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Stability of lipid membranes

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Abstract
Lipid membranes are versatile biological structures that undergo significant structural remodelling, often triggered by instabilities. Since they invariably possess heterogeneous properties, owing to the presence of multiple lipid species and their interactions with proteins, heterogeneity can have a significant impact on their equilibrium state and stability. In this work, we use curvature elasticity to derive the generalized stability criterion for heterogeneous lipid membranes. Our formulation entertains strain energies that go beyond the Helfrich energy and exhibit higher-order dependence on curvature invariants or spatially varying properties.

Keywords
Lipid membranes, stability, second variation, heterogeneity, curvature elasticity

1. Introduction

Lipid molecules are amphipathic molecules with a hydrophilic head group and hydrophobic tails. Because of these antagonistic properties, they self-assemble, in general, into bilayers, to shield the hydrophobic domains from the surrounding aqueous medium [1, 2]. These bilayers define the physical boundaries of the cells and their organelles and act as barriers, thereby maintaining ionic concentrations and pressure gradients across themselves. Lipid molecules are free to diffuse on the surface but any relative reorientation entails an energetic cost. As a result, membranes possess orientational order but lack positional order. The lipid bilayers exhibit a wide variety of morphologies, ranging from spherical vesicles and spherocylindrical shapes in mitochondria to much more complex shapes in endoplasmic reticulum. These shapes undergo drastic changes in response to mechanical, electrical or thermal stimuli during numerous cellular processes [3–5], making it pertinent to study the stability of these versatile structures to comprehend their shape evolution.

Significant work has been done on modelling the physical response of membranes [6–11] (see Deserno's excellent review [12] for more details). Canham [6] and Helfrich [7] were the first to propose a curvature elasticity theory for lipid membranes. The continuum mechanics framework that explicitly accounted for restrictions on material symmetry arising from two-dimensional fluidity of the lipids was derived by Jenkins [8] and Steigmann [11]. Several fundamental studies have investigated the stability of lipid membranes. A number of researchers have provided a rigorous derivation of the second variation for homogeneous membranes with quadratic strain energy (Helfrich–Canham energy) [13–17]. Stability analysis has been applied to investigate the shape transitions of spheres and cylinders. A classic example is the loss of stability in membrane tubules, known as the pearling instability [18, 19]. The stability of flat discs has been recently studied in the context of high density lipoproteins [20] and disc-to-vesicle shape transition [21].

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Table 1. Notation used in the text.

Symbol	Description
θ^α	Parameters describing the surface
$\mathbf{r}(\theta^\alpha)$	Position of any material point on the surface
$\mathbf{a}_\alpha$	Tangent vectors on the surface based on the parameterization θ^α
$a_{\alpha\beta}$	Covariant components of the metric tensor defined on the surface
$b_{\alpha\beta}$	Covariant components of the curvature tensor defined on the surface
E	Total energy of the membrane and the bulk fluid
W	Strain energy per unit area of the membrane in the current configuration
Ω	Reference configuration
ω	Current configuration
H	Mean curvature field on the surface
K	Gaussian curvature field on the surface
λ	Lagrange multiplier, to preserve local area
J	Determinant of the Jacobian matrix
p	Transmembrane pressure
V	Volume enclosed by the fluidic shell
Δ	Surface Laplacian

While these studies have given fundamental insights into the stability of homogeneous membranes with quadratic energy, there is a need to extend the framework to study systems with heterogeneous properties and higher-order bending energies. The impact of heterogeneity on equilibrium configurations has been revealed [22, 23]. The effect of inhomogeneity-dependent instability was recently shown to be a key factor in determining cellular transport via clathrin-mediated endocytosis [24]. In addition, generalized strain energies have been used to study lipid sorting [25, 26] (where stiffness of tubular membranes is observed to be a function of curvature) and phase transitions [27]. In this context, the Legendre–Hadamard condition for the stability of generalized fluidic shells was derived [11, 27, 28], and the stability of fluidic surfaces with multiple phases was investigated [29]. In this work, we extend the model to account for strain energy that can have arbitrary dependence on the mean curvature and the Gaussian curvature. In addition, our model allows for material properties such as bending moduli or preferred curvatures to undergo spatial variation due to heterogeneities induced by lipid composition or protein interactions [22]. We note that stability in continuous systems is only defined with respect to a particular norm and that choices of different norms could lead to different results regarding inferring the stability, the details of which have been discussed by Como and Grimaldi [30]. We, as others, use the second Gâteaux derivative of the energy functional to define the stability criterion. Thus, stability is examined with respect to a weaker norm (also known as the energy norm) for which the variations in the position and its higher-order derivatives are assumed to be bounded.

The outline of the paper is as follows: we briefly revisit the derivation of the first variation for inhomogeneous membranes in Section 2, the second variation of the energy functional is derived in Section 3, and the generalized stability criterion is reduced to the specific case of homogeneous membranes with quadratic energy in Section 4. Table 1 lists the notation used.

2. First variation

Let $\mathbf{r}(\theta^\alpha)$ be the position of a material point on the surface, where θ^α are the surface coordinates that parameterize the surface. The tangent vectors at any point on the surface are given by $\mathbf{r}_{,\alpha} = \mathbf{a}_\alpha$. The strain energy density of an isotropic membrane depends on the mean curvature H and the Gaussian curvature K [8, 11]. Owing to heterogeneities, the strain energy density can explicitly depend on θ^α [22]. In the presence of area and volume constraints, the energy of an isotropic membrane is given by

$$E = \int_\omega W(H, K; \theta^\alpha)\,\mathrm{d}a + \int_\omega \lambda(\theta^\alpha)\,\mathrm{d}a - pV(\omega), \tag{1}$$

where $\lambda(\theta^\alpha)$ is the local Lagrange multiplier associated with the local area constraint commonly known as surface tension; p is the Lagrange multiplier associated with the volume constraint and is commonly referred to as the transmembrane pressure.

The variation of the position vector is given by

$$\dot{\mathbf{r}} = \mathbf{u} = u^{\alpha}\mathbf{a}_{\alpha} + u\mathbf{n}. \tag{2}$$

Here, and henceforth, a superposed dot $\dot{()}$ signifies the derivative with respect to a parameter ϵ (at a fixed value) that generates a family of surfaces $\mathbf{r}(\theta^{\alpha};\epsilon)$. In equation (2), u^{α} are the tangential components, and u is the normal component of the variations. As derived by Steigmann et al. [31], this yields the following variations of the first and second fundamental forms

$$\begin{aligned} \dot{a}_{\alpha\beta} &= u_{\alpha;\beta} + u_{\beta;\alpha} - 2ub_{\alpha\beta}, \\ \dot{b}_{\alpha\beta} &= u^{\lambda}_{;\alpha}b_{\lambda\beta} + u^{\lambda}_{;\beta}b_{\lambda\alpha} + u^{\lambda}b_{\lambda\alpha;\beta} + u_{;\alpha\beta} - ub_{\alpha\lambda}b^{\lambda\beta}. \end{aligned} \tag{3}$$

where the subscripted semicolon $()_{;\alpha}$ denotes the covariant derivative with respect to the metric $a_{\alpha\beta}$ defined on the surface and Δ denotes the surface Laplacian, such that $\Delta() = ()_{;\alpha\beta}a^{\alpha\beta}$. Using these relations, the variations of the mean curvature, the Gaussian curvature and the Jacobian can be computed as [31]

$$\begin{aligned} \dot{H} &= u^{\alpha}H_{,\alpha} + \frac{1}{2}(\Delta u) + u(2H^2 - K), \\ \dot{K} &= u^{\alpha}K_{,\alpha} + 2HKu + \tilde{b}^{\alpha\beta}u_{;\alpha\beta}, \\ &\text{and} \\ \frac{\dot{J}}{J} &= u^{\alpha}_{;\alpha} - 2uH. \end{aligned} \tag{4}$$

With the help of these variations and the procedure outlined in Agrawal and Steigmann [22] and Steigmann et al. [31], the first variation of E can be expressed as

$$\dot{E} = \int_{\omega}\left\{-u^{\alpha}\left(\lambda_{,\alpha} + \frac{\partial W}{\partial\theta^{\alpha}}\right) + uG\right\}\mathrm{d}a, \tag{5}$$

where

$$G = \frac{1}{2}\Delta W_H + (W_K)_{;\alpha\beta}\tilde{b}^{\alpha\beta} + W_H(2H^2 - K) + 2H(KW_K - W) - 2H\lambda - p. \tag{6}$$

Here, we have suppressed the boundary terms, since we restrict our attention to closed geometries in this study.

The first variation in equation (5) then furnishes the equilibrium equations in the tangent plane

$$\lambda_{,\alpha} = -\frac{\partial W}{\partial\theta^{\alpha}}, \tag{7}$$

and along the surface normal

$$G = 0. \tag{8}$$

popularly known as the shape equation.

3. Second variation

The second variation of the position field can be expressed as

$$\ddot{\mathbf{r}} = \left.\frac{\partial^2\mathbf{r}}{\partial\epsilon^2}\right|_{\epsilon=0} = \mathbf{v} = v^{\alpha}\mathbf{a}_{\alpha} + v\mathbf{n}, \tag{9}$$

where v^{α} and v are the tangential and normal components and are independent of the vector components of the first variation. The second variation of the energy E can be computed from equation (5) and is given by

$$\ddot{E} = \int_{\omega}\left\{-\dot{u}^{\alpha}\left(\lambda_{,\alpha} + \frac{\partial W}{\partial\theta^{\alpha}}\right) - u^{\alpha}\frac{\partial\dot{W}}{\partial\theta^{\alpha}} + \dot{u}G + u\dot{G}\right\}\mathrm{d}a, \tag{10}$$

subject to the incompressibility constraint

$$\frac{\dot{J}}{J} = u^{\alpha}_{;\alpha} - 2uH = 0, \tag{11}$$

and the volumetric constraint

$$\dot{V} = \int_{\omega} u \, \mathrm{d}a = 0. \tag{12}$$

The variation of the tangential components of the first variation of the position field $\mathbf{u}$ is given by

$$\begin{aligned} \dot{u}^{\alpha} &= \dot{\mathbf{u}} \cdot \mathbf{a}^{\alpha} + \mathbf{u} \cdot \dot{\mathbf{a}}^{\alpha} \\ &= (\mathbf{v} \cdot \mathbf{a}^{\alpha}) + (\mathbf{u} \cdot \mathbf{u}_{,\beta}) a^{\alpha\beta} - (\mathbf{u} \cdot \mathbf{a}^{\gamma}) a^{\alpha\lambda} \dot{a}_{\lambda\gamma}, \end{aligned} \tag{13}$$

where

$$\begin{aligned} \dot{\mathbf{a}}^{\alpha} &= a^{\alpha\beta} \dot{\mathbf{a}}_{\beta} + \dot{a}^{\alpha\beta} \mathbf{a}_{\beta}, \\ &\text{and} \\ \dot{a}^{\alpha\beta} &= -a^{\alpha\lambda} a^{\beta\gamma} \dot{a}_{\lambda\gamma}. \end{aligned} \tag{14}$$

Using the relation

$$\mathbf{u} \cdot \mathbf{u}_{,\beta} = \frac{1}{2} (\mathbf{u} \cdot \mathbf{u})_{,\beta} = u^{\eta} u_{\eta;\beta} + u u_{,\beta}, \tag{15}$$

in equation (13), we obtain

$$\begin{aligned} \dot{u}^{\alpha} &= v^{\alpha} + a^{\alpha\beta} \Big(u^{\eta} u_{\eta;\beta} + u u_{,\beta} \Big) - u^{\gamma} a^{\alpha\lambda} \Big(u_{\lambda;\gamma} + u_{\gamma;\lambda} - 2u b_{\lambda\gamma} \Big) \\ &= v^{\alpha} + u u_{,\beta} a^{\alpha\beta} - u^{\gamma} u^{\alpha}_{;\gamma} + 2u u^{\gamma} b^{\alpha}_{\gamma}. \end{aligned} \tag{16}$$

Next, we compute the variation of the normal component of the first variation of the position field

$$\begin{aligned} \dot{u} &= \dot{\mathbf{u}} \cdot \mathbf{n} + \mathbf{u} \cdot \dot{\mathbf{n}} \\ &= v - u^{\alpha} u_{,\alpha} - u^{\alpha} u^{\gamma} b_{\gamma\alpha}. \end{aligned} \tag{17}$$

Here, the variation of the surface normal has been expressed as [32]

$$\dot{\mathbf{n}} = -(\mathbf{n} \cdot \mathbf{u}_{,\alpha}) \mathbf{a}^{\alpha}. \tag{18}$$

To proceed further, we define $I_{\alpha}(H, K; \theta^{\gamma}) = \partial W / \partial \theta^{\alpha}$ and compute its variation

$$\begin{aligned} \dot{I}_{\alpha} =& (I_{\alpha})_H \dot{H} + (I_{\alpha})_K \dot{K} \\ =& \frac{\partial W_H}{\partial \theta^{\alpha}} \left(u^{\gamma} H_{,\gamma} + \frac{(\Delta u)}{2} + u(2H^2 - K) \right) \\ &+ \frac{\partial W_K}{\partial \theta^{\alpha}} \left(u^{\gamma} K_{,\gamma} + 2uHK + \tilde{b}^{\gamma\beta} u_{;\gamma\beta} \right). \end{aligned} \tag{19}$$

Next, we compute the variation of G. We decompose its variation into tangential and normal parts, denoted $\dot{G}_{\mathrm{t}}$ and $\dot{G}_{\mathrm{n}}$, respectively.

3.1. Tangential variations

The tangential variation of the first term of G in equation (6) can be written as

$$\overline{\Delta W_H}^{\cdot} = \overline{(W_H)_{;\alpha\beta}}^{\cdot} a^{\alpha\beta} + (W_H)_{;\alpha\beta} \dot{a}^{\alpha\beta}, \tag{20}$$

where $\dot{\overline{()}}$ signifies the variation of the overall quantity within the parentheses. Expanding the first term in equation (20), we get

$$\dot{\overline{(W_H)_{;\alpha\beta}}} = \dot{\overline{(W_H)_{,\alpha\beta} - (W_H)_{,\lambda}\Gamma^{\lambda}_{\alpha\beta}}}. \tag{21}$$

Here, we note that the variational derivative (signified by the superposed dot) does not commute with the covariant derivative but it does commute with the derivative with respect to parameterizing variables θ^{α}. Thus, the relation can be rewritten as

$$\dot{\overline{(W_H)_{;\alpha\beta}}} = (\dot{W}_H)_{,\alpha\beta} - (\dot{W}_H)_{,\lambda}\Gamma^{\lambda}_{\alpha\beta} - (W_H)_{,\lambda}\dot{\Gamma}^{\lambda}_{\alpha\beta}. \tag{22}$$

Substituting equation (22) into equation (20) yields

$$\dot{\overline{\Delta W_H}} = (\dot{W}_H)_{;\alpha\beta}a^{\alpha\beta} - (W_H)_{,\lambda}\dot{\Gamma}^{\lambda}_{\alpha\beta}a^{\alpha\beta} + (W_H)_{;\alpha\beta}\dot{a}^{\alpha\beta}. \tag{23}$$

where the variation of the Christoffel symbols is given by [14]

$$\dot{\Gamma}^{\lambda}_{\alpha\beta} = \frac{1}{2}a^{\lambda\eta}\left\{\dot{a}_{\eta\beta;\alpha} + \dot{a}_{\eta\alpha;\beta} - \dot{a}_{\alpha\beta;\eta}\right\}. \tag{24}$$

The tangential variations of $a_{\alpha\beta}$, $b_{\alpha\beta}$, H, K and J are given by [31]

$$\begin{gathered} \dot{a}_{\alpha\beta} = u_{\alpha;\beta} + u_{\beta;\alpha}; \quad \dot{b}_{\alpha\beta} = u^{\lambda}_{;\beta}b_{\lambda\alpha} + u^{\lambda}_{;\alpha}b_{\lambda\beta} + u^{\lambda}b_{\lambda\alpha;\beta}; \\ \dot{H} = u^{\alpha}H_{,\alpha}; \quad \dot{K} = u^{\alpha}K_{,\alpha}; \quad \text{and} \quad \frac{\dot{J}}{J} = u^{\alpha}_{;\alpha}. \end{gathered} \tag{25}$$

Using equations (14), (24) and (25), equation (23) can be expressed as

$$\begin{aligned} \dot{\overline{\Delta W_H}} = {} & (W_{HH}\dot{H} + W_{HK}\dot{K})_{;\alpha\beta}a^{\alpha\beta} \\ & - (W_H)_{,\lambda}a^{\lambda\eta}(u_{\eta;\alpha\beta} + u_{\alpha;\eta\beta} - u_{\alpha;\beta\eta})a^{\alpha\beta} \\ & - (W_H)_{;\alpha\beta}a^{\alpha\lambda}a^{\beta\gamma}(u_{\lambda;\gamma} + u_{\gamma;\lambda}). \end{aligned} \tag{26}$$

With the help of equation (25), equation (26) can be further rearranged and expressed as

$$\begin{aligned} \dot{\overline{\Delta W_H}} = {} & \left(u^{\gamma}(W_H)_{,\gamma} - u^{\gamma}\frac{\partial W_H}{\partial\theta^{\gamma}}\right)_{;\alpha\beta}a^{\alpha\beta} - (W_H)_{,\lambda}u^{\lambda}_{;\alpha\beta}a^{\alpha\beta} \\ & - (W_H)_{,\lambda}a^{\lambda\eta}(u^{\beta}_{;\eta\beta} - u^{\beta}_{;\beta\eta}) - 2u^{\alpha}_{;\gamma}a^{\beta\gamma}(W_H)_{;\alpha\beta}. \end{aligned} \tag{27}$$

To simplify this relation, we use the definition of the Riemann curvature tensor

$$R^{\alpha}_{\beta\gamma\eta} = Ka^{\alpha\lambda}\left\{a_{\lambda\gamma}a_{\beta\eta} - a_{\lambda\eta}a_{\beta\gamma}\right\}. \tag{28}$$

and the relationship

$$R^{\alpha}_{\beta\eta\gamma}u^{\beta} = u^{\alpha}_{;\gamma\eta} - u^{\alpha}_{;\eta\gamma}. \tag{29}$$

which holds for any arbitrary vector field lying in the tangent plane. With the help of equation (29), along with the linearity of the Laplace operator and the chain rule for covariant derivatives, equation (27) can be written as

$$\begin{aligned} \dot{\overline{\Delta W_H}} = {} & \left(u^{\gamma}_{;\alpha\beta}(W_H)_{,\gamma} + 2u^{\gamma}_{;\alpha}(W_H)_{;\gamma\beta} + u^{\gamma}(W_H)_{;\gamma\alpha\beta}\right)a^{\alpha\beta} - \Delta\left(u^{\gamma}\frac{\partial W_H}{\partial\theta^{\gamma}}\right) \\ & - (W_H)_{,\lambda}u^{\lambda}_{;\alpha\beta}a^{\alpha\beta} - u^{\gamma}(W_H)_{,\lambda}a^{\lambda\eta}R^{\beta}_{\gamma\beta\eta} - 2u^{\alpha}_{;\gamma}a^{\beta\gamma}(W_H)_{;\alpha\beta} \\ = {} & u^{\gamma}((W_H)_{,\alpha}a^{\alpha\beta})_{;\gamma\beta} - \Delta\left(u^{\gamma}\frac{\partial W_H}{\partial\theta^{\gamma}}\right) - u^{\gamma}(W_H)_{,\lambda}a^{\lambda\eta}R^{\beta}_{\gamma\beta\eta}. \end{aligned} \tag{30}$$

To derive equation $(30)_2$, we have used the fact that the metric is covariant constant and torsion free. As a result, for any scalar field $f(\theta^\alpha)$ defined on the surface, $(f(\theta^\alpha))_{;\alpha\beta} = (f(\theta^\alpha))_{;\beta\alpha}$. Using the definition of the covariant derivative of a vector field, along with equations (29) and (28), equation (30) can be further reduced to

$$\begin{aligned}\overline{\Delta \dot{W}_H} &= u^\gamma \Big[((W_H)_{,\alpha} a^{\alpha\beta})_{;\gamma\beta} - ((W_H)_{,\alpha} a^{\alpha\beta})_{;\beta\gamma} \Big] + u^\gamma (\Delta W_H)_{,\gamma} - \Delta\left(u^\gamma \frac{\partial W_H}{\partial \theta^\gamma}\right) \\ &\quad - u^\gamma (W_H)_{,\lambda} a^{\lambda\eta} R^\beta_{\gamma\beta\eta} \\ &= u^\gamma (W_H)_{,\alpha} a^{\alpha\eta} R^\beta_{\eta\beta\gamma} + u^\gamma (\Delta W_H)_{,\gamma} - R^\beta_{\gamma\beta\eta} u^\gamma (W_H)_{,\lambda} a^{\lambda\eta} - \Delta\left(u^\gamma \frac{\partial W_H}{\partial \theta^\gamma}\right) \\ &= u^\gamma (\Delta W_H)_{,\gamma} - \Delta\left(u^\gamma \frac{\partial W_H}{\partial \theta^\gamma}\right).\end{aligned} \tag{31}$$

The rightmost term in this equation arises from the inhomogeneity in the lipid membrane.

Next, we compute the tangential variation of $(W_K)_{;\alpha\beta}\tilde{b}^{\alpha\beta}$ in equation (6). We use the Cayley–Hamilton theorem in the form

$$\tilde{b}^{\alpha\beta} = 2Ha^{\alpha\beta} - b^{\alpha\beta}, \tag{32}$$

to obtain

$$\overline{(W_K)_{;\alpha\beta}(2\dot{H}a^{\alpha\beta} - b^{\alpha\beta})} = 2\dot{H}\Delta W_K + 2H\overline{\Delta \dot{W}_K} - \overline{(W_K)_{;\alpha\beta}\dot{b}^{\alpha\beta}}. \tag{33}$$

By analogy with the variation of the surface Laplacian of W_H, we can write

$$\overline{\Delta \dot{W}_K} = u^\gamma (\Delta W_K)_{,\gamma} - \Delta\left(u^\gamma \frac{\partial W_K}{\partial \theta^\gamma}\right). \tag{34}$$

Substituting equations (25) and (34) into equation (33) yields

$$\overline{(W_K)_{;\alpha\beta}(2\dot{H}a^{\alpha\beta} - b^{\alpha\beta})} = 2u^\gamma \left(H\Delta W_K \right)_{,\gamma} - \overline{(W_K)_{;\alpha\beta}\dot{b}^{\alpha\beta}} - 2H\Delta\left(u^\gamma \frac{\partial W_K}{\partial \theta^\gamma}\right). \tag{35}$$

The second term on the right-hand side of equation (35) can be expressed as

$$\overline{(W_K)_{;\alpha\beta}\dot{b}^{\alpha\beta}} = \left((\dot{W}_K)_{;\alpha\beta} - (W_K)_{,\lambda}\dot{\Gamma}^\lambda_{\alpha\beta} \right) b^{\alpha\beta} + (W_K)_{;\alpha\beta}\dot{b}^{\alpha\beta}. \tag{36}$$

Next, we substitute equations (3), (14), (24) and (29) into equation (36) to obtain

$$
\begin{aligned}
\overline{(W_K)_{;\alpha\beta}b^{\alpha\beta}}^{\cdot} &= \left(u^\gamma (W_{KK}\dot{K} + W_{KH}\dot{H})\right)_{;\alpha\beta} b^{\alpha\beta} \\
&\quad - \frac{1}{2}(W_K)_{,\lambda}a^{\lambda\eta}\left\{\dot{a}_{\eta\beta;\alpha} + \dot{a}_{\eta\alpha;\beta} - \dot{a}_{\alpha\beta;\eta}\right\} \\
&\quad + (W_K)_{;\alpha\beta}\dot{b}_{\lambda\gamma}a^{\alpha\lambda}a^{\beta\gamma} + 2(W_K)_{;\alpha\beta}b_{\lambda\gamma}a^{\beta\gamma}\dot{a}^{\alpha\lambda} \\
&= \left(u^\gamma (W_K)_{,\gamma}\right)_{;\alpha\beta} b^{\alpha\beta} - \left(u^\gamma \frac{\partial (W_K)}{\partial\theta^\gamma}\right)_{;\alpha\beta} b^{\alpha\beta} \\
&\quad - (W_K)_{,\lambda}b^{\alpha\beta}a^{\lambda\eta}(u_{\eta;\beta\alpha} + u_{\beta;\eta\alpha} - u_{\alpha;\beta\eta}) \\
&\quad + (W_K)_{;\alpha\beta}a^{\alpha\lambda}a^{\beta\gamma}\left\{u^\eta_{;\gamma}b_{\eta\lambda} + u^\eta_{;\lambda}b_{\eta\gamma} + u^\eta b_{\eta\gamma;\lambda}\right\} \\
&\quad - 2(W_K)_{;\alpha\beta}b^\beta_\lambda a^{\alpha\eta}a^{\lambda\theta}(u_{\theta;\eta} + u_{\eta;\theta}) \\
&= \left(u^\gamma_{;\alpha\beta}(W_K)_{,\gamma} + 2u^\gamma_{;\alpha}(W_K)_{;\gamma\beta} + u^\gamma (W_K)_{;\gamma\alpha\beta}\right)b^{\alpha\beta} - u^\lambda_{;\alpha\beta}(W_K)_{,\lambda}b^{\alpha\beta} \\
&\quad - (W_K)_{,\lambda}b^\alpha_\beta a^{\lambda\eta}(u^\beta_{;\eta\alpha} - u^\beta_{;\alpha\eta}) + u^\eta (W_K)_{;\alpha\beta}(b^{\alpha\beta})_{;\eta} - 2u^\alpha_{;\theta}(W_K)_{;\alpha\beta}b^{\beta\theta} \\
&= u^\gamma (W_K)_{;\beta\gamma\alpha}b^{\alpha\beta} - (W_K)_{,\lambda}b^\alpha_\beta a^{\lambda\eta}R^\beta_{\gamma\alpha\eta}u^\gamma + u^\eta (W_K)_{;\alpha\beta}(b^{\alpha\beta})_{;\eta} \\
&\quad - b^{\alpha\beta}\left(u^\gamma \frac{\partial W_K}{\partial\theta^\gamma}\right)_{;\alpha\beta}.
\end{aligned} \tag{37}
$$

We use the fact that the metric is torsion free and add and subtract $u^\gamma (W_K)_{;\alpha\beta\gamma}b^{\alpha\beta}$ in equation (37) to obtain

$$
\begin{aligned}
\overline{(W_K)_{;\alpha\beta}b^{\alpha\beta}}^{\cdot} = \ & u^\gamma \left\{((W_K)_{,\beta}a^{\beta\eta})_{;\gamma\alpha} - ((W_K)_{,\beta}a^{\beta\eta})_{;\alpha\gamma}\right\}b^\alpha_\eta \\
& - (W_K)_{,\lambda}b^\alpha_\beta a^{\lambda\eta}R^\beta_{\gamma\alpha\eta}u^\gamma + u^\gamma (W_K)_{;\alpha\beta\gamma}b^{\alpha\beta} \\
& + u^\gamma (W_K)_{;\alpha\beta}(b^{\alpha\beta})_{;\gamma} - b^{\alpha\beta}\left(u^\gamma \frac{\partial W_K}{\partial\theta^\gamma}\right)_{;\alpha\beta}.
\end{aligned} \tag{38}
$$

With the help of equations (28) and (29), equation (38) can be further reduced to

$$
\overline{(W_K)_{;\alpha\beta}b^{\alpha\beta}}^{\cdot} = u^\gamma \left((W_K)_{;\alpha\beta}b^{\alpha\beta}\right)_{;\gamma} - b^{\alpha\beta}\left(u^\gamma \frac{\partial W_K}{\partial\theta^\gamma}\right)_{;\alpha\beta}. \tag{39}
$$

We then substitute equation (39) into equation (35) to obtain

$$
\begin{aligned}
\overline{(W_K)_{;\alpha\beta}(2Ha^{\alpha\beta} - b^{\alpha\beta})}^{\cdot} &= u^\gamma \left(2H\Delta W_K\right)_{,\gamma} - u^\gamma \left((W_K)_{;\alpha\beta}b^{\alpha\beta}\right)_{,\gamma} \\
&\quad + b^{\alpha\beta}\left(u^\gamma \frac{\partial W_K}{\partial\theta^\gamma}\right)_{;\alpha\beta} - 2H\Delta\left(u^\gamma \frac{\partial W_K}{\partial\theta^\gamma}\right) \\
&= u^\gamma \left((W_K)_{;\alpha\beta}\tilde{b}^{\alpha\beta}\right)_{;\gamma} - \tilde{b}^{\alpha\beta}\left(u^\gamma \frac{\partial W_K}{\partial\theta^\gamma}\right)_{;\alpha\beta}.
\end{aligned} \tag{40}
$$

As with the tangential variation of $\Delta(W_H)$, the second term in equation (40) arises because of the inhomogeneity in the membrane properties.

Next, we compute the tangential variations of the remaining terms of G in equation (6). These include

$$
\begin{aligned}
\overline{W_H(2H^2 - K)}^{\cdot} &= (W_{HH}\dot{H} + W_{HK}\dot{K})(2H^2 - K) + W_H(4H\dot{H} - \dot{K}) \\
&= u^\gamma \left(W_H(2H^2 - K)\right)_{,\gamma} - u^\gamma \left(\frac{\partial W_H}{\partial\theta^\gamma}\right)(2H^2 - K),
\end{aligned} \tag{41}
$$

$$\begin{aligned}\overline{2H(KW_K - W)}^{\boldsymbol{\cdot}} &= 2\dot{H}(KW_K - W) + 2H(\dot{K}W_K + KW_{KH}\dot{H} + KW_{KK}\dot{K} \\ &\quad - W_H\dot{H} - W_K\dot{K}) \\ &= u^\gamma\Big(2H(KW_K - W)\Big)_{,\gamma} - u^\gamma 2H\Big(K\frac{\partial W_K}{\partial\theta^\gamma} - \frac{\partial W}{\partial\theta^\gamma}\Big),\end{aligned} \tag{42}$$

and

$$\overline{2\lambda H}^{\boldsymbol{\cdot}} = 2\lambda\dot{H} = u^\gamma(2\lambda H)_{,\gamma} - 2u^\gamma\lambda_{,\gamma}H. \tag{43}$$

We now substitute equations (31), (40), (41), (42) and (43) into equation (6), to compute the tangential variation of G ($\dot{G}_\mathrm{t}$)

$$\begin{aligned}\dot{G}_\mathrm{t} = {} & u^\gamma\left\{\frac{1}{2}\Delta W_H + (W_K)_{;\alpha\beta}\tilde{b}^{\alpha\beta} + W_H(2H^2 - K) + 2H(KW_K - W) - 2\lambda H - p\right\}_{,\gamma} \\ & - \left\{\frac{1}{2}\Delta\Big(u^\gamma\frac{\partial W_H}{\partial\theta^\gamma}\Big) + \tilde{b}^{\alpha\beta}\Big(u^\gamma\frac{\partial W_K}{\partial\theta^\gamma}\Big)_{;\alpha\beta} + u^\gamma(2H^2 - K)\frac{\partial W_H}{\partial\theta^\gamma}\right. \\ & \left. + 2u^\gamma H\Big(K\frac{\partial W_K}{\partial\theta^\gamma} - \frac{\partial W}{\partial\theta^\gamma} - \lambda_{,\gamma}\Big)\right\},\end{aligned} \tag{44}$$

where we have used the fact that the pressure field is uniform on the surface. Using equation (6), equation (44) can be expressed as

$$\dot{G}_\mathrm{t} = u^\gamma G_{,\gamma} - \left\{\frac{1}{2}\Delta\Big(u^\gamma\frac{\partial W_H}{\partial\theta^\gamma}\Big) + \tilde{b}^{\alpha\beta}\Big(u^\gamma\frac{\partial W_K}{\partial\theta^\gamma}\Big)_{;\alpha\beta} + u^\gamma(2H^2 - K)\frac{\partial W_H}{\partial\theta^\gamma} + 2u^\gamma H\Big(K\frac{\partial W_K}{\partial\theta^\gamma} - \frac{\partial W}{\partial\theta^\gamma} - \lambda_{,\gamma}\Big)\right\}, \tag{45}$$

Other than the terms arising from inhomogeneity, equation (45) is similar to the tangential variations of an arbitrary scalar surface field $f(\mathbf{r}(\theta^\alpha))$ derived by Capovilla et al. [14].

3.2. Normal variations

For normal variations $\mathbf{u} = u\mathbf{n}$, equations (3) and (4) yield [31]

$$\begin{aligned}&\dot{a}_{\alpha\beta} = -2ub_{\alpha\beta}; \quad \dot{b}_{\alpha\beta} = u_{;\alpha\beta} - ub_\alpha^\gamma b_{\gamma\beta}; \\ &\dot{H} = \frac{1}{2}(\Delta u) + u(2H^2 - K); \quad \dot{K} = u_{;\alpha\beta}\tilde{b}^{\alpha\beta} + 2uHK.\end{aligned} \tag{46}$$

First, we use equation (23) to compute the normal variation of $\Delta(W_H)$. With the help of equation (46), we compute the first term on the right-hand side of equation (23) to obtain

$$\begin{aligned}(\dot{W}_H)_{;\alpha\beta}a^{\alpha\beta} &= \Big\{W_{HH}\dot{H} + W_{HK}\dot{K}\Big\}_{;\alpha\beta}a^{\alpha\beta} \\ &= \Big\{W_{HH}\Big(\frac{1}{2}\Delta u + u(2H^2 - K)\Big) + W_{HK}\Big(u_{;\alpha\beta}\tilde{b}^{\alpha\beta} + 2uHK\Big)\Big\}_{;\alpha\beta}a^{\alpha\beta}.\end{aligned} \tag{47}$$

We use equation (24) along with Cayley–Hamilton theorem and the fact that the metric tensor is covariant constant to compute the second term in equation (23)

$$\begin{aligned}-(W_H)_{,\lambda}\dot{\Gamma}^\lambda_{\alpha\beta}a^{\alpha\beta} &= (W_H)_{,\lambda}a^{\alpha\beta}a^{\lambda\eta}((2ub_{\eta\alpha})_{;\beta} - (ub_{\alpha\beta})_{;\eta}) \\ &= (W_H)_{,\lambda}\Big((2uHa^{\beta\lambda})_{;\beta} - (2u\tilde{b}^{\beta\lambda})_{;\beta}\Big).\end{aligned} \tag{48}$$

We again use equation (46) to compute the third term in equation (23)

$$(W_H)_{;\alpha\beta}\dot{a}^{\alpha\beta} = 2u(W_H)_{;\alpha\beta}b^{\alpha\beta}. \tag{49}$$

Finally, we substitute equations (47), (48) and (49) into equation (23) to obtain

$$\overline{\Delta \dot{W}_H} = \left\{ W_{HH}\left(\frac{1}{2}\Delta u + u(2H^2 - K)\right) + W_{HK}\left(u_{;\alpha\beta}\tilde{b}^{\alpha\beta} + 2uHK\right)\right\}_{;\alpha\beta} a^{\alpha\beta} + (W_H)_{,\lambda}\left((2uHa^{\beta\lambda})_{;\beta} - (2u\tilde{b}^{\beta\lambda})_{;\beta}\right) + 2u(W_H)_{;\alpha\beta}b^{\alpha\beta}. \tag{50}$$

Next, we use equation (46) to compute the normal variation of $(W_K)_{;\alpha\beta}\tilde{b}^{\alpha\beta}$, which is given by

$$\overline{(W_K)_{;\alpha\beta}(2Ha^{\alpha\beta} - b^{\alpha\beta})}^{\cdot} = (\dot{W}_K)_{;\alpha\beta}\tilde{b}^{\alpha\beta} - (W_K)_{,\lambda}\dot{\Gamma}^{\lambda}_{\alpha\beta}\tilde{b}^{\alpha\beta} + (W_K)_{;\alpha\beta}\dot{\tilde{b}}^{\alpha\beta}, \tag{51}$$

where

$$(\dot{W}_K)_{;\alpha\beta}\tilde{b}^{\alpha\beta} = \left\{ W_{KH}\left(\frac{1}{2}\Delta u + u(2H^2 - K)\right) + W_{KK}\left(u_{;\alpha\beta}\tilde{b}^{\alpha\beta} + 2uHK\right)\right\}_{;\alpha\beta}\tilde{b}^{\alpha\beta}, \tag{52}$$

$$(W_K)_{,\lambda}\dot{\Gamma}^{\lambda}_{\alpha\beta}\tilde{b}^{\alpha\beta} = -(W_K)_{,\lambda}\tilde{b}^{\alpha\beta}a^{\lambda\eta}\left[(2ub_{\eta\alpha})_{;\beta} - (ub_{\alpha\beta})_{;\eta}\right] = 0. \tag{53}$$

and

$$\begin{aligned}\dot{\tilde{b}}^{\alpha\beta} &= -\varepsilon^{\alpha\lambda}\varepsilon^{\beta\gamma}\frac{\dot{a}}{a}b_{\lambda\gamma} + \varepsilon^{\alpha\lambda}\varepsilon^{\beta\gamma}\dot{b}_{\lambda\gamma} \\ &= 4uH\tilde{b}^{\alpha\beta} + \varepsilon^{\alpha\lambda}\varepsilon^{\beta\gamma}(u_{;\lambda\gamma} - ub^{\eta}_{\lambda}b_{\eta\gamma}).\end{aligned} \tag{54}$$

Substituting equations (52), (53) and (54) into equation (51) furnishes

$$\overline{(W_K)_{;\alpha\beta}(2Ha^{\alpha\beta} - b^{\alpha\beta})}^{\cdot} = \left\{ W_{KH}\left(\frac{1}{2}\Delta u + u(2H^2 - K)\right) + W_{KK}\left(u_{;\alpha\beta}\tilde{b}^{\alpha\beta} + 2uHK\right)\right\}_{;\alpha\beta}\tilde{b}^{\alpha\beta} + 4uH(W_K)_{;\alpha\beta}\tilde{b}^{\alpha\beta} + \varepsilon^{\alpha\lambda}\varepsilon^{\beta\gamma}(u_{;\lambda\gamma} - ub^{\eta}_{\lambda}b_{\eta\gamma})(W_K)_{;\alpha\beta}. \tag{55}$$

Next, we use equation (46) to compute the normal variation of the remaining terms of G in equation (6), which are given by

$$\begin{aligned}&\overline{W_H(2H^2 - K) + 2H(KW_K - W)}^{\cdot} \\ &= \left\{W_{HH}(2H^2 - K) + 2HW_H + 2KW_K + 2HKW_{HK} - 2W\right\}\dot{H} \\ &\quad + \left\{W_{HK}(2H^2 - K) - W_H + 2HKW_{KK}\right\}\dot{K} \\ &= (\Delta u)\left\{\frac{1}{2}W_{HH}(2H^2 - K) + HW_H + HKW_{HK} + (KW_K - W)\right\} \\ &\quad + u_{;\alpha\beta}\tilde{b}^{\alpha\beta}\left\{2HKW_{KK} + (2H^2 - K)W_{HK} - W_H\right\} \\ &\quad + u\Big\{W_{HH}(2H^2 - K)^2 + W_{KK}(2HK)^2 + 4HK(2H^2 - K)W_{HK} \\ &\qquad + 4HW_H(H^2 - K) + 2(2H^2 - K)(KW_K - W)\Big\},\end{aligned} \tag{56}$$

and

$$2\lambda\dot{H} = \lambda(\Delta u + 2u(2H^2 - K)). \tag{57}$$

Combining equations (50), (55), (56) and (57) yields the total normal variation of G, which after some rearrangement can be written as

$$
\begin{aligned}
\dot{G}_{\mathrm{n}} = \; & \frac{1}{2}\Bigg\{ \Delta\Bigg[W_{HH}\Bigg(\frac{1}{2}\Delta u + u(2H^2-K)\Bigg)\Bigg] + \Delta\Bigg[W_{HK}\Bigg(u_{;\alpha\beta}\tilde{b}^{\alpha\beta} + 2uHK\Bigg)\Bigg] \\
& \quad + (W_H)_{,\lambda}\Bigg((2uH)_{;\beta}a^{\beta\lambda} - 2u_{,\beta}\tilde{b}^{\beta\lambda}\Bigg)\Bigg\} \\
& + \Bigg\{ W_{KH}\Bigg[\frac{1}{2}\Delta u + u(2H^2-K)\Bigg] + W_{KK}\Bigg[u_{;\lambda\gamma}\tilde{b}^{\lambda\gamma} + 2uHK\Bigg]\Bigg\}_{;\alpha\beta}\tilde{b}^{\alpha\beta} \\
& + \varepsilon^{\alpha\lambda}\varepsilon^{\beta\gamma}(W_K)_{;\alpha\beta}(u_{;\lambda\gamma} - ub^{\eta}_{\lambda}b_{\eta\gamma}) - u(W_H)_{;\alpha\beta}\tilde{b}^{\alpha\beta} \\
& + (\Delta u)\Bigg\{ \frac{1}{2}W_{HH}(2H^2-K) + HW_H + HKW_{HK} + (KW_K - W)\Bigg\} \\
& + u_{;\alpha\beta}\tilde{b}^{\alpha\beta}\Bigg\{ 2HKW_{KK} + (2H^2-K)W_{HK} - W_H\Bigg\} \\
& + u\Bigg\{ W_{HH}(2H^2-K)^2 + W_{KK}(2HK)^2 + 4HK(2H^2-K)W_{HK} \\
& \quad - 4H^3W_H - 2(2H^2+K)(KW_K - W)\Bigg\} \\
& - 2\lambda\Bigg\{\frac{1}{2}\Delta u - u(2H^2+K)\Bigg\} + 4uH(G+p).
\end{aligned}
\tag{58}
$$

3.3. Total variations

We now combine equations (16), (17), (19), (45) and (58) to compute the second variation of E

$$
\begin{aligned}
\ddot{E} = \int_{\omega} \Bigg\{ & -(v^{\alpha} + uu_{,\beta}a^{\alpha\beta} - u^{\gamma}u^{\alpha}_{;\gamma} + 2uu^{\gamma}b^{\alpha}_{\gamma})\Bigg(\lambda_{,\alpha} + \frac{\partial W}{\partial\theta^{\alpha}}\Bigg) \\
& - u^{\alpha}\Bigg\{ \frac{\partial W_H}{\partial\theta^{\alpha}}\Bigg(u^{\gamma}H_{,\gamma} + \frac{1}{2}\Delta u + u(2H^2-K)\Bigg) + \frac{\partial W_K}{\partial\theta^{\alpha}}\Bigg(u^{\gamma}K_{,\gamma} + 2uHK + u_{;\gamma\beta}\tilde{b}^{\gamma\beta}\Bigg)\Bigg\} \\
& + (v - u^{\gamma}u_{,\alpha} - u^{\alpha}u^{\gamma}b_{\gamma\alpha})G \\
& - uu^{\gamma}G_{,\gamma} \\
& - u\Bigg\{ \frac{1}{2}\Delta\Bigg(u^{\gamma}\frac{\partial W_H}{\partial\theta^{\gamma}}\Bigg) + \tilde{b}^{\alpha\beta}\Bigg(u^{\gamma}\frac{\partial W_K}{\partial\theta^{\gamma}}\Bigg)_{;\alpha\beta} + u^{\gamma}(2H^2-K)\frac{\partial W_H}{\partial\theta^{\gamma}} + 2u^{\gamma}H\Bigg(K\frac{\partial W_K}{\partial\theta^{\gamma}} - \frac{\partial W}{\partial\theta^{\gamma}} - \lambda_{,\gamma}\Bigg)\Bigg\} \\
& + u\Bigg[\frac{1}{2}\Bigg\{ \Delta\Bigg[W_{HH}\Bigg(\frac{1}{2}\Delta u + u(2H^2-K)\Bigg)\Bigg] + \Delta\Bigg[W_{HK}\Bigg(u_{;\alpha\beta}\tilde{b}^{\alpha\beta} + 2uHK\Bigg)\Bigg] + (W_H)_{,\lambda}\Bigg((2uH)_{;\beta}a^{\beta\lambda} - 2u_{,\beta}\tilde{b}^{\beta\lambda}\Bigg)\Bigg\} \\
& + \Bigg\{ W_{KH}\Bigg[\frac{1}{2}\Delta u + u(2H^2-K)\Bigg] + W_{KK}\Bigg[u_{;\alpha\beta}\tilde{b}^{\alpha\beta} + 2uHK\Bigg]\Bigg\}_{;\alpha\beta}\tilde{b}^{\alpha\beta} \\
& + \varepsilon^{\alpha\lambda}\varepsilon^{\beta\gamma}(W_K)_{;\alpha\beta}(u_{;\lambda\gamma} - ub^{\eta}_{\lambda}b_{\eta\gamma})d \\
& - u(W_H)_{;\alpha\beta}\tilde{b}^{\alpha\beta} \\
& + (\Delta u)\Bigg\{ \frac{1}{2}W_{HH}(2H^2-K) + HW_H + HKW_{HK} + (KW_K - W)\Bigg\} \\
& + u_{;\alpha\beta}\tilde{b}^{\alpha\beta}\Bigg\{ 2HKW_{KK} + (2H^2-K)W_{HK} - W_H\Bigg\} \\
& + u\Bigg\{ W_{HH}(2H^2-K)^2 + W_{KK}(2HK)^2 + 4HK(2H^2-K)W_{HK} - 4H^3W_H - 2(2H^2+K)(KW_K - W)\Bigg\} \\
& - 2\lambda\Bigg\{\frac{1}{2}\Delta u - u(2H^2+K)\Bigg\} + 4uH(G+p)\Bigg]\Bigg\}\mathrm{d}a.
\end{aligned}
\tag{59}
$$

Since at equilibrium, equations (7) and (8) are satisfied and $G_{,\gamma} = 0$, (as $G = 0$ is identically satisfied on all the material points on the surface), $\ddot{E}$ reduces to

$$
\begin{aligned}
\ddot{E} = \int_\omega \Bigg\{ & - u^\alpha \left\{ \frac{\partial W_H}{\partial \theta^\alpha} \left(u^\gamma H_{,\gamma} + \frac{1}{2}\Delta u + 2u(2H^2 - K) \right) \right. \\
& \left. + \frac{\partial W_K}{\partial \theta^\alpha} \left(u^\gamma K_{,\gamma} + 4uHK + u_{;\gamma\beta} \tilde{b}^{\gamma\beta} \right) \right\} \\
& - u \left\{ \frac{1}{2}\Delta \left(u^\gamma \frac{\partial W_H}{\partial \theta^\gamma} \right) + \tilde{b}^{\alpha\beta} \left(u^\gamma \frac{\partial W_K}{\partial \theta^\gamma} \right)_{;\alpha\beta} \right\} \\
& + u \left[\frac{1}{2} \left\{ \Delta \left[W_{HH} \left(\frac{1}{2}\Delta u + u(2H^2 - K) \right) \right] \right. \right. \\
& + \Delta \left[W_{HK} \left(u_{;\alpha\beta} \tilde{b}^{\alpha\beta} + 2uHK \right) \right] \\
& \left. + (W_H)_{,\lambda} \left((2uH)_{;\beta} a^{\beta\lambda} - 2u_{,\beta} \tilde{b}^{\beta\lambda} \right) \right\} \\
& + \left\{ W_{KH} \left[\frac{1}{2}\Delta u + u(2H^2 - K) \right] \right. \\
& \left. + W_{KK} \left[u_{;\alpha\beta} \tilde{b}^{\alpha\beta} + 2uHK \right] \right\}_{;\alpha\beta} \tilde{b}^{\alpha\beta} \\
& + \varepsilon^{\alpha\lambda} \varepsilon^{\beta\gamma} (W_K)_{;\alpha\beta} (u_{;\lambda\gamma} - u b^\eta_\lambda b_{\eta\gamma}) \\
& - u (W_H)_{;\alpha\beta} \tilde{b}^{\alpha\beta} \\
& + (\Delta u) \left\{ \frac{1}{2} W_{HH} (2H^2 - K) + H W_H + HK W_{HK} \right. \\
& \left. + (K W_K - W) \right\} \\
& + u_{;\alpha\beta} \tilde{b}^{\alpha\beta} \left\{ 2HK W_{KK} + (2H^2 - K) W_{HK} - W_H \right\}. \\
& + u \left\{ W_{HH} (2H^2 - K)^2 + W_{KK} (2HK)^2 + 4HK(2H^2 - K) W_{HK} \right. \\
& \left. - 4H^3 W_H - 2(2H^2 + K)(K W_K - W) \right\} \\
& - 2\lambda \left\{ \frac{1}{2}\Delta u - u(2H^2 + K) \right\} \\
& \left. + 4uHp \right] \Bigg\} \mathrm{d}a.
\end{aligned} \tag{60}
$$

This is the generalized second variation of E at an equilibrium configuration. For a system to be stable, $\ddot{E} > 0$, subject to the incompressibility constraint

$$u^\alpha_{;\alpha} = 2uH. \tag{61}$$

and the volumetric constraint

$$\int_\omega u \, \mathrm{d}a = 0. \tag{62}$$

Note that while W can have arbitrary dependence on the curvature invariants, it has to satisfy the Legendre–Hadamard stability condition derived by Agrawal and Steigmann [27].

4. Comparison with other models

We specialize equation (60) for the Helfrich–Canham energy given by

$$W = k(H - C_0(\theta^\alpha))^2 + \bar{k}K. \tag{63}$$

Here, k is the local bending modulus of the membrane and $\bar{k}$ is the Gaussian modulus. $C_0(\theta^\alpha)$ is the spatially varying spontaneous curvature field, which could arise because of a heterogeneous composition of a bilayer or spatially varying interaction with membrane remodelling proteins. Substituting equation (63) into equation (60) and invoking the Gauss–Bonnet theorem yields

$$\begin{aligned}
\ddot{E} = \int_\omega \Bigg\{ & u^\alpha \left\{ 2k \frac{\partial C_0}{\partial \theta^\alpha} \left(u^\gamma H_{,\gamma} + \frac{1}{2}\Delta u + 2u(2H^2 - K) \right) \right\} \\
& + u \left\{ k\Delta \left(u^\gamma \frac{\partial C_0}{\partial \theta^\gamma} \right) \right\} \\
& - 2u_{;\alpha\beta} k \tilde{b}^{\alpha\beta}(H - C_0) \\
& + u \Bigg[\Bigg\{ \Delta \left[k \left(\frac{1}{2}\Delta u + u(2H^2 - K) \right) \right] \\
& + 2k(H - C_0)_{,\lambda} \left((2uH)_{;\beta} a^{\beta\lambda} - 2u_{,\beta} \tilde{b}^{\beta\lambda} \right) \Bigg\} \\
& + (\Delta u) \left\{ k(2H^2 - K) + 2kH(H - C_0) - k(H - C_0)^2 - \lambda \right\} \\
& + u \Big\{ k(2H^2 - K)^2 - 4kH^3(H - C_0) \\
& + 2k(2H^2 + K)(H - C_0)^2 - 2k(H - C_0)_{;\alpha\beta} \tilde{b}^{\alpha\beta} \\
& + 2\lambda(2H^2 + K) + 4Hp \Big\} \Bigg] \Bigg\} \, \mathrm{d}a.
\end{aligned} \tag{64}$$

For a homogeneous membrane with a uniform preferred curvature C_0, equation (64) further reduces to

$$\begin{aligned}
\ddot{E} = \int_\omega \Bigg\{ & u \Bigg[\left\{ k\Delta \left[\left(\frac{1}{2}\Delta u + u(2H^2 - K) \right) \right] + kH_{,\lambda} \left((2uH)_{;\beta} a^{\beta\lambda} - 2u_{,\beta} \tilde{b}^{\beta\lambda} \right) \right\} \\
& + (\Delta u) \left\{ k(3H^2 - K) - \lambda - k(C_0)^2 \right\} \\
& - 2u_{;\alpha\beta} k \tilde{b}^{\alpha\beta}(H - C_0) \\
& + u \Big\{ kK(K - 2H^2) + 2(2H^2 + K)(\lambda + kC_0^2) + 4Hp - 4kHKC_0 \\
& - kH_{;\alpha\beta} \tilde{b}^{\alpha\beta} \Big\} \Bigg] \Bigg\} \, \mathrm{d}a.
\end{aligned} \tag{65}$$

For $C_0 = 0$ and $W = k(2H)^2$, this equation reduces to the one derived by Capovilla et al. [14]. As expected, at equilibrium, the tangential variations do not play a role in the stability criterion for a homogeneous membrane. It is, however, important to note that for the cases with incompressibility constraint, the tangential perturbations are related to the normal perturbations through equation (61) (unless the surface at equilibrium is a minimal surface with $H = 0$). As a consequence, equation (61) can be used to write the entire second variation in terms of the tangential variations and their derivatives.

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