A Phase Transition Model of Cooperative Phenomena in Membranes

Kåre Larsson and Sten Andersson

Chemical Center, University of Lund, Box 124, S-221 00 Lund, Sweden

Larsson, Kåre and Andersson, Sten. 1986. A Phase Transition Model of Cooperative Phenomena in Membranes. – Acta Chem. Scand. B 40: 1–5.

The occurrence of an intrinsic curvature of the lipid bilayer in membranes with long-range periodicity is proposed, based on a demonstration of structures of infinite periodic minimal surfaces in two dimensions. These surfaces are related to certain crystallographic planes in "normal" infinite periodic minimal surfaces, a structure type which has recently been shown to exist in lipid-water systems. The periodicity of two-dimensional lipid bilayers, curved according to minimal surfaces, provides a mechanism for communication along the membrane surface, and makes possible a long-range coupling of bilayer related membrane functions. The most important effect of the proposed phase transitions between planar and curved bilayers in membranes is the possibility of a simple mechanism for cooperative processes. Driving forces which should favour these transitions are discussed.

In the traditional structure model of membranes the continuous lipid bilayer is mainly regarded as a barrier and as a medium for various functional units. On the basis of recent results on the properties of phases with infinite periodic minimal surfaces in lipids, and from an analysis of the possibility of forming such surfaces in two dimensions, we propose in this paper that lipid bilayer phase transitions involving curved minimal surfaces occur in membranes. These phase transitions can provide cooperativeness in functions over limited regions as well as over the whole surface of the membrane. As an illustration of what is meant by cooperativeness of lipid bilayer behaviour in this context, we can consider the synaptic transmission of the nerve impulse (cf. Ref.[1]). The rate and capacity of mass fusion of synaptic vesicles with the presynaptic membrane and the recycling of bilayer material by coated vesicles indicate some type of long-range coupling effect of the lipid bilayer changes.

A most interesting study with regard to the lipid structure in membranes has recently been reported by Gulik et al.[2] In structure studies of membrane lipids from archaebacteria these authors observed new phases, and the results led them to wonder "whether a flexible fabric of lipid threads with proteins inserted in the interstices would not provide a more realistic picture of membranes than an inert bilayer". The structures proposed in the present paper point in the same direction.

Lipid phases with bilayers forming infinite periodic minimal surfaces

Periodic minimal surfaces were discovered by Schwarz[3], and their symmetry relations have been analysed by Schoen[4]. It has been shown that minimal surfaces provide a general principle for the description of interpenetrating structures, and in this connection an improved mathematical description of minimal surfaces was derived (cf. Ref.[5]).

An infinite periodic minimal surface divides space into two congruent labyrinth-systems, and the surface is free from intersections. The mean curvature of a minimum surface is zero everywhere. The Gaussian curvature (which is the product of the two principal curvatures) is thus negative or zero.

There are three fundamental cubic minimal

surfaces: The P-surface (the primitive surface according to Schwarz), the D-surface (with a diamond lattice of the labyrinth-system), and the G-surface (the gyroid, which is a helical labyrinth-system). An interesting feature relevant to lipids is that the P-surface can be bent *via* the G-surface to the D-surface (and *vice versa*) with the zero average curvature maintained, and with constant Gaussian curvature at corresponding points. The bending occurs without stretching or tearing. These so-called Bonnet transformations in cases of lipid structures, which will be discussed below, represent a unique type of phase transition with extremely small transition enthalpies.

The background for the demonstration of infinite periodic minimal surfaces in lipids is the following:

From a combined NMR- and X-ray diffraction study of the thermal transition in monoglycerides from the lamellar liquid-crystalline phase to the cubic phase[6], it was concluded that the structure is both lipid- and water-continuous and that the lipid forms bilayer units. It was later proposed that an infinite periodic minimal surface would be the ideal structure[7], and from an assumed symmetry (*Im3m*) of the observed body-centered lattice it was proposed that the structure was equivalent to Schwarz's P-surface. Then Longley and McIntosh[8] reported that a primitive cubic lattice exists when monoolein is close to its water swelling limit, and according to the space group (*Pm3n*) they proposed that the structure is an infinite periodic minimal surface of the D-type. Thus there are two cubic phases in the binary monoolein-water system[9], and we have analysed this phase transition[10]. From the agreement between the observed lattice parameters at the transition between the two cubic phases with the theoretical values, it was concluded that the phase transition is a Bonnet transformation from a G-surface to a D-surface with increasing water content. The enthalpy of the transition was below the limit of detection in the calorimeter used, which is less than a factor 10^{-2} of an ordinary liquid-crystalline phase transition in lipids[10]. It should be pointed out that a lipid bilayer centered at the G-surface has space group *Ia3d*, which Luzzati et al.[12] have observed in various lipid-water systems, and that the structure of the body-centered lattice of monoglycerides is not

the earlier assumed P-surface type7 but the G-type.

In describing these cubic lipid-water structures as infinite periodic minimal surfaces, the center of the bilayer is equivalent to the minimal surface and the polar head groups are located on parallel surfaces on each side of the minimal surface. The water fills the rest of the space of the two congruent labyrinth-systems. The two parallel surfaces are formed by transforming each point at a distance equal to half of the bilayer thickness $(d_c/2)$ along the surface normal at the actual point. An interesting property of such a parallel surface is that the mean curvature and the Gaussian curvature are related by the factor $d_c/2$ at every point.

Lipid water phases (cf. Ref.[13]) are either interpenetrating structures, i.e. they are both lipid and water continuous, or contain aggregates of one component dispersed in a continuous medium of the other. These cubic phases represent the first type and hexagonal phases (H_I and H_{II}) the second, whereas the lamellar phase falls in between. A general feature of solids consisting of interpenetrating structures is that the atoms are arranged according to minimal surfaces[5], and it is therefore not surprising that interpenetrating lipid-water structures form minimal surfaces.

Two-dimensional minimal surfaces

A planar bilayer is the trivial case of a minimal surface, which corresponds to the present view of the lipid structure in membranes. Although this bilayer is flexible and can adopt various curvatures, its spontaneous curvature is zero. Let us imagine a bilayer, which is also infinite in two dimensions but which is regularly curved according to a spontaneous curvature different from zero. If the mean curvature is zero in every point of the center of such a bilayer, we would have an infinite periodic minimal surface in two dimensions. With certain limitations it is possible to form such minimal surfaces, which will be considered below.

Starting with the P-, G- or D-surface discussed above we can choose a few crystallographic planes, which follow the minimal surface with a minimum of connections to adjacent regions of the minimal surface arranged in parallel. The two simplest surfaces of this kind are shown in Fig. 1.
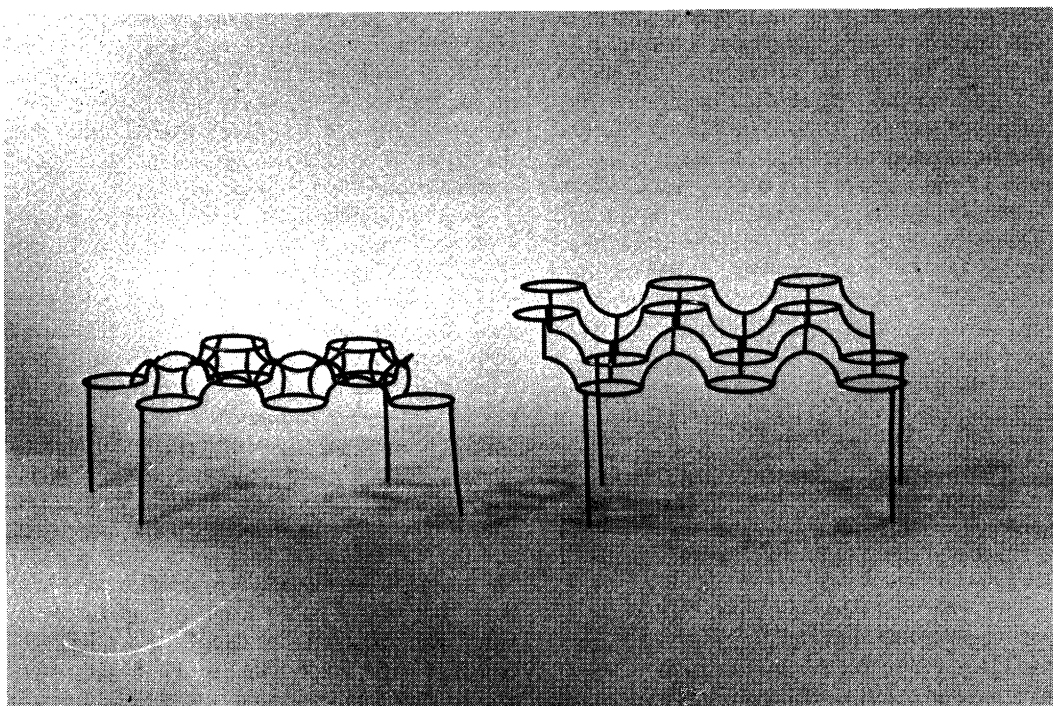
Fig. 1. Skeletal frameworks (built by circular arcs) corresponding to two-dimensional curved minimal surfaces of *P*- (to the right) and *D*-type (to the left). These surfaces follow the (100)-plane and the (111)-plane of the *P*- and *D*-lattice, respectively.

We prepared metal frames corresponding to circular arcs exhibited by the *P*- and *D*-surface, respectively. When this frame is dipped into a soap solution, a soap film will cover each frame. We consider these films as the two most important examples of two-dimensional curved minimal surfaces. The two-dimensional surface is limited by two parallel planes, and circles located in these planes are cross-sections through connecting "tubes" with parallel regions above and below of complete *P*- and *D*-surfaces. In the soap film model of Fig. 1 there will be circular discs of planar film units (which also are minimal surfaces) dispersed in a curved minimal surface.

If a lipid bilayer is arranged according to a curved minimal surface, as shown in Fig. 1, there are several alternatives for the structure of the circular discs. They might be fully covered by protein molecules. Protein molecules could form frames which shield the discs, and possible effects of net-works of clathrin molecules (cf. Ref.[14]) in

this respect will be considered in the next paragraph. The periodicity of the curved bilayer should be expected to be of the same order of magnitude as that observed in three-dimensional minimal surfaces of lipid-water or lipid-protein-water phases, i.e. a few hundred Å[10,11]. This means that the discs considered here should be some ten Ås in diameter.

An important property of a lipid bilayer curved according to a minimal surface is the difference in molecular area at the polar head groups compared to the area at the center of the bilayer. This gives a wedge shape of the molecules, with diverging chains from the polar head groups towards the methyl end groups. This wedge shape varies according to the type of minimal surface and with its periodicity. Israelachvili *et al.*[15] have analysed the relations between the wedgeshape of lipid molecules and phases formed in aqueous systems, and structural effects due to molecular geometry have been quantified. A possible driving

3

force for transition from the common planar bi-
layer structure to a curved bilayer structure is an
increase in the average molecular wedge shape.
A conformational change of a membrane pro-
tein, which increases its area in the region of the
polar head groups in relation to the area in the
center of the bilayer, will have the same effect.
There are numerous environmental factors which
should be expected to give these effects on the li-
pids of the bilayer. Changes in pH so that
charged polar heads become neutral or the pres-
ence of di- or multivalent cations capable of
crosslinking charged lipids are examples of these
factors that should give an increased average mo-
lecular wedge-shape, and thus favour transitions
towards the curved bilayers.

Another driving force from planar bilayers to-
wards curved minimal surface bilayers in a mem-
brane might be the formation of excess bilayer
material, for example in connection with vesicle
fusion.

In view of the complex phase behaviour of
lipid-water systems (cf. Ref.[13]) we consider it un-
likely that a lipid bilayer in a membrane under
varying geometrical conditions only exhibits one
type of structure. Unfortunately there are no di-
rect experimental methods available in order to
determine whether two-dimensional curved lipid
bilayers exist or not. X-ray diffraction technique
cannot be used and, for comparison, we would
know little about cubic lipid-water structures
without X-ray results.

A lipid phase transition model of membranes

Phase transitions of the lipid bilayer in mem-
branes involving curved minimal surface struc-
tures would provide interesting functions. An ob-
vious property mentioned above is the ability to
incorporate varying amounts of bilayer per unit
area of membrane front. The most important fea-
ture of phase transitions into curved bilayers,
however, is the introduction of long-range order
which can extend over the whole surface of a
membrane. Such transitions would give a direct
communication mechanism along the membrane
surface and make possible cooperativity in mem-
brane processes as exemplified in the introduc-
tion. The theoretical value which should be ex-
pected for the rate of propagation of these bi-
layer transitions is equal to the velocity of sound.

This rate of progress of phase transitions invol-
ving minimal surfaces is consistent with experi-
mental observations[16]. It is tempting to relate the
propagation of the action potential in nerves with
movement of such a phase transition band, and
the effect of anesthetic agents with blocking of
the phase transition.

A curved minimal surface bilayer can also pro-
vide the periodicity required in order to obtain
regular packing of proteins. Observed regular-
ities in packing of connexons in specialized re-
gions for cell contact[17], or the arrangement of
acetylcholine receptors in the postsynaptic mem-
brane[18] might be achieved by minimal surfaces. A
regular hexagonal arrangement of protein mole-
cules with a periodicity of about 200 Å has been
observed at the interface between the milk fat
globule and its membrane[19]. The mechanism of
adhesion between the milk fat globule membrane
and the surface of the fat globule is not known.
The electron microscopy observations by Buch-
heim[19] are in fact consistent with regions of the
membrane arranged according to a minimal sur-
face of D-type (shown in Fig. 1), which down-
wards is fused with the polar lipids on the surface
of the fat globule.

A hypothetical transport process utilizing the
proposed lipid bilayer phase transition is illus-
trated in Fig. 2. Environmental effects discussed
in the earlier paragraph may induce a phase tran-
sition from a planar to a curved bilayer structure,
and it is obvious that this change in membrane
shape will favour fusion of vesicles located close
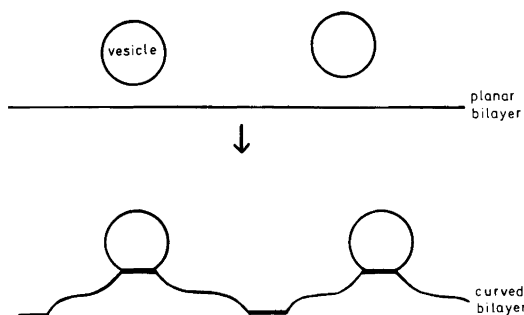to the membrane. We can also imagine a curved

Fig. 2. Schematic illustration of a cooperative fusion
process between a membrane and vesicles, induced
by a hypothetic phase transition from a planar to a
curved bilayer.

bilayer with a budding sac, which is cut off at a transition to a planar bilayer. These processes could correspond to pinocytosis and endocytosis. Although it is known that clathrin, coated pits and coated vesicles (cf. Ref.[12]) play an important role in these processes, we have only considered the lipid bilayer here in order to demonstrate how lipid phase transitions can contribute to these functions and how cooperativeness can be achieved. The ability of clathrin to form networks is certainly not enough to explain transitions between coated pits and coated vesicles.

Work by Helfrich and coworkers[20] indicates that the energy barrier is quite small against local formation of a curved bilayer structure between planar lipid bilayers in water. They have thus demonstrated that tube networks are formed during the swelling of egg lecithin in water, and the same type of networks have been identified from observations of fusion of liposomes[21]. These tube networks are in fact consistent with periodic minimal surfaces of the D-type.

In a recent study of junctions between cultured rat hepatocytes[22], it was observed that intramembrane particles (IMPs) in small gap junctions and in tight junctions occur synchronously in neighbouring cells. Cooperativeness of this kind should be expected if phase transitions are involved, and it seems very likely that IMPs are bilayer aggregates with the infinite periodic minimal surface type of structure. It should be pointed out that the structure of IMPs has been related to reversed micelles or the H_{II}-phase of lipids. In view of the present knowledge of minimal surface bilayer structures, however, they appear to be better candidates.

In a study of the luminal surface of the intestinal wall of an insect[23] a "double plasma membrane" was described. The observed ultrastructure indicates that the membrane is curved according to a periodic minimal surface. We have earlier pointed out[7] that the ultrastructure derived by Gunning[24] of the prolamellar body (a storage form of thylakoid membranes) is in perfect agreement with an infinite periodic minimum surface of the P-type. The surface layer of this membrane system is a two-dimensional minimal surface, as shown in Fig. 1, fused with the layer underneath.

This work continues with a theoretical characterization of two-dimensional periodic minimal surfaces.

References

1. Heuser, J. E. and Reese, T. E. *J. Cell biol. 57* (1973) 315.
2. Gulik, A., Luzzati, V., De Rosa, M. and Gambacorta, A. *J. Mol. Biol.* (1985). *In press.*
3. Schwarz, H. A. *Gesammelte Mathematische Abhandlung, Vol. 1,* Springer, Berlin 1890.
4. Schoen, A. H. *Nasa Technical Notes NASA TN D-5541,* 1970.
5. Andersson, S., Hyde, S. T. and von Schnering, H. G. *Z. Krist.* (1985). *In press.*
6. Lindblom, G., Larsson, K., Johansson, L., Fontell, K. and Forsén, S. *J. Am. Chem. Soc. 101* (1979) 5465.
7. Larsson, K., Fontell, K. and Krog, N. *Chem. Phys. Lipids 27* (1980) 321.
8. Longley, W. and McIntosh. J. *Nature 303* (1983) 612.
9. Larsson, K. *Nature 304* (1983) 664.
10. Hyde, S. T., Andersson, S., Ericsson, B. and Larsson, K. *Z. Krist.* (1985). *In press.*
11. Ericsson, B., Larsson, K. and Fontell, K. *Biochim. Biophys. Acta 729* (1983) 23.
12. Luzzati, V., Tardieu, A., Gulik-Krzywicki, T., Rivas, E. and Reiss-Husson, F. *nature 220* (1968) 485.
13. Luzzati, V. In Chapman, D., ed. *Biological Membranes,* Academic Press, London 1968, Vol. 1, p. 71.
14. Pearse, B. M. F. and Bretscher, M. S. *Ann. Rev. Biochem. 50* (1981) 55.
15. Israelachvili, J. N., Mitchell, D. J. and Ninham, B. W. *J. C. S. Faraday Trans. II 72* (1976) 1525.
16. Hyde, S. T. and Andersson, S. *Z. Krist.* (1985). *In press.*
17. Green, C. R. and Severs, N. J. *J. Cell Biol. 99* (1984) 453.
18. Bursztajn, S., McManaman, J. L. and Appel, S. H. *J. Cell Biol. 98* (1984) 507.
19. Buchheim, W. *Naturwissenschaften 69* (1982) 505.
20. Harbich, W., Servuss, R. M. and Helfrich, W. *Z. Naturforschn. 33a* (1978) 1013.
21. Glad, A. E., Broza, R. and Eytan, G. D. *Biochim. Biophys. Acta 556* (1979) 181.
22. Feltkamp, C. A. and van der Waerden, J. A. W. *J. Cell Sci. 63* (1983) 271.
23. Lane, N. J. and Harrison, J. B. *J. Cell Biol. 39* (1979) 353.
24. Gunning, B. E. S. *Protoplasma 60* (1965) 111.