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Long Range Protein-Protein Interaction in Membranes*

H. Gruler and E. Sackmann

Abteilung für Experimentelle Physik III, Universität Ulm, Oberer Eselsberg, D-7900 Ulm (Donau), BRD

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A model of mechanical long range protein-protein interaction in membranes is proposed: a membrane bound protein may induce a distortion of the average lipid orientation which may then be transmitted by the lipid orientational elasticity over long distances to a second protein. The elastic force field thus created may lead to both attractive and repulsive forces between the proteins. This type of interaction is a special case of the mechanical long range forces between disclination in ordered fluids. The close analogy between the lipid mediated protein-protein interaction in membranes and the attraction between disclination lines in a nematic layer is illustrated.

The long range interaction between membrane bound macromolecules may be important for the formation of enzyme complexes in biological membranes. A further pathway of enzyme--complex formation in biological membranes is the condensation of the proteins in small lipid domains that are formed upon thermal- or a charge-induced phase separation in the lipid bilayer. It is shown that the charge induced domain formation may also provide a simple mechanism of transverse protein-protein coupling accross the membrane.

CONTINUUM MODEL OF MEMBRANE

This is a rather speculative contribution on the possibility of non-Van der Waals long range protein-protein interaction in biological membranes. It is common practice to discuss long range protein-protein interactions in terms of Van der Waals forces[1]. An alternative pathway of long range interaction in membranes was proposed recently[2]. It is based on the orientational elastic force field within the lipid matrix. A membrane with intrinsic proteins may be idealized as a continous system as shown in Figure 1a. It may be considered as a shell built up of rod shaped molecules containing (in general) conical protein molecules. As pointed out by Helfrich, the elastic properties of fluid membranes (*i. e.* above the chain melting temperature) are determined primarily by orientational elasticity[3]. The basic ideas of these models are:

1. A distortion of a planar membrane (cf. Figure 2) leads to a deflection of the lipid axes from the preferred (parallel) orientation. A so called »splay deformation« is thus created within the lipid bilayer.

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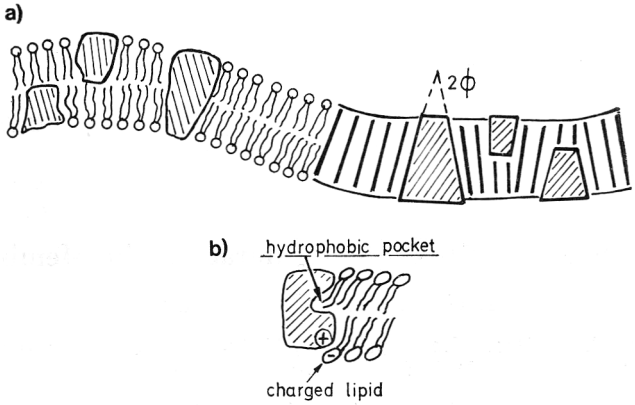

Figure 1. *a*) Idealized model of a biological membrane. Membrane bound proteins are intentionally drawn as conically shaped; b) Demonstration how a cylindrical protein molecule may induce a tilt of the boundary lipid. Physically the protein may be considered as conical.

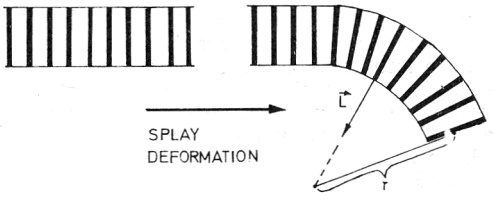

Figure 2. »Splay« deformation of lipid bilayer shown in cross section. **N** vector normal to membrane surface. **L** direction parallel to long lipid axis. Membrane considered as optically uniaxial: **N** || **L**.

2. Membranes that are asymmetric with respect to the reflection plane separating the two monolayers exhibit a spontaneous (intrinsic) curvature. Asymmetry may be realized by
   (a) different lipid compositions in the two monolayers
   (b) differences in the ionic composition or in the structure of the polar lipid/water interfaces on either side of the lipid bilayer.

These ideas may be expressed in terms of the elastic free energy g_{el}

$$g_{el} = k_{11}\, d \left(\frac{\partial L_x}{\partial x} + \frac{\partial L_y}{\partial y} - c_o\right)^2$$

The first two terms describe the energy that would be necessary for a distortion of the membrane. The term $c_o = 1/r$ accounts for the intrinsic curvature. k_{11} is the splay elastic constant of the order $k_{11} \approx 10^{-6}$ dynes and d is the bilayer thickness. $k_{11} \cdot d$ is the elastic constant for a two-dimensional system.

PROTEIN-PROTEIN INTERACTION VIA LIPID ELASTIC FORCE FIELD

The basic ideas of this model are as follows[2]: Incorporation of a non-cylindrical protein into the bilayer disturbes the lipid orientation in its neighbourhood. In order to minimize the splay energy, the membrane becomes curved as indicated in Figure 3a. This curvature allows the direction of the preferred lipid orientation, **L**, to remain parallel to the normal of the mem-

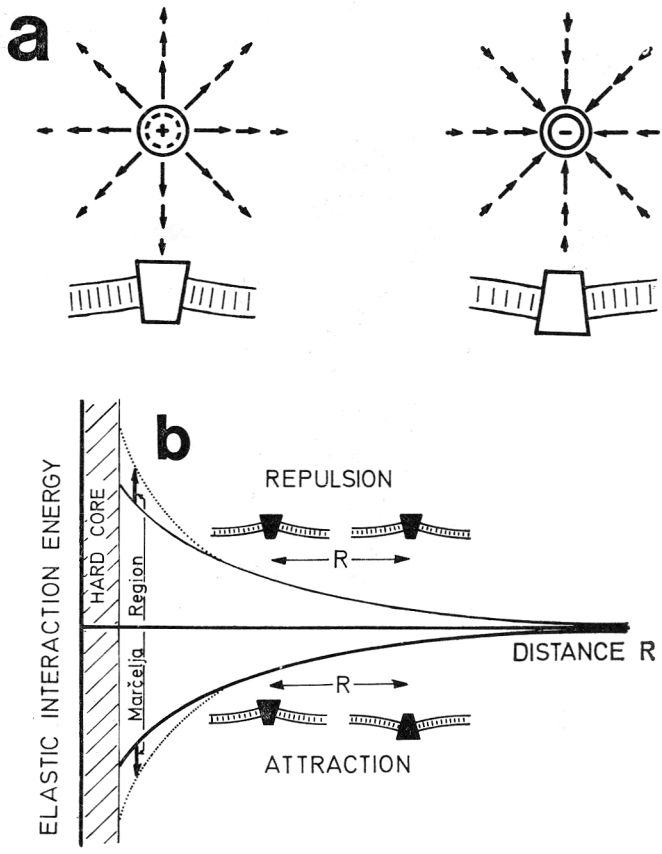

Figure 3. *a*) Schematic representation of the orientation pattern produced by incorporation of a conical body into an uniaxial membrane. The arrows characterize the projection of the optical axis (**L**) to the undeformed plane. The curvature of the membrane is shown on the lower part of the figure. The signs marked on the conical bodies distinguish between a negative and a positive disturbance (Compare Figure 4); *b*) Elastic interaction(exchange)energy of the conical bodies plotted as a function of distance *r*.

brane. Consequently a change in membrane thickness which needs much more energy is avoided. Figure 3b shows the projection of the direction of the lipid orientation, **L**, into the plane of the membrane. The orientation pattern of these projections is analogous to the orientation pattern induced by some line singularities in nematic liquid crystals[4]. Upon going around a line singularity that is orientated perpendicular to the plane of a thin nematic layer, the local direction, **L**, of the nematic orientation rotates. In the core of the disclination line, the molecular orientation is random. This core plays the same role as conical proteins in lipid bilayers.

It is the close analogy between the orientation pattern of Figure 3b in the vicinity of integral membrane proteins on one side and of disclination lines in nematic layers on the other side which strongly suggest elastic long range forces. From the study of disclination lines in nematics it is known that both attractive and repulsive forces may exist between the defects. The attractive

force between nematic defect of opposite sign is demonstrated in the photomicrograph of Figure 4 where two defects can be seen approaching each other rapidly and merging together.

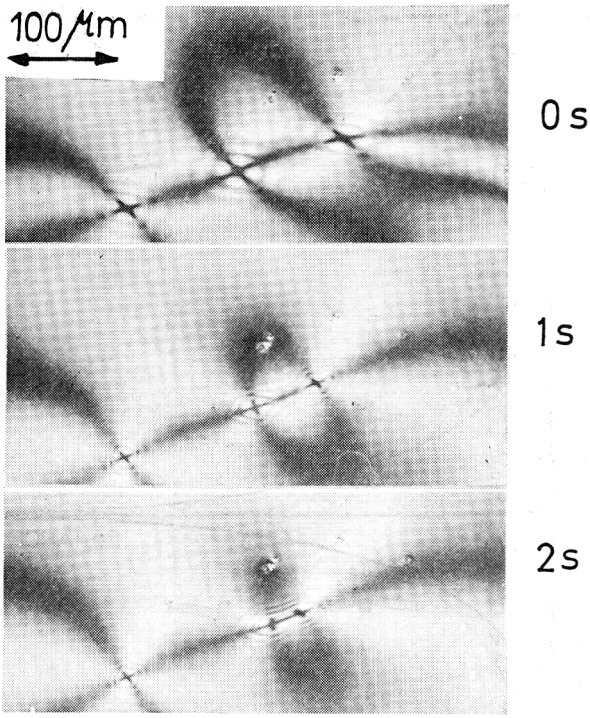

Figure 4. Model System for Long Range Elastic Force.
Demonstration of an attractive force between two disclination lines of opposite sign in a layer of nematic liquid crystal. The nematic layer (20 µm thick) is viewed between crossed polarizers in a direction perpendicular to the glass plates. Upon application of an electric field of about 3000 V/cm a high number of disclination lines is produced. These lines extend between the two glass plates and are surrounded by concentric nearly rectangular shapes. Four dark brushes protrude from each disclination line. Any two disclinations which are connected by three dark brushes attract each other and finally melt together. The approach of two such disclinatoins is illustrated in the three consecutively taken photomicrographs. The disclination line at the lower left side is repelled by the central line. The system of brushes connecting two attractive disclinations is reminiscent of the electric field between two charges of opposite sign (dipolar field). This analogy has been stressed by Nehring and Saupe[4].

The elastic theory of such defect interactions has been worked out by Nehring and Saupe[4]. They showed that in analogy to electrostatic forces, defects of opposite sign attract each other while defects of equal sign are repelled. In view of the above mentioned close analogy the results of ref. 4 may be directly applied to the problem of lipid mediated protein-protein interaction. Accordingly, the forces between proteins are expected to be inversely proportional to the distance of the centres of the proteins.

$$F_{ij} = -2\pi k_{11} \cdot d \cdot \frac{1}{r_{ij}} \cdot \sin \Phi_i \cdot \sin \Phi_j \qquad (2)$$

$2\Phi_i$ is the cone angle of the protein as defined in Figure 1. As expected the

force does not depend on the diameter of the proteins. Eq. (2) was derived for a 2-dimensional distortion[4]. Due to the slight curvature of the membrane shown in Fugure 3a it has to be treated as a weakly 3-dimensional system. Thus in a first approximation the force should be rather expressed as

$$F_{ij} \propto r_{ij}^{-(1+\delta)} \tag{3}$$

where δ may be considered as a quantity which is small compared to 1. $\delta = 1$ would correspond to the 3-dimensional case. In a true 2-dimensional system the elastic interaction energy of the lipid mediated protein coupling would decay logarithmically with distance ($W_{ij} = \int F_{ij} \cdot dr_{ij}$).

$$W_{ij} = -2\pi k_{11} \cdot d \cdot \ln\{r_{ij}/r_p\} \sin \Phi_i \sin \Phi_j \tag{4}$$

where r_p is approximately determined by the protein radius. Thus the protein interaction decays very slowly with distance. W_{ij} does not even converge. This difficulty may be overcome by considering the third dimension according to eq. (4). The interaction energy may then be written as:

$$W_{ij} = 2\pi k_{11} \cdot \left(\frac{d}{\delta}\right)^{1+\delta} \cdot r_{ij}^{-\delta} \sin \Phi_i \sin \Phi_j \tag{5}$$

Here δ is a measure for the deviation from two-dimensionality. From the above equation, the long range protein-protein interaction may be estimated, considering two protein molecules at a mutual distance of 100 Å. For a conical angle of $\Phi_i \approx 10^0$ one obtaines a force of $F_{ij} \sim 10^{-7}$ dynes by assuming two-

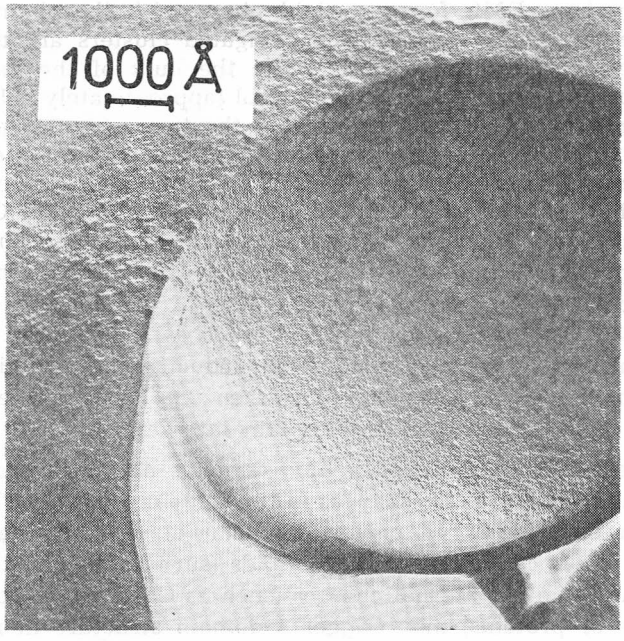

Figure 5. Electron microscope picture of a large vesicle composed of 65% dioleyl phosphatidic acid, 15% dioleyllecithin and 20% cholesterol. Preparation by freeze etching technique[6]. Clearly circular domains are formed which exhibit a pronounced local curvature. Note that the domains are kept apart by repulsive forces.

-dimensionality ($\delta = 0$). For the same protein-protein distance of 100 Å, the interaction energy varies from $W_{ij} = 0.02$ eV for $\delta = 1$ (3-dimensional case) to $W_{ij} = 0.2$ eV for $\delta = 0.1$. This result clearly shows that the proposed mechanism of protein interaction in membranes is strong enough to overcome thermal randomization ($kT \sim 0.025$ eV at 20 °C).

The lipid mediated protein-protein interaction leads to long range forces. When two protein molecules approach each other more closely, the short ranging interaction mechanism proposed in Marčelja's model[5] prevails. The proposed elastic interaction between proteins has not been observed experimentally yet. However, the domain structure of lipid lamellae shown in Figure 5 strongly suggests such repulsive forces between the domains. The lipid domains exhibit a spontaneous local curvature as can easily be seen with a stereo electron microscope[6]. Such spontaneously curved domains within a fluid membrane have exactly the same effect as a conical, membrane bound body.

PROTEIN-CONDENSATION

A second mechanism of protein association may be a co-condensation of lipids and proteins. A first suggestion for such a model originates from studies of lipid organization in membranes of binary lipid mixtures. If the external conditions (temperature, pH) are adjusted in such a way that the lipids are partially immiscible, the membrane may exhibit a domain like lipid distribution[6,7]. In favorable cases the domain structure may be observed by electron microscopy. An example is shown in Figure 6 for a mixture of dimyristoyllecithin (DML) and cholesterol. The situation shown corresponds to a temperature of 20 °C where DML forms a rigid phase with the chains tilted with respect to the membrane normal. The elongated clusters are therefore supposed to be composed of pure DML while the bulk of the membrane is a homogeneous mixture of DML and cholesterol (approximately a 1 : 1 mixture)[6]. The electron micrograph clearly shows that the two separated phases exhibit different curvatures. It is highly probable that such domains are also present in biological membranes. This is indicated by spin label studies of erythrocytes which show several conformational changes: at 18 °C, 33 °C and 46 °C[8,9]. This finding strongly suggests that at physiological temperatures the membrane is composed of rigid and fluid domains. It is easily conceivable that intrinsic proteins tend to assemble in one type of domain and are thus held in close contact. One possible example for such a model is the incorporation of the cytochrome *P450*/cytochrome *P450* reductase in a lipid domain of specific composition[8]. The assembly of this enzyme system is explained also in terms of a lipid mediated protein-protein interaction[5].

The condensation of proteins in lipid domains may also provide an effective triggering mechanism for changes in the protein coupling. This suggestion arises from recent studies[6] of chemically (charge) induced phase separation in membrane alloys containing charged lipids (such as phosphatidyl serine or cardiolipin). Addition of external charges (such as Ca^{++} ions or positively charged polylysine or proteins) may trigger a domain structure in mixtures of a zwitterionic lipid (*e. g.* lecithin) and a lipid with negative charges such as 1) phosphatidic acid at pH > 7.5 (where it is two fold charged) and 2) phosphatidyl serine. Domains of charged lipids bound by Ca^{++} or the polypeptide are formed

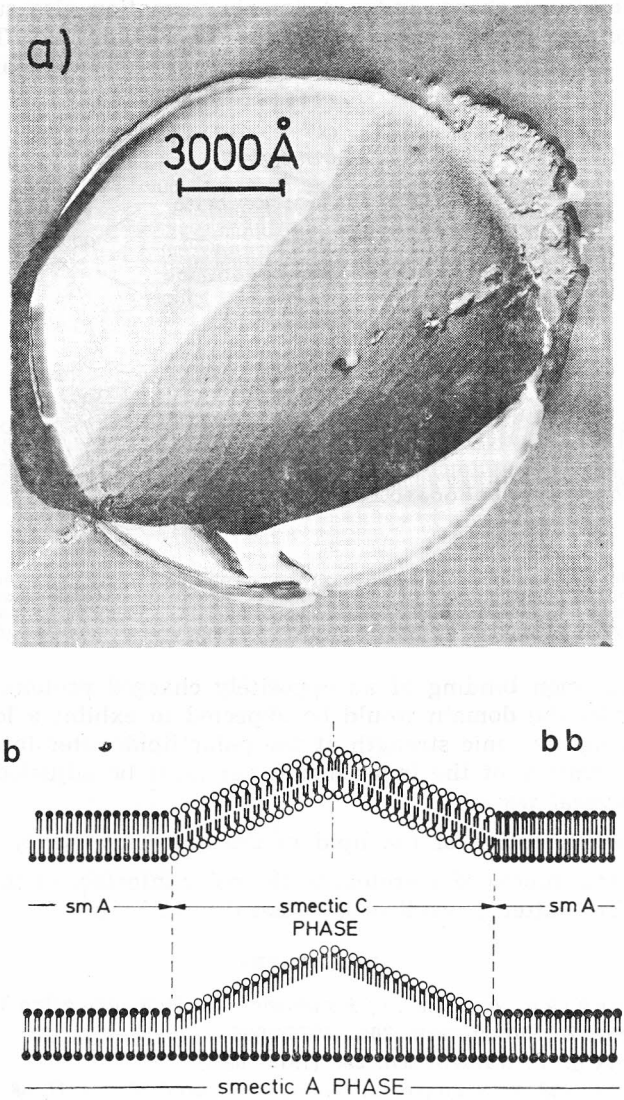

Figure 6. *a*) Electron micrograph (freeze etching technique) of large mixed vesicle of dimyristoyl lecithin (72%) and cholesterol (28%). Vesicle preparation rapidly cooled from 10 °C. Elongated domains are formed that are expected to be composed mainly of DML in a tilted rigid structure; *b*) Possible lipid orientation in elongated domains composed of dimyristoyl lecithin (DML). The long axis of the DML-molecules are tilted at an angle ϑ with respect to the plane of the membrane.

(cf. Figure 7). The charge induced redistribution of the lipids is then followed by a subsequent redistribution of the membrane bound proteins. The protein--protein interaction could then be changed considerably. Such processes are to be expected in the inner monolayer of erythrocytes with their high content of phosphatidyl serine. The inner mitochondrial membrane with its cardiolipin concentration is another possible example.

The possibility of charge induced domain formation also suggests a mechanism of protein-protein coupling across the membrane. The two step mechanism is illustrated in Figure 7. In the first step a domain of charged

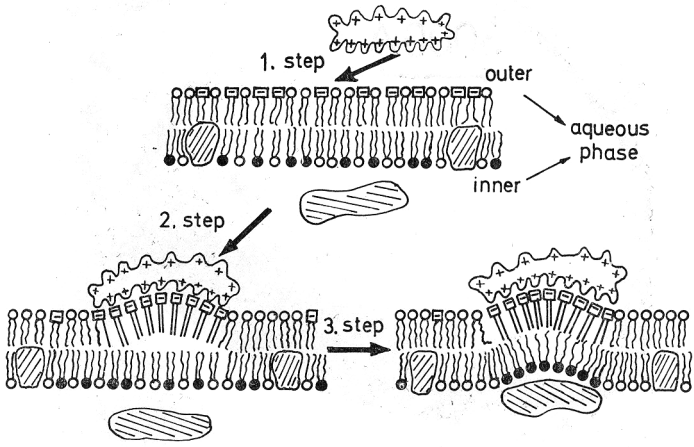

Figure 7. Possible mechanism of transverse protein-protein coupling or information transfer. Two step mechanism: 1) Attachment of charged model protein (poly lysine) leads to the formation of a spontaneous local curvature in outer monolayer. 2) Redistribution of lipids in inner monolayer or adsorption of protein to match curvature of opposing monolayer.

lipid is formed upon binding of an oppositely charged protein. According to the elastic model the domain would be expected to exhibit a local curvature due to the change in ionic strength at the polar lipid/water interface. Consequently the curvature of the inner monolayer must be adjusted. This can be achieved in several ways:

1. by a redistribution of the lipid in the inner monolayer, and
2. by the attachment of a protein to the polar interface of the inner monolayer. The latter possibility is illustrated in Figure 7.

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DISCUSSION

F. Jung:

A good experiment is always better than a theory. Have you made studies with monolayers measuring area/pressure curves with *a*) pure lipids and *b*) wedge shaped proteins and lipids? It should be possible to get an anomaly if you were to approach the condensed state.

E. Sackmann:

Experiments of this kind are currently being performed in our laboratory.

F. Jung:

In a natural membrane, there is a continuous exchange of lipids. Is this exchange not faster compared to the lateral movement of proteins?

E. Sackmann:

The phospholipid exchange between vesicles is very slow, with half life times of the order of 10 hours. The protein lateral diffusion, in vesicles of this kind, is characterized by a rather high average drift velocity (in a given direction) of the order of 5000 nm/s. This situation seems to be characteristic for fluid biological membranes such as the visual receptor membrane or microsomal membranes. In erythrocytes the situation is reverse. The proteins are nearly immobilized, while the phospholipid exchange is characterized by an exchange time of the order of an hour.

J. I. Mason:

Can changes in the annular lipid result in movement of the membrane protein out of the plane of the membrane?

E. Sackmann:

Since the conformation of integral proteins seems to depend on the physical state of the annular lipid this may well be possible. The out-of-plane movement of rhodopsin upon excitation, as observed by small angle X-ray diffraction, may be an example.

S. Maričić:

Have I correctly understood the consequences of your protein-protein interaction model? If proteins are clustered together, does this lead to a final freezing of the cluster?

E. Sackmann:

If the proteins come in contact the Marčelja model comes into play. It predicts an ordering, or if you wish, a crystallization of the boundary lipid. The whole enzyme--complex would then be incorporated in a frozen lipid halo (cf. reference 10).

S. Vuk-Pavlović:

Your model is based on the assumption that the hydrophobic portion of the integral proteins is highly asymmetric. Do you have any evidence of this?

E. Sackmann:

No. However, it should be emphasized that the effect of a »wedge« shaped protein is to induce a change in local curvature (cf. Figure 3a). It is this effect which leads to the elastic forces. A similar change in local curvature would also be created if the hydrophilic parts of the proteins differ at the two sides of the bilayer.

R. Austin:

Electrostatically bound proteins can often break lipid vesicles into much smaller spheres. Is this in any way related to a change in the natural curvature of the lipid?

E. Sackmann:

In principle this may be the case. If the curvature of a domain differs considerably from the curvature of the bulk membrane, the domain may form a vesicle that is

expelled from the membrane. Electrostatically bound proteins may thus induce phagocytosis.

R. Austin:

Ca^{++} ions can often split a single lipid phase transition into two transitions. So proteins are not exceptional in achieving this effect.

E. Sackmann:

That is true. The binding of any external charge (H^+ ions, Ca^{++}, proteins) to the surface of a membrane composed of lipids with a net charge, will shift the transition temperature. The shift induced by Ca^{++} is much larger even than that caused by H^+ or polylysine.

D. L. Williams-Smith:

Could part of the deformation caused by a wedge shaped protein be accomodated by refolding of the lipid chain?

E. Sackmann:

According to the Marčelja model, the boundary lipid is ordered by the protein surface. I think this ordering effect would rather prevent refolding.

D. L. Williams-Smith:

Is your plot of elastic exchange energy *versus* r based on calculations for a monolayer or does it include interaction with the complementary (protein free) monolayer.

E. Sackmann:

The model has only been worked out for proteins extending over both bilayers.

SAŽETAK

Interakcije dugog dosega među proteinima u membranama

H. Gruler i E. Sackmann

Opisan je model mehaničke interakcije dugog dosega među proteinima u membranama. Protein vezan u membrani remeti prosječnu orijentaciju lipida. Takva se poremetnja prenosi orijentacijskom elastičnošću lipida na velike udaljenosti do drugog proteina. Polje elastičnih sila koje time nastane može dovesti do privlačnih ili odbojnih sila između proteina. Taj je tip interakcije posebni slučaj mehaničkih sila duga dosega između disklinacija u sređenim tekućinama. Prikazana je bliska analogija između interakcije proteina u membranama preko lipida i privlačenja disklinacijskih linija u nematičkom sloju. Interakcija duga dosega između makromolekula vezanih u membranama može biti značajna za stvaranje enzimskih kompleksa u biološkim membranama. Drugi put za stvaranje enzimskih kompleksa u biološkim membranama jest kondenzacija proteina u malim lipidnim domenima koji nastaju razdvajanjem faza zbog termičkog djelovanja ili pak zbog djelovanja naboja u lipidnom dvosloju. Stvaranje domena zbog naboja može biti jednostavni mehanizam za transverzalnu spregu proteina u membranama.

ABTEILUNG FÜR EXPERIMENTELLE PHYSIK III
UNIVERSITÄT ULM
OBERER ESELSBERG, D-7900 ULM (DONAU)
B.R.DEUTSCHLAND

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