Geometric theory on the elasticity of bio-membranes

Z C Tu†‡* and Z C Ou-Yang†§
†Institute of Theoretical Physics, Chinese Academy of Sciences, P. O. Box 2735
Beijing 100080, China
‡Graduate School, Chinese Academy of Sciences, China
§Center for Advanced Study, Tsinghua University, Beijing 100084, China

Abstract. The purpose of this paper is to study the shapes and stabilities of bio-membranes within the framework of exterior differential forms. After a brief review of the current status in theoretical and experimental studies on the shapes of bio-membranes, a geometric scheme is proposed to discuss the shape equation of closed lipid bilayers, the shape equation and boundary conditions of open lipid bilayers and two-component membranes, the shape equation and in-plane strain equations of cell membranes with cross-linking structures, and the stabilities of closed lipid bilayers and cell membranes. The key point of this scheme is to deal with the variational problems on the surfaces embedded in three-dimensional Euclidean space by using exterior differential forms.

PACS numbers: 87.16.Dg, 02.30.Xx, 02.40.Hw

* Present address: Computational Material Science Center, National Institute for Materials Science, Tsukuba 305-0047, Japan. Email: tu.zhanchun@nims.go.jp
1. Introduction

Cell membranes play crucial role in living movements. They consist of lipids, proteins and carbohydrates etc. There are many simplified models for cell membranes in history [1]. Among them, the widely accepted one is the fluid mosaic model proposed by Singer and Nicolson in 1972 [2]. In this model, a cell membrane is considered as a lipid bilayer where lipid molecules can move freely in the membrane surface like fluid, while proteins are embedded in the lipid bilayer. This model suggests that the shape of the cell membrane is determined by its lipid bilayer. Usually, the thickness of lipid bilayer is about 4 nanometers which is much less than the scale of the cell (about several micrometers). Therefore, we can use a geometrical surface to describe the lipid bilayer.

In 1973, Helfrich [3] proposed the curvature energy per unit area of the bilayer

\[ f_c = \left(\frac{k_c}{2}\right)(2H + c_0)^2 + \bar{k}K, \]

where \(k_c\) and \(\bar{k}\) are elastic constants; and \(H, K, c_0\) are the mean, Gaussian, and spontaneous curvatures of the membrane surface, respectively. We can safely ignore the thermodynamic fluctuation of the curved bilayer at the room temperature because of \(k_c \approx 10^{-19} J \gg k_B T\) [4, 5], where \(k_B\) is the Boltzmann factor and \(T\) the room temperature. Based on Helfrich’s curvature energy, the free energy of the closed bilayer under the osmotic pressure \(p\) (the outer pressure minus the inner one) is written as

\[ \mathcal{F}_H = \int (f_c + \mu)dA + p \int dV, \]

where \(dA\) is the area element, \(\mu\) the surface tension of the bilayer, and \(V\) the volume enclosed within the lipid bilayer. Starting with above free energy, many researchers studied the shapes of bilayers [6, 7]. Especially, by taking the first order variation of the free energy, Ou-Yang et al. derived an equation to describe the equilibrium shape of the bilayer [8]:

\[ p - 2\mu H + k_c(2H + c_0)(2H^2 - c_0H - 2K) + k_c \nabla^2 (2H) = 0. \]

They also obtained that the threshold pressure for instability of spherical bilayer was \(p_c \sim k_c/R^3\), where \(R\) being the radius of spherical bilayer.

Recently, opening-up process of lipid bilayers by talin was observed by Saitoh et al. [9, 10], which arose the interest of studying the shape equation and boundary conditions of lipid bilayers with free exposed edges. Capovilla et al. first gave the shape equation and boundary conditions [11] of open lipid bilayers. They also discussed the mechanical meaning of these equations [11, 12]. In recent paper, we also derived the shape equation and boundary conditions in different way—using exterior differential forms to deal with the variational problems on curved surfaces [13]. It is necessary to further develop this method because we have seen that it is much more concise than the tensor method in recent book [6] by one of the authors.

In fact, the structures of cell membranes are far more complex than the fluid mosaic model. The cross-linking structures exist in cell membranes where filaments of membrane skeleton link to proteins mosaicked in lipid bilayers [14]. It is worth
discussing whether the cross-linking structures have effect on the shapes and stabilities of cell membranes.

In the following contents, both lipid bilayers and cell membranes are called bio-membranes. We will fully develop our geometric method to study the shapes and stabilities of bio-membranes. Our method might not new for mathematicians who are familiar with the work by Griffiths and Bryant et al. [15, 16]. Our method focuses on the application aspect, but the work by Griffiths and Bryant et al. emphasizes on the geometric meaning. Otherwise, we notice a nice review paper by Kamien [17], where he give an introduction to the classic differential geometry in soft materials. Here we will show that exterior differential forms not mentioned by Kamien might also be useful in the study of bio-membranes. This paper is organized as follows: In Sec 2, we briefly introduce the basic concepts in differential geometry and the variational theory of surfaces. In Sec 3, we deal with variational problems on a closed surface, and derive the shape equation of closed lipid bilayers, and then discuss the mechanical stabilities of spherical bilayers. In Sec 4, we deal with variational problems on an open surface, and then derive the shape equation and boundary conditions of open lipid bilayers as well as two-component lipid bilayers. In Sec 5, we derive the free energy of the cross-linking structure by analogy with the theory of rubber elasticity, and regard the free energy of the cell membrane as the sum of the free energy of the closed bilayer and that of cross-linking structure. The shape equation, in-plane strain equations, and mechanical stabilities of cell membranes are discussed by taking the first and second order variations of the total free energy. In Sec 6, we summarize the new results obtained in this paper.

2. Mathematical preliminaries

Here we assume that the readers are familiar with the basic concepts in differential geometry, such as manifold, differential form and Stokes theorem (see also Appendix A).

2.1. Surfaces in three-dimensional Euclidean space, moving frame method

At every point $P$ of a smooth and orientable surface $M$ in three-dimensional Euclidean space $\mathbb{E}^3$, as shown in Fig 1, we can construct an orthogonal system $\mathbf{e}_1, \mathbf{e}_2, \mathbf{e}_3$ such that $\mathbf{e}_3$ is the normal of the surface and $\mathbf{e}_i \cdot \mathbf{e}_j = \delta_{ij}, (i, j = 1, 2, 3)$. We call $\{P; \mathbf{e}_1, \mathbf{e}_2, \mathbf{e}_3\}$ a moving frame. For the point in curve $C$, we let $\mathbf{e}_1$ be its tangent vector and $\mathbf{e}_2$ point to the inner point of $M$. The difference between two frames at point $P$ and $P'$ (which is very close to $P$) is denoted by

$$d\mathbf{r} = \lim_{P \to P'} \frac{\mathbf{r}'}{P'P} = \omega_1 \mathbf{e}_1 + \omega_2 \mathbf{e}_2,$$

(4)

$$d\mathbf{e}_i = \omega_{ij} \mathbf{e}_j \quad (i = 1, 2, 3),$$

(5)

where $\omega_1, \omega_2$ and $\omega_{ij} (i, j = 1, 2, 3)$ are 1-forms.

It is easy to obtain $\omega_{ij} = -\omega_{ji}$ from $\mathbf{e}_i \cdot \mathbf{e}_j = \delta_{ij}$. Additionally, using $dd\mathbf{r} = 0$ and $d\mathbf{d} = 0$, we obtain the structure equations of the surface:

$$d\omega_1 = \omega_{12} \wedge \omega_2;$$

(6)
Geometric theory on the elasticity of bio-membranes

Figure 1. A smooth and orientable surface $M$ with an edge $C$. 

d$\omega_2 = \omega_{21} \wedge \omega_1$;  
$\omega_1 \wedge \omega_{13} + \omega_2 \wedge \omega_{23} = 0$;  
d$\omega_{ij} = \omega_{ik} \wedge \omega_{kj}$ $(i, j = 1, 2, 3)$. 

If we considering the Cartan Lemma, \[\text{eq:8}\] suggests 
$\omega_{13} = a\omega_1 + b\omega_2$ and $\omega_{23} = b\omega_1 + c\omega_2$.  

Thus we have \[\text{eq:18}\]: 
The area element: $dA = \omega_1 \wedge \omega_2$,  
The first fundamental form: $I = d\mathbf{r} \cdot d\mathbf{r} = \omega_1^2 + \omega_2^2$,  
The second fundamental form: $II = -d\mathbf{r} \cdot d\mathbf{e}_3 = a\omega_1^2 + 2b\omega_1\omega_2 + c\omega_2^2$,  
The third fundamental form: $III = d\mathbf{e}_3 \cdot d\mathbf{e}_3 = \omega_{31}^2 + \omega_{32}^2$,  
Mean curvature: $H = (a + c)/2$,  
Gaussian curvature: $K = ac - b^2$.  

2.2. Hodge star $*$ and Gauss mapping

2.2.1. Hodge star *  
Here we just show the basic properties of Hodge star $*$ on surface $M$. So as to its general definition, please see Ref. [19]. 

If $g, h$ are functions defined on 2D smooth surface $M$, then the following formulas are valid: 

\[ * f = f \omega_1 \wedge \omega_2; \]
\[ * \omega_1 = \omega_2, * \omega_2 = -\omega_1; \]
\[ d * df = \nabla^2 f \omega_1 \wedge \omega_2, \]

where $\nabla^2$ is Laplace-Beltrami operator.
It is easy to obtain the second Green identity
\[
\int_M (f d \ast h - h d \ast f) = \int_{\partial M} (f \ast h - h \ast f) \tag{17}
\]
through Stokes theorem and the integration by parts. It follows that
\[
\int_M f d \ast h = \int_M h d \ast f, \tag{18}
\]
if \(M\) is a closed surface.

2.2.2. Gauss mapping

The Gauss mapping \(G : M \to S^2\) is defined as \(G(r) = e_3(r)\), where \(S^2\) is a unit sphere. It induces a linear mapping \(G^* : \Lambda^1 \to \Lambda^1\) such that:

(i) \(G^* \omega_1 = \omega_{13}, \ G^* \omega_2 = \omega_{23}\);

(ii) if \(df = f_1 \omega_1 + f_2 \omega_2\), then \(G^* df = f_1 G^* \omega_1 + f_2 G^* \omega_2\).

Thus we can define a new differential operator \(\tilde{d} = G^* d\). Obviously, if \(df = f_1 \omega_1 + f_2 \omega_2\), then \(\tilde{d}f = f_1 \omega_{13} + f_2 \omega_{23}\). If define a new operator \(\tilde{\ast}\) such that \(\tilde{\omega}_{13} = \omega_{23}\) and \(\tilde{\omega}_{23} = -\omega_{13}\), we have

Lemma 2.1 \[\int_M (f d \tilde{\ast} \tilde{h} - h d \tilde{\ast} \tilde{f}) = \int_{\partial M} (f \tilde{\ast} \tilde{h} - h \tilde{\ast} \tilde{f}) \] for the smooth functions \(f\) and \(h\) on \(M\).

Proof: Using the integration by parts and Stokes theorem, we obtain
\[
\int_M f d \tilde{\ast} \tilde{h} = \int_{\partial M} f \tilde{\ast} \tilde{h} - \int_M df \wedge \tilde{\ast} \tilde{h}, \tag{19}
\]
\[
\int_M h d \tilde{\ast} \tilde{f} = \int_{\partial M} h \tilde{\ast} \tilde{f} - \int_M dh \wedge \tilde{\ast} \tilde{f}. \tag{20}
\]

Otherwise, if let \(df = f_1 \omega_1 + f_2 \omega_2\) and \(dh = h_1 \omega_1 + h_2 \omega_2\), we can prove \(df \wedge \tilde{\ast} \tilde{h} = dh \wedge \tilde{\ast} \tilde{f} = [a f_2 h_2 + c f_1 h_1 - b (f_1 h_2 + f_2 h_1)] \omega_1 \wedge \omega_2\) through a few steps of calculations. Therefore, We can arrive at Lemma 2.1 by (19) minus (20).

It follows that
\[
\int_M f d \tilde{\ast} \tilde{h} = \int_M h d \tilde{\ast} \tilde{f} \tag{21}
\]
for a closed surface.

Because \(d \tilde{\ast} \tilde{f}\) is a 2-form, we can define an operator \(\nabla \cdot \tilde{\nabla}\) such that \(d \tilde{\ast} \tilde{f} = \nabla \cdot \tilde{\nabla} f \omega_1 \wedge \omega_2\).

2.3. Variational theory of surface

If let \(M\) undergoes an infinitesimal deformation such that every point \(r\) of \(M\) has a displacement \(\delta r\), we obtain a new surface \(M' = \{r' | r' = r + \delta r\}\). \(\delta r\) is called the variation of surface \(M\) and expressed as
\[
\delta r = \delta_1 r + \delta_2 r + \delta_3 r, \tag{22}
\]
\[
\delta_i r = \Omega_i e_i \quad (i = 1, 2, 3), \tag{23}
\]
where the repeated subindexes do not represent Einstein summation.
Definition 2.1 If \( f \) is a generalized function of \( r \) (including scalar function, vector function, and \( r \)-form dependent on point \( r \)), define
\[
\delta_i^{(q)} f = \langle q! \rangle \mathcal{L}^{(q)}[f(r + \delta_i r) - f(r)] \quad (i = 1, 2, 3; q = 1, 2, 3, \ldots),
\]
and the \( q \)-order variation of \( f \)
\[
\delta^{(q)} f = \langle q! \rangle \mathcal{L}^{(q)}[f(r + \delta r) - f(r)] \quad (q = 1, 2, 3, \ldots),
\]
where \( \mathcal{L}^{(q)}[\cdots] \) represents the terms of \( \Omega_1^{q_1} \Omega_2^{q_2} \Omega_3^{q_3} \) in Taylor series of \( \cdots \) with \( q_1 + q_2 + q_3 = q \) and \( q_1, q_2, q_3 \) being non-negative integers.

It is easy to prove that:
(i) \( \delta_i^{(q)} \) and \( \delta^{(q)} \) \((i = 1, 2, 3; q = 1, 2, \ldots)\) are linear operators;
(ii) \( \delta_1^{(1)}, \delta_2^{(1)}, \delta_3^{(1)} \) and \( \delta^{(1)} \) are commutative with each other;
(iii) \( \delta_i^{(q+1)} = \delta_i^{(1)} \delta_i^{(q)} \) and \( \delta^{(q+1)} = \delta^{(1)} \delta^{(q)} \), thus we can safely replace \( \delta_i^{(1)}, \delta_i^{(q)}, \delta^{(1)}, \)
and \( \delta^{(q)} \) by \( \delta_1, \delta_2, \delta_3 \) and \( \delta \) \((q = 2, 3, \ldots)\), respectively;
(iv) For functions \( f \) and \( g \), \( \delta_i[f(r) \circ g(r)] = \delta_i f(r) \circ g(r) + f(r) \circ \delta_i g(r) \), where \( \circ \)
represents the ordinary production, vector production or exterior production;
(v) \( \delta_i f[g(r)] = (\partial f/\partial g) \delta_i g \);
(vi) \( \partial \delta = (\delta_1 + \delta_2 + \delta_3)^\partial \), e.g. \( \delta^2 = \delta_1^2 + \delta_2^2 + \delta_3^2 + 2\delta_1 \delta_2 + 2\delta_2 \delta_3 + 2\delta_1 \delta_3 \).

Due to the deformation of \( M \), the vectors \( e_1, e_2, e_3 \) are also changed. Denote their changes
\[
\delta_i e_i = \Omega_{iij} e_j,
\]
Obviously, \( e_i \cdot e_j = \delta_{ij} \) implies \( \Omega_{iij} = -\Omega_{ij} \). Because \( \delta_1, \delta_2 \) and \( \delta_3 \) are linear mappings from \( M \) to \( M' \), they are commutative with exterior differential operator \( d \) \([18]\). Therefore, using \( d\delta_i r = \delta_i dr \) and \( d\delta_i e_j = \delta_i de_j \), we arrive at
\[
\delta_1 \omega_1 = d\Omega_1 - \omega_2 \Omega_{121},
\]
\[
\delta_1 \omega_2 = \Omega_1 \omega_{12} - \omega_1 \Omega_{112},
\]
\[
\Omega_{113} = a\Omega_1, \quad \Omega_{123} = b\Omega_1;
\]
\[
\delta_2 \omega_1 = \Omega_2 \omega_{21} - \omega_2 \Omega_{221},
\]
\[
\delta_2 \omega_2 = d\Omega_2 - \omega_1 \Omega_{212},
\]
\[
\Omega_{213} = b\Omega_2, \quad \Omega_{223} = c\Omega_2;
\]
\[
\delta_3 \omega_1 = \Omega_3 \omega_{31} - \omega_2 \Omega_{321},
\]
\[
\delta_3 \omega_2 = \Omega_3 \omega_{32} - \omega_1 \Omega_{312},
\]
\[
d\Omega_3 = \Omega_{313} \omega_1 + \Omega_{323} \omega_2;
\]
\[
\delta_i \omega_{ij} = d\Omega_{ij} + \Omega_{ik} \omega_{kj} - \omega_{ik} \Omega_{kj}.
\]

Above equations \((24) \sim (36)\) are the fundamental equations in our paper and have not existed in previous mathematical literature \([15]\) and \([16]\). Otherwise, it is easy to deduce that \( \delta_i \tilde{d} f = \tilde{d} \delta_i f \) \((i = 1, 2, 3)\) for function \( f \).
3. Closed lipid bilayers

In this section, we will discuss the equilibrium shapes and mechanical stabilities of closed lipid bilayers. We just consider the closed surface in this section.

3.1. First order variational problems on a closed surface

In this subsection, we will discuss the first order variation of the functional

\[ \mathcal{F} = \int_M \mathcal{E}(2H[r], K[r])dA + p \int_V dV, \]

(37)

where \( H \) and \( K \) are mean and gaussian curvatures at point \( r \) in surface \( M \). \( p \) is a constant and \( V \) be the volume enclosed within the surface.

According to the variational theory of surface in Sec 2, we have \( \delta \mathcal{F} = \delta_1 \mathcal{F} + \delta_2 \mathcal{F} + \delta_3 \mathcal{F} \). Therefore, the next tasks are to calculate \( \delta_1 \mathcal{F}, \delta_2 \mathcal{F} \) and \( \delta_3 \mathcal{F} \), respectively.

3.1.1. Calculation of \( \delta_3 \mathcal{F} \)

Here, we will briefly prove 4 Lemmas and a theorem. Above all, denote

\[ \mathcal{F}_e = \int_M \mathcal{E}(2H[r], K[r])dA. \]  

(38)

**Lemma 3.1** \( \delta_3 dA = -(2H)\Omega_3 dA \).

**Proof:** \( \delta_3 dA = \delta_3(\omega_1 \wedge \omega_2) = \delta_3 \omega_1 \wedge \omega_2 + \omega_1 \wedge \delta_3 \omega_2 \). Considering (11), (12), (33) and (34), we arrive at this Lemma.

**Lemma 3.2** \( \delta_3(2H)dA = 2(2H^2 - K)\Omega_3 dA + d\ast d\Omega_3 \).

**Proof:** \( \delta_3(2H)dA = \delta_3 a\omega_1 \wedge \omega_2 + \delta_3 c\omega_1 \wedge \omega_2 \). Let \( \delta_3 \) acts on (10), we have

\[
\begin{align*}
\delta_3 \omega_{13} &= \delta_3 a\omega_1 + a\delta_3 \omega_1 + \delta_3 b\omega_2 + b\delta_3 \omega_2, \\
\delta_3 \omega_{23} &= \delta_3 b\omega_1 + b\delta_3 \omega_1 + \delta_3 c\omega_2 + c\delta_3 \omega_2.
\end{align*}
\]

If considering (12), (13), (15), (33)∼(36), we arrive at this Lemma.

**Lemma 3.3** \( \delta_3 KdA = 2KH\Omega_3 dA + d\ast d\Omega_3 \).

**Proof:** Theorem Egregium (see Appendix C) implies that \( \delta_3 KdA = \delta_3 d\omega_1 - K\delta_3 dA = -d\delta_3 \omega_1 - K\delta_3 dA \). We will arrive at this Lemma from (36) and Lemma 3.1 as well as the discussions in Sec 2.2.2.

**Theorem 3.1** \( \delta_3 \mathcal{F}_e = \int_M [(\nabla^2 + 4H^2 - 2K)\frac{\partial \mathcal{E}}{\partial (2H)} + (\nabla \cdot \hat{\nabla} + 2KH)\frac{\partial \mathcal{E}}{\partial K} - 2H\mathcal{E}]\Omega_3 dA. \)

**Proof:** Above all, we have

\[
\begin{align*}
\delta_3 \mathcal{F}_e &= \int_M \delta_3 \mathcal{E}dA + \int_M \mathcal{E} \delta_3 A \\
&= \int_M \frac{\partial \mathcal{E}}{\partial (2H)} \delta_3 (2H)dA + \int_M \frac{\partial \mathcal{E}}{\partial K} \delta_3 KdA + \int_M \mathcal{E} \delta_3 dA.
\end{align*}
\]
By using Lemmas 3.1, 3.2, 3.3, we obtain
\[ \delta_3 F_e = \int_M \left[ \left( 4H^2 - 2K \right) \frac{\partial E}{\partial (2H)} + 2KH \frac{\partial E}{\partial K} - 2H E \right] \Omega_3 dA + \int_M \left[ \frac{\partial E}{\partial (2H)} \partial E_3 + \frac{\partial E}{\partial K} \partial E_3 d* d\Omega_3 \right]. \] (39)

For the closed surface \( M \), we arrive at this theorem by considering (18) and (21).

**Lemma 3.4** \( \delta_3 \int_V dV = \int_M \Omega_3 dA \).

**Proof:** Because \( M \) is a closed surface in \( \mathbb{E}^3 \), Stokes theorem (see Appendix A) implies \( \int_V 3dV = \int_V \nabla \cdot r dV = \int_{\partial V} r \cdot n dA \), thus
\[ \delta_3 \int_V dV = \frac{1}{3} \int_M \delta_3 \left[ r \cdot e_3 (\omega_1 \wedge \omega_2) \right] = \frac{1}{3} \int_M \left[ \delta_3 r \cdot e_3 (\omega_1 \wedge \omega_2) + r \cdot \delta_3 e_3 (\omega_1 \wedge \omega_2) + r \cdot e_3 \delta_3 (\omega_1 \wedge \omega_2) \right]. \] (40)

From (33) \( \sim \) (35), we obtain
\[ \delta_3 r \cdot e_3 (\omega_1 \wedge \omega_2) = \Omega_3 \omega_1 \wedge \omega_2, \] (41)
\[ r \cdot e_3 \delta_3 (\omega_1 \wedge \omega_2) = r \cdot e_3 (-2H) \Omega_3 \omega_1 \wedge \omega_2, \] (42)
\[ r \cdot \delta_3 e_3 (\omega_1 \wedge \omega_2) = -d\Omega_3 \wedge (-r \cdot e_2 \omega_1 + r \cdot e_1 \omega_2). \] (43)

By using the integration by parts and Stokes theorem, we have
\[ -\int_M d\Omega_3 \wedge (-r \cdot e_2 \omega_1 + r \cdot e_1 \omega_2) = \int_M \Omega_3 d(-r \cdot e_2 \omega_1 + r \cdot e_1 \omega_2) = \int_M \Omega_3 \left[ 2 + r \cdot e_3 (2H) \right] \omega_1 \wedge \omega_2. \] (44)

Therefore, we will arrive at \( \delta_3 \int_V dV = \int_M \Omega_3 dA \) by using (40) \( \sim \) (44).

**3.1.2. Calculation of \( \delta_1 F \) and \( \delta_2 F \)**

**Theorem 3.2** \( \delta_1 F \equiv 0 \) and \( \delta_2 F \equiv 0 \).

**Proof:** We obtain
\[ db \wedge \omega_1 + 2bd \omega_1 = (a - c)d\omega_2 - dc \wedge \omega_2. \] (45)

from (9) and (10).

Using (27) \( \sim \) (29), (36), and (45), we arrive at
\[ \delta_1 (\omega_1 \wedge \omega_2) = d(\Omega_1 \omega_2), \] (46)
\[ \delta_1 (2H) \omega_1 \wedge \omega_2 = d(2H) \wedge \omega_2 \Omega_1 \] (47)

through a few calculations.

By analogy with the proof of Lemma 3.3, we can prove that
\[ \delta_1 K \omega_1 \wedge \omega_2 = dK \wedge \Omega_1 \omega_2. \] (48)
Therefore, we have
\[ \delta_1 F = \int_M \left[ \frac{\partial E}{\partial (2H)} \delta_1 (2H) \omega_1 \wedge \omega_2 + \frac{\partial E}{\partial K} \delta_1 K \omega_1 \wedge \omega_2 + E \delta_1 (\omega_1 \wedge \omega_2) \right] \]
\[ = \int_M \delta (E \omega_2 \Omega_1). \quad (49) \]

Similarly, we can obtain
\[ \delta_2 F = -\int_M \delta (E \omega_1 \Omega_2). \quad (50) \]

Otherwise, it is not hard to obtain
\[ \delta_1 \int_V dV = \int_M \delta (r \cdot e_3 \omega_2 \Omega_1) \]
and
\[ \delta_2 \int_V dV = -\int_M \delta (r \cdot e_3 \omega_1 \Omega_2). \]
Therefore, \( \delta_1 F = \delta_2 F = 0 \) because \( M \) is a closed surface.

### 3.1.3. Euler-Lagrange equation

Till now, we can obtain
\[ \delta F = \int_M \left[ (\nabla^2 + 4H^2 - 2K) \frac{\partial E}{\partial (2H)} + (\nabla \cdot \tilde{\nabla} + 2KH) \frac{\partial E}{\partial K} - 2HE + p \right] \Omega_3 dA. \quad (51) \]
Thus the Euler-Lagrange equation corresponding to the functional \( F \) is:
\[ \left[ (\nabla^2 + 4H^2 - 2K) \frac{\partial}{\partial (2H)} + (\nabla \cdot \tilde{\nabla} + 2KH) \frac{\partial}{\partial K} - 2H \right] \mathcal{E}(2H, K) + p = 0. \quad (52) \]
The similar equation is first found in Ref. [20].

### 3.2. Second order variation

In this subsection, we discuss the second order variation of functional \( \mathcal{F} \). This problem was also studied by Capovilla and Guven in recent paper [21]. Because \( \delta_1 F = \delta_2 F = 0 \) for closed surface \( M \), we have \( \delta^2 F = \delta \delta_3 F = 0 \), and \( \delta^2 F = \delta \delta_3 F = \delta_3^2 F \).

Form (39) and Lemma 3.4, we obtain
\[ \delta^2 F = \delta_3 \int_M \left[ (4H^2 - 2K) \frac{\partial E}{\partial (2H)} + (2KH) \frac{\partial E}{\partial K} - 2HE + p \right] \Omega_3 dA \]
\[ + \delta_3 \int_M \frac{\partial E}{\partial (2H)} d * d \Omega_3 + \delta_3 \int_M \frac{\partial E}{\partial K} d \tilde{d} \tilde{d} \Omega_3 \]
\[ = \int_M \delta_3 \left[ (4H^2 - 2K) \frac{\partial E}{\partial (2H)} + (2KH) \frac{\partial E}{\partial K} - 2HE + p \right] \Omega_3 dA \]
\[ + \int_M \delta_3 \left[ (4H^2 - 2K) \frac{\partial E}{\partial (2H)} + (2KH) \frac{\partial E}{\partial K} - 2HE + p \right] \Omega_3 dA \]
\[ + \int_M \delta_3 \left[ \frac{\partial E}{\partial (2H)} \right] d * d \Omega_3 + \frac{\partial E}{\partial (2H)} \delta_3 (d * d \Omega_3) \]
\[ + \int_M \delta_3 \left( \frac{\partial E}{\partial K} \right) d \tilde{d} \tilde{d} \Omega_3 + \frac{\partial E}{\partial K} \delta_3 (d \tilde{d} \tilde{d} \Omega_3). \quad (53) \]
Please notice that \( \Omega_3 \) can freely come into and out of the expressions acted by the operator \( \delta_3 \).
**Lemma 3.5** For every function $f$, $\delta_3 d * df = d * \delta_3 f + d(2H\Omega_3 * df) - 2d(\Omega_3 * \tilde{d}f)$.

**Proof:** Let $df = f_1 \omega_1 + f_2 \omega_2$, we have $*df = f_1 \omega_2 - f_2 \omega_1$, $d\tilde{f} = f_1 \omega_{13} + f_2 \omega_{23}$ and $*\tilde{d}f = f_1 \omega_{23} - f_2 \omega_{13}$. By using (33) and (34), we have

$$
\delta_3 * df = (\delta_3 f_1 \omega_2 - \delta_3 f_2 \omega_1) - \Omega_{312} df + \Omega_3 [f_2 (a \omega_1 + b \omega_2) - f_1 (b \omega_1 + c \omega_2)],
$$

$$
*\delta_3 df = (\delta_3 f_1 \omega_2 - \delta_3 f_2 \omega_1) - \Omega_{312} df + \Omega_3 [f_1 (b \omega_1 - a \omega_2) + f_2 (c \omega_1 - b \omega_2)],
$$

$$
\delta_3 * df - *\delta_3 df = 2H\Omega_3 * df - 2\Omega_3 * \tilde{d}f.
$$

Using the operator $d$ to act on both sides of (54) and noticing the commutativity of $d$ and $\delta_3$, we arrive at this Lemma. \(\square\)

**Lemma 3.6** For every function $f$, $\delta_3 \tilde{d} f = d[\delta_3 (2H) * df + 2H\delta_3 * df + 2K \Omega_3 * df - 2H\Omega_3 * \tilde{d}f]$. 

**Proof:** Similar to the proof of Lemma 3.5, we have

$$
\delta_3 * \tilde{d}f = \delta_3 (a f_1 + b f_2) \omega_2 - \delta_3 (b f_1 + c f_2) \omega_1 - K \Omega_3 * df - \Omega_{312} \tilde{d}f,
$$

$$
*\delta_3 \tilde{d}f = \delta_3 (a f_1 + b f_2) \omega_2 - \delta_3 (b f_1 + c f_2) \omega_1 - 2H \Omega_3 * \tilde{d}f + K \Omega_3 * df - \Omega_{312} \tilde{d}f.
$$

The difference of above two equations gives

$$
* \delta_3 \tilde{d}f - \delta_3 * \tilde{d}f = 2K \Omega_3 * df - 2H \Omega_3 * \tilde{d}f.
$$

Otherwise, It is easy to see

$$
* \tilde{d}f + \tilde{d}f = 2H * df.
$$

Using $d\delta_3$ to act on both sides of (56) and $d$ to act on both sides of (55), considering the commutative relations $d\delta_3 = \delta_3 d$ and $d\delta_3 = \delta_3 d$, we arrive at this Lemma. \(\square\)

If $df = f_1 \omega_1 + f_2 \omega_2$, we define $\nabla f = f_1 e_1 + f_2 e_2$, $\tilde{\nabla} f = (a f_1 + b f_2) e_1 + (b f_1 + c f_2) e_2$, $\tilde{\tilde{\nabla}} f = (c f_1 - b f_2) e_1 + (a f_2 - b f_1) e_2$ and $d * \tilde{d}f = (\nabla \cdot \tilde{\nabla}) f \omega_1 \land \omega_2$. It follows that, for function $f$ and $g$,

$$
\tilde{\nabla} f + \tilde{\tilde{\nabla}} f = 2H \nabla f
$$

$$
df \land * dg = (\nabla f \cdot \nabla g) \omega_1 \land \omega_2,
$$

$$
df \land * \tilde{d}g = (\nabla f \cdot \tilde{\nabla} g) \omega_1 \land \omega_2,
$$

$$
df \land \tilde{d}g = (\nabla f \cdot \tilde{\tilde{\nabla}} g) \omega_1 \land \omega_2.
$$

**Remark 3.1** The tensor expressions of $\nabla$, $\tilde{\nabla}$, $\tilde{\tilde{\nabla}}$, $\nabla \cdot \tilde{\nabla}$, $\nabla \cdot \tilde{\tilde{\nabla}}$ are developed in Appendix D.

**Theorem 3.3** The second order variation of functional (37) is

$$
\delta^2 F = \int_M \Omega^2_3 \left[(4H^2 - 2K) \frac{\partial^2 \mathcal{E}}{\partial(2H)^2} - 4KH \frac{\partial \mathcal{E}}{\partial(2H)} - 2K \frac{\partial^2 \mathcal{E}}{\partial K} + 4KH(4H^2 - 2K) \frac{\partial^2 \mathcal{E}}{\partial(2H) \partial K} + 4K^2H^2 \frac{\partial^2 \mathcal{E}}{\partial K^2} + 2KE - 2Hp \right] dA
$$

$$
+ \int_M \Omega_3 \nabla^2 \Omega_3 \left[4H \frac{\partial \mathcal{E}}{\partial(2H)} + 4(2H^2 - K) \frac{\partial^2 \mathcal{E}}{\partial(2H)^2} + K \frac{\partial \mathcal{E}}{\partial K} \right] dA
$$
Geometric theory on the elasticity of bio-membranes

\[ + 4HK \frac{\partial^2 \mathcal{E}}{\partial K \partial (2H)} - \mathcal{E} + 8H^2 \frac{\partial \mathcal{E}}{\partial K} \] \, dA

\[ + \int_M \Omega_3 \nabla \cdot \tilde{\nabla} \Omega_3 \left[ 4(2H^2 - K) \frac{\partial^2 \mathcal{E}}{\partial (2H)^2} - 4 \frac{\partial \mathcal{E}}{\partial (2H)} \right] \, dA \]

\[ + 4HK \frac{\partial^2 \mathcal{E}}{\partial K^2} - 4H \frac{\partial \mathcal{E}}{\partial K} \] \, dA

\[ + \int_M (\nabla^2 \Omega_3)^2 \left[ \frac{\partial^2 \mathcal{E}}{\partial (2H)^2} + \frac{\partial \mathcal{E}}{\partial (2H)} \right] \, dA \]

\[ + \int_M \left[ \frac{2\partial^2 \mathcal{E}}{\partial (2H)^2} \nabla^2 \Omega_3 \nabla \cdot \tilde{\nabla} \Omega_3 + \frac{\partial \mathcal{E}}{\partial (2H)} \nabla (2H \Omega_3) \cdot \nabla \Omega_3 \right] \, dA \]

\[ + \int_M \left[ \frac{\partial^2 \mathcal{E}}{\partial K^2} (\nabla \cdot \tilde{\nabla} \Omega_3)^2 - \frac{2\partial \mathcal{E}}{\partial (2H)} \nabla \Omega_3 \cdot \tilde{\nabla} \Omega_3 \, dA \right] \]

\[ + \int_M \frac{\partial \mathcal{E}}{\partial K} \left[ \nabla (8H^2 \Omega_3 + \nabla^2 \Omega_3) \cdot \nabla \Omega_3 - \nabla (4H \Omega_3) \cdot \tilde{\nabla} \Omega_3 - 4H \Omega_3 \cdot \tilde{\nabla} \Omega_3 ight. \]

\[ \left. - \nabla (2H \Omega_3) \cdot \tilde{\nabla} \Omega_3 - 2H \Omega_3 \cdot \tilde{\nabla} \Omega_3 \right] \, dA. \]

**Proof:** Replacing \( f \) by \( \Omega_3 \) in Lemmas 3.5 and 3.6 and noticing that \( \Omega_3 \) is similar to a constant relative to \( \delta_3 \), we have

\[ \delta_3 d * d \Omega_3 = [\nabla (2H \Omega_3) \cdot \nabla \Omega_3 + 2H \Omega_3 \nabla^2 \Omega_3 - 2 \nabla \Omega_3 \cdot \tilde{\nabla} \Omega_3 - 2 \Omega_3 \nabla \cdot \tilde{\nabla} \Omega_3] \, dA, \]

\[ \delta_3 d * \tilde{d} \Omega_3 = [\nabla (8H^2 \Omega_3 + \nabla^2 \Omega_3) \cdot \nabla \Omega_3 + (8H^2 \Omega_3 + \nabla^2 \Omega_3) \nabla^2 \Omega_3 
abla \Omega_3 - \nabla (4H \Omega_3) \cdot \tilde{\nabla} \Omega_3 - 4H \Omega_3 \cdot \tilde{\nabla} \Omega_3 - 2H \Omega_3 \nabla \cdot \tilde{\nabla} \Omega_3] \, dA. \]

Substituting them into (61) and using Lemmas 3.2 and 3.3, we can arrive at this theorem through expatiantory calculations. \( \square \)

In particular, if \( \partial \mathcal{E} / \partial K = \tilde{k} \) being a constant, (63) is simplified to

\[ \delta^2 \mathcal{F} = \int_M \Omega_3^2 \left[ (4H^2 - 2K)^2 \frac{\partial^2 \mathcal{E}_H}{\partial (2H)^2} - 4HK \frac{\partial \mathcal{E}_H}{\partial (2H)} + 2K \mathcal{E}_H - 2Hp \right] \, dA \]

\[ + \int_M \Omega_3 \nabla \Omega_3 \left[ 4H \frac{\partial \mathcal{E}_H}{\partial (2H)} + 4(2H^2 - K) \frac{\partial^2 \mathcal{E}_H}{\partial (2H)^2} - \mathcal{E}_H \right] \, dA \]

\[ - \int_M \frac{4\partial \mathcal{E}_H}{\partial (2H)} \Omega_3 \nabla \cdot \tilde{\nabla} \Omega_3 \, dA + \int_M \frac{\partial^2 \mathcal{E}_H}{\partial (2H)^2} (\nabla^2 \Omega_3)^2 \, dA \]

\[ + \int_M \frac{\partial \mathcal{E}_H}{\partial (2H)} \left[ \nabla (2H \Omega_3) \cdot \nabla \Omega_3 - 2 \nabla \Omega_3 \cdot \tilde{\nabla} \Omega_3 \right] \, dA, \tag{61} \]

where \( \mathcal{E}_H = \mathcal{E} - \tilde{k}K \).

### 3.3. Shape equation of closed lipid bilayers

Now, Let us turn to the shape equation of closed lipid bilayers. We take the free energy of closed lipid bilayer under the osmotic pressure as \( [2] \). Substituting \( \mathcal{E} = (k_c/2)(2H + c_0)^2 + \tilde{k}K + \mu \) into (52), we obtain the shape equation (3). This equation is the fourth order nonlinear equation. It is not easy to obtain its special solutions. We
will give three typical analytical solutions as follows. Some new important results on it can be found in recent paper by Landolfi [22].

3.3.1. Constant mean curvature surface From 1956 to 1958, Alexandrov proved an unexpected theorem: an embedded surface (i.e. the surface does not intersect with itself) with constant mean curvature in $\mathbb{R}^3$ must be a spherical surface [23]. Thus the closed bilayer with constant mean curvature must be a sphere. For a sphere with radius $R$, we have $H = -1/R$ and $K = 1/R^2$. Substituting them into (3), we arrive at

$$pR^2 + 2\mu R - k_c c_0 (2 - c_0 R) = 0. \tag{62}$$

This equation gives the spherical radius under the osmotic pressure $p$.

3.3.2. Biconcave discoid shape and $\sqrt{2}$ torus It is instructive to find some axisymmetrical solutions to the shape equation [3]. To do that, we denote $r = \{u \cos v, u \sin v, z\}$, $\psi = \arctan[u \frac{dz}{du}]$, and $\Psi = \sin \psi$. Thus (3) is transformed into

$$\left(\Psi^2 - 1\right) \frac{d^3 \Psi}{du^3} + \Psi \frac{d^2 \Psi}{du^2} - \frac{1}{2} \left(\frac{d\Psi}{du}\right)^3 - \frac{p}{k_c}$$

$$+ \frac{2(\Psi^2 - 1)}{u} \frac{d^2 \Psi}{du^2} + \frac{3\Psi}{2u} \left(\frac{d\Psi}{du}\right)^2 + \left(\frac{c_0^2}{2} + \frac{2c_0 \Psi}{u} + \frac{\mu}{k_c} - \frac{3\Psi^2 - 2}{2u^2}\right) \frac{d\Psi}{du}$$

$$+ \left(\frac{c_0^2}{2} + \frac{\mu}{k_c} - \frac{1}{u^2}\right) \frac{\Psi}{u} + \frac{\Psi^3}{2u^3} = 0. \tag{63}$$

To find the solution of (63) that satisfies $\Psi = 0$ when $u = 0$, we consider the asymptotic form of (63) at $u = 0$:

$$\frac{d^3 \Psi}{du^3} + \frac{2}{u} \frac{d^2 \Psi}{du^2} - \frac{1}{u^2} \frac{d\Psi}{du} + \frac{\Psi}{u^3} = 0. \tag{64}$$

Please notice that there are two misprints in our previous paper [13]. Above equation is the Euler differential equation and has the general solution $\Psi = \alpha_1/u + \alpha_2 u + \alpha_3 u \ln u$ with three integral constants $\alpha_1 = 0, \alpha_2$, and $\alpha_3$. The asymptotic solution hints that $\Psi = -c_0 u \ln(u/u_B)$ might be a solution to (63) which requires $p = \mu = 0$. When $0 < c_0 u_B < e$, $\Psi = -c_0 u \ln(u/u_B)$ corresponds to the biconcave discoid shape [6, 24]. Otherwise, when $\mu/k_c = -2\alpha_0 - c_0^2/2$ and $p/k_c = -2\alpha_0^2 c_0$, $\Psi = \alpha u + \sqrt{2}$ satisfies (63). This solution corresponds to a torus with the ratio of its two radii being exactly $\sqrt{2}$ if $\alpha < 0$ [6, 25].

3.4. Mechanical stability of spherical bilayers

A spherical bilayer can be described by $r = R(\sin \theta \cos \phi, \sin \theta \sin \phi, \cos \phi)$ with $R$ satisfying (62). We have $H = -1/R$, $K = 1/R^2$, $\tilde{\nabla} = -(1/R)\nabla$, $\nabla \cdot \tilde{\nabla} = -(1/R)\nabla^2$ and $\nabla^2 = \frac{1}{R^2 \sin^2 \theta} \left(\frac{\partial}{\partial \phi}\right)^2 + \frac{1}{R^2 \sin^2 \theta} \left(\frac{\partial^2}{\partial \theta^2}\right)$. If we take $E_{sh} = (k_c/2)(2H + c_0)^2 + \mu$, (61) is transformed into

$$\delta^2 \mathcal{F} = (2c_0 k_c/R + pR) \int_0^\pi \sin \theta d\theta \int_0^{2\pi} d\phi \Omega_3^2$$
Geometric theory on the elasticity of bio-membranes

\[ + (k_c c_0 R + 2k_c + p R^3 / 2) \int_0^\pi \sin \theta d\theta \int_0^{2\pi} d\phi \Omega_3 \nabla^2 \Omega_3 \]

\[ + k_c R^2 \int_0^\pi \sin \theta d\theta \int_0^{2\pi} d\phi (\nabla^2 \Omega_3)^2. \]  (65)

Expand \( \Omega_3 \) with the spherical harmonic functions [26]:

\[ \Omega_3 = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} a_{lm} Y_{lm}(\theta, \phi), \quad a_{lm}^* = (-1)^m a_{l,-m}. \]  (66)

If considering \( \nabla^2 Y_{lm} = -l(l+1)Y_{lm}/R^2 \) and \( \int_0^\pi \sin \theta d\theta \int_0^{2\pi} d\phi Y_{lm} Y_{lm'}^* = \delta_{mm'} \delta_{ll'} \), we transform (65) into

\[ \delta^2 F = (R/2) \sum_{l,m} |a_{lm}|^2 [l(l+1) - 2] \{2k_c/R^3[l(l+1) - c_0 R] - p \}. \]  (67)

Denote that

\[ p_l = (2k_c/R^3)[l(l+1) - c_0 R] \quad (l = 2, 3, \cdots). \]  (68)

When \( p > p_l \), \( \delta^2 F \) can take negative value. Therefore, we can take the critical pressure as

\[ p_c = \min \{p_l\} = p_2 = (2k_c/R^3)(6 - c_0 R). \]  (69)

In this case, the spherical bilayer will be inclined to transform into the biconcave discoid shape.

4. Open lipid bilayers

In this section, we will deal with the variational problems on surface \( M \) with edge \( C \) as shown in Fig.1 and discuss the shape equation and boundary conditions of open lipid bilayers with free edges.

4.1. First order variational problems on an open surface

In this subsection, we will discuss the first order variation of the functional

\[ F = \int_M \mathcal{E}(2H[r], K[r]) dA + \int_C \Gamma(k_n, k_g) ds. \]  (70)

Denote \( F_e = \int_M \mathcal{E}(2H[r], K[r]) dA \) and \( F_C = \int_C \Gamma(k_n, k_g) ds \).

In terms of Appendix B we have \( \omega_2 = 0, \omega_1 = a, k_n = a, k_g ds = \omega_1, \tau_g = b \) in curve \( C \). Using (27)~(36), we can arrive at

\[ \delta_1 F_C = \int_C d(\Gamma \Omega_1) = 0, \]  (71)

\[ \delta_2 F_C = \int_C \left[ \frac{d^2}{ds^2} \left( \frac{\partial \Gamma}{\partial k_g} \right) + K \frac{\partial \Gamma}{\partial k_g} - k_g \left( \Gamma - \frac{\partial \Gamma}{\partial k_g} k_g \right) \right. \]

\[ + 2(k_n - H)k_g \frac{\partial \Gamma}{\partial k_n} - \tau_g \frac{d}{ds} \left( \frac{\partial \Gamma}{\partial k_n} \right) - \frac{d}{ds} \left( \tau_g \frac{\partial \Gamma}{\partial k_n} \right) \]

\[ \left. \Omega_2 ds \right] \]  (72)
\[
\delta_3 \mathcal{F}_C = \int_C \left[ \frac{d^2}{ds^2} \left( \frac{\partial \Gamma}{\partial k_n} \right) + \frac{\partial \Gamma}{\partial k_n} (k_n^2 - \tau_g^2) + \tau_g \frac{d}{ds} \left( \frac{\partial \Gamma}{\partial k_g} \right) \\
+ \frac{d}{ds} \left( \tau_g \frac{\partial \Gamma}{\partial k_g} \right) - \left( \Gamma - \frac{\partial \Gamma}{\partial k_g} k_g \right) \right] \Omega_3 ds \\
+ \int_C \left( \frac{\partial \Gamma}{\partial k_g} k_n - \frac{\partial \Gamma}{\partial k_n} k_n \right) \Omega_{323} ds.
\] (73)

In particular, (39), (49) and (50) are still applicable. Consequently,
\[
\delta_1 \mathcal{F}_e = \int_M d(\mathcal{E} \omega_2 \Omega_1) = \int_C \mathcal{E} \omega_2 \Omega_1 = 0,
\] (74)
\[
\delta_2 \mathcal{F}_e = -\int_M d(\mathcal{E} \omega_1 \Omega_2) = -\int_C \mathcal{E} \Omega_2 ds,
\] (75)
\[
\delta_3 \mathcal{F}_e = \int_M \left[ (\nabla^2 + 4H^2 - 2K) \frac{\partial \mathcal{E}}{\partial (2H)} + (\nabla \cdot \tilde{\nabla} + 2KH) \frac{\partial \mathcal{E}}{\partial K} - 2H \mathcal{E} \right] \Omega_3 dA \\
+ \int_C \left[ \mathbf{e}_2 \cdot \nabla \left( \frac{\partial \mathcal{E}}{\partial (2H)} \right) + \mathbf{e}_2 \cdot \tilde{\nabla} \left( \frac{\partial \mathcal{E}}{\partial K} \right) - \frac{d}{ds} \left( \frac{\partial \mathcal{E}}{\partial K} \right) \right] \Omega_3 ds \\
+ \int_C \left[ -\frac{\partial \mathcal{E}}{\partial (2H)} - k_n \frac{\partial \mathcal{E}}{\partial K} \right] \Omega_{323} ds.
\] (76)

The functions \( \Omega_{323}, \Omega_2, \) and \( \Omega_3 \) can be regarded as virtual displacements. Thus \( \delta \mathcal{F} = (\delta_1 + \delta_2 + \delta_3)(\mathcal{F}_e + \mathcal{F}_C) = 0 \) gives
\[
(\nabla^2 + 4H^2 - 2K) \frac{\partial \mathcal{E}}{\partial (2H)} + (\nabla \cdot \tilde{\nabla} + 2KH) \frac{\partial \mathcal{E}}{\partial K} - 2H \mathcal{E} = 0,
\] (77)
\[
\mathbf{e}_2 \cdot \nabla \left( \frac{\partial \mathcal{E}}{\partial (2H)} \right) + \mathbf{e}_2 \cdot \tilde{\nabla} \left( \frac{\partial \mathcal{E}}{\partial K} \right) - \frac{d}{ds} \left( \frac{\partial \mathcal{E}}{\partial K} \right) + \frac{d^2}{ds^2} \left( \frac{\partial \Gamma}{\partial k_n} \right) + \frac{\partial \Gamma}{\partial k_n} (k_n^2 - \tau_g^2) \\
+ \tau_g \frac{d}{ds} \left( \frac{\partial \Gamma}{\partial k_g} \right) + \frac{d}{ds} \left( \tau_g \frac{\partial \Gamma}{\partial k_g} \right) - \left( \Gamma - \frac{\partial \Gamma}{\partial k_g} k_g \right) \middle|_C = 0,
\] (78)
\[
- \frac{\partial \mathcal{E}}{\partial (2H)} - k_n \frac{\partial \mathcal{E}}{\partial K} + \frac{\partial \Gamma}{\partial k_n} k_n - \frac{\partial \Gamma}{\partial k_n} k_n \middle|_C = 0,
\] (79)
\[
\frac{d^2}{ds^2} \left( \frac{\partial \Gamma}{\partial k_g} \right) + K \frac{\partial \Gamma}{\partial k_g} - k_g \left( \Gamma - \frac{\partial \Gamma}{\partial k_g} k_g \right) + 2(k_n - H) k_g \frac{\partial \Gamma}{\partial k_n} \\
- \tau_g \frac{d}{ds} \left( \frac{\partial \Gamma}{\partial k_n} \right) - \frac{d}{ds} \left( \tau_g \frac{\partial \Gamma}{\partial k_n} \right) - \mathcal{E} \middle|_C = 0.
\] (80)

Among above equations, (77) determines the shape of the surface \( M \), and (78)~(80) determine the position of curve \( C \) in the surface \( M \).

4.2. Shape equation and boundary conditions of open lipid bilayers

In order to obtain the shape equation and boundary conditions of an open lipid bilayer with an edge \( C \), we take \( \mathcal{E} = (k_c/2)(2H + c_0)^2 + \bar{k}K + \mu \) and \( \Gamma = \frac{1}{2}k_b(k_n^2 + k_g^2) + \gamma \) with
where $k_b$ and $\gamma$ being constants. In this case, (77)–(80) are transformed into

$$ k_c (2H + c_0)(2H^2 - c_0H - 2K) + k_c \nabla^2 (2H) - 2\mu H = 0, \quad (81) $$

$$ k_b [d^2 k_n/ds^2 + k_n(\kappa^2/2 + \tau_g^2) + \tau_g dk_n/ds + d(\tau_g k_n)/ds] $$

$$ + k_c e_2 \cdot \nabla (2H) - \bar{k} d\tau_g/ds - \gamma k_n \big|_C = 0, \quad (82) $$

$$ k_c (2H + c_0) + \bar{k} k_n \big|_C = 0, \quad (83) $$

$$ k_b [d^2 k_g/ds^2 + k_g(\kappa^2/2 + \tau_g^2) - \tau_g dk_n/ds - d(\tau_g k_n)/ds] $$

$$ - [(k_c/2)(2H + c_0)^2 + \bar{k} K + \mu + \gamma k_g] \big|_C = 0, \quad (84) $$

where $\kappa^2 = k_n^2 + k_g^2$.

In fact, the above four equations express the force and moment equilibrium equations of the surface and the edge: (81) represents the force equilibrium equation of point in the surface $M$ along $e_3$ direction; (82) represents the force equilibrium equation of point in the curve $C$ along $e_3$ direction; (83) represents the bending moment equilibrium equation of point in the curve $C$ around $e_1$ direction; (84) represents the force equilibrium equation of point in the curve $C$ along $e_2$ direction.

If $k_b = 0$, (81) and (83) remain unchanged, but (82) and (84) are simplified to

$$ k_c e_2 \cdot \nabla (2H) - \bar{k} d\tau_g/ds - \gamma k_n \big|_C = 0, \quad (85) $$

$$ (k_c/2)(2H + c_0)^2 + \bar{k} K + \mu + \gamma k_g \big|_C = 0. \quad (86) $$

### 4.3. Two-component lipid bilayer

In this subsection, we study a closed bilayer consists of two domains containing different kinds of lipid. This problem in axisymmetrical case was theoretically discussed by Jülicher and Lipowsky [27]. The shapes of two-component bilayers also were observed in recent experiment [28].

We assume that the boundary between two domains is a smooth curve and the bilayer is still a smooth surface. The free energy is written as

$$ \mathcal{F} = p \int_V dV + \int_{M_I} [(k^I_c/2)(2H + c^I_0)^2 + \bar{k}^I K + \mu^I] dA $$

$$ + \int_{M_{II}} [(k_{II}^I/2)(2H + c_{II}^I)^2 + \bar{k}^{II} K + \mu^{II}] dA + \gamma \int_C ds. \quad (87) $$

In terms of the discussions on closed bilayers in section 3 and above discussions in this section, we can promptly write the shape equations of the two-component bilayer without any symmetrical assumption as

$$ p - 2\mu^I H + k^I_c (2H + c_0)(2H^2 - c_0H - 2K) + k^I_c \nabla^2 (2H) = 0, \quad (88) $$

where the superscripts $i = I$ and $II$ represent the two lipid domains, respectively. And the boundary conditions are as follows:

$$ k^I_c e_2 \cdot \nabla (2H) - \bar{k}^I d\tau_g/ds + k^I e_2 \cdot \nabla (2H) - \bar{k}^{II} d\tau_g/ds - \gamma k_n \big|_C = 0, \quad (89) $$

$$ k^I_c (2H + c^I_0) + \bar{k}^I k_n - [k^I_c (2H + c^I_0) + \bar{k}^{II} k_n] \big|_C = 0, \quad (90) $$

$$ (k^I_c/2)(2H + c^I_0)^2 + \bar{k}^I K + \mu^I $$
\[-\left[\left(k'^I_I\right)/2\right](2H + c'^I_K) + k'^I_K \frac{c_I}{2} + \gamma k^I_I C = 0.\] (91)

In above equations, the positive direction of curve \(C\) is set to along \(e_1\) of the lipid domain consisting of component \(I\). Furthermore, \((88) \sim (91)\) are also applied to describe the closed bilayer with more than two domains. But the boundary conditions are not applied to the bilayer with a sharp angle across the boundary between domains.

5. Cell membranes with cross-linking structures

Cell membranes contain cross-linking protein structures. As is well known, rubber also consists of cross-linking polymer structures [29]. In this section, we first drive the free energy of cell membrane with cross-linking protein structure by analogy with the rubber elasticity. Secondly, we derive the shape equation and in-plane stain equations of cell membrane by taking the first order variation of the free energy. Lastly, we discuss the mechanical stability of spherical cell membrane.

5.1. The free energy of cell membrane

Above all, we discuss the free energy change of a Gaussian chain in a small strain field

\[
\epsilon = \begin{pmatrix} \varepsilon_{xx} & \varepsilon_{xy} & 0 \\ \varepsilon_{xy} & \varepsilon_{yy} & 0 \\ 0 & 0 & \varepsilon_{zz} \end{pmatrix}
\] (92)

with \(\varepsilon_{zz} = -(\varepsilon_{xx} + \varepsilon_{yy})\) expressed in an orthogonal coordinate system \(Oxyz\).

Assume that one end of the chain is fixed at origin \(O\) while another is denoted by \(R_N\) before undergoing the strains, where \(N\) is the number of the segments of the chain. The partition function of the chain can be calculated by path integrals [30]:

\[
Z = \int_{R_0}^{R_N} D[R_n] \exp \left[ -\frac{3}{2L^2} \int_0^N dn \left( \frac{\partial R_n}{\partial n} \right)^2 \right] = \sigma \exp \left[ -\frac{3(R_N - R_0)^2}{2NL^2} \right],
\]

where \(\sigma\) is a constant, and \(L\) is the segment length. After undergoing the strains, the partition function is changed to

\[
Z_\epsilon = \sigma \exp \left[ -\frac{3(R_N - R_0)^2}{2NL^2} \right].
\]

Considering the relation \((R_N - R_0)^2 = [(1 + \varepsilon) \cdot (R_N - R_0)]^2\) and the distribution function of end-to-end distance \(P(R_N - R_0) = \frac{Z}{\int_{R_N} dR_N Z}\), we can obtain the free energy change as a result of the strains:

\[
f_s = -k_BT \int dR_N (\ln Z_\epsilon - \ln Z) P(R_N - R_0)
= k_BT \left[ (\varepsilon_{xx} + \varepsilon_{yy})^2 - (\varepsilon_{xx} \varepsilon_{yy} - \varepsilon_{xy}^2) \right].\] (93)
the thickness of the membrane. (ii) There is no change of volume occupied by the cross-linking structure on deformation. On average, there are $N$ protein chains per volume. (iii) The protein chain can be regarded as a Gaussian chain with the mean end-to-end distance much smaller than the dimension of the cell membrane. (iv) The junction points move on deformation as if they were embedded in an elastic continuum (Affine deformation assumption). (v) The free energy of the cell membrane on deformation is the sum of the free energies of closed lipid bilayer and the cross-linking structure. The free energy of cross-linking structure is the sum of the free energies of individual protein chains.

If the cell membrane undergoing the small in-plane deformation $\begin{pmatrix} \varepsilon_{11} & \varepsilon_{12} \\ \varepsilon_{12} & \varepsilon_{22} \end{pmatrix}$, where “1” and “2” represent two orthogonal directions of the membrane surface, we can obtain the free energy of a protein chain on deformation with the similar form of (93) under above assumptions (i)~(iv). Using above assumption (v), we have the free energy of the cell membrane under the osmotic pressure $p$:

$$ \mathcal{F} = \int_M (\mathcal{E}_d + \mathcal{E}_H)dA + p \int_V dV, $$

where $\mathcal{E}_H = (k_c/2)(2H + c_0)^2 + \mu$ and $\mathcal{E}_d = (k_d/2)((2J)^2 - Q$ with $k_d = 2N h k_B T$, $2J = \varepsilon_{11} + \varepsilon_{22}$, $Q = \varepsilon_{11}\varepsilon_{22} - \varepsilon_{12}^2$.

**Remark 5.1** We do not write the term of $\bar{k}K$ in (94) because $\int_M \bar{k}KdA$ is a constant for closed surface (see Appendix C).

5.2. Strain analysis

If a point $\begin{pmatrix} r_0 \end{pmatrix}$ in a surface undergoing a displacement $\begin{pmatrix} u \end{pmatrix}$ to arrive at point $\begin{pmatrix} r \end{pmatrix}$, we have $\begin{pmatrix} du \end{pmatrix} = \begin{pmatrix} dr \end{pmatrix} - \begin{pmatrix} dr_0 \end{pmatrix}$ and $\delta_i d\begin{pmatrix} u \end{pmatrix} = \delta_i \begin{pmatrix} dr \end{pmatrix}$ ($i = 1, 2, 3$).

If denote $\begin{pmatrix} dr \end{pmatrix} = \omega_1 \begin{pmatrix} e_1 \end{pmatrix} + \omega_2 \begin{pmatrix} e_2 \end{pmatrix}$ and $\begin{pmatrix} du \end{pmatrix} = U_1 \omega_1 + U_2 \omega_2$ with $|U_1| \ll 1, |U_2| \ll 1$, we can define the strains [31]:

$$ \varepsilon_{11} = \left[ \frac{d\begin{pmatrix} u \end{pmatrix} \cdot \begin{pmatrix} e_1 \end{pmatrix}}{|d\begin{pmatrix} r \end{pmatrix}|} \right]_{\omega_2 = 0} \approx U_1 \cdot \begin{pmatrix} e_1 \end{pmatrix}, $$

(95)

$$ \varepsilon_{22} = \left[ \frac{d\begin{pmatrix} u \end{pmatrix} \cdot \begin{pmatrix} e_2 \end{pmatrix}}{|d\begin{pmatrix} r \end{pmatrix}|} \right]_{\omega_1 = 0} \approx U_2 \cdot \begin{pmatrix} e_2 \end{pmatrix}, $$

(96)

$$ \varepsilon_{12} = \frac{1}{2} \left[ \left( \frac{d\begin{pmatrix} u \end{pmatrix} \cdot \begin{pmatrix} e_2 \end{pmatrix}}{|d\begin{pmatrix} r \end{pmatrix}|} \right)_{\omega_2 = 0} + \left( \frac{d\begin{pmatrix} u \end{pmatrix} \cdot \begin{pmatrix} e_1 \end{pmatrix}}{|d\begin{pmatrix} r \end{pmatrix}|} \right)_{\omega_1 = 0} \right] \approx \frac{1}{2} (U_1 \cdot \begin{pmatrix} e_2 \end{pmatrix} + U_2 \cdot \begin{pmatrix} e_1 \end{pmatrix}). $$

(97)

Using $\delta_i d\begin{pmatrix} u \end{pmatrix} = \delta_i d\begin{pmatrix} r \end{pmatrix}$ and the definitions of strains (95)~(97), we can obtain the variational relations:

$$ \delta_i \varepsilon_{11} \omega_1 \wedge \omega_2 = (1 - \varepsilon_{11}) \delta_i \omega_1 \wedge \omega_2 - U_2 \cdot \begin{pmatrix} e_1 \end{pmatrix} \delta_i \omega_2 \wedge \omega_2 + \Omega_{112} U_1 \cdot \begin{pmatrix} e_2 \end{pmatrix} \wedge \omega_2 + \Omega_{113} U_1 \cdot \begin{pmatrix} e_3 \end{pmatrix} \wedge \omega_2, $$

$$ \delta_i \varepsilon_{12} \omega_1 \wedge \omega_2 = \frac{1}{2} (1 - \varepsilon_{11}) \omega_1 \wedge \delta_i \omega_1 + (1 - \varepsilon_{22}) \delta_i \omega_2 \wedge \omega_2 - U_2 \cdot \begin{pmatrix} e_1 \end{pmatrix} \omega_1 \wedge \delta_i \omega_2 $$
Geometric theory on the elasticity of bio-membranes

5.3. Shape equation and in-plane strain equations of cell membranes

Thus,

\[ \delta_i \varepsilon_{22} \omega_1 \wedge \omega_2 = (1 - \varepsilon_{22}) \omega_1 \wedge \delta_i \omega_2 - U_1 \cdot e_2 \omega_1 \wedge \delta_i \omega_1 \]
\[ + \Omega_{21} U_2 \cdot e_1 \omega_1 \wedge \omega_2 + \Omega_{23} U_2 \cdot e_3 \omega_1 \wedge \omega_2. \]

The leading terms of above relations are:

\[ \delta_i \varepsilon_{11} \omega_1 \wedge \omega_2 = \delta_i \omega_1 \wedge \omega_2, \quad \delta_i \varepsilon_{12} \omega_1 \wedge \omega_2 = \frac{1}{2} [\omega_1 \wedge \delta_i \omega_1 + \delta_i \omega_2 \wedge \omega_2], \]
\[ \delta_i \varepsilon_{22} \omega_1 \wedge \omega_2 = \omega_1 \wedge \delta_i \omega_2. \]

Thus,

\[ \delta_i (2J) \omega_1 \wedge \omega_2 = \delta_i (\varepsilon_{11} + \varepsilon_{22}) \omega_1 \wedge \omega_2 = \delta_i \varepsilon_{22} \omega_1 \wedge \omega_2 = \delta_i \omega_1 \wedge \omega_2 + \omega_1 \wedge \delta_i \omega_2, \]
\[ \delta_i Q \omega_1 \wedge \omega_2 = \delta_i (\varepsilon_{11} \varepsilon_{22} - \varepsilon_{12}^2) \omega_1 \wedge \omega_2 \]
\[ = (\varepsilon_{11} \omega_1 + \varepsilon_{12} \omega_2) \wedge \delta_i \omega_2 - (\varepsilon_{12} \omega_1 + \varepsilon_{22} \omega_2) \wedge \delta_i \omega_1. \]

Considering equations \[27\] ~ \[35\], \[101\] and \[102\], we have

\[ \delta_1 (2J) \omega_1 \wedge \omega_2 = d(\Omega_1 \omega_2), \]
\[ \delta_1 Q \omega_1 \wedge \omega_2 = (\varepsilon_{11} \omega_1 - \varepsilon_{12} \omega_1) \Omega_1 - (\varepsilon_{12} \omega_1 + \varepsilon_{22} \omega_2) \wedge d\Omega_1; \]
\[ \delta_2 (2J) \omega_1 \wedge \omega_2 = -d(\Omega_2 \omega_1), \]
\[ \delta_2 Q \omega_1 \wedge \omega_2 = (\varepsilon_{11} \omega_1 + \varepsilon_{12} \omega_2) \wedge d\Omega_2 + \Omega_2 (\varepsilon_{12} \omega_2 - \varepsilon_{22} \omega_1); \]
\[ \delta_3 (2J) \omega_1 \wedge \omega_2 = -2H \Omega_3 dA, \]
\[ \delta_3 Q \omega_1 \wedge \omega_2 = [-2H (2J) + a \varepsilon_{11} + 2b \varepsilon_{12} + c \varepsilon_{22}] \Omega_3 dA. \]

5.3. Shape equation and in-plane strain equations of cell membranes

To obtain the shape equation and in-plane strain equations of cell membranes, we must take the first order variation of the functional \[21\]. Denote \( F_d = \int_M E_d dA \) and \( F_{cp} = \int_M E_{cp} dA + p \int_V dV \).

From \[103\] ~ \[108\], we can calculate that:

\[ \delta_1 F_d = \int_M \left[ \frac{\partial E_d}{\partial (2J)} \delta_1 (2J) dA + \frac{\partial E_d}{\partial Q} \delta_1 Q dA + E_d (2J, Q) \delta_1 dA \right] \]
\[ = -\int_M d \left[ \frac{\partial E_d}{\partial (2J)} + E_d (2J, Q) \right] \wedge \omega_2 \Omega_1 + \int_M \frac{\partial E_d}{\partial Q} (\varepsilon_{11} \omega_2 - \varepsilon_{12} \omega_1) \Omega_1 \]
\[ - \int_M \Omega_1 d \left[ (\varepsilon_{12} \omega_1 + \varepsilon_{22} \omega_2) \frac{\partial E_d}{\partial Q} \right], \]
\[ \delta_2 F_d = \int_M \left[ \frac{\partial E_d}{\partial (2J)} \delta_2 (2J) dA + \frac{\partial E_d}{\partial Q} \delta_2 Q dA + E_d (2J, Q) \delta_2 dA \right] \]
\[ = \int_M d \left[ \frac{\partial E_d}{\partial (2J)} + E_d (2J, Q) \right] \wedge \omega_1 \Omega_2 + \int_M \frac{\partial E_d}{\partial Q} (\varepsilon_{12} \omega_2 - \varepsilon_{22} \omega_1) \Omega_2 \]
\[ + \int_M \Omega_2 d \left[ (\varepsilon_{11} \omega_1 + \varepsilon_{12} \omega_2) \frac{\partial E_d}{\partial Q} \right], \]
\[ \delta_3 \mathcal{F}_d = \int_M \left[ \frac{\partial \mathcal{E}_d}{\partial (2J)} \delta_3 (2J) dA + \frac{\partial \mathcal{E}_d}{\partial Q} \delta_3 Q dA + \mathcal{E}_d (2J, Q) \delta_3 dA \right] \]

\[ = \int_M \frac{\partial \mathcal{E}_d}{\partial Q} [a \varepsilon_{11} + 2b \varepsilon_{12} + c \varepsilon_{22}] \Omega_3 dA \]

\[ - \int_M 2H \left[ \frac{\partial \mathcal{E}_d}{\partial (2J)} + \mathcal{E}_d + (2J) \frac{\partial \mathcal{E}_d}{\partial Q} \right] \Omega_3 dA. \]  

(111)

Otherwise, section 5 tells us:

\[ \delta_1 \mathcal{F}_{cp} = \delta_2 \mathcal{F}_{cp} = 0, \]

\[ \delta_3 \mathcal{F}_{cp} = \int_M \left( (\nabla^2 + 4H^2 - 2K) \frac{\partial \mathcal{E}_H}{\partial (2H)} - 2H \mathcal{E}_H + p \right) \Omega_3 dA. \]

Therefore, \( \delta_i \mathcal{F} = \delta_i \mathcal{F}_d + \delta_i \mathcal{F}_{cp} = 0 \) gives:

\[ - d \left[ \frac{\partial \mathcal{E}_d}{\partial (2J)} + \mathcal{E}_d \right] \land \omega_2 + \frac{\partial \mathcal{E}_d}{\partial Q} (\varepsilon_{11} d \omega_2 - \varepsilon_{12} d \omega_1) \]

\[ - d \left[ (\varepsilon_{12} \omega_1 + \varepsilon_{22} \omega_2) \frac{\partial \mathcal{E}_d}{\partial Q} \right] = 0, \]  

(112)

\[ d \left[ \frac{\partial \mathcal{E}_d}{\partial (2J)} + \mathcal{E}_d \right] \land \omega_1 + \frac{\partial \mathcal{E}_d}{\partial Q} (\varepsilon_{12} d \omega_2 - \varepsilon_{22} d \omega_1) \]

\[ + d \left[ \frac{\partial \mathcal{E}_d}{\partial Q} (\varepsilon_{11} \omega_1 + \varepsilon_{12} \omega_2) \right] = 0, \]  

(113)

\[ (\nabla^2 + 4H^2 - 2K) \frac{\partial \mathcal{E}_H}{\partial (2H)} - 2H \left[ \mathcal{E}_H + \frac{\partial \mathcal{E}_d}{\partial (2J)} + \mathcal{E}_d + (2J) \frac{\partial \mathcal{E}_d}{\partial Q} \right] \]

\[ + p + \frac{\partial \mathcal{E}_d}{\partial Q} [a \varepsilon_{11} + 2b \varepsilon_{12} + c \varepsilon_{22}] = 0. \]  

(114)

Substituting \( \mathcal{E}_H = \frac{k}{2} (2H + c_0)^2 + \mu \) and \( \mathcal{E}_d = \frac{k}{2} [(2J)^2 - Q] \) into above three equations, we obtain:

\[ k_d [-d(2J) \land \omega_2 - \frac{1}{2} (\varepsilon_{11} d \omega_2 - \varepsilon_{12} d \omega_1) + \frac{1}{2} d(\varepsilon_{12} \omega_1 + \varepsilon_{22} \omega_2)] = 0, \]  

(115)

\[ k_d [d(2J) \land \omega_1 - \frac{1}{2} (\varepsilon_{12} d \omega_2 - \varepsilon_{22} d \omega_1) - \frac{1}{2} d(\varepsilon_{11} \omega_1 + \varepsilon_{12} \omega_2)] = 0, \]  

(116)

\[ p - 2H (\mu + k_d J) + k_c (2H + c_0) (2H^2 - c_0 H - 2K) + k_c \nabla^2 (2H) \]

\[ - \frac{k_d}{2} (a \varepsilon_{11} + 2b \varepsilon_{12} + c \varepsilon_{22}) = 0. \]  

(117)

(115) and (116) are called in-plane strain equations of the cell membrane, while (117) is the shape equation.

Remark 5.2 The higher order terms of \( \varepsilon_{ij} \ (i, j = 1, 2) \) are neglected in above three equations.

Obviously, if \( k_d = 0 \), then (115) and (116) are two identities. Moreover (117) degenerates into shape equation (3) of closed lipid bilayers in this case. Otherwise, for small strain, (117) is very close to (3), which may suggest that cross-linking structures have small effects on the shape of lipid bilayers.
It is not hard to verify that $\varepsilon_{12} = 0$, $\varepsilon_{11} = \varepsilon_{22} = \varepsilon$ satisfy (115) and (116) if $\varepsilon$ being a constant. In this case, the sphere with radius $R$ is the solution of (117) if it satisfies

$$pR^2 + (2\mu + 3k_0\varepsilon)R + k_0(c_0R - 2) = 0. \tag{118}$$

### 5.4. Mechanical stabilities of spherical cell membranes

To discuss the stabilities of spherical cell membranes, we must discuss the second order variations of the functional $F$. In mathematical point of view presented in section 2, we must calculate $\delta_i \delta_j F$ ($i, j = 1, 2, 3$). But in physical and symmetric point of view, we just need to calculate $\delta_3 F$ because we can expect that the perturbations along the normal are primary to the instabilities of spherical membranes under the osmotic pressure which is perpendicular to the sphere surfaces.

If we taking $E_H = \frac{k_0}{2}(2H + c_0)^2 + \mu$ and $E_d = \frac{k_d}{2}[(2J)^2 - Q]$, the leading term of (111) is

$$\delta_3 F_d = -\frac{k_d}{2} \int_M \left[ (a\varepsilon_{11} + 2b\varepsilon_{12} + c\varepsilon_{22}) + 2H(2J) \right] \Omega_3 dA. \tag{119}$$

Using Lemmas 3.1, 3.2 Eqs. (98) ~ (100), we can obtain

$$\delta_3^2 F_d = -\frac{k_d}{2} \int_M \left[ \left( \delta_3 a\varepsilon_{11} + 2\delta_3 b\varepsilon_{12} + \delta_3 c\varepsilon_{22} \right) + \delta_3 (2H)(2J) \right] \Omega_3 dA$$

$$-\frac{k_d}{2} \int_M \left[ \left( a\delta_3 \varepsilon_{11} + 2b\delta_3 \varepsilon_{12} + c\delta_3 \varepsilon_{22} \right) + 2H\delta_3 (2J) \right] \Omega_3 dA$$

$$-\frac{k_d}{2} \int_M \left[ \left( a\varepsilon_{11} + 2b\varepsilon_{12} + c\varepsilon_{22} \right) + 2H(2J) \right] \Omega_3 \delta_3 dA$$

$$= k_d \int_M \frac{3(1 + \varepsilon)}{R^2} \Omega_3^2 dA - \frac{3k_d\varepsilon}{2} \int_M \Omega_3 \nabla^2 \Omega_3 dA, \tag{120}$$

for the spherical cell membrane with radius $R$ and strain $\varepsilon$.

Otherwise, (111) suggests that

$$\delta_3^2 F_{cp} = \int_M \Omega_3^2 \left\{ k_0 c_0^2 / R^2 + 2\mu / R^2 + 2p / R \right\} dA$$

$$+ \int_M \Omega_3 \nabla^2 \Omega_3 \left\{ 2k_1 c_0 / R + 2k_1 / R^2 - \mu - k_0 c_0^2 / 2 \right\} dA$$

$$+ \int_M k_1 (\nabla^2 \Omega_3)^2 dA. \tag{121}$$

Therefore

$$\delta_3^2 F = \delta_3^2 F_d + \delta_3^2 F_{cp}$$

$$= \int_M \Omega_3^2 \left\{ 3k_d / R^2 + (3k_d\varepsilon + k_0 c_0^2 + 2\mu) / R^2 + 2p / R \right\} dA$$

$$+ \int_M \Omega_3 \nabla^2 \Omega_3 \left\{ 2k_1 c_0 / R + 2k_1 / R^2 - (3k_d\varepsilon + k_0 c_0^2 + 2\mu) / 2 \right\} dA$$

$$+ \int_M k_1 (\nabla^2 \Omega_3)^2 dA. \tag{122}$$
If considering (118) and expanding \( \Omega_3 \) as (68), we have

\[
\delta^3 \mathcal{F} = \int_M \Omega_3^2 \{3k_d/R^2 + (2k_c c_0/R^3) + p/R\} dA
\]

\[
+ \int_M \Omega_3 \nabla^2 \Omega_3 \{k_c c_0/R + 2k_c/R^2 + pR/2\} dA + \int_M k_c (\nabla^2 \Omega_3)^2 dA
\]

\[
= \sum_{l,m} |a_{lm}|^2 \{3k_d + [l(l+1) - 2][l(l+1)k_c/R^2 - k_c c_0/R - pR/2]\}.
\]

The zero point of the coefficient of \( |a_{lm}|^2 \) in above expression is

\[
p_l = \frac{6k_d}{l(l+1) - 2} + \frac{2k_c[l(l+1) - c_0R]}{R^3} \quad (l = 2, 3, \cdots).
\] (123)

Obviously, on the one hand, if \( k_d = 0 \), (123) is degenerated into (68) with \( l \geq 2 \). On the other hand, if \( k_d > 0 \), we must take the minimum of (123) to obtain the critical pressure.

If let \( \xi = l(l+1) \geq 6 \), we have

\[
p(\xi) = \frac{6k_d}{(\xi - 2)R} + \frac{2k_c(\xi - c_0R)}{R^3},
\] (124)

\[
\frac{dp}{d\xi} = -\frac{6k_d/R}{(\xi - 2)^2} + \frac{2k_c}{R^3},
\] (125)

\[
\frac{d^2p}{d\xi^2} = \frac{12k_d/R}{(\xi - 2)^3} > 0.
\] (126)

\( dp/d\xi = 0 \) and \( \xi \geq 6 \) imply \( \xi = 2 + R \sqrt{3k_d/k_c} \) which is valid only if \( 3k_d R^2 > 16k_c \). Therefore, the critical pressure is:

\[
p_c = \min\{p_l\} = \begin{cases} \frac{3k_d}{2R} + \frac{2k_c(6-c_0R)}{R^3} & (3k_d R^2 < 16k_c), \\ \frac{4 \sqrt{3k_d k_c}}{R} + \frac{2k_c(2-c_0R)}{R^3} & (3k_d R^2 > 16k_c). \end{cases}
\] (127)

Eq. (127) includes the classical result for stability of elastic shell. The critical pressure for classical spherical shell is \( p_c \propto Y h^2/R^2 \) \([32, 33]\), where \( Y \) is the Young’s modulus of the shell. If taking \( c_0 = 0 \), \( k_d \propto Y h \), \( k_c \propto Y h^3 \) and \( R \gg h \), our result (127) also gives \( p_c \propto Y h^2/R^2 \). As far as we know, this is the first time to obtain the critical pressure for spherical shell through the second order variation of free energy without any assumption to the shape of its losing the stability (cf. Ref. [32, 33]).

Otherwise, if we take the typical parameters of cell membranes as \( k_c \sim 20k_BT \) \([4, 5]\), \( k_d \sim 2.4 \mu N/m \) \([34]\), \( h \sim 4nm \), \( R \sim 1\mu m \), \( c_0 R \sim 1 \), we obtain \( p_c \sim 4 \) Pa from (127), which is much larger than \( p_c \sim 0.2 \) Pa without considering \( k_d \) induced by the cross-linking structures. Therefore, cross-linking structures greatly enhance the mechanical stabilities of cell membranes.

6. Conclusion

In above discussion, we deal with variational problems on closed and open surfaces by using exterior differential forms. We obtain the shape equation of closed lipid bilayers,
the shape equation and boundary conditions of open lipid bilayers and two-component lipid bilayers, and the shape equation and in-plane stain equations of cell membranes with cross-linking protein structures. Furthermore, we discuss the mechanical stabilities of spherical lipid bilayers and cell membranes.

Some new results are obtained as follows:

(i) The fundamental variational equations in a surface: Eqs. (27) ∼ (36).

(ii) The general expressions of the second order variation of the free energy for closed lipid bilayers: theorem 3.3 and Eq. (61).

(iii) The general shape equation and boundary conditions of open lipid bilayers and two-component lipid bilayers: Eqs. (77) ∼ (80) and (88) ∼ (91).

(iv) The free energy (94), shape equation and strain equations (115) ∼ (117) of the cell membranes with cross-linking protein structures.

(v) The critical pressure (127) of losing stabilities for spherical cell membranes. It includes the critical pressures not only for closed lipid bilayers, but also for the classic solid shells. Otherwise it suggests that cross-linking protein structures can enhance the stabilities of cell membranes.

In the future, we will devote ourselves to applying above results to explain the shapes of open lipid bilayers found by Saitoh et al., and predict new shapes of multi-component lipid bilayers and cell membranes. Moreover, We will discuss whether and how the in-plane modes affect the instability of cell membranes although we believe they have no qualitative effect on our results in section 5.4.

Acknowledgments

We are grateful to Prof. H W Peng for his useful discussions and to Prof. S S Chern for his advice that we should notice the work by Griffiths et al. Thank Prof. J Guven and G Landolfi for their friendly email discussions. Thank Prof. J Hu, Dr. W Zhao and R An for their kind helps.

Appendix A. Exterior differential forms and Stokes’ theorem

A manifold can be roughly regarded as a multi-dimensional surface. In the neighborhood of every point, we can construct the local coordinates \((u^1, u^2, \cdots, u^m)\), where \(m\) is the dimension of the surface. In this paper we just consider smooth, orientable manifolds and smooth functions.

We call the function \(f(u^1, u^2, \cdots, u^m)\) 0-form and \(a_i(u^1, u^2, \cdots, u^m)du^i\) 1-form, where Einstein summation rule is used and it is also used in the following contents. The \(r\)-form \((r \leq m)\) is defined as \(a_{i_1i_2\cdots i_r}du^{i_1} \wedge du^{i_2} \wedge \cdots \wedge du^{i_r}\), where the exterior production “\(\wedge\)” satisfies \(du^i \wedge du^i = -du^i \wedge du^i\). Denote \(\Lambda^r = \{\text{all } r\text{-forms}\}, \ (r = 0, 1, 2, \cdots, m\).

Definition A linear operator \(d : \Lambda^r \rightarrow \Lambda^{r+1}\) is called the exterior differential operator if it satisfies:

(i) For function \(f(u^1, u^2, \cdots, u^m)\), \(df = \frac{\partial f}{\partial u^i}du^i\) is an ordinary differential;
(ii) \( dd = 0 \);
(iii) \( \forall \omega_1 \in \Lambda^r \) and \( \forall \omega_2 \in \Lambda^k \), \( d(\omega_1 \wedge \omega_2) = \omega_1 \wedge \omega_2 + (-1)^r \omega_1 \wedge d\omega_2 \).

**Stokes theorem** If \( \omega \) is an \((m - 1)\)-form with compact support set on \( M \), and \( D \) is a domain with boundary \( \partial D \) in \( M \), then
\[
\int_D d\omega = \int_{\partial D} \omega. \tag{A.1}
\]

**Appendix B. Curves in a surface**

If a curve passes through \( P \) in the surface, we construct a Frenet frame \( \{T, N, B\} \) such that \( T \), \( N \) and \( B \) are the tangent, normal and binormal vectors of the curve, respectively. Denote \( \theta \) the angle between \( e_1 \) and \( T \). Set \( M = e_3 \times T \). Thus we have
\[
\begin{align*}
T &= e_1 \cos \theta + e_2 \sin \theta, \\
M &= -e_1 \sin \theta + e_2 \cos \theta.
\end{align*}
\]

It is not hard to calculate
\[
dT = (d\theta + \omega_{12})M + e_3(\omega_{13} \cos \theta + \omega_{23} \sin \theta).
\]

Frenet Formulas tell us \( dT/ds = \kappa N \). Therefore, we have the geodesic curvature, the geodesic torsion, and the normal curvature of the curve:
\[
\begin{align*}
k_g &= \kappa N \cdot M = (dT/ds) \cdot M = (d\theta + \omega_{12})/ds, \tag{B.1} \\
\tau_g &= -(d(e_3/ds) \cdot M = [(b\omega_1 + c\omega_2) \cos \theta - (a\omega_1 + b\omega_2)(\sin \theta)]/ds, \\
k_n &= II/I = (a\omega_1^2 + 2b\omega_1\omega_2 + c\omega_2^2)/(\omega_1^2 + \omega_2^2).
\end{align*}
\]

If the curve along \( e_1 \) such that \( \theta = 0 \), we have \( ds = \omega_1, \omega_2 = 0 \) and
\[
k_g = \omega_{12}/\omega_1, \quad \tau_g = b, \quad \text{and} \quad k_n = a. \tag{B.2}
\]

**Appendix C. Gauss-Bonnet formula**

Using (9), (10) and (13), we have
\[
d\omega_{12} = -K\omega_1 \wedge \omega_2. \tag{C.1}
\]

This formula was called *Theorem Egregium* by Gauss. From *Theorem Egregium* and (B.1), we can derive Gauss-Bonnet formula:
\[
\int_M KdA + \int_C k_g ds = 2\pi \chi(M), \tag{C.2}
\]

where \( \chi(M) \) is the characteristic number of smooth surface \( M \) with smooth edge \( C \). \( \chi(M) = 1 \) for a simple surface with an edge. For a closed surface, we have
\[
\int_M KdA = 2\pi \chi(M). \tag{C.3}
\]
Appendix D. The tensor expressions of $\nabla$, $\bar{\nabla}$, $\nabla^2$, $\nabla \cdot \bar{\nabla}$, and $\nabla \cdot \bar{\nabla}$

At every point $\mathbf{r}$ in the surface, we can take local coordinates $(u^1, u^2)$ where the first and the second fundamental form are denoted by $I = g_{ij} du^i du^j$ and $II = L_{ij} du^i du^j$, respectively. Let $(g^{ij}) = (g_{ij})^{-1}$, $(L^{ij}) = (L_{ij})^{-1}$ and $\mathbf{r}_i = \partial \mathbf{r}/\partial u^i$, thus we have

$$
\nabla = g^{ij} \mathbf{r}_i \frac{\partial}{\partial u^j},
$$

$$
\bar{\nabla} = \mathbf{r}_i (2H g^{ij} - KL^{ij}) \frac{\partial}{\partial u^j},
$$

$$
\tilde{\nabla} = KL^{ij} \mathbf{r}_i \frac{\partial}{\partial u^j},
$$

$$
\nabla^2 = \frac{1}{\sqrt{g}} \frac{\partial}{\partial u^i} \left( \sqrt{g} g^{ij} \frac{\partial}{\partial u^j} \right),
$$

$$
\nabla \cdot \bar{\nabla} = \frac{1}{\sqrt{g}} \frac{\partial}{\partial u^i} \left[ \sqrt{g} (2H g^{ij} - KL^{ij}) \frac{\partial}{\partial u^j} \right],
$$

$$
\nabla \cdot \tilde{\nabla} = \frac{1}{\sqrt{g}} \frac{\partial}{\partial u^i} \left( \sqrt{g} KL^{ij} \frac{\partial}{\partial u^j} \right).
$$

As an example, We will prove the last one of above expressions.

**Proof:** If taking the orthogonal local coordinates, we have $I = g_{11} (du^1)^2 + g_{22} (du^2)^2 = \omega_1^2 + \omega_2^2$, which implies $\omega_1 = \sqrt{g_{11}} du^1$ and $\omega_2 = \sqrt{g_{22}} du^2$. For function $f$, on the one hand, we have $df = f_1 \omega_1 + f_2 \omega_2 = f_1 \sqrt{g_{11}} du^1 + f_2 \sqrt{g_{22}} du^2$, on the other hand, we have $df = \frac{\partial f}{\partial u^1} du^1 + \frac{\partial f}{\partial u^2} du^2$. Therefore, $f_1 = \frac{1}{\sqrt{g_{11}}} \frac{\partial f}{\partial u^1}$, $f_2 = \frac{1}{\sqrt{g_{22}}} \frac{\partial f}{\partial u^2}$.

The second fundamental form $II = a \omega_1^2 + 2b \omega_1 \omega_2 + c \omega_2^2 = L_{ij} du^i du^j$ implies $a = L_{11}/g_{11}$, $b = L_{12}/\sqrt{g}$, $c = L_{22}/g_{22}$. Thus $K = ac - b^2 = (L_{11} L_{22} - L_{12}^2)/g$, and

$$
L_{11} = \frac{L_{22}}{L_{11} L_{22} - L_{12}^2} \Rightarrow L_{22} = gKL_{11};
$$

$$
L_{12} = -\frac{L_{12}}{L_{11} L_{22} - L_{12}^2} \Rightarrow L_{12} = -gKL_{12};
$$

$$
L_{22} = \frac{L_{11}}{L_{11} L_{22} - L_{12}^2} \Rightarrow L_{11} = gKL_{22}.
$$

Moreover, we have

$$
\bar{d} f = -f_2 \omega_{12} + f_1 \omega_{22} = -f_2 (a \omega_1 + b \omega_2) + f_2 (b \omega_1 + c \omega_2)
$$

$$
= \frac{1}{\sqrt{g}} \left( L_{12} \frac{\partial f}{\partial u^1} - L_{11} \frac{\partial f}{\partial u^2} \right) du^1 + \frac{1}{\sqrt{g}} \left( L_{22} \frac{\partial f}{\partial u^1} - L_{12} \frac{\partial f}{\partial u^2} \right) du^2;
$$

$$
d\bar{d} f = \left\{ \frac{\partial}{\partial u^1} \left[ \sqrt{g} (L_{11} \frac{\partial f}{\partial u^1} + L_{12} \frac{\partial f}{\partial u^2}) \right] \right. \\
\quad + \left. \frac{\partial}{\partial u^2} \left[ \sqrt{g} (L_{12} \frac{\partial f}{\partial u^1} + L_{22} \frac{\partial f}{\partial u^2}) \right] \right\} du^1 \wedge du^2.
$$

Therefore, $\nabla \cdot \bar{\nabla} f = \frac{d\bar{d} f}{\omega_1 \wedge \omega_2} = \frac{1}{\sqrt{g}} \frac{\partial}{\partial u^1} \left( \sqrt{g} KL^{ij} \frac{\partial f}{\partial u^j} \right)$. \(\blacksquare\)
Geometric theory on the elasticity of bio-membranes

References

[1] Edidin M 2003 Nature Reviews Molecular Cell Biology 4 414
[2] Singer S J and Nicolson G L 1972 Science 175 720
[3] Helfrich W 1973 Z. Naturforsch. C 28 693
[4] Duwe H P, Kaes J and Sackmann E 1990 J. Phys. Fr. 51 945
[5] Mutz M and Helfrich W 1990 J. Phys. Fr. 51 991
[6] Ou-Yang Z C, Liu J X and Xie Y Z 1999 Geometric Methods in the Elastic Theory of Membranes in Liquid Cristal Phases (Singapore: World Scientific)
[7] Lipowsky R 1991 Nature 349 475; Seifert U 1997 Adv. Phys. 46 13
[8] Ou-Yang Z C and Helfrich W 1987 Phys. Rev. Lett. 59 2486; 1989 Phys. Rev. A 39 5280
[9] Saitoh A, Takiguchi K, Tanaka Y and Hotani H 1998 Proc. Natl. Acad. Sci. 95 1026
[10] Nomura F, Nagata M, Inaba T, Hiramatsu H, Hotani H and Takiguchi K 2001 Proc. Natl. Acad. Sci. 98 2340
[11] Capovilla R, Guven J and Santiago J A 2002 Phys. Rev. E 66 021607
[12] Capovilla R and Guven J 2002 J. Phys. A: Math. Gen. 35 6233
[13] Tu Z C and Ou-Yang Z C 2003 Phys. Rev. E 68 061915; An R and Tu Z C 2003 Preprint math-phys/0307007
[14] Chirias D 2002 Human Biology: Health, Homeostasis, and the Environment, 4th ed. (Boston: Jones & Bartlett Publishers)
[15] Griffiths P 1983 Exterior Differential Systems and the Calculus of Variations (Boston: Birkhäuser)
[16] Bryant R, Chern S S, Gardner R, Goldschmidt H and Griffiths P 1991 Exterior Differential Systems (New York: Springer-Verlag)
[17] Kamien R D 2002 Rev. Mod. Phys. 74 953
[18] Chern S S and Chen W H 2001 Lectures on Differential Geometry 2nd. (Beijing: Peking University Press)
[19] Westenholz C V 1981 Differential Forms in Mathematical Physics (Amsterdam: North-Holland)
[20] Natio H, Okuda M and Ou-Yang Z C 1995 Phys. Rev. E 52 2095
[21] Capovilla R and Guven J 2004 J. Phys. A: Math. Gen. 37 5983
[22] Landolphi G 2003 J. Phys. A: Math. Gen. 36 11937
[23] Alexandrov A D 1962 Amer. Math. Soc. Transl. 21 341
[24] Naito H, Okuda M and Ou-Yang Z C 1993 Phys. Rev. E 48 2304
[25] Ou-Yang Z C 1990 Phys. Rev. A 41 4517
[26] Wang Z X and Guo D R 2000 Introduction to Special Function (Beijing: Peking University Press)
[27] Jülicher F and Lipowsky R 1996 Phys. Rev. E 53 2670
[28] Baumgart T, Hess S T and Webb W W 2003 Nature 425 821
[29] Treloar L R G 1975 The Physics of Rubber Elasticity (Oxford: Clarendon Press)
[30] Dio M and Edwards S F 1986 The Theory of protein chain Dynamics (Oxford: Clarendon Press)
[31] Wu J K and Wang M Z 1981 Introduction to Elastic theory (Beijing: Peking University Press); Wu J K and Su X Y 1994 Stabilities of Elastic Systems (Beijing: Science Press)
[32] Landau L D and Lifshitz E M 1997 Theory of Elasticity 3rd edn. (Oxford: Butterworth-Heinemann)
[33] Pogorelov A V 1989 Bending of surfaces and stability of shells (Providence, R.I.: AMS)
[34] Lenormand G et al. 2001 Biophys. J. 81 43