Molecular dynamics approaches for determination of elastic parameters of lipid membranes

Konstantin V. Pinigin

1 A.N. Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences, 31/4 Leninskiy prospekt, Moscow 119071, Russia; * Correspondence: piniginkv@gmail.com;

Abstract: Lipid membranes are abundant in living organisms, where they constitute a surrounding shell for cells and their organelles. There are a lot of circumstances in which the deformations of lipid membranes are involved in living cells: fusion and fission, membrane-mediated interaction between membrane components, lipid-protein interaction, formation of pores, etc. In all of these cases, elastic parameters of lipid membranes are important for the description of membrane deformations, as these parameters determine energy barriers and characteristic times of membrane-involved phenomena. Since the development of molecular dynamics (MD), a variety of methods have been proposed for the determination of elastic parameters of in silico simulated lipid membranes. MD approaches are characterized by their own peculiarities in comparison with experiments, allowing the consideration of details unattainable in experimental techniques. These MD approaches represent a distinct scientific field, which is rapidly developing. In this work, we review these MD approaches and describe their capabilities, applicability conditions, problems, advantages and disadvantages in comparison with each other, existing challenges, and directions for further development.

Keywords: lipid membranes, lipid bilayers, elasticity, elastic energy, elastic modulus, strain, stress, molecular dynamics

1. Introduction

Lipid membranes are ubiquitous in living systems. The structural unit of these membranes is a lipid molecule which can be divided into two parts: a hydrophobic hydrocarbon tail and hydrophilic head. Lipids tend to diminish the contact of their tails with water and therefore lipid membranes usually exist in the form of a bilayer: two layers (monolayers) of lipid molecules, the hydrophobic tails of which are hidden inside a membrane from the contact with water. The most common example of a lipid membrane is the cytoplasmic membrane which provides an almost impermeable shell for living cells, protecting them from the external environment. Except for cytoplasmic membranes, lipid membranes also constitute a boundary of cell organelles: nucleus, endoplasmic reticulum (ER), Golgi apparatus, lysosomes, mitochondria, vesicles, vacuoles, lipid droplets, etc. Also, lipid membranes are a part of an envelope of some viruses [1].

Experimentally it is known that lipid membranes possess elasticity [2–5]: a tendency to preserve shape in response to applied forces and return to its original configuration when forces are removed. There is a lot of evidence that elastic properties of lipid membranes play a key role in various deformation-involved circumstances, controlling energy barriers and characteristic times: fusion and fission [6–9], membrane-mediated interaction between membrane components [10–16], lipid-protein interaction [17–19], formation of pores [20,21], structure of the boundary of lipid domains [22,23], sorting of membrane proteins and peptides between different phase regions [23,24].

The elasticity of lipid membranes can be characterized by elastic energy, which specifies the energy cost required for a specific deformation. The magnitude of a particular
deformation is usually called a strain, while the strain coefficients in the elastic energy are called elastic moduli. While the strain is a variable quantity, elastic moduli are fixed constants and thus represent the property of a considered membrane. However, elastic moduli are not the only property, as the elastic energy can also contain spontaneous values of strain. In the following, we refer to elastic moduli and spontaneous strain as elastic parameters of lipid membranes. For example, the bending degree of freedom of lipid membranes is characterized by the bending modulus and spontaneous curvature [2]. In general, elastic parameters depend on lipid composition. It is known that there is a variety of different lipids that comprise the membranes of living cells [25], which allows cells to adjust the lipid composition of their membranes. The determination of elastic parameters is thus important for the understanding and description of how living cells regulate the course of deformation-involved processes.

Plenty of experimental techniques exist for the determination of elastic parameters of lipid membranes [26]. Since the development of MD, various methods also appeared for the determination of elastic parameters of in silico simulated lipid membrane models. MD is in an intermediate place between theory and experiment. In its essence, MD is a theoretical tool, but in its form, MD has all properties of an experiment. MD approaches for the determination of elastic properties of lipid membranes have their specific features in comparison with experimental techniques. In MD simulations it is possible to follow the movements of individual atoms. Therefore, although the size of MD systems is rather limited due to computational constraints, MD simulations allow considering details and controlling systems parameters unattainable in experiments, which led to the development of peculiar MD methods different from experimental ones.

In this work, we review the MD approaches for the determination of elastic properties of lipid membranes. Our goal is to describe the capabilities of various approaches, their applicability conditions, problems, advantages, and disadvantages in comparison with each other. We divide all the approaches into two groups: i) equilibrium force methods and ii) fluctuations-based methods. The first group of methods considers average (equilibrium) values of strain and stress by applying external forces to lipid membranes. The second group, in turn, analyzes the fluctuations of different membrane characteristics from their average values. The paper is organized as follows. In Sec. 2, we provide the theoretical preliminaries necessary for the subsequent overview of MD methods. In Sec. 3 and Sec. 4, we review the equilibrium force methods and fluctuations-based methods, respectively. In Sec. 5, we sum up the results of this review.

2. Theoretical preliminaries

2.1. 2D elasticity of lipid membranes

On a large scale, much larger than their thickness, lipid membranes can be considered infinitely thin fluid films and modeled as 2D surfaces. The consideration of all quadratic geometrical invariants of a surface \( S \) leads to the classical Helfrich Hamiltonian [2,27,28]:

\[
W = \int_S \left[ \frac{k}{2} (K - K_0)^2 + \bar{K} K_G + \sigma \right] dS
\]  

(1)

where \( W \) is the elastic energy, \( K \) is the extrinsic curvature, \( K_G \) is the Gaussian curvature, \( K_0 \) is the spontaneous curvature, \( k \) is the bending modulus, \( \bar{K} \) is the Gaussian curvature modulus and \( \sigma \) is the lateral tension. The first two energy contributions of Eq. (1) can be written in terms of principal curvatures: each point at a surface has two normal directions along the tangent plane, at which the curvatures of normal sections (intersections of a surface with planes normal to the tangent plane) obtain maximum and minimum values, \( c_1 \) and \( c_2 \), called principal curvatures. In terms of \( c_1 \), \( c_2 \), \( K = c_1 + c_2 \) and \( K_G = c_1 c_2 \). These principal curvatures, as well as \( K \) and \( K_G \), represent geometrical invariants that do not depend on a surface parametrization. For a cylindrical surface, for example, one of the principal directions is along the cylinder axis, the principal
curvature of which equals zero, while the other direction is along the circumference and equals \(1 / R\), where \(R\) is the cylinder radius. Thus, \(K = 1 / R\) and \(K_G = 0\). The last term of Eq. (1) accounts for the energy cost of pulling lipid material from a reservoir. For example, in experiments with micropipette pulling of membrane tubes, the reservoir is a lipid solution that may be employed for the formation of bilayer lipid membranes on a grid [29]. Another example is the lateral tension due to shape undulations in lipid vesicles [4], which can be considered an effective reservoir.

2.2. 3D elasticity of lipid membranes

In the Helfrich Hamiltonian, the internal structure of lipid membranes is not taken into account. The internal structure, however, is important for the consideration of membrane deformations at a nanoscale, i.e. at a scale compatible with a membrane thickness. Such nanoscopic deformations occur in a variety of cases: in the vicinity of membrane components [10–16], formation of pores [20,21], stalk formation during fusion and fission [6,7], at the boundary of liquid-ordered domains (rafts) [22,23].

To take into account the internal structure of a lipid bilayer membrane, each monolayer of a lipid bilayer can be considered a three-dimensional elastic body. As a reference configuration, from which the energy is calculated, a planar state is usually chosen. This reference configuration allows incorporating the following features of lipid monolayers: i) transverse isotropy, ii) lateral fluidity, iii) incompressibility. The first property follows from a symmetry argument: the properties of a planar lipid monolayer are the same in every direction along the plane of a monolayer surface. The second feature is implied by the lateral fluidity of lipid molecules which can freely diffuse along the plane of a monolayer. The third feature is supported by experiments [30–33] showing a rather large bulk modulus of lipid membranes. With these three properties taken into account, the classical theory of elasticity leads to the following quadratic energy density (energy per unit volume) of a lipid monolayer [13,34]:

\[
\sigma_0 (z) \varepsilon (x, y, z) + \frac{1}{2} E (z) \varepsilon (x, y, z)^2 + \frac{1}{2} \lambda_t (z) \mathbf{T}(x, y, z)^2.
\]

(2)

In Eq. (2), \(x, y, z\) are the coordinates of a Cartesian coordinate system, which is chosen in such a way that the \(z\)-axis points from the hydrophilic to the hydrophobic part along the axis of symmetry of a monolayer. Elastic strains in Eq. (2) are given by two functions: \(\varepsilon (x, y, z)\) and \(\mathbf{T}(x, y, z)\). The first one is a scalar function which represents the local lateral area expansion: any infinitesimal lateral area \(dA\) changes by \(\varepsilon \, dA\) upon a deformation. The second one is a vector field, called local tilt, which quantifies the transverse shear deformation mode. Any deformation of a lipid monolayer from the reference state to some deformed state can be given by a function \(\mathbf{X}(x, y, z)\). The tilt degree of freedom is defined as \(\mathbf{T}(x, y, z) = \nabla \cdot \mathbf{X} - 1\), where \(\mathbf{N}\) is a unit normal to a surface given by \(\mathbf{X}(x, y, z)\) with fixed \(z\). \(\mathbf{n}(x, y, z)\), being a unit tangent vector to a curve given by \(\mathbf{X}(x, y, z)\) with fixed \(x\) and \(y\), is called a lipid director and is usually assumed to be a constant function of \(z\). Note that Eq. (2) lacks the lateral shear deformation mode, reflecting the lateral fluidity of lipid monolayers. Also, there is only the local lateral strain given by \(\varepsilon (x, y, z)\) in Eq. (2), while the local longitudinal strain, \(\varepsilon_z = \mathbf{n} \cdot \nabla \cdot \mathbf{X} - 1\), is absent. The latter follows from the incompressibility assumption. Here, it is necessary to distinguish between two types of incompressibility: local and global. Local incompressibility implies that the volume of any infinitesimal monolayer patch remains constant during deformations. From experiments, however, it is only known that lipid monolayers are incompressible in the global sense: the total volume of lipid monolayers is constant, whereas local volumes may change. Actually, MD simulations show that local incompressibility does not hold in lipid monolayers.
[13,35]. Despite this, in theory, it is usually assumed for simplicity that lipid monolayers are locally incompressible, which implies that $\varepsilon$ and $\varepsilon_z$ are not independent [36] and allows the incorporation of only $\varepsilon$ to the elastic energy. In general, however, $\varepsilon$ and $\varepsilon_z$ are not independent. Nevertheless, even if global incompressibility is assumed, it was shown that $\varepsilon$ and $\varepsilon_z$ are still not independent in planar configurations of a lipid monolayer [37]. Elastic moduli in Eq. (2) are given by two functions, $E(z)$ and $\lambda_T(z)$. $E(z)$ is a coefficient of $\varepsilon^2$ and thus represents the local stretching modulus of a lipid monolayer. $\lambda_T(z)$ in turn multiplies $\sigma_0(z)$ and has the meaning of the local tilt modulus. Both $E(z)$ and $\lambda_T(z)$ do not depend on $x$ and $y$ for a lipid monolayer is transversely symmetric in the reference configuration. In contrast to the tilt degree of freedom, the local lateral stretching also has a linear term in Eq. (2), given by $\sigma_0(z)\varepsilon(x,y,z)$.

$\sigma_0(z)$ thus represents the local lateral pressure (or stress, i.e. minus the pressure) profile of a lipid monolayer in the reference configuration. Although $\sigma_0(z)$ can be measured experimentally [38,39], the experimental precision is not as high as can be provided by MD simulations. In general, the stress profile has the following characteristics: a large negative pressure at the interface between the hydrophilic and hydrophobic parts of a monolayer, i.e. in the proximity of the glycerol region of lipid molecules, and mainly a repulsive positive pressure in the head-group region and hydrophobic tails [40,41].

Both the Helfrich Hamiltonian and Eq. (2) are phenomenological relations between the elastic energy and strain since they are mainly based on symmetry arguments and do not rely on first principles. However, the level of phenomenology is different: in Eq. (2) the third dimension is additionally considered as opposed to the Helfrich Hamiltonian, wherein lipid membranes are considered as infinitely thin fluid films. Therefore, Eq. (2) is more general than Eq. (1) and, in fact, Eq. (1) can be obtained from Eq. (2). To do this, one can integrate out all energy contributions along the thickness of a monolayer. In this way, within the assumption that $n(x,y,z)$ and $T(x,y,z)$ does not depend on $z$, Hamm and Kozlov (HK in the following) derived the following quadratic energy functional [36]:

$$\bar{w}_{mono}^{2D} = \frac{1}{2} k_{A,m} \alpha^2 + \frac{1}{2} k_m (\bar{K} + K_{0,m})^2 + \bar{K}_m K_G + \frac{1}{2} k_i T_i^2,$$

(3)

where:

$$k_{A,m} = \int_{m^\perp} E(z) dz, \quad k_m = \int_{m^\perp} \frac{E(z)}{2} (z - z_0)^2 dz,$$

$$-k_m K_{0,m} = \int_{m^\perp} \sigma_0(z) (z - z_0) dz, \quad \bar{K}_m = \int_{m^\perp} \sigma_0(z) (z - z_0)^2 dz,$$

$$k_i = \int_{m^\perp} \lambda_T(z) dz,$$

(4)

In Eq. (4), $\int_{m^\perp} dz$ is the integration along the thickness from the hydrophilic to the hydrophobic part of a lipid monolayer in the reference configuration, $k_{A,m}$ is the stretching modulus, $k_m$ is the bending modulus, $K_{0,m}$ is the spontaneous curvature, $z_0$ is the position of the neutral surface, $K_G$ is the Gaussian curvature modulus, and $k_i$ is the tilt modulus. In Eq. (3), stretching $\alpha$ and tilt $T$ correspond to $\varepsilon(x,y,z)$ and $T(x,y,z)$, respectively, are evaluated at the neutral surface. $\bar{K}$ and $\bar{K}_G$, which are defined on the neutral surface, are called the effective extrinsic curvature and effective Gaussian curvature, respectively. They are constructed from the effective curvature tensor: $\bar{K}_{ij} = K_{ij} + \nabla_i T_j$, where $\nabla_i$ is the covariant derivative along the $i$-th coordinate ($x$ and $y$...
refer to the first and second coordinate, respectively, \( K_{ij} = -N \cdot \nabla e_j \) (where \( e_j = V_j X \) are local basis vectors) is the second form of the neutral surface, \( T_j = T \cdot e_j \) are the covariant components of the tilt vector. In these notations, \( \tilde{K} = g^{ij} \tilde{K}_{ij} = K + \nabla \cdot T \) and \( \tilde{K}_G = \frac{-\tilde{K}_{11}\tilde{K}_{22} - \tilde{K}_{12}\tilde{K}_{21}}{g} \), where \( g^{ij} \) is the inverse of the metric tensor \( g_{ij} = e_i \cdot e_j \) and \( g = \det(g_{ij}) = g_{11}g_{22} - g_{12}^2 \). Although the expressions for \( \tilde{K} \) and \( \tilde{K}_G \) in terms of \( \tilde{K}_{ij} \) are rather cumbersome, the actual meaning of \( \tilde{K} \) and \( \tilde{K}_G \) is more transparent when written in terms of the lipid director \( n \).

At any point of the neutral surface, a local Cartesian coordinate system can be constructed with the origin at this point and the z-axis pointed along \( N \) in the same direction. Then, \( \tilde{K} \approx \frac{\partial n_x}{\partial x} + \frac{\partial n_y}{\partial y} \), i.e., approximately equals the divergence, \( \nabla \cdot n \), of the lipid director; \( \tilde{K}_G = \frac{\partial n_x}{\partial x} \frac{\partial n_y}{\partial y} - \frac{\partial n_x}{\partial y} \frac{\partial n_y}{\partial x} = \det \left( \frac{\partial n_i}{\partial x_j} \right) \), where \((x_1, x_2) = (x, y)\).

The position of the neutral surface, \( z_m \), is defined by the equation \( \int_{z_m} E(z)(z-z_0)dz = 0 \), which implies that the stretching and bending modes are decoupled at this surface. An equivalent definition of the neutral surface is that it is such a surface the bending modulus relative to which is minimal. Indeed, the bending modulus relative to an arbitrary plane \( \tilde{z} = \text{const} \) can be written as \( k_m(\tilde{z}) = \int_{z_m} E(z)(z-\tilde{z})^2 dz \) [13]. The extremum of \( k_m(\tilde{z}) \) satisfies \( \frac{\partial k_m(\tilde{z})}{\partial \tilde{z}} = -2\int_{z_m} E(z)(z-\tilde{z})dz = 0 \), which coincides with the definition of the neutral surface. That this extremum corresponds to the minimum follows from the equality \( \frac{\partial^2 k_m(\tilde{z})}{\partial \tilde{z}^2} = 2\int_{z_m} E(z)dz = 2k_A > 0 \). Thus, at \( \tilde{z} = z_0 \) the bending modulus is minimum. This implies that when an arbitrary torque is applied, a lipid monolayer would bend relative to the neutral surface to minimize the elastic energy. Therefore, the bending modulus relative to the neutral surface is of most interest for the determination.

When \( T = 0 \), the HK Hamiltonian, Eq. (3), transforms to the Helfrich Hamiltonian amended by the elastic energy of stretching. At the macroscopic scale, the stretching deformation mode, being a rather tough one in comparison with bending, is usually omitted [2]. Thus, the HK Hamiltonian represents a generalization to the Helfrich Hamiltonian for lipid monolayers, additionally taking into account the tilt deformation mode, i.e., the deviation of the average orientation of lipid molecules from the local normal to the monolayer surface. Although the signs of spontaneous curvatures in Eqs. (2) and (3) are different, this is a matter of definition. In Eq. (3), we follow the definition usually used in experiments [42,43]: a positive spontaneous curvature implies that lipid tails tend to repel more strongly than lipid heads.

It should be noted that the HK Hamiltonian, written in the form of Eq. (3), relies on the local fluidity assumption, \( \lambda_{LS}(z) = 0 \), where \( \lambda_{LS}(z) \) is the lateral shear modulus. However, a more general assumption can be assumed, the global fluidity assumption: \( \int_{z_m} \lambda_{LS}(z)dz = 0 \). Actually, global fluidity is enough to take into account the free translational motion of lipids in the lateral direction, which is usually meant by the lateral fluidity of lipid monolayers. Along with local fluidity, HK also considered global fluidity and derived that, in the case of the global fluidity assumption, Eq. (3) should be supplemented by the twist term [36]: \( \frac{1}{2}k_{tw}(\nabla \times T)^2 \), where \( k_{tw} = \int_{z_m} \lambda_{LS}(z)(z-z_0)^2 dz \) and \( \nabla \times T = \frac{V_1 T_2 - V_2 T_1}{\sqrt{g}} \). Also, it turns out that \( \lambda_{LS}(z) \) modifies the bending and Gaussian...
curvature moduli: \( k_m \rightarrow k_m + k_{tw}, \quad k_{m} \rightarrow k_{m} - 4k_{tw} \) [36]. In a subsequent elaboration of the HK theory, it was argued [13] that stability considerations require \( \lambda_{LS}(z) \geq 0 \) and thus the global and local fluidity assumptions are equivalent: from \( \lambda_{LS}(z) \geq 0 \) and \( \int_{m_{m}} \lambda_{LS}(z) \, dz = 0 \) it follows that \( \lambda_{LS}(z) = 0 \). Moreover, it was shown that within the local fluidity assumption the HK Hamiltonian, Eq. (3), is unstable: it is possible to construct a deformation which takes the elastic energy to minus infinity, leading to unphysical results [13]. This instability occurs due to the effective Gaussian curvature term, \( \tilde{K}_G \), in Eq. (3) and it was suggested that this term should be omitted from the elastic energy [13]. Another arguments to omit \( \tilde{K}_G \) stems from the fact that the Gaussian curvature modulus, \( m_k \), equals \( (\alpha - \int_{m} \sigma_0(z)(z-z_0)^2 \, dz \) (see Eqs. (4)) and thus contains only the pre-stress \( \sigma_0(z) \). In general, however, in any quadratic theory, pre-stress contributions to the elastic energy should contain only linear terms.

In the HK theory, the local tilt field, \( T(x, y, z) \), was assumed to be a constant function of \( z \). In subsequent developments of the HK theory [13,34], a more general case was considered: the dependence of \( T(x, y, z) \) on \( z \) was taken into account, which led to the following quadratic energy functional [13]:

\[
e^{\text{mon}}_{2D} = \frac{1}{2} k_{m, \alpha} \alpha^2 + \frac{1}{2} k_m \left( \tilde{K} - K_{0,m} \right)^2 + \frac{1}{2} k_{\alpha} T^2 + k_{\tilde{K}} T \nabla \tilde{K} + \frac{k_{\nabla \tilde{K}}}{2} \left( \nabla \tilde{K} \right)^2 + B T \cdot \nabla \alpha - k_{c} \left( \nabla \alpha \right)^2 + C \nabla \alpha \cdot \left( \nabla \tilde{K} \right),
\]

where:

\[
k_{c} = -\frac{1}{2} \int_{m} \lambda_{\xi}(z)(z-z_0)^2 \, dz, \quad k_{\alpha} = \frac{1}{4} \int_{m} \lambda_{\xi}(z)(z-z_0)^3 \, dz, \quad B = \int_{m} \lambda_{\xi}(z)(z-z_0) \, dz, \quad C = \frac{1}{2} \int \nabla \lambda_{\xi}(z)(z-z_0)^3 \, dz.
\]

The HK Hamiltonian is thus supplemented by additional energy contributions which characterize the coupling between the tilt field \( T \), stretching \( \alpha \) and effective curvature \( \tilde{K} \). The tilt-curvature coupling term, for example, is given by \( k_{\xi} T \nabla \tilde{K} \), where \( k_{\xi} \) is the tilt-curvature coupling modulus. The \( T - \alpha \) and \( \alpha - \tilde{K} \) couplings are given by \( B T \cdot \nabla \alpha \) and \( C \nabla \alpha \cdot (\nabla \tilde{K}) \). Also, Eq. (5) contains the contributions from the effective curvature gradient, \( \frac{k_{\nabla \tilde{K}}}{2} \left( \nabla \tilde{K} \right)^2 \), and stretching gradient, \( -k_{c} \left( \nabla \alpha \right)^2 \). Note that when \( T = 0 \), the Hamiltonian Eq. (5), does not transform the Helfrich Hamiltonian, since \( \frac{k_{\nabla \tilde{K}}}{2} \left( \nabla \tilde{K} \right)^2 \) and \( -k_{c} \left( \nabla \alpha \right)^2 \) remain. This stems from the fact that even if at the neutral surface \( T = 0, T(x, y, z = z_0) \), in general, is not zero. In ref. [13], it was shown that the values of the moduli given in Eqs. (6) can influence the character of membrane-mediated interactions between peripheral membrane components and thus may have biological implications.

3. Equilibrium force methods

3.1. Planar lipid bilayers

Although the planar configuration of lipid bilayers is rather simple, quite a few elastic parameters can be determined in this configuration. We divide this section into two parts: macroscopic stress methods and microscopic stress methods.

3.1.1. Macroscopic stress methods
Due to the symmetry of the planar configuration, the local stress tensor of a planar bilayer in the Cartesian coordinate system, the $z$-axis of which is normal to the bilayer, can be written as:

$$
\Sigma(x, y, z) = \begin{pmatrix}
S_L(x, y, z) & 0 & 0 \\
0 & S_L(x, y, z) & 0 \\
0 & 0 & S_z(x, y, z)
\end{pmatrix},
$$

(7)

where $S_L$ and $S_z$ are the lateral and longitudinal stresses, respectively. The sign convention we use for $\Sigma$ is the following: if we choose some volume inside a lipid bilayer, then for any unit vector $\mathbf{v}$ normal to the surface of this volume, $\Sigma \mathbf{v}$ equals the force per unit area, which acts on this volume. Stability requirements demand that the divergence of $\Sigma$ equals zero:

$$
\frac{\partial S_L}{\partial x} + \frac{\partial S_L}{\partial y} + \frac{\partial S_z}{\partial z} = 0,
$$

implying $S_L$ does not depend on $x$, $y$ and $S_z$ does not depend on $z$. MD simulations allow setting up two pressure values: the longitudinal pressure, $P_z$, and lateral pressure, $P_L$, which are equal to 1 bar in normal conditions. With given $P_L$ and $P_z$, $P_L = -\frac{1}{L_z} \int_{\text{box}} S_L(z) dz$, where $\int_{\text{box}} dz$ is the integration over the box thickness, and $S_z(z) = -P_z = \text{const}$.

Perhaps, the simplest parameter to determine in planar configurations of lipid membranes is the stretching modulus, $k_{A,m}$. The elastic energy density per unit area for stretching $a$ of a lipid bilayer can be written as:

$$
\omega_A = \frac{1}{2} k_A a^2,
$$

(8)

where $k_A$ is the bilayer stretching modulus. In terms of the monolayer stretching modulus, $k_A = 2k_{A,m}$, i.e. twice the contribution from individual monolayers, see Eq. (3). The derivative of Eq. (8) with respect to $a$ equals:

$$
\sigma(a) = k_A a,
$$

(9)

which is called lateral tension. Note that $\sigma(a)$ is different from that of the Helfrich Hamiltonian, Eq. (1), which corresponds to the tension due to some reservoir and corresponds to the energy cost required to change the membrane area with a fixed area per lipid. $\sigma(a)$ of Eq. (9), in turn, accounts for the change in the area per lipid. Since $\sigma(a) da$ represents an infinitesimal work, $\sigma(a)$ can be determined, within the incompressibility assumption, from $S_L(z)$ and $S_z$ as [44]:

$$
\sigma(a) = \int (S_L(z) - S_z) dz = L_z (a)(P_z - P_L),
$$

(10)

where integration is over the bilayer thickness and $L_z(a)$ is the simulation box thickness along the $z$-axis. From Eq. (9), it follows that $k_A$ can be determined in MD simulations from the slope of $\sigma(a)$ as a function of $a$ [45–47]. This approach, however, is complicated by in-plane undulations: due to thermal motion lipid bilayers constantly undergo bending deformations and at any moment of time membrane is not flat. The latter implies that when a planar bilayer is stretched, the projected area may increase by reducing the magnitude of undulations with the area per lipid remaining the same. This effect is observed both in experiments [3,4,48] and MD simulations [49–51]. Formally, these undulations can be taken into account by introducing an additional deformation mode. The analysis of undulations of flat bilayer membranes leads to [3,28]:
\[ \alpha' = \frac{k_B T}{8\pi k} \ln \left( 1 + \frac{\sigma A}{\pi k} \right), \] (11)

where \( A \) is the initial projected area of a bilayer and \( \alpha' \) is stretching of the projected area due to undulations. It can be assumed that (by analogy with springs in series) the total strain, \( \alpha' \), is a sum of \( \alpha \) and \( \alpha' \) [28]:

\[ \alpha = \alpha + \alpha' = \alpha' = \frac{k_B T}{8\pi k} \ln \left( 1 + \frac{\sigma A}{\pi^2 k} \right) + \frac{\sigma}{k_A}. \] (12)

Note that there is not only the stretching modulus \( k_A \) but also the bending modulus \( k \) in Eq. (12). It follows, therefore, that by fitting Eq. (12) to simulation data one can obtain both \( k_A \) and \( k \) as was done in Refs. [50,51]. The same approach is employed in experiments [3,4,48], where the undulations effect is more prominent. Actually, the smaller the system the smaller the effect of undulations to stretching. Indeed, \( \alpha' \approx \frac{k_B T \sigma A}{8\pi k \pi^2 k} \) as \( \sigma \to 0 \), leading to the effective spring constant of undulations equal to \( k_{Au} = \frac{8\pi^3 k^2}{k_A T} \).

Therefore, the crossover membrane area \( A_c \), which can be estimated from the equality \( k_{Au} = k_A \), is \( A_c = \frac{8\pi^3 k^2}{k_A T} \). For the typical values of \( k \approx 20 k_B T \) and \( k_A \approx 60 k_B T / \text{nm}^2 \) [48], \( \sqrt{A_c} \approx 40 \text{ nm} \). This implies that for box side lengths larger than 40 nm, stretching at small tension is largely dominated by the reduction of undulations. For smaller systems, the area per lipid instead is mostly involved, see Fig. 2 of Ref. [50]. It should be noted the theoretical prediction for stretching due to undulations, Eq. (11), depends on a power spectrum of shape fluctuations [28]. The latter however often deviates from theoretical predictions at small wavelengths (see Sec. 4.3), which complicates a direct derivation of the expression for the undulations-involved stretching.

Apart from the stretching modulus, another important parameter, which can be determined from the macroscopic stress measurements, is the bulk modulus \( k_V \):

\[ k_V = -V \frac{dP}{dV}, \] (13)

where \( V \) is the total volume of a bilayer and \( P \) is the ambient pressure. A direct application of Eq. (13) would be the measurement of the total volume under the applied external pressure:

\[ k_V = \frac{\ln \left( \frac{V_1}{V_2} \right)}{P_2 - P_1}. \] (14)

Eq. (14) was employed in Ref. [52], wherein \( P_1 \) and \( P_2 \) were chosen to be 0 and 0.1 kbar, respectively. The external pressure was applied to lipid bilayers along the \( z \)-axis with the lateral sides of the simulation box being fixed, and \( V \) was calculated as a product of a bilayer area and thickness, which was defined as a distance between the phosphate atoms. The obtained values of \( k_V \) were 0.35 GPa and 0.21 GPa for atomistic and coarse-grained models, respectively. These are quite large values. From Eq. (13) it follows, for example, that to change the bilayer thickness by 1% it is necessary to apply pressures as large as 3.5 MPa and 2.1 MPa, respectively. Therefore, most often, lipid membranes are assumed to be incompressible.

An indirect way to determine \( k_V \) was suggested in Ref. [53]. A lipid bilayer can be simulated at different values of the cutoff radius, \( r_c \), of the Lennard-Jones interactions,
which results in different total volumes of a simulated system. It can be shown that the volume correction $\Delta V$ due to a finite value of $r_c$ is:

$$\Delta V = \frac{1}{k_V} \frac{16\pi N^2}{3} \frac{\epsilon \sigma^6}{r_c^3},$$  \hspace{1cm} (15)$$

where $\epsilon$ and $\sigma$ are the Lennard-Jones parameters, $N$ is the number of particles and $V$ is the total volume. The value of $k_V$, measured in this way, was $\approx 2.2$ GPa. This value approximately equals that of water [54] and is close to experimental results [30–33]. As was pointed in Ref. [53], the application of Eq. (15) has one caveat: interactions are not limited to only that of Lennard-Jones but also include electrostatic ones. However, the influence of electrostatic interactions on Eq. (15) can be assumed to be small, as the number of Lennard-Jones interactions is much larger than that of electrostatic ones.

3.1.2. Local stress methods

These methods require information about the local characteristics of the lateral stress profiles, not only their mean values, and are based on Eqs. (4) of the theory section. For example, from the first moment of the stress profile, it is possible to determine the product of the bending modulus and spontaneous curvature: $-k_m K_{0,m} = \int_{m} \sigma_0 (z)(z - z_0) dz$. For a symmetric lipid bilayer in a tensionless state, $\int_{m} \sigma_0 (z) dz$ equals zero and we can write:

$$-k_m K_{0,m} = \int_{m} \sigma_0 (z) zdz.$$

Although Eq. (16) provides only the product of $k_m$ and $K_{0,m}$, Eq. (16) still contains a lot of useful information. In particular, as $k_m$ is always positive, from Eq. (16) it is possible to determine the sign of the monolayer spontaneous curvature. Also, as was shown in Ref. [55], using Eq. (16) it is possible to infer the additivity or nonadditivity of the spontaneous curvature as a function of composition. Eq. (16) can also be applied to lipid bilayers, which in this case transforms to:

$$-k K_{0,b} = \int_{b} \sigma_0 (z) dz,$$

(17)

where $\int_{b} dz$ is the integration over the bilayer thickness, $k$ and $K_{0,b}$ are the bilayer bending modulus and spontaneous curvature, respectively. For symmetric bilayers, the right-hand side of Eq. (17) equals zero, implying $K_{0,b}$ is also zero. However, for asymmetric bilayers, $K_{0,b}$ can be nonzero. The latter may occur due to a different leaflet composition or different concentrations of adsorbed molecules on opposite sides of a bilayer. In Ref. [56], it was shown that the right-hand side of Eq. (17) can be expressed in terms of a difference in the leaflet lateral tensions $\Delta \Sigma$:

$$-k K_{0,b} = l_{sy} \Delta \Sigma,$$

(18)

where $l_{sy}$ is the effective leaflet thickness of the symmetric bilayer. In addition, it was shown that at a low coverage of adsorbed molecules $\Delta \Sigma \approx k_B T \Delta \Gamma$, where $\Gamma$ is the concentration of adsorbed particles, and therefore the following holds:

$$-k K_{0,b} = k_B T l_{sy} \Delta \Gamma.$$  \hspace{1cm} (19)$$

A precise determination of $l_{sy}$ requires the fitting of $l_{sy} \Delta \Sigma$ values plotted against the right-hand side of Eq. (17) [56]. However, $l_{sy}$ can also be estimated in a reasonable way, which allows the determination of $K_{0,b}$ from Eq. (19) (if the bending modulus is known).
which is much computationally easier than the calculation of the local stress, which enters Eq. (17).

Still, for the determination of spontaneous curvature values from Eq. (16), the bending modulus needs to be known. In many practical applications of Eq. (16), the bending modulus is determined with some other method [56–59], not relying on the local stress. However, a self-consistent way of determining $k$ from Eq. (16) would be the application of Eqs. (4), where $k$ is given as the second moment of the local stretching modulus $E(z)$.

In Ref. [60], Campelo et al. developed a method for calculating the local stretching modulus $E(z)$ and derived the following relation:

$$E(z) = \frac{\partial \left( S_L(\varepsilon, z) + P_z \right)}{\partial \varepsilon} \bigg|_{\varepsilon=0}, \quad (20)$$

where $S_L(\varepsilon, z)$ is the lateral part of the stress tensor at stretching $\varepsilon$, uniformly rescaled to match the reference (tensionless) configuration, assuming local incompressibility of lipid monolayers. The scaling in necessary, as due to incompressibility the membrane thickness changes upon stretching. With $E(z)$ being found out, Eqs. (4) can be used to determine the following elastic parameters: stretching modulus, neutral surface position, bending modulus, and spontaneous curvature.

In a subsequent elaboration of the Campelo et al.’s approach, an important correction to the expression of $E(z)$, given in Eq. (20), was found [37]:

$$E(z) = \frac{\partial \left( \frac{S_L(\varepsilon, z) + P_z}{1+\varepsilon} \right)}{\partial \varepsilon} \bigg|_{\varepsilon=0} = \frac{\partial \left( S_L(\varepsilon, z) + P_z \right)}{\partial \varepsilon} \bigg|_{\varepsilon=0} - \left( S_L(0, z) + P_z \right), \quad (21)$$

i.e., $E(z)$ contains not only the derivative of the lateral stress profile but also the lateral stress profile itself. If this correction is not taken into account, the systematic error of the bending modulus calculated from $E(z)$ can reach rather large values of up to $24 \pm 5\%$.

Further, in Ref. [37] a more general condition of global incompressibility was taken into account rather than local incompressibility as was in Ref. [60]. With global incompressibility taken into account, the expression for $E(z)$ transforms to:

$$E(z) = \frac{\partial \left( \frac{S_L(\varepsilon, z) + P_z}{1+\varepsilon} \beta(\varepsilon, z) - P_z \frac{\partial \beta(\varepsilon, z)}{\partial \varepsilon} \right)}{\partial \varepsilon} \bigg|_{\varepsilon=0}, \quad (22)$$

where $\beta(\varepsilon, z) = \frac{dV'(\varepsilon, z)}{dV(\varepsilon, z)}$ is the ratio of the local volumes of the lipid material at coordinate $z$ in deformed and initial states. $\beta(\varepsilon, z)$ allows determining the exact scaling map for matching the stress profiles at stretched and tensionless states [37]:

$$\zeta(\varepsilon, z) = (1 + \varepsilon)^{-1} \int_0^z \beta(\varepsilon, t) dt, \quad (23)$$

where $\zeta(\varepsilon, z)$ is the $z$-dependent thickness of a monolayer at stretching $\varepsilon$. $z = 0$ corresponds to the bilayer center. Also, $\beta(\varepsilon, z)$ permits the determination of the local Poisson’s ratio profile, $\nu(z) = \frac{1}{2} \lim_{\varepsilon \to 0} \left( \frac{\varepsilon}{\varepsilon} \right)$:
\[ \nu(z) = \frac{1}{2(1-\gamma(z))}, \quad (24) \]

where \( \gamma(z) = \frac{\partial S_L}{\partial P_z} \bigg|_{P_z=1 \text{bar}} + 1 \), i.e., the derivative of the lateral stress profile with respect to the isotropic ambient pressure. Local incompressibility implies that \( \nu(z) = 0.5 \).

Simulations of coarse-grained DPPC showed considerable deviations from 0.5 in some regions of the monolayer with the largest deviation from 0.5 being 0.12 ± 0.1 in the glycerol region. It should be noted that the stress-profile approach is not the only way to determine the local Poisson’s ratio. In Ref. [35] the authors directly calculated how the amount of material in a particular slab at coordinate \( z \) changes upon stretching. As a definition for the material amount, the authors employed the volumes of individual atomic groups comprising lipid molecules.

In addition, in Ref. [60] a more simple procedure for the determination of elastic parameters of lipid membranes was proposed: instead of calculating \( E(z) \) and then the integrals of Eqs. (4), it is possible to bypass the calculation of \( E(z) \). Actually, by definition (see Eq. (2)):

\[ E(z) = \frac{\partial^2 \omega_{\text{mono}}}{\partial \varepsilon^2} (\varepsilon, z) \bigg|_{\varepsilon=0} = \frac{\partial}{\partial \varepsilon} \sigma(\varepsilon, z) \bigg|_{\varepsilon=0}, \quad (25) \]

where \( \sigma(\varepsilon, z) \) is called the local tension profile. Eq. (25) allows replacing the integral and derivative signs after the substitution into Eqs. (4). Thus, the expression for the bending modulus, for example, becomes:

\[ k_c = \frac{\partial}{\partial \varepsilon} \int_{m} \sigma(\varepsilon, z)(z-z_0)^2 \, dz \bigg|_{\varepsilon=0}, \quad (26) \]

which is much computationally easier than firstly calculating \( E(z) \), as only one curve fitting of simulation data is required for employing Eq. (26).

It should be noted that the local stress methods have a drawback regarding ambiguity in the definition of the local stress. The problem is that a force decomposition into pairwise contributions is required for the calculation of the local stress [41,61,62]. This problem concerns multibody potentials, i.e., potentials which depend on the positions of more than two particles. Such multibody potentials are employed in MD simulations of lipids. For example, four-body potentials are used for the description of the rotations around the double bonds of hydrocarbon chains [41]. Currently, the so-called covariant central force decomposition (cCFD) is mostly employed [62]. Unlike the Goetz-Lipowsky decomposition [45], cCFD is consistent with Newton’s laws of motion and, in particular, with the conservation of the angular momentum [62]. In Ref. [63], however, it was shown that it is possible to construct another force decomposing, a force center decomposition (FCD), which is also consistent with Newton’s laws of motion. Moreover, it was shown that the elastic parameters can depend on the force decomposition employed: although the first moments of the stress profile remain invariant, the second moment is sensitive to the choice of the force decomposition [63,64].

In summary, within the equilibrium force methods, the planar configuration of lipid membranes allows the determination of all major elastic parameters: bending, stretching and bulk moduli, spontaneous curvature, and the local Poisson’s ratio. The advantage of the planar configuration is that it has a rather simple setup: a square planar lipid membrane with periodic boundary conditions. However, one should be cautious with the system size, as for large systems the effect of thermal undulations becomes important and may impact theoretical predictions. Attempts exist to explicitly take into account these
undulations, but at small wavelengths, these undulations become hardly predictable (see Sec. 4.3). It should also be emphasized that, although the macroscopic stress can be strictly defined, there is still no consensus for the definition of the local stress.

3.2. Tubular bilayers

A cylindrical geometry of lipid membrane tubes, also called tethered, is another useful configuration for the determination of lipid membranes elastic parameters. Lipid tubes are also widely used in experiments for the determination of elastic parameters [29,65,66]. In this setup, a cylindrical lipid membrane is created [67–69]. From Eq. (1), it follows that the stress-strain relation in this configuration is [70]:

$$ f = 2\pi k \left( \frac{1}{R} - K_0 \right) $$

(27)

where $f$ is the force along the tube axis, $R$ is the tube radius. For lipid bilayers, $R$ is usually chosen to be the distance from the tube axis to the midsurface between two monolayers [67]. Employing Eq. (27), it is possible to determine both the bending modulus $k$ and spontaneous curvature $K_0$ by measuring the axial force and tube radius [67–69]. Eq. (27) is usually used to determine elastic properties of symmetric lipid bilayers, which implies that $K_0 = 0$. It is to be noted that the area per lipid should be equalized in both monolayers before the measurements. The same applies to the internal (inside a tube) and external (outside a tube) pressures. However, the self-equilibration of the area per lipid takes a rather long time, as the lipid flip-flop rate is usually slow. To facilitate this process, small pores oriented perpendicular to the tube axis can be intentionally introduced [69]. As for planar bilayers, an undulation correction exists for Eq. (27) [71]:

$$ f = \frac{2\pi k}{R} \left( 1 - \frac{k_B T}{2\pi^2 k} R^2 \Lambda^2 \right) $$

(28)

where $K_0$ is assumed to be zero and $\Lambda$ is the cutoff wave vector. This correction becomes important at large $R$, see Fig. 4 of Ref. [71].

In principle, it is possible to apply the method of tubular bilayers not only to single-component membranes but also to multicomponent ones. However, as the outer and inner monolayers have different curvature moduli, the equilibrium composition of these monolayers is different due to the composition curvature coupling [72]. Therefore, the bending modulus of individual monolayers can be different, which complicates the determination of intrinsic bending moduli of constituent monolayers.

Overall, by simulating tubular bilayers, it is possible to determine the bilayer bending modulus and spontaneous curvature. In addition, if a membrane consists of only one lipid type, the monolayer bending modulus can be inferred as half of the bilayer bending modulus. However, this reasoning does not work for the monolayer spontaneous curvature. Also, the determination of elastic parameters of multicomponent monolayers is complicated due to the composition-curvature coupling effect.

3.3. Buckling

Buckling is the bulging of thin elastic materials out of a plane as a result of a significant compression in the lateral direction. Such bulging also occurs in fluid films [73] and lipid membranes [74]. As a method for the determination of lipid membranes elastic parameters, the buckling procedure was introduced in Refs. [73,74]. In this approach, the simulation starts with a planar lipid membrane along the $xy$-plane in a simulation box with side lengths $(L_x, L_y, L_z)$ with $L_x$ being several times larger than $L_y$. Then, a compressive force is applied along the $x$-direction with $L_y$ kept fixed to create a buckled configuration at some fixed value of $\gamma = \frac{L-L_x}{L}$, where $L$ and $L_x$ are the side lengths of a
simulation box along the $x$-direction of the initial and deformed state, respectively. Subsequent simulations are performed with $L_x, L_y, L_z$ being fixed. The analysis of the stress-strain relation of the buckled configuration following from the Helfrich Hamiltonian leads to the following relations [74]:

$$f_x = k \left( \frac{2\pi}{L} \right)^2 \left( 1 + \frac{1}{2} \gamma + \frac{9}{32} \gamma^2 + \ldots \right), \quad (29a)$$

$$f_y = k \left( \frac{2\pi}{L} \right)^2 \frac{L_y}{L_x} \left( 1 - \frac{5}{2} \gamma - \frac{23}{32} \gamma^2 + \ldots \right), \quad (29b)$$

where $A = LL_x$, and $f_x, f_y$ are the stresses (forces per unit length) along the $x$- and $y$-direction, respectively. Thus, by fitting experimental results by the functions given in Eqs. (29a–b), one can determine the bending modulus $k$. It should be noted however that a rather large system is required for the implementation of the buckling method ($L \approx 40$ nm, $L_y \approx 7$ nm [74]), which leads to the necessity to make undulation corrections to Eqs. (29a–b) [74]. Although the correction for $f_x$ is negligibly small, the correction for $f_y$ can be up to 10% [74]:

$$\delta f_y = -k a^T \frac{L_y}{L_x} \left( 1 + \frac{3L_y a}{2l^2} \left( 1 - \frac{11}{8} \gamma + \ldots \right) \right), \quad (30)$$

where $a$ indicates the microscopic cutoff length of membrane undulations. With this correction taken into account, both Eq. (29a) and (29b) yield consistent results for the bending modulus.

Apart from fluid symmetric bilayers, the buckling approach can also be applied to bilayers in the gel phase [75] and asymmetric bilayers [76, 77]. The theoretical analysis of these systems, however, requires significant corrections to Eqs. (29a–b) due to the softening effect: the bending modulus of these systems decreases with curvature. This softening effect can be incorporated into the elastic energy in the following phenomenological way [75]:

$$W_S = kl^{-2} \left( \sqrt{1 + l^2 K^2} - 1 \right), \quad (31)$$

where $l$ is some curvature scaling factor. With this elastic energy, the stress-strain relation of Eq. (29a) transforms to [75]:

$$f_x = k \left( \frac{2\pi}{L} \right)^2 \left( 1 + \frac{1}{2} \left( 1 - 3\delta^2 \right) \gamma + \ldots \right), \quad (32)$$

where $\delta = 2\pi l / L$ is a softening parameter, which enters the stress-strain relation as an additional fitting parameter.

The buckling method can also be applied for the determination of the tilt modulus [78]. The Euler-Lagrange equation following from the HK Hamiltonian, Eq. (3), for the tilt field can be written as:

$$T^*(s) - l^2 T(s) = -K'(s), \quad (33)$$

where $s$ is a natural parametrization parameter for the buckled configuration and $l = \left( \frac{k_m}{k_l} \right)^{1/2}$. Within the approximation $|T^*| \ll \left| \frac{T}{l^2} \right|$, it is possible to derive the following equation for the tilt modulus:
where \( z_0(\xi) \) is the pivotal plane position, determined with respect to the reference atom at a distance \( \xi \) from the midplane. The pivotal plane is a special plane parallel to the monolayer surface, at which the area per lipid remains the same during deformations. It was shown that the location of this plane can be determined from the difference in the number of lipids between the opposing leaflets in curved geometries such as spherical and cylindrical [79]. The determination of the pivotal plane is also possible in the buckled configuration by looking at the leaflet difference at different membrane sections [79]. In this framework, the number of lipids in a particular section depends on the choice of a reference atom, the coordinates of which define the position of lipids relative to a particular membrane section. This leads to a \( \xi \)-dependent position of the neutral plane, which enters Eq. (34). It should be noted that Eq. (34) follows from the HK Hamiltonian which does not take into account the tilt-curvature coupling term of a more general Hamiltonian, Eq. (5), which was subsequently discovered, and the effect of this tilt-curvature coupling term on Eq. (34) was not yet analyzed.

Currently, the theoretical framework for the buckling protocol exists only for single-component lipid bilayers [73,74]. The consideration of multi-component lipid bilayers is complicated by the composition-curvature coupling [66,80]: a lateral redistribution of lipids to regions of different curvature. This effect leads to an inhomogeneous concentration of lipid components and thus to the composition-dependent elastic parameters: \( k_m \rightarrow k_m(\mathbf{r}) \), \( K_0 \rightarrow K_0(\mathbf{r}) \), etc., where \( \mathbf{r} \) is the position vector. Formally, the buckling method can be applied to multicomponent lipid membranes [81]. The bending modulus obtained in this way reflects some mean value of the overall system. However, this value is buckling-specific, as in general \( k_m(\mathbf{r}) \) is different in other curved geometries. Position-dependent elastic parameters can be referred to as the intrinsic ones, as they reflect the parameters of a specific lipid composition at \( \mathbf{r} \). To avoid the composition-curvature coupling effect, intrinsic elastic parameters of multicomponent lipid membranes can be determined in geometries with fixed curvatures such as planar or cylindrical bilayers. The constancy of the extrinsic curvatures assures that the lipid composition remains homogeneous and the composition-curvature coupling effect is not involved.

In summary, the buckling procedure is a rather powerful technique, which permits the determination of not only the bilayer bending modulus but also the tilt modulus and pivotal plane position. However, currently, the theoretical framework for the buckling approach exists only for single-component lipid membranes: the application to multicomponent membranes is complicated by the composition-curvature coupling effect. Also, the buckling procedure does not provide the spontaneous curvature values of constituent monolayers.

3.4. Sinusoidal bilayers

In this approach [82], a lipid bilayer is sinusoidally deformed by two cylindrical guiding potentials. The potentials are given as cylindrical rigid wall potentials which interact with hydrophobic beads of a considered membrane. The cylinders are located on opposite sides of a membrane parallel to each other. For a planar membrane spanning from 0 to \( L \) along the \( x \)-axis, the axes of the cylinders are located at \( L/4 \) and \( 3L/4 \). The coordinates of the points where the cylinders touch a membrane are chosen to be equal up to a sign and are denoted by \( z_m \) and \( -z_m \). From the Euler-Lagrange equation, following from the Helfrich Hamiltonian under the assumption \( z_m/L \ll 1 \), it follows that the force \( F_z \) acting on the cylinders equals:
where \( L_y \) is the side length of the simulation box along the \( y \)-axis. Eq. (35) is valid only for \( z_m / L \ll 1 \). The exact stress-strain relation does not have a closed-form analytical solution and can be found numerically. Then, the simulation data can be fitted to this numerical solution to determine the bending modulus \( k \). In this approach, one should be cautious with the definition of \( z_m \). It was shown that \( z_m = 0 \) differs for membranes with different \( L \) due to thermal undulations \([82]\). In practice, \( z_m = 0 \) is chosen to be a limit at \( L \to 0 \) \([82]\). The approach of Ref. \([82]\) resembles that of buckling \([73,74]\). However, a lack of an analytical solution to the boundary value problem for the configuration of Ref. \([82]\) makes the buckling procedure more suitable for applications. Also, there is no need to introduce any external potentials in the buckling approach wherein lipid bilayers naturally buckle staying within the periodic boundary conditions.

3.5. Spontaneously curved state

In Ref. \([83]\), an approach was suggested for the determination of the spontaneous curvature of asymmetric bilayer membranes. In this work, lipid bilayers consisting of two lipid types, \( A \) and \( B \), were considered. Each leaflet was divided into two equal parts: the first part is composed by lipid \( A \) of ratio \( \rho \) and lipid \( B \) of ratio \( 1-\rho \), while in the second part the ratios are \( 1-\rho \) and \( \rho \) for lipid \( A \) and \( B \), respectively. The opposing leaflet is constructed similarly, such that the lipid bilayer is in an antisymmetric configuration. If the diffusion of lipids is artificially prevented, from the Helfrich Hamiltonian it follows that such a lipid bilayer relaxes to a sine-like shape with the extrinsic curvature equal to the spontaneous curvature \([83]\). Coarse-grained MD showed that in the equilibrium state the membrane divides into two regions with approximately constant curvature of opposite signs. At \( \rho = 0.5 \), the bilayer spontaneous curvature appeared to be a linear function of the head bead diameter difference between lipid \( A \) and \( B \). Also, at a constant head bead diameter difference, the spontaneous curvature showed a linear dependence on \( \rho \). The approach of Ref. \([83]\) considers only the bilayer spontaneous curvature, not the monolayer spontaneous curvature. Currently, this method was applied only to coarse-grained lipids represented by two hydrophobic and one hydrophilic bead. Note that a modification of a force field is required for preventing the diffusion of lipids. This modification is assumed to not influence spontaneous curvature values, as this modification concerns only fluidity properties of a membrane.

3.6. Collective-variable methods

In this type of the methods, a bias potential \( V_{\text{bias}}[\xi(s)] \) is introduces that depends on some collective variable \( \xi(s) \), where \( s = s(\{r_i\}) \) with \( \{r_i\} \) being the collection of particles’ positions of a considered system. \( V_{\text{bias}}(\xi) \) is usually set by a harmonic potential, \( V_{\text{bias}}(\xi) = \frac{K}{2}(\xi - \xi_0)^2 \), which permits the determination of the free energy \( F(\xi_0) \) employing the umbrella sampling \([84]\). Collective variables are frequently employed for reshaping of lipid membranes to various geometries with nonzero curvatures \([72,85–89]\). However, it is usually difficult to single out all elastic contributions corresponding to \( F(\xi_0) \), as in general each value of \( \xi \) can be characterized by the presence of different deformation modes.

In Ref. \([72]\), the authors employed the local density of hydrophobic beads as a collective variable. The local density values were sampled from the unbending of a halve cylindrical membrane. At each snapshot of the unbending trajectory, the midsurface of a lipid bilayer can be well approximated by a parabolic profile, the elastic energy of which can
be calculated from the Helfrich Hamiltonian. The bending modulus, thus, can be obtained from the comparison of this elastic energy with the calculated free energy along the unbending trajectory. To calculate the elastic energy, a truncation of the midsurface at the membrane edges is required. The choice of this truncation, however, is not strictly defined, which can contribute to the uncertainty in a measured bending modulus [72].

The same process, i.e., unbending of half-cylinders, was employed in Ref. [90]. However, the collective variable was defined differently from Ref. [72]. In Ref. [90], the authors employed the permutation reduction scheme and principal component analysis to identify a reaction coordinate (collective variable). The simulation results showed that the bending modulus increases with extrinsic curvature, i.e., the Helfrich Hamiltonian fails at small radii of extrinsic curvatures, $R \leq 10 \text{ nm}$ [86]. As in Ref. [72], in Ref. [90] the curvature elastic energy was determined via the integration over the midsurface of a bilayer. It was later shown that the interpretation of the simulation data of Ref. [86] depends on the choice of the reference surface used for the calculation of the elastic energy: it was argued that a monolayerwise application of the Helfrich Hamiltonian is more appropriate for this problem and restores the constancy of the curvature elastic energy [91].

In Refs. [92,93], a spherical deformation was applied to an initially flat bilayer patch. To accomplish this, an artificial external potential was applied to the outer tail-beads of all lipids to create a spherical cap of a fixed curvature $c$. Unlike previously described methods, umbrella sampling was not employed. Instead, the system was freed to relax to an equilibrium state, which is either a closed bilayer vesicle or planar bilayer patch. It can be shown that the elastic energy, Eq. (1), of these spherical caps can be written as:

$$\frac{\Delta W(x, \xi)}{4\pi (2k + \bar{k})} = x + \xi \left[ \sqrt{1-x} - 1 \right],$$

(36)

where $x = (Re)^2$ and $\xi = \frac{\gamma R}{2k + \bar{k}}$ with $R$ corresponding to the radius of the closed vesicle and $\gamma$ being the edge tension of the cap circumference. For each value of $x$, it is possible to determine the probability of the cap relaxation to the closed vesicle, which corresponds to $x = 1$, for which an analytical expression can be determined [92]. The approach of Refs. [92,93] was originally developed for the determination of the Gaussian curvature modulus $\bar{k}$, as a spherical configuration has two nonzero principal curvatures, unlike planar or cylindrical configurations. Therefore, the bending modulus $k$, which enters Eq. (36), was determined independently employing lipid tubes (see Sec. 3.2). The edge tension was also determined independently employing planar bilayers with edges [92]. For a Cooke model [94,95], it was shown that $\frac{\bar{k}}{k}$ lies within the range $-0.95 \pm 0.1$ [92]. However, $\bar{k}$ measured from the stress profile appeared to disagree with that of Eq. (36), which was attributed to the ambiguity in the definition of the local stress [92].

Currently, the collective-variable approaches are limited to the determination of either the bilayer bending modulus or Gaussian curvature modulus. Also, the considered collective-variable approaches were applied only for single-component membranes. The application of the collective-variable methods to multicomponent membranes can be complicated by the composition-curvature coupling. For example, during the relaxation of half-cylinders as in Refs. [72,86], the shape soon becomes parabolic, which does not have a constant curvature and can involve the redistribution of lipids to regions of different curvatures in multicomponent membranes, complicating the determination of intrinsic elastic parameters.

4. Fluctuations-based methods

In the previous section, we considered the methods which rely on the average values of stresses and strains. However, any macroscopic physical system always undergoes thermal fluctuations: random deviations of system parameters from their average values.
Lipid membranes are not an exception, and in this section, we consider MD approaches that analyze these fluctuations to infer the elastic parameters of lipid membranes.

4.1. Fluctuations of surface area

In Sec. 3, it was already shown that the stretching modulus of lipid membranes can be obtained from the dependence of the membrane area on the lateral tension. The latter approach relies on the average values of the lateral tension and membrane area. However, the membrane area constantly fluctuates around the average value. The characteristics of these fluctuations can be derived from the rules of statistical mechanics. The elastic energy of stretching of a lipid bilayer $\alpha$ equals $A_0 \frac{1}{2} k_A \alpha^2$, where $k_A$ is the bilayer stretching modulus and $A_0$ is the average area of a lipid bilayer in the reference tensionless state. According to the Boltzmann distribution, the probability of a state with stretching $\alpha$ is proportional to $\exp \left( -\frac{A_0 k_A \alpha^2}{2 k_B T} \right)$, from which it follows that [47]:

$$k_A = \frac{k_B T}{A_0} \frac{\langle \alpha^2 \rangle}{\langle \delta \alpha^2 \rangle},$$

(37)

where the triangular brackets indicate the averaging over time, $A$ is the total membrane area and $\langle \delta \alpha^2 \rangle = \langle \alpha^2 \rangle - \langle \alpha \rangle^2$. As with the stress-strain relation of Eq. (9), Eq. (37) is applicable for sufficiently small systems, wherein the contribution of thermal undulations to $k_A$ can be neglected.

In Eq. (37), $k_A$ refers to the bending modulus of a lipid bilayer. For symmetric bilayers, the equality $k_A = 2k_{A,m}$ holds. For asymmetric bilayers, it can be shown that [96]:

$$\frac{1}{k_A} = \frac{1}{2} \left( \frac{1}{k_A^u} + \frac{1}{k_A^l} \right),$$

(38)

where $k_A^u$, $k_A^l$ are the monolayer stretching moduli of the upper and lower monolayers, respectively. Note that Eq. (37) does not apply to the determination of $k_A^u$ and $k_A^l$, as both $\langle \alpha \rangle$ and $\langle \delta \alpha^2 \rangle$ are identically equal in both monolayers due to the periodic boundary conditions. Therefore, a different method was suggested for the determination of $k_A^u$ and $k_A^l$ [96]: $k_A^u$, $k_A^l$ can be determined from the fluctuations of the local thickness of monolayers. The local thickness appears more suitable for MD calculations than the local area [96]. For each leaflet, the volume incompressibility allows expressing the elastic energy of stretching through the leaflet thickness [96]:

$$E^L = \frac{1}{2} k_{A,m}^L a_0^L \left( \frac{t_0^L - t^L}{t^L} \right)^2,$$

(39)

where $k_{A,m}^L$ is the stretching modulus of a considered leaflet, $t_0^L$ is the average leaflet thickness, $a_0^L$ and $t^L$ are the average area and instantaneous thickness of some local region of a leaflet, respectively. Applying the Boltzmann distribution to the energy levels of Eq. (39), one obtains [96]:

$$-\frac{2k_B T}{a_0^L} \ln p \left( \frac{t_0^L - t^L}{t^L} \right) = k_{A,m}^L \left( \frac{t_0^L - t^L}{t^L} \right)^2 + C,$$

(40)
where \( p \) is the probability. \( k_{A,m}^L \) can also be calculated from the equipartition theorem (analogous to Eq. (37)). However, Eq. (40) has the advantage of employing the truncation for the large values of \( \frac{t^L_0 - t^L}{t^L} \) which does not follow the quadratic elastic regime. It was shown that the most relevant surface to employ for the definition of the local thickness lies approximately in the middle of the hydrocarbon chains. The local thickness, thus, is the distance between the terminal methyl carbons and this relevant surface. However, if high concentrations of cholesterol molecules are considered, this definition requires corrections to be made to the measured values of monolayer stretching moduli [96].

Recall that \( k_{A,m} \) is equal to the integral of the local stretching modulus \( E(z) \) over the monolayer thickness, \( k_{A,m} = \int E(z) \, dz \). When the fluctuations are analyzed with the help of Eq. (37) or Eq. (40), the information about \( E(z) \) remains hidden. In Sec. 3, we considered the equilibrium force approach for the determination of \( E(z) \). The question is whether \( E(z) \) can also be determined from fluctuations. In Refs. [97,98], the authors equated \( E(z) \) with the fluctuations of the depth-dependent area occupied by lipid molecules, \( A_{occ}(z) \):

\[
E(z) = \frac{k_B T \left\langle A_{occ}(z) \right\rangle}{\left\langle \delta A_{occ}^2(z) \right\rangle}.
\] (41)

\( A_{occ}(z) \) is determined by dividing the simulation box into the grid cells with side lengths of approximately 0.03 nm \( \times \) 0.03 nm \( \times \) 0.01 nm along the \( x-, y-\) and \( z- \) axis, respectively. A grid point is assumed to be occupied by a lipid molecule if any atom of this lipid lies within the van der Waals radius from the grid point. Then, \( A_{occ}(z) \) is calculated as a sum of the areas of the occupied grid cells. Thus, Eq. (41) is analogous to Eq. (37) with \( A \) replaced to \( A_{occ}(z) \) and enables the calculation of the depth-dependent stretching modulus \( E(z) \).

4.2. Fluctuations of volume

The bulk modulus \( k_V \) expressed through average values of volume and pressure is given by Eq. (13). By analogy with the stretching modulus, it is possible to derive a fluctuations-based expression for \( k_V \). The elastic energy density per unit volume is:

\[
w_V = \frac{1}{2} k_V \alpha_V^2,
\] (42)

where \( \alpha_V = \frac{V - V_0}{V_0} \) with \( V, V_0 \) being the deformed and initial volume, respectively. The relation between \( k_V \) and volume fluctuations is thus [99,100]:

\[
k_V = \frac{k_B T \left\langle V \right\rangle}{\left\langle \delta V^2 \right\rangle},
\] (43)

where \( V \) is the total volume of a lipid membrane and \( \left\langle \delta V^2 \right\rangle = \left\langle V^2 \right\rangle - \left\langle V \right\rangle^2 \). Eq. (43) is similar to Eq. (37): only the averaging values differ. Eq. (43) was employed in Ref. [99] for the determination of the bulk modulus of atomistic DPPC bilayers with the volume boundary defined at the position of phosphorus atoms, and the obtained value of \( k_V \) was 0.6 GPA. DPPC bilayers were also studied in Ref. [100] and the value of 1.5 GPa for the
bulk modulus was reported. However, in Ref. [100], Eq. (43) was applied to the simulation box as a whole, i.e., with water molecules included. Disentangling the contributions to the bulk modulus from lipids and water leads to a slightly smaller value of 1.3 GPa [35].

4.3. Fluctuations of shape

The shape of lipid membranes, as well as surface area and volume, constantly changes due to thermal motion. Like the area and surface fluctuations, the shape fluctuations can be described using the rules of statistical mechanics. Let’s consider a planar square lipid membrane of area $A$ in the $xy$ plane with periodic boundary conditions. From the Helfrich Hamiltonian, it follows that the fluctuations of the shape $h(x, y)$ of this membrane satisfy the following equation [28]:

$$
\langle h_q^2 \rangle = \frac{k_B T}{k q^4 + \sigma q^2},
$$

where $q = \frac{2\pi}{\sqrt{A}} \left( n_x, n_y \right)$, $n_x, n_y \in \mathbb{Z}$ is the wave vector, $q = |q|$ and $h = \frac{1}{\sqrt{A}} \sum_q h_q e^{i q \cdot r}$ with $r$ being the position vector. Eq. (44) follows from the equipartition theorem applied to the Helfrich Hamiltonian written in the Fourier space. Eq. (44) is a theoretical result for infinitely thin fluid films. In MD simulations, a reference surface should be chosen for the calculation of $h_q$. The first application of Eq. (44) to MD simulations of planar bilayers was introduced in Ref. [46]. The reference surface was chosen to be the midsurface between the two monolayers. Although the simulations were performed at $\sigma = 0$, the fluctuation spectrum showed two regimes: $\sim 1/q^4$ at low $q$ and $\sim 1/q^2$ at high $q$. The transition between the two regimes occurs at a wavelength comparable with the monolayer thickness, $\approx 2.5$ nm. The $1/q^2$ dependence was attributed to the protrusion modes, i.e., relative displacements of individual lipid molecules, at low wavelengths, which roughen the shape of the reference surface. It was later shown that the discrepancy from Eq. (44) at large $q$-values can be explained by the tilt degree of freedom, which if taken into account leads to the following fluctuation spectrum [101] at $\sigma = 0$:

$$
\langle h_q^2 \rangle = \frac{k_B T}{k q^4 + k_t q^2},
$$

which is the same as Eq. (44) if $\sigma$ is replaced by the tilt modulus $k_t$. The deviation from Eq. (44) at large $q$-values, however, is realized not only for 3D lipid membranes but also for 2D meshless membranes [68], which is again attributed to protrusions [68]. It was shown that a proper averaging procedure over grid cells can recover the theoretical result of Eq. (44) at large $q$-values. In general, the protrusions are coupled with the bending deformation mode. In Refs. [102,103], it was shown that the protrusions are disentangled from the coupled undulatory mode $\langle h_{qU}^2 \rangle = \langle h_{qL}^2 h_{qU}^2 \rangle$, where $h_{qL}^2$ and $h_{qU}^2$ are the Fourier amplitudes of the shape of the lower and upper monolayer, respectively, which allows a more accurate determination of the bending modulus both from the fluctuations of the membrane shape [102] and density of phosphorus atoms [103]. In practice, the bending modulus $k$ is usually determined by sampling at low $q$-values to avoid protrusions. The sampling at low $q$-values is however complicated by a slow relaxation rate of small $q$ deformation modes which scales as $1/q^3$ [5,74] due to a solvent viscosity. To enhance the sampling at low $q$-values methods have been proposed that use umbrella enhancement of large wavelength deformations [104,105].

It should be noted that the application of Eq. (44) requires the choice of the reference surface to be made. Actually, lipid bilayers consist of two monolayers and each monolayer has its reference surface. A comprehensive study of systematic errors in fluctuations-
based methods shows that the ambiguity in the choice of the reference surface can lead to the systematic errors in the bending modulus being as large as 75% (according to row 1 of Fig. 5 in Ref. [106]). It can be shown, however, that some choices are more reasonable than others. In particular, the head group region appears to be more favorable than the tails region, as the lipid tails tend to undergo quite large fluctuations [106].

In principle, Eq. (44) can be applied for the bending modulus determination not only to single-component lipid bilayers but also to multi-component bilayers. However, the composition-curvature coupling can introduce some corrections to Eq. (44) [107], leading to the effective bending modulus, which is smaller than the intrinsic bending modulus due to the presence of the additional degree of freedom (composition-curvature coupling). In fact, the comparison of the bending moduli obtained from the analysis of shape fluctuations with that obtained employing the stress-strain relation of lipid tubes, Eq. (27), shows that the former is always smaller than the latter [69]. In Ref. [69], it was also shown that it is possible to determine the composition curvature coupling in fluctuating binary lipid membranes: \( \Delta \varphi = \Lambda K \), where \( \Delta \varphi \) is a local composition difference between the opposing monolayers and \( K \) is the curvature of the midsurface. It is also possible to obtain a direct analytical expression for \( \Lambda \), which is given by a rather bulky Eq. (14) of Ref. [69] and which depends, among other things, on the spontaneous curvature of monolayers, allowing its determination from measuring \( \Lambda \).

The planar configuration is not the only one the shape fluctuations of which can be analyzed. Another rather simple configuration is a lipid nanotube. The shape \( u \) of a cylindrical tube can be parametrized by cylindrical coordinates \( 0 \leq \varphi \leq 2\pi, 0 \leq \zeta \leq L/R \), where \( L \) and \( R \) are the tube length and radius, respectively:

\[
\mathbf{r}(\varphi, \zeta) = \begin{pmatrix} x \\ y \\ z \end{pmatrix} = R \begin{pmatrix} 1 + u(\varphi, \zeta) \cos \varphi \\ 1 + u(\varphi, \zeta) \sin \varphi \\ \zeta \end{pmatrix}.
\]

(46)

The Fourier amplitudes of the Fourier series \( u(\varphi, \zeta) = \sum_{m,n} u_{mn} e^{i(m\varphi + n\zeta)} \) satisfy the following relation [108]:

\[
\left\langle |h_{m,n}|^2 \right\rangle = \frac{k_B T}{kQ^2},
\]

(47a)

\[
Q^4 = (m^2 - 1)^2 + q^2 (q^2 + 2m^2)
\]

(47b)

where \( m \in \mathbb{Z} \) and \( q = 2\pi n R / L \), \( n \in \mathbb{Z} \). In Ref. [68], the analysis of the shape fluctuations of 2D meshless membrane tubes showed that at small \( Q \)-values Eq. (47a) well describes the fluctuations, and the bending modulus value determined based on Eq. (47a) agrees with that of planar membranes obtained based on Eq. (44). At large \( Q \)-values, however, a softening similar to that of planar membranes also occurs, which can be attributed to the protrusion mode.

4.4. Fluctuations of director

Due to the thermal motion, not only the membrane shape fluctuates but also the orientation of individual lipid molecules. In Sec. 2, we showed that within the 3D classical theory of elasticity, the orientations of lipid molecules are characterized by the vector field of unit vectors \( \mathbf{n} \), called directors. The elastic energy written in terms of \( \mathbf{n} \) is given either by the HK Hamiltonian, Eq. (3), or a more general one, Eq. (5), where \( \mathbf{n} \) enters through the effective curvature as \( \tilde{K} = \nabla \cdot \mathbf{n} \), where \( \nabla \cdot \mathbf{n} \) is the divergence of \( \mathbf{n} \). Within the framework of the HK Hamiltonian, it can be shown that the director fluctuations of planar lipid bilayers satisfy the following relation [109]:
where \( K_c \) is the bilayer bending modulus and \( \hat{n}_q = \frac{\hat{n}_q \cdot \mathbf{q}}{q} \), where \( \hat{n}_q \) is the Fourier amplitude of \( \hat{n} = \frac{1}{2} \left[ \mathbf{n}^u - \mathbf{n}^l \right] \) with \( \mathbf{n}^u \) and \( \mathbf{n}^l \) being the lipid director fields of the upper and lower directors, respectively. In Refs. [109,110], it was shown that \( \langle |\hat{p}_q|^2 \rangle \) appears to be a more useful quantity than \( \langle |\hat{h}_q|^2 \rangle \) for the determination of the bending modulus, as \( q^2 \langle |\hat{p}_q|^2 \rangle \) exhibits a plateau regime at larger values of \( q \) than \( q^4 \langle |\hat{h}_q|^4 \rangle \), which allows a more accurate determination of the bending modulus for small systems, reducing the simulation time. Still, at high \( q \)-values there is a substantial discrepancy between theory and simulation data: \( q^2 \langle |\hat{p}_q|^2 \rangle \) appears to be a monotonically increasing function of \( q \) [109], not a constant as Eq. (48) predicts. In Refs. [34,111], it was proposed that the tilt-curvature coupling term \( -\mathbf{T} \cdot \nabla \hat{K} \) is responsible for this discrepancy: the inclusion of this term into the elastic energy modifies the fluctuation spectrum to a monotonically increasing function. However, in Ref. [13] it was shown that a curvature gradient term, \( -\left( \nabla \hat{K} \right)^2 \), should also be included in the elastic energy to provide the overall stability of a lipid membrane. With the curvature gradient included in the elastic energy, the theoretical prediction of the fluctuation spectrum for \( q^2 \langle |\hat{p}_q|^2 \rangle \) transforms to a monotonically decreasing function of \( q \). Therefore, still the discrepancy between the theory and fluctuation data from MD simulations exist, which might indicate the failure of the elastic theory at large \( q \)-values and a necessity to include protrusion-like deformation modes to explain the fluctuation spectrum. In this respect, the determination of the elastic moduli of small-scale deformation modes, such as the tilt modulus or tilt-curvature coupling modulus, which require the sampling at high \( q \)-values, appears to be problematic. Another difficulty with director fluctuations is ambiguity in the definition of a lipid director. As with shape fluctuations, a set of reference beads should be chosen to define the lipid director field. It was shown that the ambiguity in the choice of these reference beads can lead to a rather large systematic error up to 40% (according to row 2 of Fig. 5 in Ref. [106]).

Fluctuations of lipid directors can be considered not only in the Fourier space. In Refs. [112–114], a method was proposed wherein the director fluctuations are analyzed in real space. It was shown that the monolayer bending modulus can be determined from the probability distribution of an angle \( \alpha \) between the directors of neighboring lipids [113]:

\[
-\frac{2k_B T}{a_0} \ln \left[ \frac{P(\alpha)}{\sin \alpha} \right] = k_m \alpha^2 + C,
\]

where \( C \) is a constant and \( a_0 \) is the area per lipid. Eq. (49) is valid only for small tilt angles, \( \leq 10^\circ \) [113]. A quadratic fit to Eq. (49) thus yields the monolayer bending modulus \( k_m \). This approach can be applied not only to single-component but also to multicomponent monolayers: \( P(\alpha) \) can be calculated for every pair of lipid names and then an averaging performed [113]. Although a particular averaging type is a matter of choice and not strictly justified, it was shown that the Reuss averaging agrees well with experimental data. However, in Ref. [37] it was shown that the Reuss averaging does hold for the
DPPC/DOPC mixture. In addition to the bending modulus, the tilt modulus can be determined analogously by employing the following equation [114]:

\[
-\frac{4k_BT}{\alpha_0}\ln\left[\frac{P(\theta)}{\sin \theta}\right] = k_\theta \theta^2 + C,
\]  

(50)

where \( \theta \) is the angle between the lipid director and local monolayer normal.

### 4.5. Virtual deformations

When lipid membranes fluctuate, a lot of deformation modes are simultaneously involved. However, by a straightforward application of the rules of statistical mechanics, it is possible to single out a deformation mode of interest and study the statistics of this deformation mode, thereby virtually imposing this deformation on a membrane [64,115]. In particular, each deformation mode can be characterized by some perturbation variable \( \lambda \), which characterizes the corresponding strain of a deformation. \( \lambda \) may be, for instance, lateral stretching \( \alpha \), curvature, tilt angle, etc. The free energy \( F_\lambda \) corresponding to strain \( \lambda \) can be expressed as

\[
F_\lambda = -k_BT \ln \left( \int e^{-E/k_BT} dE \right),
\]  

(51a)

\[
S_\lambda = \frac{\partial F_\lambda}{\partial \lambda} \bigg|_{V,T} = \frac{\partial^2 E}{\partial \lambda^2} \bigg|_{V,T},
\]  

(51b)

\[
k_\lambda = \frac{\partial^2 F}{\partial \lambda^2} \bigg|_{V,T} = \frac{1}{k_BT} \left( \frac{\partial^2 E}{\partial \lambda^2} \bigg|_{V,T} \right)^2 - \frac{1}{k_BT} \left( \frac{\partial E}{\partial \lambda} \bigg|_{V,T} \right)^2,
\]  

(51c)

where \( E \) is the energy of a system. As the kinetic energy provides only a constant to \( F, E \) in Eqs. (51b–c) can be replaced with the potential energy \( U(r_1, ..., r_N) \), where \( r_i \) are the position vectors of all \( N \) particles of a system. Let’s consider, for example, the lateral stretching deformation mode with stretching \( \alpha \). The energy is given by \( A \frac{k_\lambda \alpha^2}{2} \) and \( \lambda = \alpha \). The stretching deformation can be parametrized as:

\[
r_i' = \begin{pmatrix}
1 + \frac{\varepsilon}{2} & 0 & 0 \\
0 & 1 + \frac{\varepsilon}{2} & 0 \\
0 & 0 & 1 - \varepsilon
\end{pmatrix} r_i.
\]  

(52)

Employing Eqs. (51b) and (52), we can write that the lateral tension, \( \sigma = k_\lambda \alpha \), can be expressed as:

\[
\sigma = \frac{1}{A} \sum_i \left\{ \frac{1}{2} \left( x_i \frac{\partial U}{\partial x_i} + y_j \frac{\partial U}{\partial y_j} - z_i \frac{\partial U}{\partial z_i} \right) \right\},
\]  

(53)
where \( x_i, y_i, \) and \( z_i \) are the coordinates of the position vector \( \mathbf{r}_i \).

In Ref. [64], a method was proposed for the calculation of the bending modulus and Gaussian curvature modulus employing Eq. (51c). For this, two virtual deformations were considered: spherical and cylindrical with the corresponding curvature radii of \( 1/C_{sp} \) and \( 1/C_{cy} \). As, according to the Helfrich Hamiltonian, Eq. (1), the elastic energy of these deformations equals 
\[
\left( \frac{k}{2} \left( C_{cy} - C_0 \right)^2 \right) A \quad \text{and} \quad \left[ \frac{k}{2} \left( 2C_{sp} - C_0 \right)^2 + kC_0^2 \right] A ,
\]
the elastic moduli can be expressed as [64]:
\[
k = \frac{1}{A} \frac{\partial^2 F}{\partial C_{cy}^2} \bigg|_{V,T,C_y=0},
\]
(54a)
\[
4k + 2\kappa = \frac{1}{A} \frac{\partial^2 F}{\partial C_{sp}^2} \bigg|_{V,T,C_y=0}.
\]
(54b)
The corresponding expressions through the potential energy \( U \), which can be obtained after substituting the parametrizations of the spherical and cylindrical deformations into Eqs. (51b–c), are too bulky and are given by Eqs. (26) and (27) of Ref. [64]. It was shown that the method works well for 1D meshless membranes [64]. However, for thick membranes, a further extension of the theory is required to take into account the volume fluctuations effects, as these volume fluctuations enter the parameterizations of the spherical and cylindrical deformations [64]. It was also pointed out that for the calculation of the derivatives of Eq. (51c), a choice of the geometrical center is required, the definition of which contains some ambiguity. Nevertheless, the advantage of the approach of Refs. [64,115] is that it does not require performing the force decomposition, which also leads to some ambiguity in the local-stress methods [64].

5. Discussion

The development of MD led to the appearance of approaches for the determination of elastic properties of \textit{in silico} models of lipid membranes that can be simulated by a computer. In this work, a review of these approaches was provided. All approaches were divided into two broad groups: equilibrium force methods and fluctuations-based methods. In the first group of methods, average equilibrium quantities of stress and strain are measured, while in the second group the deviations from the average values are analyzed.

In MD simulations, it is possible to measure the positions and velocities of individual atoms, which allows a more detailed consideration of the lipid membranes mechanics unattainable in experiments. At the same time, this detailed character of MD simulations also poses a difficulty. In particular, a transition from the continuum language of elastic theories to a discrete depiction of MD simulations is required. A way to accomplish this transition is not straightforward. A major challenge exists regarding the description of discrete quantities, which often leads to ambiguities. In equilibrium force approaches, for instance, the definition of the local stress is not unique (see Sec. 3.2.1) [63]. As for the fluctuations-based methods, the problems are with the definitions of lipid directors and membrane shapes (see Secs. 4.1–4.2), which lead to significant systematic errors [106]. Therefore one has to rely on reasonable choices [62,106]. Also, at small deformation scales comparable with membrane thickness the fluctuations-based methods show the discrepancy between theory and simulation data [13], which might indicate the failure of the elastic theory at short fluctuations wavelengths and makes the determination of small-scale deformation modes, such as the tilt modulus or the tilt-curvature coupling modulus, problematic. These issues represent an important problem to be solved in subsequent developments of MD methods.
Another challenge concerns the determination of elastic moduli of lipid mixtures. Elastic parameters of lipid mixtures represent large interest since cell membranes are multicomponent. However, deformations of such membranes involve the composition-curvature coupling effect. This implies that curved geometries with nonconstant extrinsic curvature, such as in the buckling procedure [73,74], are not applicable for the determination of intrinsic bending moduli and spontaneous curvatures. The same is true for the cylindrical geometry of tubular bilayers, in which the curvature radii of outer and inner monolayers are different [72]. In addition, the composition-curvature coupling is present in fluctuations-based methods [107], which also impedes the determination of intrinsic elastic parameters of multicomponent lipid membranes. The constant curvature geometry of planar bilayers still enables the determination of intrinsic parameters. However, for their determination, the calculation of the local stress is required, the definition of which is still in the state of no consensus. It is also possible to look at the local real-space fluctuations of lipid directors [113] to determine the intrinsic bending moduli of lipid mixtures. The latter approach however requires the choice of averaging made for pairwise splay moduli, which is not strictly justified. Moreover, it was shown that a particular type of averaging employed in Ref. [113], the Reuss averaging, may not always hold for lipid mixtures.

Funding: The work was supported by the Ministry of Science and Higher Education of the Russian Federation

Data Availability Statement: Not applicable.

Acknowledgment: Grateful acknowledgment to Dr. Timur R. Galimzyanov for providing critical comments on the manuscript

References

1. Yeagle, P.L. The membranes of cells; Academic Press: Oxford, 2016; ISBN 978-0-12-800047-2.
2. Helfrich, W. Elastic Properties of Lipid Bilayers: Theory and Possible Experiments. Z. Naturforsch. C 1973, 28, 693–703, doi:10.1515/znc-1973-11-1209.
3. Evans, E.; Rawicz, W. Entropy-driven tension and bending elasticity in condensed-fluid membranes. Phys. Rev. Lett. 1990, 64, 2094, doi:10.1103/PhysRevLett.64.2094.
4. Evans, E.; Rawicz, W.; Smith, B.A. Concluding remarks back to the future: mechanics and thermodynamics of lipid biomembranes. Faraday Discuss. 2013, 161, 591–611, doi:10.1039/C2FD20127E.
5. Brochard, F.; Lennon, J.F. Frequency spectrum of the flicker phenomenon in erythrocytes. J. Phys. 1975, 36, 1035–1047, doi:10.1051/jphys:0197500360110103500.
6. Kuzmin, P.I.; Zimmerberg, J.; Chizmadzhev, Y.A.; Cohen, F.S. A quantitative model for membrane fusion based on low-energy intermediates. Proc. Natl. Acad. Sci. 2001, 98, 7235–7240, doi:10.1073/pnas.121191898.
7. Kozlovsky, Y.; Kozlov, M.M. Stalk model of membrane fusion: Solution of energy crisis. Biophys. J. 2002, 82, 882–895, doi:10.1016/S0006-3495(02)75450-7.
8. Bashkirov, P. V.; Akimov, S.A.; Evseev, A.I.; Schmid, S.L.; Zimmerberg, J.; Frolov, V.A. GTPase Cycle of Dynamin Is Coupled to Membrane Squeeze and Release, Leading to Spontaneous Fission. Cell 2008, 135, 1276–1286, doi:10.1016/j.cell.2008.11.028.
9. Frolov, V.A.; Escalada, A.; Akimov, S.A.; Shnyrova, A. V. Geometry of membrane fission. Chem. Phys. Lipids 2015, 185, 129–140, doi:10.1016/j.chemphyslip.2014.07.006.
10. Fournier, J.B. Coupling between membrane tilt-difference and dilation: A new “ripple” instability and multiple crystalline inclusions phases. EPL (Europhysics Lett. 1998, 43, 725, doi:10.1209/epl/i1998-00424-4.
11. May, S.; Ben-Shaul, A. Molecular theory of lipid-protein interaction and the Lα-HII transition. Biophys. J. 1999, 76, 751–767, doi:10.1016/S0006-3495(99)77241-3.
12. Müller, M.M.; Deserno, M.; Guven, J. Interface-mediated interactions between particles: A geometrical approach. Phys. Rev.
13. Pinigin, K. V.; Kuzmin, P. I.; Akimov, S. A.; Galimzyanov, T. R. Additional contributions to elastic energy of lipid membranes: Tilt-curvature coupling and curvature gradient. *Phys. Rev. E* 2020, 102, 042406, doi:10.1103/PhysRevE.102.042406.

14. Pinigin, K. V.; Volovik, M. V.; Batishchev, O. V.; Akimov, S. A. Interaction of Ordered Lipid Domain Boundaries and Amphipathic Peptides Regulates Probability of Pore Formation in Membranes. *Biol. Membr.* 2020, 37, 337–349, doi:10.31857/S0233475520050096.

15. Pinigin, K. V.; Galimzyanov, T. R.; Akimov, S. A. Interaction of Ordered Lipid Domains in the Presence of Amphipatic Peptides. *Biol. Membr.* 2021, 38, 163–175, doi:10.1134/S0233475520050096.

16. Pinigin, K. V.; Galimzyanov, T. R.; Akimov, S. A. Amphipathic peptides impede lipid domain fusion in phase-separated membranes. *Membranes (Basel)* 2021, 11, 797, doi:10.3390/membranes11110797.

17. Campelo, F.; McMahon, H. T.; Kozlov, M. M. The hydrophobic insertion mechanism of membrane curvature generation by proteins. *Biophys. J.* 2008, 95, 2325–2339, doi:10.1529/biophysj.108.133173.

18. Sodt, A. J.; Pastor, R. W. Molecular modeling of lipid membrane curvature induction by a peptide: more than simply shape. *Biophys. J.* 2014, 106, 1958–1969, doi:10.1016/j.bpj.2014.02.037.

19. Sodt, A. J.; Beaven, A. H.; Andersen, O. S.; Im, W.; Pastor, R. W. Gramicidin A Channel Formation Induces Local Lipid Redistribution II: A 3D Continuum Elastic Model. *Biophys. J.* 2017, 112, 1198–1213, doi:10.1016/j.bpj.2017.01.035.

20. Akimov, S. A.; Volynsky, P. E.; Galimzyanov, T. R.; Kuzmin, P. I.; Pavlov, K. V.; Batishchev, O. V. Pore formation in lipid membrane I: Continuous reversible trajectory from intact bilayer through hydrophobic defect to transversal pore. *Sci. Rep.* 2017, 7, 12509, doi:10.1038/s41598-017-12127-7.

21. Akimov, S. A.; Volynsky, P. E.; Galimzyanov, T. R.; Kuzmin, P. I.; Pavlov, K. V.; Batishchev, O. V. Pore formation in lipid membrane II: Energy landscape under external stress. *Sci. Rep.* 2017, 7, 12152, doi:10.1038/s41598-017-12749-x.

22. Galimzyanov, T. R.; Molotkovsky, R. J.; Bozdaganyan, M. E.; Cohen, F. S.; Pohl, P.; Akimov, S. A. Elastic membrane deformations govern interleaflet coupling of lipid-ordered domains. *Phys. Rev. Lett.* 2015, 115, 088101, doi:10.1103/PhysRevLett.115.088101.

23. Pinigin, K. V.; Kondrashov, O. V.; Jiménez-Munguía, I.; Alexandrova, V. V.; Batishchev, O. V.; Galimzyanov, T. R.; Akimov, S. A. Elastic deformations mediate interaction of the raft boundary with membrane inclusions leading to their effective lateral sorting. *Sci. Rep.* 2020, 10, 4087, doi:10.1038/s41598-020-61110-2.

24. Kondrashov, O. V.; Pinigin, K. V.; Akimov, S. A. Characteristic lengths of transmembrane peptides controlling their tilt and lateral distribution between membrane domains. *Phys. Rev. E* 2021.

25. Symons, J. L.; Cho, K. J.; Chang, J. T.; Du, G.; Waxham, M. N.; Hancock, J. F.; Levental, I.; Levental, K. R. Lipidomic atlas of mammalian cell membranes reveals hierarchical variation induced by culture conditions, subcellular membranes, and cell lineages. *Soft Matter* 2021, 17, 288–297, doi:10.1039/D0SM00404A.

26. Dimova, R. Recent developments in the field of bending rigidity measurements on membranes. *Adv. Colloid Interface Sci.* 2014, 208, 225–234, doi:10.1016/j.cis.2014.03.003.

27. Canham, P. B. The minimum energy of bending as a possible explanation of the biconcave shape of the human red blood cell. *J. Theor. Biol.* 1970, 26, 61–81, doi:10.1016/S0022-5193(70)80032-7.

28. Helfrich, W.; Servuss, R. M. Undulations, steric interaction and cohesion of fluid membranes. *Nuovo Cim. D* 1984, 3, 137–151, doi:https://doi.org/10.1007/BF02452208.

29. Bashkirov, P. V.; Kuzmin, P. I.; Chekashkina, K.; Arrasate, P.; Vera Lillo, J.; Shnyrova, A. V.; Frolov, V. A. Reconstitution and real-time quantification of membrane remodeling by single proteins and protein complexes. *Nat. Protoc.* 2020, 15, 2443–2469, doi:https://doi.org/10.1038/s41596-020-0337-1.

30. Braganza, L. F.; Worcester, D. L. Structural Changes in Lipid Bilayers and Biological Membranes Caused by Hydrostatic Pressure. *Biochemistry* 1986, 25, 7484–7488, doi:10.1021/bi00371a034.

31. Scarlata, S. F. Compression of lipid membranes as observed at varying membrane positions. *Biophys. J.* 1991, 60, 334–340.
32. Tosh, R.E.; Collings, P.J. High pressure volumetric measurements in dipalmitoylphosphatidylcholine bilayers. *Biochim. Biophys. Acta (BBA)-Biomembranes* **1986**, *859*, 10–14, doi:10.1016/0006-3495(86)90312-3.

33. Vennemann, N.; Lechner, M.D.; Henkel, T.; Knoll, W. Densitometric Characterization of the Main Phase Transition of Dimyristoyl-Phosphatidylcholine between 0.1 and 40 MPa. *Berichte der Bunsengesellschaft für Phys. Chemie* **1986**, *90*, 888–891, doi:10.1002/bbpc.19860901011.

34. Terzi, M.M.; Ergüder, M.F.; Deserno, M. A consistent quadratic curvature-tilt theory for fluid lipid membranes. *J. Chem. Phys.* **2019**, *151*, 164108, doi:10.1063/1.5119683.

35. Terzi, M.M.; Deserno, M.; Nagle, J.F. Mechanical properties of lipid bilayers: a note on the Poisson ratio. *Soft Matter* **2019**, *15*, 9085–9092, doi:10.1039/C9SM01290G.

36. Hamm, M.; Kozlov, M.M. Elastic energy of tilt and bending of fluid membranes. *Eur. Phys. J. E* **2000**, *3*, 323–335, doi:10.1007/s101890070003.

37. Kalutsky, M.A.; Galimzyanov, T.R.; Pinigin, K.V. Local stress and elastic properties of lipid membranes obtained from elastic energy variation. *arXiv Prepr. arXiv2206.11781* **2022**, doi:https://doi.org/10.48550/arXiv.2206.11781.

38. Templer, R.H.; Castle, S.J.; Curran, A.R.; Rumbles, G.; Klug, D.R. Sensing isothermal changes in the lateral pressure in model membranes using di-pyrenyl phosphatidylcholine. *Faraday Discuss.* **1999**, *111*, 41–53, doi:10.1039/A806472E.

39. Kamo, T.; Nakano, M.; Kuroda, Y.; Handa, T. Effects of an amphipathic α-helical peptide on lateral pressure and water penetration in phosphatidylcholine and monoolein mixed membranes. *J. Phys. Chem. B* **2006**, *110*, 24987–24992, doi:10.1021/jp064988g.

40. Ollila, O.S.; Vattulainen, I. Lateral Pressure Profiles in Lipid Membranes: Dependence on Molecular Composition. In *Molecular Simulations and Biomembranes*; The Royal Society of Chemistry: Cambridge, 2010; pp. 26–55.

41. Vanegas, J.M.; Torres-Sánchez, A.; Arroyo, M. Importance of force decomposition for local stress calculations in biomembrane molecular simulations. *J. Chem. Theory Comput.* **2014**, *10*, 691–702, doi:10.1021/ct4008926.

42. Kollmitzer, B.; Heftberger, P.; Rappolt, M.; Pabst, G. Monolayer spontaneous curvature of raft-forming membrane lipids. *Soft Matter* **2013**, *9*, 10877–10884.

43. Kaltenegger, M.; Kremser, J.; Frewein, M.P.; Zihrer, P.; Bonthuis, D.J.; Pabst, G. Intrinsic lipid curvatures of mammalian plasma membrane outer leaflet lipids and ceramides. *Biochim. Biophys. Acta (BBA)-Biomembranes* **2021**, *1863*, 183709, doi:10.1016/j.bbamem.2021.183709.

44. Terzi, M.M.; Deserno, M. Lipid Membranes: From Self-assembly to Elasticity. In *The Role of Mechanics in the Study of Lipid Bilayers*; Steigmann, D.J., Ed.; Springer, Cham, 2018; Vol. 577 ISBN 978-3-319-56347-3.

45. Goetz, R.; Lipowsky, R. Computer simulations of bilayer membranes: self-assembly and interfacial tension. *J. Chem. Phys.* **1998**, *108*, 7397–7409, doi:10.1063/1.476160.

46. Goetz, R.; Gompper, G.; Lipowsky, R. Mobility and elasticity of self-assembled membranes. *Phys. Rev. Lett.* **1999**, *82*, 221, doi:10.1103/PhysRevLett.82.221.

47. Feller, S.E.; Pastor, R.W. Constant surface tension simulations of lipid bilayers: the sensitivity of surface areas and compressibilities. *J. Chem. Phys.* **1999**, *111*, 1281–1287, doi:10.1063/1.479313.

48. Rawicz, W.; Olbrich, K.C.; McIntosh, T.; Needham, D.; Evans, E.A. Effect of chain length and unsaturation on elasticity of lipid bilayers. *Biophys. J.* **2000**, *79*, 328–339, doi:10.1016/S0006-3495(00)76295-3.

49. Ayton, G.; Voth, G.A. Bridging microscopic and mesoscopic simulations of lipid bilayers. *Biophys. J.* **2002**, *83*, 3357–3370, doi:10.1016/S0006-3495(02)75336-8.

50. den Otter, W.K. Area compressibility and buckling of amphiphilic bilayers in molecular dynamics simulations. *J. Chem. Phys.* **2005**, *123*, 214906, doi:10.1063/1.2132287.

51. Waheed, Q.; Edholm, O. Undulation contributions to the area compressibility in lipid bilayer simulations. *Biophys. J.* **2009**, *97*, 2754–2760, doi:10.1016/j.bpj.2009.08.048.
52. Izvekov, S.; Voth, G.A. Solvent-free lipid bilayer model using multiscale coarse-graining. J. Phys. Chem. B 2009, 113, 4443–4455, doi:10.1021/jp810440c.

53. Berger, O.; Edholm, O.; Jähnig, F. Molecular dynamics simulations of a fluid bilayer of dipalmitoylphosphatidylcholine at full hydration, constant pressure, and constant temperature. Biophys. J. 1997, 72, 2002–2013, doi:10.1016/S0006-3495(97)78845-3.

54. Fine, R.A.; Millero, F.J. Compressibility of water as a function of temperature and pressure. J. Chem. Phys. 1973, 59, 5529–5536, doi:10.1063/1.1679903.

55. Sodt, A.J.; Venable, R.M.; Lyman, E.; Pastor, R.W. Nonadditive compositional curvature energetics of lipid bilayers. Phys. Rev. Lett. 2016, 117, 138104, doi:10.1103/PhysRevLett.117.138104.

56. Różycki, B.; Lipowsky, R. Spontaneous curvature of bilayer membranes from molecular simulations: Asymmetric lipid densities and asymmetric adsorption. J. Chem. Phys. 2015, 142, 054101.

57. Marrink, S.J.; Risselada, H.J.; Yefimov, S.; Tieleman, D.P.; De Vries, A.H. The MARTINI force field: coarse grained model for biomolecular simulations. J. Phys. Chem. B 2007, 111, 7812–7824, doi:10.1021/jp071097f.

58. Watson, M.C.; Penev, E.S.; Welch, P.M.; Brown, F.L. Thermal fluctuations in shape, thickness, and molecular orientation in lipid bilayers. J. Chem. Phys. 2011, 135, 244701, doi:10.1063/1.3660673.

59. Venable, R.M.; Brown, F.L.H.; Pastor, R.W. Mechanical properties of lipid bilayers from molecular dynamics simulation. Chem. Phys. Lipids 2015, 192, 60–74, doi:10.1016/j.chemphyslip.2015.07.014.

60. Campelo, F.; Arnarez, C.; Marrink, S.J.; Kozlov, M.M. Helfrich model of membrane bending: From Gibbs theory of liquid interfaces to membranes as thick anisotropic elastic layers. Adv. Colloid Interface Sci. 2014, 208, 25–33, doi:10.1016/j.cis.2014.01.018.

61. Admal, N.C.; Tadmor, E.B. A unified interpretation of stress in molecular systems. J. Elast. 2010, 100, 63–143, doi:10.1007/s10659-010-9249-6.

62. Torres-Sánchez, A.; Vanegas, J.M.; Arroyo, M. Geometric derivation of the microscopic stress: A covariant central force decomposition. J. Mech. Phys. Solids 2016, 93, 224–239, doi:10.1016/j.jmps.2016.03.006.

63. Nakagawa, K.M.; Noguchi, H. Nonuniqueness of local stress of three-body potentials in molecular simulations. Phys. Rev. E 2016, 94, 053304, doi:10.1103/PhysRevE.94.053304.

64. Noguchi, H. Virtual bending method to calculate bending rigidity, saddle-splay modulus, and spontaneous curvature of thin fluid membranes. Phys. Rev. E 2020, 102, 053315, doi:10.1103/PhysRevE.102.053315.

65. Tian, A.; Baumgart, T. Sorting of lipids and proteins in membrane curvature gradients. Biophys. J. 2009, 96, 2676–2688, doi:10.1016/j.bpj.2008.11.067.

66. Bashkirov, P. V.; Kuzmin, P.I.; Lillo, J. V.; Frolov, V.A. Molecular shape solution for mesoscopic remodeling of cellular membranes. Annu. Rev. Biophys. 2022, 51, 473, doi:10.1146/annurev-biophys-011422-100054.

67. Harmandaris, V.A.; Deserno, M. A novel method for measuring the bending rigidity of model lipid membranes by simulating tethers. J. Chem. Phys. 2006, 125, 204905, doi:10.1063/1.2327261.

68. Shiba, H.; Noguchi, H. Estimation of the bending rigidity and spontaneous curvature of fluid membranes in simulations. Phys. Rev. E 2011, 84, 031926, doi:10.1103/PhysRevE.84.031926.

69. Barragán Vidal, I.A.; Rosetti, C.M.; Pastorino, C.; Müller, M. Measuring the composition-curvature coupling in binary lipid membranes by computer simulations. J. Chem. Phys. 2014, 141, 194902, doi:10.1063/1.4901203.

70. Deserno, M. Fluid lipid membranes: From differential geometry to curvature stresses. Chem. Phys. Lipids 2015, 185, 11–45, doi:10.1016/j.chemphyslip.2014.05.001.

71. Barbetta, C.; Fournier, J.B. On the fluctuations of the force exerted by a lipid nanotubule. Eur. Phys. J. E 2009, 29, 183–189, doi:10.1140/epje/i2009-10468-8.

72. Smirnova, Y.G.; Müller, M. Calculation of membrane bending rigidity using field-theoretic umbrella sampling. J. Chem. Phys. 2015, 143, 243155, doi:10.1063/1.4938383.
73. Noguchi, H. Anisotropic surface tension of buckled fluid membranes. *Phys. Rev. E* 2011, 83, 061919, doi:10.1103/PhysRevE.83.061919.

74. Hu, M.; Diggins IV, P.; Deserno, M. Determining the bending modulus of a lipid membrane by simulating buckling. *J. Chem. Phys.* 2013, 138, 214110, doi:10.1063/1.4808077.

75. Diggins IV, P.; McDargh, Z.A.; Deserno, M. Curvature softening and negative compressibility of gel-phase lipid membranes. *J. Am. Chem. Soc.* 2015, 137, 12752–12755, doi:10.1021/jacs.5b06800.

76. Hossein, A.; Deserno, M. Spontaneous curvature, differential stress, and bending modulus of asymmetric lipid membranes. *Biophys. J.* 2020, 118, 624–642.

77. Hossein, A.; Deserno, M. Stiffening transition in asymmetric lipid bilayers: The role of highly ordered domains and the effect of temperature and size. *J. Chem. Phys.* 2021, 138, 014704, doi:10.1063/10.1063/5.0028255.

78. Wang, X.; Deserno, M. Determining the lipid tilt modulus by simulating membrane buckles. *J. Phys. Chem. B* 2016, 120, 6061–6073, doi:10.1021/acs.jpcb.6b02016.

79. Wang, X.; Deserno, M. Determining the pivotal plane of fluid lipid membranes in simulations. *J. Chem. Phys.* 2015, 143, 164109, doi:10.1063/1.4933074.

80. Baumgart, T.; Capraro, B.R.; Zhu, C.; Das, S.L. Thermodynamics and mechanics of membrane curvature generation and sensing by proteins and lipids. *Annu. Rev. Phys. Chem.* 2011, 62, 483, doi:10.1146/annurev.physchem.012809.103450.

81. Eid, J.; Razmazma, H.; Jraij, A.; Ebrahimi, A.; Monticelli, L. On calculating the bending modulus of lipid bilayer membranes from buckling simulations. *J. Phys. Chem. B* 2020, 124, 6299–6311, doi:10.1021/acs.jpcb.0c04253.

82. Kawamoto, S.; Nakamura, T.; Nielsen, S.O.; Shinoda, W. A guiding potential method for evaluating the bending rigidity of tensionless lipid membranes from molecular simulation. *J. Chem. Phys.* 2013, 139, 07B613_1, doi:10.1063/1.4811677.

83. Wang, H.; Hu, D.; Zhang, P. Measuring the spontaneous curvature of bilayer membranes by molecular dynamics simulations. *Commun. Comput. Phys.* 2013, 13, 1093–1106, doi:10.4208/cicp.230411.230312a.

84. Torrie, G.M.; Valleau, J.P. Nonphysical sampling distributions in Monte Carlo free-energy estimation: Umbrella sampling. *J. Comput. Phys.* 1977, 23, 187–199, doi:10.1016/0021-9991(77)90121-8.

85. Awasthi, N.; Hub, J.S. Simulations of pore formation in lipid membranes: reaction coordinates, convergence, hysteresis, and finite-size effects. *J. Chem. Theory Comput.* 2016, 12, 3261–3269, doi:10.1021/acs.jctc.6b00369.

86. Bubnis, G.; Risselada, H.J.; Grubmüller, H. Exploiting lipid permutation symmetry to compute membrane remodeling free energies. *Phys. Rev. Lett.* 2016, 117, 188102, doi:10.1103/PhysRevLett.117.188102.

87. Massone, D.; Uhart, M.; Bustos, D.M. Bending lipid bilayers: a closed-form collective variable for effective free-energy landscapes in quantitative biology. *J. Chem. Theory Comput.* 2018, 14, 2240–2245, doi:10.1021/acs.jctc.8b00012.

88. Bouvier, B. Curvature as a collective coordinate in enhanced sampling membrane simulations. *J. Chem. Theory Comput.* 2019, 15, 6551–6561, doi:10.1021/acs.jctc.9b00716.

89. Larsen, A.H. Molecular Dynamics Simulations of Curved Lipid Membranes. *Int. J. Mol. Sci.* 2022, 23, 8098, doi:10.3390/ijms23158098.

90. Bassecrueu, P.; Jin, R.; Baumgart, T.; Deserno, M.; Dimova, R.; Frolov, V.A.; Bashkirov, P. V.; Grubmüller, H.; Jahn, R.; Risselada, H.J.; et al. The 2018 biomembrane curvature and remodeling roadmap. *J. Phys. D. Appl. Phys.* 2018, 51, 343001, doi:10.1088/1361-6463/aac98.

91. Galimzyanov, T.R.; Bashkirov, P. V.; Blank, P.S.; Zimmerberg, J.; Batishchev, O. V.; Akimov, S.A. Monolayerwise application of linear elasticity theory well describes strongly deformed lipid membranes and the effect of solvent. *Soft Matter* 2020, 16, 1179–1189, doi:10.1039/C9SM02079A.

92. Hu, M.; Briguglio, J.J.; Deserno, M. Determining the Gaussian curvature modulus of lipid membranes in simulations. *Biophys. J.* 2012, 102, 1403–1410, doi:10.1016/j.bpj.2012.02.013.

93. Hu, M.; De Jong, D.H.; Marrink, S.J.; Deserno, M. Gaussian curvature elasticity determined from global shape transformations and local stress distributions: A comparative study using the MARTINI model. *Faraday Discuss.* 2013, 161,
94. Cooke, I.R.; Kremer, K.; Deserno, M. Tunable generic model for fluid bilayer membranes. *Phys. Rev. E* **2005**, *72*, 011506.
95. Cooke, I.R.; Deserno, M. Solvent-free model for self-assembling fluid bilayer membranes: stabilization of the fluid phase based on broad attractive tail potentials. *J. Chem. Phys.* **2005**, *123*, 224710, doi:10.1063/1.2135785.
96. Doktorova, M.; LeVine, M. V.; Khelashvili, G.; Weinstein, H. A new computational method for membrane compressibility: Bilayer mechanical thickness revisited. *Biophys. J.* **2019**, *116*, 487–502, doi:10.1016/j.bpj.2018.12.016.
97. Falck, E.; Patra, M.; Karttunen, M.; Hyvönen, M.T.; Vattulainen, I. Lessons of slicing membranes: interplay of packing, free area, and lateral diffusion in phospholipid/cholesterol bilayers. *Biophys. J.* **2004**, *87*, 1076–1091, doi:10.1529/biophysj.104.041368.
98. Kupiainen, M.; Falck, E.; Ollila, S.; Niemelä, P.; Gurtovenko, A.A.; Hyvönen, M.T.; Patra, M.; Karttunen, M.; Vattulainen, I. Free volume properties of sphingomyelin, DMPC, DPPC, and PLPC bilayers. *J. Comput. Theor. Nanosci.* **2005**, *2*, 401–413, doi:10.1166/jctn.2005.211.
99. Song, Y.; Guallar, V.; Baker, N.A. Molecular dynamics simulations of salicylate effects on the micro- and mesoscopic properties of a dipalmitoylphosphatidylcholine bilayer. *Biochemistry* **2005**, *44*, 13425–13438, doi:10.1021/bi0506829.
100. Venable, R.M.; Skibinsky, A.; Pastor, R.W. Constant surface tension molecular dynamics simulations of lipid bilayers with trehalose. *Mol. Simul.* **2006**, *32*, 849–855, doi:10.1080/08927020600615018.
101. May, E.R.; Narang, A.; Kopelevich, D.I. Role of molecular tilt in thermal fluctuations of lipid membranes. *Phys. Rev. E* **2007**, *76*, 021913, doi:10.1103/PhysRevE.76.021913.
102. Tarazona, P.; Chacón, E.; Bresme, F. Thermal fluctuations and bending rigidity of bilayer membranes. *J. Chem. Phys.* **2013**, *139*, 084702, doi:10.1063/1.4818421.
103. Hernández-Muñoz, J.; Bresme, F.; Tarazona, P.; Chacón, E. Bending Modulus of Lipid Membranes from Density Correlation Functions. *J. Chem. Theory Comput.* **2022**, *18*, 3151–3163, doi:10.1021/acs.jctc.2c00099.
104. den Otter, W.K.; Briels, W.J. The bending rigidity of an amphiphilic bilayer from equilibrium and nonequilibrium molecular dynamics. *J. Chem. Phys.* **2003**, *118*, 4712–4720, doi:10.1063/1.1543941.
105. Fiorin, G.; Marinelli, F.; Faraldo-Gómez, J.D. Direct derivation of free energies of membrane deformation and other solvent density variations from enhanced sampling molecular dynamics. *J. Comput. Chem.* **2020**, *41*, 449–459, doi:10.1002/jcc.26075.
106. Ergüder, M.F.; Deserno, M. Identifying systematic errors in a power spectral analysis of simulated lipid membranes. *J. Chem. Phys.* **2021**, *154*, 214103, doi:10.1063/5.0049448.
107. Leibler, S. Curvature instability in membranes. *J. Phys.* **1986**, *47*, 507–516.
108. Fournier, J.B.; Galatola, P. Critical fluctuations of tense fluid membrane tubules. *Phys. Rev. Lett.* **2007**, *98*, 018103, doi:10.1103/PhysRevLett.98.018103.
109. Watson, M.C.; Brandt, E.G.; Welch, P.M.; Brown, F.L. Determining biomembrane bending rigidities from simulations of modest size. *Phys. Rev. Lett.* **2012**, *109*, 028102, doi:10.1103/PhysRevLett.109.028102.
110. Levine, Z.A.; Venable, R.M.; Watson, M.C.; Lerner, M.G.; Shea, J.E.; Pastor, R.W.; Brown, F.L. Determination of biomembrane bending moduli in fully atomistic simulations. *J. Am. Chem. Soc.* **2014**, *136*, 13582–13585, doi:10.1021/ja507910r.
111. Terzi, M.M.; Deserno, M. Novel tilt-curvature coupling in lipid membranes. *J. Chem. Phys.* **2017**, *147*, 084702, doi:10.1063/1.4990404.
112. Khelashvili, G.; Harries, D. How cholesterol tilt modulates the mechanical properties of saturated and unsaturated lipid membranes. *J. Phys. Chem. B* **2013**, *117*, 2411–2421, doi:10.1021/jp3122006.
113. Khelashvili, G.; Kollmitzer, B.; Heftberger, P.; Pabst, G.; Harries, D. Calculating the bending modulus for multicomponent lipid membranes in different thermodynamic phases. *J. Chem. Theory Comput.* **2013**, *9*, 3866–3871, doi:10.1021/ct400492e.
114. Doktorova, M.; Harries, D.; Khelashvili, G. Determination of bending rigidity and tilt modulus of lipid membranes from real-space fluctuation analysis of molecular dynamics simulations. *Phys. Chem. Chem. Phys.* **2017**, *19*, 16806–16818, doi:10.1039/C7CP01921A.
Farago, O.; Pincus, P. Statistical mechanics of bilayer membrane with a fixed projected area. *J. Chem. Phys.* **2004**, *120*, 2934–2950, doi:10.1063/1.1639000.