

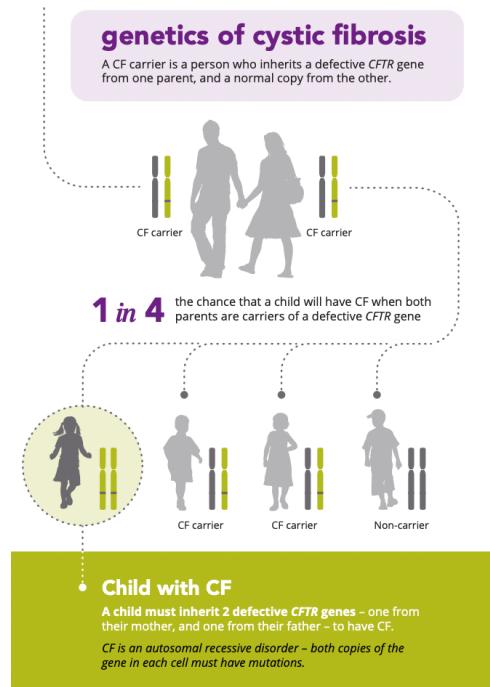
## LECTURE 1

### CYSTIC FIBROSIS (CF)

- multi-system disease that affects the **lungs, gastrointestinal tract, pancreas** and **other organs**.
- It is a **genetic disease**.
- Causes the production of thick mucus, which eventually clogs the lungs and can lead to a variety of lung infections. Furthermore, the disease also affects the pancreas, disabling digestive enzymes to break and absorb food.
- Inhaling concentrated salt water (hypertonic saline) mist provided benefits to adults and children suffering from CF.
- Inhaling salt water rehydrates the lining of the lungs and loosens the thick mucus that builds up, thus reducing recurrent infections alongside its associated lung damage and respiratory failure
- The size of the saline droplets is crucial to the success of the treatment as they need to be small enough to penetrate deep into the lungs

#### - CFTR GENE

- The most common, lethal inherited disease of caucasians
- A **recessive** genetic disorder
- Carriers of the disease don't have any symptoms
- If two carriers have children together is a 1/4 chance that the children will have cystic fibrosis



### Molecular mechanism of Cystic Fibrosis

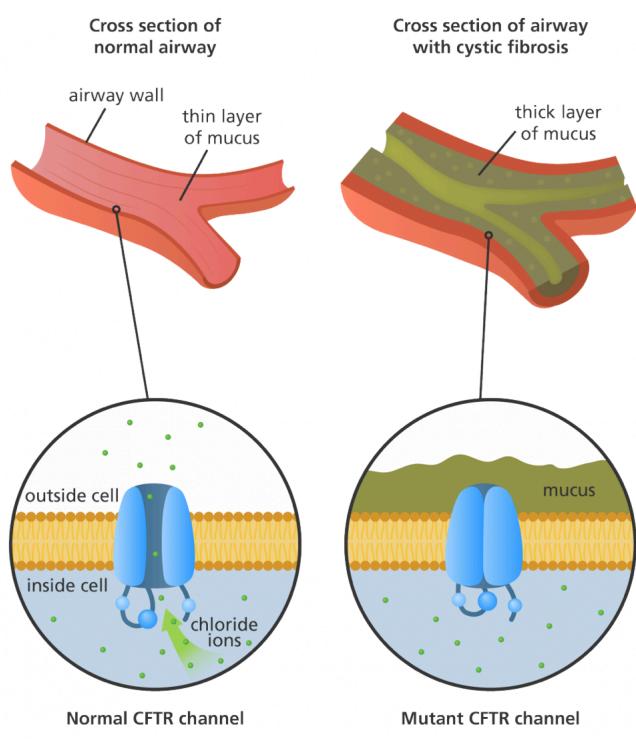
Defective Gene (mutation in the DNA) → Defective protein →

Defective ion transport in epithelial tissue → Defective water movement across the surface of cells in the lung = build up of mucus → Bronchial obstruction



### Bacterial infection.

- (Protein that allows transportation of Chloride ions) therefore can't move across membrane
- Epithelial cells line surfaces
- Shortened lifespan due to bacterial infection of the lungs colonising mucus as no flow of water as no flow of chloride as no flow of ions as defect protein channel as a result of mutated genes the environment mucus creates is optimal for bacterial growth
  
- Mucus blocks air sacs (alveoli) in the lungs (causing bronchiectasis in the long term this results in a decrease in SA:V ratio and effecting alveoli function (gas exchange) and can cause hypoxia)
- Mucus blocks pancreatic ducts (therefore pancreatic enzymes can "attack" the pancreas itself = pancreatitis)
- Due to concentration gradient, and osmosis, water follows ions



### LECTURE 2

- Cystic Fibrosis involves a defect in salt and water balance, particularly in epithelial tissues, and most particularly in the lungs
- Salt and Water = NaCl ( $\text{Na}^+$  and  $\text{Cl}^-$ ) and  $\text{H}_2\text{O}$
- To understand properly the consequences of having cystic fibrosis it is necessary to understand ions and water molecules, and the interaction between them
- The cause of cystic fibrosis is a chemical change (mutation) in the DNA, the hereditary material that is in the nucleus of all eukaryotic cells. As a result of this chemical change in the DNA there is a chemical change in one of the important proteins in the cell. This particular protein has the job of carrying  $\text{Cl}^-$  ions across membranes
- To understand the nature of the chemical defects in the DNA and in the protein it is necessary to understand a little about the chemistry of DNA and proteins. To understand this it is necessary to understand a little about the chemistry of the atoms that make up DNA, proteins and other molecules that are important in biology.
  
- An element is a substance that cannot be broken down to other substances by chemical reactions
- A compound is a substance consisting of two or more elements in a fixed ratio
  
- Around 25 of the 92 elements are essential to life
- C, H, O and N make up 96% of living matter
- Most of the remaining 4% consists of calcium, phosphorus, potassium and sulfur
- Trace elements are those required by an organism in minute quantities.
  
- Covalent bonds: a molecule consists of two or more atoms held together by covalent bonds
- A single covalent bond, or single bond is the sharing of one pair of valence electrons
- A double covalent bond, or double bond, is the sharing of two pairs of valence electrons
- Covalent bonds can form between atoms of the same element or atoms of different elements
  
- Electronegativity is an atom's attraction for the electrons in a covalent bond
- The more electronegative an atom, the more strongly it pulls shared electrons toward itself
  
- Bond polarity
  - In a non polar covalent bond, the atoms share the electron equally
  - In a polar covalent bond, one atom is more electronegative than the other, and the atoms do not share the electron equally

# Functional groups

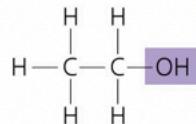
## HYDROXYL

### STRUCTURE



(may be written HO—)

### EXAMPLE



Ethanol, the alcohol present in alcoholic beverages

### NAME OF COMPOUNDS

Alcohols (their specific names usually end in *-ol*)

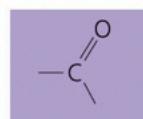
### FUNCTIONAL PROPERTIES

- ▶ Is polar as a result of the electronegative oxygen atom drawing electrons toward itself.
- ▶ Attracts water molecules, helping dissolve organic compounds such as sugars

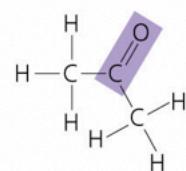
- Because electrons are negatively charged this results in an asymmetric distribution of

## CARBONYL

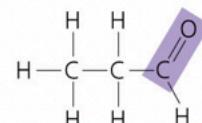
### STRUCTURE



### EXAMPLE



Acetone, the simplest ketone



Propanal, an aldehyde

### NAME OF COMPOUNDS

Ketones if the carbonyl group is within a carbon skeleton

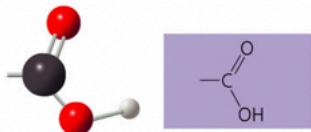
Aldehydes if the carbonyl group is at the end of the carbon skeleton

### FUNCTIONAL PROPERTIES

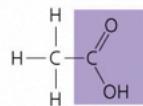
- ▶ A ketone and an aldehyde may be structural isomers with different properties, as is the case for acetone and propanal.

## CARBOXYL

### STRUCTURE



### EXAMPLE



Acetic acid, which gives vinegar its sour taste

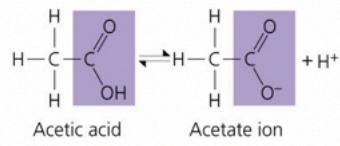
electrical charge along the length of the bond

### NAME OF COMPOUNDS

Carboxylic acids, or organic acids

### FUNCTIONAL PROPERTIES

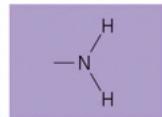
- ▶ Has acidic properties because it is a source of hydrogen ions.
- ▶ The covalent bond between oxygen and hydrogen is so polar that hydrogen ions ( $H^+$ ) tend to dissociate reversibly; for example,



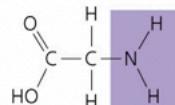
- ▶ In cells, found in the ionic form, which is called a carboxylate group.

## AMINO

### STRUCTURE



### EXAMPLE



Glycine

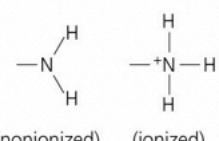
Because it also has a carboxyl group, glycine is both an amine and a carboxylic acid; compounds with both groups are called amino acids.

### NAME OF COMPOUNDS

Amine

### FUNCTIONAL PROPERTIES

► Acts as a base; can pick up a proton from the surrounding solution:



(nonionized)      (ionized)

► Ionized, with a charge of 1+, under cellular conditions.

## SULPHYDRYL

### STRUCTURE



### EXAMPLE



Ethanethiol

### NAME OF COMPOUNDS

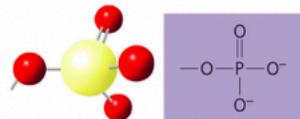
Thiols

### FUNCTIONAL PROPERTIES

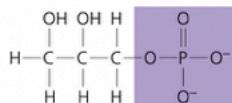
- Two sulphydryl groups can interact to help stabilize protein structure

## PHOSPHATE

### STRUCTURE



### EXAMPLE



Glycerol phosphate

### NAME OF COMPOUNDS

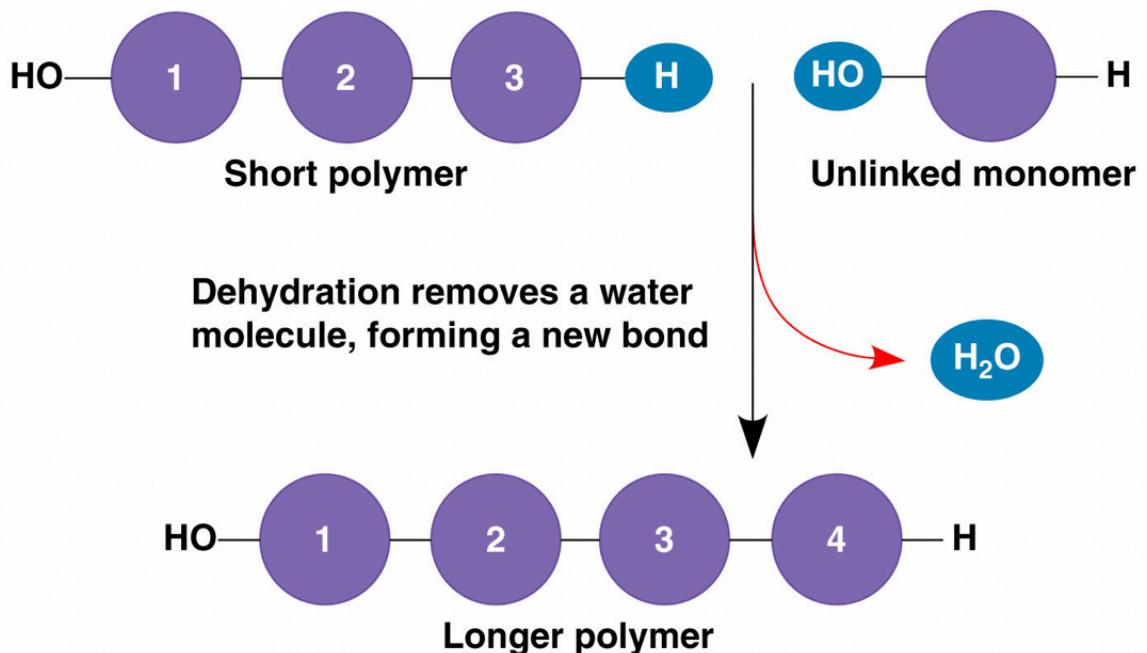
Organic phosphates

### FUNCTIONAL PROPERTIES

- Makes the molecule of which it is a part an anion (negatively charged ion).
- Can transfer energy between organic molecules.

- Covalent bonds are a primary determinant of molecular shape
- Biological molecules recognise and interact with each other with a specificity based on molecular shape
- Molecules with similar shapes can have similar biological effects
- Ionic bonds: atoms sometimes strip electrons from their bonding partners
- One example of this is the transfer of an electron from sodium to chlorine
- After the transfer of an electron, both atoms have charges
- A charged atom (or molecule) is called an ion

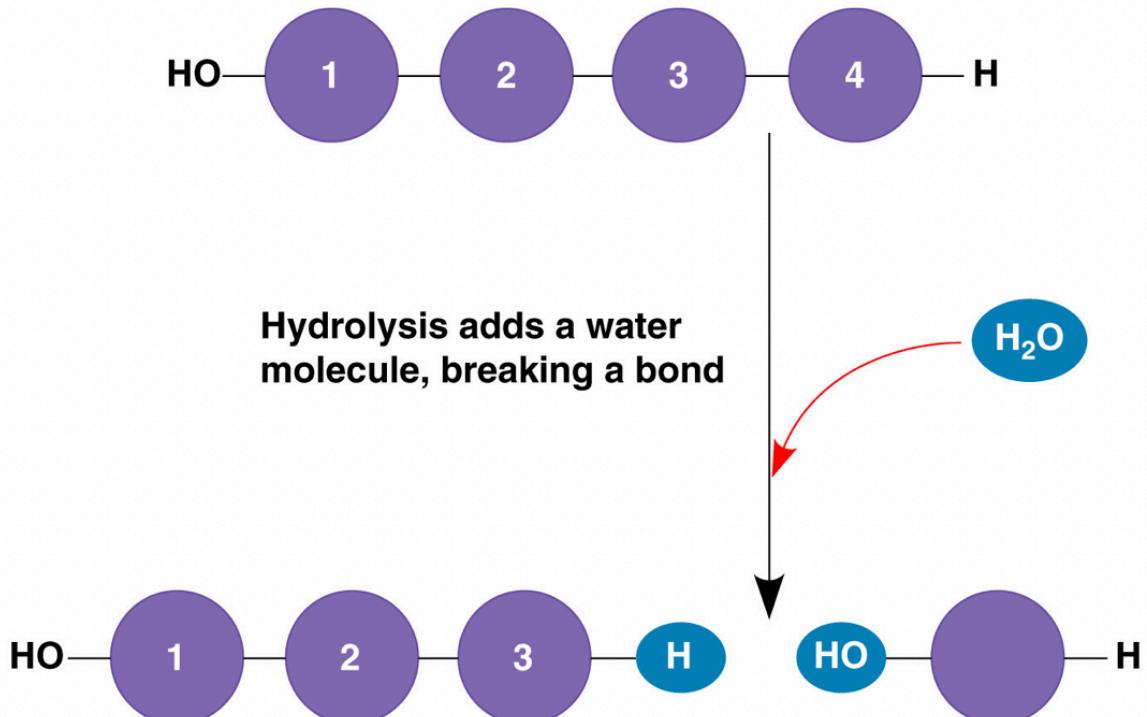
## Polymers are formed from dehydration reactions



(a) Dehydration reaction in the synthesis of a polymer

- Van der Waals Interactions

## Polymers are broken down via hydrolysis reactions



- Molecules or atoms that are very close together can be attracted by transient charge differences
- These weak attractions are called van der waals interactions
- Van der Waals interactions are the weakest of all intermolecular attractions between molecules. However, with a lot of Van der Waals forces interacting between two objects, the interaction can be very strong
  
- Strongest bonds in organism are covalent bonds that form molecules
- Weak Chemical bonds, such as ionic and hydrogen bonds are also important as reinforce shapes of large molecules and help molecules adhere to each other.

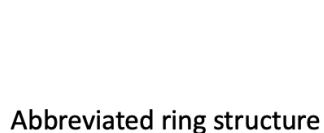
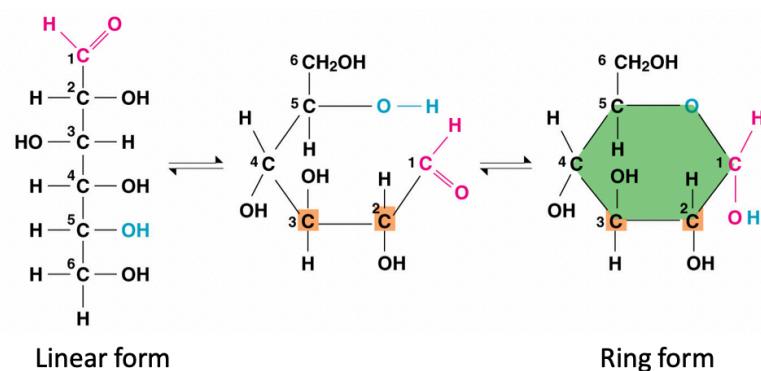
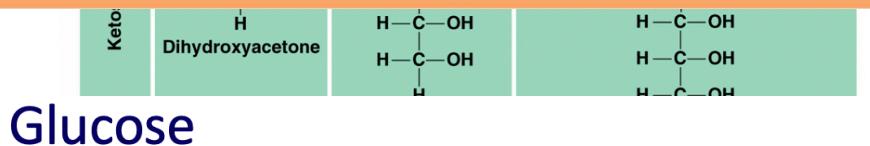
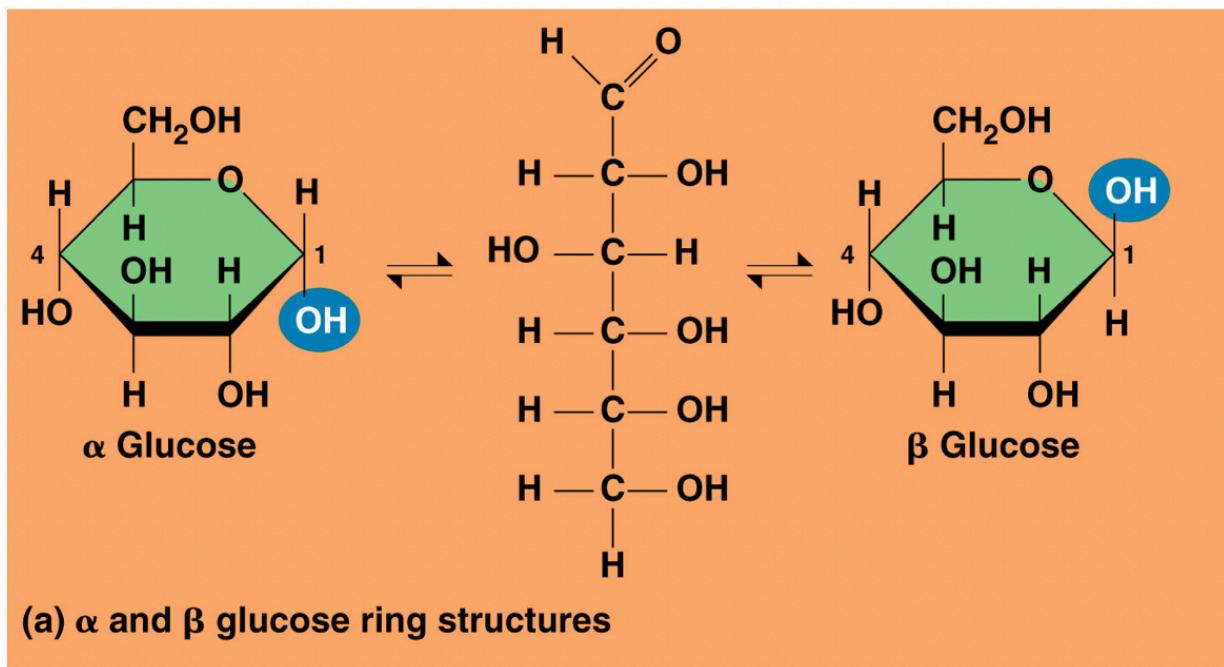
### LECTURE 3

- Cells use four main types of small molecules:
  - Sugars
  - Amino acids
  - Nucleotides
  - Fatty Acids
- These molecules are found free in solution, both inside cells and in the extracellular compartments of multicellular organisms.
- They are essential intermediates in the biochemical reactions necessary for supporting life.
- They are also used extensively in the construction of large polymers (i.e. many small molecules of one type linked together by covalent bonds) known as **macromolecules**.
- These typically contain thousands of atoms. e.g. haemoglobin is a **protein**, inside your red blood cells. It is a polymer of amino acids and contains more than 6000 atoms
- The protein that is defective in cystic fibrosis is another one that contains thousands of atoms. It only takes for a few of these to be the wrong ones, or missing, for the protein to not function properly.
  
- In living systems there are three classes of macromolecule:
  - **Proteins (polymers of amino acids)**
  - **Nucleic Acids (DNA and RNA; polymers of nucleotides)**
  - **Polysaccharides (polymers of sugars)**
- Lipids (containing fatty acids) are a fourth type of molecule that play crucial roles in biological systems. But although lipids form very large structures (membranes) these are not held together by covalent bonds and they are not true macromolecules

### **Carbohydrates: sugars and sugar polymers**

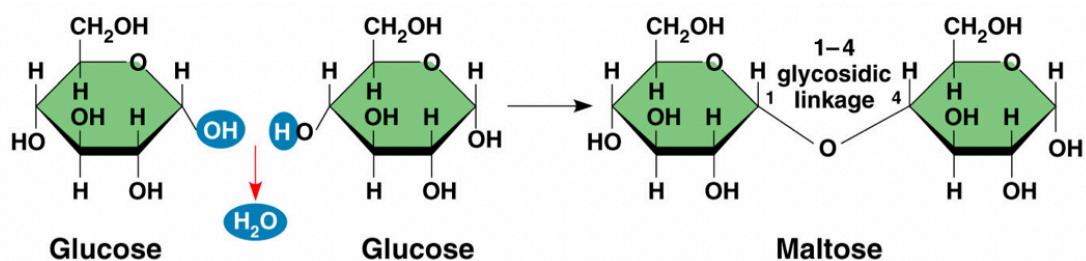
- One of the most dangerous symptoms of cystic fibrosis is accumulation of mucus in the lungs
- The build up of mucus facilitates the colonisation of the lungs by pathogenic bacteria
- Some of the major components of mucus are sugar polymers
- Sugar polymers perform a diverse array of other functions in biological systems
- But first we consider simple sugars, the building blocks for these larger molecules.
- **Monosaccharides**
- **Glucose** is the most abundant monosaccharide in biological systems

## The $\alpha$ and $\beta$ ring forms of glucose

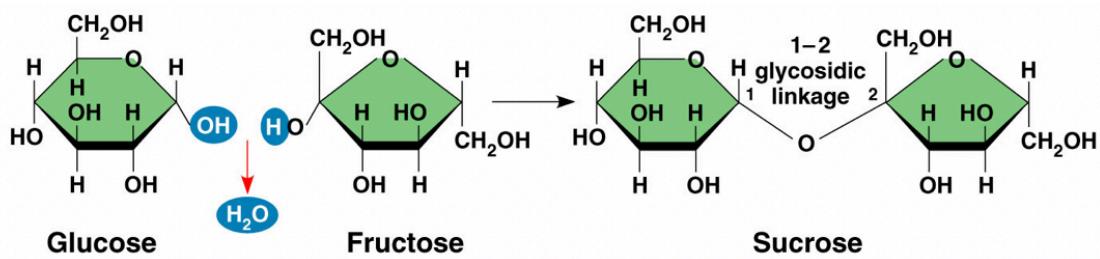


- Formation of disaccharides via dehydration reactions
    - **Disaccharides** (two monosaccharides linked together) are formed by **glycosidic linkages**
    - The formation of a glycosidic linkage entails the loss of a water ( $H_2O$ ) molecule and is therefore an example of a **dehydration reaction**

## Synthesis of maltose

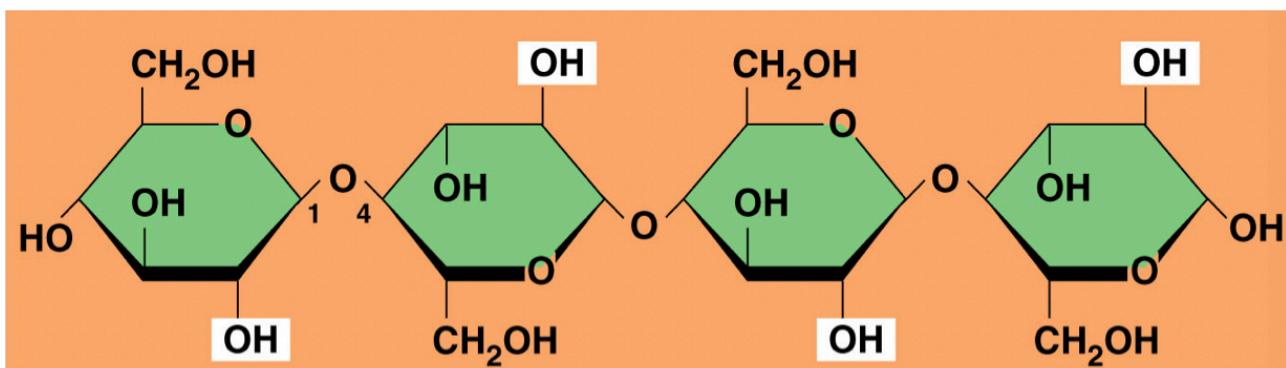


## Synthesis of sucrose



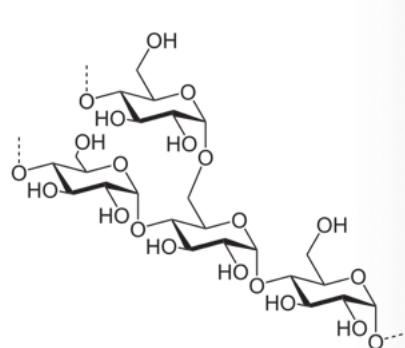
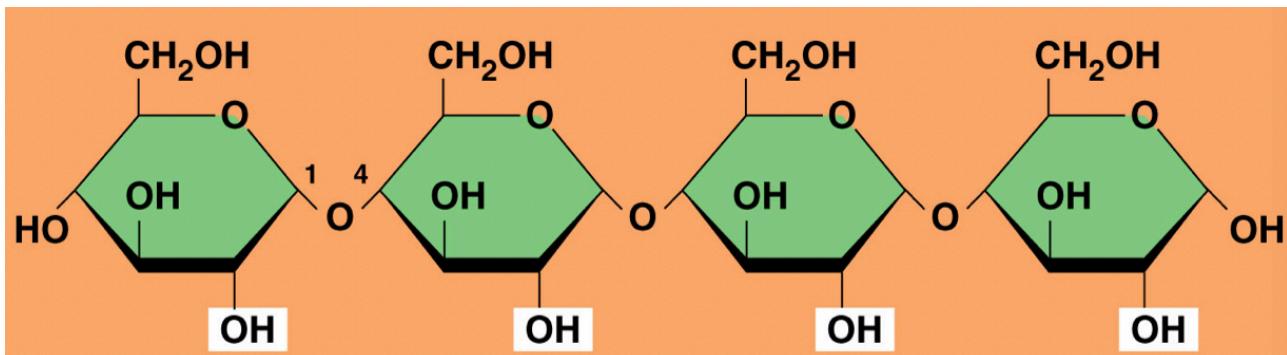
## Cellulose

- Cellulose is an **unbranched** polymer composed of beta glucose molecules
  - It is formed through 1-4 linkage of  $\beta$  glucose monomers



## Starch

- Starch is a storage form of energy in plants
- It contains two polymers composed of glucose units: amylose (unbranched) and amylopectin (branched)
- These polymers are formed through 1-4 linkage of a glucose monomers



Branching in amylopectin takes place with  $\alpha(1 \rightarrow 6)$  bonds occurring every 24 to 30 glucose units

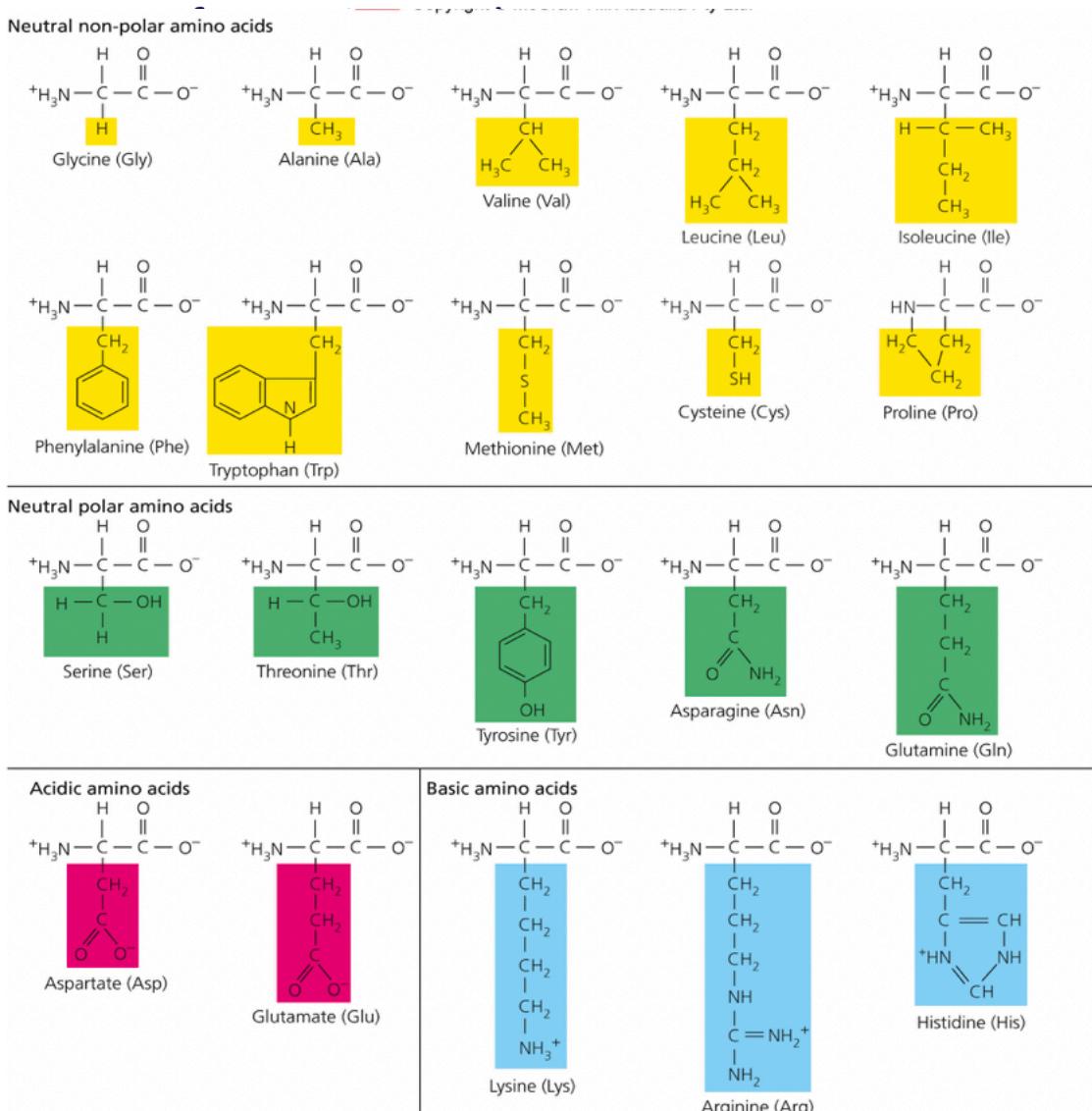
## Glycogen

- Glycogen is a storage form of energy in animals it is more highly branched than amylopectin

## Proteins: Polymer of Amino Acids

- In patients with cystic fibrosis there is a defect in a protein that is located in the plasma membrane of epithelial cells, such as those lining the inner surface of the lung
- Proteins are formed from linear polymers of often hundreds (or even thousands) of **amino acid**.
- In most people with CF, just one of these amino acids in this one particular protein is missing
- There are 20 different amino acids commonly found in proteins and they all have the same basic structure

# The

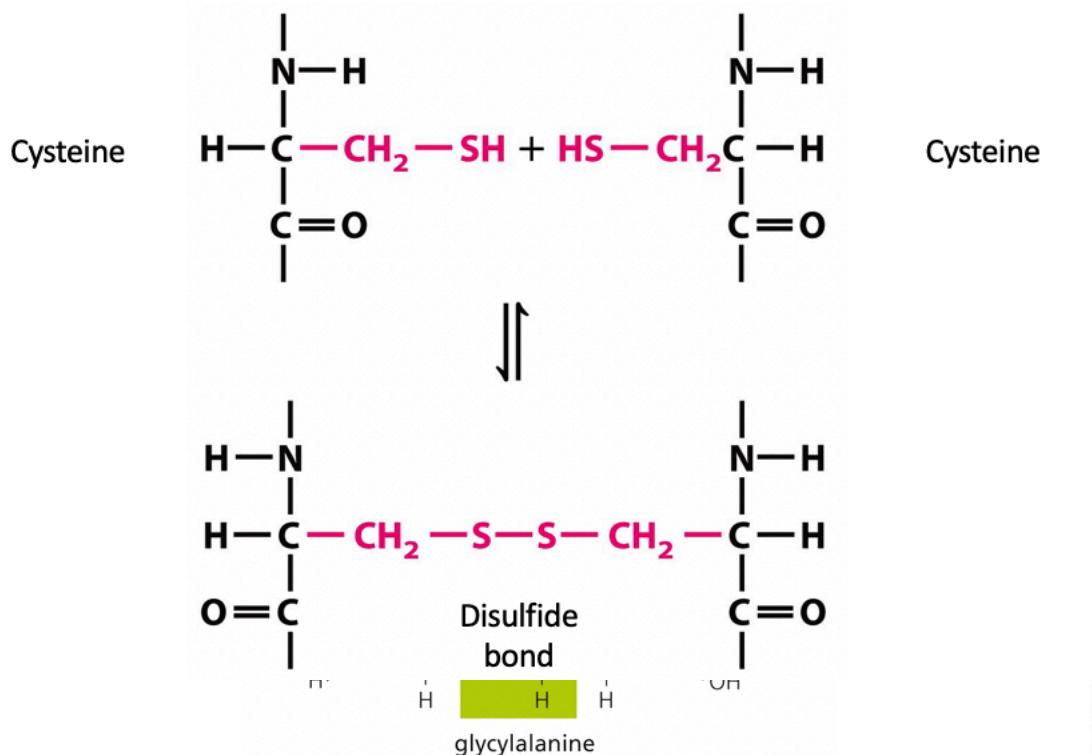


## LECTURE 4

### Peptides

- Two amino acids linked by a **peptide bond** are referred to as a **dipeptide**, three as a **tripeptide**
- Up to about 12 amino acids linked by peptide linkages are referred to as an **oligopeptide**
- More than this and the term **polypeptide** is used. Proteins are formed from one or more polypeptides
- Proteins are always formed from linear polypeptide chains - there are no branches
- However two cysteine side chains (each with an -SH group on the end) can react to form a covalent bond called **disulfide bond or a disulfide bridge**
- Proteins carry out diverse roles
  - Structural support
  - Movement
  - Recognition, protection, defence
  - Transport of molecules and ions across membranes (**transporters and channels**)
  - Catalysis of biochemical reactions (**enzymes**)
- Protein structure
  - One of the keys to the ability of proteins to perform a diverse range of functions lies in their ability to adopt a wide range of structures (shapes)

# Disulfide bonds form between cysteine molecules



- Protein structure is extremely complex and is described in terms of four different levels

## 1° Structure

- Precise sequence of amino acids

## 2° Structure

- Consists of regular patterns or shapes adopted by different regions of a polypeptide chain. α helix and β pleated sheet. (initial 3D conformation). Held in place by hydrogen bonds between the amino acids

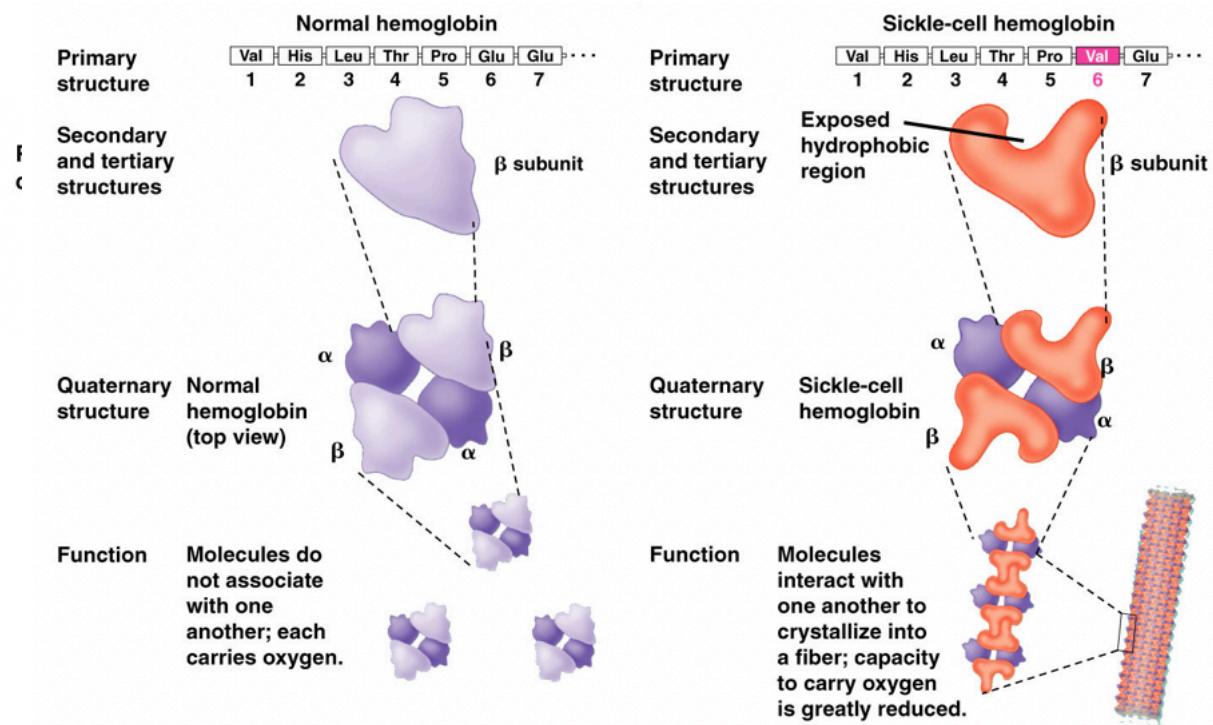
## 3° Structure

- Overall shape or structure of a polypeptide in a protein. A complete description of the tertiary structure specifies the location of every atom in the polypeptide in 3D space. (final 3D conformation) (maintained by a combination of different types of chemical bonds and interactions between the component amino acids)

## 4° Structure

- How the different peptides (subunits) of a multi-polypeptide protein fit together. Some proteins consist of a single polypeptide in which case they don't have a quaternary structure (the membrane protein that goes wrong in cystic fibrosis is an example of this) whereas some proteins are made up of multiple polypeptides (in which case they do).
- Haemoglobin is an example of a protein with a quaternary structure
- A haemoglobin molecule has **four (polypeptide) subunits** and is therefore referred to as a **tetramer**
- The quaternary structure of a protein is maintained by a combination of the same types of chemical bonds and interactions that hold the tertiary structure in place

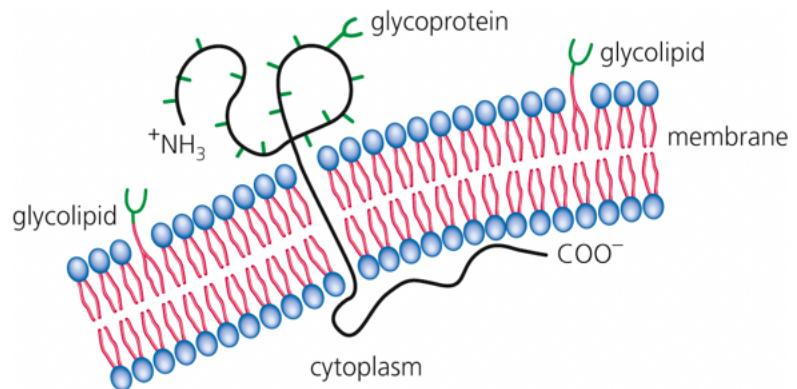
# Sickle cell disease



## - Sickle cell disease

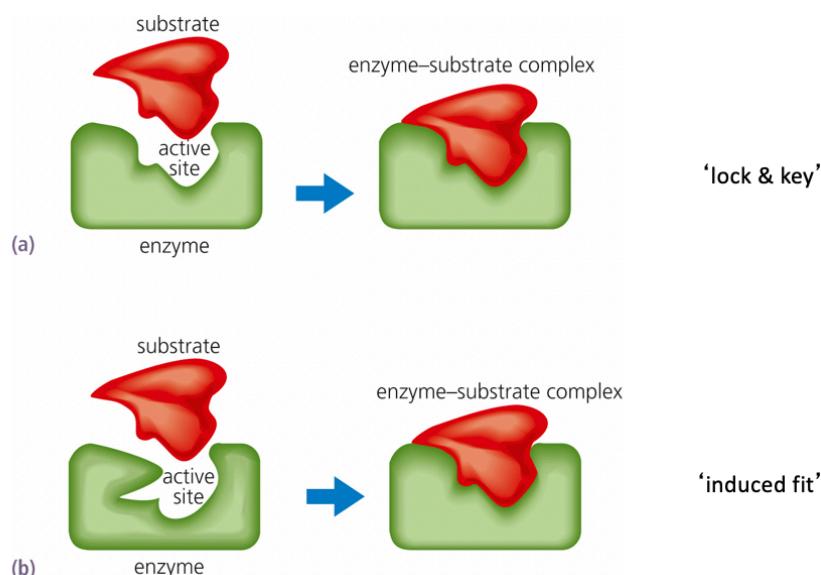
Protein structure:

- In **globular proteins** the polypeptide backbone tends to be folded so that amino acids with non polar (hydrophobic) side chains are buried in the interior of the molecule, away from the aqueous environment
- The surface of globular proteins tend to be rich in polar and charged amino acids which readily interact with water molecules
- The tertiary and quaternary structures of proteins are stabilised by non-covalent **hydrogen bonds, ionic bonds, hydrophobic interactions between non polar regions and van der waals interactions** and in some cases, by covalent **disulfide bonds**
- Membrane- spanning proteins (**integral membrane proteins**) tend to have stretches of non-polar amino acids in the portions that extend across the membranes



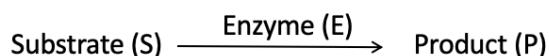
## LECTURE 5

- Enzymes are (in most, but not all cases) proteins that have the ability to **catalyse** chemical reactions
- These important macromolecules lie at the heart of all of biochemistry
- An enzyme **binds specifically** reacting molecules
- The reacting molecules are called **reactants or substrates**
- The substrate(s) binds to a groove or cleft on the enzyme, called the active site
- The binding of a substrate (e.g. sugar molecule) to an enzyme depends on their having **complementary shapes** which fit together, like a **lock and key**
- In some cases the binding of a substrate might **induce** the enzyme to adjust its shape, leading to a better fit

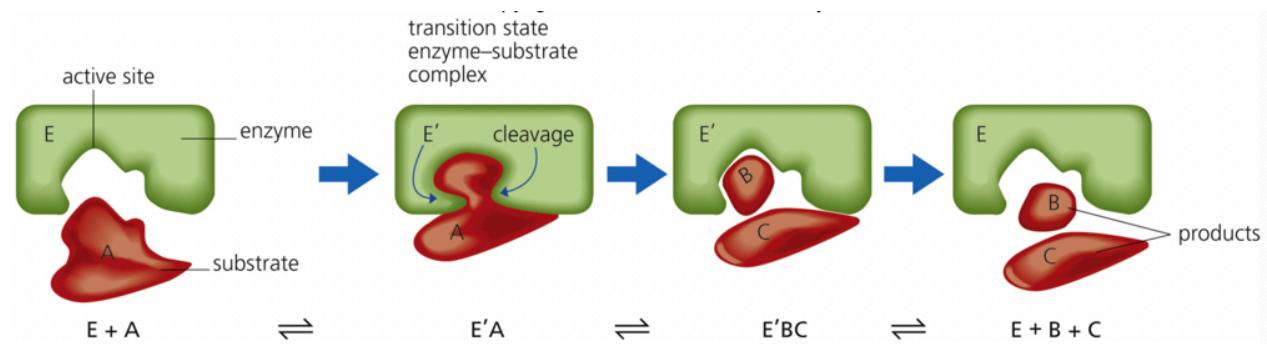
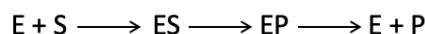


- Enzymes convert **substrates** to **products** by breaking, rearranging, reforming chemical bonds
- The product is released and the enzyme is then ready to catalyse another reaction

- The overall process can be summarised as:

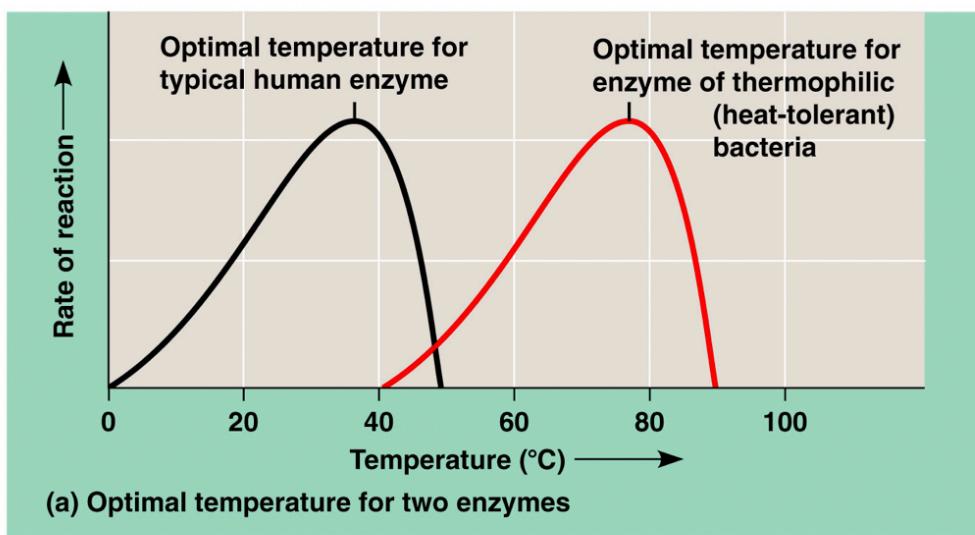


- It is often represented schematically as:



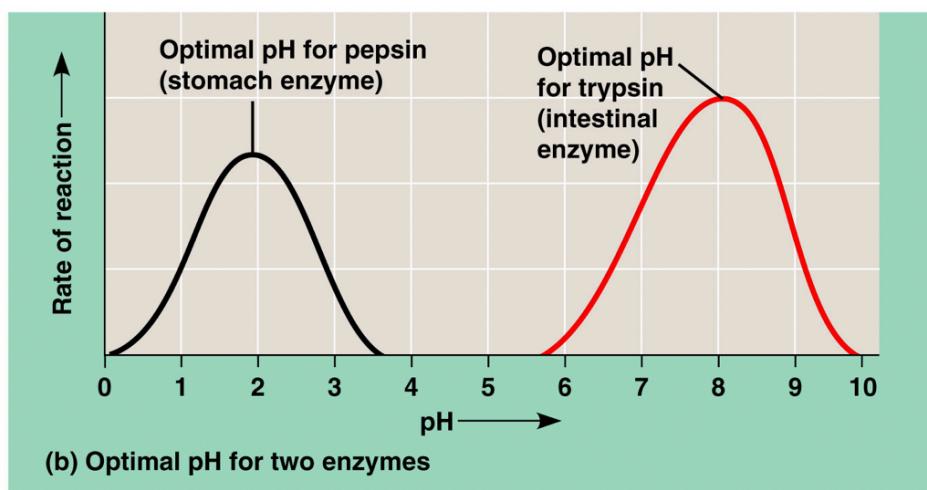
- In every cell there are many different types of enzymes, each capable of catalysing a specific type of reaction
- Many enzymes convert a single substrate molecule to a single product molecule
- Others combine two or more substrates and/or produce two or more products
- Enzymes are astonishingly quick. Some enzymes bind substrate molecules, convert them to product and release them more than 100'000 times a second
- Enzymes are usually named on the basis of the reaction that they carry out and are often, though not always given names ending in “**ase**”.

## Enzymes have optimal temperatures

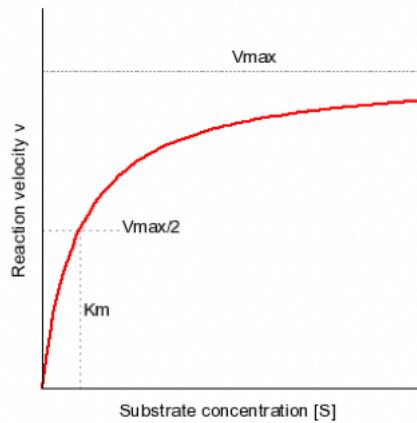


- Enzymes are sensitive to **pH**
- Changes in pH can change the charge on amino acids of which enzymes are composed, and this can perturb the ionic interactions that contribute to holding enzymes in a particular shape
- Changes in the charges of the amino acids at the active site of an enzyme can have a particularly dramatic effect
- Enzymes have an **optimal pH**, at which they work most efficiently
- **When changes of temperature or pH destroy the tertiary structure** of a protein, the protein is said to be **denatured**

## Enzymes have optimal pH values



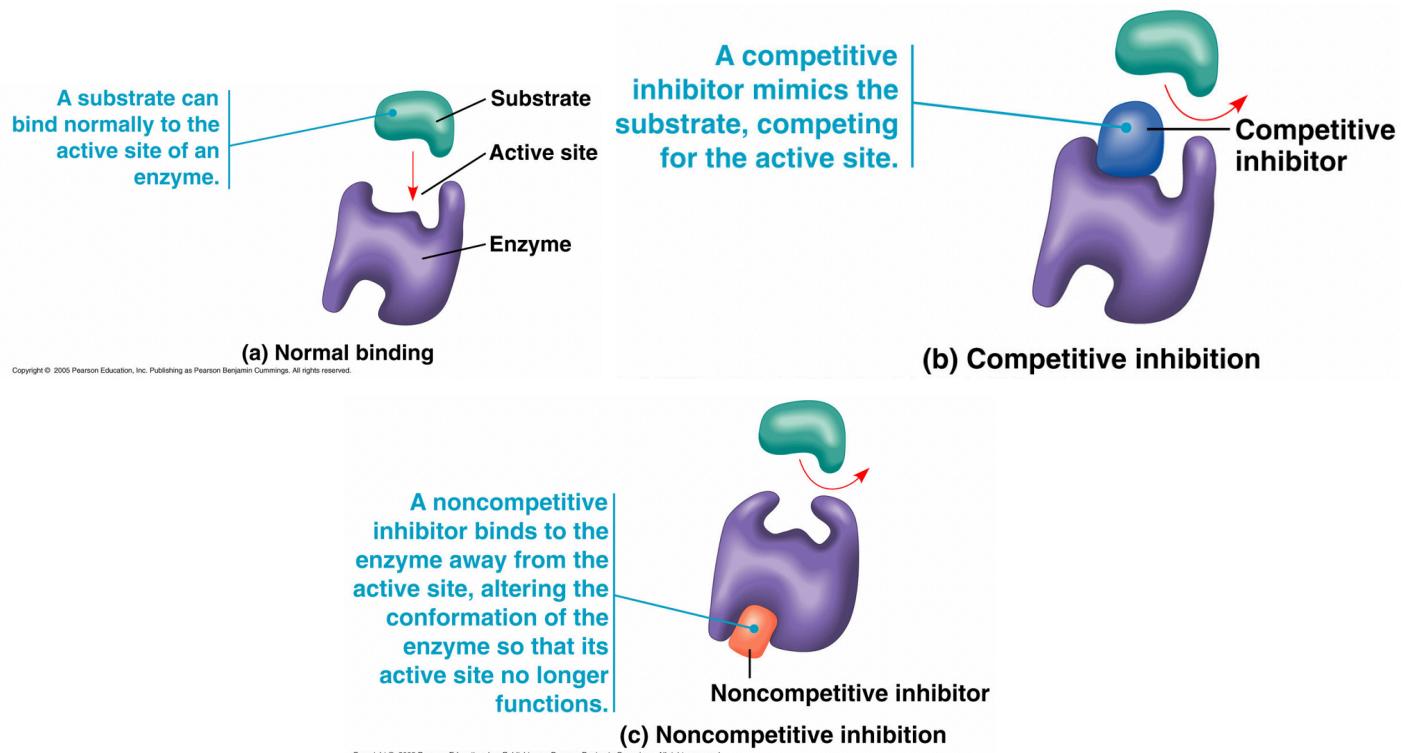
- Many enzymes have ‘cofactors’
- **Cofactors** are typically small, non-protein molecules which bind to enzymes and thereby facilitate their activity
- Some cofactors are inorganic metal ions; e.g. zinc, copper or iron atoms
- Other organic molecules (carbon containing molecules such as vitamins) in which case they are called **coenzymes**
- Enzymes can be ‘saturated’ with substrate
- When all the enzyme present is working at a maximum rate it is said to be **saturated**



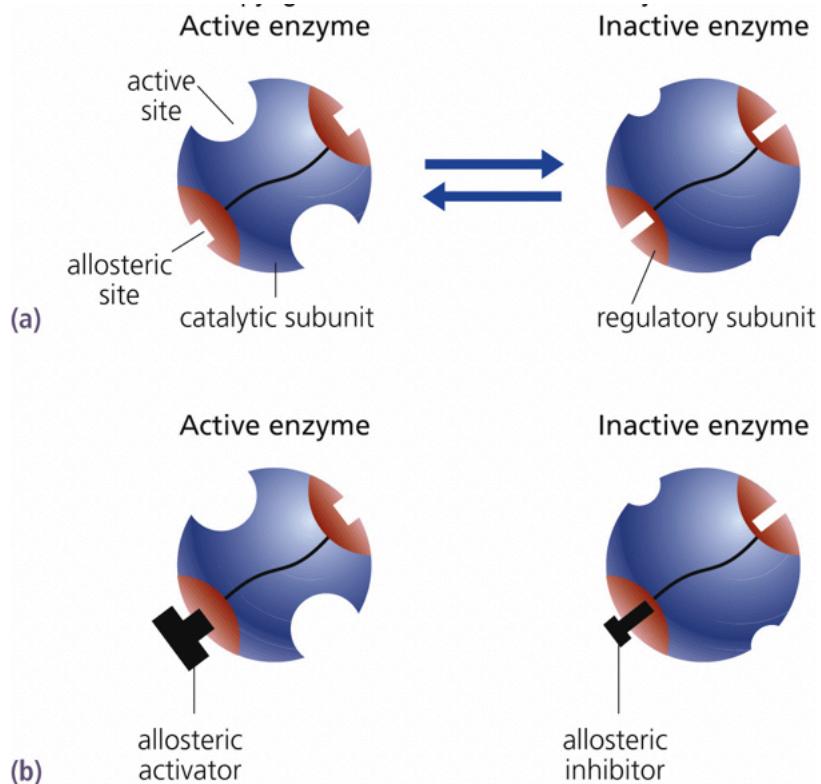
- This behaviour is summarised by the **Michaelis-Menten** equation:  $V = V_{max} [S] / (K_M + [S])$

This gives the velocity of a reaction ( $V$ ) as a function of the concentration of substrate ( $[S]$ ), the maximal velocity of the reaction ( $V_{max}$ ), i.e. the velocity when all of the enzyme present is working at a maximum rate and the substrate molecules are having to wait around to undergo their conversion) and  $K_M$  a “dissociation constant” which provides a measure of how tightly the enzyme can bind to the substrate (the lower the  $K_M$ , the tighter the binding).

## Enzymes can be inhibited



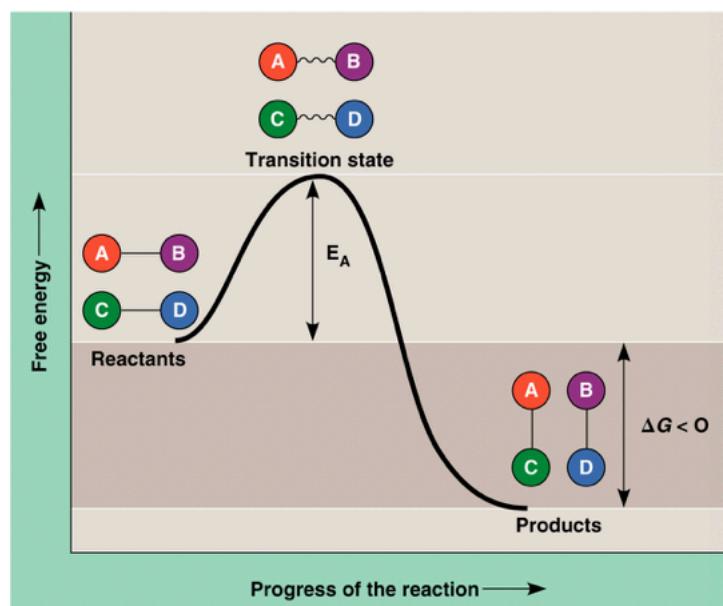
- Many drugs are enzyme inhibitors (i.e. aspirin)
- Enzymes may have one or more allosteric sites
- It would be wasteful to have all the enzymes in a cell working as fast as they can, all the time
- Cells use a variety of approaches to control both the amount of enzyme being produced and the activity of enzymes already present
- Many enzymes are subject to **allosteric regulation**, which involves molecules or ions bringing to a site different from the active site and causing the activity of the enzyme to **increase** or **decrease**.



- enzymes are components of metabolic pathways
- To understand how enzymes are able to speed up chemical reactions (and thereby allow biochemical processes to occur on the time-scale that is necessary for life to exist) it is necessary to understand a little about the energy associated with chemical reactions.
- All chemicals have a certain amount of **energy**
- The energy of a particular molecule depends on, amongst other things, the nature of the chemical bonds that it contains, and the degree of stress or strain on the bonds in the molecule.
- The more **stressed or strained** a molecule is, the **less stable** it is, and the **higher its energy**.
- In talking about the energy of molecules in this way chemists (and biochemists) define something called the **free energy** (referred to as **G**)
- This is a measure of the total energy available from a molecule (or a number of molecules) under the conditions of a chemical reaction
- For every chemical or biochemical reaction it is possible to calculate the total change in free energy (i.e.  $\Delta G$ ) associated with the conversion of reactants to products

$$\Delta G = G_{\text{products}} - G_{\text{reactants}}$$

- The conversion of reactants (substrates) to products requires the reactants to have a minimum **activation energy**.
- In being converted from reactants to products the reactants have to pass through a form that is intermediate between the two
- This is a strained, stressed molecule that has a **higher free energy** than either the reactants or products
- It is referred to as the **transition state**
- The **activation energy** for a reaction is the **difference** between the free energy of the reactants and the free energy of the transition state
- Only those reactant molecules with sufficient energy to adopt the stressed transition state form are able to undergo the conversion to products.
- The higher the activation energy, the fewer the number of reactant molecules that will, at any given time, have sufficient energy to overcome this barrier, and the slower the reaction will proceed
- If the activation energy is very high then almost no molecules will have sufficient energy to surmount it and the reaction will proceed very slowly, if at all



- Enzymes lower the activation energy for a reaction
  1. Substrates enter active site changes shape to embrace substrate
  2. Substrate held in active site by weak interactions, H bond, ionic bond
  3. Active site can lower Ea and speed up reaction by
    - Acting as template
    - Stressing substrate and stabilising transition state
    - Providing favourable microenvironment
    - Participating directly in catalytic reaction
  4. Substrate converted to product
  5. Product released
  6. Active site is available for new substrate

## Lecture 6

### Nucleotides and Nucleic Acids

- DNA: Deoxyribonucleic acid; Linear, non-branching: Polynucleotide
- RNA: Ribonucleic acid (single-stranded)
- Nucleotides: phosphate group, pentose sugar (deoxyribose), Nitrogenous base

### Nitrogenous Base

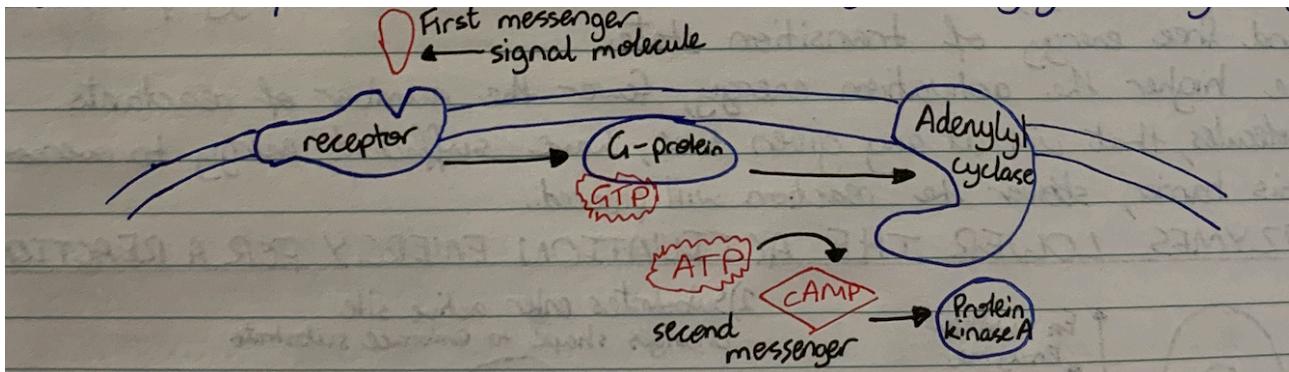
- Pyrimidines: single six-membered ring (Uracil, Thymine, Cytosine)
- Purine: Double ring, six-membered and five-membered (adenine, Guanine)
- Nucleotide without phosphate group-nucleotide
- Major source of energy, energy released by breaking phosphate-phosphate bonds of ATP



### Cyclic AMP (cAMP)

- Is formed from ATP and serves as 'second messenger' inside cell

- Conversion of ATP to cAMP is via enzyme adenylate cyclase



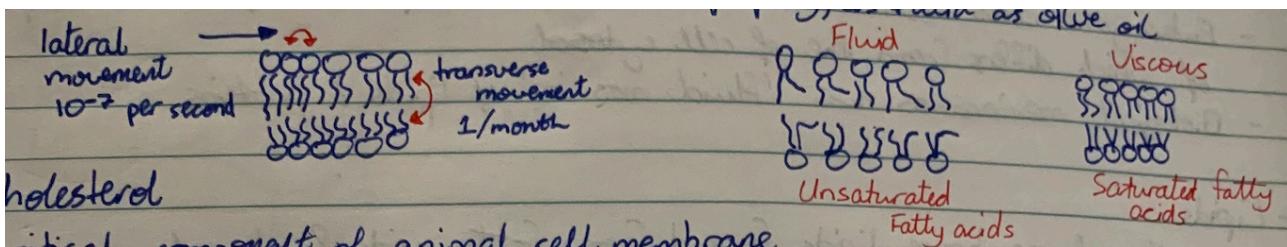
- Polynucleotides: Adjacent nucleotides are joined by covalent bond between -OH group on 3' Carbon of one nucleotide and phosphate on the 5' carbon (phosphodiester bond)

## Lecture 7

- Membrane structure and function
- Cystic Fibrosis
  - The protein that is defective in people with CF, is Cl<sup>-</sup> ion channel; integral membrane protein that carries Cl<sup>-</sup> ions
- Cell Membranes
  - Plasma membrane is boundary that separates interior of living cell from non living surrounding
  - Membrane exhibits selective permeability
  - Eukaryotic cells - contain membrane-bound compartments, internal composition of which differs from the of cell cytosol
  - Biological membranes are 'fluid mosaics' of lipids and proteins
- Lipids
  - Majority of membrane lipids formed from fatty acids and glycerol
- Phospholipids
  - Most abundant lipid
  - Amphipathic molecules, hydrophobic and hydrophilic regions
  - Hydrophobic region composed of fatty acid 'tail'
  - Hydrophilic region composed of phosphate group 'head'

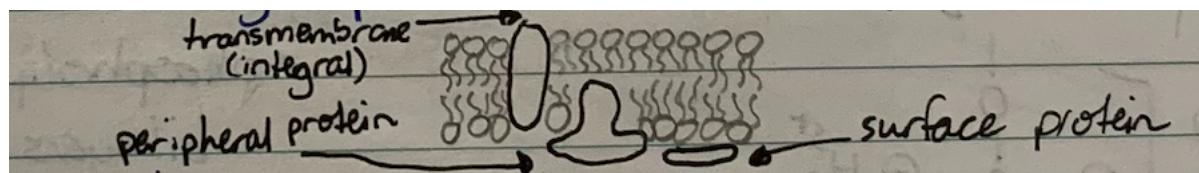
hydrophilic region composed of phosphate group  
 $\text{CH}_2 - \overset{\substack{+ \\ \text{H-bonding}}}{\text{N}(\text{CH}_3)_3} \quad \text{H}^+$   
 $\text{CH}_2$   
 phosphate [  $\text{O} = \text{P}(\text{O}^-)(\text{H}^+) \text{OS}^-$  ]  
 $\text{CH}_2 - \underset{\substack{\text{H-bonding} \\ \text{H}^+}}$   $\text{CH} - \text{CH}_2$  Glycerol  
 hydrophobic - non-polar  
 C=O C=O  
 unsaturated fat  
 saturated fat

- phospholipids spontaneously form bilayers { bilayer
- to minimise exposure of hydrophobic 'tail' to hydrophilic
- Coalescence of phospholipid into bilayers, minimises exposure of water molecules to hydrophobic tail of lipid
- dynamic layer



- Membrane Fluidity
    - Phospholipids in membrane move within bilayer, spin around long axis
    - Most of lipids and some proteins, diffuse laterally in bilayer
    - Only very rarely does molecule flip between layers (transversely) across membrane
    - As temperature at which membrane switch from fluid state to solid state
    - The temperature at which membrane solidifies depends on type of lipid
    - Membranes rich in unsaturated fatty acids are more fluid than those rich in saturated fatty acids
    - Membrane must be fluid to work properly, as fluid as olive oil
  - Cholesterol
    - Critical component of animal cell membrane
    - Controls and maintains membrane fluidity - 37°C restrains phospholipid movement
    - Within membrane, OH- group facing water, hydrocarbon tail-hydrophobic

- Membrane protein
  - Membrane collage of different proteins embedded in fluid metric of lipid bilayer
  - Determine most of membranes specific functions
  - Peripheral proteins one not embedded
  - Integral proteins penetrate hydrophobic core and often span the membrane



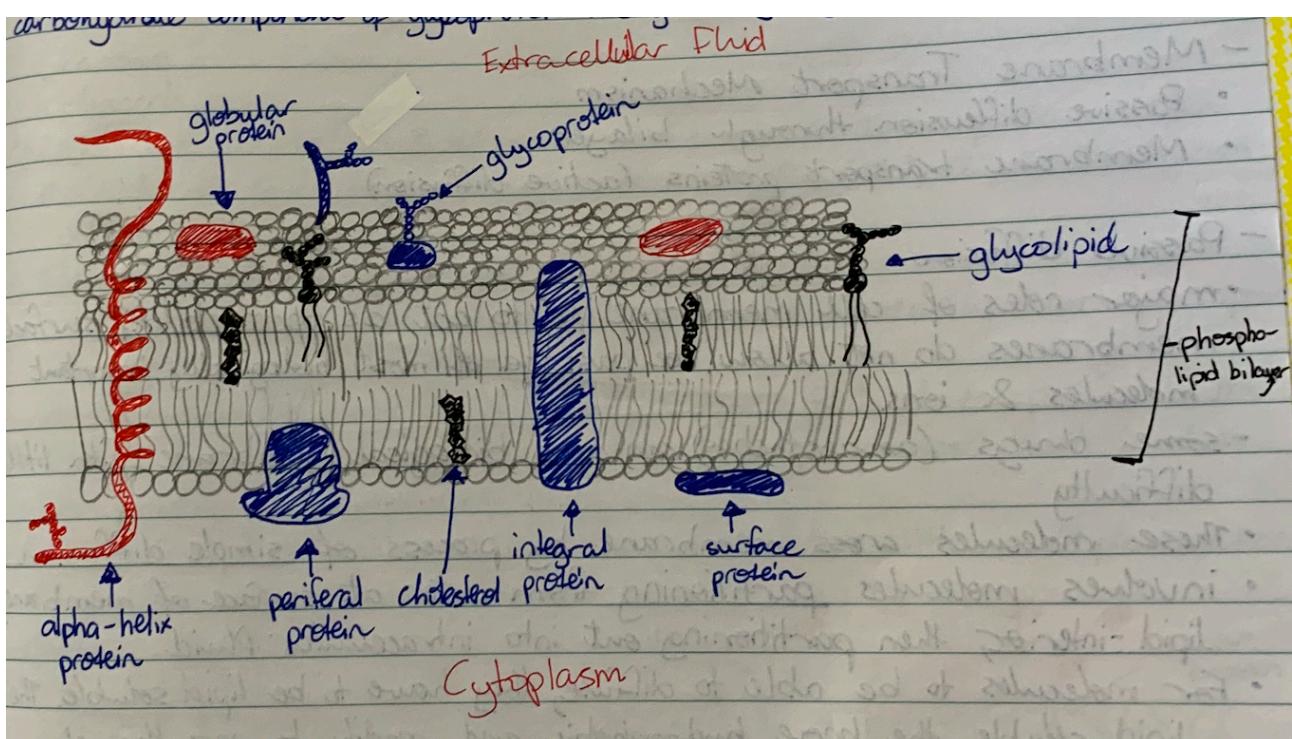
- Integral membrane protein
  - Transmembrane protein
  - Hydrophobic regions of integral protein consists of one or more stretches of non-polar amino acids, often coiled into α helices

- Diffusion of proteins within membrane bilayer

- some diffuse within bilayer
- proteins much larger than lipids move slowly
- the diffusion of proteins in cell membrane is demonstrated and measured by technique known as Fluorescent Recovery After Photobleach (FRAP)

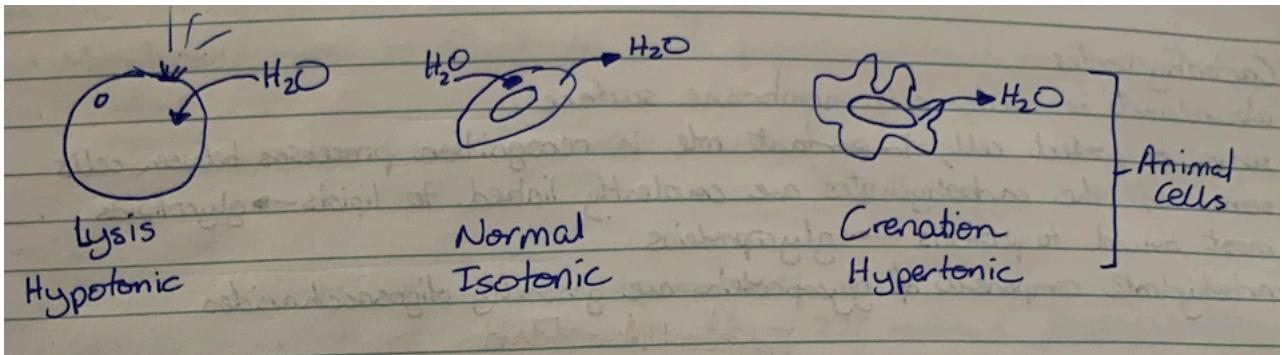
- Major function of membrane protein
  - Transport
  - Enzymatic activity
  - Signal transduction
  - Cell-cell recognition
  - Intercellular joining
  - Attachment to cytoskeleton and extracellular matrix

- Carbohydrates
  - Abundant on outer cell membrane surface
  - Serve to protect cell, important role in recognition processes between cells
  - Some of the carbohydrates are covalently linked to lipids - glycolipids
  - Most bound to proteins - glycoproteins
  - Carbohydrate component of glycoprotein are generally oligosaccharides

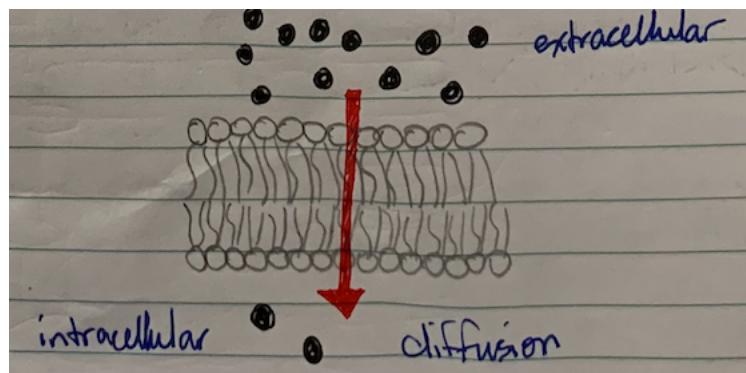


## Lecture 8

- Membrane Transport
  - Concentration gradient: Molecules have natural tendency to move down concentration gradient. [HIGH] to [LOW]
  - Energy required to move up concentration gradient of molecules
  - Regions of high solute concentration, have high **osmolarity**
  - Process of water moving to regions with higher osmolarity is **osmosis**
    - Water has natural tendency to move to high conc solute.



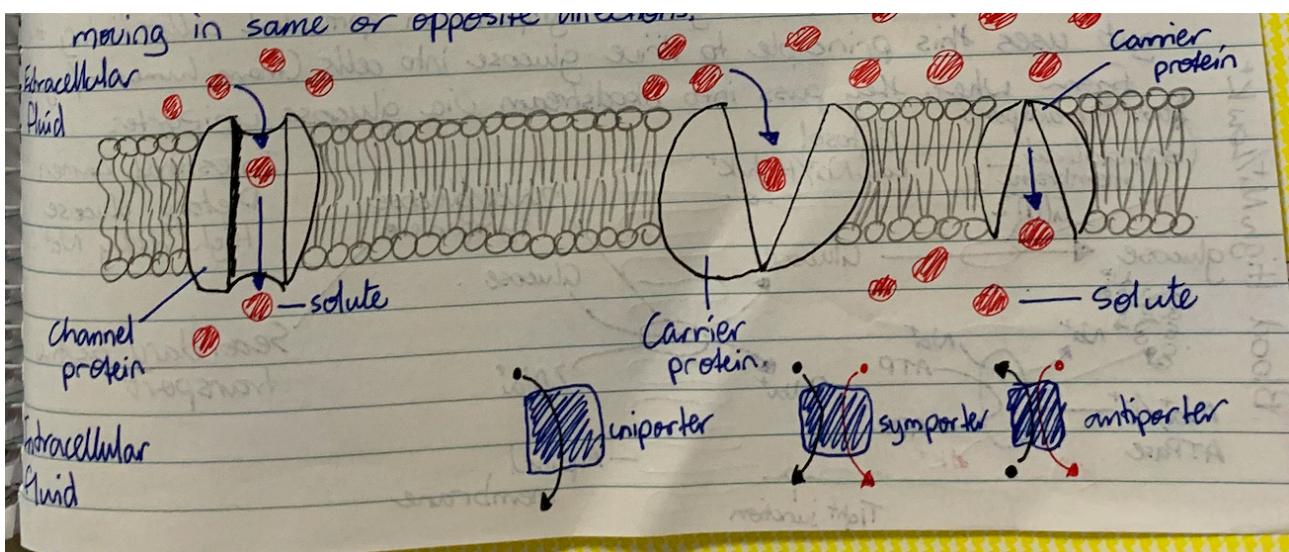
- Membrane transport Mechanism
  - Passive diffusion through bilayer
  - Membrane transport Proteins (active diffusion)
- Passive diffusion
  - Major roles of cell membrane is to act as **permeability barrier**
  - Membranes do not allow free passage of most biologically important molecules and ions
  - Some drugs (anaesthetics) can cross biological membrane with little difficulty
  - These molecules across membrane by process of **simple diffusion**
  - Involves molecules **partitioning** from water at surface of membrane, into **lipid-interior**, then partitioning out into intracellular fluid
  - For molecules to be able to diffuse, they have to be lipid soluble. The more lipid soluble, the more hydrophobic and readily to pass through.



### Membrane Transport Proteins

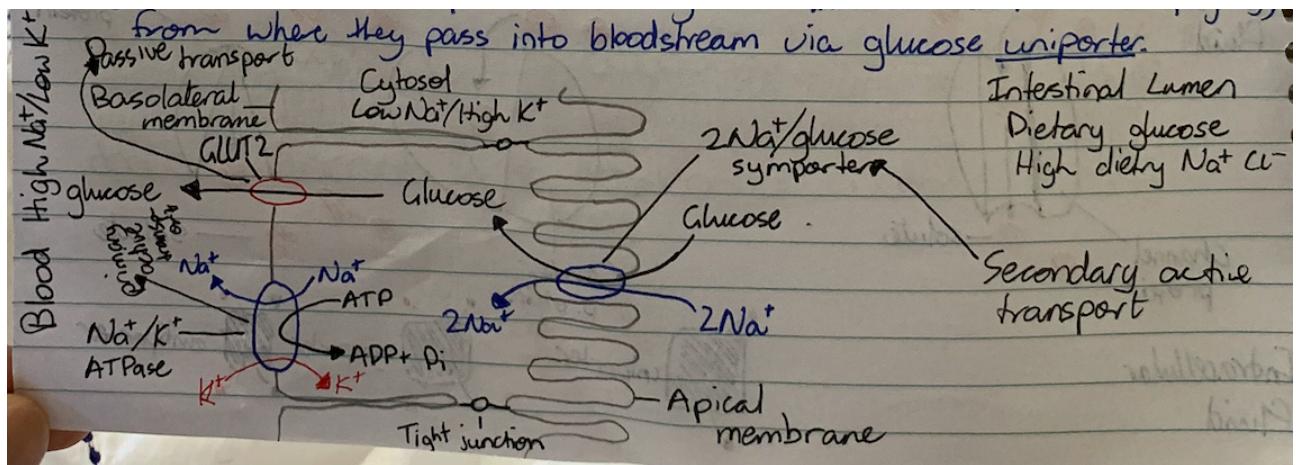
- Many important biological molecules have very low or no solubility in lipids - **hydrophilic** (due to polar bonds and/or +ve and -ve charges)
- Organic molecules such as these include
  - $Cl^-$
  - $Na^+$

- K<sup>+</sup>
- Ca<sup>2+</sup>
- For such solutes, rate of diffusion across lipid phase of membranes is negligible
- However, solutes such as these cross membrane with help of membrane transport proteins
- Membrane proteins are integral (transmembrane) proteins
- Two Classes:
  - Carriers
  - Channels
- Channels are simplest sort of membrane proteins. These proteins, within their structure, pose through which solutes (Na<sup>+</sup>, Cl<sup>-</sup>, K<sup>+</sup> and Ca<sup>2+</sup>) can diffuse
- Such channels, where present are not open all the time
- Their opening and closure is tightly regulated and usually occurs in response to specific signals
- Carriers are more complicated. They recognise and bind to specific solutes and transport them across the membrane through process that involves protein undergoing conformational change
- Carrier-mediated transport can be complicated with multiple substrate moving in same or opposite directions



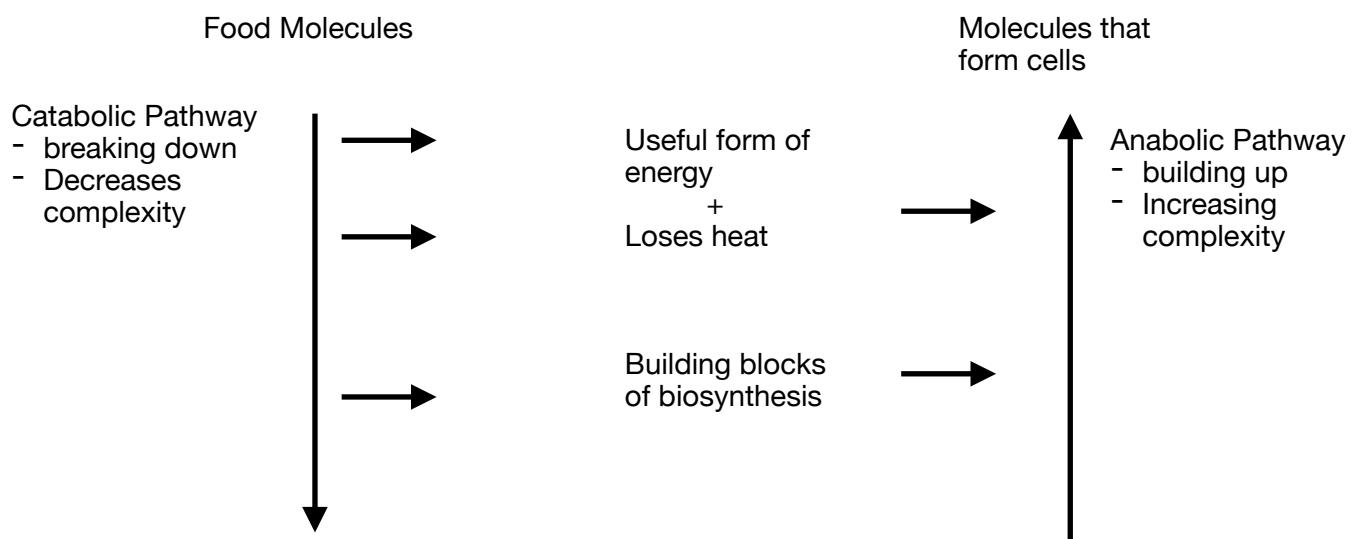
- Passive vs Active Transport
  - Molecules and ions have tendency to move down concentration gradient, from regions of HIGH concentration to LOW concentration
  - Passive transport is movement of molecules or ions in accordance with its natural tendency
  - The simple diffusion of substances across membrane is example of passive transport
  - The diffusion of ions through channels is another example of passive transport
  - Some carriers (uniporters) also mediate passive transport (e.g. glucose carrier of red blood cell membranes)
  - The passive transport of solute via channel or carrier is referred to as facilitated transport
  - An active transport process is one in which the cell expends energy in transporting molecules or ions against gradient (i.e. in reversing natural tendency of solutes to move down concentration gradient)
  - Primary active transporters require direct participation of ATP
    - Proteins hydrolyse ATP and use the energy released in this process to pump ions or molecules against a gradient
  - For example, the Na<sup>+</sup> - K<sup>+</sup> pump forces K<sup>+</sup> ions into cell and Na<sup>+</sup> ions out of cell
  - As a result, the cell cytoplasm has high [K<sup>+</sup>] and low [Na<sup>+</sup>]
  - Secondary active transporters are symporters or antiporters
    - By coupling the transport of one solute to transport of an ion down its gradient, the first solute can be transported against or up a concentration gradient.

- For example the sodium: glucose symporter in epithelial cells lining the gut uses this principle to drive glucose into cells (from lumen of gut), from where they pass into bloodstream via glucose uniporter.



## LECTURE 9

- Energy and metabolism
- First law of Thermodynamics
  - Heat is form of energy and thermodynamic processes are therefore subject to principle of conservation of energy
- Second law of Thermodynamics
  - There is natural tendency of any isolated system to degenerate into a more disordered state
- Metabolism



- Energy can be gained from transfer of electrons
  - Sharing electrons = covalent bond
  - Transfer electrons = ionic bond
- Electronegativity
  - Oxidation = loss of electrons
  - Reduction = gain of electrons
  - Redox reactions, one molecule loses electrons, the other gains electrons
- Generating energy from inorganic compounds



- Gaining energy from organic compounds

Reduction



Oxidation

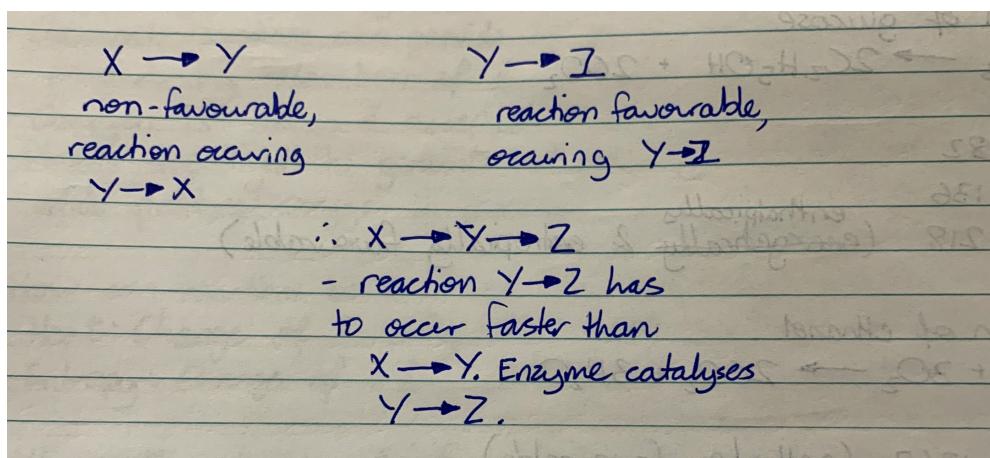
- Gaining energy from light
  - Photosynthesis



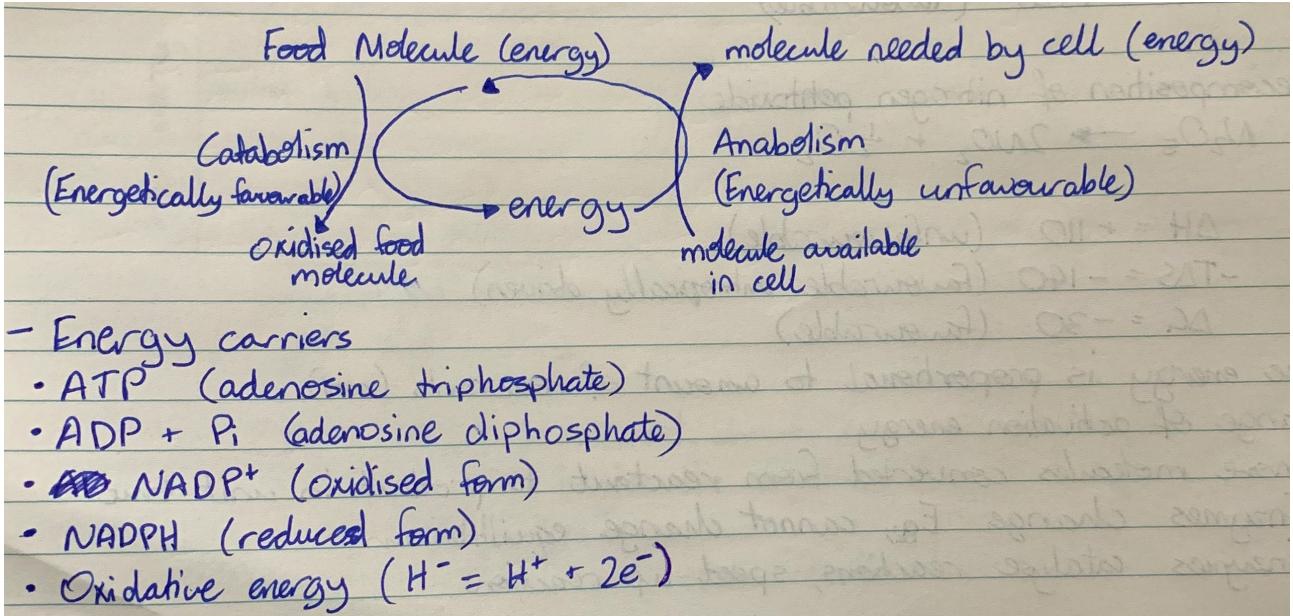
- Summary (I)
  - Living matter is generated by multitude of chemical reactions (metabolic pathways). Generating ordered structures requires energy
  - Chemical reactions can provide energy to sustain life. These reactions are powered by relocation of electrons
  - Oxygen is main electron acceptor in energy yielding reactions
  - Plants use light to generate reduced organic matter from  $\text{CO}_2$  and  $\text{H}_2\text{O}$ , and produce oxygen in process
- How can reactions be described macroscopically
  - Heat: Change of electrons configuration ( $\Delta H$ )
  - Entropy: Change of order ( $\Delta S$ )
- All reactions have activation barrier
- Reactions in equilibrium cannot drive reactions (no energy production)
- $\Delta H$ : Change in enthalpy, higher the difference between two energy barriers, less energy production
- Activation barriers prevent spontaneous reaction from occurring

## Lecture 10

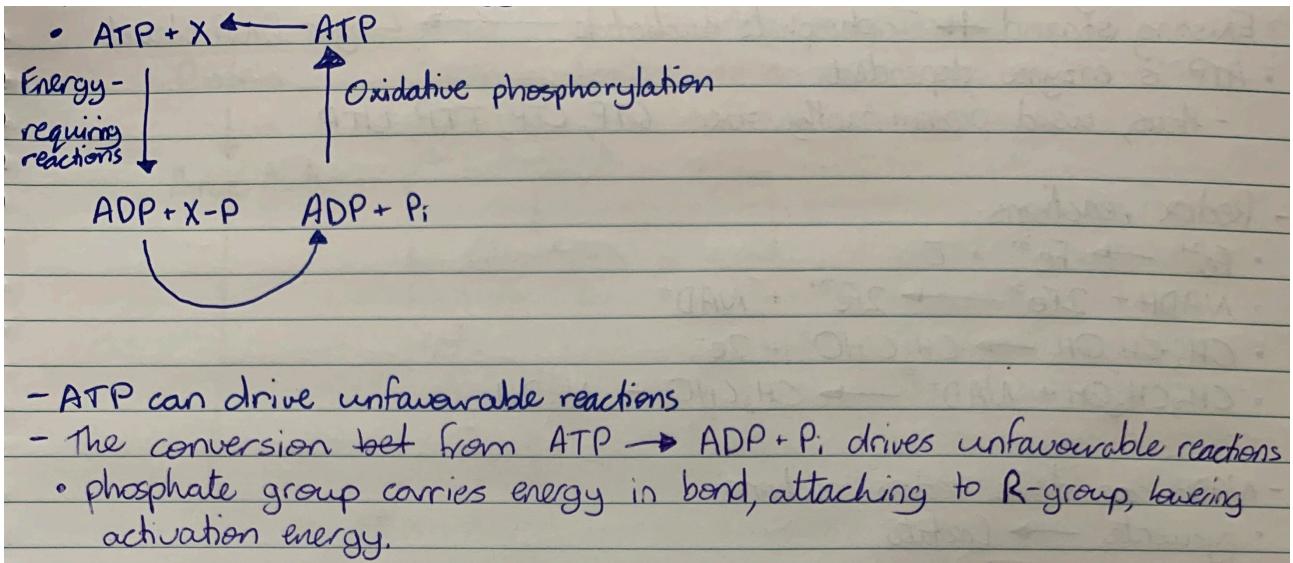
- Change of Entropy ( $\Delta S$  = order)
- High levels of entropy, high levels of freedom, vice versa
- $\Delta G = \Delta H - T\Delta S$ 
  - G: Gibbs free energy
  - $\Delta H$ : Change in enthalpy
  - T: Temperature (Kelvin)
  - $\Delta S$ : Change in entropy
- Free energy in equilibrium
  - $\Delta G$  (-ve): Product driven
  - $\Delta G$  (+ve): Reactant driven
  - $\Delta G$  (0) : Equilibrium
- Example
  - Fermentation of Glucose
    - $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$
    - $\Delta H = -82$
    - $-T\Delta S = -136$
    - $\Delta G = -218$  (enthalpically and entropically favourable)
  - Combustion of Ethanol
    - $C_2H_5OH + 3O_2 \rightarrow 2CO_2 + 3H_2O$
    - $\Delta H = -1367$  (enthalpy favourable)
    - $-T\Delta S = +41$  (entropy unfavourable)
    - $\Delta G = -1326$  (favourable)
  - Decomposition of nitrogen pentoxide
    - $N_2O_5 \rightarrow 2NO_2 + 0.5O_2$
    - $\Delta H = +110$  (unfavourable)
    - $-T\Delta S = 140$  (favourable: entropically driven)
    - $\Delta G = -30$  (favourable)
- Free energy is proportional to amount of reactants (mols)
- Change of activation energy
  - More molecules converted from reactant to product in unit time
  - Enzymes change activation energy, not equilibrium
  - Enzymes catalyse reactions, speed up reaction
- Fast diffusion allows enzymatic catalysis
- Free energy and equilibrium
  - Energetically favourable reaction (reactant - product)
    - Free energy of reactants is greater than free energy of products
    - Therefore  $\Delta G < 0$ , disorder of universe increases during reaction
    - Occurs spontaneously
  - Energetically unfavourable (product - reactant)
    - If reaction X-Y occurred,  $\Delta G$  would be  $>0$
    - Universe become more ordered
    - Only occur if it is coupled to second more energetically favourable reaction
- Driving non-favourable reactions



- Coupled Chemical reactions



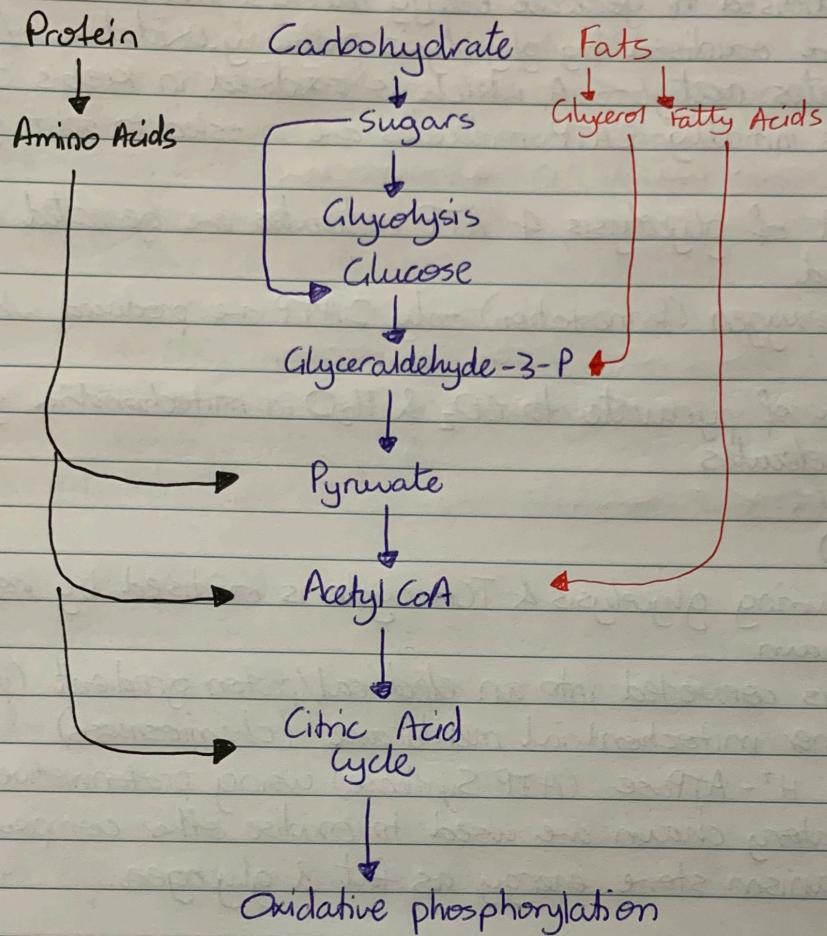
- How does ATP store energy



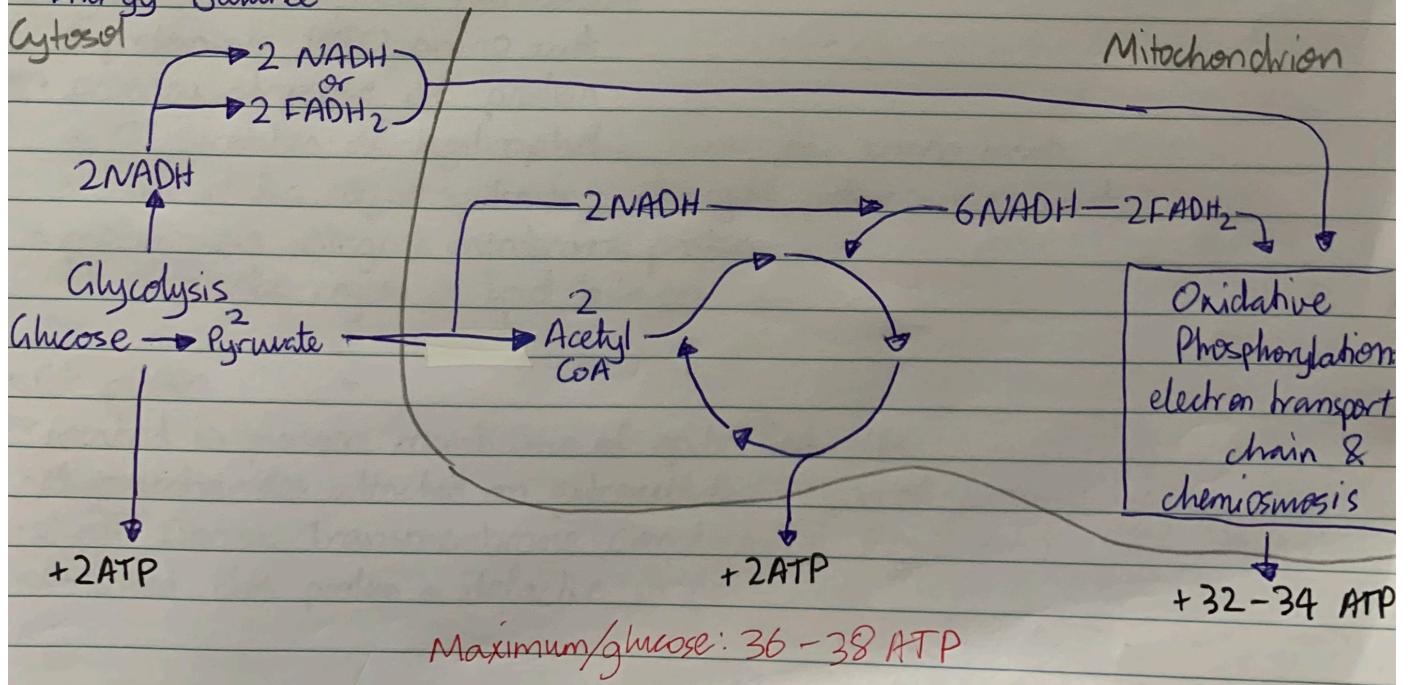
## Lecture 11

- DNA biosynthesis
- Existing strand - trisphosphate nucleotide
  - Diphosphate released, energy used for synthesis
  - Longer DNA strand
- ATP is enzyme dependent
  - Thus, used predominantly over GTP, CTP, TTP, UTP
- Redox reactions
  - $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + e^-$
  - $\text{NADH} + 2\text{Fe}^{3+} \rightarrow 2\text{Fe}^{2+} + \text{NAD}^+$
  - $\text{CH}_3\text{CH}_2\text{OH} \rightarrow \text{CH}_3\text{CHO} + 2e^-$
  - $\text{CH}_3\text{CH}_2\text{OH} + \text{NAD}^+ \rightarrow \text{CH}_3\text{CHO} + \text{NADH}$
- NADH can drive redox reactions
  - Pyruvate - lactate
- Summary
  - Free energy of reaction determined by amount of heat produced and amount of entropy generated
  - Reactions with  $\Delta G < 0$  proceed towards products, reactions with  $\Delta G > 0$  proceed towards substrates
  - Chemical reactions require activation energy to proceed even if reaction is overall favourable
  - To facilitate metabolic reactions at ambient temperature, enzymatic catalysis is used
  - Enzymes reduce activation energy but do not change chemical equilibrium
  - Unfavourable reactions can be completed, by coupling to reactions that have  $\Delta G < 0$
  - Living cells make use of energy carriers to couple unfavourable with favourable reactions
  - The major energy carriers are ATP and NAD/NADH
  - ATP stores energy by keeping its hydrolysis out of equilibrium

## - Overview of metabolism



## - Energy Balance



- Summary: Energy generation in heterotrophs
  - Nutrients are oxidised to generate energy carriers ATP and NADH
  - Carbohydrates are oxidised by glycolysis pathway. Oxidation of all nutrients generates acetyl-CoA, which is oxidised in Krebs cycle
  - During glycolysis initially 2ATP molecules are invested to split glucose into 2 halves
    - In second part of glycolysis, 4 ATP molecules are generated and 2NADH are produced
  - In absence of oxygen (fermentation) only 2ATP are produced, while NADH is recycled
  - Further oxidation of pyruvate to CO<sub>2</sub> and H<sub>2</sub>O in mitochondria yields up to 36 ATP molecules
- NADH produced during glycolysis and TCA cycle is oxidised by iron complexes of respiratory chain
- Oxidation energy is converted into an electrical proton gradient (protonmotive force) across inner mitochondrial membrane (chemiosmosis)
- ATP produced by H<sup>+</sup> - ATPase (ATP synthase) using proton motive force
- Elements of respiratory chain are used to oxidise other compounds
- Heterotrophic organism store energy as fat and glycogen.

## LECTURE 12

- Review CYSTIC FIBROSIS
- HISTORY
  - 1938: First clinical description of CF
  - 1953: Realisation that sufferers had abnormally high Na<sup>+</sup> and Cl<sup>-</sup> in sweat, now forms basis for clinical diagnosis
  - 1980s: Demonstration of defective Cl<sup>-</sup> channel (membrane ion channel with preference for Cl<sup>-</sup> and other -ve charge ions) in epithelial tissue from CF sufferers, causing defective salt absorption and secretion in airways cells, this involves reduced salt and water secretion, resulting in build-up of mucus, lungs become colonised with pathogenic bacteria, leading to respiratory infection and chronic airway inflammation
  - 1989: Identification of CF gene (chromosome 7)
- Regulation of affected Cl<sup>-</sup> channel by cAMP
  - The Cl<sup>-</sup> channel which is defective in cystic fibrosis is not open all time. As with other channels, opening and closing tightly regulated
  - Particular channel is activated in response to increase levels of cAMP within cells
  - Cyclic AMP (cAMP) is nucleotide-containing molecule plays important role in signal transduction in cells
- CF gene
  - Gene is 250000 nucleotides
    - Protein is 1480 amino acids
  - Primary structure of protein
    - 12 stretches of hydrophobic non polar amino acids
    - Hydrophilic regions, having charged polar amino acids
  - Protein was integral membrane protein
    - Hydrophobic region in lipid bilayer
- CF protein
  - Located in plasma membrane of epithelial cells
  - Oligosaccharides attached on extracellular region of protein therefore glycoprotein
  - CF Transmembrane Conductance Regulator (CFTR)
  - Confirmed, this protein is defective in CF patients
- Mutations
  - CF occurs in people who have mutations in both copies of CFTR gene
  - Mutation = alteration in nucleotide sequence in DNA

- >70% of people with CF have mutation  $\Delta F508$ 
  - CFTR missing phenylalanine
  - Protein does not reach apical plasma membrane
  - Or, protein functions abnormally
- Treatment
  - Lumacaftor helps CFTR protein reach membrane
  - Kalydeco, interacts with surface protein CFTR, increases Cl<sup>-</sup> regulation across membrane
  - Orkambi = lumacaftor + kalydeco.

## Lecture 13

### CELL STRUCTURE and FUNCTION

#### The Cell Theory

- All living organisms are made up of cells and the materials produced by them
- All cells come from preexisting cells
- The cell is the smallest organisational unit
- A note on viruses
  - Viruses can be purified like chemicals. The definition of living cells excludes viruses which are non-living infectious particles.

#### Cells

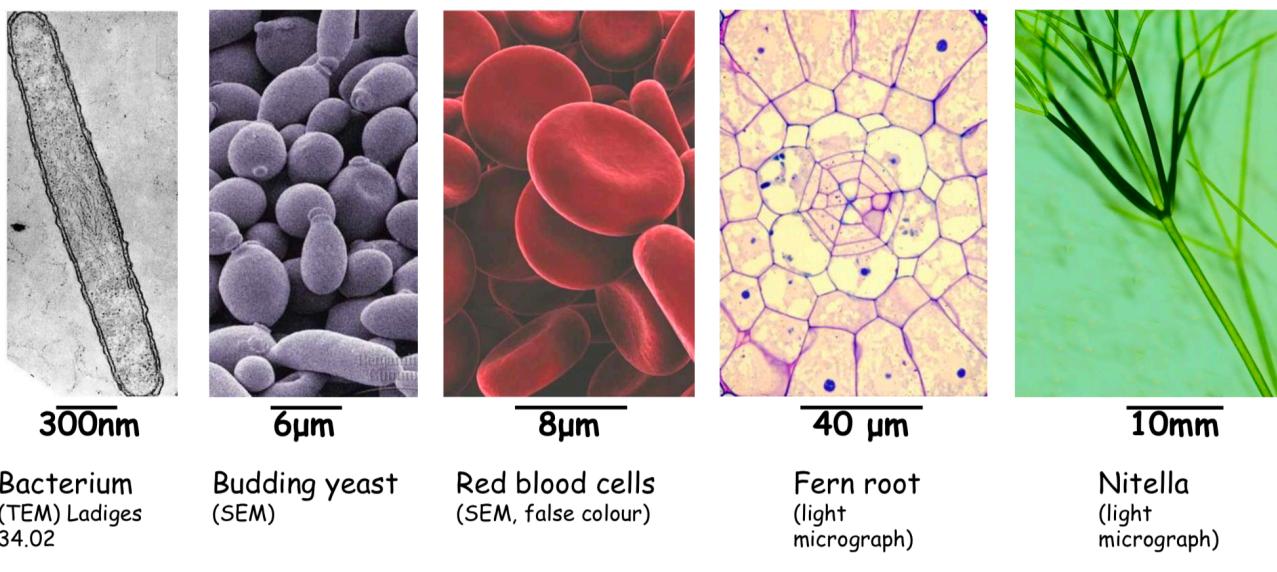
- Cells are small, **membrane bound** compartments that contain ions, lipids, proteins, carbohydrates and many other molecules in solution and forming sub cellular structures
- Most biological reactions occur within cells, often within specialised sub cellular compartments
- All cells are enclosed by a membrane called the **plasma membrane**
- All cells contain cytoplasm which is a semifluid matrix called **cytosol** containing a variety of subcellular **organelles**.

#### Prokaryotic Cells

- The first cells to evolve were bacteria
- Bacteria are prokaryotic cells (from greek, pro = before and karyon = nucleus)
- Have a simple structure and lack internal compartments
- The DNA lies within the bacterial cell in an area called the nucleoid
- Bacteria have a variety of sizes and shapes **spherical, rod-shaped and spiral**
- Some bacteria have flagella and are motile

#### Eukaryotic Cells

- More complex than bacterial cells
- Eu = true
- The DNA is enclosed within a membrane bound sub cellular compartment called the nucleus
- Eukaryotic cells contain a variety of sub cellular organelles in addition to the nucleus
- Algae, fungi, plants and animals are all eukaryotes
- Prokaryotic are usually less than one micron diameter
- Eukaryotic cells may be as small as a few microns in diameter or as large as one millimetre in diameter and several centimetres in length



- Protist: single celled eukaryotic
- multicellular organisms arose: about 800 million years ago, instead of separating after replication, some cells remained attached, giving rise to the first multicellular organisms.

#### Cell Differentiation

- During evolution, cells in multicellular organisms became different from one another. In other words they differentiated into different cell types.
- During continued evolution, the differentiated cells, became organised into tissues and organs
- Multicellularity requires signalling and communication between cells and control of cell adhesion, division and death

#### ADVANTAGES of multicellularity

- The development of a large body size - bigger organisms can occupy different environmental niches
- Distribution of labour - different cells develop different capabilities
- Resilience - some cells can die without the organism as a whole being killed
- Surface area increase - division into many cells results in a large increase in total cell surface area of the organism

- Microscopy
  - Scientists use microscopes to visualise cells too small to see with the naked eye
  - There are two main types of microscopes
    - Light microscopes (LM)
    - Electron microscope (EM)
- Light Microscopy
  - Light microscopes use glass lenses to illuminate and collect the light from an object
  - Using green light with a wavelength of about 500nm, the limit of resolution is 200nm (i.e. 800 times better than the human eye)
- Robert Hook
  - 1665: first to observe and describe cells
- Antonie van Leeuwenhoek
  - 1674: first to observe and describe a component inside a cell. He described the spiral chloroplasts in the green alga (spirogyra)
- Robert Brown
  - 1828: Nucleus was first described

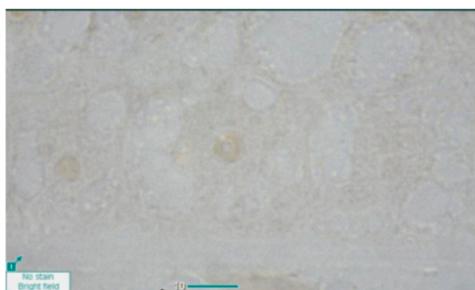
- He was a botanist and early describer of Australian plant species
- He visited Australia with Matthew Flinders in 1801
- Discovered Brownian motion, the random movement of small particles
- Microscope image quality depends on:
  - Magnification - the ratio of image size to actual size
  - Resolution - the minimum distance between two distinguishable points
  - Contrast - Visible differences in parts of the sample
- Size and Magnification
  - **Magnification** is the enlargement factor between the image and the original sample
  - Light microscopes can usefully magnify the image of objects to about 1000 times their actual size.
  - In LAB make sure to write magnification factor under images

MAKE SURE TO INCLUDE SCALE BAR TO DRAWINGS

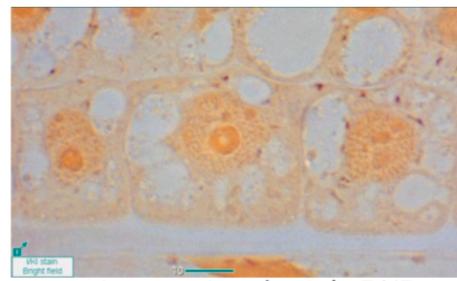
- Resolution
  - Cells and subcellular components are usually too small to see unaided because of the limit of resolution of our eyes.
  - Resolution is the ability to see two particles as separate entities and depends on the wavelength of illuminating light and the numerical aperture of the lens collecting the light.
  - For the human eye, the limit of resolution is about 200 µm (0.2 mm).
- Sample contrast
  - In addition to resolution, an important factor that determines whether an object is visible or not is its contrast relative to that of its surroundings.
  - For example, little detail can be seen in the untreated section of a bean root tip below.

## Generating sample contrast

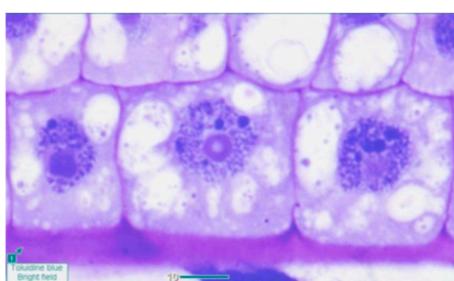
- Contrast can be increased by differential staining of cell components.



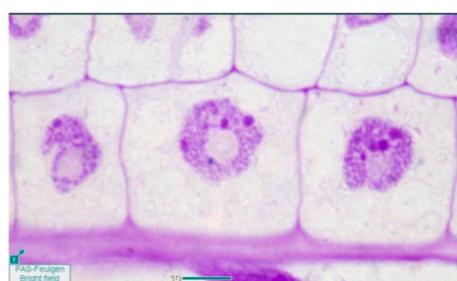
Unstained section



Section stained with I姬



Section stained with Toluidine blue



Section stained with PAS-Feulgen  
Gunning DVD 2.2.1

## Physical Sectioning

- Samples can be sections by hand
- Many samples require the use of a microtome to cut the sections
- Cryosections are often used to section biopsies in pathology labs

•Samples can be embedded in plastic resins that support the material and allow thin slices to be cut.

•For light microscopy, sections are typically 1-5  $\mu\text{m}$  thick.

## Optical sectioning

- If the samples are not too large, they can sometimes be viewed using optical sectioning.
- Often, using a standard light microscope, the sample thickness and density makes it difficult to obtain clear images.
- A solution is to use a confocal microscope.