PRINCIPLES AND METHODS OF LIQUID CRYSTAL PHYSICS APPLIED TO THE STRUCTURE AND FUNCTIONS OF BIOLOGICAL MEMBRANES*

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The application of liquid crystal physics to biological membranes is successful because they can be considered as two-dimensional smectic liquid crystals. Molecular order and phase changes in homogeneous and mixed lipid bilayers are discussed on this basis. It is stressed that the hydrophobic core possesses properties completely different from that of a bulk hydrocarbon due to the long range order of lipid chains. The application of the continuum theory to the membranes turns out to be an especially fruitful way of describing the membrane system as a whole. Examples such as bending elasticity and flexoelectricity are described in detail. The connection between continual and molecular properties is studied. The application of the liquid crystal approach is stressed not only for pure lipid bilayers but also for lipid-protein structures. Many biological implications are considered. The synthesis of amphiphilic molecules at model prebiotic conditions is studied and the importance of liquid-crystal line structures for the origin of life is discussed.

1. Introduction

The concepts of liquid crystal physics are well developed in the case of thermotropic mesophases and have a great number of successful applications. Good results have also been obtained for lyotropic liquid crystals. In this investigation efforts are concentrated on the application of these principles to biological membranes.

A natural basis for such an application in membranology was provided only after the introduction of the model of Singer and Nicolson [1] — the so-called Fluid lipid--globular protein mosaic model of membrane structure, demonstrated on Fig. 1. The skeleton of the biomembrane is a lipid bilayer, which is an example of two-dimensional LC structure — a single piece of the three-dimensional lipid-water lamelar phase [2]. From the general statistical-mechanical point of view it is known that pure two-dimensional

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structures cannot exist because fluctuations in such a structure have a divergent character. However, a structure like a lipid bilayer, composed of amphiphile molecules surrounded by water, could not be destroyed because of the hydrophobic effect — contact between

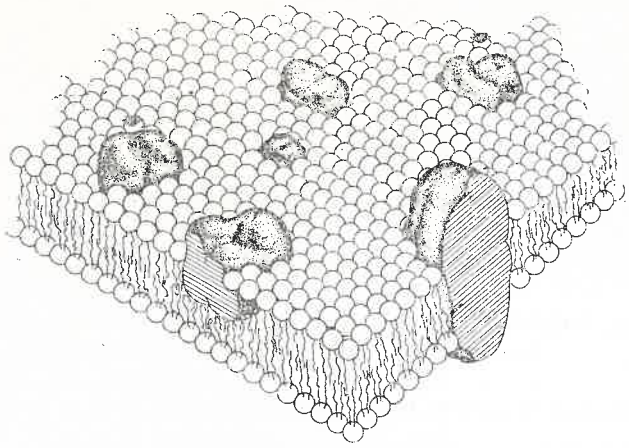

Fig. 1. The Singer-Nicolson model — a schematic representation of the three-dimensional organization of the mosaic structure with phospholipids forming the matrix of the membrane. The globular protein molecules are partially embedded in the membrane and partially protrude from it. The distribution of membrane proteins is random at long range, although some specific protein or lipoprotein aggregates may exist due to the protein-protein interaction (see in this connection Sections 2.2 and 3.3). (With the permission of New York Academy of Sciences, Ref. [1'])

water and the hydrophobic part of the lipid molecule would increase the free energy of the system very strongly.

This is also valid for globular proteins embedded in the lipid matrix. They also have a polar and a nonpolar part and the degree of their dipping in the membrane depends on the protein conformation, which gives a definite proportion between hydrophilic and hydrophobic parts. Two different examples are shown in Fig. 1.

Still the fluctuations in biomembranes are huge and they are limited by the bilayer elasticity and surface tension, as we will see later on.

The Singer and Nicolson model is well confirmed at present by many experimental facts. The physics of liquid crystals is capable of giving a theoretical background for this model and of making possible the quantitative treatment of the processes taking place on the membrane surface. In the following we shall consider some examples. We have selected from the voluminous biomembrane literature contributions, given mainly by scientists who had worked in the field of liquid crystals beforehand, for it is always easier for a LC man to follow the ideas developed in a frame familiar to him.

2. Molecular order and phase changes

At normal body temperature the lipid bilayer in Singer's model is fluid. The lateral diffusion of lipid molecules is very fast ($D \sim 10^{-8}$ cm^2 s^{-1}). The integral proteins "float" in this lipid sea as well with a mobility only one order of magnitude smaller ($D \sim 10^{-9}$ cm^{2}s^{-1}).

Often this liquid character in the plane of the membrane is also ascribed to the hydrophobic membrane core. We shall see that such point of view is not correct because a definite long range order exists in the membrane interior making the properties of the hydrophobic core completely different from a bulk hydrocarbon. Let us consider first the case of homogeneous bilayers, built up from only one type lipid molecules.

2.1. Homogeneous systems

There are many experimental techniques for studying the phase transitions in lipid bilayers such as X-ray scattering [3], differential scanning calorimetry [4], IR-spectroscopy [5], laser-Raman spectroscopy [6], EPR [7], NMR [8], fluorescence [9] and so on and a number of well documented facts exist. Perhaps the most simple technique was developed recently by Harbich, Servuss and Helfrich [10]. It is a direct optical method utilizing a phase-contrast microscope. It turned out to be possible to observe *visually* the melting process in large lecithin vesicles (diam. 10 to 30 μm) obtained by the swelling of anhydrous lecithin in water (freezing point was demonstrated by changes in the smooth contours of the vesicles which become rough displaying more or less pronounced ridges). The melting point was found to be dependent on the number of lipid bilayers making the wall of a vesicle, being $\sim 1°C$ lower in the case of only one (single) bilayer. This was the first optical observation of two-dimensional melting. It is a convenient way for determining the number of bilayers in big vesicles.

The phase transition we speak about is often called the "gel-liquid crystal" transition, and the transition temperature — Krafft-Chapman point [11]. It is well established that at low temperatures there is a two-dimensional translational order of lipids and their aliphatic chains are in a fully extended rigid conformation, often tilted. At the transition temperature (e. g. for DPL it is 41°C) the translational order disappears and some kinks appear in the chains giving rise to different rotational isomers which easily change from one to another: the chains become fluid. In reality this is not so. They are preferentially oriented normal to the membrane and this orientation can be described by the well known order parameter S_i

$$S_i = \langle \tfrac{3}{2} \cos^2 \theta_i - \tfrac{1}{2} \rangle, \tag{1}$$

θ_i being the angle between the membrane normal and the normal to the plane spanned by the C–H bonds of each C-atom. The averaging is performed over all C atoms of the same index i [12].

A statistical theory of chain ordering in lipid bilayers was developed by Marčelja [13] based on a mean field approximation analogical to the Maier-Saupe theory of thermotropic LC's. A parameter of the theory is the lateral surface pressure P experienced by each aliphatic chain, due to the close packing of polar heads in order to avoid the entering of water molecules into the hydrophobic core. At low values of this parameter (dyne/cm) a typical first order transition between the ordered and disordered phase is obtained (this situation can be realized in monolayer experiments only). Higher lateral pressures impose increasing order on the chain, resulting in a more and more continuous change of the order with temperature. A comparison between the values of the order parameter along

the chain calculated and measured by Seelig and Niderberger [8] was made. The agreement was almost complete for a lateral pressure of 25 dynes/cm. This is a way of determining this parameter.

We are just at the point of noting that such an agreement between the theory, predicting an almost constant value of the order parameter along the chain with the exception of the terminal atoms, and the experiment was found only in more recent DMR experiments using deuterated chains [8, 14]. In former experiments with selectively spin labelled chains [7] a completely different profile was established which was an approximately

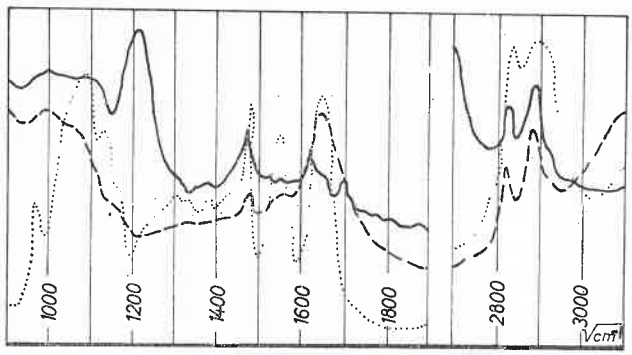

Fig. 2. I R spectra of brain cerebron in different systems: ..... solid cerebron suspended in KBr tablets, - - - - - - 20% cerebron solution in H_2O, ——— 20% cerebron solution in D_2O (99.8% purity)

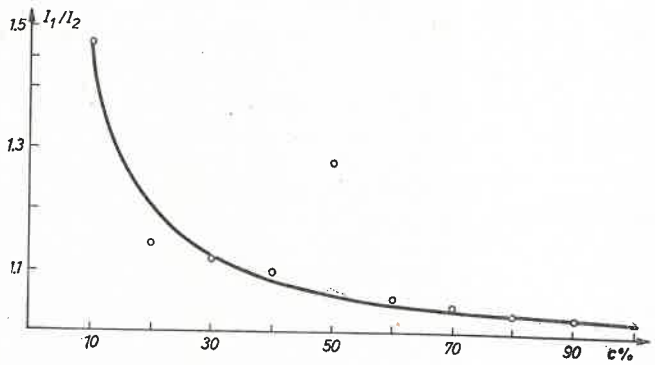

Fig. 3. Dependence of the intensity ratio of valent vibrations 2830 cm^{-1} over 2890 cm^{-1} on the cerebron concentration. The increase of this ratio at low lipid concentrations reflects the increased disorder in aliphatic chains at low packing densities (see the text)

exponentially decreasing S_i like that in [13] when $P = 0$. This was explained by the disturbance caused by the large size spin label and its hydrophilic character. The circumstance that these two methods work on a different time scale should be taken into account as well [15].

The fact, that steric restrictions in the case of long chain molecules attached by one

end to an interface will impose a non zero chain alignment even if the chains are very flexible, was first recognized in the original letter of de Gennes (1974) "General features of lipid organization" [16].

Increasing the area per molecule clearly leads to decreased order along the chain. This effect was studied recently by Seleznev, Feodorov and Usoltseva [17] in the system cerebron-water by IR spectroscopy (Fig. 2). The dependence of the intensity ratio of symmetric (2830 cm^{-1}) to antisymmetric (2890 cm^{-1}) vibrations of the chains on the cerebron concentration, shown in Fig. 3, reflects the increased number of gauche-configurations in the chains at low lipid concentration.

2.2. Heterogeneous systems

The native membranes contain many types of lipids with different head groups and chains of different length and degree of saturation (e. g. a human erythrocyte contains phosphatidyl choline, phosphatidyl ethanolamine, sphingomyelin and phosphatidyl serine in a ratio close to 1:1:1:0.5, small amounts of phosphatidyl inositol, phosphatidic acid and lysolecithin [18]).

At temperatures between the Kraft-Chapman points of each of the components of a multicomponent mixture the formation of clusters or domains of one ingredient with

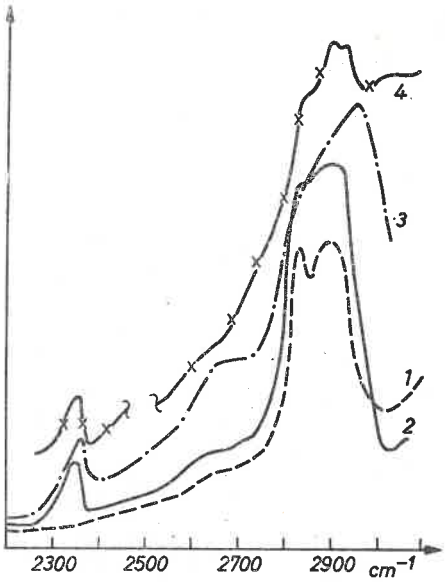

Fig. 4. I R spectra of solid cerebron (*1*), mixture with 20.2 mol% cholesterin (*2*), mixture with 41.4 mol% cholesterin (*3*), a preparation of erythrocyte membrane lipids (*4*). One can see that in (*4*) maxima similar to the mixed systems appear

ordered chains in another with disordered is characteristic. It is necessary, however, to have a sufficient difference in the chain length (at least two CH_2 groups, the half thickness of the disordered middle part). The bigger the length difference, the more pronounced

is the phase separation. It is possible that increasing of the number of crystal-like domains in the membrane is a pathogenic mechanism for cell ageing. This process was studied in a model cerebron-water system by Seleznev et al. [19].

We are not going to speak here in detail on the plastifying effect of cholesterol. We could only notice that a relatively rigid cholesterol molecule restricts the freedom of neighbouring chains in the fluid state and increases molecular order. However, for steric reasons the interaction in the "gel" state between a chain and a cholesterol molecule cannot be as strong as the interaction among ordered all-trans chains. The order in the "gel" phase is therefore decreased. The difference between the "gel" and "liquid crystal" phase become smaller and so does the latent heat of the transition [13]. At a lecithin-cholesterol ratio 1:1 the cooperative phase transition disappears completely.

The interaction of cholesterol with cerebron in the solid state was studied very recently by Seleznev and Fedorov by means of IR spectroscopy. The spectra in the region 2300 cm^{-1}–3000 cm^{-1} are shown in Fig. 4. These spectra demonstrate that besides the influence of cholesterol on the state of chains the formation of a strong hydrogen bond between the polar parts is possible (bands at 2340 cm^{-1} and 2700 cm^{-1}).

The problem of the solvent properties of hydrophobic core deserves special attention. Because of the order already described, it is internally anisotropic. This is not important when the solute molecules are small — then the bulk assumption can be fulfilled but the bulk assumption is clearly questioned if the molecular dimension is of the order of the alkyl chain length. If there are no polar groups to anchore such a molecule at the water-lipid interface (as in the case of cholesterol) its dissolving will not be favourable because of the severe restrictions of its motion when placing it in a relative ordered fluid like membrane interior. Then these molecules collect in lenses or in the central part of the bilayer which is more fluid [21] and do not mix with the bilayer. Some examples are n-alkanes [21] and squalene [22]. It was demonstrated that a linear chain of more than 21 carbons is completely insoluble in a BLM. These results suggest that the information about lipid interior obtained by fluorescent measurements of nonpolar molecules like pirene must be reconsidered [22].

Very interesting is the effect of integral proteins on the lipid chains order. In a recent work of Marčelja this problem was solved theoretically [23]. The result demonstrates that the rigid proteic molecules increase their order in adjacent lipid chains, this order decreases rapidly with distance (see Fig. 9a). Because of the changes in the lipid "solvent" a rise in the free energy of the system takes place and when two protein molecules are near one another, they tend to come together in order to diminish the region with increased order. This is an example of indirect solute-solute interaction via changes in the solvent.

The conclusion to be drawn from these examples is that a biomembrane is fluid in its plane but ordered along its thickness. This order brings about the anisotropy of many macroscopic membrane properties just as in the case of ordinary liquid crystals. That is why some people say that the membranes are fluid in two dimensions and solid in the third. We could simply state the same by saying that the membranes are two-dimensional smectic *A* liquid crystals in the case of normal fluid chains or smectic *C* when the chains are tilted. A similar view point was expressed by Fergason and Brown ten years

ago [24] and by Ambrose in 1971 [25]. New important developments of these ideas in connection with domain formation in bilayers were made by Sackmann et al. [26] and Gruler et al. [27].

3. Continuum properties

The capabilities and the importance of the liquid crystal approach become extremely obvious when we start looking for a way to describe the membrane system as a whole. The level of liquid crystal physics at present is rather high and it is very effective in describing the continual properties of ordinary mesophases [28]. The necessary conditions for the application of the continuum description to biomembranes are discussed by Evans [29]. The membrane is imagined as a two-dimensional continuum. We shall consider here two continual properties — elastic and flexoelectric properties. It must be noted beforehand that the continuum theory of liquid crystals can be developed in two different manners: a pure phenomenological theory, the coefficients of which are taken from experiment, or a molecular theory expressing these coefficients from the molecular properties and intermolecular interactions with the help of statistical mechanics. Examples of both types are well known in thermotropics [30]. We shall try to do the same for bilayer systems.

3.1. Elastic properties

There are several types of possible deformations of a membrane: volume compression, stretching, shear and bending described very well by Evans [29] with the corresponding elastic constants. In the following we shall consider only the curvature elasticity, for it is an elasticity of the LC type. In fact, in a smectic *A* the only possible deformation is that of splay — when smectic layers are curved, the orientation of the molecules varies in space. This change in the orientation give rise to a torque tending to restore parallel molecular alignment and at the same time the planar state of the layer.

A phenomenological expression for the curvature elastic energy of a bilayer was derived by Helfrich [31, 32]. The energy per unit area is given by

$$g = \tfrac{1}{2}K(c_1+c_2-c_0)^2+\overline{K}c_1c_2, \tag{2}$$

where $c_1 = 1/R_1$, $c_2 = 1/R_2$; R_1, R_2 being the principal radii of the curvature, c_0 the so-called "spontaneous curvature", allowing for bilayers whose two sides are chemically different or facing different environments, or contain a different number of molecules. The two terms in the formula are analogous to the splay and saddle splay in the Frank's elastic theory. If $c_0 \neq 0$, the equilibrium configuration of the membrane is nonplanar.

Expression (2) was used by Helfrich and Deuling to obtain theoretical forms for red blood cells (RBC) [33, 34] and explained as a result of negative spontaneous curvature of RBC membrane. In the case of lecithin vesicles [35] and discocytes [34] the agreement between theory and experiment is perfect. The central role in the explanation of the biconcave form of the discocyte in the theory of Helfrich and Deuling is played by the spontaneous curvature c_0 which is expected to be negative in order to produce a curvature opposite to that of the sphere. In another theoretical treatment Jenkins succeeded

in producing biconcave shapes without assuming spontaneous curvature [36]. Different theories of red blood cell shape have been compared recently by Skalak [37]. The difference between theory and experiment in the case of some complicated erythrocyte forms like cups [34] is expected to be connected with the shear elasticity, neglected in these calculations. The fluid lipid membrane itself cannot sustain shear. But at the cytoplasmic membrane face there is a network of a peripheral protein, spectrin [38], giving rise to non zero shear rigidity [29].

Another problem successfully treated by the continuum theory is the fliker effect in RBC [39] — fluctuations in the form, which turned out to be thermally created like orientational fluctuations in nematics [40]. Because of the smallness of the curvature elastic constant the energy kT can produce very large curvature fluctuations in the case of vanishing surface tension, characteristic for discocyte forms [41]. The frequency spectrum predicted by the theory corresponds well with experiment. A $\omega^{-4/3}$ law was established [39]. The experiment was performed by means of phase contrast microscopy of a single cell recording the intensity fluctuations at a given point of the image and performing frequency analysis (J. Lennon, private communication). The form of the spatial correlation function of fluctuations at different points of the erythrocyte membrane is also very well predicted. It must be noted, however, that the form of this function applies only to living cells. A dead cell does not follow it. From the analysis of the experimental data it was possible to extract information about the curvature elastic constant of RBC membranes of human, frog and chicken erythrocytes. The order of magnitude was the same: $K \sim 2.10^{-13}$ erg. This technique for measuring elastic constants is very promising because it uses only a dynamical information from the fluctuations of the system near its equilibrium and does not require static stresses or torques which can easily destroy or alter in a nonreversible way the delicate membrane system. Helfrich and co-workers used the same idea to measure the curvature elasticity of large tubular lecithin vesicles, by studying the fluctuations of their shape in time [42]. They found a value one order of magnitude bigger: $K = 2.3 \pm 0.3 \times 10^{-12}$ erg. The influence of fluctuations on the stretching membrane elasticity was treated by Helfrich [43].

As an important stage in the development of the elastic theory, the concept of "edge" formation must be considered [44]. If, for some reason, the continuity of the bilayer is disrupted and conditions for contact between water and the hydrophobic core arise, a hydrophilic "edge" must be formed connecting the two monolayers (Fig. 7). Along this edge, however, the orientation of lipids changes considerably at a small distance which leads to increase of the energy per unit length of the edge γ. The tendency for the length of this edge to decrease in the case of disc bilayer fragments obtained after sonication of the lamelar phase leads to the curving of these fragments and finally to their closing in vesicles. Such a tendency is opposed by the curvature energy, so a formula for the minimal vesicle radius can be derived [44]

$$r_{0\,\min} = 2(2K + \overline{K})/\gamma. \tag{3}$$

This approach to the problem of a vesicle dimension is to be contrasted with the packing considerations of Israelashwili and co-workes who look for the packing restrictions in the

outer monolayer of the vesicle only, entirely neglecting the elasticity of the inner monolayer [45–47].

The concept of edge energy also permits an understanding of the existence of metastable pores in the membranes [48]. Comparison between the gain in energy by increasing the pore radius when the membrane is under tension Γ, and the loss in energy, because of the increase in edge length, shows that the pore will tend to "reseal" up to the critical radius

$$r_c = \gamma/\Gamma. \tag{4}$$

r_c decreases when Γ increases. This explains the hypotonic hemolysis of RBC with a subsequent resealing of the ghost. The energy for creating a critical radius pore is

$$E_c = \pi\gamma^2/\Gamma. \tag{5}$$

If the tension Γ is large enough, the thermal energy kT is capable of creating fluctuating pores which open and close statistically. In such experiments with lecithin vesicles subjected to a large difference in osmotic pressures [49] it was possible to measure the edge energy γ for DPL: $\gamma = 0.65 \times 10^{-6}$ erg/cm and practically the same for egg lecithin. The important difference with egg lecithin is that the number of defects in the bilayer acting as pore nucleation points is 200 times smaller. The "edge" concept could play an important role in elucidation of some features in membrane functioning (see below).

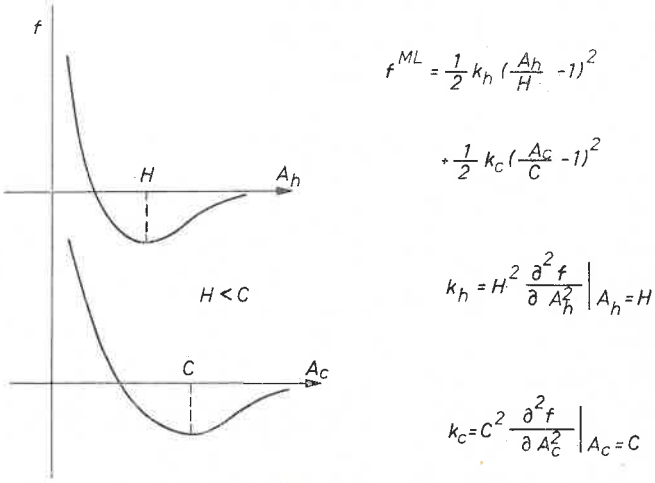

Fig. 5. Schematic form of the dependence of the energy of polar heads' system on the area per head (A_h) and the energy of chains' system on the area per chain (A_c). Definition of the parameters of the theory: The minima points H and C do not coincide in general. The same is valid for the force constants k_h and k_c expressed by the second derivatives of the energy profile in the minima points

The phenomenological theory of elasticity presented up to now can be developed on a molecular basis. An attempt in this direction was made by us in 1975 [50]. We put in the foundation of the theory an expression for the energy of a monolayer as a function of the area per lipid molecule. This expression is a sum of the energy of heads and chains.

Considering the energy of the system of heads, we can expect that for a given area H it will have a minimum (Fig. 5). The same will be true for the system of chains. Then, making a series expansion around the points of minima in the lowest order, we will have:

$$f^{\mathrm{ML}} = \tfrac{1}{2} k_{\mathrm{h}} \left(\frac{A_{\mathrm{h}}}{H} - 1 \right)^2 + \tfrac{1}{2} k_{\mathrm{c}} \left(\frac{A_{\mathrm{c}}}{C} - 1 \right)^2, \tag{6}$$

where k_{h} and k_{c} are second derivatives representing the curvature of the energetic profile in the minima points. The values H and C are the equilibrium areas of the head and the nonpolar part. If they are equal: $H = C = A_0$, the equilibrium state of the monolayer must be planar. Then (6) becomes identical to the formula given by Marčelja [13]. But it is not necessary for H and C to be equal. In this manner the concept of intrinsic asymmetry of a lipid molecule was introduced: positive ($H > C$) and negative ($H < C$). H depends on the parameters of the head group such as conformation, charge, dipole moment ..., so it can change with pH, ionic strength, temperature and so on. C depends first of all on the number of chains, their conformation and degree of unsaturation. It also can change with temperature. We must point out that the four parameters of our theory are in principle predictable from quantum-chemical calculations of the type developed by Frischleder and co-workers (private communication).

A schematic representation of asymmetric lipid molecules is shown in Fig. 6. The equilibrium form of a monolayer built up from asymmetrical molecules will be curved.

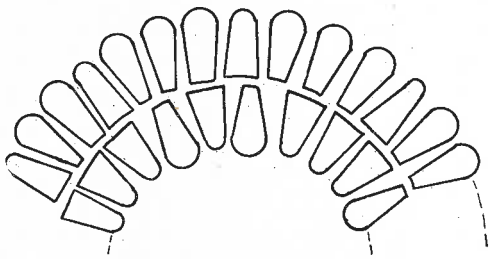

Fig. 6. Distribution of asymmetric lipids in a curved bilayer — these with positive asymmetry ($H > C$) must be concentrated in the outer monolayer while those with negative ($H < C$) — in the inner. Examples of lipids with positive asymmetry are lysolecithin and gangliosides; with negative asymmetry — phosphatidilethanolamine and cholesterol

If the monolayer is forced to be in a planar state, e. g. in a bilayer, then the bilayer structure will be internally stressed. The presence of internal stresses in the mebranes is a feature more connected with their liquid-crystalline properties.

The expression obtained for K for a bilayer with two equal monolayers is

$$K = \frac{d^2}{2} \left(\frac{k_{\mathrm{h}}}{H^2} + \frac{k_{\mathrm{c}}}{4C^2} \right) \left(\frac{k_{\mathrm{h}}}{H} + \frac{k_{\mathrm{c}}}{C} \right)^2 \left(\frac{k_{\mathrm{h}}}{H^2} + \frac{k_{\mathrm{c}}}{C^2} \right)^{-2}. \tag{7}$$

Putting $k_c = 0$, in (6) we obtain a special case of the Evans' formula [51] (if $k_h^{out} = k_h^{in}$)

$$K = d^2 \frac{k_h^{out} k_h^{in}}{k_h^{out} + k_h^{in}} = \frac{d^2 k_h}{2}. \tag{8}$$

In fact, he has considered only head interactions.

A new result of our approach is an expression for the saddle splay elastic coefficient $\overline{K}$. It is obtained by including a higher order term d^2/R_1R_2 in the area vs curvature dependence and reads [52]

$$\overline{K} = -\frac{3d^2}{8} \frac{k_h k_c}{H^2 C^2} \left(\frac{k_h}{H} + \frac{k_c}{C}\right)\left(\frac{k_h}{H^2} + \frac{k_c}{C^2}\right)^{-2} (H - C). \tag{9}$$

Its sign turned out to be just the opposite of that, which the molecular asymmetry has. So, the saddle splay elasticity is the most direct continual demonstration of molecular asymmetry. If $\overline{K}$ exceeds a threshold positive value, a special kind of saddle splay instability arises, recently observed by Kleman and co-workers in lamelar lipid-water phases with low water content [53]. Comparison with that experiment suggests that the asymmetry of lecithin can change with the degree of hydration and shows that the order of $\overline{K}$ is again 10^{-12} erg, so it can be rather important in determining for instance r_0 of vesicles. In fact a small increase was registered by means of the light beating method in mixed systems with cholesterol and ethanolamine. Both admixtures are believed to have negative asymmetry and consequently a positive value of $\overline{K}$. This leads to an increasing of r_0 (if $\gamma = \text{const}$) according to (3). All these results are described in detail by Mitov (this conference) [54].

Strictly speaking, a description of similar phenomena in mixed systems calls for a theory. The development of such a theory will be very important for native membranes which are a complicated mixture of number of lipids. Some preliminary results were obtained by us, based on a generalization of (6) in the form [55]

$$F^{ML} = \tfrac{1}{2} \sum_{i,j=1}^{n} k_{ij}^H \left(\frac{A_H}{H_i} - 1\right)\left(\frac{A_H}{H_j} - 1\right) f_i f_j + \tfrac{1}{2} \sum_{i,j=1}^{n} k_{ij}^C \left(\frac{A_C}{C_i} - 1\right)\left(\frac{A_C}{C_j} - 1\right) f_j f_i. \tag{10}$$

H_i, C_i are the areas for lipid i, k_{ii} — coefficient of the pure system, f_i — partial number of lipid i, k_{ij} — mixing effects.

The problem of lysis was considered and a formula for a critical lysolecithin concentration obtained (assuming $k_{11}^{H,C} = k_{22}^{H,C} = k_{12}^{H,C}$, which is probably justified for lecithin and lysolecithin having the same head group and similar chains)

$$f_{lyso} = \left(1 - \frac{H_l C_l}{H_{lyso} C_{lyso}} \frac{H_{lyso} - 2C_{lyso}}{H_l - 2C}\right)^{-1}. \tag{11}$$

Using the experimental result of van Deenen et al. $f_{lyso} = 50\%$ for mixed vesicles, we obtained positive asymmetry of lecithin

$$H_1/C_1 = 4/3,$$

then

$$H_{\text{lyso}}/C_{\text{lyso}} = 8/3. \tag{12}$$

The influence of lipids, with strong positive asymmetry like lysolecithin, on the value of edge energy has a very important application of the stability of pores. In fact, it was found by Seeman that lysolecithin and saponin create constant pores in a RBC membrane which do not tend to reseal [56]. This is to be explained by the fact that the edge energy is proportional to the difference $(H - 2C)^2$, so it is practically zero — there is no tendency for the pore to close.

The future development of the theory of a mixed system must explain the asymmetric distribution of zwiterionic lipids between the two sides of strongly curved lamela. The qualitative explanation is obvious (Fig. 6).

3.2. Flexoelectric properties

The only possible flexoelectric polarization in a membrane is of the splay type, as is the corresponding deformation. As we noticed the components of the splay are represented by the inverse radii of curvature $1/R_1$ and $1/R_2$, so the expression for curvature-induced polarization per unit area has the form [57]

$$P = e(1/R_1 + 1/R_2), \tag{13}$$

e is a flexoelectric coefficient, dimension of charge.

Analogically to the situation in thermotropics two molecular mechanisms giving rise to such a polarization can be considered: dipolar and quadrupolar. The dipolar mechanism was proposed by us in 1974 [57]. Some new results were reported at the previous conference in Halle (1976). The polarization arises as a result of a transition of lipid dipoles from the inner monolayer of a curved membrane sector where their density increases to the outer resulting in an uncompensated dipole moment per unit area. The expression for a flexoelectric coefficient of cylindrical lipids ($H = C$) is [50]

$$e^D = \tfrac{1}{2}\, \nu_0 \mu_l d\, \frac{2k_h + k_c}{k_h + k_c}, \tag{14}$$

ν_0 is a mean surface density of lipids, μ_l — normal component of a lipid dipole.

Dipolar mechanism is closely connected with the transbilayer lipid transitions — a process called "flip-flop". Usually this process is rather slow [58] — the barrier for the transition of a polar head through the hydrophobic core is too high $\sim 12\ kT$. So in the usual cases the flexoelectric polarization will rise very slowly and its biological significance will be strongly reduced.

A drastic change in the speed occurs, however, when conditions for the formation of hydrophylic pores exist. Here the crucial role of the pore edge was recognized [49] as establishing a bridge between the two monolayers and by means of fast lateral diffusion, permitting an increase of exchange between inside and outside, or an apparently increased flip-flop rate. So, appropriate conditions for a demonstration of the dipolar flexoeffect exist when the membrane is curved and at the same time stretched and when the number

of deffects acting as nucleation sites for forming fluctuation pores is large. Such deffects as lipids with large positive asymmetry as we have already seen, or integral proteins as we shall see, could decrease the edge energy γ locally diminishing in such a way the energy for the opening of a pore: $E_c = \pi\gamma^2/\Gamma$ (Fig. 7).

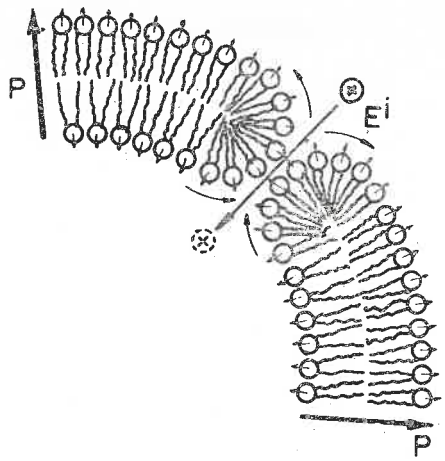

Fig. 7. Flexoelectric model for active transport — Ref. [77]. A cross section of the lipid bilayer through a hydrophilic pore is shown and the change of the lipid orientation at the pore edge is represented. The depolarizing electric field E^i moves the ions through the pore. At the same time the pore edge provides a path for flipping of lipid dipoles from the inner monolayer to the outer and in this way a path for faster establishment of the membrane polarization. See the text for further details

The quadrupolar model involves polarization as a result of the gradients in the orientation of molecular quadrupoles in a medium with uniaxial symmetry — in our case the hydrophobic core. It is much faster because no flip-flop is necessary. Specifying the consideration of Prost [59] for a membrane, we could utilize for the splay flexocoefficient the expression

$$e^Q = -\tfrac{1}{3} L_{zz} N S \theta_a, \tag{15}$$

where L_{zz} is the z component of the Lorentz tensor, N — number of quadrupoles per unit area, S — degree of uniaxial order of the quadrupoles, $\theta_a = \theta_{zz} - \frac{1}{2}(\theta_{xx} + \theta_{yy})$ — the anisotropy of the tensor of quadrupole moment $\{\theta_{ij}\}$, z — membrane normal.

Let us consider the possibility of an experimental investigation of the flexoelectric polarization in membrane systems. In our previous publications on this subject [55, 50] we quoted two papers in which two types of artificial membranes: brain lipid extract modified with dibarenilmercury [60] and lecithin-cholesterol, nonmodified [61] were found to be capable of generating an electric current upon periodical deformation by means of hydrostatic pressure. This deformation involves both stretching and bending strains. The current was interpreted by us as a displacement current due to the oscillation of the membrane polarization with changes in curvature. If the establishment of the polarization

at a jump of the curvature takes place with a relaxation time τ according to the law

$$P(t) = P_m(1-e^{-t/\tau}),$$

where

$$P_m = 2ec_m = 2e(1/R_m), \tag{16}$$

then in the case of oscillating curvature

$$c = c_m \cos \omega t,$$

the current density will be given by the expression

$$i = \frac{d}{dt}\frac{P}{d} = -\frac{ec_m}{d}\frac{\omega}{\sqrt{1+\omega^2\tau^2}} \sin(\omega t - \varphi), \tag{17}$$

where

$$\operatorname{tg} \varphi = \omega\tau.$$

We see that the investigation of the frequency dependence of this current will provide important information about the relaxation time τ and in this way about the molecular mechanism of the effect. However, such investigations are not easy to perform due to the mechanical and hydrodynamical resonances in the vibrating system which prevents from keeping a frequency independent value of c_m.

In the experiment of Ochs and Burton [61] the amplitude of the current was 10^{-10}A. Our own experiments with egg lecithin-n-decan BLM (Derzhanski, Pavlov, Petrov, unpublished) confirm this value of the effect. With the assumption that $\omega \ll 1/\tau$ (in [61] $\omega/2\pi = 50$ Hz) we have reported a value of the flexoelectric coefficient of the Ochs and Burton's membrane $e = 1.5 \times 10^{-9}$ statcoul [50]. With a mean surface density of lipids $\nu_0 = 2 \times 10^{14}$ cm^{-2} making an extremal estimation for μ_l with $k_h \gg k_c$ and $d = 100$ Å, we obtain that $\mu_l \geqslant 7.5$ debye. Such a value appears to be rather high. For instance, it is not in accordance with the surface potential measurements in monolayers which are curently assumed to be capable of providing information about the normal component of the lipid dipole [62–64]. The mean value of the lecithin dipole inferred from these measurements is about 0.5 debye. On the other hand the total dipole moment of lecithin is 25 debye [65]. That is why it is assumed that the orientation of the choline-phosphate dipole is nearly parallel to be plane of the mebrane [62]. However, in paper [66] it was concluded that the surface potential in the case of zwitterionic lipids is not connected with the presence of dipoles and therefore cannot provide any information about the normal component of lecithin dipole moment and consequently the orientation of the choline-phosphate group.

With the same surface density of quadrupoles in one monolayer, assuming $L_{zz} = 1$, $S = 0.5$, we get for θ_a

$$\theta_a = 2250 \times 10^{-26} \text{ statcoul cm}^2$$

per molecule lipid. Such a value also appears to be very high even if we take into account the presence of cholesterol. But if one considers any two lipid dipoles of 25 D at each

side of the bilayer separated by a distance 100 Å, as one quadrupole, one gets just this order of magnitude for the quadrupole moment (Prost, private communication).

According to formula (14) the dipolar flexoelectric coefficient depends strongly on the normal component of the dipole moment which can change when polar head conformation changes. Such changes were postulated as produced by tempearature and NaCl addition [67, 68] hydratation [69, 70], threevalent ions binding [71]. It was proposed earlier [57] that this dependence can have a regulatory function in the processes where the flexoelectric effect is involved (see below). One can look also for changes of μ_1 as a result of the curvature; at the conditions of blocked flip-flop the area per lipid in the outer and inner monolayer will differ. The resulting difference in dipole moments will also bring a non-zero polarization per unit area [72]. This effect which is not a pure flexoeffect but merely a piezoeffect in a bimorph plate is characterized by a "flexoelectric" coefficient

$$e^{\mathrm{B}} = \frac{d\mu_1}{ds}\, d, \tag{18}$$

where $\frac{d\mu_1}{ds}$ is the derivative of the dependence $\mu_1(s)$, s — area per lipid molecule.

It is known that usual piezoelectric do not give polarization at bending but bimorphs composed of two plates with polar axes in opposite directions do. Like quadrupolar the "bimorph" effect operates without flip-flop, so it can be much faster than the dipolar one.

In the absence of any other information, data for $\mu_1(s)$ could be taken from monolayer experiments [64]. In the vicinity of 50 Å^2 the results are $\mu_1(60\ \text{Å}^2) = 0.72$ debye; $\mu_1(44\ \text{Å}^2) = 0.64$ debye so $\frac{d\mu_1}{ds} \approx \frac{\mu_1^2 - \mu_1^1}{s_2 - s_1} = 5 \times 10^{-5}$ statcoul cm^{-1} and $e^{\mathrm{B}} = 5 \times 10^{-11}$ statcoul.

We see that all possible effects which can contribute to the curvature-induced polarization give a somewhat smaller value for e than 10^{-9} statcoul. Perhaps the reason is that in experiments with artificial membranes not only the flexoeffect but some pure charge effects contribute, like selective dragging of cations with the water flow passing through the pores [73] opened by stetching or facilitated transport of cations using lipid molecules in the process of their "flip-flop" through the edges of the pores (Derzhanski, unpublished).

Now we will leave artificial membranes and will take a closer look at natural membrane systems. Changes in the membrane curvature are common effects in many membranes during their functioning. In principle, the flexoelectric coefficient of a biomembrane can be measured by means of curving it with a microelectrode before its puncture. In similar experiments with plant cells, a potential of ~ 10 mV was registered [74].

Some words are necessary about the mechanism producing membrane curvature in living cells. This role can be played by contractile actin-like peripheral proteins attached to the integral ones and in this way to the membrane bilayer. Their contraction can be a result of ATP-hydrolysis or Ca^{++} ions binding. Examples are known when the pH

change creates contraction of a spectrin network causes even a tearing away of spherical bulbs from an erythrocyte membrane [75].

Many facts about the influence of the subsurface microfilament network on the cell membrane are described in a recent paper by Abrose [76].

In 1974 we proposed a flexoelectric model for active transport involving an integral protein as a charge carrier. On the picture (Fig. 7) another variant of this model is represented [77]. The motive power for ion movement is the depolarizing electric field tending to cancel the polarization of a curved sector. The value of this field if there is no external potential difference is given from the continuity condition for electric displacement

$$D^i = E^i + 4\pi P/d = D^e = 0,$$

so

$$E^i = -4\pi P/d = -8\pi e/Rd. \tag{19}$$

The estimations at $R = 1000$ Å and $e = 10^{-10}$ esu give $E^i = 0.75 \times 10^5$ V/cm. This field is quite capable of moving ions against a concentration gradient. This motion will stop when the external field from the displaced ions becomes equal to the internal. It corresponds to a potential difference of 75 mV while the usual membrane potentials are of the same order. So it becomes evident, the capability of our mechanism to act against the existing gradients, even at relatively weak curvatures ($R = 1000$ Å $= 10$ d).

3.2.1. Experimental confirmation

The ion motion across the membrane can take place through the hydrophilic pores, the edges of which provide at the same time a bridge for lipid flipping. The fact that deformed dog red blood cells (deformation being produced by Ca^{++} binding and a muscle-like contraction of peripheral proteins) are 5–6 times more permeable for Na^+ than nondeformed is interesting in association with this [78]. In a recent study of Elford [79] this fact was confirmed once again. Quite different factors (hypertonic shrinking in sucrose and dimethyl sulphoxide action) both resulting in an increasing of Na^+ influx were shown to be connected with the same change in the conformation of RBC membrane — discocyte-echinocyte transition.

If after reaching the condition $E^e = -E^i$ the membrane remains curved, we will get a concentration difference without voltage difference. After the second step of our flexoelectric machine — making the membrane planar — a voltage difference will arise as well. In such a way the mechanical energy of deformation involved in these two steps is converted into electrical. The efficiency of this conversion is given by the coefficient of electromechanical coupling $\kappa^2 = e^2/4\pi\varepsilon Kd$, where K is bending elastic modulus. There are many facts suggesting that such a machine could be involved in the function of mitochondria [80].

Concluding this section, we must stress that the flexoelectric effect connects mechanical and electrical characteristics of membranes. If a membrane possesses flexoelectric properties, its mechanical and electrical degrees of freedom cannot be considered separately. Flexoelectricity provides an efficient way for energy transformation because the

electromechanical coupling coefficient can easily reach a value of 100%. Flexoelectricity above all gives us new ideas for understanding the nature of the electro-mechano-chemical membrane machine.

3.3. Lipid-protein interaction

It is well known that many molecules, placed in a liquid crystal matrix can take only one possible orientation and orientational changes in the matrix influence their orientation as well ("guest-host" effect).

This idea is applied by us to the integral protein orientation. In the case of such big molecules it is the elastic energy change which dictates their orientation. An expression

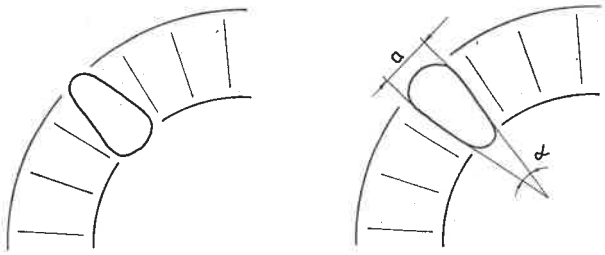

Fig. 8. Two orientations of a wedge-like protein in curved lipid lamela. In some cases the energy difference between these orientations could lead to a reorientation of the protein as a result of the change of the membrane curvature — "guest-host" effect

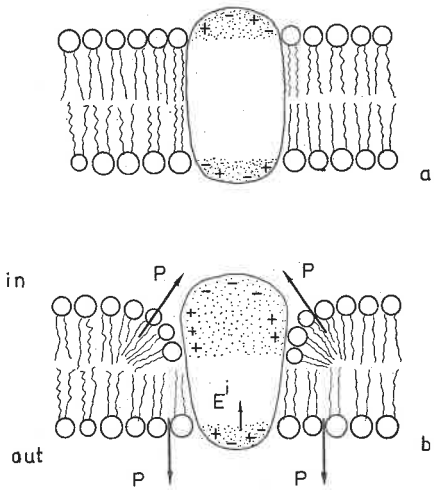

Fig. 9. Lipid-protein interaction and K-Na ATPase functioning. The change of the ensime conformation after the binding of ATP (b) connected with a bigger dipping of its hydrophilic part in the membrane core compared to the nonenergized state (a) must lead to the formation of an curved edge (in this case a half edge) around the protein. The edge polarization changes its direction as flexoelectric notions tell us, so that an unbalanced polarization of the outer annulus arises, creating a depolarizing field E^i in the protein centre. This field would drive the influx of K^+ ions. So the ATP energy is required for the first step of the process only — the expelling of Na^+ ions out of the cell (Ref. [83, 77])

for the elastic energy difference between two orientations of a wedge-like protein depending both on the protein asymmetry and the membrane curvature was given by us in 1974 [57] (see Fig. 8)

$$\Delta U = \frac{K}{N}\,\frac{\alpha}{a}\,\frac{2}{R}, \tag{20}$$

where K is the elastic constant, N — surface lipid density, α — protein wedge angle, a — protein diameter, R — radius of membrane curvature.

Changes in the protein conformation, expressed by a change in the wedge angle α or the value or the sign of the curvature, are the essence of the "guest-host" effect in membranes proposed here.

Pershan and Prost [81] and Gruler [82] suggested later on that long range elastic strains created by proteins with different asymmetry can result in their long range attraction or repulsion. Another mechanism — solute-solute interaction of Marčelja — we have already considered.

The recognition of the role of edge formation around a protein after changing the dipping of its polar part in the core was presented by us at this Conference [83] (Fig. 9) with the application to the ATPase functioning (reported earlier in Wisla (February 1977) [77]). Similar ideas were communicated to us later on by Israelashwili (Fig. 10) [84]. All these pictures can be considered as an important refinement of the Fluid–Mosaic model of Singer and Nicolson which we started with.

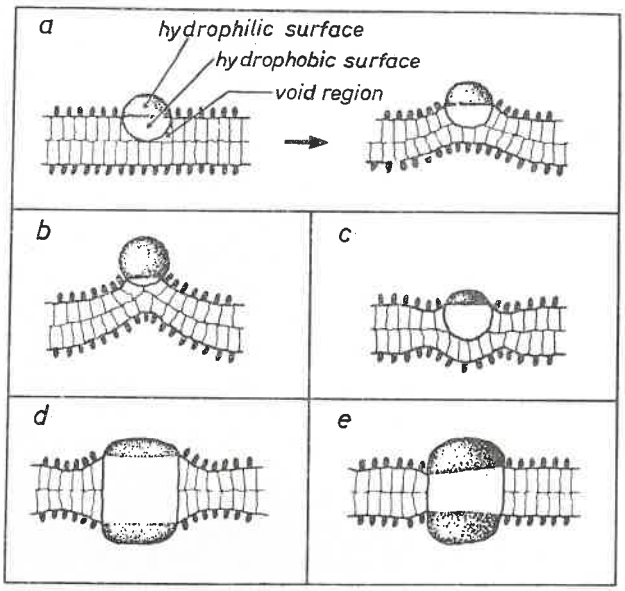

Fig. 10. a(right) — arrangement of lipids around a protein consistent with packing requirements. b — e, diagrammatic illustration of the way lipid bilayers structure around different globular proteins consistent with both packing and thermodynamic restrictions. Note the analogy between (e) and Fig. 9b (With the permission of Jacob N. Israelachvili and Elsevier, North-Holland Biomedical Press, Ref. [84]

So we can see that the liquid crystal approach can provide a consistent description of not only pure lipid bilayers but also of lipid-protein assemblies like native membranes and this is a very big advantage.

4. Conclusion

At the end of this talk let us return to the origin of life on the earth. Even at the first stages of evolution the principles of liquid crystal physics can be followed. Using model prebiotic conditions — UV irradiation of thin layer of parafins on the surface of sea water — amphiphile molecules — were synthesized by Selezne vet al. [85]. The product was found to be capable of forming lamelar smectic systems — protomembranes. After their formation the surface of these structures could be involved in the matrix synthesis of the first protein molecules as a nonspecific catalyst [87] because the capability of colloidal particles to increase the rate of some reactions up to 3 orders of magnitude is well known [86]. This is due to the specific orientation of reactants at the polar-nonpolar interface. In principle, LC systems could represent a new class of prebiotic systems and can help us to understand the next stages of the evolution process e. g. the origin of biopolymers.

Let us finish with a quotation from a report of Ambrose at the Bangalore Liquid Crystal Coference [88]: "... thinking in terms of the biochemistry of the genetic code, synthesis of nucleid acids, proteins, phospholipids, polysacharides etc., tell us very little. The building units of life are remarkably similar from bacteria to man. This is where the study of liquid crystals becomes important; these studies show that the long range order of liquid crystals can be controlled extremely accurately by simple physico-chemical parameters such as: 1. Concentration of molecules; 2. Relative proportions of the components; 3. Ionic environment ...".

What we can add to these words? Clearly we need a theory capable of giving complete description of a membrane system, paying attention at the same time to its molecular constitution. The physics of liquid crystals belongs today to the well developed branches of the physical science. So, we believe that it is that physical theory which must establish itself in modern membranology, because it provides a general theoretical framework which includes, in a natural way, the two-dimensional LC systems — biological membranes. The examples given above are only the first steps in a long way towards the complete understanding of the structure, functions and evolution of different membrane systems in the living cell.

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