

# Environmental Microbiology

## Chapter 2 Bacterial Cell Molecules



**Li Tianxin**

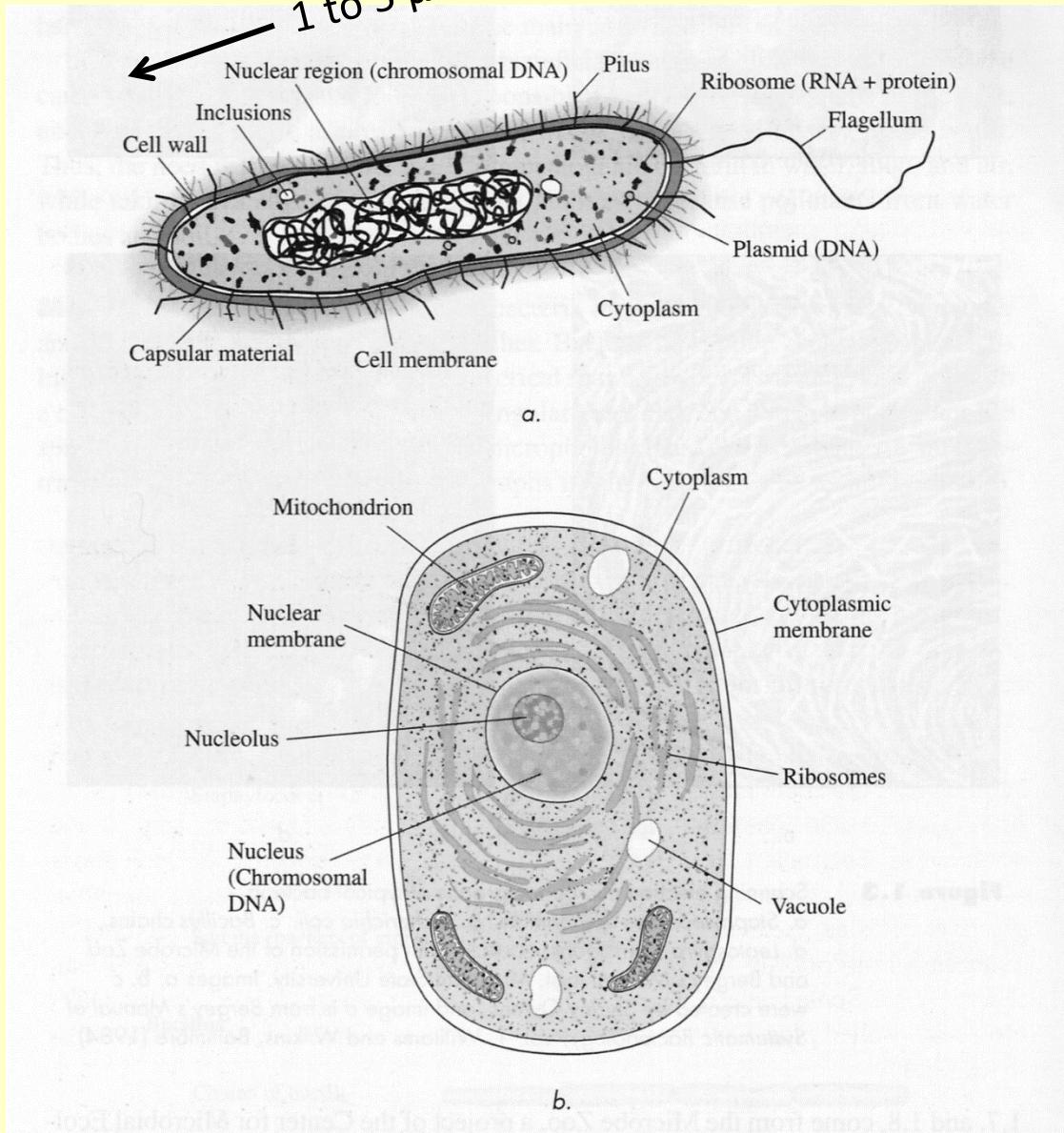
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University of Science and Technology Beijing**

# **General Heterotrophic Bacterial Cell**

# Bacterial Cell

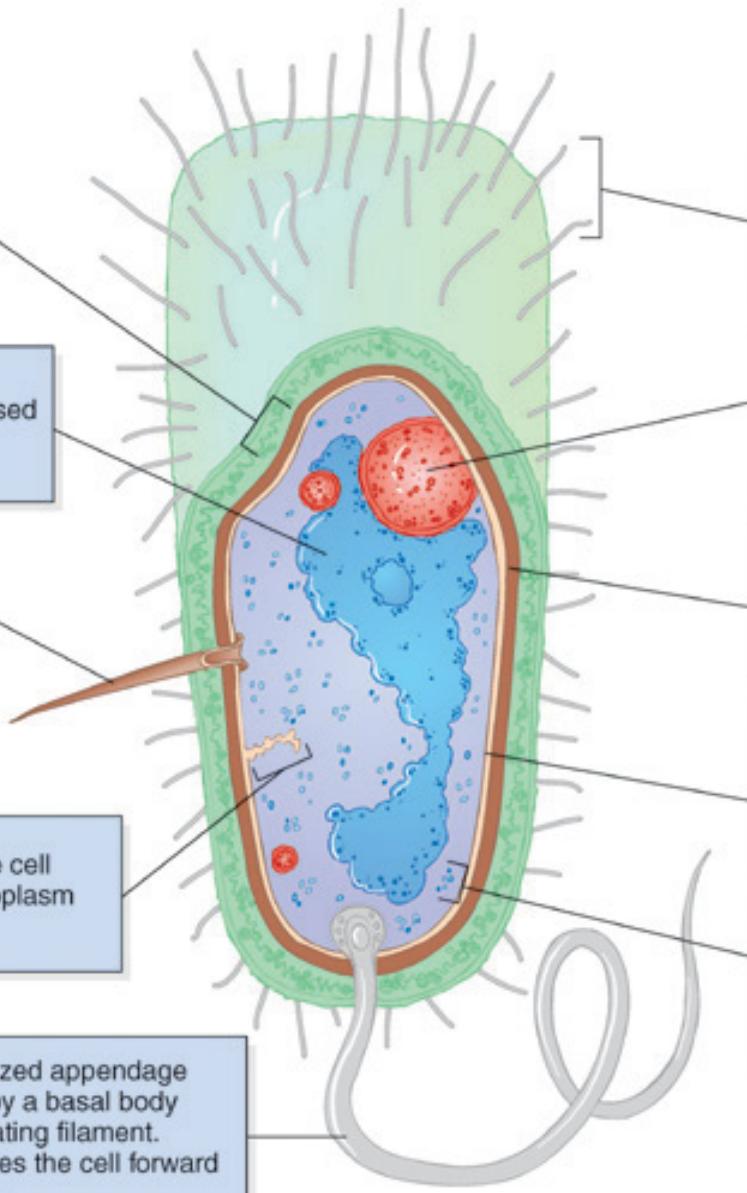
$10^{12}$  bacteria  
in a gram of  
dry weight

0.5 to 2  $\mu\text{m}$



**Figure 1.4**

The structure of typical prokaryotic and eukaryotic cells.  
a. Prokaryotic cell. b. Eukaryotic cell.



**Glycocalyx**—A coating or layer of molecules external to the cell wall. It serves protective, adhesive, and receptor functions.

**Bacterial chromosome or nucleoid**—The site where the large DNA molecule is condensed into a packet. DNA is the code that directs all genetics and heredity of the cell.

**Pilus**—An elongate, hollow appendage used in transfers of DNA to other cells and in cell adhesion.

**Mesosome**—An extension of the cell membrane that folds into the cytoplasm and increases surface area.

**Flagellum**—Specialized appendage attached to the cell by a basal body that holds a long rotating filament. The movement pushes the cell forward and provides motility.

**Fimbriae**—Fine, hairlike bristles from the cell surface that help in adhesion to other cells and surfaces.

**Inclusion/Granule**—Stored nutrients such as fat, phosphate, or glycogen deposited in dense crystals or particles that can be tapped into when needed.

**Cell wall**—A semirigid casing that provides structural support and shape for the cell.

**Cell membrane**—A thin sheet of lipid and protein that surrounds the cytoplasm and controls the flow of materials into and out of the cell pool.

**Ribosomes**—Tiny particles composed of protein and RNA that are the sites of protein synthesis.

# A bacteria cell includes:

1. Capsular, Glucocalyx, or slime layer – extracellular polysaccharides for food storage, protection and interact with outside environment
2. Flagellum – cell mobility
3. Pili, pilus – genetic material transfer (sexual)
4. Cell wall – protection and provide rigidity
5. Cell membrane – contain cytoplasm and facilitate transfer nutrients in and out of cell
6. Cytoplasm – All the cell synthesis and functions are happening
  - ✓ Nuclear region – chromosomal DNA (vital genetic information storage and replication, millions of base pair)
  - ✓ Plasmid (DNA) – secondary genetic information several thousands base pairs: biodegradation, antibiotic resistant
  - ✓ Ribosome (RNA + protein) – enzyme synthesis
  - ✓ Inclusions – food or energy storage, gas vesicles

TABLE 2.1 Overall Macromolecular Composition of an Average *E. coli* B/r Cell

Macromolecule	Percentage of total dry weight	Weight per cell ( $10^{15} \times$ weight, grams)	Molecular weight	Number of molecules per cell	Different kinds of molecules
Protein	55.0	155.0	$4.0 \times 10^4$	2,360,000	1050
RNA	20.5	59.0			
23S rRNA		1.0	$1.0 \times 10^6$	18,700	1
16S rRNA		16.0	$5.0 \times 10^5$	18,700	1
5S rRNA		1.0	$3.9 \times 10^4$	18,700	1
Transfer RNA		8.6	$2.5 \times 10^4$	205,000	60
Messenger RNA		2.4	$1.0 \times 10^6$	1380	400
DNA	3.1	9.0	$2.5 \times 10^9$	2.13	1
Lipid	9.1	26.0	705	22,000,000	4
Lipopolysaccharide	3.4	10.0	4346	1,200,000	1
Peptidoglycan	2.5	7.0	(904) <sub>n</sub>	1	1
Glycogen	2.5	7.0	$1.0 \times 10^6$	4360	1
Total macromolecules	96.1	273.0			
Soluble pool	2.9	8.0			
Building blocks		7.0			
Metabolites, vitamins		1.0			
Inorganic ions	1.0	3.0			
Total dry weight	100.0	284.0			
Total dry weight/cell		$2.8 \times 10^{-13}$ g			
Water (at 70% of cell)		$6.7 \times 10^{-13}$ g			
Total weight of one cell		$9.5 \times 10^{-13}$ g			

Adapted with permission from Neidhardt et al., 1990.

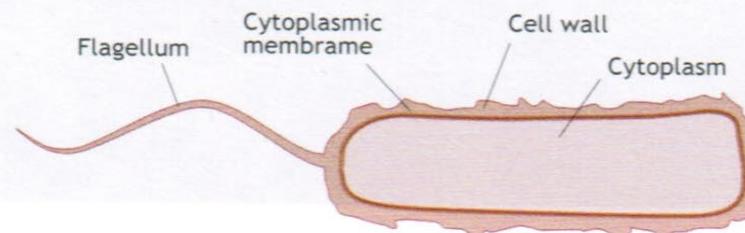
# Bacterial Molecules

**Most bacterial functional molecules are polymers. They are made up of various monomers. It takes energy to produce polymers from monomer. Conversely, when polymers disintegrate into monomers energy will be released.**

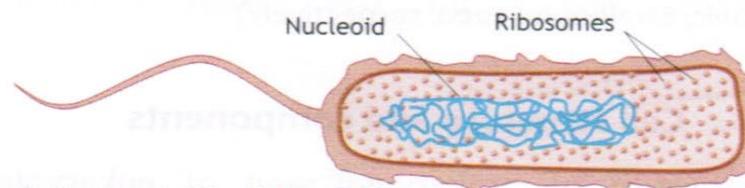
**In their metabolism, bacteria will breakdown large molecules step wise to useful monomers and then to CO<sub>2</sub> and water to obtain energy. They also general cell material from the monomer pool by a step wise manner. Here energy will be expended.**

**The important molecules are:**

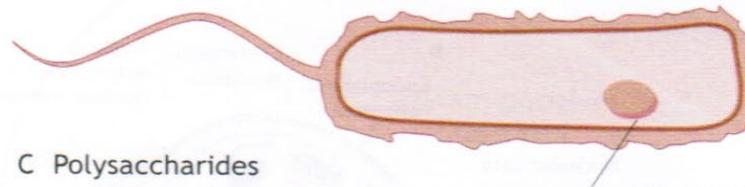
<b>1. Proteins</b>	<b>55%</b>
<b>2. Polysaccharides</b>	<b>5-15%</b>
<b>3. Lipids</b>	<b>9.1%</b>
<b>4. Nucleic acids</b>	<b>23.6%</b>
<b>5. Others monomers</b>	<b>6.3%</b>
<b>6. Inorganic ions</b>	<b>1%</b>



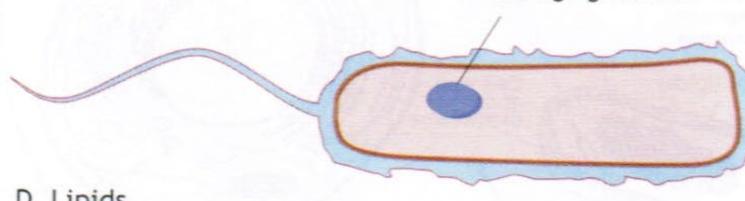
A Proteins



B Nucleic Acids: DNA RNA



C Polysaccharides



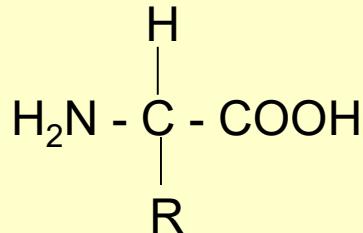
D Lipids

**Figure 2.7** Bacterial macromolecules and location in the cell. (A) Proteins are found in the flagellum, the cytoplasmic membrane, the cell wall and the cytoplasm; (B) nucleic acids (DNA and RNA) are found in the nucleoid and ribosomes; (C) polysaccharides are found in the cell wall and sometimes in storage granules and (D) lipids are found in the cytoplasmic membrane, the cell wall and in storage granules (adapted from Madigan and Martinko, 2006)

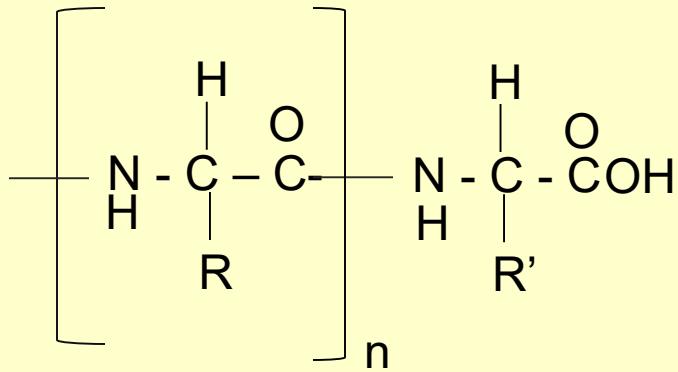
# **Proteins**

# 1. Proteins : (Mostly function as enzymes, some in cell membrane)

- Proteins are polymers made of monomeric amino acid chains:
- Amino acids are: about 20 natural amino acids used by bacteria



- Protein is a polymer made up by series of amino acids  $n = 100$  to  $1000$



- The major function of protein in bacteria is acting as enzymes, catalyst for biological reactions. They speed up the reactions without being consumed.

•The important characteristic of enzymes are:

- ✓ Rapidity of catalysis, as high as  $10^{12}$  times or ( $10^3$  to  $10^6$  molecular transformation/sec).
- ✓ Specificity: most can only catalyze one single reaction step of a long reaction chain.

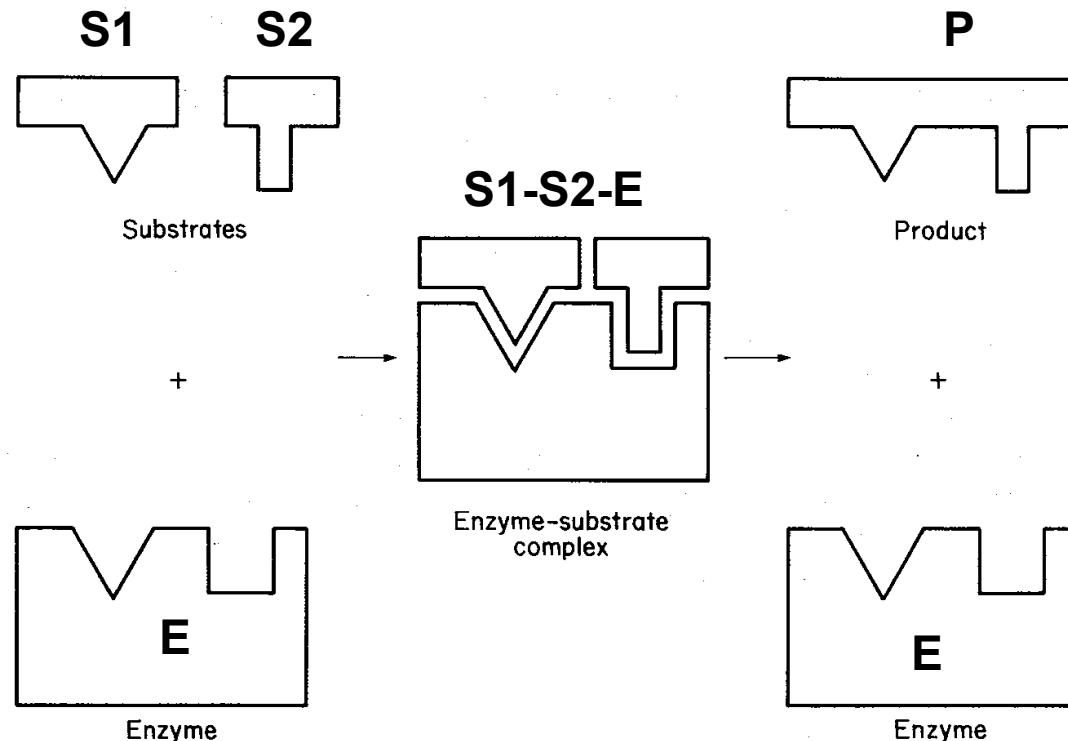
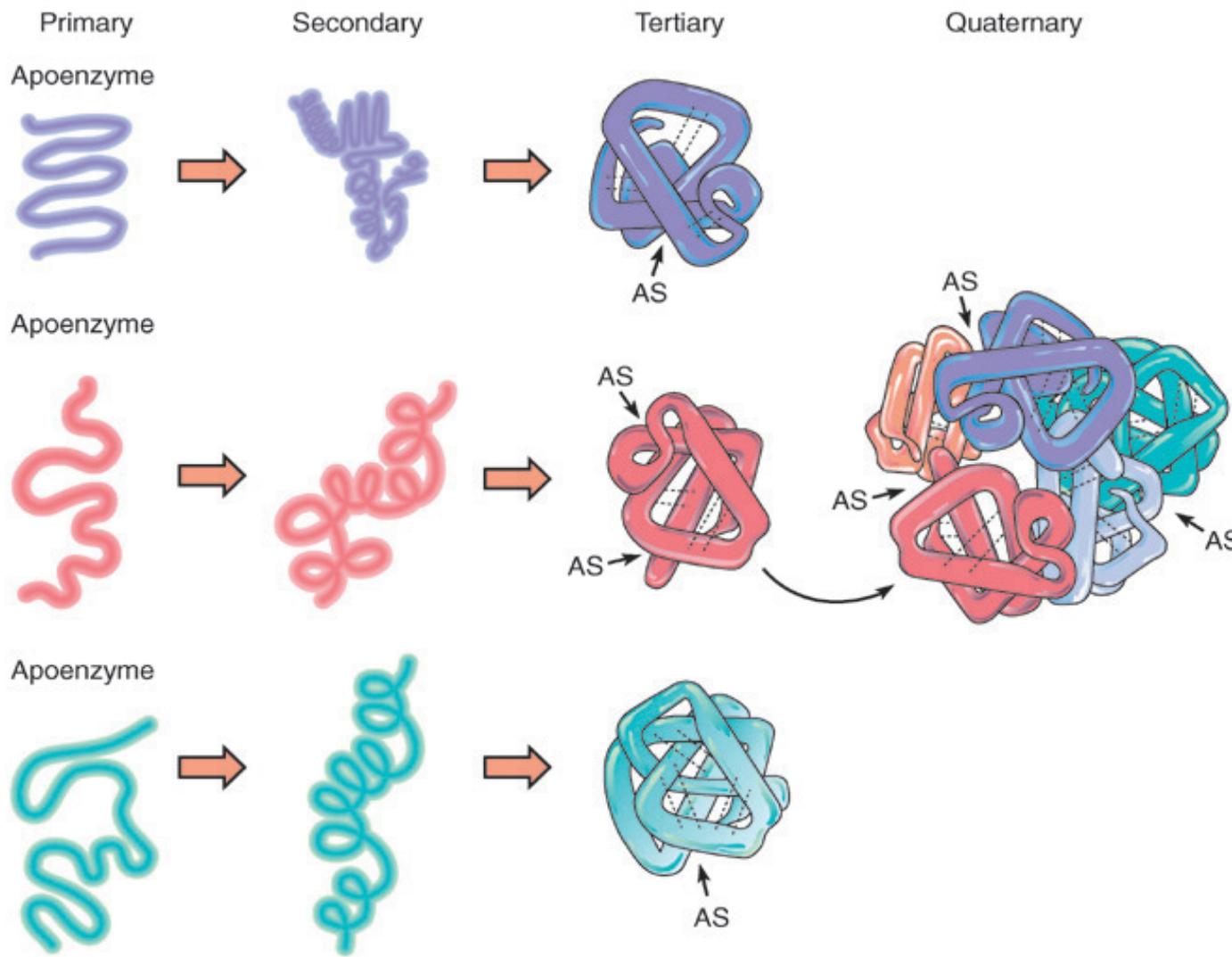


Fig. 6.3 Schematic of enzyme action.

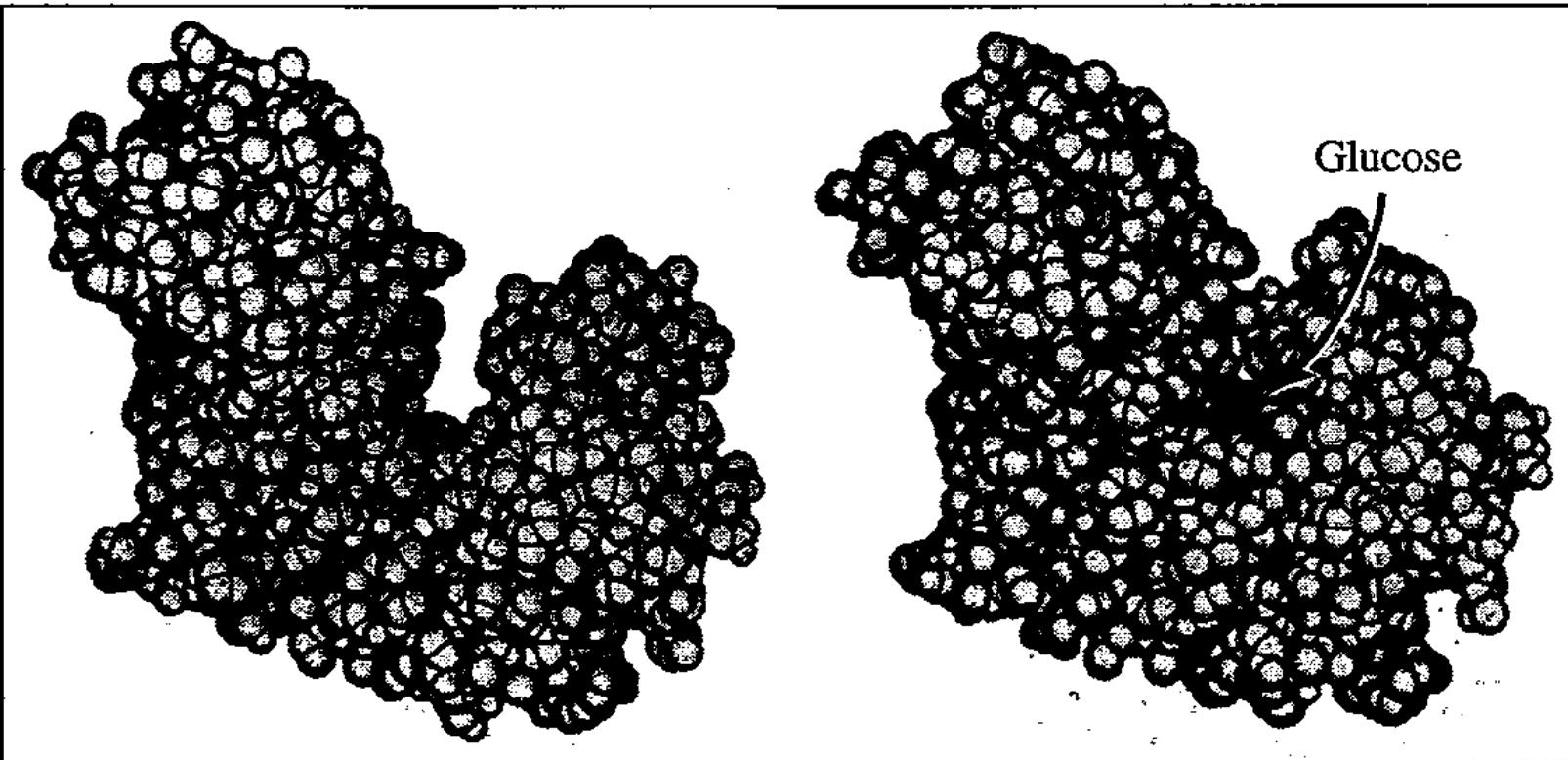
### Levels of Structure

(a) As the polypeptide forms intrachain bonds and folds, it assumes a three-dimensional (tertiary) state with numerous surface features.



(b) Because each different polypeptide folds differently each apoenzyme will have differently shaped active sites (AS)

(c) Some enzymes have more than one active site; others have sites to attach cofactors and regulatory compounds; and more complex enzymes have a quaternary structure consisting of several polypeptides bound by weak forces, as in (b)

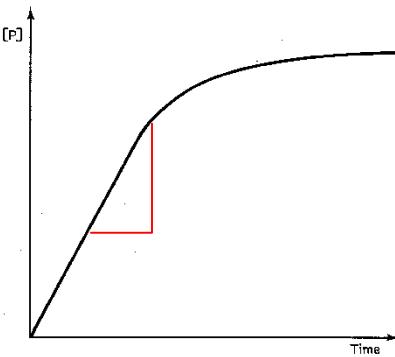


**Figure 1.11**

Computer-generated structure of the enzyme hexokinase that converts glucose and ATP into glucose 6-phosphate during the first step of glycolysis. Shown is the location where the glucose molecule fits into the enzyme structure and the resulting change in the enzyme configuration.  
Courtesy of Dr. Thomas Steitz.

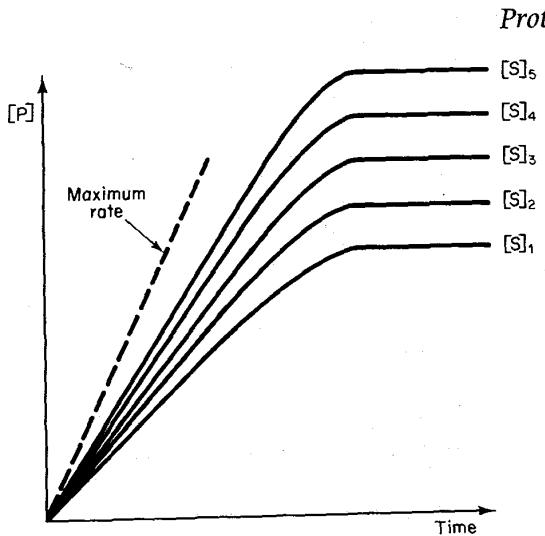
Enzymes contains metal cofactors include: Co, Cu, Fe, Mn, Mo, Ni, Se V, W, Zn

•Enzyme Kinetics: The rate of substrate decrease or product formation under the catalysis of a enzyme system can be expressed as:



Rate of reaction  $V_0$

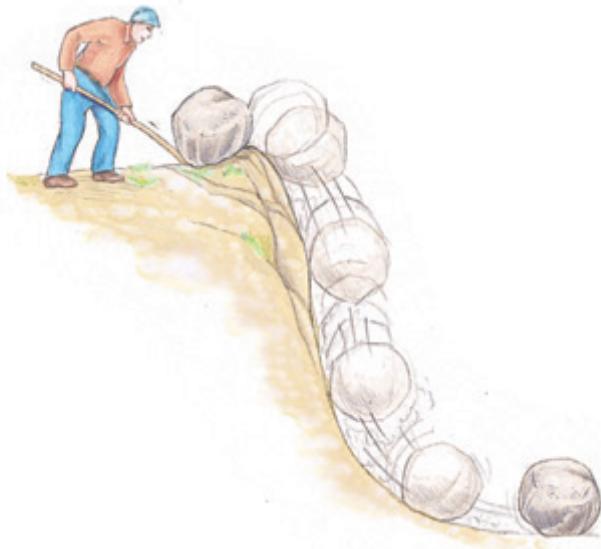
Fig. 6.4 Time course of an enzyme-catalyzed reaction.



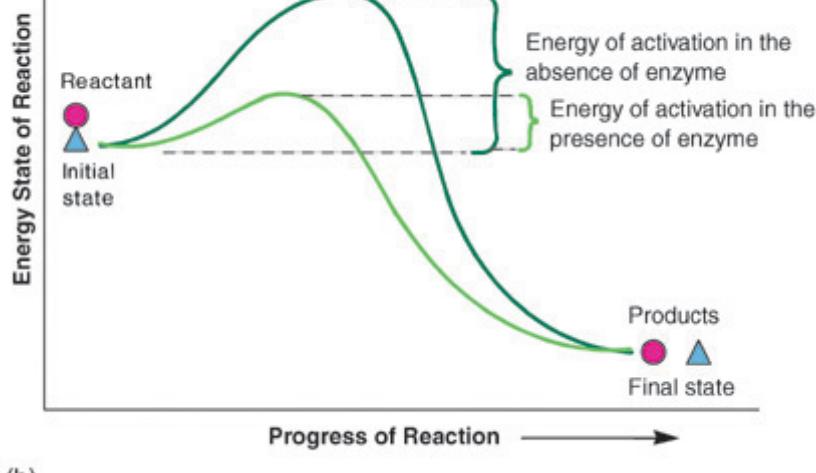
At different initial S concentration

Fig. 6.5 Time course of enzyme-catalyzed reactions with different substrate concentrations.

# Analogy demonstrating the influence of enzymes on chemical reactions



(a)



- a) A boulder can represent potential energy available for a chemical reaction.
- b) Graph of chemical reaction, with and without an enzyme. Energy (called energy of activation) is required in both cases to convert a reactant molecule to products. But in an enzyme-catalyzed reaction, the enzyme significantly lowers this energy of activation and allows the reaction to proceed more readily and rapidly.

$$V = K S_1 S_2$$

second order reaction

$$V_o = \frac{V_{\max} * [S]}{K_m + [S]} * [E]$$

When  $[S] \ll K_m$

$$V_o = V_{\max} * [S] / K_m$$

$$\text{Or } V_o = K [S]$$

A first order reaction

When  $[S] \gg K_m$   
The enzyme is saturated

$$V_o = V_{\max}$$

a zero order reaction

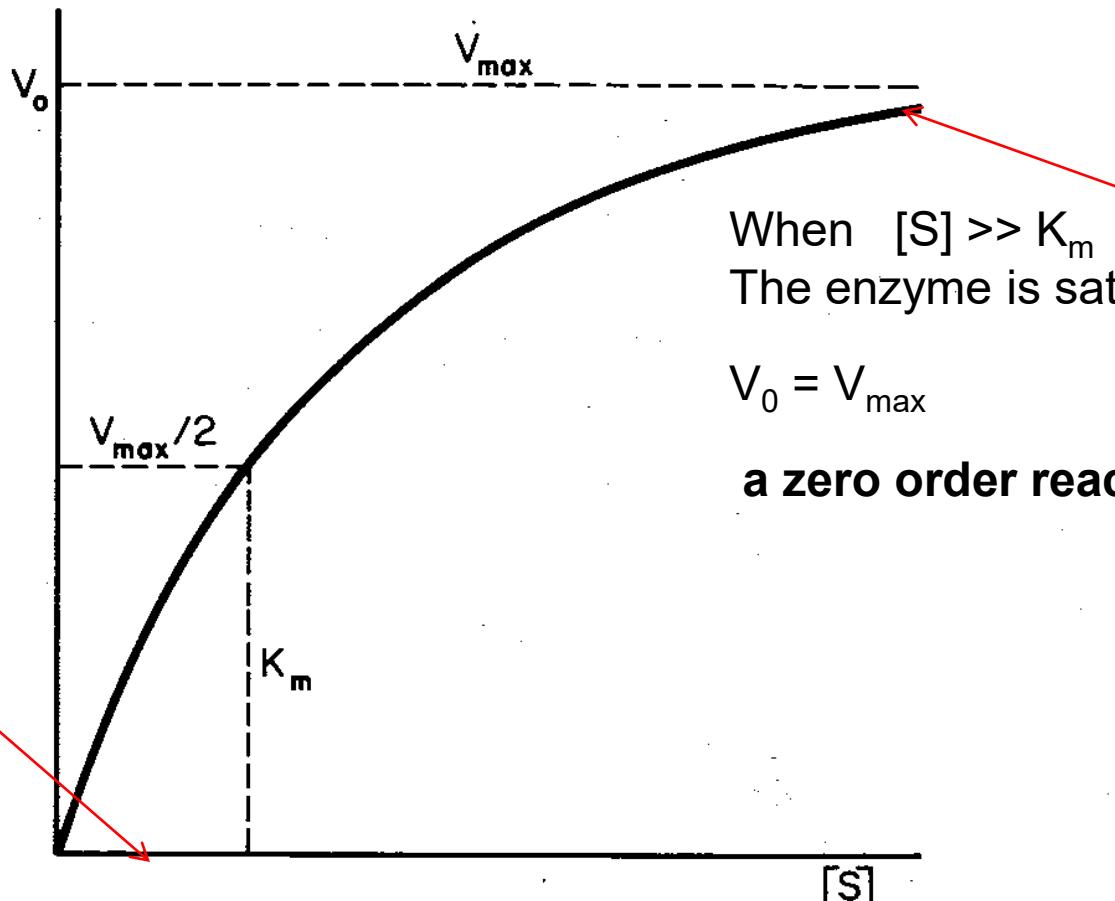
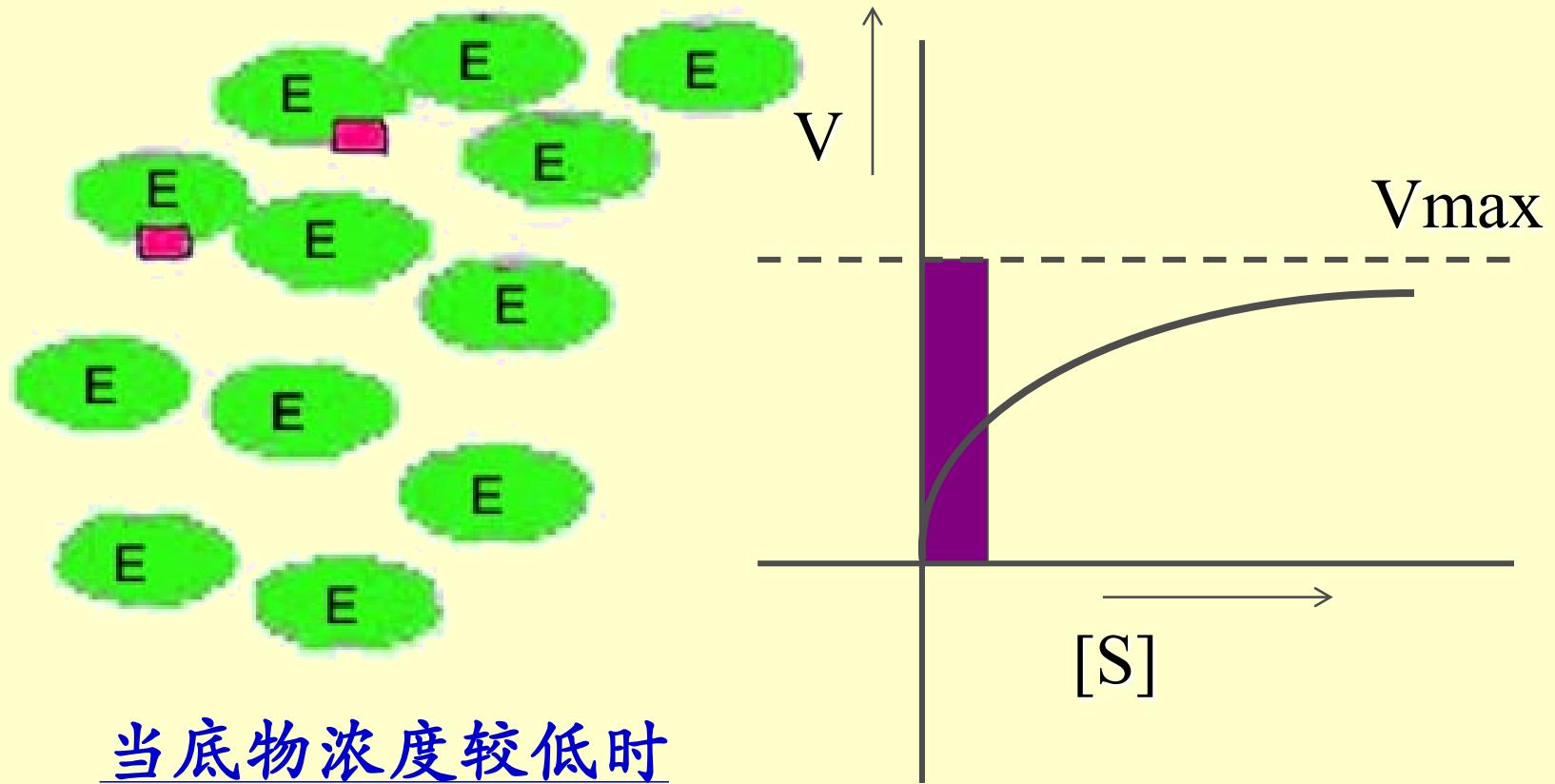
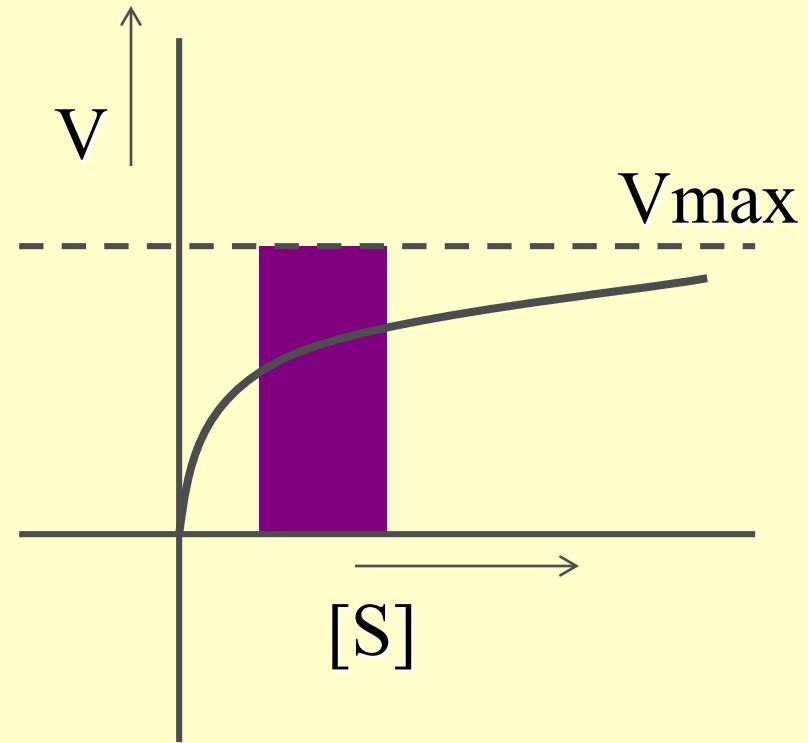
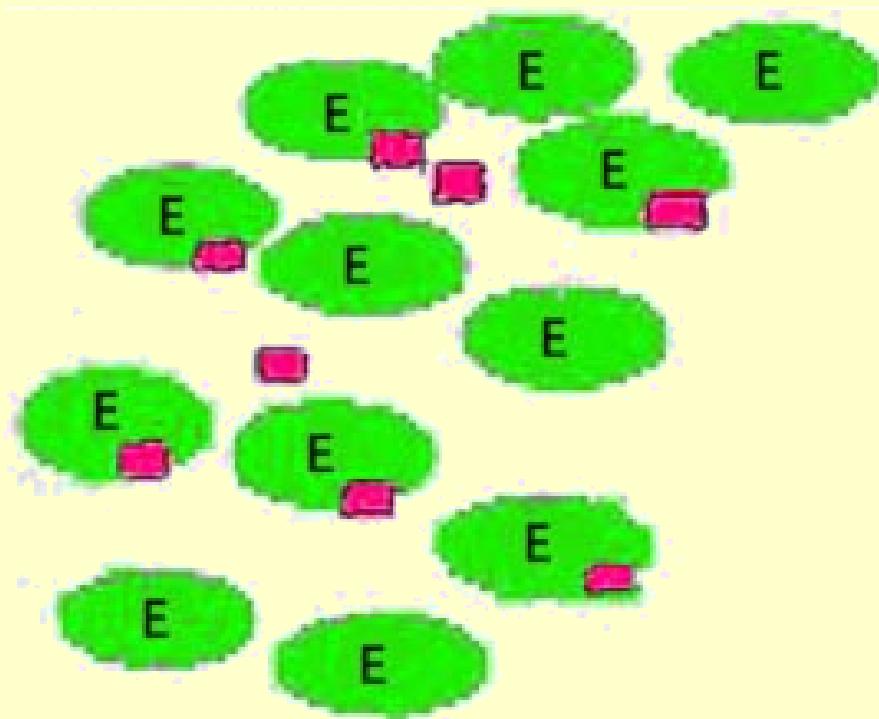


Fig. 6.6 Effect of substrate concentration on reaction velocity.



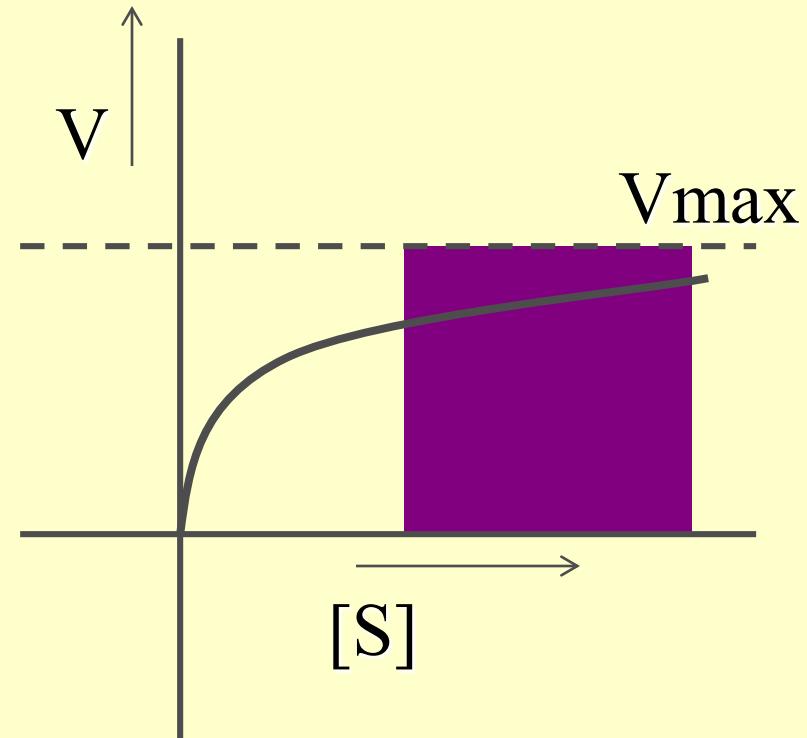
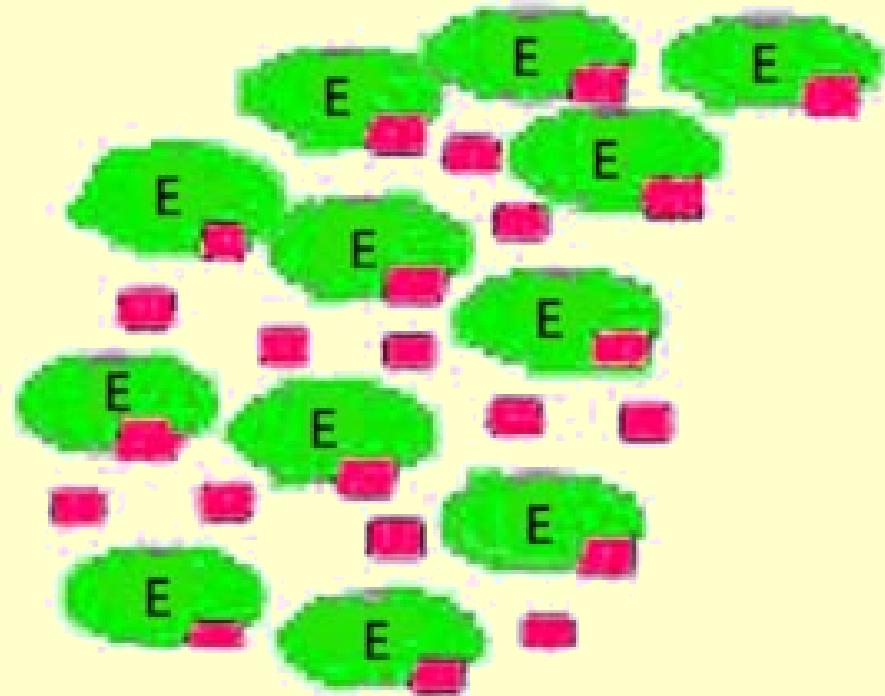
当底物浓度较低时

反应速度与底物浓度成正比；反  
应为一级反应。



随着底物浓度的增高

反应速度不再成正比例加速；反应  
为混合级反应。



当底物浓度高达一定程度

反应速度不再增加，达最大速度；  
反应为零级反应

breaks down to form free enzyme and products  $P$ :



Both reactions are considered reversible, and the various  $k$  values are rate coefficients for each of the four possible reactions. In the Briggs and Haldane development,  $E$  equals the total enzyme concentration,  $ES$  is the concentration of enzyme-substrate complex, and the difference between the two,  $E - ES$ , is the concentration of free enzyme.

The rate of formation of  $ES$  from  $E + S$  is thus given by

$$\frac{dES}{dt} = k_1(E - ES)S \quad [1.5]$$

The rate of formation of  $ES$  from  $E + P$  is very small and neglected. The rate of breakdown of  $ES$  is thus given by:

$$-\frac{dES}{dt} = k_{-1}ES + k_2ES \quad [1.6]$$

When the rate of formation of  $ES$  just equals its rate of breakdown, the system is at steady state with respect to  $ES$  concentration, and

$$k_1(E - ES)S = k_{-1}ES + k_2ES \quad [1.7]$$

Rearranging gives

$$\frac{S(E - ES)}{ES} = \frac{k_{-1} + k_2}{k_1} = K_M \quad [1.8]$$

The coefficient  $K_M$ , which represents a composite of the three rate coefficients, is called the *Michaelis-Menten coefficient*. Equation 1.8 may be solved for the concentration of the  $ES$  complex,

$$ES = \frac{E \cdot S}{K_M + S} \quad [1.9]$$

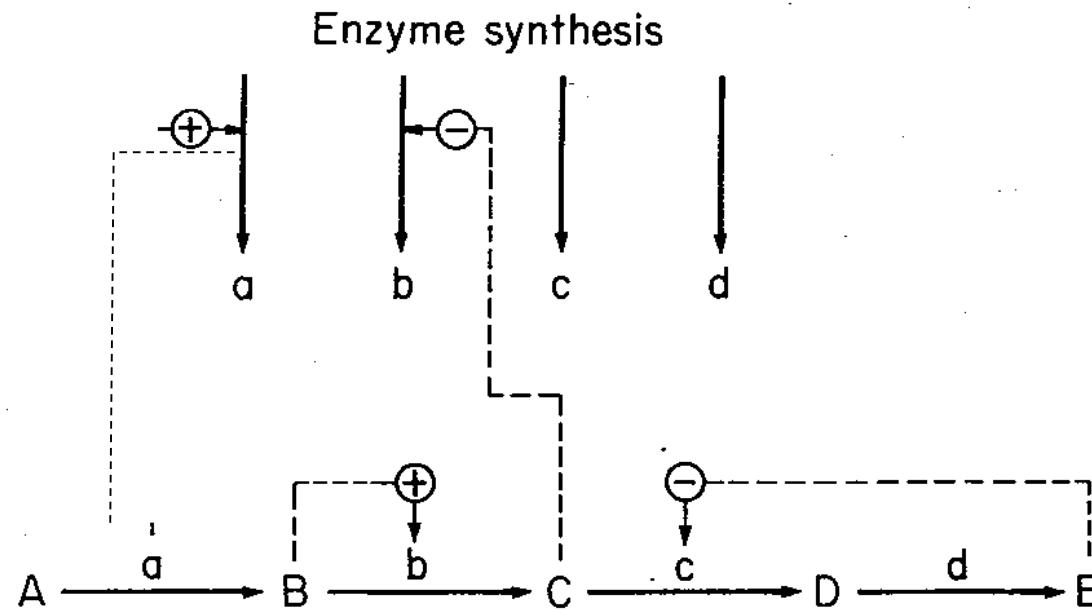
Our interest is in the overall rate of the reaction, in other words, the rate of formation of product  $P$ . The velocity of this reaction  $v$  is given by

$$v = k_2ES \quad [1.10]$$

Combining this with Equation 1.9 yields

$$v = \frac{k_2 \cdot E \cdot S}{K_M + S} \quad [1.11]$$

- ✓ cell reactions can be controlled easily through manipulate enzyme functions : substrate induction or substrate or product inhibition



Capital letters: substrates

Lower case letters: enzymes

$\oplus$  = Enhancement

$\ominus$  = Inhibition

Fig. 6.2 Schematic of control mechanisms for enzyme-catalyzed reactions.

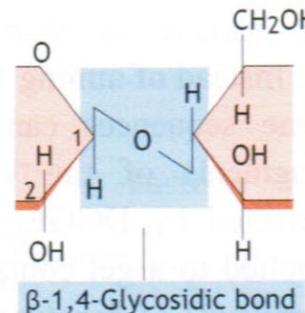
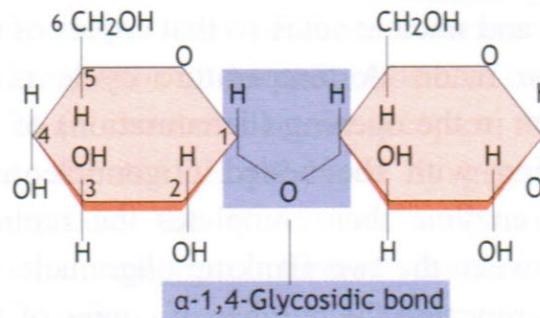
# **Sugars**

# **Polysaccharides**

## 2. Polysaccharides : (Mostly function as food storage, cell walls, part of nucleic acids structures )

- Only three general types of sugar monomer are used by cells.  
 $(C_nH_{2n}O_n)$  n=4,5,6
- Some bacteria can produce significant amount of extra-cellular polysaccharides for food storage, connecting with the environment and protection

A



B

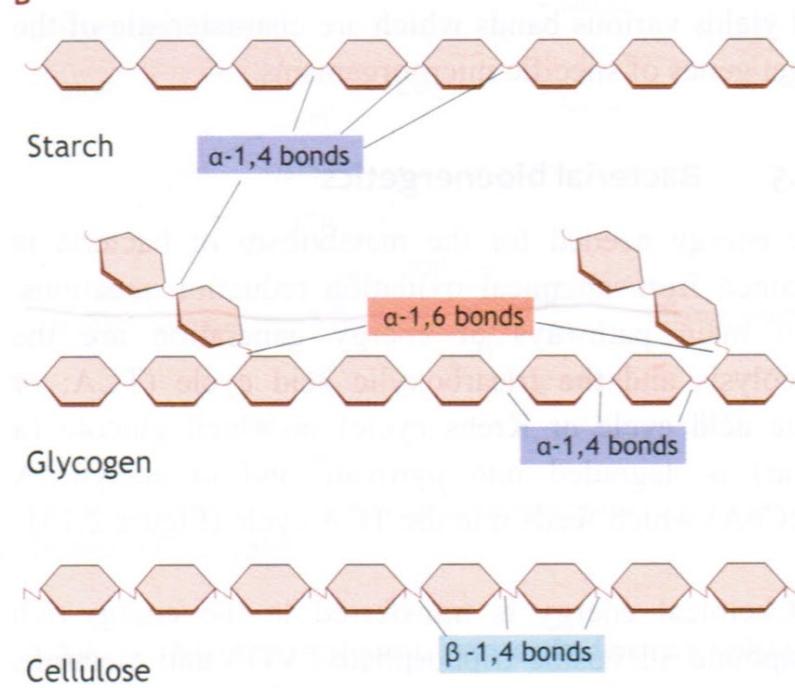
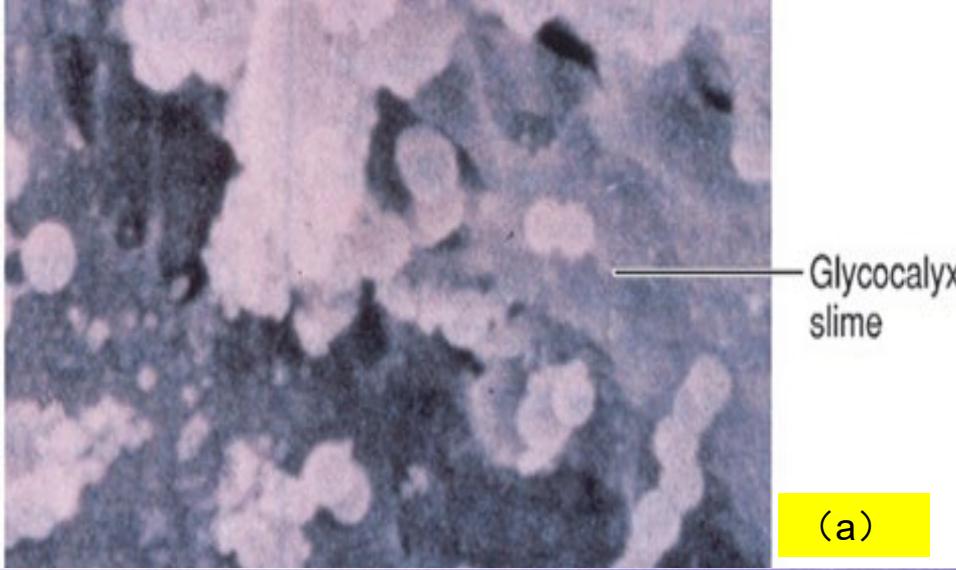
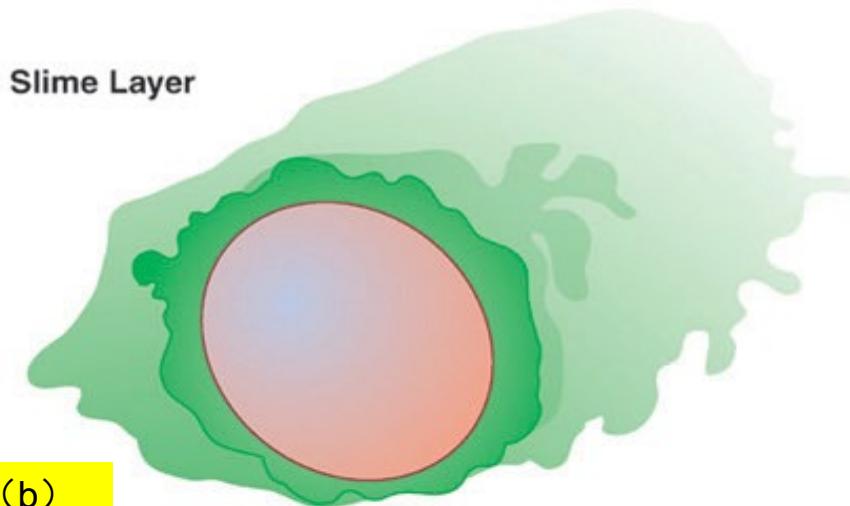


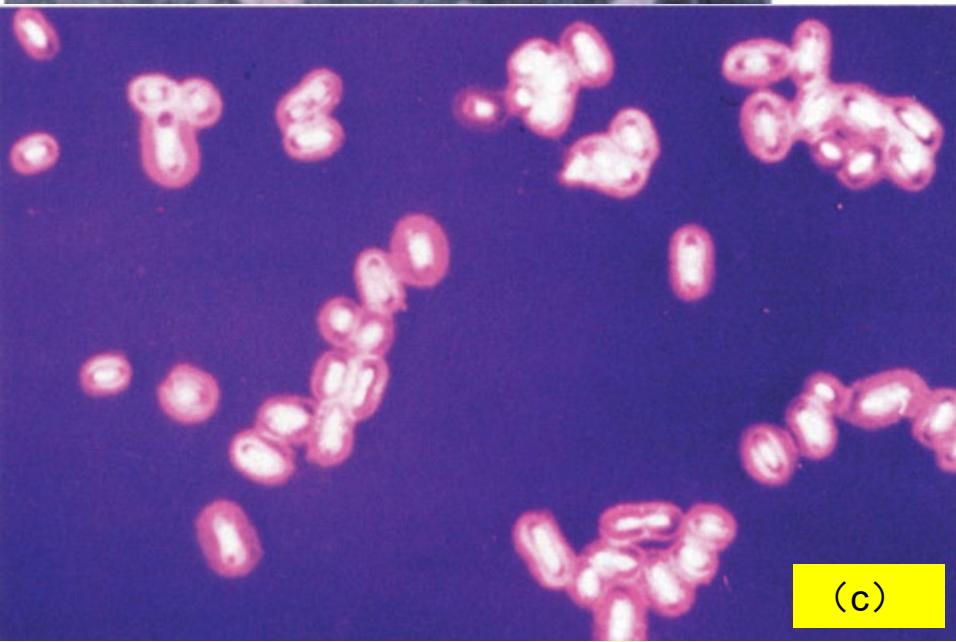
Figure 2.9 Structure of the polysaccharides. (A) Differences in the glycosidic bonds in the position of linkage between glucose molecules and in geometry ( $\alpha$  and  $\beta$ ). (B) Structure of starch, glycogen (a bacterial storage polymer) and cellulose (adapted from Madigan and Martinko, 2006)



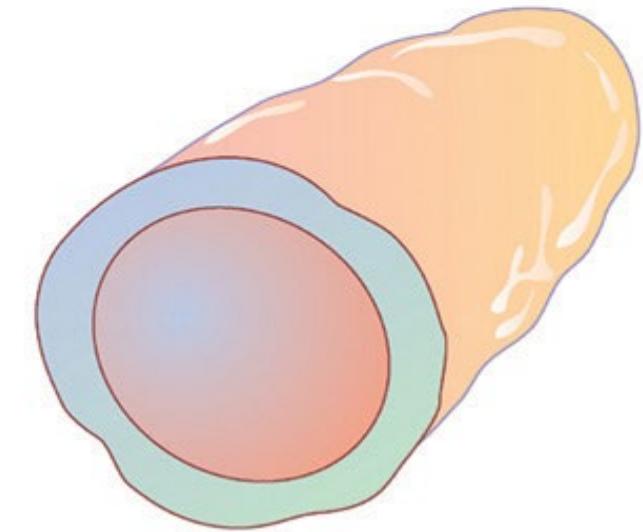
(a)



(b)



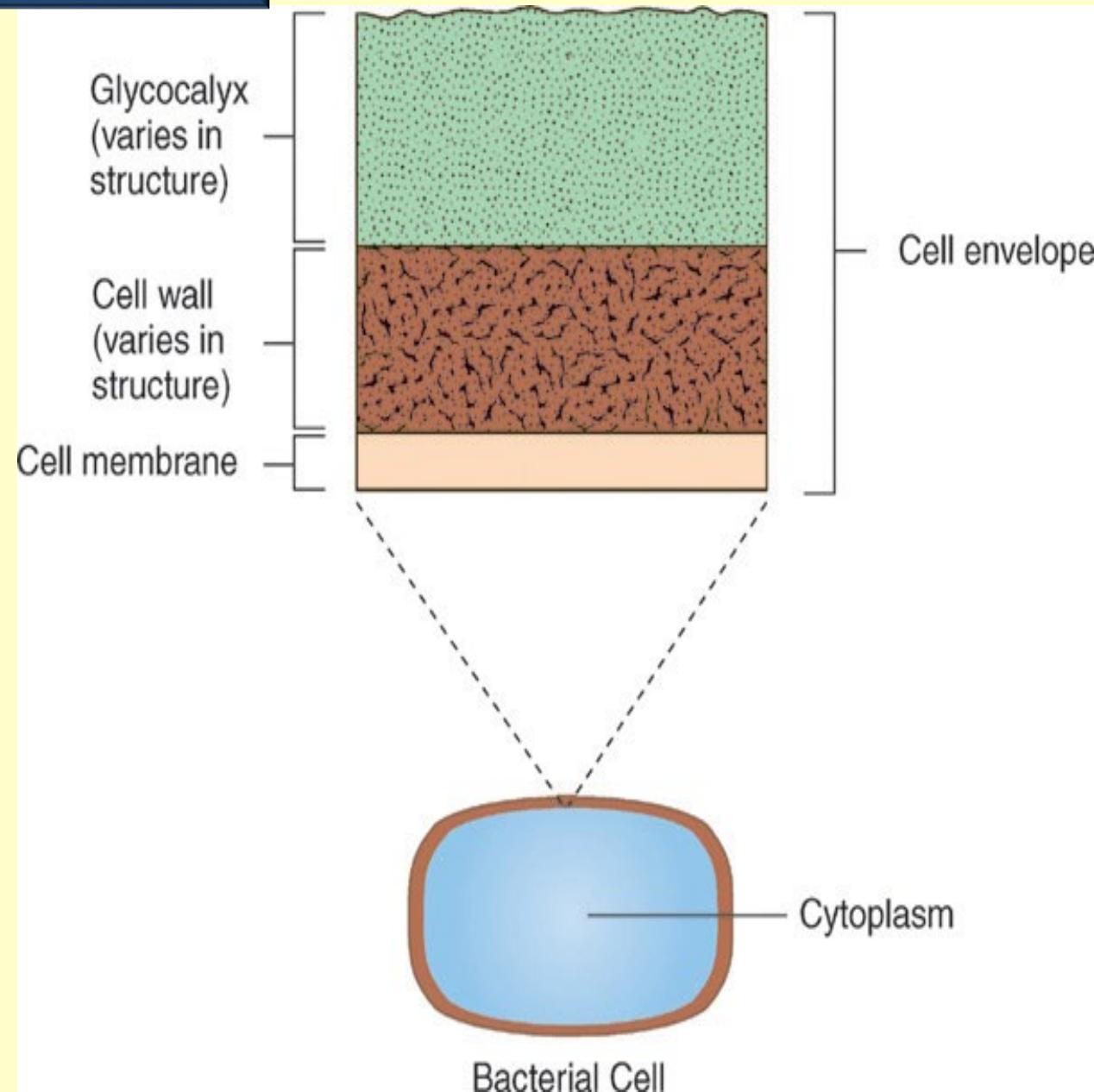
(c)



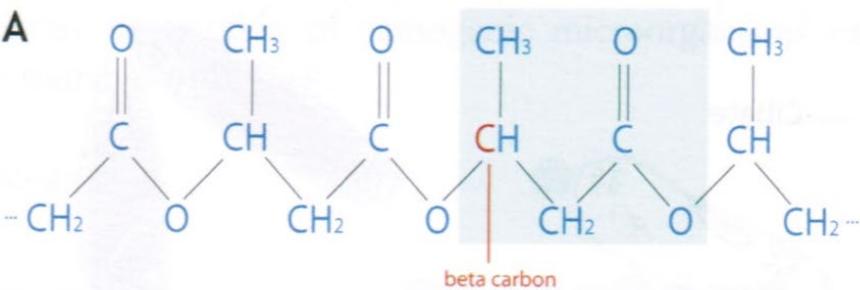
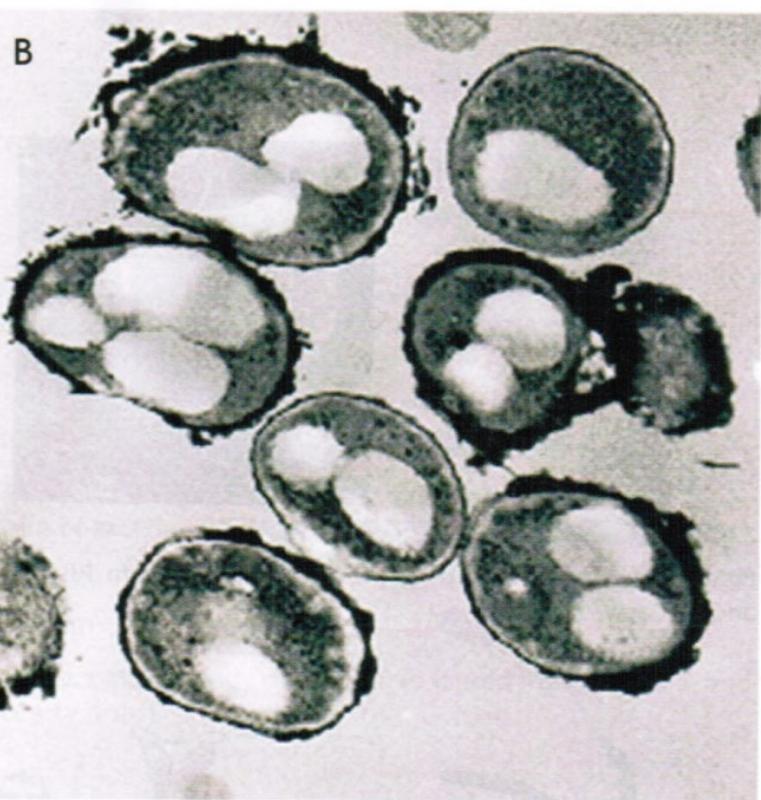
(d)

(a) Glycocalyx slime; (b) Slime Layer; (c) fluorescence microphoto; (d) capsule

# Glycocalyx



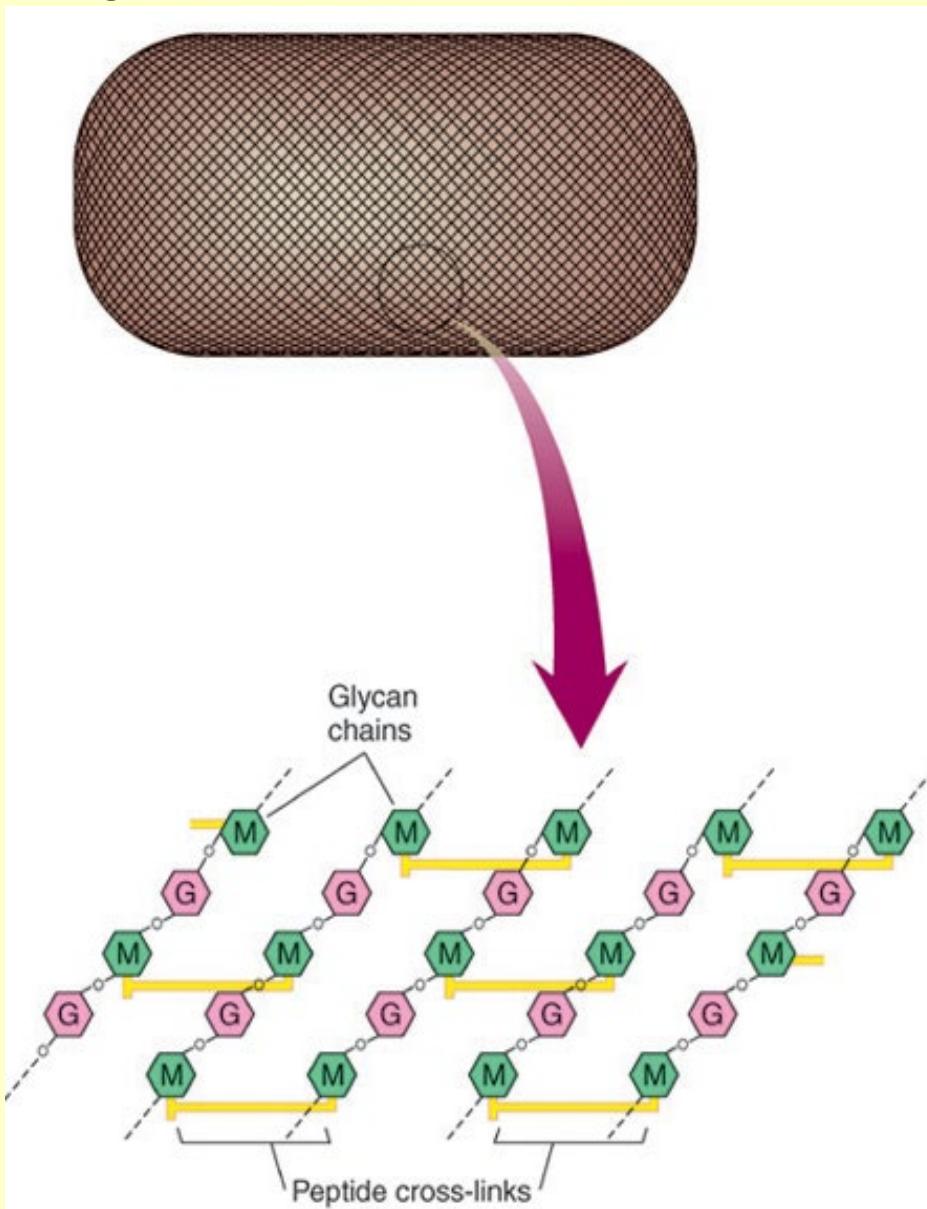
## Other type of storage material PHB, poly- $\beta$ -hydroxybutyrate



The PHB material is important in bio-enhanced Phosphate removal

Figure 2.8 (A) Structure of poly- $\beta$ -hydroxybutyrate (PHB). In poly- $\beta$ -hydroxyvalerate (PHV), the  $-\text{CH}_3$  group is replaced by  $-\text{CH}_2\text{CH}_3$  group. PHB and PHV are the two most common poly- $\beta$ -hydroxyalcanoates (PHAs). (B) White granules of PHA stored inside the cells (cell size approximately 1  $\mu\text{m}$ ) (photo: M.C.M. van Loosdrecht)

# Cell Wall – dye stain of the cell wall used to be one of the key factors in identify the species



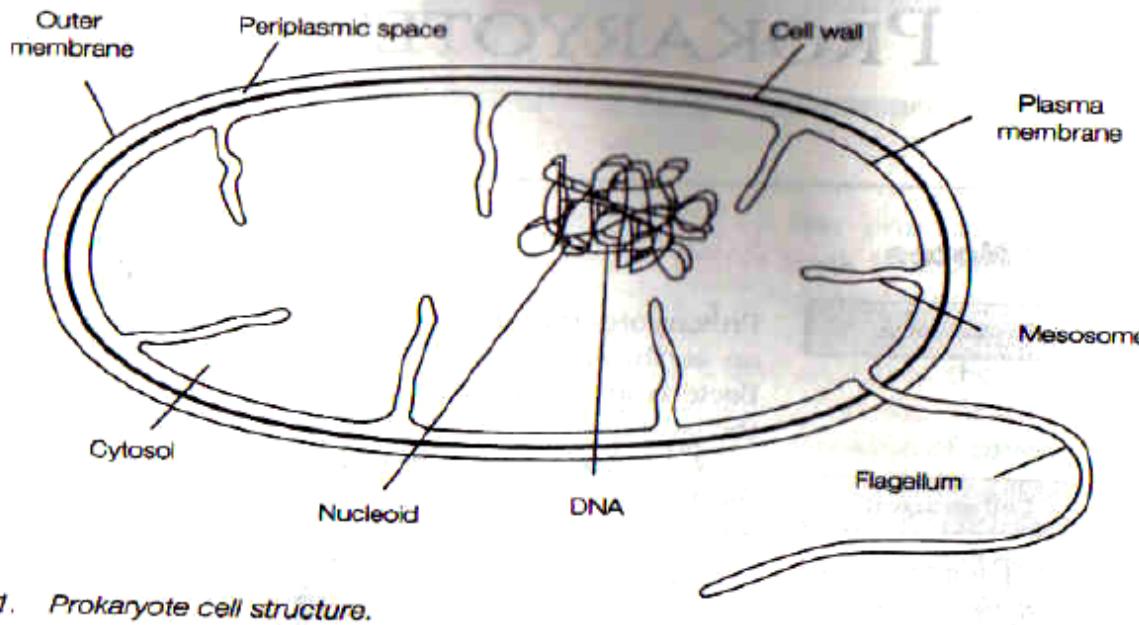


Fig. 1. Prokaryote cell structure.

To protect the cell from mechanical injury and osmotic pressure, most prokaryotes are surrounded by rigid 3-25nm thick cell wall. The cell wall is composed of peptidoglycan, a complex of oligosaccharides and proteins. The presence of D-amino acids in the peptidoglycan chains renders the cell wall resistant to the action of proteases which act on the more commonly occurring L- amino acids, but provide a unique target for the action of certain antibiotics such as penicillin.

# Cell Wall

## ■ Functions

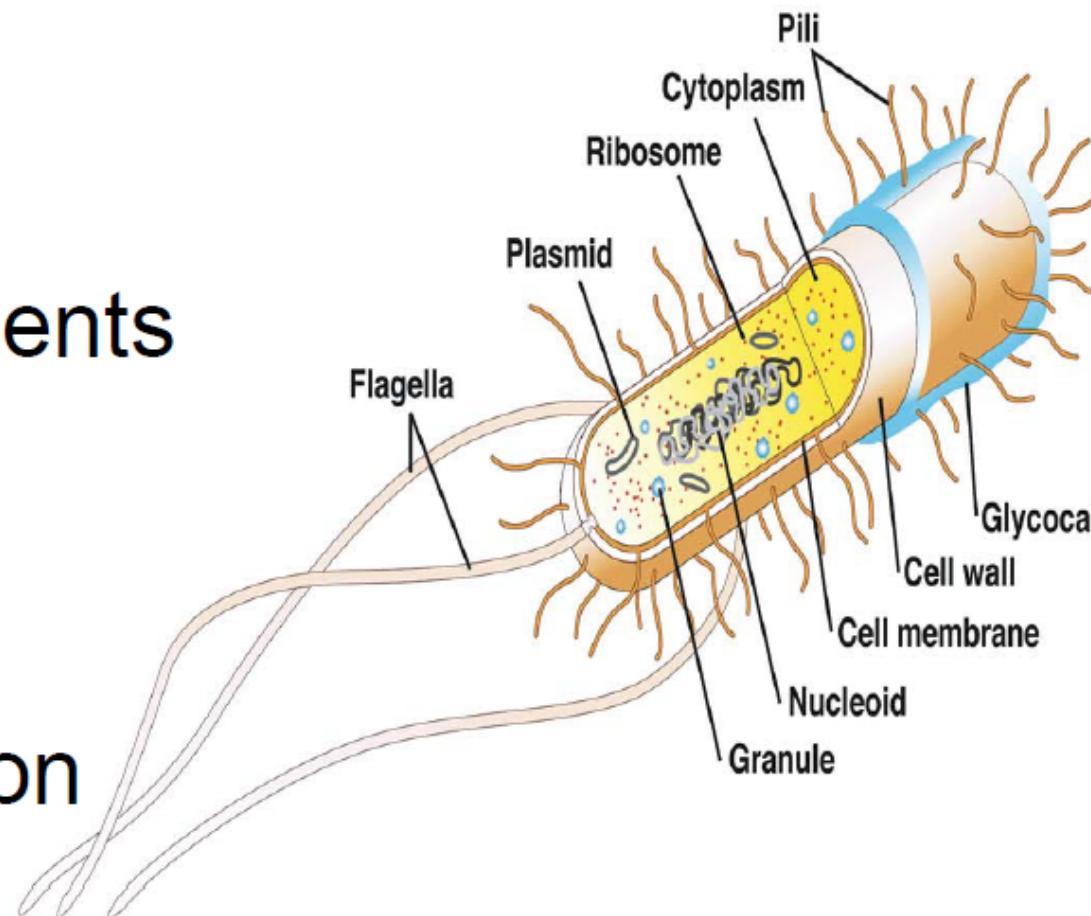
- Protection
- Cell shape

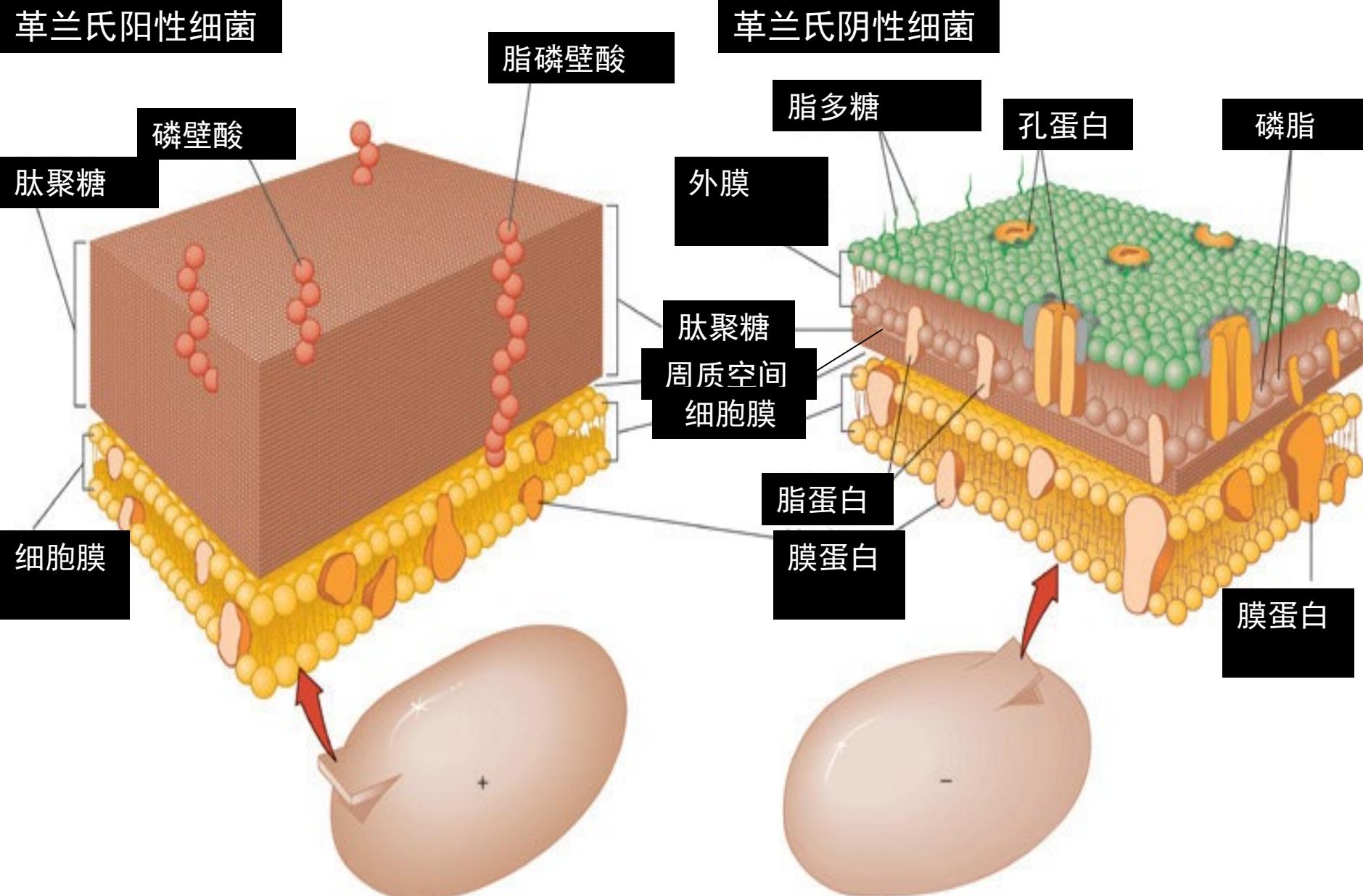
## ■ Chemical constituents

- Peptidoglycan
- Teichoic acid
- LPS

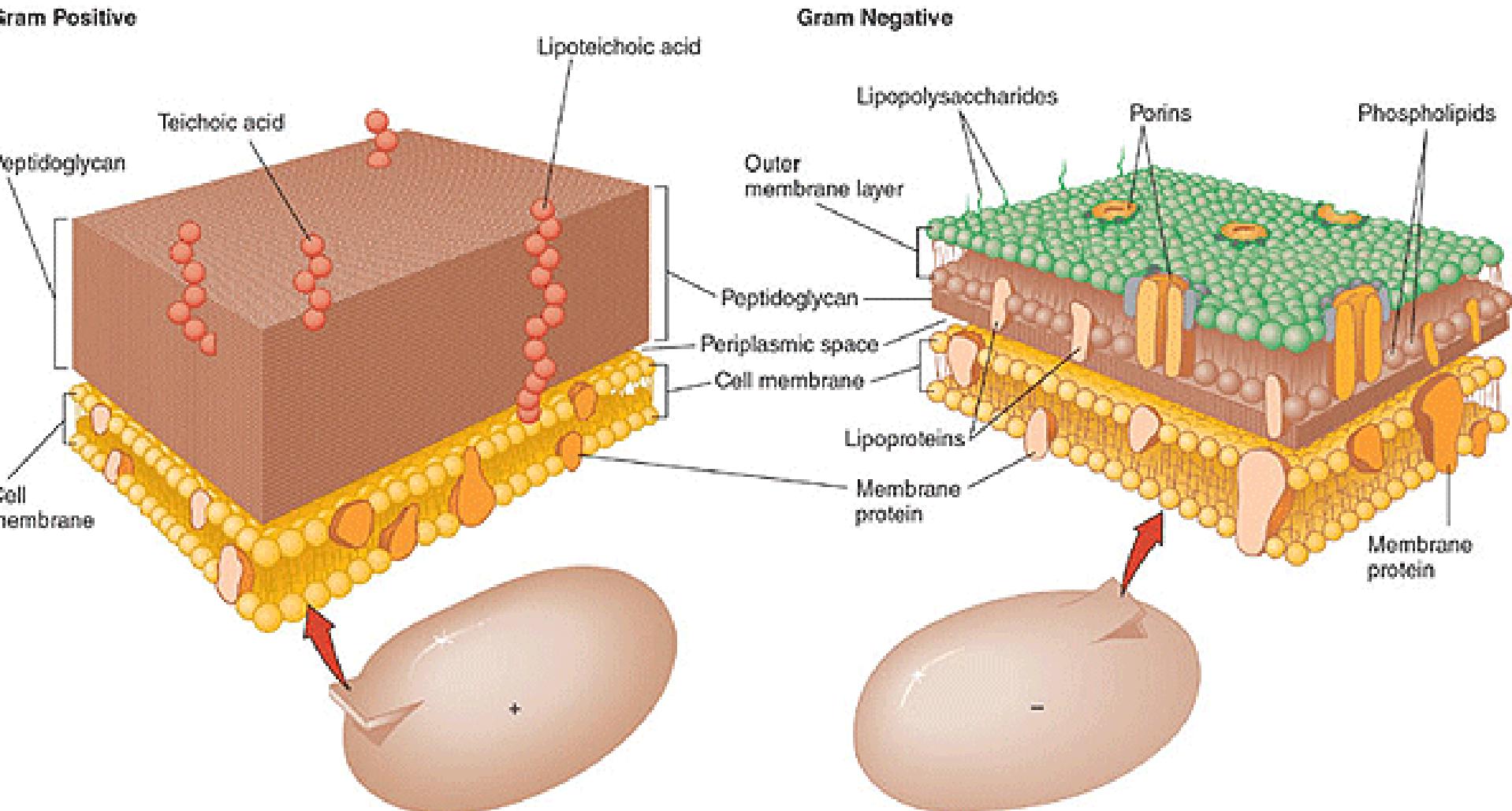
## ■ Antimicrobial Action

- Penicillin
- Lysozyme





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# Types(Gram staining) **G<sup>+</sup>** & **G<sup>-</sup>** bacteria

Gram (+)



Gram (-)



Gram (+)



Gram (-)



Both cell walls affix the dye



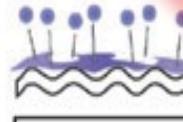
Dye crystals  
trapped in wall



No effect  
of iodine



Crystals remain  
in cell wall



Cell wall partially  
dissolved, loses  
dye



Red dye has  
no effect



Red dye stains  
the colorless cell

Structure of cell wall  
about gram-positive  
bacteria and gram-  
negative bacteria

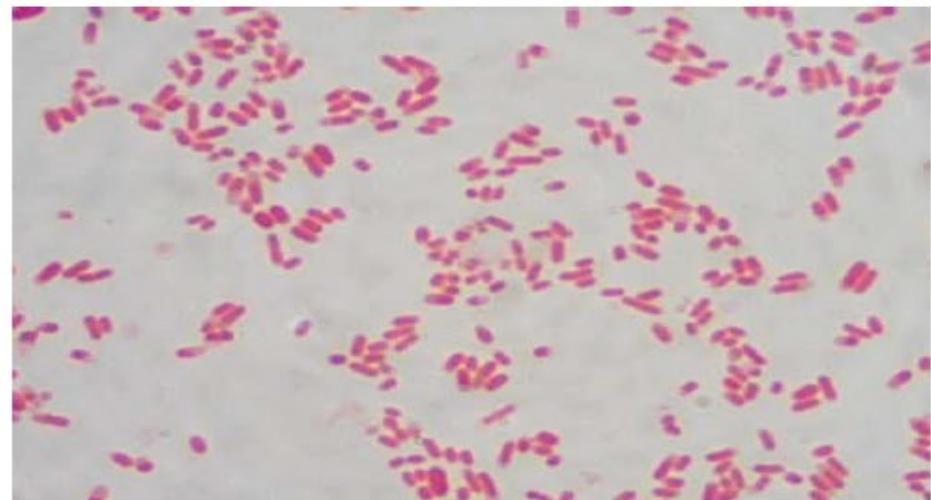
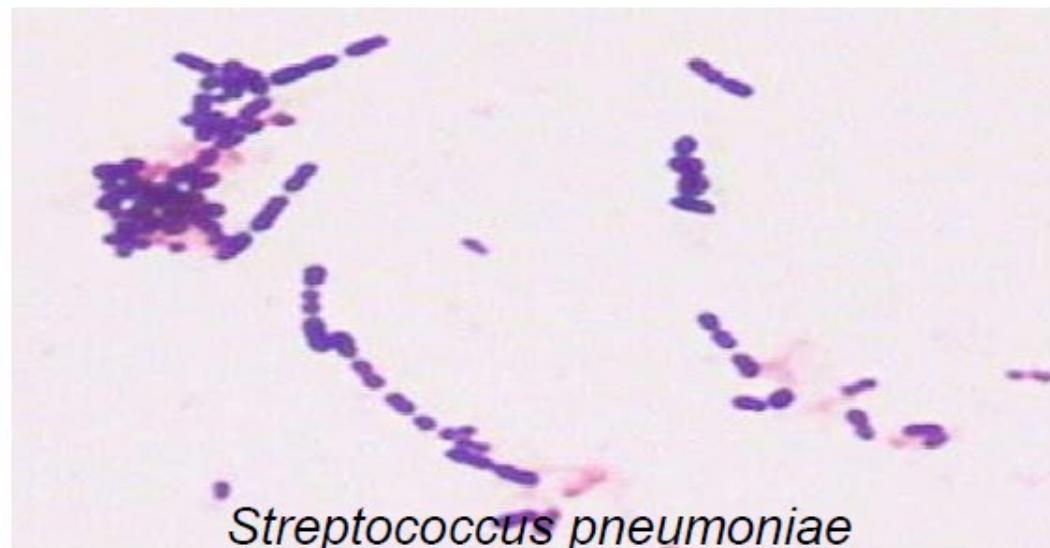
Colomn

In 1884, Doctor  
Hans Christian  
Gram (Sweden)  
discovered the  
staining  
technique.

# Cell Wall and the Gram Stain

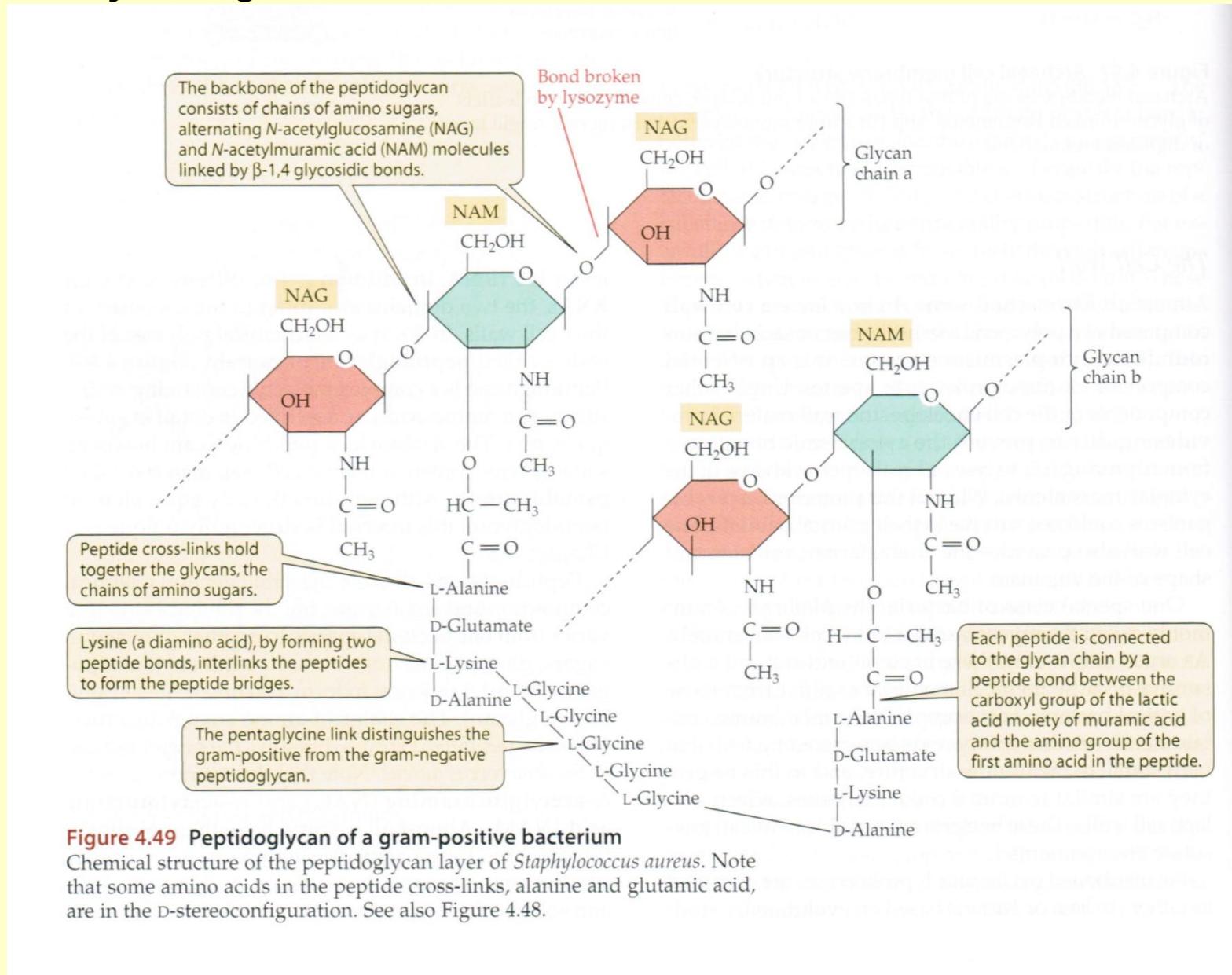
Staining technique  
used

to determine cell wall  
components



*E. coli*

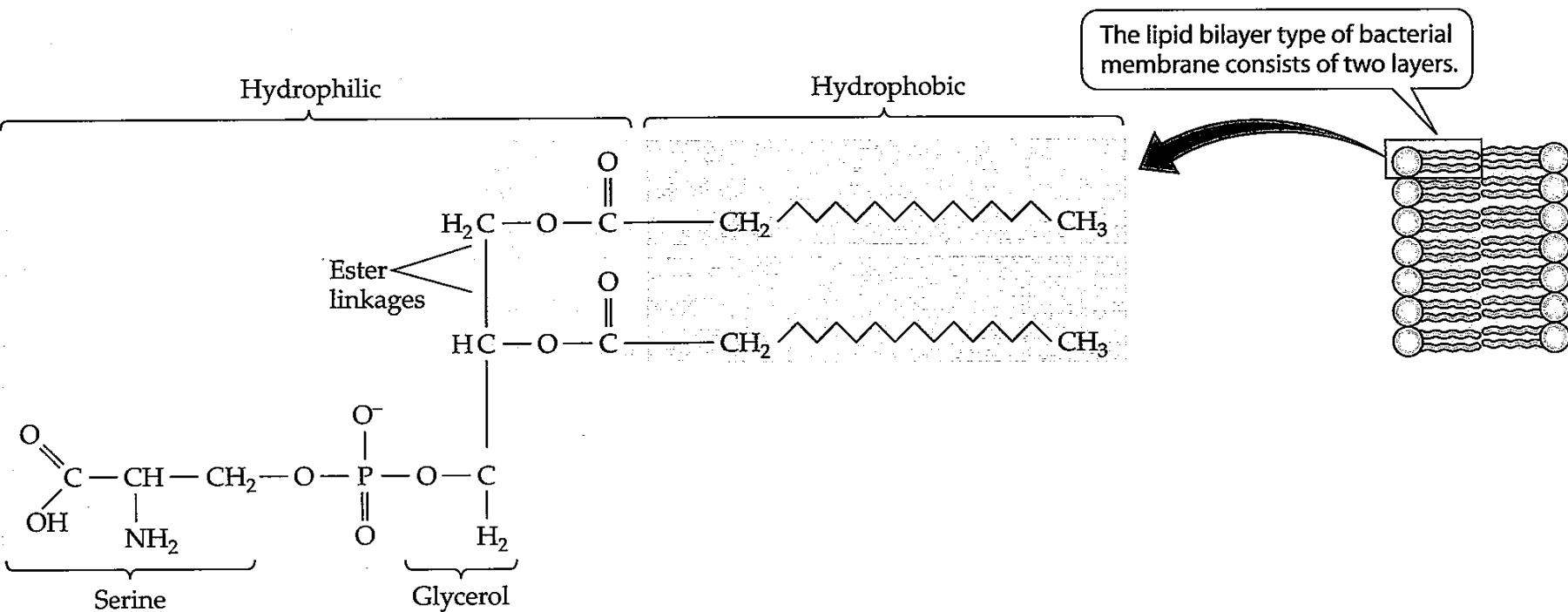
# Cell walls are made of peptidoglycan polymers and they are more complicated and structurally strong



# **Lipids or Fats**

### 3. Lipids or fat

- Mostly function as cell membrane: 40%-50% lipids and 50% - 60% protein
- Internal storage material for algae and some bacteria

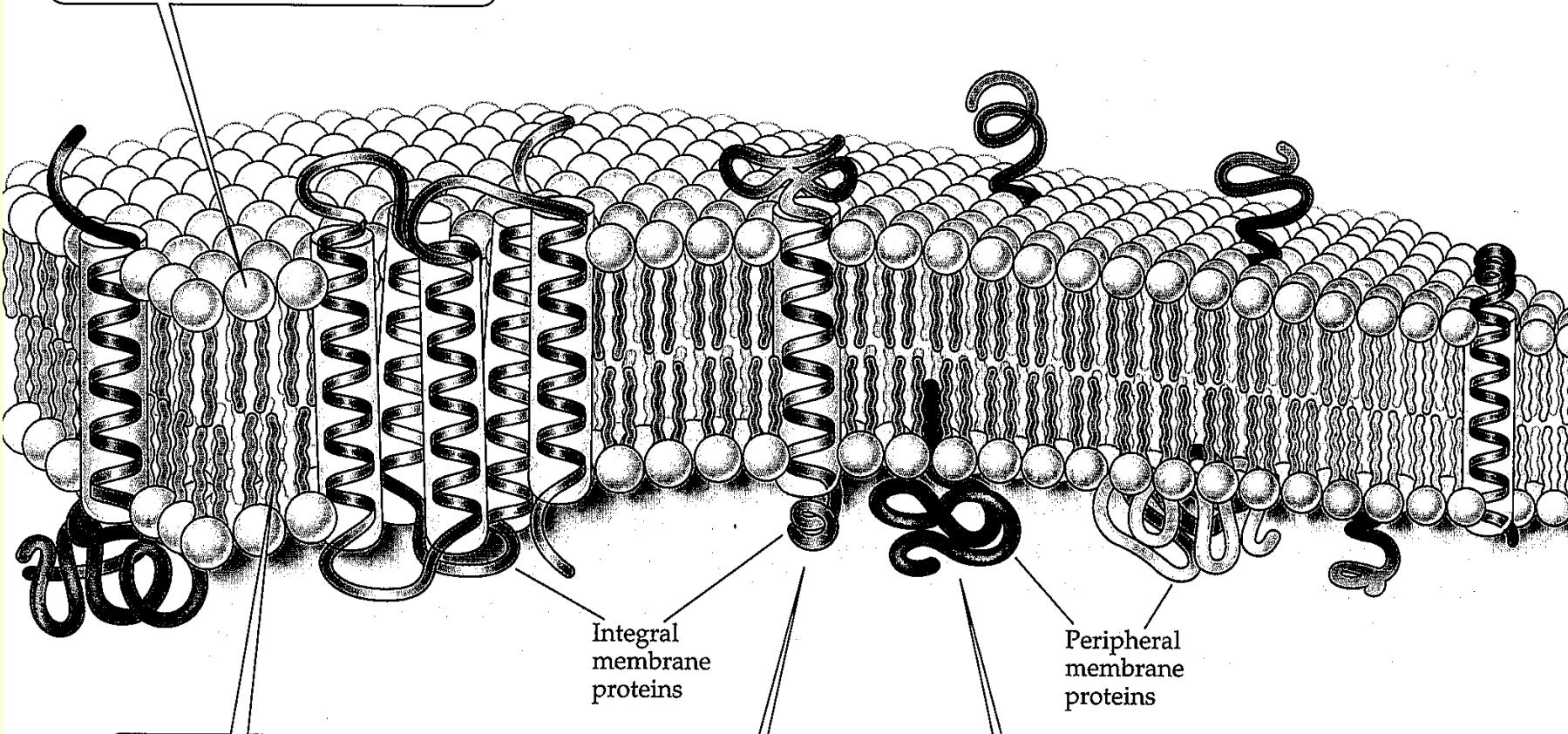


**Figure 4.45 Phospholipid**

Chemical structure of phosphatidyl serine, a typical phospholipid. The hydrocarbon side chains are fatty acids, typically containing 12 to 18, sometimes more, carbon atoms. Each is attached to glycerol by an ester linkage. Serine is attached to the phosphate moiety and forms the polar "head" group.

The hydrophilic glycerol phosphate moieties face away from the membrane into the aqueous environment inside and outside the cell.

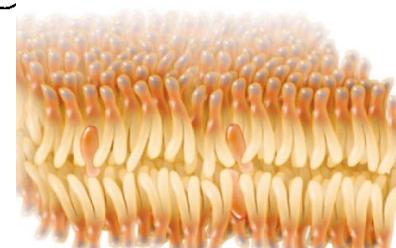
Outside of cell



The hydrophobic tails of the phospholipid molecules in each leaflet of the membrane face inward to form a hydrophobic layer.

Protein molecules, with charged, globular structures, are embedded in the phospholipid bilayer through their hydrophobic segments.

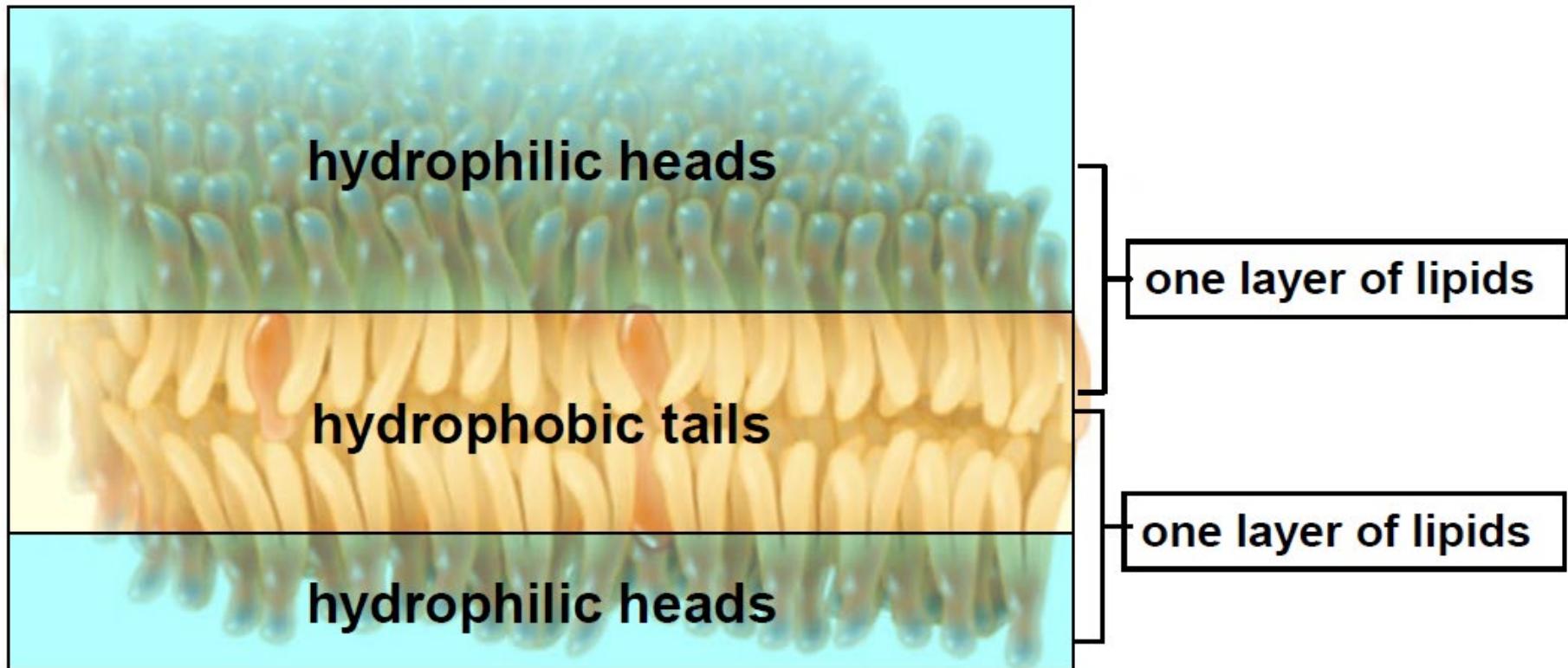
Cytoplasm



**Figure 4.44 Bacterial cell membrane structure**

The fluid mosaic model of cell membrane structure. The bilayer structure consists of two phospholipid leaflets.

# Phospholipids



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hydro = water

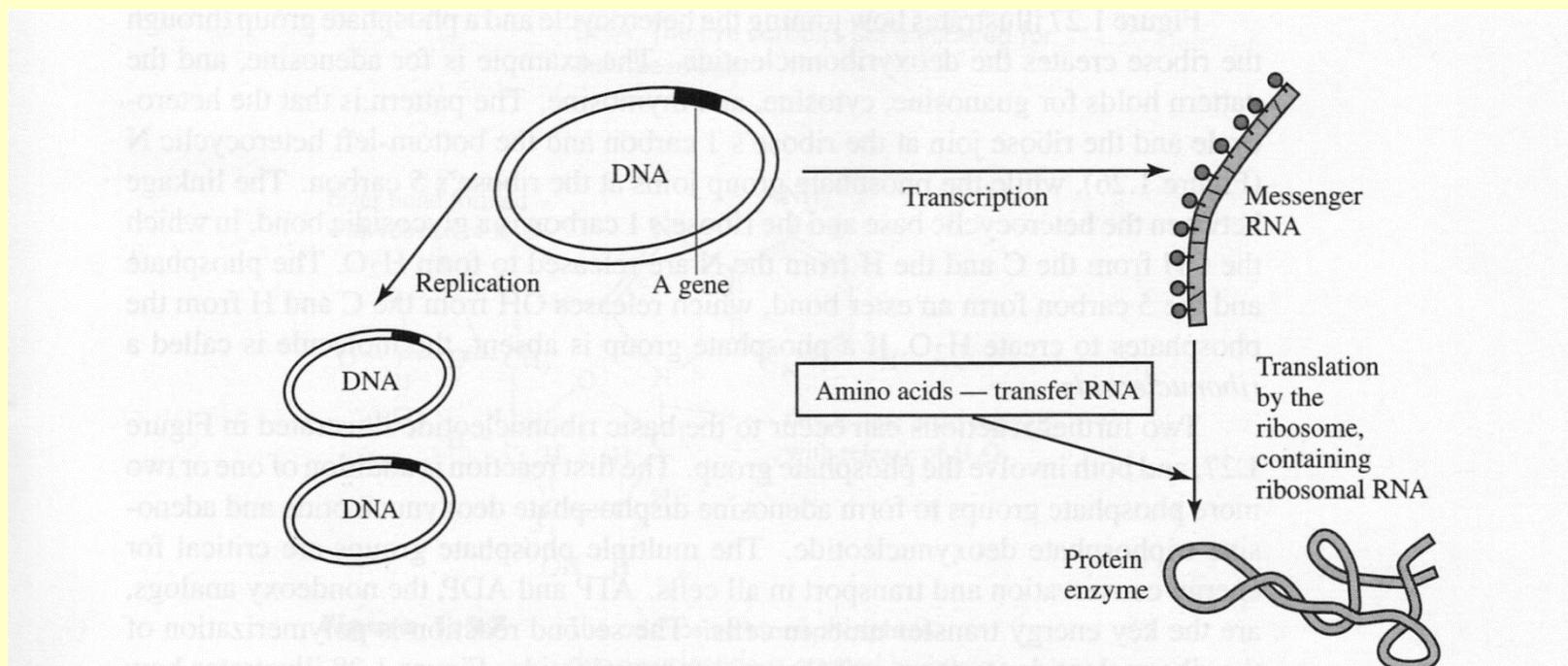
philic = loving

phobic = fearing

# Nucleic Acids

**Nucleic acids : Basic information to be used in cell function and genetically transferred to the daughter cells.**

- ✓ **DNA (Gene code) – deoxyribonucleic acid**
- ✓ **RNA (Using the code to generate enzymes) – Ribonucleic acids**
  - messenger RNA , m-RNA
  - ribosomal RNA, r-RNA
  - transfer RNA, t-RNA



**Figure 1.25**

Summary of information flow from the gene (in DNA) to the working enzyme catalyst.

# DNA Structure

## Deoxyribose units

## Purine and Pyrimidine bases

Phosphate Groups

Nucleic acids 1

A

Adenine

T

Thymine

G

Guanine

C

Cytosine

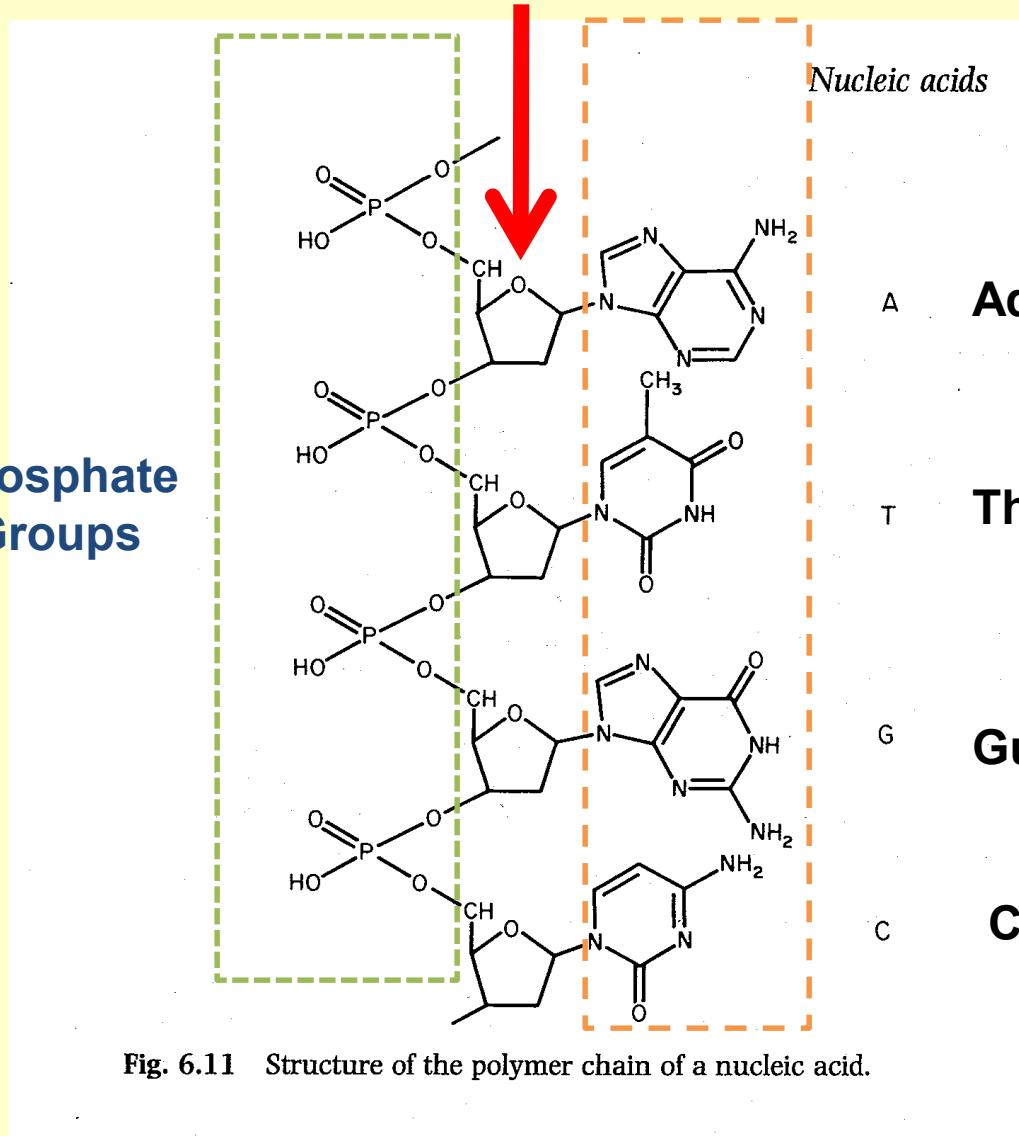
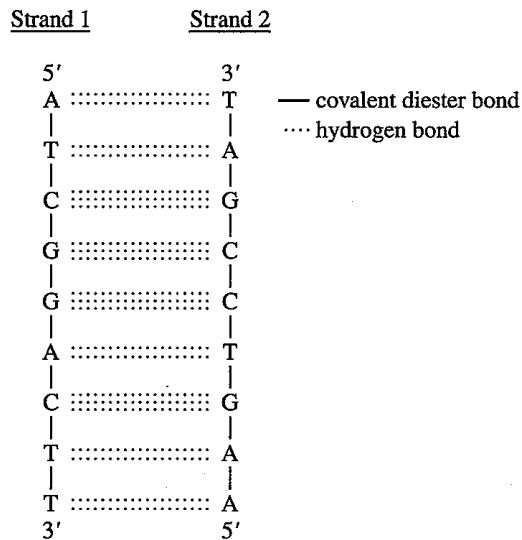


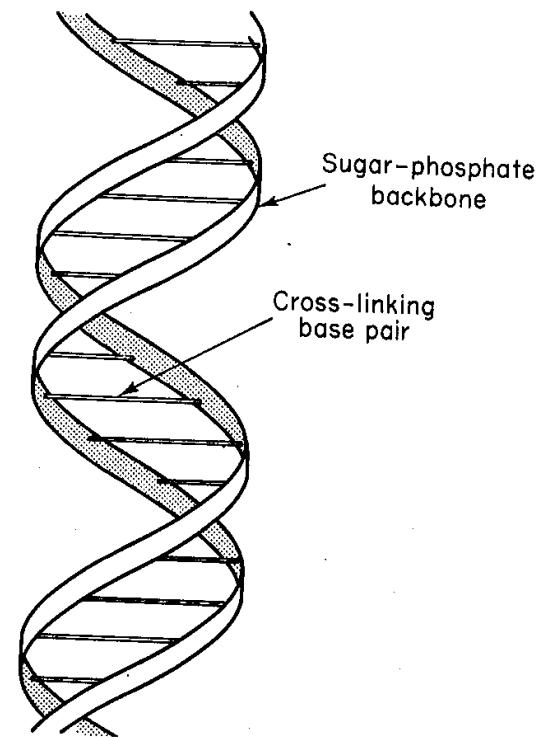
Fig. 6.11 Structure of the polymer chain of a nucleic acid.

**A typical bacteria chromosome double-stranded DNA can have  $5 \times 10^6$  base pairs of nucleotides. The sequence of these ATCG units forms the genetic code**



**Figure 1.30**

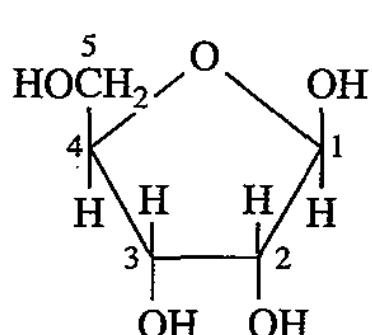
DNA strands are held together very strongly by hydrogen bonds when the bases are complementary in the opposing directions.



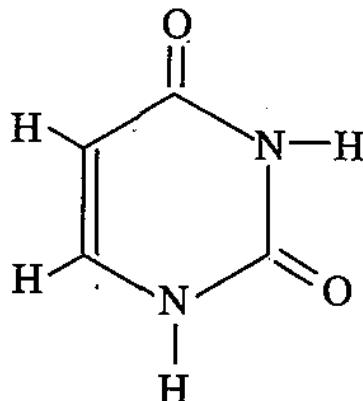
**Fig. 6.13** Schematic of the DNA double helix.

**Plasmids DNA: A circular double strained smaller DNA with  $10^3$  to  $10^5$  base pairs**

**RNA are polymers structurally very similar to DNA, except the base sugar is the ribose unit and the uracil base replace the thymine.**



Ribose unit



Uracil (U)

### **Figure 1.31**

The components of RNA. The ribose sugar on the left has an -OH group on its 2 carbon. The uracil base on the right replaces the thymine base found in DNA.

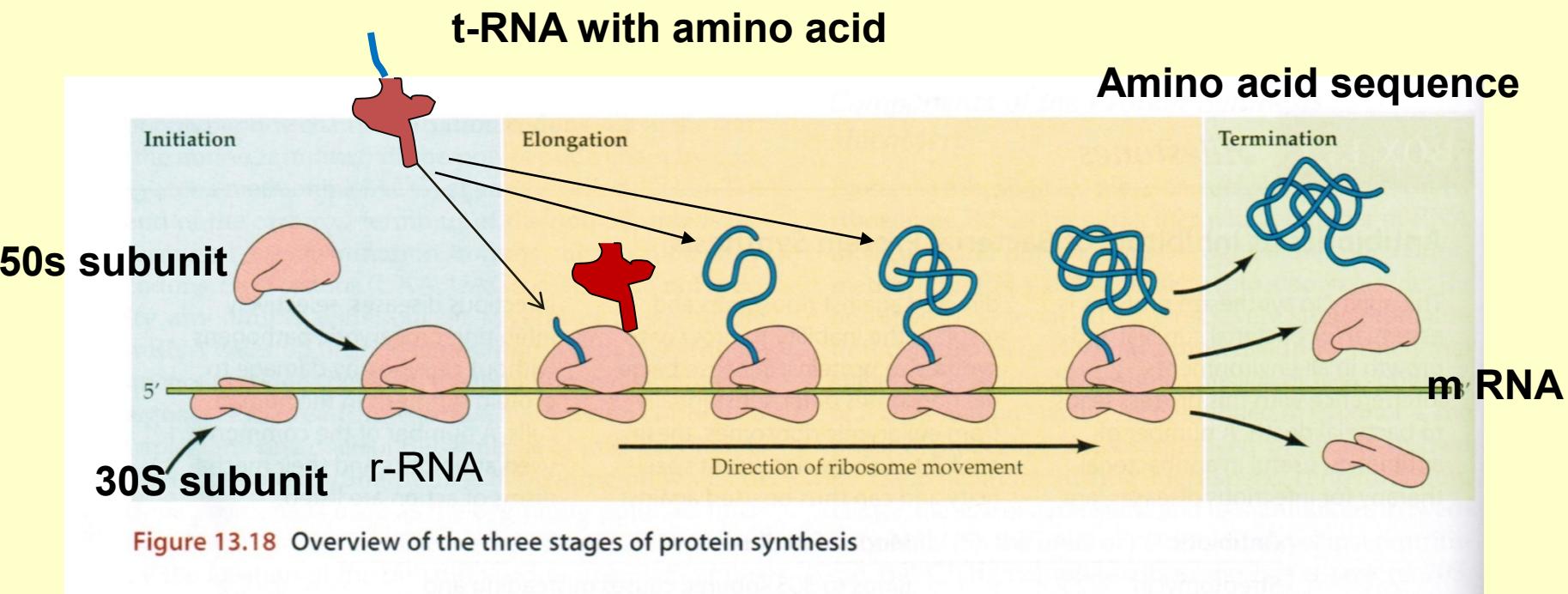
**Adenine**

**Uracil**

**Guanine**

**Cytosine**

**1. Messenger RNA – transcribed from a portion of DNA for the synthesis of a special protein. It is then transported to the ribosome where its code is translated into a amino acid polymer. The translation is directed by the r-RNA.**



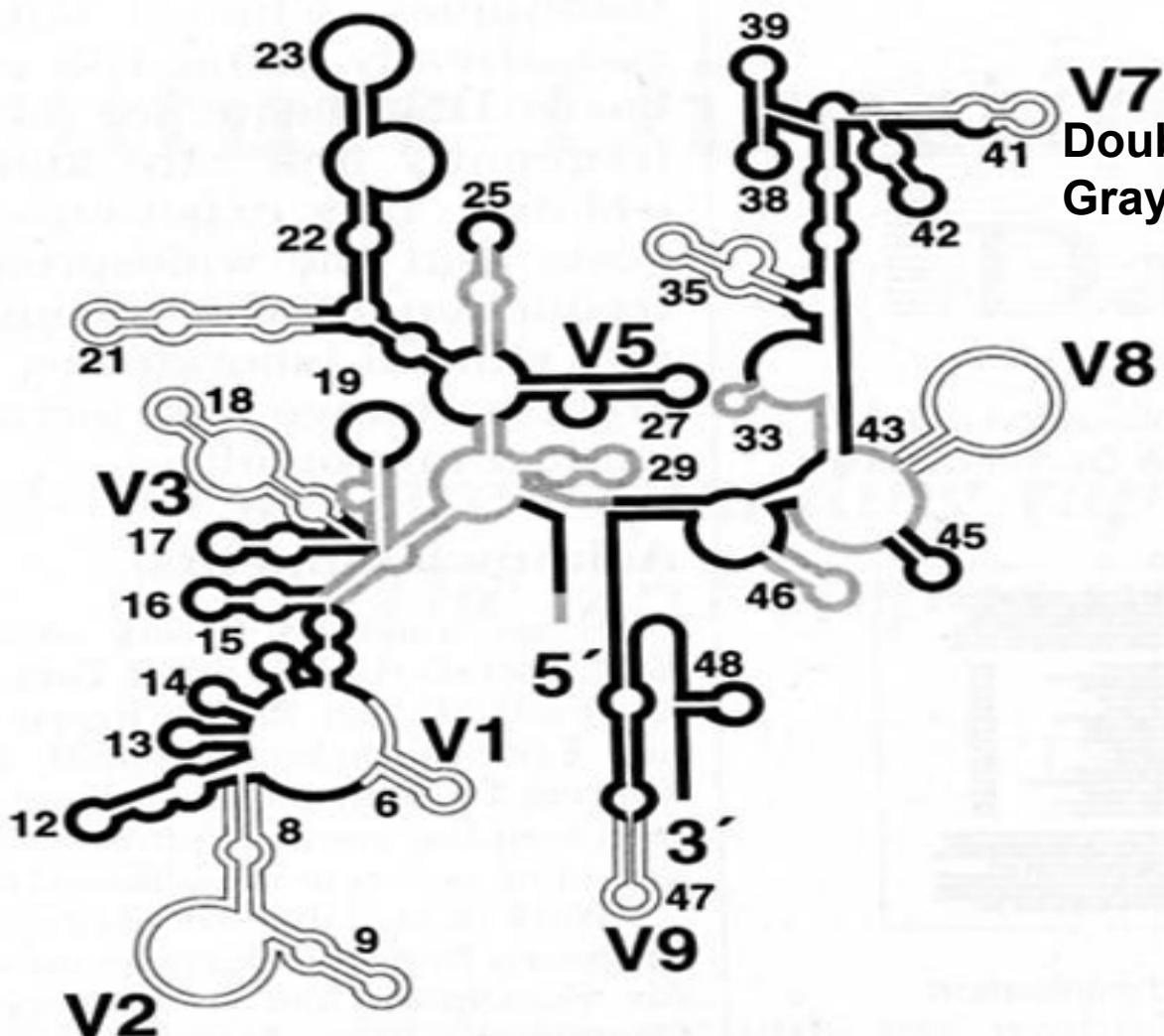
**2. Ribosomal-RNA: Direct the synthesis of proteins. Usually have two parts. The 16s-rRNA contains approximately 1500 based units which has been used for the phylogenetic classification of bacterial species.**

**TABLE 13.3** The structural components of *E. coli* ribosomes

	Ribosome	Small Subunit	Large Subunit
Sedimentation coefficient	70S	30S	50S
Mass (kD)	2520	930	1590
Major RNAs		16S = 1542 nt	23S = 2904 nt
Minor RNAs			5S = 120 nt
RNA mass (kD)	1664	560	1104
RNA proportion	66%	60%	70%
Protein number		21 polypeptides	31 polypeptides
Protein mass (kD)	857	370	487
Protein proportion	34%	40%	30%

From: Garrett and Grisham, *Biochemistry*, 1995. Figure 32.1, page 1041.

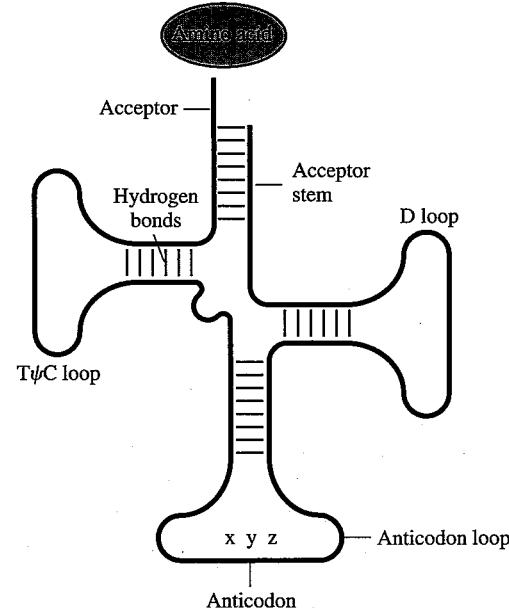
**Figure 2. Secondary-Structure Model of the 16S rRNA**



**V7**  
Double lines= variable  
Gray lines= conserved

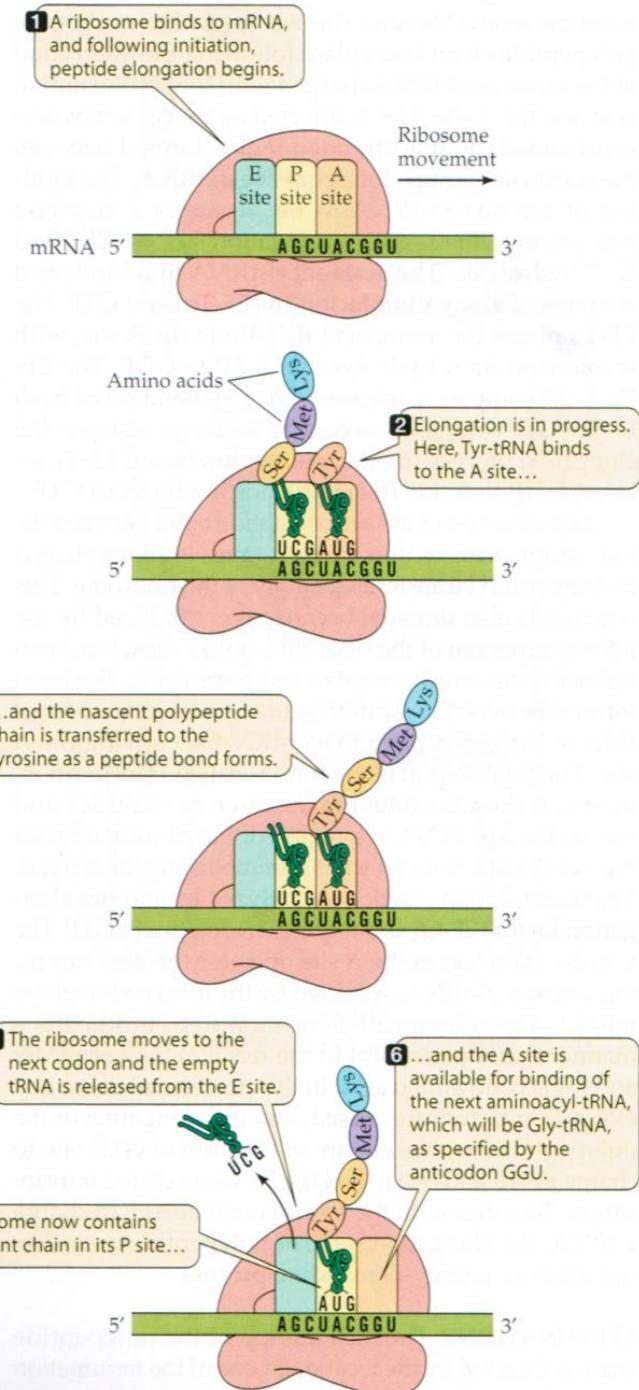
The degree of conservation is indicated as follows: double lines, variable and hypervariable; grey lines, highly conserved. The major variable regions are numbered V1 to V9.

**3. Transfer-RNA: They are shuttles for amino acids. Each amino acid has a special carrier t-RNA. The coded t-RNA will deliver the right amino acid to the proper sequence according to the code on the m-RNA.**



**Figure 1.32**

Cloverleaf representation of the tRNA molecule shows the three loops, the anticodon, the acceptor, and an amino acid attached to the acceptor.



## Development of high-rate anaerobic ammonium-oxidizing (anammox) biofilm reactors

Ikuo Tsushima<sup>a</sup>, Yuji Ogasawara<sup>a</sup>, Tomonori Kindaichi<sup>b</sup>, Hisashi Satoh<sup>a</sup>, Satoshi Okabe<sup>a,\*</sup>

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<sup>b</sup>Department of Social and Environmental Engineering, Graduate School of Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashihiroshima 739-8527, Japan

### 2.7. Cloning and sequencing of 16S rRNA gene and phylogenetic analysis

Using Polymerase Chain Reaction (PCR) Technique to amplify the specific 16S RNA and compare the sequence in gel electrophoresis against existing RNA ladder to identify the genetic position of the species in question.

# **Elucidation of the microbial community structure within a laboratory-scale activated sludge process using molecular techniques**

**P Padayachee, A Ismail\* and F Bux**

*Centre for Water and Wastewater Technology, Department of Biotechnology, Durban University of Technology,  
PO Box 1334, Durban 4000, South Africa*

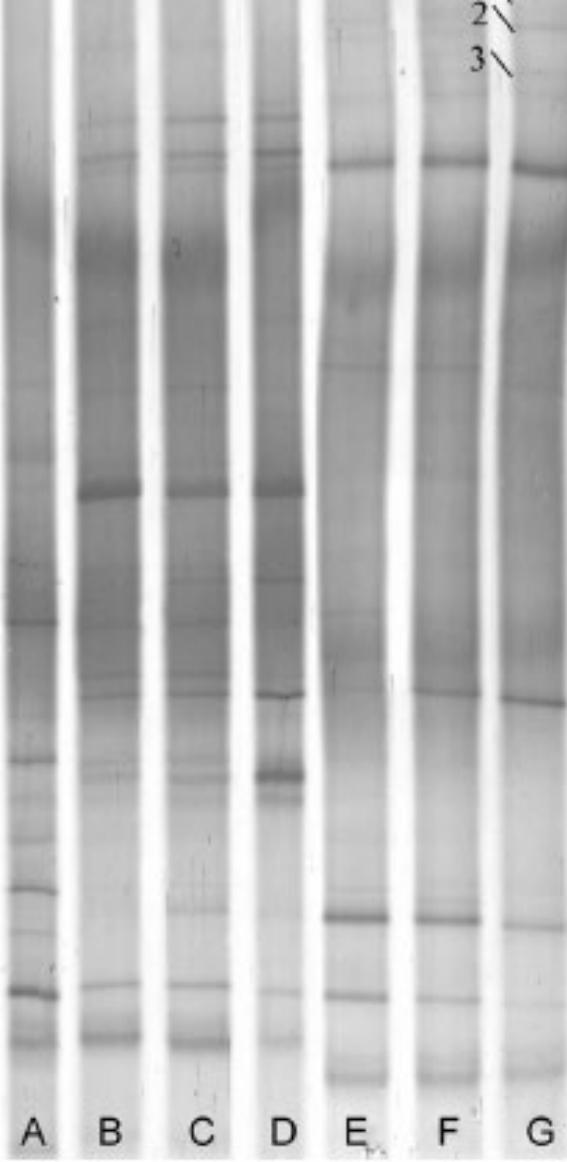


Fig. 4 - DGGE profile (47% denaturing agent on top, 70% denaturing agent on bottom) of 16S rDNA PCR fragments (U968 bacterial primer) of inoculum (A) and sludges after 65, 95 and 150 days of operation (R1: lanes B-D; R2: lanes E-G). DNA fragments resembled to sequences of (1) *Dendrosporobacter querciculus* and bacterial clones (2) B2 and (3) BA149.

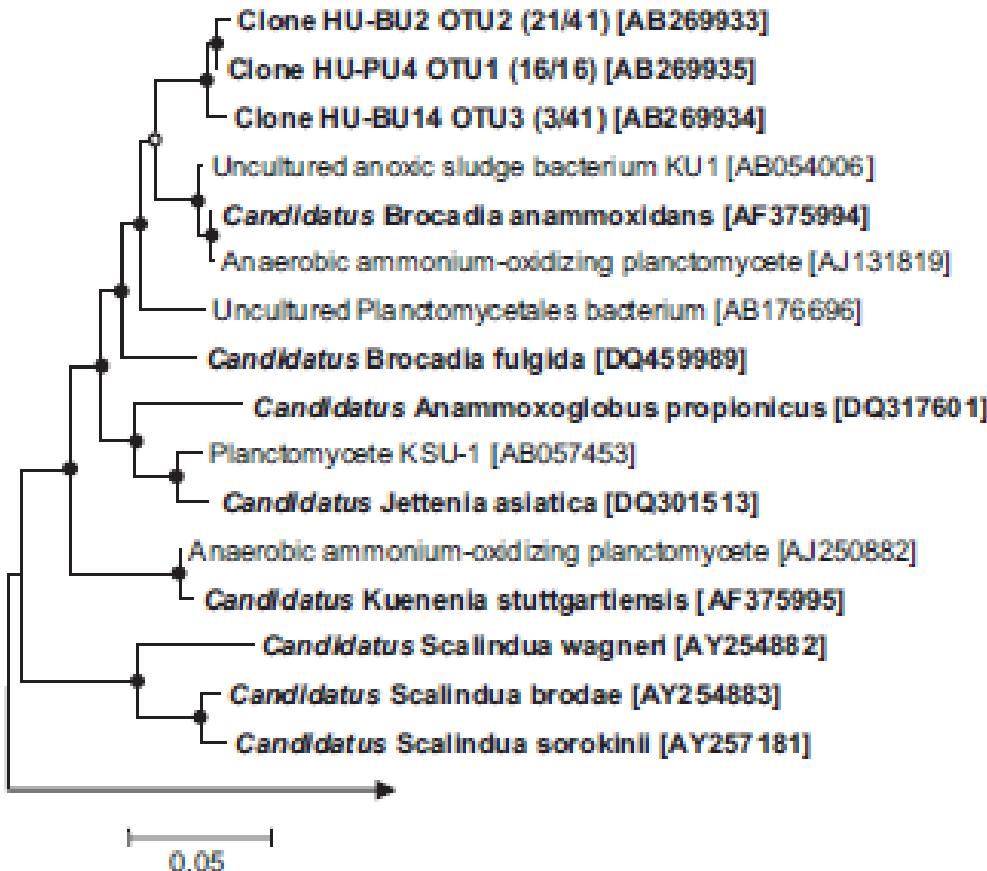
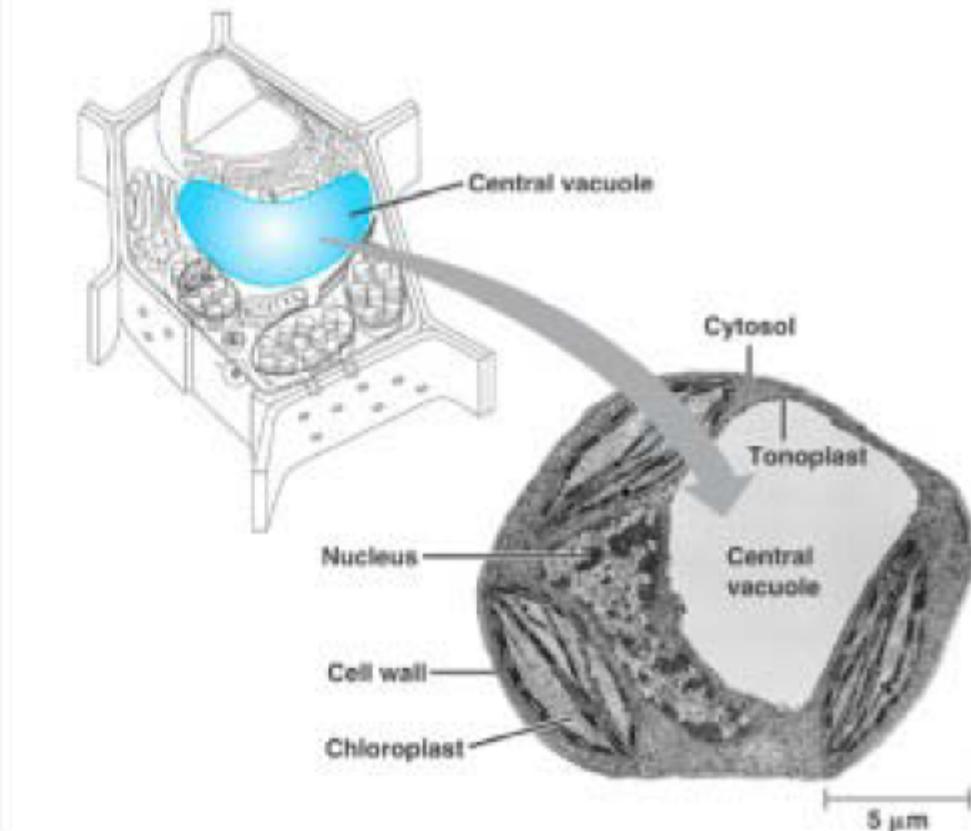


Fig. 6 – Phylogenetic tree of anammox bacteria showing the positions of the clones obtained from the biofilm in reactor I after 392-day operation. The tree was generated by using 1429 bp of the 16S rRNA and neighbor-joining method. The scale bar represents 5% sequence divergence. The filled and empty circles at the nodes represent bootstrap values higher than 95% and 80%, respectively (100 times resampling analysis). The *Aquifex aeolicus* sequence served as the outgroup for rooting the tree. Numbers in parentheses indicate the frequency of appearance of the identical clones in the total clones analyzed with specific primer set.

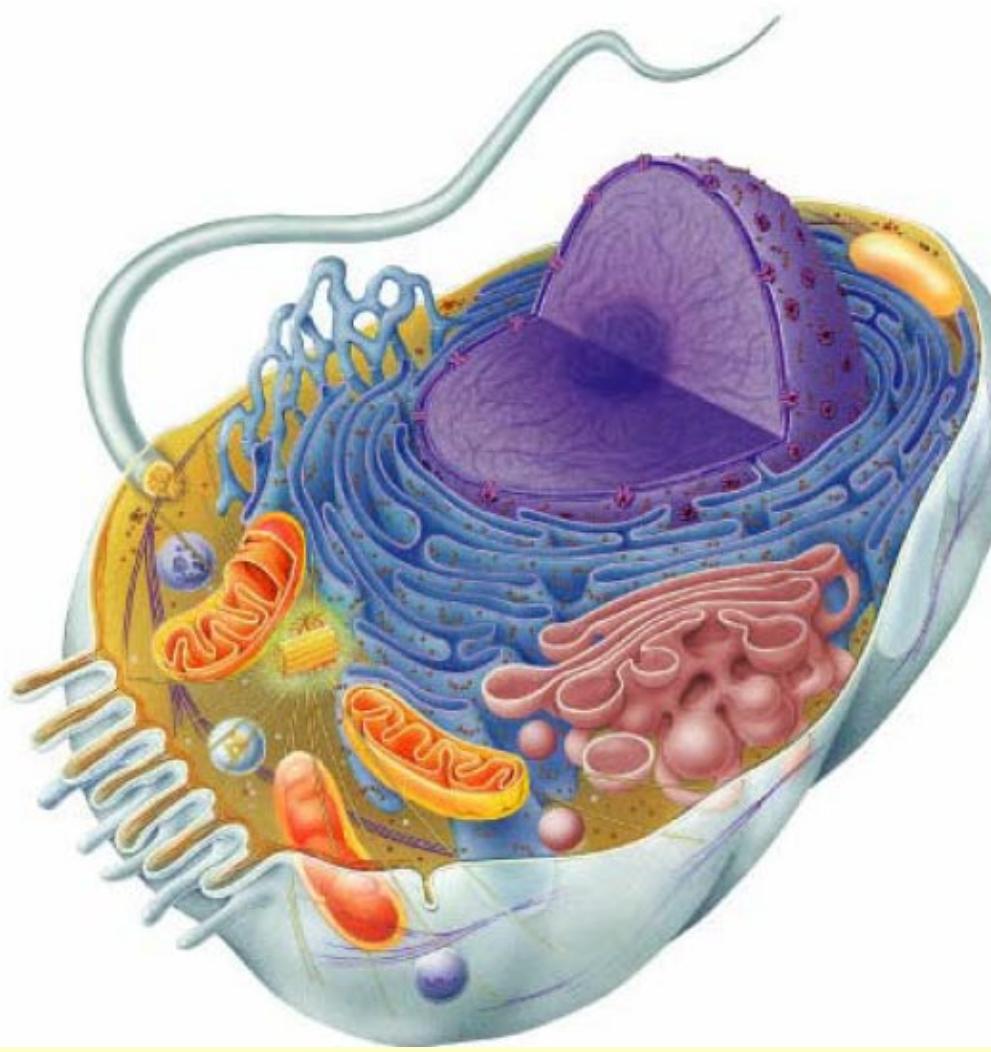
# Organelles: Vacuoles

- Mature plant cells usually have a large central vacuole enclosed by tonoplast
- Storage site for ions, by-products of metabolism, pigments, poisons

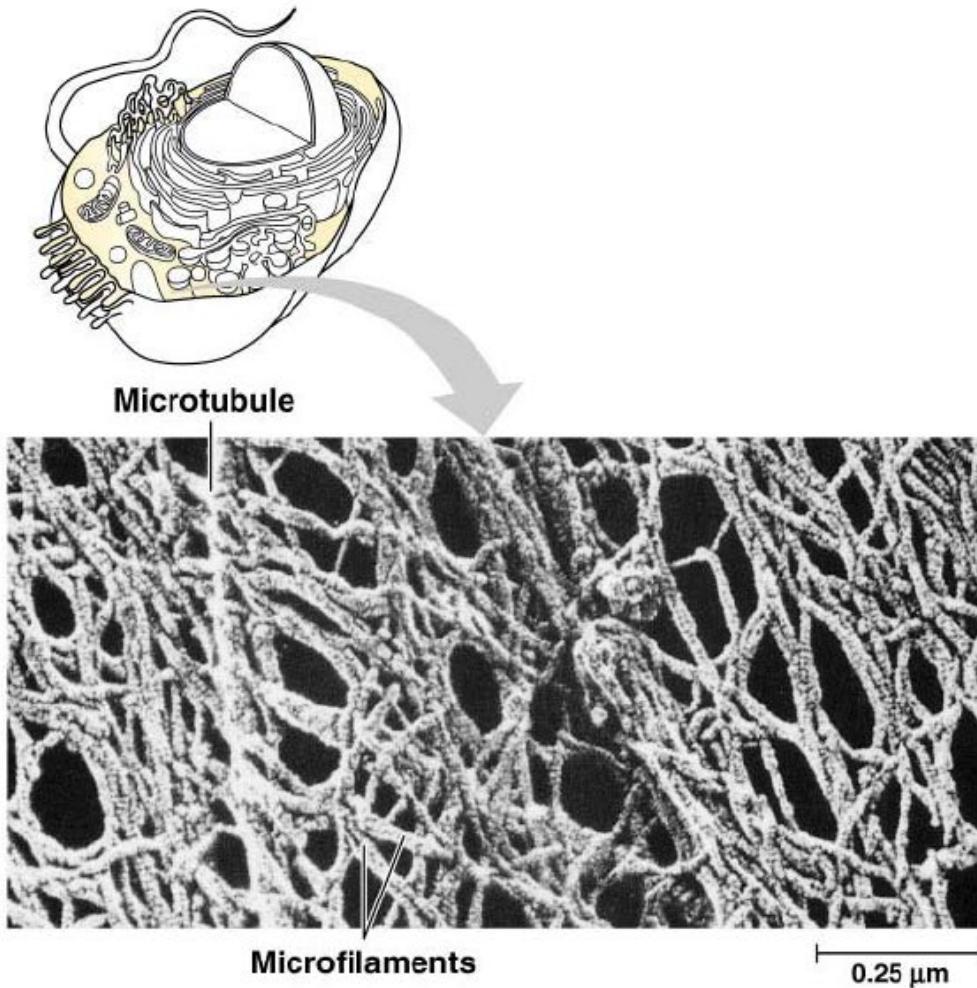


# Endomembrane system

Plasma membrane  
Nuclear envelope  
Endoplasmic reticulum  
Golgi apparatus  
Lysosomes  
Vacuoles

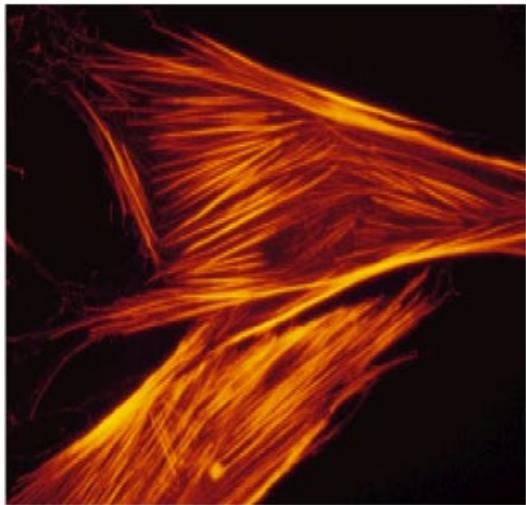


# Inside the cell: the cytoskeleton

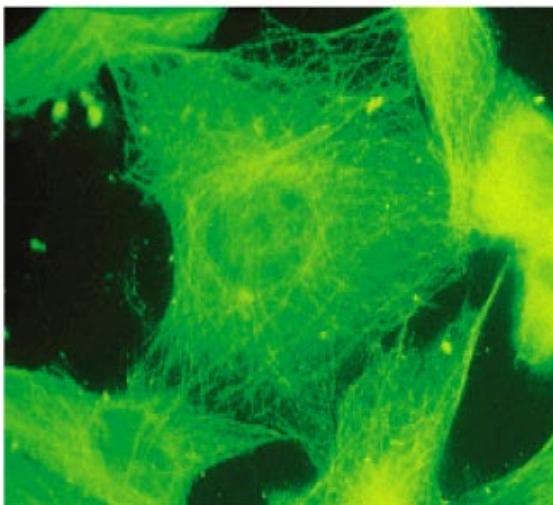


- Provides support for the organelles
- Enables the cell to move
- Made up of microtubules, microfilaments, and intermediate filaments

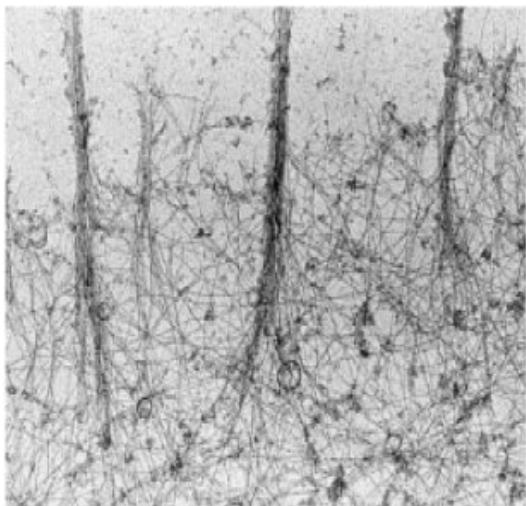
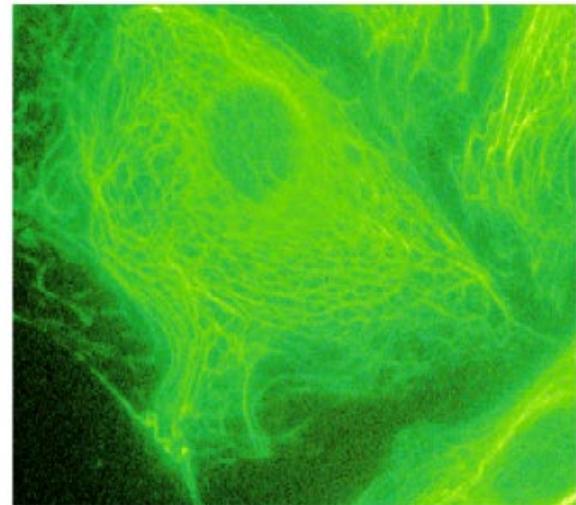
Cytoskeleton: actin  
stress fibers



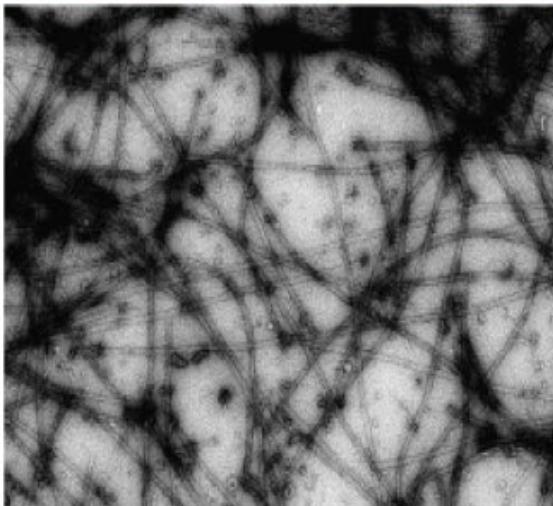
Cytoskeleton:  
microtubules



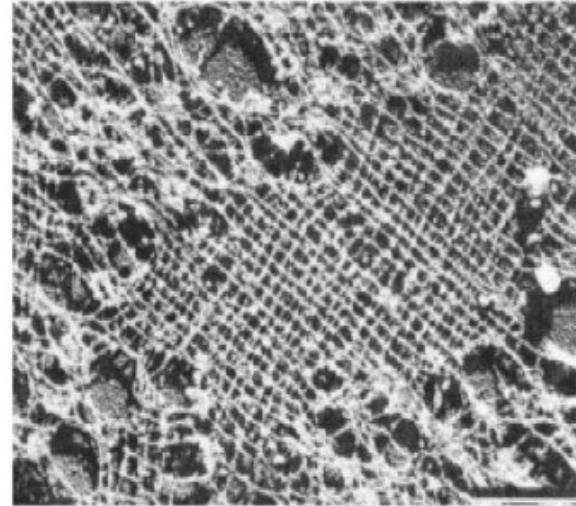
Cytoskeleton:  
intermediate filaments



Actin stress fibers  
(a)



Microtubules  
(b)



Intermediate filaments  
(c)

## Composition of normal bacteria cell

**Cellular composition of bacteria which provides a recipe for providing a balanced microbial growth media (feed to the biological wastewater treatment systems)**

**Some bacteria will require Vitamin B for growth which they can't synthesis themselves. But this compounds can be supplied by other organisms in the environment.**

**Empirical cell formula:**

**$C_5H_7O_2N$  or**

**$C_{60}H_{87}O_{23}N_{12}P$**

**Other trace metals: Zn, Mn, Mo, Se, Co, Cu, Ni may be required**

**Table 2.1** Typical composition of bacteria (adapted from Metcalf & Eddy 2003)

Constituent or element	%TSS	Empirical formula for cells $C_5H_7O_2N$
Major cellular constituents		
Protein	55.0	
Polysaccharides	5.0	
Lipid	9.1	
DNA	3.1	
RNA	20.5	
Other (sugars, amino acids)	6.3	
Inorganic ions	1.0	
As cell elements		%VSS
Organic (VSS)	93.0	
Carbon	50.0	53.1
Oxygen	22.0	28.3
Nitrogen	12.0	12.4
Hydrogen	9.0	6.2
Inorganics (FSS)	7.0	
Phosphorus	2.0	
Sulfur	1.0	
Potassium	1.0	
Sodium	1.0	
Calcium	0.5	
Magnesium	0.5	
Chlorine	0.5	
Iron	0.2	
Other trace elements	0.3	

**Analysis of the Chemical Composition  
of an *Escherichia coli* Cell**

	% Total Weight	% Dry Weight
<b>Organic Compounds</b>		
Proteins	15	50
Nucleic acids		
RNA	6	20
DNA	1	3
Carbohydrates	3	10
Lipids	2	Not determined
Miscellaneous	2	Not determined
<b>Inorganic Compounds</b>		
Water	70	
All others	1	3
<b>Elements</b>		
Carbon (C)		50
Oxygen (O)		20
Nitrogen (N)		14
Hydrogen (H)		8
Phosphorus (P)		3
Sulfur (S)		1
Potassium (K)		1
Sodium (Na)		1
Calcium (Ca)		0.5
Magnesium (Mg)		0.5
Chloride (Cl)		0.5
Iron (Fe)		0.2
Manganese (Mn), zinc (Zn), molybdenum (Mo), copper (Cu), cobalt (Co), zinc (Zn)		0.3

Mineral Elements {

Trace Elements {

} 78%