Spontaneous and Intrinsic Curvature of Lipid Membranes: Back to the Origins

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Abstract We review the background behind the notions of spontaneous and intrinsic curvatures of lipid membranes with a goal to make clear a fundamental physical difference between them. We recall the underlying mechanical and thermodynamic models for intrinsically curved lipid monolayers, whose geometry is described by the intrinsic curvature, and for flat monolayers whose elastic stresses are captured by the spontaneous curvature. We describe the existing ideas concerning the spontaneous and intrinsic curvatures of mixed lipid membranes. We mention the conditions upon which the values of the spontaneous and intrinsic curvatures are expected to be similar and the specific systems for which each of the notions is relevant.

Keywords Membrane bending elasticity · Spontaneous curvature · Intrinsic curvature · Bending modulus

1 Introduction

Biological membranes form physical boundaries between the inner volume of a biological cell and the external medium as well as, within the cell, between the lumens of intracellular organelles and cytosol. The structural base of any biological membrane is a lipid bilayer—an about 3–4 nm thick film consisting of two monolayers of amphiphilic phospholipid molecules referred to below as the lipids. As described in more detail below, the lipid monolayer formation and coupling into bilayers in aqueous solution are driven by the hydrophobic effect [1].

Upon common physiological conditions, each monolayer has properties of a two-dimensional fluid, which is due to the ability of the lipid molecules to undergo two-dimensional diffusion in the membrane plane referred to as the lateral diffusion. Moreover, while being coupled in the direction perpendicular to the membrane

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P. Bassereau, P. Sens (eds.), *Physics of Biological Membranes*,
https://doi.org/10.1007/978-3-030-00630-3_12

plane, the two monolayers are free to slip with respect to each other in the in-plane direction to the extent permitted by external geometrical constraints. In addition, flipping of lipid molecules between the monolayers is possible and referred to as flip-flop [2], which also contributes to an effective in-plane decoupling of the monolayers. Because of this mechanical decoupling in the lateral (in-plane) direction, a lipid monolayer represents the basic mechanical units of the membrane, whereas the mechanical properties of a bilayer can be derived from those of its constituent monolayers. Therefore, this review will be concerned, mostly, with elastic properties of lipid monolayers, which, using a loose terminology, will be also referred to as the membranes.

Since early 70th, acquiring by membranes of curved shapes and the underlying physical properties of lipid bilayers and monolayers became one of the central topics of soft matter physics. This was motivated, on the one hand, by the attempts to understand the physical mechanisms behind the shapes adopted by erythrocytes [3, 4] and, on the other hand, by X-ray investigations of three-dimensional phases formed by lipids in aqueous solutions [5]. About 30 years later, the interest to membrane curvature expanded to bona fide cell biology (see for recent review [6]), which has been motivated by the necessity to understand the intricate and strongly bent shapes of membrane-bound intracellular organelles such as endoplasmic reticulum (ER), Golgi complex, mitochondria, and transport intermediates [7, 8].

A central notion used in the curvature-related fields of membrane physics and biology is that of *spontaneous curvature*, which has been commonly meant to describe the inherently preferable membrane shapes. In parallel, although less commonly, the term of *intrinsic curvature* has been used in the membrane literature to describe, basically, the same membrane property. In some cases, the two notions appeared interchangeably in the same article. The goal of this review is to go back to the original physical contents of the concepts of spontaneous curvature, as defined by Wolfgang Helfrich [4], and of intrinsic curvature as introduced, originally, by Sol Gruner [9]. We will make clear that there is a fundamental rather than semantic difference between the two notions.

We will show that the spontaneous curvature does not have a direct meaning of a geometrical characteristic of the membrane surface but rather describes the stresses existing within a flat membrane and provides tools for computing the energy of the membrane deviation from the flat shape. In contrast, the intrinsic curvature does have a geometrical connotation describing the local membrane shape in the mechanically relaxed and, hence, energetically preferable state. We will indicate the conditions under which the spontaneous and intrinsic curvatures are expected to have approximately equal values meaning that, for practical purposes, one can be used instead of the other.

This consideration will give us a reason to come back to the “first principles” of the two alternative approaches to physics of membrane bending and discuss, specifically, the current views on the bending elastic properties and, in particular, on the spontaneous/intrinsic curvatures of membranes with mixed lipid compositions.

Although the concept of spontaneous curvature was formulated more than 10 years ahead of introduction of the intrinsic curvature, we will first overview the latter notion, which is more intuitive, and then the former, whose physical content is somewhat more involved.

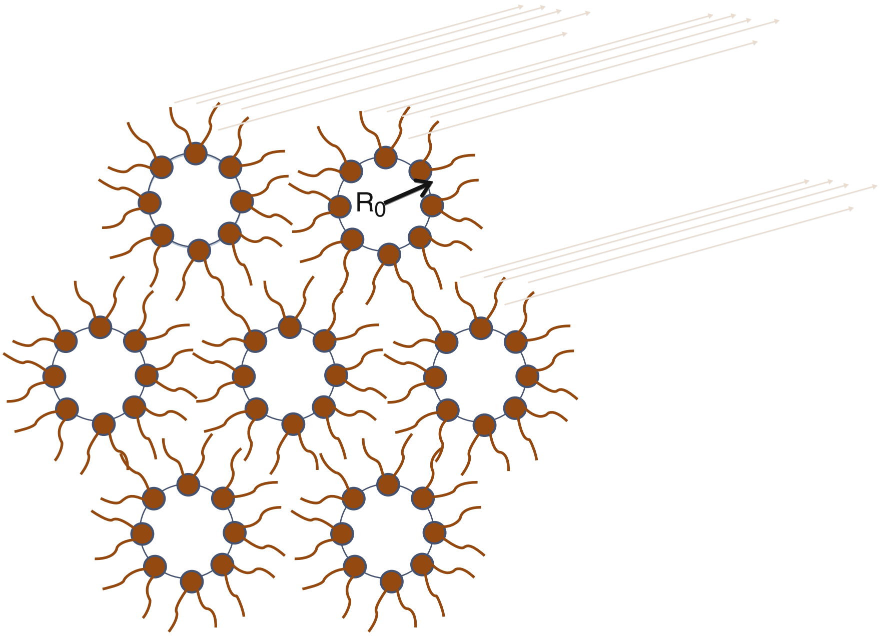

Fig. 1 Illustrations of the structure of H_{II}-phase, R_0 being the intrinsic radius of the monolayer so that the intrinsic curvature is $J_0 = 1/R_0$

2 Intrinsic Curvature

The concept of intrinsic curvature as a quantitative structural characteristic of lipid monolayers has originated from understanding the so-called lipid mesophases, which result from lipid self-assembly in aqueous surrounding [5]. Usually, mesophases are formed by continuous hydration of initially dry lipid samples up to a saturating limit. Driven by the hydrophobic forces, lipid molecules self-assemble into monolayers, which segregate in such a way that their hydrocarbon moieties are shielded from the aqueous surrounding by the layers of polar heads. Depending on the lipid composition, the monolayers adopt a plethora of shapes [10–12], the most familiar of which is the flat shape. Flat monolayers form planar bilayers, which in turn pack into stacks where they are separated by few nanometer-thick layers of water. The resulting mesophase is called the lamellar phase (see, e.g., [5, 12]).

Lipids, which do not form flat bilayers, are often called the non-bilayer lipids. The most common monolayer shape formed by such lipids is that of a narrow cylindrical tube whose internal surface of few nanometer cross-sectional diameter is covered by the lipid polar heads and engulfs a water cylinder. These lipid tubes get oriented in parallel, contact each other along the hydrophobic surfaces, and pack in such a way that their cross-sections form a two-dimensional hexagonal lattice (Fig. 1). The resulting structure is referred to as the inverted hexagonal (H_{II}) phase [11].

In addition to the H_{II}- phase, few other non-lamellar mesophases characterized by curved monolayer shape can form such as H_I- phase consisting of cylindrical micelles packed in hexagonal lattice and bicontinuous and micellar cubic phases. Here we discuss only H_{II}-phases, whereas thorough description of other phases can be found in numerous reviews (see, e.g., [10, 12]).

Essentially, a lipid monolayer within H_{II}-phase is almost free to acquire the most favorable shape dictated by the monolayer intrinsic properties. Ideally, this is possible if the inter-monolayer spatial gaps, which unavoidably form as a result of the monolayer packing within the phase, can be filled by non-lipid substances coming from external reservoirs. In a fully hydrated H_{II}-mesophase, the water cylinders inside the lipid tubes unrestrictedly exchange water molecules with the external aqueous medium. Filling of the hydrophobic gaps between the lipid tubes, which form as a result of the hexagonal packing and are referred to as the hydrophobic interstices [13], is more problematic since, usually, there are no required reservoirs of hydrophobic substances in contact with the mesophases. However, even these restrictions can be reduced or even lifted by introduction into the system of hydrophobic substances, such as tetra- or hexadecane, which redistribute into the hydrophobic interstices [9, 14–16]. As a result, the shapes adopted by lipid monolayers within H_{II}-phases can be considered as representing the intrinsically favored shapes.

The intrinsic curvature, J_0 [9], is the inverse of the radius, R_0 (Fig. 1), of a cylindrical tube formed by an unrestricted monolayer of H_{II}-phase, $J_0 = 1/R_0$. It has to be noted that the intrinsic curvature value must be related to a specific surface chosen within the monolayer as a reference plane [17]. The most convenient reference plane is the neutral surface, for which the deformations of the membrane stretching and bending are energetically decoupled [17, 18].

Due to three-dimensional long-range order of lipid packing, the structural parameters of lipid mesophases in general and of H_{II}-phases, in particular, can be measured with high precision by X-ray scattering [13]. This enables quantitative determination of the monolayer intrinsic curvatures, which needs, however, some theoretical treatment of the measured parameters [18–20].

2.1 Intrinsic Curvatures of Individual Lipids and Lipid Mixtures

A direct measurement of intrinsic curvature of a specific individual lipid requires generation of unconstrained H_{II}-phase of this lipid. In practical terms, only one such lipid, dioleoylphosphatidylethanolamine (DOPE) [13], has been found to date, which is considered to be a "king" of non-bilayer lipids [21]. The radius of water cylinder, ρ_W, within the DOPE phase is close to 2 nm so that the hydrophobic interstices, whose dimension is proportional to ρ_W, are relatively small and do not require much of the hydrophobic solvent to be introduced. The intrinsic curvature of DOPE measured at its neutral plane located close to the level glycerol backbones of the lipid molecules [19, 20] was found to constitute $J_0^{\mathrm{DOPE}} = 1/2.75$ nm for the room temperature [22].

The $\mathrm{H_{II}}$-phase of DOPE enables also determination of the intrinsic curvature values of a series of other biologically relevant lipids, such as phosphatidylcholine (PC), diacylglycerol (DAG), lysolipids, and others, which are commonly found within biological membranes but do not form $\mathrm{H_{II}}$-phases by themselves. This is based on the ability of the DOPE monolayers to accommodate some amounts of these lipids. Whereas the resulting lipid mixtures keep forming the inverted hexagonal phase, the radii of the monolayer tubes deviate from that of the purely DOPE monolayer and depend on the composition of the mixed monolayer. Essentially, although not expected a priori, the dependence of the intrinsic curvature of a mixed cylinder on the mole fraction, ϕ, of the added lipid, $J_0(\phi)$, has a linear character for all investigated lipid mixtures $J_0\,(\phi) = J_0^{\mathrm{DOPE}} + \phi J_0^L$ [20, 23–26]. The slope of this linear dependence, J_0^L, has been defined as the intrinsic curvature of the lipid under question. The values of the intrinsic curvatures of quite a few biologically relevant lipids measured by the described method can be found in several reviews (see, e.g., [27].). It has to be emphasized that the resulting J_0^L values may not have a universal character since the conformation of a lipid molecule must depend to some extent on the surrounding lipids within the mixture, as recently confirmed by numerical simulations [28]. Therefore, strictly speaking, the intrinsic curvature values, J_0^L, obtained for individual lipids by the described method of mixed $\mathrm{H_{II}}$-phases, have to be considered within the DOPE context.

2.2 *Bending Elasticity of Lipid Monolayers in the Intrinsically Curved State*

Experimentation with $\mathrm{H_{II}}$-phases led to understanding the structure and mechanics of lipid monolayer in their intrinsic state beyond determination of the lipid intrinsic curvatures. The experiments consisted in application to the lipid sample of external compressing pressures and measuring the resulting deformations of $\mathrm{H_{II}}$-phases. These pressures, which can have either a gravimetric (within an aqueous vapor atmosphere) (see, e.g., [13]) or osmotic (in bulk water) (see, e.g., [16]) character, change the lipid tube radii [13, 16, 19, 20] . The obtained dependence of the monolayer radius change on the applied pressure represents a stress-strain relationship, which, generally, enables determination of the system elastic constants. For weak compressions leading to small deviations of the monolayer curvature, J, from the intrinsic value, J_0, the elastic energy, F_{B}, accumulated with the monolayer was presented as [9]

$$F_B = \frac{1}{2}\,K_B(J - J_0)^2, \tag{1}$$

where K_B is the membrane bending modulus determined for the intrinsic state. While this equation captured the essential physics of the system, treatment of the experimental data required a more rigorous approach taking into account that the bending modulus must be an intensive rather than extensive thermodynamic value and that the choice of the dividing surface within the membrane becomes of primary importance for the strongly curved monolayers of H_{II}-phases. This approach was developed in a series of articles [18–20], which enabled determination of the monolayer bending moduli in the intrinsic state for pure DOPE and mixed monolayers [20, 22–26, 29]. In all cases the value of the bending modulus was close to $10k_BT \approx 4 \times 10^{-20}$ J (where k_BT is the product of Boltzmann constant and the absolute temperature).

3 Spontaneous Curvature

The concept of membrane spontaneous curvature was introduced by W. Helfrich in his seminal article establishing a model for bending elasticity of nearly flat membranes [4].

The way of reasoning, which underlies Helfrich's derivation of the energy associated with bending deformations of membrane surface, is analogous to and, possibly, inspired by the previous consideration by F.C. Frank of three-dimensional bending deformations of nematic and cholesteric liquid crystals [30]. Specifically, the notion of the membrane spontaneous curvature is parallel to that of a nematic elastic parameter defined by Frank, which can be referred to as the spontaneous splay.

The analogy between Helfrich's and Frank's approaches is based on a fundamental physical similarity existing between the two systems in spite of the fact that the nematic liquid crystal is a three-dimensional phase characterized by bulk properties (Fig. 2a), whereas a lipid membrane has been described as a surface, i.e., a two-dimensional system immersed in three-dimensional space (Fig. 2b). This similarity includes the physical variables by which the nematics and the lipid membranes can be described and the intrinsic material properties, which determine the common symmetry rules underlying the physical models of the two systems.

Specifically, the bulk of a uniaxial nematic liquid crystal, on one hand, and the membrane surface, on the other, can be described by, basically, the same physical variable, whose essence is the direction of the local orientation. (It has to be emphasized that we consider only membranes in high-temperature phase exhibiting laterally isotropic behavior and do not describe the low-temperature gel phase of lipid characterized by a collective tilting of the lipid hydrocarbon chains within the monolayer and the related lateral anisotropy of the membrane properties.) For a nematic, this variable, referred to as the director, is a unit vector, $\vec{L}$, which determines the direction of the molecular orientation in any infinitesimal volume element of the bulk (Fig. 2a) [30]. Similarly, the membrane surface can be described at every point by a unit normal vector,

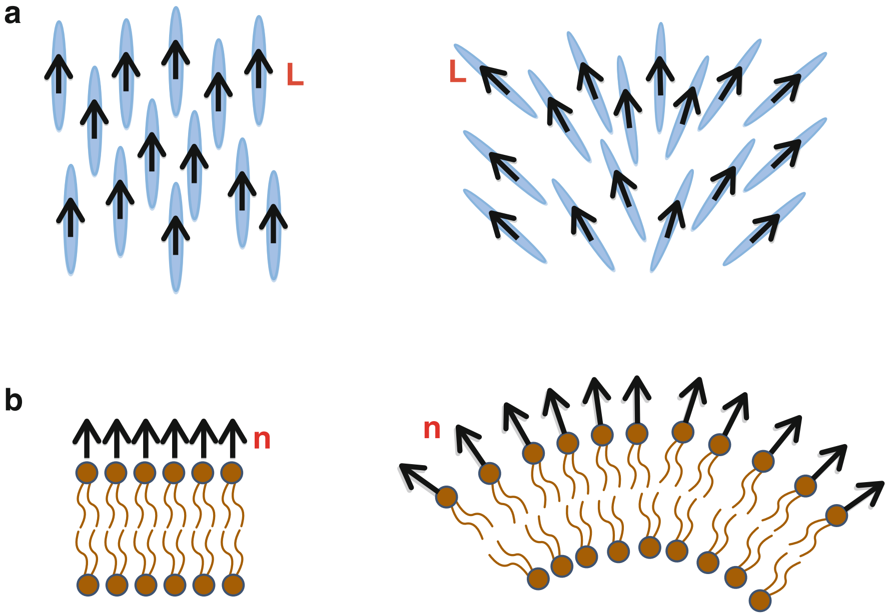

Fig. 2 Illustration of (**a**) the nematic liquid crystal characterized by the director $\vec{L}$ and (**b**) a lipid membrane described by the unit normal vector, $\vec{n}$

$\vec{n}$, characterizing the local surface orientation (Fig. 2b). Further, provided that the initial state of the liquid crystal is the state where the director, $\vec{L}$, is uniformly oriented all over the volume, the essence of the nematic bending deformation is a generation of differences in the director orientations between the adjacent volume elements throughout the system (Fig. 2a, right panel) [30]. Analogously, if the membrane is considered to be, originally, flat with the normal vector, $\vec{n}$, uniformly oriented all over the membrane surface (Fig. 2b, left panel), the membrane bending leads to deviations of $\vec{n}$ belonging to every pair of adjacent membrane elements from the initial parallel orientation (Fig. 2b, right panel).

In terms of the material properties, both nematic liquid crystals and lipid membranes in high-temperature phase exhibit a liquid-like behavior. The molecules constituting a nematic phase can switch their positions within the available volume, which is not accompanied by any stress generation. The same is true for swapping of the lipid molecule positions in the membrane plane within the given membrane area. As a result, neither nematics nor lipid membranes resist the shear deformations. A nematic phase does not develop any stress against three-dimensional shear, whereas a lipid membrane behaves as a two-dimensional liquid complying without resistance with in-plane shear.

Our goal here is to thoroughly describe the physics behind the notion of membrane spontaneous curvature. Therefore, we start with the elements of Frank's analysis of nematics [30] and then present the essence of Helfrich's consideration of the membrane elastic parameters [4, 31] by mentioning the analogy and the differences between the two systems.

3.1 *Spontaneous Splay and Twist of Nematic Liquid Crystal*

As already mentioned, the initial state of a nematic bulk is considered to exhibit a uniform orientation of the director, $\vec{L}$, throughout the whole system (Fig. 2a, left panel). Generally, any deformation imposed on a three-dimensional elastic phase by external forces acting on its boundaries leads to development at any point of the phase of the internal stresses, σ_{ik}, and strains, u_{ik}, so that the volumetric density of the deformation energy can be presented as [32]

$$f = \int \sigma_{ik} du_{ik}, \tag{2}$$

where the integration is performed from zero to a final strain. The total energy, F, is given by integration of the energy density, f, over the whole volume of the system $F = \oint dV(\int \sigma_{ik} du_{ik})$. Here and below we use the conventions of summation over the repeated indices.

To proceed, based on Eq. (1), one needs to specify the types of the strains, u_{ik}, and stresses, σ_{ik}, developing in the system and establish the explicit stress-strain relationships, $\sigma_{ik}(u_{ik})$.

Since the essence of the nematic bending deformations is the deviation of the director, $\vec{L}$, from the initial uniform orientation (Fig. 2a, right panel), the resulting strains, e_i, can be represented by the local gradients of $\vec{L}$. The only stresses emerging as a result of generation of the director gradients are the local torques, τ_i, counteracting the local rotation of the director, $\vec{L}$ [30]. In general, the three-dimensional bending of the nematic phase leads also to the shear stresses, but, due to the liquid nature of the system, the corresponding stresses must relax due to a rearrangement of the constituent molecules.

To express the strain components explicitly, we introduce at any point of the nematic phase a local Cartesian coordinate system, $\{z, x, y\}$, with z-axis parallel to $\vec{L}$ in the origin, $x = 0$, $y = 0$. Since our goal here is to follow the similarity between the nematics and the membranes, we consider only the deformations for which the director does not change in z-direction so that $\frac{\partial L_x}{\partial z} = \frac{\partial L_y}{\partial z} = 0$. Therefore, the relevant for our consideration strain components are the two “splay”,

$$e_{sx} = \frac{\partial L_x}{\partial x},$$
$$e_{sy} = \frac{\partial L_y}{\partial y}, \tag{3}$$

and two “twist”,

$$e_{tx} = -\frac{\partial L_y}{\partial x},$$
$$e_{ty} = \frac{\partial L_x}{\partial y}, \tag{4}$$

components [30]. In the initial state of uniform director, $\vec{L}$, both splay and twist components vanish, $e^0_{sx} = e^0_{sy} = e^0_{tx} = e^0_{ty} = 0$.

The stress-strain relationships, $\tau_i(e_j)$, which have to be used for computation of the energy density according to Eq. (2), are set by the molecular interaction within the system and have, generally, a nonlinear character. However, they can be presented in a simple form for small strains. The smallness of the strains means, specifically, that the dimensionless parameters equal to the products of the absolute values of the strain components, $|e_j|$, and the internal molecular scale of the system, δ, whose essence in the effective size of molecules constituting the nematic phase remain much smaller than one,

$$\left|e_j\right| \cdot \delta \ll 1, \tag{5}$$

which means that the angles, θ_i, by which the directors deviate from the initial orientations remain small, $\theta_i \ll 1$. In this case, it is possible to use the approximate stress-strain relationships, accounting only for the contributions up to the first order in the small parameters, $|e_j| \cdot \delta$, and neglecting the higher-order terms,

$$\tau_i = \tau_i^0 + \epsilon_{ij} \cdot \delta \cdot e_j. \tag{6}$$

This expression [Eq. (6)] represents the Hooke’s approximation for the relationships between the torque and the strain components.

In Eq. (6) the first contributions, τ_i^0, are the torques existing within the nematic phase in the initial state of the uniform orientation of the director, $\vec{L}$, before the onset of the bending deformations. The values of the initial torques, τ_i^0, depend on the intermolecular interactions of the specific liquid crystal. In case of vanishing initial torques, $\tau_i^0 = 0$, the uniform state is free of the torque stresses, meaning that there is no intrinsic tendency of the director, $\vec{L}$, to splay and/or twist. In the case of nonvanishing initial torques, $\tau_i^0 \neq 0$, the inter-molecular interactions favor a deviations of the liquid crystal from the initial state of uniformly oriented director, $\vec{L}$.

The coefficients, ϵ_{ij}, in Eq. (6) represent the elastic parameters of the system. Commonly, the molecular length, δ, is included into the definition of the system elastic parameters so that one uses $\kappa_{ij} = \epsilon_{ij} \cdot \delta$, instead of ϵ_{ij}, and the torque-curvature relationships are introduced in the form

$$\tau_i = \tau_i^0 + \kappa_{ij} \cdot e_j. \tag{7}$$

Symmetry considerations taking into account the liquid-like properties of the system reduce the number of the independent initial torques to two, τ_s^0 and τ_t^0, associated with the splay and twist, respectively [30]. The number of the independent and nonvanishing elastic parameters, κ_{ij}, is reduced to four associated with the splay-only, twist-only, and mixed splay-twist deformations [30]. Using these conclusions and [Eq. (7)], the integration [Eq. (2)] from zero to the final values of the splay and twist components of the strains results in the explicit expression for the energy density, f, which can be presented in the form

$$\begin{aligned} f = {} & \tau_s^0 \left(e_{sx} + e_{sy}\right) + \tau_t^0 \left(e_{tx} + e_{ty}\right) + \tfrac{1}{2}\kappa_{ss}\left(e_{sx} + e_{sy}\right)^2 + \tfrac{1}{2}\kappa_{tt}\left(e_{tx} + e_{ty}\right)^2 + \\ & \kappa_{st} \left(e_{sx} + e_{sy}\right) \left(e_{tx} + e_{ty}\right) + \overline{\kappa} \left(e_{sx}e_{sy} + e_{tx}e_{ty}\right). \end{aligned} \tag{8}$$

The first two terms of [Eq. (8)] correspond, respectively, to the thermodynamic work performed against the initial splaying torque, τ_s^0, on generation of the total splay strain, $e_s = e_{sx} + e_{sy}$, and against the initial twisting torque, τ_t^0, on producing the total twist strain, $e_t = e_{tx} + e_{ty}$. The third and fourth terms are quadratic in the total splay, e_s, and twist, e_t, meaning that they represent the elastic contributions to the energy with the corresponding elastic moduli of the pure splay, κ_{ss}, and the pure twist, κ_{tt}. Fundamentally, these two elastic moduli must be positive, $\kappa_{ss} > 0$, $\kappa_{tt} > 0$, to guarantee the thermodynamic stability of the system. The fifth contribution to Eq. (8) is determined by the interplay between the splay and twist deformations, the coefficient, κ_{st}, setting the extent to which the splaying torque, τ_s, is influenced by the total twist strain, e_t, and vice versa, how the twist stress, τ_t, depends on the total splay strain, e_s. Finally, the sixth contribution has a more complicated geometrical origin depending on the product of the splay components, $e_{sx}e_{sy}$, and the twist components, $e_{tx}e_{ty}$. This energy contribution does not vanish only if the deformation occurs simultaneously in x- and y-directions so that both the x- and y-components of the splay and twist strains differ from zero. If the deformation is unidirectional, the last term in Eq. (8), which can be referred to as the saddle-splay energy, does not contribute. The coefficients, κ_{st} and $\overline{\kappa}$, may adopt positive as well as negative values, which do not violate the requirement of the thermodynamic stability of the system.

The expression [Eq. (8)] can be presented in a more compact form by introducing the parameter combinations

$$e_{s0} = -\frac{\tau_s^0}{\kappa_{ss}} \tag{9}$$

and

$$e_{t0} = -\frac{\tau_t^0}{\kappa_{tt}}$$

and changing the energy of the reference state by a constant value, $-\frac{1}{2}\,\kappa_{ss}{e_{s0}}^2 - \frac{1}{2}\,\kappa_{tt}{e_{t0}}^2$. The energy density is then presented as

$$f = \frac{1}{2}\kappa_{ss}(e_s - e_{s0})^2 + \frac{1}{2}\kappa_{tt}(e_t - e_{t0})^2 + \kappa_{st}e_s e_t + \overline{\kappa}\left(e_{sx}e_{sy} + e_{tx}e_{ty}\right). \tag{10}$$

The first two terms of Eq. (10) have a familiar form of Hooke's law for the total splay, e_s, and the total twist, e_t, respectively. The parameters e_{s0} and e_{t0} play the roles of apparent equilibrium values of the local total splay and twist for which Hooke's splay and twist stresses would vanish. Therefore, e_{s0} and e_{t0} can be, formally, considered as structural parameters determining the stress-free state of the nematic and referred to as the spontaneous splay and twist, respectively.

It is important to emphasize, however, that the real meaning of e_{s0} and e_{t0} is directly related to the stresses existing in the uniform state of the nematic, as it follows from the derivation above and the expressions [Eq. (9)]. In other words, the spontaneous splay and twist do not really describe the structure of the stress-free state of the system but rather quantify, together with the elastic moduli, κ_{ss} and κ_{tt}, the internal stresses existing within the liquid crystal in the uniform state.

This point has not just a semantic meaning but rather an important physical content. To illustrate that, let us consider, for simplicity, a nematic with vanishing elastic moduli, corresponding to the third and fourth contributions to Eq. (10), $\kappa_{st} = 0$, $\overline{\kappa} = 0$. The energy density, f, is determined in this case by Hooke's law only. Assume that a sample of such liquid crystal is not subjected to any constraints imposed on its boundaries and there are no external forces acting on the system. A practical question would be: is such liquid crystal expected to adopt the state with total splay and twist having the spontaneous values e_{s0} and e_{t0}? The answer is that, generally, this is not the case. Indeed, our consideration above was not limited to small values of the initial torques, τ_s^0 and τ_t^0. Therefore, the spontaneous splay and twist, e_{s0} and e_{t0}, do not have to be small in the sense that their products with the inverse molecular dimension, $|e_{s0}|\delta$ and $|e_{t0}|\delta$, can be comparable to or even larger than one. In such cases, adopting by the system a state characterized by e_{s0} and e_{t0} would mean a strong deviation from the initial uniform state so that the resulting strains do not satisfy the condition of smallness [Eq. (5)], and, hence, the quadratic formula for the energy [Eq. (9)] is invalid a priori. Specifically, in the case of large initial torques, τ_s^0 and τ_t^0, the relaxation of the system from the state of uniform director, $\vec{L}$, generates large strains for which the nonlinear contributions to the stress-strain relationships [Eq. (6)] are, generally, expected to become essential. This will lead to the additional substantial contributions to the energy density [Eq. (10)] of higher than quadratic orders in splay and twist resulting in an equilibrium state different from that characterized by e_{s0} and e_{t0}.

3.2 Spontaneous Curvature of Lipid Membranes

The physical meaning of the membrane spontaneous curvature, as defined by Helfrich [4], is, basically, analogous to that of the spontaneous splay of a nematic liquid crystal described above. We present here the major steps of introduction of Helfrich model of membrane bending elasticity, which includes definition of the membrane spontaneous curvature [4]. We use, explicitly, the analogy between the physical ideas behind modeling the elastic properties of a membrane with those presented above for description of bending of a nematic liquid crystal.

The membrane is described by a surface whose infinitesimal elements are characterized by the area, da, and the orientation of the unit normal vector, $\vec{n}$, playing a role of the director (Fig. 2b). Since we are interested only in the curvature effects, we do not address here the deformations of area stretching-compression.

In the initial state, the membrane is considered to be flat so that the normal vector, $\vec{n}$, is uniformly oriented throughout the whole membrane surface (Fig. 2b, left panel). Bending of the membrane surface results in two kinds of local deformations. First, the surface elements change their shapes in the membrane plane without changing their areas, which constitutes the lateral shear deformations. Second, the normal vector, $\vec{n}$, deviates from the uniform orientation (Fig. 2b, right panel). Since, as mentioned above, the membrane has properties of a two-dimensional fluid, no in-plane shear stresses develop in the system. Therefore, the lateral shear strains do not contribute to the membrane energy. By contrast, generation of inhomogeneity in $\vec{n}$ orientation resulting in a reciprocal rotation of the normal vectors, $\vec{n}$, of neighboring membrane elements (Fig. 2b) does cost energy. The corresponding strains are represented by the components of the gradient of $\vec{n}$ determined along the surface plane. The stresses associated with these strains are the torques, τ_i, opposing the mutual turning of the adjacent surface elements.

To express, explicitly, the strains we choose at every point of the membrane surface a local Cartesian system of coordinates, $\{z, x, y\}$, with z-axis parallel to the normal vector, $\vec{n}$, in the origin ($x = 0$, $y = 0$). The membrane shape in the vicinity of the chosen point is determined by a function $z(x, y)$. Analogously to the liquid crystal model, the membrane strain, e_j, can be presented as having two “splay”,

$$e_{sx} = \frac{\partial n_x}{\partial x} \text{ and } e_{sy} = \frac{\partial n_y}{\partial y},$$

and two “twist”,

$$e_{tx} = -\frac{\partial n_y}{\partial x} \text{ and } e_{ty} = \frac{\partial n_s}{\partial y}, \tag{11}$$

components.

The definition of the strain components [Eq. (11)] relates them directly to the central geometrical characteristics of the membrane surface, the covariant components of the shape tensor (second fundamental form), $b_{\alpha\beta}$ [33],

$$b_{xx} = \frac{\partial n_x}{\partial x}, b_{yy} = \frac{\partial n_y}{\partial y}, b_{yx} = \frac{\partial n_y}{\partial x}, \text{ and } b_{xy} = \frac{\partial n_x}{\partial y}. \tag{12}$$

It can be demonstrated that at the origin of the local Cartesian coordinate system, ($x = 0$, $y = 0$), where $\partial z/\partial x = 0$ and $\partial z/\partial y = 0$, the covariant, contravariant, and mixed components of the shape tensor are equal, so that $b_{xx} = b_x^x$, $b_{yy} = b_y^y$, $b_{xy} = b_x^y$, and $b_{yx} = b_y^x$.

As a result, the splay [Eq. (11)] can be represented by the diagonal,

$$e_{sx} = b_x^x, e_{sy} = b_y^y, \tag{13}$$

and the twist is given by the non-diagonal,

$$e_{t1} = -b_y^x, e_{t2} = b_x^y, \tag{14}$$

components of the mixed shape tensor, b_α^β.

Analogously to the above consideration of the bending energy of liquid crystals, the membrane bending energy can be computed by integration of the torques over the strains. In the membrane case however, this integration results in the energy per unit area of the membrane surface rather than the volumetric energy density. Since the torque-strain relationships, $\tau_k(e_i)$, are, generally, nonlinear and unknown, to proceed in the energy determination, we have to make an assumption of smallness of the strains and use the approximate linear relationships between the torques and the strains [Eq. (6) or (7)]. In the case of membrane bending, the role of the characteristic length setting the scale in the system is played by the membrane thickness, d. The smallness of the strains means that the absolute values of the shape tensor components are much smaller than the inverse membrane thickness,

$$\left|b_x^x\right| d \ll 1, \quad \left|b_y^y\right| d \ll 1, \quad \text{and} \quad \left|b_x^y\right| d = \left|b_y^x\right| d \ll 1. \tag{15}$$

Taking into account the relationships [Eq. (12)], smallness of the strains [Eq. (15)] means that the angles, $\theta_i = n_i$, generated as a result of membrane bending between the normal vectors, $\vec{n}$, in the adjacent membrane points, remain much smaller than one, $\theta_i \ll 1$.

The number of the nonvanishing elastic moduli relating the torques and the strains in the linear approximation [Eqs. (6) and (7)] is determined based on the same symmetry consideration as in the case of liquid crystals [30].

Integration of the torques over the strains accounting for [Eq. (7)], the condition [Eq. (15)], and the relationships [Eqs. (13) and (14)] results in the expression analogous to [Eq. (8)] relating the energy density to the total splay, $e_s = e_{sx} + e_{sy}$, the total twist, $e_t = e_{tx} + e_{ty}$, and the products of the strain components, $e_{sx}e_{sy}$ and $e_{tx}e_{ty}$.

It has to be taken into account that the shape tensor, by definition, statisfies, $b_x^y = b_y^x$ [33]. Therefore, the total twist, $e_t = e_{tx} + e_{tx}$, which is equal, according to Eq. (14), to the difference between the non-diagonal components of the shape tensor, vanishes,

$$e_t = b_x^y - b_x^y = 0, \tag{16}$$

which is not necessarily the case for nematics [30].

As a result, the expression for the density of the membrane energy simplifies to

$$f = \tau_s^0 \left(b_x^x + b_y^y\right) + \frac{1}{2}\kappa_{ss}\left(b_x^x + b_y^y\right)^2 + \overline{\kappa}\left(b_x^x b_y^y - b_y^x b_x^y\right), \tag{17}$$

where τ_s^0 is the splaying torque existing in the initial flat state of the membrane and κ_s and $\overline{\kappa}$ are the remaining elastic parameters relating the torque components to the strains in the linear approximation.

The first and second contributions to the density of the membrane bending energy [Eq. (17)] depend only on the total splay, $e_s = e_{sx} + e_{sy} = b_x^x + b_y^y$, which can be presented as the trace of the shape tensor, $e_s = Tr\left(b_\alpha^\beta\right)$, referred to as the total curvature of the surface, $J = Tr\left(b_\alpha^\beta\right)$ [33]. The third contribution depending on the products of the shape tensor components is proportional to the determinant of the shape tensor, $\det\left(b_\alpha^\beta\right) = b_x^x b_y^y - b_y^x b_x^y$, referred to as the Gaussian curvature of the surface, $\mathrm{K} = \det\left(b_\alpha^\beta\right)$ [33]. It has to be noted that in the mathematical literature, the notion of the mean curvature, $H = -\frac{1}{2}J$, is more common than that of the total curvature, J. According to their definitions, the total and Gaussian curvatures are surface scalars, meaning that their values do not depend on the orientation of the local x , y axes in the membrane plane. For the special local coordinate system, where x , y-orientation is such that the shape tensor has a diagonal form, $b_y^x = b_x^y = 0$, the diagonal components of the shape tensor are called the principal curvatures of the surface, $c_x = b_x^x$, $c_y = b_y^y$. The corresponding x- and

y-directions are called the principle directions [33]. The principle curvatures have a straightforward geometrical meaning of curvatures of the lines formed by crossing the membrane surface by perpendicular planes in the principle directions. Therefore, a frequent presentation of the total and Gaussian curvatures in the literature is as a sum, $J = c_x + c_y$, and a product, $K = c_x \cdot c_y$, of the principal curvatures, respectively.

Using the introduced definitions, the energy density [Eq. (17)] can be expressed through the mean and Gaussian curvatures as

$$f = \tau_s^0 J + \frac{1}{2}\kappa_{ss} J^2 + \overline{\kappa} K. \tag{18}$$

The total bending energy of the membrane, F_B, is given by integration of the energy density [Eq. (18)] over the whole membrane surface,

$$F_B = \oint f dA. \tag{19}$$

Analogously to the above consideration of the elastic model of liquid crystals [Eqs. (8) and (10)], the elastic coefficient, κ_{ss}, coupled to the total curvature square, J^2, has a meaning of an elastic modulus or, equivalently, the membrane susceptibility with respect to the total curvature, J. In the literature, this elastic modulus is referred to as the membrane bending modulus and denoted by κ_{B} instead of κ_{ss} [4, 31]. The bending modulus must be positive to guarantee the stability of the membrane with respect to bending deformations. The bending modulus, κ_B, has been measured by different methods for lipid bilayers and monolayers of various compositions. The characteristic value of this modulus constitutes 10 $\mathrm{k_B T}$ and 20 $\mathrm{k_B T}$ for a monolayer and a bilayer, respectively, $\mathrm{k_B T} \approx 0.6$ kcal/mole being the product of the Boltzmann constant and the absolute temperature (see for recent review [34]). The elastic coefficient, $\overline{\kappa}$, related to the Gaussian curvature, K, is referred to as the modulus of Gaussian curvature or the saddle-splay modulus. This elastic coefficient determines the dependence of the energy density on the first power of the Gaussian curvature, K, and, therefore, does not have a meaning of susceptibility with respect to K. Hence, the membrane mechanical stability does not require $\overline{\kappa}$ to be positive, and, in fact, this modulus was shown to be negative in a few cases where it was accessible for the experimental determinations [35–37]. It has to be noted that as long as the modulus of Gaussian curvature, $\overline{\kappa}$, has a constant value all over the membrane, it becomes relevant only for membrane processes, which include topological remodeling of the membrane by fission, self-fusion, fusion with other membranes, and/or formations of holes in the membrane surface accompanied by deformation of surface regions bound by the hole rims. The reason for that is Gauss-Bonnet theorem according to which integral of Gaussian curvature, K, which has to be computed for determination of the total bending energy [Eq. (19)], is independent of the surface shape as long as the surface remains closed and varies only upon changes of the surface connectivity through topological rearrangements [33]. Importantly, in the cases where the value of the modulus of

Gaussian curvature, $\overline{\kappa}$, changes along the membrane surface, which may be the case in biological membranes, the energy contribution of the Gaussian curvature affects the membrane shapes also in the absence of topological transformations (see, e.g., [38]).

The spontaneous curvature of the surface, J_s, is defined through the initial splaying torque, τ_s^0, and the bending modulus, κ_B, by

$$J_s = -\frac{\tau_s^0}{\kappa_B}. \tag{20}$$

Using this definitions [Eq. (20)] and changing the energy of the reference flat state by a constant, $-\frac{1}{2}\kappa_B {c_0}^2$, we obtain the familiar Helfrich form for the area density of the membrane bending energy,

$$f = \frac{1}{2}\kappa_B (J - J_s)^2 + \overline{\kappa} K. \tag{21}$$

Importantly, the dependence of Helfrich bending energy [Eqs. (18) and (21)] on the total, J, and Gaussian, K, curvatures rather than, separately, on each of the principle curvatures of the surface, c_x and c_y, or on different components of the shape tensor, b_α^β, has a fundamental physical meaning. As mentioned above, according to their definitions through, respectively, the trace and the determinant of the shape tensor, the total and Gaussian curvatures are the surface scalars. The energy dependence only on the surface scalars is the consequence of the membrane properties of an isotropic two-dimensional fluid with no designated structural direction in the membrane plane.

In this context and to conclude this section, we mention the relationship between the Helfrich model of membrane bending elasticity given by Eq. (21) and that suggested by Canham [3] for explanation of red blood cell shapes [3]. The reason for this discussion is a frequent reference in the literature to the energy [Eq. (21)] as the Canham-Helfrich Hamiltonian. Canham [3] considered the membrane as a homogeneous isotropic solid sheet having no elastic stresses in the flat state rather than a two-dimensional fluid layer subject to torques while being flat. For derivation of the membrane bending energy, Canham used the common methods of the thin-shell mechanics (see, e.g., [32]). As a result, the Canham energy accounts neither for the membrane spontaneous curvature nor for the contribution of the Gaussian curvature determined by a separate elastic constant. Canham's approach can be extended to include the missing contributions, but in that case the analog of Helfrich's modulus of Gaussian curvature turns out to be proportional to the lateral shear modulus, which must vanish for fluid membranes. Summarizing, because of the substantial differences in the physical properties of membranes assumed by Helfrich and Canham's approaches and due to the crucial factors, the spontaneous curvature and the modulus of Gaussian curvature, accounted by Helfrich's but not Canham's formula, we find unjustified the association of the model [Eq. (21)] with Canham's name.

3.3 Meaning of Spontaneous Curvature

There is a substantial difference between the notion of the spontaneous curvature, as set by its definition [Eq. (20)], and the concept of the intrinsic curvature, J_0, described in the previous section. The analogous issue related to the spontaneous splay and twist of nematic liquid crystals was already addressed above. Nevertheless, because of its importance and frequent misinterpretation in the literature, we are going to discuss that matter again in a specific relation to the membrane spontaneous curvature, J_s.

The first contribution to the density of the membrane bending energy,f, presented by Helfrich's formula [Eq. (21)], has a form of Hooke's law with the spontaneous curvature, J_s, playing a role of a geometrical characteristic of the relaxed membrane state. Therefore, commonly, the spontaneous curvature, J_s, would be associated with the total curvature the membrane tended to adopt spontaneously in case there were no external forces acting on the membrane surface and the membrane did not undergo topological transformations by fusion, fission, and rupture. In other words, the spontaneous curvature, J_s, is often identified with the intrinsic curvature, J_0, defined above. Such equating of the two concepts is, generally, misleading since they have a priori different physical contents. The spontaneous curvature is merely a measure of the stress, τ_s^0, existing within the membrane in the initial flat state rather than a direct characteristic of the membrane geometry. In contrary, the intrinsic curvature, J_0, is a geometrical feature of a relaxed membrane, which does not carry any information about the membrane stresses.

At the same time, for applications, it is important to know, whether and under which conditions, in spite of the difference in the physical meaning, the value of the spontaneous curvature, J_s, can become similar to that of the intrinsic curvature, J_0. A short answer is that such concurrence can be expected in two situations: either the spontaneous and intrinsic curvatures are both much smaller than the inverse membrane thickness, $|J_s|d \ll 1$, $|J_0|d \ll 1$, or the torque-strain relationship of the membrane, $\tau_i(e_i)$, remains linear [Eqs. (6) and (7)] not only for small but also for large membrane curvatures comparable to the inverse membrane thickness.

In general case, however, the spontaneous and intrinsic curvatures are not expected to have equal or even close values. Indeed, depending on the membrane lipid composition, the initial stress, τ_s^0, can be arbitrarily large so that the spontaneous curvature determined according to Eq. (20) can adopt values comparable with or larger than the inverse membrane thickness, $|J_s|d \geq 1$. This means that, in order to reach a shape with the total curvature equal to J_s, the membrane has to deviate from the initial flat state by the extent violating the requirement of smallness of membrane strains [Eq. (16)]. In that case the Helfrich's expression for the membrane energy [Eq. (21)] has to be complemented by the terms of higher orders in the product of the curvature and the membrane [39]. Because of these additional energy contributions, the intrinsic curvature, J_0, resulting from the complete energy minimization and describing the equilibrium membrane shape, is not expected to equal the spontaneous curvature, J_s, determined by Eq. (20).

It has to be kept in mind, however, that some membranes or, more precisely, lipid monolayers of, at least, certain lipid compositions appear to demonstrate an unexpectedly long-reaching linearity in the torque-strain relationship, which, as mentioned above, can lead to the value similarity between the spontaneous, J_s, and intrinsic, J_0, curvatures. This conclusion is based on the addressed above measurements of the bending moduli of monolayers forming the strongly curved tubes of H_{II}-phases [20, 23–26]. The intrinsic curvatures of these monolayers are, practically, equal to the inverse monolayer thicknesses, $1/d \approx 1/2\text{nm}$. Deformation of these monolayers in the vicinity of their strongly curved intrinsic shapes revealed the values of the bending moduli of about 10 k_BT, which is very close to the bending moduli determined for nearly flat monolayers, albeit of different lipid compositions [34]. This consistency of the bending modulus values determined experimentally over a very broad range of membrane curvatures indicates, although does not prove, that the bending energy of lipid monolayers is quadratic in the total curvature in an extremely wide range of the bending deformations including those for which the curvature radii are comparable to the monolayer thickness. In other words, this means that the dependence of the monolayer torque on the total curvature may remain linear up to very large deformations. In this case, the numbers obtained experimentally for the intrinsic curvatures can be used as substitutions for the spontaneous curvature in spite of the difference between the physical meanings of the two notions.

3.4 Spontaneous Curvature of Mixed Lipid Monolayers

As, in contrast to the membrane intrinsic curvature, J_0, the spontaneous curvature, J_s, does not have a direct geometrical meaning but is rather related to the torque in the flat membrane state, τ_0, understanding how J_s depends on the membrane lipid composition requires a thermodynamic analysis rather than a purely geometrical consideration. Since, as already mentioned, the elastic properties of a lipid bilayer are determined by the properties of its constituent monolayers, we present here the thermodynamic description of mixed, weakly, and homogeneously curved lipid monolayers [40]. For constant temperature, the variation of the monolayer free energy can be written as [41, 42]

$$dF = \gamma dA + A\tau\, dJ + A\overline{\kappa}\, dK + \Sigma \mu_i\, dN_i, \tag{22}$$

where γ is the Gibbs tension; A is the monolayer area; N_i is the number of lipid molecules of i-th type; μ_i is the chemical potential of i-th lipid type; J and K are, respectively, the total and Gaussian curvatures of the monolayer surface; τ is the torque; and $\overline{\kappa}$ is the modulus of Gaussian curvature of the monolayer. The summation in the last term of Eq. (22) is taken over all types of the membrane lipid components. Eq. (22) provides a thermodynamic definition for the membrane torque, τ, and modulus of Gaussian curvature, $\overline{\kappa}$, through the derivatives of the free energy with respect to the total, J, and Gaussian, K, curvatures, respectively. These thermodynamic definitions of τ and $\overline{\kappa}$ are complementary to the discussed above definitions by Helfrich [4].

All the thermodynamic functions and variables related to the curvatures depend on the choice within the membrane of Gibbs dividing surface (reference plane) [17, 18]. Generally, one can choose any arbitrary plane parallel to the membrane-bulk interface as the dividing surface. The thermodynamic description simplifies for several special dividing surfaces such as Gibbs' surface of tension [41] or the neutral surface [17, 18]. For our purposes, the most convenient is the neutral surface, for which the deformation of membrane area stretching-compression and the deformation of changing the total curvature, J, are energetically decoupled. The position of the neutral surface has been determined for a series of monolayers of different compositions and appears to be located near the interface between the polar heads and the hydrophobic moieties of the constituent lipid molecules [20].

The free energy, F, must be a first-order homogeneous function of its extensive thermodynamic variables, A and N_i. It can be, therefore presented, according to Euler's theorem, as

$$F = \gamma A + \Sigma \mu_i \, N_i, \tag{23}$$

where the tension, γ, and the chemical potentials, μ_i, are functions of the curvatures. The Gibbs-Duhem-type equations resulting from Eqs. (22) and (23)] relate the differentials of the chemical potentials, the tension, and the curvatures,

$$\Sigma \; N_i d\mu_i = -A d\gamma + A\tau \; dJ + A\overline{\kappa} dK. \tag{24}$$

From Eq. (24) we can derive a general relationship between the torque and the chemical potentials of the membrane components,

$$\tau = \left(\frac{\partial \gamma}{\partial J}\right)_{A,N_i} + \frac{1}{A}\Sigma \; N_i \left(\frac{\partial \mu_i}{\partial J}\right)_{A,N_i}, \tag{25}$$

where the subscripts indicate the values, which are kept constant through the differentiation. In the following we skip this explicit indication. To present Eq. (25) in a more useful form, we apply Maxwell relationships between the derivatives of the intensive thermodynamic functions, which follow from the independence of the mixed derivatives of the energy [Eq. (22)] of the order of differentiation. The Maxwell relations we substitute in Eq. (25) are

$$A\frac{\partial \tau}{\partial N_i} = \frac{\partial \mu_i}{\partial J}. \tag{26}$$

Moreover, since we describe the lipid monolayer by its neutral surface, the derivative of the tension with respect to the total curvature vanishes, $\frac{\partial \gamma}{\partial J} = 0$. Taking this and Eq. (26) into account, we obtain from Eq. (25) for the case of constant area

$$\tau A = \Sigma\, N_i A \frac{\partial \tau}{\partial N_i}. \tag{27}$$

It follows from Eq. (27) that for fixed monolayer area, A, the product of the torque and the area, τA, is a first-degree homogeneous function of the molecular numbers of the components, N_i, and, hence, can be presented in the form

$$\tau A = (\Sigma\, N_i) \cdot \Phi\left(\frac{N_2}{N_1}, \frac{N_3}{N_1}, \ldots, \frac{N_n}{N_1}\right). \tag{28}$$

It has to be emphasized that Eqs. (27) and (28) are general thermodynamic relationships, which must be satisfied by any specific model of the elastic parameters of mixed monolayers.

Importantly, the relationships [Eqs. (27) and (28)] impose constraints on the models for the torques, $\tau = -J_s\kappa$, rather than, separately, for the spontaneous curvature, J_s, and bending modulus, κ. In the literature, however, specific models for J_s and κ have been suggested. These models operated with the notions of spontaneous curvatures, J_s^i, and bending moduli, κ_B^i, of individual components, whose meanings were the elastic characteristics of monolayer with uniform composition consisting only of the i-th lipid. Namely, the spontaneous curvature of a mixed monolayer containing N_i molecules of i-th type has been suggested to be equal to a sum of spontaneous curvatures of the individual components, J_s^i, weighted with their molar ratios within the membrane, $\phi_i = \frac{N_i}{\Sigma N_i}$, so that $J_s = \Sigma \phi_i J_s^i$. The same assumption was made concerning the inverse bending modulus, $\frac{1}{\kappa_B} = \Sigma \frac{\phi_i}{\kappa_B^i}$ [43]. A slight development of this model taking into account a possibility of differences between the in-plane molecular areas of the components, a_i, presents the spontaneous curvature and the inverse bending modulus as sums of the characteristics of individual components weighted with their relative areas, $J_s = \frac{1}{\Sigma N_i a_i} \Sigma N_i \cdot a_i \cdot J_s^i$, $\frac{1}{\kappa_B} = \frac{1}{\Sigma N_i a_i} \Sigma N_i \cdot a_i \cdot \frac{1}{\kappa_B^i}$ [40]. The background for these models was a reasoning based on a mechanical meaning of the intrinsic rather than spontaneous curvature and ignoring a fundamental difference between the physical contents of the two notions. It is easy to see that both versions of the model do not satisfy the thermodynamic constraints [Eqs. (27) and (28)]. For example, in the latter version of the model, the torque in the flat membrane state equals $\tau_s^0 = -J_s \kappa_B = -\frac{\Sigma J_s^i N_i a_i}{\Sigma \frac{1}{\kappa_B^i} N_i a_i}$, which does not fulfill [Eq. (28)].

A thermodynamically correct model for the spontaneous curvature and bending modulus of a mixed monolayer, where the contributions of the individual components are assumed to be additive, has to propose that J_s and κ_B satisfy [40],

$$J_s = \frac{1}{A} \Sigma N_i \cdot a_i \cdot J_s^i \tag{29}$$

$$\frac{1}{\kappa_B} = \frac{1}{\Sigma N_i a_i} \Sigma N_i \cdot a_i \cdot \frac{1}{\kappa_B^i}. \tag{30}$$

The difference between the model presented by Eqs. (29) and (30) and the previous ones consists in an assumption that the change of the monolayer area, A, upon a condition of constant number of all components, leads to variation of the spontaneous curvature [Eq. (29)] but not of the bending modulus [Eq. (30)]. The resulting expression for the bending moment in the flat membrane state,

$$\tau_s^0 = -J_s \kappa_B = -\frac{\Sigma N_i a_i}{A} \cdot \frac{\Sigma J_s^i N_i a_i}{\Sigma \frac{1}{\kappa_B^i} N_i a_i}, \tag{31}$$

fulfills (Eqs. (27) and (28)]. Obviously, also more complex relationships accounting for nonadditive contributions of different components to the monolayer elastic characteristics, J_s and κ_B [28], are allowed by the thermodynamic relationships [Eqs. (27) and (28)].

4 Conclusions

The goal of this review was to recall the physical background behind the notion of the membrane spontaneous curvature introduced by Helfrich [4], which has been ubiquitously used in the membrane literature over the last few decades, and to illustrate its essential difference from the intrinsic curvature defined by Gruner [9] for lipid monolayers. We emphasized that, while the intrinsic curvature is a geometrical characteristic of a lipid monolayer determined by X-ray studies of the inverted hexagonal phase, the spontaneous curvature characterizes the stress existing in a flat membrane and does not have a direct geometrical meaning. The spontaneous and intrinsic curvatures may have similar values, if both of them are much smaller than the inverse membrane thickness.

Which notion is more useful for the practical characterization of the membrane elastic behavior? Obviously, the answer depends on the specific membrane system. In a lipid bilayer constituting the matrix of any biological membrane, the two monolayers are coupled in the transverse direction so that acquisition of curvature by one of them leads to acquirement of an opposite curvature by the other. In case the lipid compositions of the two membrane monolayers are similar, the bilayer and, hence, the monolayers acquire a flat shape independent of the monolayer intrinsic curvatures. In this situation, each monolayer is elastically frustrated and is characterized by a bending stress described by the spontaneous curvature. Thus, the latter is the relevant monolayer characteristic. Within the membrane structures, where the two monolayers, locally, deviate from the mutually parallel orientation, the appropriate value determining the membrane behavior may be the intrinsic rather than the spontaneous curvature. Examples are the intermediate structures formed in the course of membrane fusion and fission, the most common of which is membrane stalk [44].

While the present article discussed the spontaneous and intrinsic curvatures of purely lipid monolayers, similar ideas have been used to describe the effects of proteins on the membrane curvature and elastic behavior.

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