Appendix A:

Energy of anisotropic membrane constituents

The membrane components are in general anisotropic with respect to the axis perpendicular to the membrane surface [22–24,27,29,45]. The intrinsic shape of anisotropic membrane constituents, which may be single molecules (Fig. A1) or small complexes of molecules (nanodomains) (Fig. A2), energetically prefer a local shape, described by the two different intrinsic principal curvatures $C_{1m}$ and $C_{2m}$ [21,29]. The intrinsic principal curvatures $C_{1m}$ and $C_{2m}$ may be used to describe also the intrinsic shape of a small flexible nanodomain (Fig. A2) or spontaneous curvatures of anisotropic flexible nanodomain [29,33].

**Figure A1:** Different possible intrinsic shapes (isotropic and anisotropic) of small membrane constituents in relation to intrinsic principal curvatures $C_{1m}$ and $C_{2m}$ [21,24].
Figure A2: Schematic representation of different intrinsic shapes of larger membrane constituents (so called flexible membrane nanodomains described by the two intrinsic principal (spontaneous) curvatures $C_{1m}$ and $C_{2m}$ [29].

Due to their structure involving two tails and a headgroup (which is generally non-axisymmetric), also the shape of lipid molecules is in general anisotropic (Fig. A1) [22,23,25,28,45]. It was shown that the intrinsic anisotropy of detergents, lipids or lipid nanodomains can explain the stability of nanotubular protrusions of liposomes [24] and cells [22,23], and a continuous shape transition between different pear-shaped giant lipid vesicles [26,28] which can not be predicted within the models based on the isotropic elasticity of the lipid bilayer [57]. Also the stability of the inverted hexagonal phase of lipids and the transition from the lamellar to the inverted hexagonal phase can be better explained by taking into account the anisotropic shape of lipid molecules [21]. In addition, it was shown that after their intercalation in the outer membrane layer, isotropic detergents ($C_{1m} = C_{2m} > 0$, Fig. A1) induce spherical buds of the membrane, while anisotropic detergents with a dimeric headgroup or anisotropic dimeric detergents ($C_{1m} \approx 0$ and $C_{2m} > 0$, Fig. A1) always induce long tubular membrane buds [23,29,68] which can be theoretically explained by considering the preference of anisotropic dimeric detergent for cylindrically shaped membrane of high curvature [22,23,29].

The energy ($E_{ij}$) of a small anisotropic membrane constituent of the type $i$, where $j = \text{in, out}$ denotes the membrane constituents in the inner and outer layer, derives from the mismatch between the actual local curvature of the membrane (Fig. 1) and the intrinsic (spontaneous) curvature of the constituents (Figs. A1, A2) which can be characterized by the mismatch tensor $M_{ij} = R C_{m,ij} R^{-1} - C_j$ [25,28,29]. Here the tensor $C_j$ describes the actual curvature (see Fig. 1), while the tensor $C_{m,ij}$ describes the intrinsic curvature of the constituents:

\[
\begin{align*}
C_{1m} > 0 & \quad & C_{1m} = 0 & \quad & C_{1m} > 0 \\
C_{2m} = 0 & & C_{2m} = 0 & & C_{2m} < 0
\end{align*}
\]
\[
\mathbf{C}_j = \begin{bmatrix} C_{1,j} & 0 \\ 0 & C_{2,j} \end{bmatrix}, \quad \mathbf{C}_{m,i} = \begin{bmatrix} C_{1m,i} & 0 \\ 0 & C_{2m,i} \end{bmatrix},
\]

(A.1)

where \( j = \text{in, out} \). The rotation matrix is:

\[
\mathbf{R} = \begin{bmatrix} \cos \omega & -\sin \omega \\ \sin \omega & \cos \omega \end{bmatrix},
\]

(A.2)

The angle \( \omega \) describes the orientation of the principal axes system of a single membrane constituent with respect to the local principal axes system of the membrane [20,22]. In the respective principal systems the matrices that represent curvature tensors \( \mathbf{C}_j \) and \( \mathbf{C}_{m,i} \) include only the diagonal elements (for tensor \( \mathbf{C}_j \) the principal curvatures \( C_{1,j} \) and \( C_{2,j} \) (Fig. 1) and for tensor \( \mathbf{C}_{m,i} \) the intrinsic principal curvatures \( C_{1m,i} \) and \( C_{2m,i} \)).

The energy of the membrane constituent \((E_{i,j})\) expressed in terms of invariants of the mismatch tensor \( \mathbf{M}_{i,j} \) should be a scalar quantity. In this work, the energy density of the single constituent is approximated up to the second order in the trace and the determinant of the tensor \( \mathbf{M}_{i,j} \) [24,25,28,29]:

\[
E_{i,j} = \frac{K}{2} \left( \text{Tr} \mathbf{M}_{i,j} \right)^2 + K (\text{Det} \mathbf{M}_{i,j}),
\]

(A.3)

where \( K \) and \( \bar{K} \) are constants. Taking into account the definition of the tensor \( \mathbf{M}_{i,j} \) it follows from Eq. (A.3) that the elastic energy of the single membrane constituent can be written as [25,28,29]:

\[
E_{i,j} = \left[ \left( 2K + \bar{K} \right) \left( H_j - H_{m,i} \right)^2 - \bar{K} \left( D_j^2 - 2D_jD_{m,i} \cos 2\omega + D_{m,i}^2 \right) \right],
\]

(A.4)

where \( H_j = (C_{1,j} + C_{2,j})/2 \) and \( D_j = \left| C_{1,j} - C_{2,j} \right|/2 \) are the mean curvature and the curvature deviator of the membrane (see also Fig. 1), \( H_{m,i} = (C_{1m,i} + C_{2m,i})/2 \) is the intrinsic (spontaneous) mean curvature and \( D_{m,i} = \left| C_{1m,i} - C_{2m,i} \right|/2 \) is the intrinsic (spontaneous)
curvature deviator. The constants $K$ and $\bar{K}$ are proportional to the area of the single membrane constituent [28,29,68]. In the case of a simple flexible membrane nanodomain composed of a rigid core (protein) and the surrounding lipids which are distorted in order to fit with the rigid core, the constants $K$ and $\bar{K}$ were estimated using a microscopic model [68] while in the case lipid molecules they were estimated from the bending constant [21,28]. The optimal values of the membrane mean curvature $H$, the curvature deviator $D$ and the membrane constituent orientation angle $\omega$ corresponding to the minimum of the function $E_{i,j}$ can be calculated from the necessary and sufficient conditions for the extremum of $E_{i,j}$ [29]:

$$H_j = H_{m,j}, \quad D_j = D_{m,j}, \quad \omega = 0, \pi, 2\pi,$$

where $\omega = 0$ and $\omega = 2\pi$ describe the same orientation and where $K > \bar{K} / 2, \quad \bar{K} < 0$.

Since all orientations of anisotropic constituents do not have the same energy (see Eq. (A.4)), the anisotropic membrane constituent exhibits an average orientation:

$$\langle \cos(2\omega) \rangle = \frac{2\pi}{2\pi} \cos(2\omega) \exp \left( -\frac{E_{i,j}(\omega)}{kT} \right) d\omega = \frac{I_1 \left( \frac{-2\bar{K}D_j D_{m,j}}{kT} \right)}{I_0 \left( \frac{2\bar{K}D_j D_{m,j}}{kT} \right)},$$

where $I_0$ and $I_1$ are modified Bessel functions. Here $kT$ is the thermal energy. The value of $\langle \cos(2\omega) \rangle$ increases with increasing $\left| -2\bar{K}D_j D_{m,j} / kT \right|$. The partition function of a single anisotropic membrane constituent is [20,22]:

$$Q_{i,j} = \frac{1}{\omega_o} \int_0^{2\pi} \exp \left( -\frac{E_{i,j}(\omega)}{kT} \right) d\omega.$$

where $\omega_o$ is normalization constant. The free energy of the single anisotropic constituent ($f_{i,j}$) is then obtained by considering that $f_{i,j} = -kT \ln Q_{i,j}$ [28,29]:

$$f_{i,j} = \left( 2K + \bar{K} \right) \left( H_j - H_{m,j} \right)^2 - \bar{K} \left( D_j^2 + D_{m,j}^2 \right) - kT \ln \left( I_0 \left( \frac{2\bar{K}D_j D_{m,j}}{kT} \right) \right),$$

In the limit of small $\left| 2\bar{K}D_j D_{m,j} / kT \right|$, Eq. (A.7) transforms into:
\[ f_{i,j} = \left(2K - \frac{\bar{K}^2D_{m,i}}{kT}\right)(H_j - H_0)^2 + \left(\bar{K} + \frac{\bar{K}^2D_{m,i}}{kT}\right)C_{1,j}C_{2,j}, \quad (A.8) \]

\[ H_0 = \frac{H_{m,i}(2K + \bar{K})}{2K - \frac{\bar{K}^2D_{m,i}}{kT}}, \quad (A.9) \]

where we took into account \( \ln I_0(x) \approx x^2/4 \) for \( x \ll 1 \) and \( D_j^2 = H_j^2 - C_{1,j}C_{2,j} \). The constant term was omitted in Eq. (A.8). For \( |2\bar{K}D_{j,D_{m,i}/kT}| > 1 \), Eq. (A.7) becomes:

\[ f_{i,j} = (2K + \bar{K})(H_j - H_{m,i})^2 - \bar{K}(D_j - D_{m,i})^2, \quad j = \text{in, out}, \quad (A.10) \]

where we took into account that \( \ln I_0(x) \approx |x| \) for \( x > 1 \) and \( \bar{K} < 0 \).