Do We Need a New Biomembrane Model?

OLE G. MOURITSEN *and* OLAF S. ANDERSEN

Department of Chemistry
Technical University of Denmark
DK-2800 Lyngby, Denmark
e-mail: ogm@kemi.dtu.dk

Department of Physiology and Biophysics
Cornell University Medical College
New York, NY 10021, USA
e-mail: sparre@mail.med.cornell.edu

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For 25 years the Singer-Nicolson fluid mosaic model of biological membranes (Singer and Nicolson, 1972) has been a central paradigm in membrane science. The simple, yet powerful conceptual framework it provided continues to have an enormous impact on the field of biomembranes. As a key property, the Singer-Nicolson model assigned to the membrane's lipid bilayer component a certain degree of "fluidity". The fluidity concept was meant to characterize the lipid bilayer as a kind of pseudo-two-dimensional liquid in which both lipids and membrane-associated proteins display sufficient lateral mobility to allow for function. The overall random appearance of this lipid-protein fluid composite made the membrane look like a mosaic. Except in cases where sterols or unsaturated lipid acyl chains might alter the bilayer "fluidity", the conspicuous diversity in the chemical structures of lipids, which is actively maintained by cells, had little significance in the model. This lipid diversity, together with the varying (but characteristic) lipid composition of different types of cells and organelles, have become an increasing puzzle, which was exacerbated by the enhanced understanding of the variation in physical properties among different lipids and lipid assemblies (Gennis, 1989; Kinnunen, 1991; Bloom et al., 1991).

When Singer and Nicolson proposed the fluid-mosaic model in 1972, membrane modeling already had come a long way, see Fig. 1. The first important step was taken by Gorter and Grendel (1925), who showed experimentally that the membrane is very thin, being a bimolecular layer (Fig. 1a). The association of membrane proteins with the lipid bilayer was introduced in the Danielli-Davson model (1935), as a spread on the lipid polar head groups at the two sides of the lipid bilayer (Fig. 1b). A related version of membrane organization appears in Robertson's unit membrane model (1966) in which the proteins are pictured as stratified layers sandwiching the lipid bilayer (Fig. 1c). In the Singer-Nicolson fluid-mosaic model (1972) shown in Fig 1d, the proteins are grouped in two classes: integral membrane proteins, which traverse the bilayer and primarily interact with the bilayer through hydrophobic forces; and peripheral membrane proteins, that are peripherally associated with the lipid bilayer and primarily interact with the bilayer through polar (electrostatic and hydrogen bond) interactions. In either case, the proteins "float" in a fluid sea. Refinements of the fluid mosaic model

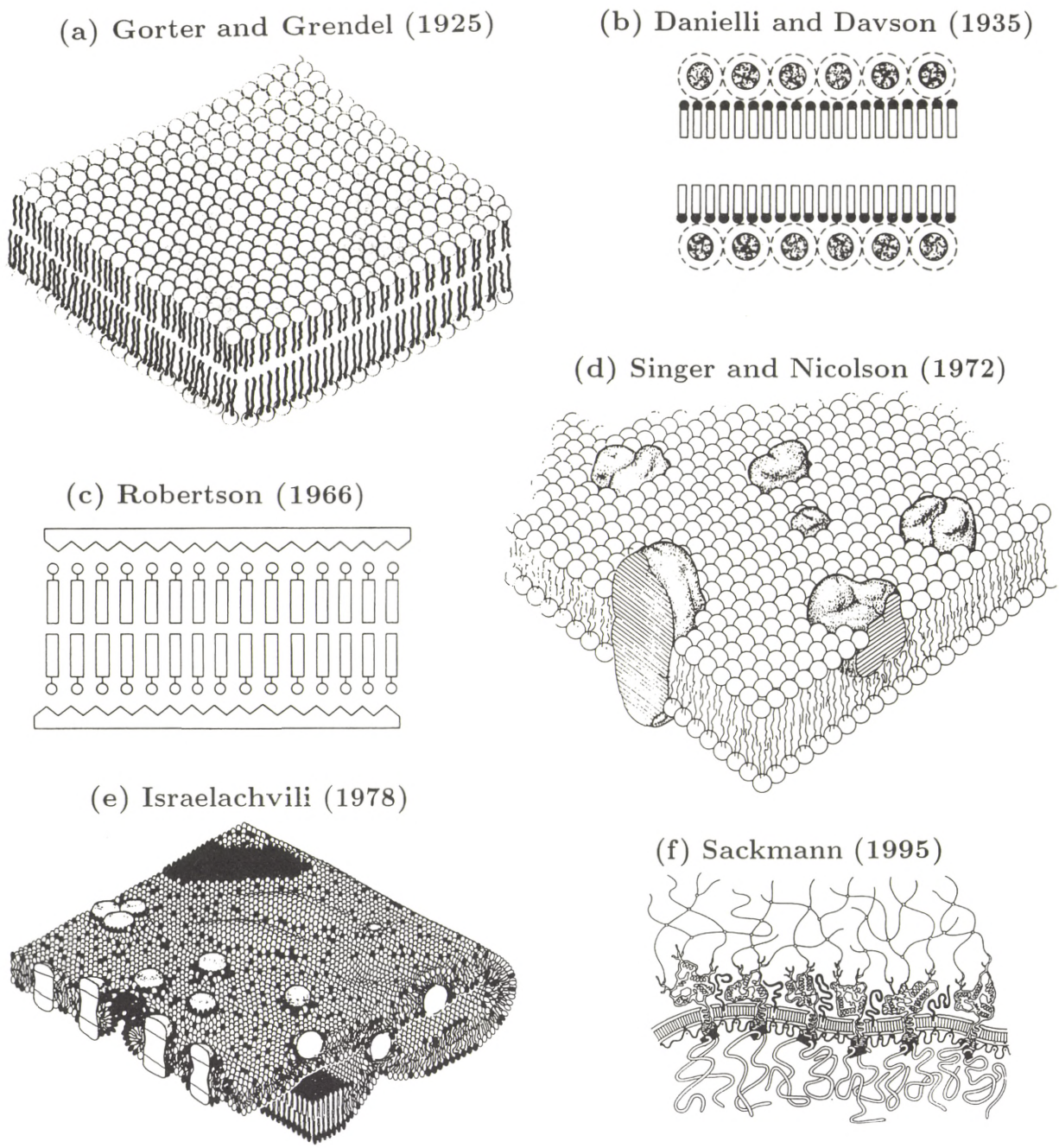

Figure 1. Historic picture gallery of membrane modeling.

have been suggested from time to time, usually inspired by new insights obtained by focusing on some specific, or specialized, membrane feature. One example is the model by Israelachvili (1977; 1978), who refined the Singer-Nicolson model to account for the need for membrane proteins and lipids to adjust to each other, and also incorporated membrane folding, pore formation, and thickness variations as well some degree of heterogeneity (Fig. 1e). Another elaboration of the Singer-Nicolson model, which emphasized the importance of the cytoskeleton and glycocalyx, was developed by Sackmann (Fig. 1f).

The notion of membrane fluidity, which was embodied in the Singer-Nicolson fluid-mosaic model (Fig. 1d), was important because it served to emphasize that membranes are dynamic structures. Unfortunately, many subsequent investigators assumed, explicitly or implicitly, that fluidity implies randomness. This assumption neglects that fluids may be structured on length scales between the molecular scale and scales that are accessible by the microscopic, spectroscopic, or scattering methods commonly used to study the lateral organization of membranes. Also, structuring in time, in particular the correlated dynamical phenomena characteristic of liquid crystals, were not appreciated as being important for membrane function. Importantly, however, such lively dynamics is perhaps the most conspicuous feature of a fluid membrane. In fact, the many-body nature inherent in the molecular assembly of a membrane insures that local order and structure develop naturally from an initially disordered fluid. Finally, the fluid-mosaic model in Fig. 1d pictured the membrane as a flat, pseudo-two-dimensional layer. This may be an artistic simplification; but it nevertheless de-emphasizes the transverse dynamical modes of individual lipid molecules as well as the existence of large-scale excursions into the third dimension with the ensuing curvature-stress fields, instabilities toward non-lamellar symmetries, and coupling between internal membrane structure and molecular organization on the one hand and membrane shape and shape transformations on the other (Lipowsky and Sackmann, 1995).

It is now recognized that the randomness implied in the fluid-mosaic membrane model does not exist. This recognition builds on a wealth of experimental results, which show that the lateral distribution of molecular components in membranes is heterogeneous, both statically and dynamically - corresponding to an organization into compositionally distinct domains and compartments. In addition to immobilization and domain formation due to interactions between the cytoskeleton or the extracellular matrix and the membrane, several physical principles generate dynamic lateral heterogeneity of both lipids and proteins in fluid (liquid-crystalline) membranes. This non-random organization imposed by the fluid membrane means that biomembrane functions do not need to depend on random collisions and interactions among reactants, but may be steered in a well-defined manner that allows for a considerable mobility of the individual constituents. This dynamic organization of the membrane makes it sensitive to perturbations by both physical (e.g. temperature and pressure) as well as chemical (e.g. drugs and metabolites) factors, which thus provides an exceptional vehicle for biological triggering and signaling processes. Specifically, local changes in organization, brought about by physiological perturbants, are likely to evolve and result in relevant changes in the function of the entire system.

The principles underlying these novel features of membrane organization are well known to scientists familiar with the physical properties of membranes, but they are only beginning to be appreciated by scientists more familiar with the function of biological membranes. Similarly, physical scientists often are unfamiliar with the functional properties of biological membranes. The aim of the present volume is to discuss and focus attention on these issues in order to bridge the gap between the different physical and biological approaches to biomembranes and their functions. To the extent this is accomplished, it will have implications for the future progress in biomembrane research.

In retrospect, one may ask why a more refined model, which incorporated the dynamic order and fluctuations of the biological membrane,

was not formulated long ago. Paradoxically, this may be due to the tremendous success of modern structural molecular biology and its focus on structure-function relationships that involve well-defined static (crystal) structures of proteins, nucleic acids, and lipids. From a static structure point of view, once the stable lipid bilayer has been established, the assembly of the lipid molecules in the membrane may appear featureless - like that of a passive solvent. The physical properties of a fluid membrane are not coded for directly by the genes, which together with the legitimate preoccupation with static structures, may have hampered the acceptance of membranes as dynamic entities in which lipids play active and important roles for structure, function, and regulation.

A key element in the formulation of a model is to find the proper balance between general principles and specific detail - or to balance the sometimes conflicting demands for truth and clarity. On the one hand, too many details will render the model applicable only to specific cases, and the details may obscure the generic underlying principles of organization. On the other hand, a too general model may provide little mechanistic insight, which makes the model less useful for the design of new critical experiments. Moreover, the important elements of a model are likely to depend on which length- and time-scales that are relevant for describing the problem of interest. This becomes a particular difficulty when building membrane models because many membrane properties are controlled by phenomena that take place over a range of scales, which are mutually coupled. Given the current, rather advanced, state of knowledge in the field it this is likely that one will be best served by working with a set of membrane models, chosen according to the particular type of question under consideration - and which time- and length scales that are likely to be relevant. This is not an easy task. Two examples of such models are illustrated in Fig. 2.

Figure 2. Cartoons illustrating different aspects of biomembrane structure and dynamics.

(a): A plasma membrane model that highlights the membrane as a stratified composite involving the central lipid bilayer, which is sandwiched between a rubber-like cytoskeleton, attached to the cytoplasmatic inner surface, and the glycocalyx carbohydrate network on the outer surface. The membrane displays undulations; the lipid bilayer displays lateral heterogeneity, lipid domain formation, and thickness variations - close to the integral proteins. Whereas the lipid molecules in this representation are given with some structural details, the membrane-associated proteins remain fairly featureless. In order to capture many different features in the same illustration, the different membrane components are not drawn to scale. The picture was drawn without consideration of time scales and can best be considered as an instantaneous snapshot. (Illustration by Ove Broo Sørensen, Technical University of Denmark.)

(b): A membrane model that highlights the lipid bilayer component and details of the molecular structure of a membrane protein (bacteriorhodopsin). The picture is drawn to scale and it reflects averaging over fast dynamical modes. A 200×200Å slap of a 50Å thick lipid bilayer is shown. The time scale of view is in the range of 10^{-3} to 10^{-6} seconds. On this scale most molecular processes will appear blurred, but not totally indiscernible. For example, the very rapidly moving chains seen on the edges of the lipid bilayer are indicated by subtle texturing parallel to the chain axis. The scale of the texture is on the order of the lipid chains, but the chains themselves are not seen. The membrane edge shading is based on information obtained from X-ray and neutron scattering. The shading used on the headgroup surfaces suggest the presence of small lipid domains. The lipid bilayer displays large-scale bending fluctuations. The transmembrane proteins are modeled by use of the X-ray coordinates for bacteriorhodopsin. Consistent with the slow time scale characterizing this picture, the protein surfaces have been slightly blurred. (Illustration and text by Bruce Paul Gaber, Laboratory for Molecular Interfacial Interactions, Center for Bio/Molecular Science and Engineering, Naval Research Laboratory, Washington, DC 20375.)

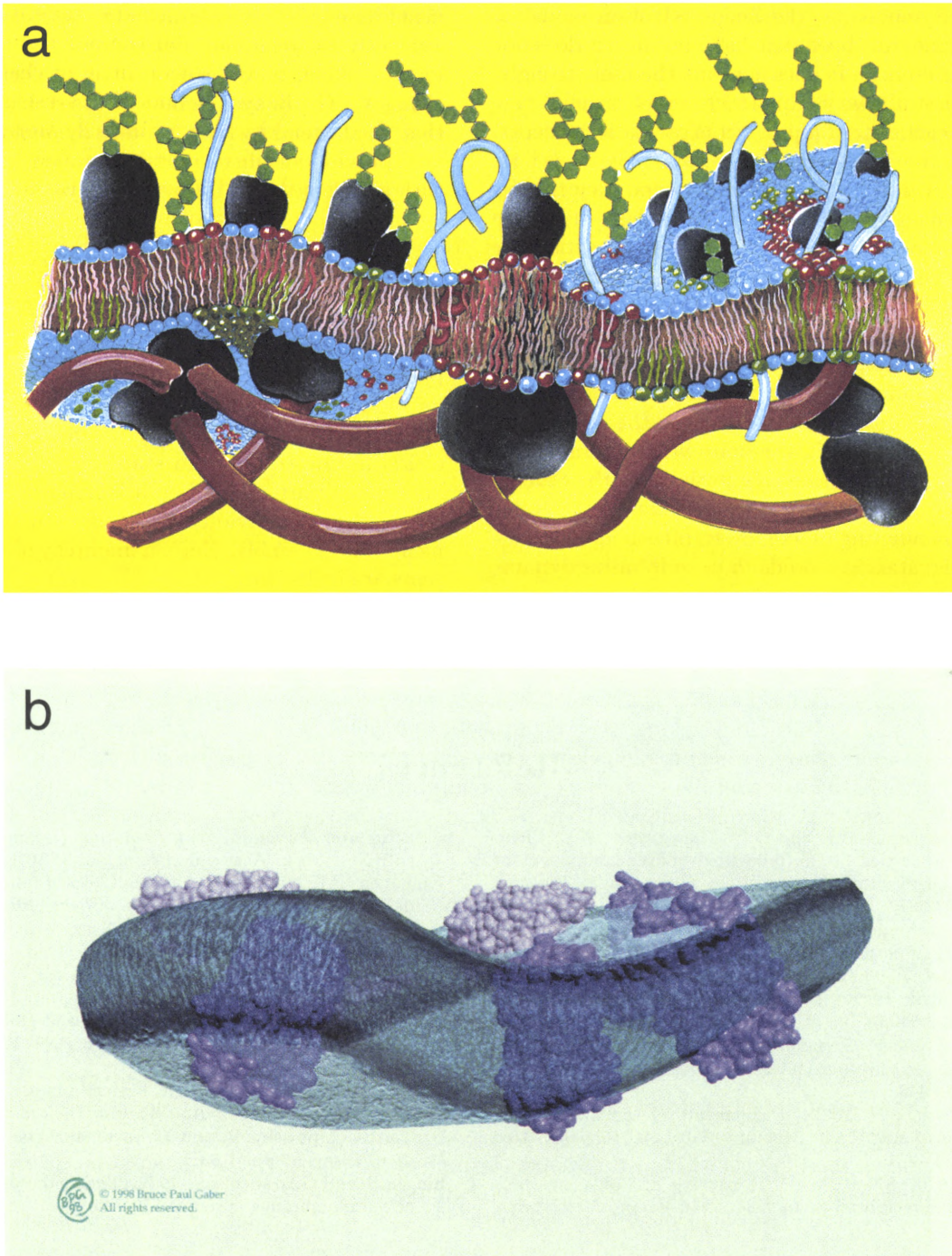

Figure 2.

As was brought out during the final discussions at the Symposium, the Singer-Nicolson model of membranes has been successful because it does not say (too) much. It does not bias the user strongly, and hence allows for broad interpretations of new experimental data and novel theoretical concepts. This is the strength of the model. It is also its weakness, as it in many cases is not very helpful when asking questions about membrane structure and, in particular, about membrane function. For these purposes the model is too generic - in part because it provides too little, or no, insight into membrane protein assembly, lipid bilayer heterogeneity, monolayer or bilayer curvature, and bilayer bending and thickness fluctuations. (One should not forget, however, that the membrane model(s) we use in discussions with our colleagues tend to be even more schematic than the Singer-Nicolson model.)

Moreover, the model, by emphasizing (thermodynamic) stability, tends to de-emphasize dynamics: it does not address the issues relating to conformational transitions in membrane proteins and, just as importantly, the model does not address the conflict between the need for bilayer stability (the membrane must be a permeability barrier and consequently be relatively defect-free) and the need for the bilayer to adapt to protein conformational changes . The bilayer cannot be too stable because that would tend to limit protein dynamics, which may provide insights into the prevalence of lipids with a propensity to form non-bilayer structures.

Finally, a major problem in membrane modeling is how to deal with phenomena that take place far from thermodynamic equilibrium. Non-equilibrium properties are not captured by models like those shown in Fig. 1. Still, non-equilibrium phenomena are the rule, rather than the exception, for biological membranes. The principles of membrane organization, and emergent membrane properties, in non-equilibrium states are basically unknown – in particular when it is important to consider not only individual molecules but the whole membrane assembly. Here, a hierarchy of new concepts are called for.

The answer to the question posed in the title will have to be both a "no" and a "yes".

References

Bloom, M., E. Evans, and O.G. Mouritsen. 1991. Physical properties of the fluid lipid-bilayer component of cell membranes: a perspective. *Quart. Rev. Biophys.* 24:293-397.

Danielli, J.F. and H. Davson. 1935. A contribution to the theory of permeability of thin films. *J. Cellular Comp. Physiol.* 7:393-408.

Gennis, R.B. 1989. *Biomembranes.* London: Springer-Verlag. 530pp.

Gorter, E. and F. Grendel. 1925. On bimolecular layers of lipoids on chromatocytes of blood. *J. Exp. Medicine* 41:439-443.

Israelachvili, J.N. 1977. Refinements of the fluid-mosaic model of membrane structure. *Biochim. Biophys. Acta* 469:221-225.

Israelachvili, J.N. 1978. The packing of lipids and proteins in membranes. In *Light Transducing Membranes: Structure, Function, and Evolution* (Deamer, D.W., ed.) New York: Academic Press. pp. 91-107.

Kinnunen, P.K.J. 1991. On the principles of functional ordering in biological membranes. *Chem. Phys. Lipids* 57:375-399.

Lipowsky, R. and E. Sackmann (eds.) 1995. Structure and dynamics of membranes. In *Handbook of Biological Physics. Vol.1A&B* Amsterdam: Elsevier. 1020pp.

Robertson, J.D. 1966. Granulo-fibrillar and globular substructure in unit membranes. *Ann. NY Acad. Sci.* 137:421-440.

Sackmann, E. 1995. Biological membranes. Architecture and function. In *Handbook of Biological Physics. Vol.1A* (Lipowsky, R. and E. Sackmann, eds.) Amsterdam: Elsevier. pp. 1-63.

Singer, S. and G.L. Nicolson. 1972. The fluid mosaic model of cell membranes. *Science* 172:720-730.