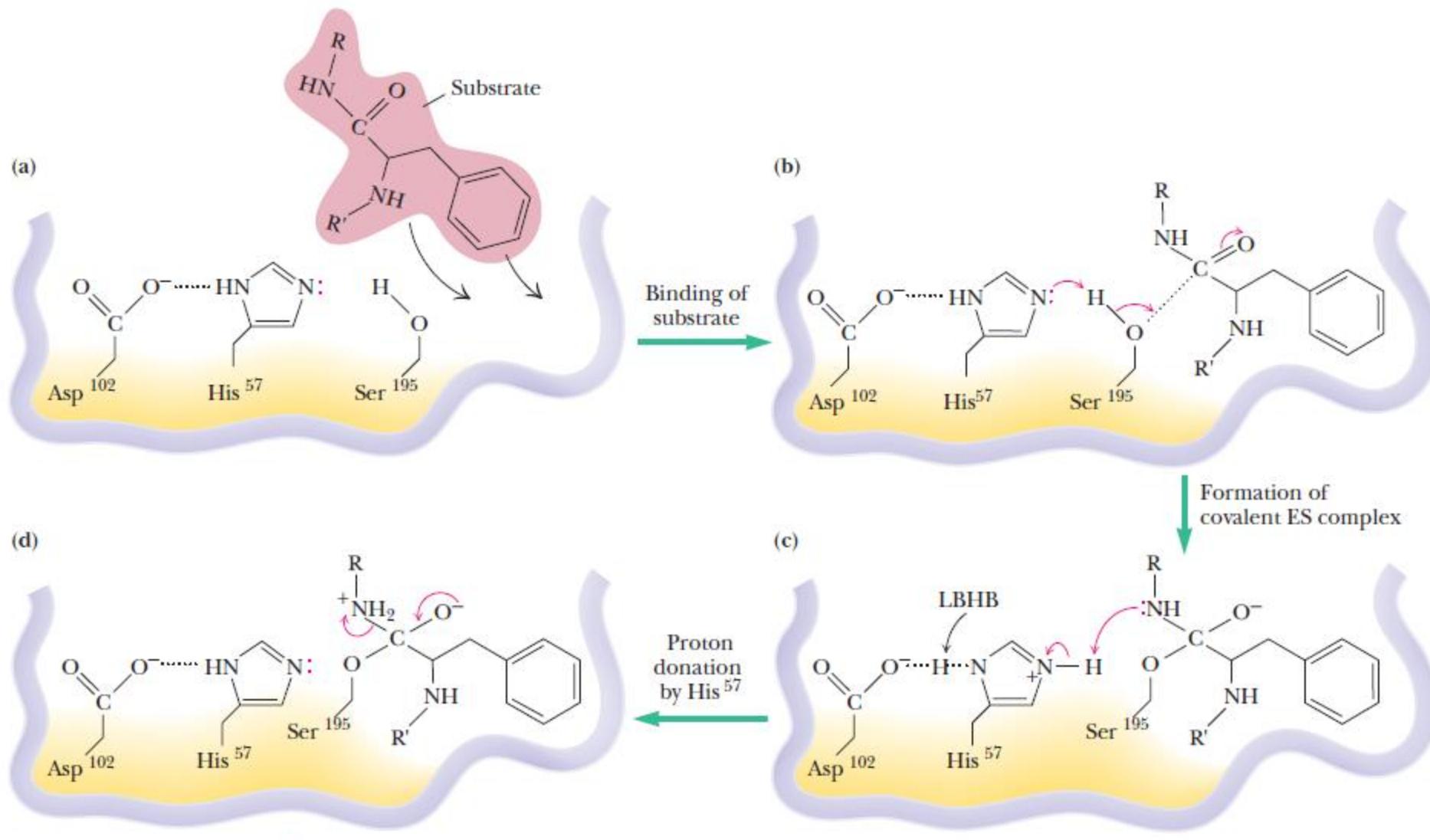
FIGURE 14.17 The catalytic triad of chymotrypsin.

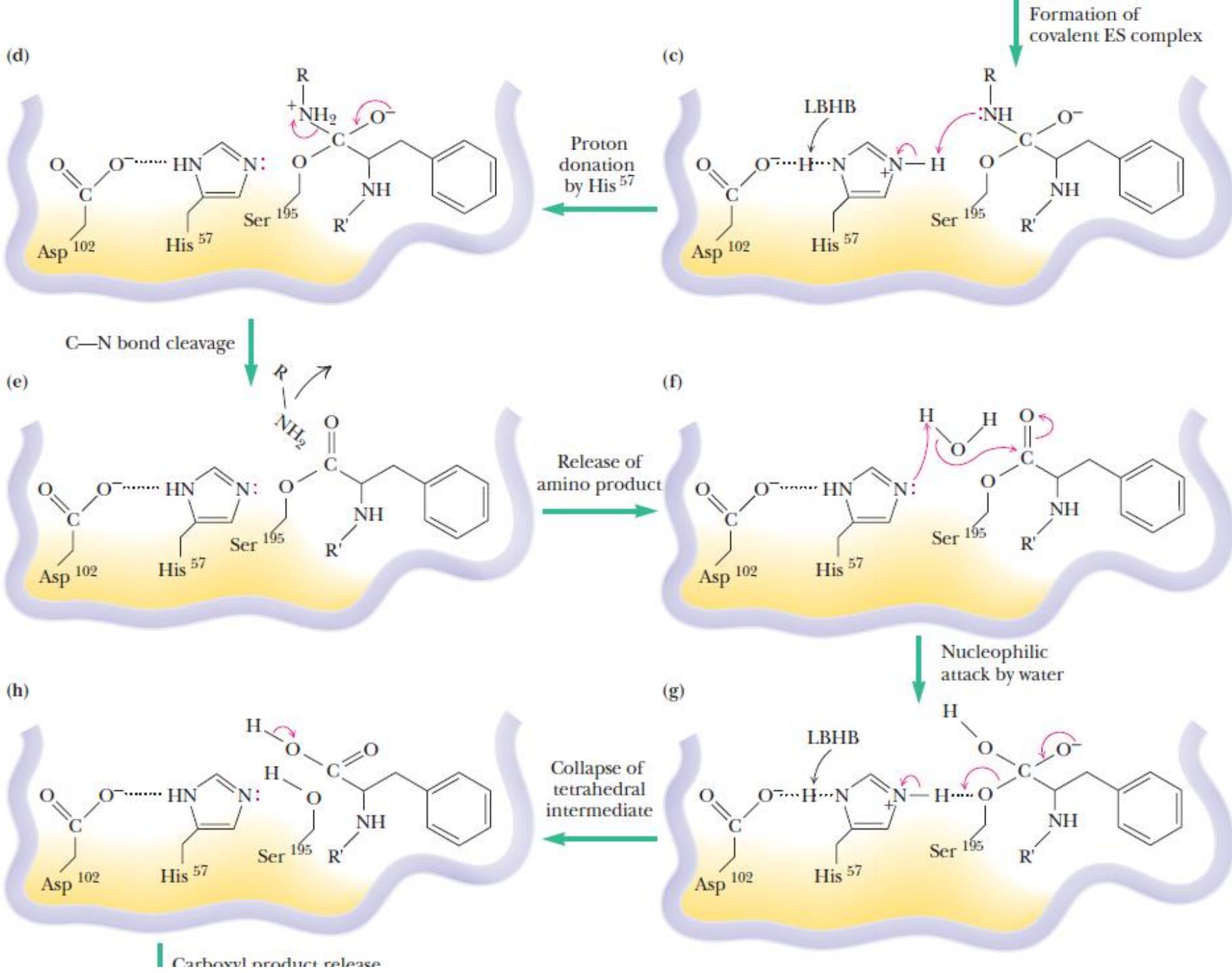
(a)



(b)







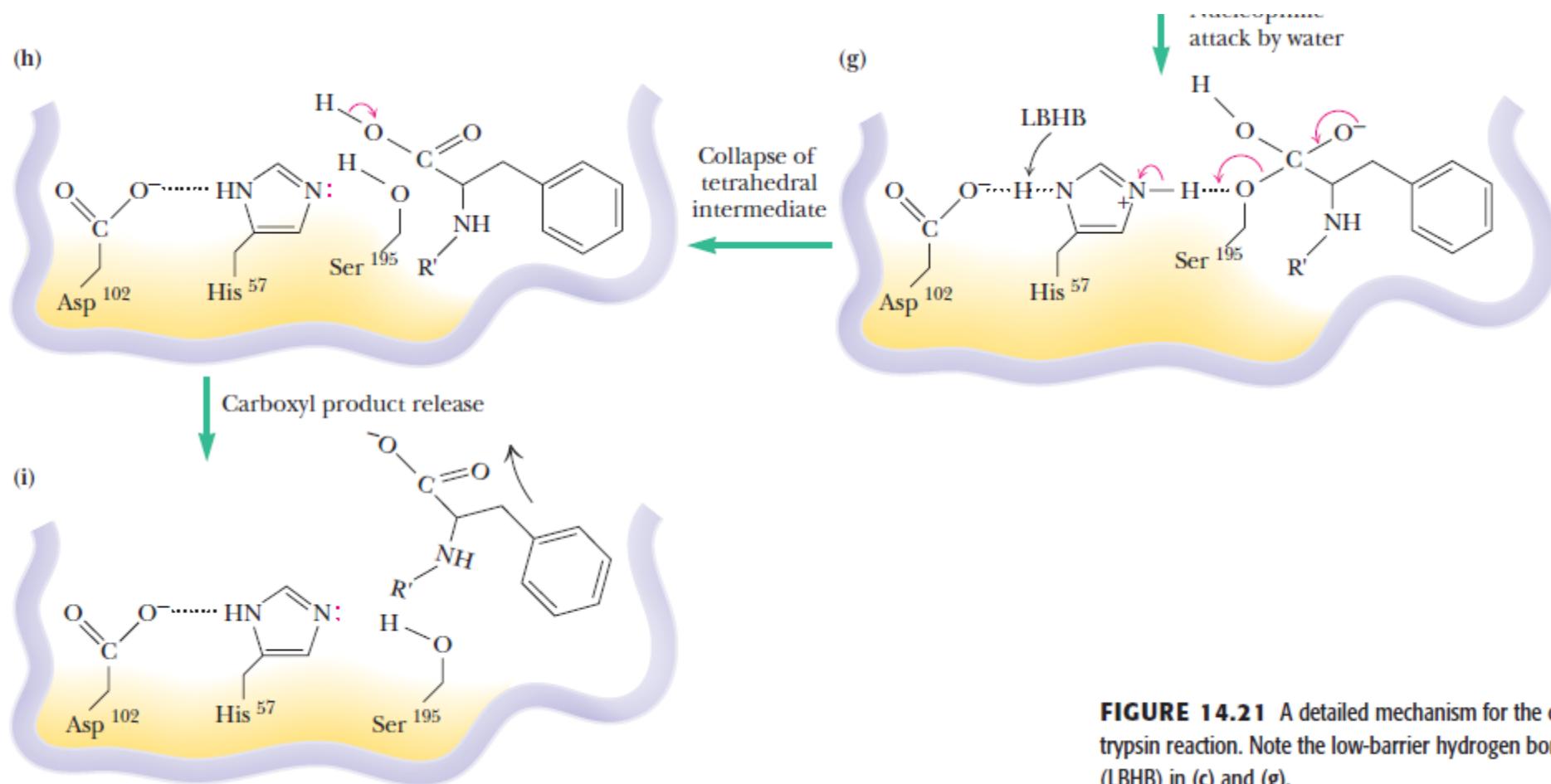
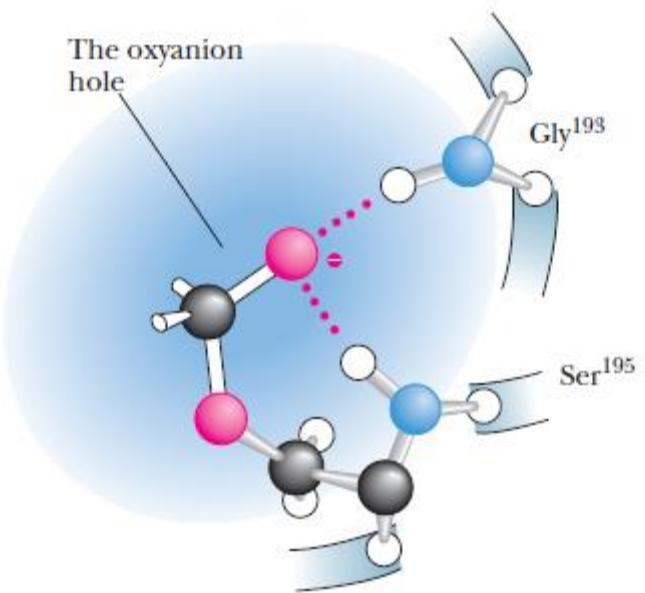
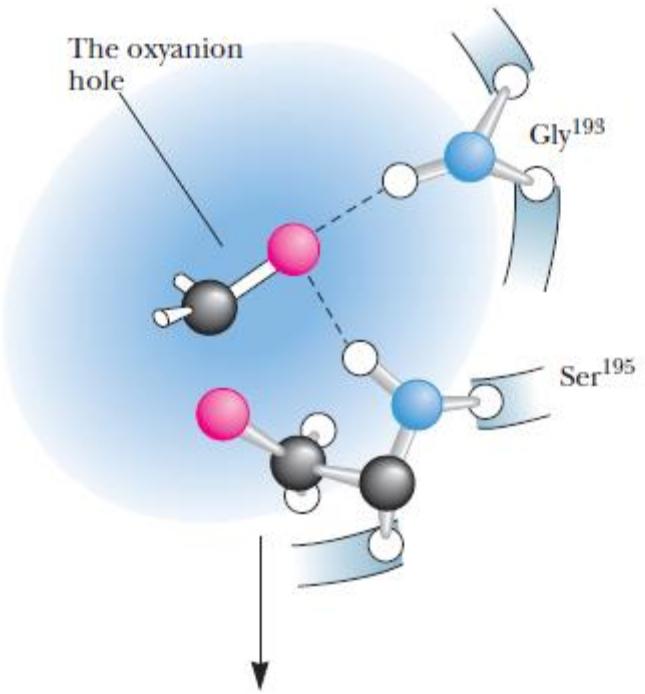


FIGURE 14.21 A detailed mechanism for the chymotrypsin reaction. Note the low-barrier hydrogen bond (LBHB) in (c) and (g).



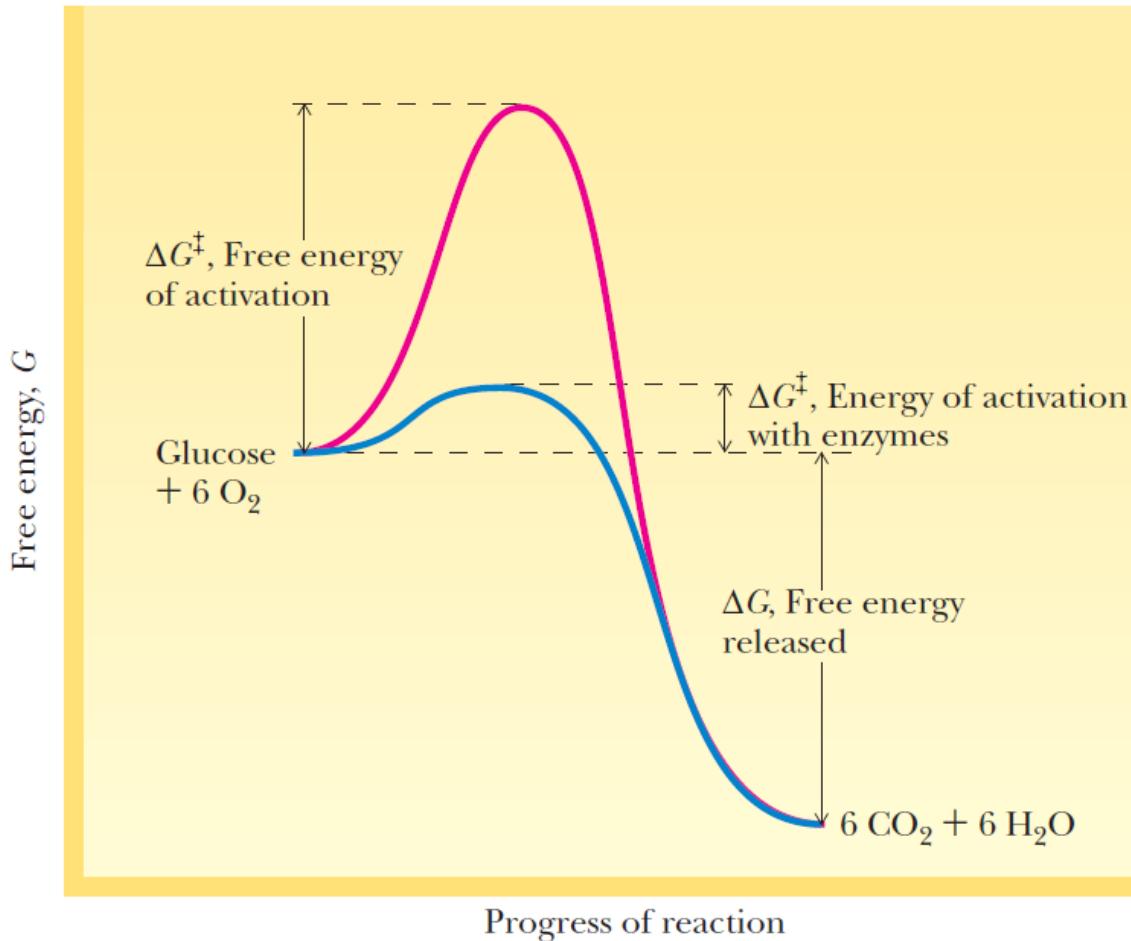
L7 Enzyme functional nature: Mechanism

Ravikrishnan Elangovan,
Department of Biochemical Engg and Biotechnology
Indian Institute of Technology - Delhi

Enzyme; Functional nature and mechanism

Enzymes are catalyst,
That accelerate chemical reactions

Mostly enzymes are proteins,
some RNA enzymes also exist



Gibbs free energy (G)

- Thermodynamics: changes in free energy, entropy, ...

$$\Delta G = \Delta H - T \cdot \Delta S$$

$$\Delta G = (\Delta U + P \cdot \Delta V) - T \cdot \Delta S$$

- For nearly all biochemical reactions ΔV is small and ΔH is almost equal to ΔU
- Hence, we can write:

$$\boxed{\Delta G = \Delta U - T \cdot \Delta S}$$

If ΔG is negative

Energy was released, products are simpler, greater entropy (2nd Law of Thermodynamics)
Exergonic / exothermic reaction (spontaneous)

If ΔG is positive

Energy input, product more complex, energy needed to go against 2nd Law
Endergonic / endothermic (non-spontaneous)

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- Changes in bonding
 - van der Waals
 - Hydrogen bonding
 - Charge interactions

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- Reflects the degrees of freedom
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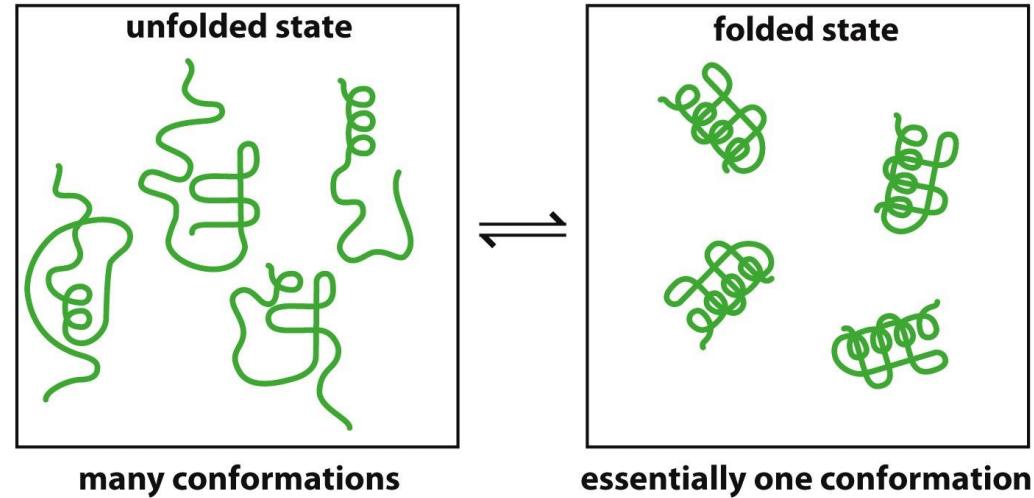


Figure 10.13 The Molecules of Life (© Garland Science 2013)

Protein folding is a spontaneous process?

$$\Delta G < 0 ???$$

Protein folding



$$k_{\text{folding}} = \frac{(F)}{(U)} = \frac{1}{k_{\text{unfolding}}}$$

$$\Delta G^{\circ}_{\text{unfolding}} = \Delta H^{\circ} - T \Delta S^{\circ}$$

Enthalpy change

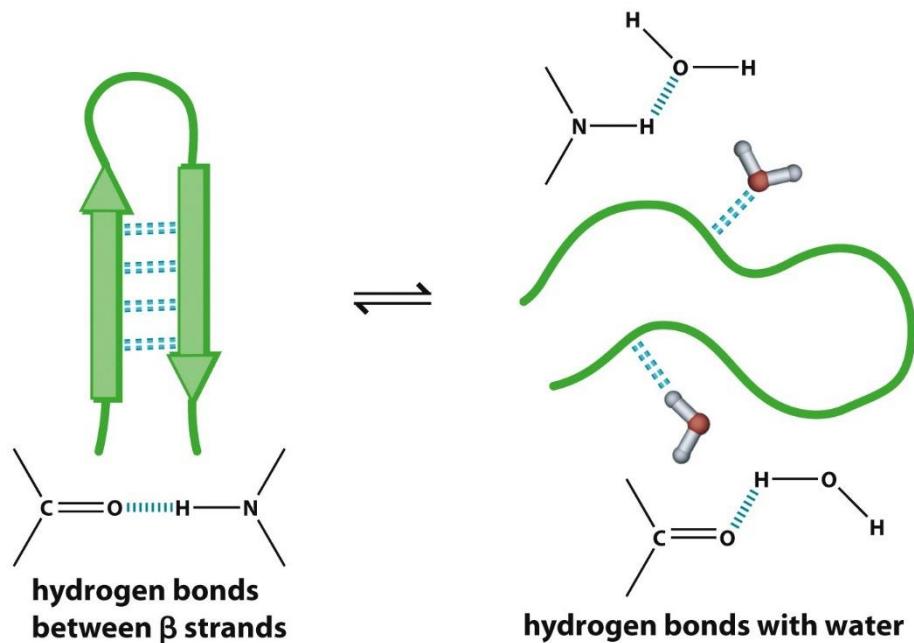


Figure 10.14 The Molecules of Life (© Garland Science 2013)

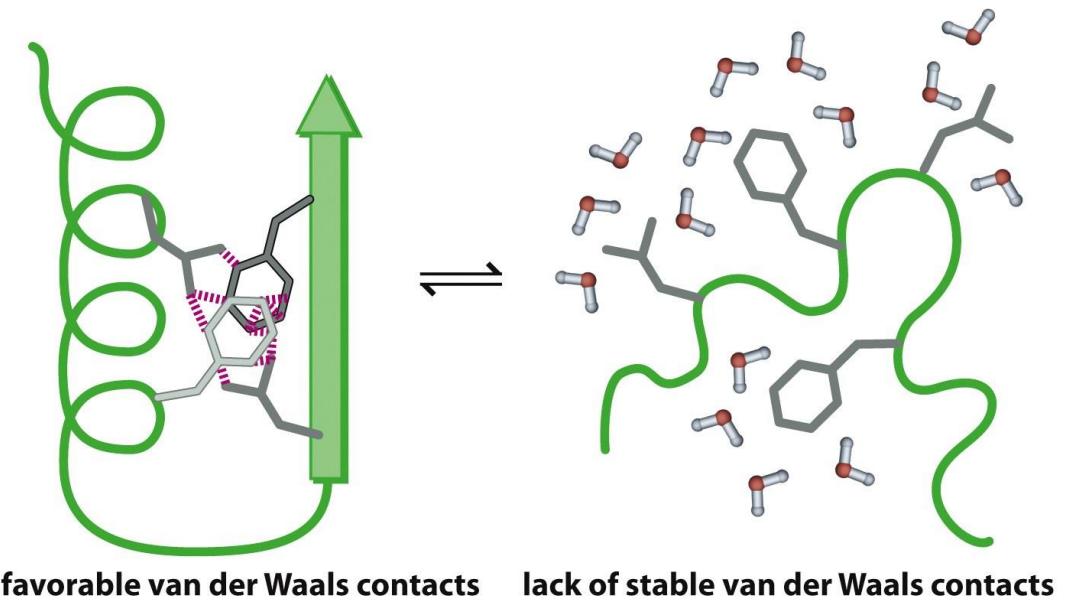


Figure 10.15 The Molecules of Life (© Garland Science 2013)

Entropy contribution

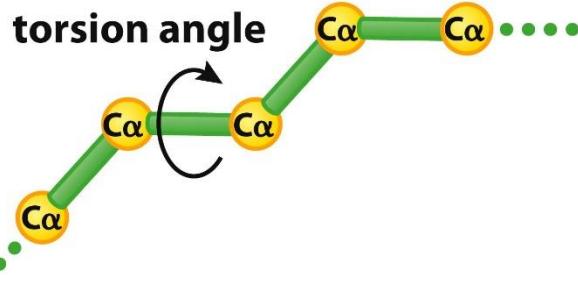


Figure 10.18 The Molecules of Life (© Garland Science 2013)

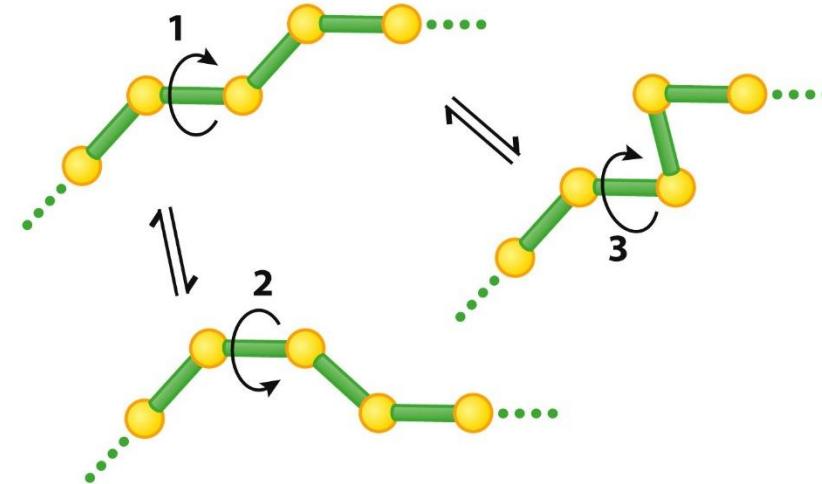


Figure 10.19 The Molecules of Life (© Garland Science 2013)

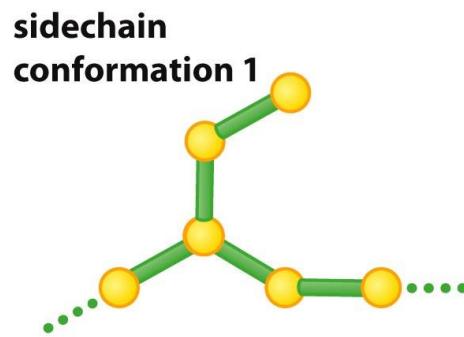
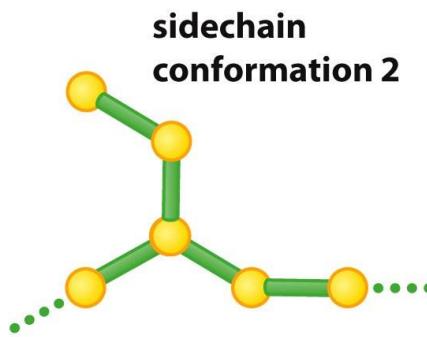


Figure 10.20 The Molecules of Life (© Garland Science 2013)



sidechain
conformation 2

Entropy contribution from water

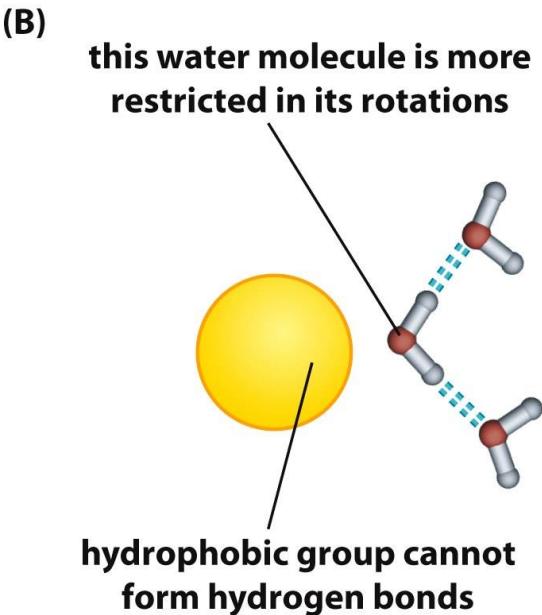
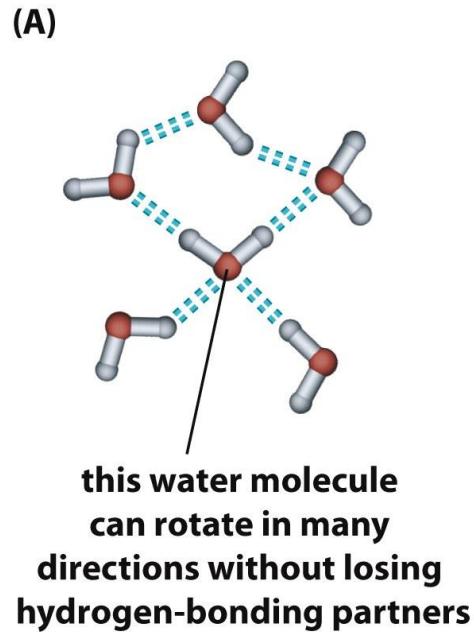


Figure 10.21 The Molecules of Life (© Garland Science 2013)

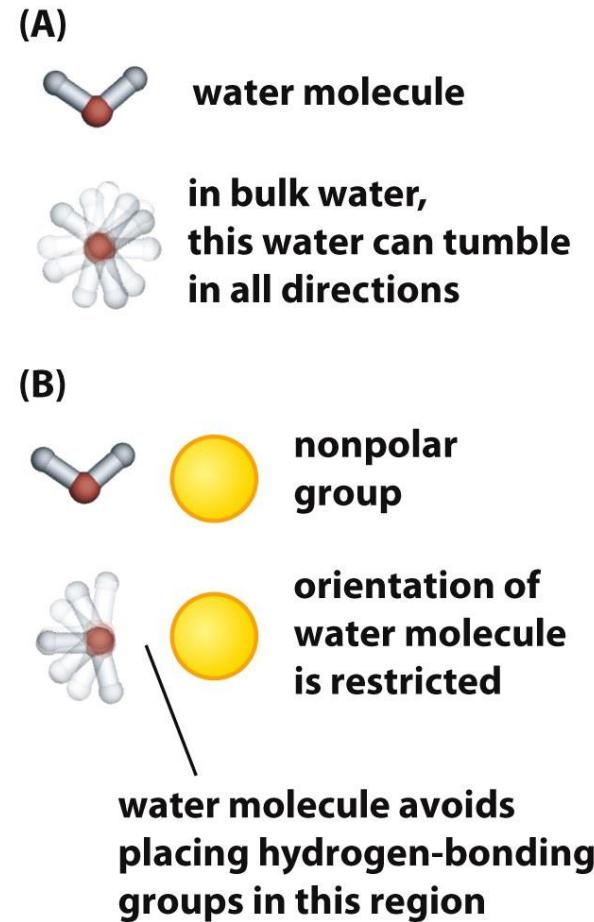


Figure 10.22 The Molecules of Life (© Garland Science 2013)

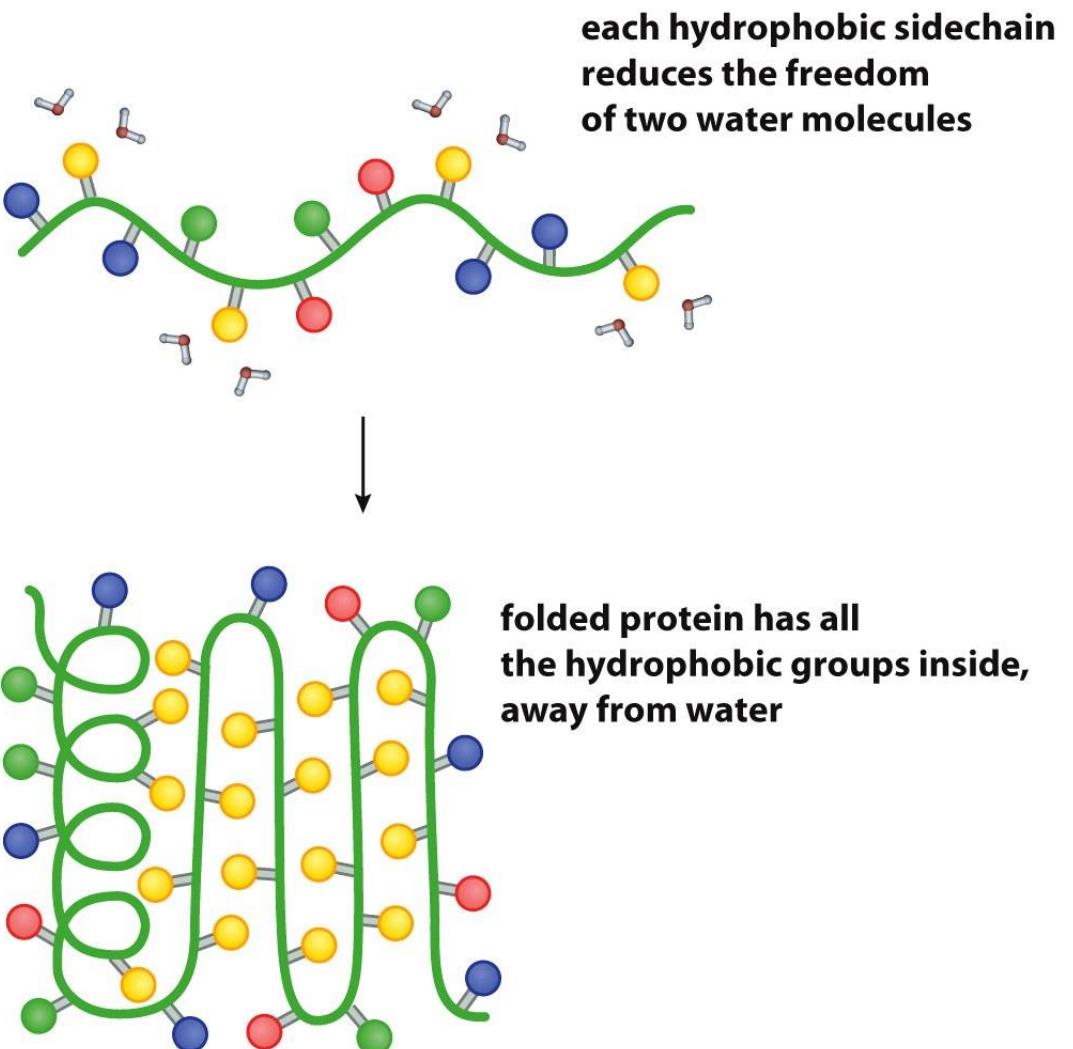
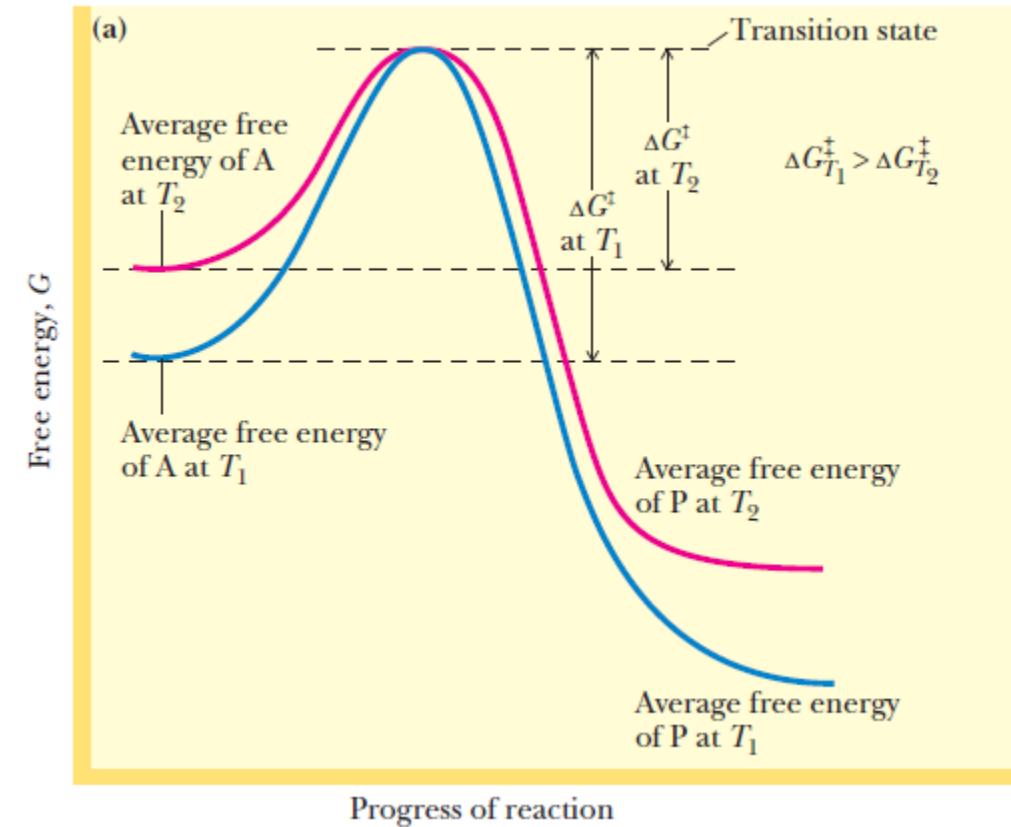
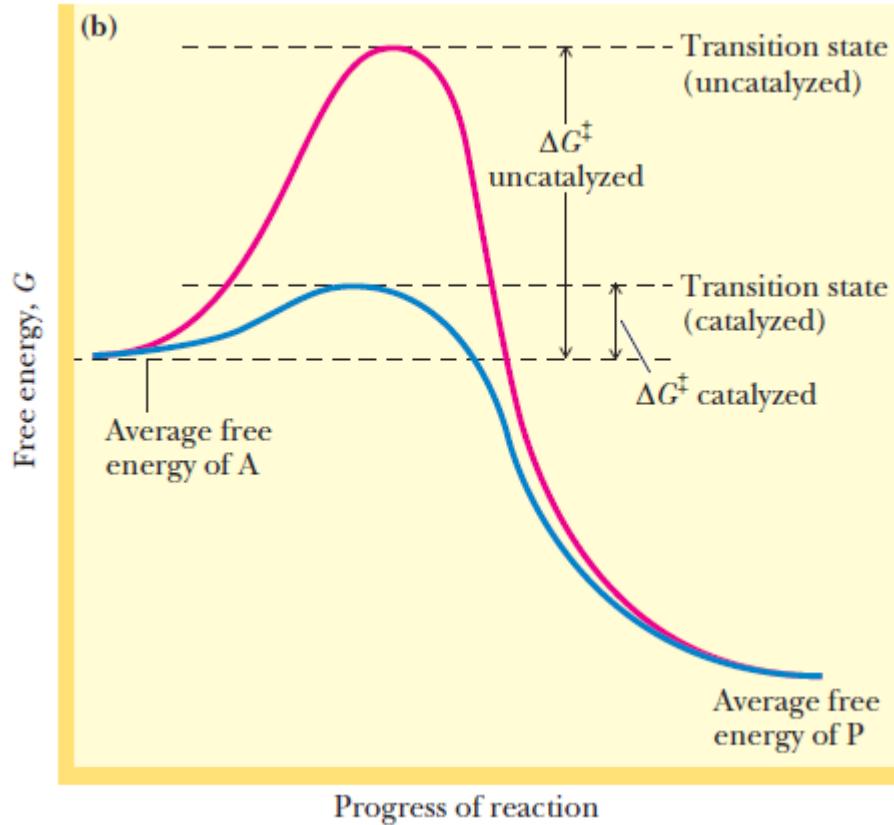


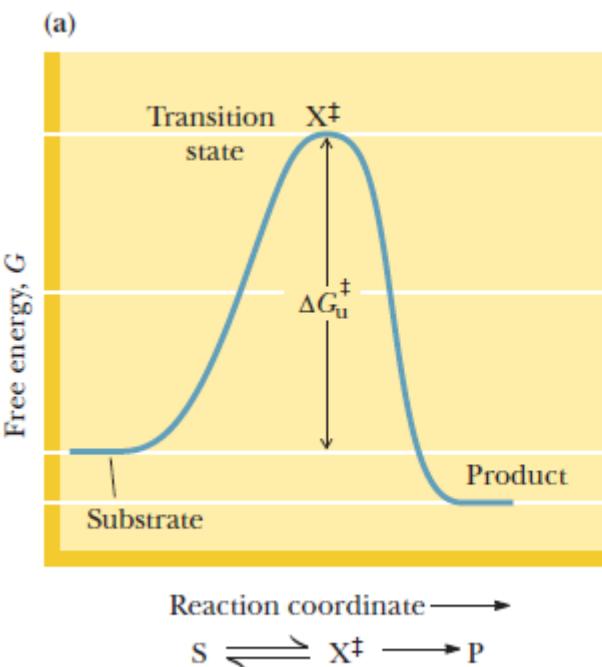
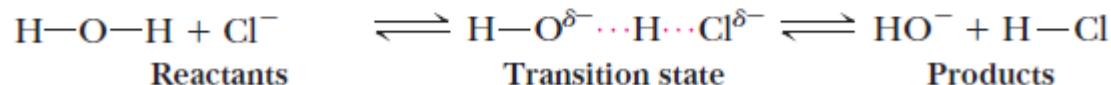
Figure 10.23 The Molecules of Life (© Garland Science 2013)

Mechanism :Catalysts Lower the Free Energy of Activation for a Reaction

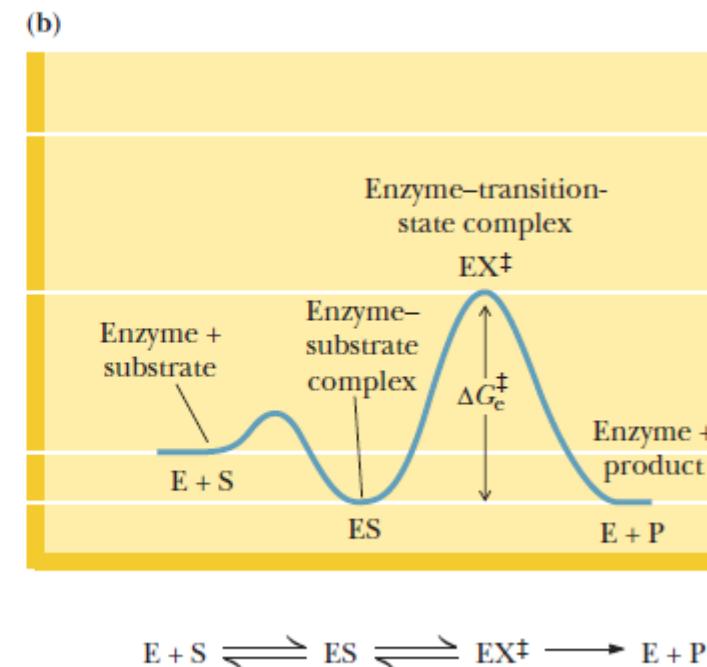


Transition intermediate in chemical reactions!

In all chemical reactions, the reacting atoms or molecules pass through a state that is intermediate in structure between the reactant(s) and the product(s). Consider the transfer of a proton from a water molecule to a chloride anion:



Without enzyme



With enzyme

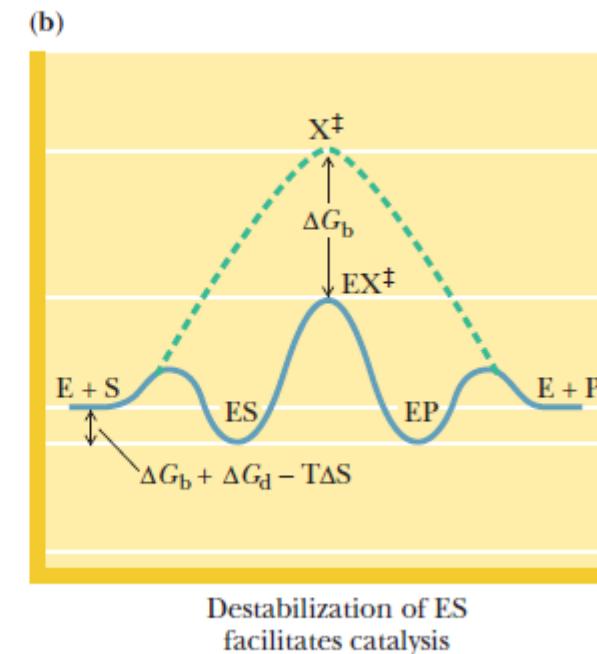
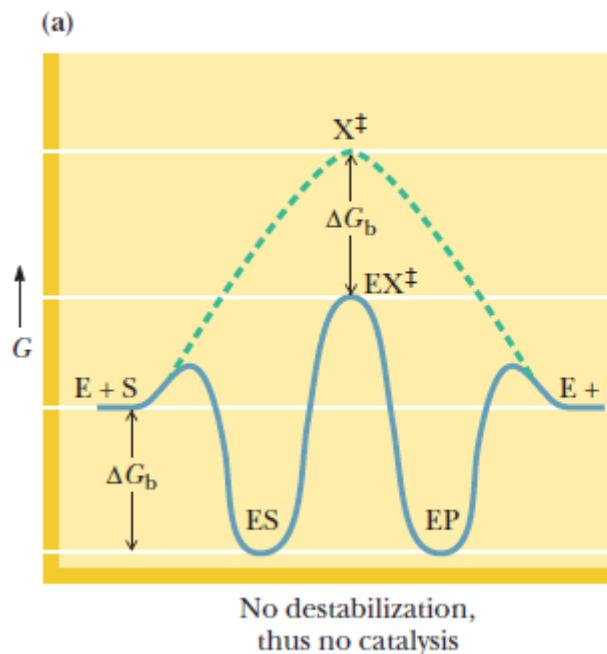
Enzyme substrate complex is partially destabilized!



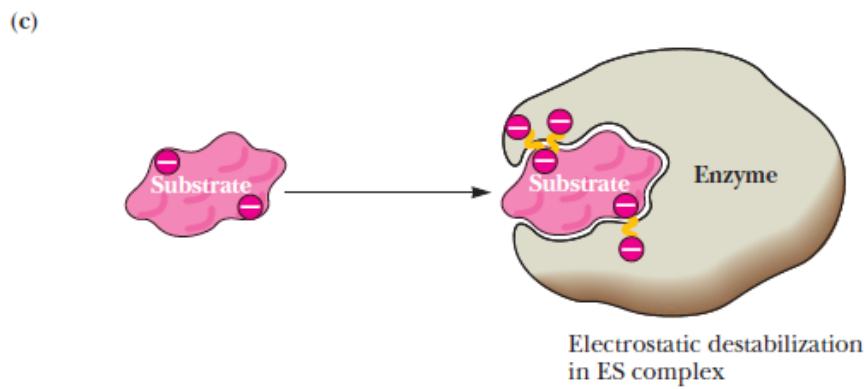
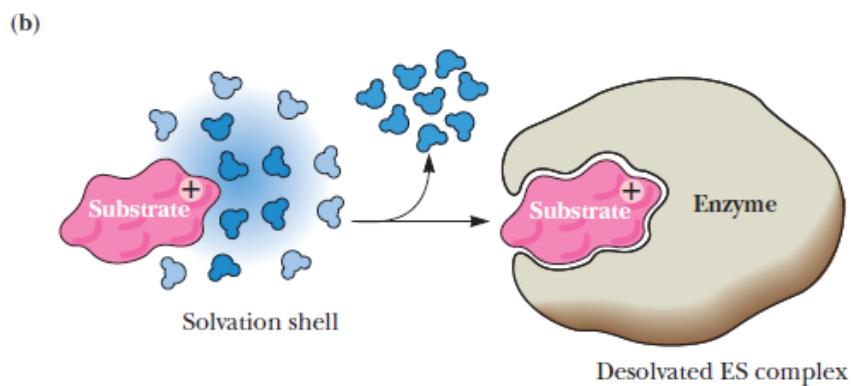
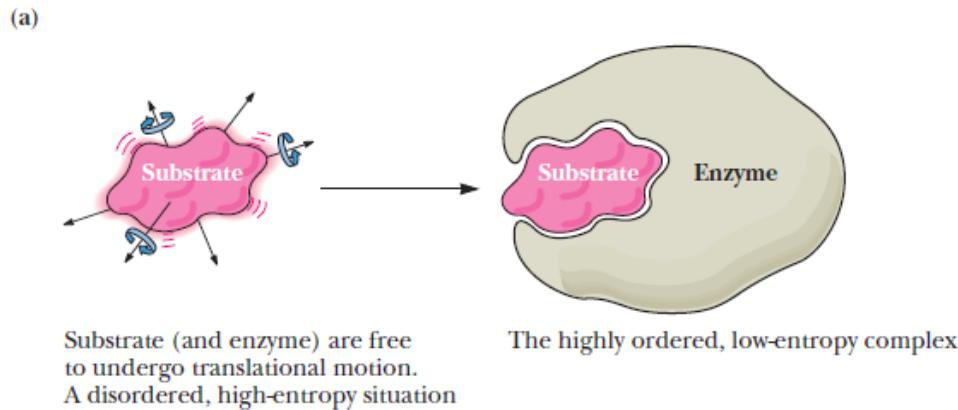
$$K_S = \frac{[E][S]}{[ES]}$$

$$K_T = \frac{[E][X^\ddagger]}{[EX^\ddagger]}$$

$$k_e/k_u \approx K_S/K_T$$



Partially de-stabilize ES complex



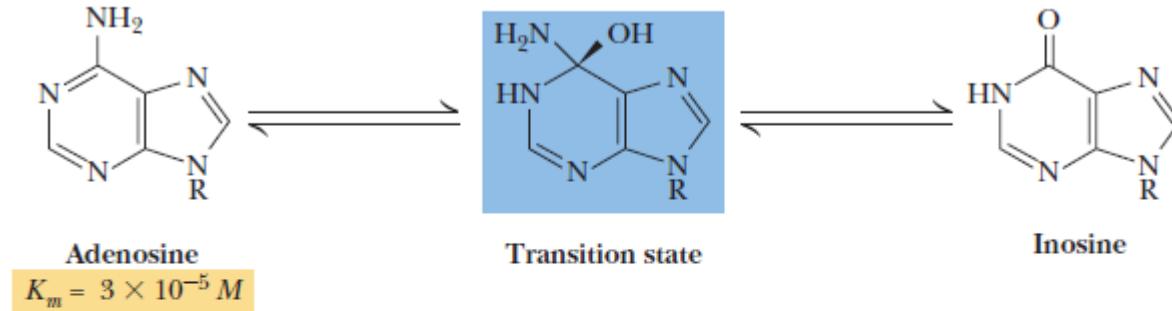
How analogs of transition state exploited?

The value of K_T for fructose-1,6-bisphosphatase is an astounding $7 \times 10^{-26} M$!

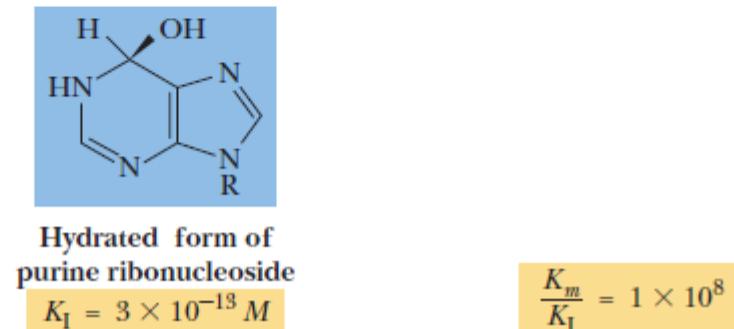
This low value for binding constant means very tight binding between enzyme and transition complex

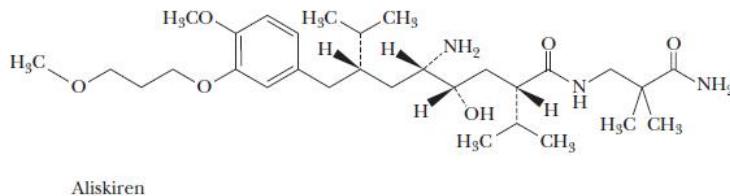
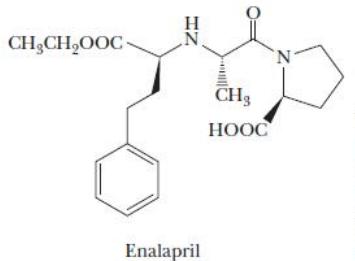
If you can mimic the transition state with similar compound, it will bind the enzyme complex very tightly.
This idea is exploited very well and many known drug today have designed on this principle

(b) Calf intestinal adenosine deaminase reaction



$$K_m = 3 \times 10^{-5} M$$

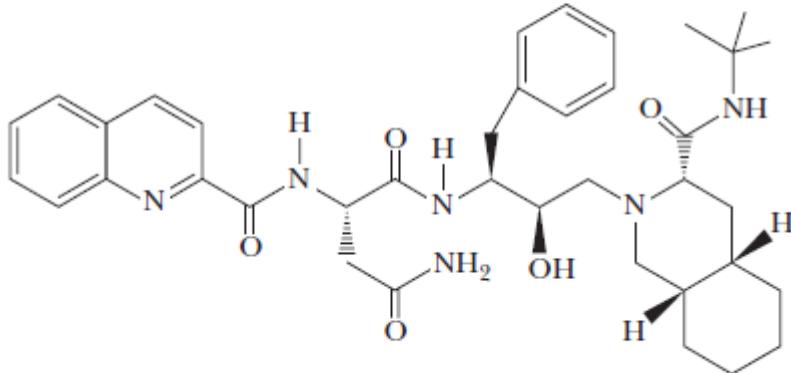




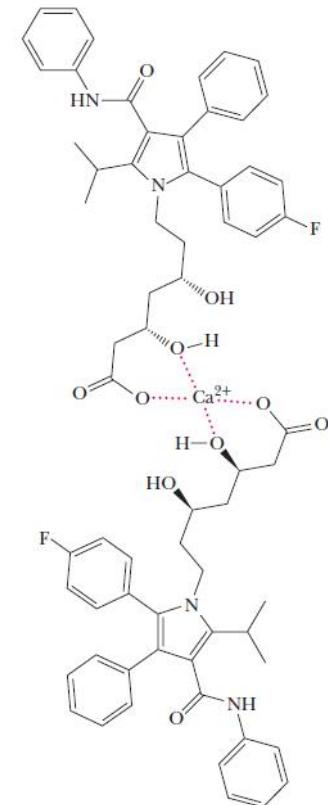
Angiotensin Converting Enzyme (ACE)

Protease Inhibitors Are AIDS Drugs

Crixivan (indinavir) by Merck, Invirase (saquinavir) by Roche, and similar “protease inhibitor” drugs are transition-state analogs for the HIV-1 protease, discussed on pages 470–471.



Saquinavir

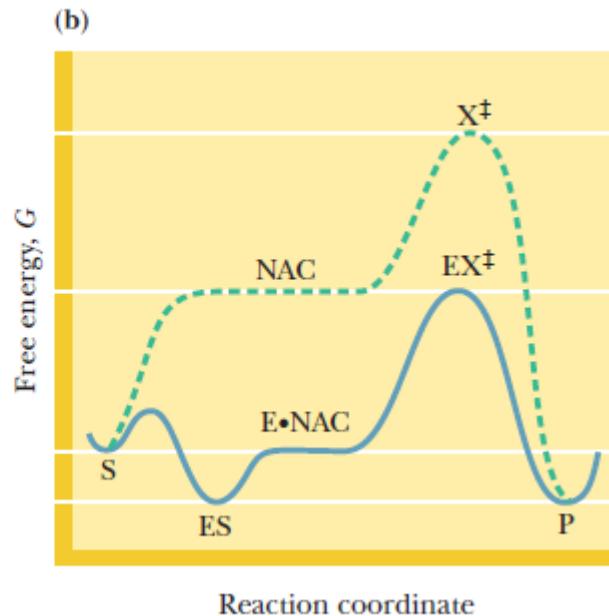
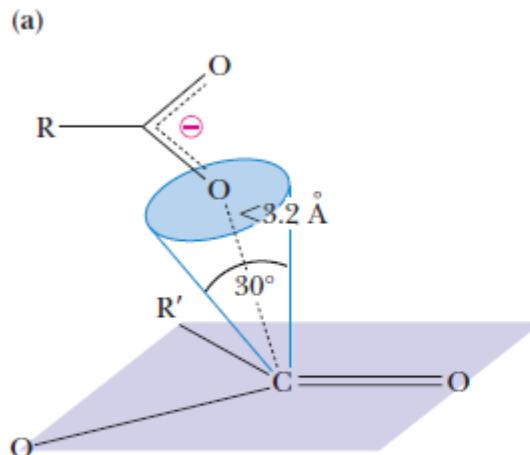


HMG-CoA reductase

Enzymes Facilitate Formation of Near-Attack Conformations

Near attack conformations

Reacting atoms are in *Van der waals* contact and at an angle resembling the bond to be formed



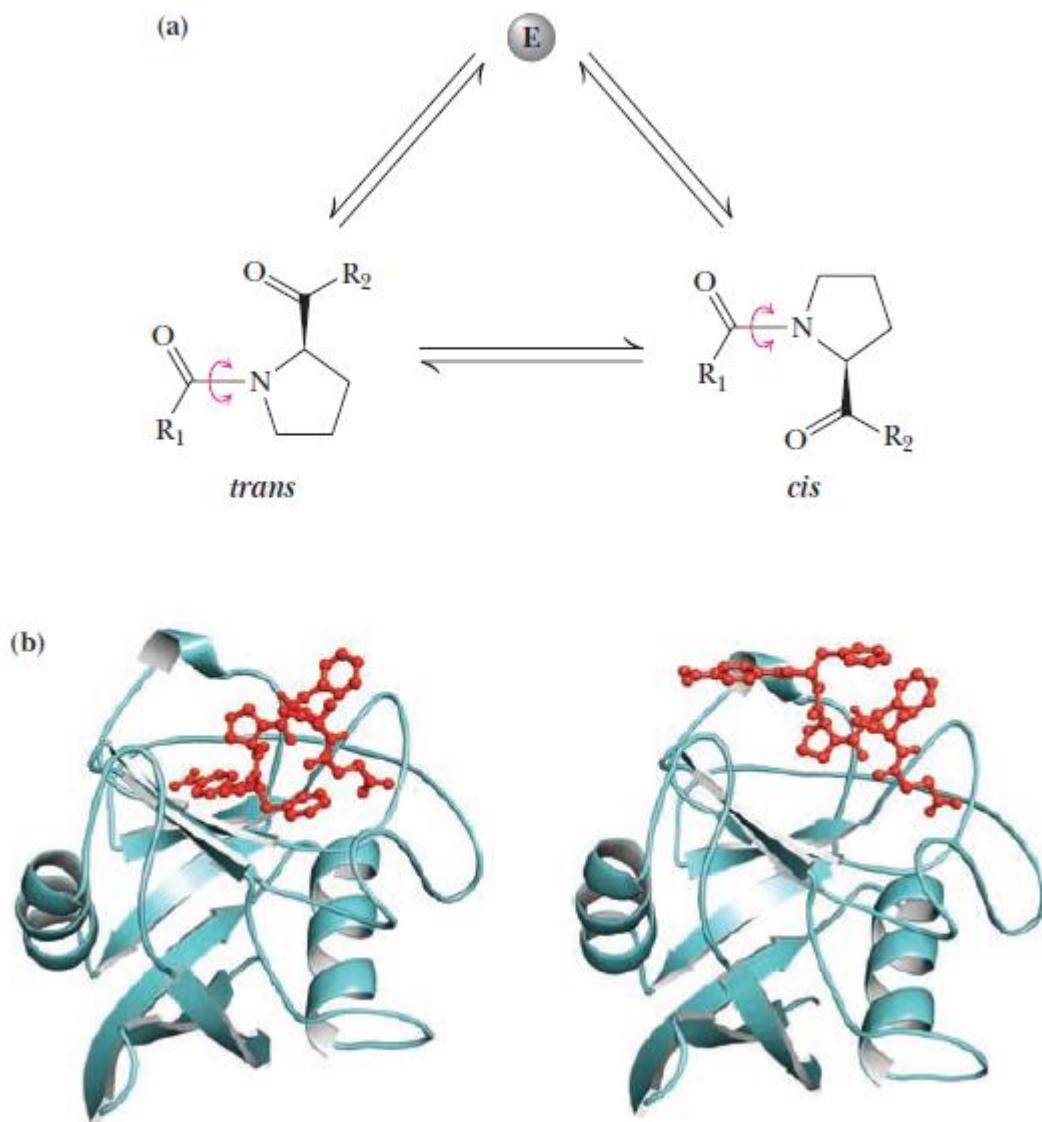
For reactions involving bonding between O, N, C & S atoms: NAC are characterized as Distance within 3.2 Å and ± 15° angle

What exactly happens at protein active site??

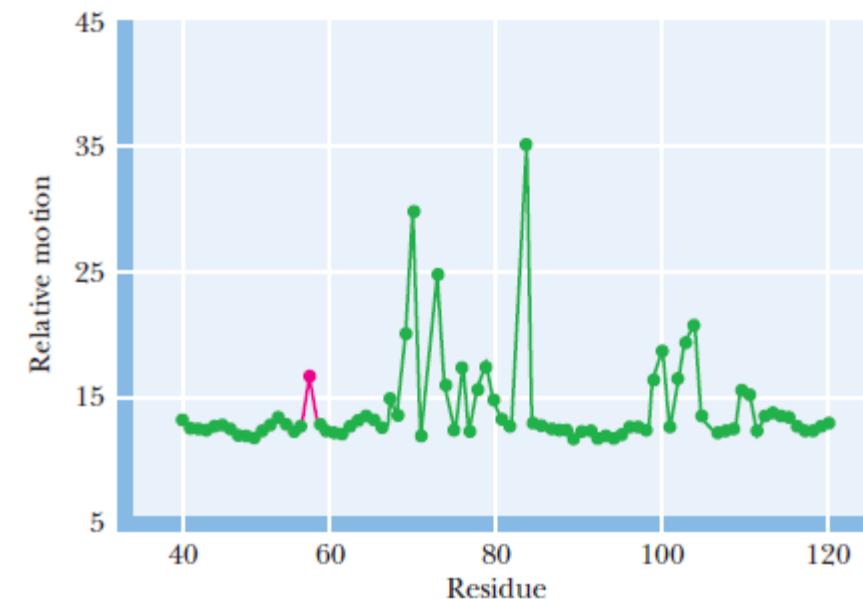
Different amino-acids in protein active site are in constant motions and they assist in

1. Substrate binding
2. Bring catalytic group into position around a substrate
3. Induce formation of NAC
4. Assist in bond making and bond breaking
5. Facilitate conversion of substrate to product

example



Human cyclophilin A is a prolyl isomerase, which catalyzes the interconversion between *trans* and *cis* conformations of proline in peptides.



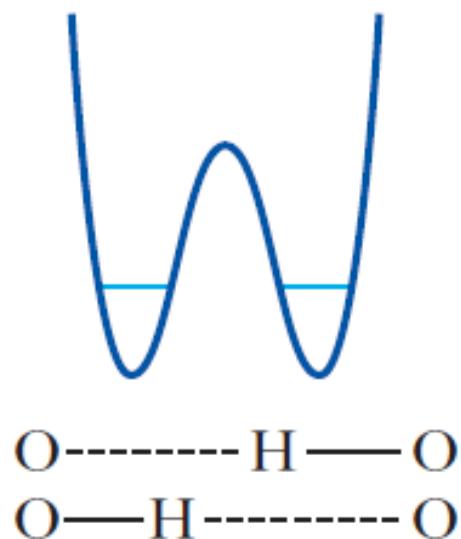
This network extends from the active site to the surface of the protein, and the motions in this network span time scales of femtoseconds to milliseconds. Such extensive networks of motion make it likely that the entire folded structure of the protein may be involved in catalysis at the active site.

Enzymes catalyze reactions by utilizing the same general reactions as studied in organic chemistry:

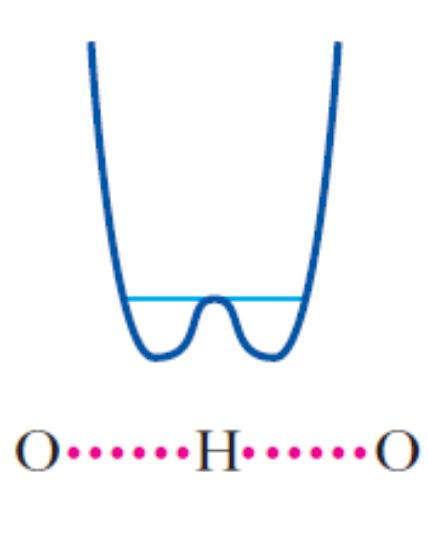
- Acid-base catalysis
- Covalent catalysis
- Metal ion catalysis
- Catalysis by alignment (approximation)

Low barrier hydrogen bonds & Proton tunneling

(a)



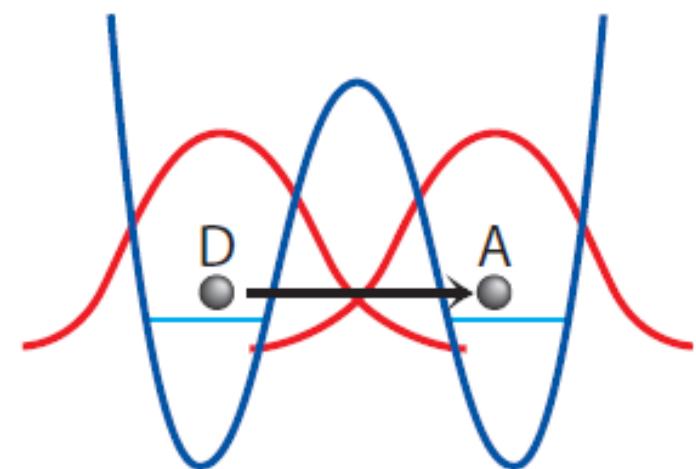
(b)



(c)



(d)



Weak bond

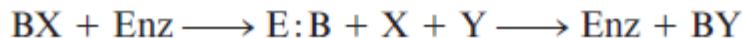
Strong bond

Covalent Catalysis

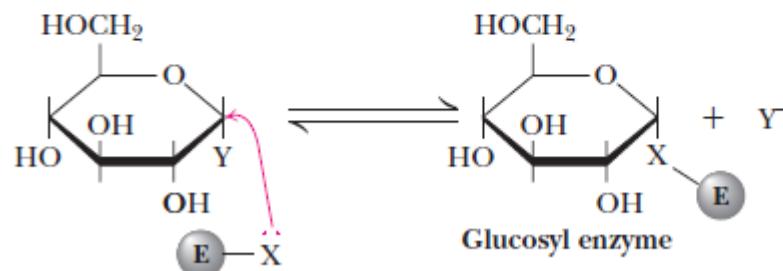
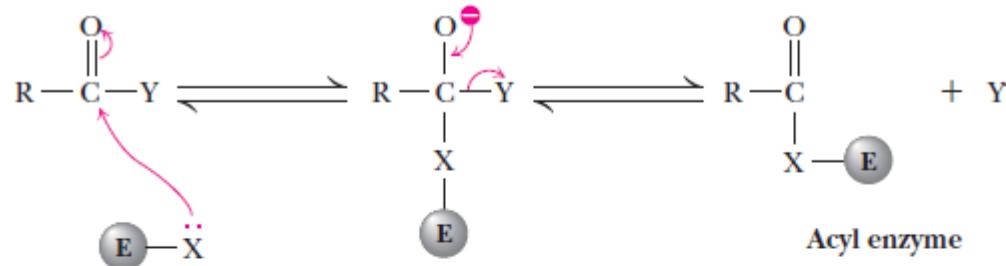
Some enzyme reactions derive much of their rate acceleration from the formation of covalent bonds between enzyme and substrate. Consider the reaction:



and an enzymatic version of this reaction involving formation of a covalent intermediate:



examples



Digestive serine protease

Ex., trypsin, chymotrypsin, thrombin, tissue plasminogen activator

Catalytic mechanism based on active site serine

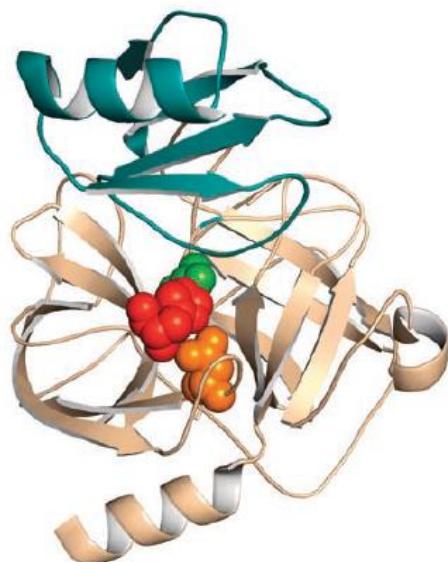


FIGURE 14.16 Structure of chymotrypsin (white) in a complex with eglin C (blue ribbon structure), a target protein. The residues of the catalytic triad (His⁵⁷, Asp¹⁰², and Ser¹⁹⁵) are highlighted. His⁵⁷ (red) is flanked by Asp¹⁰² (gold) and by Ser¹⁹⁵ (green). The catalytic site is filled by a peptide segment of eglin. Note how close Ser¹⁹⁵ is to the peptide that would be cleaved in the chymotrypsin reaction (pdb id = 1ACB).

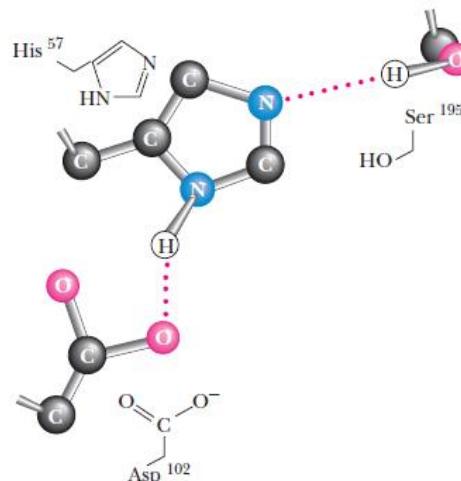
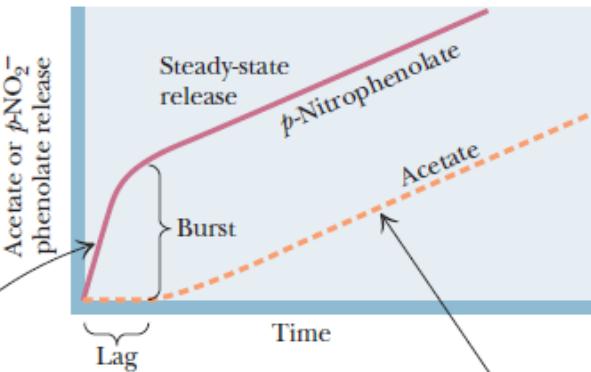
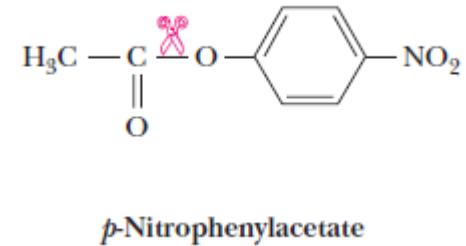
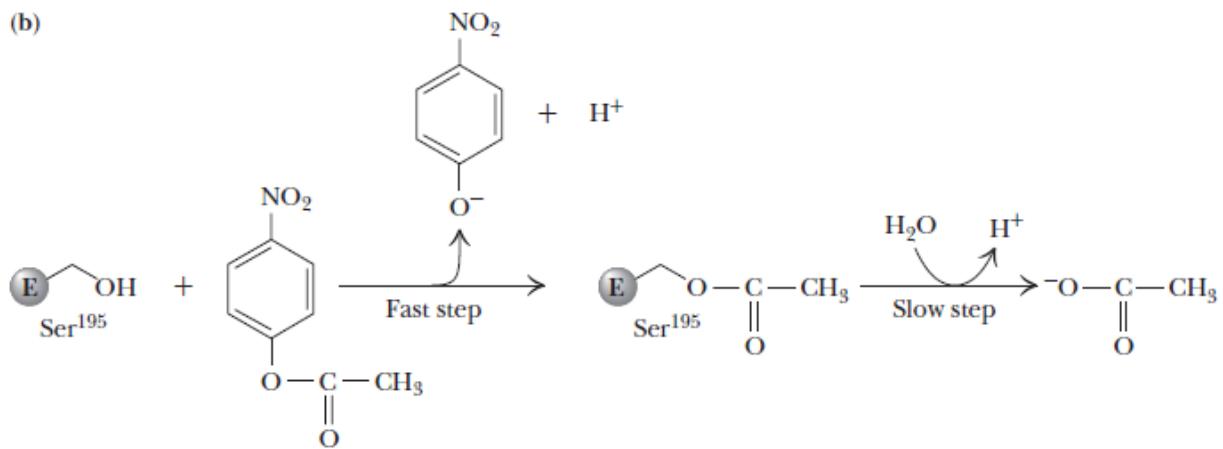


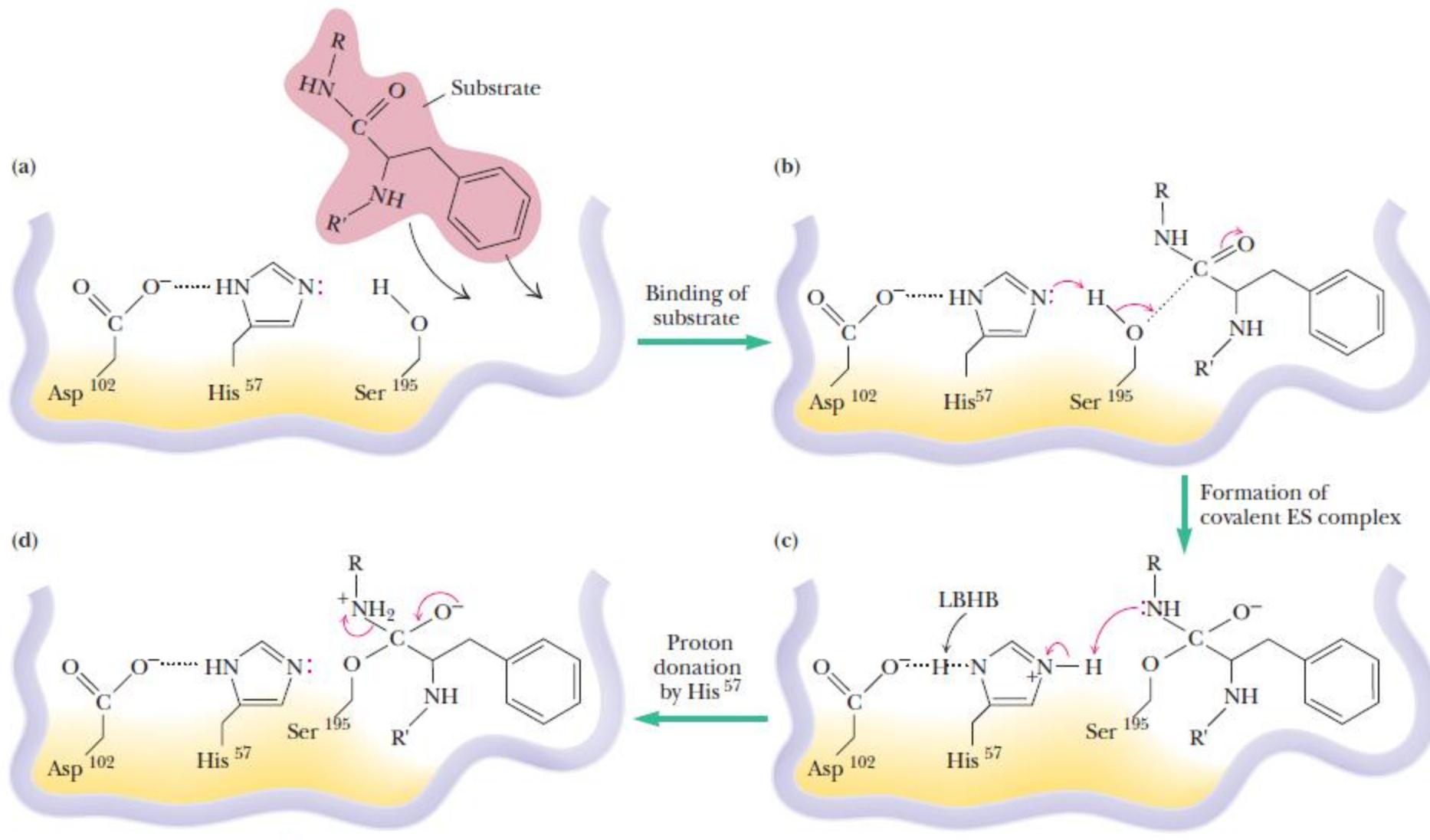
FIGURE 14.17 The catalytic triad of chymotrypsin.

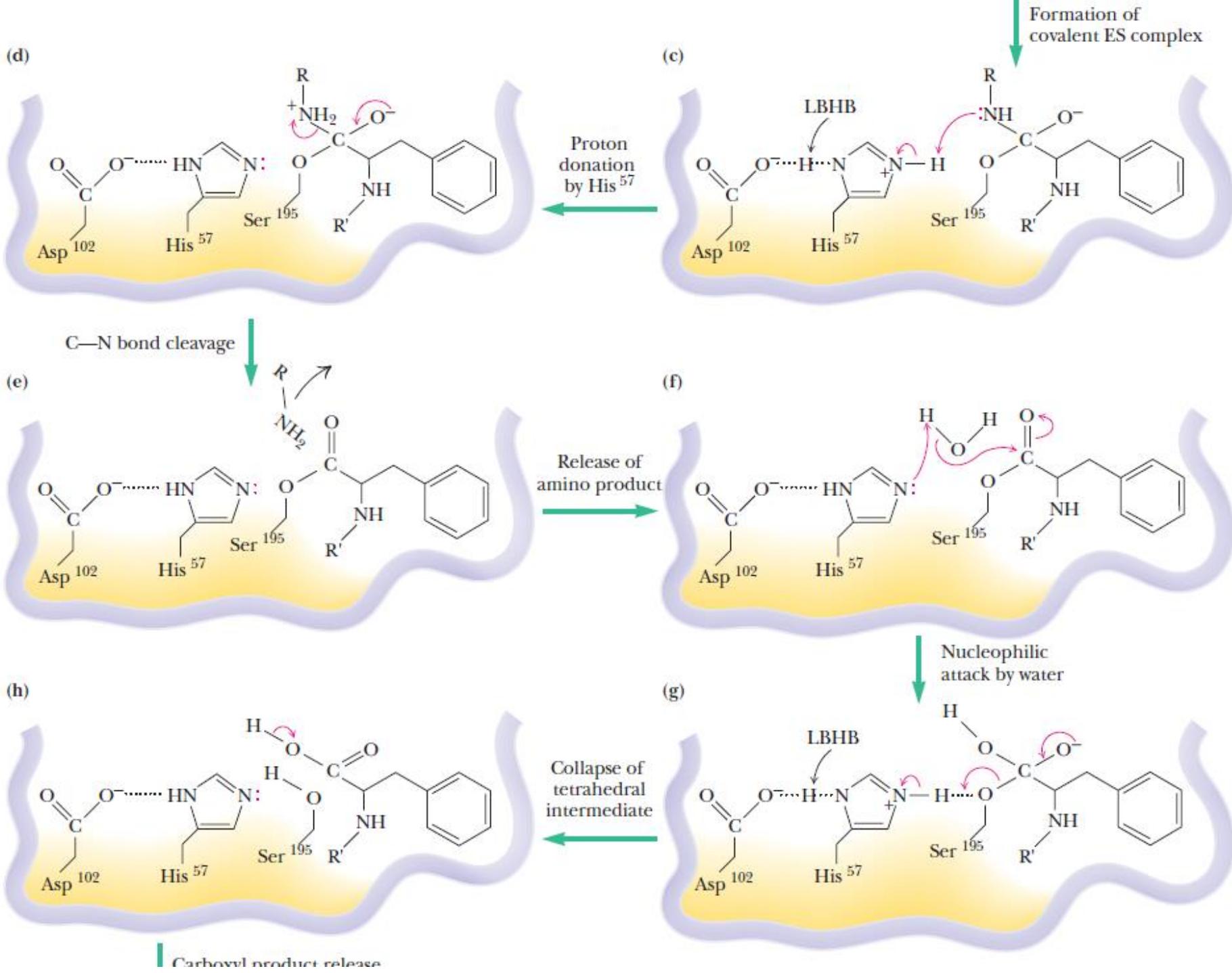
(a)



(b)







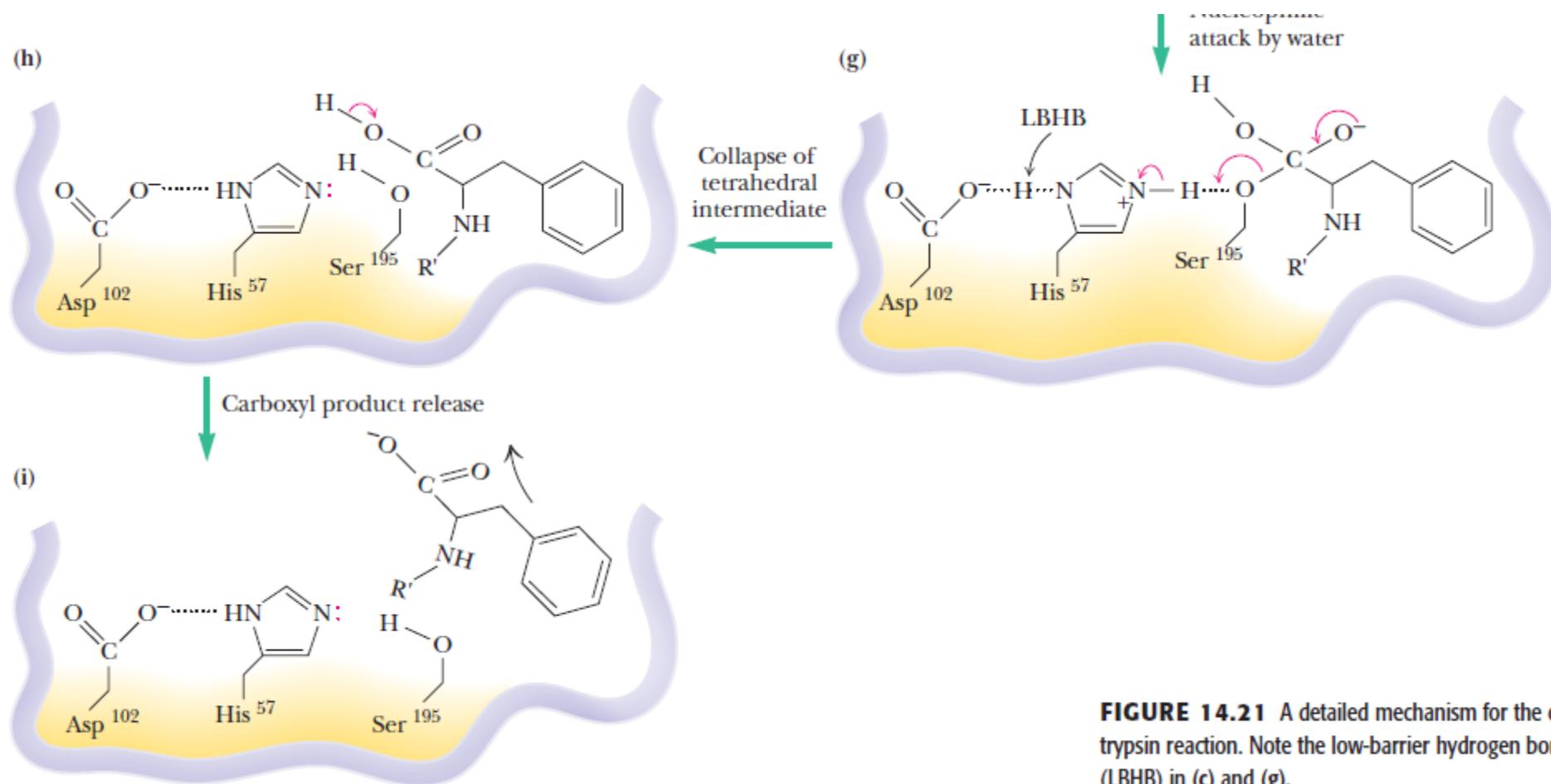


FIGURE 14.21 A detailed mechanism for the chymotrypsin reaction. Note the low-barrier hydrogen bond (LBHB) in (c) and (g).

L5 +6 Enzyme functional nature: Mechanism continued

Ravikrishnan Elangovan,
Department of Biochemical Engg and Biotechnology
Indian Institute of Technology - Delhi

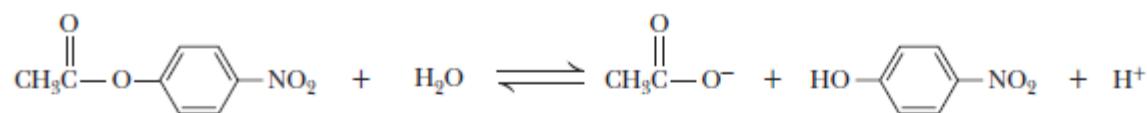
Acid-Base Catalysis occurs by Proton Transfer

General acid catalysis: Proton transfer from an acid lowers the free energy of a reaction's transition state

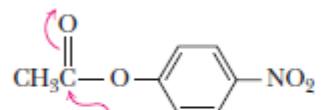
Specific base catalysis

General base catalysis

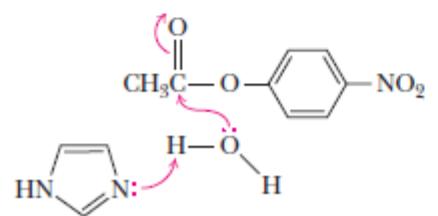
Reaction



Specific base mechanism



General base mechanism



The enzyme α -glucosidase (EC 3.2.1.20) catalyzes the hydrolysis of maltose into glucose. α -glucosidase is competitively inhibited by the product glucose and inhibited at high maltose concentrations in a partial uncompetitive mode. Determine a kinetic rate expression in terms of the dissociation constants for: the secondary enzyme–substrate complex (K_M), the secondary enzyme–product complex (K_P), the tertiary enzyme–substrate–substrate complex (K_0), and the maximum reaction rates of product formation from the enzyme–substrate active complex (V_{max}) and the enzyme–substrate–substrate partially active complex (V_{max}^0). The molar concentrations of maltose and glucose are $[M]$ and $[G]$, respectively.

Effects of pH on Enzyme Activity

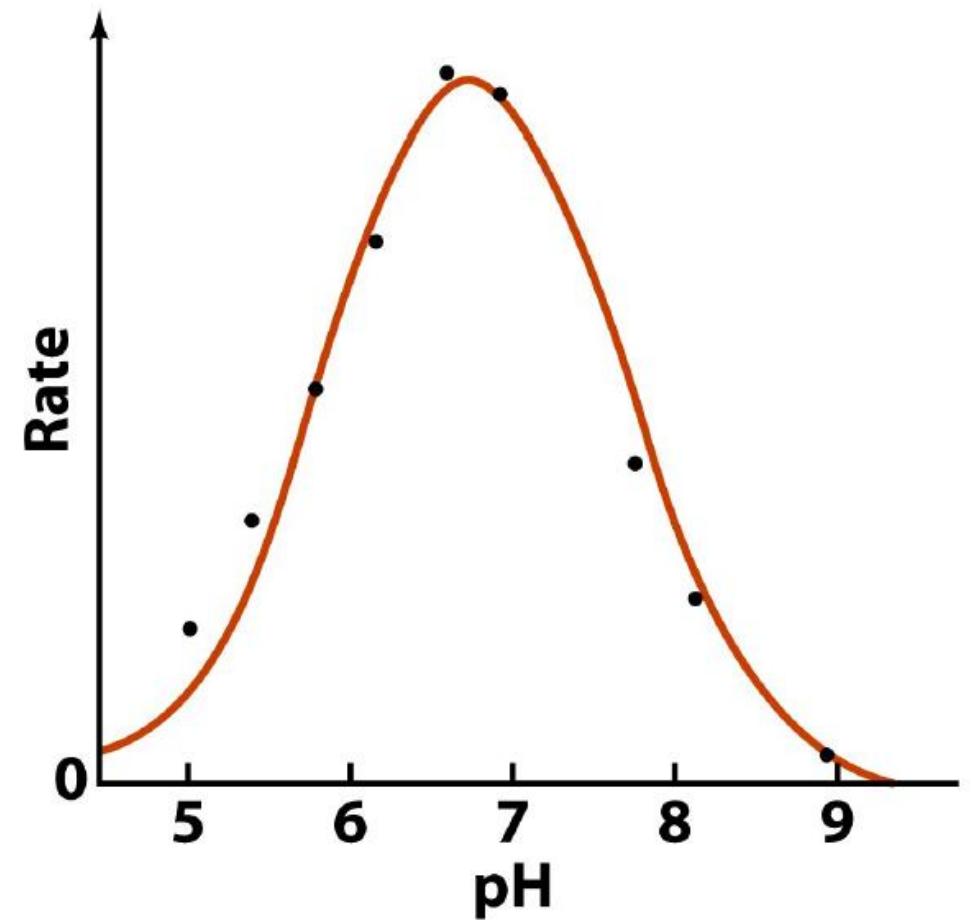
Most enzymes are active only within a narrow pH range of 5-9.

Reaction rates exhibit bell-shaped curves in dependence of pH (reflects ionization state of important residues)

pH optimum gives information about catalytically important residues, if 4/5 -> Glu, Asp; 6->His, 10->Lys

pK_a of residues can vary depending on chemical environment +/- 2

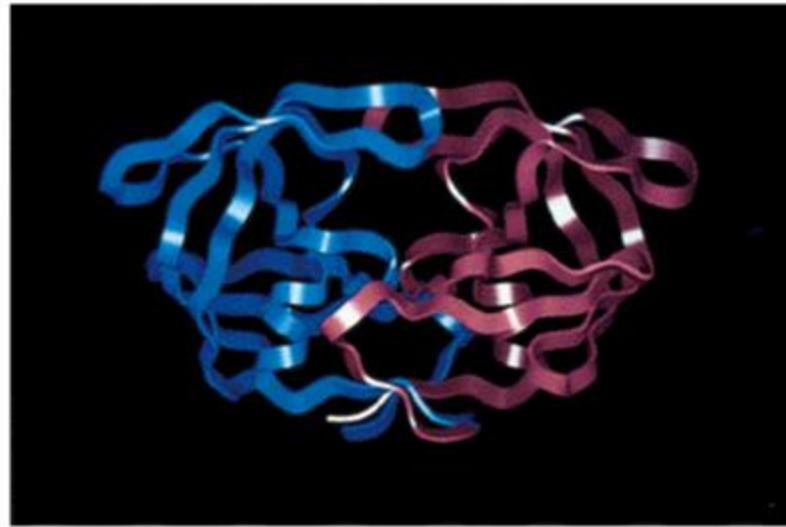
pH Optimum of Fumarase



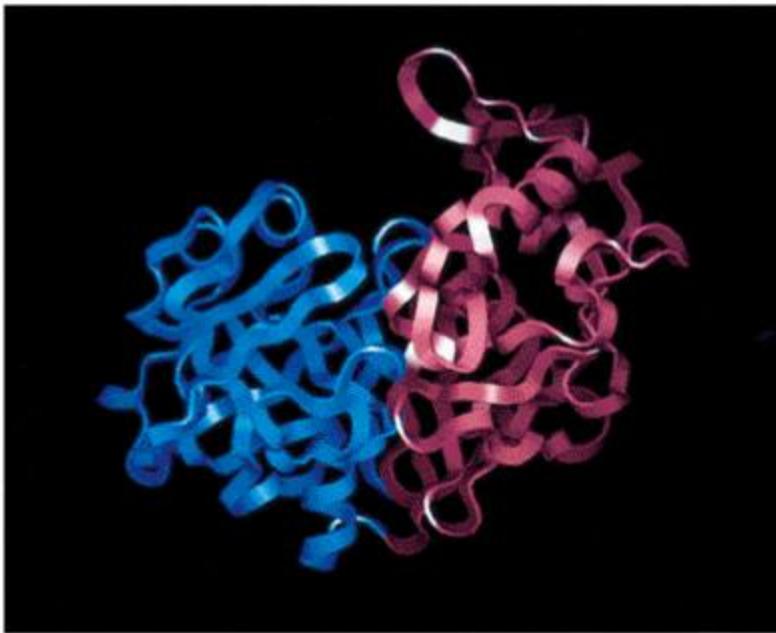
The Aspartic Proteases

Pepsin, chymosin, cathepsin D, renin and HIV-1 protease

- All involve two Asp residues at the active site
- Two Asps work together as general acid-base catalysts
- Most aspartic proteases have a tertiary structure consisting of two lobes (N-terminal and C-terminal) with approximate two-fold symmetry
- HIV-1 protease is a homodimer



(a)

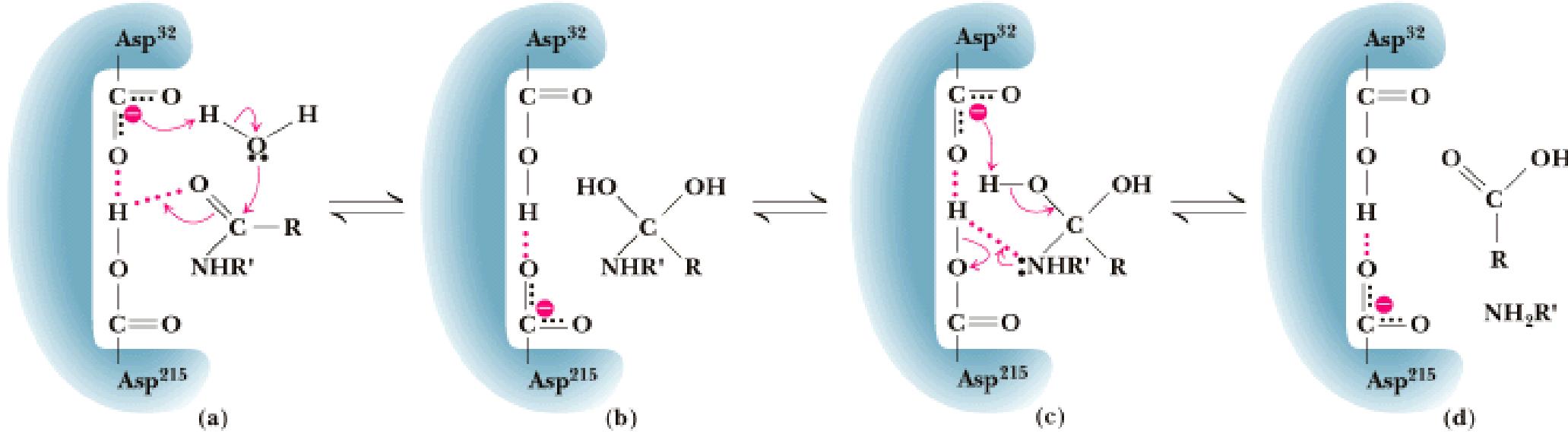


(b)

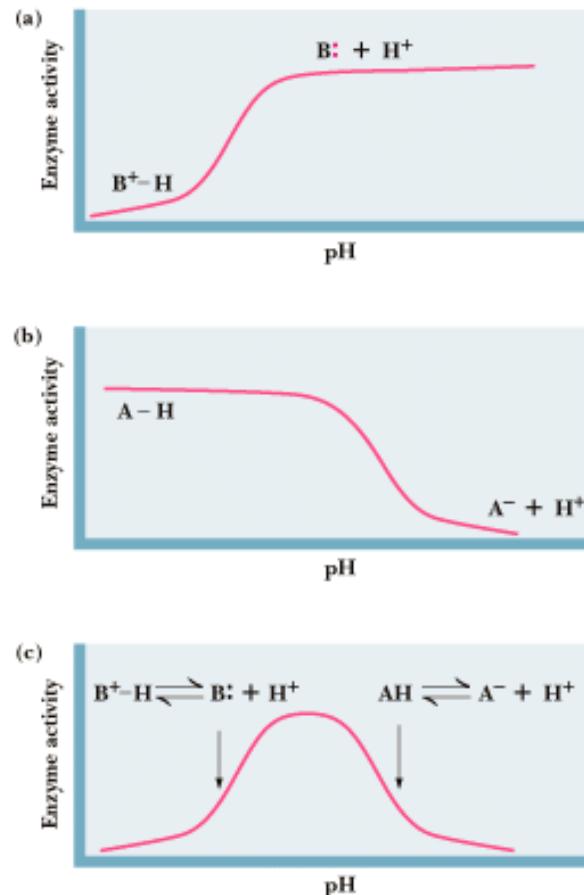
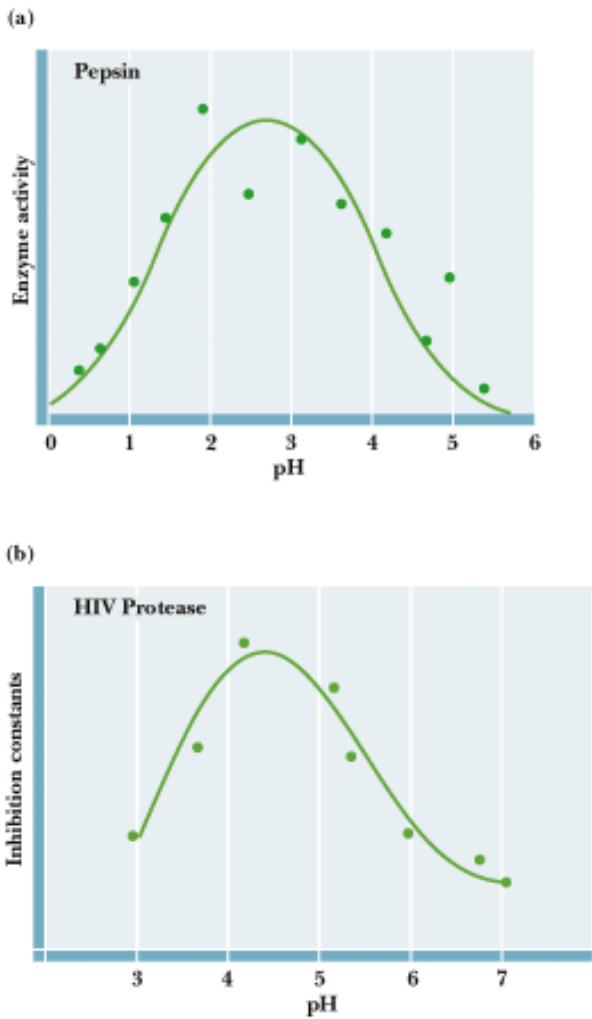
Aspartic Protease Mechanism

The pK_a values of the Asp residues are crucial

- One Asp has a relatively low pK_a , other has a relatively high pK_a
- Deprotonated Asp acts as general base, accepting a proton from HOH, forming OH⁻ in the transition state
- Other Asp (general acid) donates a proton, facilitating formation of tetrahedral intermediate



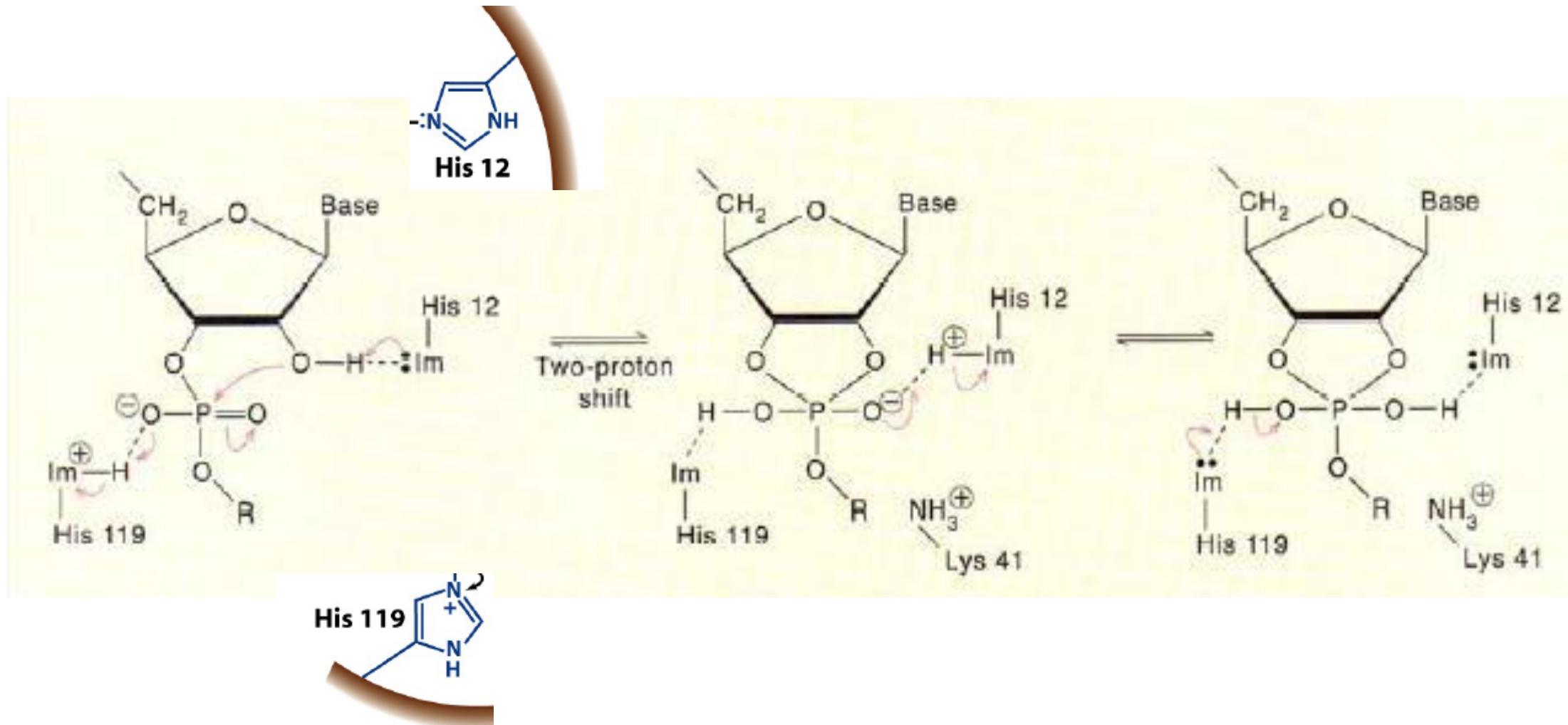
Garrett & Grisham: Biochemistry, 2/e
Unnumbered Figure p.525

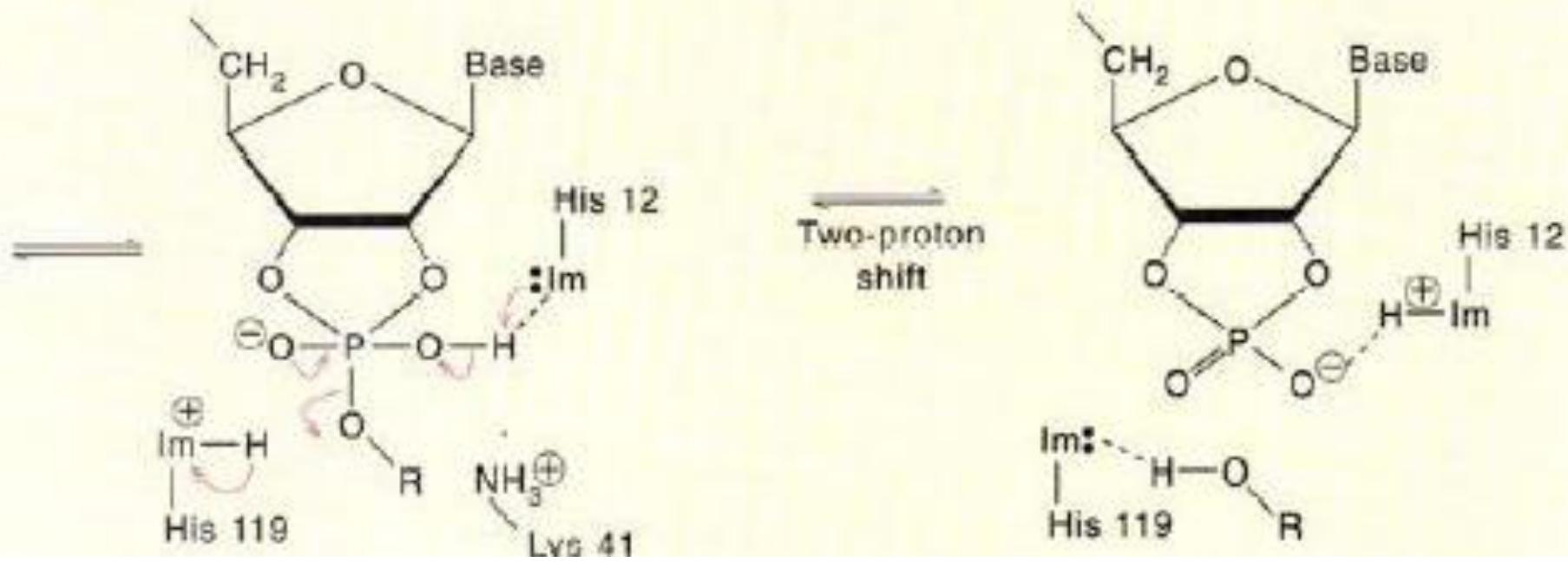


RNase A is an acid-base catalyst

Bovine pancreatic RNase A: Digestive enzyme secreted by pancreas into the small intestine

2',3' cyclic nucleotides isolated as intermediates pH-dependence indicates 2 important His, 12, 119 that act in a concerted manner as general acid and base catalysts to catalyze a two-step reaction



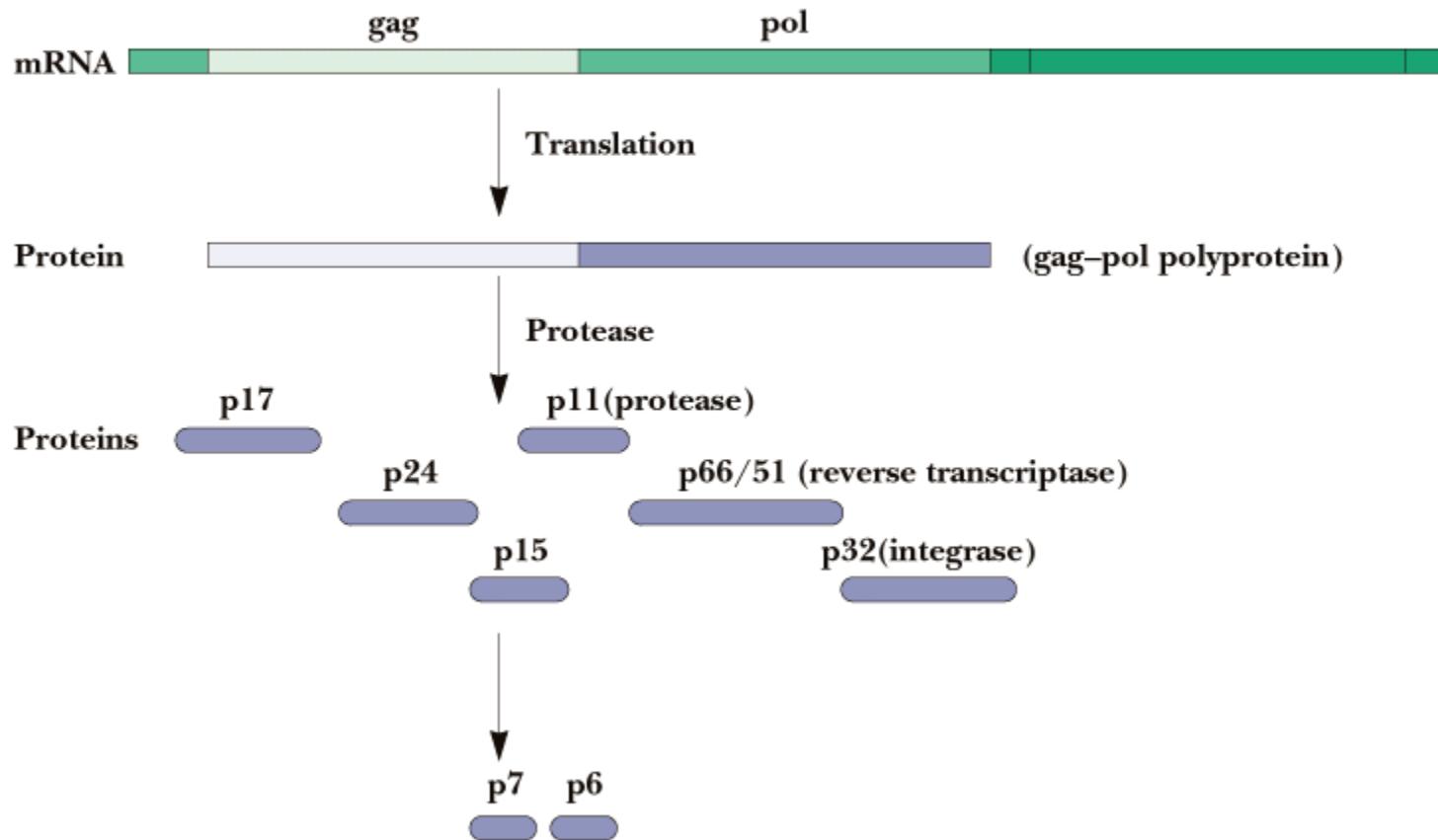


HIV-1 Protease

A novel aspartic protease

- HIV-1 protease cleaves the polyprotein products of the HIV genome
- This is a remarkable imitation of mammalian aspartic proteases
- HIV-1 protease is a homodimer - more genetically economical for the virus
- Active site is two-fold symmetric
- Two Asp residues - one high pK_a , one low pK_a

**Garrett & Grisham: Biochemistry, 2/e
Figure 16.28**



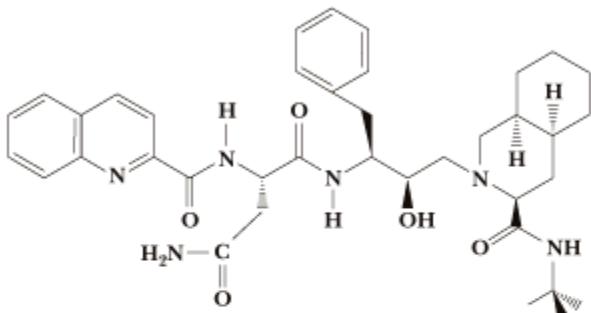
Saunders College Publishing

Therapy for HIV?

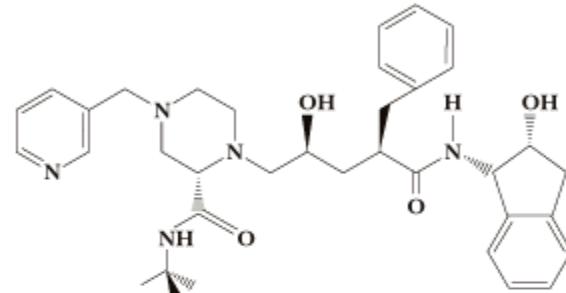
Protease inhibitors as AIDS drugs

- If the HIV-1 protease can be selectively inhibited, then new HIV particles cannot form
- Several novel protease inhibitors are currently marketed as AIDS drugs
- Many such inhibitors work in a culture dish
- However, a successful drug must be able to kill the virus in a human subject without blocking other essential proteases in the body

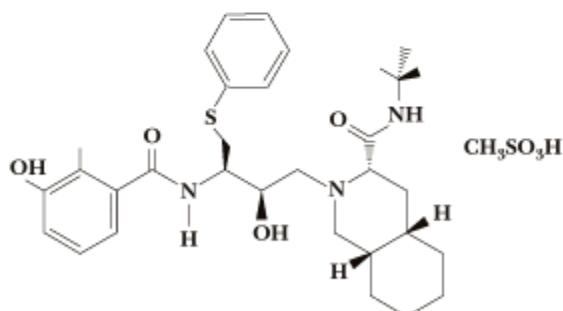
Garrett & Grisham: Biochemistry, 2/e
Unnumbered Figure p.524



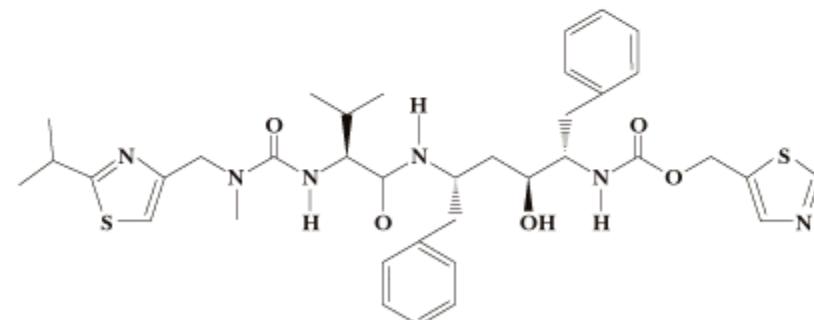
Invirase (Saquinavir)



Crixivan (Indinavir)

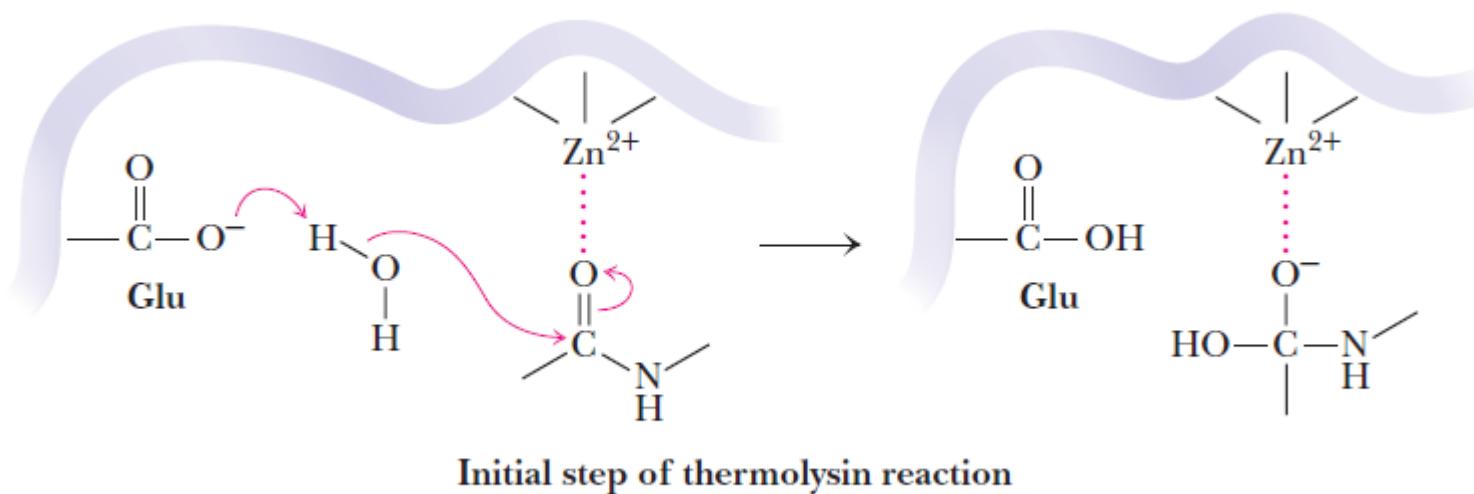


Viracept (Nelfinavir mesylate)



Norvir (Ritonavir)

Metal Ion Catalysis

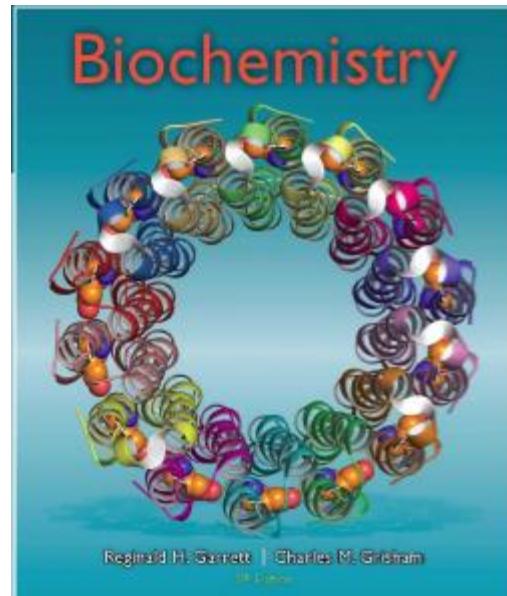


One role for metals in metal-activated enzymes and metalloenzymes is to act as electrophilic catalysts, stabilizing the increased electron density or negative charge that develop during reaction

Another potential function of metal ions is to provide a powerful nucleophile at neutral pH.

Enzyme regulation

Chapter 15, Biochemistry



Reginald Garrett & Charles Grisham • University of Virginia

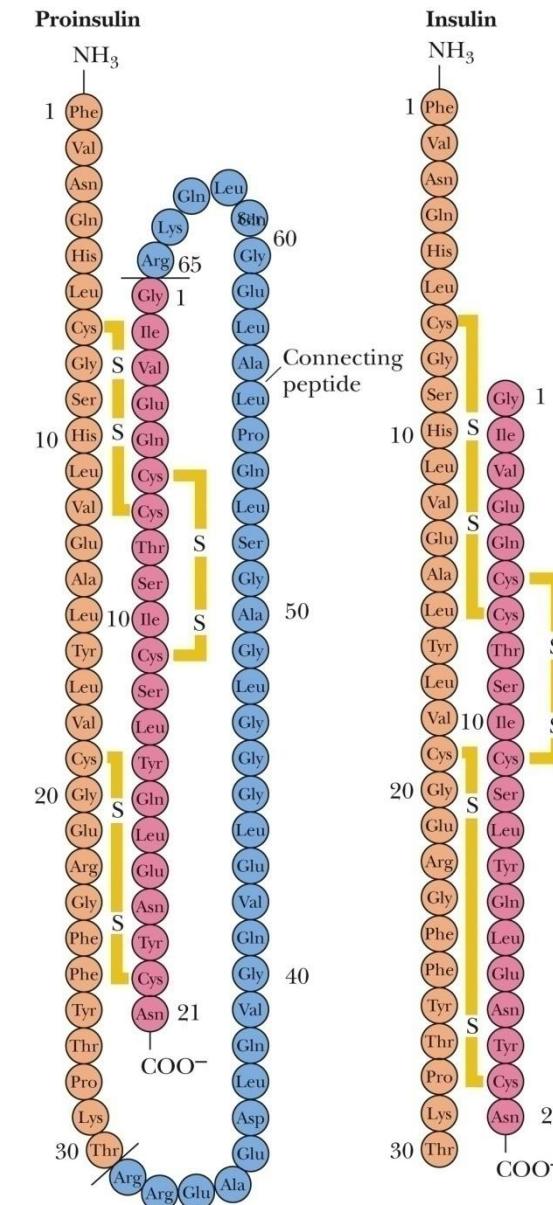
Different kinds of regulation

- Product inhibition
- Substrate/co-factor availability
- Gene controls induction/repression
- allosteric regulation
- Covalent modification
- Zymogens/Isozymes

Zymogens are inactive precursors of enzymes. Typically, proteolytic cleavage produces the active enzyme

Example 1

Proinsulin is an 86-residue precursor to insulin



Example 2

Chymotrypsinogen (inactive zymogen)



Cleavage at Arg¹⁵
by trypsin

π -Chymotrypsin (active enzyme)

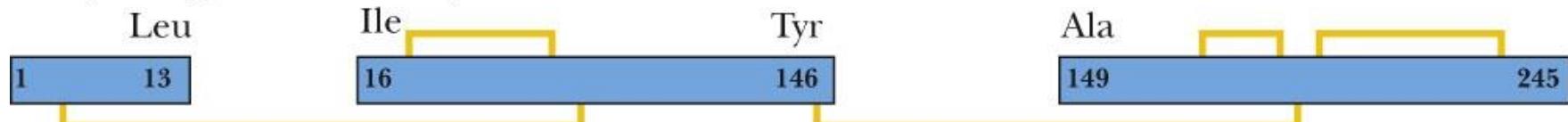


Self-digestion at Leu¹³,
Tyr¹⁴⁶, and Asn¹⁴⁸ by
 π -chymotrypsin

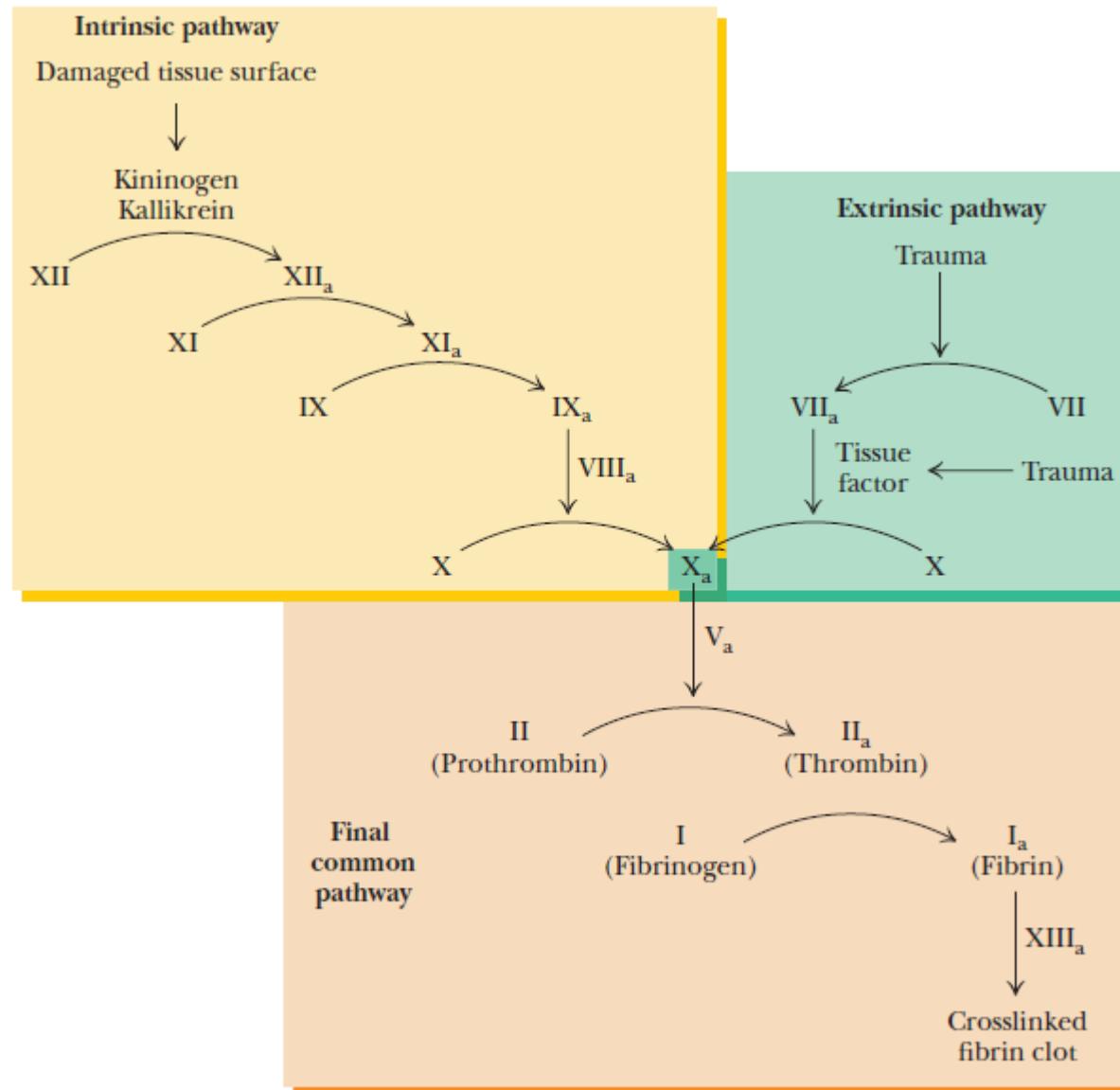
14 15
Ser Arg

147 148
Thr Asn

α -Chymotrypsin (active enzyme)

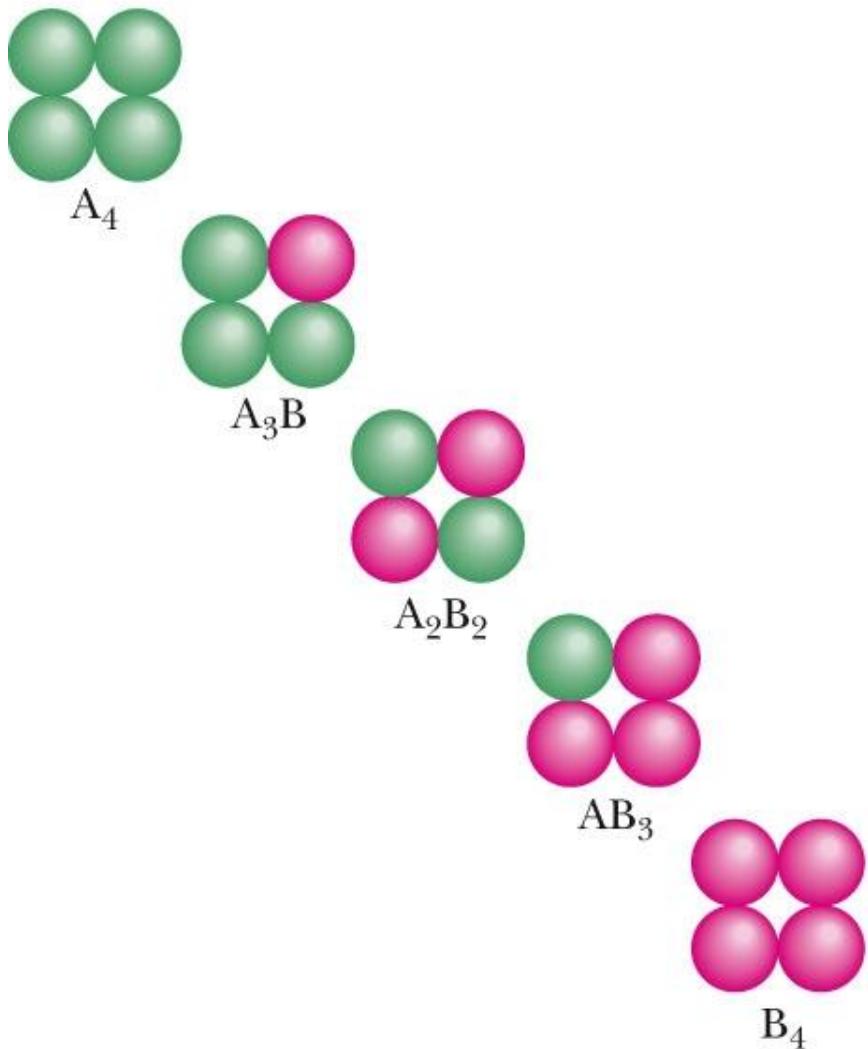


Blood clotting is regulated by
Series of zymogen



Isozymes Are Enzymes With Slightly Different Subunits

(a) The five isomers of lactate dehydrogenase



The isozymes of lactate dehydrogenase (LDH).

(b)

$A_4 \quad A_3B \quad A_2B_2 \quad AB_3 \quad B_4$

Liver

Muscle

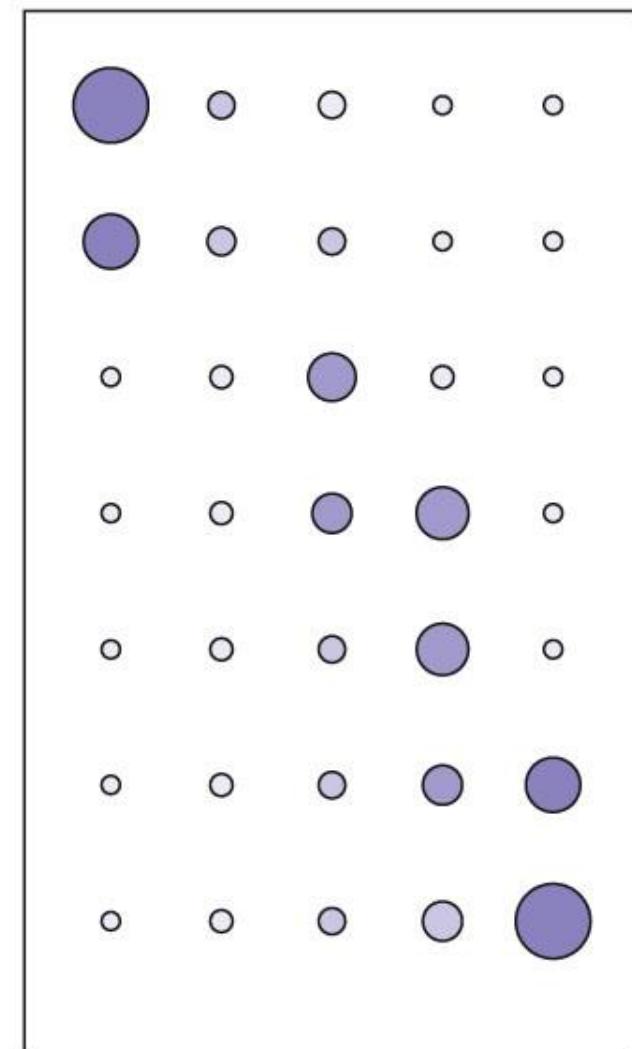
White cells

Brain

Red cells

Kidney

Heart



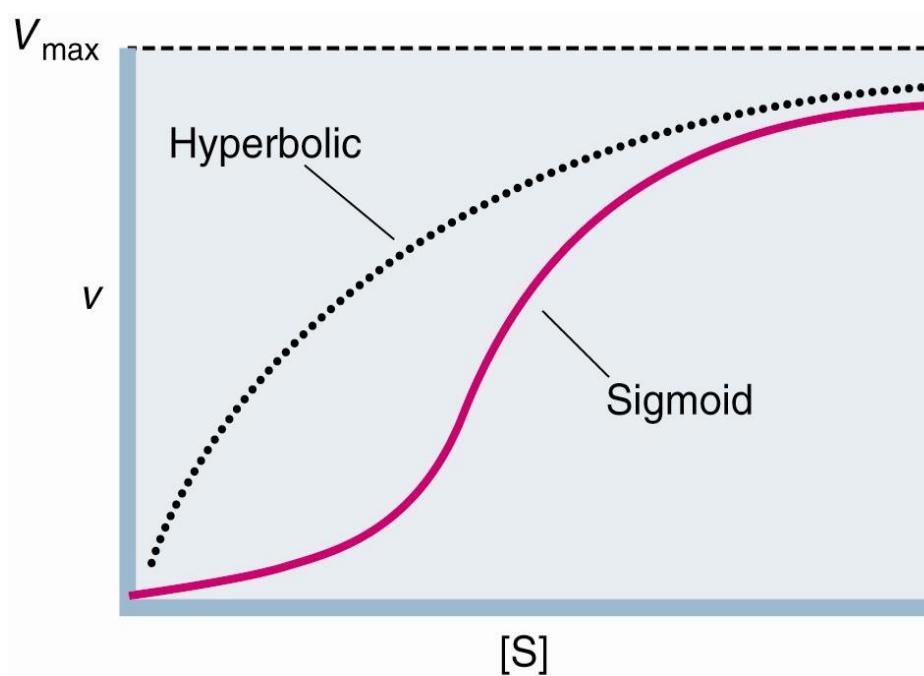
Allsteric Regulation?

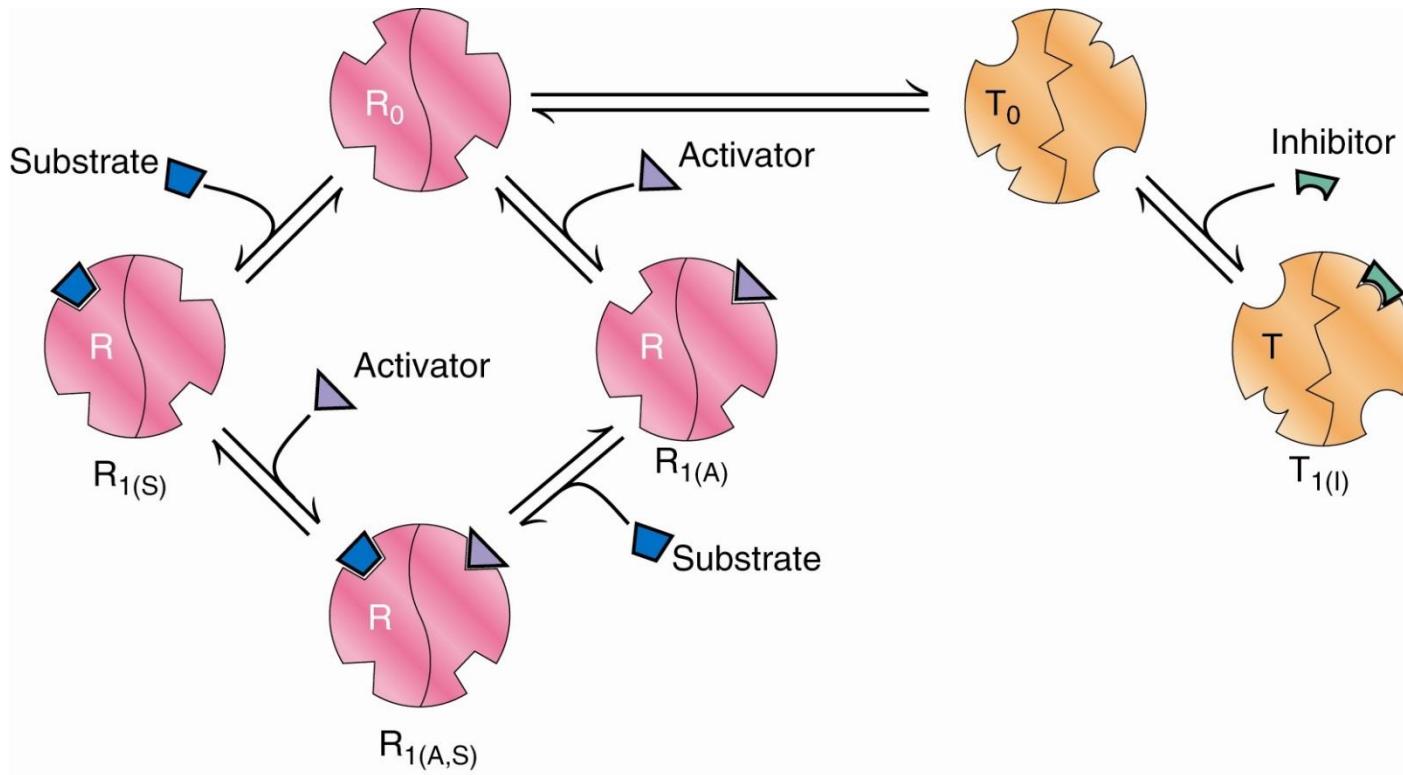
Action at "another site"

Enzymes situated at key steps in metabolic pathways are modulated by allosteric effectors

These effectors are usually produced elsewhere in the pathway

Effectors may be feed-forward activators or feedback inhibitors
Kinetics are sigmoid ("S-shaped")





Cooperativity is achieved because S binding increases the population of R, which increases the sites available to S

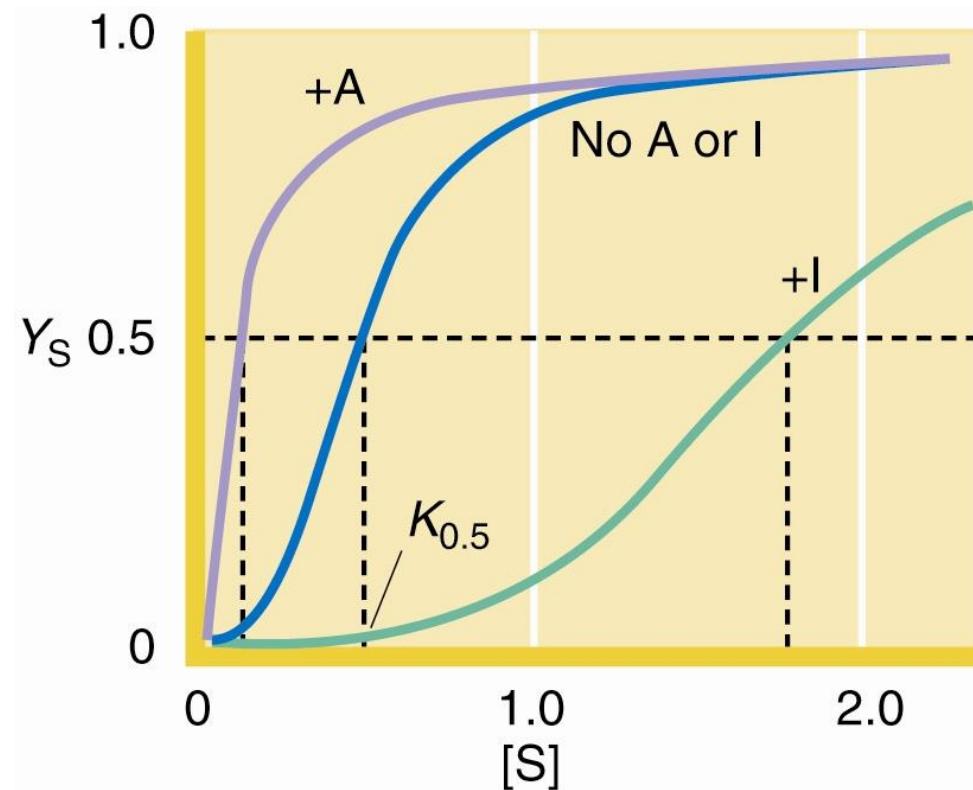
Effects of I:

$I + T_0 \rightarrow T_{1(I)}$
Increase in number of T-conformers (decrease in R₀ as R₀ \rightleftharpoons T₀ to restore equilibrium)

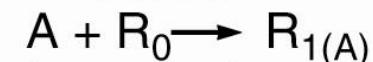
Thus, I inhibits association of S and A with R by lowering R₀ level. I increases cooperativity of substrate saturation curve.

Monod, Wyman, Changeux (MWC) Model:

- Allosteric proteins can exist in two states: R (relaxed) and T (taut)
- In this model, all the subunits of an oligomer must be in the same state
- T state predominates in the absence of substrate S
- S binds much tighter to R than to T



Effects of A:



Increase in number of R-conformers shifts $R_0 \rightleftharpoons T_0$ so that $T_0 \rightarrow R_0$

- (1) More binding sites for S made available.
- (2) Decrease in cooperativity of substrate saturation curve.

Covalent Modification Regulate the Activity of Enzymes?

Phosphorylation:

- Enzyme activity can be regulated through reversible phosphorylation
- This is the most prominent form of covalent modification in cellular regulation
- Phosphorylation is accomplished by protein kinases
- Each protein kinase targets specific proteins for phosphorylation
- Phosphoprotein phosphatases catalyze the reverse reaction – removing phosphoryl groups from proteins
- Kinases and phosphatases themselves are targets of regulation

- Protein kinases phosphorylate Ser, Thr, and Tyr residues in target proteins
- Kinases typically recognize specific amino acid sequences in their targets
- In spite of this specificity, all kinases share a common catalytic mechanism based on a conserved core kinase domain of about 260 residues (see Figure 15.9)
- Kinases are often regulated by **intrasteric control**, in which a regulatory subunit (or domain) has a **pseudosubstrate sequence** that mimics the target sequence, minus the phosphorylatable residue

Thank you