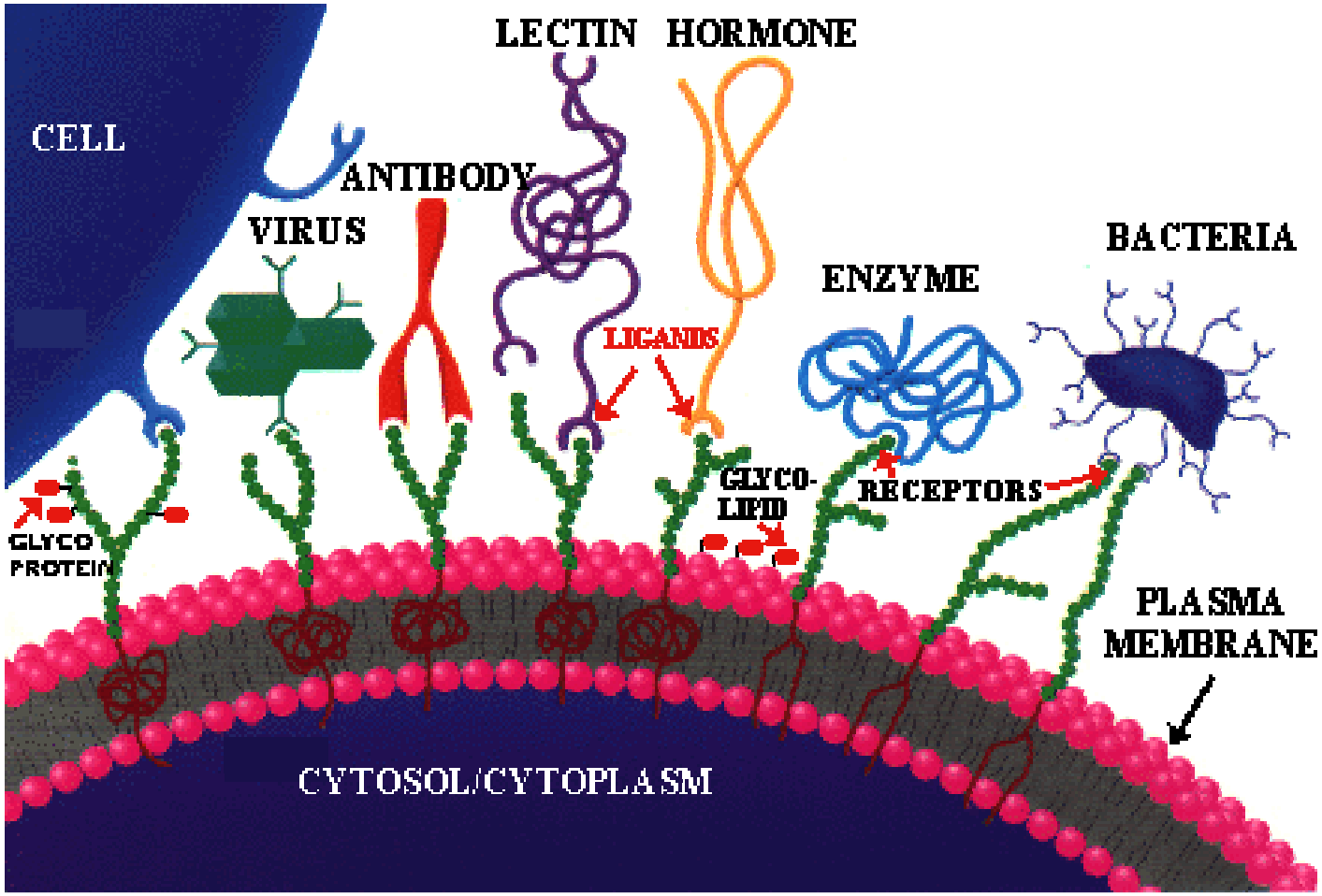
Overview of Microbiology and the major indicator organisms

SCI5508


LECTIN
HORMONE
CELL
ANTIBODY
VIRUS
BACTERIA
ENZYME
LIGANDS
GLYCO-
LIPID
RECEPTORS
GLYCO
PROTEIN
PLASMA
MEMBRANE
CYTOSOL/CYTOPLASM

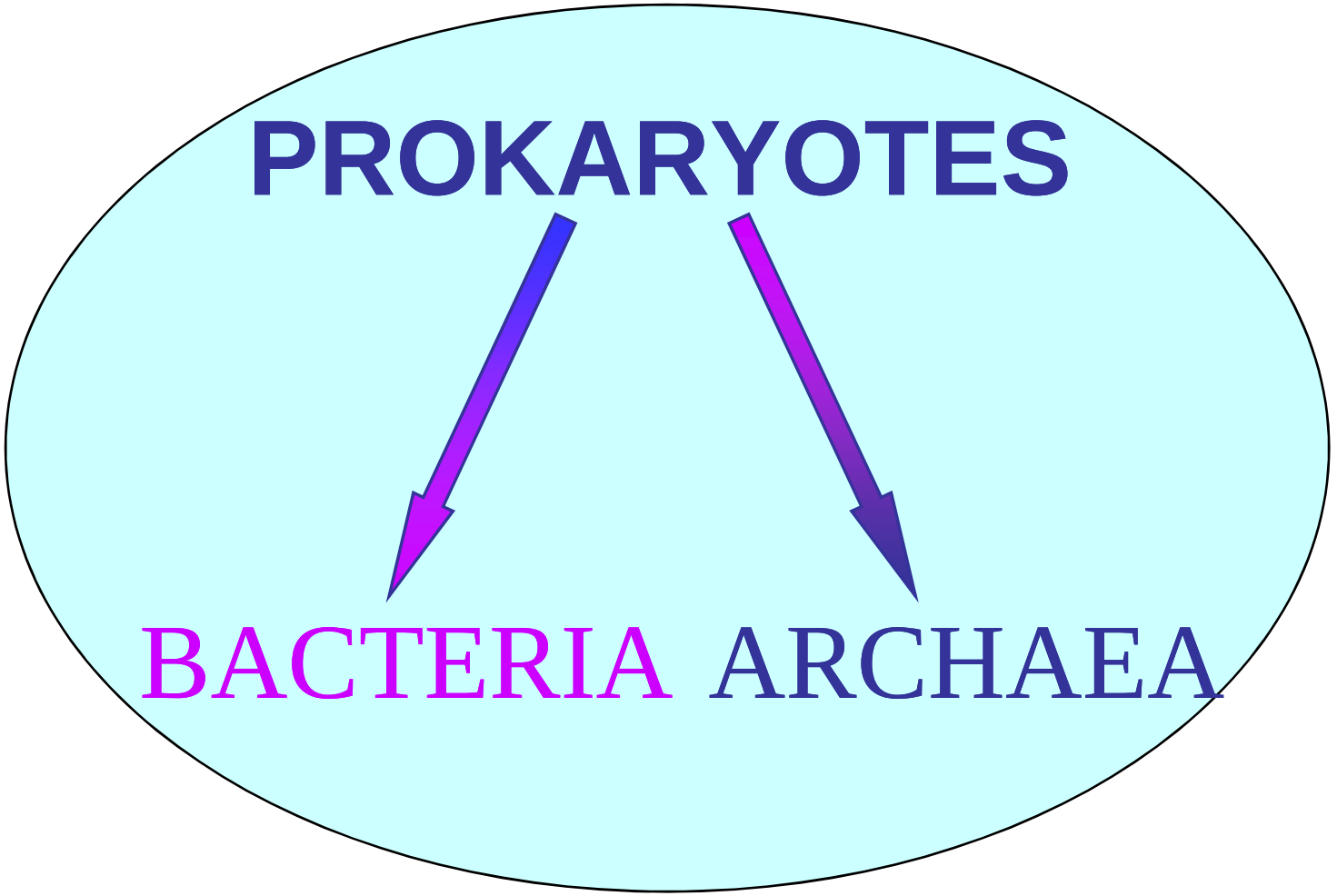
Organization

- **The cell is a unit of organization**
- Cells are classified:
  - By the way they obtain energy.
  - Into Kingdoms:
  - As prokaryotes [Monera (Eubacteria) and Archaea]
  - As eukaryotes [Protista, Plantae, Fungae, Animalia.]

EUKARYOTIC vs. PROKARYOTIC CELLS

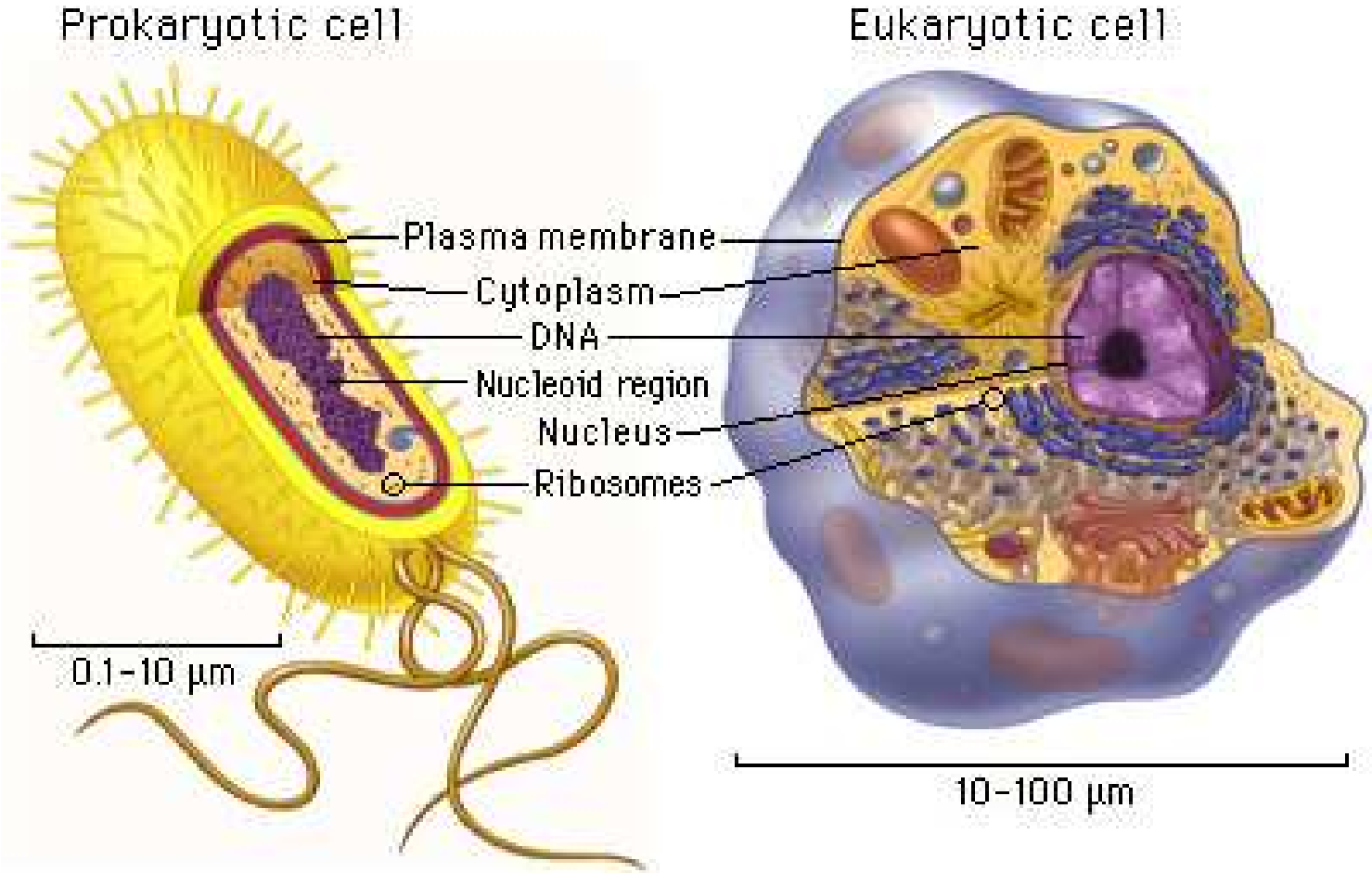
- All cellular life has the following characteristics in common.
- **CELL MEMBRANE**
- **CONTAINS DNA → RNA, PROTEINS**,
- same **BASIC CHEMICALS**:.
- All cells **REGULATE** the flow
- All cells **REPRODUCE**
- All cells require a **SUPPLY OF ENERGY**.
- All cells are **HIGHLY REGULATED** by **ELABORATE SENSING SYSTEMS**
- information is continually **PROCESSED** to make metabolic decisions.

EUKARYOTES



Concept 1: Common Features of All Cells

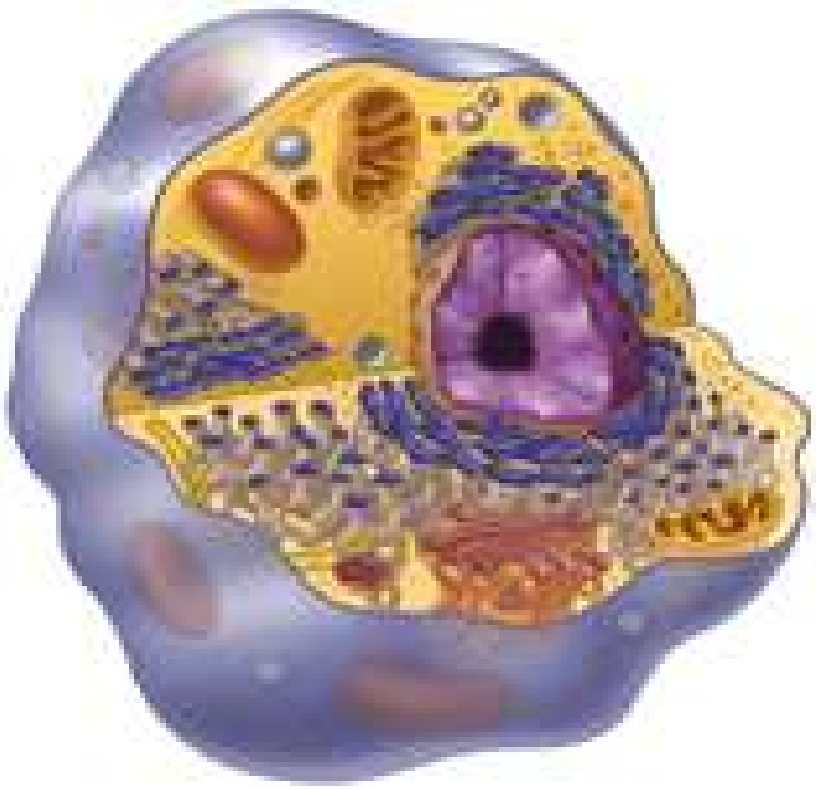
All cells, whether they are prokaryotic or eukaryotic, have some common features.



Concept 2: Features of Prokaryotic Cells

Prokaryotes, which include all bacteria and archaea (archaebacteria), are the simplest cellular organisms.

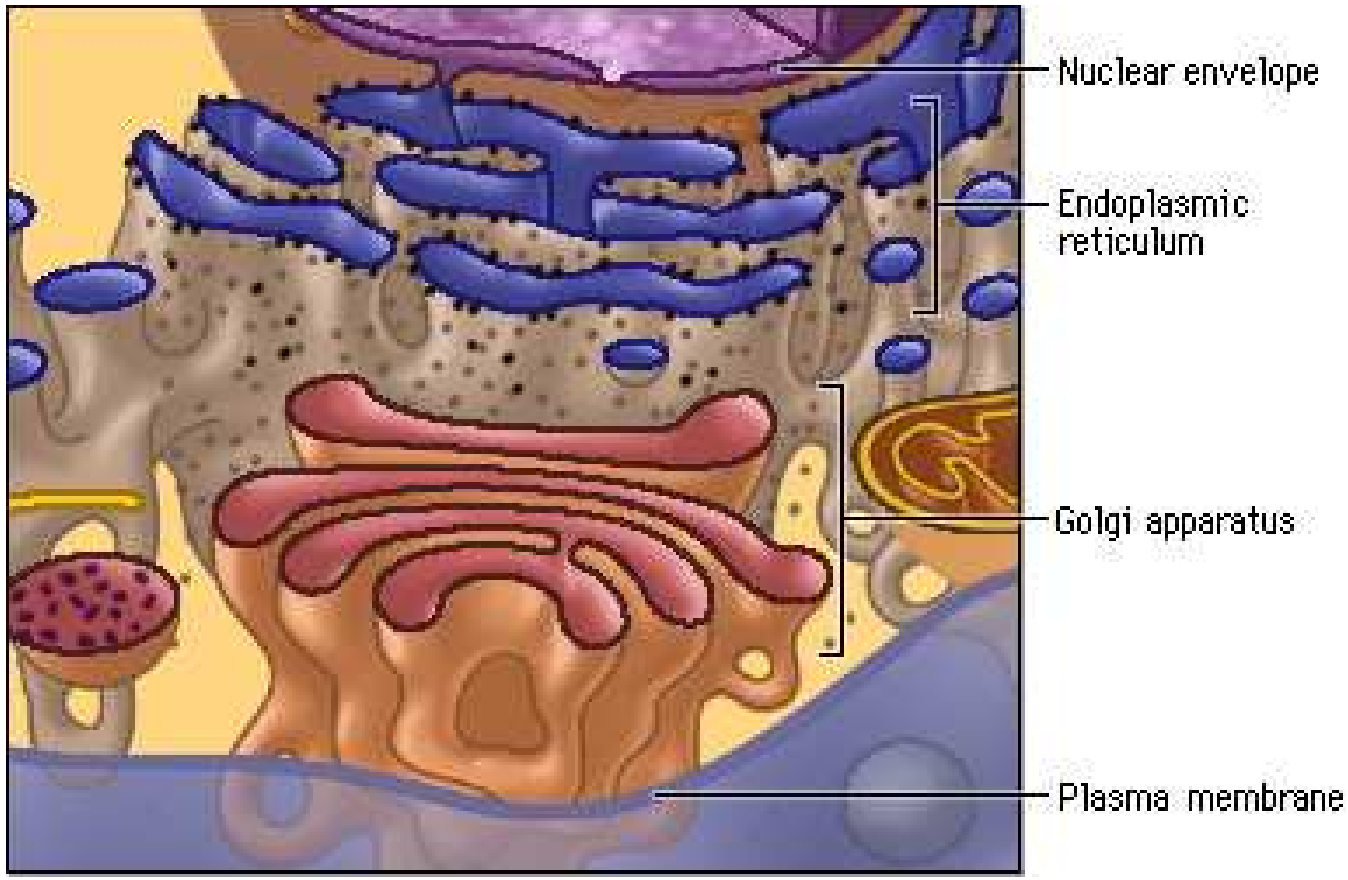
Concept 3: Features of Eukaryotic Cells



Eukaryotic cells contain a membrane-bound nucleus and numerous membrane-enclosed organelles
(e.g., mitochondria, lysosomes, Golgi apparatus) not found in prokaryotes.

Concept 4: The Endomembrane System in Eukaryotic Cell

All eukaryotic cells have an endomembrane system consisting of the nuclear envelope, ER and Golgi apparatus, vesicles and other organelles derived from them, and the plasma membrane.



THE TWO GROUPS OF PROKARYOTES

- the **EUBACTERIA** and the **ARCHAEA** **or ARCHAEBACTERIA**.
- The Archaea
  - found in **environmental extremes**,
  - no reports of pathogenic/disease forming activity

Prokaryotes (Bacteria)

- ***Eubacter*** **"True" bacteria**
  - **human pathogens**
  - **clinical or environmental**
  - ***Archaea***
  - **Environmental organisms**

Eukaryotes

- **Other cell-based life e.g.**
  - **Protista**
  - **plantae**
  - **animalia**
  - **fungae**

Prokaryotic Cell versus Eukaryotic Cell

- **Not compartmentalized**
- **Cell membranes lack sterols (e.g. cholesterol)**
- **Single circular chromosome**
- **Ribosomal are 70S**
  - **subunits**
    - **30S (16S rRNA)**
    - **50S (5S & 23S rRNA)**

Bacteria versus Archaebacteria

- **Eubacteria**
  - **peptidoglycan (murein)**
  - **muramic acid**

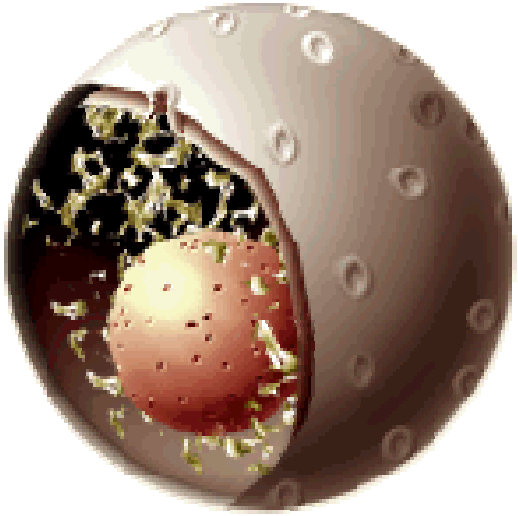
- **Archaebacteria**
  - **pseudomurein**
  - **no muramic acid**

Eukaryotes

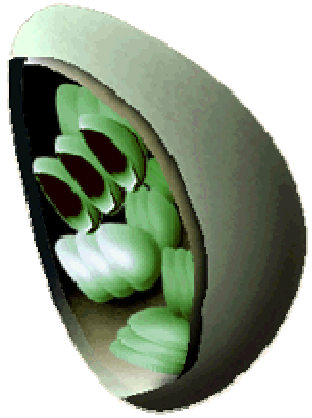
1. plasma membrane
2. glycocalyx
3. cytoplasm
4. cytoskeleton

Plasma Membrane

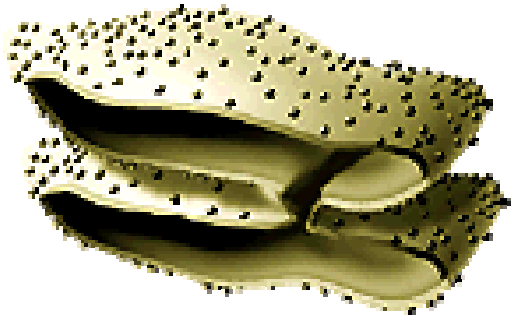
Mitochondria



Nucleus

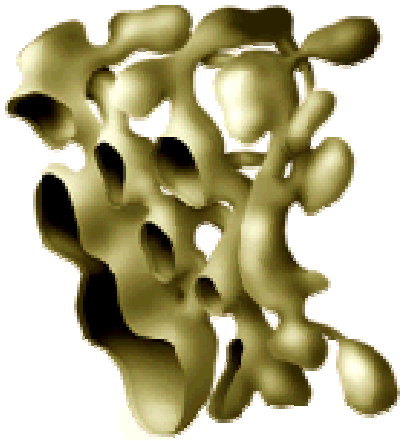


Chloroplasts (plastids)

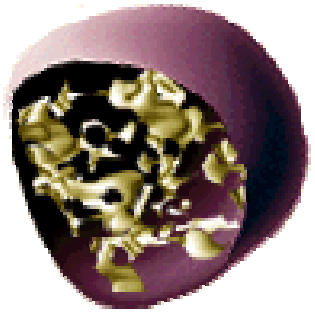


Rough endoplasmic reticulum (RER)

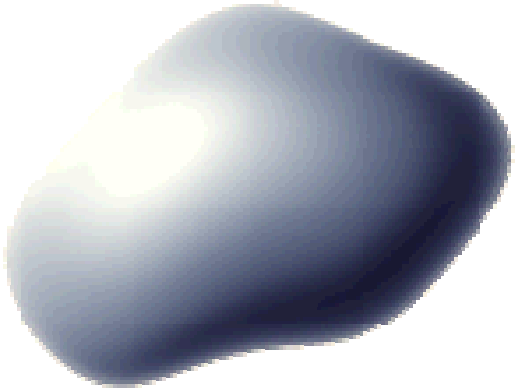
Golgi apparatus



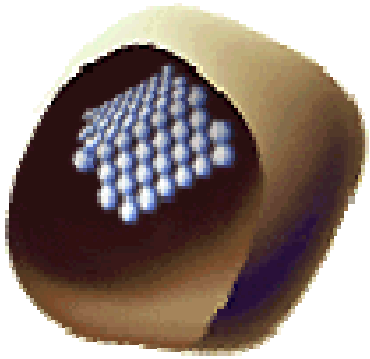
Smooth endoplasmic reticulum (SER)



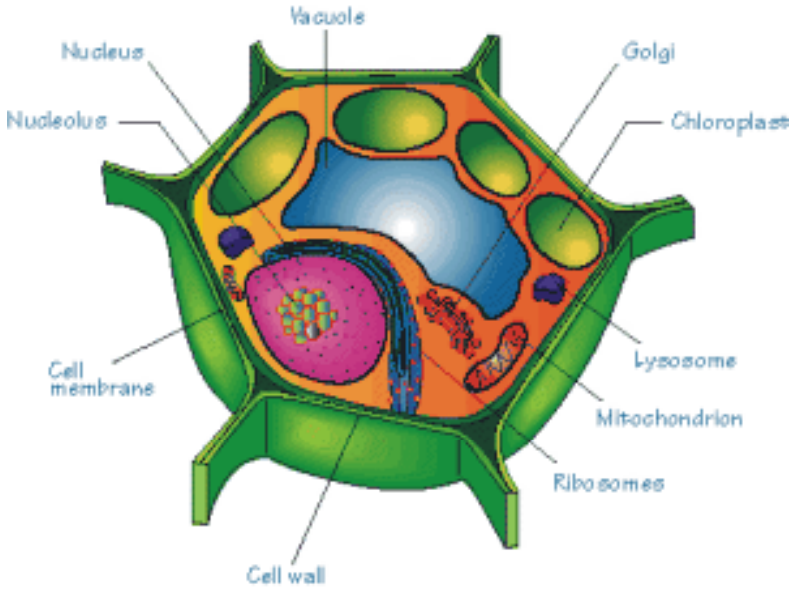
Lysosymes



Vacuoles



Peroxisomes or Microbodies



Plant Cell with a cell wall

Bacteria

- **Plasmids**
  - **Extra-chromosomal DNA**
  - **multiple copy number**
  - **coding pathogenesis and antibiotic resistance factors**
  - **bacterial replication**

The Bacteria

Cocci

Rods

Ovoids

Spira

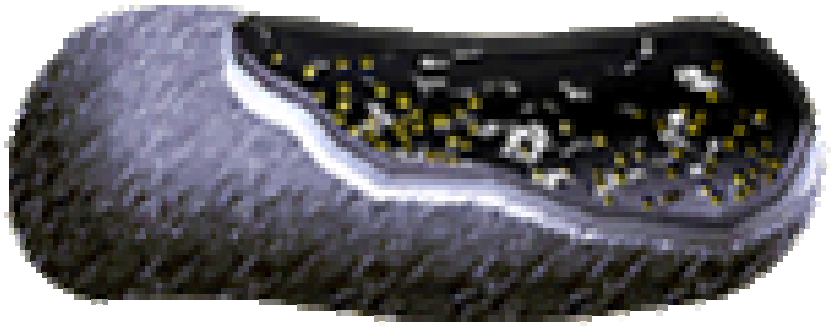
Curved Rods

Curved Rods

Spirochaetes

Filamentous

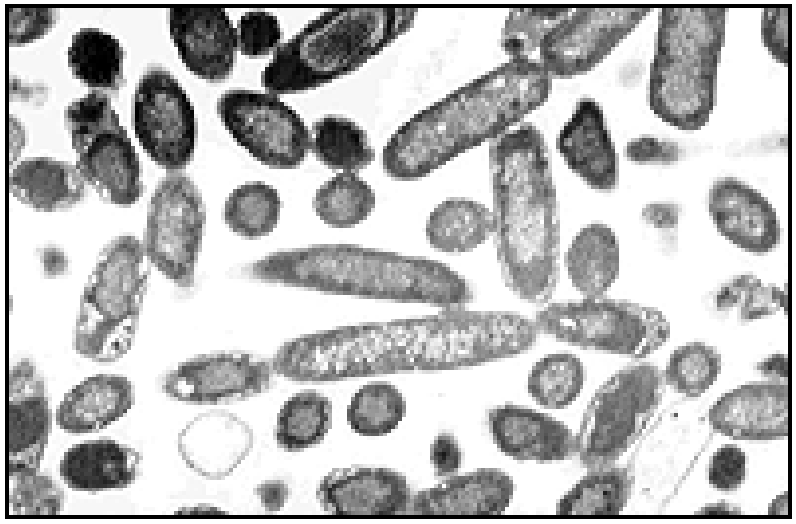
Prokaryotes



- Monera (simple bacteria) and Archaea
- Lack subcellular membrane enclosed ''organelles

- Pigments
- Flagella
- Pili

Bacteria & antibiotics



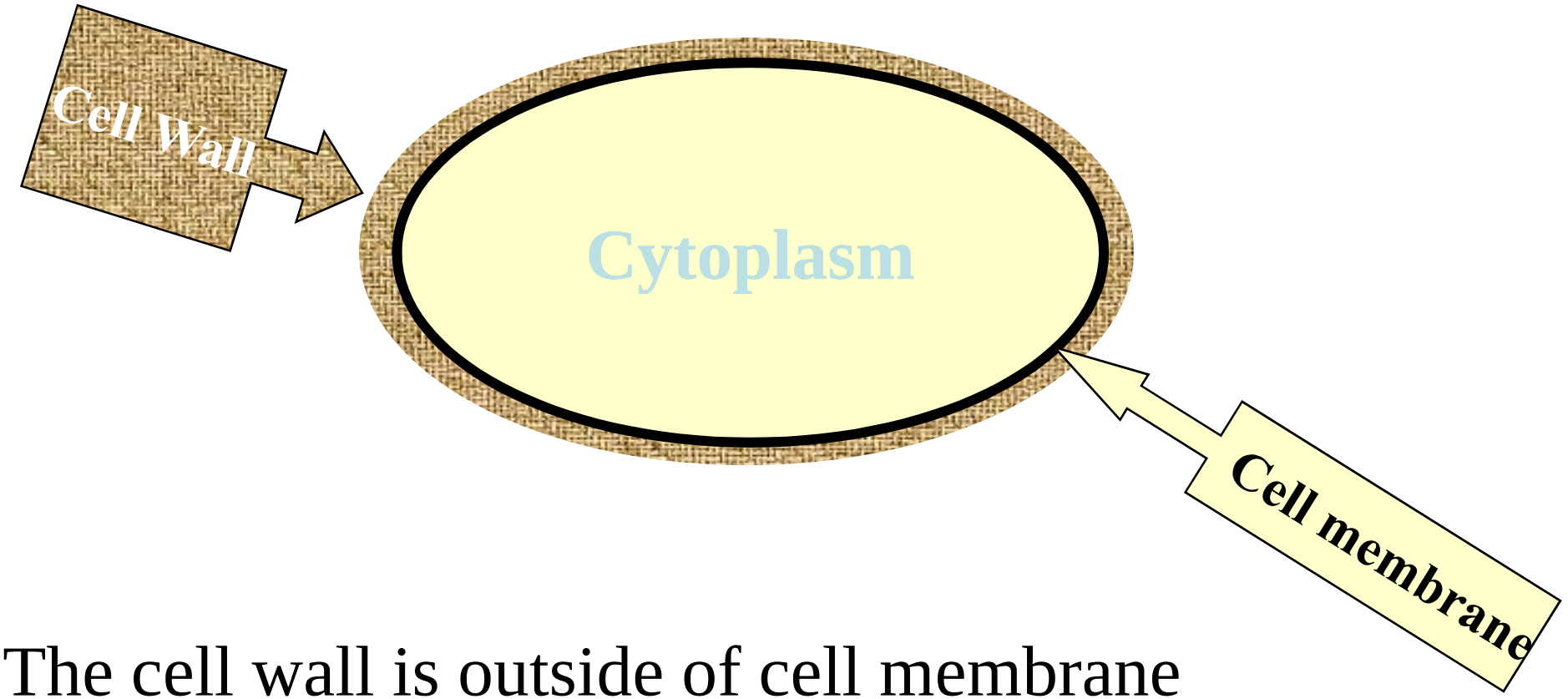
Bacteria: Facts and figures

Found everywhere
Ground, air, ice

- Wide range of size ➔ Normally 0.1-0.2 µm
- *Epulopiscium approx 200* µm

Cell Envelope

- Cell membrane + cell wall (+ plus outer membrane)
- Cell wall
  - peptidoglycan
  - attached structures.

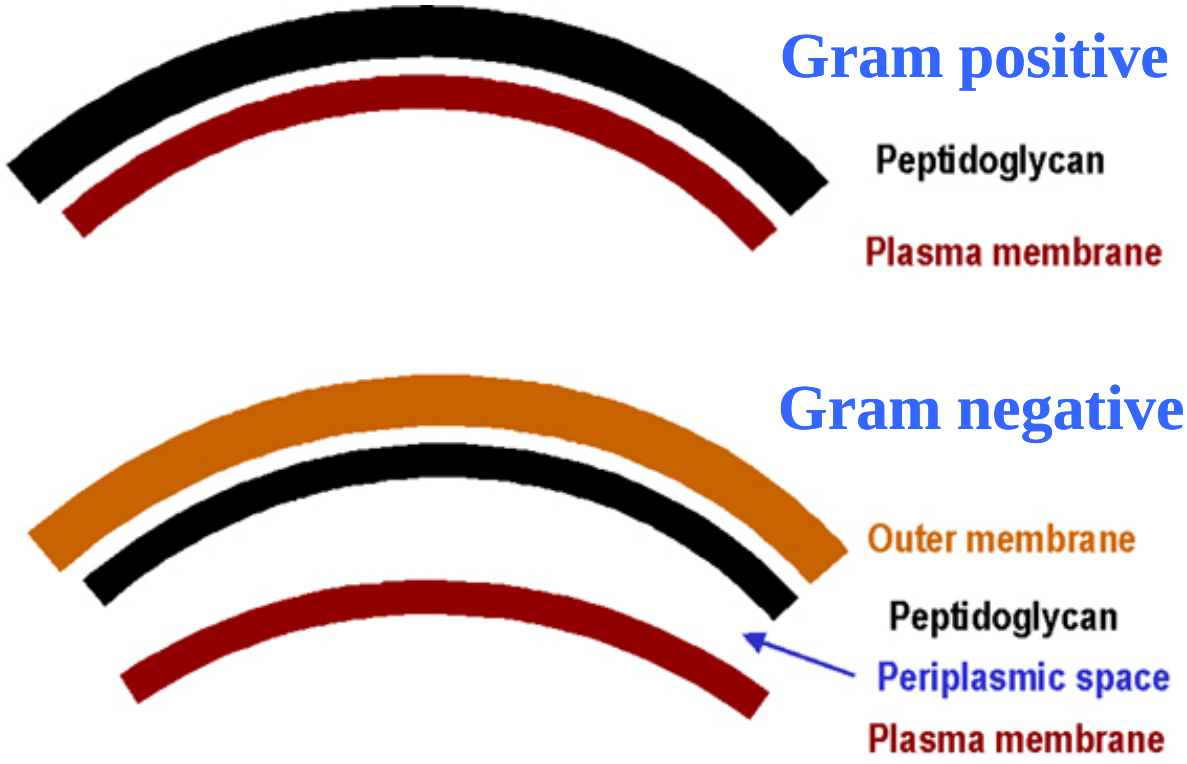


The cell wall is outside of cell membrane

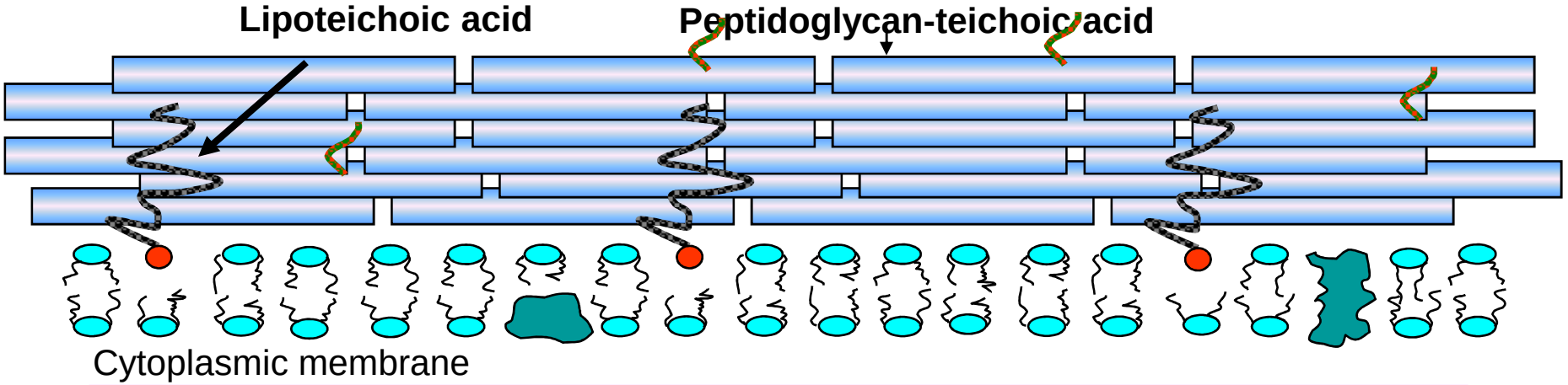
– rigid, protecting cell from osmotic lysis.

The Cell Envelope

Gram Positive **Gram Negative**

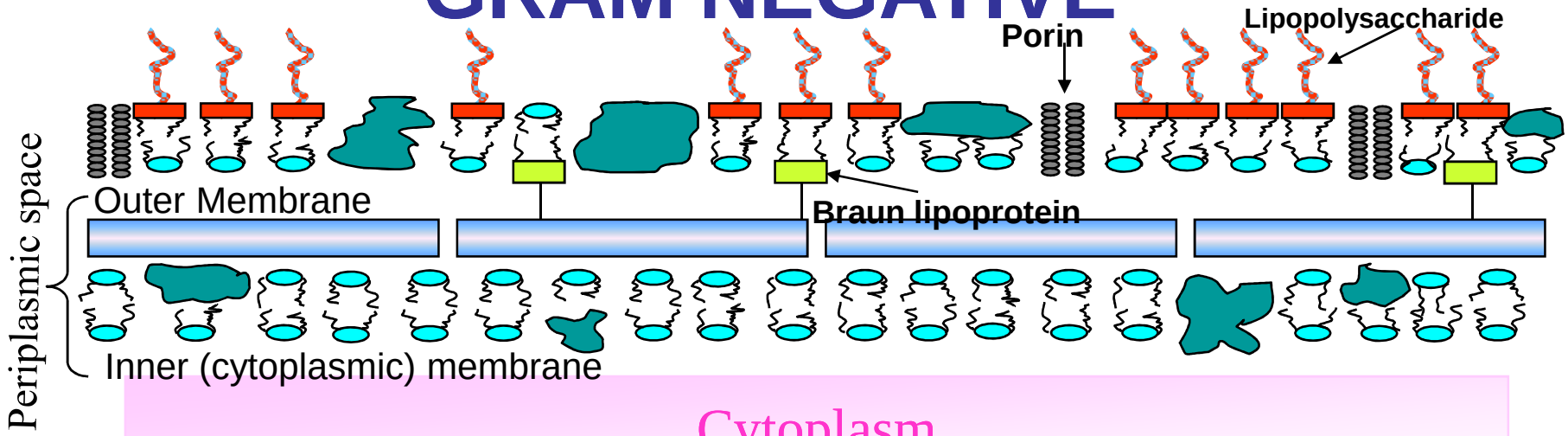

Gram positive
Peptidoglycan
Plasma membrane
Gram negative
Outer membrane
Peptidoglycan
Periplasmic space
Plasma membrane

GRAM POSITIVE



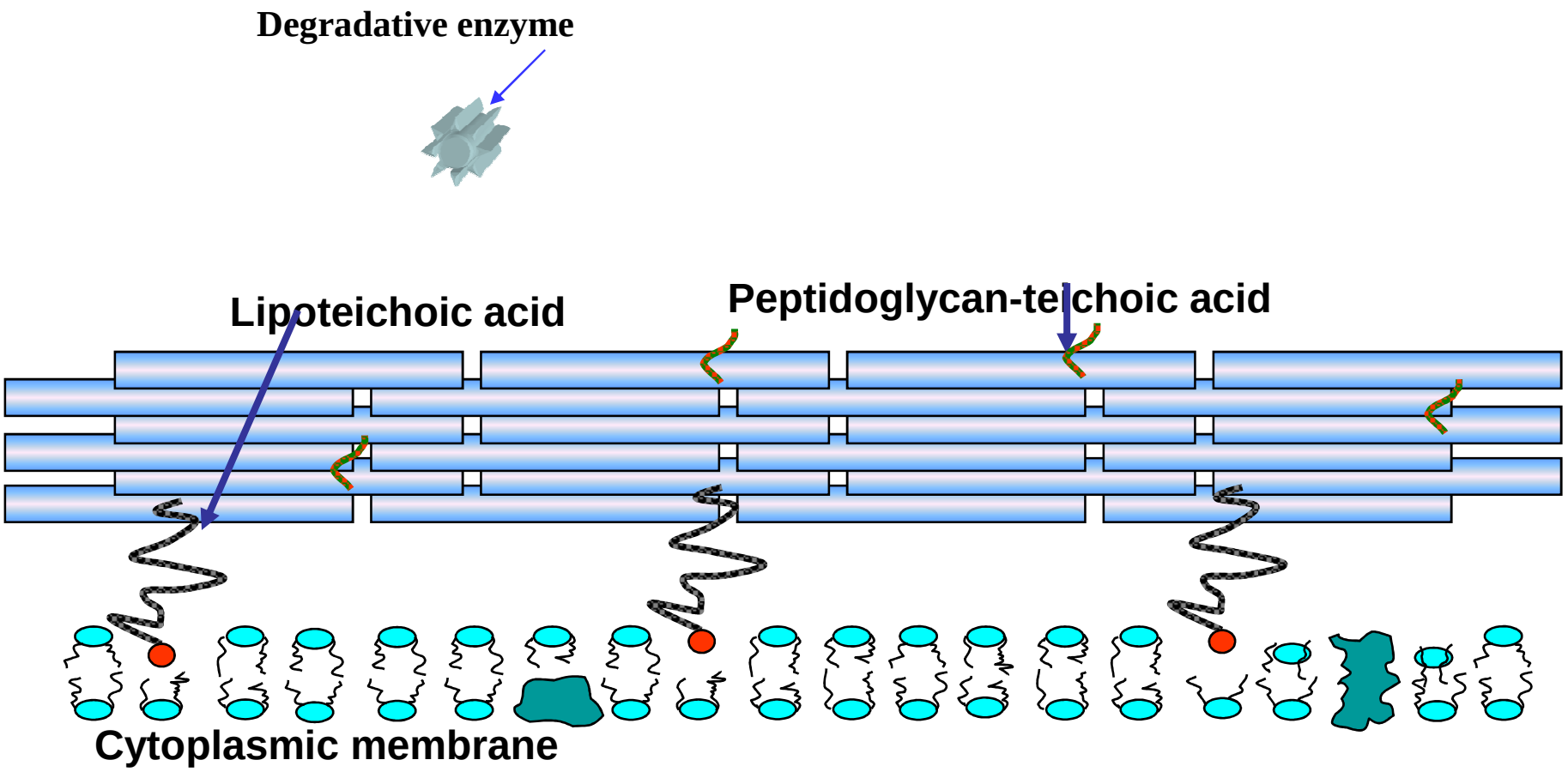
Cytoplasm

GRAM NEGATIVE

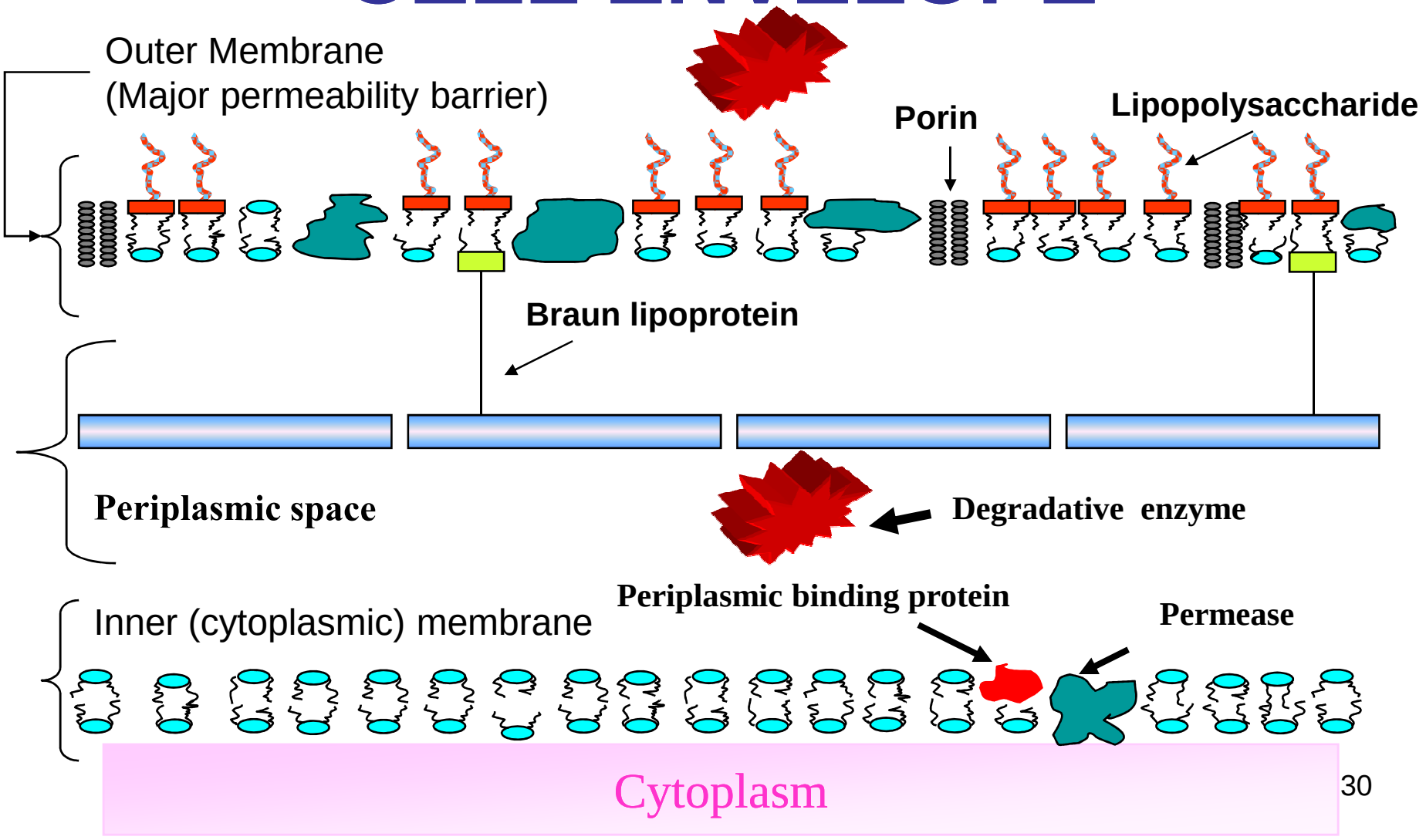


Cytoplasm

GRAM POSITIVE CELL ENVELOPE



GRAM NEGATIVE CELL ENVELOPE



Outer Membrane

Gram negative bacteria

- **major permeability barrier**
- **space between inner and outer membrane**
  - **periplasmic space**
    - **store degradative enzymes**
- **Gram positive bacteria**
- **no periplasmic space**

Bacterial cell envelopes

Gram stain (+ or -) correlates with structure

Peptidoglycan

- **single macromolecule**
- **highly cross-linked**
- **surrounds cell**
- **provides rigidity**

Peptidoglycan

- **glycan backbone**
  - **muramic acid**
  - **glucosamine**

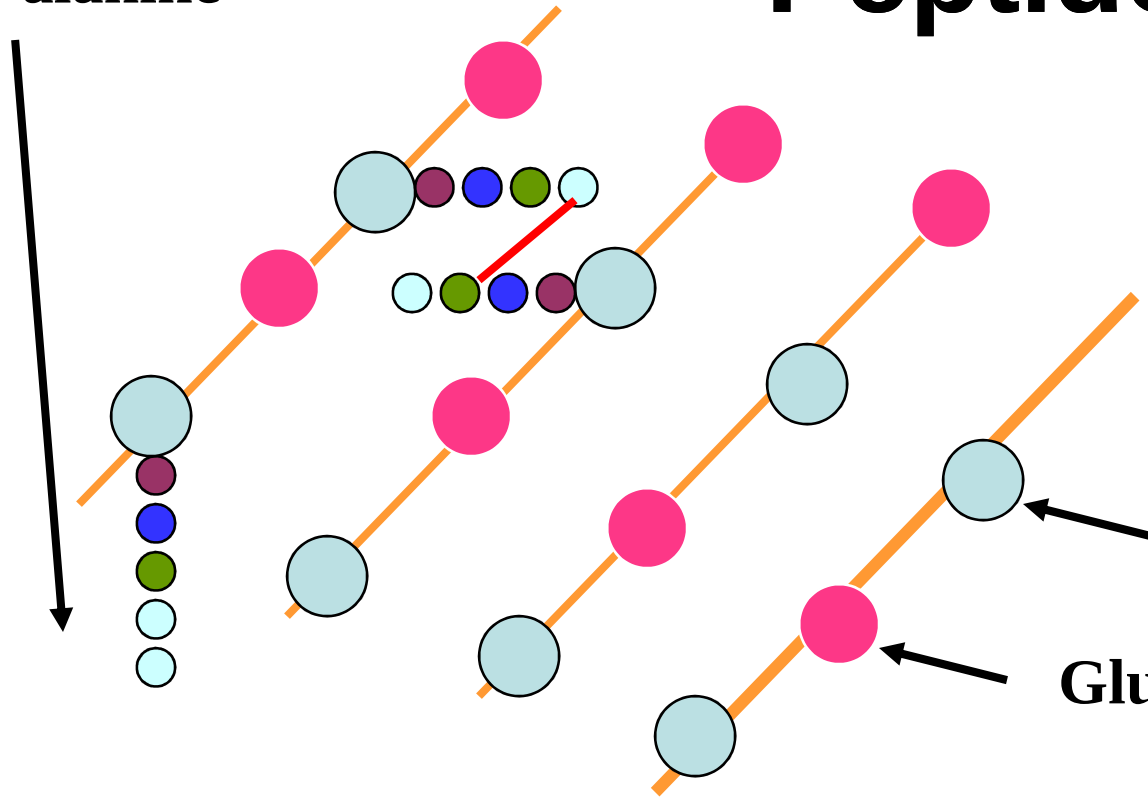
- **peptide side chain**
- **peptide cross-bridge**
  - **D- and L- amino acids**
  - **diaminopimelic acid**

Muramic acid, D-amino acids diaminopimelic acid

– <u>not</u> synthesized by mammals

L-alanine
D-glutamic acid
L-lysine/Diaminopimelic acid
D-alanine
D-alanine

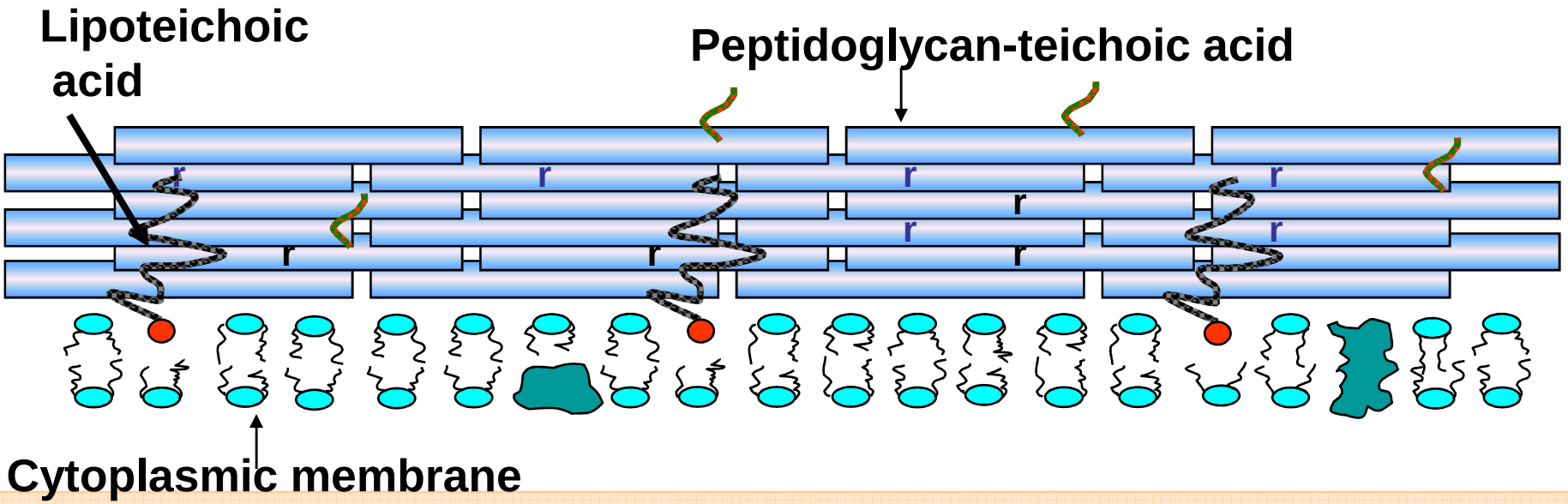
Peptidoglycan



Muramic acid

Glucosamine

Gram Positive Cell Envelope



Cytoplasm

Gram Positive Cell Envelope

- **Teichoic acid**
  - **polymer**
  - **phosphorus**
  - **ribitol or glycerol backbone**
  -
- **Teichuronic acid**
  - **polymer**
  - **no phosphorus**
  - **glucuronic acid backbone**

Teichoic and teichuronic acids

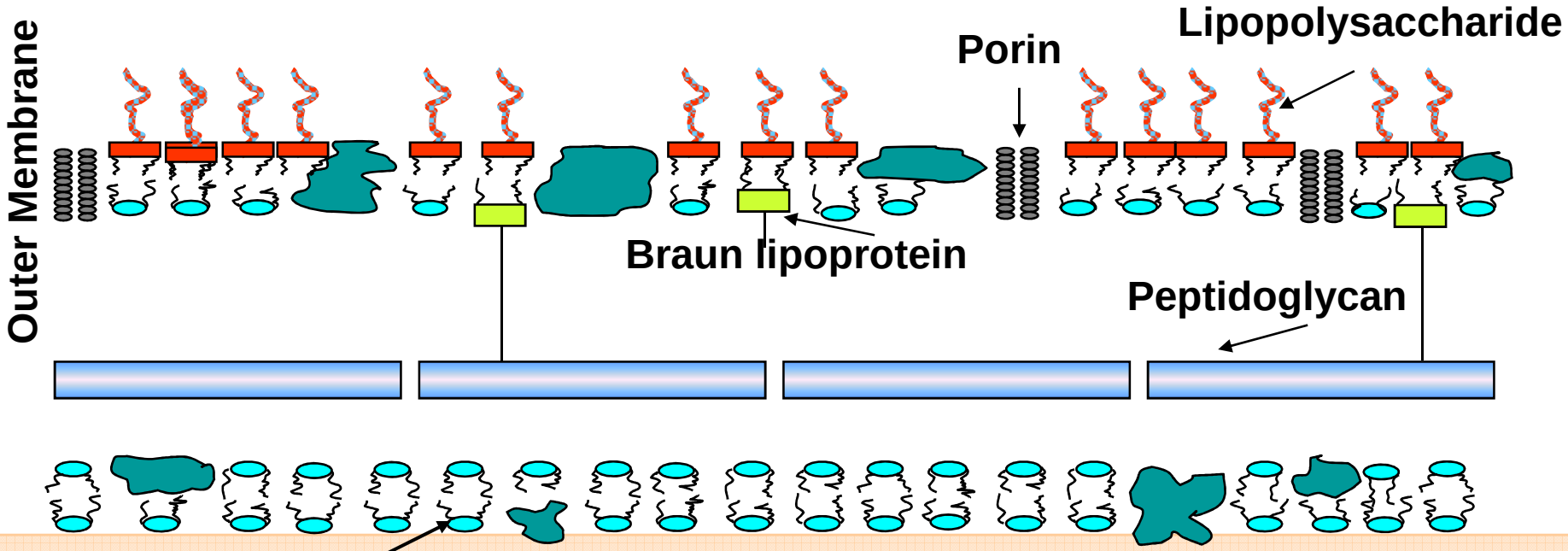
- **Metal ion uptake**

- **Direct autolytic enzymes**
  - **holes punched in cell wall**
  - **allows insertion cell wall (synthesis)**

Lipoteichoic acids

- **cell membrane**
- **autolysins kept from cell wall**

Gram Negative Cell Envelope



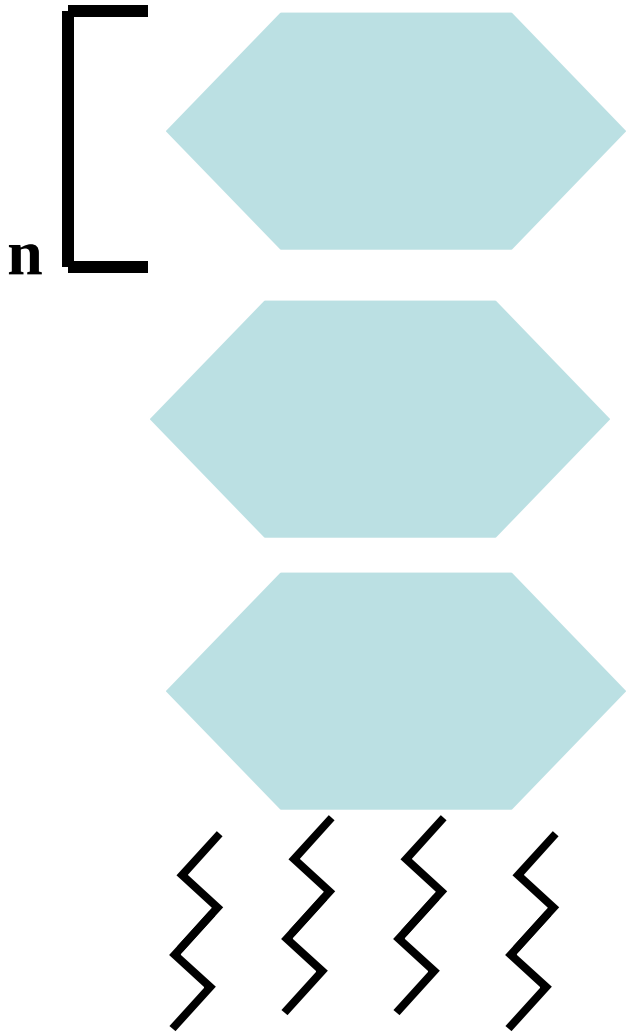
Gram Negative Peptidoglycan

- **Braun lipoprotein**
  - **binds cell wall to outer membrane**

Outer Membrane

- **lipopolysaccharide**
- **phospholipids**
- **Proteins**
  - **porins**

Lipopolysaccharide

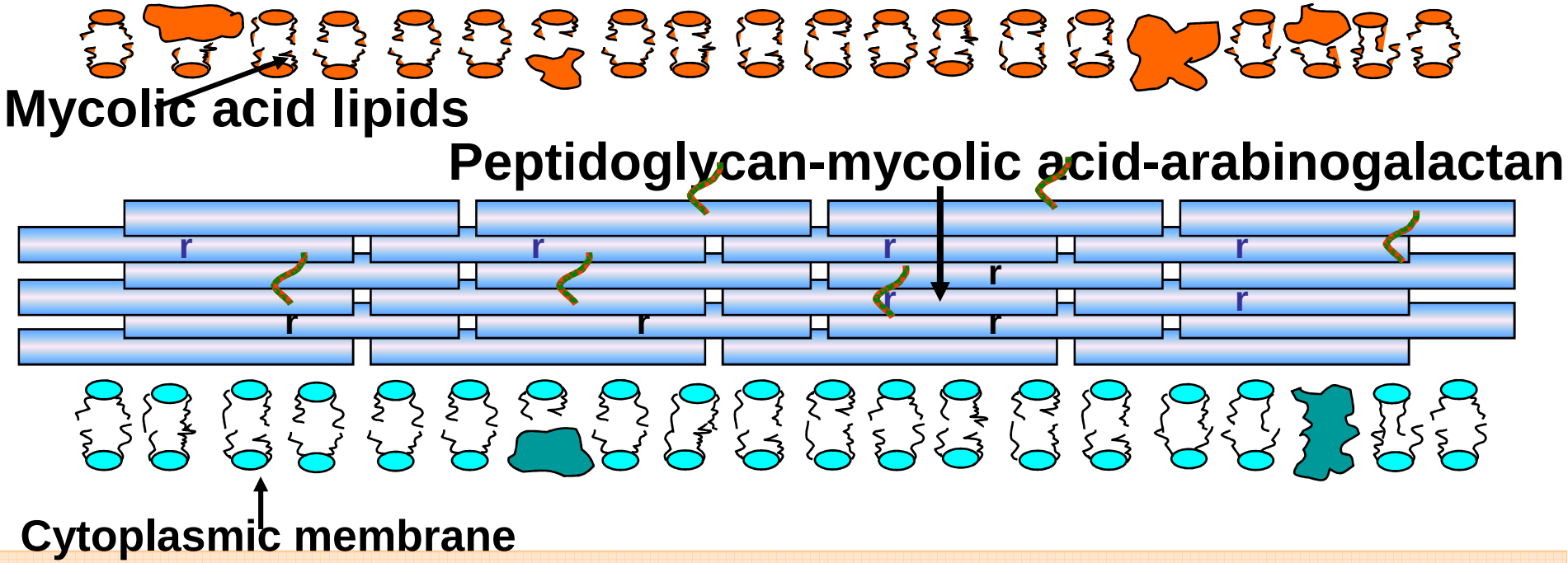


O-antigen
Highly variable

Core
- **Heptoses**
- **Ketodeoxyoctonic acid**

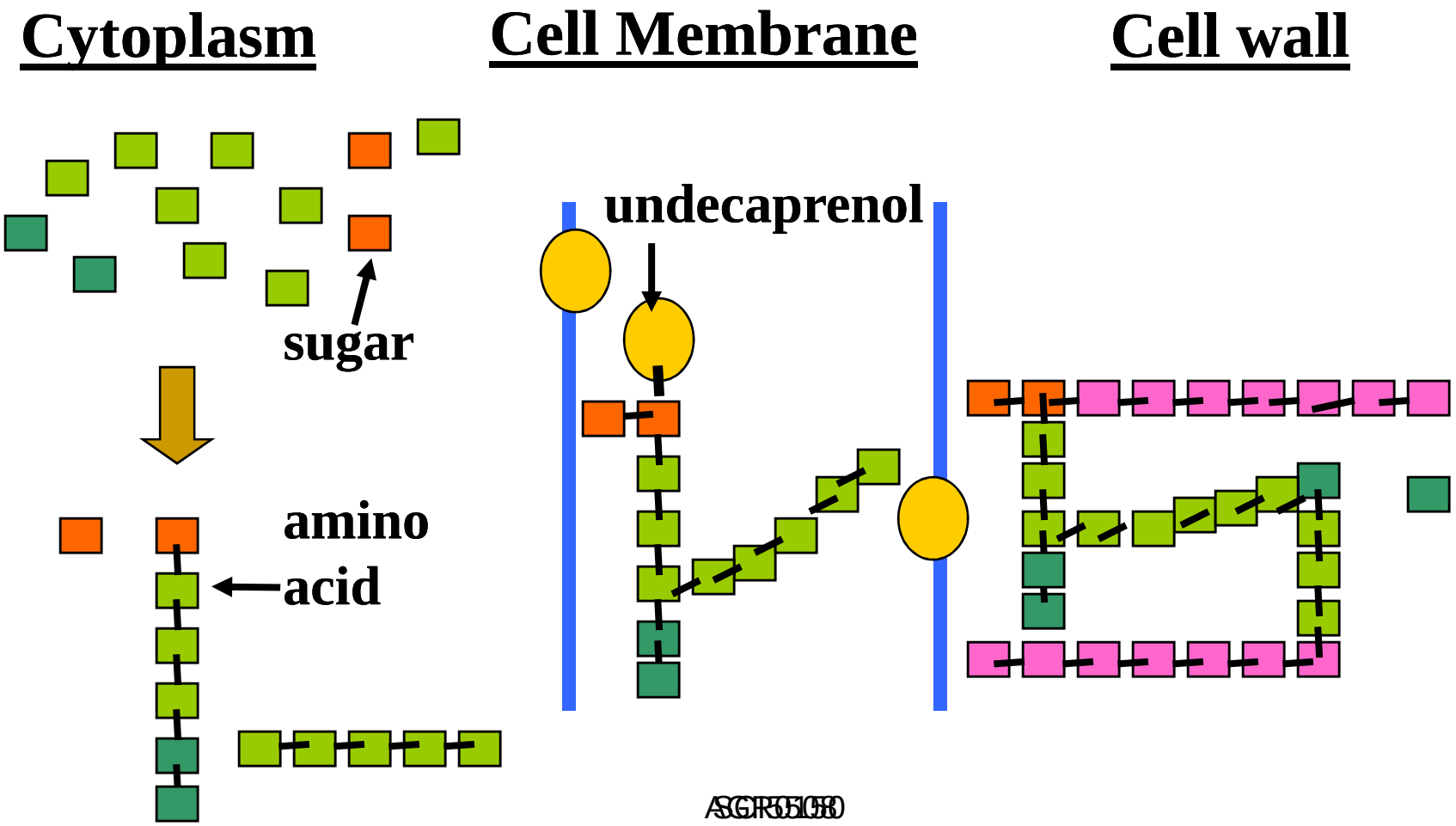
Lipid A
- **Glucosamine disaccharide**
- **Beta hydroxy fatty acids**

Acid Fast Cell Envelope



Cytoplasm

Peptidoglycan synthesis



Lipopolysaccharide

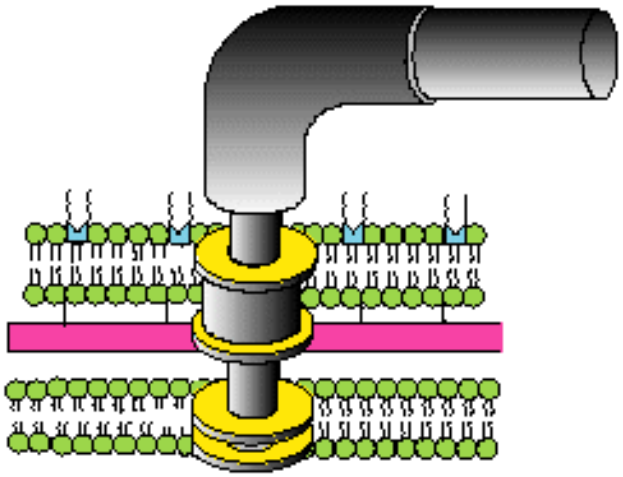
- synthesis similar to peptidoglycan
- also on undecaprenol carrier

Capsules and slime layers

- **outside cell envelope**
- **well defined: capsule**
- **not defined: slime layer or glycocalyx**
- **usually polysaccharide**
- **often lost on *in vitro* culture**
- **protective *in vivo***

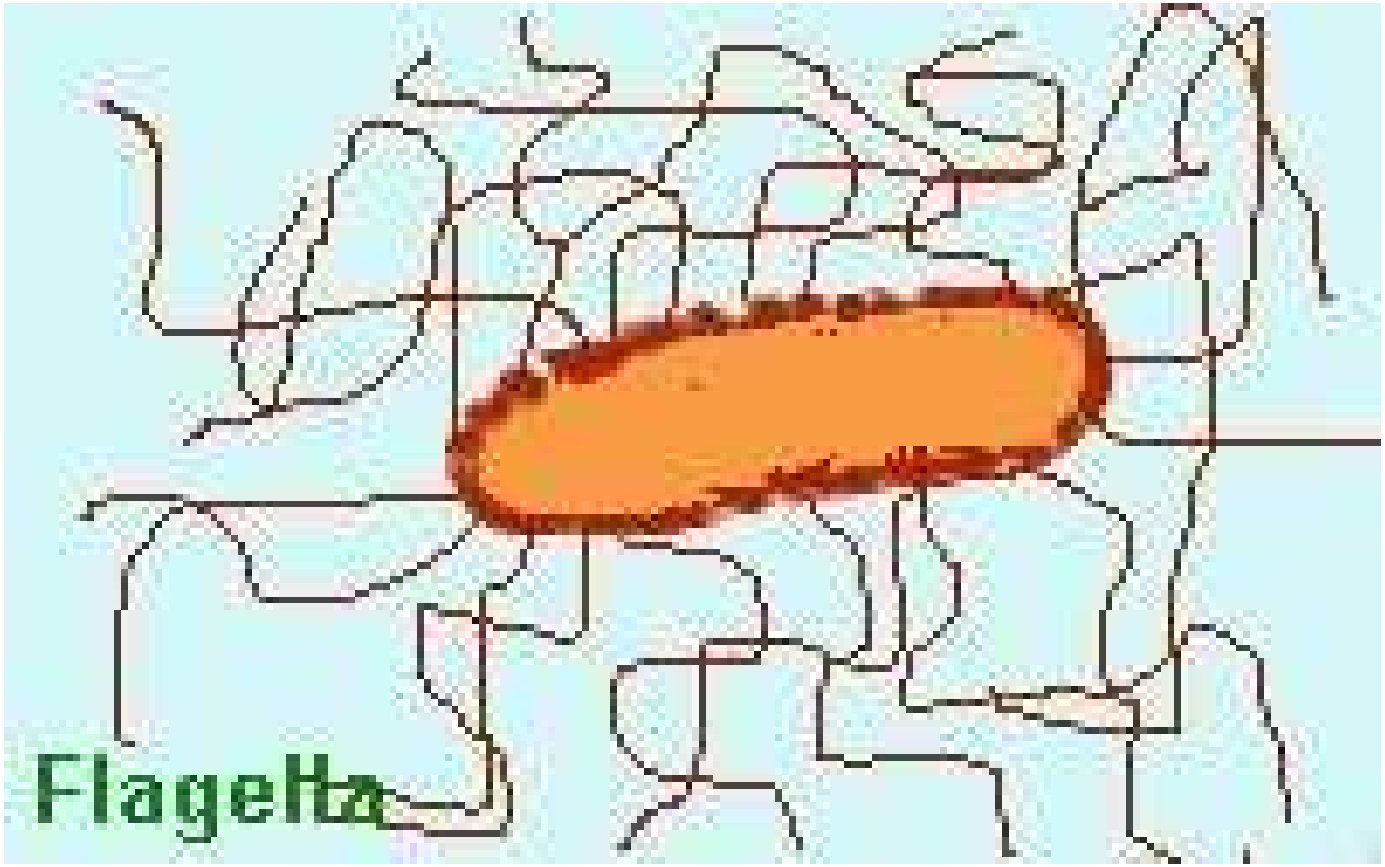
FLAGELLA

- **Some bacteria are motile**
  - **flagella**
- **Taste environment**
  - **Respond to food/poison**
  - **chemotaxis**

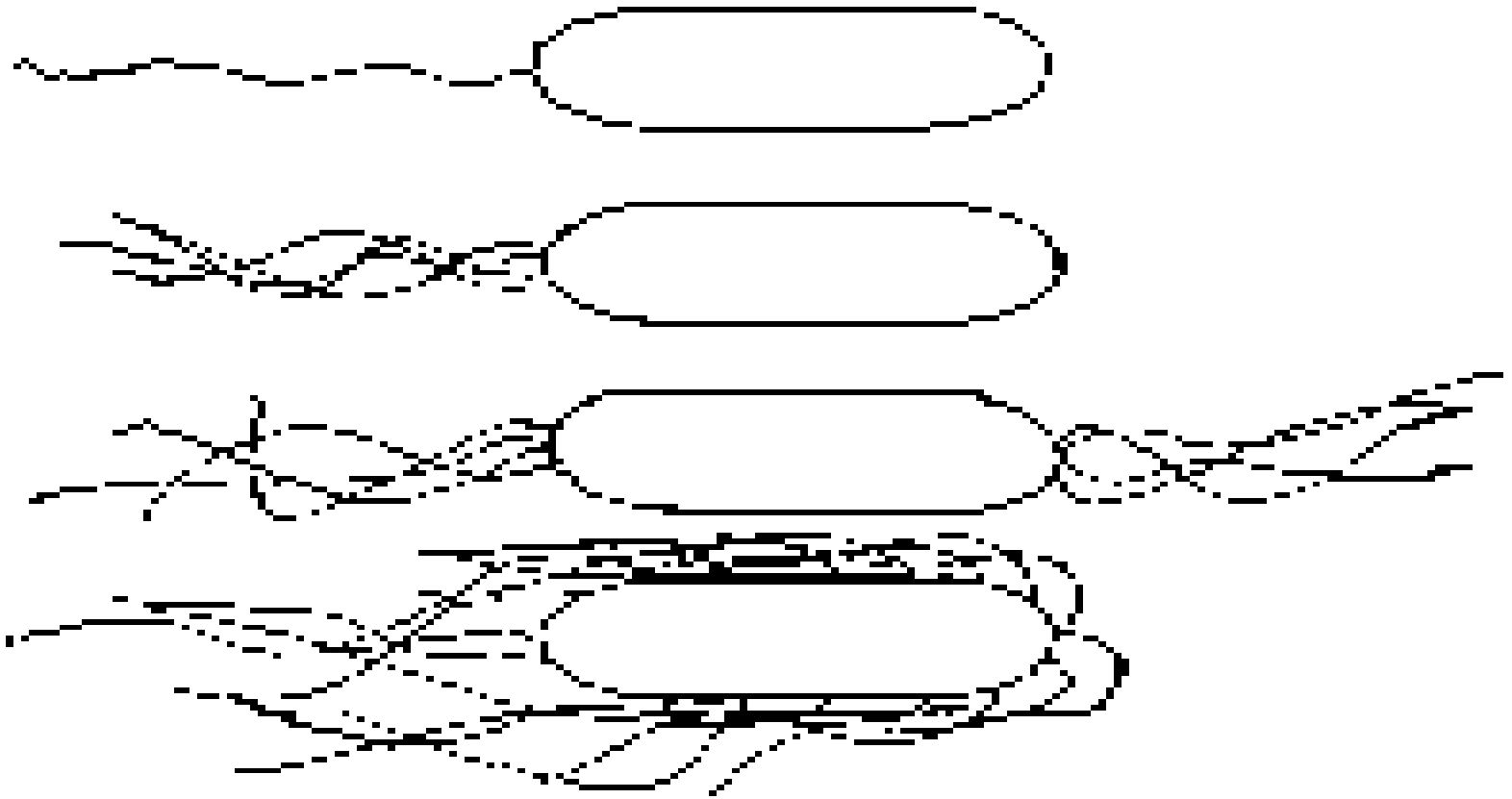


- **Flagella**
  - **embedded in cell membrane**
  - **project as strand**
  - **Flagellin (protein) subunits**
  - **move cell by propeller like action**

Fast Movers



Flagella



Axial filaments

- spirochetes
- similar function to flagella
- run lengthwise along cell
- snake-like movement

Making Wall-less forms

- **Result from action of:**
  - **enzymes lytic for cell wall**
  - **antibiotics inhibiting peptidoglycan biosynthesis**

- **Usually non-viable**

- **Wall-less bacteria that don't replicate:**
  - **spheroplasts (with outer membrane)**
  - **protoplasts (no outer membrane).**

- **Wall-less bacteria that replicate**
  - **L forms**

Naturally Wall-less Genus

- ***Mycloplasma***

Pili (fimbriae)

- **hair-like projections of the cell**
- **sexual conjugation**
- **adhesion to host epithelium**

Endospores (spores)

- **Dormant cell**
- **Produced when starved**
- **Resistant to adverse conditions**
  - high temperatures
  - organic solvents
- **contain calcium dipicolinate**
- ***Bacillus* and *Clostridium***

Spore

- **Modified Gram positive bacteria cell**
  - **unusual cell envelope**
    - **cell membrane**
    - **outer membrane**

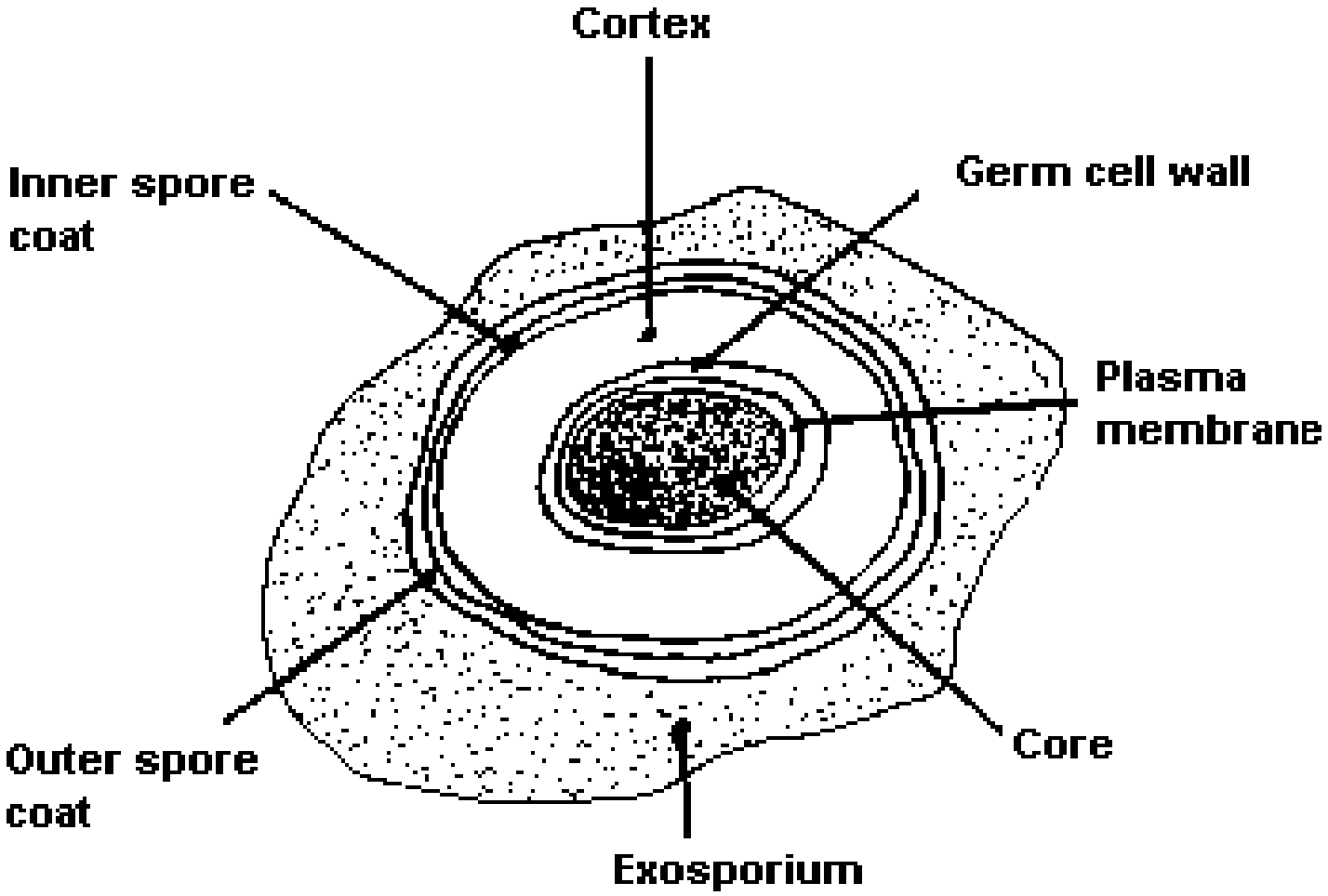
Spore

- **Peptidoglycan layer**
  - **cortex**
  - **between two membranes**
  - **less cross-linked**
  - **dehydrated muramic acid (lactam)**

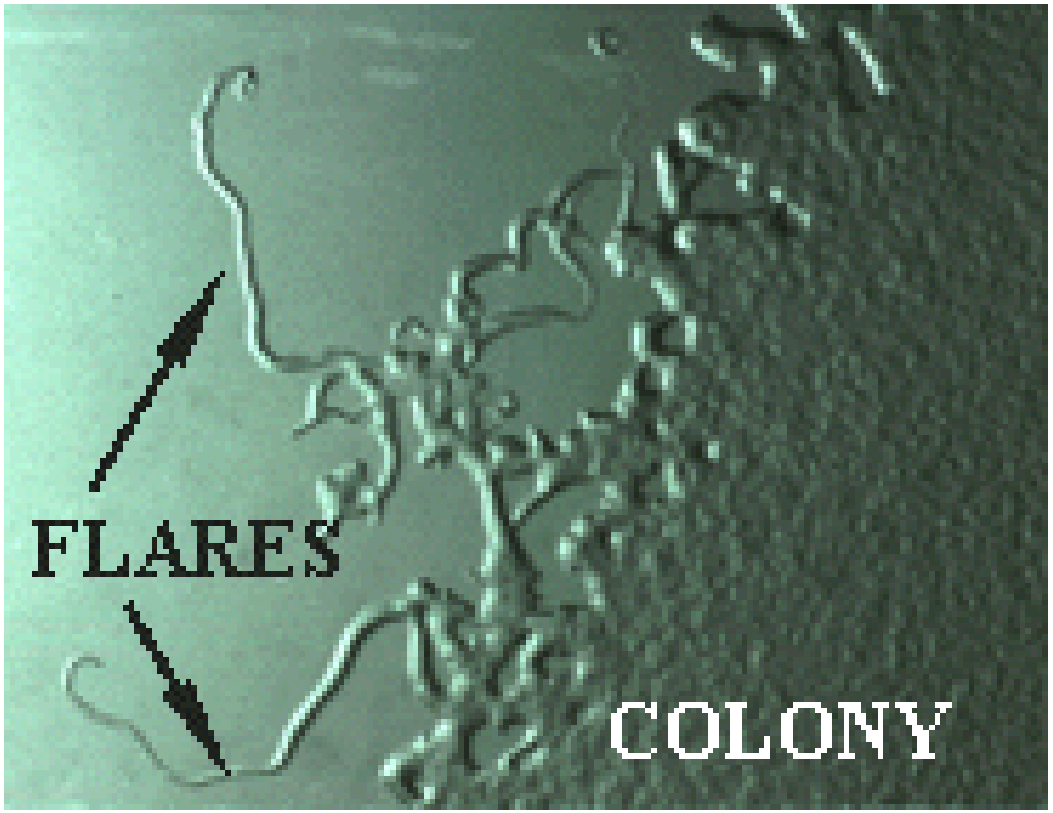
Surviving the Bad Times

- Bacillus and Clostridium
- Endospore
- inactive,
- they are a bit like seeds, except that one bacterium can only become one endospore

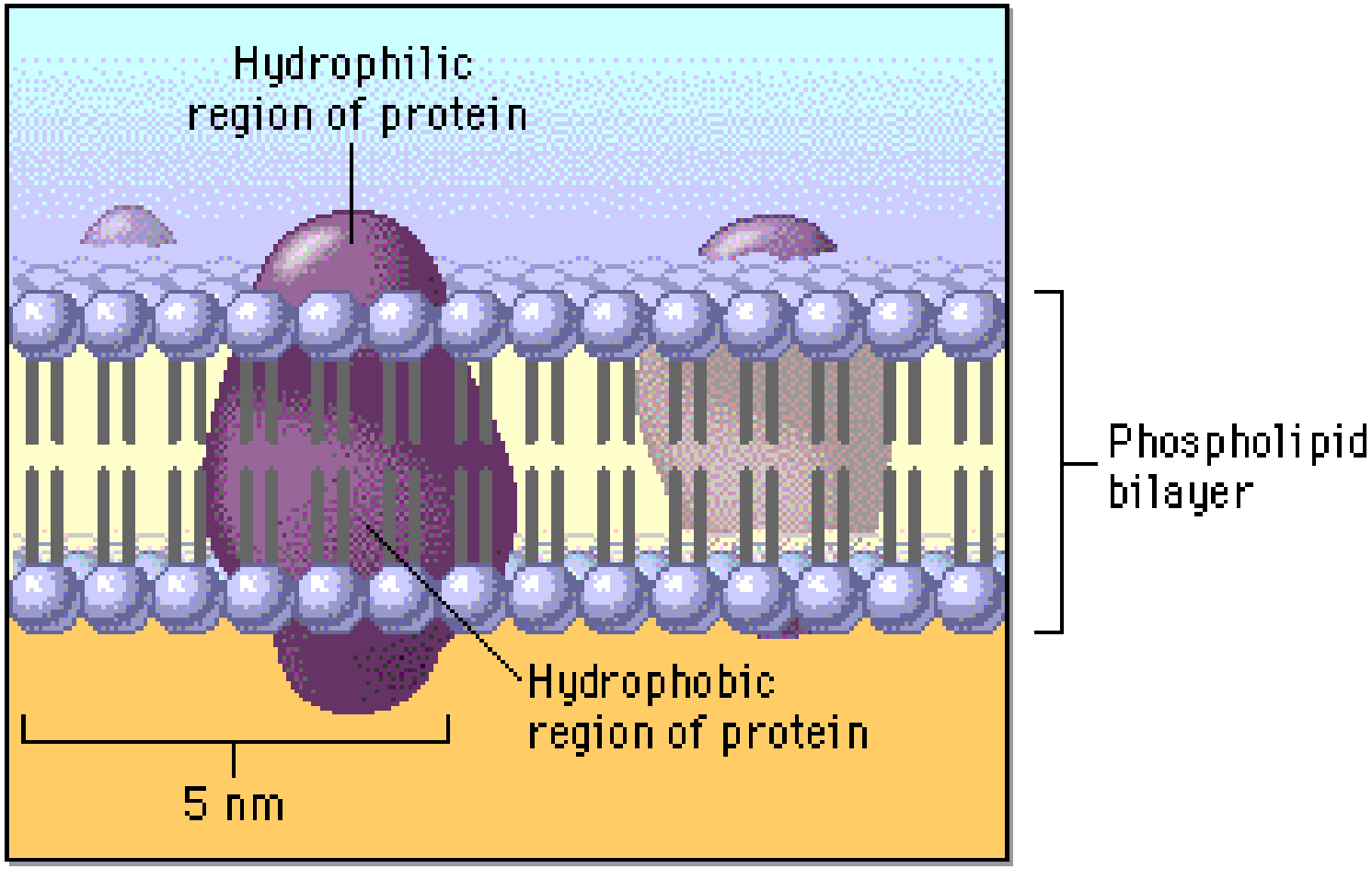
The endospore



Cellular differentiation in Bacteria



Plasma membrane



Permeability

- cell membrane is semi permeable
- Can go through➔ Water, Carbon dioxide, Oxygen , very small polar molecules
- Can't ➔ Lipids , All ions, mid to large polar molecules, Amino acids, macromolecules
- Passive transport mechanisms do not require a source of energy
- Active transport mechanisms involve the cell to use cellular energy

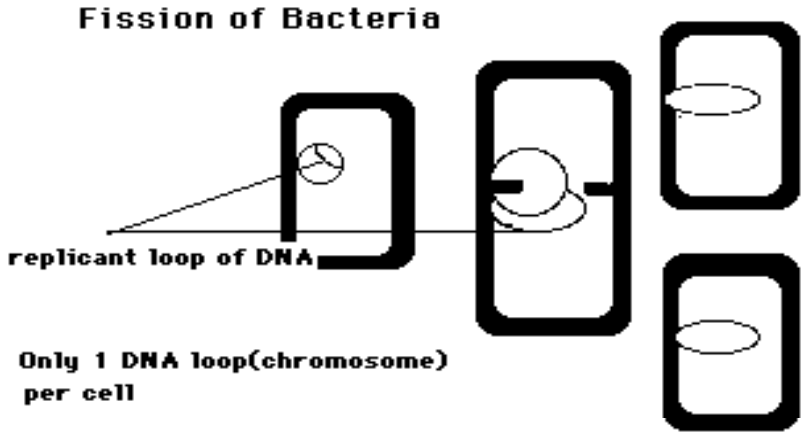
How Bacteria Eat

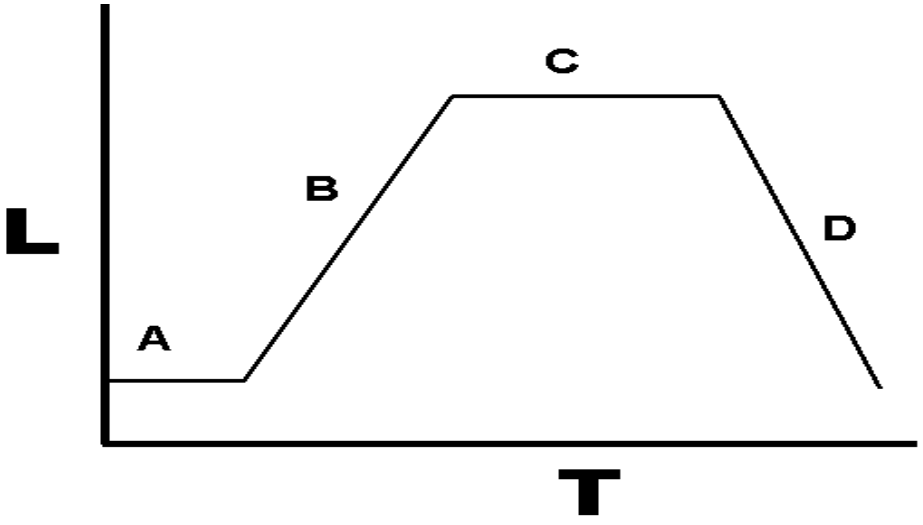
- channels in their cell walls and cell membranes
- Once inside the cell
  - broken down into their componant parts
  - rebuilt into the macromoloecules
- Enzyme secretion

Reproduction in Bacteria

- In optimal conditions they can reach maturity in 20 minutes
- The simplest form of bacterial reproduction is called binary fission → asexual reproduction
- Sexual Reproduction → transfer DNA

Bacterial Reproduction





Bacterial Ecology

- All organisms need energy
- Phototrophic = Getting energy from the sun
- Chemoorganotrophic = Getting energy from organic molecules
- Chemolithotrophic = Getting energy from inorganic molecules
- Autotrophic = Able to live with CO2 as the only carbon source

Ecologically Important

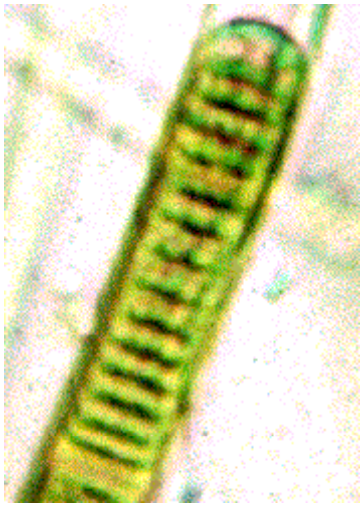
- Bacteria are important in soil
  - live by degrading organic compounds are called Chemooganotrophs.
- The carbon dioxide produced during respiration by chemoorganotrophic bacteria is converted to carbonic acid which is an important agent in the break down of rocks.

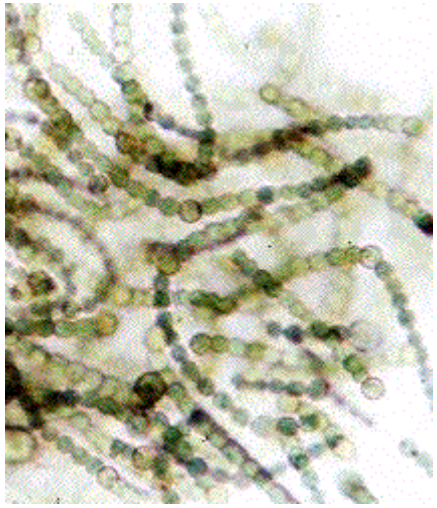
- Aquatic environments
  - cyanobacteria, sometimes called Blue-Green Algae because of their colour, are the most important primary producers. They contain chlorophyll and trap energy from the sun in the form of light.

Introduction to the Cyanobacteria
Architects of earth's atmosphere

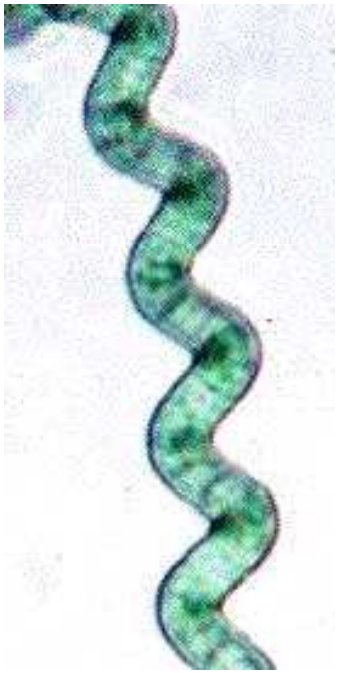
- Cyanobacteria are aquatic and photosynthetic,
- small and usually unicellular,
- often grow in colonies large enough to see.
- They have the distinction of being the oldest known fossils,

Cyanobacteria





- larger than other bacteria,
- morphologies in the group have remained much the same for billions of years.
- no nucleus or internal membrane systems.
- In many species, however, the external membrane has been folded to increase total surface area.
- movement



Spirulina

it is high in protein,

can be cultivated in ponds quite easily.

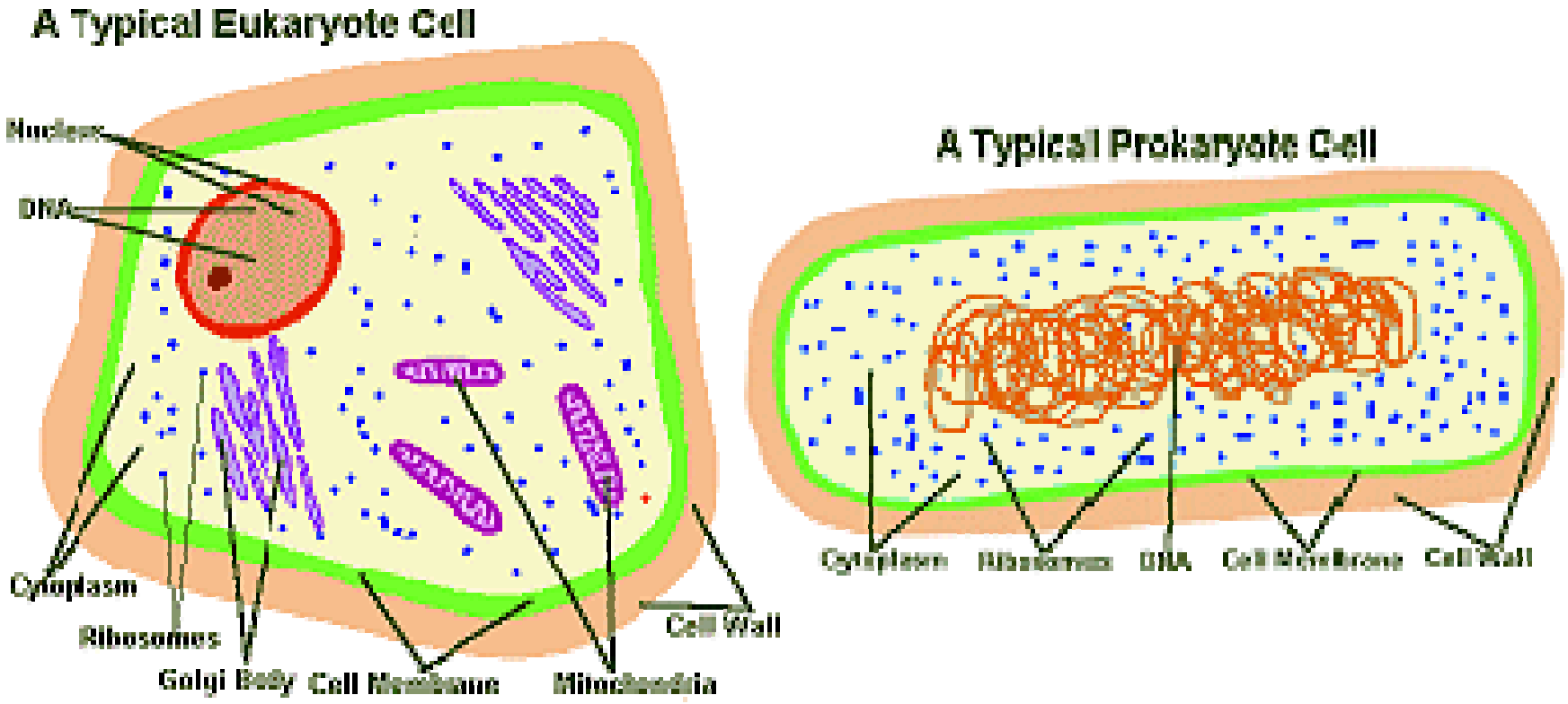
In tropical countries, it may be a very important part of the diet, and was eaten regularly by the Aztecs; it is also served in several Oriental dishes.

In the US, the popularity of *Spirulina* is primarily as a "health food", being sold in stores as a dried powder or in tablet form.

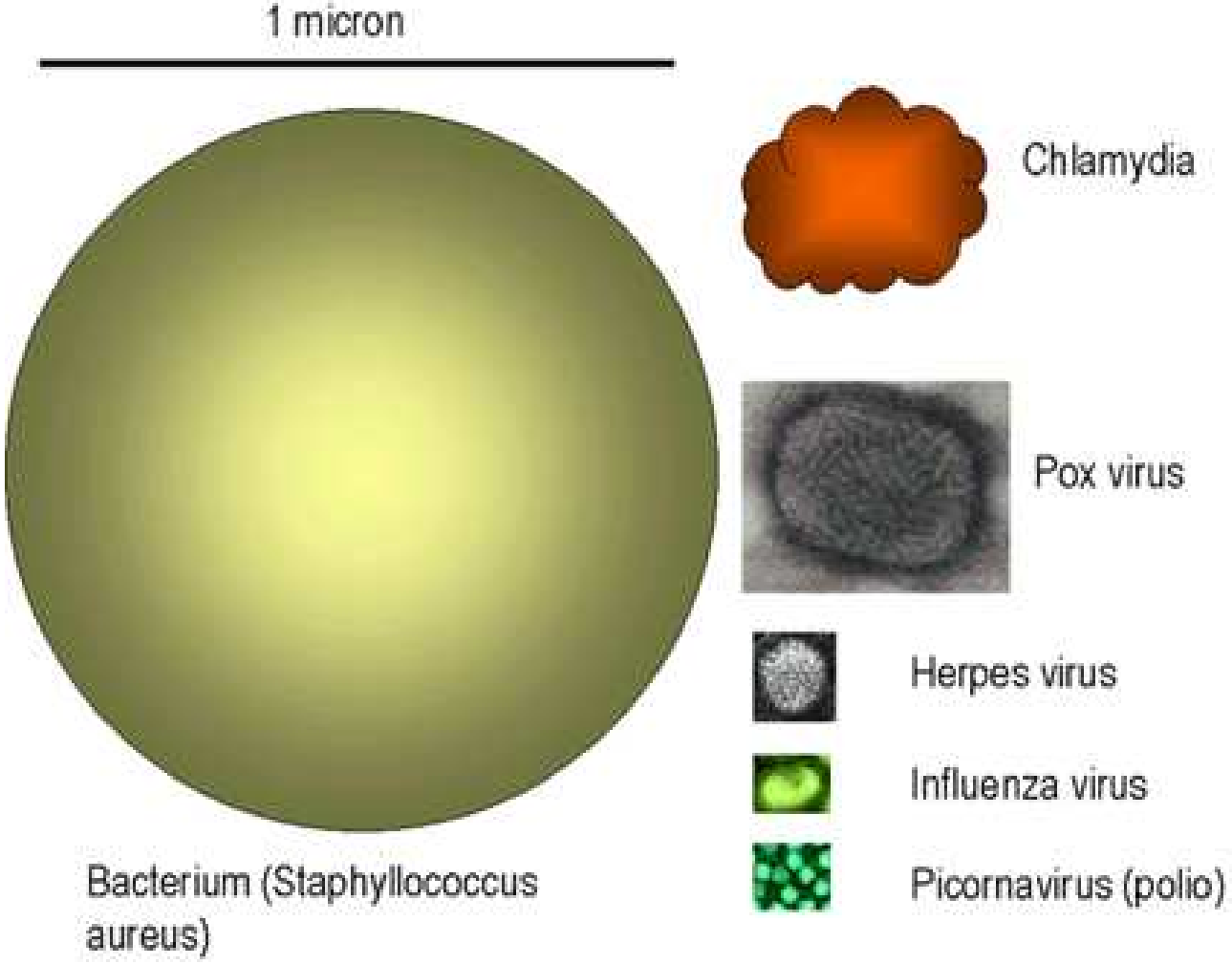
Bacterial and technology

- mining, medicine, food culture, plastics synthesis and sewage control.

- production of complex organic molecules
- genetics


A Typical Eukaryote Cell
Nucleus
DNA
Cytoplasm
Ribosomes
Golgi Body
Cell Membrane
Mitochondria
Cell Wall
A Typical Prokaryote Cell
Cytoplasm
Ribosomes
DNA
Cell Membrane
Cell Wall

Viruses


1 micron
Chlamydia
Pox virus
Herpes virus
Influenza virus
Picornavirus (polio)
Bacterium (Staphyllococcus aureus)

	Growth on artificial media	Division by binary fission	Whether they have both DNA and RNA	Whether they have ribosomes	Whether they have muramic acid	Their sensitivity to antibiotics
Bacteria	Yes	Yes	Yes	Yes	Yes	Yes
Mycoplasma	Yes	Yes	Yes	Yes	No	Yes
Rickettsia	No	Yes	Yes	Yes	Yes	Yes
Chlamydia	No	Yes	Yes	Yes	No	Yes
Viruses	No	No	No	No *	No	No

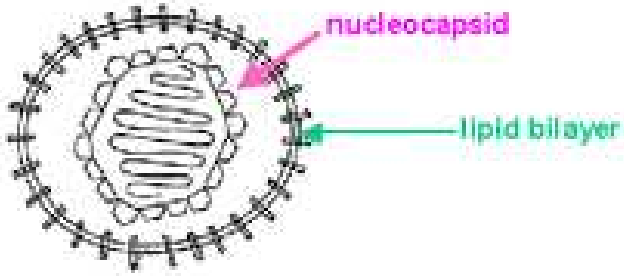
* The arenavirus family (an RNA virus family) appears to package ribosomes 'accidentally'. The packaged ribosomes appear to play no role in viral protein synthesis.

VIRUS STRUCTURE

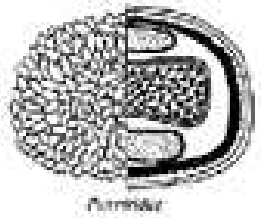
- range in size
- All viruses contain
  - a nucleic acid genome (RNA or DNA) and
  - a protective protein coat (called the capsid).
- may or may not have an envelope

5 BASIC TYPES OF VIRAL SYMMETRY

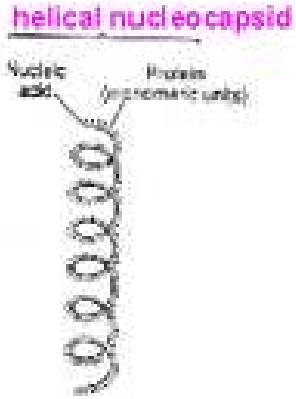
ICOSAHEDRAL



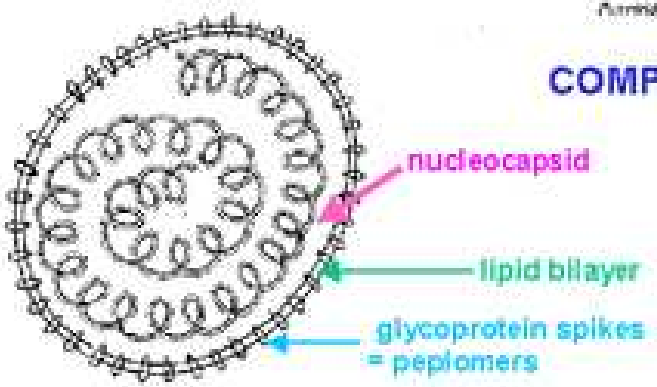
ENVELOPED ICOSAHEDRAL



COMPLEX



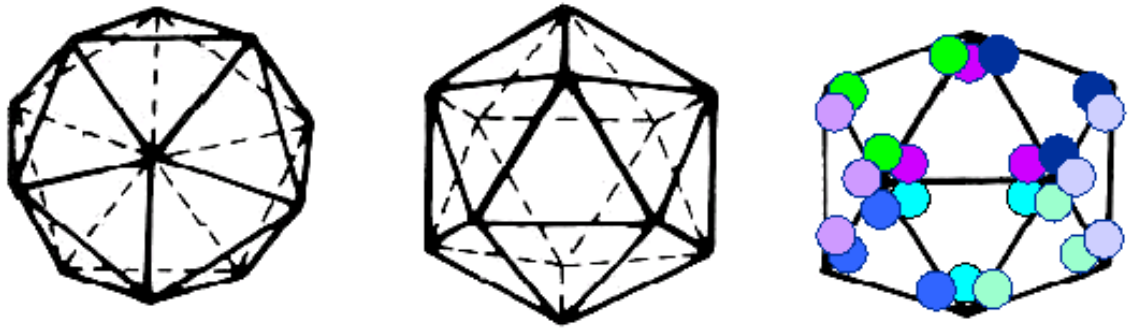
HELICAL



ENVELOPED HELICAL

Adapted from Schaeter et al., Mechanisms of Microbial Disease

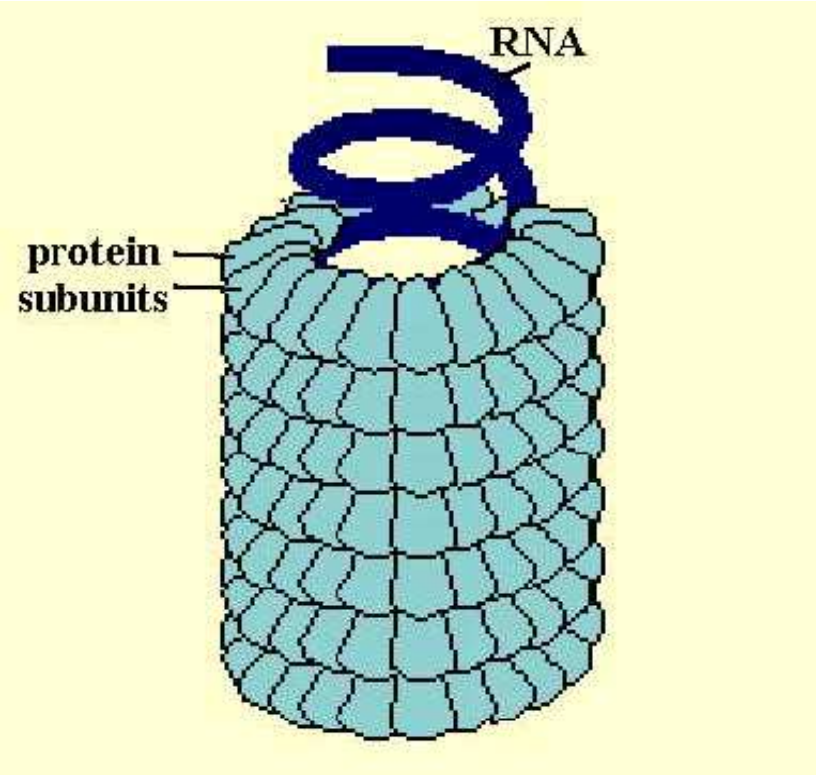
ICOSAHEDRAL SYMMETRY

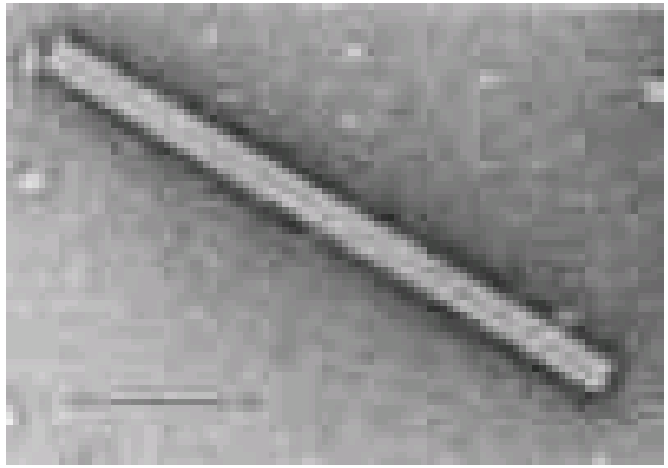


ICOSAHEDRAL SYMMETRY

The icosahedral shape of a soccer ball.

penton subunits (black)
and
hexon subunits (white)


RNA
protein
subunits



Virus Facts

- viruses do not **respire**,
- nor do they display **irritability**;
- they do not **move**
- and nor do they **grow**,
- however, they do most certainly **reproduce**, and may adapt to new hosts.

Viruses

- Viruses may be **defined** as **acellular organisms** whose **genomes** consist of **nucleic acid**,
- obligately **replicate inside host cells** using **host metabolic machinery** and **ribosomes** to form a **pool of components**
- which assemble into particles called **VIRIONS**, which serve to **protect the genome** and to **transfer it to other cells**.
- They are distinct from other so-called **VIRUS-LIKE AGENTS** such as **VIROIDS and PLASMIDS and PRIONS**
-

Other Autonomous or Semi-Autonomously Replicating Genomes

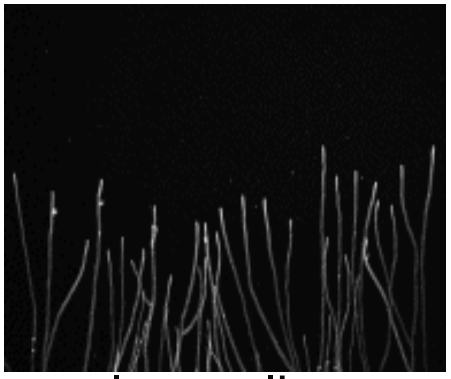
Retroid Elements and Retroviruses

Prions (Diseases)

- **"small proteinaceous infectious particles which resist inactivation by procedures that modify nucleic acids".**
- **spongiform encephalopathies**

What are fungi

- Fungi belong to their own special ‘kingdom’ as they differ from both plants and animals.
- The fungal kingdom is largely hidden from our view underground and we usually only see the "fruit" of a fungus.
- Fungi can exist as single cells or chains of cells together.
- The living body of a fungus is called a mycelium and is made up of a branching network of filaments known as hyphae.



Fungal hyphae growing - contain chains of cells.

- Fungal mycelia are usually hidden in a food source like wood and we only know they are there when they develop mushrooms or other fruiting bodies. Some fungi only produce microscopic fruiting bodies and we never notice them.

- **Fungi** feed by absorbing nutrients from the organic material that they live in.
- They digest their food before they absorb it by secreting acids and hydrolytic enzymes.
- Different fungi have evolved to live on various types of organic matter, some live on plants eg.*Phytopthora infestans* - the potato blight fungus, as seen here;

Some live on animals eg.the athlete's foot fungus and some live on insects eg.*Cordyceps australis.*

- Most of us use fungi every day without knowing it. We eat mushrooms and Quorn (a vegetarian fungal protein), but we also prepare many other foods using fungi.

- The yeast *Saccharomyces cerevisiae* is used to ferment sugar to alcohol and carbon dioxide – the process used to make beer and wine and also to make bread rise.

- The fungi *Aspergillus oryzae* and *Aspergillus sojae* are used in the production of the oriental foods soy sauce and miso. We also use fungi to produce flavourings, vitamins and enzymes and to mature many cheeses.

- Fungi play an essential role in both the Nitrogen and

- We get some important drugs from fungi such as the antibiotic penicillin and cyclosporin A - a drug that stops organ rejection after transplantation.

- Research scientists use several fungi to investigate basic functions that occur in all cells because they are simple and easy to grow; some cancer research is done using fungi.

- Fungi are responsible for breaking down dead organic matter which allows nutrients to be cycled through the ecosystem.

- Without fungi we would not have bread, beer, wine or antibiotics, but more importantly without the nutrient recycling and plant nutrition provided by fungi - we probably could not survive at all.
- In humans, fungi cause skin infections such as ringworm and athlete's foot, but they also cause several deadly diseases which can be hard to treat. Fungi that can cause life-threatening infections in people include *Aspergillus fumigatus, Candida albicans* and *Cryptococcus neoformans* – they are called pathogens.

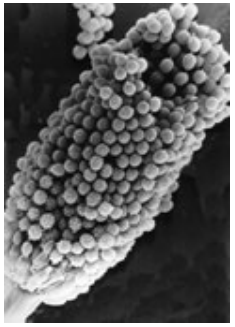
Ringworm infection on leg

SCI5508

Most patients with deadly fungal diseases do not have a fully functional immune system. They may have leukaemia or AIDS or they may be taking drugs to suppress their immune system because of organ transplantation.

Although there are drugs to treat fungal infections these drugs can have some nasty side-effects because they are often toxic to people as well as to fungi. There is a desperate need for new and better anti-fungal agents.

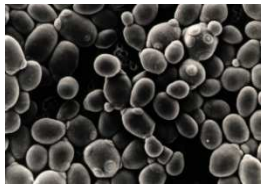
Aspergillus fumigatus
spore forming head (Electron micrograph)



SCI5508

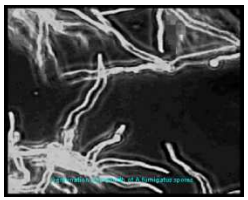
What is Aspergillus

- It is a genus of around 200 fungi (moulds) found worldwide.
- Fungi are identified in the lab by their structure and appearance. They may appear as round single cells like yeast, or made of chains of cells called hyphae.



Yeast

- Aspergillus is a filamentous fungus as opposed to yeast which is single celled.



Aspergillus hyphae

- Fungi reproduce by forming tiny spores which can easily be airborne.

Conidial head or fruiting body of Aspergillus - producing spores

When was aspergillus first identified?

- In 1729 Aspergillus was first catalogued by an Italian biologist - P Micheli.
- The first known case of infection was in a jackdaw in 1815 and in a human in 1842.
- In the 19th century it was an occupational hazard amongst wig combers when it caused allergic disease of the lungs.

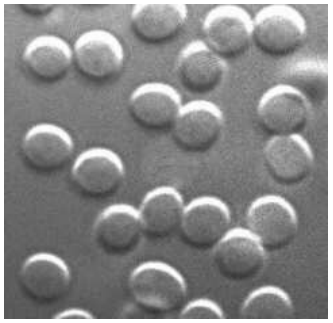
Why is Aspergillus important?

The following slides focus on Aspergillus because this fungus illustrates a spectrum of positive and negative aspects of fungi with respect to the environment and disease.

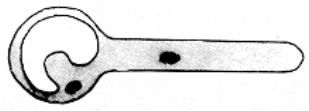
- Some *Aspergillus* species cause serious disease in humans and animals – it is pathogenic.
- Some *Aspergillus* species produce enzymes which have important industrial applications.
- *Aspergillus* can produce mycotoxins – these are often found in contaminated foodstuff and are hazardous to the consumer.

Where is Aspergillus found?

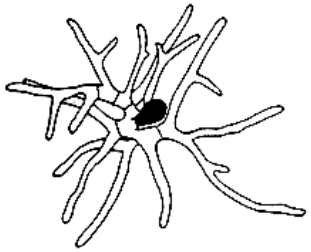
- Its natural habitat is in hay and compost.
- *Aspergillus* spores are easily airborne and we normally breathe in 100-200 spores daily.
- Some species withstand heat eg; *Aspergillus fumigatus* (pathogenic type) these are commonly found in compost.



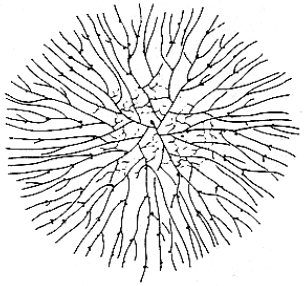
Spores inhaled



Germination



Hyphal elongation
and branching

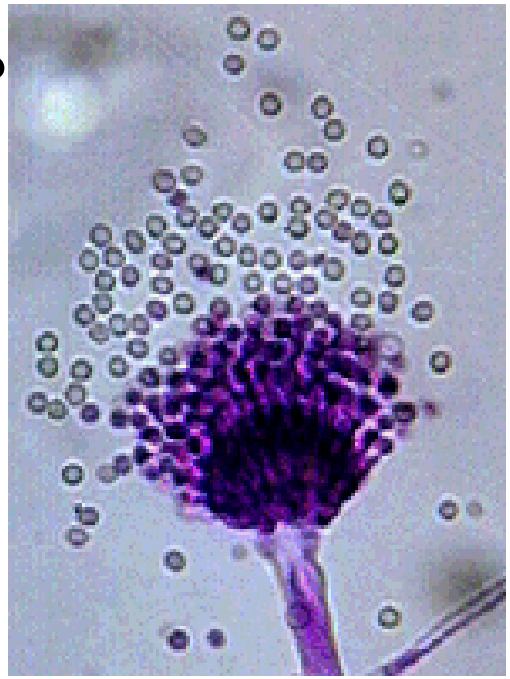


Mass of hyphae
(plateau phase)

Sources of Infection?

Aspergillus species are found in :

- Soil
- Air; spores may be inhaled
- Water / storage tanks in hospitals etc
- Food
- Compost and decaying vegetation
- Fire proofing materials
- Bedding, pillows
- Ventilation and air conditioning systems
- Computer fans



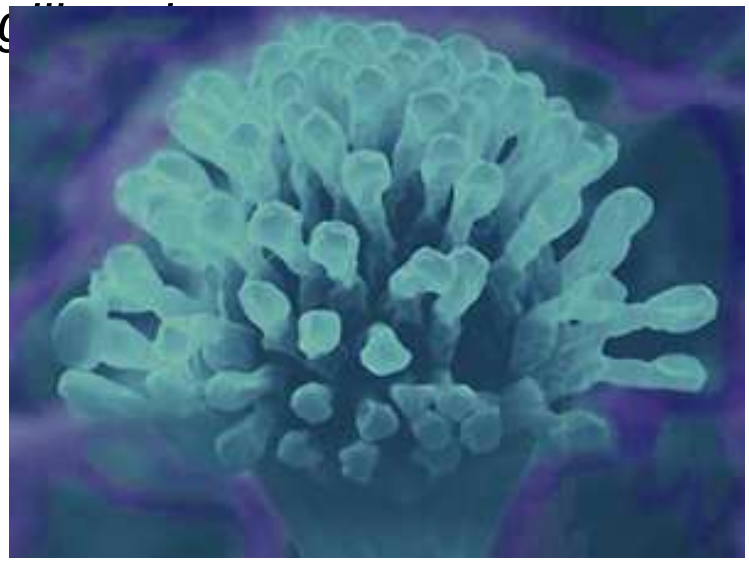
Aspergillus spores

Which species of Aspergillus are pathogens?

- The most common causing invasive disease are *Aspergillus fumigatus* and *Aspergillus flavus.*

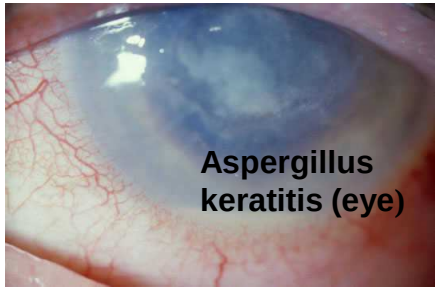
- The most common causing allergic disease are *Aspergillus fumigatus* and *Asperg*

EM of Aspergillus clavatus



Aspergillus as a pathogen in man-

- Aspergillosis is a group of diseases caused by *Aspergillus*. The symptoms – fever, a cough, chest pain or breathlessness occur in many other illnesses so diagnosis can be hard.
- Usually only patients with already weakened immune systems or who suffer other lung conditions are susceptible.

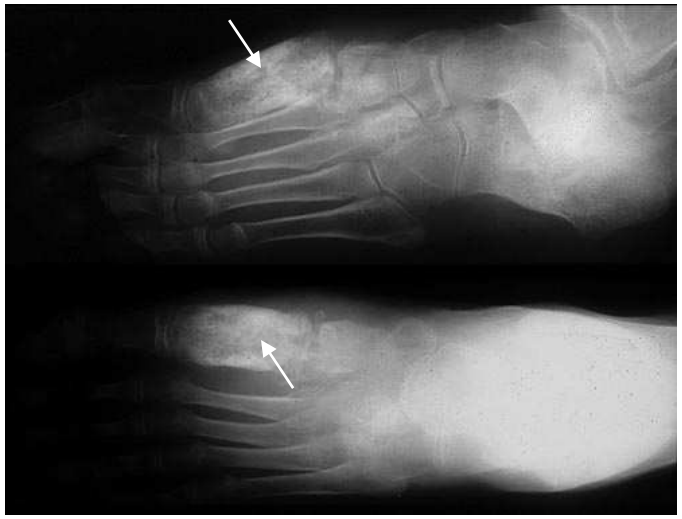


- In man the major forms of disease are:

1. Allergic aspergillosis (affects asthma, cystic fibrosis and sinusitis patients).
2. Acute invasive aspergillosis (risk increases if patient has weakened immunity such as some cancer patients and those on chemotherapy).
3. Disseminated invasive aspergillosis (widespread through body).

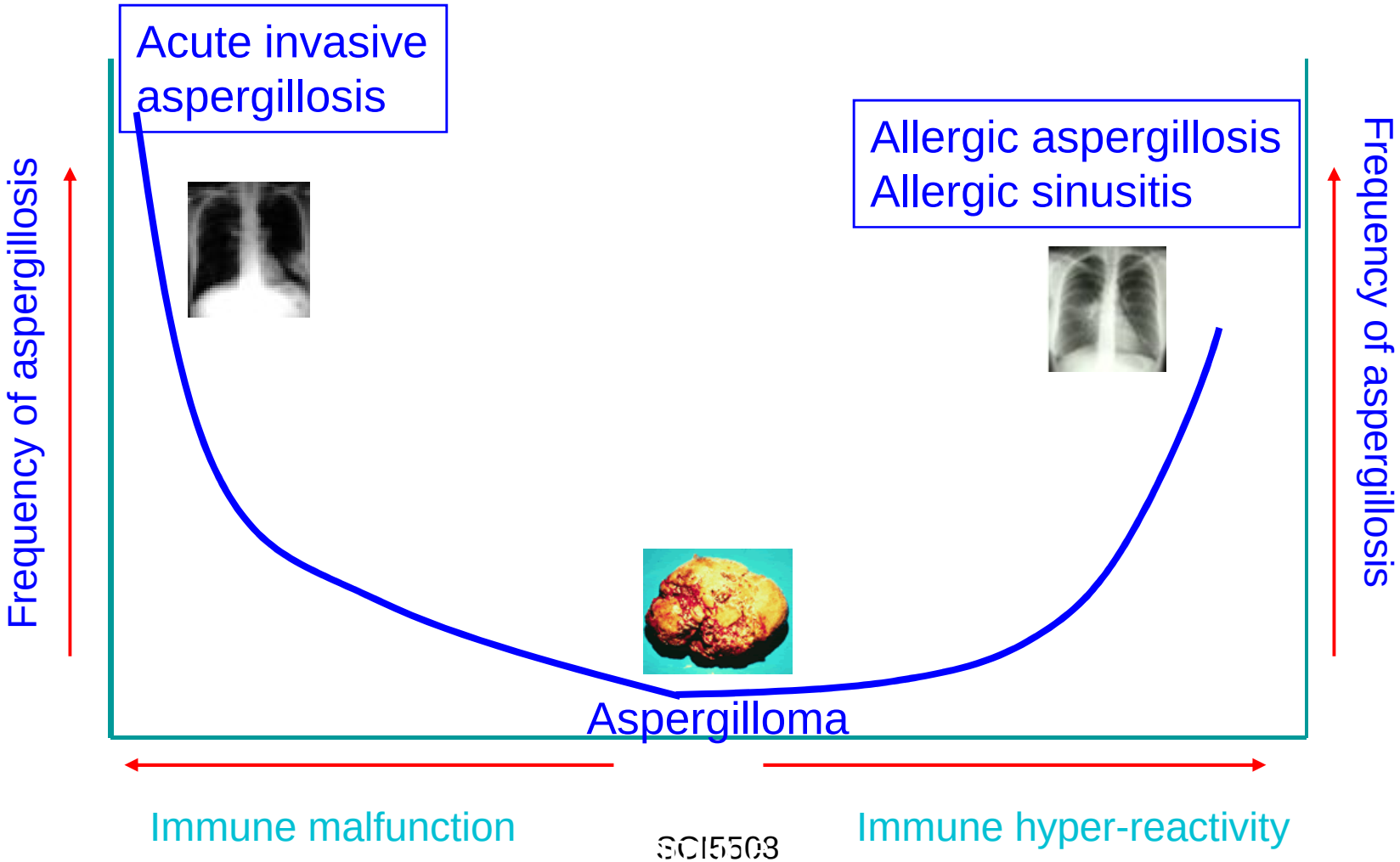
An example of invasive aspergillosis

Aspergillus bone and soft tissue infection in a patient with the inherited condition chronic granulomatous disease (CGD).



Relative risk of Aspergillus infection

Patients whose immune system is already weakened are most susceptible. Those most at risk include some cancer and leukaemia patients, those on chemotherapy and transplant patients.



Aspergillus as a pathogen in animals

- All domestic mammals, birds and numerous wild species can get aspergillosis.

- Birds such as penguins and falcons when stressed by malnutrition or capture are particularly susceptible to aspergillosis.
- Spores of *Aspergillus fumigatus* cause lung infections – leading to death.
- *Aspergillus fumigatus* spores are often present on the surface of eggs after laying. The spores may penetrate the shell pores and contaminate newly hatched chicks.

- Genetically modified *A. oryzae* is used for the large scale production of lipases used in biological washing powders.

Aspergillus species secrete a number of enzymes

- *A.niger* is used in the commercial production of citric acid, which is widely used in the food industry.

- Fermentation of genetically modified *A.oryzae* is the major source of recombinant chymogen which is used to curdle milk to make hard cheeses.

SC

Aspergillus Mycotoxins

- Mycotoxins are chemical products of fungi that have the capacity to damage animal health and contaminate crops.
- Repeated aflatoxin ingestion in man has been linked to liver cancer.
- Mycotoxins (aflatoxins) produced by *Aspergillus parasiticus* and *A. flavus* are commonly found to contaminate corn, peanuts, and other crops used for animal feedstuff. High temperature and humidity increase chances of contamination.

Why sequence the Aspergillus genome?

- Aspergillus contains about 10,000 genes compared to the possible 33,000 genes or more found in humans – how many of these genes are shared with humans?
- 50% of the fungal genes identified so far are completely new to science, implying they are unique to fungi.
- So far genetic analysis shows fungi may contain many unique coding sequences – do these encode unique genes which may be useful to mankind?
- Can we identify fungal genes which also function or malfunction in man? Yes - *Aspergillus nidulans* has been a successful genetic model for the identification of genes responsible for alkaptonuria – a metabolic disorder.

Comparison of the size of different genomes

Species	**Approx. Size**	**Type**
Human	$3{,}300 \times 10^6$	Mammal
Aspergillus fumigatus	30×10^6	Multi-cellular
Mycobacterium tuberculosis	4×10^6	Single cellular (complex)
Mycoplasma pneumoniae	400,000	Smallest independent life form
Haemophilus influenzae	1.2×10^6	Single cellular
Malaria	30×10^6	Single and multi-cellular forms
Worm	100×10^6	Multi-cellular

How will the sequence of Aspergillus be useful?

- Genome sequencing of a harmful pathogen allows us to compare DNA sequences with other Aspergillus species which are not pathogenic.
- That information will enable an understanding of why *Aspergillus fumigatus* can cause infection resulting in allergic or invasive disease.
- New drug targets will emerge for use in medicine and agriculture.
- New diagnostic tools will be developed - early detection of infection is critical for a better outcome for the patient.

Aspergillus is a remarkable member of the fungal kingdom, with a wide diversity of uses and effects on mankind.

- In the environment it plays a role in both the Carbon and Nitrogen cycles and in the breakdown of organic material into compost.
- It is a pathogen and allergen in humans and animals.
- *Aspergillus nidulans* has played a crucial role as a genetic model including identifying the genes responsible for alkaptonuria.
- The biotechnology industry has harnessed it's potentially useful enzymes for the food industry and commercial uses.

The future understanding of these fungal genomes will hopefully pave the way for understanding the role of aspergillus species as pathogens and to enable the development of effective and perhaps less toxic medicines for the treatment of aspergillosis.