

16/08/2021

# CH-1 FLOW AND FORM

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## CH40001 Biochemical Engineering

### Chapter 1. Introduction to Biochemical Energy: Flow & Form, Chaos & Organization, Evolution & Extinction

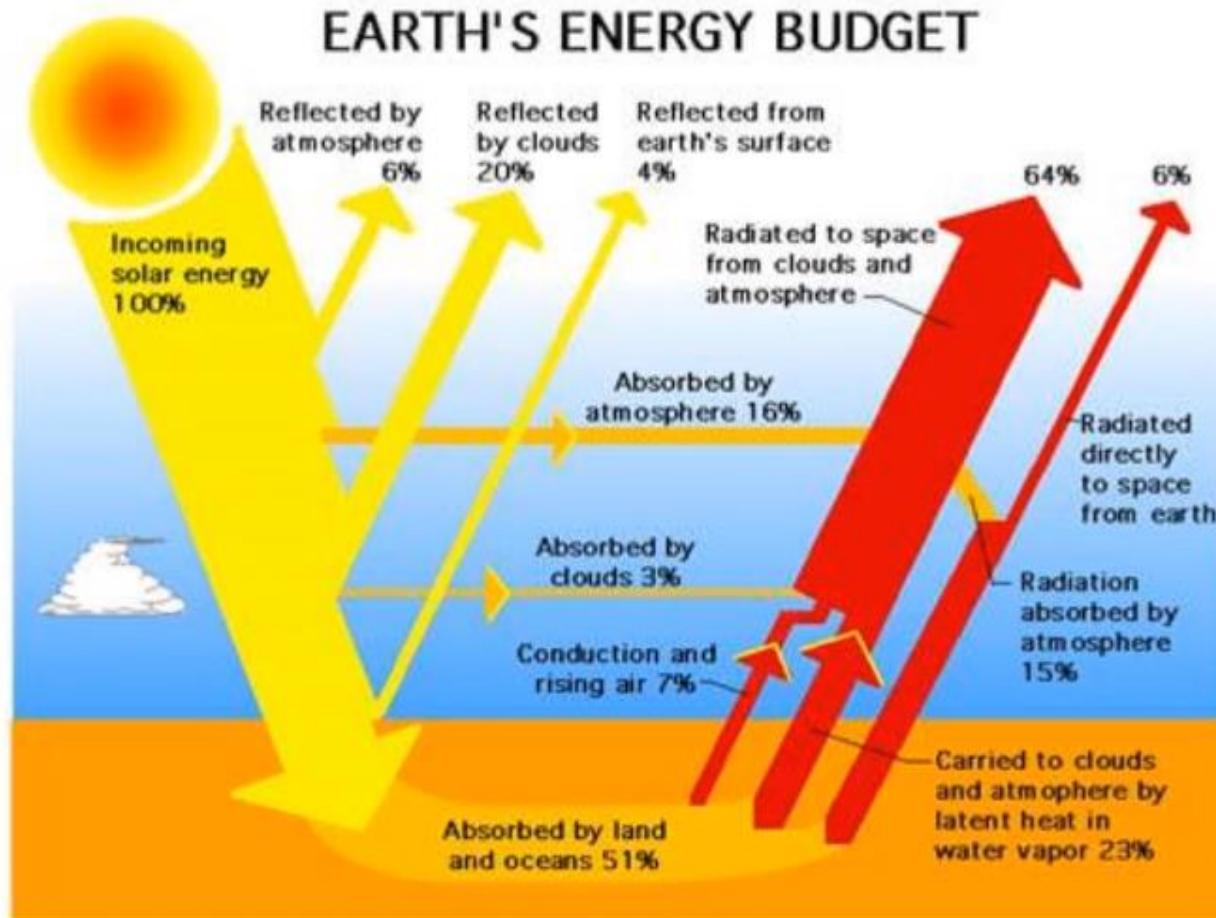
Saikat Chakraborty  
Department of Chemical Engineering,  
Indian Institute of Technology

# Flow and Form

Plato made the connection between motion and form in 360 BC in *Cratylus*

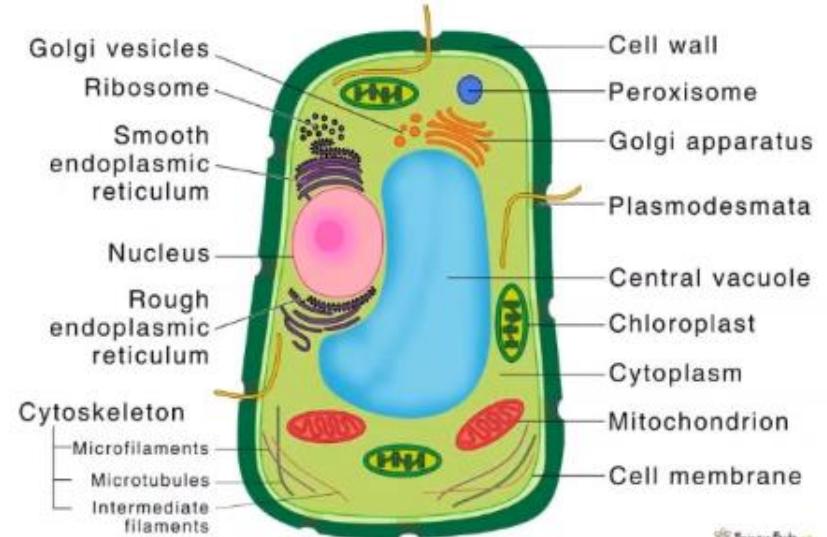


Solar Energy

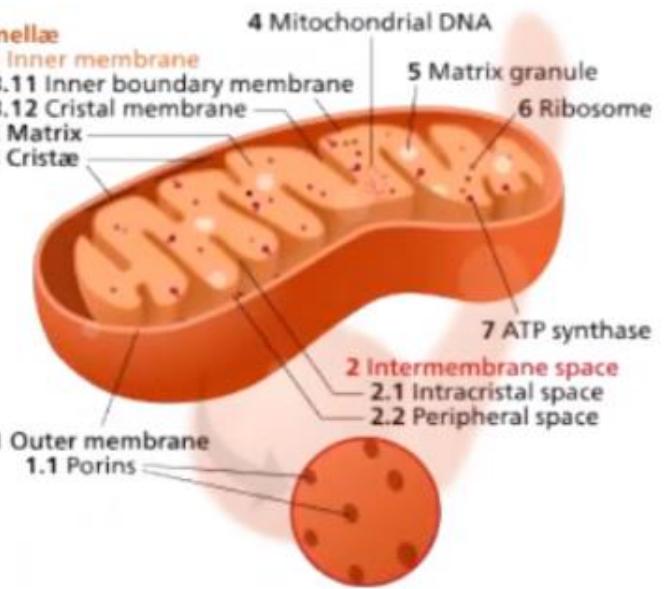
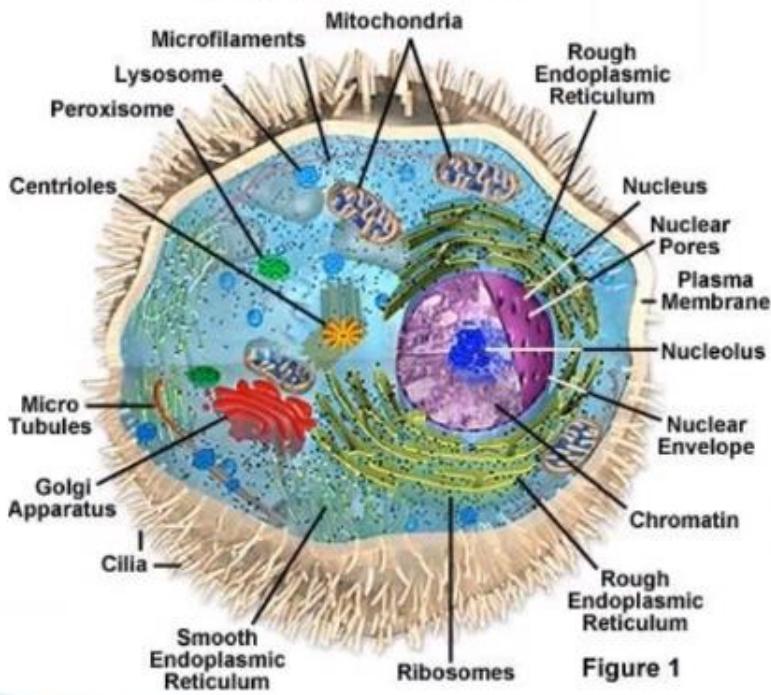


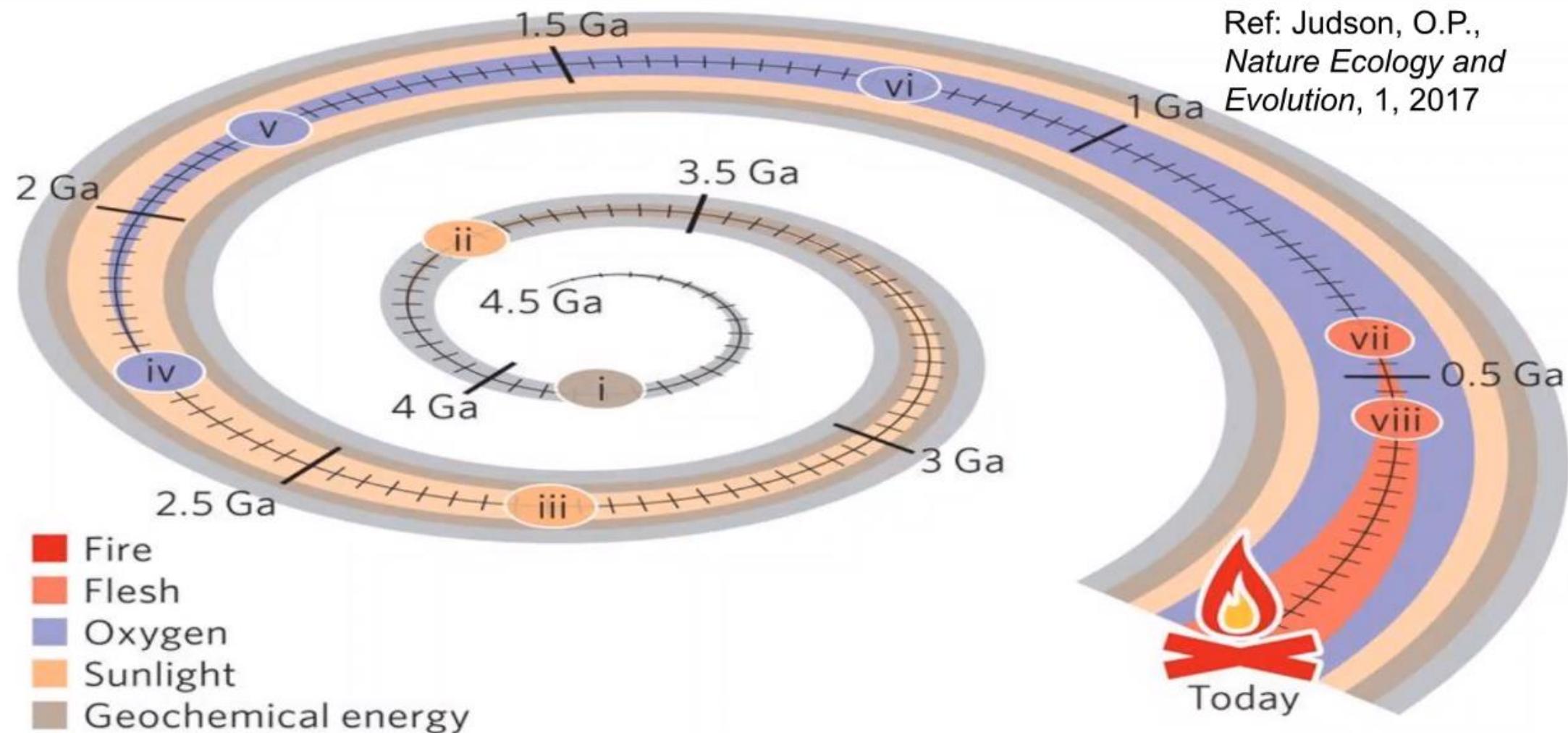
Flow of Solar Energy across the scales: through  
Radiation, Convection, and Conduction/Diffusion

# Plant Cell



Anatomy of the Animal Cell



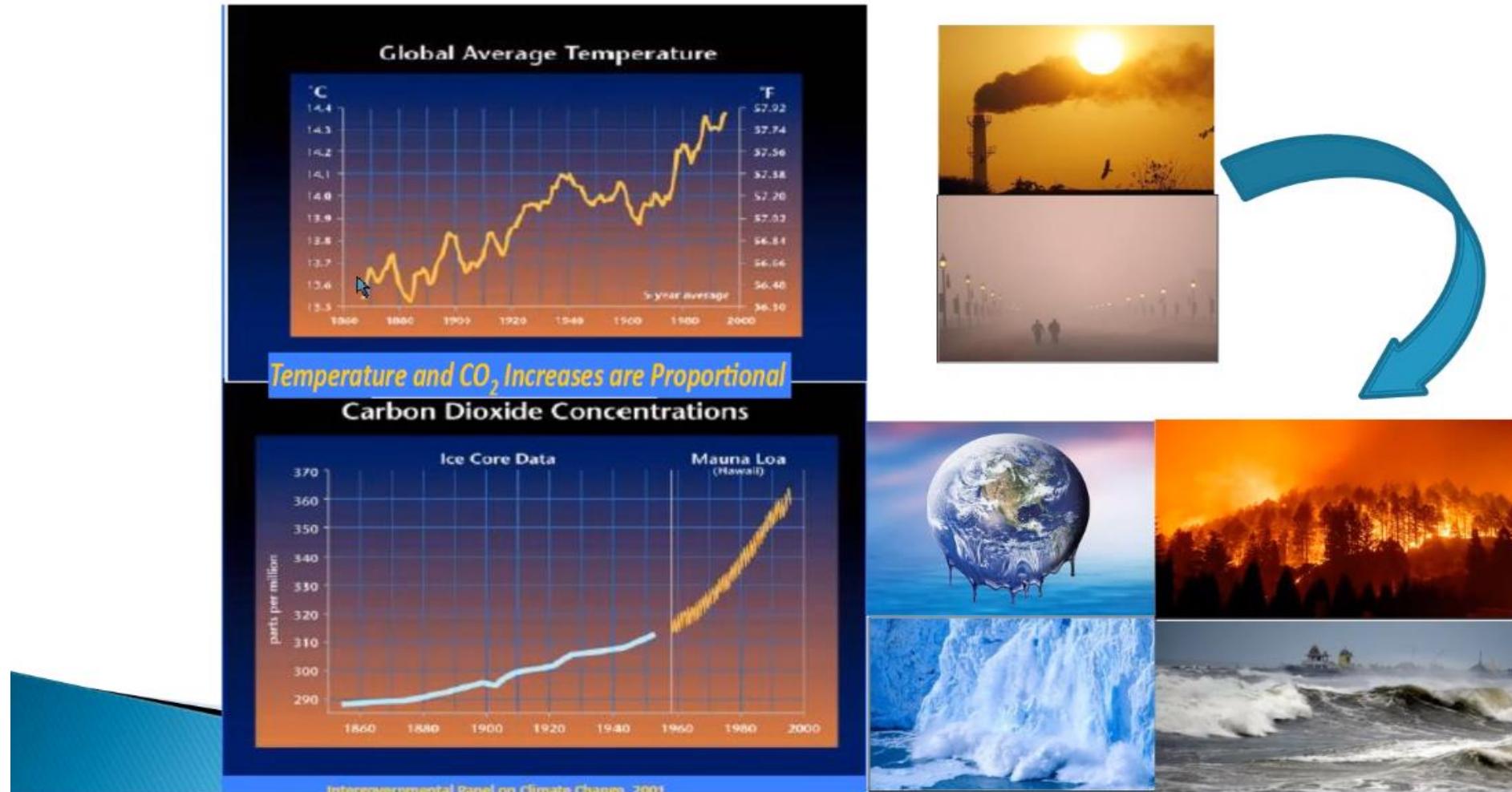


(i) Life emerges; epoch of geochemistry begins. (ii) Anoxygenic photosynthesis: start of energy epoch 2, sunlight. (iii) Emergence of cyanobacteria. (iv) Great Oxidation Event: energy epoch 3, oxygen. (v) Probable eukaryotic fossils appear. (vi) Fossils of red algae appear. (vii) Start of energy epoch 4, flesh. (viii) Vascular plants colonize land; fire appears on Earth. Finally, the burning logs indicate the start of energy epoch 5, fire. The dates of (i)–(iii) are highly uncertain. For (i) I have taken the earliest date for which there is evidence consistent with life<sup>20</sup>. For (ii) I have taken the earliest date for which there is evidence consistent with photosynthesis<sup>18,19,21</sup>. For (iii), I have marked the date currently supported by fossil evidence for the presence of cyanobacteria (see main text, ‘Cyanobacteria and the oxygenation of the air’). Tick marks represent intervals of 25 million years.

# Six Mass Extinctions

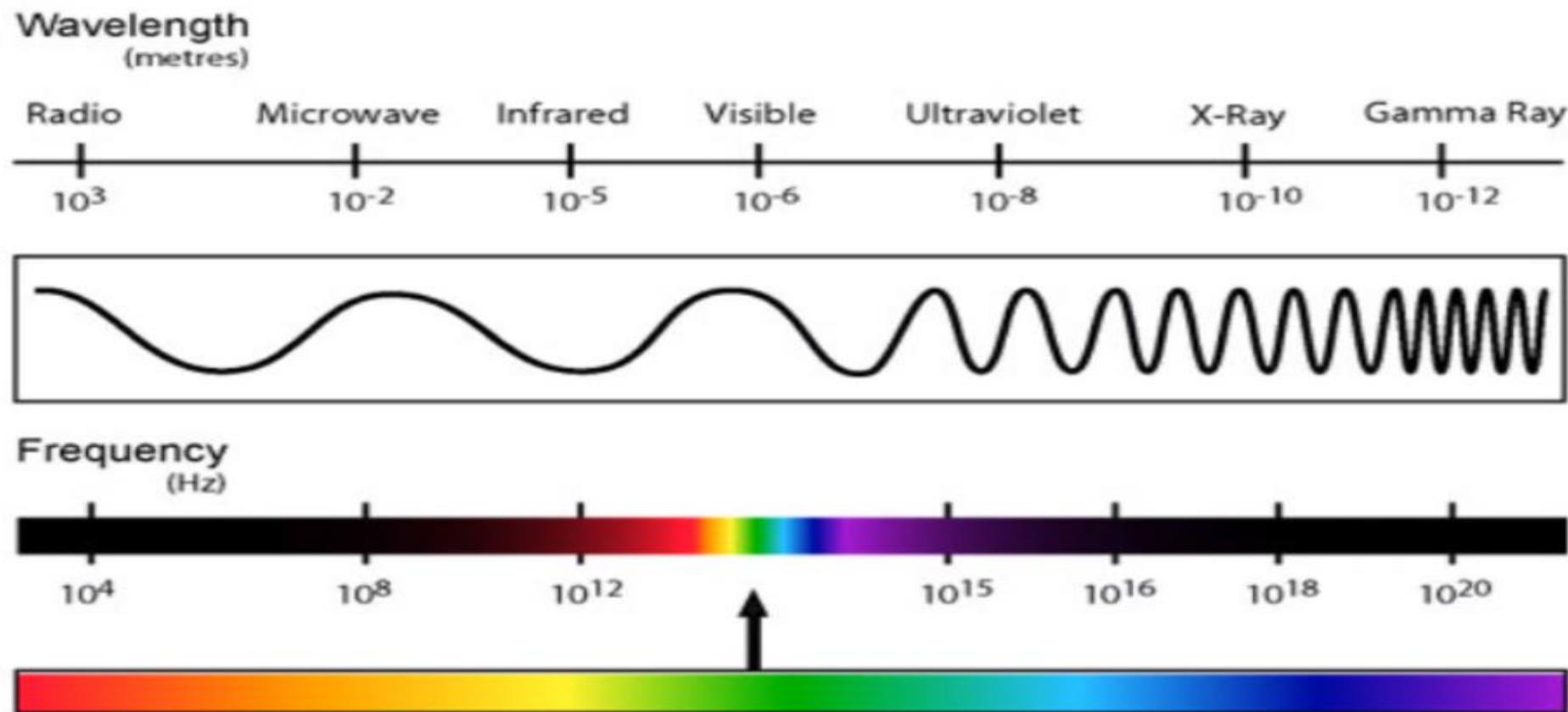
- ▶ **1<sup>st</sup> Mass Extinction:** The Great Oxidation Event occurred 2.05 to 2.4 billion years ago on our planet, resulting in a sudden increase in our planet's oxygen levels (known as “oxygen overshoot”) due to photosynthesis of ancient microorganisms and the natural weathering of rocks. However, the oxygen-producing organisms rapidly exhausted their nutrient supplies and oxygen levels in the oceans, which led to the first mass extinction two billion years ago that killed 99% of all life on Earth.
- ▶ **2<sup>nd</sup> Mass Extinction** occurred 450 million years ago, wiping off 86% of all living species.
- ▶ **3<sup>rd</sup> Mass Extinction** happened 380 million years ago, destroying 75% of all species.
- ▶ **4<sup>th</sup> Mass Extinction** happened 250 million years ago, when a sudden increase in the carbon dioxide levels raised the planet's temperature by 5°C, killing 96% of all species.
- ▶ **5<sup>th</sup> Mass Extinction** occurred 200 million years ago, destroying 80% of all species.
- ▶ **6<sup>th</sup> Mass Extinction** occurred 65 million years due to an asteroid strike on Earth, which wiped off 75% of all species, including all the dinosaurs, off the face of this planet.
- ▶ **All but one of the above 6 mass extinctions resulted from Climate Change.**

# Climate Change & the 7<sup>th</sup> Mass Extinction?



## **Heat Transfer by Radiation is an Electromagnetic Wave**

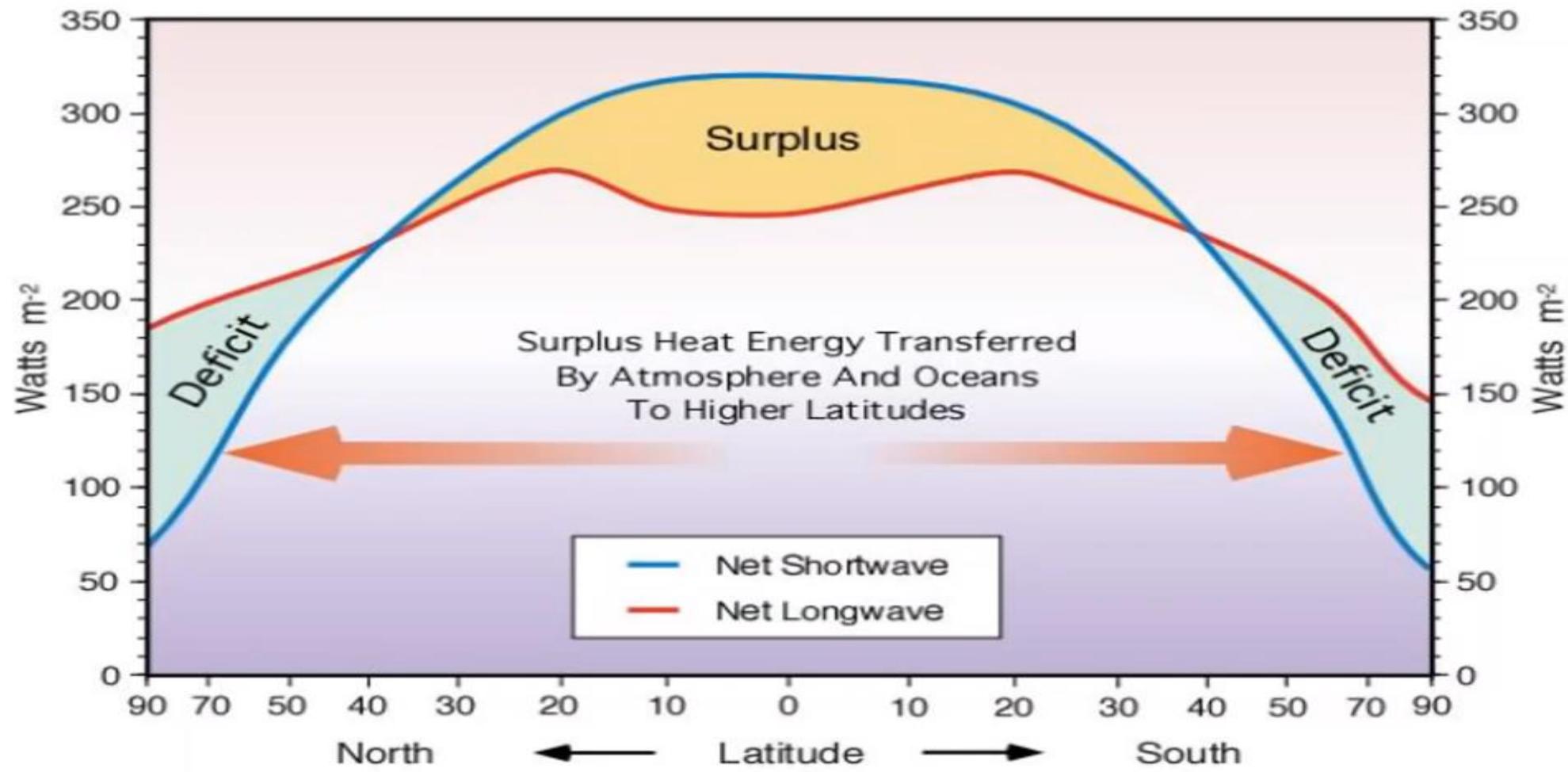
### THE ELECTRO MAGNETIC SPECTRUM



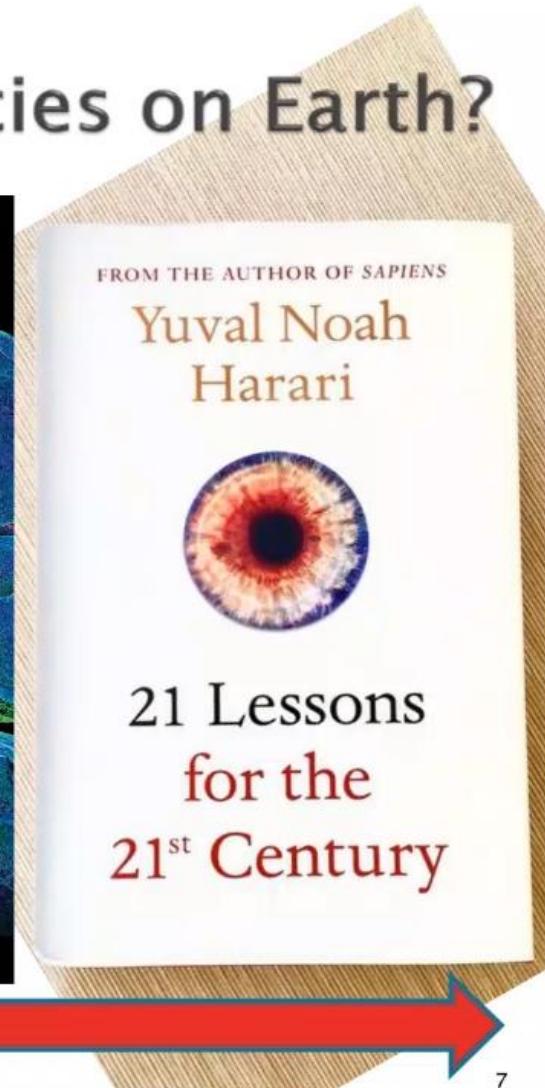
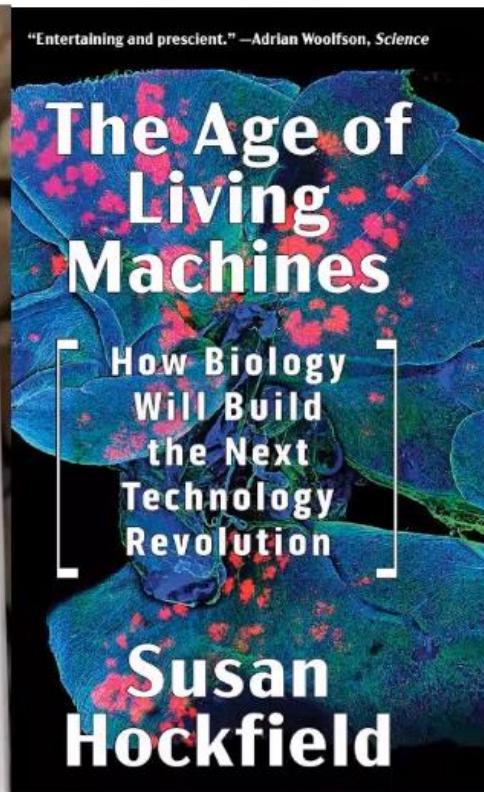
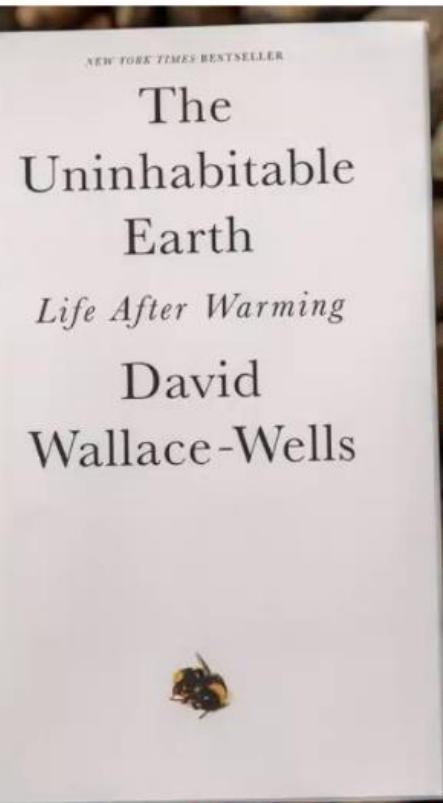
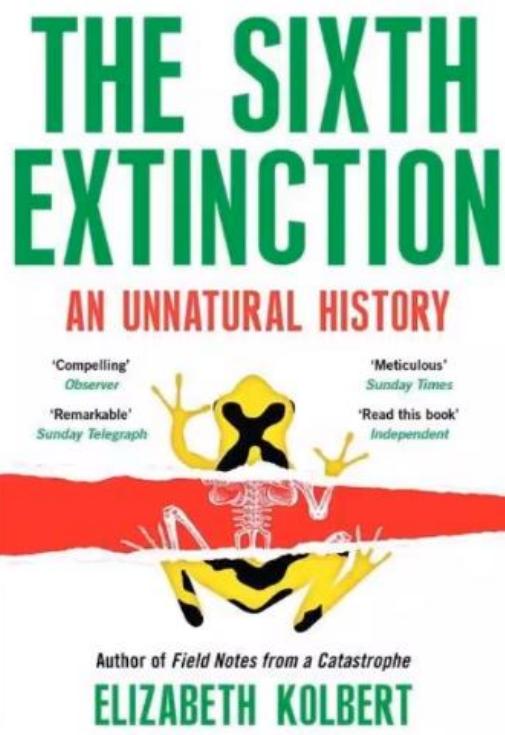
## Green House Effect

**Downward shortwave radiation comes in, but  
outgoing upward**

**Longwave radiation is trapped by atmospheric  
gasses**



# 7<sup>th</sup> Mass Extinction and/or New Species on Earth?



Time in the 21<sup>st</sup> Century

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# Evidence for early life in Earth's oldest hydrothermal vent precipitates

Matthew S. Dodd, Dominic Papineau, Tor Grenne, John F. Slack, Martin Rittner, Franco Pirajno, Jonathan O'Neil & Crispin T. S. Little

*Nature* 543, 60–64 (02 March 2017) doi:10.1038/nature21377

Although it is not known when or where life on Earth began, some of the earliest habitable environments may have been submarine-hydrothermal vents. Here we describe putative fossilized microorganisms that are at least 3,770 million and possibly 4,280 million years old in ferruginous sedimentary rocks, interpreted as seafloor-hydrothermal vent-related precipitates, from the Nuvvuagittuq belt in Quebec, Canada. These structures occur as micrometre-scale haematite tubes and filaments with morphologies and mineral

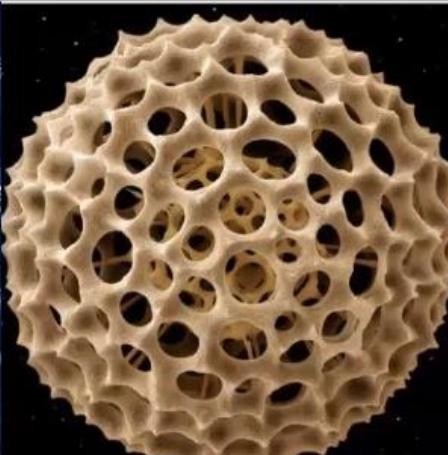
**a–f**, Tubes from the NSB. **a**, Tubes associated with iron oxide band. **b**, Depth reconstruction of tubes with haematite filament (arrow). Inset, image of tubes at the surface. **c**, Tube showing a twisted filament (red arrow) and walls (black arrow). **d**, Strongly deformed tubes. **e**, Depth reconstruction of tubes. **f**, Two tubes attached to terminal knob (arrows); lower image taken in false colour. **g, h**, Tubes from the Løkken jaspers. **g**, Tube showing filament (red arrow) and walls (black arrow). **h**, Aligned tubes (green arrows).



Ice Crystal



Turbulent Water



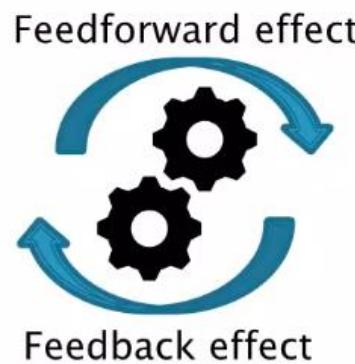
Fossilized Ancient Single Cell



Plant Cell Assembly

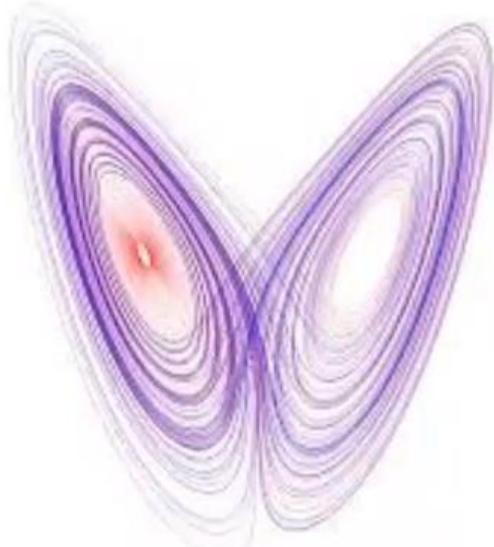
## Chemical Engineering of Life Processes:

Transport  
Processes  
(Flow)

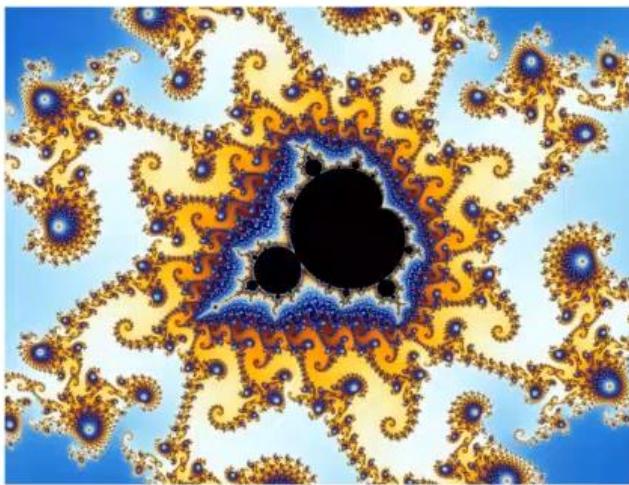


Reaction  
Processes  
(Form)

“In this way, we introduce in physics and chemistry a “historical” element, which until now seemed to be reserved for sciences dealing with biological, social, and cultural phenomena.”  
Professor Ilya Prigogine in his 1977 Nobel Prize lecture titled ‘*Time, Structure and Fluctuations*’.



Lorenz's Butterfly Attractor  
J. of Atmos. Sci., 1963



Mandelbrot Set  
Fractals: Form, Chance,  
Dimension, 1977



Jackson Pollock's Fractals;  
Dimension: 1 to 1.72

17/08/2021



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Press Ctrl+Shift+M to unmute your microphone.

# Dissipative Structures in Non-equilibrium Thermodynamics (Ilya Prigogine, Nobel Prize in Chemistry, 1977)

## Characteristics of complex systems

- ▶ they are open systems, as opposed to the closed systems of equilibrium thermodynamics that attain maximum entropy or disorder at the state of equilibrium
- ▶ they are non-linear;
- ▶ they are auto-regulatory, i.e., they regulate the very thing they arise from;
- ▶ they can attain chaos because of their sensitivity to initial conditions.

Discovered by the Belgian chemist Ilya Prigogine in 1967, dissipative structures are coherent states formed far from equilibrium through continuous input of energy. However, dissipative structures are not always coherent; small fluctuations in the system can lead to bifurcations that produce unstable, incoherent or chaotic states.

This phenomenon of non-equilibrium thermodynamics applies to all living systems, whose direction – Jeremy England (MIT) shows – determines the direction of evolution.

**CH40001 Biochemical Engineering**

**Chapter 2.**

**(a) Origin of Bacterial and Photosynthetic Life,  
(b) Cell Physiology**

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Department of Chemical Engineering

Indian Institute of Technology

# Origin of life

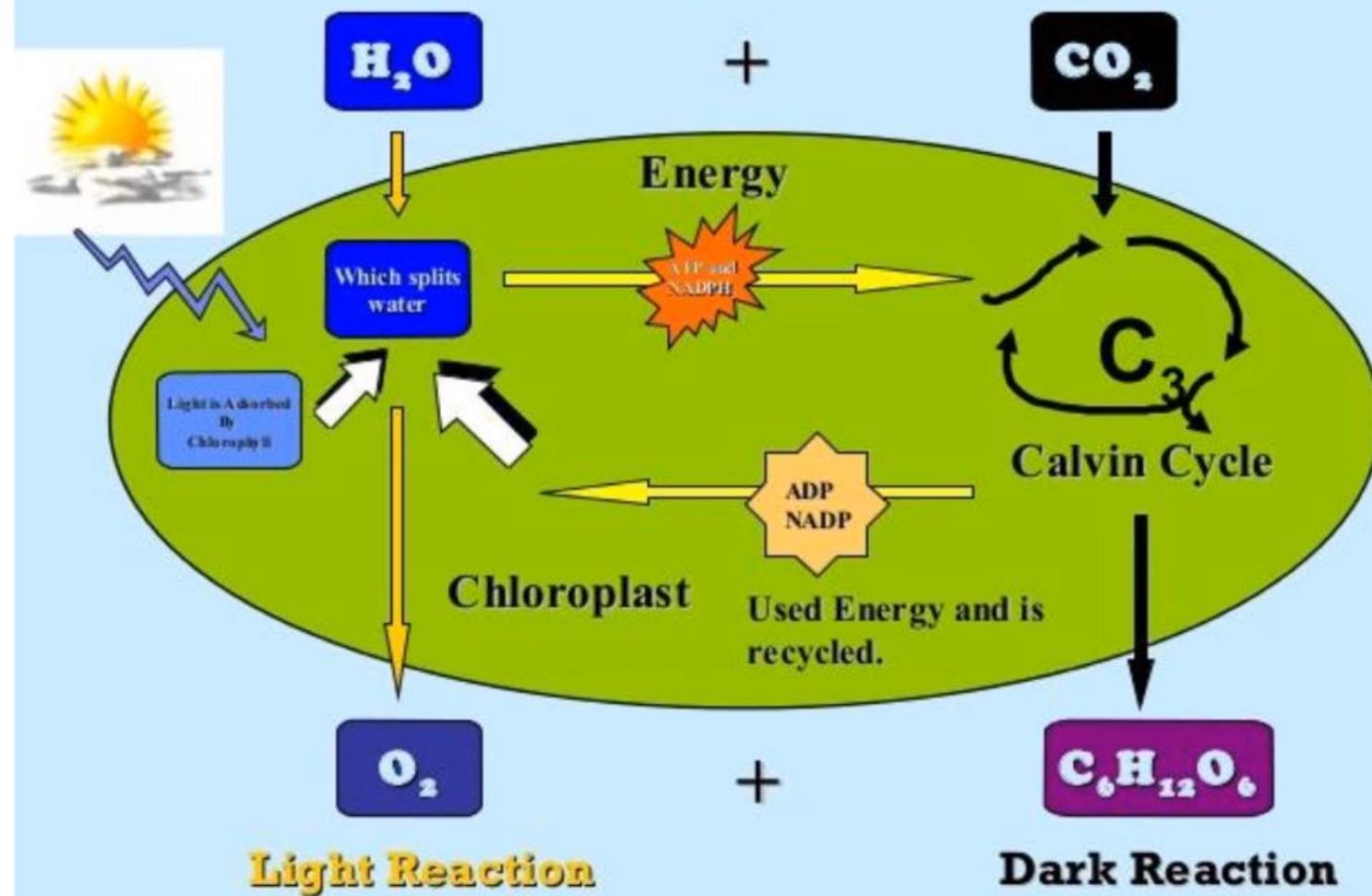
- ▶ Bacteria that thrived 4.28 billion years ago – merely 320 million years after the birth of our planet – existed at 60°C in water near volcanic ocean surfaces.
- ▶ All information point to the fact that the origin of life is rooted in the availability of heat as well as water.
- ▶ But where did this heat come from?
- ▶ 2 fundamental molecules of life – the single-strand short nucleic acid RNA that is a precursor to DNA molecules in life's evolution – are unparalleled absorbers of ultra-violet (UV) light, which in the presence of water, could rapidly convert sunlight to heat.

- The polymerization of mononucleotides to polynucleotides and the replication of life molecules RNA and DNA are endothermic processes that require heat energy.
- The RNA and DNA strands would absorb the intense UV radiation reaching the sea surface of the early Earth, and rapidly convert light into heat in the presence of seawater, thereby coupling with the water cycle and promoting evaporation.
- The presence of heat would make the endothermic process of RNA–DNA replication thermodynamically feasible. Moreover, UV light can destroy other organic molecules that catalyse the breakdown of RNA and DNA.
- Thus, life couldn't have occurred on earth without the sun's UV rays and water, with the immense heat being generated by UV rays accelerating the water cycle of seawater evaporation and rainwater precipitation.

- ▶ Illya Prigogine showed in 1967 that an entropy of a non-equilibrium process in an **open system** can decrease at the cost of another (Nobel Prize, Chemistry, 1977)
- ▶ A decrease of entropy represents localization of information, greater probability of locating a molecule in a certain region in space.
- ▶ The replication of the two life molecules RNA and DNA entailed decrease in entropy since it resulted in a higher probability of locating life in a certain region in time and space.
- ▶ But the decrease of entropy resulting in the replication of life was paid for by the acceleration of the water cycle that enhanced the disorder and randomness of water molecules in the Earth's atmosphere.

- ▶ Thus, life originated in an open system that allowed constant inflow of solar energy in the form of UV radiation followed by vibrational cooling of the UV energy by RNA and DNA molecules.
- ▶ The entropy of life molecules reduced, while the surrounding water gained disorder and entropy by evaporating from the heat dumped on it by the RNA and DNA molecules.
- ▶ While the net entropy change of the solar radiation in the atmosphere, the water molecules in sea and air, and the RNA and DNA molecules would still be positive, that of life molecules alone would be negative.

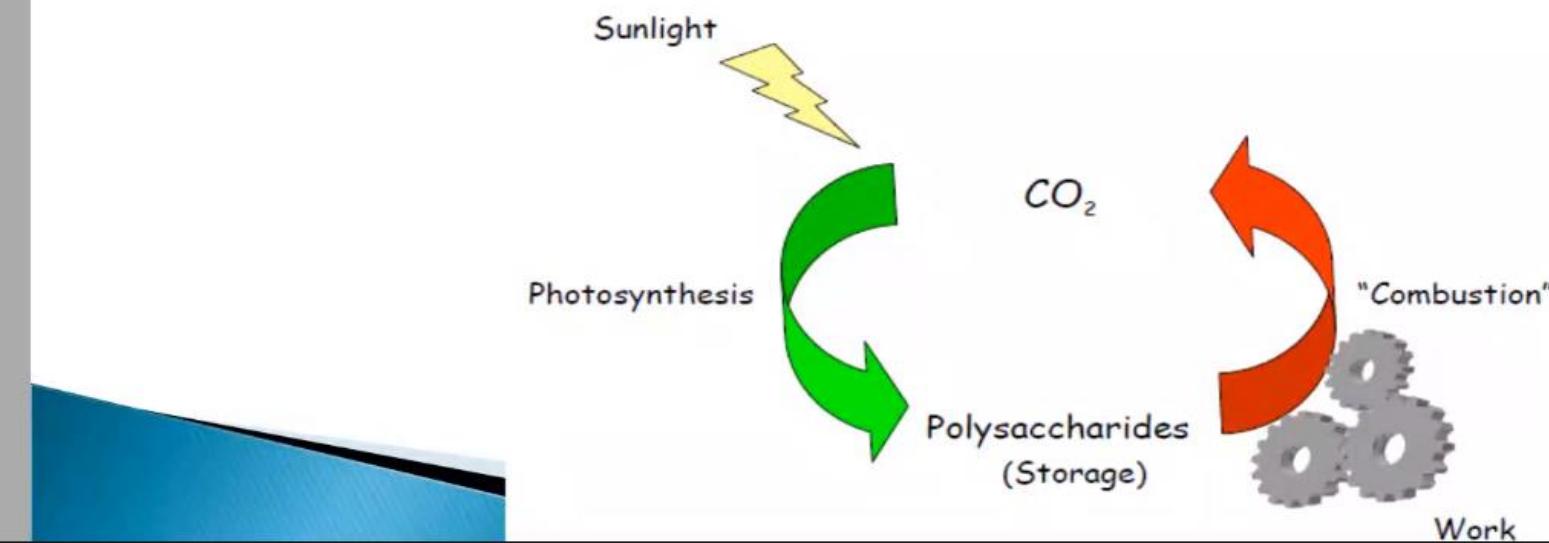
# Photosynthesis



# Thermodynamics of Photosynthesis

- ▶ In 1944, the famous quantum physicist Erwin Schrödinger in his book *What is Life?* asked, How do plants store the sun's energy in its leaves as carbohydrates?
- ▶ Plants do so by the virtue of being open systems that exhibit a non-equilibrium process (photosynthesis), which occurs far from equilibrium.
- ▶ A plant absorbs the intense solar radiation, concentrates this absorbed energy as carbohydrates through photosynthesis using water and carbon dioxide, stores this solar energy as long chain carbohydrates of simple sugars, and ejects the remaining energy as less concentrated low-energy infrared radiation to the surrounding air.
- ▶ The carbohydrates are stored in the plant cell walls as highly ordered cellulose polymer molecules and semi-ordered hemicellulose polymers.

- ▶ Plants reduce their own entropy – Schrödinger showed – by the virtue of being open systems.
- ▶ In this process of photosynthesis that occurs far from equilibrium, the plant's entropy decreases and its coherence increases, while the entropy of the atmosphere increases.
- ▶ This phenomenon of non-equilibrium thermodynamics applies to all living systems, whose direction – Jeremy England (MIT) shows – determines the direction of evolution.

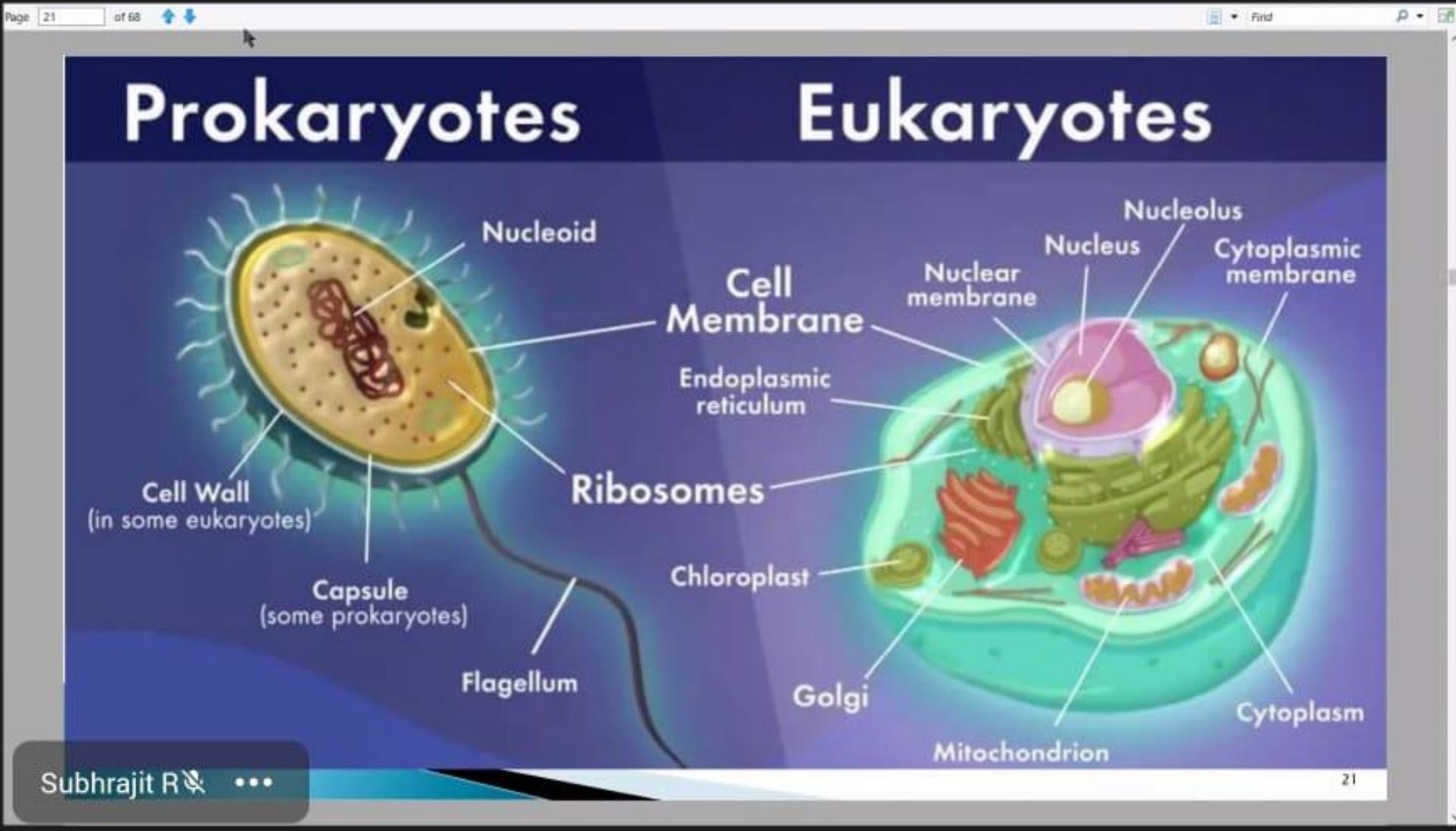


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**23/8/2021**



## Anoxygenic Photosynthesis by prokaryotes (3.7 Ga)

- ▶ Anoxygenic photosynthesis by prokaryotic bacterial cells started 3.7 Ga, which converted solar energy to intracellular chemical energy by fixing atmospheric carbon dioxide in the presence of sunlight and electron donors such as hydrogen sulfide (instead of water) to produce organic carbon and elemental sulfur (instead of oxygen).
- ▶ The energy of the photons from sunlight impinging upon its photosystem is transferred to electrons, whereby the excited electrons are harnessed to initiate the electron transport chain with the help of electron carriers such as ferrodoxin and plastoquinone, and synthesize – via cyclic photophosphorylation – a nucleic acid called Adenosine triphosphate (ATP) capable of temporarily storing intracellular chemical energy
- ▶ Anoxygenic photosynthesis might have been accompanied by photoheterotrophy in marine prokaryotes that converts organic carbon molecules in the presence of sunlight to ATP. Clearly, the two complexities that emerged in this second phase of life's evolution are: (a) the photosynthetic conversion of inorganic carbon (carbon dioxide) to organic carbon, and (b) the temporary storage of solar energy to intracellular chemical energy in the form of ATP for immediate use by the cell.

## Oxygenic Photosynthesis by marine cyanobacteria (2.75 Ga)

- a) a brand new light harvesting mechanism that consisted of photons from sunlight hydrolyzing water molecules to produce oxygen and hydrogen, and transferring the solar energy of photons to free electrons for subsequent storage in ATP molecules
- b) the release of oxygen (instead of sulfur, as in anoxygenic photosynthesis) as a product of the light reactions of photosynthesis into the seawater, while the hydrogen was transferred to methane ( $\text{CH}_4$ ) through methanogenesis
- c) the emergence of RubSiCO (ribulose-1,5-bisphosphate carboxylase oxygenase) – the planet's oldest enzyme – in the cyanobacteria's cytosol, which accelerated the rate of carbon dioxide fixation to organic carbon (3PGA) several folds by lowering the activation energy of the metabolic reactions in the Calvin cycle.
- d) the oxygen produced by photosynthesis by marine cyanobacteria dissolved in seawater where it reacted with reduced metamorphic and volcanic gases, and with oceanic cations such as  $\text{Fe}^{2+}$  to form oxides of iron, thus buffering the level of oxygen in oceans, while the methane escaped with a flux of 200 to 3000 ppmv to the upper atmosphere where it was reduced back to hydrogen by the sun's UV rays.
- e) The Great Oxidation Event occurred over 130 million years between 2.45 Gyr and 2.32 Gyr ago. The oxygen content of the Earth's atmosphere jumped from  $10^{-5}$  times the present atmospheric level of 21% before The Great Oxidation Event to 0.1-1% of the present level by 2 Gyr ago.

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Ribulose-1,5-bisphosphate carboxylase oxygenase

Carbon fixation  
done by the  
enzyme RuBisCO

photosynthesis:

$$2n \text{ CO}_2 + 4n \text{ H}_2\text{O} \xrightarrow{\text{light}} 2(\text{CH}_2\text{O})_n + 2n \text{ O}_2 + 2n \text{ H}_2\text{O}$$

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Rubisco's role is as a catalyst for the fixing of carbon by RuBP.

The diagram illustrates the process of photosynthesis within a chloroplast. Light energy from the Sun strikes the chlorophyll in the thylakoid membranes, causing the splitting of water ( $H_2O$ ) into oxygen ( $O_2$ ) and electrons. These electrons enter the electron transport chain, which includes NADP<sup>+</sup> + H<sup>+</sup> and ADP. The chain also involves ATP and NADPH. The electrons return to the chlorophyll, completing the cycle. The energy from this chain drives the Calvin cycle. In the Calvin cycle,  $CO_2$  is fixed onto RuBP (ribulose bisphosphate) to form PGA (3-phosphoglycerate). PGA is then converted into Sugars. The diagram also shows the Stroma (gelatinous matrix inside membranes), Intermembrane space, Outer membrane, and Grana (stack of thylakoids).

- Rubisco is thought to be the most abundant protein in the world since it is present in every plant that undergoes photosynthesis and molecular synthesis through the Calvin cycle.
- It makes up 20-25% of soluble protein in leaves and is made on the Earth @ 1000 kg/s. They estimate that every person on Earth is supported by about 44 kg of rubisco!
- Rubisco has a molecular weight of 490,000 Daltons and is composed of 8 large (in grey & white) & 8 small (in blue & orange) chains/ subunits.
- It can survive on its own without the need of the plant so even if it is dead it remains and helps decomposition. This is due to it not being much affected by temperature or pH.

Subhrajit R ✎ ...

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### Phototrophic C assimilation and Photorespiration in the Stroma of the Chloroplast

The diagram illustrates the process of phototrophic C assimilation and photorespiration in the stroma of the chloroplast. It shows the RuBisCo enzyme catalyzing the fixation of CO<sub>2</sub> into 3-phosphoglycerate (3-PGA). This is followed by two phases: Phase 1 (Carbon Fixation) leading to 1,3-bisphosphoglycerate, and Phase 2 (Reduction) leading to Glycerdehyde 3-phosphate (G3P). G3P is then converted into inorganic phosphate and used for the regeneration of RuBP. The diagram also shows the conversion of G3P into CH<sub>2</sub>O. A separate section on photorespiration shows the cycle involving Rubisco, O<sub>2</sub>, and CO<sub>2</sub>, producing PGA and regenerating RuBP.

**Central Metabolic Pathways**

**RuBisCo**

**Phase 1: Carbon Fixation**

**Phase 2: Reduction**

**CO<sub>2</sub>**

**3-PGA**

**3-phosphoglycerate**

**Glyceraldehyde 3-phosphate (G3P)**

**Inorganic phosphate**

**ATP**

**ADP**

**RuBP**

**Ribulose 1,5-bisphosphate**

**Ribulose 5-phosphate**

**1,3-bisphosphoglycerate**

**NADPH**

**CH<sub>2</sub>O**

**C<sub>3</sub> carbon fixation**

**Photorespiration**

**Rubisco**

**ATP**

**NADPH**

**CO<sub>2</sub>**

**O<sub>2</sub>**

**RuBP**

**PGA**

**PG + PGA**

**ATP**

**NADPH**

**26**

**3 Thylakoid**

- 3.1 Thylakoid space (lumen)
- 3.2 Thylakoid membrane

**2 Chloroplast envelope**

- 2.1 Outer membrane
- 2.2 Intermembrane space
- 2.3 Inner membrane

**1 Granum**

**4 Stromal thylakoids (lamellae or frusts)**

**5 Granal thylakoids**

**6 Stroma**

**7 Nucleoid (DNA rings)**

**8 Ribosome**

**9 Plastoglobulus**

**10 Starch granule**

Subhrajit R

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LTEVo  
LTE

4G+

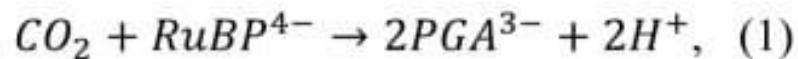


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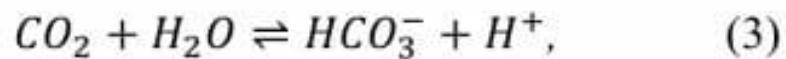
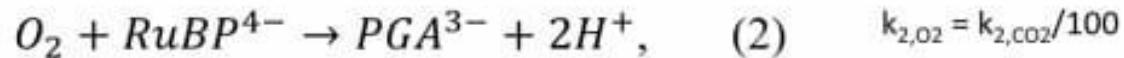
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## Biochemical reactions showing how photorespiration competitively inhibits RuBisCo-catalyzed photosynthesis



$k_{2,CO_2}=1-10\text{ s}^{-1}$ ,  $k_{2,CO_2}/K_m,CO_2=0.5-5\times 10^5\text{ M}^{-1}\text{ s}^{-1}$   
( $2.5-5\times 10^5\text{ M}^{-1}\text{ s}^{-1}$  in higher plants).



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Apurva R

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Ritam M

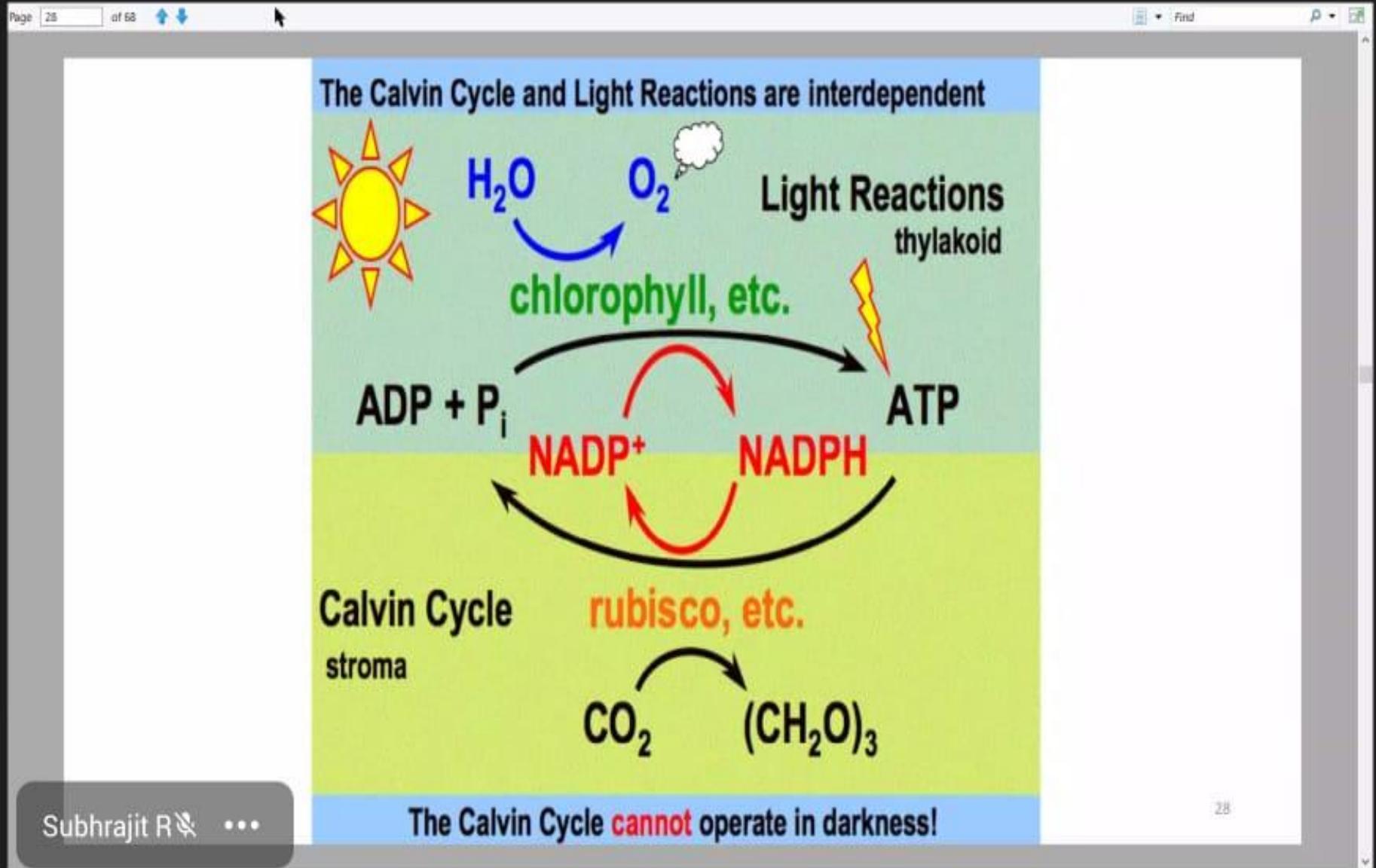


Saikat C

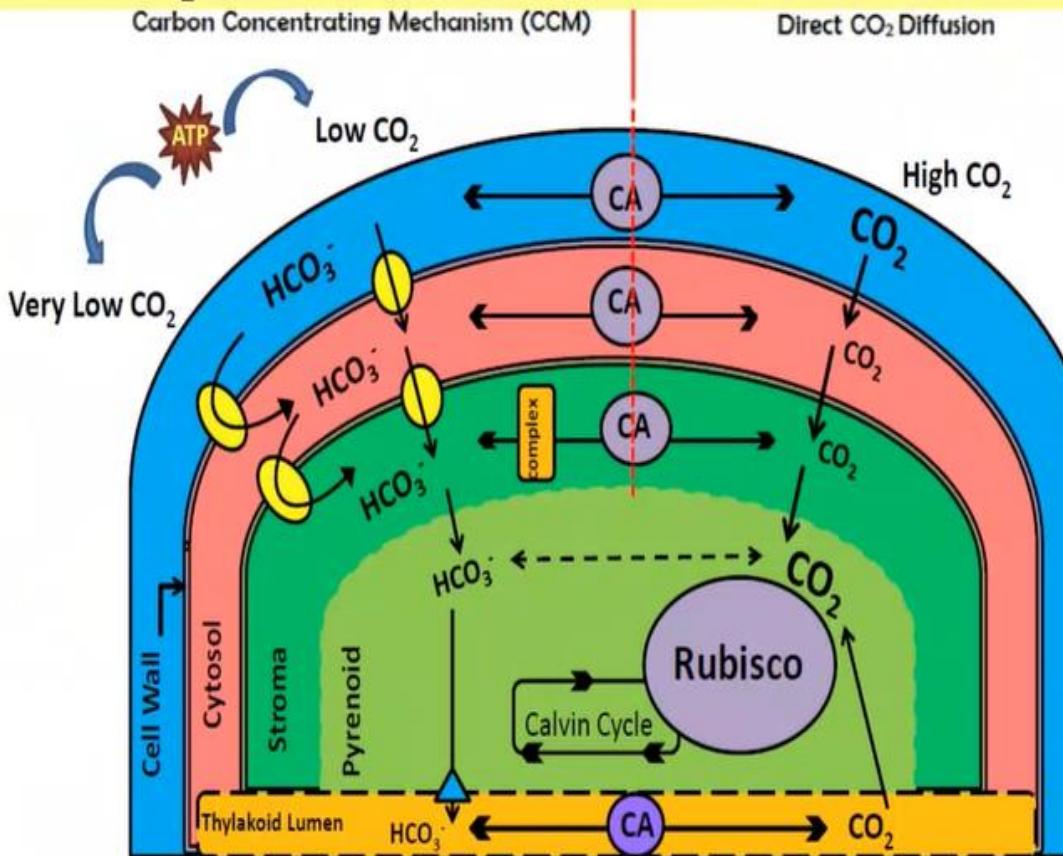


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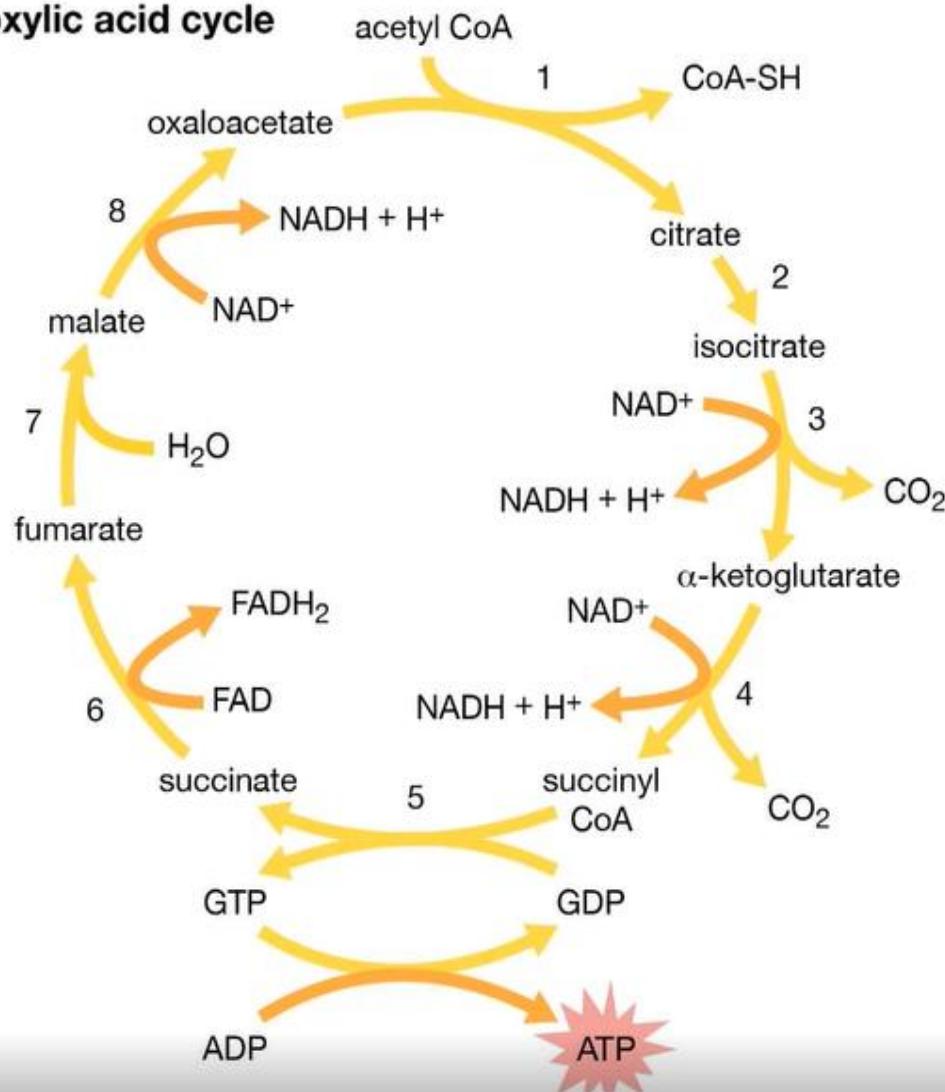
## Photosynthesis: CO<sub>2</sub> Acclimatization & Carbon Concentrating Mechanism (CCM)



**CCM** comprises of three energized, functionally interactive systems:

- inorganic carbon ( $\text{C}_i$ ) uptake systems,
- enzymatic systems interconverting different  $\text{C}_i$  species
- microcompartments where Rubisco is sequestered.

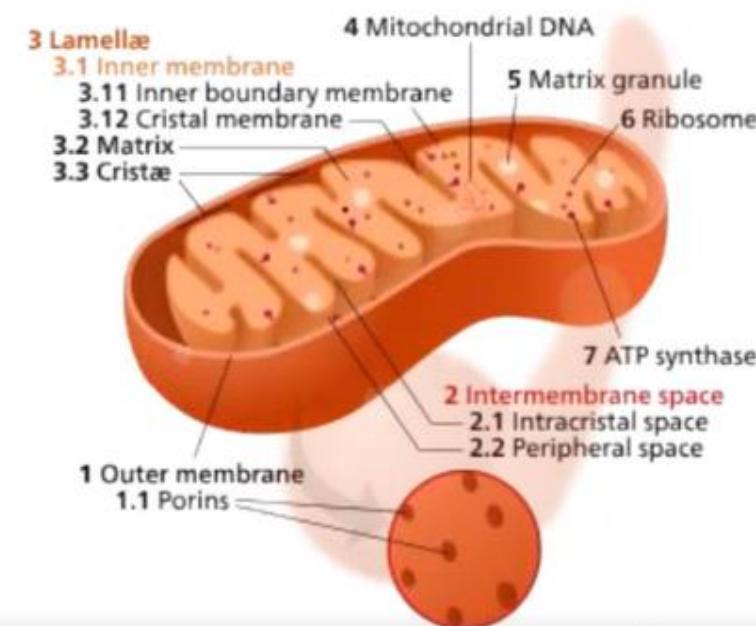
### Tricarboxylic acid cycle

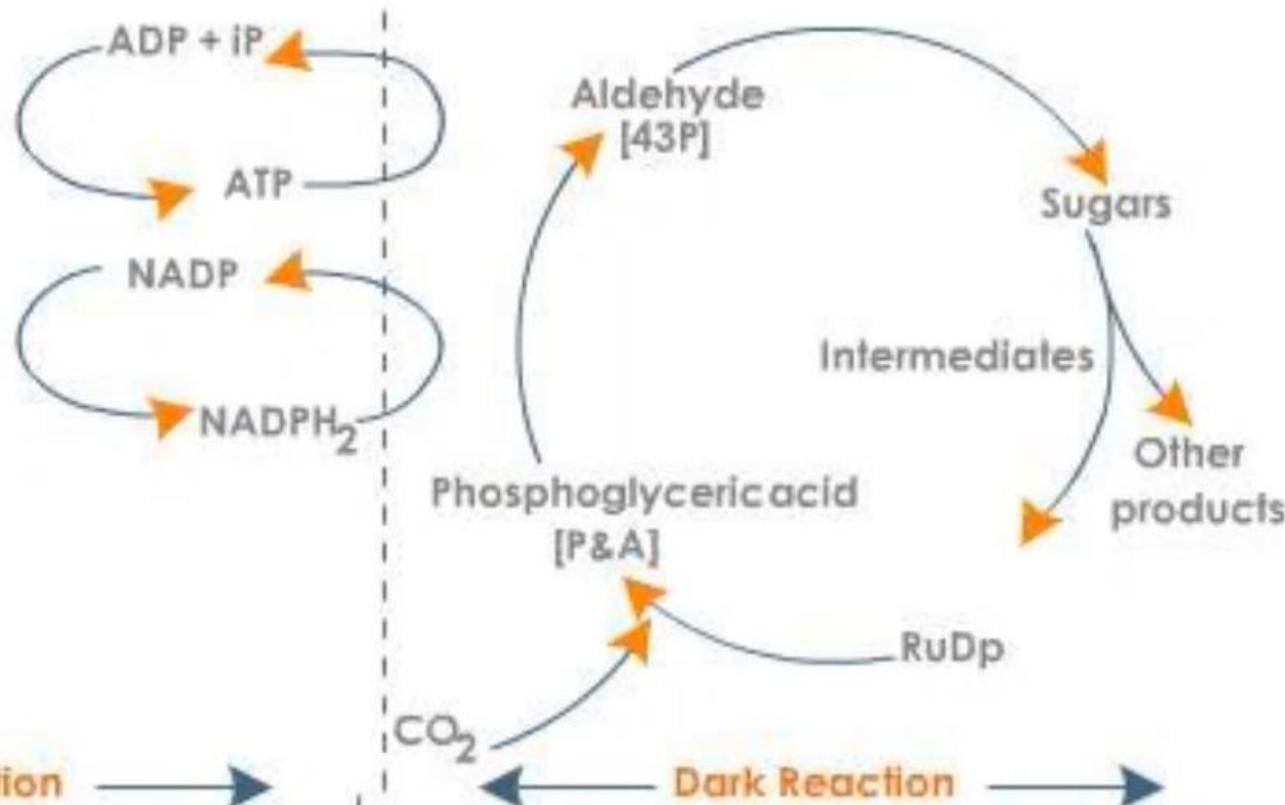
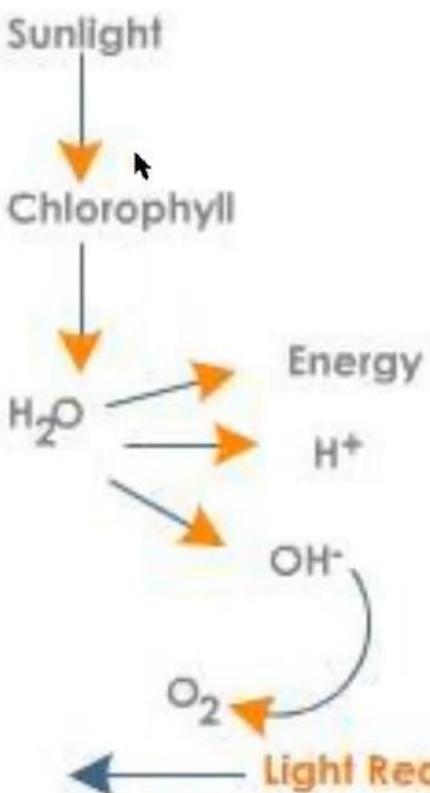


## CELLULAR RESPIRATION in the MITOCHONDRION in EUKARYOTES

TCA (Tricarboxylic Acid Cycle) or Krebs

Cycle occurs in the mitochondrion in eukaryotes, and in the cytosol in prokaryotes

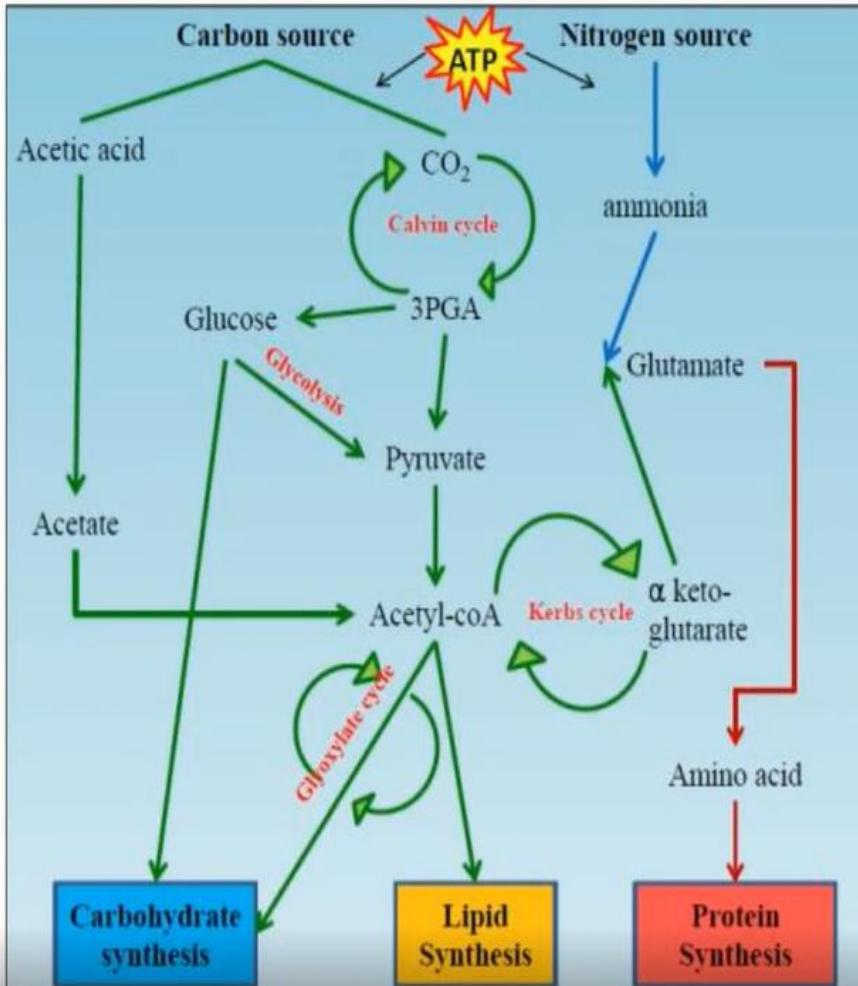




### Summary of photosynthesis



## Nutrient Flow Pathway in Photosynthetic Dark Reactions



- The environment of photosynthetic cells is responsible for its yield and macromolecular composition.
- Variation of parameters (such as sunlight, CO<sub>2</sub> levels, Nitrogen and Organic carbon sources and concentrations) can direct metabolic fluxes towards targeted metabolite production

24/08/2021

# Chapter 2b. Cell Physiology

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# The cell: an introduction

A detailed diagram of a eukaryotic cell with various organelles labeled:

- mitochondrion
- ribosome
- rough endoplasmic reticulum
- plasma membrane
- cytoplasm
- microtubules (part of cytoskeleton)
- lysosome
- nucleus
- nucleolus
- chromatin
- nuclear pore
- nuclear envelope
- Golgi complex
- smooth endoplasmic reticulum
- free ribosome
- centriole

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Saika



Marapal

SR

## Range of cell sizes

Cell Type	Diameter
Viruses (not a cell)	0.03 - 0.1 $\mu\text{m}$
Mycoplasmas	0.1 - 1.0 $\mu\text{m}$
Most bacteria	1.0 - 10 $\mu\text{m}$
Most human cells	10 - 100 $\mu\text{m}$



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Marapaka I

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## How we study cells:Cell Fractionation

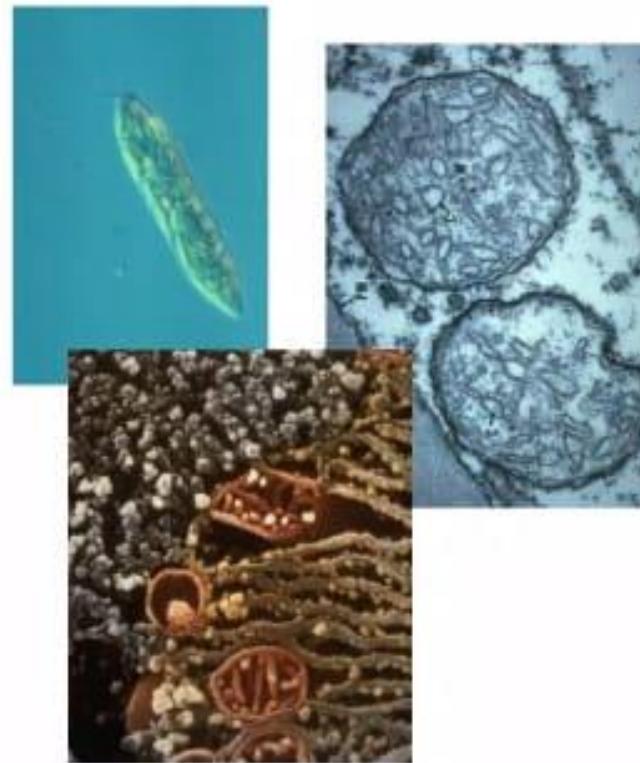
- Disrupt Cells using a blender or sonication
- Centrifuge slowly to sediment larger components (nuclei)
- Centrifuge rapidly to sediment smaller components (mitochondria)
- Supernatant (residual fluid) contains soluble cell components

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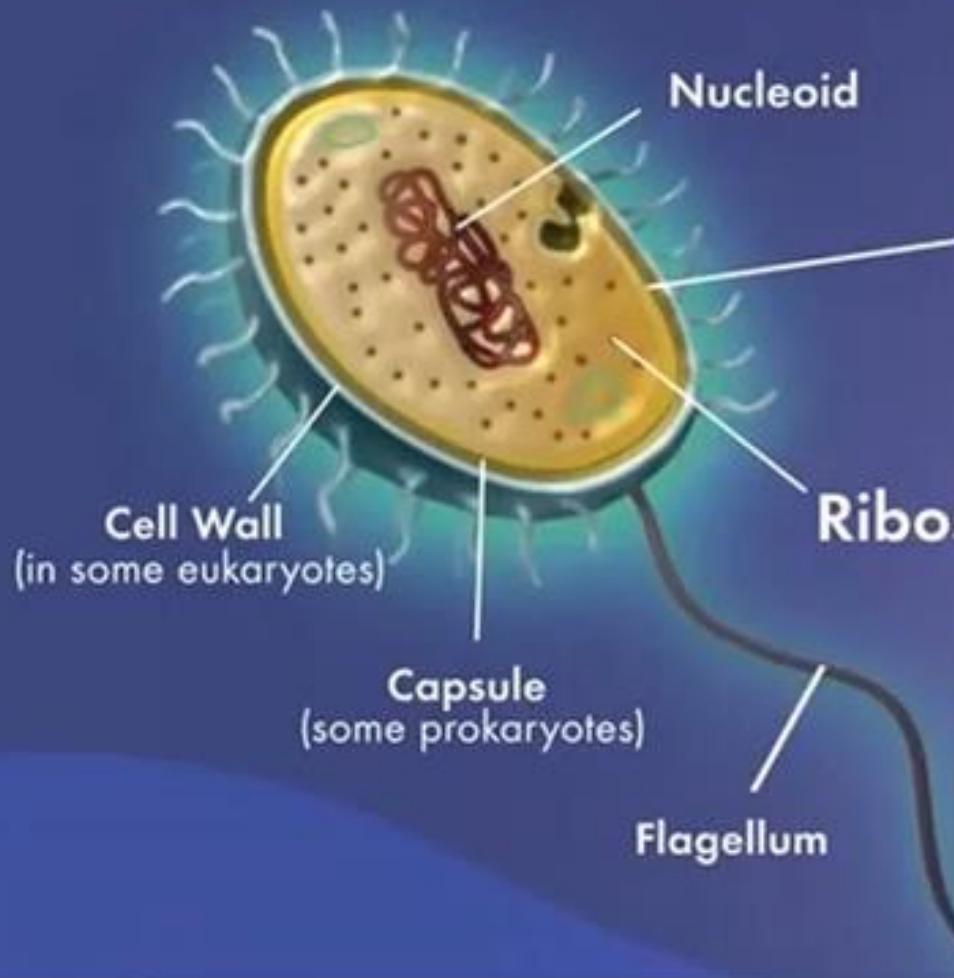
40

## How we study cells: Microscopy

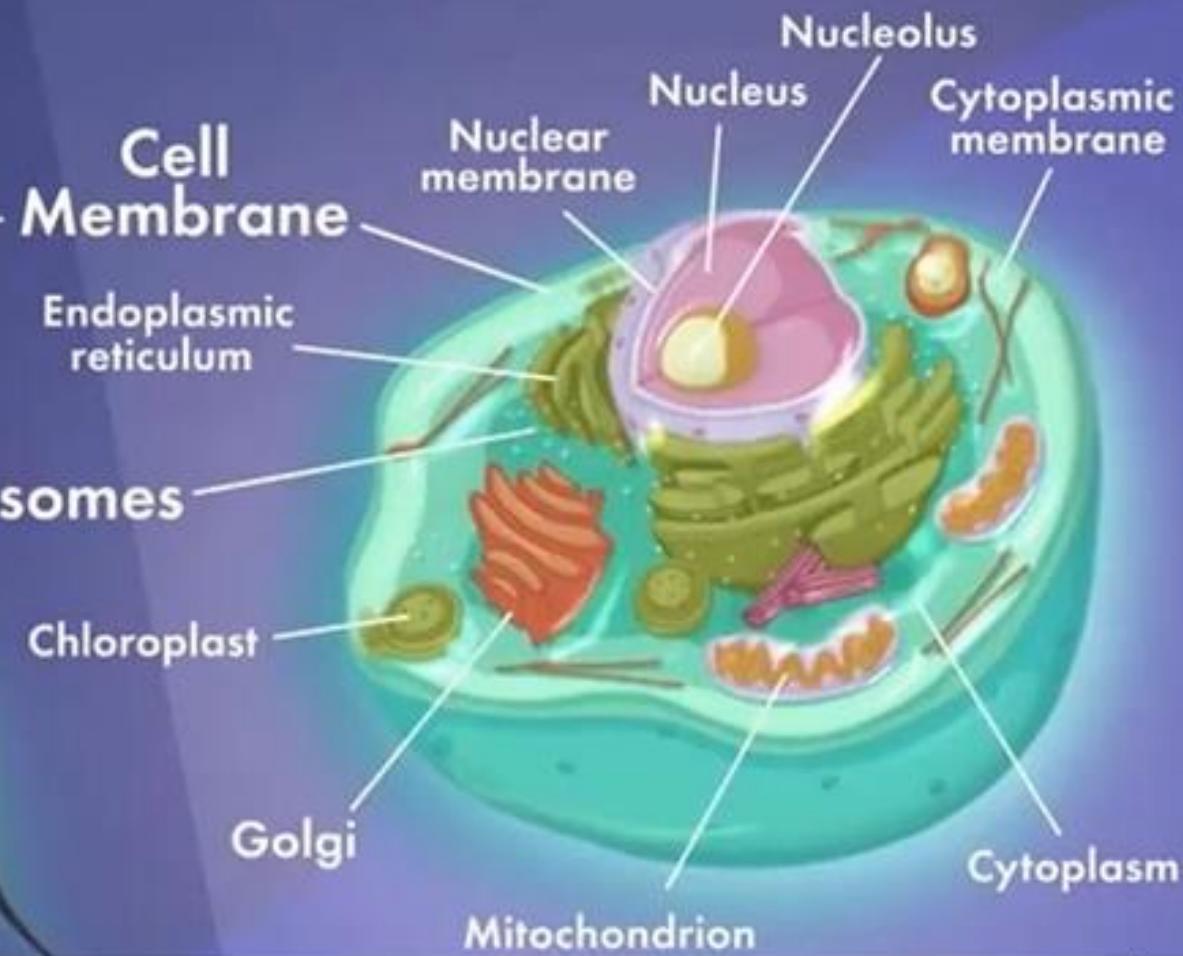
- Light microscopy
- Transmission electron microscopy (TEM)
- Scanning electron microscopy (SEM)



# Prokaryotes



# Eukaryotes



## Prokaryotic Cells: Structure and Function (e.g., bacteria, archaea)

Capsules and slime layers	Resistance to phagocytosis, adherence to surfaces
Cell wall	Gives bacteria shape and protection from lysis in dilute solutions
Endospore	Survival under harsh environmental conditions
Fimbriae and pili	Attachment to surfaces, bacterial mating
Flagella	Provides the power of motility or self-propulsion
Gas vacuole	Buoyancy for floating in aquatic environments.
Inclusion bodies	Storage of carbon, phosphate, and other substances
Nucleoid	Localization of genetic material (DNA)
Periplasmic space	Contains hydrolytic enzymes and binding proteins for nutrient processing and uptake'
Plasma membrane	Selectively permeable barrier, mechanical boundary of cell, nutrient and waste transport, location of many metabolic processes (respiration, photosynthesis), detection of environmental cues for chemotaxis
Ribosomes	Protein synthesis



Eukaryotic Cells: Structure & Function (e.g., fungi, protozoa, algae, plants, animals)	
<b>Cell wall and pellicle</b>	Strengthen and give shape to the cell
<b>Chloroplasts</b>	Photosynthesis—trapping light energy and formation of carbohydrate from CO <sub>2</sub> and water
<b>Cilia and flagella</b>	Cell movement
<b>Cytoplasmic matrix</b>	Environment for other organelles, location of many metabolic processes
<b>Endoplasmic reticulum</b>	Transport of materials, protein and lipid synthesis
<b>Golgi apparatus</b>	Packaging and secretion of materials for various purposes, lysosome formation
<b>Lysosomes</b>	Intracellular digestion
<b>Microfilaments, intermediate filaments, and microtubules</b>	Cell structure and movements, form the cytoskeleton
<b>Mitochondria</b>	Energy production through use of the tricarboxylic acid cycle, electron transport, oxidative phosphorylation, and other pathways
<b>Nucleolus</b>	Ribosomal RNA synthesis, ribosome construction
<b>Nucleus</b>	Repository for genetic information, control centre for cell
<b>Plasma membrane</b>	Mechanical cell boundary, selectively permeable barrier with transport systems, mediates cell-cell interactions and adhesion to surfaces, secretion
<b>Ribosomes</b>	Protein synthesis
<b>Vacuole</b>	Temporary storage and transport, digestion (food vacuoles), water balance (contractile vacuole)

Characteristics	Prokaryote	Eukaryote
Size	Typically 1 – 5 $\mu\text{m}$	Normally greater than 10 $\mu\text{m}$
Cell nucleus	Do not possess a true nucleus	Have a nucleus surrounded by a nuclear membrane
Location of chromosomes	In the cytoplasm, usually attached to the cell membrane	Within a true nucleus separated from the cytoplasm by a nuclear membrane
Nuclear division and reproduction	Mitosis and meiosis are absent so reproduction is asexual	Exhibit both mitosis and meiosis, so reproduction may be sexual or asexual or both depending on species
Nucleolus	Absent	Present
Genetic variation	Resulting largely from mutations	Resulting both from mutations and the creation of new gene combinations during sexual reproduction
Mitochondria, chloroplasts and ribosomes	Mitochondria and chloroplasts absent; ribosome size is 70s	Mitochondria and chloroplasts may be present; ribosomes larger: 80s
Chemical composition	Do not possess sterols in the cell membrane but do usually have peptidoglycan in the cell walls	Do possess sterols in the cell membrane but no peptidoglycan in the walls
Flagella	Structurally simple	Structurally complex
Pili	Present	Absent
Storage compounds	Poly - $\beta$ - hydroxybutyrate often present	Poly - $\beta$ - hydroxybutyrate absent

## EUKARYOTIC CELL ORGANELLES

- Cell Membrane
- Nucleus
- Endoplasmic reticulum
- Golgi complex
- Mitochondria
- Lysosomes
- Microfilaments and microtubules
- Vesicles



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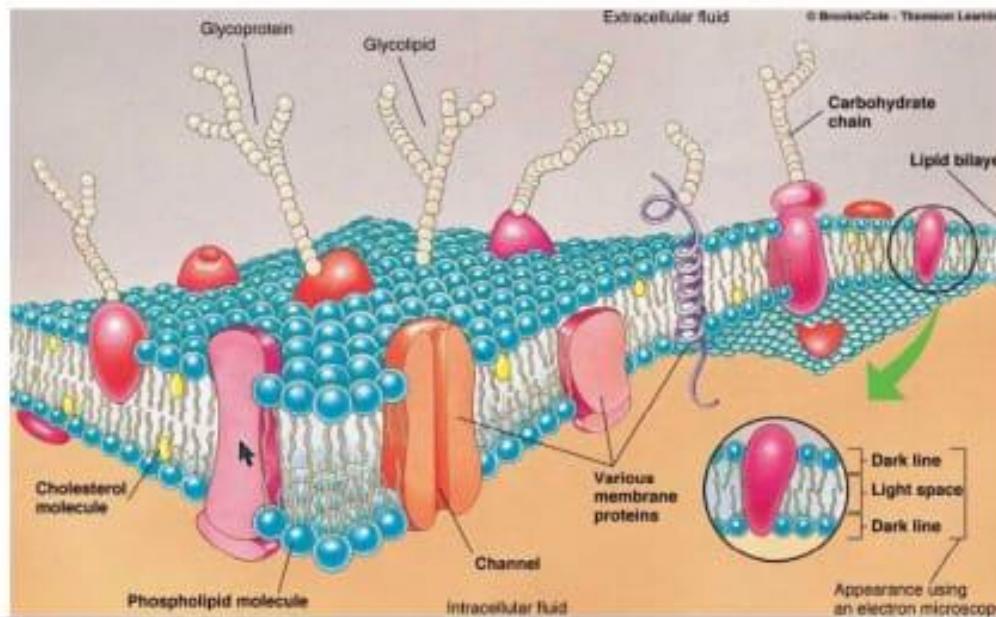


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## The plasma membrane is a fluid lipid bilayer embedded with proteins

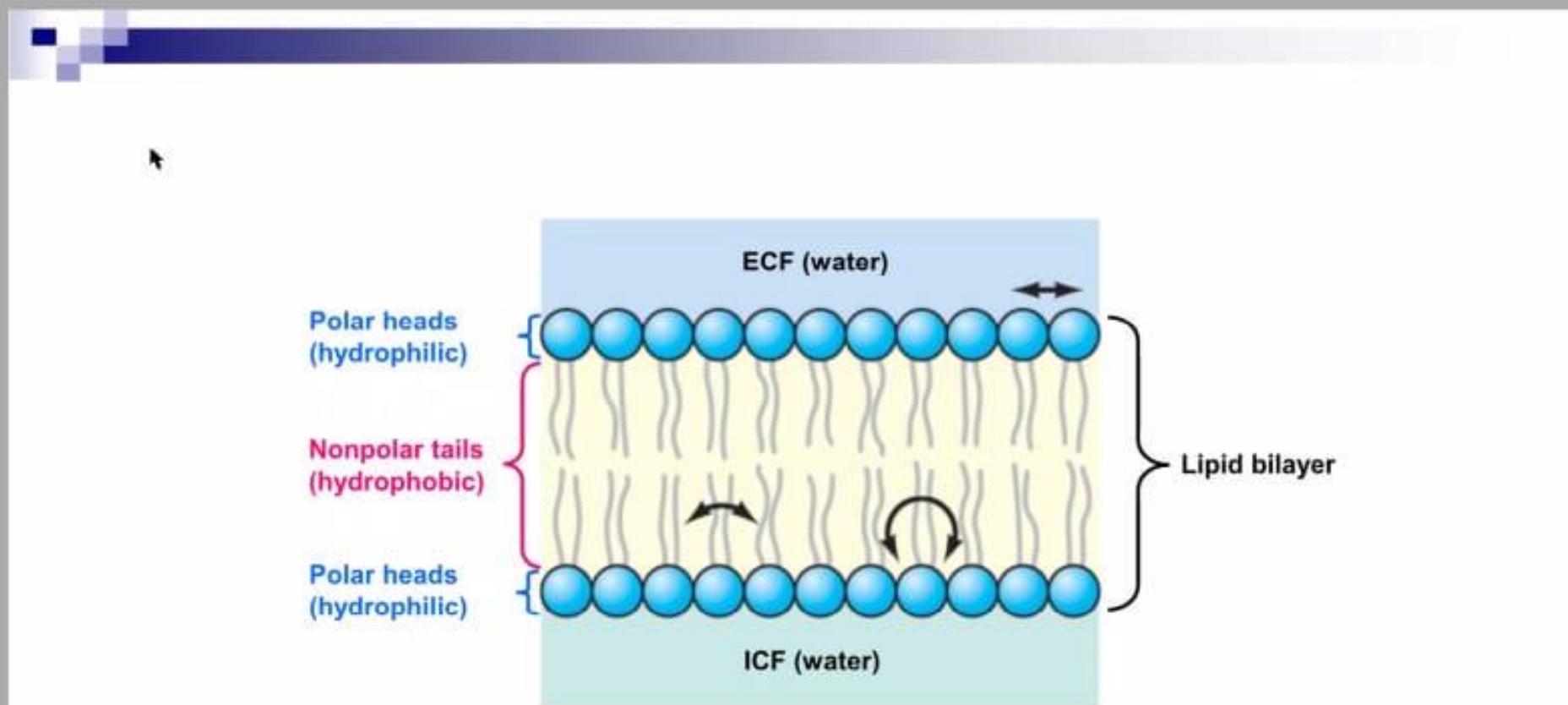
- *Phospholipids form a bilayer. The bilayer has a hydrophobic interior. This interior is sandwiched between hydrophilic inner and outer surfaces.*
- *Carbohydrates are attached to its outer surface.*
- *Cholesterol molecules are tucked between the phospholipid molecules.*
- *Its EM appearance is trilaminar.*



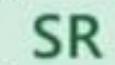
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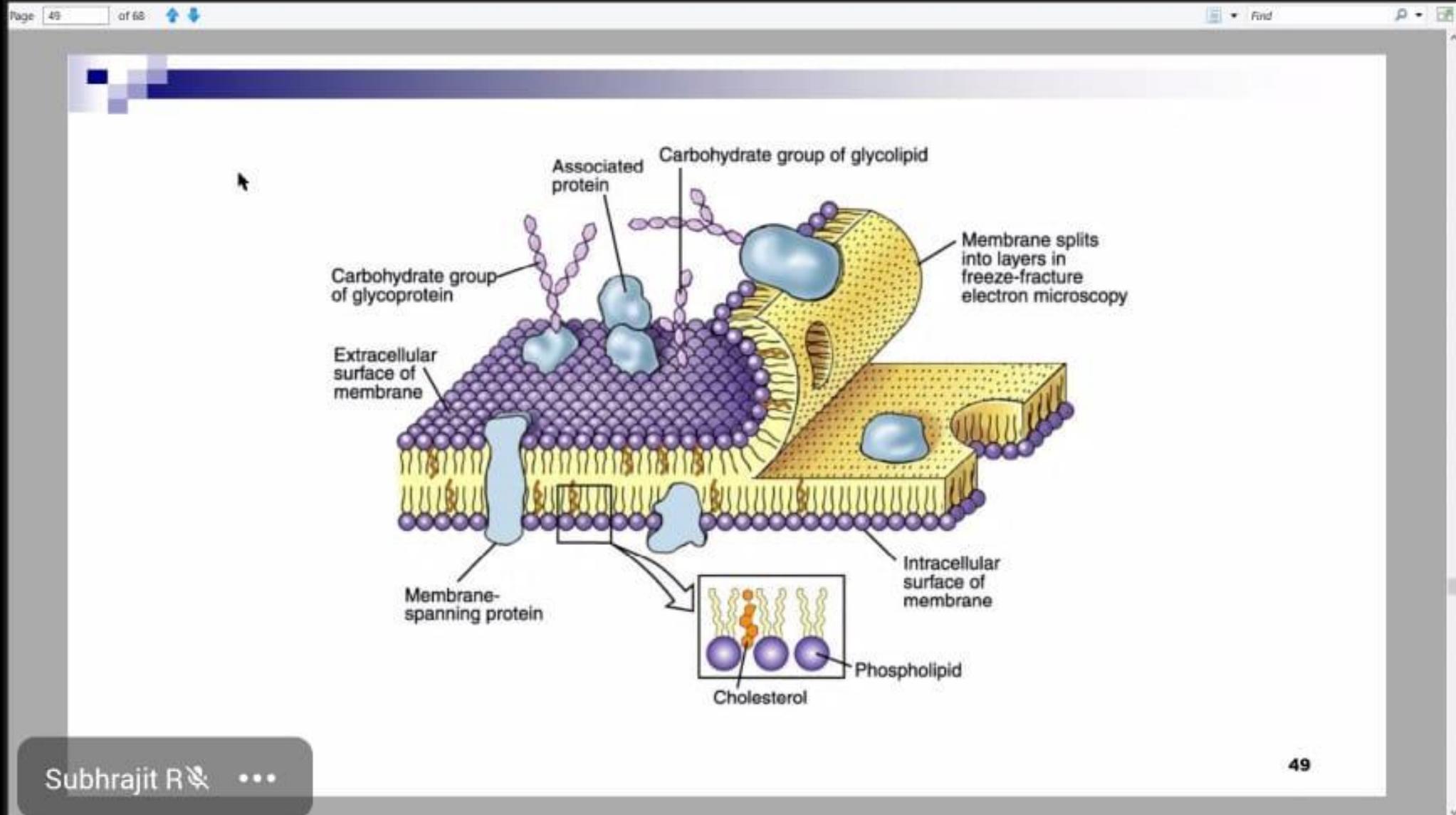


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Subhrajit





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## The lipid bilayer has three functions

- *It forms the basic structure of the membrane.*
- *Its hydrophobic interior is a barrier to water-soluble substances.*
- *It allows the membrane to be fluid.*

### **The membrane proteins have many functions.**

- *Some span the membrane as water-filled channels.*
- *Others serve as carrier molecules.*
- *Some serve as docking-marker acceptors.*
- *Membrane-bound enzymes are on the surface.*
- *Receptor sites are proteins that bind with specific molecules.*
- *Some proteins are cell adhesion molecules.*

# Membrane proteins

Proteins can also attach to the membrane via covalently linked fatty acid moieties

The diagram illustrates several types of membrane proteins embedded in a lipid bilayer. Some proteins have long, hydrophobic alpha-helical regions spanning the bilayer, while others are peripheral proteins associated with the membrane. One protein is shown with a covalently linked fatty acid moiety attached to a phosphate group (P) on the cytosolic side. The diagram includes labels for the 'lipid bilayer', 'CYTOSOL', and numbered proteins (1, 2, 3, 4, 5).

lipid bilayer

CYTOSOL

COOH

NH<sub>2</sub>

① ② ③ ④ ⑤

Membrane-spanning regions are  $\alpha$ -helical, made of hydrophobic AA's

Peripheral membrane proteins are associated with the membrane, but are not directly attached

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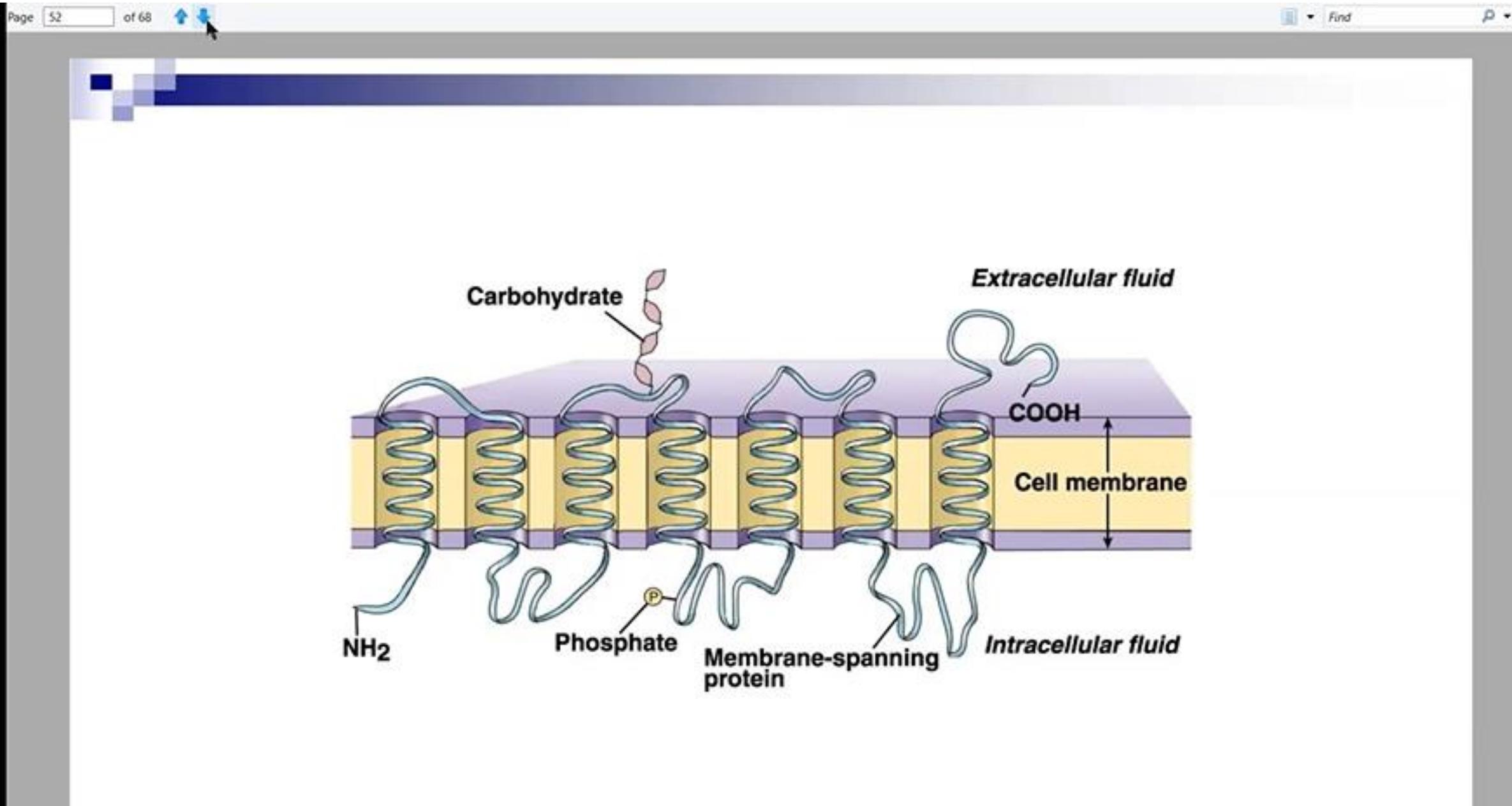


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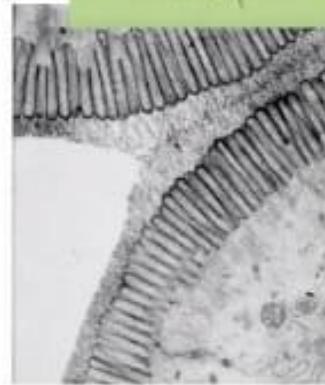
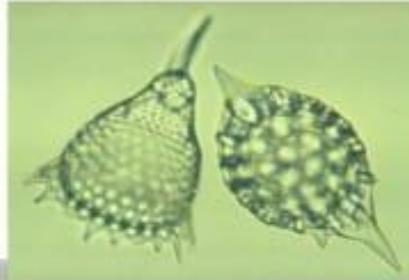




Page 54 of 68 ⏷ ⏵

# The cell surface

- Most cells have some coat external to the plasma membrane.
- Frequently these coats are of extreme importance to the cell.
  - Protection
  - Recognition
- Cell walls (plants)
- Glycocalyx (animals)



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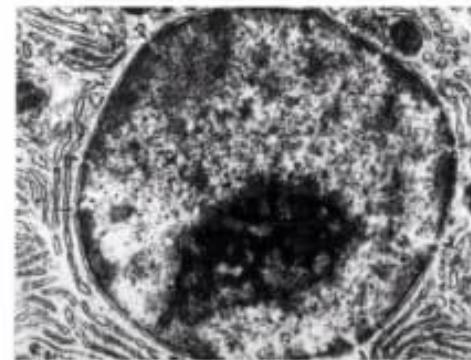


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## The nucleus: Information central

- Controls operation of eukaryotic cell
- Largest “organelle”
- Houses **chromosomes**
- **Genes**



A circular profile picture of a person with glasses and a blue shirt.

A circular profile picture of a person with dark hair and a plaid shirt.

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A green circular icon containing the letters "SR".

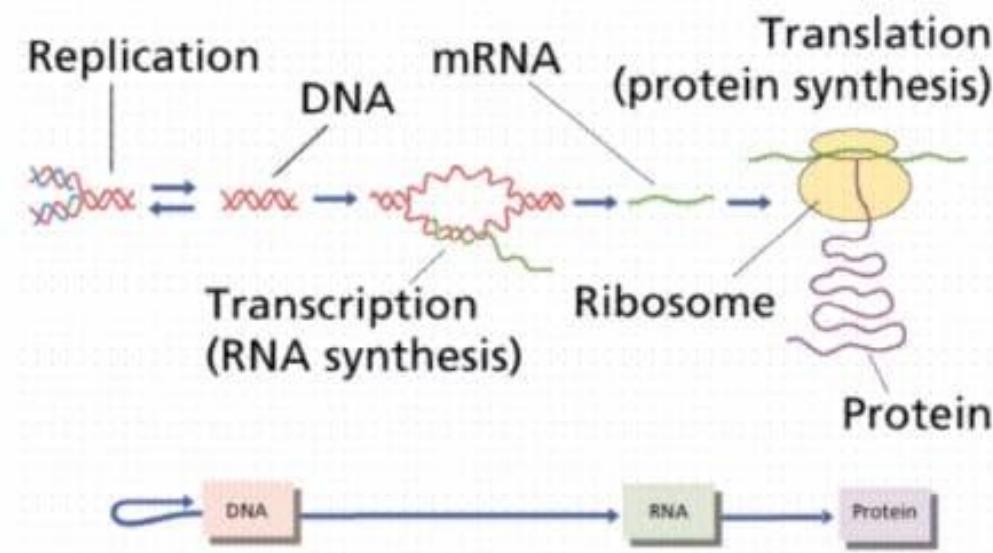
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A circular profile picture of a person with dark hair and a dark shirt.

16 messages | 50% Daily Data quota used as on 24-Aug-21 15...



## Protein synthesis in the cell



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## Forms of Biological Information

### DNA

- Information is contained in the primary structure (the sequence of bases).

### Protein

- Information is contained at multiple structural levels (primary, secondary, tertiary, quaternary)

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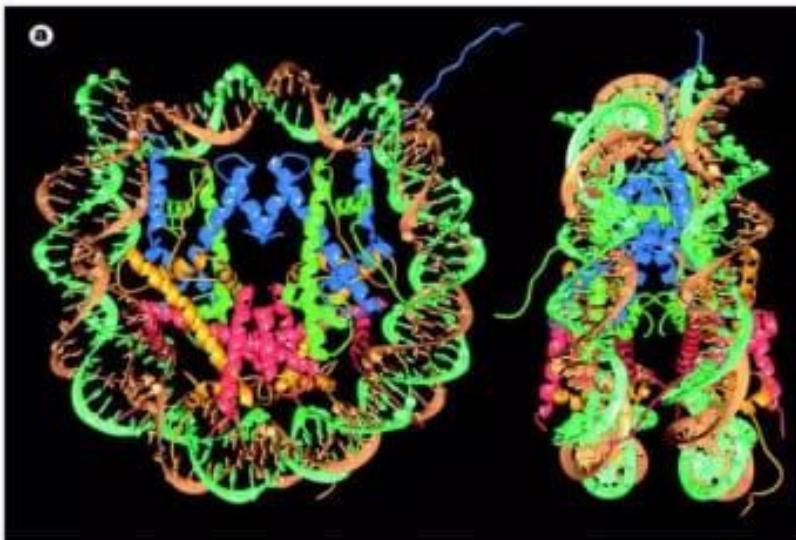
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## Three dimensional protein structures – relationship to function

The nucleosome  
core particle

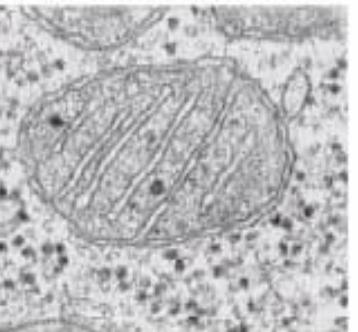


*K. Luger et al, Nature 1997*

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# Mitochondria

- Site of cellular respiration
- In virtually all eukaryotes (animals and plants)
- Number in cell varies widely



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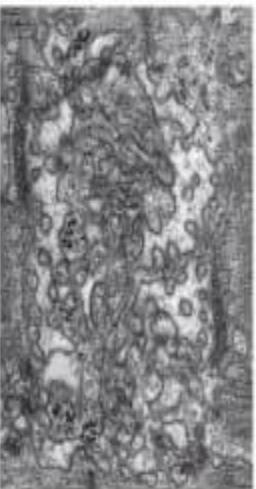


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# The endoplasmic reticulum



- Functions of the endoplasmic reticulum
  - Membrane Protein Synthesis for Export (RER)
  - Membrane Lipid Synthesis (SER)
  - Carbohydrate Addition and Modification (RER/SER)
  - Detoxification Reactions (SER)



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# The cytoskeleton

- Components of the cytoskeleton
  - Microtubules
  - Microfilaments
  - Intermediate filaments
- Functions of the cytoskeleton
  - Mechanical support to maintain shape
  - Motive force for cell shape
  - Cell motility



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# *Physiology:*

**the study of the mechanisms  
by which biological systems  
function and how those  
systems are controlled.**



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**30/8/2021**

# CH40001 Biochemical Engineering

## Chapter 3 Enzyme Kinetics

Saikat Chakraborty  
Department of Chemical Engineering,  
Indian Institute of Technology

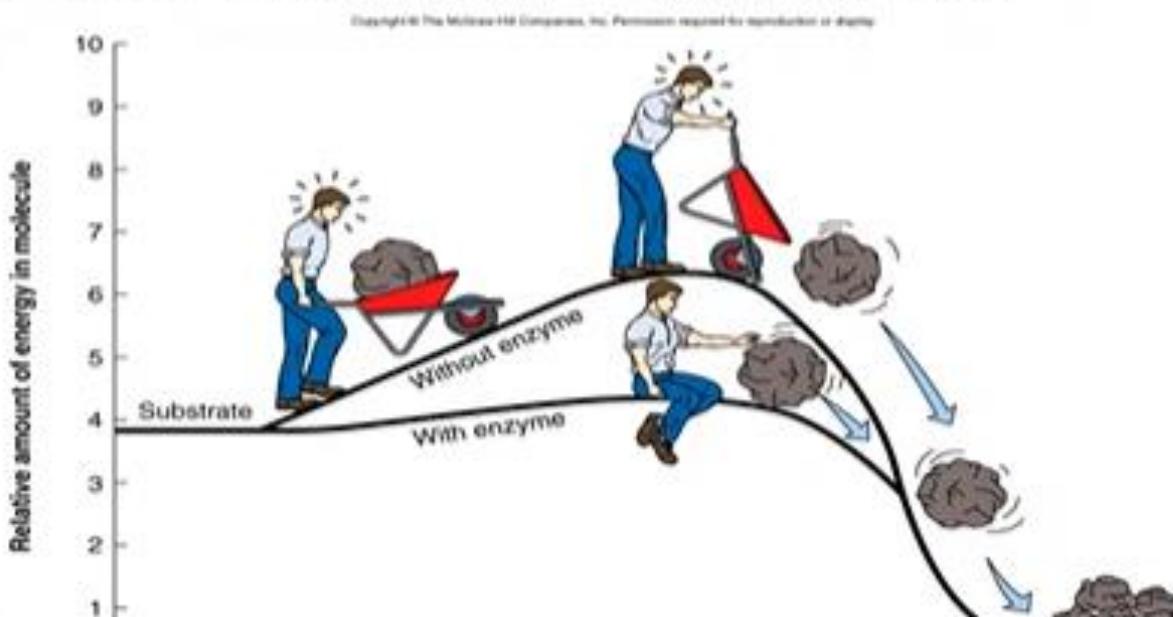
**Epistemic Qs 1.**  
**How do enzymes/catalysts act, thermodynamically?**



# Energy

- ▶ All living things require energy.
  - *Nutrients* are one source of energy, as well as being molecules organisms require to grow, reproduce or repair
- ▶ *Biochemical reactions* are the processes used for the formation, breakdown and rearrangement of molecules to provide organisms with energy

*Activation Energy* is needed to start the reaction.



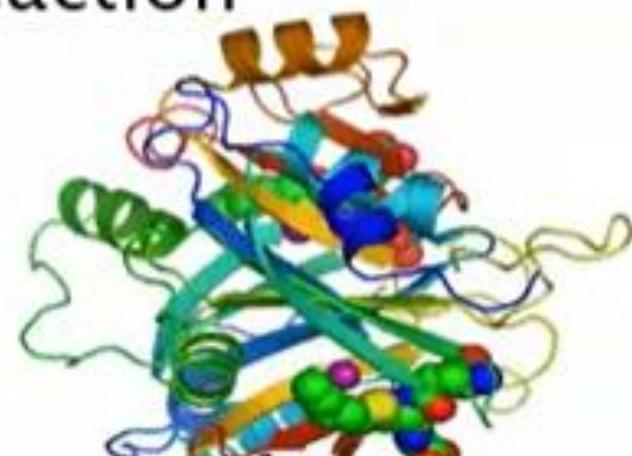
# Enzymes are biological catalysts

- ▶ A *catalyst* is a chemical that speeds up the reaction but is not used up in the reaction
  - Lowers the activation energy needed to start a reaction
  - Is not used up during the reaction
  - Is unchanged after a reaction
- ▶ *Enzymes* act as catalysts. Enzymes are proteins that speed up a rate of reaction
  - Found in cells throughout the body
  - Lowers activation energy
  - Names of enzymes will end in -ase
  - They are specific.



# Enzymes are biological catalysts

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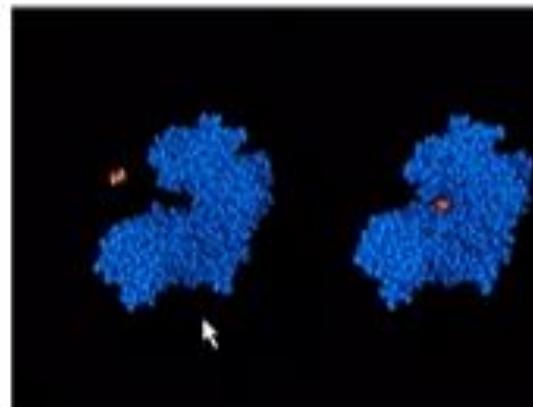
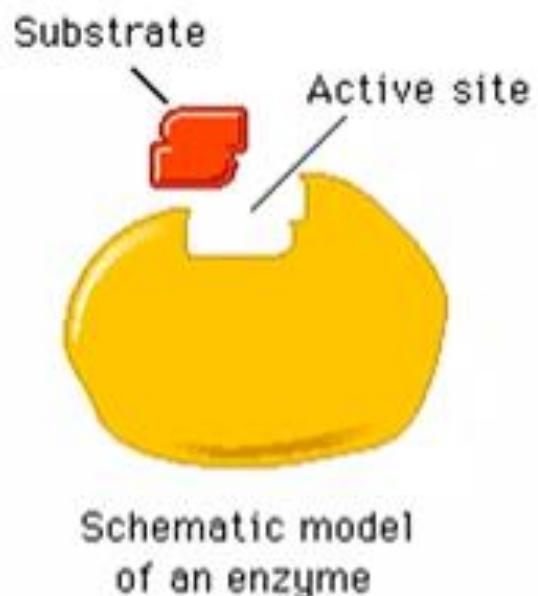
## Enzymes

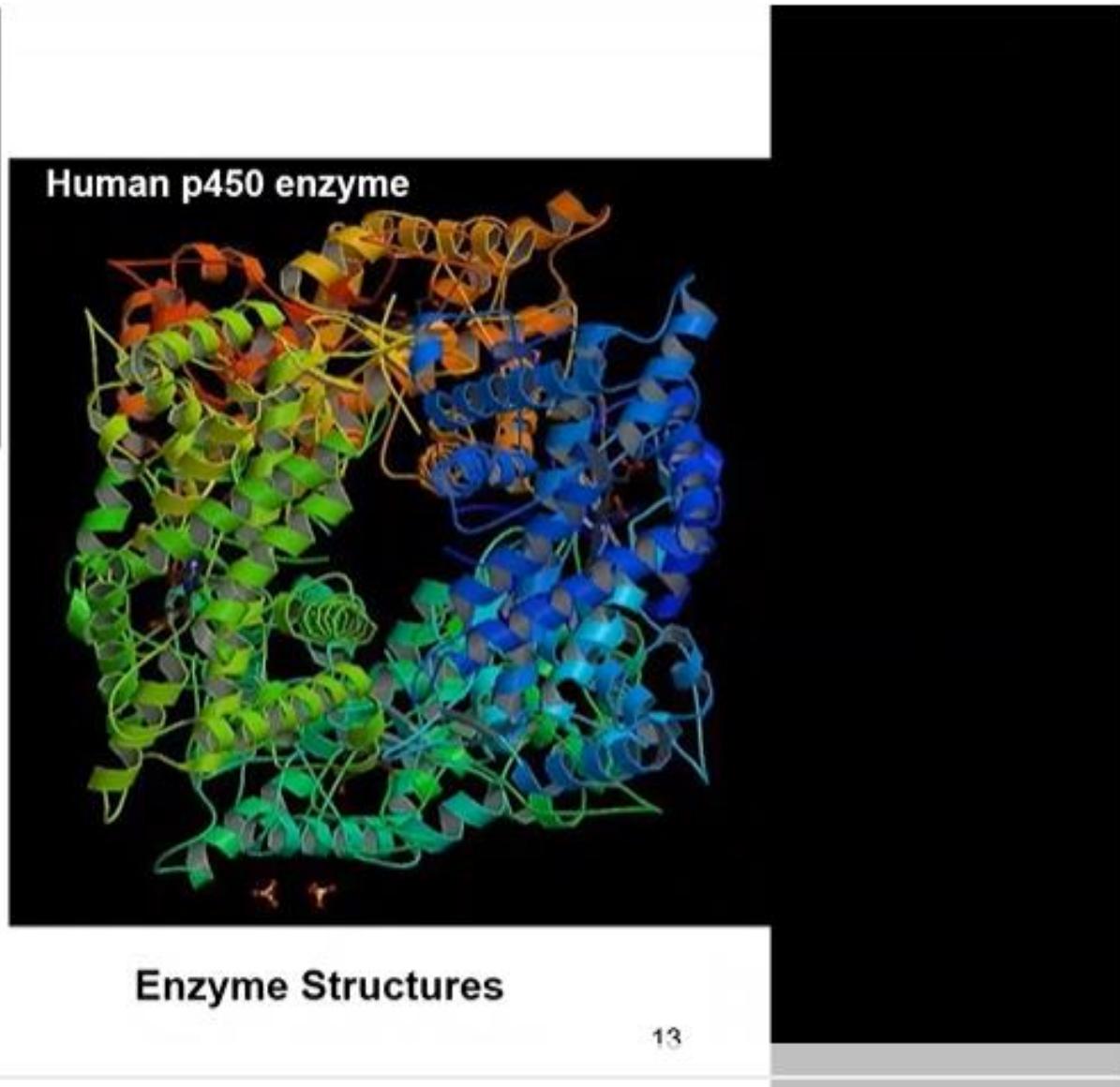
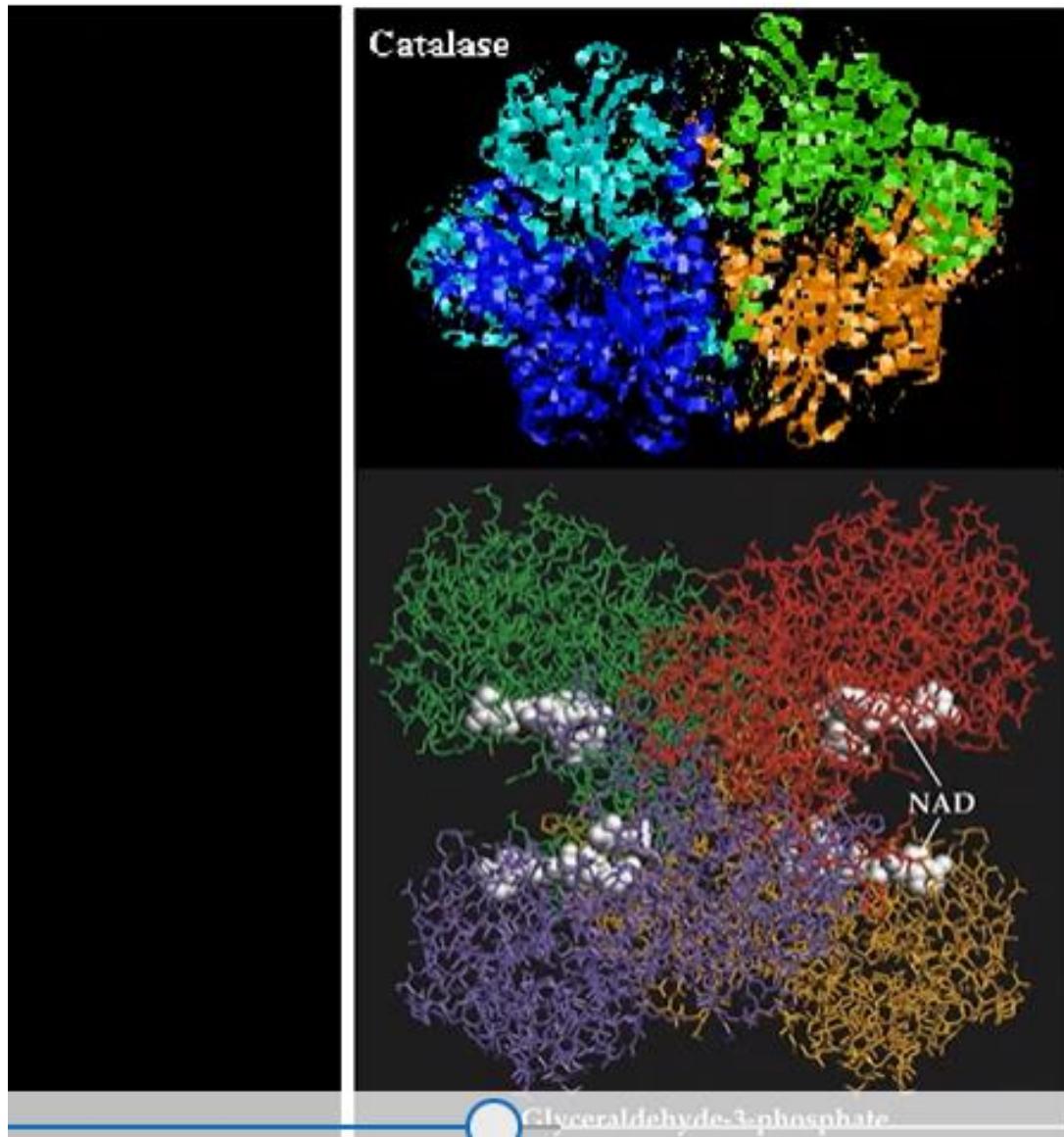
- They are proteins, RNA or DNA that catalyze biological reactions.
- It increases the rate of biological reactions  $10^4$  to  $10^{12}$  times the rate in the absence of the catalyst (many important biochemical reactions do not occur appreciably in the absence of an enzyme).
- Specificity: Enzymes are specific to reactant molecules known as substrates which interact at a specific site on the enzyme, often with high affinity. The high affinity finding allows enzymes to function effectively in a solution containing a large no. of biological molecules at low concentration.
- The activity of enzymes can be regulated in several ways, offering control over the rate and amount of product formed . Examples of regulation include
  - (a) cofactor , which binds to the enzyme.
  - (b) using a reaction product that inhibits the reaction.

**Epistemic Qs 6.**  
**Do enzymes/catalysts take part in the reaction?**

# How Enzymes work : Biochemistry of Enzyme Function

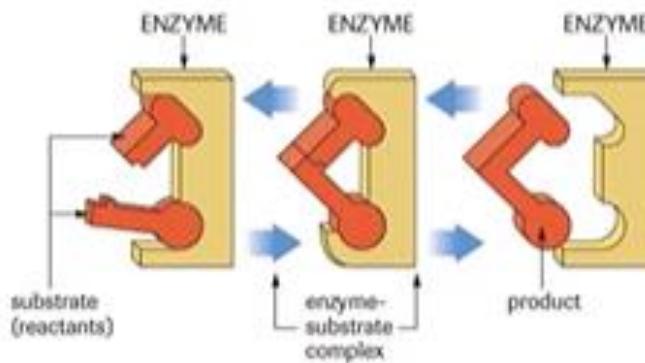
- Enzymes catalyze biochemical reactions following the binding of one or more substrates to the active site of the enzyme. An active site interacts specifically with the substrate, provides appropriate orientation of the reacting molecules and alters the local electro-dynamic environment to make the occurrence of the reaction more favorable.
- Specific amino-acid side chains serve as catalytic agents facilitating bond breakage or formation of the product.
- Molecular biology techniques (such as site-directed mutagenesis) are used to determine the specific structure-function relationship).





# CATALYSTS AND REACTION RATE

- How do catalysts work??
- Scientists do not really understand the actual mechanism. Catalysts are also usually discovered through trial and error.
- What they do know is that they provide an alternative, lower energy pathway from reactants to products.
- Most of the catalysts (**enzymes**) for biological reactions work by shape and orientation. They fit substrate proteins into locations on the enzyme as a key fits into a lock, enabling only specific molecules to link or detach on the enzyme.
- Almost all enzymes catalyze only one specific reaction

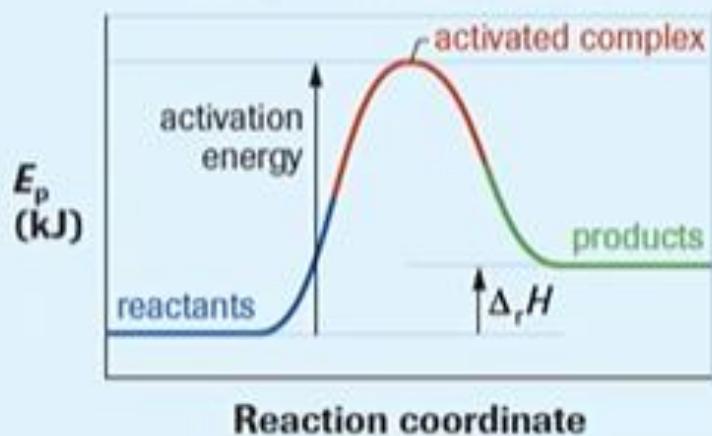


# LET'S SEE IF YOU GET IT

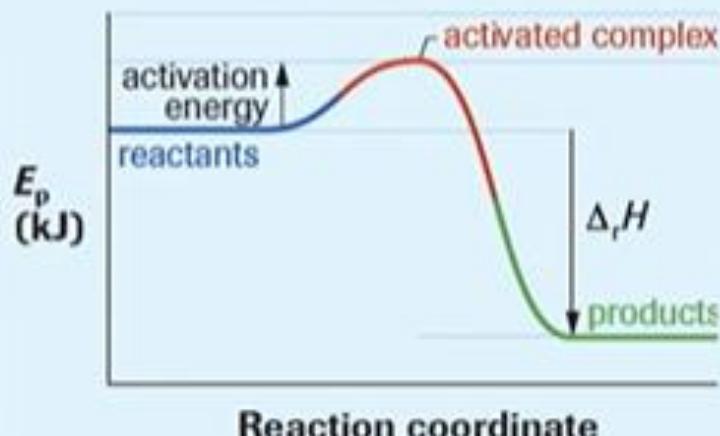
Draw energy pathway diagrams for general endothermic and a general exothermic reaction. Label the reactants, products, enthalpy change, activation energy, and activated complex.

## *Solution*

Potential Energy Changes During  
an Endothermic Reaction



Potential Energy Changes During  
an Exothermic Reaction



**Epistemic Qs 3.**

**How does enzymes/catalyst act stereo-chemically?**

**Epistemic Qs 7.**

**What's an enzymes relationship with it's substrates?**

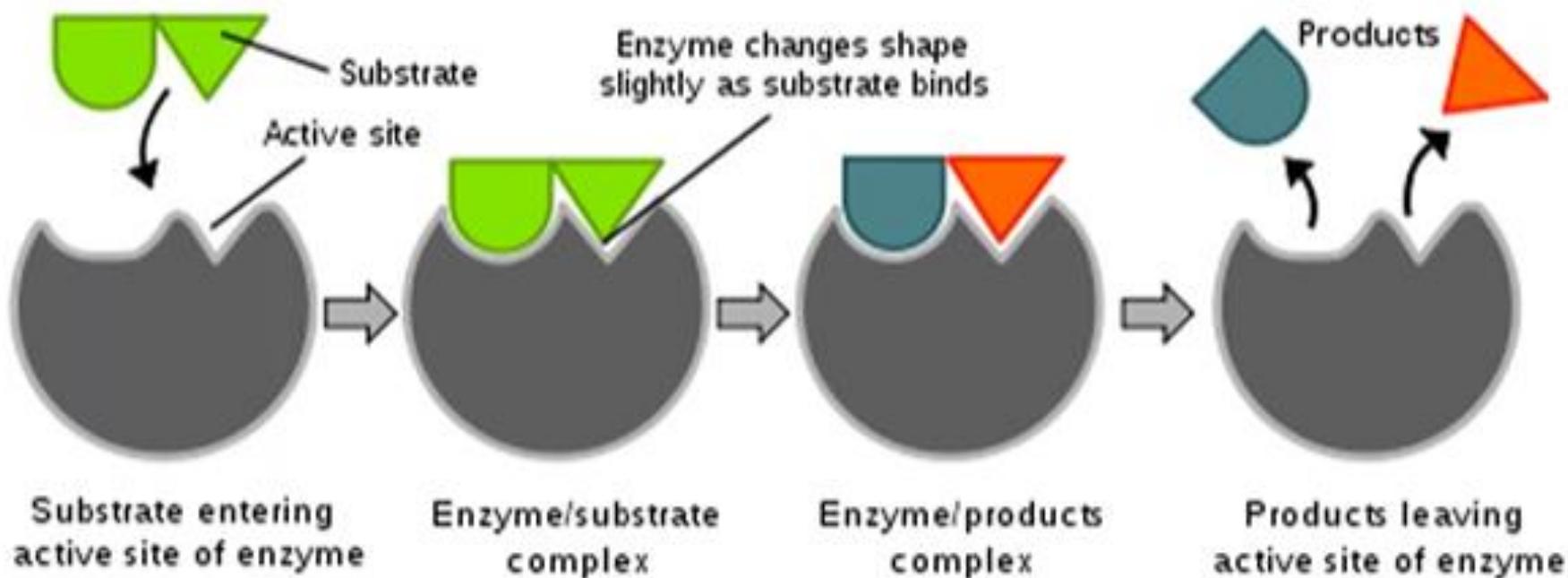
# Enzyme Form and Function

- **Lock & key Model:** The shape of an enzyme allows it to do a specific job much like a lock and key.



# Induced Fit Model

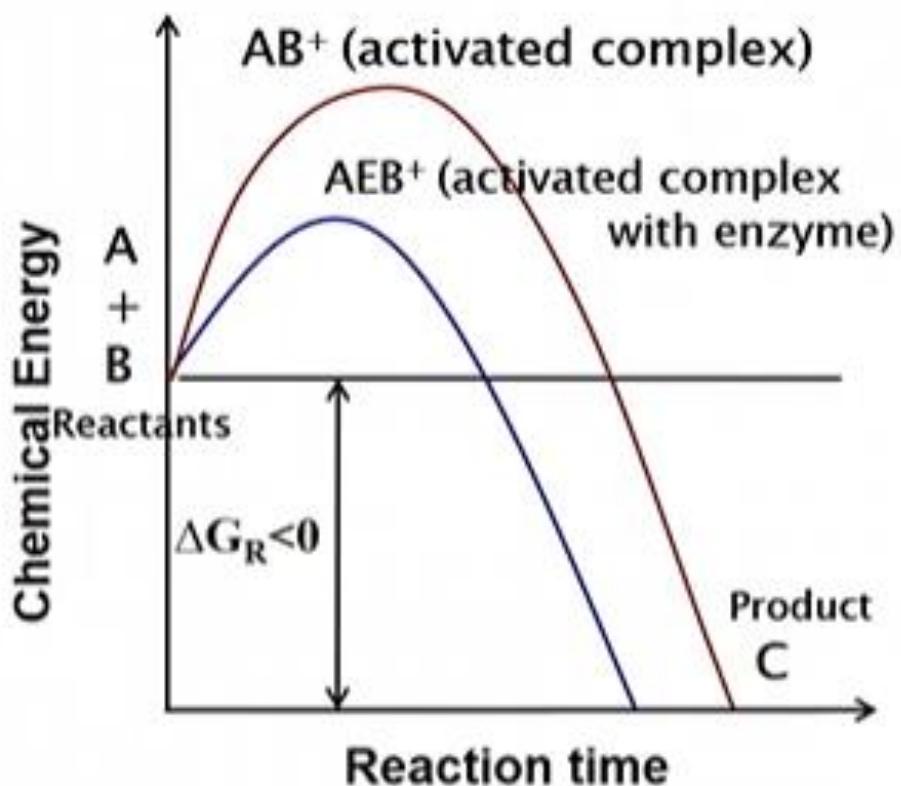
- Enzymes can form to the shape of its substrate.



[http://en.wikipedia.org/wiki/File:Induced\\_Fit\\_diagram.svg](http://en.wikipedia.org/wiki/File:Induced_Fit_diagram.svg)

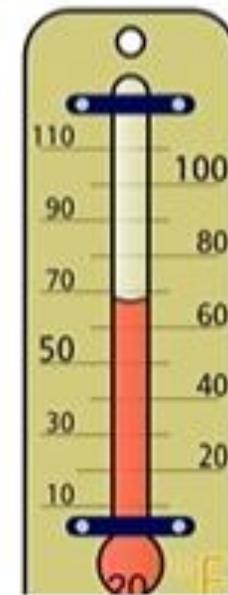
## How enzymes work: the Thermodynamics of Enzyme Activity

- For any reaction to be thermodynamically favorable ,  $\Delta G < 0$ .
- However even for reactions with  $\Delta G < 0$ , they are often limited by the energy barrier needed to form an activated state. As a result these reactions do not occur without *heat or catalyst*.
- Enzymes provide an alternate reaction pathway that produces an activated state of the reactants with lower energy barrier. As a result , the rate of reaction is increased significantly but the overall change in energy between the reactants and the products is not altered.



# Denaturing Enzymes

- When an enzyme is denatured it is damaged.
- Denaturing changes the shape.
- Without the correct shape enzymes won't function properly.
- HOW are enzymes denatured?
  - Temperature
  - pH



# **What other factors control the action of enzymes?**

- Temperature
- Water Content
- pH
- Chemicals
- Alteration of Substrates
- Alteration of Products



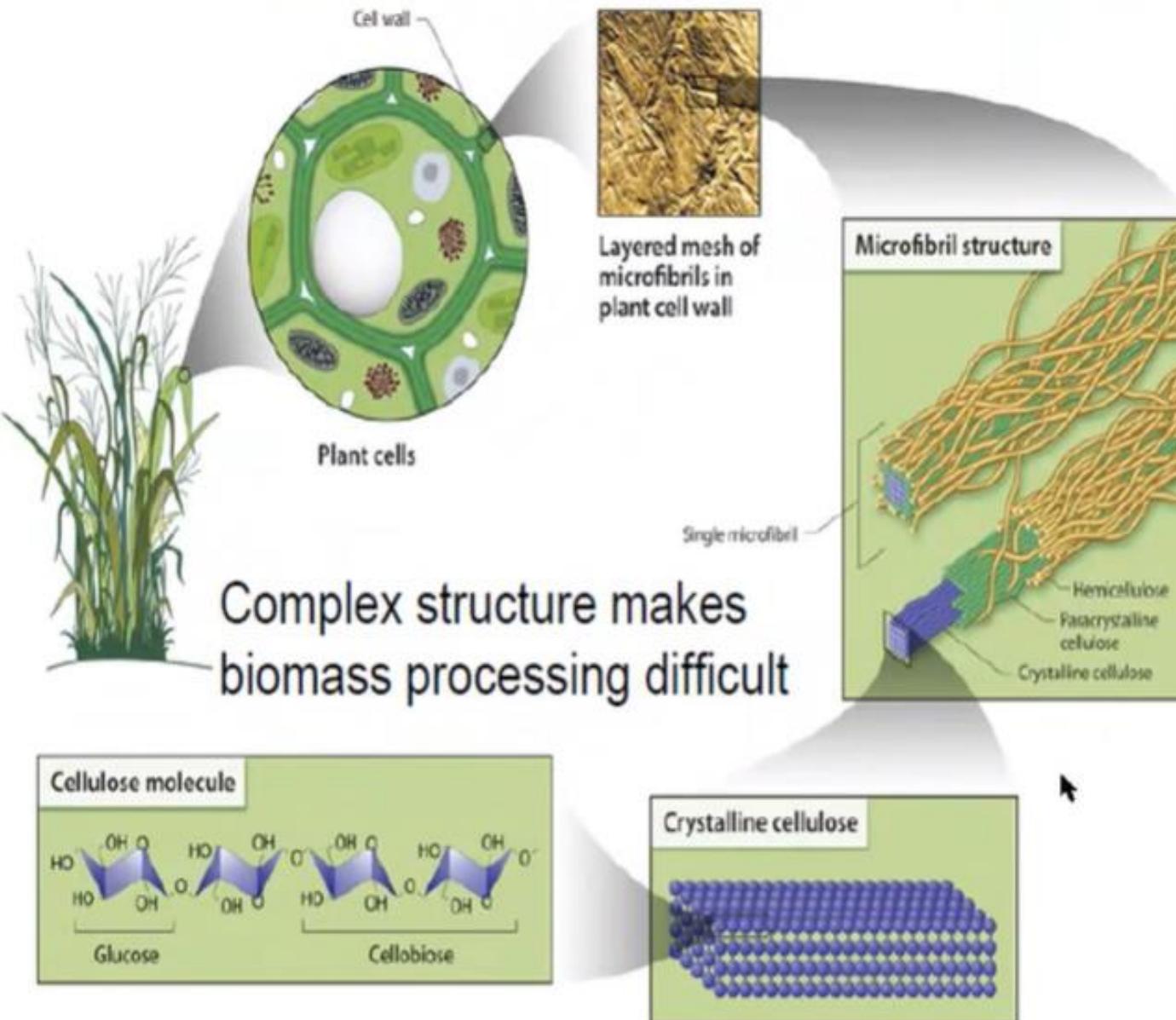
**Epistemic Qs 8.**

**What's the role of evolutionary biology in determining  
the structure of lignocelluloses in plant cell walls?**

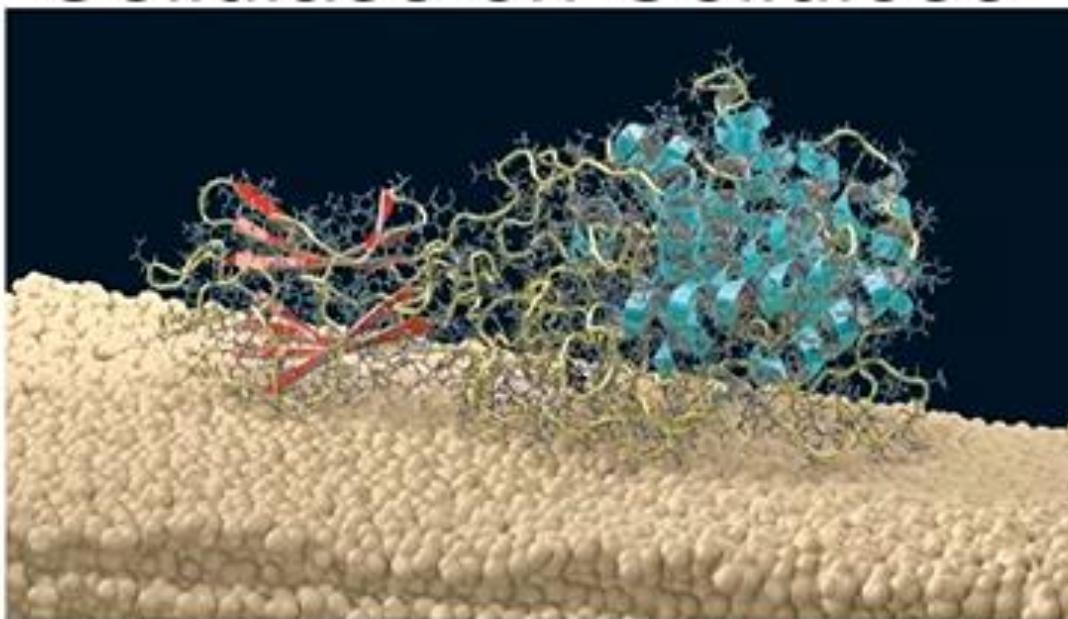
**Epistemic Qs 9.**

**How do enzymes connect to evolutionary biology?**

# Complex Biomass Structure

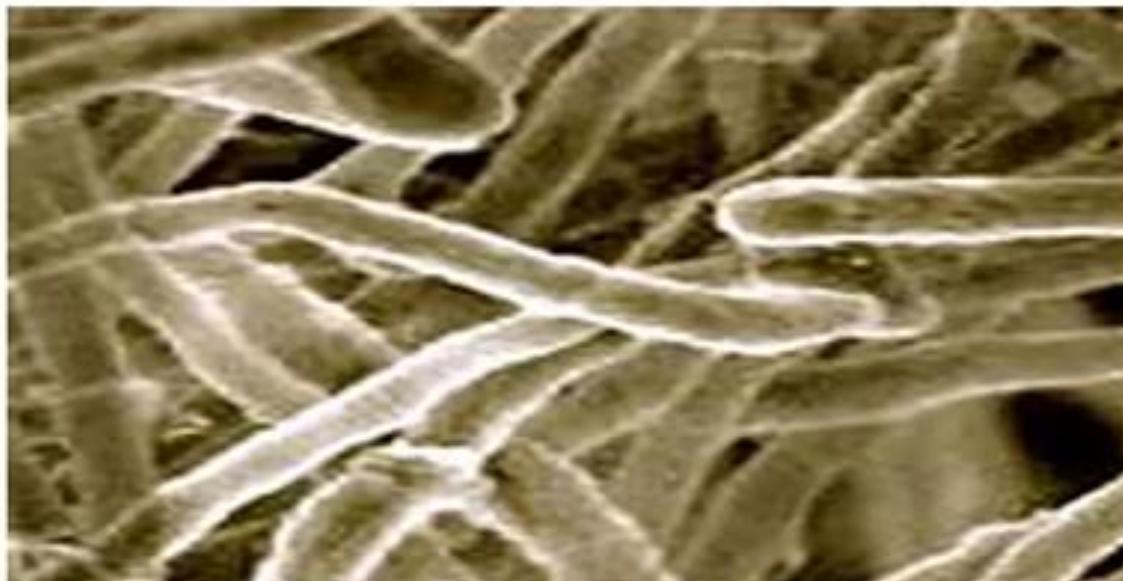


# Cellulase on Cellulose

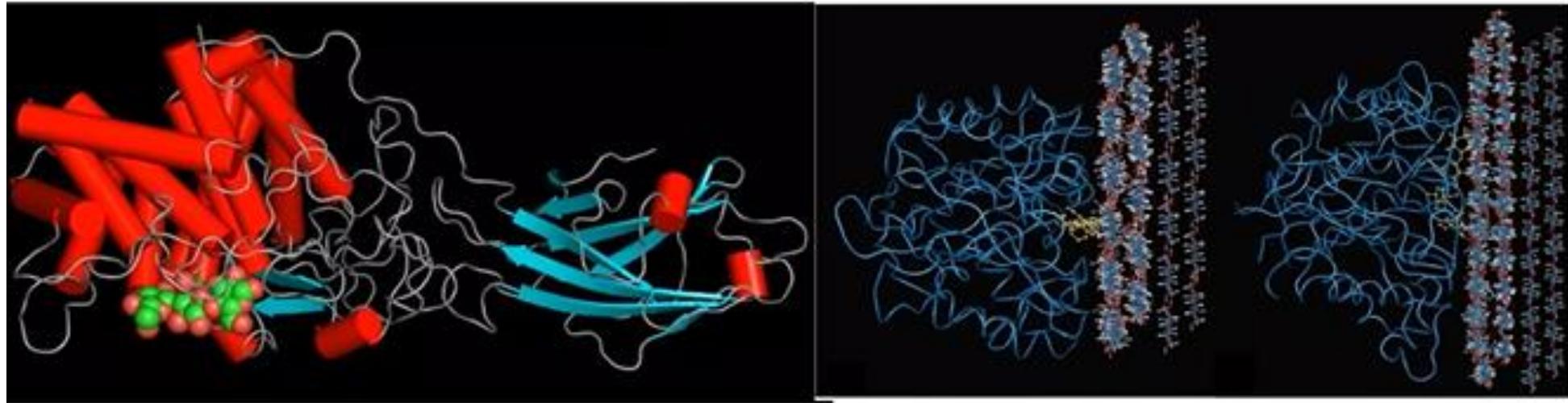


## *Trichoderma reesei* Rut C30

A mesophilic and filamentous fungus has been used for cellulase enzyme production

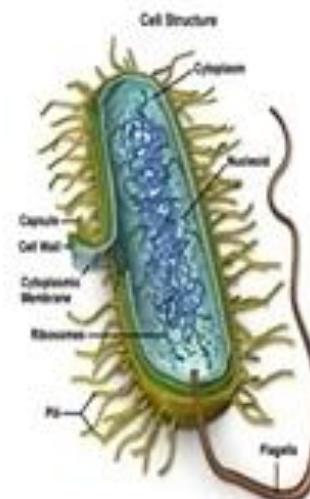


# Cellulase



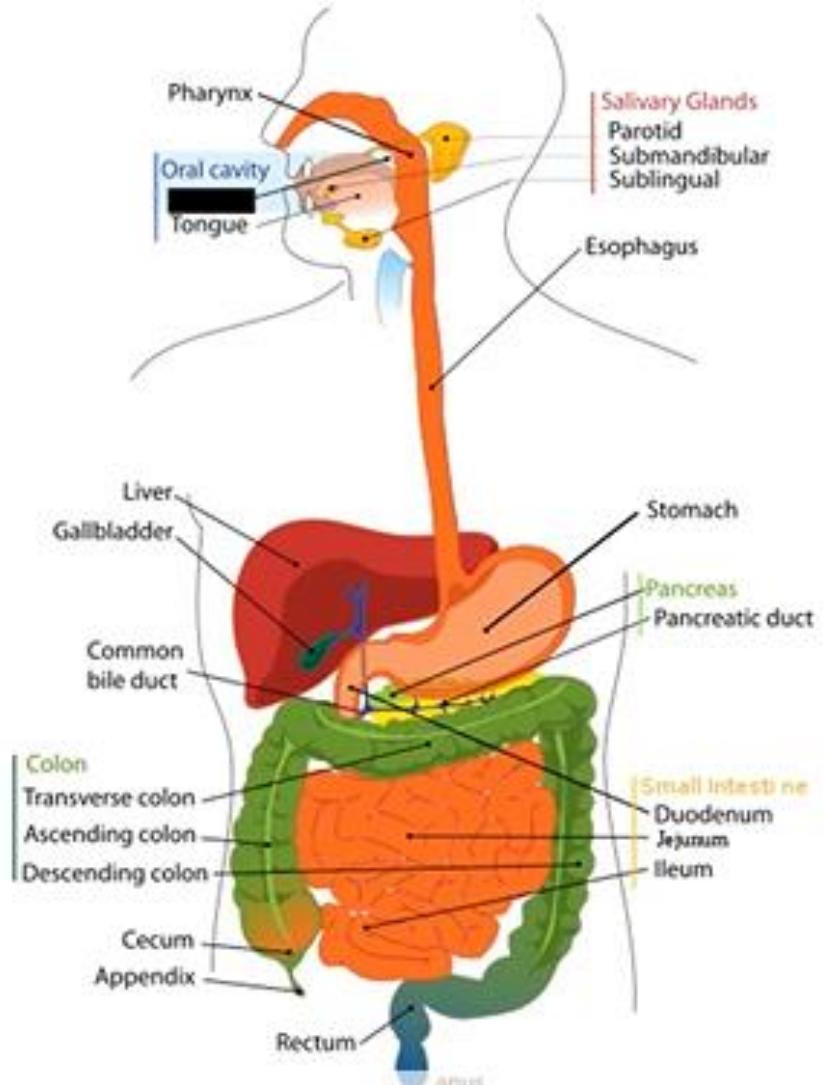
Cellulase is composed of three different type of enzymes:  
Endo-glucanase  
Exo-glucanase (CBH-I and CBH-II)  
and  
 $\beta$ -glucosidase

# Sources of enzymes



- Cellulase obtained from sources such as bacteria, fungi or archaea
- Thermophilic/ Mesophilic cellulase to be important
- Relative concentration of enzyme in the mixture & their kinetic parameters matched to their values in cellulase from *T.reesei* (65-70% CBHI ,10-15% EG) or *T.viride*

# Enzymes are used all over your body!



# Major Digestive Enzymes

Enzyme	Produced In	Site of Release	pH Level
<b>Carbohydrate Digestion:</b>			
Salivary amylase	Salivary Glands	Mouth	Neutral
Pancreatic amylase	Pancreas	Small Intestine	Basic
Maltase	Small intestine	Small intestine	Basic
<b>Protein Digestion:</b>			
Pepsin	Gastric glands	Stomach	Acidic
Trypsin	Pancreas	Small intestine	Basic
Peptidases	Small intestine	Small intestine	Basic
<b>Nucleic Acid Digestion:</b>			
Nuclease	Pancreas	Small intestine	Basic
Nucleosidases	Pancreas	Small intestine	Basic
<b>Fat Digestion:</b>			
Lipase	Pancreas	Small intestine	Basic

# Enzyme Deficiency

## Protease Deficient:

Cannot digest protein

- causes blood to be more acidic
- Can't produce glucose
- Inadequate hydration in the body

Problems include:

- Arthritis
- Bone spurs
- Hypoglycemia
- Edema (swelling)
- Toxic Colon
- Ear infections in children
- Compromised Immune System



# Enzyme Deficiency

## Lipase Deficient:

Lipase digests fats and fat-soluble vitamins

## Problems include:

- High Cholesterol
- Difficulty losing weight
- High triglycerides
- Decreased cell permeability(can't get glucose out of cells)
- Muscle Spasms
- Chronic Fatigue Syndrome
- Spastic Colon
- Vertigo
- Early Menopause



# Enzyme Deficiency



## Amylase Deficient:

Amylase digests starches and polysaccharides – end result is glucose

Also digests dead white blood cells (pus)

Can be cause by excessive consumption of Carbohydrates

## Problems Include:



- Skin problems: Abscesses, Psoriasis, Eczema, allergic reactions to bee stings
- Lung problems: asthma and emphysema
- Phosphorus deficiency
  - Thick blood

# Enzyme Deficiency

## Sucrase Deficient

Cannot split sucrose into glucose

Glucose is basically brain food

- Problems include: moodiness, depression,
- panic attacks, manic and schizophrenic behavior, severe mood swings and seizures.



## Lactase Deficient

Cannot digest lactose

- Causes cramping and diarrhea



## **Flow & Form: Engineering the interactions between Transport and Reactions**

**Epistemic Qs 15.**

**How does mixing/transport limitation influence reaction rate (kinetics)?**

**Epistemic Qs 16.**

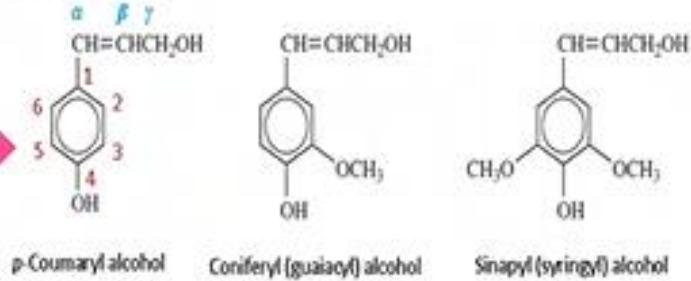
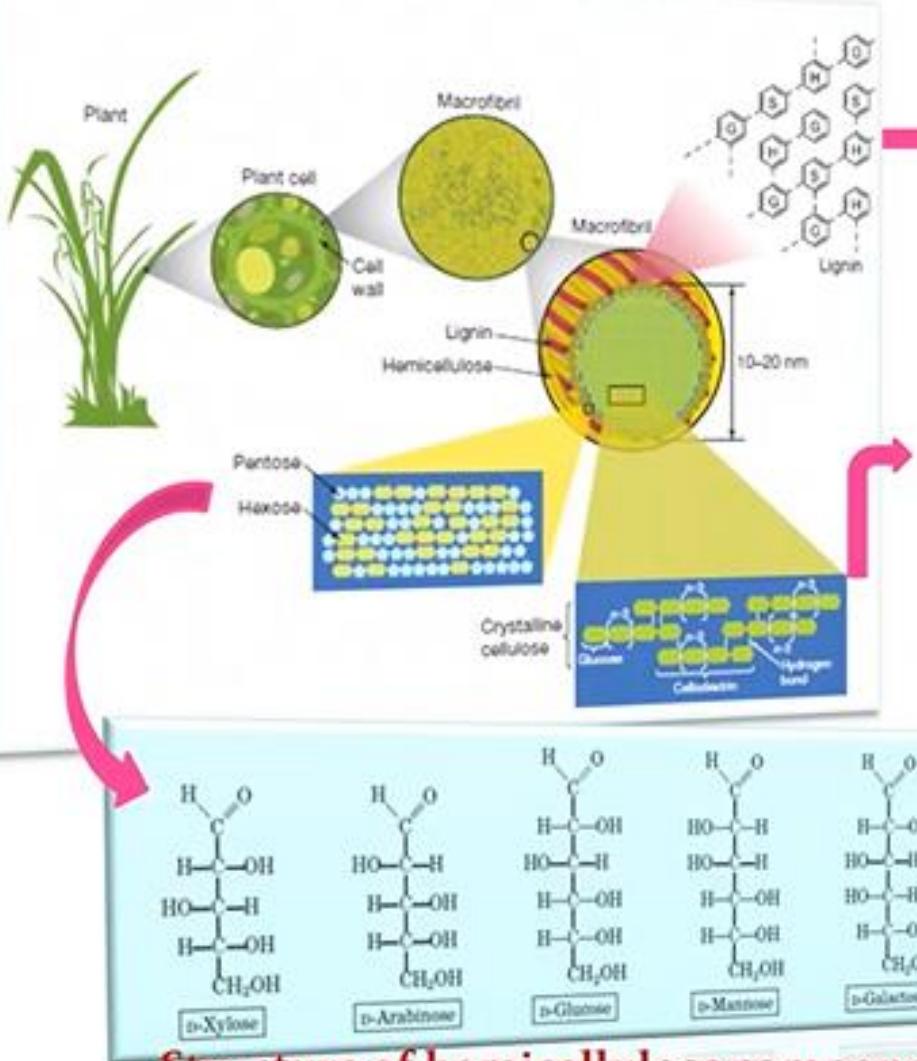
**How to manipulate reactor mixing/type to regulate product distribution?**

**Epistemic Qs 20.**

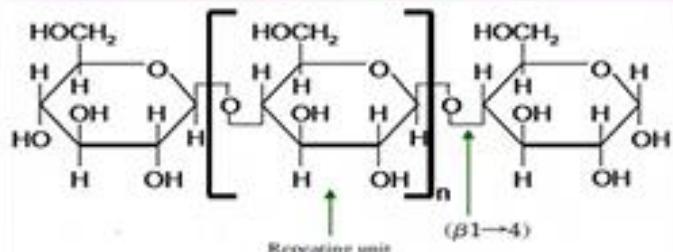
**What determines the rate of transport of enzymes to solid substrate surface in a well-mixed reactor?**

**31/8/2021**

# Structure of Lignocellulosic Biomass



## Structure of lignin components



## Structure of cellulose

<b>Cellulose</b>	<b>15 – 55%</b>
<b>Hemicelluloses</b>	<b>10 – 50%</b>
<b>Lignin</b>	<b>7 – 35%</b>

Average value

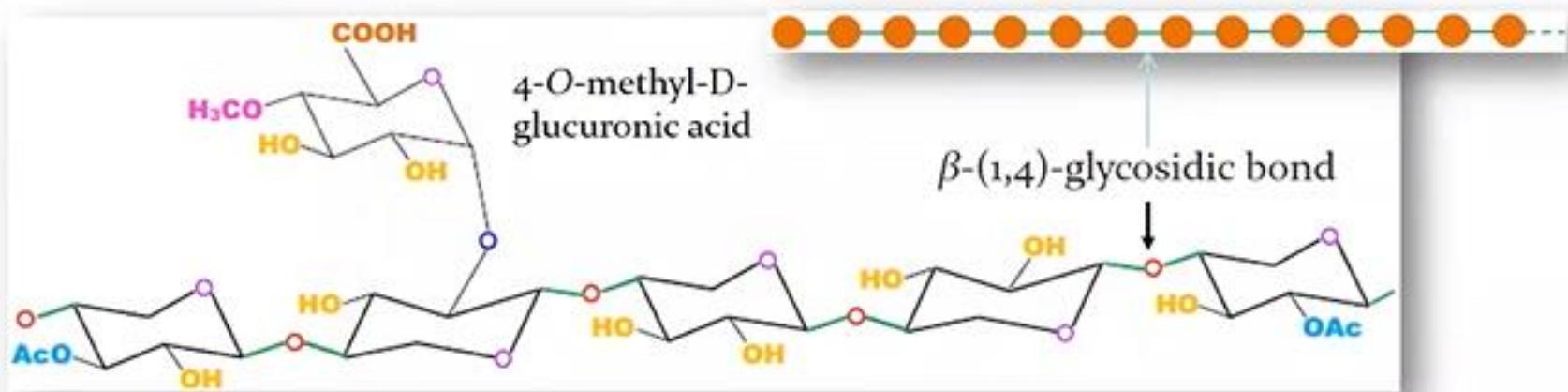
Rubin, E.M., 2008. *Nature Rev.*, 454, 841–845.

Jorgensen et al., 2007. *Biofuels, Bioprod. Bioref.* 1,

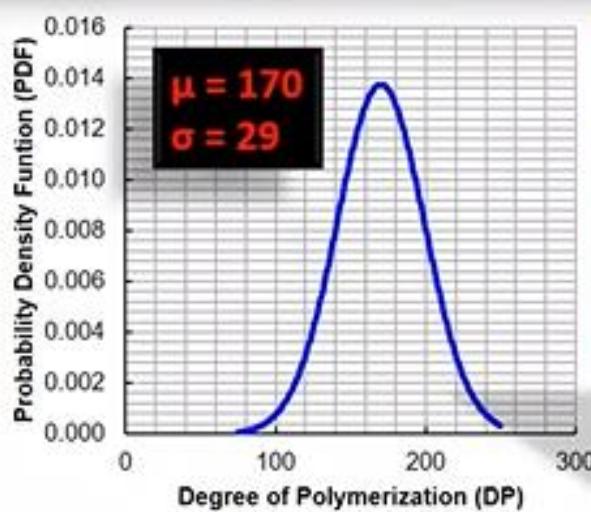
110–127

# Hemicellulose Hydrolysis: at a glance

## Structure of beechwood xylan



Sugar	%
Xylose	90.8
Arabinose	1.1
Glucose	4.7
Uronic acid	3.4

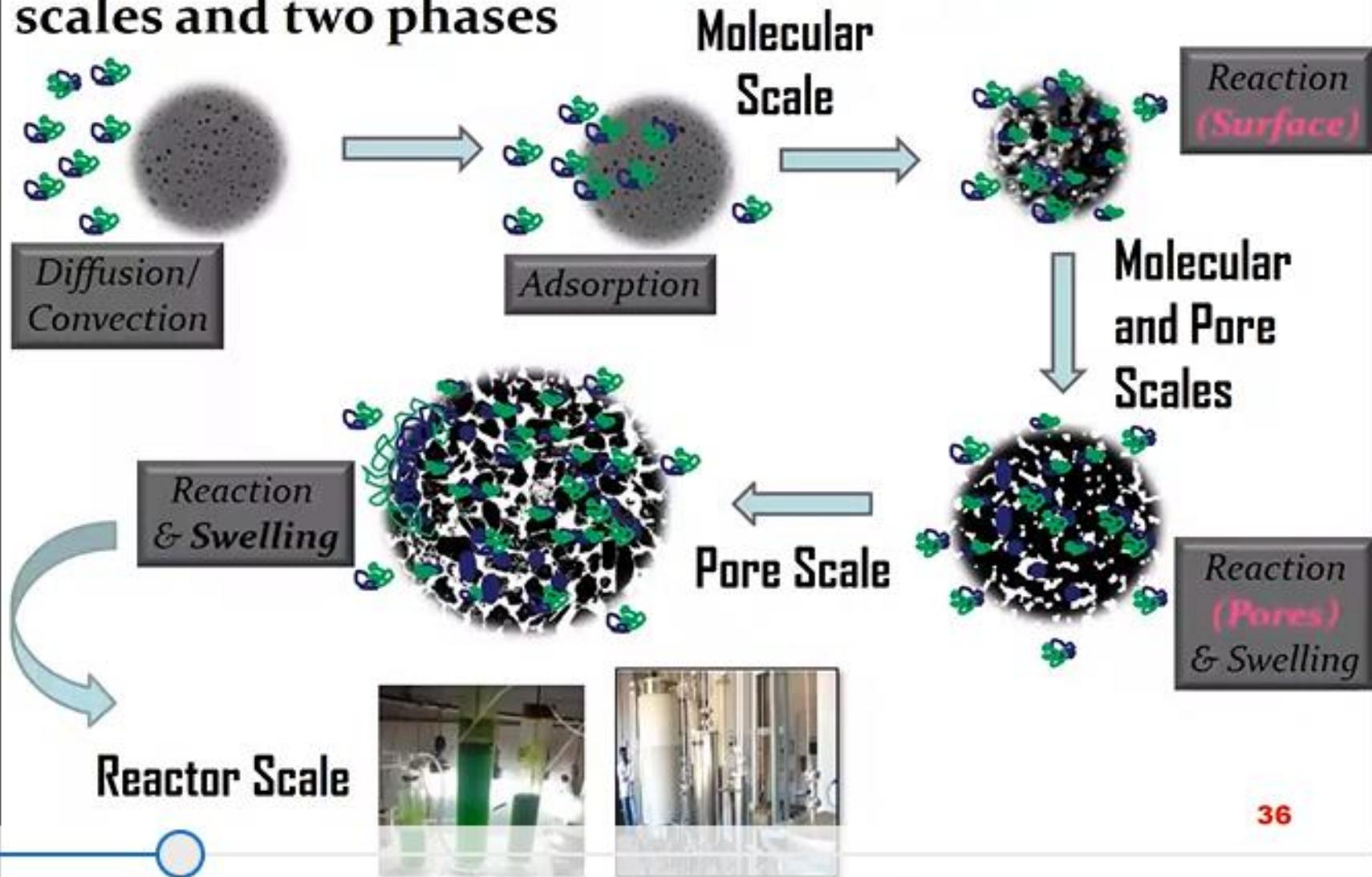


Xylose diameter	0.64 nm
Molecular size of xylan	48 - 160 nm
Average size	109 nm

Timell and Syracuse, 1967. *Wood Sci. Technol.* 1, 45–70.

Dusterhöft et al., 1997. *Enzyme Microb. Technol.* 20,

# Complexities of the Multiscale System: coupled Transport and Reaction occur across three scales and two phases



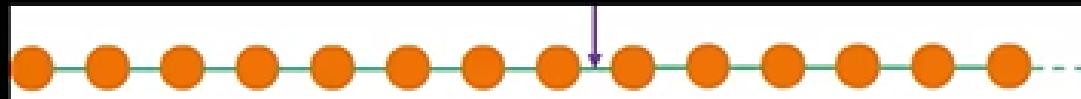
Pre-treatment of Biomass

Enzymatic Hydrolysis

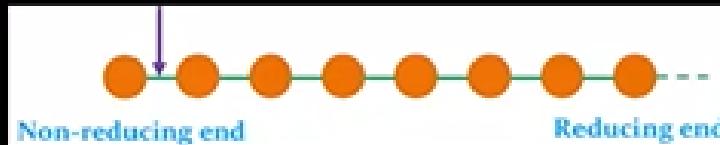
Slowest & Rate Limiting Step

Fermentation

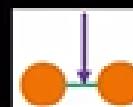
- ✓ Endoxylanase randomly cleaves  $\beta$ -(1 $\rightarrow$ 4) glycosidic bonds



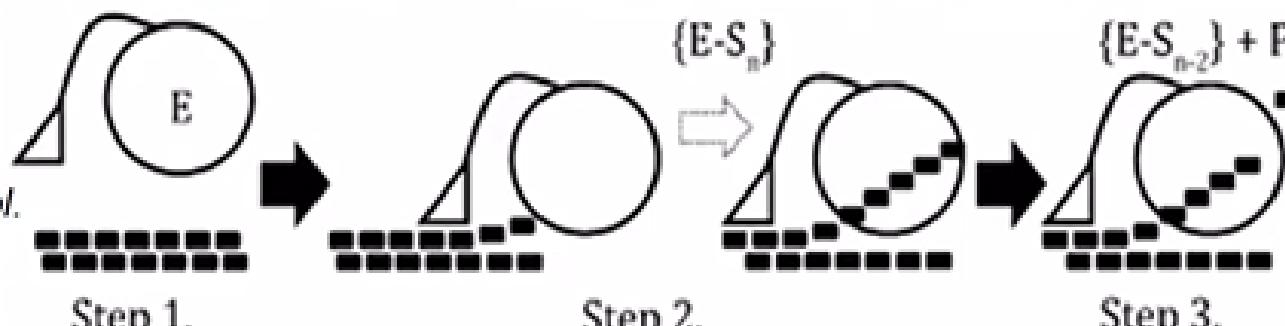
- ✓ Exoxylanase releases xylose from xylan and xylo-oligosaccharides from *non-reducing ends*



- ✓  $\beta$ -xylosidase cleaves xylobiose and xylo-oligosaccharides from *non-reducing ends*



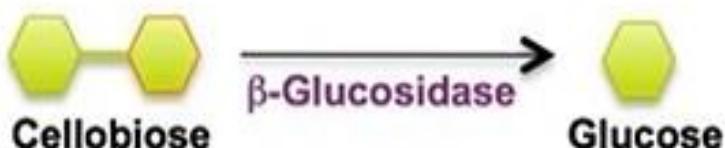
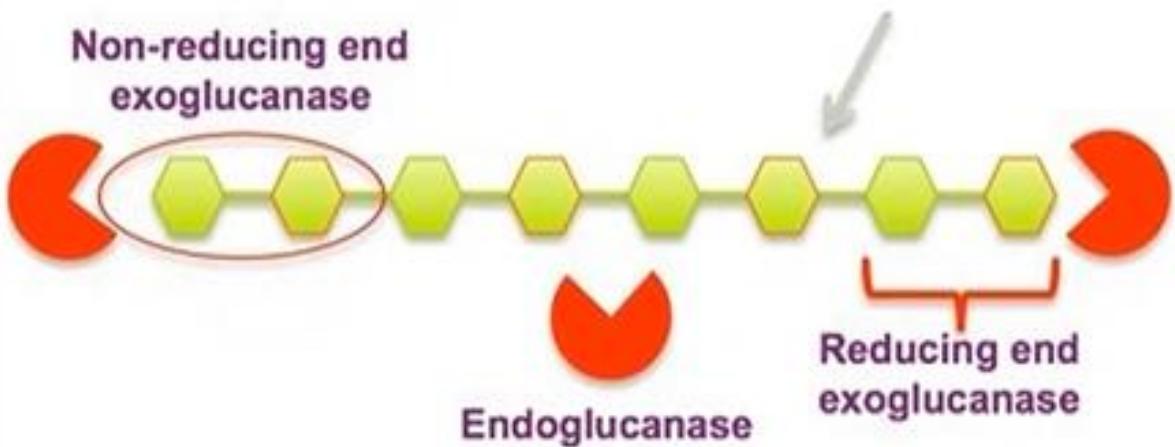
Carbohydrate Binding Doman (CBD) & Catalytic Doman (CD)



# Cellulose to Glucose via Enzymatic Hydrolysis Depolymerization

**Substrates**  
**DP = 60-20,000**

PASC DP = 60  
Avicel DP = 300  
Filter Paper DP = 750  
CMC DP = 1500  
Cotton DP = 3000

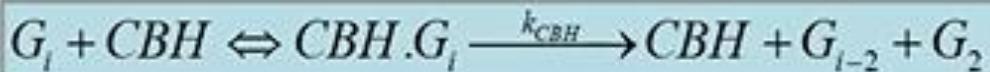


# Kinetics of Enzymatic Hydrolysis of Cellulose using Cellulase Enzyme

- Enzymes are a part of cellulases obtained from several organisms, including *T.reesei* : Endoglucanase, Exoglucanase &  $\beta$ -glucosidase
  - The endoglucanase cuts the cellulose chain rapidly at random points



- The exoglucanase(CBH) forms complexes with either reducing or the non-reducing end of the cellulose chain cleaving them into primarily cellobiose



- The  $\beta$ -glucosidase forms glucose by cleaving cellobiose.



## Kinetics of Enzyme Reactions

- The enzymes provides an alternate reaction pathway that produces an activated state of the reactants with lower energy barrier.



- E= enzyme, S= substrate, P= product.
- The enzyme accelerates the reaction but is not consumed in the reaction.
- The enzyme affects the rate of reaction, but does not affect/alter the equilibrium.

# Michaelis-Menten Kinetics

For most enzymes involving single substrates, expts. show that rate of consumption of substrate is given by,

$$R_S = \frac{R_{\max} C_S}{K_M + C_S} \dots \quad (1)$$

$R_S$  = Rate of disappearance of substrate/reactant

$K_M$  = Michaelis constant =  $C_S$  at which,  $R_S = R_{\max} / 2$

$R_{\max}$  =Maxm. reaction. rate

case 1.

## Invertase-catalyzed hydrolysis of sucrose into glucose and fructose.

*Leonor Micahelis &  
Maud Menten, 1913*

case2.

## **Derivation fo M-M Kinetics: Reversibility, Parameter Lumping, Asymptotic Solutions**

**Epistemic Qs 4.**

**What's the difference between reversible and irreversible reactions?**

**Epistemic Qs 11.**

**Why lumped kinetics is more advantageous than detailed kinetics?**

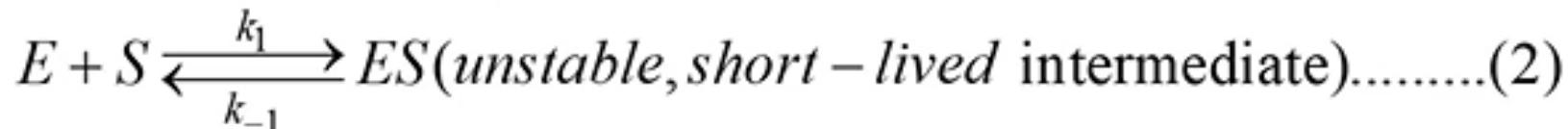
**Epistemic Qs 12.**

**What are the uses of asymptotic solutions to equations?**

6/09/2021

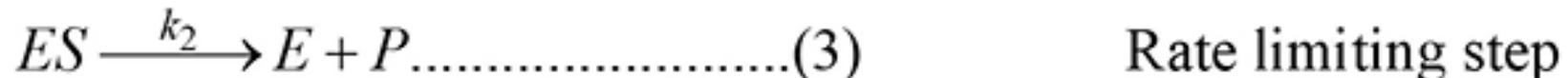
# Derivation of Michaelis-Menten Kinetics

- Enzyme (E) reacts with substrate (S) to form a complex (ES)



Lois Generales de l'Action des Diastases, **Victor Henri, 1903**

In the second step this unstable , the short-lived intermediate complex dissociates yielding the enzyme(E) and the product(P).



Rate of disappearance of substrate S =

$$-R_S = \frac{dC_S}{dt} = -k_1 C_S C_E + k_{-1} C_{ES} \text{.....(4)}$$

## Derivation of Michaelis-Menten Kinetics...Contd

Rate of formation of 'ES' =

As enzyme is not consumed,

total enzyme = free enzyme + enzyme in complex (ES) form

## Constraint eqn.

# Derivation of Michaelis-Menten Kinetics...Contd

Rate of product formation =

## Key Assumption:

Quasy steady state(Complex(ES)is unstable breaks down rapidly, its rate of accumulation is zero)

## Derivation of Michaelis-Menten Kinetics...Contd

using eqn.8 on eqn 5.  $C_{ES} = \frac{k_1 C_S C_E}{k_{-1} + k_2}$ .....(9)

Substituting eqn 9 into eqn.6

$$C_E = \frac{(k_{-1} + k_2)C_{E0}}{(k_{-1} + k_2) + k_1 C_S} .....(10)$$

$$C_{ES} = \frac{k_1 C_S C_E}{k_{-1} + k_2} = \frac{k_1 C_S C_{E0}}{(k_{-1} + k_2) + k_1 C_S} .....(11)$$

# Derivation of Michaelis-Menten Kinetics... Evaluating the Rxn. Rate in terms of known variables

Substituting eqn 10 & 11 into eqn.4

$$\begin{aligned}-R_S &= \frac{dC_S}{dt} = -k_1 C_S C_E + k_{-1} C_{ES} \\&= \frac{k_1 k_2 C_S C_{E0}}{(k_{-1} + k_2) + k_1 C_S} \\&= \frac{k_2 C_S C_{E0}}{\frac{(k_{-1} + k_2)}{k_1} + C_S} \quad \dots \dots \dots (12)\end{aligned}$$



# Derivation of Michaelis-Menten Kinetics...Evaluating Michaelis Constants

Comparing:

$$R_S = \frac{R_{\max} C_S}{K_M + C_S}$$

with

$$R_S = \frac{k_2 C_S C_{E0}}{\frac{(k_{-1} + k_2)}{k_1} + C_S}$$

$$\left\{ \begin{array}{l} R_{\max} = k_2 C_{E0} \\ K_M = \frac{(k_{-1} + k_2)}{k_1} \end{array} \right\}$$

$$\begin{cases} R_{\max} = k_2 C_E 0 \\ K_M = \frac{(k_{-1} + k_2)}{k_1} \end{cases}$$

case1.

$C_S \gg K_M$ ,

$R_S = R_{\max}$

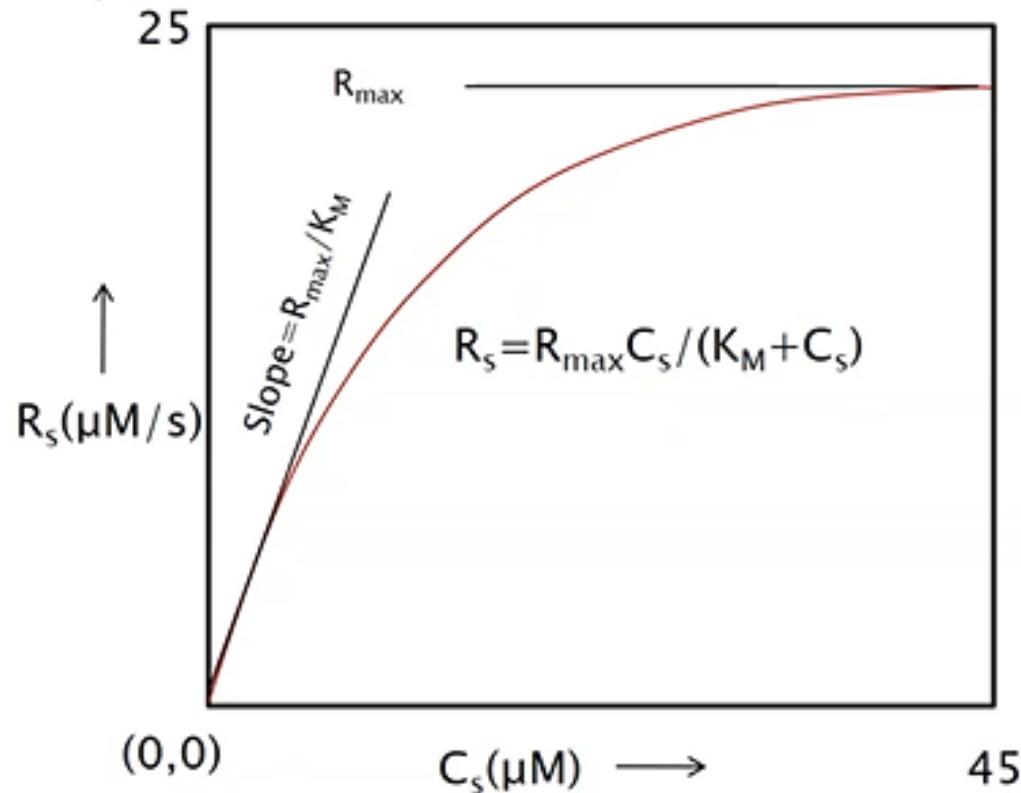
(reaction is '0'th order)

case2.

$C_S \ll K_M$ ,

$$R_S = \frac{R_{\max} C_S}{K_M}$$

(reaction is 1st order)

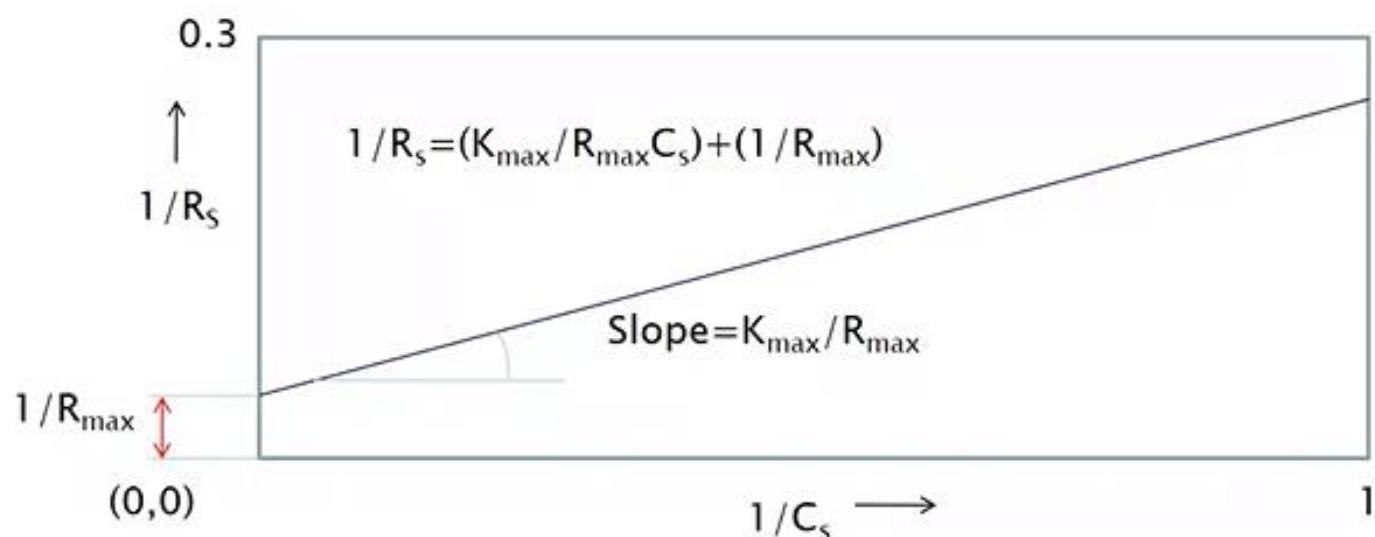


### Evaluation of $R_{max}$ & $K_M$

### Lineweaver-Burk Equation::

$$\frac{1}{R_S} = \frac{K_M}{R_{\max} C_S} + \frac{1}{R_{\max}} \dots \quad (12)$$

plot  $\frac{1}{R_S}$ (y axis) vs.  $\frac{1}{C_S}$ (x-axis) is a straight line.intercept on y-axis=  $\frac{1}{R_{\max}}$ .



## Evaluation of $R_{\max}$ & $K_M$ (contd..)

Two other important plots.

1. Scatchard Equation:  $\frac{C_S}{R_S} = \frac{K_M}{R_{\max}} + \frac{C_S}{R_{\max}}$  .....(13)

2. Eadie-Hofstee Eqn.:  $R_S = R_{\max} - K_M \frac{R_S}{C_S}$  .....(14)

## **States of a Reactor: Steady, Unsteady and Quasi-steady**

**Epistemic Qs 10.**

**What kind of reactors can attain steady state?**

**Epistemic Qs 13.**

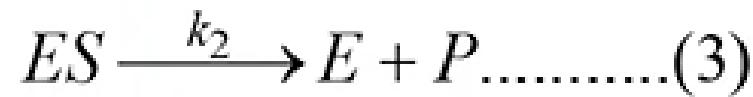
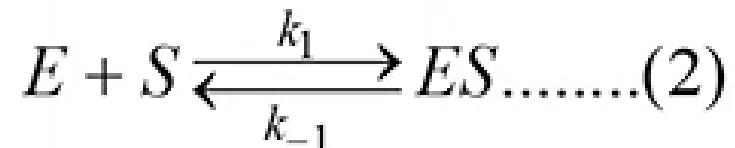
**When is Quasi-steady state (QSS) assumption valid for a species (or several species) participating in a reaction network?**

# When is the Quasi-Steady State Assumption Valid?

## Assumptions:

1. Fast step corresponding to initial formation of ES complex.
  2. Quasi-steady state Complex (ES) is unstable,  
breaks down rapidly, its rate of accumulation is zero

$$\frac{dC_{ES}}{dt} \approx 0$$



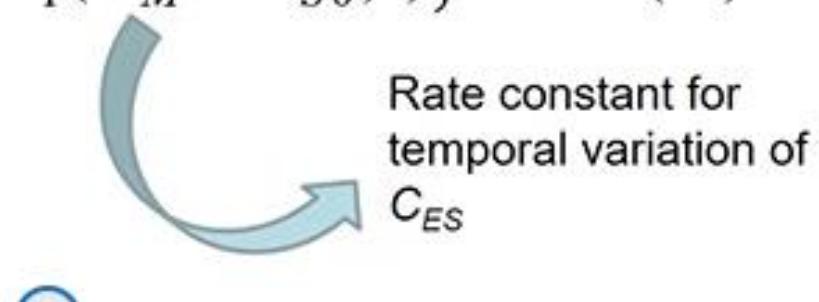
In this period, assume

# When is the Quasi-Steady State Assumption Valid?...Contd.

### **Balance Equation for unstable complex ES:**

solving eqn. (17) with initial condition at  $t=0$ ,  $C_{ES}=0$ .

$$C_{ES} = \frac{C_{S0} C_{E0}}{K_M + C_{S0}} \left\{ 1 - \exp(-k_1(K_M + C_{S0})t) \right\} \dots \dots \dots (18)$$



Rate constant for first phase (step 'a') =  $k_1(K_M + C_{S0})$

For second phase, (step 'b')

$$\frac{dC_S}{dt} = -\frac{k_2 C_{E0} C_S}{K_M + C_{S0}}$$

Rate constant of this process during initiation =  $\frac{k_2 C_{E0}}{K_M + C_{S0}}$

For quasi steady state assumption to be valid:

Step 'a' must be much faster than Step 'b'.

∴ Rate constant for step (b) << rate constant for step (a)

$$\text{or, } \frac{k_2 C_{E0}}{K_M + C_{S0}} \ll k_1(K_M + C_{S0})$$

$$\text{or, } \frac{C_{E0}}{K_M + C_{S0}} \ll (1 + \frac{k_{-1}}{k_2})(1 + \frac{C_{S0}}{K_M})$$

Rate constant for first phase (step 'a') =  $k_1(K_M + C_{S0})$

For second phase, (step 'b')

$$\frac{dC_S}{dt} = -\frac{k_2 C_{E0} C_S}{K_M + C_{S0}}$$

Rate constant of this process during initiation =  $\frac{k_2 C_{E0}}{K_M + C_{S0}}$

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or,  $\frac{k_2 C_{E0}}{K_M + C_{S0}} \ll k_1(K_M + C_{S0})$

or,  $\frac{C_{E0}}{K_M + C_{S0}} \ll (1 + \frac{k_{-1}}{k_2})(1 + \frac{C_{S0}}{K_M})$

## Regulation of enzyme activity

- Need for inhibition: To control the amount of product formed by rxn. Because
  1. Product may stimulate a biochemical pathway that is needed at certain times (e.g. growth).
  2. Product may be biologically active over a narrow concentration range.
  3. Excess accumulation of products may require too much energy or interfere with other pathway.

# Inhibition

*How does one inhibit?*

by regulating substrate, products or other molecules  
that interact with the enzymes.

*What does regulation lead to?*

- a. inhibition (intrinsic as well as extrinsic)
- b. activation (extrinsic)

*What are the types of inhibition?*

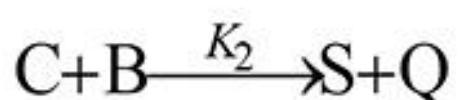
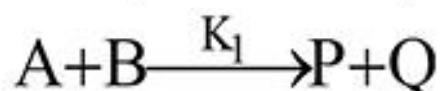
- 1. Competitive inhibition
- 2. a. Un-competitive inhibition
  - b. Non-competitive inhibition
- 3. Substrate inhibition.

## Epistemic Qs 14.

### Reaction networks: series/parallel/series-parallel?

#### Examples of competitive Reactions

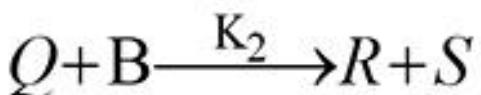
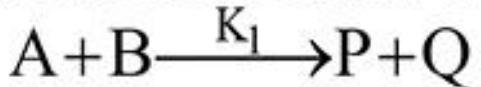
- Competitive parallel :  $(A:B)_{fed}=1:1$  S:desired product



Case 1:  $K_2 >> K_1$  no P

Case 2:  $K_1 >> K_2$  no S

- Competitive Consecutive:  $(A:B)_{fed}=1:1$  S:desired product

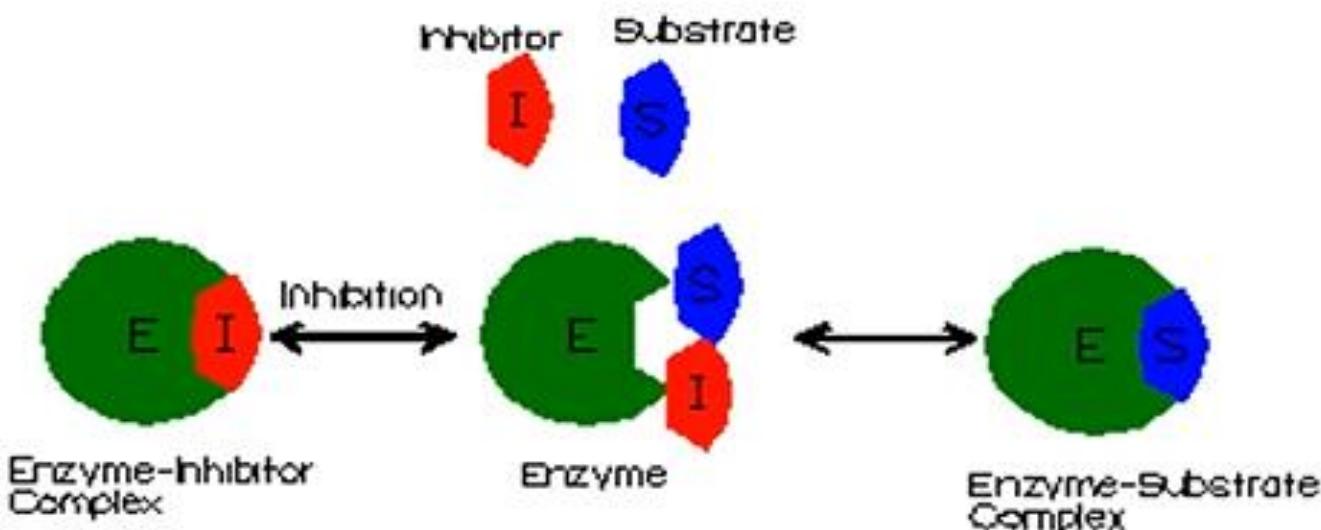
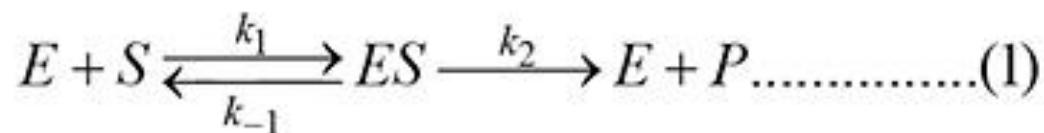


If  $\frac{K_1}{V} \rightarrow \infty$ , no S is formed.



## Competitive inhibition

Inhibitor (I) blocks active sites of enzymes



## Derivation

## Assumptions:

## 1. Quasi-steady state for ES

$$\frac{dC_{ES}}{dt} \approx 0$$

$$or, k_1 C_S C_E - (k_{-1} + k_2) C_{ES} = 0$$

$$or, C_{ES} = \frac{k_1 C_S C_E}{k_{-1} + k_2} = \frac{C_E C_S}{K_M} \dots \dots \dots (3)$$

## 2. Reaction 2 attains equilibrium

$$k_i C_E C_J = k_{-i} C_{EJ}$$

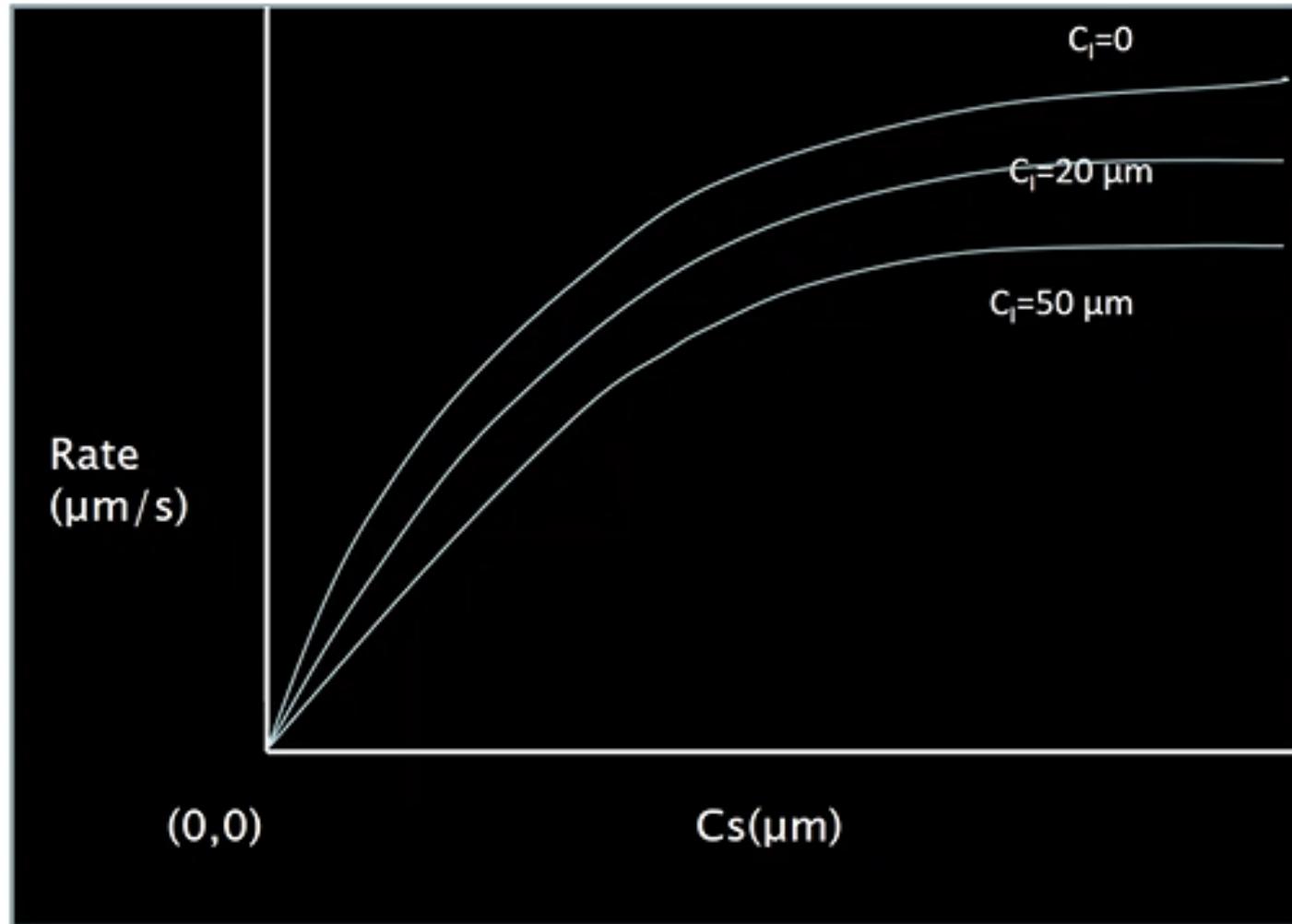
### 3. Constraint equation:

## Competitive inhibition (contd..)

Substituting eqn. (6) in eqn(3):

where,  $R_{\max} = k_2 C_{E0}$  &  $\bar{K}_M = K_M \left(1 + \frac{C_I}{K_I}\right)$

since,  $C_I, K_I > 0, \bar{K}_M > K_M$ .



$$R_{\max} = 25 \mu\text{m}/\text{s},$$

$$K_M = 5 \mu\text{m},$$

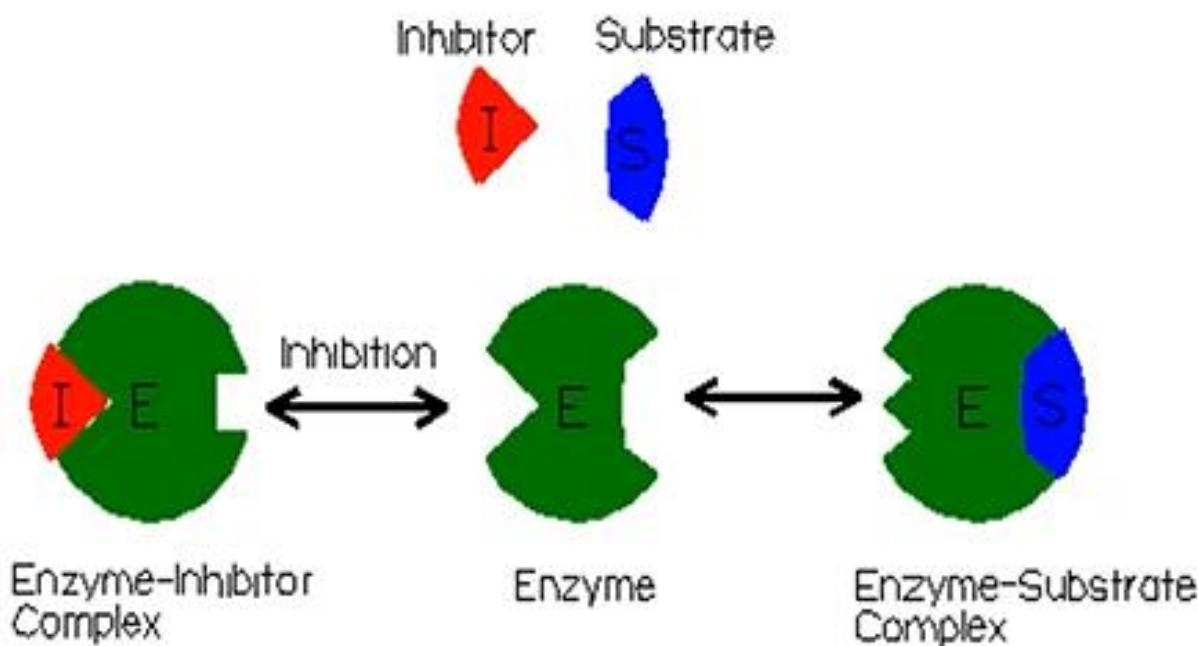
$$K_I = 10 \mu\text{m}$$

$$\text{At } C_s \rightarrow 0, \text{slope} = \frac{R_{\max}}{K_M}$$

Note: as  $K_M$  decrease,  
slopes increase

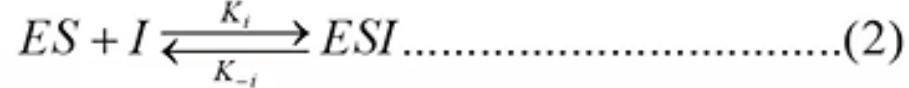
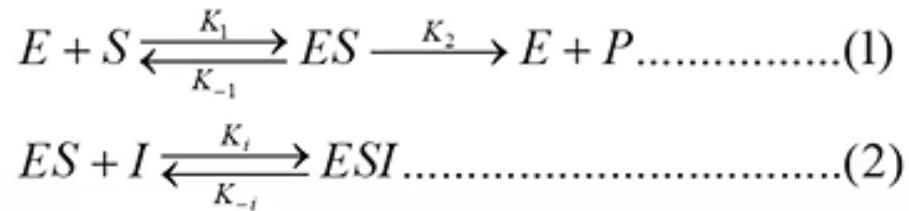
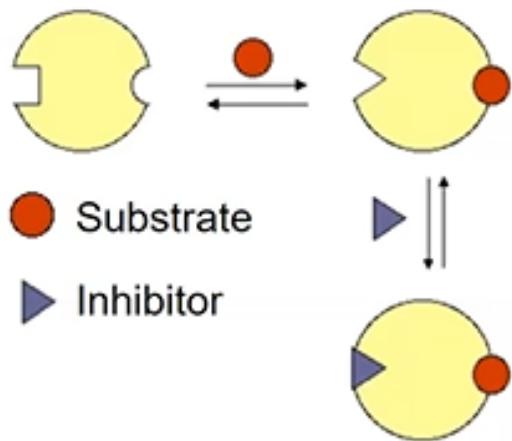
## Uncompetitive Inhibition

Instead of binding to the active sites inhibition can bind to other sites on the enzymes, reducing the reaction rate.



## Uncompetitive inhibition

- It does not directly block active sites of enzymes but interacts with enzyme bounded substrate on another sites, reducing reaction rate.



1. Assumption: Reaction (2) is in equilibrium:

2. Constraint eqn:

$$C_{EQ} = C_E + C_{ES} + C_{ESI} \dots\dots(4)$$

### 3. Quasi-steady state assumption for 'ES'

$$\frac{dC_{ES}}{dt} = K_1 C_E C_S - K_{-1} C_{ES} - K_2 C_{ES} - \cancel{K_3 C_{ES} C_I} + \cancel{K_4 C_{ESI}} = 0$$

$$(K_{-1} + K_2)C_{ES} = K_1 C_E C_S$$

## Uncompetitive Inhibition (Contd....)

Substituting (3) & (5) in (4)

$$C_{EO} = C_E \left( 1 + \frac{K_1}{K_{-1} + K_2} C_s + \frac{K_1}{K_{-1} + K_2} C_s \frac{C_I}{K_I} \right);$$

$$C_E = \frac{C_{EO}}{1 + \frac{C_s}{K_M} + \frac{C_s}{K_M} \frac{C_I}{K_I}}$$

$$C_{ES} = \frac{C_E C_s}{K_M} = \frac{C_{EO} C_s}{K_M + C_s \left( 1 + \frac{C_I}{K_I} \right)}$$

### Uncompetitive Inhibition (Contd....)

Substituting (3) & (5) in (4)

$$C_{EO} = C_E \left( 1 + \frac{K_1}{K_{-1} + K_2} C_s + \frac{K_1}{K_{-1} + K_2} C_s \frac{C_I}{K_I} \right);$$

$$C_E = \frac{C_{EO}}{1 + \frac{C_S}{K_M} + \frac{C_S}{K_M} \frac{C_I}{K_I}}$$

$$C_{ES} = \frac{C_E C_S}{K_M} = \frac{C_{EO} C_S}{K_M + C_S \left( 1 + \frac{C_I}{K_I} \right)}$$

## Uncompetitive Inhibition (Contd....)

$$Rate = \frac{dc_p}{dt} = K_2 C_{ES} = \frac{K_2 C_{EO} C_S}{K_M + \left(1 + \frac{C_I}{K_I}\right) C_S}$$

$$= \frac{\frac{K_2 C_{EO}}{C_I/K_I} C_S}{\frac{K_M}{C_I/K_I} + C_S} = \frac{\tilde{R}_{\max} C_S}{\tilde{K}_M + C_S}$$

$$\tilde{K}_M = \frac{K_M}{1 + \frac{C_I}{K_I}}$$

$$\tilde{R}_{\max} = \frac{K_2 C_{EO}}{1 + \frac{C_I}{K_I}}$$

## Uncompetitive Inhibition (Contd....)

$$\tilde{K}_M = \frac{K_M}{1 + \frac{C_I}{K_I}}$$

$$\tilde{R}_{\max} = \frac{K_2 C_{EO}}{1 + \frac{C_I}{K_I}}$$

$$C_I, K_I > 0, \quad \tilde{K}_M < K_M, \tilde{R}_{\max} < R_{\max}$$

$$\text{However, } \frac{\tilde{R}_{\max}}{\tilde{K}_M} = \frac{K_2 C_{EO}}{K_M} = \frac{R_{\max}}{K_M}$$

For,  $C_s \rightarrow 0$ , slope remains unchanged;

However maxm. rxn. rate  $R_{\max}$  decreases.