SCIE1106 Notes

LECTURE 2 & 3

- PROKARYOTES / EUKARYOTES
- STRUCTURE / FUNCTION OF TYPICAL CELL COMPONENTS
- CELLULAR RESPIRATION / PHOTOSYNTHESIS

All cells have the same common ancestor that was prokaryotic and originated 3.5 billion years ago.

PROKARYOTES

- 2 groups:
  - ↳ Division between these two groups is based on molecular biological characteristics.
  - ↳ They are as different to each other as either are to eukaryotes.
  1. **Eubacteria**
- True bacteria.
- Found in environments familiar to us.
  2. **Archea bacteria**
- Found in hostile environments as well as in more familiar ones.

FEATURES OF PROKARYOTES

- Smallest cellular organisms (0.4um to 5um long).
- Cells lack nuclei and organelles.
- Plasma membrane.
- Tough cell walls.
- Circular DNA free in cytosol.
- Ribosomes.
- May have flagellum.
- Can reproduce quickly.
- Successfully inhabit many different environments.
- Exhibit many different growth forms.
  - ↳ Spherical, rod-shaped, spiral.
  - ↳ Chains, clusters, other organised multicellular structures.
- May be:
  - ↳ Organotrophic
  - ↳ Phototrophic
  - ↳ Lithotrophic

EUKARYOTES

- **Unicellular**
  - ↳ Most protests
  - ↳ Yeast
- **Multicellular**
  - ↳ Animals
  - ↳ Plants
  - ↳ Fungi

FEATURES OF EUKARYOTES

- Organisms with a membrane bound nucleus.
- Have membrane bound organelles.

PHOTOSYNTHESIS

Two Components of Photosynthesis

1. **LIGHT DEPENDENT**: occur within the thylakoids.

- Light energy is used by the light reactions to produce ATP and NADPH (and O_2 is formed).
- Depends on pigments

2. **LIGHT INDEPENDENT**: occur within the stroma.

- ATP and NADHP is used to fix **CO_2** into **ribulose bisphosphate** in the stroma.

LIGHT DEPENDENT REACTIONS

- Harvest light energy and convert it into NADH and ATP.

PHOTOSYSTEMS

= protein complexes responsible for light reactions.

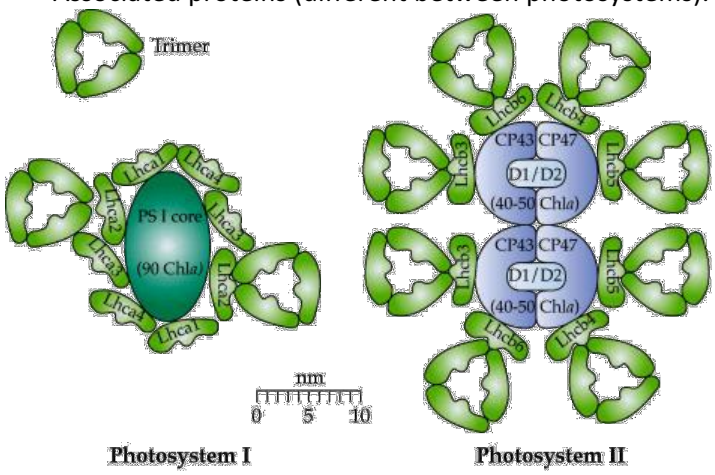
- Consists of:

↳ Light harvesting complexes (chlorophyll-a, accessory pigments, including chlorophyll b): protein bound chlorophyll molecules → light intercepting **antennae**.

↳ **Reaction centre** (chlorophyll-a only).

↳ Electron acceptor molecules.

↳ Associated proteins (different between photosystems).



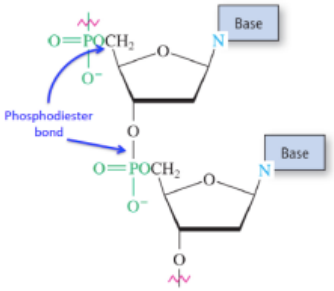
Photosystem I Photosystem II

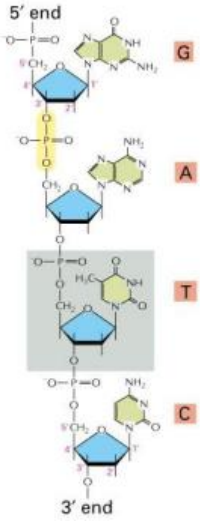
Process

- When a chlorophyll molecule absorbs a **photon** it becomes excited.
  - ↳ One of its electrons moves to a higher energy level.
- Excited chlorophyll molecule interacts with neighbour, transferring energy and excitation.
  - ↳ Excited electrons not transferred.
- Excitation is channelled to the **reaction centre**.
  - ↳ Chlorophyll molecules in reaction centre become excited and expel an electron.
- Electron acceptor in stromal side of thylakoid membrane (but still within photosystem) receives the electron.
  - ↳ Produces a charge separation across the thylakoid membrane.

Structure of Nucleic Acids

- Nucleotides are joined together through condensation reactions.
- **Phosphodiester bonds** link adjacent nucleotides in nucleic acids.
  - ↳ Hydroxyl group on 3' carbon of pentose sugar is covalently linked to phosphate group attached to 5' carbon of adjacent pentose sugar.
  - ↳ Forms **sugar-phosphate backbone**.
- RNA is usually single-stranded.
- DNA is nearly always double-stranded.



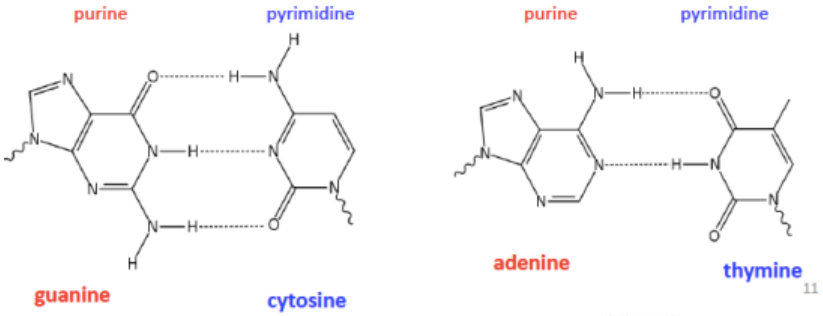


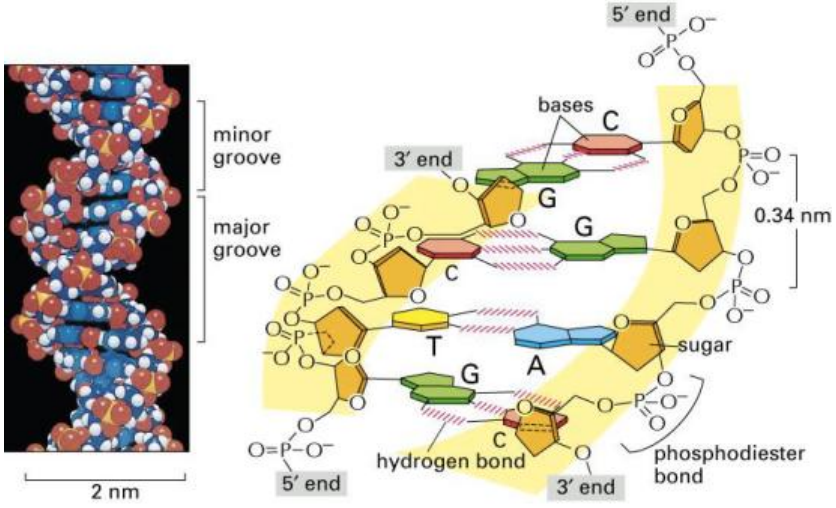
Sequence and Directionality

- RNA and DNA have **directionality**.
- By convention, a nucleic acid strand is read from the 5' to the 3' end.

Secondary Structure of DNA

- Consists of two strands of nucleic acids.
  - ↳ **Sugar-phosphate backbone** on outside and bases projecting inward.
  - ↳ One **coding** strand and another **non-coding** strand.
- Strands are **anti-parallel** (run in opposite directions).
- Strands held together by **hydrogen bonds** between bases.
- **Complementary base pairing** (Chargaff's Rules) = pyramidine always pairs with a purine.
  - ↳ Adenine pairs with Thymine (2 H-bonds).
  - ↳ Guanine pairs with Cytosine (3 H-bonds).
- Arrangement results in **double helix** = helix model.
  - ↳ Looking down, double helix follows a clockwise path → right-handed convention.

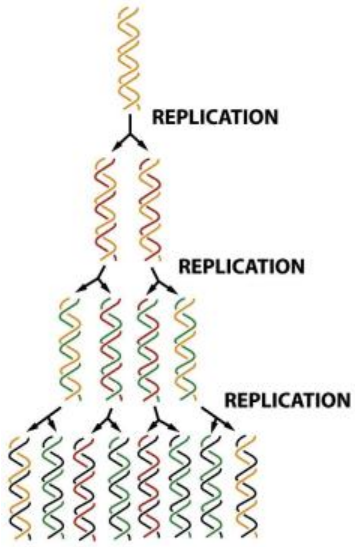




LECTURE 7

- Define the terms describing DNA replication: semiconservative, origin, bidirectional, replication fork, Okazaki fragment.
- Understand the mechanism of leading and lagging strand replication and role of the RNA primer.
- Understand the functions of the proteins at the DNA replication fork.
- List major DNA polymerases of prokaryotes and eukaryotes and their functions.

DNA REPLICATION



- 3 proposed models:
  1. **Semiconservative (accepted)**
  2. Conservative: copy would have two new strands.
  3. Dispersive
- Doubles the DNA content in a cell.
- **"Semi-conservative"**
  - ↳ The **parent** molecule is unwound so that each strand becomes a **template** for replication.
  - ↳ The **daughter** molecules have each got one parent strand and one newly synthesized strand.
  - ↳ Accuracy and speed – 1000 nucleotides per second almost without error.
- Occurs by the sequential addition of nucleotides from energy-charged building blocks = **DEOXYRIBONUCLEOSIDE TRIPHOSPHATES (dNTPs)** = a deoxyribose sugar covalently attached through the 1' C to either A, T, G or C, and through the 5' C to 3 phosphate groups.
  - ↳ dATPs
  - ↳ dTTPs
  - ↳ dCTPs
  - ↳ dGTPs
- **Bidirectional** = replication moves in both directions from the origin, creating two replication forks moving in opposite directions.

BASIC OVERVIEW

1. DNA replication begins at specific sites = **origins**.
2. Bonds broken between adjacent nucleotides.
3. Double helix is unwound.
   - ↳ Generation of **REPLICATION FORK** = a point in a chromosome at which DNA strands separate to allow replication of each strand.

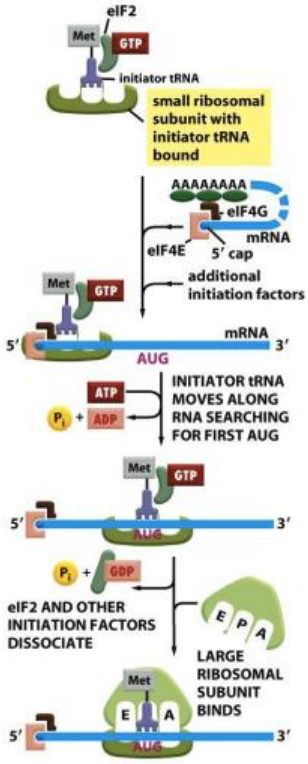
ABSOLUTE REQUIREMENTS

1. A pre-existing DNA template (single stranded complementary sequence).
2. A pre-existing free 3'hydroxyl group on a short double stranded region (achieved by a primer or Okazaki fragment).
3. A protein catalyst (an enzyme) = **DNA polymerase**
4. dNTP precursors (building blocks)

Initiation in Eukaryotes

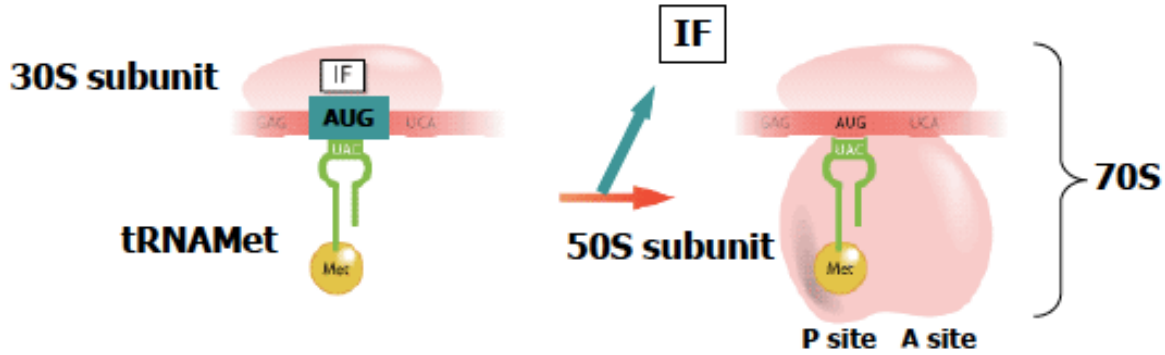
- Initiation site is crucial as it will define the correct open reading frame.

1. **Initiator tRNA-methionine complex (Met-tRNAi)** is first loaded into the 'P' site of small ribosomal subunit along with **eukaryotic initiation factors** (**eIF2-GTP** (guanosine triphosphate)).
   ↳ Only aminoacyl-tRNA capable of tightly binding to the small subunit without the complete ribosome being present.
2. The loaded small ribosomal subunit attaches to the 5' end of the mRNA (recognised by its 5' end cap).
3. It then scans along the mRNA (5' to 3' direction) until it identifies the first AUG codon, surrounded by a long consensus sequences (**Kozac sequence**).
   ↳ If the Kozac sequence is degenerate, the ribosome will initiate translation from multiple start codons producing truncated polypeptides → not the case for prokaryotes.
4. The initiation factors detach, allowing the large subunit to bind and complete the ribosomal complex.
   ↳ Initiator tRNA remains in the 'P' site, leaving the 'A' site vacant.



Initiation in Prokaryotes

1. 16S rRNA (30S), bound with initiation factors, forms base pairs with the **Shine Dalgarno** sequence upstream of the AUG start codon.
2. Initiator tRNA (**fMet-tRNAi**) aligns and binds with start codon (AUG) in mRNA at 'p' site.
   ↳ Small ribosomal subunit binds first and then the tRNA binds to the complex.
3. 50S associates with 30S, releasing IF and forming 70S complex.
   ↳ tRNA occupies **peptidyl** (P) site of 50S subunit (**aminoacyl** (A) site is empty).



Initiation: tRNA-Met – Prokaryotes vs Eukaryotes

PROKARYOTES

- The initiator tRNA in bacteria has a **formylated methionine** (N-formyl-methionine tRNA) or **^{f}Met-tRNAi.**

EUKARYOTES

- The initiator tRNA in eukaryotes is **Met-tRNAi** (**not formylated**).

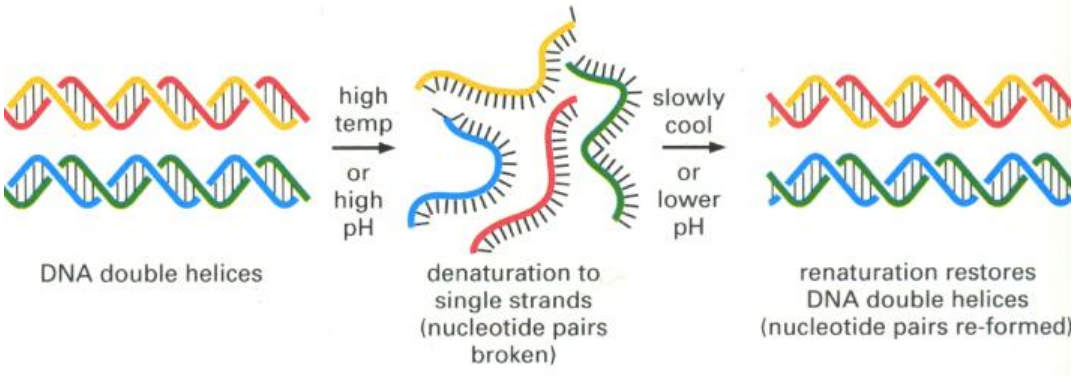
ELONGATION

- For both PROKARYOTES & EUKARYOTES:
  ↳ When a **methionine** codon appears inside a coding region, an elongation **Met-tRNA** is used.
  ↳ Met-tRNA has a different **stem loop structure** that preferentially binds to elongation co-factors.
- Three step cycle which is repeated over and over during the synthesis of a protein chain.
- Always in the 5' to 3' direction of the mRNA.

LECTURE 17: CHAPTER 8 – HYBRIDISATION TECHNIQUES

DNA Denaturation & Renaturation

Process



- Hybridisation will also occur between:
  - ↳ Complementary RNA strands → double stranded RNA molecules.
  - ↳ Complementary DNA and RNA strands → RNA/DNA hybrids.

Probes

- Single-stranded.
- 15 to 1000's of nucleotides long.
  - ⇒ **HOMOLOGOUS:** to detect identical nucleic acid molecules.
  - ⇒ **HETEROLOGOUS:** to detect related molecules.

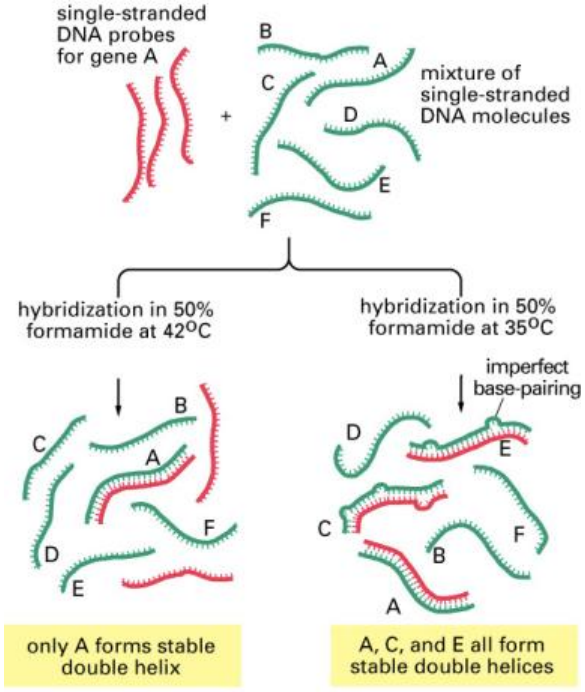


Figure 8–25. Molecular Biology of the Cell, 4th Edition.

- ↳ **Formamide** functions to preferentially allow the double stranded molecules to stay together if there is complementary base pairing.
- ↳ Left: only A pairs (higher temperature – hybridisation less favourable)
- ↳ Right: A, C and E pair (lower temperature – hybridisation more favourable); C & E have 'bubbles' (where there is no complementary base pairing).

LECTURE 21: CHAPTER 7 – THE LACTOSE OPERON

- Draw a graph to illustrate usage of glucose and lactose in E. coli.
- List the enzymes of the lactose operon and their functions.
- Describe the lactose operon and its negative regulation, using diagrams.
- Explain the terms inducer, inducible, on–off regulation and diauxic growth.

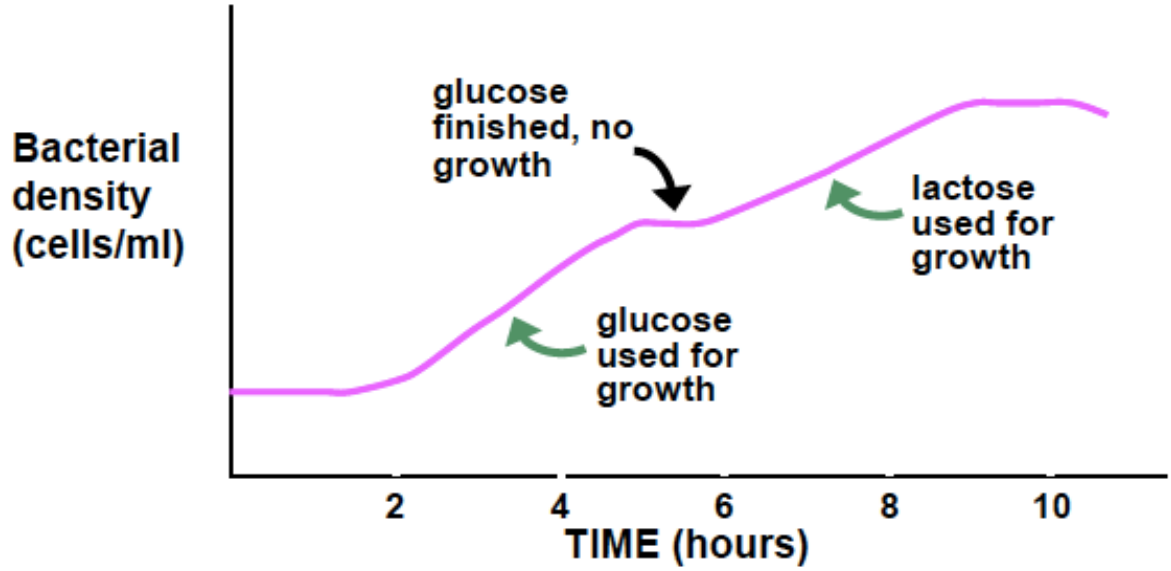
THE LACTOSE (LAC) OPERON

= an operon which is responsible for the transport and metabolism of the sugar lactose in E. Coli.

E. *coli.* ENERGY SOURCE

- Lactose is one of many organic molecules E. coli can use as a carbon and energy source.
- Glucose is the preferred C source for E. coli.
- If we supply E. coli with both glucose and lactose, the cells use the glucose until it is exhausted, stop growing briefly, then start growing again using the lactose.

GROWTH OF E. *coli*



  - ↳ Linear phase (1) – glucose.
  - ↳ Small stationary phase.
  - ↳ Linear phase (2) – lactose.
- E. coli cells are grown on a medium containing both glucose and lactose, and the bacterial density (number of cells/ml) is measured.
- **Diauxic** growth is observed → cellular growth in two phases.
- During the 'no growth' period, the cells have been adjusting to the new nutrient source by turning on the *lac* operon and accumulating the enzymes needed to break down the lactose.

ENZYMES NEEDED FOR LACTOSE METABOLISM IN E.COLI

lactose permease	Transports lactose into the cell
β-galactosidase	Breaks lactose down to its component sugars : glucose and galactose

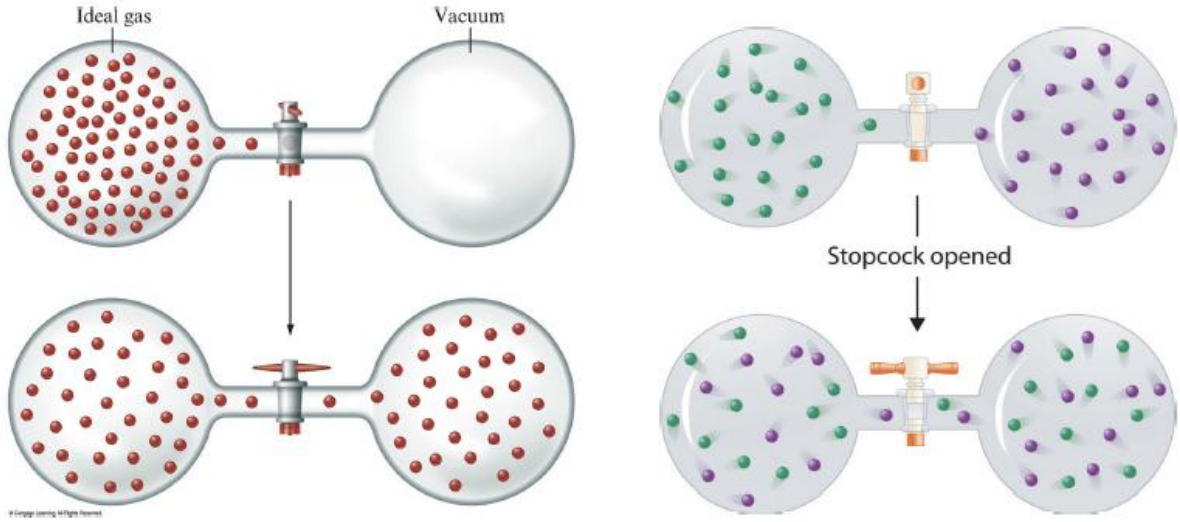
SIMPLE DIFFUSION

Brownian Motion, Entropy & Diffusion

- Molecules in solution are in constant random motion.
- Diffusion: molecules diffuse from a region of high concentration to a region of low concentration.
- 2nd Law = systems spontaneously evolve towards thermodynamic equilibrium.
- Disorder = maximum entropy.

Simple Passive Diffusion

- Molecules diffuse down their own concentration gradient.



Fick's Law of Diffusion: Flux

- Flux (J) = number of molecules passing through a certain area in a given amount of time (mol cm^{-2} s^{-1}).
  - ↳ J is directly proportional to concentration gradient (ΔC).
  - ↳ J is indirectly proportional to distance (membrane thickness, Δx).
  - ↳ J is indirectly proportional to molecular weight.
- Diffusion coefficient (D) = $nRT/(8\pi r^3)$.
  - ↳ N = fluid viscosity
  - ↳ R = Universal Gas Constant
  - ↳ T = absolute temperature
  - ↳ R = the molecular radius (molecular weight).

$$\text{Flux } (J) = -D \frac{\Delta C}{\Delta x}$$

Factors Affecting Membrane Permeability of Substance

- Polarity
  - ↳ More polar = less permeable.
- Lipid solubility
  - ↳ More lipid soluble = more permeable.
- Molecular weight
  - ↳ Lower molecular weight = more permeable.