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A geometric theory on the elasticity of bio-membranes

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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 of 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 surfaces embedded in three-dimensional Euclidean space by using exterior differential forms.

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1. Introduction

Cell membranes play a 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 most 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 the lipid bilayer is about 4 nanometres which is much less than the scale of the cell (about several micrometres). Therefore, we can use a geometrical surface to describe the lipid bilayer.

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

$$f_c = (k_c/2)(2H + c_0)^2 + \bar{k}K, \tag{1}$$

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 room temperature because $k_c \approx 10^{-19} J \gg k_B T$ [4, 5], where k_B is the Boltzmann factor and T is the room temperature. Based on Helfrich's curvature energy, the free energy of the closed bilayer under osmotic pressure p (the outer pressure minus the inner one) is written as

$$\mathcal{F}_H = \int (f_c + \mu)\,\mathrm{d}A + p \int \mathrm{d}V, \tag{2}$$

where $\mathrm{d}A$ is the area element, μ is the surface tension of the bilayer, and V is the volume enclosed within the lipid bilayer. Starting with above free energy, many researchers studied the shapes of bilayers [6, 7]. Particularly, 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]:

$$p - 2\mu H + k_c(2H + c_0)(2H^2 - c_0 H - 2K) + k_c \nabla^2(2H) = 0. \tag{3}$$

They also showed that the threshold pressure for instability of a spherical bilayer was $p_c \sim k_c/R^3$, where R is the radius of the spherical bilayer.

Recently, the opening-up process of lipid bilayers by talin was observed by Saitoh *et al* [9, 10], which raised 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 a recent paper, we derived the shape equation and boundary conditions in a 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 described in a recent book [6] by one of the authors.

In fact, the structures of cell membranes are far more complex than the fluid mosaic model. 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 an effect on the shapes and stabilities of cell membranes.

In the following, 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 be new for mathematicians who are familiar with the work by Griffiths and Bryant *et al* [15, 16]. However, our method focuses on the application aspect, while the work by Griffiths and Bryant *et al* emphasizes the geometric meaning. Also, we notice a review paper by Kamien [17], where he gave 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 section 2, we briefly introduce the basic concepts in differential geometry and the variational theory of surfaces. In section 3, we deal with variational problems on a closed surface, derive the shape equation of closed lipid bilayers, and then discuss the mechanical stabilities of spherical bilayers. In section 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 section 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

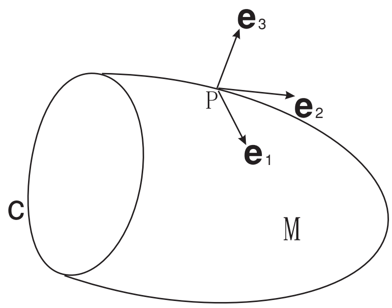

Figure 1. A smooth and orientable surface M with an edge C.

the first and second order variations of the total free energy. In section 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 figure 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

$$\mathrm{d}\mathbf{r} = \lim_{P \to P'} \overrightarrow{PP'} = \omega_1 \mathbf{e}_1 + \omega_2 \mathbf{e}_2, \tag{4}$$

$$\mathrm{d}\mathbf{e}_i = \omega_{ij} \mathbf{e}_j \qquad (i = 1, 2, 3), \tag{5}$$

where ω_1, ω_2 and ω_{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 $\mathrm{d}\,\mathrm{d}\mathbf{r} = 0$ and $\mathrm{d}\,\mathrm{d}\mathbf{e}_i = 0$, we obtain the structure equations of the surface:

$$\mathrm{d}\omega_1 = \omega_{12} \wedge \omega_2; \tag{6}$$

$$\mathrm{d}\omega_2 = \omega_{21} \wedge \omega_1; \tag{7}$$

$$\omega_1 \wedge \omega_{13} + \omega_2 \wedge \omega_{23} = 0; \tag{8}$$

$$\mathrm{d}\omega_{ij} = \omega_{ik} \wedge \omega_{kj} \qquad (i, j = 1, 2, 3). \tag{9}$$

If we consider Cartan's lemma, (8) suggests

$$\omega_{13} = a\omega_1 + b\omega_2 \quad \text{and} \quad \omega_{23} = b\omega_1 + c\omega_2. \tag{10}$$

Thus we have [18]:

$$\begin{aligned}
&\text{the area element:} \quad \mathrm{d}A = \omega_1 \wedge \omega_2,\\
&\text{the first funsdamental form:} \quad I = \mathrm{d}\mathbf{r} \cdot \mathrm{d}\mathbf{r} = \omega_1^2 + \omega_2^2,\\
&\text{the second fundamental form:} \quad II = -\mathrm{d}\mathbf{r} \cdot \mathrm{d}\mathbf{e}_3 = a\omega_1^2 + 2b\omega_1\omega_2 + c\omega_2^2,\\
&\text{the third fundamental form:} \quad III = \mathrm{d}\mathbf{e}_3 \cdot \mathrm{d}\mathbf{e}_3 = \omega_{31}^2 + \omega_{32}^2,
\end{aligned} \tag{11}$$

mean curvature: $H = (a + c)/2$, (12)

Gaussian curvature: $K = ac - b^2$. (13)

2.2. Hodge star (∗) and Gauss mapping

2.2.1. Hodge star (∗). Here we just show the basic properties of the Hodge star (∗) on surface M. As to its general definition, please see [19].

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

$$*f = f\omega_1 \wedge \omega_2; \tag{14}$$

$$*\omega_1 = \omega_2, *\omega_2 = -\omega_1; \tag{15}$$

$$\mathrm{d} * \mathrm{d} f = \nabla^2 f \omega_1 \wedge \omega_2, \tag{16}$$

where ∇^2 is the Laplace–Beltrami operator.

It is easy to obtain the second Green identity

$$\int_M (f\,\mathrm{d} * \mathrm{d}h - h\,\mathrm{d} * \mathrm{d}f) = \int_{\partial M} (f * \mathrm{d}h - h * \mathrm{d}f) \tag{17}$$

through Stokes' theorem and integration by parts. It follows that

$$\int_M f\,\mathrm{d} * \mathrm{d}h = \int_M h\,\mathrm{d} * \mathrm{d}f, \tag{18}$$

if M is a closed surface.

2.2.2. Gauss mapping. The Gauss mapping $\mathcal{G} : M \to S^2$ is defined as $\mathcal{G}(\mathbf{r}) = \mathbf{e}_3(\mathbf{r})$, where S^2 is a unit sphere. It induces a linear mapping $\mathcal{G}^\star : \Lambda^1 \to \Lambda^1$ such that:

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

(ii) if $\mathrm{d}f = f_1\omega_1 + f_2\omega_2$, then $\mathcal{G}^\star\,\mathrm{d}f = f_1\mathcal{G}^\star\omega_1 + f_2\mathcal{G}^\star\omega_2$.

Thus we can define a new differential operator $\tilde{\mathrm{d}} = \mathcal{G}^\star\,\mathrm{d}$. Obviously, if $\mathrm{d}f = f_1\omega_1 + f_2\omega_2$, then $\tilde{\mathrm{d}}f = f_1\omega_{13} + f_2\omega_{23}$. If we define a new operator $\tilde{*}$ such that $\tilde{*}\omega_{13} = \omega_{23}$ and $\tilde{*}\omega_{23} = -\omega_{13}$, we have

Lemma 2.1. $\int_M (f\,\mathrm{d}\tilde{*}\tilde{\mathrm{d}}h - h\,\mathrm{d}\tilde{*}\tilde{\mathrm{d}}f) = \int_{\partial M}(f\tilde{*}\tilde{\mathrm{d}}h - h\tilde{*}\tilde{\mathrm{d}}f)$ *for the smooth functions* f *and* h *on* M.

Proof. Using integration by parts and Stokes' theorem, we obtain

$$\int_M f\,\mathrm{d}\tilde{*}\tilde{\mathrm{d}}h = \int_{\partial M} f\tilde{*}\tilde{\mathrm{d}}h - \int_M \mathrm{d}f \wedge \tilde{*}\tilde{\mathrm{d}}h, \tag{19}$$

$$\int_M h\,\mathrm{d}\tilde{*}\tilde{\mathrm{d}}f = \int_{\partial M} h\tilde{*}\tilde{\mathrm{d}}f - \int_M \mathrm{d}h \wedge \tilde{*}\tilde{\mathrm{d}}f. \tag{20}$$

If we let $\mathrm{d}f = f_1\omega_1 + f_2\omega_2$ and $\mathrm{d}h = h_1\omega_1 + h_2\omega_2$, we can prove $\mathrm{d}f \wedge \tilde{*}\tilde{\mathrm{d}}h = \mathrm{d}h \wedge \tilde{*}\tilde{\mathrm{d}}f = [af_2h_2 + cf_1h_1 - b(f_1h_2 + f_2h_1)]\omega_1 \wedge \omega_2$ through a few steps of calculations. Therefore, we can arrive at lemma 2.1 by (19) minus (20). □

It follows that

$$\int_M f\,\mathrm{d}\tilde{*}\tilde{\mathrm{d}}h = \int_M h\,\mathrm{d}\tilde{*}\tilde{\mathrm{d}}f \tag{21}$$

for a closed surface.

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

2.3. Variational theory of surface

If we let M undergo an infinitesimal deformation such that every point $\mathbf{r}$ of M has a displacement $\delta\mathbf{r}$, we obtain a new surface $M' = \{\mathbf{r}' \mid \mathbf{r}' = \mathbf{r} + \delta\mathbf{r}\}$. $\delta\mathbf{r}$ is called the variation of surface M and is expressed as

$$\delta\mathbf{r} = \delta_1\mathbf{r} + \delta_2\mathbf{r} + \delta_3\mathbf{r}, \tag{22}$$

$$\delta_i\mathbf{r} = \Omega_i\,\mathbf{e}_i \qquad (i = 1, 2, 3), \tag{23}$$

where the repeated subindexes do not represent Einstein summation.

Definition 2.1. *If f is a generalized function of* $\mathbf{r}$ *(including scalar function, vector function, and r-form dependent on point* $\mathbf{r}$*), define*

$$\delta_i^{(q)} f = (q!)\mathcal{L}^{(q)}[f(\mathbf{r} + \delta_i\mathbf{r}) - f(\mathbf{r})] \qquad (i = 1, 2, 3;\ q = 1, 2, 3, \ldots), \tag{24}$$

and the q-order variation of f

$$\delta^{(q)} f = (q!)\mathcal{L}^{(q)}[f(\mathbf{r} + \delta\mathbf{r}) - f(\mathbf{r})] \qquad (q = 1, 2, 3, \ldots), \tag{25}$$

where $\mathcal{L}^{(q)}[\cdots]$ represents the terms of $\Omega_1^{q_1}\Omega_2^{q_2}\Omega_3^{q_3}$ in a 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 $\delta_i, \delta_i^q, \delta$, and $\delta^q (q = 2, 3, \ldots)$ respectively;
(iv) for functions f and g, $\delta_i[f(\mathbf{r}) \circ g(\mathbf{r})] = \delta_i f(\mathbf{r}) \circ g(\mathbf{r}) + f(\mathbf{r}) \circ \delta_i g(\mathbf{r})$, where $\circ$ represents the ordinary production, vector production or exterior production;
(v) $\delta_i f[g(\mathbf{r})] = (\partial f/\partial g)\delta_i g$;
(vi) $\delta^q = (\delta_1 + \delta_2 + \delta_3)^q$, 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 $\mathbf{e}_1, \mathbf{e}_2, \mathbf{e}_3$ are also changed. Denote their changes

$$\delta_l\,\mathbf{e}_i = \Omega_{lij}\mathbf{e}_j. \tag{26}$$

Obviously, $\mathbf{e}_i \cdot \mathbf{e}_j = \delta_{ij}$ implies $\Omega_{lij} = -\Omega_{lji}$. Because δ_1, δ_2 and δ_3 are linear mappings from M to M', they are commutative with the exterior differential operator d [18]. Therefore, using $\mathrm{d}\delta_l\mathbf{r} = \delta_l\,\mathrm{d}\mathbf{r}$ and $\mathrm{d}\delta_l\,\mathbf{e}_j = \delta_l\,\mathrm{d}\mathbf{e}_j$, we arrive at

$$\delta_1\omega_1 = \mathrm{d}\Omega_1 - \omega_2\Omega_{121}, \tag{27}$$

$$\delta_1\omega_2 = \Omega_1\omega_{12} - \omega_1\Omega_{112}, \tag{28}$$

$$\Omega_{113} = a\Omega_1, \qquad \omega_{123} = b\Omega_1; \tag{29}$$

$$\delta_2\omega_1 = \Omega_2\omega_{21} - \omega_2\Omega_{221}, \tag{30}$$

$$\delta_2\omega_2 = \mathrm{d}\Omega_2 - \omega_1\Omega_{212}, \tag{31}$$

$$\Omega_{213} = b\Omega_2, \qquad \Omega_{223} = c\Omega_2; \tag{32}$$

$$\delta_3\omega_1 = \Omega_3\omega_{31} - \omega_2\Omega_{321}, \tag{33}$$

$$\delta_3\omega_2 = \Omega_3\omega_{32} - \omega_1\Omega_{312}, \tag{34}$$

$$\mathrm{d}\Omega_3 = \Omega_{313}\omega_1 + \Omega_{323}\omega_2; \tag{35}$$

$$\delta_l\omega_{ij} = \mathrm{d}\Omega_{lij} + \Omega_{lik}\omega_{kj} - \omega_{ik}\Omega_{lkj}. \tag{36}$$

The above equations (27)–(36) are the fundamental equations in our paper and do not exist in previous mathematical literature ([15, 16]). Otherwise, it is easy to deduce that $\delta_i\,\tilde{\mathrm{d}}f = \tilde{\mathrm{d}}\delta_i f (i = 1, 2, 3)$ for function f.

3. Closed lipid bilayers

In this section, we will discuss the equilibrium shapes and mechanical stabilities of closed lipid bilayers. We only 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

$$\mathcal{F} = \int_M \mathcal{E}(2H[\mathbf{r}], K[\mathbf{r}])\,\mathrm{d}A + p\int_V \mathrm{d}V, \tag{37}$$

where H and K are mean and Gaussian curvatures at point $\mathbf{r}$ in surface M. p is a constant and V is the volume enclosed within the surface.

According to the variational theory of surface in section 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 four lemmas and a theorem. Firstly, denote

$$\mathcal{F}_e = \int_M \mathcal{E}(2H[\mathbf{r}], K[\mathbf{r}])\,\mathrm{d}A. \tag{38}$$

Lemma 3.1. $\delta_3\,\mathrm{d}A = -(2H)\Omega_3\,\mathrm{d}A$.

Proof. $\delta_3\,\mathrm{d}A = \delta_3(\omega_1\wedge\omega_2) = \delta_3\omega_1\wedge\omega_2 + \omega_1\wedge\delta_3\omega_2$. By considering (10), (12), (33) and (34), we arrive at this lemma. □

Lemma 3.2. $\delta_3(2H)\,\mathrm{d}A = 2(2H^2 - K)\Omega_3\,\mathrm{d}A + \mathrm{d}*\mathrm{d}\Omega_3$.

Proof. $\delta_3(2H)\,\mathrm{d}A = \delta_3 a\omega_1\wedge\omega_2 + \delta_3 c\omega_1\wedge\omega_2$. Letting δ_3 act on (10), we have

$$\delta_3\omega_{13} = \delta_3 a\omega_1 + a\delta_3\omega_1 + \delta_3 b\omega_2 + b\delta_3\omega_2,$$
$$\delta_3\omega_{23} = \delta_3 b\omega_1 + b\delta_3\omega_1 + \delta_3 c\omega_2 + c\delta_3\omega_2.$$

If we consider (12), (13), (15) and (33)–(36), we arrive at this lemma. □

Lemma 3.3. $\delta_3 K\,\mathrm{d}A = 2KH\Omega_3\,\mathrm{d}A + \mathrm{d}\tilde{*}\tilde{\mathrm{d}}\Omega_3$.

Proof. *Theorem Egregium* (see appendix C) implies that $\delta_3 K\,\mathrm{d}A = -\delta_3\,\mathrm{d}\omega_{12} - K\delta_3\,\mathrm{d}A = -\mathrm{d}\delta_3\omega_{12} - K\delta_3\,\mathrm{d}A$. We arrive at this lemma from (36) and lemma 3.1, as well as from the discussions in section 2.2.2. □

Theorem 3.1. $\delta_3\mathcal{F}_e = \int_M [(\nabla^2 + 4H^2 - 2K)\frac{\partial\mathcal{E}}{\partial(2H)} + (\nabla\cdot\tilde{\nabla} + 2KH)\frac{\partial\mathcal{E}}{\partial K} - 2H\mathcal{E}]\Omega_3\,\mathrm{d}A$.

Proof. Firstly, we have

$$\begin{aligned}\delta_3\mathcal{F}_e &= \int_M \delta_3\mathcal{E}\,\mathrm{d}A + \int_M \mathcal{E}\delta_3 A\\ &= \int_M \frac{\partial\mathcal{E}}{\partial(2H)}\delta_3(2H)\,\mathrm{d}A + \int_M \frac{\partial\mathcal{E}}{\partial K}\delta_3 K\,\mathrm{d}A + \int_M \mathcal{E}\delta_3\,\mathrm{d}A.\end{aligned}$$

By using lemmas 3.1, 3.2 and 3.3, we obtain

$$\delta_3\mathcal{F}_e = \int_M \left[(4H^2 - 2K)\frac{\partial \mathcal{E}}{\partial(2H)} + 2KH\frac{\partial \mathcal{E}}{\partial K} - 2H\mathcal{E}\right]\Omega_3\,\mathrm{d}A + \int_M \left[\frac{\partial \mathcal{E}}{\partial(2H)}\,\mathrm{d}*\mathrm{d}\Omega_3 + \frac{\partial \mathcal{E}}{\partial K}\,\mathrm{d}\tilde{*}\tilde{\mathrm{d}}\Omega_3\right]. \quad (39)$$

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

Lemma 3.4. $\delta_3 \int_V \mathrm{d}V = \int_M \Omega_3\,\mathrm{d}A$.

Proof. Because M is a closed surface in $\mathbb{E}^3$, Stokes' theorem (see appendix A) implies $\int_V 3\,\mathrm{d}V = \int_V \nabla\cdot\mathbf{r}\,\mathrm{d}V = \int_{\partial V}\mathbf{r}\cdot\mathbf{n}\,\mathrm{d}A$, thus

$$\delta_3\int_V \mathrm{d}V = \frac{1}{3}\int_M \delta_3[\mathbf{r}\cdot\mathbf{e}_3(\omega_1\wedge\omega_2)] = \frac{1}{3}\int_M [\delta_3\mathbf{r}\cdot\mathbf{e}_3(\omega_1\wedge\omega_2) + \mathbf{r}\cdot\delta_3\,\mathbf{e}_3(\omega_1\wedge\omega_2) + \mathbf{r}\cdot\mathbf{e}_3\delta_3(\omega_1\wedge\omega_2)]. \quad (40)$$

From (33)–(35), we obtain

$$\delta_3\mathbf{r}\cdot\mathbf{e}_3(\omega_1\wedge\omega_2) = \Omega_3\omega_1\wedge\omega_2, \quad (41)$$

$$\mathbf{r}\cdot\mathbf{e}_3\delta_3(\omega_1\wedge\omega_2) = \mathbf{r}\cdot\mathbf{e}_3(-2H)\Omega_3\omega_1\wedge\omega_2, \quad (42)$$

$$\mathbf{r}\cdot\delta_3\,\mathbf{e}_3(\omega_1\wedge\omega_2) = -\mathrm{d}\Omega_3\wedge(-\mathbf{r}\cdot\mathbf{e}_2\omega_1 + \mathbf{r}\cdot\mathbf{e}_1\omega_2). \quad (43)$$

By using integration by parts and Stokes' theorem, we have

$$-\int_M \mathrm{d}\Omega_3\wedge(-\mathbf{r}\cdot\mathbf{e}_2\omega_1 + \mathbf{r}\cdot\mathbf{e}_1\omega_2) = \int_M \Omega_3\,\mathrm{d}(-\mathbf{r}\cdot\mathbf{e}_2\omega_1 + \mathbf{r}\cdot\mathbf{e}_1\omega_2) = \int_M \Omega_3[2 + \mathbf{r}\cdot\mathbf{e}_3(2H)]\omega_1\wedge\omega_2. \quad (44)$$

Therefore, we arrive at $\delta_3\int_V \mathrm{d}V = \int_M \Omega_3\,\mathrm{d}A$ by using (40)–(44). □

3.1.2. Calculation of $\delta_1\mathcal{F}$ and $\delta_2\mathcal{F}$.

Theorem 3.2. $\delta_1\mathcal{F} \equiv 0$ *and* $\delta_2\mathcal{F} \equiv 0$.

Proof. We obtain

$$\mathrm{d}b\wedge\omega_1 + 2b\,\mathrm{d}\omega_1 = (a - c)\,\mathrm{d}\omega_2 - \mathrm{d}c\wedge\omega_2 \quad (45)$$

from (9) and (10).

Using (27)–(29), (36) and (45), we arrive at

$$\delta_1(\omega_1\wedge\omega_2) = \mathrm{d}(\Omega_1\omega_2), \quad (46)$$

$$\delta_1(2H)\omega_1\wedge\omega_2 = \mathrm{d}(2H)\wedge\omega_2\Omega_1 \quad (47)$$

through a few calculations.

By analogy with the proof of lemma 3.3, we can prove that

$$\delta_1 K\omega_1\wedge\omega_2 = \mathrm{d}K\wedge\Omega_1\omega_2. \quad (48)$$

Therefore, we have

$$\delta_1\mathcal{F}_e = \int_M \left[\frac{\partial\mathcal{E}}{\partial(2H)}\delta_1(2H)\omega_1\wedge\omega_2 + \frac{\partial\mathcal{E}}{\partial K}\delta_1 K\omega_1\wedge\omega_2 + \mathcal{E}\delta_1(\omega_1\wedge\omega_2)\right]$$
$$= \int_M \mathrm{d}(\mathcal{E}\omega_2\Omega_1). \quad (49)$$

Similarly, we can obtain

$$\delta_2\mathcal{F}_e = -\int_M \mathrm{d}(\mathcal{E}\omega_1\Omega_2). \quad (50)$$

It is also not hard to obtain $\delta_1\int_V \mathrm{d}V = \int_M \mathrm{d}(\mathbf{r}\cdot\mathbf{e}_3\omega_2\Omega_1)$ and $\delta_2\int_V \mathrm{d}V = -\int_M \mathrm{d}(\mathbf{r}\cdot\mathbf{e}_3\omega_1\Omega_2)$.

Therefore, $\delta_1\mathcal{F} = \delta_2\mathcal{F} \equiv 0$ because M is a closed surface. □

3.1.3. Euler–Lagrange equation. We can obtain

$$\delta\mathcal{F} = \int_M \left[(\nabla^2 + 4H^2 - 2K)\frac{\partial\mathcal{E}}{\partial(2H)} + (\nabla\cdot\tilde{\nabla} + 2KH)\frac{\partial\mathcal{E}}{\partial K} - 2H\mathcal{E} + p\right]\Omega_3\,\mathrm{d}A. \quad (51)$$

Thus the Euler–Lagrange equation corresponding to the functional $\mathcal{F}$ is:

$$\left[(\nabla^2 + 4H^2 - 2K)\frac{\partial}{\partial(2H)} + (\nabla\cdot\tilde{\nabla} + 2KH)\frac{\partial}{\partial K} - 2H\right]\mathcal{E}(2H, K) + p = 0. \quad (52)$$

A similar equation is first found in [20].

3.2. Second order variation

In this subsection, we discuss the second order variation of functional (37) . This problem was also studied by Capovilla and Guven in a recent paper [21]. Because $\delta_1\mathcal{F} = \delta_2\mathcal{F} \equiv 0$ for closed surface M, we have $\delta\delta_1\mathcal{F} = \delta\delta_2\mathcal{F} = 0$, and $\delta^2\mathcal{F} = \delta\delta_3\mathcal{F} = \delta_3^2\mathcal{F}$.

From (39) and lemma 3.4, we obtain

$$\delta^2\mathcal{F} = \delta_3\int_M \left[(4H^2 - 2K)\frac{\partial\mathcal{E}}{\partial(2H)} + (2KH)\frac{\partial\mathcal{E}}{\partial K} - 2H\mathcal{E} + p\right]\Omega_3\,\mathrm{d}A$$
$$+\ \delta_3\int_M \frac{\partial\mathcal{E}}{\partial(2H)}\,\mathrm{d}*\mathrm{d}\Omega_3 + \delta_3\int_M \frac{\partial\mathcal{E}}{\partial K}\,\mathrm{d}\,\tilde{*}\,\tilde{\mathrm{d}}\Omega_3$$
$$= \int_M \delta_3\left[(4H^2 - 2K)\frac{\partial\mathcal{E}}{\partial(2H)} + (2KH)\frac{\partial\mathcal{E}}{\partial K} - 2H\mathcal{E} + p\right]\Omega_3\,\mathrm{d}A$$
$$+ \int_M \left[(4H^2 - 2K)\frac{\partial\mathcal{E}}{\partial(2H)} + (2KH)\frac{\partial\mathcal{E}}{\partial K} - 2H\mathcal{E} + p\right]\Omega_3\delta_3\,\mathrm{d}A$$
$$+ \int_M \delta_3\left[\frac{\partial\mathcal{E}}{\partial(2H)}\right]\mathrm{d}*\mathrm{d}\Omega_3 + \frac{\partial\mathcal{E}}{\partial(2H)}\delta_3(\mathrm{d}*\mathrm{d}\Omega_3)$$
$$+ \int_M \delta_3\left(\frac{\partial\mathcal{E}}{\partial K}\right)\mathrm{d}\,\tilde{*}\,\tilde{\mathrm{d}}\Omega_3 + \frac{\partial\mathcal{E}}{\partial K}\delta_3(\mathrm{d}\,\tilde{*}\,\tilde{\mathrm{d}}\Omega_3). \quad (53)$$

Please notice that Ω_3 can freely come into and out of the expressions acted on by the operator δ_3.

Lemma 3.5. *For every function* f, $\delta_3\,\mathrm{d}*\mathrm{d}f = \mathrm{d}*\mathrm{d}\delta_3 f + \mathrm{d}(2H\Omega_3 * \mathrm{d}f) - 2\mathrm{d}(\Omega_3\,\tilde{*}\,\tilde{\mathrm{d}}f)$.

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

$$\begin{aligned}
&\delta_3 * \mathrm{d}f = (\delta_3 f_1\omega_2 - \delta_3 f_2\omega_1) - \Omega_{312}\,\mathrm{d}f + \Omega_3[f_2(a\omega_1 + b\omega_2) - f_1(b\omega_1 + c\omega_2)],\\
&*\delta_3\,\mathrm{d}f = (\delta_3 f_1\omega_2 - \delta_3 f_2\omega_1) - \Omega_{312}\,\mathrm{d}f + \Omega_3[f_1(b\omega_1 - a\omega_2) + f_2(c\omega_1 - b\omega_2)],\\
&\delta_3 * \mathrm{d}f - *\delta_3\,\mathrm{d}f = 2H\Omega_3 * \mathrm{d}f - 2\Omega_3\,\tilde{*}\,\tilde{\mathrm{d}}f.
\end{aligned} \tag{54}$$

Using the operator d to act on both sides of (54) and noticing the commutativity of d and δ_3, we arrive at this lemma. □

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

Proof. Similar to the proof of lemma 3.5, we have

$$\begin{aligned}
&\delta_3 * \tilde{\mathrm{d}}f = \delta_3(af_1 + bf_2)\omega_2 - \delta_3(bf_1 + cf_2)\omega_1 - K\Omega_3 * \mathrm{d}f - \Omega_{312}\,\tilde{\mathrm{d}}f,\\
&*\delta_3\tilde{\mathrm{d}}f = \delta_3(af_1 + bf_2)\omega_2 - \delta_3(bf_1 + cf_2)\omega_1 - 2H\Omega_3 * \tilde{\mathrm{d}}f + K\Omega_3 * \mathrm{d}f - \Omega_{312}\,\tilde{\mathrm{d}}f.
\end{aligned}$$

The difference of the above two equations gives

$$*\delta_3\tilde{\mathrm{d}}f - \delta_3 * \tilde{\mathrm{d}}f = 2K\Omega_3 * \mathrm{d}f - 2H\Omega_3 * \tilde{\mathrm{d}}f. \tag{55}$$

It is also easy to see

$$*\tilde{\mathrm{d}}f + \tilde{*}\tilde{\mathrm{d}}f = 2H * \mathrm{d}f. \tag{56}$$

Using $\mathrm{d}\delta_3$ to act on both sides of (56) and d to act on both sides of (55), and by considering the commutative relations $\tilde{\mathrm{d}}\delta_3 = \delta_3\,\tilde{\mathrm{d}}$ and $\mathrm{d}\delta_3 = \delta_3\,\mathrm{d}$, we arrive at this lemma. □

If $\mathrm{d}f = f_1\omega_1 + f_2\omega_2$, we define $\nabla f = f_1\,\mathbf{e}_1 + f_2\,\mathbf{e}_2$, $\bar{\nabla} f = (af_1 + bf_2)\,\mathbf{e}_1 + (bf_1 + cf_2)\,\mathbf{e}_2$, $\tilde{\nabla} f = (cf_1 - bf_2)\,\mathbf{e}_1 + (af_2 - bf_1)\,\mathbf{e}_2$ and $\mathrm{d} * \tilde{\mathrm{d}}f = (\nabla\cdot\bar{\nabla})f\omega_1\wedge\omega_2$. It follows that for function f and g,

$$\tilde{\nabla} f + \bar{\nabla} f = 2H\nabla f \tag{57}$$

$$\mathrm{d}f \wedge *\mathrm{d}g = (\nabla f\cdot\nabla g)\omega_1\wedge\omega_2, \tag{58}$$

$$\mathrm{d}f \wedge *\tilde{\mathrm{d}}g = (\nabla f\cdot\bar{\nabla} g)\omega_1\wedge\omega_2, \tag{59}$$

$$\mathrm{d}f \wedge \tilde{*}\tilde{\mathrm{d}}g = (\nabla f\cdot\tilde{\nabla} g)\omega_1\wedge\omega_2. \tag{60}$$

Remark 3.1. The tensor expressions of $\nabla, \bar{\nabla}, \tilde{\nabla}, \nabla^2, \nabla\cdot\bar{\nabla}, \nabla\cdot\tilde{\nabla}$ are developed in appendix D.

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

$$\begin{aligned}
\delta^2\mathcal{F} = &\int_M \Omega_3^2\left[(4H^2 - 2K)^2\frac{\partial^2\mathcal{E}}{\partial(2H)^2} - 4KH\frac{\partial\mathcal{E}}{\partial(2H)} - 2K^2\frac{\partial\mathcal{E}}{\partial K}\right.\\
&\left. + 4KH(4H^2 - 2K)\frac{\partial^2\mathcal{E}}{\partial(2H)\partial K} + 4K^2H^2\frac{\partial^2\mathcal{E}}{\partial K^2} + 2K\mathcal{E} - 2Hp\right]\mathrm{d}A\\
&+\int_M \Omega_3\nabla^2\Omega_3\left[4H\frac{\partial\mathcal{E}}{\partial(2H)} + 4(2H^2 - K)\frac{\partial^2\mathcal{E}}{\partial(2H)^2} + K\frac{\partial\mathcal{E}}{\partial K}\right.\\
&\left. + 4HK\frac{\partial^2\mathcal{E}}{\partial K\partial(2H)} - \mathcal{E} + 8H^2\frac{\partial\mathcal{E}}{\partial K}\right]\mathrm{d}A
\end{aligned}$$

$$
\begin{aligned}
&+\int_M \Omega_3\nabla\cdot\tilde{\nabla}\Omega_3\left[4(2H^2-K)\frac{\partial^2\mathcal{E}}{\partial(2H)\partial K}-4\frac{\partial\mathcal{E}}{\partial(2H)}\right.\\
&\left.+4HK\frac{\partial^2\mathcal{E}}{\partial K^2}-4H\frac{\partial\mathcal{E}}{\partial K}\right]\mathrm{d}A+\int_M(\nabla^2\Omega_3)^2\left[\frac{\partial^2\mathcal{E}}{\partial(2H)^2}+\frac{\partial\mathcal{E}}{\partial K}\right]\mathrm{d}A\\
&+\int_M\left[\frac{2\partial^2\mathcal{E}}{\partial(2H)\partial K}\nabla^2\Omega_3\nabla\cdot\tilde{\nabla}\Omega_3+\frac{\partial\mathcal{E}}{\partial(2H)}\nabla(2H\Omega_3)\cdot\nabla\Omega_3\right]\mathrm{d}A\\
&+\int_M\left[\frac{\partial^2\mathcal{E}}{\partial K^2}(\nabla\cdot\tilde{\nabla}\Omega_3)^2-\frac{2\partial\mathcal{E}}{\partial(2H)}\nabla\Omega_3\cdot\tilde{\nabla}\Omega_3\,\mathrm{d}A\right]\\
&+\int_M\frac{\partial\mathcal{E}}{\partial K}[\nabla(8H^2\Omega_3+\nabla^2\Omega_3)\cdot\nabla\Omega_3-\nabla(4H\Omega_3)\cdot\tilde{\nabla}\Omega_3-4H\Omega_3\nabla\cdot\tilde{\nabla}\Omega_3\\
&-\nabla(2H\Omega_3)\cdot\bar{\nabla}\Omega_3-2H\Omega_3\nabla\cdot\bar{\nabla}\Omega_3]\mathrm{d}A.
\end{aligned}
$$

Proof. Replacing f by Ω_3 in lemmas 3.5 and 3.6 and noticing that Ω_3 is similar to a constant relative to δ_3, we have

$$
\begin{aligned}
&\delta_3\,\mathrm{d}*\mathrm{d}\Omega_3=[\nabla(2H\Omega_3)\cdot\nabla\Omega_3+2H\Omega_3\nabla^2\Omega_3-2\nabla\Omega_3\cdot\tilde{\nabla}\Omega_3-2\Omega_3\nabla\cdot\tilde{\nabla}\Omega_3]\,\mathrm{d}A,\\
&\delta_3\mathrm{d}\,\tilde{*}\,\tilde{\mathrm{d}}\Omega_3=[\nabla(8H^2\Omega_3+\nabla^2\Omega_3)\cdot\nabla\Omega_3+(8H^2\Omega_3+\nabla^2\Omega_3)\nabla^2\Omega_3\\
&-\nabla(4H\Omega_3)\cdot\tilde{\nabla}\Omega_3-4H\Omega_3\nabla\cdot\tilde{\nabla}\Omega_3-\nabla(2H\Omega_3)\cdot\bar{\nabla}\Omega_3-2H\Omega_3\nabla\cdot\bar{\nabla}\Omega_3]\,\mathrm{d}A.
\end{aligned}
$$

Substituting them into (53) and using lemmas 3.2 and 3.3, we can arrive at this theorem through expatiatory calculations. □

In particular, if $\partial\mathcal{E}/\partial K=\bar{k}$ is a constant, (53) is simplified to

$$
\begin{aligned}
\delta^2\mathcal{F}=&\int_M\Omega_3^2\left[(4H^2-2K)^2\frac{\partial^2\mathcal{E}_H}{\partial(2H)^2}-4HK\frac{\partial\mathcal{E}_H}{\partial(2H)}+2K\mathcal{E}_H-2Hp\right]\\
&+\int_M\Omega_3\nabla^2\Omega_3\left[4H\frac{\partial\mathcal{E}_H}{\partial(2H)}+4(2H^2-K)\frac{\partial^2\mathcal{E}_H}{\partial(2H)^2}-\mathcal{E}_H\right]\mathrm{d}A\\
&-\int_M\frac{4\partial\mathcal{E}_H}{\partial(2H)}\Omega_3\nabla\cdot\tilde{\nabla}\Omega_3\,\mathrm{d}A+\int_M\frac{\partial^2\mathcal{E}_H}{\partial(2H)^2}(\nabla^2\Omega_3)^2\,\mathrm{d}A\\
&+\int_M\frac{\partial\mathcal{E}_H}{\partial(2H)}\left[\nabla(2H\Omega_3)\cdot\nabla\Omega_3-2\nabla\Omega_3\cdot\tilde{\nabla}\Omega_3\right]\mathrm{d}A,
\end{aligned}
\tag{61}
$$

where $\mathcal{E}_H=\mathcal{E}-\bar{k}K$.

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 a closed lipid bilayer under osmotic pressure as in (2). Substituting $\mathcal{E}=(k_c/2)(2H+c_0)^2+\bar{k}K+\mu$ into (52), we obtain the shape equation (3). This equation is a 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 this can be found in a recent paper by Landolfi [22].

3.3.1. Constant mean curvature surface. Between 1956 and 1958, Alexandrov proved an unexpected theorem: an embedded surface (i.e. the surface does not intersect with itself) with constant mean curvature in $\mathbb{E}^3$ 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 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 this, we denote $\mathbf{r} = \{u\cos v, u\sin v, z\}$, $\psi = \arctan\left[\frac{dz(u)}{du}\right]$, and $\Psi = \sin\psi$. Thus (3) is transformed into

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

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 note that there are two misprints in our previous paper [13]. The 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 α_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 $\mu/k_c = -2\alpha c_0 - c_0^2/2$ and $p/k_c = -2\alpha^2 c_0$, $\Psi = \alpha u + \sqrt{2}$ 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 $\mathbf{r} = R(\sin\theta\cos\phi, \sin\theta\sin\phi, \cos\theta)$ 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 $\nabla^2 = \frac{1}{R^2\sin\theta}\frac{\partial}{\partial\theta}\left(\sin\theta\frac{\partial}{\partial\theta}\right) + \frac{1}{R^2\sin^2\theta}\frac{\partial^2}{\partial\phi^2}$. If we take $\mathcal{E}_H = (k_c/2)(2H + c_0)^2 + \mu$, (61) is transformed into

$$\delta^2\mathcal{F} = (2c_0k_c/R + pR)\int_0^\pi \sin\theta\,d\theta\int_0^{2\pi} d\phi\,\Omega_3^2 + (k_c c_0 R + 2k_c + pR^3/2)\int_0^\pi \sin\theta\,d\theta\int_0^{2\pi} d\phi\,\Omega_3\nabla^2\Omega_3 + k_c R^2\int_0^\pi \sin\theta\,d\theta\int_0^{2\pi} d\phi\,(\nabla^2\Omega_3)^2. \tag{65}$$

Expand Ω_3 with the spherical harmonic functions [26]:

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

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

$$\delta^2\mathcal{F} = (R/2)\sum_{l,m}|a_{lm}|^2[l(l+1) - 2]\{2k_c/R^3[l(l+1) - c_0R] - p\}. \tag{67}$$

Denote that

$$p_l = (2k_c/R^3)[l(l+1) - c_0R] \qquad (l = 2, 3, \ldots). \tag{68}$$

When $p > p_l$, $\delta^2\mathcal{F}$ can take a negative value. Therefore, we can take the critical pressure as

$$p_c = \min\{p_l\} = p_2 = (2k_c/R^3)(6 - c_0R). \tag{69}$$

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 figure 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

$$\mathcal{F} = \int_M \mathcal{E}(2H[\mathbf{r}], K[\mathbf{r}])\,\mathrm{d}A + \int_C \Gamma(k_n, k_g)\,\mathrm{d}s. \tag{70}$$

Denote $\mathcal{F}_e = \int_M \mathcal{E}(2H[\mathbf{r}], K[\mathbf{r}])\,\mathrm{d}A$, and $\mathcal{F}_C = \int_C \Gamma(k_n, k_g)\,\mathrm{d}s$.

From the calculations of appendix B, we have $\omega_2 = 0$, $\mathrm{d}s = \omega_1$, $k_n = a$, $k_g\,\mathrm{d}s = \omega_{12}$, $\tau_g = b$ in curve C. Using (27)–(36), we can arrive at

$$\delta_1\mathcal{F}_C = \int_C \mathrm{d}(\Gamma\Omega_1) = 0, \tag{71}$$

$$\begin{aligned}\delta_2\mathcal{F}_C = \int_C \Bigg[&\frac{\mathrm{d}^2}{\mathrm{d}s^2}\left(\frac{\partial\Gamma}{\partial k_g}\right) + K\frac{\partial\Gamma}{\partial k_g} - k_g\left(\Gamma - \frac{\partial\Gamma}{\partial k_g}k_g\right)\\ &+ 2(k_n - H)k_g\frac{\partial\Gamma}{\partial k_n} - \tau_g\frac{\mathrm{d}}{\mathrm{d}s}\left(\frac{\partial\Gamma}{\partial k_n}\right) - \frac{\mathrm{d}}{\mathrm{d}s}\left(\tau_g\frac{\partial\Gamma}{\partial k_n}\right)\Bigg]\Omega_2\,\mathrm{d}s,\end{aligned} \tag{72}$$

$$\begin{aligned}\delta_3\mathcal{F}_C = \int_C \Bigg[&\frac{\mathrm{d}^2}{\mathrm{d}s^2}\left(\frac{\partial\Gamma}{\partial k_n}\right) + \frac{\partial\Gamma}{\partial k_n}(k_n^2 - \tau_g^2) + \tau_g\frac{\mathrm{d}}{\mathrm{d}s}\left(\frac{\partial\Gamma}{\partial k_g}\right)\\ &+ \frac{\mathrm{d}}{\mathrm{d}s}\left(\tau_g\frac{\partial\Gamma}{\partial k_g}\right) - \left(\Gamma - \frac{\partial\Gamma}{\partial k_g}k_g\right)k_n\Bigg]\Omega_3\,\mathrm{d}s + \int_C\left(\frac{\partial\Gamma}{\partial k_g}k_n - \frac{\partial\Gamma}{\partial k_n}k_g\right)\Omega_{323}\,\mathrm{d}s.\end{aligned} \tag{73}$$

Additionally, (39), (49) and (50) are still applicable. Consequently,

$$\delta_1\mathcal{F}_e = \int_M \mathrm{d}(\mathcal{E}\omega_2\Omega_1) = \int_C \mathcal{E}\omega_2\Omega_1 = 0, \tag{74}$$

$$\delta_2\mathcal{F}_e = -\int_M \mathrm{d}(\mathcal{E}\omega_1\Omega_2) = -\int_C \mathcal{E}\Omega_2\,\mathrm{d}s, \tag{75}$$

$$\begin{aligned}\delta_3\mathcal{F}_e = \int_M &\left[(\nabla^2 + 4H^2 - 2K)\frac{\partial\mathcal{E}}{\partial(2H)} + (\nabla\cdot\tilde{\nabla} + 2KH)\frac{\partial\mathcal{E}}{\partial K} - 2H\mathcal{E}\right]\Omega_3\,\mathrm{d}A\\ &+ \int_C\left[\mathbf{e}_2\cdot\nabla\left[\frac{\partial\mathcal{E}}{\partial(2H)}\right] + \mathbf{e}_2\cdot\tilde{\nabla}\left(\frac{\partial\mathcal{E}}{\partial K}\right) - \frac{\mathrm{d}}{\mathrm{d}s}\left(\frac{\partial\mathcal{E}}{\partial K}\right)\right]\Omega_3\,\mathrm{d}s\\ &+ \int_C\left[-\frac{\partial\mathcal{E}}{\partial(2H)} - k_n\frac{\partial\mathcal{E}}{\partial K}\right]\Omega_{323}\,\mathrm{d}s.\end{aligned} \tag{76}$$

The functions Ω_{323}, Ω_2, and Ω_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

$$(\nabla^2 + 4H^2 - 2K)\frac{\partial\mathcal{E}}{\partial(2H)} + (\nabla\cdot\tilde{\nabla} + 2KH)\frac{\partial\mathcal{E}}{\partial K} - 2H\mathcal{E} = 0, \tag{77}$$

$$\mathbf{e}_2\cdot\nabla\left[\frac{\partial\mathcal{E}}{\partial(2H)}\right] + \mathbf{e}_2\cdot\tilde{\nabla}\left(\frac{\partial\mathcal{E}}{\partial K}\right) - \frac{\mathrm{d}}{\mathrm{d}s}\left(\tau_g\frac{\partial\mathcal{E}}{\partial K}\right) + \frac{\mathrm{d}^2}{\mathrm{d}s^2}\left(\frac{\partial\Gamma}{\partial k_n}\right) + \frac{\partial\Gamma}{\partial k_n}\left(k_n^2 - \tau_g^2\right)$$
$$+ \tau_g\frac{\mathrm{d}}{\mathrm{d}s}\left(\frac{\partial\Gamma}{\partial k_g}\right) + \frac{\mathrm{d}}{\mathrm{d}s}\left(\tau_g\frac{\partial\Gamma}{\partial k_g}\right) - \left(\Gamma - \frac{\partial\Gamma}{\partial k_g}k_g\right)k_n\bigg|_C = 0, \tag{78}$$

$$-\frac{\partial\mathcal{E}}{\partial(2H)} - k_n\frac{\partial\mathcal{E}}{\partial K} + \frac{\partial\Gamma}{\partial k_g}k_n - \frac{\partial\Gamma}{\partial k_n}k_g\bigg|_C = 0, \tag{79}$$

$$\frac{\mathrm{d}^2}{\mathrm{d}s^2}\left(\frac{\partial\Gamma}{\partial k_g}\right) + K\frac{\partial\Gamma}{\partial k_g} - k_g\left(\Gamma - \frac{\partial\Gamma}{\partial k_g}k_g\right) + 2(k_n - H)k_g\frac{\partial\Gamma}{\partial k_n}$$
$$-\tau_g\frac{\mathrm{d}}{\mathrm{d}s}\left(\frac{\partial\Gamma}{\partial k_n}\right) - \frac{\mathrm{d}}{\mathrm{d}s}\left(\tau_g\frac{\partial\Gamma}{\partial k_n}\right) - \mathcal{E}\bigg|_C = 0. \tag{80}$$

In the 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 $\Gamma = \frac{1}{2}k_b\left(k_n^2 + k_g^2\right) + \gamma$ with k_b and γ being constants. In this case, (77)–(80) are transformed into

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

$$k_b\left[\mathrm{d}^2k_n/\mathrm{d}s^2 + k_n\left(\kappa^2/2 - \tau_g^2\right) + \tau_g\,\mathrm{d}k_g/\mathrm{d}s + \mathrm{d}(\tau_g k_g)/\mathrm{d}s\right]$$
$$+ k_c\mathbf{e}_2\cdot\nabla(2H) - \bar{k}\,\mathrm{d}\tau_g/\mathrm{d}s - \gamma k_n|_C = 0, \tag{82}$$

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

$$k_b\left[\mathrm{d}^2k_g/\mathrm{d}s^2 + k_g\left(\kappa^2/2 - \tau_g^2\right) - \tau_g\,\mathrm{d}k_n/\mathrm{d}s - \mathrm{d}(\tau_g k_n)/\mathrm{d}s\right]$$
$$- [(k_c/2)(2H + c_0)^2 + \bar{k}K + \mu + \gamma k_g]|_C = 0, \tag{84}$$

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 a point on the surface M along the $\mathbf{e}_3$ direction; (82) represents the force equilibrium equation of a point on the curve C along the $\mathbf{e}_3$ direction; (83) represents the bending moment equilibrium equation of a point on the curve C around the $\mathbf{e}_1$ direction; (84) represents the force equilibrium equation of a point on the curve C along the $\mathbf{e}_2$ direction.

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

$$k_c\mathbf{e}_2\cdot\nabla(2H) - \bar{k}\,\mathrm{d}\tau_g/\mathrm{d}s - \gamma k_n|_C = 0, \tag{85}$$

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

4.3. Two-component lipid bilayer

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

$$\mathcal{F} = p\int_V \mathrm{d}V + \int_{M_I}\left[\left(k_c^I/2\right)\left(2H + c_0^I\right)^2 + \bar{k}^I K + \mu^I\right]\mathrm{d}A \\ + \int_{M_{II}}\left[\left(k_c^{II}/2\right)\left(2H + c_0^{II}\right)^2 + \bar{k}^{II} K + \mu^{II}\right]\mathrm{d}A + \gamma\int_C \mathrm{d}s. \tag{87}$$

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

$$p - 2\mu^i H + k_c^i(2H + c_0)\left(2H^2 - c_0^i H - 2K\right) + k_c^i\nabla^2(2H) = 0, \tag{88}$$

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

$$k_c^I\mathbf{e}_2\cdot\nabla(2H) - \bar{k}^I\,\mathrm{d}\tau_g/\mathrm{d}s + k_c^{II}\mathbf{e}_2\cdot\nabla(2H) - \bar{k}^{II}\,\mathrm{d}\tau_g/\mathrm{d}s - \gamma k_n|_C = 0, \tag{89}$$

$$k_c^I\left(2H + c_0^I\right) + \bar{k}^I k_n - \left[k_c^{II}\left(2H + c_0^{II}\right) + \bar{k}^{II}k_n\right]|_C = 0, \tag{90}$$

$$\left(k_c^I/2\right)\left(2H + c_0^I\right)^2 + \bar{k}^I K + \mu^I - \left[\left(k_c^{II}/2\right)\left(2H + c_0^{II}\right)^2 + \bar{k}^{II}K + \mu^{II}\right] + \gamma k_g|_C = 0. \tag{91}$$

In the above equations, the positive direction of curve C is set along $\mathbf{e}_1$ of the lipid domain consisting of component I. Furthermore, (88)–(91) are also applied to describe the closed bilayer with more than two domains. However, 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 derive the free energy of a cell membrane with cross-linking protein structure by analogy with rubber elasticity. Secondly, we derive the shape equation and in-plane strain equations of cell membranes by taking the first order variation of the free energy. Lastly, we discuss the mechanical stability of spherical cell membranes.

5.1. The free energy of cell membranes

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

$$\epsilon = \begin{pmatrix} \varepsilon_{xx} & \varepsilon_{xy} & 0 \\ \varepsilon_{xy} & \varepsilon_{yy} & 0 \\ 0 & 0 & \varepsilon_{zz} \end{pmatrix} \tag{92}$$

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

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

$$Z = \int_{\mathbf{R}_0}^{\mathbf{R}_N} D[\mathbf{R}_n] \exp\left[-\frac{3}{2L^2}\int_0^N \mathrm{d}n \left(\frac{\partial \mathbf{R}_n}{\partial n}\right)^2\right] = \sigma \exp\left[-\frac{3(\mathbf{R}_N - \mathbf{R}_0)^2}{2NL^2}\right],$$

where σ is a constant, and L is the segment length. After undergoing the strains, the partition function is changed to

$$Z_\epsilon = \sigma \exp\left[-\frac{3(\mathbf{R}_N - \mathbf{R}_0)_\epsilon^2}{2NL^2}\right].$$

Considering the relation $(\mathbf{R}_N - \mathbf{R}_0)_\epsilon^2 = [(1+\epsilon)\cdot(\mathbf{R}_N - \mathbf{R}_0)]^2$ and the distribution function of end-to-end distance $P(\mathbf{R}_N - \mathbf{R}_0) = \frac{Z}{\int \mathrm{d}\mathbf{r}_N Z}$, we can obtain the free energy change as a result of the strains:

$$\begin{aligned} f_s &= -k_B T \int \mathrm{d}\mathbf{R}_N (\ln Z_\epsilon - \ln Z) P(\mathbf{R}_N - \mathbf{R}_0) \\ &= k_B T\left[(\varepsilon_{xx} + \varepsilon_{yy})^2 - \left(\varepsilon_{xx}\varepsilon_{yy} - \varepsilon_{xy}^2\right)\right]. \end{aligned} \tag{93}$$

For the cell membrane with cross-linking structure, we assume the following. (i) The membrane is a smooth surface and junction points between protein chains are confined in the vicinity of the surface and freely depart from it within the range of $\pm h/2$, where h is the thickness of the membrane. (ii) There is no change of volume occupied by the cross-linking structure on deformation. On average, there are $\mathcal{N}$ 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 the closed lipid bilayer and the cross-linking structure. The free energy of the cross-linking structure is the sum of the free energies of individual protein chains.

If the cell membrane undergoes a 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 in a similar form to (93) under above assumptions (i)–(iv). Using the above assumption (v), we have the free energy of the cell membrane under osmotic pressure p:

$$\mathcal{F} = \int_M (\mathcal{E}_d + \mathcal{E}_H)\, \mathrm{d}A + p\int_V \mathrm{d}V, \tag{94}$$

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

Remark 5.1. We do not write the term of $\bar{k}K$ in (94) because $\int_M \bar{k}K\, \mathrm{d}A$ is constant for a closed surface (see appendix C).

5.2. Strain analysis

If a point $\mathbf{r}_0$ in a surface undergoes a displacement $\mathbf{u}$ to arrive at point $\mathbf{r}$, we have $\mathrm{d}\mathbf{u} = \mathrm{d}\mathbf{r} - \mathrm{d}\mathbf{r}_0$ and $\delta_i\, \mathrm{d}\mathbf{u} = \delta_i\, \mathrm{d}\mathbf{r}$ $(i = 1, 2, 3)$.

If we denote $\mathrm{d}\mathbf{r} = \omega_1\, \mathbf{e}_1 + \omega_2\, \mathbf{e}_2$ and $\mathrm{d}\mathbf{u} = \mathbf{U}_1\omega_1 + \mathbf{U}_2\omega_2$ with $|\mathbf{U}_1| \ll 1$, $|\mathbf{U}_2| \ll 1$, we can define the strains [31]:

$$\varepsilon_{11} = \left[\frac{\mathrm{d}\mathbf{u}\cdot\mathbf{e}_1}{|\mathrm{d}\mathbf{r}_0|}\right]_{\omega_2=0} \approx \mathbf{U}_1\cdot\mathbf{e}_1, \tag{95}$$

$$\varepsilon_{22} = \left[\frac{\mathrm{d}\mathbf{u}\cdot\mathbf{e}_2}{|\mathrm{d}\mathbf{r}_0|}\right]_{\omega_1=0} \approx \mathbf{U}_2\cdot\mathbf{e}_2, \tag{96}$$

$$\varepsilon_{12} = \frac{1}{2}\left[\left(\frac{\mathrm{d}\mathbf{u}\cdot\mathbf{e}_2}{|\mathrm{d}\mathbf{r}_0|}\right)_{\omega_2=0} + \left(\frac{\mathrm{d}\mathbf{u}\cdot\mathbf{e}_1}{|\mathrm{d}\mathbf{r}_0|}\right)_{\omega_1=0}\right] \approx \frac{1}{2}\left(\mathbf{U}_1\cdot\mathbf{e}_2 + \mathbf{U}_2\cdot\mathbf{e}_1\right). \tag{97}$$

Using $\delta_i\,\mathrm{d}\mathbf{u} = \delta_i\,\mathrm{d}\mathbf{r}$ and the definitions of strains (95)–(97), we can obtain the variational relations:

$$\begin{aligned}
\delta_i\varepsilon_{11}\omega_1\wedge\omega_2 &= (1-\varepsilon_{11})\delta_i\omega_1\wedge\omega_2 - \mathbf{U}_2\cdot\mathbf{e}_1\delta_i\omega_2\wedge\omega_2\\
&\quad + \Omega_{i12}\mathbf{U}_1\cdot\mathbf{e}_2\omega_1\wedge\omega_2 + \Omega_{i13}\mathbf{U}_1\cdot\mathbf{e}_3\omega_1\wedge\omega_2,\\
\delta_i\varepsilon_{12}\omega_1\wedge\omega_2 &= \tfrac{1}{2}[(1-\varepsilon_{11})\omega_1\wedge\delta_i\omega_1 + (1-\varepsilon_{22})\delta_i\omega_2\wedge\omega_2 - \mathbf{U}_2\cdot\mathbf{e}_1\omega_1\wedge\delta_i\omega_2\\
&\quad - \mathbf{U}_1\cdot\mathbf{e}_2\delta_i\omega_1\wedge\omega_2 + \Omega_{i21}(\varepsilon_{11}-\varepsilon_{22})\omega_1\wedge\omega_2\\
&\quad + \Omega_{i23}\mathbf{U}_1\cdot\mathbf{e}_3\omega_1\wedge\omega_2 + \Omega_{i13}\mathbf{U}_2\cdot\mathbf{e}_3\omega_1\wedge\omega_2],\\
\delta_i\varepsilon_{22}\omega_1\wedge\omega_2 &= (1-\varepsilon_{22})\omega_1\wedge\delta_i\omega_2 - \mathbf{U}_1\cdot\mathbf{e}_2\omega_1\wedge\delta_i\omega_1\\
&\quad + \Omega_{i21}\mathbf{U}_2\cdot\mathbf{e}_1\omega_1\wedge\omega_2 + \Omega_{i23}\mathbf{U}_2\cdot\mathbf{e}_3\omega_1\wedge\omega_2.
\end{aligned}$$

The leading terms of the above relations are:

$$\delta_i\varepsilon_{11}\omega_1\wedge\omega_2 = \delta_i\omega_1\wedge\omega_2, \tag{98}$$

$$\delta_i\varepsilon_{12}\omega_1\wedge\omega_2 = \tfrac{1}{2}[\omega_1\wedge\delta_i\omega_1 + \delta_i\omega_2\wedge\omega_2], \tag{99}$$

$$\delta_i\varepsilon_{22}\omega_1\wedge\omega_2 = \omega_1\wedge\delta_i\omega_2. \tag{100}$$

Thus,

$$\delta_i(2J)\omega_1\wedge\omega_2 = \delta_i(\varepsilon_{11}+\varepsilon_{22})\omega_1\wedge\omega_2 = \delta_i\omega_1\wedge\omega_2 + \omega_1\wedge\delta_i\omega_2,$$

$$\delta_i Q\omega_1\wedge\omega_2 = \delta_i(\varepsilon_{11}\varepsilon_{22}-\varepsilon_{12}^2)\omega_1\wedge\omega_2 \tag{101}$$

$$= \left(\varepsilon_{11}\omega_1+\varepsilon_{12}\omega_2\right)\wedge\delta_i\omega_2 - (\varepsilon_{12}\omega_1+\varepsilon_{22}\omega_2)\wedge\delta_i\omega_1. \tag{102}$$

Considering equations (27)–(35), (101) and (102), we have

$$\delta_1(2J)\omega_1\wedge\omega_2 = \mathrm{d}(\Omega_1\omega_2), \tag{103}$$

$$\delta_1 Q\omega_1\wedge\omega_2 = (\varepsilon_{11}\,\mathrm{d}\omega_2 - \varepsilon_{12}\,\mathrm{d}\omega_1)\Omega_1 - (\varepsilon_{12}\omega_1+\varepsilon_{22}\omega_2)\wedge\mathrm{d}\Omega_1; \tag{104}$$

$$\delta_2(2J)\omega_1\wedge\omega_2 = -\mathrm{d}(\Omega_2\omega_1), \tag{105}$$

$$\delta_2 Q\omega_1\wedge\omega_2 = (\varepsilon_{11}\omega_1+\varepsilon_{12}\omega_2)\wedge\mathrm{d}\Omega_2 + \Omega_2(\varepsilon_{12}\,\mathrm{d}\omega_2 - \varepsilon_{22}\,\mathrm{d}\omega_1); \tag{106}$$

$$\delta_3(2J)\omega_1\wedge\omega_2 = -2H\Omega_3\,\mathrm{d}A, \tag{107}$$

$$\delta_3 Q\omega_1\wedge\omega_2 = [-2H(2J) + a\varepsilon_{11} + 2b\varepsilon_{12} + c\varepsilon_{22}]\Omega_3\,\mathrm{d}A. \tag{108}$$

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 (94). Denote $\mathcal{F}_d = \int_M \mathcal{E}_d\,\mathrm{d}A$ and $\mathcal{F}_{cp} = \int_M \mathcal{E}_H\,\mathrm{d}A + p\int_V \mathrm{d}V$.

From (103)–(108), we can calculate that:

$$\delta_1\mathcal{F}_d = \int_M \left[\frac{\partial\mathcal{E}_d}{\partial(2J)}\delta_1(2J)\,\mathrm{d}A + \frac{\partial\mathcal{E}_d}{\partial Q}\delta_1 Q\,\mathrm{d}A + \mathcal{E}_d(2J, Q)\delta_1\,\mathrm{d}A\right]$$
$$= -\int_M \mathrm{d}\left[\frac{\partial\mathcal{E}_d}{\partial(2J)} + \mathcal{E}_d(2J, Q)\right]\wedge\omega_2\Omega_1 + \int_M \frac{\partial\mathcal{E}_d}{\partial Q}(\varepsilon_{11}\,\mathrm{d}\omega_2 - \varepsilon_{12}\,\mathrm{d}\omega_1)\Omega_1$$
$$- \int_M \Omega_1\mathrm{d}\left[(\varepsilon_{12}\omega_1 + \varepsilon_{22}\omega_2)\frac{\partial\mathcal{E}_d}{\partial Q}\right], \tag{109}$$

$$\delta_2\mathcal{F}_d = \int_M \left[\frac{\partial\mathcal{E}_d}{\partial(2J)}\delta_2(2J)\,\mathrm{d}A + \frac{\partial\mathcal{E}_d}{\partial Q}\delta_2 Q\,\mathrm{d}A + \mathcal{E}_d(2J, Q)\delta_2\,\mathrm{d}A\right]$$
$$= \int_M \mathrm{d}\left[\frac{\partial\mathcal{E}_d}{\partial(2J)} + \mathcal{E}_d(2J, Q)\right]\wedge\omega_1\Omega_2 + \int_M \frac{\partial\mathcal{E}_d}{\partial Q}(\varepsilon_{12}\,\mathrm{d}\omega_2 - \varepsilon_{22}\,\mathrm{d}\omega_1)\Omega_2$$
$$= + \int_M \Omega_2\,\mathrm{d}\left[\frac{\partial\mathcal{E}_d}{\partial Q}[(\varepsilon_{11}\omega_1 + \varepsilon_{12}\omega_2)\right], \tag{110}$$

$$\delta_3\mathcal{F}_d = \int_M \left[\frac{\partial\mathcal{E}_d}{\partial(2J)}\delta_3(2J)\,\mathrm{d}A + \frac{\partial\mathcal{E}_d}{\partial Q}\delta_3 Q\mathrm{d}A + \mathcal{E}_d(2J, Q)\delta_3\,\mathrm{d}A\right]$$
$$= \int_M \frac{\partial\mathcal{E}_d}{\partial Q}[a\varepsilon_{11} + 2b\varepsilon_{12} + c\varepsilon_{22}]\Omega_3\,\mathrm{d}A$$
$$- \int_M 2H\left[\frac{\partial\mathcal{E}_d}{\partial(2J)} + \mathcal{E}_d + (2J)\frac{\partial\mathcal{E}_d}{\partial Q}\right]\Omega_3\,\mathrm{d}A. \tag{111}$$

Otherwise, section 3 tells us:

$$\delta_1\mathcal{F}_{cp} = \delta_2\mathcal{F}_{cp} = 0,$$
$$\delta_3\mathcal{F}_{cp} = \int_M \left[(\nabla^2 + 4H^2 - 2K)\frac{\partial\mathcal{E}_H}{\partial(2H)} - 2H\mathcal{E}_H + p\right]\Omega_3\,\mathrm{d}A.$$

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

$$-\mathrm{d}\left[\frac{\partial\mathcal{E}_d}{\partial(2J)} + \mathcal{E}_d\right]\wedge\omega_2 + \frac{\partial\mathcal{E}_d}{\partial Q}(\varepsilon_{11}\,\mathrm{d}\omega_2 - \varepsilon_{12}\,\mathrm{d}\omega_1) - \mathrm{d}\left[(\varepsilon_{12}\omega_1 + \varepsilon_{22}\omega_2)\frac{\partial\mathcal{E}_d}{\partial Q}\right] = 0, \tag{112}$$

$$\mathrm{d}\left[\frac{\partial\mathcal{E}_d}{\partial(2J)} + \mathcal{E}_d\right]\wedge\omega_1 + \frac{\partial\mathcal{E}_d}{\partial Q}(\varepsilon_{12}\,\mathrm{d}\omega_2 - \varepsilon_{22}\,\mathrm{d}\omega_1) + \mathrm{d}\left[\frac{\partial\mathcal{E}_d}{\partial Q}(\varepsilon_{11}\omega_1 + \varepsilon_{12}\omega_2)\right] = 0, \tag{113}$$

$$(\nabla^2 + 4H^2 - 2K)\frac{\partial\mathcal{E}_H}{\partial(2H)} - 2H\left[\mathcal{E}_H + \frac{\partial\mathcal{E}_d}{\partial(2J)} + \mathcal{E}_d + (2J)\frac{\partial\mathcal{E}_d}{\partial Q}\right]$$
$$+ p + \frac{\partial\mathcal{E}_d}{\partial Q}[a\varepsilon_{11} + 2b\varepsilon_{12} + c\varepsilon_{22}] = 0. \tag{114}$$

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

$$k_d[-\mathrm{d}(2J)\wedge\omega_2 - \frac{1}{2}(\varepsilon_{11}\,\mathrm{d}\omega_2 - \varepsilon_{12}\,\mathrm{d}\omega_1) + \frac{1}{2}\,\mathrm{d}(\varepsilon_{12}\omega_1 + \varepsilon_{22}\omega_2)] = 0, \tag{115}$$

$$k_d[\mathrm{d}(2J)\wedge\omega_1-\frac{1}{2}(\varepsilon_{12}\,\mathrm{d}\omega_2-\varepsilon_{22}\,\mathrm{d}\omega_1)-\frac{1}{2}\,\mathrm{d}(\varepsilon_{11}\omega_1+\varepsilon_{12}\omega_2)]=0, \tag{116}$$

$$p-2H(\mu+k_dJ)+k_c(2H+c_0)(2H^2-c_0H-2K)+k_c\nabla^2(2H)$$
$$-\frac{k_d}{2}(a\varepsilon_{11}+2b\varepsilon_{12}+c\varepsilon_{22})=0. \tag{117}$$

(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 ε_{ij} $(i, j = 1, 2)$ are neglected in the above three equations.

Obviously, if $k_d = 0$, then (115) and (116) are identities. Moreover, (117) degenerates into the shape equation (3) of closed lipid bilayers in this case. Otherwise, for small strains, (117) is very close to (3), which may suggest that cross-linking structures have little effect 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 ε is a constant. In this case, the sphere with radius R is the solution of (117) if it satisfies

$$pR^2+(2\mu+3k_d\varepsilon)R+k_cc_0(c_0R-2)=0. \tag{118}$$

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 $\mathcal{F}$. From the mathematical point of view presented in section 2, we must calculate $\delta_i\delta_j\mathcal{F}$ $(i, j = 1, 2, 3)$. But from the physical and symmetric points of view, we just need to calculate $\delta_3^2\mathcal{F}$ because we can expect that the perturbations along the normal are primary to the instabilities of spherical membranes under osmotic pressure, which is perpendicular to the sphere surfaces.

If we take $\mathcal{E}_H = \frac{k_c}{2}(2H + c_0)^2 + \mu$ and $\mathcal{E}_d = \frac{k_d}{2}[(2J)^2 - Q]$, the leading term of (111) is

$$\delta_3\mathcal{F}_d=-\frac{k_d}{2}\int_M[(a\varepsilon_{11}+2b\varepsilon_{12}+c\varepsilon_{22})+2H(2J)]\,\Omega_3\,\mathrm{d}A. \tag{119}$$

Using lemmas 3.1, 3.2 and equations (98)–(100), we can obtain

$$\begin{aligned}\delta_3^2\mathcal{F}_d&=-\frac{k_d}{2}\int_M[(\delta_3a\varepsilon_{11}+2\delta_3b\varepsilon_{12}+\delta_3c\varepsilon_{22})+\delta_3(2H)(2J)]\,\Omega_3\,\mathrm{d}A\\&\quad-\frac{k_d}{2}\int_M[(a\delta_3\varepsilon_{11}+2b\delta_3\varepsilon_{12}+c\delta_3\varepsilon_{22})+2H\delta_3(2J)]\,\Omega_3\,\mathrm{d}A\\&\quad-\frac{k_d}{2}\int_M[(a\varepsilon_{11}+2b\varepsilon_{12}+c\varepsilon_{22})+2H(2J)]\,\Omega_3\delta_3\,\mathrm{d}A\\&=k_d\int_M\frac{3(1+\varepsilon)}{R^2}\Omega_3^2\,\mathrm{d}A-\frac{3k_d\varepsilon}{2}\int_M\Omega_3\nabla^2\Omega_3\,\mathrm{d}A,\end{aligned} \tag{120}$$

for a spherical cell membrane with radius R and strain ε.

Additionally, (61) suggests that

$$\begin{aligned}\delta_3^2\mathcal{F}_{cp}&=\int_M\Omega_3^2\{k_cc_0^2/R^2+2\mu/R^2+2p/R\}\,\mathrm{d}A\\&\quad+\int_M\Omega_3\nabla^2\Omega_3\{2k_cc_0/R+2k_c/R^2-\mu-k_cc_0^2/2\}\,\mathrm{d}A+\int_Mk_c(\nabla^2\Omega_3)^2\,\mathrm{d}A.\end{aligned} \tag{121}$$

Therefore

$$\begin{aligned}\delta_3^2\mathcal{F} &= \delta_3^2\mathcal{F}_d + \delta_3^2\mathcal{F}_{cp}\\ &= \int_M \Omega_3^2\big\{3k_d/R^2 + \big(3k_d\varepsilon + k_c c_0^2 + 2\mu\big)\big/R^2 + 2p/R\big\}\,\mathrm{d}A\\ &\quad + \int_M \Omega_3\nabla^2\Omega_3\big\{2k_c c_0/R + 2k_c/R^2 - \big(3k_d\varepsilon + k_c c_0^2 + 2\mu\big)\big/2\big\}\,\mathrm{d}A\\ &\quad + \int_M k_c(\nabla^2\Omega_3)^2\,\mathrm{d}A. \end{aligned} \tag{122}$$

In considering (118) and expanding Ω_3 as (66), we have

$$\begin{aligned}\delta_3^2\mathcal{F} &= \int_M \Omega_3^2\{3k_d/R^2 + (2k_c c_0/R^3) + p/R\}\,\mathrm{d}A\\ &\quad + \int_M \Omega_3\nabla^2\Omega_3\{k_c c_0/R + 2k_c/R^2 + pR/2\}\,\mathrm{d}A + \int_M k_c(\nabla^2\Omega_3)^2\,\mathrm{d}A\\ &= \sum_{l,m}|a_{lm}|^2\{3k_d + [l(l+1)-2][l(l+1)k_c/R^2 - k_c c_0/R - pR/2]\}.\end{aligned}$$

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

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

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

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

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

$$\frac{\mathrm{d}p}{\mathrm{d}\xi} = -\frac{6k_d/R}{(\xi-2)^2} + \frac{2k_c}{R^3}, \tag{125}$$

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

$\mathrm{d}p/\mathrm{d}\xi = 0$ and $\xi \geqslant 6$ imply $\xi = 2 + R\sqrt{3k_d/k_c}$ which is valid only if $3k_dR^2 > 16k_c$. Therefore, the critical pressure is:

$$p_c = \min\{p_l\} = \begin{cases} \frac{3k_d}{2R} + \frac{2k_c(6-c_0R)}{R^3} < \frac{2k_c(10-c_0R)}{R^3} & (3k_dR^2 < 16k_c),\\ \frac{4\sqrt{3k_dk_c}}{R^2} + \frac{2k_c}{R^3}(2-c_0R) & (3k_dR^2 > 16k_c). \end{cases} \tag{127}$$

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

Also, if we take the typical parameters of cell membranes as $k_c \sim 20k_BT$ [4, 5], $k_d \sim 2.4\ \mu\mathrm{N\,m^{-1}}$ [34], $h \sim 4\,\mathrm{nm}$, $R \sim 1\,\mu\mathrm{m}$ and $c_0R \sim 1$, 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 stability of cell membranes.

6. Conclusion

In the 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 strain 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: equations (27)–(36).
(ii) The general expressions of the second order variation of the free energy for closed lipid bilayers: theorem 3.3 and equation (61).
(iii) The general shape equation and boundary conditions of open lipid bilayers and two-component lipid bilayers: equations (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) for losing stability of spherical cell membranes. The result includes the critical pressures not only for closed lipid bilayers, but also for the classic solid shells. It also suggests that cross-linking protein structures can enhance the stability of cell membranes.

In the future, we will devote ourselves to applying the above results to explain the shapes of open lipid bilayers found by Saitoh *et al*, and to 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.

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Appendix A. Exterior differential forms and Stokes' theorem

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

We call the function $f(u^1, u^2, \ldots, u^m)$ 0-form and $a_i(u^1, u^2, \ldots, u^m)\,\mathrm{d}u^i$ 1-form, where Einstein's summation rule has been used and is also used in the following. The r-form ($r \leqslant m$) is defined as $a_{i_1 i_2 \cdots i_r}\mathrm{d}u^{i_1} \wedge \mathrm{d}u^{i_2} \wedge \cdots \wedge \mathrm{d}u^{i_r}$, where the exterior production '$\wedge$' satisfies $\mathrm{d}u^i \wedge \mathrm{d}u^j = -\mathrm{d}u^j \wedge \mathrm{d}u^i$. Denote $\Lambda^r = \{\text{all } r\text{-forms}\}$, $(r = 0, 1, 2, \ldots, m)$.

Definition. *A linear operator* $d : \Lambda^r \to \Lambda^{r+1}$ *is called the exterior differential operator if it satisfies:*

(i) for function $f(u^1, u^2, \ldots, u^m)$, $\mathrm{d}f = \frac{\partial f}{\partial u^i}\mathrm{d}u^i$ *is an ordinary differential;*

(ii) $\mathrm{dd} = 0$*;*

(iii) $\forall\omega_1 \in \Lambda^r$ *and* $\forall\omega_2 \in \Lambda^k$, $\mathrm{d}(\omega_1 \wedge \omega_2) = \mathrm{d}\omega_1 \wedge \omega_2 + (-1)^r \omega_1 \wedge \mathrm{d}\omega_2$.

Stokes' theorem. *If* ω *is an* $(m-1)$*-form with compact support set on* M*, and* $\mathcal{D}$ *is a domain with boundary* $\partial\mathcal{D}$ *in* M*, then*

$$\int_{\mathcal{D}} \mathrm{d}\omega = \int_{\partial\mathcal{D}} \omega. \tag{A.1}$$

Appendix B. Curves in a surface

If a curve passes through a point P on the surface, we construct a Frenet frame $\{\mathbf{T}, \mathbf{N}, \mathbf{B}\}$ such that $\mathbf{T}$, $\mathbf{N}$ and $\mathbf{B}$ are the tangent, normal and binormal vectors of the curve respectively. Denote θ the angle between $\mathbf{e}_1$ and $\mathbf{T}$. Set $\mathbf{M} = \mathbf{e}_3 \times \mathbf{T}$. Thus we have

$$\begin{cases} \mathbf{T} = \mathbf{e}_1 \cos\theta + \mathbf{e}_2 \sin\theta, \\ \mathbf{M} = -\mathbf{e}_1 \sin\theta + \mathbf{e}_2 \cos\theta. \end{cases}$$

It is not hard to calculate

$$\mathrm{d}\mathbf{T} = (\mathrm{d}\theta + \omega_{12})\mathbf{M} + \mathbf{e}_3(\omega_{13}\cos\theta + \omega_{23}\sin\theta).$$

Frenet formulae tell us $\mathrm{d}\mathbf{T}/\mathrm{d}s = \kappa\mathbf{N}$. Therefore, we have the geodesic curvature, the geodesic torsion, and the normal curvature of the curve:

$$\begin{aligned} k_g &= \kappa\mathbf{N}\cdot\mathbf{M} = (\mathrm{d}\mathbf{T}/\mathrm{d}s)\cdot\mathbf{M} = (\mathrm{d}\theta + \omega_{12})/\mathrm{d}s, \\ \tau_g &= -(\mathrm{d}\mathbf{e}_3/\mathrm{d}s)\cdot\mathbf{M} = [(b\omega_1 + c\omega_2)\cos\theta - (a\omega_1 + b\omega_2)(\sin\theta)]/\mathrm{d}s, \\ k_n &= II/I = \left(a\omega_1^2 + 2b\omega_1\omega_2 + c\omega_2^2\right)\big/\left(\omega_1^2 + \omega_2^2\right). \end{aligned} \tag{B.1}$$

If the curve is along $\mathbf{e}_1$ such that $\theta = 0$, we have $\mathrm{d}s = \omega_1$, $\omega_2 = 0$ and

$$k_g = \omega_{12}/\omega_1, \qquad \tau_g = b, \qquad \text{and} \qquad k_n = a. \tag{B.2}$$

Appendix C. Gauss–Bonnet formula

Using (9), (10) and (13), we have

$$\mathrm{d}\omega_{12} = -K\omega_1 \wedge \omega_2. \tag{C.1}$$

This formula was called *Theorem Egregium* by Gauss. From *Theorem Egregium* and (B.1), we can derive the Gauss–Bonnet formula:

$$\int_M K\,\mathrm{d}A + \int_C k_g\,\mathrm{d}s = 2\pi\chi(M), \tag{C.2}$$

where $\chi(M)$ is the characteristic number of the smooth surface M with smooth edge C. $\chi(M) = 1$ for a simple surface with an edge. For a closed surface, we have

$$\int_M K\,\mathrm{d}A = 2\pi\chi(M). \tag{C.3}$$

Appendix D. The tensor expressions of $\nabla, \bar{\nabla}, \tilde{\nabla}, \nabla^2, \nabla\cdot\bar{\nabla}$, and $\nabla\cdot\tilde{\nabla}$

At every point **r** in the surface, we can take local coordinates (u^1, u^2) where the first and the second fundamental form are denoted by $I = g_{ij}\,\mathrm{d}u^i\,\mathrm{d}u^j$ and $II = L_{ij}\,\mathrm{d}u^i\,\mathrm{d}u^j$ 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

$$\begin{aligned}
\nabla &= g^{ij}\mathbf{r}_i\frac{\partial}{\partial u^j},\\
\bar{\nabla} &= \mathbf{r}_i(2Hg^{ij} - KL^{ij})\frac{\partial}{\partial u^j},\\
\tilde{\nabla} &= KL^{ij}\mathbf{r}_i\frac{\partial}{\partial u^j},\\
\nabla^2 &= \frac{1}{\sqrt{g}}\frac{\partial}{\partial u^i}\left(\sqrt{g}g^{ij}\frac{\partial}{\partial u^j}\right),\\
\nabla\cdot\bar{\nabla} &= \frac{1}{\sqrt{g}}\frac{\partial}{\partial u^i}\left[\sqrt{g}(2Hg^{ij} - KL^{ij})\frac{\partial}{\partial u^j}\right],\\
\nabla\cdot\tilde{\nabla} &= \frac{1}{\sqrt{g}}\frac{\partial}{\partial u^i}\left(\sqrt{g}KL^{ij}\frac{\partial}{\partial u^j}\right).
\end{aligned}$$

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

Proof. If we take orthogonal local coordinates, we have $I = g_{11}(\mathrm{d}u^1)^2 + g_{22}(\mathrm{d}u^2)^2 = \omega_1^2 + \omega_2^2$, which implies $\omega_1 = \sqrt{g_{11}}\,\mathrm{d}u^1$ and $\omega_2 = \sqrt{g_{22}}\,\mathrm{d}u^2$. For function f, on the one hand, we have $\mathrm{d}f(u^1, u^2) = f_1\omega_1 + f_2\omega_2 = f_1\sqrt{g_{11}}\,\mathrm{d}u^1 + f_2\sqrt{g_{22}}\,\mathrm{d}u^2$; on the other hand, we have $\mathrm{d}f = \frac{\partial f}{\partial u^1}\,\mathrm{d}u^1 + \frac{\partial f}{\partial u^2}\,\mathrm{d}u^2$. 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 $II = a\omega_1^2 + 2b\omega_1\omega_2 + c\omega_1^2 = L_{ij}\,\mathrm{d}u^i\,\mathrm{d}u^j$ implies $a = L_{11}/g_{11}, b = L_{12}/\sqrt{g}, c = L_{22}/g_{22}$. Thus $K = ac - b^2 = \left(L_{11}L_{22} - L_{12}^2\right)/g$, and

$$\begin{aligned}
L^{11} &= \frac{L_{22}}{L_{11}L_{22} - L_{12}^2} \quad\Rightarrow\quad L_{22} = gKL^{11};\\
L^{12} &= -\frac{L_{12}}{L_{11}L_{22} - L_{12}^2} \quad\Rightarrow\quad L_{12} = -gKL^{12};\\
L^{22} &= \frac{L_{11}}{L_{11}L_{22} - L_{12}^2} \quad\Rightarrow\quad L_{11} = gKL^{22}.
\end{aligned}$$

Moreover, we have

$$\begin{aligned}
\tilde{*}\tilde{\mathrm{d}}f &= -f_2\omega_{13} + f_1\omega_{23} = -f_2(a\omega_1 + b\omega_2) + f_2(b\omega_1 + c\omega_2)\\
&= \frac{1}{\sqrt{g}}\left(L_{12}\frac{\partial f}{\partial u^1} - L_{11}\frac{\partial f}{\partial u^2}\right)\mathrm{d}u^1 + \frac{1}{\sqrt{g}}\left(L_{22}\frac{\partial f}{\partial u^1} - L_{12}\frac{\partial f}{\partial u^2}\right)\mathrm{d}u^2;\\
\mathrm{d}\tilde{*}\,\tilde{\mathrm{d}}f &= \left\{\frac{\partial}{\partial u^1}\left[\sqrt{g}K\left(L^{11}\frac{\partial f}{\partial u^1} + L^{12}\frac{\partial f}{\partial u^2}\right)\right]\right.\\
&\quad\left. + \frac{\partial}{\partial u^2}\left[\sqrt{g}K\left(L^{12}\frac{\partial f}{\partial u^1} + L^{22}\frac{\partial f}{\partial u^2}\right)\right]\right\}\mathrm{d}u^1\wedge\mathrm{d}u^2.
\end{aligned}$$

Therefore, $\nabla\cdot\tilde{\nabla}f = \frac{d\tilde{*}\tilde{\mathrm{d}}f}{\omega_1\wedge\omega_2} = \frac{1}{\sqrt{g}}\frac{\partial}{\partial u^i}\left(\sqrt{g}KL^{ij}\frac{\partial f}{\partial u^j}\right)$. □

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