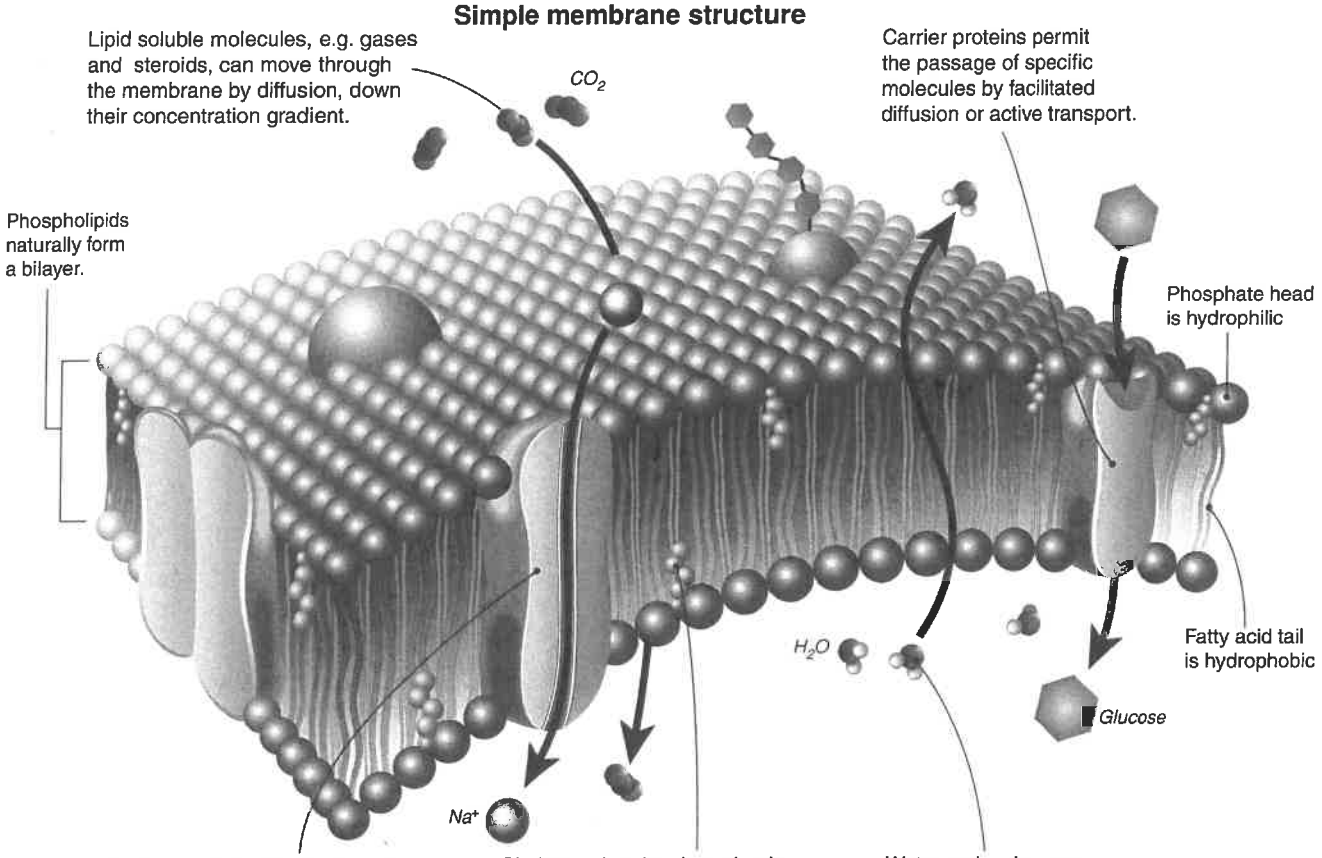
59 The Structure of Membranes

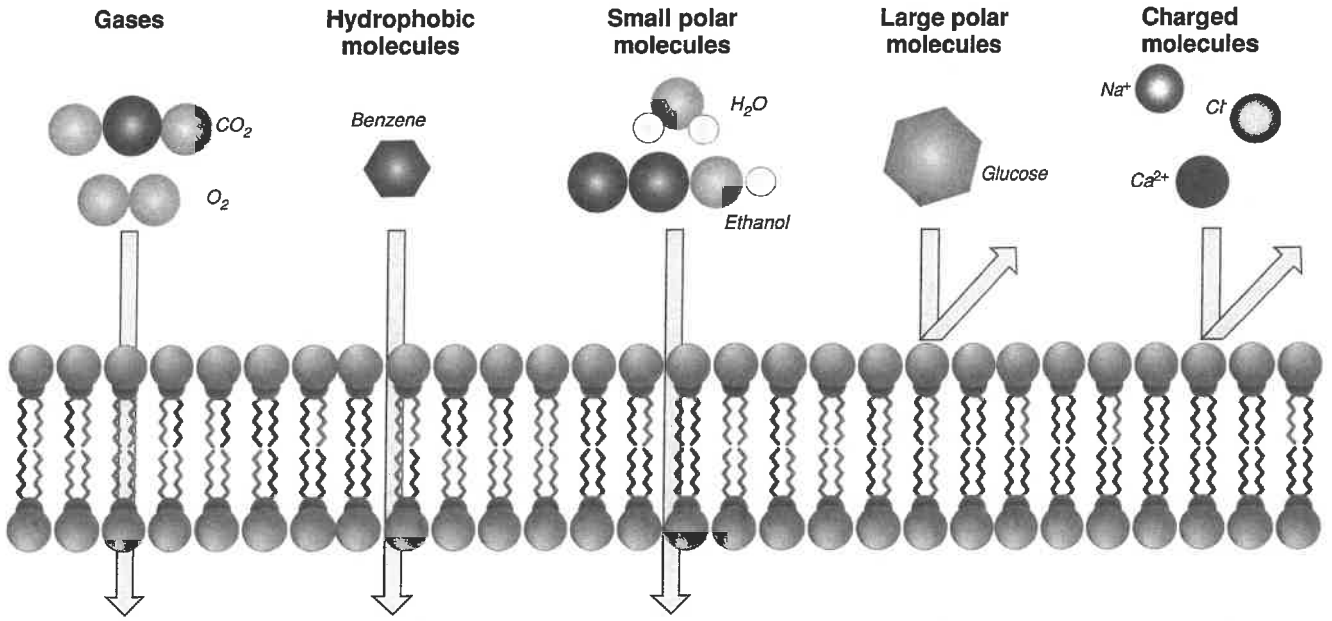
Key Idea: A cellular membrane is made of a phospholipid bilayer with proteins of different sorts embedded in it.

The cell surface (or plasma) membrane encloses the cell's contents and regulates many of the cell's activities. Importantly, it controls what enters and leaves the cell by the use of carrier and channel proteins.

Simple membrane structure

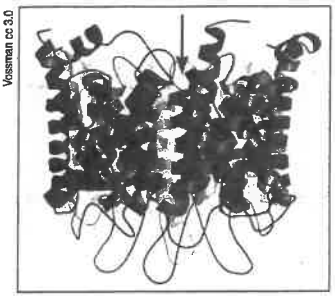


What can cross a lipid bilayer?

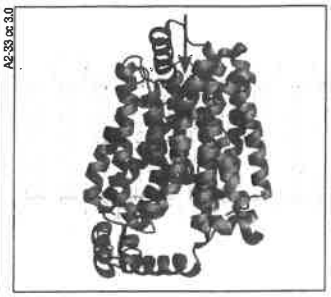


What do proteins in the cell surface membrane really look like?

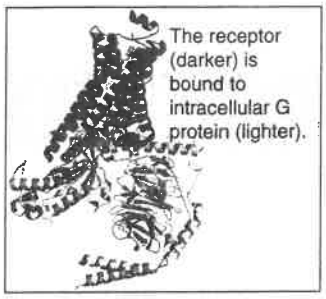
The structure of membrane proteins enables them to perform their particular function in transport, cell signaling, or cell recognition. The proteins are integral to the membrane, and often have parts of their structure projecting from both internal and external sides of the membrane.



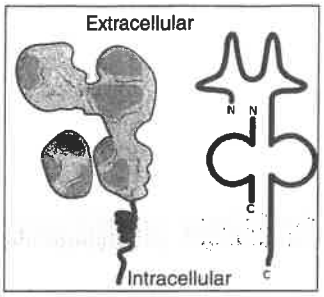
Aquaporins are a special type of channel protein that speed up the passage of water molecules across the membrane. Their tertiary structure creates a pore through the center of the protein through which molecules can pass.



The GLUT1 glucose transporter is a carrier protein that facilitates the transport of glucose across the cell surface membranes of mammalian cells. It enables glucose to be transported into cells at a rate high to supply the cell's energy needs (50,000x >0).



G-protein coupled receptors are proteins involved in signaling pathways. A signal molecule binds to the receptor protein outside the cell to trigger a reaction involving intracellular G protein. The receptor in this example binds to adrenaline.



Cell surface antigens provide an identifiable cell signature so that the body can distinguish between its own cells and foreign molecules. They are often glycoproteins. The image above shows how the antigens project from the membrane.

1. What is the purpose of carrier proteins in the membrane? _____

2. What is the purpose of channel proteins in the membrane? _____

3. Identify the molecule(s) that:

   (a) Can diffuse through the plasma membrane on their own: _____

   (b) Can diffuse through the membrane via channel proteins: _____

   (c) Must be transported across the membrane by carrier proteins: _____

4. Describe the role of the following proteins in the plasma membrane:

   (a) Aquaporins: _____

   (b) GLUT1 protein: _____

   (c) G protein: _____

   (d) Cell surface antigens: _____

60 How Do We Know? Membrane Structure

Key Idea: The freeze-fracture technique for preparing and viewing cellular membranes has provided evidence to support the fluid mosaic model of the plasma membrane.

Cellular membranes play many extremely important roles in cells and understanding their structure is central to understanding cellular function. Moreover, understanding the structure and function of membrane proteins is essential to understanding cellular transport processes, and cell recognition and signaling. Cellular membranes are far too small to be seen clearly using light microscopy, and certainly any detail is impossible to resolve. Since early last century, scientists have known that membranes were composed of a lipid bilayer with associated proteins. The original model of membrane structure, proposed by Davson and Danielli, was the unit membrane (a lipid bilayer coated with protein). This model was later modified by Singer and Nicolson after the discovery that the protein molecules were embedded *within* the bilayer rather than coating the outside. But how did they find out just how these molecules were organized?

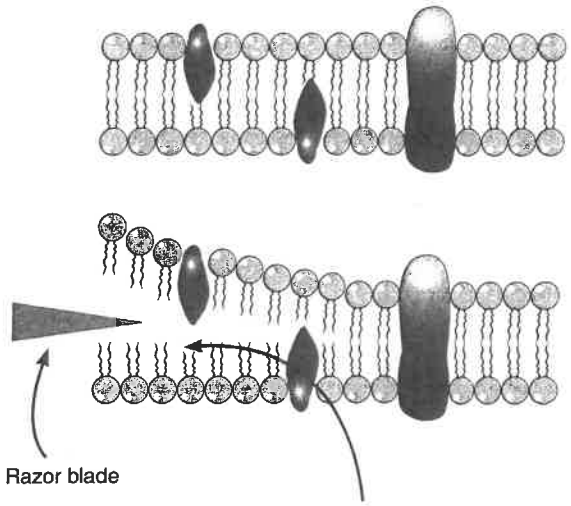
The answers were provided with electron microscopy, and one technique in particular – **freeze fracture**. As the name implies, freeze fracture, at its very simplest level, is the freezing of a cell and then fracturing it so the inner surface of the membrane can be seen using electron microscopy. Membranes are composed of two layers of phospholipids held together by weak intermolecular bonds. These split apart during fracture.

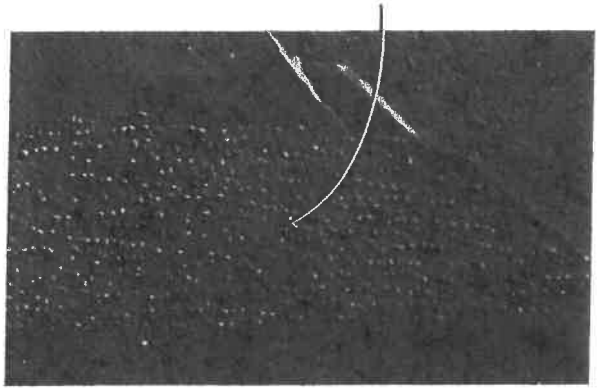
The procedure involves several steps:

- Cells are immersed in chemicals that alter the strength of the internal and external regions of the plasma membrane and immobilize any mobile macromolecules.
- The cells are passed through a series of glycerol solutions of increasing concentration. This protects the cells from bursting when they are frozen.
- The cells are mounted on gold supports and frozen using liquid propane.
- The cells are fractured in a helium-vented vacuum at -150°C. A razor blade cooled to -170° C acts as both a cold trap for water and the fracturing instrument.
- The surface of the fractured cells may be evaporated a little to produce some relief on the surface (known as etching) so that a three-dimensional effect occurs.
- For viewing under an electron microscope (EM), a replica of the cells is made by coating them with gold or platinum to ~3 nm thick. A layer of carbon around 30 nm thick is used to provide contrast and stability for the replica.
- The samples are then raised to room temperature and placed into distilled water or digestive enzymes, which separates the replica from the sample. The replica is then rinsed in distilled water before it is ready for viewing.

The freeze fracture technique provided the necessary supporting evidence for the current fluid mosaic model of membrane structure. When cleaved, proteins in the membrane left impressions that showed they were embedded into the membrane and not a continuous layer on the outside as earlier models proposed.

Cleaving the membrane





50 nm —

Photo: Louisa Howard and Chuck Daghlian, Dartmouth College

1. Explain how freeze-fracture studies provided evidence for our current model of membrane structure: _____

2. The Davson and Danielli model of membrane structure was the unit membrane; a phospholipid bilayer with a protein coat. Explain how the freeze-fracture studies showed this model to be flawed:

63 Factors Affecting Membrane Permeability

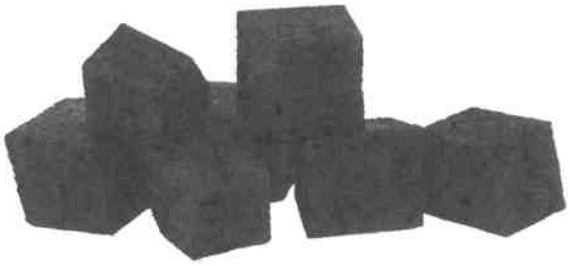
Key Idea: Temperature and solvents can disrupt the structure of cellular membranes and alter their permeability.

Membrane permeability can be disrupted if membranes are subjected to high temperatures or solvents. At temperatures above the optimum, the membrane proteins become denatured. Alcohols, e.g. ethanol, can also denature proteins. In both instances, the denatured proteins no longer function properly and the membrane loses its selective permeability and becomes leaky. In addition, the combination of alcohol and high temperature can also dissolve lipids.

The aim and hypothesis

To investigate the effect of ethanol concentration on membrane permeability. The students hypothesized that the amount of pigment leaking from the beetroot cubes would increase with increasing ethanol concentration.

Beetroot cubes



Background

Plant cells often contain a large central vacuole surrounded by a membrane called a **tonoplast**. In beetroot plants, the vacuole contains a water-soluble red pigment called betacyanin, which gives beetroot its color. If the tonoplast is damaged, the red pigment leaks out into the surrounding environment. The amount of leaked pigment relates to the amount of damage to the tonoplast.

Method for determining effect of ethanol concentration on membrane permeability

Raw beetroot was cut into uniform cubes using a cork borer with a 4 mm internal diameter. The cubes were trimmed to 20 mm lengths and placed in a beaker of distilled water for 30 minutes. The following ethanol concentrations were prepared using serial dilution: 0, 6.25, 12.5, 25, 50, and 100%.

Eighteen clean test tubes were divided into six groups of three and labeled with one of the six ethanol concentrations. Three cm^3 of the appropriate ethanol solution was placed into each test tube. A beetroot cube (dried by blotting) was added to each test tube. The test tubes were covered with parafilm (plastic paraffin film with a paper backing) and left at room temperature. After one hour the beetroot cubes were removed and the absorbance measured at 477 nm. Results are tabulated, below.

Absorbance of beetroot samples at varying ethanol concentrations				
Ethanol concentration (%)	**Absorbance at 477 nm**			**Mean**
	Sample 1	**Sample 2**	**Sample 3**	
0	0.014	0.038	0.038	
6.25	0.009	0.015	0.023	
12.5	0.010	0.041	0.018	
25	0.067	0.064	0.116	
50	0.945	1.100	0.731	
100	1.269	1.376	0.907	

1. Why is it important to wash the beetroot cubes in distilled water prior to carrying out the experiment? _____

2. Complete the table above by calculating the mean absorbance for each ethanol concentration:

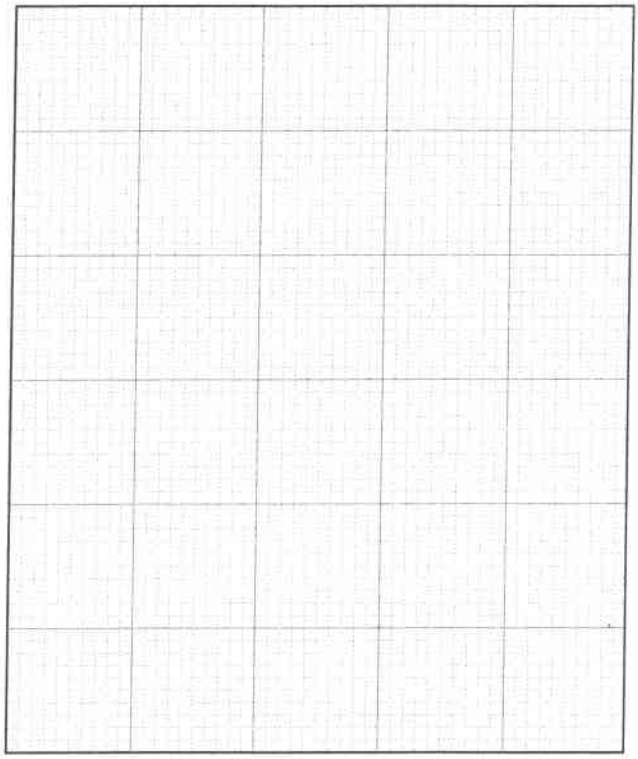
3. What is absorbance measuring and why is it increasing with increasing ethanol concentration? _____

4. What was the purpose of the 0% ethanol solution in the experiment described opposite? _____

5. (a) Why do you think the tubes were covered in parafilm?

(b) How could the results have been affected if the test tubes were not covered with parafilm?

6. (a) Plot a line graph of ethanol concentration against mean absorbance on the grid (right):



(b) Describe the effect of ethanol concentration on the membrane permeability of beetroot:

7. Some students wanted to find out how temperature affected membrane permeability. They prepared the beetroot cubes the same way as in the previous experiment.

(a) Write a hypothesis for their experiment: _____

(b) List an appropriate range of temperatures for the experiment: _____

(c) Write the method for the experiment: _____

(d) Make a prediction about the results based on your understanding of cellular processes: _____