CHAPTER 5

Physical Basis of Self-Organization and Function of Membranes: Physics of Vesicles

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1. Introduction

A continuous discussion between cell biologists and membrane biophysicists is whether lipids play an active role in biochemical membrane processes such as the formation of functional complexes (e.g., activation of adenylate cyclase by G-protein after hormone binding, cf. chapter 1) or whether they provide just an inert matrix of the right fluidity for the real functional entities: the proteins. Arguments for the former view are for instance:

- that the average lipid composition of cells but also the distribution of the various lipids among the cellular organelles are well preserved despite a rapid material exchange within the cell (cf. chapter 1);
- that deviation from the natural lipid composition may lead to severe health problems (such as the Tay–Sachs disease, cf. chapter 1).

Arguments for the latter view are that the activity of ion channels (such as band III) does not depend very critically on the lipid chainlength provided the bilayer is in a fluid state. The growth of cells (e.g., mycoplasts) requires that the lipid bilayer moiety is in a fluid state whereas the growth rate does not depend critically on the lipid chain structure [1]. This unspecific effect is also the basis of the adaption of the lipid composition of plant cells to the environment.

Whatever the answer, there is little doubt that nature was extremely clever by choosing lipids as basic building unit of membranes. Thus,

- Two-chain lipids associate in water at extremely low concentrations (∼$10^{-11}$ M) to form bilayer vesicles.
- The vesicles are extremely stable for instance if the bilayer undergoes fluid-to-solid phase transition as is shown in the examples of fig. 1a.
- Lipid bilayers are quite permeable for water but simultaneously rather impermeable for ions which allows the rapid establishment of the osmotic equilibrium of cells (as discussed in chapter 1) and renders bilayers simultaneously excellent insulating properties.
- The two-dimensional-fluid character of bilayers entails a large increase in efficiency of diffusion controlled processes and the lateral organization of membranes may be controlled externally (e.g., by the adsorption of proteins [2, 3]).
- Bilayers are extremely soft with respect to bending but essentially incompressible under lateral tension. It is this unique combination of elastic properties of the lipid bilayer which allows migrating cells (such as erythrocytes) to travel for several hundred km through narrow body channels without loss of ions [2].

The intention of this chapter is to review phenomenological physical properties of isolated lipid bilayers: namely vesicles. The first part deals with the structural dynamic and elastic properties of fluid and solid bilayer vesicles together with the
thermodynamics of the thermotropic phase transitions. In the second part the advantage of lipid bilayers as two dimensional solvent are described and the physics of lipid mixtures is discussed on a thermodynamic basis. In all parts we endeavour to point out how nature could exploit the exceptional physical properties of lipid bilayers to control the self-organization and function of cell membranes. Even more important is to show that lipid bilayers are exciting as model systems to explore the novel and rich physical properties of soft two-dimensional materials.

Main emphasis is laid on essential phenomenological physical properties of isolated vesicles. Concerning the molecular structure of membranes, recent reviews are now available [4–6]. Aspects treated in other chapters of this volume are only indicated.
2. Self-assembly and stability of lipid-bilayer-vesicles

One of the most important applications of the hydrophobic effect by nature for the design of living materials is the self-association of two-chain lipids occurring already at very small critical concentrations of association of the order of \((C^* \approx 10^{-12}\text{ M})\). This is a consequence of the well-known exponential dependence of \(C^*\) on the work (= chemical potential \(\Delta\mu\) ) required to transfer a lipid molecule from a bilayer (or a micelle) into the aqueous phase [7]

\[
C^* = c_0 \exp\{-\Delta\mu/k_B T\}, \tag{1}
\]

\(c_0\) is the concentration of the amphiphile in the aggregate (that is \(c_0 \sim 1\)), \(\Delta\mu\) is roughly proportional to the total area of the surface of the hydrocarbon chains and
thus to the chain length \( n_{\text{CH}_2} \). For one chain amphiphiles (e.g., fatty acids) the empirical rule

\[
\Delta \mu = (11 - 3n_{\text{CH}_2}) \text{ kJ/M}
\]

holds which allows to estimate the values of \( C^* \). Thus for lyso-DPPC one estimates \( C^* \approx 2 \times 10^{-5} \text{ M} \), compared to the measured value of \( 10^{-4} \text{ M} \), and for DPPC the estimate is \( C^* = 10^{-12} \text{ M} \).

The low lipid solubility can have severe biological consequences. The biological control requires that phospholipids and sphingolipids (e.g., gangliosides) are continuously synthesized and decomposed. The decomposition occurs by phospholipases in the case of phospholipids while for gangliosides another enzyme, hexosaminidase, is required. While phospholipases can pull out lipids from the bilayer (in particular in the presence of defects, cf. fig. 24) the hexosaminidases require associated helper proteins for this task. Failures in the structure of the latter by genetic mutations causes their desactivation and leads to the accumulation of the gangliosides in the lysosomes resulting in their swelling and causing the death of the infants within days (cf. [8]). At excess water the lipid bilayers form spontaneously closed shells exhibiting an astonishingly high stability with respect to shape changes (cf. fig. 1). Lipid aggregates which do not swell to form vesicles above their respective chain melting transition (in the older literature called Krafft point) in most cases do so by admixture of other lipids. Thus PE’s or sphingomyelins which tend to form compact micellar aggregates readily form vesicles by admixture of a few mole percent of PC’s. The formation of stable closed vesicles is a consequence of the unique elastic properties of the lipid films: (1) a very low bending stiffness (cf. fig. 13 below) (2) a high intrinsic lateral pressure (of some 30 mN/m) and (3) the possibility to create an intrinsic bending moment by introduction of a slight asymmetry (= spontaneous curvature, cf. fig. 2).

Vesicle formation can be described as an interplay between the energy required to bend the bilayer and the interfacial energy, \( \gamma \), associated with the formation of a large opening (pore) in the vesicle exposing the hydrophobic interior to water [9]. The total energy per unit area of the bilayer (of thickness \( d_m \)) can be expressed as

\[
g_{\text{el}} = \frac{1}{2} \kappa \left( \frac{2}{R} \right)^2 - 2\pi \gamma \frac{\rho}{4\pi R^2}, \tag{2a}
\]

where \( \kappa \) is the bending stiffness (cf. section 5), \( \gamma \) is the line tension along the rim of the pore of radius \( \rho \) and \( R \) the radius of curvature of the piece of bilayer. The equilibrium vesicle radius (cf. section 5), \( \gamma \) is the line tension along the rim of the pore of radius \( \rho \) and \( R \) the radius of curvature of the piece of bilayer. The equilibrium vesicle radius \( R_0 \) is determined by the minimum of \( g_{\text{el}} \) with respect to \( R \). Assuming \( \rho \approx R \) one finds

\[
R_0 \approx 8\kappa / \gamma; \tag{2b}
\]

\( \gamma \) can be estimated from the hydrophobic chemical potential defined in eq. (1) by assuming that half of the surface of the lipid chains is exposed to water. For DMPC,
eq. (1) yields $\Delta \mu \approx +5 \times 10^{-20}$ J per molecule or $\gamma \approx 5 \times 10^{-11}$ N. With $\kappa \approx 10^{-19}$ J one obtains $R_0 \approx 10$ nm. This corresponds to the average radius found after extended sonification.

For a given bending stiffness, the vesicle radius and stability can be controlled by the line tension ($\gamma$). It can be drastically reduced by fatty acids forming a micellar cap at the openings, thus preventing the access of water to the hydrophobic chains. Indeed large pores and even disc shaped bilayers can be formed by cholates [9b].

Fig. 2. Illustration of the creation of intrinsic bending moments by a gradient in the lateral pressure, $\Pi(z)$ across the bilayer. The spontaneous curvature is determined by the interplay of the trans-bilayer pressure gradient in the hydrocarbon chain and headgroup region.

The most subtle and versatile control is achieved by introduction of a spontaneous curvature $c_0 = 1/R_0$ reducing the bending energy to $g_{el} = (1/2)\kappa (2/R - 2/R_0)^2$. $c_0$ can be adjusted by any lateral pressure gradient $d\Pi/dz$ in the direction of the membrane normal (denoted as $z$) which causes a bending moment

$$M_0 = \int_{-d_m/2}^{+d_m/2} z\pi(z)\,dz$$

resulting in a spontaneous curvature $c_0 = M_0/\kappa$ as is well known from the elasticity of shells (cf. [10], vol. VII, § 12). The composition of the phospholipids from two independent parts (the chain and the headgroup) is ideally suited to vary the spontaneous curvature of bilayers in many ways; in particular by adsorption of proteins or by changes in the surface charge.
3. Polymorphism and structural phase transitions of lipid bilayers

Most spectacular and fascinating from the physicists point of view are the structural phase transitions of isolated lipid bilayers and monolayers (also denoted as thermotropic polymorphism). The best studied examples are the diacyl-phospholipids for which many data have been collected since the pioneering work of Luzatti. They exhibit the richest behaviour found yet: one fluid phase denoted as $L_\alpha$-phase (or smectic A) and three crystalline phases denoted as $P_\beta'$, $L_\beta'$ and $L_c$-phases (cf. figs 3–5). Owing to a high degree of disorder (caused by frustrations) the two former solid phases are also denoted as gel-phases. In principle fluid phases with tilted chains are also possible (corresponding to smectic C phases of thermotropic liquid crystals). Potential examples are dioleyl (= C18:1) -phospholipids and polymerizable lipids containing butadiene and diacetylene groups within the chain (cf. [12]).

3.1. Thermodynamic properties of structural phase transitions

The thermodynamic and structural properties of the lipid bilayers and monolayers have been studied by most physical techniques available. Besides the traditional methods as calorimetry (cf. fig. 3) and X-ray diffraction, the most powerful methods are NMR- and FTIR-spectroscopy [13]. In cases where the phases exhibit some surface texture the lipid phases can be well characterized by freeze fracture EM. This technique gives simultaneously insight into the defect structure of the crystalline phases which is essential for the stability of vesicles in the gel state. Examples are shown in fig. 6.

The essential features of the bilayer polymorphism may be summarized as follows:

1) The transitions between all four phases are of first order in bilayers but may become of second order at high packing densities such as monolayers under high pressure (cf. Landau theory of chain melting transition, below). The first order character of the main and pre-transition is for instance clearly seen from NMR measurements of the vesicle suspension as shown in fig. 4a.

2) The $L_\alpha \rightarrow P_\beta'$-transition is associated with the transition of the hydrocarbon chains from a disordered to an essentially all-trans state and it is therefore also called chain melting or main transition. The $P_\beta' \rightarrow L_\beta'$ (solid-solid) transition is often denoted as pre-transition. It is generally found for lipids with large head groups such as PC as well as single charged (PG$^-$) or two fold charged lipids (PA$^{2-}$). The so called sub-transition $L_\beta' \rightarrow L_c$ is kinetically hindered and its formation may require days (cf. ref. [2, 14]). Figure 4a shows that the formation of the low temperature solid phase $L_\beta'$ from $P_\beta'$ may be kinetically hindered as a consequence of defect healing.

3) The transition temperatures of all three transitions of diacylphospho-lipids increase monotonically with chain length (cf. fig. 3). This is the consequence...
Fig. 3a. Summary of transition temperatures of main transition of diacyl-phospholipids namely phosphatidyl cholines (PC), phosphatidyl ethanolamines (PE), phosphatidyl serine (PS). Simultaneously shown is the pre-and sub-transition of PC as well as the variation of the transition temperature of the C14:0-PE after gradual methylation of the NH$_3^+$ group leading to PC (points ⊗).

Fig. 3b. Variation of chain melting temperatures of dioctadecenoyl-PE (C18:1-PC) bilayers with position of double bond within the chain. Most common is the oleate-chain with the double bond at C$_9$–C$_{10}$.

Fig. 4. a) Observation of the $L_\alpha \rightarrow P_{\beta'}$ (at the temperature $T_m$) and $P_{\beta'} \rightarrow L_{\beta'}$-transition (at $T = T_p$) of DPPC bilayers by $^2$H-NMR. The phase transitions are revealed by abrupt changes in the quadrupolar-splitting of the $-\mathrm{N}^+(\mathrm{CD}_3)_3$-group signal. Note the broadening of the $P_{\beta'} \rightarrow L_{\beta'}$-phase change at decreasing temperature which is a consequence of defect healing $T_m$. b) Visualization of main- (at $T_m$) and pre-transition (at $T_v$) of DMPG by temperature dependence of specific heat.

of a remarkable incremental increase of the heats, $\Delta H_t$, and entropies, $\Delta S_t$, of transition with each additional CH$_2$-groups. Thus, for the main transition of the diacyl-phosphatidylethanol-amines, the incremental variations are

$$\frac{\Delta H_t}{\Delta n_{\text{CH}_2}} = 4.5 \pm 0.5 \text{ kJ M}^{-1}; \quad \frac{\Delta S_t}{\Delta n_{\text{CH}_2}} = 12 \pm 2 \text{ J M}^{-1} \text{ K}^{-1}.$$ (4)

Since the transition temperature is related to the two quantities by

$$T_t = \frac{\Delta H_t}{\Delta S_t},$$ (5)
it increases monotonically with the chain length. The incremental increases of $\Delta H_i$ and $\Delta S_i$ (and therefore $T_i$) are remarkably similar for PC's and PE's showing that the chain melting transition is mainly determined by the cohesion of the hydrocarbon chains. It is for that reason that the molecular statistical theories based on the consideration of the chain configurations alone works so well (cf. chapter 7 and [15]).

4) It is often helpful to realize that the main transition temperature $T_i$ increases with increasing packing density of the lipid molecules. This rule of thumb explains the strong increase of $T_m$ when lipid multilayers are dehydrated (cf. ref. [6]) or it is often helpful to estimate the effect of charges as will be shown below.

5) The introduction of a non-saturated bond in the hydrocarbon chains has dramatic effects on the chain-melting transition. Depending on the position of the double bond, $T_m$ may be suppressed between 1°C and −60°C as has been demonstrated for bilayers of dioctadecanoyl-PC (C18:1-PC) (cf. fig. 3b). The largest depression of −60°C is observed for the double bond in the center of the chains.

6) The $\text{L}_\alpha \rightarrow \text{P}_{\beta'}$ transition may be suppressed if the escape of the membrane into the third dimension is prevented. This can occur by deposition of bilayers onto solid substrates such as glass beads [23b]; or in a more controllable way by suction of vesicles into glass-pipettes [17].

3.2. Structure and surface topology of crystalline vesicles

As noted above the solid phases are often called gel-states since they may be strongly disordered as a consequence of a rich defect structure. This is attributed to the frustration caused (1) by the different packing constraints of the hydrocarbon chains and the head groups (as shown in fig. 5 and fig. 20 below) and (2) the strongly hindered rotation of the fatty acid chains about the carboxyl bond (cf. ref. [18]).

In the $\text{P}_{\beta'}$-phase the hydrocarbon chains form a triangular lattice with a lattice constant $a = 0.42$ nm [2]. The long axes of the chains are tilted with respect to the local plane of the membrane by about 30 degrees. The most remarkable feature is the corrugated surface profile also called ripple structure (hence the name ‘ripple phase’). The freeze fracture electron micrographs of the $\text{P}_{\beta'}$-phase reveal a beautiful wave-like texture (fig. 6a). Indeed, two coexistent ripple structures are observed: the $\Lambda$-phase and the $\Lambda/2$-phase which differ in wavelength by a factor of two. The former consists of roof-like ripples exhibiting mirror symmetry with a depression in the center while the $\Lambda/2$-phase exhibits a saw-tooth (that is polar)-profile with a ratio of the projected widths of $a : b = 3$. The $\Lambda$-texture can be generated from $\Lambda/2$ by rotation of every second ripple by 180 degrees. As fig. 6a shows, the two textures are clearly distinguished by their defect structure. The texture of the $\Lambda$-phase exhibits disclinations originating in point defects ($-1/2$ and $+1/2$ in fig. 6a) which clearly show that the chains are not tilted with respect to the average membrane plane. In contrast, the $\Lambda/2$-phase exhibits Néel-type walls along lines where two sawtooth-domains of opposite polarity meet (cf. reference [20] for detailed discussion).
Fig. 5. Schematic view of structure, orientation (a) and crystal lattices (b) of hydrocarbon chains of the fluid and the various crystalline phases of diacyl phospholipids. Note that $L_{\beta'}$ and $P_{\beta'}$ exhibit tilted hydrocarbon chains with respect to local plane of membrane, while the chains in $L_\alpha$ and $L_c$ are oriented in the normal direction. In $P_{\beta'}$ and probably $L_{\beta'}$ the chains form a triangular lattice (b) and in the $L_c$ an orthorhomic 2D-lattice (c).

The $\Lambda$-phase prevails in small vesicle while the $\Lambda/2$-phase is most abundant in giant vesicles ($\gtrsim 10 \, \mu m$) or multilamellar liposomes. The stabilization of the $\Lambda/2$-phase with decreasing overall curvature of the vesicles has been justified theoretically by the bilayer elasticity theory [20].

The $\Lambda/2$-phase is stabilized by even small concentrations ($\sim 1\%$) of solutes (cf. fig. 28 and [19]). The hydrocarbon chains of the lipids within the Néel-walls and along the depressions in the center of the $\Lambda$-ripples are substantially disordered. These defects provide fluid paths for rapid lateral diffusion of solutes similar to short-circuit diffusion in dislocation cores of ordinary 3D-solids (cf. [2b]).

In the $L_{\beta'}$- and $L_c$-phases the hydrocarbon chains form an orthorhombic lattice (sometimes called distorted hexagonal, [14]). The chains are tilted in the $L_{\beta'}$-phase but point in the direction normal to the plane of the membrane in the $L_c$-phase (cf.
Fig. 6a. Characterization of $P_{β'}$, $L_{β'}$ and $L_{c}$-phases by surface texture of freeze fracture electron micrographs. a) $L_{β'}$-phase of DPPC-vesicle exhibiting corrugated surface with coexistence of two types of ripple structures: the saw-tooth like $A/2$-phase (with wavelength of 12.3 nm) and the symmetric $A$-phase (wavelength: 25 nm). Note that the textures of the two phases exhibit defects of different symmetry: namely Néel-walls in the case of the $A/2$-phase and simple disclinations in case of the $A$-phase [19]. Middle trace: Reconstruction of surface profile by analysis of platinum layer thickness following the Erastosthenes technique (for references cf. [21b]). Bottom trace: Schematic structure of $A$- and $A/2$-phase.
Fig. 6b,c. Characterization of $P_{β′}$, $L_{β′}$ and $L_c$-phases by surface texture of freeze fracture electron micrographs. b) Surface profile of $L_{β′}$-phase exhibiting a spiral-type defect. These are supposed to be caused by breaks in the bilayer. Note the (Chinese) hat-like surface profile in the center of the spiral which is due to escape of bilayer into third dimension; thus minimizing the elastic energy of the defect. Bottom trace exhibits schematic structure of bilayer along section a–b showing mutual shift of bilayer in normal direction. c) Smooth surface texture of $L_c$-phase with residual defects.
fig. 5). As shown in fig. 6b, the tilt of the Lβ′-phase leads again to a characteristic surface texture of the freeze fracture micrographs. The irregular ripple texture is however due to ‘breaks’ along which the bilayers are mutually shifted in the normal direction. Most characteristic is the spiral-shape of these breaks. Moreover surface profile reconstructions by the ‘Erasosthenes technique’ [21b] shows that the bilayer escapes into the third dimension at the origin of the spiral, forming a Chinese hat. In this way the energy of the defects caused by chain tilting is minimized [21].

3.3. Pertinent properties of the Lα-phase

A). Lipid structure and thickness parameters

The Lα-phase is completely disordered. The area per molecule is of the order of $A = 0.65 \text{ nm}^2$ but varies with chain length. As shown in fig. 7a, the two chains of the lipids are mutually shifted so that one carboxyl bond is closer to the aqueous phase than the other. Water can penetrate rather deeply into the hydrocarbon region and it is therefore not possible to define a sharp interface between the polar and apolar region of the bilayer. Figure 7b shows the positions of some selected protons of DPPC as determined by neutron diffraction [22] and neutron surface scattering [23]. As illustrated in fig. 15a (below) the carboxyl groups are expected to create a localized electric double layer rather deep within the hydrophobic domain.

It is important to realize that the lipid molecule can also assume an expanded state where both carboxyl bonds are at about the same vertical position [23a]. This structure could for instance be assumed in the expanded fluid phase of lipid monolayers.

B). Equation of state of the Lα-phase

Area (A) to lateral pressure ($\pi$)-diagrams (the equation of state) can be measured for monolayers by film balance experiments, albeit not for vesicles. The total internal lateral tension $\pi$ (e.g., of an osmotically swollen spherical vesicle) is determined by the interfacial energy (per unit area), $\gamma$, of the interaction of water with the bilayer surface and the (repulsive) interaction energy (per unit area), $\delta$, between the hydrocarbon chains [6, 24]

$$\pi = \gamma - \delta.$$  \hspace{1cm} (6)

In a flaccid vesicle the bilayer is not subjected to tension and the two contributions cancel each other. In other words the total free energy of the bilayer is a minimum with respect to the area. Unfortunately, neither $\gamma$ nor $\delta$ are known. The interfacial energy has been estimated from the hydrophobic energy density [6] suggesting $\delta = \gamma = 35 \text{ mN/m}$. Interestingly, this value agrees well with the lateral pressure $\pi$ at which monolayers of diacyl-PC exhibit the same chain melting transition temperatures as monolayers: Thus, $T_m = 23^\circ C$ for DMPC-monolayers at 33 mN/m [25]. This is certainly an upper limit for $\delta$ since it ignores the cohesion energy between the monolayers which reduces. As shown below, application of the Landau theory suggests a lower value $\delta = 20 \text{ mN/m}$. 
Fig. 7. a) Likely structure of phospholipids in condensed (left) and expanded phases (right). Note that left structure implies that carboxyl bond penetrates some 0.5 nm into hydrophobic part resulting in a large dipole moment (cf. also fig. 15). The expanded structure is possibly assumed in the expanded fluid phase of the lipid monolayers at air water interface [23a]. b) Position of various protons of DPPC measured from methyl end (in Å) and apparent thicknesses of hydrophobic core in L_α\textsuperscript{–}-phase as determined by neutron diffraction [22] and neutron surface scattering [23].

C). Area expansivity
The thermal expansion coefficient of the L_α-phase is anisotropic. The volume expansivity is

$$\alpha_0 = \alpha_\perp + \alpha_\parallel \approx 3 \times 10^{-3} \text{ K}^{-1},$$

where $\alpha_\parallel$ is the area and $\alpha_\perp$ is the thickness expansion coefficient. Values of $\alpha_\parallel$ have been determined for many lipids by the micropipette aspiration technique [17]. They lie in the range $1 \times 10^{-3} \leq \alpha_\parallel \leq 6 \times 10^{-3} \text{ K}^{-1}$ (e.g., $\alpha_\parallel \approx 6 \times 10^{-3} \text{ K}^{-1}$ for DMPC bilayers at 30°C). The transverse coefficient has been measured by neutron [26] and X-ray diffraction [6] and is negative: $\alpha_\perp = -3 \times 10^{-3} \text{ K}^{-1}$ for DMPC. Together with the in-plane (lateral) compressibility moduli, $K$, of the lipids these data allow to estimate the change in lateral tension $\pi(T)$, within spherical vesicles at increasing the temperature by $\Delta T$:

$$\pi(T) = \pi(T_0) - K\alpha_\parallel \Delta T.$$  \hspace{1cm} (7)
The most important message of this equation is that spherical and non-spherical (that is flaccid) vesicles may behave quite differently. Consider for instance the reversion of phase separation in bilayers of lipid mixtures during heating. Only in flaccid vesicles with large excess area one would run along an isobar of the $\Pi - T$ composition phase diagram. In spherical vesicles the melting of crystalline domains is impeded by the increase in lateral tension.

D). The order parameter
The hydrocarbon chain order parameter is one of the important quantities to characterize the state of the $L_\alpha$-phase. It is measured by deuterium NMR-experiments (cf. Bloom et al. [4] and Seelig [33] for details and relevant literature) and ESR-spectroscopy using spin-labelled lipids as spectroscopic probes (cf. [2a] and [27]). With both methods one can measure the average orientation of the nth CH$_2$-segments along the chain which is expressed by the classical liquid crystal order parameter

$$S_n = \frac{1}{2} \langle 3 \cos^2 \vartheta_n - 1 \rangle,$$

where $\vartheta_n$ is the angle between a local axis (such as the C–C or C–H-bond) and the membrane normal, $\langle ... \rangle$ denotes the ensemble (or time average) over all thermodynamically accessible configurations of the chains. An average order parameter can be defined according to

$$\langle S \rangle = \frac{1}{N} \sum_{n=1}^{N} S_n,$$

where $N$ is the number of CH$_2$-groups.

The two methods yield different results; not only because of the perturbation introduced by the spin probe but also since one averages over different time scales (cf. [27] for a discussion).

The most remarkable finding of both types of experiments is that $S_n$ exhibits a plateau for the first eight CH$_2$-groups following the COO-group and falls then off rapidly by a factor of five towards the terminal methyl group (cf. fig. 8 and [4]). This behaviour is a consequence of the motional restrictions of the chain segments at the carboxyl end [18] and is an important test for the validity of molecular statistical theories (cf. reference [15, 16]) or Monte Carlo simulations of the hydrocarbon chain configurations. It is particular useful in order to select the most important chain configurations such as in the Pink model [28]. Below we will introduce another way to characterize the chain order in terms of the mean square displacements of the CH$_2$-segments which can be measured by incoherent quasielastic neutron scattering [34].

3.4. Landau theory of $L_\alpha \rightarrow L_\beta$-phase transition
Many features of the bilayer phase transitions, properties of the $L_\alpha$-phase or effects of solutes (e.g., proteins) can be explained in terms of mean field statistical theories [15,
In order to understand more subtle properties associated with fluctuation effects more refined models are required. The most extensive studies are based on Monte Carlo simulations based on the so-called Pink- model which takes into account the ten most important conformational states. These efforts have been extensively reviewed [4, 28].

In order to get a more direct and intuitive insight into the physics of the phase transition or pretransitional effects it is often more helpful to apply the classical Landau theory [25, 29]. It is also traditionally applied in order to consider (1) the effect of local order fluctuations on the thermodynamic and other physical properties or (2) the lipid mediated protein-protein interaction. The chain order in the bilayer can be characterized in several ways:

- The orientational order of the hydrocarbon chains determined by the average over the segmental order parameter, $S_n$, defined in eqs (8) and (9). This order parameter serves as basis for the simple mean molecular field theories and was used to describe (1) the chain melting transition of lipid monolayers [25], (2) pre-critical phenomena and (3) the effect of proteins on the bilayer phase transitions [29].
- In order to account for the large change in lipid density at the phase transition (15–20%) or for the local deformation of the lipid bilayer by insertion of proteins (cf. fig. 32) the lateral packing density $\rho(\vec{r})$ must be considered as additional order parameter [25]. It is related to the chain order parameter
by $\rho \propto S^2$ (cf. reference [29]). Alternatively, the reduced area per molecule defined as

$$\eta(\vec{r}) = \frac{A(\vec{r}) - A_f}{A_f - A_c},$$

(10a)

can be introduced [30]. $A(\vec{r})$ is the area of the perturbed membrane. $A_f$ and $A_c$ are the areas (per molecule) of the fluid and of the solid phase, respectively. $A(\vec{r})$ is assumed to depend on the lateral position within the membrane in order to account for local fluctuations.

In order to characterize the collective tilt of the lipid chains (as in $P_{\beta'}$ or $L_{\beta'}$-phases or about proteins inducing a local tilt) a tilt order parameter

$$\Omega(\vec{r}) = \theta(\vec{r}) - \theta_0$$

(10b)

is introduced where $\theta(\vec{r})$ is the tilt angle – between two phases (e.g., $L_{\alpha}$ and $L_{\beta}$) or characterizes the local variation of the chain tilting about a protein. As suggested by de Gennes, the tilt and lateral packing density can also be combined in a stretching vector (cf. Albrecht et al. [25]).

A consistent description of the phase transition and structural changes with temperature or lateral pressure is achieved by the famous Landau expansion of the free energy (per unit volume) in terms of powers of the relevant order parameter $\eta$

$$G(\eta) = G_0 + \sum_n A_n(\Pi, T)\eta^n + \frac{1}{2} \beta(\Pi, T)(\nabla \eta)^2;$$

(11)

where $G(\eta)$ is the free energy of the phase of lower symmetry ($L_{\beta}$ or $P_{\beta'}$) and $G_0$ that of higher symmetry ($L_{\alpha}$). The gradient term is essential if local variations in the lipid order are considered. It accounts for the interfacial energy at interfaces between two phases (for instance for the elastic deformation energy arising if the two phases exhibit different bilayer thicknesses). Its effect is to smear out such interfaces. As is excellently described in the classical textbook of Landau and Lifshitz (ref. [10a], § 143–145) the phase transition is of 2nd order if the power expansion contains only quadratic and quartic terms. In order to account for first order transitions, additional cubic ($A_3 \neq 0$) and/or 6th order contributions ($A_6 \neq 0$) are required. Only in this case the free energy-versus $\eta$ curve can exhibit a double minimum. The two minima of $G(\eta)$ at $\eta = 0$ and $\eta = \eta_0$ are separated by a maximum of $G$ and the transition is discontinuous (cf. fig. 9a).

To obtain a phase transition it is supposed that the 2nd order term changes from a positive to a negative value at some temperature $T^*$ and it is useful to replace $A_2$ in eq. (11) by

$$A_2 = a(T - T^*).$$

(12)

Two situations are possible:
Fig. 9a. Free energy curve of Landau expansion eq. (11) composed of contributions of 2nd, 4th and 6th order. It is shown in order to demonstrate the existence of two characteristic temperatures: $T_t$ at which the minima of the two states exhibit equal heights and $T^*$ at which the activation mountain between the two states has just vanished. For $T^* > T_t$ the transition is of 2nd order. It is of first order if $T_t$ is above $T^*$ as shown in the example.

Fig. 9b. Variation of lateral lipid packing density of monolayer of DMPC with temperature for three lateral pressures: 12.5 mN/m, 22.5 mN/m and 30 mN/m. Data obtained from isobars in reference [25]. Note the narrowing of the transition regime (characterized by straight line $m - m'$) with increasing pressure indicating the transition to a tricritical point where the chain melting transition becomes of 2nd order.

- If $A_4 > 0$ one has only one minimum at $\eta = 0$ for $T > T^*$, which changes smoothly to $\eta_0 = (A_2/2A_4)^{1/2}$ for $T \leq T^*$. The transition is clearly continuous.
If $A_4 < 0$ one has two minima and (as depicted in fig. 9) there exist two characteristic temperatures $T_c$ and $T^*$. The phase transition occurs at the temperature $T = T_c$ where the minima exhibit equal heights. Since the states $\eta = 0$ and $\eta = \eta_0$ are separated by an activation well the transition is of first order. Stability requires a 6th order term with $A_6 > \sigma$.

Consider now the lipid phase transition. In order to account for the change in density and tilt of PC-bilayers the free energy difference between the $\alpha$ and $\beta$-phase can be divided into two parts (ignoring gradient terms) [25]:

(1) a power expansion in terms of the difference, $\rho = \rho_\alpha - \rho_\beta$, in lateral packing density between the two phases.

$$\Delta G_\rho(\Pi, \rho, T) = \frac{1}{2} a(T - T^*)\rho^2 + \frac{1}{4} A_4(\Pi, T)\rho^4 + \frac{1}{6} A_6(\Pi, T)\rho^6.$$  \hspace{1cm} (13)

(2) a contribution accounting for the change in tilt angle $\theta$ and the coupling between chain tilting and packing density $\rho$

$$\Delta G_\theta(\pi, \theta, T) = \frac{1}{2} K_{11}(\theta_\beta - \theta_\alpha)^2 + t(\pi, T)\rho^2(\theta_\beta - \theta_\alpha).$$ \hspace{1cm} (14)

$K_{11}$ is the orientational or splay elastic constant of the bilayer. It is related to the membrane bending modulus $\kappa$ as $K_{11} = \kappa/d_m$, where $d_m$ is the bilayer thickness. (Note that the energy per unit area is obtained by replacing $\kappa$ for $K_{11}$).

The 2nd term on the right side of eq. (14) accounts for the coupling between tilt and density and it is an adjustable parameter. The key step is to express the tilt angle difference in terms of the density order parameter $\rho$ by minimizing the total free energy $\Delta G = \Delta G_\rho + \Delta G_\theta$ with respect to $\theta_\beta$. A simple calculation yields [25]

$$\Delta G(\pi, T) = \frac{1}{2} a(T - T^*)\rho^2 + \frac{1}{4} (A_4 - 2t^2/K_{11})\rho^4 + \frac{1}{6} A_6\rho^6.$$ \hspace{1cm} (15)

From this expression we gain the following insights into the nature of the phase transition:

1) Both $K_{11}$ and $t$ are functions of the lateral pressure $\pi$. It is obvious that with increasing pressure, $K_{11}$ increases. In contrast, the coupling parameter $t$ (between tilt and density) is expected to decrease, since the density change at the transition decreases with increasing packing density (of the $L_{\alpha}$-phase). The resulting decrease of the new coefficient of the 4th order term with increasing pressure has the following consequence: At small $\pi$, $\Delta G(\pi, T)$ is dominated by the 4th order contribution. It is negative and according to the above rules the 6th order term, $A_6$, comes into play and the transition, is of first order. At large $\pi$-values, the 4th order term becomes positive and $\Delta G$ is determined by the 2nd and 4th order terms. Therefore the transition becomes 2nd order. This change in order has indeed been observed in monolayer phase transitions [25].
which led to the above model. Similar cross-overs from 1st to 2nd order are observed in nematic-smectic A or the paramagnetic-ferromagnetic transition where it is called Rodbell–Bean effect (cf. [25] for references). It is important to realize that due to the change from a random to a hexagonal packing of the hydrocarbon chains at the $L_{\alpha} \rightarrow L_{\beta}$-transition, the critical point is tricritical.

2) The advantage of the Landau theory is that the expansion coefficients can be related to measurable quantities. If the pressure is high enough and the transition (at $T_c$) is of 2nd order, the ratio $a^2/A_2$ is obtained from the jumps in specific heat, $\Delta C$,$\pi$, according to

$$a^2/A_2 = \frac{2\Delta C_{\pi}}{T_c}.$$  

In monolayers studies, $a^2/A_2$ is obtained from the observable jumps of the thermal expansion coefficients ($\Delta \alpha$) or the lateral compressibility modulus ($\Delta K$) (cf. [25] for a more detailed discussion).

3) Provided the coefficients $A_2$, $A_3$ and $t$ are only weakly temperature dependent, the coefficient $a$ is directly related to the heat of transition, $\Delta H_t$, [25] according to

$$a = \frac{\delta \Delta G(\pi, T)/\delta T}{2\Delta H_t/(T_m \Delta \rho_t^2)}.$$  

$\Delta H_t$ is obtained from the density jump, $\Delta \rho_t$, at the transition and can be measured in the monolayer experiments. Note that $\Delta H_t$ should be large and weakly temperature dependent for low lateral pressures (large density jumps) but approach zero at high $\pi$ (where the transition becomes more 2nd order). This prediction has also been verified in [25].

4) By equalizing the jumps of the molecular areas at the chain melting transitions of bilayers ($\Delta A \approx 1$ nm$^2$ for DPPC) and monolayers one arrives at the conclusion that the intrinsic pressure of bilayers is $\pi = 20$–30 mN/m [25]. However, the transition temperature of the DPPC monolayer at this pressures is much lower (30–35°C) than that of the bilayer ($T_m = 42^\circ{}C$). This discrepancy can be explained by the Landau theory in terms of the additional adhesion energy (per unit area) ($G_{adh}$) which shifts the transition of bilayers to higher temperatures [25].

4. Molecular dynamics of bilayers

As already noted, vesicles in the fluid state are extremely soft (hyperelastic) shells. Their dynamical behaviour is determined by a whole hierarchy of thermal excitations (cf. fig. 10) with correlation times ranging from $10^{-11}$ s (characteristic for the jump frequency of defects along the chain) to the order of seconds (corresponding to the long wave length bending undulations of the bilayer). The transitions between conformational states of the chains are actually characterized by a broad spectrum of correlation times ranging from the above mentioned $10^{-11}$ s to $10^{-8}$ s as suggested
by NMR T1-measurements (see references [4] and [32]). The best studied dynamical process is, however, the lateral diffusion which has been measured by many techniques in particular by fluorescence methods and more recently by NMR (cf. [4] and [35c] for references). In view of the important role of this dynamic process in biomembranes it will be treated in chapter 6.

A powerful technique for the exploration of molecular motions in complex fluids is the incoherent quasielastic neutron scattering (QENS). This technique has only recently been applied to membrane physics (cf. König et al. [34]) although it offers many advantages:

- Both correlation times and the mean square displacements of the different motions can be measured.
- The motion of selected parts of the molecules can be observed by deuteration of the other moiety since deuterons contribute very weakly to the incoherent scattering intensity.
- Using ordered multilamellar systems, the direction of momentum transfer with respect to the membrane normal can be selected in order to distinguish between different types of motion (e.g., lateral or rotational diffusion).
- By choosing different instruments the time window can be selected in order to probe different time regimes of a given type of motion.

The quantities measured as a function of the scattering wave vector $Q$ are:

1) The elastic incoherent structure factor (EISF) which gives information on the spatial extension of the motion and shows whether it is restricted (like diffusion in a box) or free (like lateral diffusion).

2) The line width $\Delta \omega_Q$, which yields the correlation time of the dominant motion selected.

Fig. 10. Hierarchy of motions in membranes comprising 1) chain conformational transitions and chain defect motions; 2) rotational diffusion of lipid molecules about long axes; 3) in-plane lateral diffusion which is composed of local diffusion in solvent cage and jumps between different sites; 4) head group motion; 5) out-of-plane vibrational motion of center of mass of lipid; 6) collective undulation of the bilayer. The latter three processes define the dynamic surface roughness of the bilayer.
In practice the two quantities (EISF, $\Delta \omega Q$) are obtained by computer simulation of the measured dynamic structure factor, $S(Q, \omega)$ using a priori information of the dominant motional process. Usually $S(Q, \omega)$ is expressed as a truncated series expansion of Fourier lines, the zero order term of which is the EISF [34].

4.1. Softness of the gel phase

The example of fig. 11b shows the EISF-versus-$Q$ plots of partially hydrated DPPC-multilayers (13% water) for temperatures below and above the chain melting transition which occurs at $T_m = 56^\circ C$. The time-of-flight instrument monitors motions with correlation times smaller $10^{-10}$ sec and the EISF of fig. 11b is thus dominated by the conformational transitions of the hydrocarbon chains. Above the chain melting transition the EISF decays rapidly with increasing $Q$ to a constant value at $Q \sim 1.5 \AA^{-1}$, which defines the range of the diffusion of the CH$_2$-segments. It corresponds roughly to the lateral displacement of the chains by a gtg-kink. Most remarkable is, however, that even in the gel-phase, the EISF decays remarkably fast for $Q \rightarrow 1.5 \AA^{-1}$, demonstrating an astonishingly high degree of freedom for short range motions, most probably diffusion of low order chain defects (e.g., gtg-kinks) along the chain.

4.2. Dynamic measure of chain order

The incoherent QENS measurements allow an alternative description of the chain ordering than the NMR. It is characterized quantitatively in terms of the mean square displacements ($\Delta x_i^2, \Delta y_i^2$) of the segments (cf. fig. 11a). Each segment exhibits an essentially isotropic diffusion coefficient $D_i = \Delta x_i^2 / 2 \tau_i$ [34]. Due to the coupling of the segments the jump time $\tau_i$ is essentially constant and $D_i \propto \Delta x_i^2$. To a first approximation, the data suggest a linear increase of the lateral mean square amplitudes ($\Delta x_i^2$) and thus of the diffusion coefficients $D_x$ from the carboxyl to the methyl end. $\Delta x_i$ ranges from $\Delta x_{min} = 0.03$ nm to $\Delta x_{max} = 0.6$ nm for fully hydrated DPPC (≈ 23% water) in the L$\alpha$-phase (60°C). In fact, this linear increase of the lateral displacement is completely equivalent to the plateau behaviour of the NMR-order parameter (cf. chapter 7). The very small value of $\Delta x_{min}$ shows that on the time scale of the time-of-flight instrument ($10^{-11}$ s) the center of mass of the lipid molecules is laterally practically fixed.

The measurements give also new insight into the structure and dynamics of the gel-phase. The number and mobility of these chain defects increase continuously if the L$\beta'$-phase is heated from low temperatures ($\sim 0^\circ C$) through the chain melting transition. Only long wavelength motions (with $Q < 1.9 \AA^{-1}$) show a discontinuity at the transition [34]. The softening of the gel phase has also been observed by Raman scattering experiments which provided some evidence for soft mode behaviour of the L$\beta' \rightarrow$ L$\alpha$ transition [18].

4.3. Local and delocalized lateral diffusion: the free volume model

The evaluation of the dynamics structure factor in the $10^{-8}$–$10^{-10}$ sec time regime (back scattering spectrometer) shows clearly that the in-plane motion of the whole
Fig. 11a. Characterization of dynamics of chain conformations in terms of mean square displacement of chain segments in horizontal and normal direction (∆x_i^2, ∆y_i^2). Note that the segment motions are not independent and the chain motion can be roughly characterized by a segment-dependent diffusion coefficient, D_i and a roughly segment independent correlation time, τ, thus that 2τD_i = ⟨∆x_i^2⟩. Thus D_{x,i} and D_{y,i} increase from the head groups to the chain end [34].

Fig. 11b. Scattering wave vector dependence of the elastic incoherent structure factor (EISF) of DPPC multilayers (at water content of 13% by weight) measured for temperatures ranging from 2 to 60 °C (note symbols in insert). The L_α → L_β'-transition temperature is T_m = 56 °C. Note that complete immobilization would correspond to horizontal line with EISF = 1. The remarkable decay of the EISF at Q ≈ 1.5 Å in the gel-phase shows that the chains exhibit a remarkable freedom of short range motions in the gel-phase; in particular determined by diffusional jumps of gtg-kinks along the chain.
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lipids consists of two parts: a local diffusion within their solvent cage (= cylinder)
and the well known long range diffusional jumps between adjacent sites. The corre-
sponding diffusion coefficients ($D_{\text{loc}}$ and $D_{\text{lat}}$) for DPPC at 12 wt% water and 60°C are

$$D_{\text{loc}} = 2 \times 10^{-7} \text{ cm}^2/\text{sec}, \quad D_{\text{lat}} = 1 \times 10^{-7} \text{ cm}^2/\text{sec}.$$ 

The value of $D_{\text{lat}}$ agrees well with other measurements ([32b, 35]). Superimposed
to this motion of the center of mass one has the diffusion of the chains which is
characterized by a maximum value of $D_{\text{chain}} = 6 \times 10^{-6} \text{ cm}^2/\text{s}.$

The superposition of a local and a long range lateral diffusion of the lipid molecules
is indeed predicted by the free volume model [35a]. This model is further verified
by the finding that the long range diffusion coefficient $D_{\text{lat}}$ depends strongly on the
lateral density [34].

Another remarkable result of the detailed QENS studies is that the rotational
diffusion of the whole lipid molecules is negligible in the time range of $10^{-8}$ to
$10^{-10}$ sec. Only a torsional rotational motion of the individual chains is allowed.

4.4. Dynamic surface roughness

Measurements with both instruments reveal a remarkable out-of-plane vibrational
motion of the centers of mass of the lipid molecules. The amplitudes, $u_\perp,$ and
jump frequencies, $v_\perp,$ depend of course on the time window of observation. The
time-of-flight instrument yields

$$u_\perp \approx 0.05 \text{ nm}, \quad v_\perp \approx 10^{10} \text{ Hz},$$

and the backscattering instrument

$$u_\perp \approx 0.25 \text{ nm}, \quad v_\perp \approx 5 \times 10^8 \text{ Hz}.$$ 

The exact nature of the out-of-plane motion is still unclear. One possiblility is
that it is a consequence of membrane undulations in the nm wavelength regime
(see ref. [34b]). Whatever the origin of this dynamic surface roughness the most
intriguing aspect is that it may contribute strongly to short range repulsion forces. As
pointed out by Israelachvili and Wennerstroem [36], out-of-plane vibrations of the
lipid molecules would also lead to an exponential potential in analogy to hydration
forces. For distances larger than about 3 Å they could even play a dominant role.
Indeed, the mean amplitude of the slower motion $u_\perp = 0.25 \text{ nm}$ is about equal to
the decay length of the hydration force ($\sim 0.3$ nm). The dynamic surface roughness
due to the bending undulations will be briefly addressed below and will be treated
in the article by Lipowsky.
5. Bilayer elasticity and thermomechanical properties of vesicles

Since the pioneering work of Helfrich [37] and Evans [38] the outstanding role of bending elasticity for the shape of vesicles and their interaction with hard or soft interfaces has become increasingly evident. The non-classical and scale dependent elastic properties of fluid or solid-like (tethered) membranes due to thermal excitations of long wavelength in-plane phononon and bending excitations have been recognized [39, 40, 43] resulting in a new theory of the elasticity of shells. This aspect will be covered in chapter 11. The most exciting aspects is that these new concepts of the elasticity of shells can explain many properties of cells such as (1) the shape stabilization or transformation of cells, (2) local instabilities associated with the vesicle mediated transport in cells or (3) many aspects of the cellular locomotion. Finally the bilayer elasticity is essential for the lipid/protein interaction or the behaviour of mixtures (as will be discussed below); but also for lyotropic phase transitions (e.g., bilayer \( \rightarrow \) inverted hexagonal, [11]). For the above reasons, high precision measurements of elastic properties of lipid bilayers have become an important task of membrane physics. Such measurements provide, however, also an important tool for the investigation of structural and dynamic properties of membranes; in particular by performing temperature dependent measurements and applying the classical laws of thermoelasticity (cf. references [10a, 24]).

5.1. Outline of elastic properties of vesicles

The elastic properties of vesicles are in general determined by all three classical modes of deformation: (1) pure extensial deformations, (2) pure shearing and (3) pure bending. The elasticity of vesicles in the fluid-state is determined essentially by the first and last contribution, while all three have to be considered if one deals with erythrocytes or fluid phase vesicles from polymerized lipids [3, 43].

In the energy representation of Hook’s law the shear elastic energy per unit area may be expressed as

\[ g_{sh} = \frac{1}{2} \mu (\lambda^2 - \lambda^{-2} - 2), \tag{17} \]

where \( \mu \) is the shear elastic modulus (in units of erg/cm\(^2\)) and \( \lambda = L/L_0 \) is the lateral extension ratio which characterizes the transformation of a square-shaped area element (of length \( L_0 \)) into a rectangle of length \( L \) (leaving the area constant). It is often helpful to express Hook’s law in terms of the force equation

\[ \tau_1 - \tau_2 = \mu (\lambda + \lambda^{-1}), \tag{18} \]

where \( \tau_2 \) and \( \tau_1 \) are the tensions (measured in mN/m) which have to be applied at the elongated and compressed sides of the area element in order to stabilize the rectangular shape.
The energy density associated with an isotropic lateral tension (acting on each of the four sides of the quadratic area element) is given as

\[ g_{\text{ext}} = \frac{1}{2} K (\delta A / A)^2, \]  

(19)

where \( K \) is the area compressibility modules and \( \delta A / A \) the relative area change.

The situation for bending is more complex and depends on the constraint: that is whether one deals with a piece of bilayer with free ends (and freely sliding monolayers) or a closed vesicle. In the former case the bending is equivalent to the splay deformation of a single smectic layer. The main contribution comes from the mutual tilting of adjacent lipid molecules and the bending elastic modules, \( \kappa \), is equal to the splay elastic constant \( (K_{11}) \) times the bilayer thickness \( (\kappa = K_{11}d_m) \). By expressing the bending deformation in terms of the associated displacements, \( u \), of the bilayer in the normal direction (cf. fig. 12), the well-known Helfrich equation holds for the elastic energy per unit area:

\[ g_{\text{bend}} = \frac{1}{2} \kappa \left( \frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} - c_0 \right)^2, \]  

(20)

where \( c_x = \frac{\partial^2 u}{\partial x^2} \), \( c_y = \frac{\partial^2 u}{\partial y^2} \) are the principal radii of curvature and \( c_0 \) is the spontaneous curvature introduced above.

In the classical theory of shells \( \kappa \) is related to the Young modulus \( E \) and the Poisson ratio \( \nu \) by \( \kappa = Ed_m^3 / 12(1 - \nu^2) \) and is called the flexural rigidity [10b]. This relation is helpful to compare the elastic moduli of synthetic and biological material (cf. table 2).

The behaviour of closed vesicles towards bending is in addition determined by the constraints of fixed volume, \( V \), fixed average area, \( \langle A \rangle \), of the membrane and fixed area difference, \( \Delta A \), between inner and outer monolayer which is given by

\[ \Delta A \approx d_m \int dA (c_x + c_y)^2 \]  

(21)

where the integration goes over both monolayers. Equation (21) is called bilayer coupling constraint.

The bending stiffness, \( \kappa' \), is different from that of eq. (20) and can be expressed in terms of the lateral compressibilities of the two monolayers [38]:

\[ \kappa' = d_m^2 K_i K_0 / (K_i + K_0). \]

Since also the splay energy can be expressed in terms of the variation of the lateral compressibilities in the direction of the membrane normal [15, 42] \( \kappa' \) and \( \kappa \) are expected to be similar. Since \( \kappa' \) is determined by the global constraint of fixed average area \( \langle A \rangle \) it is called the global bending modulus to distinguish it from the local modulus \( \kappa \). The above concepts form the basis for the calculation of shapes and
shape transitions of vesicles and cells and will be discussed in detail in the article by Lipowsky and Seifert.

The bilayer coupling concept appears to be essential for the behaviour of the red blood cells which is determined by the coupling of the lipid/protein bilayer and the cytoskeleton [46, 53]. In this case the bending modules is determined by the lateral compressibilities of the bilayer and the macromolecular network.

In general one has to consider also a contribution due to the Gaussian curvature ($c_G^2 = c_x \times c_y$) but since $c_G$ is constant for closed vesicles it can be ignored. This contribution plays however an important role for the lyotropic phase transitions at high lipid concentrations and microemulsions. A highly interesting case where
the Gaussian curvature becomes important are the torus-shaped vesicles discovered recently [45].

Various methods for the measurement of elastic properties have been developed such as the micropipette aspiration technique [24] the electric field deformation technique [47, 48] the cell poking [50] and the vesicle flickering spectroscopy [51, 52]. Up to the present the first technique is certainly the most versatile (albeit difficult) since it enables simultaneous measurements of all elastic constants and thermomechanical properties (cf. Bloom et al. [4] for a review of recent literature). The flicker spectroscopy measures forces in the Femto-Newton regime and is most appropriate to explore subtle effects of solutes on the bending stiffness [51] or to evaluate contributions of local and non-local bending [53].

5.2. Elastic parameters of lipid vesicles and intercorrelations

In tables 1 and 2 and fig. 13 some representative elastic parameters for bilayers are presented which yield simultaneously interesting correlations between the different

| Type of Lipid | temperature \[
\degree C\] | area compressibility modulus, K \[mN/m\] | tensile strength, \(\tau_{\text{lys}}\) \[mN/m\] | critical area strain, \(\delta A/A_{\text{max}}\) |
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<tr>
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<td>30</td>
<td>145</td>
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<td>–</td>
</tr>
<tr>
<td>DMPC/cholesterol 3:2</td>
<td>15</td>
<td>647</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
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<td>685</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DMPG (L_{\beta}-phase)</td>
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<td>855</td>
<td>–</td>
<td>–</td>
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<td>200</td>
<td>2</td>
<td>0.030</td>
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<td>335</td>
<td>–</td>
<td>–</td>
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<td>20</td>
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<td>4.5</td>
<td>0.043</td>
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<tr>
<td>Erythrocyte lipid extract (50% cholesterol)</td>
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<td>780</td>
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<th>Bending [Joule]</th>
<th>Compression [mN/m]</th>
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<td>(2 \times 10^{-17})</td>
<td>(5 \times 10^3)</td>
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<tr>
<td>Red-Blood Cell</td>
<td>(6 \times 10^{-3})</td>
<td>(5 \times 10^{-20})</td>
<td>(1 \times 10^3)</td>
</tr>
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</table>
moduli (for more detailed information see Bloom et al. [4] or [17] and references cited therein). Some pertinent features of bilayer elasticity are:

- The lateral compressibility modules, $K$, of the L$_{\alpha}$-phase of lipids with saturated chains is about 150 mN/m and increases slightly with increasing chain length. $K$ can become much smaller at high degree of unsaturation (cf. the case of DAPC in table 1).
- The ratio of the bending to the compressibility modules $K/\kappa$ is nearly constant ($K/\kappa = 3\times10^{-15}$ cm$^{-2}$ [4]). In fact the elasticity theory predicts $K/\kappa \approx d_\text{m}^{-2}$ for two-dimensional shells. (cf. reference [4]).
- Addition of cholesterol to fluid bilayers increases $K$ (and therefore also $\kappa$) drastically as shown for the case of DMPC in table 1 and fig. 13. The situation is much more complicated (and interesting) below the chain melting transition of the phospholipids. Thus for DMPC at $x_\text{chol} < 0.12$ ($x_\text{chol} =$ cholesterol molar fraction) a small shear rigidity is observed while the bilayers appear fluid above this threshold [54]. Above a third threshold ($x_\text{chol} \approx 0.3$), $K$ increases
again with $x_{\text{chol}}$ (as at $T > T_m$). This is a consequence of a miscibility gap extending from 8 to 25 mole% of cholesterol (see fig. 14 below). The increase of $K$ by cholesterol in the fluid state is a consequence of the well known condensing effect. This correlation between $K$ or $\kappa$ and the chain packing is well predicted by molecular field theories (cf. chapter 7).

- A special advantage of the micropipette technique is that it allows to measure the tension, $\tau_{\text{max}}$, at which the vesicles rupture as well as the corresponding maximum area expansions. The rupture tension is a few mN/m and is related to the compressibility modulus by a square root law: $\tau_{\text{max}} \propto \sqrt{K}$ [17]. The maximum area expansion is astonishingly small varying between 2 and 5% [17].

- Similar to cholesterol, other solutes can alter the elastic properties of bilayers drastically. Thus a few (some 2–5) mole% of a short bipolar lipid (called bola lipid) or small peptides (e.g., valinomycin) may reduce the bending stiffness $\kappa$ (and thus $K$) by an order of magnitude (cf. fig. 13 and [51]) and $\kappa$ can even become smaller than $k_B T$. As has been shown by molecular field theories (cf. chapter 7), this effect of solutes may be related to variations in bilayer thickness.

- A most interesting case is the red blood cell. The bending stiffness is comparable to that of pure lipid bilayers (cf. table 2 and fig. 13 for case of DMPC). This is most remarkable in view of the high cholesterol content ($\sim 50$ mole%) of the lipid protein bilayer of the red blood cell and of the fact that it is coupled to the cytoskeleton. This anomalous flexibility and its biological role is still an enigma [53, 55].

- Thermomechanical experiments with single bilayer vesicles (e.g., based on micropipette experiments) provide in principle a valuable tool to evaluate phase diagrams of individual bilayers of lipid mixtures. Only one example has been systematically studied: namely the SOPC-cholesterol mixture. Figure 14 shows a plot of the compressibility modulus $K$ as a function of the cholesterol molar fraction, $x_{\text{chol}}$, for 15°C (where SOPC is in a solid state). $K$ increases slightly up to about 25 mole% (from 193 to 240 mN/m). Then a more pronounced increase (from 300 to 600 mN/m) up to $x_{\text{chol}} \approx 0.4$ is observed and finally a rapid rise to a saturation value of $K = 1200$ mN/m is reached at 58%. The breaks define the phase boundaries.

- The shear elastic modulus of the solid phases has not been measured yet although it would give most valuable insight into the defect structure of gel-phases and possible hexatic phases. Only the yield shear has been determined. For DMPC it is $\tilde{\mu} = 0.03$ mN/m for the $L_{\beta'}$-phase and $\tilde{\mu} = 0.01$ mN/m for the $P_{\beta'}$-phase. $\tilde{\mu}$ is an upper limit of the shear modulus $\mu$. 
5.3. Bending excitations of vesicles (‘flickering’): application and consequences

The thermal excitation of bending undulations of vesicles and cells is a most exciting and biologically important type of fluctuation [3, 57–59]. It is a consequence of the extreme softness of fluid bilayers with respect to bending. In recent years it has been recognized that the dynamic surface roughness of bilayers associated with these bending excitations has many important consequences. It leads to a dynamic lateral tension [60–62] may result in repulsive undulation forces between vesicles and a rigid wall which can compete with weak Van der Waals attraction forces [61, 62]. Last not least, shape fluctuations can be exploited for (perturbationless) high precision measurements of membrane bending moduli [51, 52, 58]. These aspects will be briefly addressed below:

**Measurement of bending elastic moduli**

For the quantitative description of undulations of flat membranes (of dimension $L \times L$) one can expand the undulations in plane waves

$$u(\vec{r}, t) = \sum_q u_q(t) \exp(\vec{q} \cdot \vec{r}), \tag{22}$$

where $\vec{q}$ is the in-plane wave vector: $\vec{q} = (q_x, q_y)$. The familiar equipartition theorem yields for the mean square amplitude of each mode $q$

$$\langle \upsilon_q^2 \rangle = \frac{k_B T}{\kappa q^4 + \sigma q^2} \tag{23}$$

Fig. 14. Variation of elastic area expansion modulus of SOPC vesicles with increasing cholesterol content. At the measuring temperature $T = 15^\circ C$ the SOPC is in the gel state ($T_m = 30^\circ C$).
where $\sigma q^2$ accounts for a possible lateral tension.

The undulations are completely overdamped by coupling of the undulations to hydrodynamic excitations in the surrounding aqueous phase (as carefully described in the pioneering paper of Brochard and Lennon [57]), that is

$$u_q(t) = \sqrt{\langle u^2_q \rangle} e^{-\omega_q t}.$$  \hspace{1cm} (24)

The damping constant $\omega_q$ has been calculated for two limiting cases: an isolated piece of bilayer (of dimension $L \times L$) and two bilayers separated by a distance, $d$, which is small compared to the dimension $L$. The damping constants exhibit completely different $q$-dependencies for the uncoupled ($\omega^{uc}_q$) and the coupled ($\omega^c_q$) case, namely [57]:

$$\omega^{uc}_q = \frac{\kappa q^3}{2\eta}, \quad \omega^c_q = \frac{\kappa q^6 d^3}{24\eta},$$ \hspace{1cm} (25)

where $\eta$ is the water viscosity. A general dynamic theory of these planar systems stems from Frey and Nelson [60]. Monte Carlo methods have been developed to calculate the mean square amplitudes also for membranes exhibiting shear rigidity such as erythrocyte membranes (cf. ref. [39a] and chapter 8).

This bilayer approach can be applied in order to evaluate the bending excitations of vesicles or red blood cells at wavelengths short compared to the vesicle radius. This approach has been adopted to measure the bending modules of erythrocytes and vesicles by reflection interference microscopy [55, 57]. For the evaluation of long wavelength excitation the situation is much more complicated. In order to calculate the mean square amplitudes and the damping constants, the differential equations describing the elastic and hydrodynamic excitations would have to be solved for a very complex topology considering the constraint (1) of constant area and volume and (2) of constant area difference. According to Peterson (cf. [53] for reference) the problem can in principle be solved numerically by application of topological methods.

For the measurement of bending elastic moduli of vesicles, the quasispherical approach can often be adopted if the constraint of finite volume is considered by introduction of an apparent tension [51, 59]. The vesicle is considered to exhibit on the average a spherical shape of equivalent radius $R_0$. The deviation from the spherical shape is described in terms of a Fourier expansion into spherical harmonics. It has been shown that the flickering of vesicles can be analysed by only considering the fluctuations of the contour of the vesicle observed by phase contrast microscopy over a sufficient period of time [51, 52]. The bending moduli given in fig. 13 have been obtained in this way.

The quasispherical approach can also be applied to erythrocytes of discocyte shape [53, 64]. In this case the flickering is analyzed by evaluation of the thickness fluctuations of the cell by phase contrast microscopy [53, 57]. The mean square amplitudes and decay times of several lowest order spherical harmonics modes can be
directly determined by a correlation analysis of the thickness fluctuations [53]. This allows simultaneous measurement of the bending and shear modules. An astonishing result of this analysis is that the shear modules obtained in this way is much smaller than the statically measured value [53] suggesting that the red blood cell exhibits a shear free deformation regime.

Dynamically induced tension [61–63]
It is intuitively clear that the membrane undulations result in a dynamic lateral tension since work is required in order to pull out the wrinkles caused by the undulations. The lateral tension, \( \sigma \), arising if the relative area of the vesicle is increased by a fraction \( \delta A / A \) during the pulling out of the ‘wrinkles’ follows an exponential law

\[
\sigma = \frac{\pi \kappa}{a^2} \exp \left\{ -\frac{8\pi \kappa \delta A}{k_B T A} \right\}
\]

(26)

where \( a \) is the smallest excitable wavelength which is of the order of the bilayer thickness (\( a \approx 1 \text{ nm} \)). For a fractional area change of \( \delta A / A = 0.01 \) and \( \kappa = 10^{-12} \text{ ergs} \) the tension is of the order of \( \sigma \sim 2 \text{ mN/m} \) and is thus quite substantial for the deformation of vesicles. Based on this idea a method for the measurement of the bending elastic modulus by the vesicle aspiration technique has been developed [62].

Undulation forces
Another spectacular effect of the bending undulations is the dynamic repulsive force arising if a flaccid vesicle approaches a rigid surface to a distance \( d \), smaller than its diameter \( L \), or if vesicles are confined between two plates of a distance \( d \leq L \). If the vesicles approach the surface the long wavelength modes gradually freeze out. This results in a repulsive force (per unit area) which scales as \( d^{-3} \). It corresponds to an interaction potential per unit area of [61].

\[
V_{\text{rep}}(d) \approx \frac{(k_B T)^2}{\kappa} \frac{1}{d^3}.
\]

(27)

One remarkable aspect of this purely entropic interaction potential is that it exhibits the same power law as the Van der Waals interaction between interfaces. For normal phospholipids (e.g., DMPC) it could exhibit the same order of magnitude [61, 62]. It is strong enough to overcome Van der Waals attraction.

6. Membrane electrostatics and phase transitions
It has long been recognized that electrostatic effects due to the diffuse electric double layer play a decisive role for the conformational states and phase transitions of membranes containing charged lipids [65–67]. But only recently it has become clear that the electrostatic properties of membranes are also strongly influenced by the polarization of the (so called bound) water arising by the electric field of the
double layer [68, 69]. This so-called hydration effect is important for phospholipid membranes since the thickness of the polar head group layer is rather large (some 0.7 nm) owing to the deep penetration of the carboxyl groups into the hydrocarbon regime (cf. figs 7b and 15). Indeed, from an energetically point of view the hydration effects are much stronger. Thus the lateral pressure associated with the hydration effect is $\pi_h \sim 50 \text{ mN/m}$ as compared to a value of $\pi_{el} \sim 20 \text{ mN/m}$ for the electrostatic pressure at a very high membrane potential of 150 mV [69]. The situation is further complicated by the correlation effects leading to large local charge fluctuations (cf. review by G. Cevc [69]). Despite of these problems the classical Gouy–Chapman–Debye theory works remarkable well for the explanation of electrostatic effects in vesicle suspensions. One reason is that the two electrostatic contributions are characterized by two very different length scales. The electrostatic potential of the water polarization decays exponentially with a screening lengths of $\lambda_{\text{hyd}} \sim 0.1-0.4 \text{ nm}$ while the Debye-length characterizing the range of the diffuse double layer potential is $\kappa^{-1} \geq 1 \text{ nm}$ under normal conditions. The correlation effects are only important for high concentrations of bivalent ions ($\sim 0.1 \text{ M}$) which are normally avoided in vesicle studies and are biologically irrelevant. The hydration effect can play, however, a dominant role for partially hydrated multilayers. These are not considered in this article and the reader is referred to the excellent review of Cevc [69].

Before proceeding, it should be mentioned, however, that hydration effects may become essential also for vesicle at high water excess if the ions (such as organic salts) penetrate into the head group regime changing the degree of hydration. Such effects may in particular be essential for situations of protein adsorption to charged membranes associated with the penetration of hydrophobic chains into the bilayer (cf. fig. 32 below). In this case the strong osmotic effects exerted by proteins may drastically alter the lipid hydration. Moreover, hydration effects become essential for the fusion of vesicles mediated by Ca$^{++}$ or macromolecules. Finally it should be mentioned that the membrane electrostatics could also be remarkably influenced by flexoelectric effects which are well known from liquid crystal physics [42]. Thus local budding results in a curvature induced polarization and membrane potential (cf. ref. [2] for relevant literature) which may be quite substantial. As shown in fig. 15b the polarization is a consequence of the different dipole densities in the two opposing monolayers. For a radius of local curvature of $R_{\text{loc}} \approx 100 \text{ nm}$ a membrane potential of $V_m \approx 10 \text{ mV}$ is expected. For tilted chains a structure ferroelectricity is expected (even in fluid phases) since the electric dipole moments of the glycerol backbone are not completely averaged out.

6.1. Charge induced phase transition

Charge induced and thus isothermal phase transitions may be induced in various ways: (1) by variations in the pH or ionic strength and (2) by the adsorption of charged macromolecules or bivalent ions (such as Ca$^{++}$). The results are often conflicting since at high lipid and salt concentrations multilammelar systems or other lyotropic phases may form by vesicle fusion leading to high temperature shifts by dehydration. This occurs in particular if the free solvent volume available for the
Fig. 15a. Schematic view of non-homogeneous electrical potential decay across membrane containing charged lipids. $V$ denotes the total potential drops across the Gouy–Chapman–Stern layer at the outer and two inner surfaces. Note the accumulation of negative lipid on positive side.

Fig. 15b. Curvature induced space charge formation and membrane potential. $\vec{P}_0$ and $\vec{P}_i$ are the time averages of the dipole moments (per unit area or per lipid) in the outer and inner monolayer, respectively. Note the formation of positive and negative excess space charges according to $\sigma = \text{div} \vec{P}$.
lipid is reduced by polymers. New phases may arise. An example is polylsine which intercalates between bilayers.

All charge induced effects (including those caused by associated changes of the degree of hydration) may be traced back to the variation in the lateral pressure, \( \Delta \pi \), since the transition temperature shift, \( \Delta T_m \), depends on the change in molar area, \( \Delta A \), according to

\[
\Delta T_m = \Delta A \times \Delta \pi / \Delta S.
\] (28)

For monovalent counterions the purely electrostatic lateral pressure is given by the well known relationship (in c.g.s.-units; cf. \([2, 67, 69]\) for references).

\[
\Delta \pi_{el} = 6.1 \sqrt{c} \left( \cosh \frac{e \psi_0}{k_B T} - 1 \right),
\] (29)

where the numerical factor holds for \( T = 300 \) K and \( \varepsilon = 80 \); \( c \) is the concentration (in Mol/l) of the (monovalent) counterions and \( \psi_0 \) is the surface potential.

The surface potential may be expressed in terms of the surface charge density \( \sigma \)

\[
\psi_0 = \frac{2k_B T}{e} \text{arcsinh} \frac{\sigma}{\sqrt{8 \varepsilon n_0 k_B T}},
\] (30)

where \( n_0 \) is the number density of counter ions (cations/cm\(^3\)).

By introduction of the fraction, \( \alpha \), of charged lipids (which corresponds to the degree of dissociation for a membrane composed of a single lipid with a dissociable group) it is useful to write

\[
\psi_0 = \frac{2k_B T}{e} \text{arcsinh} \left\{ \frac{134 \alpha}{A \sqrt{\varepsilon}} \right\},
\] (31)

where the numerical coefficient holds again for 25\(^\circ\)C and an aqueous solution (\( \varepsilon = 80 \)). \( A \) is the area per lipid. It is helpful to note that the Debye screening length for a 1 : 1 electrolyte is related to the electrolyte concentration by

\[
\kappa^{-1} = 0.308 / \sqrt{c} \text{ [nm]}.\] (32)

Let us consider now two simple situations:

- \( pH \)-dependence of the phase transition (fig. 16): The \( pH \) dependence of the lipid phase transitions is a consequence of the \( pH \)-dependence of the degree of dissociation (cf. eq. (31)) according to the well known relationship

\[
\alpha/(1 - \alpha) = K_0 [H_s]^{-1}
\] (33)

where \( K_0 \) is the dissociation constant of the lipid head groups in the bulk and \([H_s]\) the proton concentration at the surface. It is essential to realize that the
proton concentration at the membrane surface depends on the surface potential (according to Boltzmann’s law) and can thus differ very drastically from the bulk proton concentration.

- **Ionic strength dependence of phase transition (fig. 17).** This is a more subtle effect as follows from the finding that the transition temperature may increase or decrease with increasing ionic strength as shown in fig. 17. It arises at low ionic strengths (< 0.2 M) where (according to eq. (31)) $\psi_0$ is strongly dependent on the ionic strength. The direction of the shift depends upon whether the lipids are partially or totally dissociated.

In the former case the salt effect is a result of the dependence of the dissociation constant $K_0$ on the surface potential:

$$K = K_0 \exp\left\{e\psi_0/k_B T\right\}. \quad (34)$$

At low ionic strength $c$, the negative surface potential $\psi_0$ and therefore the proton concentration at the membrane surface is high and $K$ is low. At increasing $c$ the surface potential is screened, impeding the access of protons. This leads to an increase in the degree of dissociation and thus a reduction in $T_m$ (as indeed shown by upper curve of fig. 17).

In the case of fully dissociated lipids successive addition of counterions results in a reduction of the repulsive electrostatic pressure (according to eqs (29) and (31)) and hence in an increase of $T_m$ (as in the case of the lower curve of fig. 17).

### 6.2. Hysteresis effects of charge-induced conformational changes of membranes

Membrane electrostatics has many other interesting and still unexplored effects on membrane structure such as hysteresis phenomena. They are typical for charge
induced phase transitions of membranes [65, 66]. The hysteresis is most pronounced in the pH-regime of the dissociation equilibrium. Of particular interest are such charge induced hysteresis effects in mixed vesicles of neutral and charged lipids. In the example shown in fig. 18, the temperature was adjusted near the chain melting transition of the charged lipid DPPA \( (T_m = 50^\circ C \text{ at pH 7}) \) so that it could be induced by changing the pH. The fraction of solidified lipid is monitored by the excimer technique [66]. A pronounced hysteresis extending over 1.5 pH units is observed. The observation of a small loop by changing the pH in a cyclic way over a small range shows that the hysteresis is not a kinetic effect but is due to domain formation [66]. As will be discussed below charge induced domain formation in segregated membranes is well established. The hysteresis is a consequence of the difference of the dissociation constant of DPPA\(^-\) in the fluid and solid state. Since the negative potential is more negative in the dense phase (cf. eq. (31)), the surface concentration of H\(^+\) is higher and one has to go to higher pH values in order to achieve dissociation than when starting from the fluid phase.

An intriguing question is whether such hysteresis effects play a role in biological membranes. As is well known they may be exploited for information storage or to switch between two states of activity. A likely candidate is the Ca\(^{2+}\)-induced phase separation. If the suspension of DPPA\(^-\) and DPPC is titrated with Ca\(^{2+}\) instead of H\(^+\) the same strong hysteresis is observed as in fig. 18. Many membrane processes are triggered by a transient rise in Ca\(^{2+}\)-level: (such as the fusion of synaptic vesicles with the presynaptic membrane). If the local transient increase in the Ca\(^{2+}\)-level would be sufficient to cause the binding of the synaptic vesicle to
Fig. 18. Hysteresis of pH-induced phase separation in equimolar mixture of DPPA and DPPC. At pH > 8.5 the DPPA is double negative and in a fluid state while at low pH it is onefold negative and in the gel-state. The temperature was adjusted in such a way that the chain melting transition of DPPA occurs at pH 7.5. The broadness of the transition is due to lateral phase separation which leads to a domain structure of the membrane (cf. fig. 30).

the presynaptic membrane the hysteresis effect could easily prevent the sequestering or pumping out of the excess Ca\(^{2+}\) bound at the membrane contact sides.

As has been pointed out long ago, by A. Katchalsky hysteresis effects are common for all charge induced conformation transitions; in particular also for proteins (cf. [2b] for further references). Unfortunately, the possible role of charge induced hysteresis effects for biological processes has not been explored yet.

7. Defects in membranes

Synopsis

Two-dimensional crystals of phospholipids have an exceptionally rich defect structure for several reasons:

1) The solid phases are highly frustrated since the hydrocarbon chains and the polar head group (glycerol backbone and phosphate group) tend to form different lattices. The chains form a triangular lattice while the heads can at most form an oblique 2D-lattice (cf. figs 5 and 19a). In cases with tilted chains (L\(_\beta'\), P\(_\beta'\)) the two parts of the lipid can in addition have different orientations. The frustration of high temperature solid phases is most directly revealed by the diffuseness of the X-ray [14] and electron diffraction pattern [71–73].

2) The high elastic energy associated with defects can be relaxed in isolated lipid layers by their escape into the third dimension [2, 74].

3) The defects act as strongly attractive traps for solutes. The associated reduction in the defect core energy causes a strong increase in the defect density.
Fig. 19a. Dislocation in two dimensional triangular lattice of $P_{\beta'}$-phase. Note that its core is composed of a $+\pi/3$-disclination (heptagone) and $-\pi/3$-disclination (pentagon). At the top the oblique lattice preferred by the head groups is indicated by thick lines.

Fig. 19b,c. Disclinations formed by removal ($-\pi/3$-type) or addition ($+\pi/3$-type) of 60 wedge in a Volterra process. c) Formation of a quasi-spherical cap in a crystalline bilayer caused by buckling after formation of a $-\pi/3$-disclination (effected by removal of an edge of 60°C). Note that a disc of radius $r$ is displaced in vertical direction by $u(r) = r/\sqrt{3}$ (after Nelson [75]).
For that reason the Landau theory is not well suited to describe sub-transitional phenomena of solutions.

Defects in bilayers are of interest for several reasons:
- due to their richness, free and supported bilayers are very attractive model systems to study fundamental physical properties of defects in low-dimensionality solids,
- due to their role as attractive centers defects could play an important role for biological membrane processes: a point extensively discussed below. In this context it is interesting to note that networks of linear defects in vesicles and supported bilayers act as pathways for rapid lateral diffusion of solutes [2b, 35].

7.1. Classification of defects

Defects arise on several levels (cf. classification of defects in [74]):
1) In isolated bilayers one has of course the classical defects within the 2D-lattice: such as edge dislocations and disclinations (shown in fig. 19a).
2) A new manifold of defects arises in tilted phases. Firstly, point disclinations of various topology which are well known from liquid crystal physics (cf. fig. 6 for examples). Secondly, line defects such as Bloch- and Néel-walls. Both types may arise at boundaries between tilted and non-tilted phases or between domains of opposite tilt. Up to the present only Néel-walls have been observed.
3) Another manifold of defects is exhibited by the surface texture of vesicles in the $P_{β'}$- and the $L_{β'}$-phase (cf. fig. 6) from which important information on the molecular organization of the lipids can be obtained [21].
4) In multilayers one finds (in addition to the defects discussed above) pair like arrangements of screw dislocations or edge dislocations [18].

7.2. Formation of buckled states in bilayers

As was first pointed out in the famous paper by Kosterlitz and Thoughless [75] the work required to form an edge dislocations in a 2D-crystal is strongly reduced by the entropy arising by the fact that a dislocation can be formed at $(L/a)^2$ positions of a membrane of size $L \times L$ and lattice constant $a$. The Gibbs free energy is

$$G_{\text{disloc}} = K_0 \vec{b}^2 / 8\pi \ln(L/a) - 2k_B T \ln(L/a),$$  \hspace{1cm} (35)

where the first term is the well-known elastic energy of an edge dislocation (cf. reference [10b] and [75]) while the second represents the entropy (and follows directly from the above argument). $\vec{b}$ is the Burgers vector and $K_0$ is the Young modulus for 2D-systems. Since the lateral compressibility modulus is large compared to the shear modulus $\mu$, $K_0$ may be approximated by $K_0 \approx 4\mu$. Equation (35) predicts that the density of defects diverges at a transition temperature of

$$T_{KT} \approx \frac{\mu \vec{b}^2}{4\pi k_B}$$  \hspace{1cm} (36)
and the crystal structure becomes unstable. As shown in the key paper of Nelson and Halperin [41], the new soft phase arising at \( T > T_K \) is not fluid but hexatic. In this phase the spatial long range order has broken down, while the long range orientational order (that is the orientation of the crystal axes) is still maintained. The requirement for the formation of a true hexatic phase is, however, that the disclinations making up the dislocations (fig. 19a) dissociate. In a flat membrane (e.g., a supported bilayer) of dimension \( L \times L \), the energy of formation of an isolated (say \( \pm 1 \)- or \( \pm \pi/3 \) = 60°C-) disclination is of the order

\[
W_{\text{discl}} \approx 4\mu s^2/32\pi = \mu L^2/8\pi \tag{37}
\]

and is much larger than the elastic energy of a dislocation.

In a flat membrane in the \( \text{L}_{\beta}' \)-phase (\( \mu \approx 100 \text{ mN/m} \)), the energy of an isolated dislocation is of the order \( \approx 10^3 k_B T \), while \( W_{\text{discl}} \approx 10^8 k_B T \); that is both values are excessively high. However, as shown by Nelson and Halperin a gas of unbound dislocations can screen the strain fields of the disclinations and the disclination energy depends only logarithmically on the size \( L \):

\[
W_{\text{discl}} \propto K_A \ln(L/b) \tag{38}
\]

where \( K_A \) is the so called hexatic stiffness constant (introduced in [41]). Owing to this logarithmic law, the disclinations can dissociate at a finite temperature, resulting in a transition to the hexatic phase. This phase is shear free but can be very viscous. It may therefore be difficult to distinguish hexatic phases from solid ones by mechanical measurements. This may be the reason why evidence for the existence of hexatic phases in lipid monolayers was provided by X-ray diffraction (cf. chapter 4) but not by mechanical measurements yet [72].

The situation improves further if the bilayer can escape into the third dimension [75]. Then the disclination energy is drastically reduced by buckling. The \( -\pi/3 \)-disclination can form a quasi-spherical cap (as shown in fig. 19c) and the \( +\pi/3 \)-type a saddle type of surface profile. The energy cost for the formation of a disclination is now determined by the bending energy. It can easily be calculated for a \( -\pi/3 \)-type of disclination as follows: The vertical displacement of the quasi-spherical cap as a function of the radius vector is \( u(r) \approx \hat{r}/\sqrt{3} \). The curvature is \( \nabla^2 u(r) \approx 1/r \) and the energy becomes

\[
W_{\text{discl}} \approx \frac{1}{2} \kappa \int_0^L \left(\nabla^2 u\right)^2 d^2 \hat{r} = \kappa \ln \frac{R}{a} \tag{39}
\]

(Where \( \kappa \) is the bending elastic modulus). Taking again a disc of \( L \approx 1 \mu \text{m} \) which \( a = 1 \text{ nm} \) and \( \kappa \approx 10^{-11} \text{ ergs} \) one arrives at a value of

\[
W_{\text{discl}} \approx 5 \times 10^{-11} \text{ erg} \approx 10^3 k_B T.
\]

This is still a very high energy. However, following Nelson and Peliti [40] the strain fields of two oppositely charged disclinations cancelled at large distances \( R_c \).
thus reducing $W_{\text{discl}}$. The key point is that the strain field of the two disclinations forming a dislocation (as in fig. 19a) is strongly reduced by buckling (in a spherical and a saddle-like cap). As a consequence the strain fields are cancelled at a critical distance $R_c$. According to Nelson and Peliti [40] one expects $R_c \approx 60a$. The energy per dislocation is reduced to
\[
E_D = \frac{2\mu b^2}{2\pi \ln L/a}
\]
and the number density of defects becomes:
\[
n_D = \frac{1}{a^2} \exp\left\{ -\frac{E_D}{k_B T} \right\}.
\]
Judged from freeze fracture electron micrographs of PC vesicles, the density of hat-like protrusion on vesicles in the gel-phase is of the order of $1 \mu\text{m}^{-2}$. This would correspond to an energy per dislocation of $E_D \sim 10k_B T$. With the critical distance of about $R_c \sim 60a$ one estimates a shear elastic modulus of the bilayer of $\mu \sim 50 \text{ mN/m}$. This value agrees reasonably well with that found for lipid monolayers in the condensed phase ($\mu \sim 100 \text{ mN/m}$ [72]).

7.3. On the possible role of defects as attractive centers for enzymatic processes

As shown above, defects play an important role for the stabilization of vesicles in the gel states. Various defects may also arise in fluid or fluid-solid coexistence phases. As defect we define a local perturbation of the order of the bilayer associated with elastic strain fields. In this sense the deformation region about integral proteins associated with a mismatch between the thicknesses of the hydrophobic domain of the protein and the bilayer is considered as a defect. Alternatively, they could be described in terms of local variations of the lateral packing densities between the two monolayers. Another important class are orientational defects arising by any local perturbation of the lipid equilibrium orientation (resulting in an elastic splay deformation). Orientational defects may be introduced (1) by the incorporation of non-cylindrical integral proteins (cf. fig. 20a) or by proteins penetrating only into one monolayer (cf. fig. 20b); (2) by local formations of small buds and (3) at interfaces between tilted and non-tilted phases (cf. fig. 21).

The exciting aspect of orientational defects is that the elastic strain field may be released in various ways (cf. fig. 21): (1) by local curvature (that is escape into the third dimension) (2) by the accumulation of solutes or (3) the adsorption of proteins. This suggests that defects in fluid bilayers can play a key role for the self organization and function of biomembranes. In this section some properties of such defects are discussed together with examples from in-vitro studies.

**Defects as attractive centers**

The behaviour of the defects is determined by their elastic strain field. In the liquid crystal approach the potential of the strain field of the orientational defects in
Fig. 20. a) Orientational defects about non-cylindrical protein leading to local chain tilting. The arrows show the projection of the average chain orientation onto the membrane plane. b) Schematic view of two possible mechanisms of relaxation of elastic stress at orientational defects (created by partially penetrating protein). I: Escape into third dimension by redistribution of long chain lipids in opposite monolayer. II: Accumulation of hydrophobic solutes (e.g., substrates).

smectic layers depends logarithmically on the distance from the center of the point disclination according to [2, 76]

\[ W(r) = \pi \kappa \sin^2 \theta \ln\{r/R_p\} \text{ (in J)} \]  \hspace{1cm} (42)

where \( \theta \) is the maximum tilt angle at the surface of the protein of radius \( R_p \). The
splay elastic constant has been replaced by the bending elastic modules \( \kappa = K_{11} d_m \), where \( d_m \) is the thickness of the perturbed monolayer or bilayer.

Note, that such a logarithmic law implies a long range orientational order of the chains which is only clearly existent in gel phases or in cases of critical demixing where one phase is tilted (cf. fig. 21). In fluid phases the decay of the strain field is expected to be faster, but it is certainly not short range as in the case of precritical fluctuations. A theory on this decay in two-dimensions is still missing. In the molecular field approach the strain field could be characterized in terms of the local variation of the trans-membrane pressure gradient [15] since the lipid head-groups are compressed at the side of the larger diameter of the conical protein and expanded in the other. Irrespective of the type and the range of decay, local defects will act as attractive centers for solutes which will accumulate about the defect core. Entropy will oppose a strict localization and a Boltzmann type concentrate distribution

\[
c(r) = c_0 \exp\left\{-W(r)/k_B T\right\}
\]

will result, where \( W(r) \) is the elastic potential of the defect. Here is an interesting analogy to metallurgy where this behaviour is known as Cottrell effect and plays a decisive role for the elastic response of alloys (such as Creep).

The elastic potential depends on the type of defects. Let us consider an orientational defect of a tilt angle of \( \theta \approx 10^\circ \) (which would correspond to a difference of \( \delta R_p \sim 0.2 \text{ nm} \) between the maximum and minimum radius of the membrane protein). A wedge shaped hydrophobic solute (or another protein) of a similar opening angle would be attracted with a force of

\[
F = -2\pi \kappa \sin^2 \theta / r \approx -10^{-12} \text{ N}
\]
at a distance of 10 nm from the defect (corresponding to an interaction energy of $W(r) \approx 10k_B T$). The elastic force implies a fast directed diffusion with a drift velocity of $v_d = D_{lat} F/k_B T$. The directed transport to such attractive centers is thus several orders of magnitude faster than by random walk. Solute clouds about enzymes could thus help to accelerate enzymatic processes. Another interesting consequence would be the accumulation of local anesthetics in the neighbourhood of proteins. This could in particular explain the strong effect at very small concentrations (cf. [2b] for further examples).

**Defects and enzymatic control**

There are many intriguing reports about the modulation of the enzyme activity and membrane fluidity, mainly based on in vitro studies. Indeed, the adaption of the lipid composition to the environmental temperature by variation of the degree of lipid chain unsaturation shows that such ‘fluidity effects’ are of great biological importance. It appears that at least some of these effects are rather due to the membrane defect structure (e.g., associated with phase separation). Figure 22a shows as an example the rate of lipid exchange between vesicles of DPPC and DSPC by an exchange protein [77]. The enzyme activity exhibits pronounced peaks at the liquidus and solidus line, respectively, and is much higher in the whole coexistence region than either in the fluid or in the crystalline state. Analogous effects have been observed for the activity of phospholipases by the Holland group [78]; notably phospholipase A which cleaves phospholipids into a lyso compound and free fatty acid.

The increased enzyme activity in the coexistence region is a consequence of a parallel divergence of the lateral compressibility (such as shown in fig. 22b) suggesting that the primary sites of attack are the interfaces between the two phases. This has been directly demonstrated for the case of phospholipase by fluorescence-microscopy [79]. As is illustrated in fig. 23a,b, the lipid is primarily cleaved at the sites where the solid domains are expected to exhibit defects. In some cases one can observe that the enzyme works along defect-lines within the solid domains [79].

Another interesting consequence of the cleavage of lipids by phospholipases is that the lateral organization of bilayers may be drastically altered during the enzymatic activity. An example is shown in fig. 23b where the characterizing effects of cholesterol on the line tension of solid DMPC domains vanishes rapidly after the phospholipase activity sets in. This is an example of the tight coupling between enzyme activity and membrane structure alterations.

The high local compressibility of defects or of membranes in the fluid/solid coexistence is particularly interesting in cases where the activation of the enzyme is accompanied by a volume change. This volume change may be quite large: e.g., 0.1% in the case of sodium channels [81, 82]. If it is associated with a change, $\Delta A$, in the area of the enzyme, one expects a relative change in the Michaelis–Menten constant of [3]:

$$K_M = K_{M,O} \cdot \exp \left\{ -\frac{1}{2} K \cdot \Delta A^2 / k_B T \right\}. \quad (45)$$
Fig. 22a. Temperature dependence of the rate of lipid exchange between vesicles of DPPC and DSPC by exchange proteins. The divergence of the exchange rates at the onset (liquidus) and end point (solidus) of the phase separation of the mixture is a drastic demonstration of the role of phase boundaries for enzymatic processes.

Fig. 22b. On the divergence of the lateral compressibility modulus of monolayers of DMPA and a 9:1 mixture of DMPC and DMPA at the chain melting transition of the pure DMPA or during crossing the liquidus or solidus line of the mixture.
Fig. 23a. Direct visualization of decomposition of phospholipids by phospholipase A2 in fluid-gel coexistence of monolayers by fluorescence microscopy. Example: DPPC at lateral pressure of 20 mN/m and 25 °C. Dark domains correspond to solid phase (devoid of fluorescence labelled lipid).

Fig. 23b. Illustration of initial decomposition of lipid at the defects of domains which are composed of sub domains as indicated in the schematic image. (Source: Ch. Dietrich, Diplomarbeit TUM, 1991.)

Fig. 23c. Rapid reorganization of lateral domain structure of Lα-DPPC monolayers (6 mN/m) containing 0.5 mole% of cholesterol after 5 min of phospholipase A2 action showing feedback between enzyme action and membrane structure.
Due to the exponential law, the effect of local defects on the activity can be dramatic. One can easily see, that this dependence would even result in a coupling between the activities of two membrane bound enzymes. Thus, two enzymes with opposite pressure dependencies would form an unit of positive cooperativity.

7.4. Lateral phase separation and enzyme activity [2b]

It is often observed in biological membranes that a certain fraction of membrane proteins is inactive such as in the case of $\beta$-adrenergic hormone receptors (cf. chapter 1, fig. 26). In this particular case it is not clear whether this is due to the internalization of the proteins or whether the inactive protein fraction remains in the bilayer. However, the well known breaks in the Arrhenius plots of the activity of membrane bound enzymes or transport proteins show that the fraction of active enzymes may be controlled by phase separation. Figure 24 shows some examples. These demonstrate that the temperature at which the apparent activation energies exhibit a break depends on the lipid composition and the bilayer structure. For the adenylat-cyclase activity in Chang liver cells the break is shifted from $30^\circ C$ to $22^\circ C$ if DMPC is incorporated into the natural membranes. The break in the activity of the P450 reductase shown in fig. 24 is only observed for lipophilic or hydrophobic substrates (which approach the enzyme from the bilayer moiety) while the reduction of water soluble molecules exhibits the lowest value of the activation energy at all temperatures showing that the effect is indeed related to a conformational change of the bilayer.

Two types of conformational changes are possible (1) local melting of the lipid surrounding the protein and (2) lateral phase separation into fluid and rigidified domains: In both cases the variation of the average enzyme activity with temperature is due to the formation of an inactive fraction (imbedded in the solid phase) and a more active fraction (surrounded by fluid lipid). While some evidence for local melting was provided for the P450 reductase, the most likely cause for the breaks is in general lateral phase separation. Thus, the rate of sugar transport exhibits two breaks attributed to the onset and end of the phase separation (cf. fig. 24).

In summary, the experiments of fig. 24 show that the effective activity of membrane bound enzymes can be varied over a large range via the structure and lateral organization of the lipid bilayer moiety. The biological relevance of these findings is still an open question. Below (cf. 8.7.2) we will describe experiments where the decomposition of proteins into an active and an inactive form caused by lateral phase separation is clearly demonstrated.

7.5. Fluctuation in membranes: precritical phenomena and lipid mediated protein-protein interactions

One major advantage of the Landau theory is that it allows to deal with fluctuations in the order parameter. Far away from true critical points they can be described in terms of the classical Ornstein–Zernicke theory (cf. Landau and Lifshitz [10a], § 146). It is based on the ideas (1) that local fluctuations $\delta \eta$ in the order parameter considered can be described in terms of a Fourier expansion in planar waves of
Fig. 24. Breaks in Arrhenius plots of enzyme activity and lipid bilayer conformational change. The reaction rates, $k$, are plotted as a function of temperature. (-----) Rate of reduction of spin labelled fatty acid by cytochrome P450 in rat liver microsomes. (----) Adenylate cyclase activity in Chang liver cells. (——) Rate of active sugar transport in Escherichia coli bacteria by sugar transport protein. Reproduced from ref. [2b] where references to original work are given.

The wavevector $\vec{q} = (q_x, q_y)$ and (2) that the mean square amplitude of each mode is determined by the equipartition theorem. The Landau theory then yields

$$\langle |\delta \eta(q)|^2 \rangle = \frac{k_B T}{2A(\beta q^2 + a(T - T^*))}$$  \hspace{1cm} (46)

where $\beta$ and $a$ are defined in eqs (11) and (12) and where $A$ is the membrane area. More interesting is the spatial variation of the fluctuation which is described by the correlation function $K_\eta(\xi)$. It is obtained by 2D Fourier transformation of eq. (46) and is for $T > T_c$

$$K_\eta(\xi) = \text{const} \cdot k_B T \sqrt{\frac{\xi}{r}} e^{-r/\xi}$$  \hspace{1cm} (47)
with
\[ \xi = \sqrt{\frac{\beta}{a}} \frac{(T - T_c)}{1}. \] (48)

The most useful result is the introduction of the correlation length \( \xi \) which determines the distance over which a certain fluctuation extends. \( \xi \) increases drastically if one approaches the critical temperature \( T_c \). Equation (46) holds only far away from a true critical point. If such a point is approached, the fluctuations decay even much more slowly with distance, namely
\[ K_\eta(\xi) \propto \left( \frac{r}{\xi} \right)^{-1/4} \] (49)
(cf. reference [122]).

Let us consider two examples of fluctuation controlled membrane processes.

1) Lipid mediated protein-protein interaction

The exponential law of eq. (15) has been verified by Owicki and McConnell [30] in numerical calculations of local thickness fluctuations of bilayers (e.g., about an integral protein). It has been further verified in molecular field calculations of the orientational order parameter (cf. chapter 7).

The perturbation of the lipid order results in a protein-protein interaction for the following reason: If the perturbed lipid halos of two proteins overlap a force arises which is expected to decay exponentially. The interaction potential is of the form [83]
\[ W_{ij}(r) = -\text{const} \cdot \delta S_i \delta S_j \frac{R_p}{\sqrt{r}} \exp \left\{ \frac{-2(r - R_p)}{\xi} \right\}, \] (50)
where \( R_p \) is the radius of the protein and \( \delta S_i \) is the fluctuation of the order parameter. The range of interaction is determined by the coherence length which is only of the order of two to four lattice constants (\( \sim 30 \) Å) far away from critical points. Obviously the force is attractive if the fluctuations of the order parameters have the same sign, e.g., if the proteins are identical. Another intriguing mechanism of long range protein-protein interaction mediated by the thermally driven undulations has recently proposed by Bruinsma et al. [64]. Evidence was provided that it can be stronger and of longer range than the other lipid mediated forces. These forces are generated by the resistance of the membrane against bending. They may lead to the aggregation of many proteins into patches; for instance plasma membrane receptors bending to a surface (e.g., anotherall or a solid surface). In the presence of an additional repulsive disjoining pressure between the two surfaces (e.g., based on undulation forces or electrostatic forces) the cell adheres to the surface locally but is decoupled between the strongly adhering patches. Examples of such situations are gap-junctions and focal adhesion plaques.
2) Lateral density fluctuations determine passive permeability of solutes (e.g., water) and ions

Fluctuations in the density order parameter have been proposed to play a role for the passive transport of solutes (such as water) or ions through bilayers [85]. They create free volume for the uptake of the solutes which are then driven through the bilayer by the diffusion of the chain defects (e.g., g-t-g-kinks) along the chain [86]. The permeability, \( P_i \), is proportional to the square of the density fluctuation [85]:

\[
P_i \propto \langle |\delta \rho|^2 \rangle
\]

According to the thermodynamic theory of fluctuations the relative density fluctuation is

\[
\frac{\langle |\delta \rho|^2 \rangle}{\rho^2} = k_B T \rho / K
\]

where \( K \) is again the area compressibility modulus. Therefore \( P \propto K^{-1} \). The relative density fluctuation in the L_α-phase (\( \rho = 1.5 \times 10^{14} / \text{cm}^2 \), \( K = 150 \ \text{mN/m} \)) is \( \delta \rho / \rho \approx 4\% \). Since the passive permeability, \( P \), is proportional to \( (\delta \rho / \rho)^2 \), it is quite small. Indeed, the high lateral tension within fluid bilayers guarantees a self-healing effect and prevents the passive ion efflux through cell membranes (e.g., of erythrocytes) even under high elastic strain. It has been postulated that one primary role of cholesterol is actually to reduce the density fluctuations [4]. The situation changes completely at chain melting transitions or during phase separation. Then the lateral compressibility diverges (as shown in Fig. 22b) and therefore the passive ion permeability becomes very high (cf. reference [4], Fig. 33). The ion flux then occurs actually through defects at interfacial boundaries. Since cholesterol tends to accumulate in defects at phase boundaries (cf. Fig. 28c) it would help to reduce ion losses in two ways: (1) by increase of \( K \) and (2) by plugging of the defects for instance at the boundary between proteins and the lipid matrix.

8. Structure and properties of 2D lipid-lipid and lipid-protein alloys

8.1. Lipid bilayer as 2D-solvent

One major task of lipid bilayers is to provide a solvent for small amphiphilic and hydrophobic molecules (metabolites, drugs) and for membrane associated proteins. The solubility of small hydrophobic molecules (e.g., aromatics as pyrene) in the hydrophobic core is very high (10 to 20 mole\% ) and even that of strongly polar molecules (such as water) is substantial (\( \sim 10^{-3} \) M). In fact, the solubilization of non-polar substances by membranes may be quite problematic, since it leads to the long-time accumulation of toxic substances (e.g., organic lead compounds) in cell membranes if they cannot be rapidly transformed into polar water soluble products (e.g., by the P450-oxidase/reductase system of the liver, cf. Fig. 24).

Owing to its two-dimensionality and liquid crystalline character lipid bilayers are outstanding solvents. Proteins may be solubilized in membranes by complete embedding but they can also be integrated by adsorption (as shown in Fig. 32b). Judged
from in vitro experiments, elements of the cytoskeleton (talin, α-actinin, spectrin) but also phospholipase are important examples of the latter type of ‘solubilization’.

From the point of view of polymer physics most interesting is the solubilization of macrolipids in lipid bilayer in order to prepare two-dimensional macromolecular solutions and gels (cf. fig. 25). These are presently mainly of academic interest in order to test scaling theories of low dimensional macromolecular solutions and gels. They are also of great potential practical interest, e.g., for the stabilization of vesicles or of supported lipid bilayers for biosensor designs. A biophysical aspect of partially polymerized vesicles of the type of fig. 25a is their application as mechanical models of the erythrocyte compound membrane.

8.2. Regular solution theory of lipid-lipid and lipid-protein mixtures

Solutions of proteins in bilayers or mixtures of lipids of different shape (e.g., chain length) are strongly non-ideal and two-dimensional. Nevertheless, their behaviour can be well described by the classical theories of solutions and mixtures such as the regular solution theory of Hildebrand, or the lattice models of Bragg and Williams or Flory and Huggins. These simple models have a common basis and have been astonishingly successful for the description of such different systems as metal alloys and polymer solutions. The main reason for their success is that they provide a unified model for the understanding of the physics of all kinds of many-component systems. Thus membrane physics can profit from decades of research in metal and polymer physics. The thermodynamic theory of mixtures are well described in the classical monographs of Lewis and Randal [87], Guggenheim [88] or Flory [89]. However, while the classical theories are well applicable to all lipid-lipid mixtures and lipid-protein solutions with predominant hydrophobic interaction they have to be modified for situations of adsorbed proteins. A general theory of this situation is still missing.

Both the regular solution theory and the lattice models assume that the entropy of mixings, $\Delta S_m$, is ideal while the non-ideality is determined by an excess heat of mixing, $\Delta H_m$ (which could be measured in principle in a mixing calorimeter). It is customary to express the total free energy of the mixtures, $\Delta G_m$, in terms of the contribution of the pure components and the excess quantities. For a binary system in a phase $\varepsilon$ (e.g., $L_\alpha$, $L_\beta$ etc.), $\Delta G_m$ is

$$\Delta G_m = n_1 \mu_{1,0} + n_2 \mu_{2,0} + \Delta H_m - T \Delta S_m. \tag{52}$$

In this equation 1 and 2 denote the components and $\mu_{i,0}$ are the chemical potentials ($= \text{partial molar Gibbs free energies}$) of the pure components ($i = 1, 2$).

$$\Delta G_{m,ex} = \Delta H_m - T \Delta S_m \tag{53}$$

is the excess free energy of mixing.

It is important to distinguish between mixtures of components of equal and very different size which will be treated separately below.
Fig. 25a. Two examples of two dimensional polymer solution or gels: Linear macrolipids of amphiphiles interconnected by hydrophilic chains which may be cross-linked by amphiphiles with two functional groups at the head (indicated by arrow).

Fig. 25b. Two examples of two dimensional polymer solution or gels: 2D-sponge phase prepared by photochemical cross-linking of diacetylene-phosphatidylcholine dissolved in phospholipid (DPPC) membrane (after reference [12]).
Equal-size lipid mixtures

Since the two types of lipid molecules occupy roughly the same molecular area within the bilayer, the lattice model can be applied and the partial excess free energy is given by the well-known expression

$$\frac{1}{N} \Delta G_{ex}^{\epsilon} = k_B T (x_1 \ln x_1 + x_2 \ln x_2 - w x_1 x_2)$$  \hspace{1cm} (54)$$

where $N$ is the total number of lipid molecules $N = n_1 + n_2$, $x_i$ is the molar fraction of component $i$ ($x_i = n_i/(n_1 + n_2)$) and

$$w = 1/2 Z (2 w_{12} - w_{11} - w_{22})$$  \hspace{1cm} (55)$$
is a measure for the difference of the intermolecular forces between the two components (cf. for instance Lewis and Randall [87], chapter 21, for a derivation of $w$) $Z$ is the coordination number of the lattice of lipid molecules.

The key point is that the interaction parameter $w$ is the only free parameter which determines the behaviour of the mixture (e.g., random mixing or phase separation). It can be determined from many measurable thermodynamic properties, such as:

1) The affinities in the various phase which are given by

$$RT \ln a_i = (\mu_i - \mu_i^0) = RT \ln x_i + wx_i^2.$$  \hspace{1cm} (56)$$
(Note that the suffix of the phase has been deleted and $j = 2$ if $i = 1$ and vice versa.) As shown below $a_i$ can be measured by evaluation of the freezing point depression; provided the solubility of the solute in the fluid and solid phases (e.g., $L_{\alpha}, L_{\beta}$) are known.

2) The heat of mixing which is given by

$$\Delta H_{mix} = \Delta G_{mix} + T \Delta S_{mix} = Nx_1 x_2 \left( w - T \frac{dw}{dT} \right)$$  \hspace{1cm} (57)$$
and which will be considered below (cf. eq. (62)).

3) The most rigorous way to determine the interaction parameters and to elucidate deviations from the regular solution behaviour is by computer simulation of the phase diagrams of the lipid mixtures, and comparison of calculated and observed phase diagrams (cf. fig. 29).

Mixtures of different size components (protein solutions)

Here the situation is much more complicated since both the difference in the area occupied by the lipids and the proteins within the bilayer as well as the shape of the macromolecule have to be considered.

In the classical book on mixtures by Guggenheim [88b], the modification of eq. (54) has been calculated for several simple cases: (1) oligomers occupying three or four adjacent lattice sites or (2) linear chains. For the general case of integral
proteins penetrating the bilayer with several α-helices it is more appropriate to apply
the more crude Scatchard-model based on following ideas:
The molar fractions $x_i$ are replaced by the area fractions

$$\phi_L = \frac{n_L A_L}{n_L A_L + n_P A_P}; \quad \phi_P = \frac{n_P A_P}{n_L A_L + n_P A_P}.$$  \hspace{1cm} (58)

$L$ and $P$ stand for lipid and protein, respectively; $A_P$ and $A_L$ are the molecular areas
of the protein and the lipid, respectively. It is often helpful to introduce the area
ratio

$$g = A_P / A_L$$  \hspace{1cm} (59)

which is a measure for the number of lipid sites occupied by the protein. With these
definitions the (ideal) entropy of mixing and the excess enthalpy of mixing are

$$\Delta S_{\text{mix}} = N R \left[ \phi_L \ln \phi_L + \frac{\phi_P}{g} \ln \phi_P \right],$$  \hspace{1cm} (60)

$$\Delta H_{\text{mix}}^{\text{ex}} = N W_{LP} \phi_L \phi_P$$

where $N = n_L + g n_P$ is the total number of lattice sites and where $W_{LP}$ is again
the interaction parameter characterizing the difference of the interaction between
two lipids and between a lipid and the protein. For $g = 1$ these equations become
identical with the corresponding function in eq. (54). Since the area ratio $g$ is in
general given, the only free quantity is again the interaction parameter $W_{LP}$. This
parameter as well as $\omega$ in eq. (54) could be expressed in units of $k_B T$ as normally
done in dealing with polymer mixtures. The partial free energies of these types of
solutions can again be obtained by application of the classical rules (cf. for instance
Guggenheim [88a], chapter 5). Thus the activity coefficient for the lipid is

$$\ln a_L = \ln \phi_L + (1 - g^{-1}) \phi_P + W_{LP}^2 \phi_P^2.$$  \hspace{1cm} (61)

The diameter of an α-helix is roughly 1 nm and large membrane spanning proteins
of the type of fig. 32a (such as band III) occupy about 10 lattice sites in the bilayer
and the mixing entropy is thus considerably reduced. For that reason these proteins
are expected to aggregate already at volume fractions of $\phi \sim 0.1$ (molar fractions
$x_P \sim 10^{-3}$).

Heat of transition and determination of number of lipids interacting with a protein
Based on the following idea, measurements of the reduction of the heat of transition
as a function of the protein concentration have been used to obtain information on the
number of lipids interacting with a protein molecule (cf. Kurrle et al. [90] for further
references). Ignoring the temperature dependence of the interaction parameter $W_{LP}$,
the molar heat of \((L_{\alpha} \to P_{\beta'})\) transition as measured by differential calorimetry is

\[
\frac{1}{N} \Delta H_1 = \varphi_L \Delta H^0_L(T_m) + \varphi_P \Delta H^0_P(T_m) + \frac{1}{g} (W_{LP}^a - W_{LP}^b) \varphi_L \cdot \varphi_P.
\]

\(N\) is again the number of lattice sites (analogous to Flory–Huggins model). \(\Delta H^0_L(T_m)\) is the heat of transition of the pure lipid (at the chain melting transition) and \(\Delta H^0_P\) the heat content of the protein-lipid aggregate at the chain melting transition temperature. It would correspond to the heat of transition of a saturated protein solution. Unfortunately, \(\Delta H^0_P\) and the interaction parameters in the third term are not known. However, at very small protein concentrations the molar heat of transition decreases linearly with the area fraction of unaffected lipid. The value of \(1 - x_P\) obtained by extrapolation of the \(\Delta H_m\)-versus-\(x_p\) plots to \(\Delta H_m = 0\) is an approximate measure of the number of lipids interacting with the protein.

8.3. Lipid incompatibility and segregation

The evaluation of the thermodynamic and structural properties of lipid/lipid and lipid/protein alloys is a primary and long time task of membrane physics. It is believed that it is essential for the understanding of the physical basis of self organization and function of biological membranes. It is of practical importance for instance for the application of vesicles as drug delivery systems or for the design of biosensors. Last not least, mixtures of amphiphiles exhibit a manifold of exciting new physical properties.

Three purposes of the strategy of nature to use many different lipids are obvious: (1) the stabilization of bilayer structures (2) the adaption of the membrane consistency (fluidity) to the environmental conditions and (3) the control of the lateral organization and trans-membrane asymmetry (organization) of the membranes. As in conventional mixtures, phase incompatibility is expected if the components exhibit phases of different symmetry (e.g., tilted and non-tilted) or differ strongly in their molecular structure (e.g., chain length).

The situation is very complicated since phase separation may not only occur within the bilayers (and is then called lateral phase separation). It can secondly lead to a redistribution of the components between the monolayers or may thirdly result in a decomposition of the bilayers. We call the former two types homogeneous and the latter heterogeneous phase separation. Moreover, the behaviour may be different for low and high lipid concentrations. In this article we restrict on the lateral phase separation in vesicles which is considered as the most interesting situation from the point of view of biomembrane physics.

Many methods have been applied to study phase separation in bilayers. Firstly, the classical methods as calorimetry [90, 91], densitometry [92] or X-ray diffraction. Secondly, many spectroscopic techniques such as fluorescence- [65, 66], FTIR- [90], spinlabel- [95–95] and NMR spectroscopy (cf. review by Bloom et al. [4] and [100]). Thirdly, freeze fracture electron microscopy [21, 97] and fourthly, small
Fig. 26. Hypothetical phase diagram of binary mixture of lipids with moderately different transition temperatures of pure components. It exhibits in general peritectic behaviour of the fluid-solid coexistence \((\alpha + \beta)\) if the solid-solid miscibility gap \((\beta_1 + \beta_2)\) penetrates into the \(\alpha + \beta\)-coexistence. The critical point \(T^*\) is for instance shifted to higher temperatures with increasing difference in chain length. For PC’s the situation is more complex owing to the solid-solid \((P_{\beta'} \rightarrow L_{\beta'})\)-transitions (cf. example of DMPC-cholesterol, fig. 28).

angle neutron scattering (SANS) [98, 99]. Only NMR and SANS appear to be suited to study fluid-fluid or solid-solid immiscibility.

The phase diagrams of binary lipid mixtures have in general the shape shown in fig. 26. If the lipids exhibit only a small difference in chain length (\(\leq\) two CH\(_2\)-groups per chain) and thus in the transition temperatures, one observes a cigar-like phase diagram (of the fluid-solid coexistence) while complete miscibility prevails in the fluid and the solid states (with the possible exception of the \(L_c\)-state). At increasing chain length differences, a miscibility gap appears below the cigar, the critical point of which eventually extends into the fluid solid coexistence with increasing shape difference. Under these conditions a peritectic or an eutectic behaviour may result. Up to the present only peritectic systems have been clearly established. An example of this type of mixture is the DMPC-DSPC system shown in fig. 27. Its most outstanding feature is an immiscibility above the liquidus line which is attributed to a critical point hidden within the fluid-solid coexistence-region [99]. This behaviour will be further discussed below.

Phase diagrams with fluid-fluid miscibility gaps are in principle also possible and are in particular expected for mixtures of lipids with large structural differences or if
Fig. 27. Phase diagram of DMPC/DSPC mixed vesicles. Full lines: mixture of protonated lipids; broken lines: mixture of protonated DSPC but chain deuterated DMPC-\textsubscript{d54} used for SANS evaluation of phase diagram. \(L_{\alpha} \rightarrow P_{\beta}^{'}\)-transition at \(T_{m} = 23^\circ\text{C}\) for DMPC and \(T_{m} = 17^\circ\text{C}\) for DMPC-\textsubscript{d54}. The liquidus lines \((T_l)\) and the solidus lines \((T_s)\) were obtained by calorimetry, and densitometry. The break in the liquidus provides clear indication for peritectic behaviour.

one component forms a tilted phase. The most prominent examples of the former type are mixtures of cholesterol with phosphatidylcholines; in particular DMPC (which will be discussed below) and DPPC [4]. Evidence for another fluid fluid immiscibility has been reported for DEPC (cis C18 : 1 PC) and DPPE [93]).

Figure 28 shows the phase diagram of the DMPC-cholesterol mixture as revealed by (1) the SANS contrast variation technique of fully hydrated vesicles [23b, 98], (2) SANS studies at 17 weight \% of water [26], (3) calorimetry [91], (4) ESR-studies [103] and (5) theoretical considerations [102]. The former methods have been reviewed recently [23b]. Some typical calorimetric curves are shown in fig. 28b. The cholesterol-lecithin phase diagram has also been extensively evaluated by monolayer studies [104, 105]. The salient features of the phase diagrams as suggested by these combined studies are:

1) Judged from SANS studies three solutions exist at low temperature \((T < T_m)\): (1) a (homogeneous) solid solution for \(x_{\text{chol}} < 0.08\) (called \(L_{\beta}^{'}\)); (2) a stoichiometric mixture \((L_{st})\) containing about 25–28\% cholesterol; (3) a
cholesterol rich solution (Lα) for $0.25 \leq x_{\text{chol}} \leq 0.45$. Above $x_{\text{chol}} \sim 0.45$ saturation is reached and cholesterol precipitates (both below and above $T_1$) either (laterally) within the bilayer or as crystallites in water.

2) For $x_{\text{chol}} < 0.04$ the $L_{\beta'} \rightarrow P_{\beta'}$-transition is still visible. In the $P_{\beta'}$-phase the $\Lambda/2$-superstructure is stabilized exhibiting a remarkable regular defect texture (cf. fig. 28c).

3) The $L_{\beta'}$- and $L_s$-phase coexist for $0.08 \leq x_{\text{chol}} \leq 0.25$. SANS suggests [98] that the stoichiometric mixture and the (homogeneous) saturated solution $L_s$ coexist for $0.25 \leq x_{\text{chol}} \leq 0.45$.

4) A fluid-fluid miscibility gap is strongly suggested by calorimetry (cf. fig. 28b) by considering the analogy to the behaviour found for the DPPC-cholesterol mixture [100, 101] and by Monte Carlo studies [102]. Figure 28b shows some specific heat-versus-$T$ plots. The high temperature end of the broad band at $T_u$ marks the upper phase boundary of the miscibility gap. The sharp band ranging from 23 to $18^\circ$C corresponds to the transition into the gel state. Its upper and lower end define the phase boundary of the transition into the solid
miscibility gap which is directly observed by SANS [98b]. Evidence for the fluid fluid miscibility gap is also provided by the anomalously large thermal expansion coefficient observed by SANS [23a]. It has finally be verified by lateral diffusion measurements [95].

5) The phase diagram of fig. 28 appears to be universal for all PC’s including those with non-saturated chains (e.g., SOPC). There may be chain length effects at high cholesterol concentrations. Thus a broad specific heat peak extending form 25 to 50\degree C has been observed for DPPC [106]. Accompanying NMR-studies suggest that this is due to a displacement of cholesterol from the center of the bilayer to the head group region at increasing temperature which is accompanied by a decrease in bilayer thickness of 0.4 nm.

The most interesting aspect of the phase separation in the fluid state is the expected critical behaviour above the critical point (which is expected at $T_c \approx 45 \pm 5\degree C$, $x_{chol}^c \approx 0.3 \pm 0.05$). First experimental evidence was provided by monolayer studies [105]. In these the domain structure of fluid monolayers is analyzed by fluorescence microscopy in a temperature and pressure regime where the critical point is expected. By approaching the critical point from the two-phase region (where a condensed and an expanded fluid phase coexist), it is found that the initially circular domains of the condensed phase become ragged very elongated and eventually dis-
Fig. 28c. Freeze fracture electron micrographs of DMPC-cholesterol mixtures I) DMPC + 4% cholesterol frozen from $P_{\beta'}$-phase (15°C) and $L_{\beta'}$-phase (4°C). Note stabilization of defect structure of surface texture. II) DMPC + 28% cholesterol frozen from 15°C. III) Model of gel phases containing < 8% cholesterol (top) and 15DMPC.
Fig. 29. Comparison of experimental (triangles) and calculated (drawn lines) cigar-like phase diagram of DMPC/DMPS mixed vesicles. The dashed lines would correspond to the ideal mixture. The chain melting transition temperatures are $T_{m}(\text{DMPC}) = 23^\circ\text{C}$ and $T_{m}(\text{DMPS}) = 35^\circ\text{C}$. The heats of transitions of the pure components are: $\Delta H_{m}(\text{DMPC}) = 28\text{kJ/M}$ and $\Delta H_{m}(\text{DMPS}) = 29.2\text{kJ/M}$. The excess free energies are $\Delta G_{ex,\alpha}^{m} = 4.0\text{kJ/M}$ for the $L_{\alpha}$ and $\Delta G_{ex,\beta}^{m} = 4.8\text{kJ/M}$ for the $L_{\beta'}$-phase. The lines 1,2 and $1',2'$ are calculated shifts of the liquidus and solidus-line, respectively, caused by the adsorption of increasing amounts of spectrin [107c]. The points marked by (●) correspond to observed shifts of the phase boundaries caused by the adsorption of spectrin (molar fraction $x_{p} = 10^{-4}$ with respect to lipid).

solve. Such a behaviour is indeed expected for a critical state where the correlation lengths of the two phases diverge according to $(T - T_{c})^{\alpha}$ (cf. also reference [102]).

Another intriguing finding of the monolayer [105] and Monte Carlo studies [102] is that cholesterol appears to reduce the interfacial energy between fluid and solid domains of PC which may even become negative. This is inferred from the very fine mosaic structure (composed of interwoven elongated domains as shown in fig. 23c) which cholesterol induces in PC monolayers within the fluid solid coexistence (cf. chapter 4). It is also most probably responsible for the very elongated domains in the freeze fracture electron micrograph [23b]. A second interesting finding is the above mentioned reduction of the bilayer thickness by about 0.4 nm at $x_{\text{chol}} > 0.25$ (as compared to $x_{\text{chol}} < 0.1$). The size of the domains after separation into a cholesterol-rich and a cholesterol-poor phase ($L_{0}$ and $L_{s}$ in fig. 28a) would thus be determined by the balance of the chemical and the elastic interfacial energy. While the former favours very thin domains the latter counteracts this effect.
8.4. Calculation of phase diagrams by regular solution theory

The phase diagrams of lipid mixtures can be remarkably well interpreted quantitatively by the regular solution theory. Several examples have been calculated [107, 108]. The case of the DMPC/DSPS mixtures is shown in fig. 29. Good agreement between observed and calculated phase diagrams is observed.

As mentioned above, the establishment of phase diagrams of lipid mixtures and their simulation in order to determine the interaction parameters is a tedious but essential task. It is the only way for a quantitative characterization of the lipid-lipid interaction in order to understand the physical basis of self organization of membranes or to explore the molecular basis of diseases based on malfunctions of the lipid metabolism.

Another benefit of such work is that once the parameters characterizing the lipid-lipid interaction are known, the selective lipid-protein interaction can be studied by application of a perturbation technique [107c]. The shifts of the phase boundaries after reconstitution of very small molar fractions of the protein in the mixed vesicles are measured as function of the lipid-composition. Comparison of measured and calculated phase diagrams yields then the interaction parameters characterizing the selective interaction of the protein with the two lipid components. A first step in this direction is shown in fig. 29 where the shift of the liquidus line of the DMPC-DMPS mixture after addition of spectrin is shown together with the corresponding interaction parameter.

8.5. Domain formation in mixed membranes, spinodal decomposition and coupling between phase separation and bending

One of the most intriguing aspects of phase separation in vesicles is the formation of a stable domain structure discovered in 1977 [21] which is observed in many cases of thermally or chemically induced (e.g., by protein adsorption) phase separation. Some examples are shown in fig. 30. It plays a central role for stabilization of vesicles in the fluid-solid coexistence [12] or the fission of small vesicles after budding. As demonstrated in fig. 30b, a large part of one lipid component forms buds and is detached from the mother vesicle until a stable vesicle with domain structure results. It is very likely that the coupling, budding and phase separation plays an important role for the budding-fission-fusion sequence of events during membrane trafficking in cells and the selective redistribution of lipids and proteins during this process (cf. chapter 1).

In most cases the domain formation appears to be caused by the coupling of phase separation and bending of the bilayer and is closely related to the possibility of bilayers to escape easily into the third dimension. However, as already suggested above, a difference in the thickness of the two phases would be another possibility in cases where the chemical interfacial energy between the phases is very small or negative (as in the cholesterol/DMPC mixture).

One major question is, why the coarsening of the domains formed in the initial stage of the phase separation process does not proceed until both phases are macroscopically separated. The stabilization of domains has been explained in terms of
the splay deformation arising at the interface between the two phases and it has been shown that the total interfacial energy of a domain is proportional to the square of the domain radius $\rho$ [21, 109]: $g_{sf} \propto \kappa \cdot \rho^2$. In fact, domain sizes of the order of some 10 nm have been calculated by this model.

The coupling of phase separation and curvature can be described in a very general way by an extension of the celebrated Cahn–Hilliard mean field theory of spinodal decomposition. The process of phase separation (in a binary mixture) is determined by the Landau type of chemical free energy density

$$g_{\text{chem}}(r) = \frac{1}{2} A(\pi, T) \psi(r)^2 + \frac{1}{2} B(\pi, T) \psi(r)^4 + \frac{1}{2} C(\pi, T) (\nabla \psi(r))^2$$

(63)

where $\psi(r)$ is the normalized difference of the concentration of the two components, ($\psi = c_A - c_B$) and is a function of the position within the plane of the membrane.
Fig. 30c. Formation of domain structure and stability of vesicles undergoing lateral phase separation as observed by freeze fracture electron microscopy: Elongated domains formed in \( L_\alpha/P_{\beta'} \)-coexistence regime of vesicle composed of DPPC and diacetylene-PC. Possible example of facette formation.

\( \nabla \psi(r) \) denotes again the gradient of the local concentration variation. It is absolutely necessary in order to introduce a scale factor describing the correlation length of the concentration fluctuations [110–113].

As is well known from metallurgy the process of phase separation is described by a generalized diffusion equation with an effective diffusion coefficient

\[
D_{\text{eff}} = \beta \cdot \partial^2 g_{\text{chem}} / \partial \psi^2
\]  

(64)

where \( g_{\text{chem}} \) is the chemical energy per unit area and \( \beta \) is the mobility of the lipid molecules which is related to the local diffusion coefficient, \( D_0 \), as \( \beta = (k_B T)^{-1} \cdot D_0 \). \( D_{\text{eff}} \) becomes negative within the concentration regime of the spinodale (defined by the condition \( \partial^2 g_{\text{chem}} / \partial x^2 \leq 0 \)) and the components segregate by uphill diffusion. The process is described by a generalized diffusion equation [110].

\[
\frac{\partial \psi}{\partial t} = \beta \Delta (\delta G_{\text{chem}} / \delta \psi),
\]  

(65)

where \( \delta G_{\text{chem}} / \delta \psi \) is the variation of the total energy \( G_{\text{chem}} = \int g_{\text{chem}} \, dA \) with respect to \( \psi \).
Fig. 30d. Formation of domain structure and stability of vesicles undergoing lateral phase separation as observed by freeze fracture electron microscopy: Domain formation in 1:1 mixture of DMPC and DMPE. Vesicles were quenched from a temperature where a fluid phase enriched in DMPC (80%) and a crystalline phase rich in DMPE (80%) coexist. Simultaneously shown is the surface profile indicating that fluid domains are slightly curved while the crystalline domains are flat (reproduced from [19]).

Fig. 30e. Formation of domain structure and stability of vesicles undergoing lateral phase separation as observed by freeze fracture electron microscopy: Fission of budded vesicles from giant vesicles of 60:40 DMPC/cholesterol mixtures after expansion of area-to-volume ratio by osmotic deflation. The budding-fission process occurs repeatedly.
In the regime where $D_{\text{eff}}$ is negative, an initially homogeneous solution is unstable to infinitesimal concentration fluctuations and concentration waves start to form by uphill diffusion according to

$$\psi(r, t) = \sum_{q} \Psi_q \exp\{R(q)t\} \cos \vec{q} \cdot \vec{r}$$

(66)

where

$$R(q) = -\beta q^2 \left( \frac{\partial^2 g_{\text{chem}}}{\partial \psi^2} + C(\pi, T)q^2 \right).$$

(67)

Clearly all long wavelength fluctuations with

$$q \leq q^* = \sqrt{-\frac{\partial^2 g_{\text{chem}}}{\partial \psi^2}/C}$$

(68)

grow exponentially while those with $q \geq q^*$ decay. The initial step of phase separation is therefore very fast and cannot be treated as a normal diffusion process. The fastest growing wave is that with wavelength $\lambda_{\text{max}} = \sqrt{8\pi/q^*}$.

The above perturbation approach can only describe the initial process of phase separation while the generalized diffusion equation cannot be solved for the subsequent growth of the concentration waves (the so called coarsening) since it is highly non-linear. It has been shown, however, that the final growth of the domains follows a $t^{1/3}$-law [110]. Therefore a domain structure may persist over a long time period.

The spinodal approach is very useful to understand the stabilization of the domain structure by the elastic deformation as follows: In order to account for the deformation of the vesicle during phase separation one has also to consider the change of the bending elastic energy of the vesicle as well as the coupling of curvature and phase separation. The former is given by eq. (20). The coupling is again considered by introduction of a coupling term following the rules of the Landau theory. The lowest order term would be [112, 113]

$$g_{\text{coupl}} = \frac{1}{2} \gamma \left( \frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} \right)^2 \psi(r),$$

(69)

where $g_{\text{coupl}}$ is an energy density. Higher order terms have been considered by Andelman and Leibler [113] but will be ignored here.

The general diffusion equation is now obtained from eq. (63) by replacing $G_{\text{chem}}$ by the total energy

$$G_{\text{tot}} = \int (g_{\text{chem}} + g_{\text{bend}} + g_{\text{coupl}}) \, dA$$

(70)

and is even more complicated than the classical Cahn–Hillard–Langer theory. One can however see the following:
If we consider the elastic coupling term of eq. (69) and a one-dimensional situation, the wave vector dependent growth frequency \( R(q) \) is now approximately of the form (cf. Hilliard [111] for similar calculation).

\[
R'(q) = -\beta q^2 \left( \frac{\partial^2 g_{\text{chem}}}{\partial \psi^2} + Cq^2 + H\gamma \right).
\]  

(71)

If the coupling constant \( \gamma \) is positive it counteracts the driving force of the negative term: \( \partial^2 g_{\text{chem}} / \partial \psi^2 \). Under this condition, the elastic distortion leads to a slowing down of the growth and changes the wavelength of the fastest growing waves. In the same way the coarsening is slowed-down drastically.

Up to the present the modified Cahn–Hilliard–Langer equation has only been solved for the one dimensional case. Leibler and Andelmann [113] calculated phase diagrams of stable domain structures as a function of temperature and composition. They found two types of stable domain structures: (1) stripe-like arrangements of two-dimensional precipitates and (2) hexagonal arrangements of circular domains. Both types of domain structures have indeed been observed (cf. [21] and fig. 30). Plane-wave domains have only been found for membranes exhibiting fluid-gel coexistence [3], whereas circular domains are commonly observed in cases of fluid-fluid demixing, for instance induced by adsorption of proteins [3, 21].

Starting from eq. (65), Wonneberger [112] showed for a slightly different coupling term that the modified Cahn equation is isomorphic to the Ginzburg–Landau equation and exhibits stationary sinusoidal decomposition profiles. Another interesting prediction is that an initially flat membrane becomes globally curved; in addition to the local ripple formation following the sinusoidal concentration profiles. This may indeed be the reason why the domain structure formation is so typical for vesicles. Only for these, an escape into the third dimension is easily possible. It should be noted, however, that phase separation induced curvature can always be avoided by suitable redistribution of the components in the two leaflets of the bilayer.

An important experimental finding is that the circular domains escaping into the third dimension exhibit a rather regular hexagonal arrangement (cf. case of polylysine induced phase separation in fig. 30). This is clearly a consequence of the elastic interaction between the domains. An interesting consequence of this interaction is that it may lead to an additional shear elasticity.

The fission of budded vesicles which is seldom observed for one-component fluid vesicles (cf. [56]) is quite common for mixed vesicles. One example is shown in fig. 30b. Another of more biological relevance is shown in fig. 30e.

There are two mechanisms which may drive vesicle fission. One is the accumulation of a component in the necks connecting the bud and the mother vesicle. A second mechanism was proposed by Lipowsky [114a]. It is based on the idea that the chemical interfacial energy (the 3rd term in eq. (63)) is reduced by formation of a local bud with the interface at the neck. The radius, \( R_B \), of the bud is so determined by the condition that the bending energy cost to form the bud is compensated by the gain in interfacial energy. For that reason \( R_B \) is given by eq. (2b).

This process will be described in detail in the article by Lipowsky. The fission of
PC-cholesterol vesicles by osmotic deflation (fig. 30e) is most probably driven by the first type of mechanism. According to SANS [98b] cholesterol precipitates in fluid DMPC bilayers at $x_{\text{chol}} > 0.4$ while large scale phase separation has not been observed. Since the lipid packing density is smaller in the neck than in the bulk of the vesicle, cholesterol is expected to accumulate there. The pinch-off is driven by thermal fluctuations. An example for the second mechanism is the fission of small vesicles from giant vesicles of brain sphingomyelin during heating [114b]. This lipid exhibits lateral phase separation between 30 and 50°C (that is under physiological conditions). Heating through this regime (that is increasing the excess area) leads to the continuous detachment of small vesicles, the lipid composition of which differs from that of the mother vesicle. Such processes of preferred budding and fission of a certain lipid fraction could well play a role for the selective lipid (and protein) trafficking in cells, e.g., the recycling of SPHM into the plasma membrane by selective fission from CURL-vesicles.

In summary, the coupling of phase separation and shape transitions of vesicles is a most fascinating albeit complex problem. A more systematic study of these processes is certainly crucial in order to understand many essential cellular processes. However, it is certainly also of great scientific interest to explore the coupling of phase separation and curvature in a two-dimensional space. Fortunately, better theories are gradually developed (cf. article by Lipowsky).

8.6. Thermodynamics of lipid-protein interactions

8.6.1. Mechanisms and consequences of selective lipid-protein interactions
An essential and still widely open question concerns the selectivity of lipid-protein interaction and (provided it exists) the consequences for the molecular organization
of multicomponent membranes. Established selective interaction mechanisms are (1) the elastic mechanism leading to the selective interaction of proteins with length-adapted lipids (cf. fig. 32a and [2–4]) and (2) the electrostatic mechanism which is restricted to partially charged bilayers (cf. fig. 32b and [3, 107]). The former mechanism determines the membrane coupling of proteins penetrating the bilayer with several α-helices such as ion channels (e.g., band III, β-adrenergic receptors or rhodopsin). The latter mechanisms was observed for hormone receptors with excess positive charge of the cytoplasmic or extracellular head groups [90] and for proteins mediating the coupling of actin networks to membranes (such as talin and hisactophilin, [119]). In fact the hormone receptors are examples where both mechanisms may contribute about equally (cf. fig. 33a and [90]). The electrostatic coupling may be further enforced by nonspecific adsorption of the protein head groups to the bilayer surface (cf. [116]).

In fact, at the present state of our knowledge of lipid-protein multicomponent systems one can only speculate on the biological role of the selective lipid-protein interaction. Judged form the model membrane studies it is expected to play an important role for the local formation of functional complexes or specialized regions (e.g., in the grana and stroma fractions of the thylakoid membranes). It could help to control the concerted synthesis of appropriate amounts of certain types of proteins and lipids during membrane biosynthesis and could thus account for the fact that the lipid and protein composition of the various cell organelles is so astonishingly well controlled (cf. example of sphingomyelin discussed in chapter 1 and above).

In order to explore the lipid-protein selectivity, systematic (albeit laborious) studies of the thermodynamic and structural properties of lipid-protein mixtures are required. Measurements of partial excess free energies of the proteins (µP) and the lipids (µL) in lipid-protein mixtures is an absolute necessity in order to quantify the strength of lipid-protein interaction and to compare or judge data obtained by different laboratories. Unfortunately, only a few examples have been studied yet. Some methods and examples are summarized below.

8.6.2. Determination of partial excess free energies as a quantitative measure of selective lipid-protein interaction

a). Freezing point depression measurements. The thermodynamic basis of this classical method has been described above. It is very sensitive but yields in general only relative values of the chemical potentials. First, the activity of the lipid is determined by making use of the well-known relationship for the shift of the chain melting temperature, \( T_m \), of the pure lipid (cf. [87, 88] for an excellent review).

\[
\left( \frac{1}{T_m} - \frac{1}{T_{m,0}} \right) \frac{\Delta H_m^0}{R} = \ln \left\{ \frac{a_L^\alpha}{a_L^\beta} \right\},
\]

where \( \Delta H_m^0 \) is the heat of melting of the pure lipid. For an ideal solution (\( a_L = x_L \)) the well-known Raoult law holds:

\[
\Delta T = \frac{RT_{m,0}^2}{\Delta H_m^0} \ln \left\{ \frac{x_L^\alpha}{x_L^\beta} \right\}.
\]
Fig. 32. Types of selective lipid/protein interaction mechanisms. (a) Example of elastic mechanism of selectivity. Solutions of intrinsic membrane proteins in lipid bilayer stressing the fact that the proteins tend to surround themselves with length-adapted lipid in order to minimize elastic distortion energy. (b) Case of integration of proteins into membranes by adsorption (electrostatic mechanism of selectivity). Binding occurs (1) by electrostatic interaction mediated by charged lipid constituent and (2) by partial penetration of hydrophobic protein into demipolar regime. (c) Combination of both mechanisms (e.g., of transferrin receptor). The interaction is determined by the hydrophobic effect and by the electrostatic coupling of the accumulation of charged amino acids to charged lipids. In addition, evidence for a physical adsorption of the large extracellular head group to the bilayer surface was found [90].
It should be realized that the sign of the shift depends on the relative solubility of the protein in the two phases. Only if it is insoluble in the gel \( \beta \)-phase, the familiar equation \( \Delta T = \frac{RT^2_{m,0}/\Delta H^0_{m}}{x^0_p} \) holds.

According to the definition

\[
\mu^\varepsilon_L - \mu^0_{0,L} = RT \ln (a^\varepsilon_L),
\]

\( a^\varepsilon_L \) is a measure for the work require to transfer a lipid molecule from some standard state to the real solution. It is customary to use the pure lipid (in the \( \alpha \) of \( \beta \) phase) as standard state so that \( \mu^\varepsilon_L \) is the excess partial free energy of mixing in the phase \( \varepsilon \).

Once, \( \ln(a^\varepsilon_L) \) has been measured as a function of the protein composition, the activity of the protein can be determined from the Gibbs–Duhem relationship

\[
\delta \ln a_p^\beta = -\frac{x^\varepsilon_L}{x^\alpha_p} \delta \ln a^\varepsilon_L.
\]

Unfortunately, only ratios of the activity in the two phases can be determined from freezing point depressions. In order to determine absolute values it would be necessary to measure simultaneously the concentrations \( x^\varepsilon_{\alpha,\beta} \) of the proteins in the two phases in thermodynamic equilibrium (that is in a state of fluid solid coexistence). In this case one would have an independent measurement of \( a^\varepsilon_{\alpha}/a^\beta_{\varepsilon} \) according to (cf. Guggenheim [88])

\[
a^\varepsilon_L/a^\beta_L = \exp \left\{ -\frac{\mu^\varepsilon_{L,0}(T) - \mu^\beta_{L,0}}{RT} \right\} = \exp \left\{ \frac{\Delta S_m(T - T_{l,0})}{RT_{l,0}} \right\},
\]

where \( \Delta S_m \) is the molar entropy of melting. Provided the distribution of the protein in the two phases does not depend on the concentration \( x_p \) the Gibbs–Duhem relationship of eq. (74) would also hold for the ratio \( a^\varepsilon_{\alpha}/a^\beta_{\varepsilon} \).

Since measurements of the protein distributions have not been performed yet, values of \( \mu_p \) can only be estimated by assuming that the protein solubility in one of the phases is negligible. In many cases this holds for the solid phase.

The most thorough and systematic study of selective lipid-protein interaction was performed by Möhwald et al. [117] who studied the freezing point depression of solutions of the photosynthetic reaction center in bilayers of diacyl-PC of various chain lengths. Some of their data are represented in table 3. A high temperature shift \( (\Delta T_m > 0) \) of the chain melting transitions is found for short chains and a depression \( (\Delta T_m < 0) \) for long chains. This is indeed expected from the mismatch (mattress) model. Since the thickness of the hydrophobic layer of C16:0-PC \( (d_m \approx 3.2 \text{ nm}) \) is larger than that of the protein \( (d_p = 2.8 \text{ nm}) \) it has to be compressed and therefore the solubility of the protein is higher in the \( L_\alpha \)-phase. Incorporation of the protein in the C12:0- and C14:0-PC requires drastic stretching of the chains: hence a higher solubility in the gel phase is expected. In table 3 estimates of excess molar energies are given which were obtained by assuming that the protein is insoluble in the \( L_\alpha \)-phases of the C12:0- and C14:0-PC and the gel phase of the C16:0-PC.
Table 3

Data characterizing the interaction of photosynthetic reaction centers in bilayers of saturated diacyl-PC’s of various chain lengths. Molar fraction of protein: \( x = 2 \times 10^{-4} \); \( T_{m,o} \): chain melting temperatures of pure lipids; \( \Delta H_{o,o} \): heats of transition of pure lipids; \( \Delta T \): shifts of transition temperature; \( \delta d \): mismatch of thicknesses of hydrophobic domains of protein (\( d_p = 2.8 \) nm) and lipid. \( \mu^* \) are maximal absolute values for the chemical potentials of the lipid and the protein obtained by assuming that the protein is insoluble in the gel phase (C16-PC) or in the L\(_{α}\)-phase (C12- and C14-PC).

<table>
<thead>
<tr>
<th></th>
<th>C 12:0 PC</th>
<th>C 13:0 PC</th>
<th>C 14:0 PC</th>
<th>C 15:0 PC</th>
<th>C 16:0 PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_{m,o} [°C] )</td>
<td>–1</td>
<td>12.5</td>
<td>23</td>
<td>34</td>
<td>42</td>
</tr>
<tr>
<td>( \Delta H_{o,o} [kJ/M] )</td>
<td>18</td>
<td>–</td>
<td>26</td>
<td>32</td>
<td>37</td>
</tr>
<tr>
<td>( \delta d [Å] )</td>
<td>–5 ± 1</td>
<td>–3 ± 1</td>
<td>–1 ± 1</td>
<td>0 ± 1</td>
<td>+3 ± 1</td>
</tr>
<tr>
<td>( \Delta T [°C] )</td>
<td>+8 ± 3</td>
<td>+2 ± 1</td>
<td>+1 ± 1</td>
<td>0 ± 1</td>
<td>–3 ± 1</td>
</tr>
<tr>
<td>( \mu^*_L ) (units of ( k_BT ))</td>
<td>0.4</td>
<td>0.1</td>
<td>0.05</td>
<td>0</td>
<td>0.15</td>
</tr>
<tr>
<td>( \mu^*_P ) (units of ( k_BT ))</td>
<td>4000</td>
<td>1000</td>
<td>500</td>
<td>0</td>
<td>1500</td>
</tr>
</tbody>
</table>

b). Protein induced perturbation of binary lipid mixtures [90, 116]. A rigorous way to determine the strength and selectivity of lipid protein interaction is to evaluate ternary lipid-lipid-protein mixtures using a perturbation approach. The effect of very small protein concentrations (\( x_p \leq 10^{-3} \)) on the phase diagram of a binary lipid mixture is determined. Since contributions of the additional entropy of mixing and of the protein-protein interaction to the excess free energy of mixing, \( \Delta G_{ex} \), can be neglected. The excess energies of lipid-protein interaction (that is the interaction parameters \( W_{PL,i} \)) can be determined by measuring the shifts of the solidus and liquidus lines of the lipid mixtures for various compositions. For that purpose the phase diagram of the binary lipid mixture is first calculated by the regular solution theory and the parameters \( W_{PL,i} \) are then obtained by comparing measured and calculated deviations of the phase diagram. This procedure was applied to measure the strength of the selective interaction of spectrin with phosphatidyl serine vesicles (cf. Maksymiv et al. [116b] and fig. 29). An interaction energy per lipid (PS) of \( \mu_p = 0.3k_BT \) and for the spectrin molecule of \( \mu_L = 10^3k_BT \) was found.

c). Spectroscopic techniques. Several spectroscopic techniques have been applied to probe the lipid protein interaction.

– The most prominent is the spin-label ESR spectroscopy (cf. Marsh [118], for review), which allows to determine association constants characterizing the exchange of (spin-labelled) lipids between the protein-lipid interface and the bulk lipid. Moreover, the number of lipid molecules which are motionally restricted due to their interaction with the proteins (and are often denoted as boundary lipids) may be estimated. By comparing different types of spin-labelled lipids, the selectivity of lipid-protein interaction may be characterized in terms of relative association constants. Thus, it was shown that for Na\(^+\)-K\(^+\)-ATPase, cardiolipid has a 4-fold higher association contact than PC which is a consequence of the electrostatic effect. By this method it has also been shown that the residence time of a lipid at the protein surface is \( \tau_r \approx 10^{-6} \)– \( 10^{-7} \) sec, compared to a value of \( \tau_r = 10^{-8} \) sec in the bulk [35a].
Fig. 33a. Calorimetric scanning curve of a 1:1 DMPC/CMPG mixture in absence (---) and presence (-----) of $x_P = 4 \times 10^{-4}$ transferrin receptor. Note the simultaneous shift of the liquidus line to higher and of the solidus line to lower temperatures ($T^*$). Inset shows region of main transition of bilayers containing $x_P = 3 \times 10^{-4}$ after removal of charged head group of receptor by proteases.

- A second method is FTIR-spectroscopy [90] which allows to observe the effect of lipid-protein interaction on the phase transition temperature of individual lipid components in a lipid-lipid-protein mixture. For that purpose one of the lipids is deuterated. An example is shown in fig. 33b. As can be clearly seen, the variation of the FTIR spectra (of the C–H stretching vibration) with temperature is only changed for the charged lipid (DMPG) but not for the neutral DMPC. Unfortunately, the method does not yield quantitative data.

- The application of NMR-spectroscopy to probe selective lipid-protein interaction has been reviewed recently [4]. The sensitivity of this method is strongly reduced by the fact that the residence time of the lipids at the protein $\tau$ is short compared to the NMR-time scales. Therefore one has to work at such high protein concentrations that protein-protein interaction effects become dominant.

- Finally it should be mentioned that small angle neutron scattering would be a potentially most powerful technique. By application of contrast matching techniques it should be possible to determine variations of the lipid composition about membrane proteins.

Theoretical estimations. The partial free energy of the protein due to the mismatch of the hydrophobic thicknesses can also be estimated by application of the thickness variation model [30]. The work associated with a relative mismatch $\delta d/d_m$ of the
Fig. 33b. FTIR spectroscopy as method to probe the selective lipid/protein interaction. Temperature dependence of FTIR spectra of 1:1 DMPC-\(d_{54}\)/DMPG-mixture in presence and absence of transferrin receptor (\(x_P = 3.7 \times 10^{-4}\)). Top: Plots of positions of symmetric C-H-stretching vibration of DMPG (protonated). Bottom: Same curves for chain deuterated DMPC-\(d_{54}\). (+): pure lipid mixture; (□): Presence of \(x_P = 3.7 \times 10^{-4}\) receptor. Note the clear indication of the pretransition (at \(T_P\)) in both cases. The positions of the liquidus line in the absence (\(T_l\)) and presence (\(T_{l*}\)) of the protein are indicated by arrows. The modifies solidus line cannot be localized.

thicknesses is:

\[
\mu_P = \pi K \int_{R_P}^{\infty} (\delta d/d_m)^2 r \, dr = \pi K \int_{R_P}^{\infty} \left( \frac{\delta \rho}{\rho} \right)^2 r \, dr, \tag{76a}
\]

where \(\delta \rho/\rho\) is the relative change in the lipid packing density (at the distance \(r\) from the protein center). Application of the exponential law (eq. (50)) yields

\[
\mu_P = \pi K \left( \frac{\delta d}{d_m} \right)^2 \zeta (R_P + \zeta). \tag{76b}
\]

The correlation length is typically \(\zeta \approx 2\) nm and \(K \approx 200\) mN/m. For a maximum mismatch of \(\delta d/d_m \approx 0.5\) nm one obtains \(\mu_P \approx 120\ k_B T\). This is in good agreement
with the value obtained by molecular field theories, where a value of $\mu_p \approx 100 k_B T$ was obtained for the same mismatch (cf. chapter 7).

8.6.3. Charge induced selective lipid-protein interactions: density control and induced phase separation

This type of interaction has been established for the transferrin receptor [90] for spectrin [116b] and for proteins mediating the coupling of actin filaments to the cell plasma membrane such as talin [115, 119] and hisactophilin (Dietrich and Sackmann, unpublished).

The binding of spectrin to partially charged membranes (containing PS or PG) was studied by the calorimetric perturbation technique (cf. fig. 29) and by the neutron surface scattering technique [120]. The neutron scattering studies clearly show that the protein penetrates substantially into the head group region of the bilayer since about 22% of its volume is occupied by the protein. The most likely explanation is that the flexible loops interconnecting the rigid domains (supposedly composed of triple helices) penetrate into the bilayer. Even stronger electrostatic interactions with partially charged bilayers have been observed for talin [115] and hisactophilin (Dietrich and Sackmann, unpublished).

The electrostatic coupling is strongly dependent on the lateral pressure of the lipid layer and increases the average area per lipid molecule remarkably showing again that the binding is associated with the partial penetration of the protein side chains into the lipid monolayers. The example of hisactophilin is particular interesting in this respect. This 13.5kDa protein composed of 118 amino acids contains 31 histidine residues which are mostly located in flexible loops protruding from one side of the disc-shaped molecule [121]. With these loops it penetrates into the bilayer up to lateral pressures of 30 nN/m. Above this pressure the interaction is strongly impeded. Interestingly, this limit is about equal to the intrinsic pressure of lipid bilayers. Therefore the coupling of the protein to the inner leaflet of the plasma membrane could be well modulated by local density fluctuations.

The most intriguing aspect is that the binding is pH-dependent and occurs only at pH-values below 7 (pK_a of histidin 6.5). Thus it can mediate the binding of actin to the inner leaflet of plasma membranes in a pH-dependent manner. The general view is that hisactophilin mediates the coupling of actin filaments to the plasma membrane by binding to the cytoplasmatic domain of a cell surface receptor; similar to the coupling of F-actin to receptors of the integrin family via talin and vinculin (cf. reference [119]). Nevertheless, a direct coupling of the filaments to the lipid bilayer moiety may well be essential. Thus it has been demonstrated that talin couples F-actin directly to vesicles containing PS, while the (negatively charged) actin filaments are repelled by the charged surface. Another possibility is that the electrostatic effect serves the accumulation of the actin binding proteins at the inner membrane surface of the cell in order to facilitate the rapid binding of the cytoskeleton to the plasma membrane during cell locomotion.

An example of the charge induced interaction of a cell surface receptors with lipid bilayers is the transferrin receptor. This 220kD protein is a homodimer. Each monomer penetrates the lipid bilayer with one hydrophobic domain of 28 amino
acids and carries on the extracellular side a palmitic acid chain which may help to anchor the protein in the bilayer. The cytoplasmatic domains exhibit a high density of positively charged amino acids which are located near the membrane surface. As shown in fig. 33 calorimetric studies and FTIR demonstrate a strong selective interaction of the receptor head group with charged lipids such as PS or PG leading to induced lateral phase separation. A binding energy of $10^3 k_B T$ per receptor was found [90].

The charge induced lipid-protein interaction has many interesting consequences:
- It leads to an induced lateral microphase separation and can thus control the lateral organization of membranes.
- Since the binding energy per lipid molecule is of the order of $k_B T$ it can help to maintain the lipid asymmetry.
- It can help to accumulate proteins at the inner surface of the plasma membrane and can thus accelerate the coupling of the cytoskeleton to the membrane during pseudopodia formation.
- It is associated with a partial penetration of flexible loops of the protein into the bilayer which may even lead to a partial denaturing (and thus activation) of the proteins. Since the degree of penetration is strongly dependent on the lipid packing density, the binding of proteins (of the cytoskeleton) to the inner leaflet of the plasma membrane could be controlled by local fluctuations in the lipid packing density (e.g., caused by the action of phospholipases).

Certainly more systematic studies are required to explore the richness of the charge induced lipid-protein interaction in biomembranes.

8.7. Critical phenomena in mixed membranes

8.7.1. Critical behaviour of lipid mixtures

Critical phenomena in bilayers of lipid mixtures are interesting because of their low dimensionality and since they imply the possibility of long range ordering effects in fluid phases. Three cases have been found yet which will be discussed below.

The best studied example is the mixture of DMPC and DSPC which exhibits critical demixing above the liquidus line as demonstrated by SANS (applying the highly sensitive inverse contrast variation technique [98]). This is astonishing since the critical point of the solid-solid miscibility gap is hidden within the fluid-solid coexistence region (cf. fig. 27).

The critical behaviour has been analysed in the usual way by measuring the neutron scattering intensity, $I(q)$, as a function of the scattering vector, $q$, in the low angle regime for various temperatures and hydrostatic pressures [123] (cf. fig. 34). In all cases $I(q)$ obeys the Ornstein-Zernicke law [122]:

$$I(q) = \frac{\zeta(P, T)}{1 + q^2 \zeta^2(P, T)}$$

(77)

where $\zeta(p, T)$ is the temperature and pressure dependent correlation length of the concentration fluctuations. Figure 34b shows as an example a series of $I(q)$-versus-$q^2$ plots as a function of pressure demonstrating the validity of eq. (77). It was not
Fig. 34a. $q$-dependence of scattering intensity $I(q)$ of 1:1 DMPC-$d_{54}$-DSPC mixture for 58°C, 48.5°C and 45°C. Note that liquidus line is located at 44°C. The inset shows a schematic view of the momentaneous structure of the critical mixture. The correlation length is $\xi = 50$ nm for 45°C and $\xi = 30$ nm for 49°C (from reference [99]).

Fig. 34b. Ornstein-Zernicke plots of inverse scattering intensity $I(q)^{-1}$ versus $q^2$ of DMPC-$d_{54}$-DSPC mixture of various hydrostatic pressure, $p$, (indicated at the right side). Straight lines are observed demonstrating that the system obeys the Ornstein-Zernicke law reasonably well. Reproduced from Knoll et al. [123].
possible yet to measure at scattering angles $q < 0.006 \, \text{Å}^{-1}$ to see whether deviations from the Ornstein–Zernicke law characteristic for two-dimensional systems at $T \to T_c$ occur (cf. Stanley [122], chapter 7). Nevertheless, the pressure and temperature dependencies of the correlation length, $\zeta$, obey the laws

$$\zeta \propto (T - T_c)^{-0.7 \pm 0.3}; \quad \zeta \propto (P - P_c)^{-0.5 \pm 0.1}$$

which are close to the theoretical predictions of the Ising model for 2D-systems:

$$\zeta \propto (T - T_c)^{-0.92}; \quad \zeta \propto (P - P_c)^{-0.5}.$$

Judged from the phase diagrams of the mixtures of PC with cholesterol this system should exhibit a critical point some 20°C above the $L_\alpha \to P_{\beta'}$-transitions. As noted already above, evidence for critical behaviour was provided by monolayer studies [105]. Biological membranes contain in general more than 30% of cholesterol and are thus not in a critical regime.

### 8.7.2. Critical demixing and long range interactions between proteins

The intriguing aspect of critical demixing in bilayers is that it implies a mechanism of long range protein-protein interaction (cf. [2] and [3] for reference). It is evident that even a slight preference of a protein (P_1) for one of the lipid components (say A) will stabilize the phase rich in A even at temperature well above the critical point. It will surround itself with a cloud of A-molecules, that is the concentration varies as $c_A + \delta c_A$ (where $c_A$ is the average concentration of lipid A and $\delta c_A$ the enrichment in A about the protein). A second protein, P_2, which has also a preference for A will gain energy if it shares a domain with P_1 hence an attraction results. If protein P_2 exhibits a preference for the lipid component B it will be repelled from P_1.

![Fig. 35a. Schematic view of channel formation by transient association of two gramicidin A monomers.](image-url)
Fig. 35b. Comparison of conductance distribution functions of channels in homogeneous phase of 1:1 mixed PA/PC membrane with Ca$^{++}$ (10$^{-4}$ M) sequestered by EDTA (shaded histogram) and in presence of 10$^{-4}$ M Ca$^{++}$ (histogram with white bars). $P(\Lambda)$ is the probability of single channel conductance, $\Lambda$. Note the anomalous broad conductance distribution in the latter case.

Fig. 36. Phase diagram of PC-PG-Ca$^{++}$-system as obtained from single channel conductivity histograms. Note that increasing Ca$^{++}$-concentration corresponds to decreasing temperature.
The interaction energy is simply equal to the free energy it costs to create the concentration fluctuation $\delta c_A$ and is thus

$$W_{12} \propto \delta \mu_A = k_B T \ln \delta c_A \approx -k_B T \delta c_B. \quad (78)$$

The concentration fluctuation can be estimated on the basis of the Ornstein–Zernicke theory. Very near the critical point $\delta c_A$ and thus the protein-protein interaction energy will decay very slowly with the distance $[2, 122]$

$$W_{12} = Br^{-\eta} \quad (79)$$

with $\eta \sim 1/4$.

Since the lipid chain lengths in biological membranes differ drastically (C16 to C24) they are expected to exhibit similar behaviour as the DMPC-DSPC mixture, i.e. that is critical demixing well above their chain melting transitions. Thus lipid mediated long range forces due to the stabilization of domains of specific composition are well conceivable also in biomembranes.

### 8.7.3. Ca$^{++}$-induced lateral phase separation and protein activity

As mentioned in chapter 1 the down-regulation of the enzyme activity in biomembranes could be effected by segregation of the protein into solid domains where they are not active. Such a behaviour was demonstrated in beautiful in-vitro experiments by the Knoll group $[123, 124]$. These authors studied the single channel conductance of gramicidin A in black lipid membranes composed of mixtures of a neutral (PC) and a charged lipid (PA$^-\$). The effect of phase separation on the activity of the channels was studied by evaluation of the single channel conductance fluctuations as a function of Ca$^{++}$ concentration. They found:

1. For situations were Ca$^{++}$-induced phase separation has been clearly established for vesicles, one observes two clearly separated population of channels: a population with conductivities characteristic for channels in fluid phases ($\Lambda \approx 80$ pS) and a fraction with a strongly reduced value of $\Lambda$. The latter can be attributed to channels residing in a more condensed phase.

2. An even more interesting situation is shown in fig. 35b. A 1 : 1 PA$^-$/PC-mixture in the presence of $10^{-4}$ M Ca$^{++}$ exhibits an anomalous broad conductivity distribution reminiscent of critical behaviour, whereas in the absence of Ca$^{++}$ a sharp distribution is observed. Indeed, by a systematic study of conductivity histograms as a function of the lipid composition and Ca$^{++}$ concentration, the ternary phase diagrams of the mixtures of PC with charged lipids (PA$^-\$ and PG$^-\$) and Ca$^{++}$ were established. The case of the PC-PG-Ca$^{++}$-system is shown in fig. 36. It exhibits a critical point at $x_{PC} \sim 0.5$ and $10^{-7}$ M Ca$^{++}$. Remarkably, the latter value is close to the resting state Ca$^{++}$-concentration of cells. For PC/PA mixtures the critical Ca$^{++}$-concentration is $10^{-4}$ M. Judged from dynamic measurements, one deals with a fluid-fluid miscibility gap exists.
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