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TIMELINE

Lipids on the frontier: a century of cell-membrane bilayers

Michael Edidin

Our present picture of cell membranes as lipid bilayers is the legacy of a century's study that concentrated on the lipids and proteins of cell-surface membranes. Recent work is changing the picture and is turning the snapshot into a video.

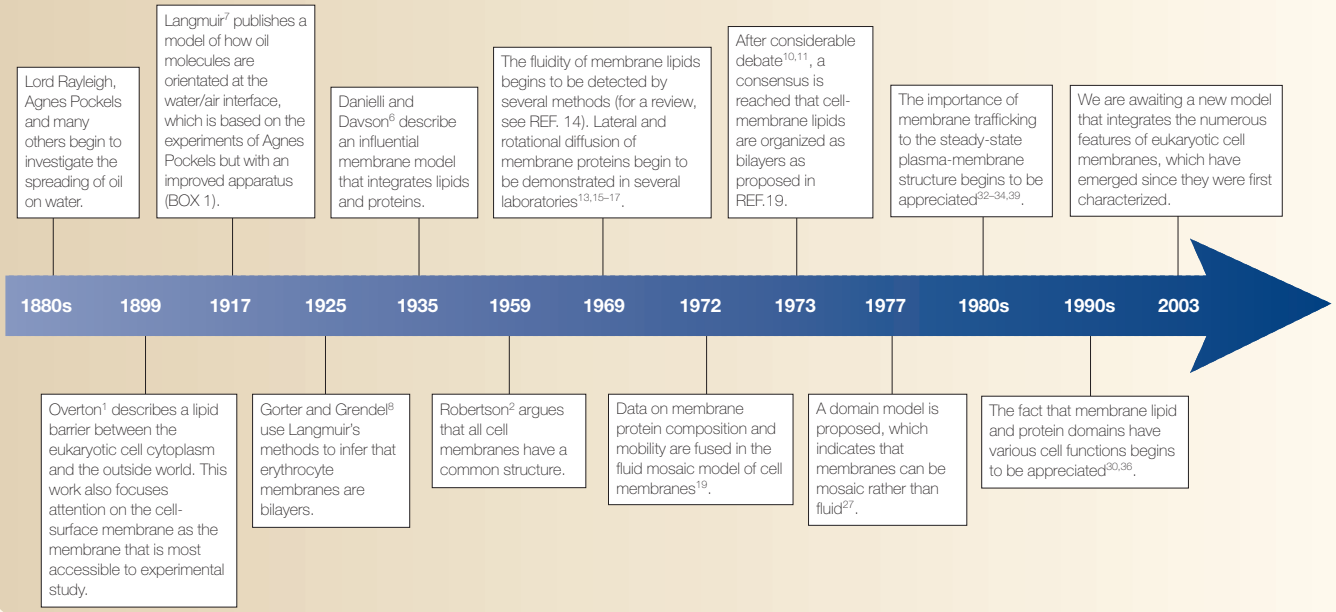
All of the membranes of eukaryotic cells separate functional compartments, but the cell-surface membrane — the plasma membrane — is an extreme. It is the frontier between the cell and its environment. Exploration of this frontier has revealed its physical and functional properties. The plasma membrane is a lipid bilayer, the composition of which regulates frontier crossings by molecules between a cell's surroundings and its interior, and the properties of the bilayer are different from those of any of its components alone.

Explorers of the cell frontier draw their resources from the physical chemistry of pure lipid ensembles, that is, model membranes made *in vitro* from just one or two kinds of lipid. The data from these simplified membranes allow the exploration of more complicated cell membranes that are rich in proteins and that contain a bewildering array of lipids. The approach of physical chemistry provides information on how lipids associate with one another and on their dynamic interplay. However, it is hard to capture the dynamic interplay between the components of cell membranes. We have information on the interactions of membrane lipids with one another and with membrane proteins, but, until recently, it has not been easy to apply this information to the membranes of living cells. Often, spatial resolution has been sacrificed for the sake of temporal resolution and vice versa. However, in recent years, new techniques have allowed us to visualize cell-membrane structure and dynamics on scales that match those of studies of model membranes. The next step to take is one towards a new integrated model of membrane structure and dynamics, that is, towards a model that spans many timescales and spatial scales. Here, I look back and discuss the way in which the lipid-bilayer model developed over the past one-hundred years (TIMELINE). Then, I look forward and suggest some elements for a dynamic model of the plasma membrane.

Membrane history: cells and models

Cell boundaries and cell permeability. To use the style of Rudyard Kipling, "In the high and far off times cells, O best beloved, had no plasma membranes". They had only an 'end layer' — an outer layer of protoplasm of unknown composition and properties,

Timeline | **A century of cell-membrane bilayers**

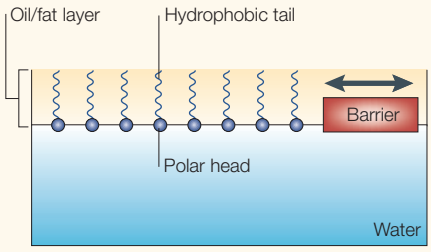


which was often described in nineteenth-century literature as a precipitate[1]. This end layer was explored by physiologists, chemists and morphologists (for a review, see REF. 2). Physiologists characterized the cell surface in terms of its functions; they measured the ease or difficulty with which migrant molecules and ions crossed the frontier. These physiological measurements showed that fat-soluble molecules generally crossed the frontier more easily than water-soluble molecules and ions. The cell-surface barrier was therefore inferred to be a lipid of some sort — in the words of the pioneering study, a "fatty oil" — rich in cholesterol and phospholipids[1]. Later, physiological and biophysical experiments developed this initial model into a combined chemical and morphological model that was a layer, just a few lipids thick, which was coated with proteins. In the 1920s and 1930s, measurements of cell-membrane capacitance by Fricke[3] indicated that the plasma membrane was only 4-nm thick, and measurements of the surface tension of many kinds of cells by Harvey (see REF. 4 for his 1935 paper with Danielli, which includes earlier results) and Cole[5] indicated that the surface was covered with proteins rather than being naked lipid. The model was elaborated in a 1935 review by Danielli and Davson[6].

Lipid monolayers and membrane structure. Membrane chemistry and physics as we know them today began with observations of the spreading of oils and fats on water — observations that go back to Babylon in the eighteenth century BC (BOX 1). In 1917, Irving Langmuir improved Agnes Pockels' method for measuring the pressure that is exerted by molecular films as they spread on water. In a splendid paper, he showed that lipids that spread in this way form a monomolecular layer on the surface of water. Simple arithmetic gave the area per lipid molecule and also showed that the hydrocarbon chains of the lipids were flexible; they did not extend straight out from the surface of the water, but were bent[7].

This work paved the way for the resolution of the bilayer structure of the plasma membrane. The first step in this resolution came when Langmuir's methods for measuring the area per lipid molecule were applied to lipid extracts of erythrocyte membranes by Gorter and Grendel in 1925 (REF. 8). Using

Box 1 | **Oil spreading on water, Ms Agnes Pockels and the Langmuir trough**



A Langmuir trough is a simple device for controlling the spreading of an oil or fat on a water surface (see figure). The molecules in the film become orientated so that their hydrophobic tails are in the air and their polar heads are in the water. A key part of this device is a method for moving the barrier to cause a defined lateral pressure against the oil layer. This was Langmuir's great improvement on Ms Agnes Pockels' apparatus (see below).

Oil films on water have been used and characterized in many different ways:

- **Eighteenth century BC: Babylonians spread oil for divination.**
- **1770: Benjamin Franklin experimented with the damping of surface waves by spreading olive oil on the surface of an English pond.**
- **Late nineteenth century: Lord Rayleigh worked on surface tension and received a letter from Agnes Pockels who developed the Langmuir trough in her family's kitchen. You can read more about Ms Pockels at the Contributions of 20th century women to physics web site (see Online links).**
- **Early twentieth century: Langmuir[7] provided detailed explanations of the thickness of oil layers and the orientation of molecules. He developed the Langmuir film balance to measure surface tension.**

The diagram and information in this box were provided courtesy of M. Dennin, Department of Physics and Astronomy, University of California, Irvine, USA.

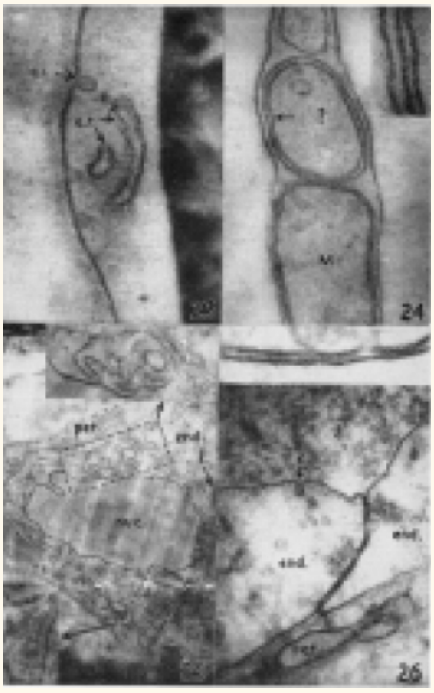


Figure 1 | **The unit membrane concept.** This figure reproduces work that was published in a paper by Robertson[2], in which he both summarized the available data and used many new examples to make the point that all cell membranes have a common structure. In electron micrographs of osmium-fixed cells, this common structure appears as the well-known trio of two dark lines separated by a clear region. Although this structure is hard to resolve in the images shown, it can be seen by close examination of the originals. Reproduced with permission from REF. 2 © the Biochemical Society (1959).

'Langmuir's trough' (BOX 1), they measured the area occupied by lipids that were extracted from a known number of erythrocytes. Then, they measured the surface area of whole erythrocytes and calculated that the lipids of a single erythrocyte could be accommodated by a lipid bilayer. After summarizing their measurements and calculations for the erythrocytes — or, as they referred to them, chromocytes — of six different mammalian species, they concluded that, "It is clear that all our results fit in well with the supposition that the chromocytes are covered by a layer of fatty substances that is two molecules thick". Although Gorter and Grendel made several experimental mistakes[9], the errors cancelled one another out and the authors reached the correct conclusion. So, the lipid-bilayer membrane was born.

The 1950s to 1980s: fluid membranes. Optical imaging of membrane morphology had to wait for the advent of electron microscopy and the resolution that it can obtain. However, once a structure that corresponded to a bilayer had been imaged, it became clear that it was not only the plasma membrane that had this 75-Å-thick structure and, by 1959, it was being argued by Robertson that all cell-organelle membranes had a common structure[2] (FIG. 1). Even ten years later, though, the bilayer was not accepted as the basic structure of cell membranes, and an important review by Stoeckenius and Engelman[10] was devoted to weighing up the evidence for the bilayer structure against the possibility that cell membranes were made of discrete, globular subunits. An even later review offered various models for protein insertion into the bilayer[11]. However, within a few more years, the reinterpretation of older work on the X-ray diffraction patterns of membranes[12] and the accumulation of new evidence on the physical state of membrane lipids[13] consolidated the bilayer model for membranes. Rapidly evolving magnetic resonance methods — NMR and electron spin resonance — showed that bilayer lipids were in motion over numerous scales of time and distance, flexing and diffusing in the plane of the membrane. In short, the bilayer was more like a fluid than a solid. This work, which was mainly from the laboratories of McConnell and Chapman, is summarized in a contemporary review[14]. The review also mentions the possibility that bilayer lipids are asymmetrically distributed — that is, that the two membrane leaflets have a different lipid composition and fluidity — which was first shown to be the case for erythrocyte membranes, and was predicted to be the case for all membrane bilayers, by Bretscher[11].

Solutes diffuse in a fluid and, in the early 1970s, Cone and Poo and Frye and I showed that some proteins can readily diffuse in lipid bilayers[15–17]. The diffusion coefficients indicated that there was an average viscosity for the bilayer that was 100-times greater than the viscosity of water. The commonplace view now is that the average bilayer lipid viscosity is similar to that of olive oil — a more 'exotic' standard is the viscosity of crocodile fat on a warm summer's day.

"The commonplace view now is that the average bilayer lipid viscosity is similar to that of olive oil — a more 'exotic' standard is the viscosity of crocodile fat on a warm summer's day."

It proved harder to characterize the properties of membrane proteins than those of membrane lipids. Many of the difficulties were encountered because membrane proteins are poorly soluble in water. Studies of erythrocyte membrane proteins[11,18] and surveys of proteins that were extracted from various other membranes led Singer and Nicolson[19] to make a crucial distinction between integral and peripheral membrane proteins in 1972. This took us to the model that is still the way most of us see membranes — the fluid mosaic model (FIG. 2). The mosaic is made of proteins that are inserted into the fluid, which is the lipid bilayer. The model is more of a cartoon than a predictive model, but it successfully managed to capture and integrate diverse experiments on membrane physics and chemistry.

The history of the lipid-bilayer membrane cannot be discussed without commenting on the forces that hold the bilayer together. The main force that shapes a bilayer from a mixture of amphipathic lipids is the hydrophobic force[20], that is, lipids form bilayers to minimize their contact with water. This principle also applies to the insertion of membrane proteins into the bilayer — the proteins are usually arranged so that their hydrophobic surfaces are buried in the lipid. These amino-acid sequences are often flanked by charged or other polar residues that interact with the watery environment of the bilayer surface[21,22].

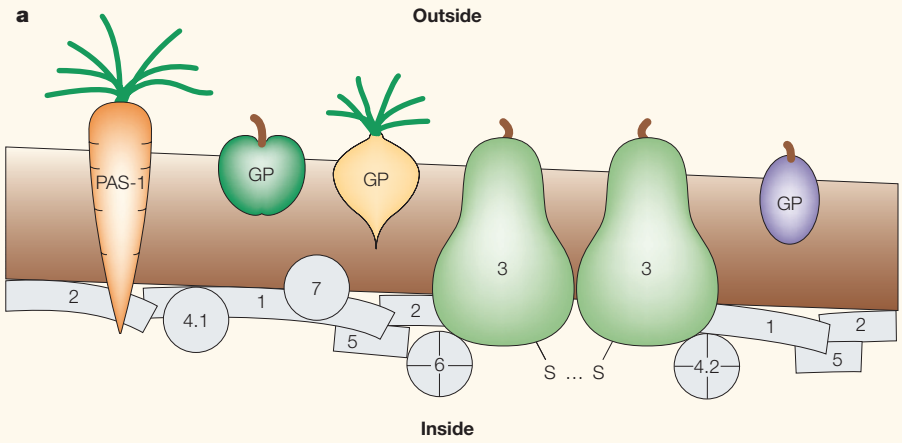
In the bilayer membrane model of the 1980s, cell membranes were based on a largely fluid lipid bilayer in which proteins were embedded. The bilayer was highly dynamic; lipids[23] and proteins[24] could flex, rotate and diffuse laterally in a two-dimensional fluid. The fluid was isotropic, that is, the diffusion of the proteins and lipids was random unless it was constrained by the cytoskeleton or by the high concentration of membrane proteins. The lipids immediately surrounding a membrane protein could affect the function of the protein, which might be one explanation for the large number of lipid species (some 500–1,000 different kinds of lipids) that are present in a single membrane. There were numerous ideas about the coupling of reactions by diffusion[25,26], but often the diffusion measurements were made on a μm scale, when the relevant reactions occurred on a scale of 10s of Å. The 1980s model captures the complexity of the fluid bilayer and the possibilities for molecular interactions in it by diffusion and collision. Although there had been a brief

interest in detecting lipid-phase transitions in cell membranes, by 1980 the model largely neglected the possibility that lipids might not be randomly distributed in the bilayer and also understated the degree of local order that might be possible in membranes.

The 1990s: membrane domains. As the fluid mosaic picture was being assimilated by cell biologists, another picture was being sketched in which membranes contained patches of lipids, the composition and physical state of which differed from the average for the bilayer. This sketch by Jain and White started with model membranes[27], and was followed by a lot of work on the formation of lipid patches in model membranes. The lipids were said to form 'domains', which implies that the patches are not at equilibrium and so are not as stable or as long-lived as separated phases, which are at equilibrium. At first, these experiments used mixtures of gel and fluid, such as that shown in FIG. 3, but they evolved to use systems of immiscible fluid lipids, which are appropriate models for biological membranes. Some measurements on whole cells and intact membranes also detected lipid domains (for examples, see REFS 28,29), although some cell-membrane domains seemed to be larger than those of the model membranes. However, this difference might be a result of the resolution limits that affect studies of cell membranes versus model membranes[30,31].

Lipid domains were proposed to solve the problem of sorting and trafficking lipids and lipid-anchored proteins in polarized epithelial cells[32]. These molecules are differentially presented on the apical surface of morphologically polarized cells, which indicates that the cytoplasmic cell-sorting machinery can recognize them, even though they are on the inner surface of trafficking vesicles[33]. The 'lipid-raft' model proposed that lipids that are to be sorted segregate into a raft, which is rich in cholesterol and sphingolipids. The entire raft is then recognized for trafficking either because it also contains transmembrane proteins or because the state of the raft lipids is somehow detected by cytoplasmic proteins.

In 1992, the first, careful test of the raft hypothesis by Brown and Rose showed that a lipid-anchored protein could indeed enter a cholesterol- and sphingolipid-rich lipid domain, which could be isolated in cold detergent[34]. Later work, which was often less careful (see the comments in a recent review[35]), found that many other molecules, such as signalling kinases, could be isolated in this detergent-insoluble complex and attention therefore shifted from lipid rafts as trafficking units to lipid rafts as signalling platforms[36]. In my opinion, great confusion has arisen from the idea that rafts represent relatively large and stable lipid domains that are 10s or 100s of nm in diameter. A loose analogy for this membrane bilayer picture would be thousands of small blocks of butter floating in a sea of heavy cream. A more realistic picture, however, might be a mixture of heavy and light cream that is on the verge of blending into a single fluid, but that is refreshed by new deliveries of one type of cream or the other.



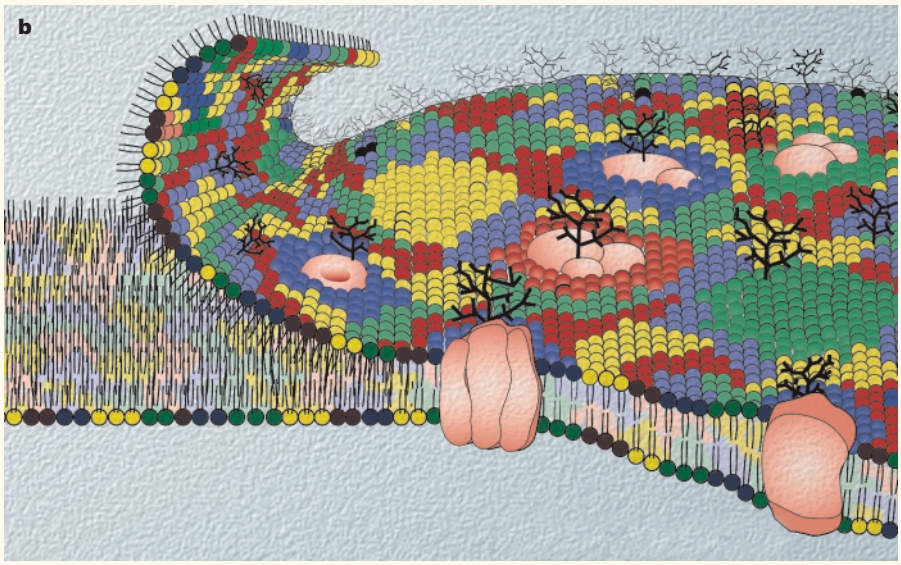


Figure 2 | **The fluid mosaic membrane of Singer and Nicholson.** In contrast to the Danielli and Davson membrane model[6], which used membrane function to indicate membrane structure, the fluid mosaic model[19] began with membrane chemistry and proposed function. **a** | This figure is modified from one in a review on erythrocyte proteins by Steck[41]. It is interesting to see that the lipid bilayer is shown only as two parallel lines and to see the distinction between integral and peripheral proteins. The integral proteins, which are represented by fruits and vegetables, are inserted into the bilayer. The proteins of the membrane skeleton, which are drawn as boxes and are numbered, have been applied to the inner surface of the membrane. Modified with permission from REF. 41 © the Rockefeller University Press (1974). **b** | This is a more exuberant version of the fluid mosaic model, which shows the lipids in more detail. Different lipid species are shown in different colours. This figure was created by P. Kinnunen (University of Helsinki, Finland) and was kindly provided by Kibron Inc., Helsinki, Finland.

Modern times

So, what is missing from our picture of the cell frontier and why does it matter? The first missing element is dynamics. We've noted that lipids 'dance to many tempos'; the problem is therefore to keep track of all the dancers and to see how they change their dance from one tempo to another (for example, from disordered and closely apposed to diffusing among other lipids, and then to diffusion in and out of a lipid domain that persists for a few seconds). Single-particle tracking methods offer a way to visualize

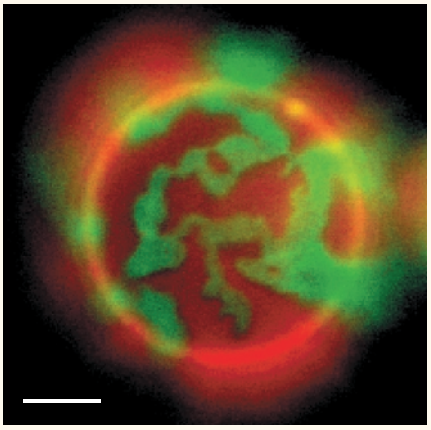


Figure 3 | **Membrane domains.** The image shows domains of gel/fluid lipid segregation in a model membrane vesicle, which is a mixture of fluid dilaurylphosphatidylcholine phospholipids with short, disordered chains and gel dipalmitoylphosphatidylcholine phospholipids with long, ordered chains. A red fluorescent lipid analogue (DiIC18) partitions into the more ordered lipids, whereas a green fluorescent lipid analogue (BODIY PC) partitions into domains of more fluid lipids. Further details of this system can be found in REF. 42. These domains in a model membrane are much larger than the domains of cell membranes. Notice the irregularity of the domain boundaries, and the fact that there is heterogeneity of fluorescence in a single domain. The scale bar represents 5 μm. This image was kindly provided by J. Heetderks and P. S. Weiss (Departments of Chemistry and Physics, The Pennsylvania State University, State College, Pennsylvania, USA).

many scales of lateral motion and confinement in a sequence of images[37].

The second missing element is traffic to and from the frontier. Over 20 years ago, Steinman showed that, in the course of one hour, all of the plasma membrane of some cells is turned over by endocytosis and exocytosis[38]. This traffic creates membrane patches that can look like stable domains, but that, in fact, disperse in 10s of seconds[39]. It can also disrupt membrane-resident domains. Although there is a great deal of study of membrane traffic, there has been little work on the way in which the constant membrane turnover randomizes membrane molecules; if there are no restraining factors then perhaps the bilayer is an isotropic fluid in which the molecules are randomly distributed. Total internal reflection microscopy offers a way to investigate this possibility[40].

A third missing element is the association of the cytoskeleton with the bilayer. There is a large amount of literature on this topic, but it has not been integrated into a new membrane model. I think that a new plasma-membrane model will be the morphologists' model after all — a 'greater membrane' that takes into account not only the bilayer and its embedded proteins, but also the asymmetry of the lipid distribution between the two leaflets of the plasma-membrane bilayer and the way that this asymmetry is used to connect the frontier membrane to the rest of the cell. When we have this model, we can move from the plasma-membrane frontier to a new frontier — the membranes of eukaryotic cell organelles — and I predict pleasant surprises there.

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Online links

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http://www.physics.ucla.edu/~cwp/Phase2/Pockels,_Agnes@871234567.html
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