The University of Burdwan
3-Year Degree/4-Year Honours in Zoology (NEP-CCFUP)
ZOOL3012 (Cell Biology)

# Membrane Architecture

Based on Fluid Mosaic Model


## DR. SAGAR ADHURYA

ASSISTANT PROFESSOR IN ZOOLOGY
WEST BENGAL EDUCATION SERVICE

# Learning objectives

- **Describe the historical context** leading to the development of the Fluid Mosaic Model and the limitations of earlier membrane models.
- **Explain the core principles** of the 1972 Singer-Nicolson Fluid Mosaic Model, including membrane fluidity and the mosaic arrangement of proteins.
- **Analyse experimental evidence** that supported the original Fluid Mosaic Model, including cell fusion experiments and fluorescence recovery after photobleaching (FRAP).
- **Compare and contrast** the original 1972 model with Nicolson's 2014 updated model, emphasising discoveries about lipid rafts, membrane domains, and protein mobility restrictions.
- **Evaluate the functional significance** of membrane organisation, including the roles of lipid-lipid, protein-protein, and lipid-protein interactions in cellular processes.

# Historical Context - Pre-1972 Membrane Models

Gorter and Grendel (1925) - Lipid Bilayer Hypothesis

Davson-Danielli Model (1935)

Robertson's Unit Membrane Model (1959)

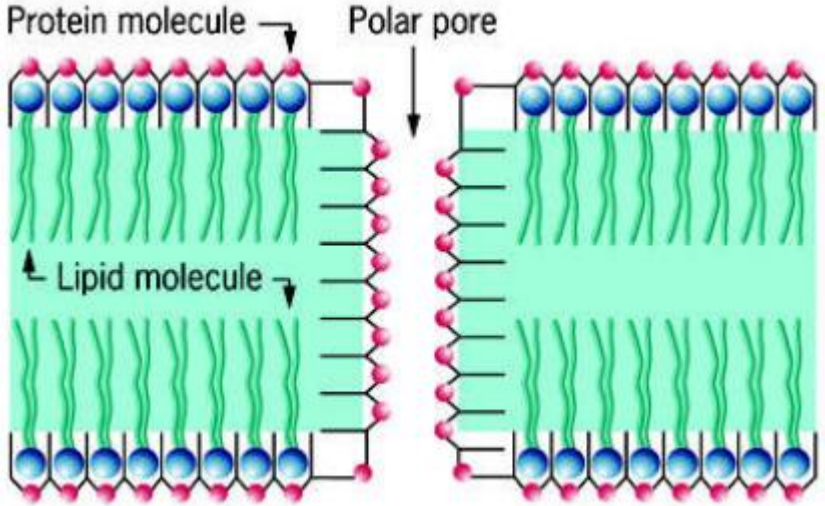
# Lipid Bilayer Hypothesis

- **Before 1925:** Scientists knew cells were bounded by membranes, but the molecular structure of that membrane was unknown.
- The first proposal that cellular membranes might contain a lipid bilayer was made in 1925 by two Dutch scientists, Evert Gorter and François Grendel.
- **Experiment:** They extracted lipids from a known number of RBCs of different mammalian sources, such as humans, goats, sheep, etc. and then spread the extracted lipids as a monolayer on a water surface.
- **Findings:** The surface area of the monolayer is approximately twice (1.8x – 2.2x) that of the RBC cell membrane.
- **Conclusion:** The plasma membrane is composed of a lipid bilayer where hydrophobic tails remain inside and polar head groups face outward.
- **Limitation:** (i) Did not account for membrane proteins; (ii) could not explain selective permeability; (iii) The surface tension and thickness of the pure bilayer are lower compared to the actual biomembrane; (iv) could not explain the functional diversity of different biomembranes.

**Suggested reading:** Gorter and Grendel (1925) Journal of Experimental Medicine (Rockefeller University Press); Karp (2010) Cell and Molecular Biology 6th ed., pp. 123-124

# Sandwich Model

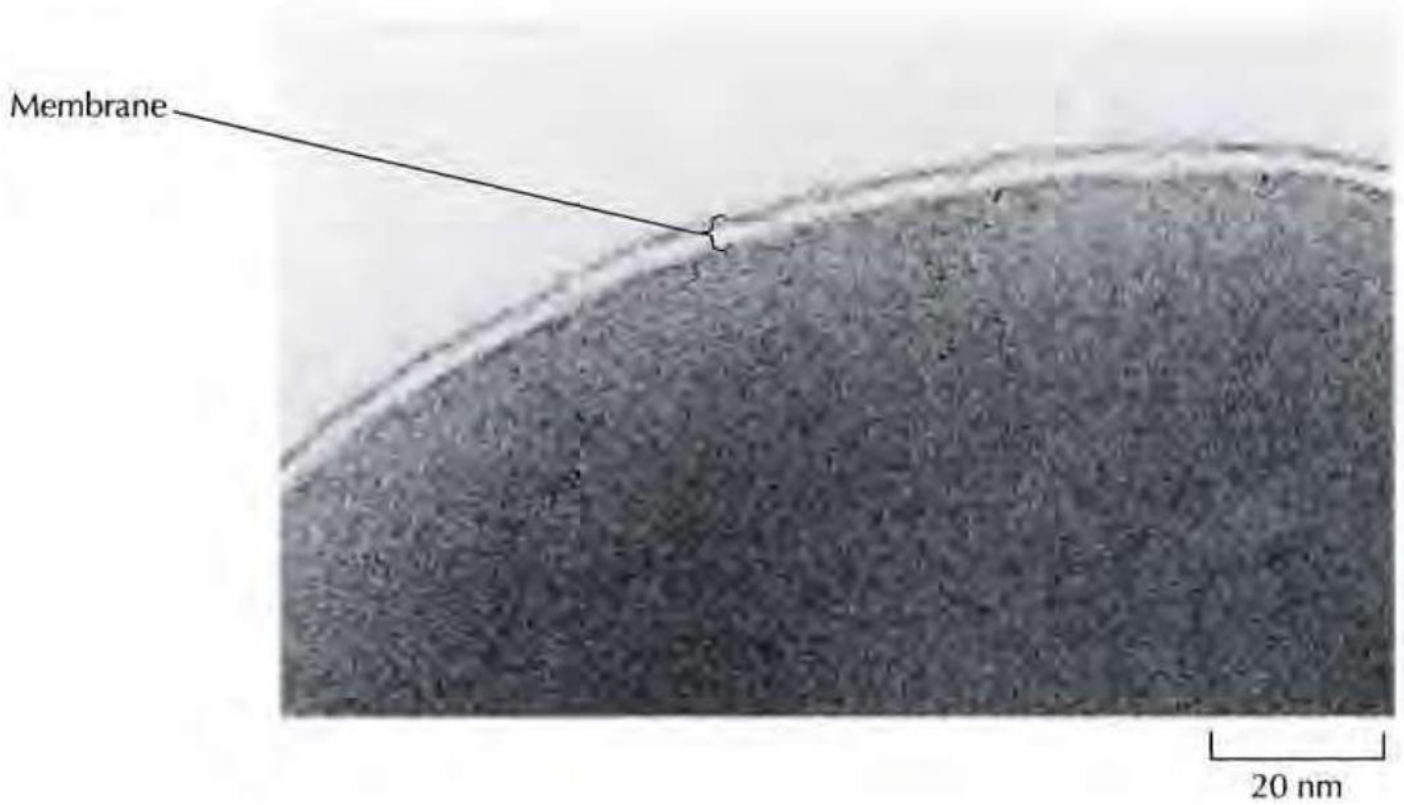
- Hugh Davson and James Danielli proposed a model to explain the plasma membrane structure, where a **lipid bilayer is sandwiched between two continuous globular protein layers** to explain membrane properties.
- The proteins are thought to interact with the polar head group of lipid through electrostatic interaction via charged amino acid molecules.
- The lipid bilayer was also penetrated by protein-lined pores, which could provide conduits for polar solutes and ions to enter and exit the cell.
- The model can explain the selective permeability and low surface tension of the membrane.
- **Limitation:**
  - Could not explain membrane protein mobility and diversity.
  - The 'pore' model failed to explain the active transport of molecules across the membrane.



# Unit Membrane Model

- J. David Robertson, in 1959, studied different kinds of biological membranes using improved electron microscopy with potassium permanganate staining.
- **Key Observation:** All biological membranes examined showed a **universal trilaminar ("railroad track") appearance:**
  - Two **dark outer lines** (electron-dense): protein layers + polar lipid head groups
  - One **light central region** (electron-lucent): hydrophobic fatty acid chains (repel heavy metal stains)
  - **Total thickness:** approximately **75-100 Ångströms (7.5-10.0 nm)**
- **Unit Membrane Hypothesis:**
  - All cellular membranes share the **same fundamental "unit membrane" structure**
  - Organellar membranes (nuclear envelope, ER, Golgi, mitochondria, lysosomes) all show an identical trilaminar pattern
  - Functional diversity arises from **different protein compositions**, not different basic architecture
- Pattern essentially a **modified Davson-Danielli model** with electron microscopic confirmation

# Unit Membrane Model




**Bilayer structure of the plasma membrane**

Electron micrograph blood cell. Note the *railroad track* appearance of the plasma membrane. (Courtesy J. David Robertson, Duke University Medical Center)

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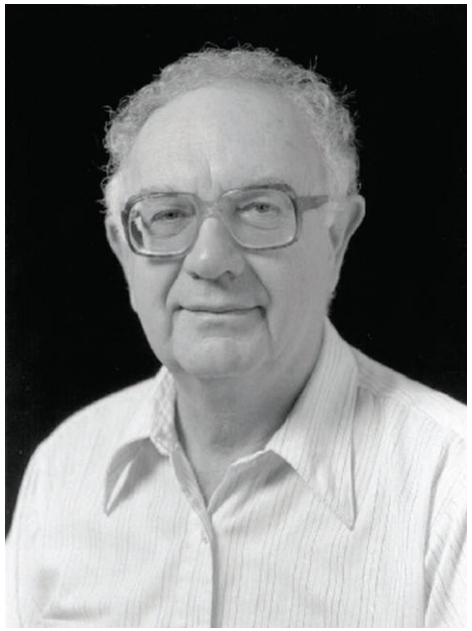
# The Paradigm Shift - Fluid Mosaic Model

# The Fluid Mosaic Model

- This model was proposed by S Jonathan Singer and Garth Nicolson of the University of California in 1972.
- They proposed the model based on data from enzymatic studies, freeze-fracture studies and ESR spectroscopic studies.
- Key terms:
  - Fluid: The membrane has a fluid nature.
  - Mosaic: the proteins are embedded in the lipid bilayer in a mosaic manner.

Mosaic floor

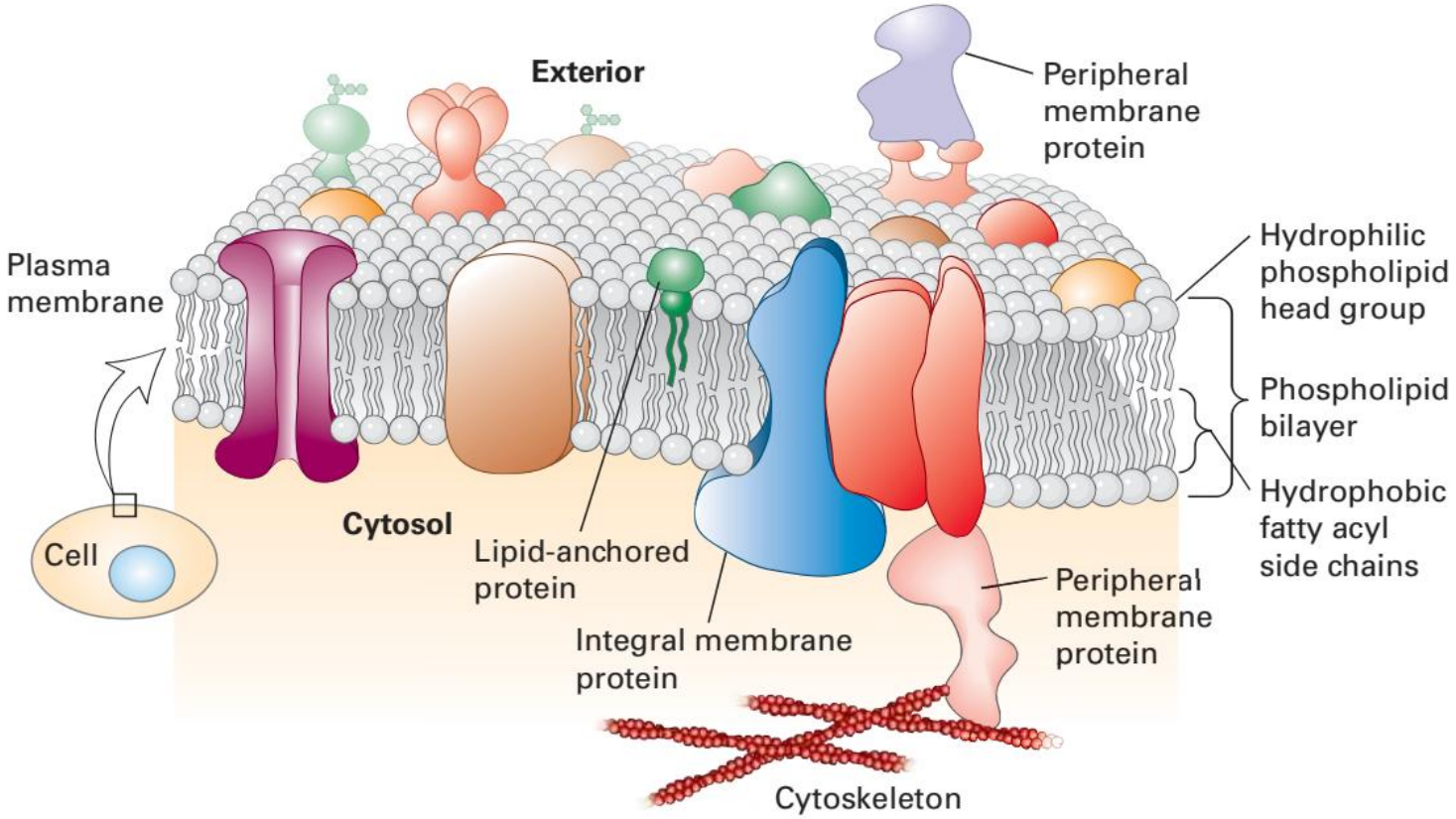


SJ Singer

G Nicolson

# The Fluid Mosaic Model

*Biological membranes consist of a* ***fluid lipid bilayer*** *in which* ***membrane proteins are embedded*** *as a* ***mosaic of discrete particles****, giving rise to a* ***mobile, dynamic structure****.*



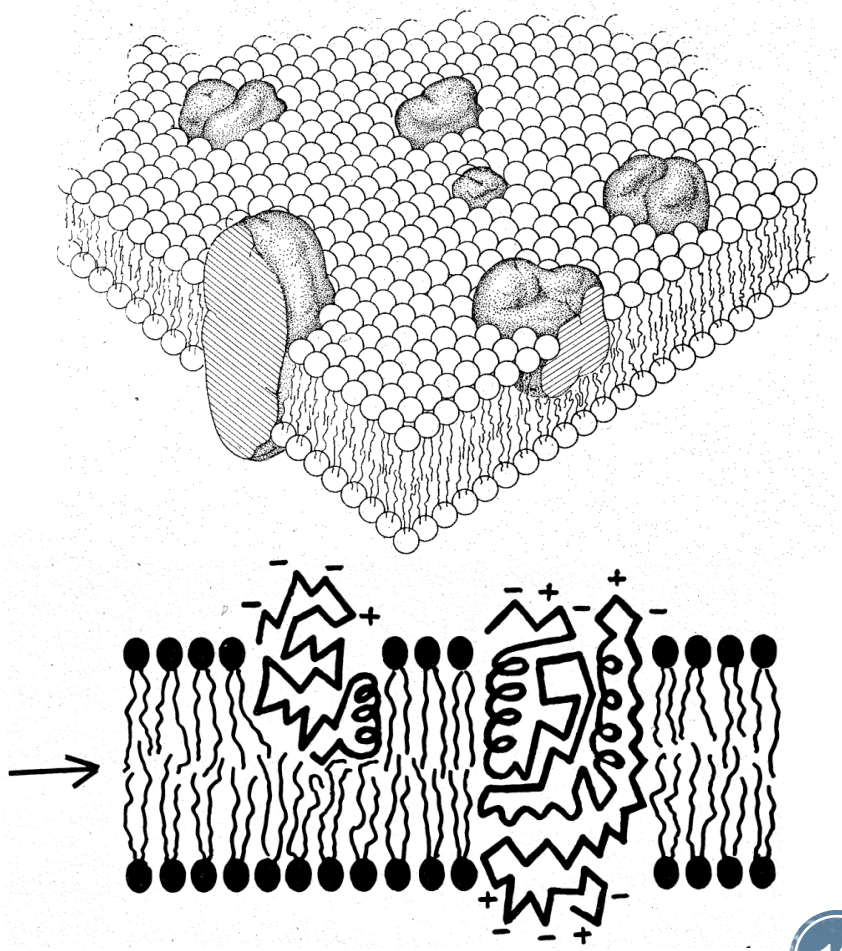



Image source: Lodish et al., 2016. Molecular Cell Biology, 8th Edn.

Image source: Singer, S. J.; Nicolson, G. L. . (1972). *The Fluid Mosaic Model of the Structure of Cell Membranes. Science, 175(4023), 720–731.* doi:10.1126/science.175.4023.720

# Core Principles

- **The Lipid Bilayer is FLUID**
  - **Not rigid or crystalline** - behaves as a two-dimensional liquid
  - Individual phospholipids can:
    - **Rotate** around their long axis (~$10^7$ times per second)
    - **Move laterally** within the same leaflet (diffusion coefficient ~$10^{-8}$ $cm^2$/sec)
    - Traverse length of bacterial cell (~2 µm) in ~1 second
  - **Flip-flop** (movement between leaflets) is extremely rare (~hours to days)
  - Membrane viscosity comparable to olive oil
- **Proteins Form a MOSAIC**
  - Proteins appear as **discontinuous particles** in freeze-fracture electron microscopy
  - **Non-uniform distribution** creates a patchwork appearance
  - Different proteins are concentrated in different membrane regions
  - Mosaic reflects functional specialisation
- **Lateral Mobility**
  - Both lipids and proteins are capable of **lateral diffusion** in the membrane plane
  - Mobility depends on:
    - Molecular size (larger molecules diffuse more slowly)
    - Temperature (higher temperature = higher fluidity)
    - Lipid composition (saturation, chain length)
  - Some proteins are more mobile than others
- **Asymmetry**
  - Two leaflets have **different lipid compositions**
  - Proteins have **unique orientation** (inside-out vs. outside-in)
  - Carbohydrate chains are always on the **extracellular (exoplasmic) surface**
  - Asymmetry is functionally important
- **Noncovalent Interactions**
  - Components held together by:
    - **Hydrophobic interactions** (lipid tails, protein transmembrane domains)
    - **Electrostatic interactions** (charged head groups)
    - **van der Waals forces** (weak attractions)
  - **No covalent bonds** between lipid and protein (except lipid-anchored proteins)
  - Allows dynamic assembly/disassembly

# The mobility of lipid bilayer

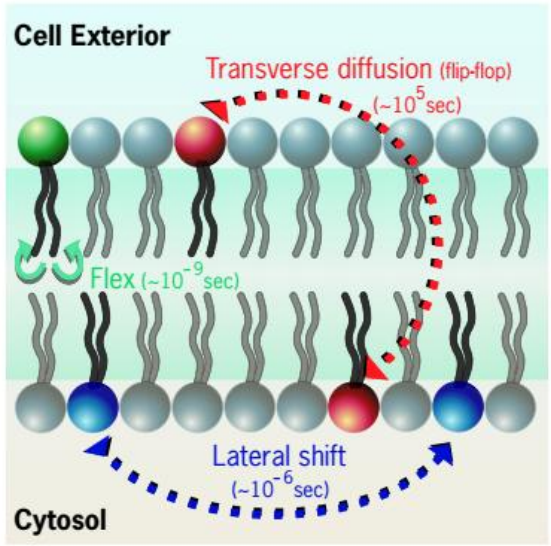



*Figure 4.25* **The possible movements of phospholipids in a membrane.** The types of movements in which membrane phospholipids can engage and the approximate time scales over which they occur. Whereas phospholipids move from one leaflet to another at a very slow rate, they diffuse laterally within a leaflet rapidly. Lipids lacking polar groups, such as cholesterol, can move across the bilayer quite rapidly.

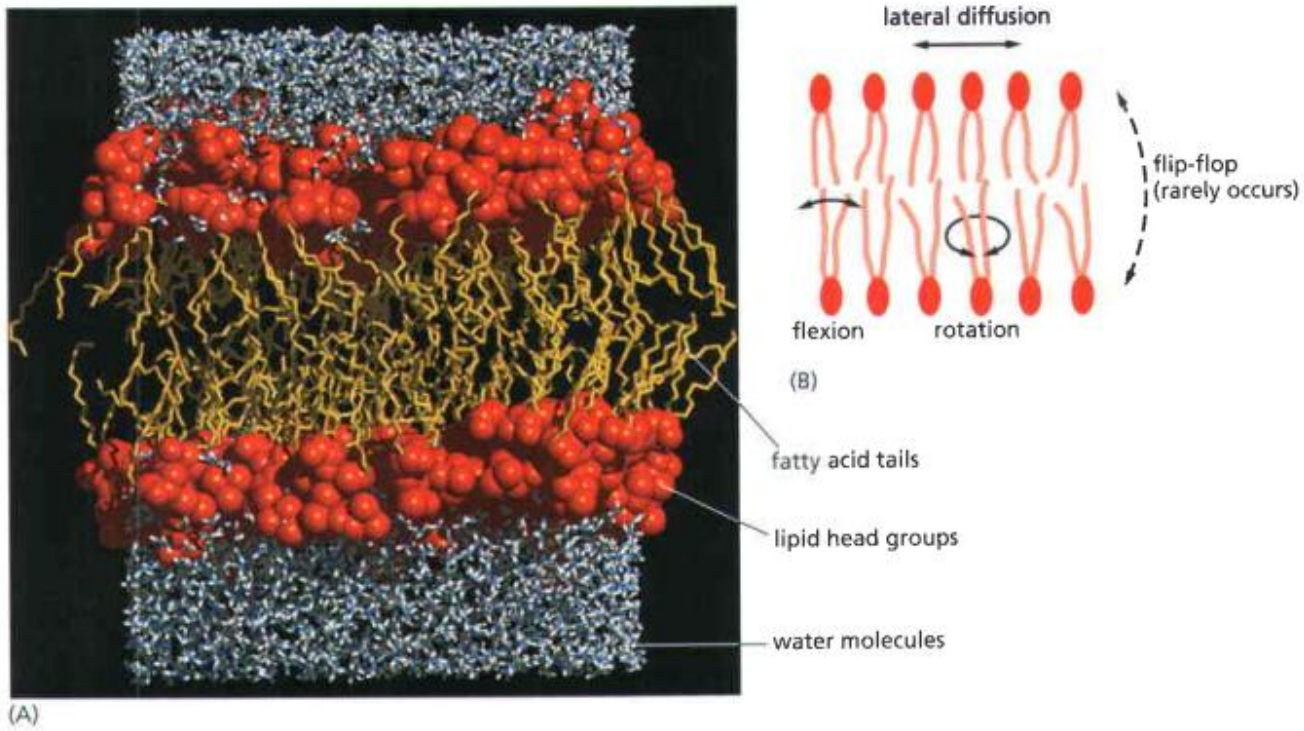



**Figure 10–11 The mobility of phospholipid molecules in an artificial lipid bilayer.** <CACA> Starting with a model of 100 phosphatidylcholine molecules arranged in a regular bilayer, a computer calculated the position of every atom after 300 picoseconds of simulated time. From these theoretical calculations (taking weeks of processor time in 1995), a model of the lipid bilayer emerges that accounts for almost all of the measurable properties of a synthetic lipid bilayer, such as its thickness, number of lipid molecules per membrane area, depth of water penetration, and unevenness of the two surfaces. Note that the tails in one monolayer can interact with those in the other monolayer, if the tails are long enough. (B) The different motions of a lipid molecule in a bilayer. (A, based on S.W. Chiu et al., *Biophys. J.* 69:1230–1245, 1995. With permission from the Biophysical Society.)

# Key innovations over previous models

- Lipid bilayer as fluid matrix NOT static structure
  - Phospholipid bilayer forms basic structural framework
  - Amphipathic lipids spontaneously form bilayers in an aqueous environment
  - Provides a permeability barrier to water-soluble molecules
- **Proteins EMBEDDED, Not Just Coating**
  - Integral proteins embedded in or span the bilayer
  - Peripheral proteins associated with membrane surfaces
  - Proteins distributed non-uniformly (mosaic pattern)
- **Fluidity Enables Function**
  - Both lipids and proteins move laterally
  - **Membrane flexibility** is necessary for critical cell functions like cell division, vesicle formation and budding, membrane fusion, protein-protein interactions etc.
- **Explains Selective Permeability**
  - Lipid bilayer is **impermeable** to most water-soluble molecules
  - **Proteins mediate selective transport** via:
    - **Carrier proteins** (facilitated diffusion, active transport)
    - **Ion channels** (rapid ion flow with selectivity)
    - **Receptor proteins** (ligand binding, signalling)
- **Accommodates Functional Diversity**
  - Different membranes have **different protein compositions** and lipid ratios
  - **Same basic structure** (bilayer + embedded proteins) supports diverse functions
  - Examples:
    - Myelin: 18% protein (insulation)
    - Inner mitochondrial membrane: 76% protein (energy production)
    - Plasma membrane: ~50:50 lipid:protein by mass

# Thermodynamic basis

- **The Hydrophobic Effect — Universal Driving Force**
  - When dispersed in water, phospholipids spontaneously organise
  - Hydrophobic tails cluster together, excluding water
  - Hydrophilic heads face aqueous environment
  - **Most stable configuration**: bilayer structure
- **Thermodynamic Favourability:**
  - **Enthalpy**: Favourable hydrogen bonding between water and head groups
  - **Entropy**: Minimises ordered water "cages" around hydrophobic groups
  - **Free energy minimisation** drives bilayer formation
  - Process is **spontaneous** - requires no energy input
- **Membrane Integrity:**
  - Bilayer has **no free edges** (energetically unfavourable)
  - Spontaneously forms **sealed compartments**
  - If torn, bilayer **self-heals** to eliminate edges
  - Provides basic structure for all cellular membranes
- **Protein Integration:**
  - **Transmembrane proteins** are amphipathic:
    - Hydrophobic regions embedded in lipid bilayer core
    - Hydrophilic regions exposed to aqueous environment on both sides
  - Proteins insert based on **hydrophobic matching**
  - Stable integration without covalent attachment

# Revolutionary Paradigm Shift

| Aspect | Pre-1972 Models | 1972 Fluid Mosaic Model |
|---|---|---|
| Membrane Nature | Static, rigid, protein-coated | Dynamic, fluid, protein-embedded |
| Protein Location | Surface only (continuous layers) | Embedded/spanning bilayer (discontinuous) |
| Lipid Organization | Static, uniform | Fluid, dynamic, asymmetric |
| Component Movement | Minimal or none | Lateral diffusion of lipids and proteins |
| Permeability Basis | Simple pores through proteins | Complex membrane-protein systems |
| Flexibility | Absent | Essential for cellular functions |

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# Key Experimental Evidences

Freeze-Fracture Electron Microscopy (1960s-1970s)

Cell Fusion - Frye and Edidin (1970)

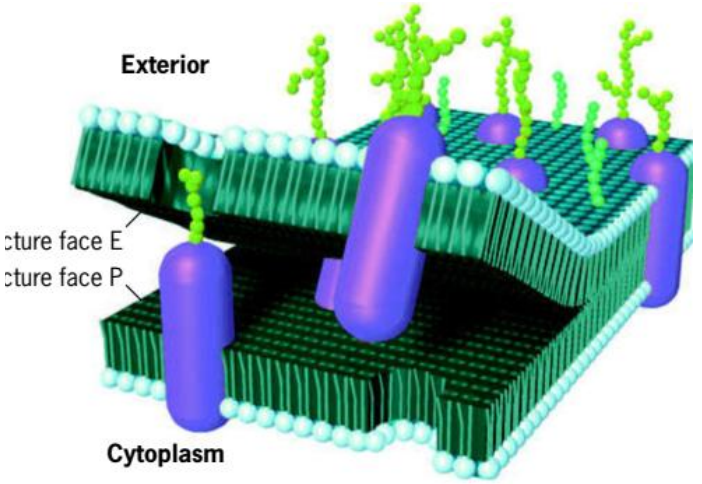
Fluorescence Recovery After Photobleaching (FRAP)

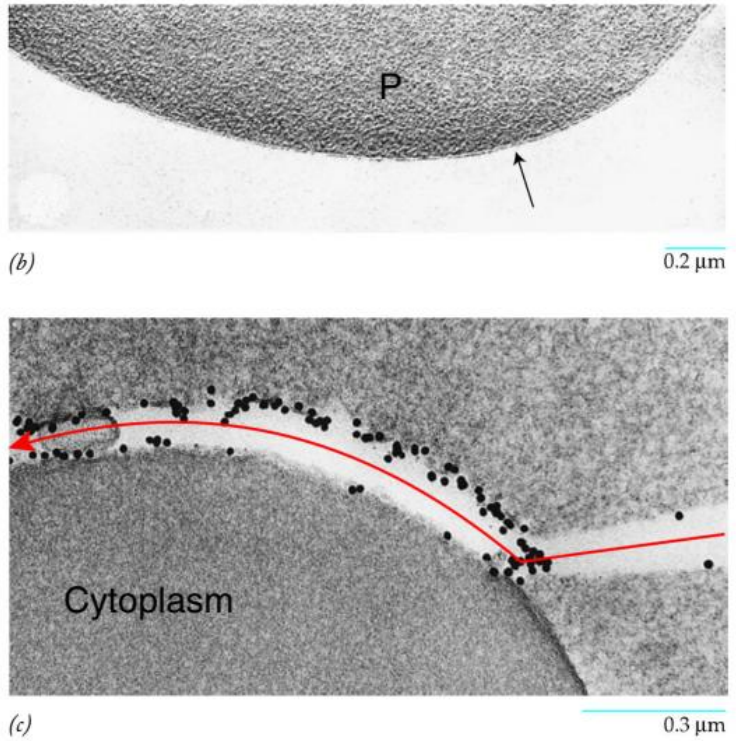
# Freeze-Fracture Electron Microscopy (1960s-1970s)

- **Technique:**
  - Rapidly freeze membrane at liquid nitrogen temperature (< -196°C)
  - When the frozen solid tissues are hit with a blade, it usually fractures between the lipid bilayer.
  - Once fractured, metals deposited into the fractured surfaces, which creates replica for EM study.
- **Observations:**
  - Membrane splits between the two leaflet layers
  - **Intramembrane particles** visible on fractured surfaces
  - Particles represent **transmembrane proteins**
  - Protein distribution shows great asymmetry.
- **Conclusion:**
  - Proteins **penetrate** lipid bilayer (not just coating surface)
  - Directly contradicts Davson-Danielli model
  - Supports Singer-Nicolson model of embedded proteins

# Freeze-Fracture Electron Microscopy (1960s-1970s)

***Figure 4.15*** **Freeze fracture: a technique for investigating cell membrane structure.** (*a*) When a block of frozen tissue is struck by a knife blade, a fracture plane runs through the tissue, often following a path that leads it through the middle of the lipid bilayer. The fracture plane goes around the proteins rather than cracking them in half, and they segregate with one of the two halves of the bilayer. The exposed faces within the center of the bilayer can then be covered with a metal deposit to form a metallic replica. These exposed faces are referred to as the E, or ectoplasmic face, and the P, or protoplasmic face. (*b*) Replica of a freeze-fractured human erythrocyte. The P fracture face is seen to be covered with particles approximately 8 nm in diameter. A small ridge (arrow) marks the junction of the particulate face with the surrounding ice. (*c*) This micrograph shows the surface of an erythrocyte that was frozen and then fractured, but rather than preparing a replica, the cell was thawed, fixed, and labeled with a marker for the carbohydrate groups that project from the external surface of the integral protein glycophorin (Figure 4.18). Thin sections of the labeled, fractured cell reveal that glycophorin molecules (black particles) have preferentially segregated with the outer half of the membrane. The red line shows the path of the fracture plane. (B: FROM THOMAS W. TILLACK AND VINCENT T. MARCHESI, J. CELL BIOL. 45:649, 1970; C: FROM PEDRO PINTO DA SILVA AND MARIA R. TORRISI, J. CELL BIOL. 93:467, 1982; B,C: REPRODUCED WITH PERMISSION OF THE ROCKEFELLER UNIVERSITY PRESS.)

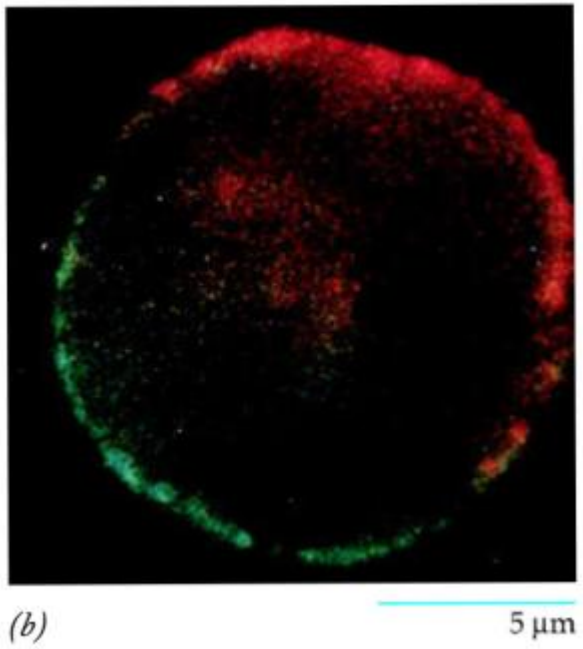


# Cell Fusion - Frye and Edidin (1970)

- **Design:**
  - Human cells labelled with **red fluorescent antibody** (against human membrane proteins)
  - Mouse cells labelled with **green fluorescent antibody** (against mouse membrane proteins)
  - Cells fused using Sendai virus or polyethene glycol (PEG)
  - Monitored protein distribution over time
- **Initial Observation (t = 0):**
  - Mouse proteins: confined to the mouse half of the hybrid cell (red hemisphere)
  - Human proteins: confined to the human half (green hemisphere)
  - **Clear segregation** of the two protein populations
- **After 40 Minutes at 37°C:**
  - Mouse and human proteins are **completely intermixed**
  - Entire cell surface shows **uniform yellow** colour (red + green)
  - Random distribution across the entire membrane
- **Control:**
  - At **4°C**: NO intermixing after many hours
  - Confirms mobility is **temperature-dependent**
  - Low temperature increases membrane viscosity
- **Conclusion:**
  - Membrane proteins are **mobile** in plane of the bilayer
  - Movement is **temperature-dependent** (requires membrane fluidity)
  - Random **lateral diffusion** driven by thermal energy
  - **Directly confirms** Fluid Mosaic Model predictions

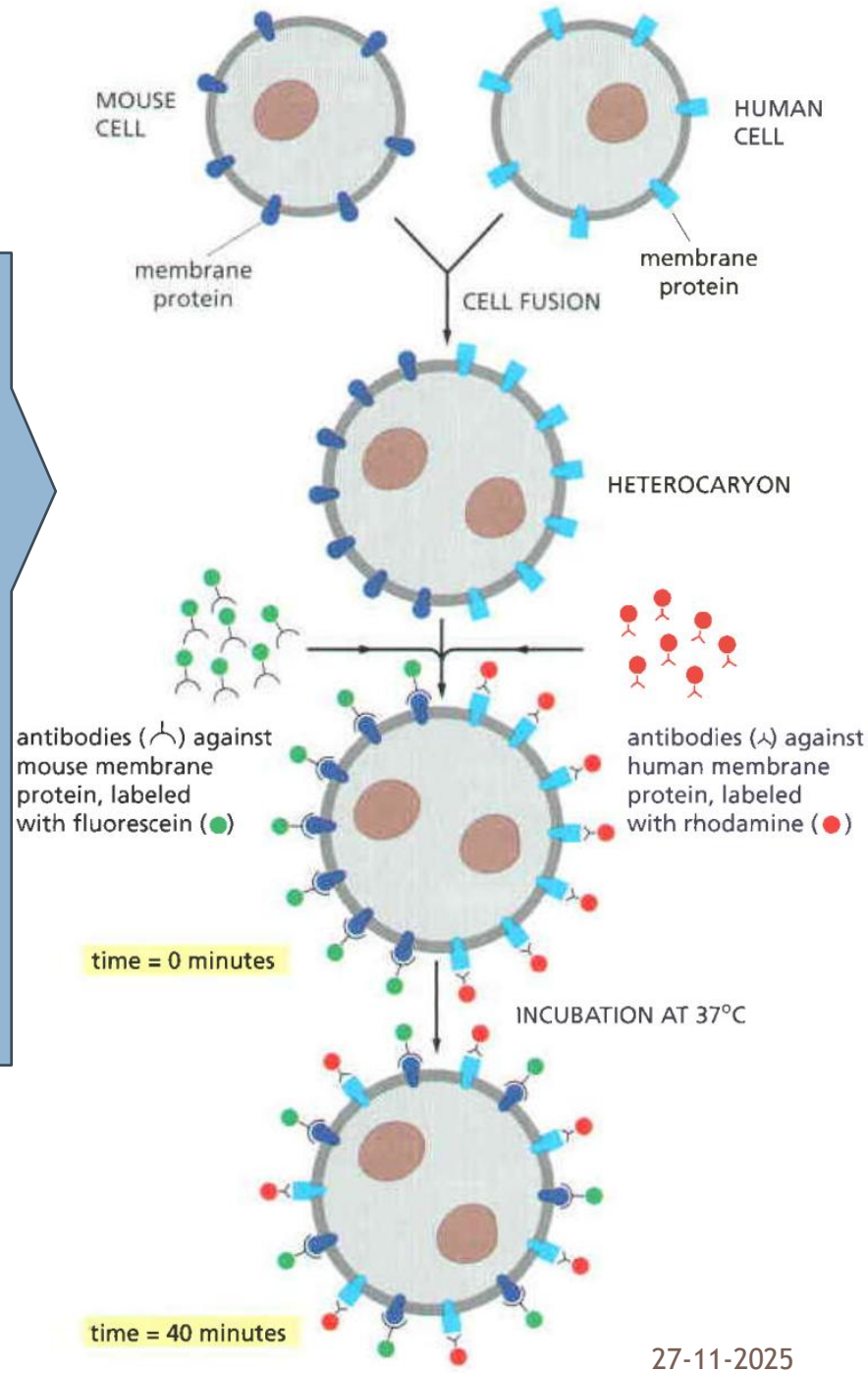
# Cell Fusion - Frye and Edidin (1970)




Micrograph showing a fused cell in which mouse and human proteins are still in their respective hemispheres .
Image source: Karp (2010) Cell and Molecular Biology 6th ed., p. 141

**Figure 10–35 An experiment demonstrating the diffusion of proteins in the plasma membrane of mouse–human hybrid cells.** The mouse and human proteins are initially confined to their own halves of the newly formed heterocaryon plasma membrane, but they intermix over time. The two antibodies used to visualize the proteins can be distinguished in a fluorescence microscope because fluorescein is green and rhodamine is red. (Based on L.D. Frye and M. Edidin, *J. Cell Sci.* 7:319–335, 1970. With permission from The Company of Biologists.)

Image source: Alberts et al. (2008) Molecular Biology of the Cell 5th ed.



# Fluorescence Recovery After Photobleaching (FRAP)

❖**Principle:**
- ❑Label membrane proteins with **fluorescent dye**
- ❑**Bleach** a small area (~1 μm diameter) with a focused laser beam
- ❑Monitor **fluorescence recovery** in the bleached area over time
- ❑Recovery indicates lateral diffusion of unbleached proteins into the area

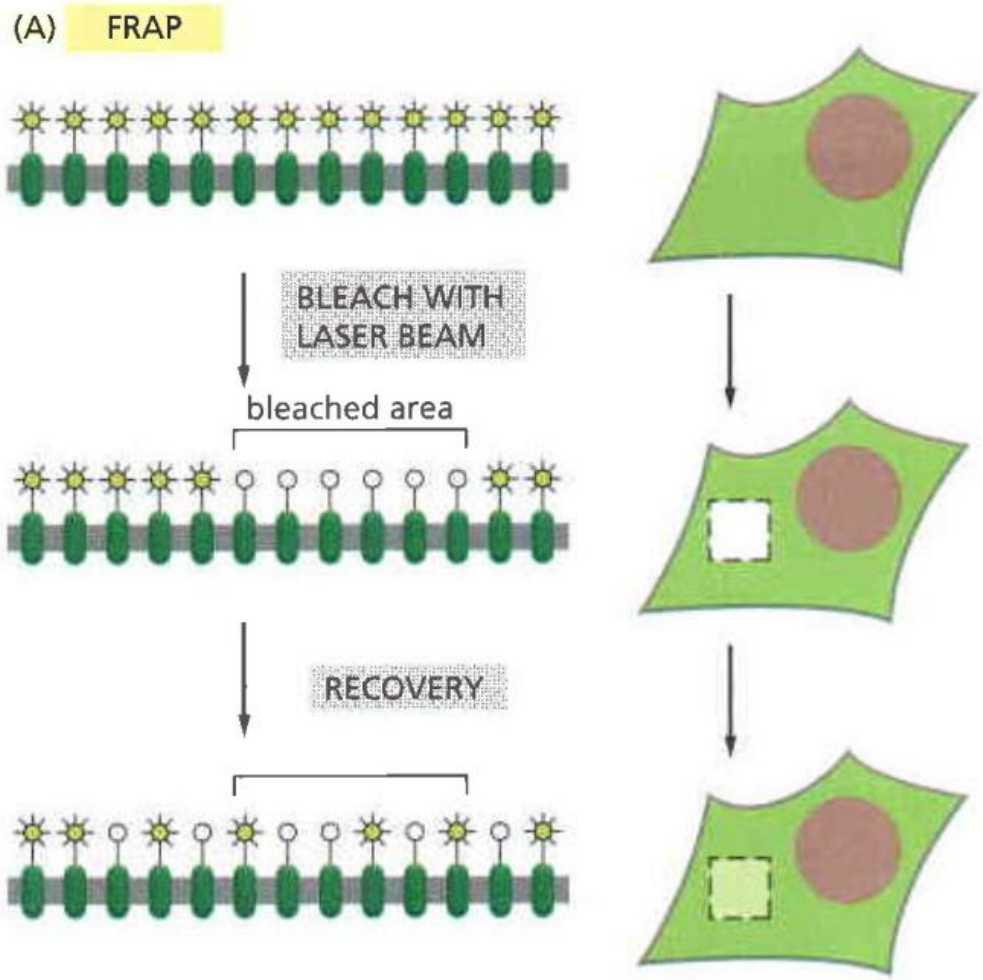
❖**Method:**
- ❑Label membrane protein with antibody-fluorophore or Green Fluorescent Protein (GF)P fusion
- ❑Bleach with intense laser pulse (~1 second)
- ❑Measure fluorescence recovery using a scanning microscope
- ❑Calculate **diffusion coefficient** (D) from recovery curve

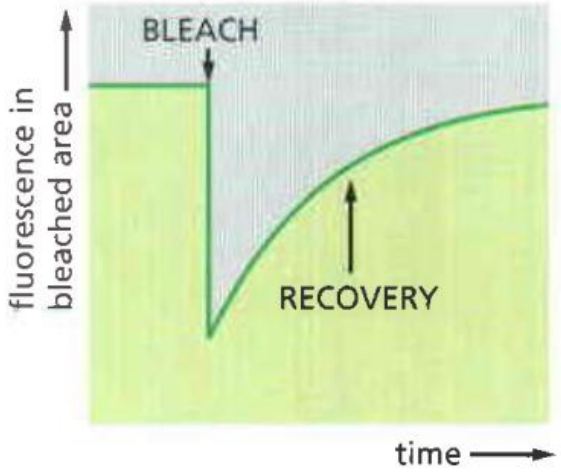
❖**Results:**
- ❑Rapid initial recovery (seconds to minutes)
- ❑Recovery follows **diffusion kinetics**
- ❑Different proteins show different recovery rates

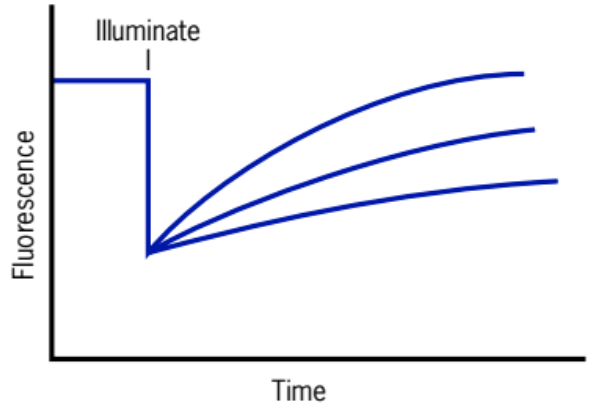
❖**Typical Diffusion Coefficients:**
- ❑**Lipids:** 2-8 $\mu m^2$/second (very mobile)
- ❑**Small proteins:** 0.1-1 $\mu m^2$/second (moderately mobile)
- ❑**Large protein complexes:** 0.001-0.1 $\mu m^2$/second (restricted)
- ❑**Some proteins:** <0.001 $\mu m^2$/second (essentially immobile)

❖**Interpretation:**
- ❑Quantifies protein **lateral diffusion**
- ❑Demonstrates **variable mobility** among proteins
- ❑Shows some proteins have **restricted movement**
- ❑First hint that the membrane is MORE organised than simple fluid

# Fluorescence Recovery After Photobleaching (FRAP)



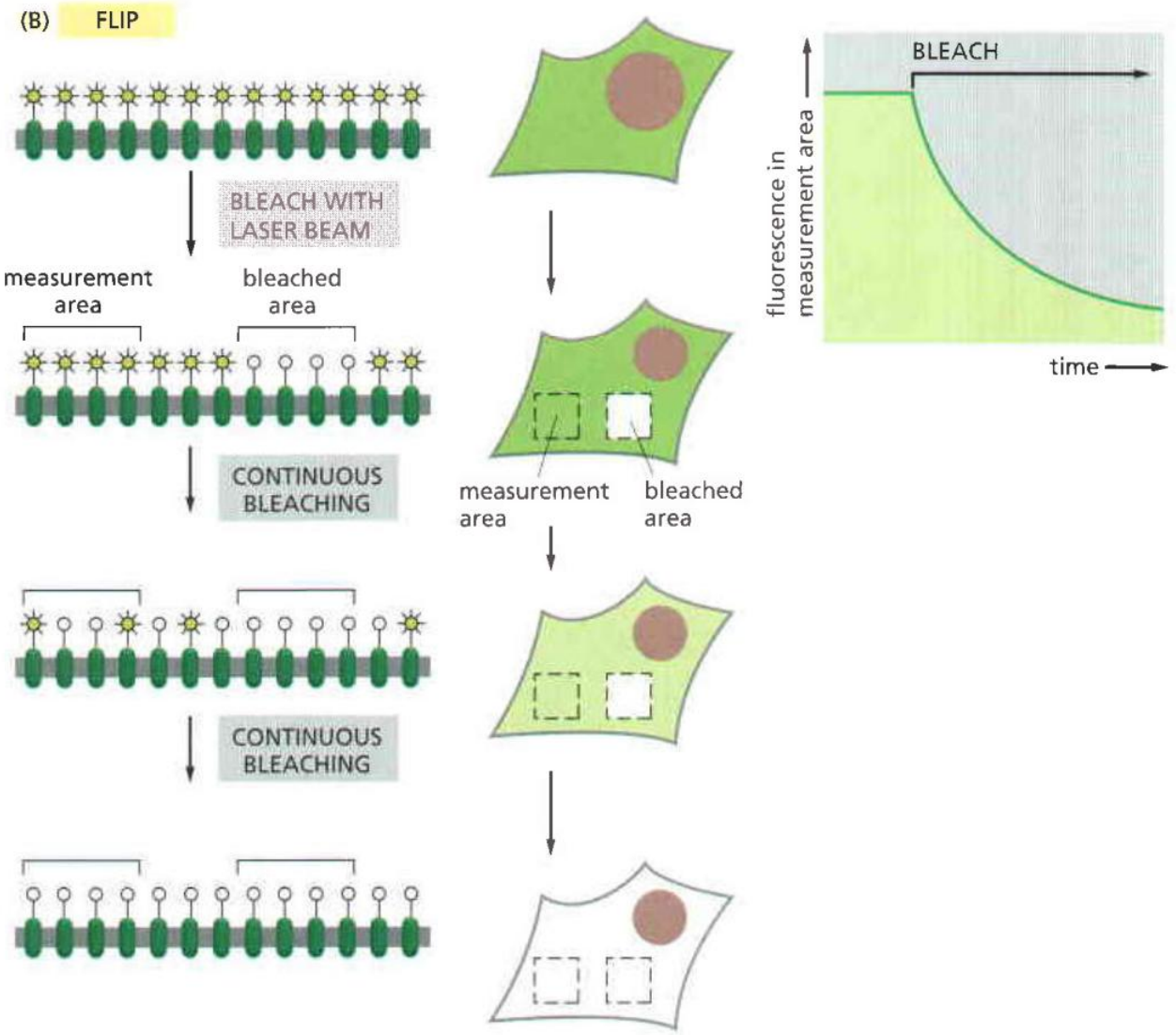




*Recovery rate varies with the protein being tracked*

# FLIP - Fluorescence Loss in Photobleaching

- A fluorescence microscopy technique complementary to FRAP, used to study membrane continuity and molecular exchange between cellular regions.
- **Key Difference from FRAP:**
  - **FRAP**: Single bleach → monitors fluorescence **recovery** in the bleached region
  - **FLIP**: Repeated bleaching → monitors fluorescence **loss** in non-bleached regions
- **Experimental Method**
  - **Label** membrane proteins or lipids with fluorescent tags (GFP, dyes)
  - **Repeatedly bleach** a selected region at regular intervals
  - **Monitor fluorescence loss** in distant, non-bleached regions
  - **Analyse** connectivity and molecular exchange
- **What FLIP Reveals**
  - **If fluorescence decreases in distant regions:**
    - Compartments are **connected**
    - Molecules **freely exchange** between regions
    - Membrane is **continuous**
  - **If fluorescence is retained in distant regions:**
    - Compartments are **separate** (barriers present)
    - Molecules are **confined** or **immobile**
    - Diffusion is **restricted**

# FLIP - Fluorescence Loss in Photobleaching



# Applications of FLIP in Membrane Biology

- **Membrane Compartmentalisation:**
  - Demonstrates **apical and basolateral membranes** are separate in epithelial cells
  - Tight junctions create barriers preventing lipid/protein exchange
- **Lipid Raft Dynamics:**
  - Assesses whether raft proteins are confined or exchange with bulk membrane
- **Cytoskeletal Barriers:**
  - Identifies "**fences**" or "**corrals**" created by membrane skeleton
- **ER Network Continuity:**
  - Confirms endoplasmic reticulum is a continuous network throughout cell
- **Summary**
  - **FLIP complements FRAP to provide comprehensive membrane dynamics understanding:**
    - **FRAP**: How fast do molecules move **into** a region? (local mobility)
    - **FLIP**: How fast do molecules move **away** from a region? (global connectivity)
  - **Together**: Reveal membrane fluidity, protein mobility, compartmentalisation, and barriers to diffusion.

# Factors Affecting Membrane Fluidity

- **Temperature**
  - **Higher temperature:** increased molecular motion → INCREASED fluidity
  - **Lower temperature:** decreased motion → DECREASED fluidity
  - **Transition temperature (Tm):** temperature at which the membrane converts from **Liquid crystalline phase** (fluid) → **Gel phase** (solid/rigid)
  - Sharp phase transition in pure lipid bilayers
- **Fatty acid saturation**
  - One double bond can lower Tm by ~60°C
  - Example: Stearic acid (18:0) Tm = 70°C; Oleic acid (18:1) Tm = 13°C

| Fatty Acid Type | Structure | Effect on Fluidity | Melting Point |
|---|---|---|---|
| **Saturated** | No double bonds; straight chains | **Decreased** fluidity (tight packing) | **Higher** Tm |
| **Unsaturated (cis)** | One or more C=C bonds; kinked chains | **Increased** fluidity (loose packing) | **Lower** Tm |

# Factors Affecting Membrane Fluidity

- **Fatty Acid Chain Length**
  - **Longer chains:** more van der Waals interactions → LOWER fluidity
  - **Shorter chains:** fewer interactions → HIGHER fluidity
  - Most membrane lipids: 16-22 carbons
- **Cholesterol Content**
  - Cholesterol constitutes up to **50% of lipid molecules** in animal plasma membranes
  - Reduces permeability to small water-soluble molecules
  - Maintains relatively constant fluidity across temperature ranges

| Temperature | Effect of Cholesterol | Mechanism |
|---|---|---|
| **High temperature** | **Decreases** fluidity | Rigid steroid rings restrict phospholipid movement |
| **Low temperature** | **Increases** fluidity | Prevents tight packing; prevents crystallisation |
| **Overall effect** | Creates **intermediate** fluidity | "Buffers" membrane fluidity |

# Cellular Regulation of Membrane Fluidity

- **Homeoviscous Adaptation:**
  - Organisms adjust membrane lipid composition to maintain optimal fluidity
- **Cold Adaptation:**
  - Temperature drops → membrane would solidify
  - Cells respond by **increasing unsaturation** of fatty acids
  - **Desaturase enzymes** add double bonds to saturated chains
  - Result: maintains fluidity at lower temperatures
- **Heat Adaptation:**
  - Temperature rises → membrane becomes too fluid
  - Cells respond by **increasing saturation**
  - Remove double bonds or synthesise saturated lipids
  - Result: prevents excessive fluidity
- **Examples:**
  - **Bacteria in hot springs**: highly unusual lipid compositions
  - **Arctic fish**: exceptionally high polyunsaturated fatty acids
  - **Hibernating animals**: increase unsaturation during hibernation

# Functional Importance of Fluidity

- **Membrane Protein Function**
  - Many proteins require fluid environment
  - Enzymes, transporters cease functioning if membrane too rigid
- **Membrane Fusion and Budding**
  - Vesicle formation requires membrane flexibility
  - Exocytosis, endocytosis depend on fluidity
- **Cell Division**
  - Membrane must be flexible to allow cell division
  - Rigid membranes prevent cytokinesis
- **Protein-Protein Interactions**
  - Allows proteins to come together temporarily
  - Formation of functional complexes (receptors, signalling)
- **Membrane Assembly**
  - New lipids and proteins insert into fluid membrane
  - Growth of membrane requires fluidity

# Key predictions from Fluid Mosaic Model

- **Prediction 1: Membrane Functions from Protein Components**
  - **Lipid bilayer:** provides impermeability, compartmentalisation, fluidity
  - **Proteins:** provide specificity, catalysis, recognition, transport
  - Different membranes have different protein complements
  - Protein diversity explains functional diversity
- **Prediction 2: Selective Permeability**
  - Lipid bilayer **impermeable** to most polar molecules
  - Transport proteins required for ions, sugars, amino acids
  - Selectivity arises from protein specificity
  - Energy-dependent transport via protein pumps
- **Prediction 3: Membrane Flexibility and Dynamics**
  - Components can rearrange within membrane plane
  - Protein-protein interactions can form transiently
  - Functional complexes assemble on demand
  - Regulation through protein recruitment/release
- **Prediction 4: Temperature Effects on Membrane Function**
  - Increased temperature → increased fluidity → altered function
  - Decreased temperature → decreased fluidity → phase transition
  - Cells must adapt lipid composition to maintain function
- **Prediction 5: Basis of Membrane Diseases**
  - Abnormal lipid composition → membrane dysfunction
  - Protein mutations → loss of function, aggregation
  - Explains hereditary disorders affecting membranes

# Impact of the Model

## Scientific Impact

- **Unified understanding** of all biological membranes
- Explained both structure and dynamics
- Became "central dogma" of membrane biology
- Generated decades of productive research

## Limitations Recognised Later

- Underestimated **protein-protein interactions**
- Overestimated **protein mobility** (many proteins more restricted than predicted)
- Underestimated **membrane organisation** (domains, rafts not included)
- Underestimated role of **cytoskeleton** in restricting movement
- "Lipid sea" more structured than originally thought

# Transition to the Updated Model (2014)

Why an update was needed? Four decades of new research (1972-2014)

## New Technologies

- Advanced fluorescence microscopy (confocal, super-resolution)
- Single-particle tracking
- X-ray crystallography of membrane proteins
- Atomic force microscopy
- Lipidomics and proteomics
- Computational modelling

## New discoveries

- **Lipid rafts** - ordered membrane domains
- **Membrane skeleton** - cytoskeletal restrictions on mobility
- **Protein complexes** - proteins function in assemblies, not isolation
- **Membrane domains** - organised, functional compartments
- **Restricted mobility** - many proteins NOT freely diffusing
- **Lipid-protein interactions** - specific and functional

**Key Insight:** Membranes are **MORE CROWDED** and **MORE ORGANISED** than the 1972 model suggested.

**Fundamental Shift:**

•Original emphasis: **"FLUID"** matrix
•Updated emphasis: **"MOSAIC"** organisation and domains

# Nicolson's 2014 Updated Model

*"The basic structural principles of the Fluid Mosaic Model remain valid, BUT the model requires updates to reflect new knowledge about membrane organisation, domains, and restricted mobility"*

- **What Remains Valid (1972 → 2014):**
  - Lipid bilayer forms basic structure
  - Proteins embedded in bilayer
  - Amphipathic nature of components
  - Some lateral movement occurs
  - Components held by noncovalent bonds
  - Membrane is dynamic

**Overall Assessment**

The Fluid Mosaic Model remains **fundamentally correct**, but modern understanding emphasises **ORGANISATION** over **RANDOMNESS**.

**What changed?**

- **Emphasis Shift: "FLUID" → "MOSAIC"**
  - Membrane is MORE organised, LESS randomly fluid
  - Proteins densely packed in many regions
  - Interaction networks create organisation
- **Lipid Domains and Rafts**
  - Membrane NOT uniformly composed
  - Specialised lipid-protein domains exist
  - Lipid rafts organise signalling and transport
- **Membrane Skeleton and Cytoskeletal Interactions**
  - Extensive cytoskeletal network beneath membrane
  - Creates "fences" and "corrals" that restrict movement
  - Organises membrane into compartments
- **Protein Complexes**
  - Proteins function in multi-subunit assemblies
  - Complexes more stable than individual proteins
  - Coordinated, cooperative functions
- **Restricted Lateral Mobility**
  - Many proteins confined to specific domains
  - Movement more restricted than originally thought
  - Dynamic but ORGANISED, not random

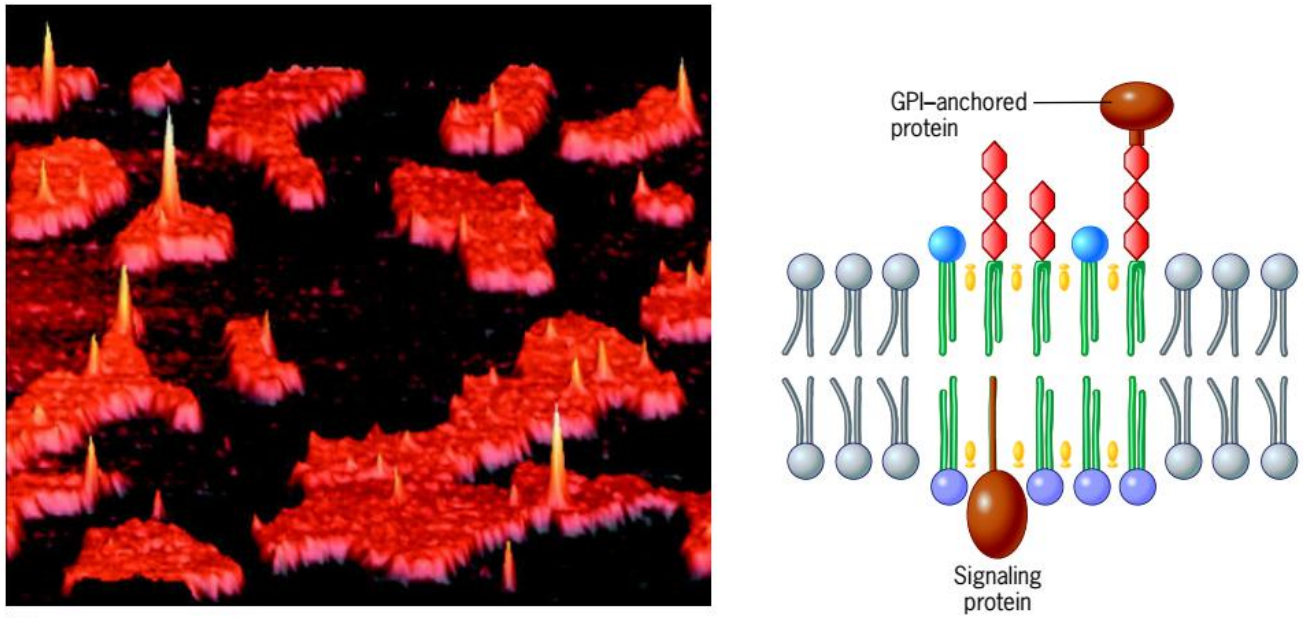
# Comparing 1972 and 2014 Models

| Aspect | 1972 Singer-Nicolson Model | 2014 Nicolson Updated Model |
|---|---|---|
| **Lipid Distribution** | Relatively uniform bilayer | Organised into lipid rafts and domains |
| **Protein Distribution** | Random, sparse mosaic | Crowded, organised complexes and assemblies |
| **Protein Mobility** | Free lateral diffusion | Restricted; compartmentalised by cytoskeleton |
| **Membrane Organisation** | Minimal structure; fluid matrix | Hierarchical organisation; multiple domains |
| **Lipid-Protein Ratio** | ~50 lipid per protein (by number) | Highly variable by region; some areas very crowded |
| **Emphasis** | **"FLUID"** matrix with embedded proteins | **"MOSAIC"** of organised, functional domains |
| **Cytoskeleton Role** | Not emphasised | Critical for organising and restricting mobility |
| **Protein Function** | Individual proteins | Protein complexes and assemblies |
| **Membrane Dynamics** | Primarily lateral diffusion | Regulated mobility; domain-specific functions |

# Lipid Rafts and Membrane Domains

- **Discovery of Lipid Rafts (1990s-2000s)**
- **Definition:** Lipid rafts are small (10-200 nm), dynamic, cholesterol- and sphingolipid-enriched microdomains that float within the more fluid, disordered membrane environment.
- **Composition:**
  - Very high cholesterol (15-30%) - provides rigidity
  - High sphingolipids (sphingomyelin, glycolipids) - long, saturated chains
  - Saturated phospholipids - ordered arrangement
  - GPI-anchored proteins - preferentially partition into rafts
- **Physical Properties:**
  - More ordered, gel-like
  - Thicker than surrounding bilayer (~0.6-1.5 nm difference)
  - Lower permeability to small molecules
  - Dynamic: form and dissolve on milliseconds to seconds timescales
- **Functions of Lipid Rafts:**
  - **Signal Transduction Platform** - concentrates receptors and signalling proteins
  - **Protein Trafficking and Sorting** - selective environment for protein function
  - **Membrane Organisation** - creates structural compartments
  - **Pathogen Entry** - some viruses use rafts (influenza, HIV); cholera toxin binds GM1 ganglioside in rafts

# Lipid Rafts and Membrane Domains

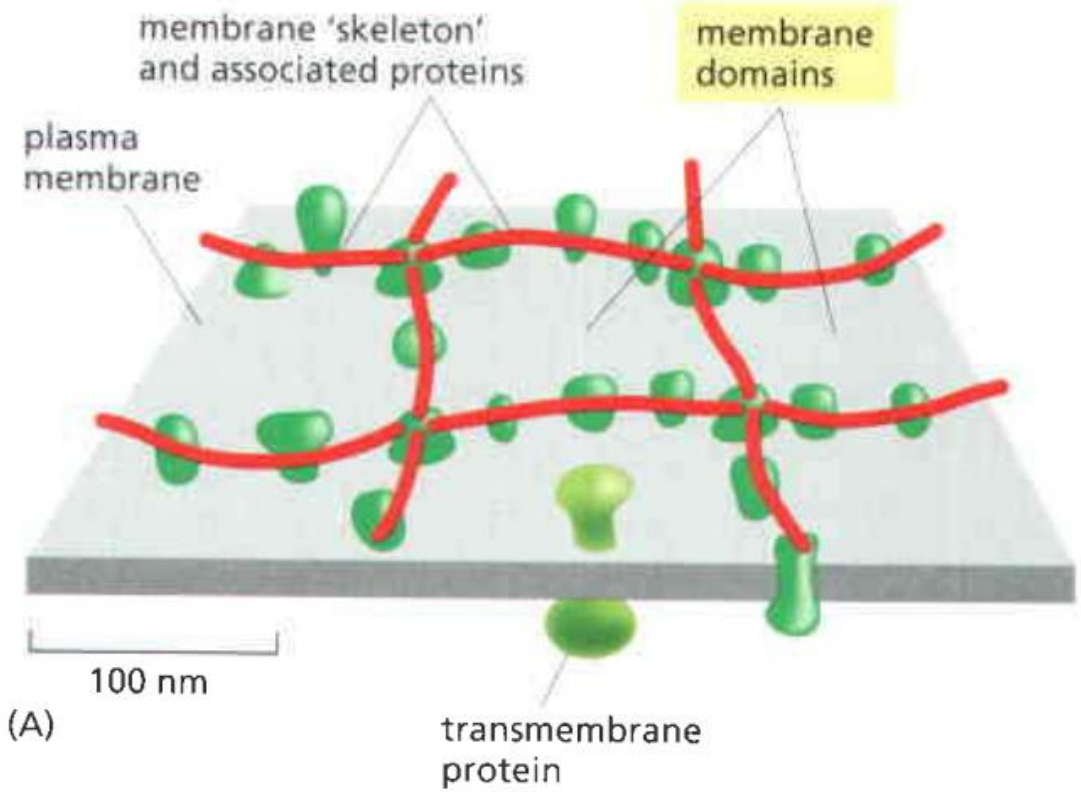



(a) (b)

*Figure 4.24* **Lipid rafts.** (*a*) Image of the upper surface of an artificial lipid bilayer containing phosphatidylcholine, which appears as the black background, and sphingomyelin molecules, which organize themselves spontaneously into the orange-colored rafts. The yellow peaks show the positions of a GPI-anchored protein, which is almost exclusively raft-associated. This image is provided by an atomic force microscope, which measures the height of various parts of the specimen at the molecular level. (*b*) Schematic model of a lipid raft within a cell. The outer leaflet of the raft consists primarily of cholesterol (yellow) and sphingolipids (red head groups). Phosphatidylcholine molecules (blue head groups) with long saturated fatty acids also tend to concentrate in this region. GPI-anchored proteins are thought to become concentrated in lipid rafts. The lipids in the outer leaflet of the raft have an organizing effect on the lipids of the inner leaflet. As a result, the inner-leaflet raft lipids consist primarily of cholesterol and glycerophospholipids with long saturated fatty acyl tails. The inner leaflet tends to concentrate lipid-anchored proteins, such as Src kinase, that are involved in cell signaling. (The controversy over the existence of lipid rafts is discussed in *Nature Revs. Mol. Cell Biol.* 11:688, 2010 and *Science* 334:1046, 2011.) (A: FROM D. E. SASLOWSKY, ET AL., J. BIOL. CHEM. 277, COVER OF #30, JULY 26, 2002; COURTESY OF J. MICHAEL EDWARDSON © 2002 THE AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY.)

# Membrane Skeleton and Cytoskeletal Fences

The **membrane skeleton** is a **fibrillar network of peripheral proteins** located on the **cytosolic surface** of the plasma membrane, providing **mechanical support** and **organising membrane proteins**.



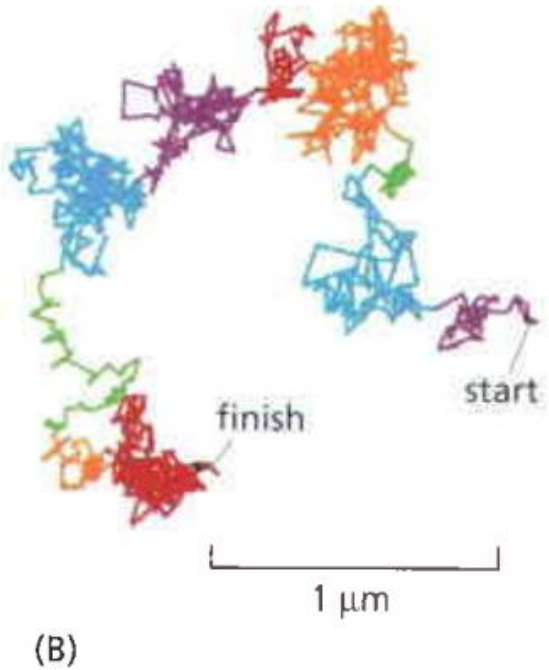
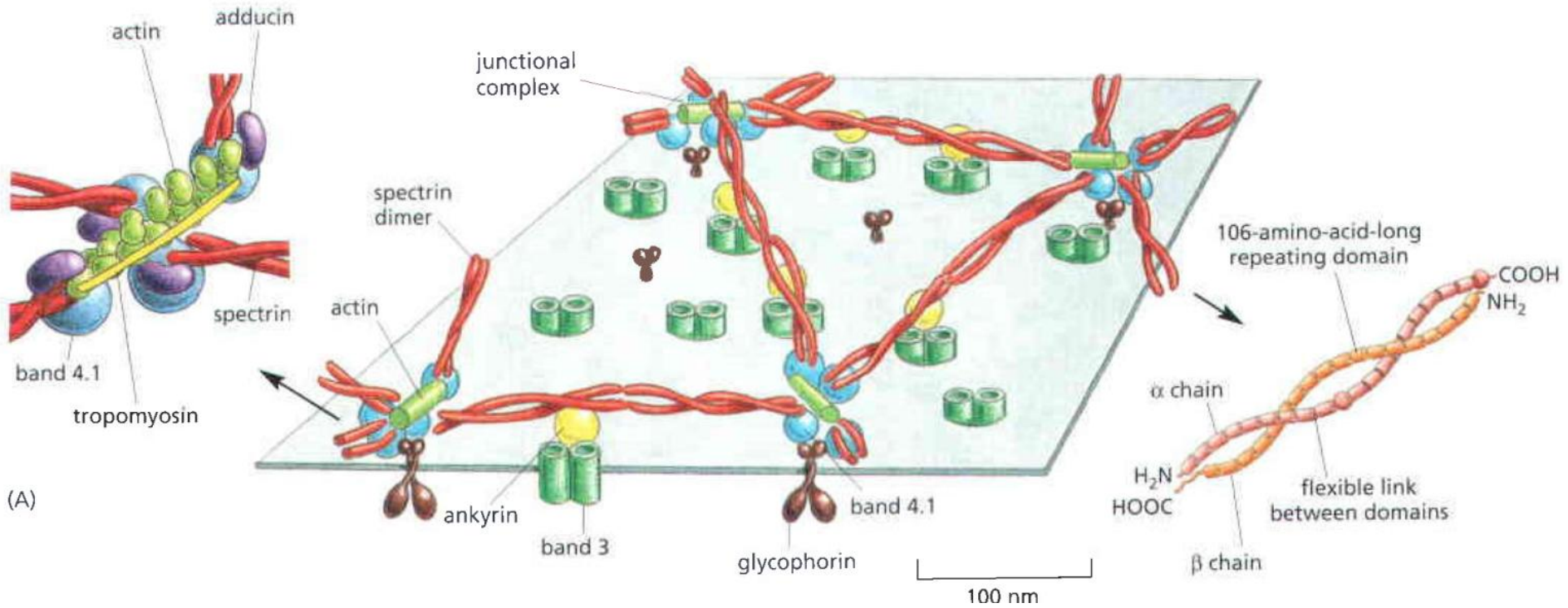




**Figure 10–42 Corraling of membrane proteins by cortical cytoskeletal filaments.** (A) How cytoskeletal filaments are thought to provide diffusion barriers that divide the membrane into small domains, or corrals. (B) High-speed, single-particle tracking was used to follow the paths of a fluorescently-labeled membrane protein over time. The trace shows that membrane proteins diffuse within a tightly delimited membrane domain (shown by different colors of the trace) and only infrequently escape into a neighboring domain. (Adapted from A. Kusumi et al., *Annu. Rev. Biophys. Biomol. Struct.* 34:351–378, 2005. With permission from Annual Reviews.)

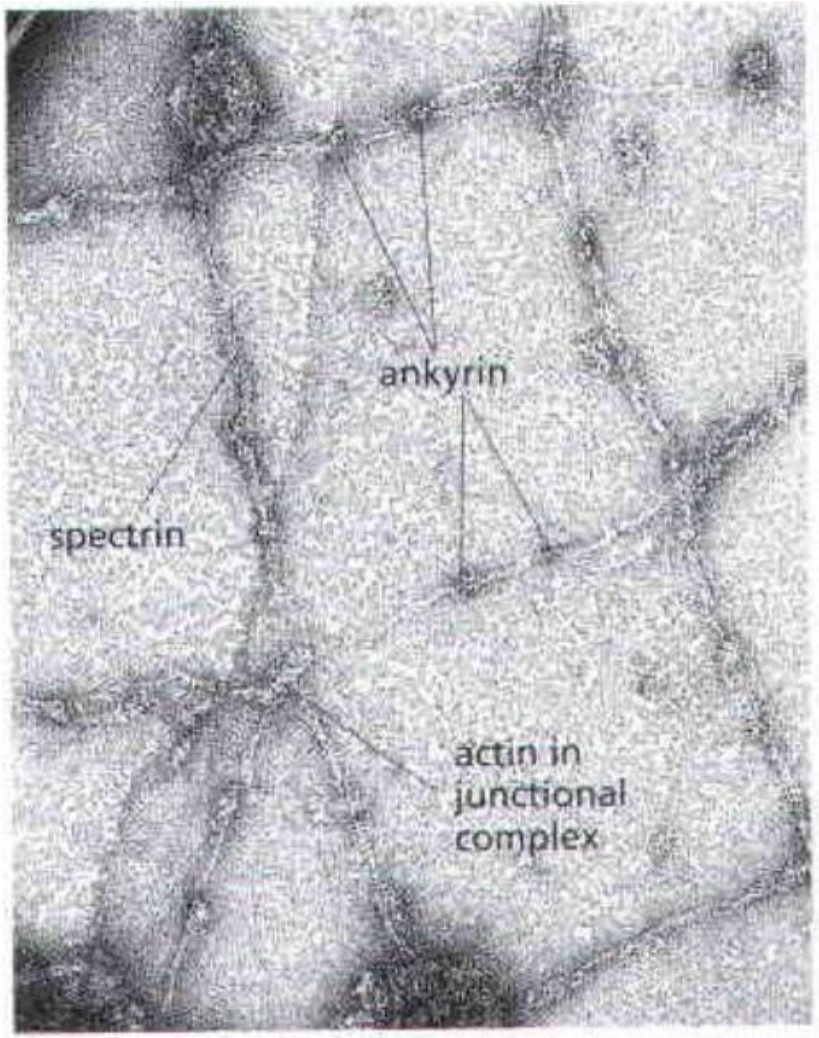
# RBC Model system




- Spectrin network - long, flexible rods forming a meshwork
- Actin - forms junctional complexes
- Ankyrin - links spectrin to Band 3 (integral protein)
- Band 4.1 - links spectrin to glycophorin
- Coverage: ~1 spectrin skeleton per 10-100 $\mu m^2$

# The RBC Model system

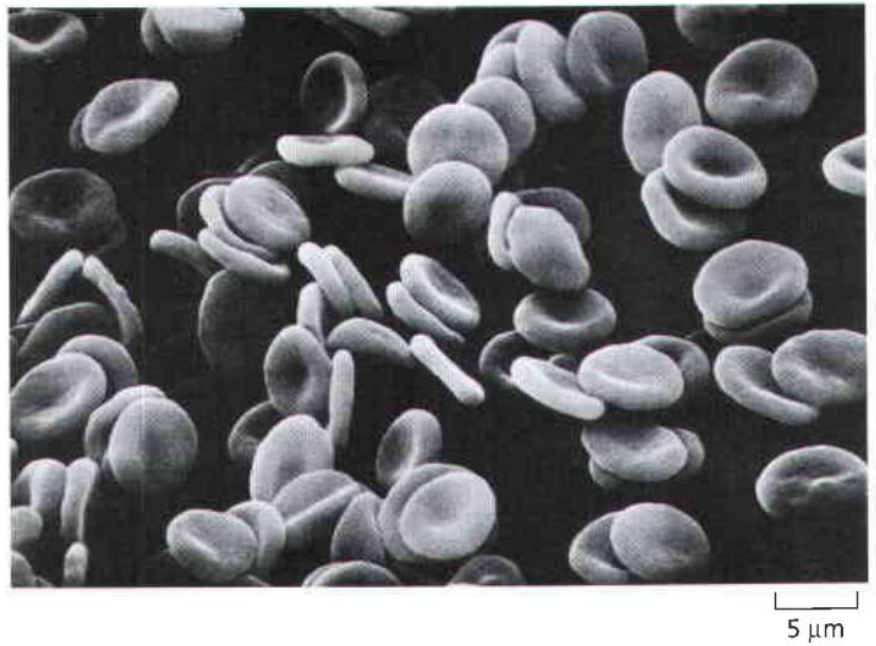
Electron micrograph showing cytosolic side of a RBC membrane after fixation and negative staining. The spectrin meshwork has been purposely stretched out to allow the details of its structure to be seen. In normal cell, the meshwork shown would be much more crowded and occupy only one-tenth of this area.
Source: Alberts et al. (2008) Molecular Biology of the Cell 5th ed.



# Functions of membrane domains

- **Mechanical Support**
  - Maintains cell shape (biconcave disc in erythrocytes)
  - Provides elasticity and deformability
  - Clinical relevance: Hereditary spherocytosis (spectrin deficiency → spherical cells → haemolysis)

- **Restriction of Lateral Protein Mobility - "Picket Fence" Model**
  - Spectrin network creates barriers ("fences") beneath the membrane
  - Transmembrane proteins act as "fence posts" anchored to the skeleton
  - Proteins diffuse rapidly WITHIN compartments (~100-200 nm)
  - Rarely cross into adjacent compartments
  - Creates a functional organisation




Scanning electron micrograph of human RBC.
Source: Alberts et al. (2008) Molecular Biology of the Cell 5th ed.

# Protein Complexes and Assemblies

- **Key Concept:** Membrane proteins rarely function as isolated individuals. Most form stable or dynamic complexes that provide specialised, cooperative functions.
- **Major Categories:**
  - **Energy Transduction Complexes**
    - Photosystem II: 19 protein subunits, >60 transmembrane helices
    - Electron transport chain complexes (mitochondria)
    - Organised into "supercomplexes" or "respirosomes"
    - More efficient than individual enzymes
  - **Ion Channel Complexes**
    - $K^+$ channels with accessory β subunits
    - Modulate gating kinetics, selectivity, localisation
  - **Receptor Complexes**
    - Receptor tyrosine kinases (RTKs) - dimerisation upon ligand binding
    - G protein-coupled receptors (GPCRs) - associate with G proteins
    - Create docking sites for adaptor proteins
  - **Cell Adhesion Complexes**
    - Integrin complexes - link extracellular matrix to cytoskeleton
    - Adherens junctions - cadherins recruit catenins
- **Consequences:**
  - Spatial organisation at specific locations
  - Temporal organisation (assemble/disassemble in response to signals)
  - Hierarchical assembly (modules combine)

# Functional Significance of Membrane Organisation

- **Signal Transduction**
  - Lipid rafts concentrate receptors and signalling proteins
  - Example: T-cell receptor signalling - TCR recruited to rafts → rapid phosphorylation cascade
- **Membrane Trafficking and Protein Sorting**
  - Apical vs. basolateral sorting in epithelial cells
  - Raft association determines destination
- **Cell Polarity**
  - Epithelial cells: apical domain (microvilli) vs. basolateral domain (different transporters)
  - Tight junctions maintain separation
  - Enables vectorial transport (intestine, kidney)
- **Clinical Significance - Membrane Disorders**
  - Hereditary spherocytosis: spectrin deficiency → abnormal erythrocyte shape
  - Cystic fibrosis: CFTR protein mislocalisation
  - Alzheimer's disease: altered membrane lipid composition affects amyloid processing
  - Cancer: aberrant lipid raft organisation in tumour cells

# Key References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