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David E. Green, *Conference Cochairman*

The Conference on Membrane Structure and Its Biological Applications, held in New York City from June 2–4, 1971, under the auspices of The New York Academy of Sciences, marked a turning point in one of the central problems of biology. A distinguished group of participants with an unusually high representation from abroad were brought together for this conference. Rear Admiral Lawrence R. Neville, Associate Executive Director of the Academy, spared no effort to ensure the smooth running of the conference. B. Morgan Boland, Managing Editor of the Annals of the Academy, whipped the manuscripts into shape for final publication with exemplary skill and patience. My secretary, Charlotte Sweet of the University of Wisconsin, shouldered the burden of the vast correspondence and the endless details which a conference of this magnitude entails.

Federal agencies, private foundations, and private companies made contributions to the support of the conference. To these contributors, listed opposite, the chairmen wish to express their profound thanks. In addition, Academic Press, Inc. and the Perkin-Elmer Corporation generously supported a reception-dinner for all the participants.

MOLECULAR ARCHITECTURE OF BIOLOGICAL MEMBRANES *

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My two objectives for this paper are: first, to present a geometrical model for membrane structure,[1–4] and second, to derive some fundamental principles of membrane structure that will have wide applicability. Two particular membrane systems will be used as examples: the retinal rod disk membrane and the cytochrome oxidase membrane. These two were chosen since, although they are both relatively simple, they differ in significant ways and thus illustrate different features of membranes.

Membranes are composed primarily of two major classes of compounds, lipids and proteins. If we are to understand the structure of membranes, we must first understand the structure and properties of these component classes of molecules. In what follows therefore, I will briefly discuss the structure of lipids, then consider the shape and properties of membrane proteins, and finally take up the problem of the arrangement of the lipids and proteins in membranes.

Lipids

A large number of different kinds of lipids are found in membranes, but for structural considerations they may all be treated as a class, since they are all amphipathic or bimodal rodlike molecules; one end of the rod is hydrophilic, and the remainder is hydrophobic. The hydrophobic tails tend to align themselves in such a way as to minimize their contacts with water, while the hydrophilic heads interact favorably with water. Of the few conceivable geometrical patterns by which these energetic requirements can be satisfied, the lipid bilayer is probably the most stable arrangement for the kinds of lipids normally found in membranes, when present in an aqueous environment. Many lines of evidence indicate not only that the membrane lipids are stable by themselves as bilayers, but that the bilayer arrangement is also in fact the predominant state of the lipids in membranes. Some of these evidences will be elaborated upon in the following papers. For the present development of a membrane model, I will accept the bilayer arrangement of the lipid as given, and not attempt at this time to substantiate the point.

Proteins

Whereas a considerable amount of information on the structure and properties of the lipids has been available for many years already, it has only been

* This work was supported by grant GM-12847 from the National Institute of General Medical Sciences, United States Public Health Service, Bethesda, Md.

in the last few years that some knowledge has been gained on the properties of membrane proteins. In fact, the former lack of knowledge about membrane proteins prevented anything more than speculation about their role in membrane structure. Fortunately, however, a few membrane proteins have now been isolated, apparently without having been severely or irreversibly damaged in the process, and from these we can begin to obtain direct information on the shape and properties of membrane proteins, as well as on the role they play in membrane structure. Two of the best characterized membrane proteins are rhodopsin, which is the visual pigmented protein found in the retinal rods of the eye, and cytochrome oxidase, one of the components of the mitochondrial electron transfer chain. Rhodopsin is a monomeric protein with a molecular weight[5–7] of about 28,000, whereas cytochrome oxidase is a highly complex multimeric protein with an aggregate molecular weight[8, 9] of about 200,000 to 250,000, based on the assumption of two heme a molecules per complex.[9, 10] Both of these proteins require lipid for full activity,[8, 11] and it has been demonstrated, at least for cytochrome oxidase, that the isolated protein reforms membranes when it is combined with lipids.[12, 13]

Several lines of evidence all indicate that rhodopsin is a fairly compact, globular protein.[3] These include data from electron microscopy of positively[14] and negatively[15] stained material, x-ray diffraction analysis,[15, 16] and hydrodynamic studies[5] on the isolated molecule. All of these methods yielded the result that rhodopsin has a diameter of 40–46 Å. Since the minimum equivalent sphere diameter of a protein with the molecular weight of rhodopsin is 40.5 Å, the actual shape of the molecule must not deviate greatly from a sphere.

Turning now to cytochrome oxidase, physical and chemical studies again indicate that this multiprotein complex also has a compact, globular structure,[9, 13] but at this point only one strong but indirect argument will be given, which is applicable to other systems as well. This is the simple observation that cytochrome oxidase is a highly specific enzyme. All indications are that enzyme proteins must have a very well-defined three-dimensional structure in order to be functional, and I can see no reason to believe that membrane enzymes should violate this rule.

In summary then, the available evidence on these two well-characterized membrane proteins indicates that they both have compact, globular shapes, and do not exist in extended or fibrous conformations, such as were proposed for membrane proteins in some earlier membrane models.

Lipids and Proteins in Membranes

Having dealt independently with the structures of the lipids and proteins, I will go on to consider the manner in which these components interact in membranes. The question to be answered now is, what is the geometrical relationship of the globular proteins to the lipid bilayer? There are two aspects to this question: the first pertains to the distribution of the protein molecules across the thickness of the membrane, and the second concerns the distribution of proteins and lipids in the plane of the membrane. FIGURE 1 shows the essence of our answer to the question of the distribution of protein across the thickness of the membrane.[2] This figure may be viewed in two ways; either that the lipid fills the spaces between the proteins, or that the proteins penetrate deeply into

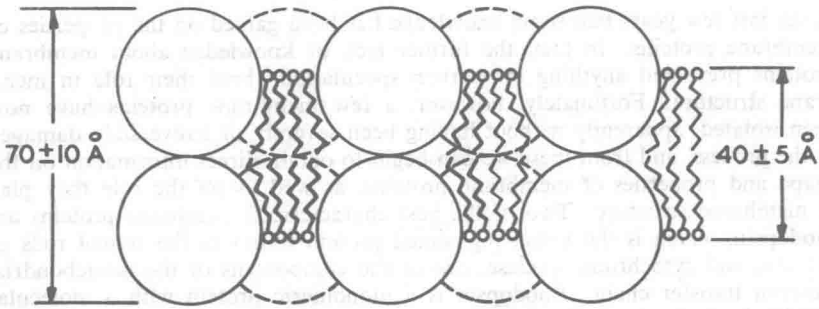

FIGURE 1. The protein crystal model for membranes. The large circles represent proteins; the dashed circles are understood to be proteins behind the plane of the section. The small circles represent the polar lipid heads and the wavy lines the nonpolar lipid tails (From Vanderkooi and Green.[2] Reprinted by permission of the Proceedings of the National Academy of Sciences of the United States of America.)

a lipid bilayer. Chemical, electron microscopic, and x-ray diffraction data may be offered in support of this placement of the proteins. The relevant data of a chemical nature are the observations indicating the importance of hydrophobic forces in the interaction between the lipids and the membrane proteins. Lenaz [17–19] demonstrated this particularly clearly in his studies of the effect of high ionic strength on the reconstitution of lipid-extracted membranes. He found that half-molar salt did not prevent the recombination of lipid with lipid-extracted mitochondrial membranes, although this level of salt is known to prevent the ionic interaction of cytochrome c with acidic lipids. On the basis of experiments such as these, the conclusion has been reached that hydrophobic forces are important in the lipid-protein interactions. Since hydrophobic forces occur between predominately nonpolar parts of the molecules, this must mean that a portion of the protein surface is relatively nonpolar, and that this surface is in contact with the nonpolar tails of the lipid, in the interior of the bilayer.

Electron microscopy also indicates that the proteins make up part of the membrane continuum. A decade ago, Fernández-Morán [14] published a micrograph of thin-sectioned, positively stained retinal rod, which showed a double row of 40-Å particles, presumed to be proteins. The thickness of this membrane, as determined both by x-ray diffraction and by electron microscopy, is 75–80 Å; this means that in order to accommodate a double layer of 40-Å proteins within the measured thickness, the proteins must penetrate roughly to the middle of the membrane.[3] FIGURE 2 illustrates the model developed by myself and Dr. M. Sundaralingam [3] for the retinal rod membrane. The large circles are the proteins, with the cross hatching indicating that the protein surface in the interior of the membrane is relatively nonpolar, whereas the other hemisphere, which is in contact with the aqueous environment, is more highly polar. These proteins evidently have a bimodal or amphipathic nature, in an analogous manner to the amphipathic nature of the lipid molecules. In the case of rhodopsin, this bimodality will keep the protein oriented in the same direction at all times relative to the direction of an incident light beam on the retina of the eye; this orientation may be of importance in a physiological sense.

On the right-hand side of FIGURE 2 is shown a crude calculation of the electron density distribution across the model (the solid line), together with the Fourier synthesis map for the retinal rod published by Blaurock and Wilkins [20] (the dashed line). The point of this diagram is to show that one would expect a membrane of this type to give two high electron density peaks separated by a trough, just as was found for it in the analysis of the diffraction data.

Before proceeding to the next major point, I should digress for a moment to point out that the proteins being discussed here are what we might call intrinsic, rather than extrinsic, membrane proteins. I define the intrinsic proteins as those which make up part of the membrane continuum and are intimately associated with the lipid. In this sense, rhodopsin and cytochrome oxidase are both intrinsic proteins. Extrinsic membrane proteins, on the other hand, are those which are associated with the membrane, but do not make up part of the continuum. Probably the majority of protein species referred to loosely as "membrane proteins" are of the extrinsic type, with only a minority being intrinsic. This distinction is of particular relevance in the case of the erythrocyte ghost membrane, where there is a large amount of membrane-associated protein.

Protein Distribution

A priori, we might conceive of either an ordered or a disordered arrangement of the proteins in the plane of the membrane. In fact, both of these possibilities are found. The retinal rod disk membrane evidently has a dis-

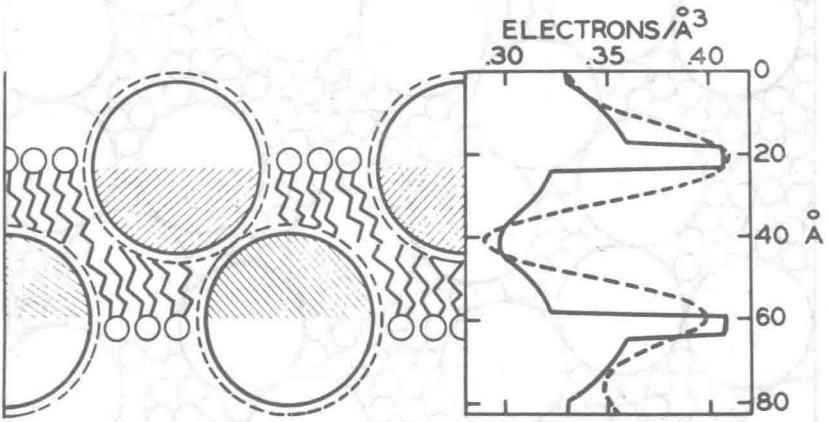

FIGURE 2. Detail of a section of retinal rod disk membrane, and the calculated electron density distribution across the membrane. The large circles in the left-hand part of the figure represent the proteins, the small circles the phospholipid heads, and the wavy lines the nonpolar phospholipid tails. The cross-hatching in the proteins denotes a predominantly nonpolar surface. The solid line in the graph at right is the calculated electron density distribution across this model, and the dashed curve is the Fourier synthesis map for this membrane obtained by Blaurock and Wilkins.[20] See the original article [3] for the details of the calculation of the electron density distribution (From Vanderkooi and Green.[4] Reprinted by permission of BioScience.)

ordered protein arrangement, whereas the cytochrome oxidase membrane can display an ordered arrangement. Blasie and colleagues [15, 16] studied the diffraction of x rays by retinal rod disk membranes, with the x-ray beam oriented perpendicularly to the plane of the membrane. The diffraction pattern so obtained was cylindrically symmetrical; this observation, taken together with their study of the effect of temperature on the diffraction pattern, as well as their use of antirhodopsin serum to identify the diffracting particles, demonstrated fairly conclusively that the rhodopsin molecules are in constant thermal motion in the plane of the membrane. This conclusion was further substantiated by the photodichroism studies of Cone [21] and of Brown [22]; these workers provided evidence indicating that the rhodopsin molecules are free to rotate about an axis perpendicular to the plane of the membrane. FIGURE 3 illustrates our conception of the surface of a disk membrane.[3] The large circles are the proteins, and the small circles are the phospholipids that fill the spaces between the proteins. The proteins were all purposefully drawn as being in contact with other proteins, since attractive protein-protein interactions are assumed to be present and to stabilize the membrane.

The Cytochrome Oxidase Membrane

In contrast to the retinal rod disk membrane, the cytochrome oxidase membrane can form a highly ordered array. This was shown by Oda,[23] using the

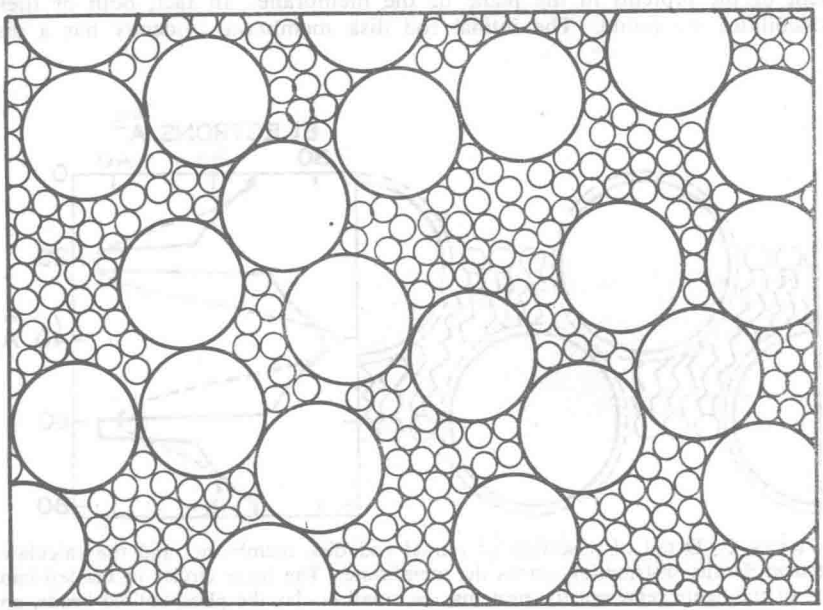

FIGURE 3. Surface view of the retinal rod disk membrane, showing the random arrangement of the proteins and lipids. The large circles represent the proteins and the small circles the lipids (From Vanderkooi and Sundaralingam.[3] Reprinted by permission of the Proceedings of the National Academy of Sciences of the United States of America.)

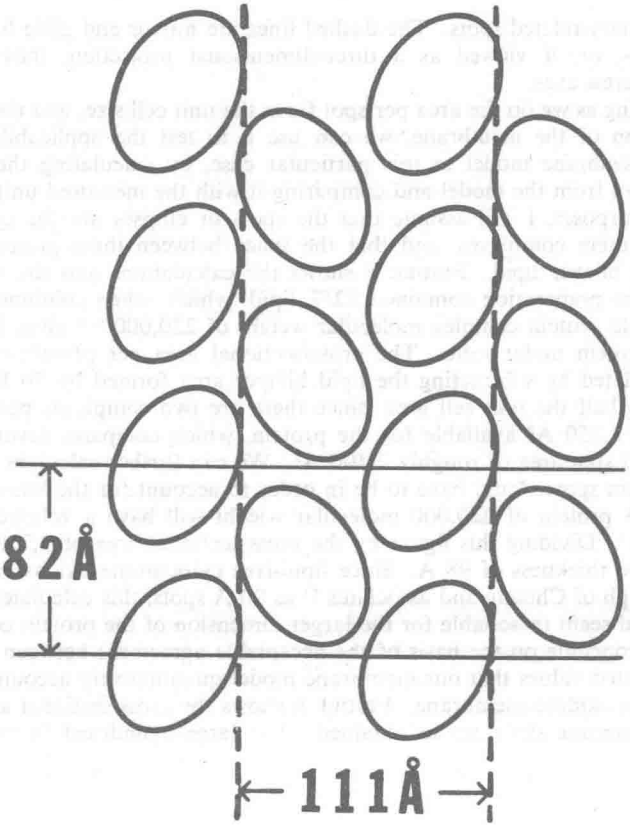

FIGURE 4. A diagram of the lattice structure of the cytochrome oxidase membrane. The ellipses represent the spots of the micrograph, which are interpreted to be proteins.

partially purified "green membranes" obtained from mitochondria (Reference 23, Figures 3–23 & 3–24). Similar results have recently been obtained in our laboratory by Wakabayashi and associates,[24] using purified "membranous cytochrome oxidase" prepared by a slight modification of the procedure given by Sun and colleagues.[8] This material was fixed with glutaraldehyde and stained with phosphotungstic acid. The resulting micrographs displayed a very high order of regularity extending over the entire surfaces of the vesicles, making possible the careful measurement of repeat distances. The spots were arranged in a herringbone pattern, just as was found by Oda [23] for the "green membrane." Analysis and measurement of the pattern on the micrographs of "membranous cytochrome oxidase" [24] showed that it belongs to one of the two-dimensional rectangular-space groups, denoted by **pg** in the International Tables for X-Ray Crystallography, Volume I. This group has two symmetry-related centers per unit cell. A diagram of the lattice is shown in FIGURE 4. The ellipses in this figure represent the spots on the micrographs. The vertical dashed lines and the two horizontal lines enclose one unit cell, which contains