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Stability of lipid membranes with orthotropic symmetry

Nikhil Walani
Universitat Politècnica de Catalunya, BarcelonaTech, Barcelona, Spain

Ashutosh Agrawal
Department of Mechanical Engineering, University of Houston, Houston, TX, USA

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Abstract
Lipid membranes routinely undergo protein-mediated morphological remodeling during vital processes such as cellular transport and division. These membrane remodeling proteins can be broadly classified into two categories: one that generates a spherical shape and another that generates a cylindrical shape. To gain physical insights into membrane shape transitions, it is important to investigate the stability of membranes in the presence of these two types of proteins. However, the existing membrane theory is mostly restricted to the class of membranes that interact with the sphere shape-generating proteins and possess isotropic symmetry. In this work, we use curvature elasticity of the lipid membranes to derive the stability criterion for membranes that interact with the cylindrical-shape-generating proteins that possess orthotropic symmetry. We derive the convexity condition followed by the stability criterion for a generalized form of strain energy that can entertain material heterogeneity. The proposed framework would allow for a rigorous analysis of a broader set of membrane–protein interactions during key cellular processes.

Keywords
Stability, elastic fluid shells, lipid membranes, orthotropic symmetry, BAR proteins

1. Introduction

Various proteins regulate membrane remodeling events during key cellular processes such as transport of macromolecules and division of organelles. One of the primary mechanisms by which proteins deform membranes is via curvature generation. There are two main types of curvature-generating proteins: one that generates isotropic spherical curvature such as clathrin and another that generates orthotropic cylindrical curvatures such as BIN-amphiphysin-Rvs (BAR) proteins. In this article, we focus our attention on BAR and similarly shaped proteins [1-3] that have been shown to play a vital role in vesicle formation and scission during clathrin-mediated endocytosis (CME). These proteins are rod-shaped, peripheral proteins with or without small wedge-like insertions into the underlying lipid membrane. In a previous study, BAR proteins were shown to drive vesicle formation in conjunction with actin filaments via instability in a high membrane tension environment [4]. In a recent study, conical lipids and cylindrical-shaped proteins have been shown to trigger instabilities during mitochondrial

Corresponding authors:
Ashutosh Agrawal, Department of Mechanical Engineering, University of Houston, 4800 Calhoun Road, Houston, TX 77204, USA.
Email: ashutosh@uh.edu

Nikhil Walani, Universitat Politècnica de Catalunya, BarcelonaTech, Barcelona, Spain
Email: nikhilwalani@gmail.com

fission [5]. In order to gain mechanistic insights into such pronounced shape transitions, it is important to study the stability of membranes coated with BAR-like proteins.

The energetics of membranes coated with isotropic proteins is well captured by a Helfrich–Canham model which depends on the two curvature invariants: the mean curvature and the Gaussian curvature [6]. However, owing to the interactions with directional proteins such as BAR-like proteins, isotropic symmetry is lost and the strain energy depends on an additional invariant, called the curvature deviator. This form of energy has been used in the context of rigid anisotropic inclusions and penalizes the deviation of the normal curvature of the surface from the curvature in the direction of the protein [7-9]. In [10], we formalized the mechanical model to account for interaction of BAR-like proteins with the lipid membrane. In addition, numerical studies have also been performed using similar energetics to investigate the response of BAR-coated membranes [11-15]. Mathematical models describing membrane–protein interactions for both isotropic and anisotropic cases have been reviewed in [16].

The stability of isotropic membranes has been a part of many fundamental studies. The second variation of Helfrich–Canham energy has been derived in [17-21]. The stability of isotropic membranes with multiple phases was derived in [22] and for membranes with heterogeneities and higher-order curvature dependence was derived in [23]. However, a mathematical framework to investigate the stability of orthotropic membranes is still lacking. In this article, we build upon these fundamental works to derive the stability criterion for orthotropic membranes. We allow the strain energy to have arbitrary functional dependence on the mean curvature, the Gaussian curvature and the curvature deviator fields and derive the necessary criterion for the existence of stable configurations. Next, we perform the linearized stability of the membrane about a given shape, distribution, and alignment of proteins and compute the second variation of the energy functional. The outline of the article is as follows: in Section 2 we briefly revisit the derivation of the first variation for orthotropic membranes; in Section 3 we discuss the convexity criterion of the energy density; in Section 4 we derive the second variation of the energy functional; and in Section 5 we discuss the key findings and draw conclusions.

A summary of the notation used in the text is given in Table 1. Let $\mathbf{r}(\theta^\alpha)$ be the position of a material point on the 2D surface embedded in 3D space, where $\theta^\alpha = (\theta^1, \theta^2)$ are the coordinates that parametrize the surface. We follow the Einstein's summation convention and use the Greek indices to define the range over the set $\{1, 2\}$. The tangent vectors at any point on the surface are given by $\mathbf{r}_{,\alpha} = \frac{\partial \mathbf{r}}{\partial \theta^\alpha} = \mathbf{a}_\alpha$. This yields a metric tensor on the surface whose components are obtained as $a_{\alpha\beta} = \mathbf{a}_\alpha \cdot \mathbf{a}_\beta$. The covariant derivative of the tangent vectors yield the components of the second fundamental form such that $\mathbf{a}_{\alpha;\beta} = b_{\alpha\beta}\mathbf{n}$, where $\mathbf{n}$ is the unit normal to the surface.

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 along with 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
D	Deviatoric curvature field on the surface
$\boldsymbol{\lambda}$	Direction of alignment of protein on the surface
$\boldsymbol{\mu}$	Direction perpendicular to $\boldsymbol{\lambda}$ on the surface
σ	Lagrange multiplier to preserve local area
J	Determinant of the Jacobian matrix
p	Transmembrane pressure across the membrane
V	Volume enclosed by the fluidic shell
Δ_s	Surface Laplacian

Steigmann [24] defined in-plane fluidity of surfaces through material symmetry and showed that the strain energy density could only depend on the mean curvature H, the Gaussian curvature K, and the areal stretch ratio J. These fields can be written in terms of the components of first and second fundamental forms as

$$\begin{aligned} 2H &= b_{\alpha\beta}a^{\alpha\beta}, \\ 2K &= \varepsilon^{\alpha\gamma}\varepsilon^{\beta\theta}b_{\alpha\beta}b_{\gamma\theta}, \\ 2J^2 &= 2a/A = e^{\alpha\gamma}e^{\beta\theta}a_{\alpha\beta}a_{\gamma\theta}/A, \end{aligned} \tag{1}$$

where $\varepsilon^{\alpha\beta} = e^{\alpha\beta}/\sqrt{a}$ with $e^{\alpha\beta}$ being the permutation tensor, a is the determinant of the metric tensor in the current configuration, and A is the determinant of the metric tensor in the reference configuration.

In the present context, the attached BAR-like proteins are assumed to have a directionality, which at each material point is assumed to be represented by a unit tangential vector field $\boldsymbol{\lambda}(\theta^\alpha)$. The direction orthogonal to $\boldsymbol{\lambda}$ and in the plane of the surface is defined such that

$$\boldsymbol{\mu} = \mathbf{n} \times \boldsymbol{\lambda}. \tag{2}$$

Thus, $\{\boldsymbol{\lambda}, \boldsymbol{\mu}\}$ form an orthonormal basis on the tangent plane of the surface. These vectors can be used to capture the in-plane orthotropic symmetry of the surface in the current configuration by allowing the strain energy density to depend on a structural tensor $\mathbf{S} = \boldsymbol{\lambda} \otimes \boldsymbol{\lambda} - \boldsymbol{\mu} \otimes \boldsymbol{\mu}$ (see [10]). Enforcing the Galilean invariance on the energy density then yields the additional dependence on a new invariant D, called the curvature deviator, such that

$$2D = b_{\alpha\beta}(\lambda^\alpha\lambda^\beta - \mu^\alpha\mu^\beta), \tag{3}$$

where

$$\lambda^\alpha = \boldsymbol{\lambda} \cdot \mathbf{a}^\alpha, \quad \mu^\alpha = \boldsymbol{\mu} \cdot \mathbf{a}^\alpha. \tag{4}$$

In the presence of constraints on the local area and the enclosed volume (V), the energy functional for a closed surface (ω) is expressed as

$$E = \int_\omega W(H, D, K; \theta^\alpha)\, da + \int_\omega \sigma(\theta^\alpha)\, da - pV(\omega), \tag{5}$$

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

To evaluate the Euler–Lagrange equations associated with the equilibrium of a patch of membrane $\pi \subset \omega$, we consider the variation of the position vector given by

$$\dot{\mathbf{r}} = \frac{\partial \mathbf{r}(\theta^\alpha; \epsilon)}{\partial \epsilon}\Big|_{\epsilon=0} = \mathbf{u} = u^\alpha \mathbf{a}_\alpha + u\mathbf{n} = \mathbf{u} + u\mathbf{n}. \tag{6}$$

Here and henceforth, the superposed dot $\dot{()}$ signifies the derivative with respect to a parameter ϵ (evaluated at $\epsilon = 0$) that generates a family of surfaces $\mathbf{r}(\theta^\alpha; \epsilon)$. In (6), $\mathbf{u} = u^\alpha \mathbf{a}_\alpha$ is the tangential variation and $u\mathbf{n}$ is the normal variation. As derived in [25], this yields the following variations of the first and second fundamental form

$$\begin{aligned} \dot{a}_{\alpha\beta} &= u_{\alpha;\beta} + u_{\beta;\alpha} - 2ub_{\alpha\beta}, \\ \dot{b}_{\alpha\beta} &= u^\lambda_{;\alpha}b_{\lambda\beta} + u^\lambda_{;\beta}b_{\lambda\alpha} + u^\lambda b_{\lambda\alpha;\beta} + u_{;\alpha\beta} - ub_{\alpha\lambda}b^\lambda_\beta, \end{aligned} \tag{7}$$

where a subscripted semi-colon $()_{;\alpha}$ denotes the covariant derivative with respect to the metric $a_{\alpha\beta}$. Using these relations, the variations of the mean curvature, the Gaussian curvature, and the Jacobian can be evaluated as [25]

$$\dot{H} = u^\alpha H_{,\alpha} + \frac{1}{2}(\Delta_s u) + u(2H^2 - K),$$
$$\dot{K} = u^\alpha K_{,\alpha} + 2uHK + u_{;\alpha\beta}\tilde{b}^{\alpha\beta}, \quad \text{and} \tag{8}$$
$$\frac{\dot{J}}{J} = u^\alpha_{;\alpha} - 2uH.$$

Here, Δ_s represents the surface Laplacian, which for a scalar field f is given by $\Delta_s f = f_{;\alpha\beta}a^{\alpha\beta}$.

Next, we derive the variation of the alignment vector of the protein $\boldsymbol{\lambda}$ and the curvature deviator (D). We note that the orientation of the protein in the tangent plane is assumed to be not convected to the surface. This is because the lipids underneath the orthotropic proteins are fluidic in nature. Thus, as the surface is given a virtual displacement, there is no variation in $\boldsymbol{\lambda}$ along the surface and, hence,

$$\dot{\boldsymbol{\lambda}} \cdot \mathbf{a}_\alpha = 0. \tag{9}$$

However, any virtual displacement should enforce that the surface protein is aligned perpendicular to the surface normal such that,

$$\boldsymbol{\lambda} \cdot \mathbf{n} = 0. \tag{10}$$

Thus, the variation of $\boldsymbol{\lambda}$, if any, occurs when the surface variation leads to a variation in the normal and has to satisfy the relation

$$\dot{\boldsymbol{\lambda}} = -(\boldsymbol{\lambda} \cdot \dot{\mathbf{n}})\mathbf{n}. \tag{11}$$

Hence, the variation of $\boldsymbol{\lambda}$ only occurs in the direction normal to the surface at the material point in consideration. Similarly, we can obtain the variation of $\boldsymbol{\mu}$ as

$$\dot{\boldsymbol{\mu}} = -(\boldsymbol{\mu} \cdot \dot{\mathbf{n}})\mathbf{n}. \tag{12}$$

We note that this aspect of variation was not presented by us previously [10], where it was assumed that $\dot{\boldsymbol{\lambda}} = 0$. Despite this difference, variations of the covariant or contravariant components of the protein alignment vector and the curvature deviator are not affected and can still be written as

$$\dot{\lambda}^\alpha = \boldsymbol{\lambda} \cdot \dot{\mathbf{a}}^\alpha = \lambda^\gamma(-u^\alpha_{;\gamma} + ub^\alpha_\gamma),$$
$$\dot{\mu}^\alpha = \boldsymbol{\mu} \cdot \dot{\mathbf{a}}^\alpha = \mu^\gamma(-u^\alpha_{;\gamma} + ub^\alpha_\gamma), \tag{13}$$
$$2\dot{D} = u^\eta b_{\alpha\beta;\eta}(\lambda^\alpha\lambda^\beta - \mu^\alpha\mu^\beta) + (u_{;\alpha\beta} + ub_{\alpha\gamma}b^\gamma_\beta)(\lambda^\alpha\lambda^\beta - \mu^\alpha\mu^\beta).$$

As vector fields $\boldsymbol{\lambda}$ and $\boldsymbol{\mu}$ are linearly independent and span the tangent plane at each material point, we can transform the basis and the components of the metric and its dual such that

$$\mathbf{a}^\alpha = \lambda^\alpha\boldsymbol{\lambda} + \mu^\alpha\boldsymbol{\mu}, \quad a^{\alpha\beta} = \lambda^\alpha\lambda^\beta + \mu^\alpha\mu^\beta, \quad \text{and} \quad a_{\alpha\beta} = \lambda_\alpha\lambda_\beta + \mu_\alpha\mu_\beta. \tag{14}$$

In addition, because $\boldsymbol{\lambda}$ and $\boldsymbol{\mu}$ are orthonormal, we note the useful identities,

$$\boldsymbol{\lambda} \cdot \boldsymbol{\mu} = \lambda^\alpha\mu_\alpha = \lambda_\beta\mu^\beta = 0,$$
$$\boldsymbol{\lambda} \cdot \boldsymbol{\lambda} = \lambda^\alpha\lambda^\beta a_{\alpha\beta} = \boldsymbol{\mu} \cdot \boldsymbol{\mu} = \mu^\alpha\mu^\beta a_{\alpha\beta} = 1. \tag{15}$$

Using the Cayley–Hamilton theorem in the form

$$b^{\alpha\beta} = 2Ha^{\alpha\beta} - \tilde{b}^{\alpha\beta}, \tag{16}$$

where $\tilde{b}^{\alpha\beta}$ is the contravariant adjugate of the curvature tensor such that

$$\tilde{b}^{\alpha\beta}b_{\beta\eta} = \delta^\alpha_\eta K, \tag{17}$$

we can write

$$b_{\alpha\gamma}b^{\gamma}_{\beta} = 2Hb_{\alpha\beta} - Ka_{\alpha\beta}. \tag{18}$$

Using the above results from (14)–(18) and the fact that the metric is covariant constant ($a_{\alpha\beta;\gamma} = 0$), the variation of D in (13) can be reduced to

$$\dot{D} = u^{\eta}D_{,\eta} - u^{\eta}b_{\alpha\beta}(\lambda^{\alpha}\lambda^{\beta})_{;\eta} + (u_{;\alpha\beta})(\lambda^{\alpha}\lambda^{\beta}) - \frac{1}{2}\Delta_s u + 2uHD. \tag{19}$$

In the above equation, the first two terms are due to the tangential variations and the remaining three terms are due to the normal variations of the surface. We would like to note that the tangential variation of D is not just $u^{\eta}D_{,\eta}$ as is the case with the scalar fields obtained only from the map $\mathbf{r}(\theta^{\alpha})$, such as those of H and K in (8), but also depends on the given orientation of the protein $\boldsymbol{\lambda}$.

With the help of the variations obtained in (8), (19), and the procedure outlined in [10], the first variation of E can be expressed as

$$\dot{E} = \int_{\pi}\{-u^{\alpha}(\sigma_{,\alpha} + \frac{\partial W}{\partial\theta^{\alpha}} + N_{\alpha}) + uG\}\, da + \dot{E}_B \tag{20}$$

where

$$\begin{aligned} N_{\alpha} &= W_D b_{\beta\eta}(\lambda^{\beta}\lambda^{\eta})_{;\alpha}, \\ G &= (W_D\lambda^{\alpha}\lambda^{\beta})_{;\beta\alpha} - \frac{1}{2}\Delta_s(W_D) + 2HDW_D + \frac{1}{2}\Delta_s W_H + (W_K)_{;\beta\alpha}\tilde{b}^{\beta\alpha} \\ &\quad + W_H(2H^2 - K) + 2H(KW_K - W) - 2H\sigma - p, \text{and} \\ \dot{E}_B &= \int_{\partial\pi}[(W+\sigma)u^{\alpha}\nu_{\alpha} + \frac{1}{2}(W_H - W_D)\nu^{\alpha}u_{,\alpha} - \frac{1}{2}(W_H - W_D)_{,\alpha}\nu^{\alpha}u \\ &\quad + (W_K\tilde{b}^{\alpha\beta} + W_D\lambda^{\alpha}\lambda^{\beta})\nu_{\beta}u_{,\alpha} - [(W_K)_{,\alpha}\tilde{b}^{\alpha\beta} + (W_D\lambda^{\alpha}\lambda^{\beta})_{;\alpha}]\nu_{\beta}u\}\, ds. \end{aligned} \tag{21}$$

The first variation in (20) furnishes the equilibrium equations in the tangent plane that describe the gradient in the surface tension field if the material is heterogeneous or has directional proteins,

$$\sigma_{,\alpha} = -\frac{\partial W}{\partial\theta^{\alpha}} - N_{\alpha}. \tag{22}$$

Here $\frac{\partial W}{\partial\theta^{\alpha}}$ represents the explicit derivative of strain energy density with respect to the parameterizing variables. For equilibrium in the direction normal to the surface, we require that

$$G = 0, \tag{23}$$

and can be termed as the *modified shape equation* in the presence of orthotropic proteins [10].

In $(21)_3$, $\dot{E}_B$ corresponds to the boundary terms of the variation of the energy functional for the membrane patch π, s represents the arc length that parameterizes the edge $\partial\pi$ and ν_{α}, and ν^{α} are the covariant and the contravariant components of the in-plane normal ($\boldsymbol{\nu}$) to the edge, respectively. Similarly, τ_{α} and τ^{α} are the covariant and the contravariant components of the unit tangent to the boundary ($\boldsymbol{\tau}$), respectively. The components of the symmetric curvature tensor can be expressed as

$$\begin{aligned} &\kappa_{\nu} = b_{\alpha\beta}\nu^{\alpha}\nu^{\beta}, \quad \kappa_{\tau} = b_{\alpha\beta}\tau^{\alpha}\tau^{\beta} \text{and} \\ &\tau = b_{\alpha\beta}\tau^{\alpha}\nu^{\beta}. \end{aligned} \tag{24}$$

Using the relation presented in [26], we decompose the derivatives of normal perturbation with respect to the parameterization θ^{α} such that

$$u_{,\alpha} = \tau_{\alpha}u' + \nu_{\alpha}u_{,\nu}. \tag{25}$$

The boundary terms can then be recast to obtain the edge forces and moment such that

$$\dot{E}_B = \int_{\partial\pi} (F_\nu \boldsymbol{\nu} + F_\tau \boldsymbol{\tau} + F_n \mathbf{n}) \cdot \mathbf{u} ds - \int_{\partial\pi} M \boldsymbol{\tau} \cdot \boldsymbol{\omega} ds, \tag{26}$$

where,

$$\begin{aligned} M &= \frac{1}{2} W_H + \kappa_\tau W_K + \frac{1}{2}(W_D 2(\boldsymbol{\lambda} \cdot \boldsymbol{\nu})^2 - 1), \\ F_\nu &= W + \sigma - \kappa_\nu M, \\ F_\tau &= - \tau M, \\ F_n &= (\tau W_K)' - \frac{1}{2}(W_H)_{,\nu} - (W_K)_{,\beta} \tilde{b}^{\alpha\beta} \nu_\alpha \\ &\quad + \frac{1}{2}(W_D)_{,\nu} - (W_D \lambda^\alpha \lambda^\beta)_{;\beta} \nu_\alpha - (W_D \lambda^\alpha \lambda^\beta \nu_\beta \tau_\alpha)'. \end{aligned} \tag{27}$$

Here, M is the bending moment per unit length, F_ν is the in-plane normal force per unit length, F_τ is the in-plane shear force per unit length, and F_n is the transverse shear force per unit length. Primes $()'$ represent the partial derivative of the concerned entity () with respect to arc length s. The details of the above derivation have been presented in [10] for orthotropic lipid membranes and in [26] for isotropic ones.

1.1. Orientation of proteins

Until now, no assumptions have been made about the orientation of the proteins $\boldsymbol{\lambda}$. If we assume that the protein orientation is regulated by the membrane curvature, we can obtain $\boldsymbol{\lambda}$ by minimizing the energy functional in (5) with respect to $\boldsymbol{\lambda}$

$$\delta E = \int_\Omega W_D (\frac{\partial D}{\partial \boldsymbol{\lambda}} \cdot \delta \boldsymbol{\lambda}) \, ds = 0. \tag{28}$$

Here, δE is the variation in the energy corresponding to a variation in the orientation of the proteins $\delta\boldsymbol{\lambda}$. From the definition of the curvature deviator (3) and the symmetry of the curvature tensor $\mathbf{b}$, we obtain

$$\frac{\partial D}{\partial \boldsymbol{\lambda}} = 2\mathbf{b}\boldsymbol{\lambda}. \tag{29}$$

We note that the variation in the orientation of the proteins ($\delta\boldsymbol{\lambda}$) is not arbitrary and has to satisfy the condition

$$\boldsymbol{\lambda} \cdot \delta \boldsymbol{\lambda} = 0. \tag{30}$$

Thus, for stationarity of the energy functional with respect to the orientation of the proteins in (28), we require that

$$2 W_D (\mathbf{b}\boldsymbol{\lambda} \cdot \delta\boldsymbol{\lambda}) = 0 \tag{31}$$

holds true locally in Ω. This would mean that either

$$W_D = 0, \tag{32}$$

indicating that the proteins are aligned in the direction of their preferred curvature on the surface, or they are aligned along a principal direction $\boldsymbol{g}$ (say) of the curvature tensor of the surface such that

$$\boldsymbol{b}\boldsymbol{\lambda} \cdot \delta\boldsymbol{\lambda} = \kappa_g \boldsymbol{\lambda} \cdot \delta\boldsymbol{\lambda} = 0. \tag{33}$$

2. Convexity criterion

Now we derive the necessary condition for stability to restrict the allowable functional forms of strain energy density in the same spirit as that derived in [27] for bendable surfaces. These surfaces are piecewise smooth such that the Euler–Lagrange equations (22) and (23) are satisfied at all points except at the curves that sustain the jumps in the curvature. Such curves have been termed as phase boundaries for 2D systems [28]. It was shown in [27] that the jump in the curvature has to be of the form

$$[\mathbf{r}_{,\alpha\beta}] = \mathbf{u}\nu_\alpha \nu_\beta. \tag{34}$$

Here, $\mathbf{u}$ is any arbitrary vector in 3D space and ν_α are the covariant components of the in-plane unit normal $\boldsymbol{\nu}$ to the curve. The square brackets indicate a jump in the quantity across the phase boundary. For a phase boundary to be a solution of an energy minimizing configuration, it is required that the force and moment across it should be continuous, i.e.,

$$\begin{aligned} &[\mathbf{f}] = [F_\nu \boldsymbol{\nu} + F_\tau \boldsymbol{\tau} + F_n \mathbf{n}] = \mathbf{0}, \text{ and} \\ &[M] = 0. \end{aligned} \tag{35}$$

Following [28], we derive the conditions for the strong relative minimizers where the perturbations in $\mathbf{r}$ and $\mathbf{r}_{,\alpha}$ are bounded and for the weak minimizers for which, in addition, perturbations in $\mathbf{r}_{,\alpha\beta}$ are bounded. To this end, we derive the Weierstrass–Erdmann condition for the jump condition of strain energy density using the Weierstrass–Graves convexity criterion [29], which requires that for a scalar potential U,

$$U(\mathbf{r}_{,\alpha}; \boldsymbol{\lambda}; \mathbf{r}_{,\alpha\beta} + \mathbf{c}d_\alpha d_\beta) - U(\mathbf{r}_{,\alpha}; \boldsymbol{\lambda}; \mathbf{r}_{,\alpha\beta}) \geqslant \mathbf{c} \cdot \frac{\partial U}{\partial \mathbf{r}_{,\alpha\beta}} d_\alpha d_\beta \tag{36}$$

for every material point in consideration except at the phase boundary. The above equation has to hold for any arbitrary vector c and d_α. Arguments conjecturing the usage of the above equation for inextensible surfaces has been presented in [28]. For $U = W(H, D, K)$, we evaluate the right-hand side of the above equation by fixing $\boldsymbol{\lambda}$, $\mathbf{r}_{,\alpha}$ and computing the variation

$$\dot{U} = \dot{W} = W_H \dot{H} + W_D \dot{D} + W_K \dot{K}, \tag{37}$$

where

$$\begin{aligned} &\dot{H} = \frac{1}{2} a^{\alpha\beta} (\mathbf{n} \cdot \dot{\mathbf{r}}_{,\alpha\beta}), \\ &\dot{D} = \frac{1}{2} (\lambda^\alpha \lambda^\beta - \mu^\alpha \mu^\beta)(\mathbf{n} \cdot \dot{\mathbf{r}}_{,\alpha\beta}), \text{ and} \\ &\dot{K} = \tilde{b}^{\alpha\beta} (\mathbf{n} \cdot \dot{\mathbf{r}}_{,\alpha\beta}). \end{aligned} \tag{38}$$

Using the relation

$$\dot{U} = \frac{\partial U}{\partial \mathbf{r}_{,\alpha\beta}} \cdot \dot{\mathbf{r}}_{,\alpha\beta}, \tag{39}$$

and (37) and (38), we obtain that

$$\frac{\partial U}{\partial \mathbf{r}_{,\alpha\beta}} = \left(\frac{1}{2} W_H a^{\alpha\beta} + \frac{1}{2} W_D (\lambda^\alpha \lambda^\beta - \mu^\alpha \mu^\beta \right) + W_K \tilde{b}^{\alpha\beta}) \mathbf{n}. \tag{40}$$

Subsequently, on using the relation $\lambda^\alpha \lambda^\beta + \mu^\alpha \mu^\beta = a^{\alpha\beta}$, we obtain

$$\mathbf{c} \cdot \frac{\partial U}{\partial \mathbf{r}_{,\alpha\beta}} d_\alpha d_\beta = \left(\frac{1}{2} W_H + \frac{1}{2} W_D (2\eta - 1) + \zeta W_K \right) (\mathbf{c} \cdot \mathbf{n}). \tag{41}$$

In the above equation, we have

$$\begin{aligned}\eta &= \lambda^\alpha \lambda^\beta d_\alpha d_\beta = (\boldsymbol{\lambda}\cdot\mathbf{d})^2\\ \zeta &= \tilde{b}^{\alpha\beta} d_\alpha d_\beta.\end{aligned} \tag{42}$$

To derive (41), we used the normalization condition such that $a^{\alpha\beta}d_\alpha d_\beta = 1$. This would mean that the vector $\mathbf{d} = d_\alpha \mathbf{a}^\alpha$, tangential at the material point in consideration, is of unit magnitude.

We use (34) and (36) and approach the boundary in consideration from either side (+ or −) with $\mathbf{c} = \mathbf{u}, d_\alpha = \nu_\alpha$ for $\mathbf{r}_{,\alpha\beta} = \mathbf{r}^-_{,\alpha\beta}$ and $\mathbf{c} = -\mathbf{u}, d_\alpha = \nu_\alpha$ for $\mathbf{r}_{,\alpha\beta} = \mathbf{r}^+_{,\alpha\beta}$. This yields

$$[W] = M^{\pm}\mathbf{u}\cdot\mathbf{n}. \tag{43}$$

Here, M with superscripted + or − represents the limiting bending moment on either side of the boundary. We then use (24) to derive the jump in normal curvature of the interface

$$[\kappa_\tau] = (\mathbf{u}\cdot\mathbf{n})(\boldsymbol{\nu}\cdot\boldsymbol{\tau})^2 = 0 \tag{44}$$

and the jump in normal curvature perpendicular to the interface

$$[\kappa_\nu] = (\mathbf{u}\cdot\mathbf{n}). \tag{45}$$

Hence, the jumps in curvature fields are given by

$$\begin{aligned}[H] &= \frac{1}{2}\mathbf{u}\cdot\mathbf{n},\\ [D] &= \frac{1}{2}(\mathbf{u}\cdot\mathbf{n})(2\eta-1), \text{ and}\\ [K] &= \kappa_\tau(\mathbf{u}\cdot\mathbf{n}).\end{aligned} \tag{46}$$

Using the above relations, we can write

$$[W] = W_H^{\pm}[H] + W_D^{\pm}[D] + W_K^{\pm}[K] = [\kappa_\nu]M^{\pm}, \tag{47}$$

where the same superscripts (either + or −) have to be used for all the terms on the right-hand side. This jump condition in strain energy density furnishes the Weierstrass–Erdmann condition for fluid membranes with orthotropic symmetry.

The Weierstrass–Graves inequality as derived in [28] can be extended to the present case of orthotropic shells by fixing the tangent vectors $\mathbf{r}_{,\alpha}$ and $\boldsymbol{\lambda}$, and perturbing $\mathbf{r}_{,\alpha\beta} \to \mathbf{r}_{,\alpha\beta} + \mathbf{c}d_\alpha d_\beta$ to obtain $\Delta H = \frac{1}{2}\mathbf{n}\cdot\mathbf{c}$, $\Delta D = \Delta H(2\eta - 1)$, and $\Delta K = 2\zeta\Delta H$. This reduces (36), with the help of (41), to

$$W(H+\Delta H, D+\Delta D, K+\Delta K) - W(H,D,K) \geqslant W_H\Delta H + W_D\Delta D + W_K\Delta K. \tag{48}$$

The above equation can be linearized with respect to $\phi = \mathbf{n}\cdot\mathbf{c}$ by fixing H, D, K, η, ζ, and setting $P_1(\phi) = W(H+\phi/2, D+\phi/2(2\eta-1), K+\zeta\phi)$. This yields that the convexity criterion in the above equation is equivalent to the convexity of $P_1(\phi)$ at $\phi = 0$, which, in turn, implies that $P_1''(0) \geqslant 0$. Thus, we obtain the Legendre–Hadamard condition for convexity

$$\frac{1}{4}W_{HH} + \frac{1}{4}(2\eta-1)^2 W_{DD} + \zeta^2 W_{KK} + \frac{1}{2}(2\eta-1)W_{HD} + \zeta W_{HK} + \zeta(2\eta-1)W_{DK} \geqslant 0, \tag{49}$$

where ζ in the above equation is bounded by extremal of the eigenvalues of the cofactor of the curvature tensor and η is bounded such that $0 \leqslant \eta \leqslant 1$.

To maintain continuity of traction across the phase boundary, we use (35) and (27) and require that

$$[F_\nu] = [W+\sigma] - [\kappa_\nu M] = 0. \tag{50}$$

Equations (47) and (50) then imply that the jump in the Lagrange multiplier across the phase boundary vanishes, i.e., $[\sigma]=0$. As $[\tau]=0$, from (27) we establish that the tractions along $\boldsymbol{\tau}$ are continuous. For the tractions along $\mathbf{n}$ to be continuous, we require

$$\begin{aligned}&[(\tau W_K)' - \frac{1}{2}(W_H)_{,\nu} - (W_K)_{,\beta}\tilde{b}^{\alpha\beta}\nu_\alpha + \frac{1}{2}(W_D)_{,\nu}\\ &- (W_D\lambda^\alpha\lambda^\beta)_{;\beta}\nu_\alpha - (W_D\lambda^\alpha\lambda^\beta\nu_\beta\tau_\alpha)'] = 0.\end{aligned} \tag{51}$$

We note here that (35) required for the continuity in traction and moment across the phase boundary is necessary for both the weak and the strong relative minimizers. Equations (47) and (48) are necessary for the strong relative minimizers whereas (49) replaces (48) as the necessary condition for the weak minimizers.

2.1. Quadratic energy density

For a quadratic energy density of the form as considered in [10],

$$W = k_1(H-H_0)^2 + k_2(D-D_0)^2 + 2k_{12}(H-H_0)(D-D_0) + \bar{k}K, \tag{52}$$

we observe that the Legendre–Hadamard condition for stability requires

$$(2\eta-1)^2 k_2 + 2(2\eta-1)k_{12} + k_1 \geqslant 0 \quad \forall\ \eta \in [0,1]. \tag{53}$$

If we assume that the protein orientation can be solved for by minimizing the energy functional with respect to the perturbations in $\boldsymbol{\lambda}$, then we require the Weierstrass–Graves inequality to hold for the perturbations in $\boldsymbol{\lambda}$ as well. For this, we fix $\mathbf{r}_{,\alpha}$, $\mathbf{r}_{,\alpha\beta}$ and vary $\boldsymbol{\lambda} \to \boldsymbol{\lambda} + \mathbf{q}$ where $\mathbf{q} = q^\alpha \mathbf{a}_\alpha$ is such that new orientation of the proteins differ from the original by a rotation and, hence, satisfies the criterion $\mathbf{q}\cdot\mathbf{q} = -2\boldsymbol{\lambda}\cdot\mathbf{q}$. This yields

$$\Delta D = (\mathbf{b}+\mathbf{b}^T)\boldsymbol{\lambda}\cdot\mathbf{q} = 2(\mathbf{b}\boldsymbol{\lambda})\cdot\mathbf{q}. \tag{54}$$

For such perturbations in the orientation of the proteins, we can write the Weierstrass–Graves inequality as

$$W(H, D+\Delta D, K) - W(H, D, K) \geqslant W_D \Delta D. \tag{55}$$

The Legendre–Hadamard condition associated with the above equation after linearization with respect to $\Delta D = \xi$ and setting $P_2(\xi) = W(H, D+\xi, K)$ yields $P_2''(0) \geqslant 0$. Thus, along with (49), we require that $W_{DD} \geqslant 0$. Moreover, because (49) is quadratic in $(\eta-1)/2$, a stricter criterion for convexity requires that its discriminant is non-positive such that

$$(W_{HD} + 2\zeta W_{DK})^2 - W_{DD}(W_{HH} + 4\zeta^2 W_{KK} + 4\zeta W_{HK}) \leqslant 0. \tag{56}$$

The above equation does not depend on the orientation of the proteins for the quadratic form of the strain energy considered in (52) and can be written as

$$k_{12}^2 - k_1 k_2 \leqslant 0 \tag{57}$$

subject to $k_2 \geqslant 0$.

3. The second variation

Now, to obtain the linearized stability about a given configuration, we write the second variation of the energy functional considered in (5). To obtain this, we make the assumption that the surface Ω is closed. This allows us to simplify the resulting expressions using the Stokes divergence theorem.

The second variation of the position field can be expressed as

$$\ddot{\mathbf{r}} = \frac{\partial^2 \mathbf{r}}{\partial \epsilon^2}\Big|_{\epsilon=0} = \mathbf{v} = v^\alpha \mathbf{a}_\alpha + v\mathbf{n}, \tag{58}$$

where v^α and v are the tangential and normal components of $\mathbf{v}$. These are related to the first variation through the constraints of areal and volume incompressibility. Otherwise, they are arbitrary. The second variation of the energy E can be computed by taking the variation of the first variation in (20) such that

$$\ddot{E} = \int_\omega \{-\dot{u}^\alpha (\sigma_{,\alpha} + \frac{\partial W}{\partial \theta^\alpha} + N_\alpha) - u^\alpha \frac{\partial \dot{W}}{\partial \theta^\alpha} - u^\alpha \dot{N}_\alpha + \dot{u}G + u\dot{G}\}\, da \tag{59}$$

subject to the areal incompressibility constraint,

$$\frac{\dot{J}}{J} = u^\alpha_{;\alpha} - 2uH = 0, \tag{60}$$

and the volumetric constraint,

$$\dot{V} = \int_\omega u\, da = 0. \tag{61}$$

The stability of such a surface at equilibrium requires that

$$\ddot{E} > 0 \tag{62}$$

for the variations that satisfy (60), (61), along with the constraints $\ddot{J} = 0$ and $\ddot{V} = 0$. The constraints on the second variation of the areal stretch ratio ($\ddot{J}$) and the volume enclosed by the membrane ($\ddot{V}$) yield the relation between the second variation $\mathbf{v}$ and the first variation $\mathbf{u}$. These relations are not presented here as the second variation of the energy functional in its entirety can be written as a functional of the first variation in position and its derivatives at equilibrium. To compute the second variation of the energy functional in (59), we need to evaluate $\{\dot{u}^\alpha, \dot{u}, \frac{\partial \dot{W}}{\partial \theta^\alpha}, \dot{N}_\alpha, \dot{G}\}$. We obtain each of these variations in the subsequent sections.

3.1. Tangential displacement

The variation of the tangential components of u is given by

$$\dot{u}^\alpha = \dot{\mathbf{u}} \cdot \mathbf{a}^\alpha + \mathbf{u} \cdot \dot{\mathbf{a}}^\alpha = (\mathbf{v} \cdot \mathbf{a}^\alpha) + (\mathbf{u} \cdot \mathbf{u}_{,\beta}) a^{\alpha\beta} - (\mathbf{u} \cdot \mathbf{a}^\gamma) a^{\alpha\lambda} \dot{a}_{\lambda\gamma}, \tag{63}$$

where the variations in the co-tangent vectors and the inverse metric are expressed as

$$\dot{\mathbf{a}}^\alpha = a^{\alpha\beta} \dot{\mathbf{a}}_\beta + \dot{a}^{\alpha\beta} \mathbf{a}_\beta \quad \text{and} \quad \dot{a}^{\alpha\beta} = -\, a^{\alpha\lambda} a^{\beta\gamma} \dot{a}_{\lambda\gamma}. \tag{64}$$

Using the relation

$$\mathbf{u} \cdot \mathbf{u}_{,\beta} = \frac{1}{2} (\mathbf{u} \cdot \mathbf{u})_{,\beta} = u^\eta u_{\eta;\beta} + u u_{,\beta} \tag{65}$$

in (63), we obtain the desired variation of the contravariant components $\mathbf{u}$

$$\begin{aligned} \dot{u}^\alpha &= v^\alpha + a^{\alpha\beta} (u^\eta u_{\eta;\beta} + u u_{,\beta}) - u^\gamma a^{\alpha\lambda} (u_{\lambda;\gamma} + u_{\gamma;\lambda} - 2u b_{\lambda\gamma}), \\ &= v^\alpha + u u_{,\beta} a^{\alpha\beta} - u^\gamma u^\alpha_{;\gamma} + 2u u^\gamma b^\alpha_\gamma. \end{aligned} \tag{66}$$

3.2. Normal displacement

To compute the variation $\dot{u}$, we use the fact that $\mathbf{n}$ at each point is a unit vector and its variation would lie in the tangent plane such that [30]

$$\dot{\mathbf{n}} = -(\mathbf{n} \cdot \mathbf{u}_{,\alpha})\mathbf{a}^{\alpha}. \tag{67}$$

This yields the variation

$$\begin{aligned} \dot{u} &= \dot{\mathbf{u}} \cdot \mathbf{n} + \mathbf{u} \cdot \dot{\mathbf{n}}, \\ &= v - u^{\alpha} u_{,\alpha} - u^{\alpha} u^{\gamma} b_{\gamma\alpha}. \end{aligned} \tag{68}$$

3.3. Heterogeneity

We define the explicit derivative of the strain energy density (W) associated with the heterogeneity of the material surface as $I_{\alpha}(H, K; \theta^{\gamma}) = \frac{\partial W}{\partial \theta^{\alpha}}$. We assume that similar to (W), its explicit derivatives with respect to (θ^{α}) are also a function of the curvature invariants and material heterogeneity, i.e., ($H, D, K; \theta^{\eta}$). This furnishes

$$\begin{aligned} \dot{I}_{\alpha} &= (I_{\alpha})_H \dot{H} + (I_{\alpha})_K \dot{K} + (I_{\alpha})_D \dot{D} \\ &= \frac{\partial W_H}{\partial \theta^{\alpha}} \left[u^{\eta} H_{,\eta} + \frac{(\Delta u)}{2} + u(2H^2 - K)\right] + \frac{\partial W_K}{\partial \theta^{\alpha}} \left[u^{\eta} K_{,\eta} + 2uHK + \tilde{b}^{\gamma\beta} u_{;\gamma\beta}\right] \\ &+ \frac{\partial W_D}{\partial \theta^{\alpha}} \left[u^{\eta} D_{,\eta} - u^{\eta} b_{\gamma\beta} (\lambda^{\gamma} \lambda^{\beta})_{;\eta} + u_{;\eta\beta} \lambda^{\eta} \lambda^{\beta} - \frac{1}{2} \Delta u + 2uHD\right]. \end{aligned} \tag{69}$$

We can re-arrange the above equation and separate out the tangential and the normal components to obtain

$$\begin{aligned} \dot{I}_{\alpha} = u^{\eta} &\left[\frac{\partial (W_{,\eta})}{\partial \theta^{\alpha}} - \frac{\partial^2 W}{\partial \theta^{\alpha} \theta^{\eta}}\right] + \frac{(\Delta u)}{2} \left(\frac{\partial W_H}{\partial \theta^{\alpha}} - \frac{\partial W_D}{\partial \theta^{\alpha}}\right) + u_{;\eta\beta} \left(\frac{\partial W_K}{\partial \theta^{\alpha}} \tilde{b}^{\eta\beta} + \frac{\partial W_D}{\partial \theta^{\alpha}} \lambda^{\beta} \lambda^{\eta}\right) \\ &+ 2uH \frac{\partial}{\partial \theta^{\alpha}} (W_H H + W_K K + W_D D) - u \frac{\partial W_H}{\partial \theta^{\alpha}} K - u^{\eta} \frac{\partial W_D}{\partial \theta^{\alpha}} b_{\gamma\beta} (\lambda^{\gamma} \lambda^{\beta})_{;\eta}. \end{aligned} \tag{70}$$

To compute the variation $\dot{N}_{\alpha}$ and $\dot{G}$, we decompose the variation into tangential and normal parts denoted by ($\dot{N}_{\alpha_t}, \dot{G}_t$) and ($\dot{N}_{\alpha_n}, \dot{G}_n$), respectively.

3.3.1. Tangential variations.

The variations of the first and second fundamental forms for tangential variations $\mathbf{u} = u^{\alpha} \mathbf{a}_{\alpha}$ can be obtained using (7)

$$\dot{a}_{\alpha\beta} = u_{\alpha;\beta} + u_{\beta;\alpha}; \quad \dot{b}_{\alpha\beta} = u^{\lambda}_{;\beta} b_{\lambda\alpha} + u^{\lambda}_{;\alpha} b_{\lambda\beta} + u^{\lambda} b_{\lambda\alpha;\beta}. \tag{71}$$

The above equation can be used to obtain the variations in the mean, deviatoric, and Gaussian curvatures

$$\dot{H} = u^{\alpha} H_{,\alpha}, \quad \dot{D} = u^{\eta} D_{,\eta} - u^{\eta} b_{\alpha\beta} (\lambda^{\alpha} \lambda^{\beta})_{;\eta}, \quad \dot{K} = u^{\alpha}.K_{,\alpha}. \tag{72}$$

Using Palatini's identity, we can compute the variation of the Christoffel symbols

$$\begin{aligned} \dot{\Gamma}^{\gamma}_{\alpha\beta} &= \frac{1}{2} a^{\gamma\eta} [(\dot{a}_{\eta\beta})_{;\alpha} + (\dot{a}_{\eta\alpha})_{;\beta} - (\dot{a}_{\alpha\beta})_{;\eta}] \\ &= \frac{1}{2} a^{\gamma\eta} (u_{\eta;\alpha\beta} + u_{\eta;\beta\alpha} + u_{\alpha;\eta\beta} - u_{\alpha;\beta\eta} + u_{\beta;\eta\alpha} - u_{\beta;\alpha\eta}). \end{aligned} \tag{73}$$

The variations in the contravariant components of $\boldsymbol{\lambda}$ and $\boldsymbol{\mu}$ are given by (using (13)),

$$\dot{\lambda}^{\alpha} = -u^{\alpha}_{;\gamma}\lambda^{\gamma}, \quad \dot{\mu}^{\alpha} = -u^{\alpha}_{;\gamma}\mu^{\gamma}. \tag{74}$$

Now, the tangential variation of N_α ($\dot{N}_{\alpha_t}$) as defined in $(21)_1$ can be written as

$$\dot{N}_{\alpha_t} = \dot{W}_D b_{\beta\eta}(\lambda^{\beta}\lambda^{\eta})_{;\alpha} + W_D \dot{b}_{\beta\eta}(\lambda^{\beta}\lambda^{\eta})_{;\alpha} + W_D b_{\beta\eta}\dot{\overline{(\lambda^{\beta}\lambda^{\eta})_{;\alpha}}}. \tag{75}$$

Here the dot above the overbar is used to represent the variation of all the fields beneath the overbar. For computing the first term on the right-hand side of the above equation, we write the variation in W_D as

$$\dot{W}_D = W_{DH}\dot{H} + W_{DD}\dot{D} + W_{DK}\dot{K}, \tag{76}$$

which, after using (72), reduces to

$$\dot{W}_D = u^{\gamma}(W_D)_{,\gamma} - u^{\gamma}\frac{\partial W_D}{\partial \theta^{\gamma}} - u^{\gamma}W_{DD}b_{\phi\psi}(\lambda^{\phi}\lambda^{\psi})_{;\gamma}. \tag{77}$$

The variation of the second term in right-hand side of (75) is computed with the help of the Manardi–Codazzi equations ($b_{\alpha\beta;\eta} = b_{\alpha\eta;\beta}$) and (71)

$$W_D \dot{b}_{\beta\eta}(\lambda^{\beta}\lambda^{\eta})_{;\alpha} = W_D[(u^{\gamma}b_{\gamma\eta})_{;\beta} + u^{\gamma}_{;\eta}b_{\gamma\beta}](\lambda^{\beta}\lambda^{\eta})_{;\alpha}. \tag{78}$$

As the curvature tensor is symmetric, the variation of the last term in the right-hand side of (75) can be written as

$$W_D b_{\beta\eta}\dot{\overline{(\lambda^{\beta}\lambda^{\eta})_{;\alpha}}} = 2W_D b_{\beta\eta}\dot{\overline{\lambda^{\beta}_{;\alpha}\lambda^{\eta}}} = 2W_D b_{\beta\eta}(\dot{\overline{\lambda^{\beta}_{;\alpha}}}\lambda^{\eta} + \lambda^{\beta}_{;\alpha}\dot{\lambda}^{\eta}). \tag{79}$$

To derive $\dot{\overline{\lambda^{\beta}_{;\alpha}}}$, we use the fact that $\boldsymbol{\lambda}$ lies in the tangent plane of the surface which enables us to write

$$\dot{\overline{\lambda^{\beta}_{;\alpha}}} = \dot{\overline{(\boldsymbol{\lambda}\cdot\mathbf{a}^{\beta})_{;\alpha}}} = \dot{\overline{\boldsymbol{\lambda}_{,\alpha}\cdot\mathbf{a}^{\beta}}}. \tag{80}$$

Using (11) and (67) and the assumption that the variation of $\boldsymbol{\lambda}$ is along the normal, we obtain

$$\begin{aligned}\dot{\overline{\lambda^{\beta}_{;\alpha}}} &= (\boldsymbol{\lambda}\cdot\mathbf{a}^{\gamma})(\mathbf{n}\cdot\mathbf{u}_{,\gamma})(\mathbf{n}_{,\alpha}\cdot\mathbf{a}^{\beta}) + (\boldsymbol{\lambda}_{,\alpha}\cdot\mathbf{u}_{,\gamma})a^{\beta\gamma} + (\boldsymbol{\lambda}_{,\alpha}\cdot\mathbf{a}_{\gamma})\dot{a}^{\beta\gamma}\\ &= -\lambda^{\gamma}u^{\phi}b_{\phi\gamma}b^{\beta}_{\alpha} + \lambda^{\eta}_{;\alpha}u_{\eta;\gamma}a^{\beta\gamma} + \lambda^{\eta}u^{\phi}b^{\beta}_{\phi}b_{\eta\alpha} - \lambda^{\eta}_{;\alpha}a^{\beta\phi}(u_{\phi;\eta} + u_{\eta;\phi})\\ &= \lambda^{\gamma}u^{\phi}(b^{\beta}_{\phi}b_{\gamma\alpha} - b_{\phi\gamma}b^{\beta}_{\alpha}) - \lambda^{\gamma}_{;\alpha}u^{\beta}_{;\gamma}.\end{aligned} \tag{81}$$

We have used (64) and (71) to write the variation of components of the dual metric $a^{\alpha\beta}$ above. Now, using the above relation and (74), Equation (79) can be arranged as

$$W_D b_{\beta\eta}\dot{\overline{(\lambda^{\beta}\lambda^{\eta})_{;\alpha}}} = 2W_D b_{\beta\eta}[\lambda^{\eta}\lambda^{\gamma}u^{\phi}(b^{\beta}_{\phi}b_{\gamma\alpha} - b_{\phi\gamma}b^{\beta}_{\alpha}) - u^{\beta}_{;\gamma}(\lambda^{\eta}\lambda^{\gamma})_{;\alpha}]. \tag{82}$$

Hence, substituting variations from (77), (78), and (82) into (75) we obtain the variation $\dot{N}_{\alpha_t}$ as

$$\begin{aligned}\dot{N}_{\alpha_t} &= u^{\gamma}(N_{\alpha})_{;\gamma} - u^{\gamma}W_D b_{\beta\eta}(\lambda^{\beta}\lambda^{\eta})_{;\alpha\gamma} - u^{\gamma}b_{\beta\eta}(\lambda^{\beta}\lambda^{\eta})_{;\alpha}(\frac{\partial W_D}{\partial\theta^{\gamma}} + W_{DD}b_{\phi\psi}(\lambda^{\phi}\lambda^{\psi})_{;\gamma})\\ &\quad + 2u^{\phi}W_D b_{\beta\eta}\lambda^{\eta}\lambda^{\gamma}(b^{\beta}_{\phi}b_{\gamma\alpha} - b_{\phi\gamma}b^{\beta}_{\alpha}),\end{aligned} \tag{83}$$

and subsequently obtain

$$u^\alpha \dot{N}_{\alpha_t} = u^\alpha u^\gamma (N_\alpha)_{;\gamma} - u^\alpha u^\gamma W_D b_{\beta\eta}(\lambda^\beta\lambda^\eta)_{;\alpha\gamma} - u^\alpha u^\gamma b_{\beta\eta}(\lambda^\beta\lambda^\eta)_{;\alpha}\left(\frac{\partial W_D}{\partial\theta^\gamma} + W_{DD} b_{\phi\psi}(\lambda^\phi\lambda^\psi)_{;\gamma}\right). \tag{84}$$

Before deriving the tangential variation of G in $(21)_2$, we note some of the important relations arising from the fact that surface is a 2D Riemannian manifold embedded in 3D space. Here, Riemann curvature tensor quantifies the non-commutability of the covariant derivative of any surface vector d_α (see [31]) such that

$$d^\beta_{;\gamma\alpha} - d^\beta_{;\alpha\gamma} = R^\beta_{\eta\alpha\gamma} d^\eta, \tag{85}$$

and the Riemann tensor is intrinsic to the surface to yield

$$R_{\alpha\beta\gamma\eta} = b_{\alpha\gamma}b_{\beta\eta} - b_{\alpha\eta}b_{\beta\gamma} = K(a_{\alpha\gamma}a_{\beta\eta} - a_{\alpha\eta}a_{\beta\gamma}). \tag{86}$$

This enables us to obtain the relation

$$d^\alpha_{;\beta\alpha} - d^\alpha_{;\alpha\beta} = K d_\beta. \tag{87}$$

Now, we write the expansion of first term in G from $(21)_2$ to be

$$\overline{(W_D\lambda^\alpha\lambda^\beta)_{;\beta\alpha}}^{\cdot} = \overline{(W_D)_{;\beta\alpha}\lambda^\alpha\lambda^\beta}^{\cdot} + \overline{2(W_D)_{,\beta}(\lambda^\alpha\lambda^\beta)_{;\alpha}}^{\cdot} + \overline{(W_D)(\lambda^\alpha\lambda^\beta)_{;\beta\alpha}}^{\cdot}. \tag{88}$$

Variation of the first term in right-hand side of the above equation is

$$\overline{(W_D)_{;\beta\alpha}\lambda^\alpha\lambda^\beta}^{\cdot} = (\dot{W}_D)_{;\beta\alpha}\lambda^\alpha\lambda^\beta - (W_D)_{,\lambda}\dot{\Gamma}^\lambda_{\beta\alpha}\lambda^\alpha\lambda^\beta + 2(W_D)_{;\beta\alpha}\dot{\lambda}^\alpha\lambda^\beta. \tag{89}$$

Using (77), (73), (74), (85)–(87) and calculations detailed in Appendix A, we obtain this to be

$$\overline{(W_D)_{;\beta\alpha}\lambda^\alpha\lambda^\beta}^{\cdot} = u^\gamma[(W_D)_{;\beta\alpha}]_{;\gamma}\lambda^\alpha\lambda^\beta - \left[u^\gamma\frac{\partial W_D}{\partial\theta^\gamma} + u^\gamma W_{DD} b_{\phi\psi}(\lambda^\phi\lambda^\psi)_{;\gamma}\right]_{;\beta\alpha}\lambda^\alpha\lambda^\beta. \tag{90}$$

The second term on the right-hand side of (88) is

$$\overline{2(W_D)_{,\beta}(\lambda^\alpha\lambda^\beta)_{;\alpha}}^{\cdot} = 2(\dot{W}_D)_{,\beta}(\lambda^\alpha\lambda^\beta)_{;\alpha} + 2(W_D)_{,\beta}\overline{(\lambda^\alpha)_{;\alpha}}^{\cdot}\lambda^\beta + 2(W_D)_{,\beta}\lambda^\alpha\overline{(\lambda^\beta)_{;\alpha}}^{\cdot} + 2(W_D)_{,\beta}(\lambda^\alpha)_{;\alpha}\dot{\lambda}^\beta + 2(W_D)_{,\beta}(\lambda^\beta)_{;\alpha}\dot{\lambda}^\alpha. \tag{91}$$

Using relations from (81) and (86), we obtain that

$$\overline{(\lambda^\alpha)_{;\alpha}}^{\cdot} = \lambda^\gamma u^\phi(b^\alpha_\phi b_{\gamma\alpha} - b_{\phi\gamma}b^\alpha_\alpha) - \lambda^\gamma_{;\alpha}u^\alpha_{;\gamma} = -u^\gamma\lambda_\gamma K - \lambda^\gamma_{;\alpha}u^\alpha_{;\gamma} \tag{92}$$

and further using (77) (elaborated upon in Appendix A), we simplify the variation in (91) to be

$$\overline{2(W_D)_{,\beta}(\lambda^\alpha\lambda^\beta)_{;\alpha}}^{\cdot} = 2u^\gamma[(W_D)_{,\beta}(\lambda^\beta\lambda^\alpha)_{;\alpha}]_{;\gamma} - 2(W_D)_{,\beta}(u^\gamma(\lambda^\beta\lambda^\alpha)_{;\gamma})_{;\alpha} - 2\left(u^\gamma\frac{\partial W_D}{\partial\theta^\gamma} + u^\gamma W_{DD}b_{\phi\psi}(\lambda^\phi\lambda^\psi)_{;\gamma}\right)_{,\beta}(\lambda^\alpha\lambda^\beta)_{;\alpha}. \tag{93}$$

Variation of the last term on right-hand side in (88) is

$$\overline{(W_D)(\lambda^\alpha\lambda^\beta)_{;\beta\alpha}}^{\cdot} = (\dot{W}_D)(\lambda^\alpha\lambda^\beta)_{;\beta\alpha} + (W_D)(\overline{\lambda^\alpha_{;\beta\alpha}\lambda^\beta + \lambda^\alpha_{;\beta}\lambda^\beta_{;\alpha} + \lambda^\alpha_{;\alpha}\lambda^\beta_{;\beta}}^{\cdot} + \overline{\lambda^\alpha\lambda^\beta_{;\beta\alpha}}^{\cdot}), \tag{94}$$

where from (87) we obtain

$$\overline{\lambda^\alpha_{;\beta\alpha}}^{\cdot} = \overline{\lambda^\alpha_{;\alpha\beta}}^{\cdot} + \dot{K}\lambda_\beta + K\dot{\lambda}_\beta. \tag{95}$$

and to evaluate the variation $\overline{\lambda^\alpha_{;\alpha\beta}}^{\cdot}$, we note that because $\lambda^\alpha_{;\alpha}$ is a scalar, we can write

$$\overline{\lambda^\alpha_{;\alpha\beta}}^{\cdot} = (\overline{\lambda^\alpha_{;\alpha}}^{\cdot})_{;\beta}. \tag{96}$$

Using (92) we obtain

$$\overline{\lambda^\alpha_{;\alpha\beta}}^{\cdot} = [-u^\gamma\lambda_\gamma K - (u^\gamma\lambda^\alpha_{;\gamma})_{;\alpha} + u^\gamma\lambda^\alpha_{;\gamma\alpha}]_{;\beta}, \tag{97}$$

which, after using (87), is arranged as

$$\overline{\lambda^\alpha_{;\alpha\beta}}^{\cdot} = u^\gamma(\lambda^\alpha_{;\alpha\beta})_{;\gamma} + u^\gamma_{;\beta}\lambda^\alpha_{;\alpha\gamma} - (u^\gamma\lambda^\alpha_{;\gamma})_{;\alpha\beta}. \tag{98}$$

Using the above relation, variation of $\lambda^\alpha_{;\beta\alpha}$ can be obtained as

$$\overline{\lambda^\alpha_{;\beta\alpha}}^{\cdot} = u^\gamma(\lambda^\alpha_{;\beta\alpha})_{;\gamma} - u^\gamma K\lambda_{\beta;\gamma} + u^\gamma_{;\beta}\lambda^\alpha_{;\gamma\alpha} - (u^\gamma\lambda^\alpha_{;\gamma})_{;\alpha\beta}. \tag{99}$$

Similarly, we can evaluate the variations using (81) and (92)

$$\overline{\lambda^\alpha_{;\beta}\lambda^\beta_{;\alpha}}^{\cdot} = 2\lambda^\alpha_{;\beta}\overline{\lambda^\beta_{;\alpha}}^{\cdot} = u^\gamma(\lambda^\beta_{;\alpha}\lambda^\alpha_{;\beta})_{;\gamma} - 2\lambda^\beta_{;\alpha}(u^\gamma\lambda^\alpha_{;\gamma})_{;\beta}, \tag{100}$$

and

$$\overline{\lambda^\alpha_{;\alpha}\lambda^\beta_{;\beta}}^{\cdot} = 2\overline{\lambda^\alpha_{;\alpha}}^{\cdot}\lambda^\beta_{;\beta} = u^\gamma(\lambda^\alpha_{;\alpha}\lambda^\beta_{;\beta})_{;\gamma} - 2\lambda^\beta_{;\beta}(u^\gamma\lambda^\alpha_{;\gamma})_{;\alpha}. \tag{101}$$

Thus, using (98)–(101), we obtain

$$\overline{(\lambda^\alpha\lambda^\beta)_{;\beta\alpha}}^{\cdot} = u^\gamma[(\lambda^\alpha\lambda^\beta)_{;\beta\alpha}]_{;\gamma} - 2[(u^\gamma\lambda^\alpha_{;\gamma})_{;\alpha}\lambda^\beta]_{;\beta} - 2u^\gamma\lambda^\alpha_{;\alpha\beta}\lambda^\beta_{;\gamma} - (u^\gamma\lambda^\alpha_{;\gamma})_{;\beta}\lambda^\beta_{;\alpha}. \tag{102}$$

We note a useful relation arising from the fact that $\boldsymbol{\lambda}$ is a unit vector

$$(\lambda^\beta\lambda_\beta)_{;\gamma} = 0 \Rightarrow \lambda^\beta_{;\gamma}\lambda_\beta = -\lambda^\beta\lambda_{\beta;\gamma} = 0 \tag{103}$$

and combine the variations obtained (as detailed in Appendix A) in (77), (81), (92), (98)–(102) and substituted in (88) to obtain

$$\overline{(W_D)(\lambda^\alpha\lambda^\beta)_{;\beta\alpha}}^{\cdot} = u^\gamma[W_D(\lambda^\alpha\lambda^\beta)_{;\beta\alpha}]_{;\gamma} - [u^\gamma\frac{\partial W_D}{\partial\theta^\gamma} + u^\gamma W_{DD}b_{\phi\psi}(\lambda^\phi\lambda^\psi)_{;\gamma}](\lambda^\alpha\lambda^\beta)_{;\beta\alpha}. \tag{104}$$

Combining the variations derived in (90), (93), (104), and substituting in (88) we obtain the tangential variation in first term of G as

$$\dot{\overline{(W_D\lambda^\beta\lambda^\alpha)_{;\beta\alpha}}} = u^\gamma[(W_D\lambda^\alpha\lambda^\beta)_{;\beta\alpha}]_{;\gamma} - [u^\gamma W_D(\lambda^\alpha\lambda^\beta)_{;\gamma}]_{;\alpha\beta} - \{[u^\gamma \frac{\partial W_D}{\partial\theta^\gamma} + u^\gamma W_{DD} b_{\phi\psi}(\lambda^\phi\lambda^\psi)_{;\gamma}]\lambda^\alpha\lambda^\beta\}_{;\beta\alpha}. \tag{105}$$

To find the variation of $\Delta_s W_D$, we follow the similar exercise as in [23] to derive the tangential variation for $\Delta_s W_H$ to write

$$\dot{\overline{\Delta_s W_D}} = (\dot{W}_D)_{;\alpha\beta}a^{\alpha\beta} - (W_D)_{,\gamma}\dot{\Gamma}^\gamma_{\alpha\beta}a^{\alpha\beta} + (W_D)_{;\alpha\beta}\dot{a}^{\alpha\beta}. \tag{106}$$

On using the variations in (71)–(73), variation of W_D in (77), and the properties of the Riemann curvature tensor from (85)–(87), we simplify (106) to

$$\dot{\overline{\Delta_s W_D}} = u^\gamma(\Delta_s W_D)_{,\gamma} - \Delta_s(u^\gamma \frac{\partial W_D}{\partial\theta^\gamma}) - \Delta_s[u^\gamma W_{DD} b_{\alpha\beta}(\lambda^\alpha\lambda^\beta)_{;\gamma}] \tag{107}$$

The variation of third term of G in (21) is

$$\dot{\overline{2HDW_D}} = 2\dot{H}DW_D + 2H\dot{D}W_D + 2HD\dot{W}_D \tag{108}$$

Using the (72) and (77) we obtain

$$\dot{\overline{2HDW_D}} = u^\gamma(2HDW_D)_{,\gamma} - 2u^\gamma HD\frac{\partial W_D}{\partial\theta^\gamma} - 2u^\gamma H(\lambda^\alpha\lambda^\beta)_{;\gamma}b_{\alpha\beta}(W_D + DW_{DD}). \tag{109}$$

Similar to variation of $\Delta_s W_D$, we obtain that the variation of $\Delta_s W_H$ can be written as

$$\dot{\overline{\Delta_s W_H}} = u^\gamma(\Delta_s W_H)_{,\gamma} - \Delta_s(u^\gamma \frac{\partial W_H}{\partial\theta^\gamma}) - \Delta_s[u^\gamma W_{HD} b_{\alpha\beta}(\lambda^\alpha\lambda^\beta)_{;\gamma}]. \tag{110}$$

The tangential variation of the fourth term in G can be expressed as

$$\dot{\overline{(W_K)_{;\alpha\beta}(2Ha^{\alpha\beta} - b^{\alpha\beta})}} = \dot{\overline{2H\Delta_s W_K}} - \dot{\overline{(W_K)_{;\alpha\beta}b^{\alpha\beta}}}, \tag{111}$$

where variation of the first term in the above equation, similar to (110) and using (77), is

$$\dot{\overline{2H\Delta_s W_K}} = u^\gamma(2H\Delta_s W_K)_{,\gamma} - 2H\Delta_s(u^\gamma \frac{\partial W_K}{\partial\theta^\gamma}) - 2H\Delta_s[u^\gamma W_{KD} b_{\alpha\beta}(\lambda^\alpha\lambda^\beta)_{;\gamma}]. \tag{112}$$

The variation of the second term in (111) can be written as [23]

$$\dot{\overline{(W_K)_{;\alpha\beta}b^{\alpha\beta}}} = u^\gamma[(W_K)_{;\alpha\beta}b^{\alpha\beta}]_{;\gamma} - b^{\alpha\beta}(u^\gamma \frac{\partial W_K}{\partial\theta^\gamma})_{;\alpha\beta} - b^{\alpha\beta}[u^\gamma W_{KD} b_{\theta\phi}(\lambda^\theta\lambda^\phi)_{;\gamma}]_{;\alpha\beta}. \tag{113}$$

Thus, we can use the above two equations and substitute into (111) to obtain

$$\dot{\overline{(W_K)_{;\alpha\beta}(2Ha^{\alpha\beta} - b^{\alpha\beta})}} = u^\gamma[(W_K)_{;\alpha\beta}\tilde{b}^{\alpha\beta}]_{,\gamma} - \tilde{b}^{\alpha\beta}(u^\gamma \frac{\partial W_K}{\partial\theta^\gamma})_{;\alpha\beta} - \tilde{b}^{\alpha\beta}[u^\gamma W_{KD} b_{\theta\phi}(\lambda^\theta\lambda^\phi)_{;\gamma}]_{;\alpha\beta}. \tag{114}$$

Similarly, the tangential variation of the remaining terms in G can be obtained using (72) such that

$$\dot{\overline{W_H(2H^2 - K)}} = u^\gamma[W_H(2H^2 - K)]_{,\gamma} - u^\gamma(\frac{\partial W_H}{\partial\theta^\gamma})(2H^2 - K) - u^\gamma[W_{HD} b_{\alpha\beta}(\lambda^\alpha\lambda^\beta)_{;\gamma}](2H^2 - K), \tag{115}$$

$$\dot{\overline{2H(KW_K - W)}} = u^\gamma[2H(KW_K - W)]_{,\gamma} - 2u^\gamma H(K\frac{\partial W_K}{\partial\theta^\gamma} - \frac{\partial W}{\partial\theta^\gamma}) - 2u^\gamma H[KW_{KD} b_{\alpha\beta}(\lambda^\alpha\lambda^\beta)_{;\gamma} - W_D b_{\alpha\beta}(\lambda^\alpha\lambda^\beta)_{;\gamma}], \tag{116}$$

and

$$\overline{2\sigma H}^{\cdot} = 2\sigma \dot{H} = u^{\gamma}(2\sigma H)_{,\gamma} - 2u^{\gamma}\sigma_{,\gamma}H. \tag{117}$$

Thus, tangential variation of G ($\dot{G}_t$) can be written using (105), (107), (109), (110), (114), (115), (116), and (117) as

$$\dot{G}_t = u^{\gamma}G_{;\gamma} + \text{termsduetoheterogeneityandorthotropy}. \tag{118}$$

For brevity we have combined all the terms in the tangential variation of G in Appendix B. We note here that as the protein orientation and material heterogeneity are decoupled from the kinematical description of membrane, they affect the tangential variation of the terms in the *shape equation*. In addition, because surface tension σ and pressure p are considered to be the Lagrange multiplier enforcing the first-order constraints on local area and volume enclosed at equilibrium, their variations have not been considered.

3.4. Normal Variations

For normal variations $\mathbf{u} = u\mathbf{n}$. Using (7), (13), we compute the variations in the metric tensor, the curvature tensor and the protein alignment as

$$\begin{aligned} \dot{a}_{\alpha\beta} &= -2ub_{\alpha\beta}, \quad \dot{b}_{\alpha\beta} = u_{;\alpha\beta} - ub^{\gamma}_{\alpha}b_{\gamma\beta}, \\ \dot{\lambda}^{\alpha} &= u\lambda^{\gamma}b^{\alpha}_{\gamma}, \quad \dot{\mu}^{\alpha} = u\mu^{\gamma}b^{\alpha}_{\gamma}. \end{aligned} \tag{119}$$

These can be used to derive the variations in the curvature invariants (H, K, D) as

$$\begin{aligned} \dot{H} &= \frac{1}{2}(\Delta_s u) + u(2H^2 - K), \quad \dot{K} = u_{;\alpha\beta}\tilde{b}^{\alpha\beta} + 2uHK, \\ \dot{D} &= \frac{1}{2}(\lambda^{\alpha}\lambda^{\beta} - \mu^{\alpha}\mu^{\beta})(u_{;\alpha\beta} + ub_{\alpha\gamma}b^{\gamma}_{\beta}) = u_{;\alpha\beta}(\lambda^{\alpha}\lambda^{\beta}) - \frac{1}{2}(\Delta_s u) + 2uHD. \end{aligned} \tag{120}$$

Variations in the components of the Christoffel symbols can be evaluated from the variations of the metric in (119) and substituting them in $(73)_1$

$$\dot{\Gamma}^{\gamma}_{\alpha\beta} = -a^{\gamma\theta}[(ub_{\alpha\theta})_{;\beta} + (ub_{\beta\theta})_{;\alpha} - (ub_{\alpha\beta})_{;\theta}]. \tag{121}$$

To obtain $\overline{\lambda^{\beta}_{;\alpha}}^{\cdot}$, we note

$$\lambda^{\beta}_{;\alpha} = \lambda^{\beta}_{,\alpha} + \lambda^{\gamma}\Gamma^{\beta}_{\gamma\alpha} \tag{122}$$

and using the commutativity of the variational and the spatial derivative, we obtain

$$\overline{\lambda^{\beta}_{;\alpha}}^{\cdot} = (\dot{\lambda}^{\beta})_{;\alpha} + \lambda^{\gamma}\dot{\Gamma}^{\beta}_{\gamma\alpha}. \tag{123}$$

Substituting the variation $(\dot{\lambda}^{\beta})$ from (119) and that of the Christoffel symbol from (121), we obtain the above variation as

$$\overline{\lambda^{\beta}_{;\alpha}}^{\cdot} = (\dot{\lambda}^{\beta})_{;\alpha} - (ub^{\beta}_{\gamma})_{;\alpha}\lambda^{\gamma} - u_{,\gamma}\lambda^{\gamma}b^{\beta}_{\alpha} + u_{,\theta}\lambda^{\gamma}b_{\alpha\gamma}a^{\beta\theta}. \tag{124}$$

This shows how the gradient of a protein's orientation changes with a normal variation to the surface. The first component specifies change with respect to the metric whereas the rest of the term are because of change in metric connection. The above equation can hence be used to obtain the variation

$$\overline{\lambda^{\alpha}_{;\alpha}}^{\cdot} = (\dot{\lambda}^{\alpha})_{;\alpha} + \lambda^{\gamma}\dot{\Gamma}^{\alpha}_{\gamma\alpha} = (\dot{\lambda}^{\alpha})_{;\alpha} - \lambda^{\gamma}(2uH)_{,\gamma}. \tag{125}$$

Similar to (124), the above equation shows how the divergence of the protein's orientation changes due to a normal variation to the surface.

Thus, normal variation of N_α as mentioned in (21), i.e., $(\dot{N}_\alpha)_n$ can be written as in (75) with superposed dots now signifying the variational derivative for normal variations to the surface. Using (119) and (124) and the fact that curvature tensor is symmetric, we obtain

$$\begin{aligned}(\dot{N}_\alpha)_n = &(\dot{W}_D) b_{\beta\eta}(\lambda^\beta\lambda^\eta)_{;\alpha} + u_{;\beta\eta} W_D (\lambda^\beta\lambda^\eta)_{;\alpha} \\ &+ 2W_D b_{\beta\eta}(-u_{,\gamma} b^\beta_\alpha \lambda^\gamma\lambda^\eta + u_{,\theta} b_{\alpha\gamma} a^{\beta\theta}\lambda^\gamma\lambda^\eta) + u W_D b_{\beta\eta} b^\eta_\gamma (\lambda^\gamma\lambda^\beta)_{;\alpha}.\end{aligned} \tag{126}$$

Variation $\dot{W}_D$ in the above equation can be expanded using (76) and (120) to be

$$\dot{W}_D = W_{DD}(u_{;\eta\gamma}\lambda^\eta\lambda^\gamma - \tfrac{1}{2}\Delta_s u + 2uHD) + W_{DH}[\tfrac{1}{2}\Delta_s u + u(2H^2 - K)] + W_{DK}(u_{;\eta\gamma}\tilde{b}^{\eta\gamma} + 2uHK). \tag{127}$$

We represent the normal variation of G as $\dot{G}_n$. To obtain this, we write the variation of the first term as

$$\overline{(W_D\lambda^\alpha\lambda^\beta)_{;\beta\alpha}}^{\cdot} = \overline{(W_D)_{;\alpha\beta}\lambda^\alpha\lambda^\beta}^{\cdot} + 2\overline{(W_D)_{,\beta}(\lambda^\alpha\lambda^\beta)_{;\alpha}}^{\cdot} + \overline{W_D(\lambda^\alpha\lambda^\beta)_{;\beta\alpha}}^{\cdot}. \tag{128}$$

In the above equation, variation of the first term on right-hand side can be written as that in (89) and can be evaluated using (119) and (121) to be

$$\overline{(W_D)_{;\beta\alpha}\lambda^\alpha\lambda^\beta}^{\cdot} = (\dot{W}_D)_{;\beta\alpha}\lambda^\alpha\lambda^\beta + 2(W_D)_{;\beta\alpha}\dot{\lambda}^\alpha\lambda^\beta + (W_D)_{,\gamma}\lambda^\alpha\lambda^\beta[(ub^\gamma_\alpha)_{;\beta} + u_{,\beta}b^\gamma_\alpha - u_{,\theta}b_{\alpha\beta}a^{\gamma\theta}]. \tag{129}$$

On changing of dummy indices in the last term, we can write the above equation as

$$\overline{(W_D)_{;\beta\alpha}\lambda^\alpha\lambda^\beta}^{\cdot} = (\dot{W}_D)_{;\beta\alpha}\lambda^\alpha\lambda^\beta + 2(W_D)_{;\beta\alpha}\dot{\lambda}^\alpha\lambda^\beta + (W_D)_{,\beta}\lambda^\alpha\lambda^\gamma[(ub^\beta_\gamma)_{;\alpha} + u_{,\gamma}b^\beta_\alpha - u_{,\theta}b_{\alpha\gamma}a^{\beta\theta}]. \tag{130}$$

Variation of the second term on the right-hand side of (128) is obtained using (91), (119), (124), and (125) such that

$$\begin{aligned}2\overline{(W_D)_{,\beta}(\lambda^\alpha\lambda^\beta)_{;\alpha}}^{\cdot} = &2(\dot{W}_D)_{,\beta}(\lambda^\alpha\lambda^\beta)_{;\alpha} + 2(W_D)_{,\beta}(\overline{\lambda^\alpha\lambda^\beta}^{\cdot})_{;\alpha} \\ &- 2(W_D)_{,\beta}[\lambda^\beta\lambda^\gamma(2uH)_{,\gamma} + \lambda^\alpha\lambda^\gamma(ub^\beta_\gamma)_{;\alpha} + u_{,\gamma}\lambda^\alpha\lambda^\gamma b^\beta_\alpha - u_{,\theta}\lambda^\alpha\lambda^\gamma b_{\alpha\gamma}a^{\beta\theta}].\end{aligned} \tag{131}$$

Variation of the third term on the right-hand side of (128) can be expanded as

$$\overline{(W_D)(\lambda^\alpha\lambda^\beta)_{;\beta\alpha}}^{\cdot} = (\dot{W}_D)(\lambda^\alpha\lambda^\beta)_{;\beta\alpha} + (W_D)(\overline{\lambda^\alpha_{;\beta\alpha}\lambda^\beta + \lambda^\alpha_{;\beta}\lambda^\beta_{;\alpha} + \lambda^\alpha_{;\alpha}\lambda^\beta_{;\beta}}^{\cdot} + \overline{\lambda^\alpha\lambda^\beta_{;\beta\alpha}}^{\cdot}). \tag{132}$$

To evaluate it, we use Eq. (125) and the fact that $\lambda^\alpha_{;\alpha}$ is scalar and obtain

$$\overline{\lambda^\beta_{;\beta\alpha}}^{\cdot} = (\overline{\lambda^\beta_{;\beta}}^{\cdot})_{;\alpha} = (\dot{\lambda}^\beta)_{;\beta\alpha} - [\lambda^\gamma(2uH)_{,\gamma}]_{;\alpha}. \tag{133}$$

Using (87) and the relation above, we then obtain

$$\overline{\lambda^\alpha_{;\beta\alpha}}^{\cdot} = \overline{\lambda^\alpha_{;\alpha\beta}}^{\cdot} + \overline{K\lambda_\beta}^{\cdot} = (u\lambda^\gamma b^\alpha_\gamma)_{;\alpha\beta} - [\lambda^\gamma(2uH)_{,\gamma}]_{;\beta} + \overline{K\lambda_\beta}^{\cdot}. \tag{134}$$

Considering $d^\alpha = u\lambda^\gamma b^\alpha_\gamma$, the above equation can be simplified after adding and subtracting the vector field $d_\beta = u\lambda^\gamma b_{\beta\gamma}K$

$$\begin{aligned}\overline{\lambda^\alpha_{;\beta\alpha}}^{\cdot} &= (u\lambda^\gamma b^\alpha_\gamma)_{;\beta\alpha} - u\lambda^\gamma b_{\beta\gamma}K - [\lambda^\gamma(2uH)_{,\gamma}]_{;\beta} + \overline{K\lambda_\beta}^{\cdot} \\ &= (\dot{\lambda}^\alpha)_{;\beta\alpha} - u\lambda^\gamma b_{\beta\gamma}K - [\lambda^\gamma(2uH)_{,\gamma}]_{;\beta} + \overline{K\lambda_\beta}^{\cdot}.\end{aligned} \tag{135}$$

Substituting the variations obtained from (119), (120), (124), (125), (133), and (135) into (132), we obtain

$$\overline{W_D(\lambda^\alpha\lambda^\beta)_{;\beta\alpha}}^{\cdot} = \dot{W}_D(\lambda^\alpha\lambda^\beta)_{;\beta\alpha} + W_D\overline{(\lambda^\alpha\lambda^\beta)}^{\cdot}_{;\beta\alpha} - 2uW_D\lambda^\beta\lambda^\gamma b_{\beta\gamma}K + (u_{;\alpha\beta}\tilde{b}^{\alpha\beta} + 2uHK)W_D - 2W_D\lambda^\alpha_{;\beta}[(ub^\beta_\gamma)_{;\alpha}\lambda^\gamma + u_{,\gamma}\lambda^\gamma b^\beta_\alpha - u_{,\theta}\lambda^\gamma b_{\alpha\gamma}a^{\beta\theta}] - 2W_D[\lambda^\gamma\lambda^\beta(2uH)_{,\gamma}]_{;\beta}. \tag{136}$$

Combining (130), (131), and (136) and substituting into (128), then yields

$$\overline{(W_D\lambda^\alpha\lambda^\beta)_{;\beta\alpha}}^{\cdot} = \overline{(W_D\lambda^\beta\lambda^\alpha)}^{\cdot}_{;\beta\alpha} + uW_DK(2uH - b_{\beta\gamma}\lambda^\beta\lambda^\gamma) + u_{;\alpha\beta}\tilde{b}^{\alpha\beta}W_D - [(W_D)_{,\beta}\lambda^\alpha\lambda^\gamma + 2W_D\lambda^\alpha_{;\beta}\lambda^\gamma][(ub^\beta_\gamma)_{;\alpha} + u_{,\gamma}b^\beta_\alpha - u_{,\theta}b_{\alpha\gamma}a^{\beta\theta}] - 2[W_D\lambda^\beta\lambda^\gamma(2uH)_{,\gamma}]_{;\beta}. \tag{137}$$

The above expression can be further simplified using the symmetry of α and γ to

$$\overline{(W_D\lambda^\alpha\lambda^\beta)_{;\beta\alpha}}^{\cdot} = \overline{(W_D\lambda^\beta\lambda^\alpha)}^{\cdot}_{;\beta\alpha} + uW_DK(2uH - b_{\beta\gamma}\lambda^\beta\lambda^\gamma) + u_{;\alpha\beta}\tilde{b}^{\alpha\beta}W_D - (W_D\lambda^\alpha\lambda^\gamma)_{,\beta}[(ub^\beta_\gamma)_{;\alpha} + u_{,\gamma}b^\beta_\alpha - u_{,\theta}b_{\alpha\gamma}a^{\beta\theta}] - 2[W_D\lambda^\beta\lambda^\gamma(2uH)_{,\gamma}]_{;\beta}. \tag{138}$$

Here the normal variations of W_D and λ^α can be obtained from (120) and (127).

Normal variation of the surface Laplacian of W_D is given by

$$\overline{\Delta_s W_D}^{\cdot} = (\dot{W}_D)_{;\alpha\beta}a^{\alpha\beta} - (W_D)_{,\gamma}\dot{\Gamma}^\gamma_{\alpha\beta}a^{\alpha\beta} + (W_D)_{;\alpha\beta}\dot{a}^{\alpha\beta}. \tag{139}$$

Variation of W_D is obtained in (127), whereas from (121) we have

$$\begin{aligned}-(W_D)_{,\gamma}\dot{\Gamma}^\gamma_{\alpha\beta}a^{\alpha\beta} &= (W_D)_{,\gamma}a^{\alpha\beta}a^{\gamma\eta}[(2ub_{\eta\alpha})_{;\beta} - (ub_{\alpha\beta})_{;\eta}] \\ &= (W_D)_{,\gamma}[(2ub^{\beta\gamma})_{;\beta} - (2uH)_{;\eta}a^{\gamma\eta}].\end{aligned} \tag{140}$$

Using (64) and (119) we obtain

$$(W_D)_{;\alpha\beta}\dot{a}^{\alpha\beta} = 2u(W_D)_{;\alpha\beta}b^{\alpha\beta}. \tag{141}$$

Thus, the variation in (139) can be obtained from (127), (140), and (141) as

$$\overline{\Delta_s W_D}^{\cdot} = (\dot{W}_D)_{;\alpha\beta}a^{\alpha\beta} + (W_D)_{,\gamma}[(2ub^{\beta\gamma})_{;\beta} - (2uH)_{;\eta}a^{\gamma\eta}] + 2u(W_D)_{;\alpha\beta}b^{\alpha\beta}. \tag{142}$$

Next, using (120) and (127), we can compute the variation

$$\overline{2HDW_D}^{\cdot} = 2\dot{H}DW_D + 2H\dot{D}W_D + 2HD\dot{W}_D. \tag{143}$$

Similar to the normal variation of $\Delta_s(W_D)$ as obtained in (139)–(141), the normal variation of $\Delta_s(W_H)$ can be written as

$$\overline{\Delta_s W_H}^{\cdot} = (\dot{W}_H)_{;\alpha\beta}a^{\alpha\beta} + (W_H)_{,\gamma}[(2ub^{\beta\gamma})_{;\beta} - (2uH)_{;\eta}a^{\gamma\eta}] + 2u(W_H)_{;\alpha\beta}b^{\alpha\beta}. \tag{144}$$

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

$$\overline{(W_K)_{;\alpha\beta}(2Ha^{\alpha\beta} - b^{\alpha\beta})}^{\cdot} = (\dot{W}_K)_{;\alpha\beta}\tilde{b}^{\alpha\beta} - (W_K)_{,\lambda}\dot{\Gamma}^\lambda_{\alpha\beta}\tilde{b}^{\alpha\beta} + (W_K)_{;\alpha\beta}\dot{\tilde{b}}^{\alpha\beta}, \tag{145}$$

where, with the help of (120), we have

$$\dot{W}_K = W_{KH}[\frac{1}{2}\Delta_s u + u(2H^2 - K)] + W_{KK}(u_{;\eta\gamma}\tilde{b}^{\eta\gamma} + 2uHK) + W_{KD}(u_{;\eta\gamma}\lambda^\eta\lambda^\gamma - \frac{1}{2}\Delta_s u + 2uHD). \tag{146}$$

Using (121) and the procedure highlighted in Appendix C, we then obtain

$$(W_K)_{,\lambda}\dot{\Gamma}^{\lambda}_{\alpha\beta}\tilde{b}^{\alpha\beta} = -u(W_K)_{,\lambda}K_{,\eta}a^{\lambda\eta} \tag{147}$$

and

$$\dot{\tilde{b}}^{\alpha\beta} = 4uH\tilde{b}^{\alpha\beta} + \varepsilon^{\alpha\lambda}\varepsilon^{\beta\gamma}(u_{;\lambda\gamma} - ub^{\eta}_{\lambda}b_{\eta\gamma}). \tag{148}$$

Using these relations, we finally compute

$$\overline{(W_K)_{;\beta\alpha}(2Ha^{\beta\alpha} - b^{\beta\alpha})}^{\cdot} = (\dot{W}_K)_{;\beta\alpha}\tilde{b}^{\beta\alpha} - u(W_K)_{,\lambda}K_{,\eta}a^{\lambda\eta} + 4uH(W_K)_{;\beta\alpha}\tilde{b}^{\beta\alpha} + \varepsilon^{\alpha\lambda}\varepsilon^{\beta\gamma}(u_{;\lambda\gamma} - ub^{\eta}_{\lambda}b_{\eta\gamma})(W_K)_{;\beta\alpha}. \tag{149}$$

We use (120) to compute the normal variation of the remaining terms of G in $(21)_2$ and group them using (138), (142), (143), (144), and (149) in Appendix D.

3.5. Total variations

Having derived all the ingredients for computing the second variation of the energy functional, at equilibrium, we can write

$$\ddot{E} = \int_{\omega} -u^{\alpha}(\dot{I}_{\alpha} + (\dot{N}_{\alpha})_t + (\dot{N}_{\alpha})_n) + u(\dot{G}_t + \dot{G}_n) \tag{150}$$

subject to the constraints area and volume incompressibility. Further, we also use the fact that at equilibrium, Equations (22), (60) hold whereas G and $G_{,\alpha}$ vanish at each point of the surface. To simplify the following equations we have used the fact that the surface is closed, and so the divergence terms can be equated to zero by invoking the divergence theorem. This allows us to write for any scalar field f

$$\int_{\omega} u\Delta_s f\, da = \int_{\omega} (\Delta_s u) f\, da. \tag{151}$$

The integrand in (150) can be expanded and written in terms of the tangential variations (u^{α}) and the normal variations (u) in the following way.

- The terms with coefficient $u^{\alpha}u^{\gamma}$, computed with the help of (70) and (84), are given by

$$\begin{aligned}&\sigma_{;\alpha\gamma} + \frac{\partial^2 W}{\partial\theta^{\alpha}\partial\theta^{\gamma}} + 2(\frac{\partial W_D}{\partial\theta^{\alpha}})b_{\beta\eta}(\lambda^{\beta}\lambda^{\eta})_{;\gamma} + W_D b_{\beta\eta}(\lambda^{\beta}\lambda^{\eta})_{;\alpha\gamma}\\ &+ W_{DD}b_{\beta\eta}b_{\phi\psi}(\lambda^{\beta}\lambda^{\eta})_{;\gamma}(\lambda^{\phi}\lambda^{\psi})_{;\alpha}.\end{aligned} \tag{152}$$

- The terms with coefficients $u^{\alpha}u$ obtained using (70), (126), and (217) and the relation $G_{,\gamma} = 0$, are given by

$$\begin{aligned}&-4H\frac{\partial}{\partial\theta^{\alpha}}(W_H H + W_K K + W_D D) + 2\frac{\partial W_H}{\partial\theta^{\alpha}}K + 2H(\frac{\partial W}{\partial\theta^{\alpha}} + \sigma_{,\alpha})\\ &-(4H(W_{DH}H + W_{DD}D + W_{DK}K) - 2KW_{HD})b_{\beta\eta}(\lambda^{\beta}\lambda^{\eta})_{;\alpha} - W_D b_{\beta\eta}b^{\eta}_{\gamma}(\lambda^{\gamma}\lambda^{\beta})_{;\alpha}.\end{aligned} \tag{153}$$

- The terms with coefficient $u^{\alpha}\Delta_s u$, obtained using (70), (126), and (217), include

$$-\{\frac{\partial W_H}{\partial\theta^{\alpha}} - \frac{\partial W_D}{\partial\theta^{\alpha}}\} - (W_{DH} - W_{DD})b_{\beta\eta}(\lambda^{\beta}\lambda^{\eta})_{;\alpha}. \tag{154}$$

- The terms with coefficient $u^{\alpha}u_{;\beta\eta}$ obtained using (70), (126), and (217), include

$$-2(\frac{\partial W_K}{\partial \theta^\alpha}\tilde{b}^{\eta\beta}+\frac{\partial W_D}{\partial \theta^\alpha}\lambda^\beta\lambda^\eta)-2(W_{DK}\tilde{b}^{\eta\beta}+W_{DD}\lambda^\beta\lambda^\eta)b_{\phi\psi}(\lambda^\phi\lambda^\psi)_{;\alpha}-2W_D(\lambda^\beta\lambda^\eta)_{;\alpha}. \tag{155}$$

- Terms with coefficient $u^\alpha u_{,\gamma}$ obtained using (126), are given by

$$=2W_D\{b_{\beta\eta}b^\beta_\alpha\lambda^\gamma\lambda^\eta-b_{\alpha\beta}b^\gamma_\eta\lambda^\beta\lambda^\eta\}. \tag{156}$$

- The terms with coefficient u^2, obtained using (231), are given by

$$\begin{aligned}&W_DK(2H-2K-b_{\beta\gamma}\lambda^\beta\lambda^\gamma)-(W_D\lambda^\alpha\lambda^\gamma)_{;\beta}b^\beta_{\gamma;\alpha}-2(W_D\lambda^\alpha\lambda^\gamma)(2H)_{;\gamma\alpha}(W_H-W_D)_{;\alpha\beta}b^{\alpha\beta}-(W_K)_{,\lambda}K_{,\eta}a^{\lambda\eta}\\&+4H(W_K)_{;\beta\alpha}\tilde{b}^{\beta\alpha}-\varepsilon^{\alpha\lambda}\varepsilon^{\beta\gamma}(W_K)_{;\alpha\beta}b^\eta_\lambda b_{\eta\gamma}+4H^2(D^2W_{DD}\\&+H^2W_{HH}+K^2W_{KK}+2HDW_{HD}+2DKW_{DK}+2HKW_{HK}+DW_D+HW_H+KW_K-W-\sigma)\\&-K[W_{HH}(4H^2-K)+4HDW_{HD}+4HKW_{HK}+4HW_H+2KW_K+2W+2\sigma].\end{aligned} \tag{157}$$

- The terms with coefficient $uu_{,\alpha}$, obtained using (231), include

$$-2(W_D\lambda^\gamma\lambda^\alpha)_{;\beta}b^\beta_\gamma+(W_D\lambda^\eta\lambda^\gamma)_{;\beta}b_{\eta\gamma}a^{\beta\alpha}-4(W_D\lambda^\alpha\lambda^\gamma)_{;\gamma}H+(W_H-W_D)_{,\gamma}(Ha^{\alpha\gamma}+\tilde{b}^{\alpha\gamma})-8W_D\lambda^\alpha\lambda^\gamma H_{,\gamma}. \tag{158}$$

- The terms with coefficient $uu_{;\alpha\beta}$, obtained using (231), include

$$\begin{aligned}&2\lambda^\alpha\lambda^\beta[2HDW_{DD}+W_{HD}(2H^2-K)+2HKW_{KD}]\\&+\tilde{b}^{\alpha\beta}[4HDW_{KD}+4HKW_{KK}+2(2H^2-K)W_{HK}+W_D-W_H]\\&+\varepsilon^{\alpha\lambda}\varepsilon^{\beta\gamma}(W_K)_{;\lambda\gamma}-4W_DH\lambda^\beta\lambda^\alpha+2W_D\lambda^\alpha\lambda^\gamma b^\beta_\gamma.\end{aligned} \tag{159}$$

- The terms with coefficient $u(\Delta_s u)$, obtained using (231), are given by

$$HW_H+DW_D+2HD(W_{HD}-W_{DD})+(W_{HH}-W_{HD})(2H^2-K)+2HK(W_{HK}-W_{DK})+KW_K-W-\sigma. \tag{160}$$

- The terms with coefficient $u_{;\alpha\beta}u_{;\gamma\eta}$, obtained using (231), include

$$W_{DD}\lambda^\alpha\lambda^\beta\lambda^\gamma\lambda^\eta+2W_{DK}\tilde{b}^{\gamma\eta}\lambda^\alpha\lambda^\beta+W_{KK}\tilde{b}^{\alpha\beta}\tilde{b}^{\gamma\eta}. \tag{161}$$

- The terms with coefficient $u_{;\alpha\beta}(\Delta_s u)$, obtained using (231), include

$$\lambda^\alpha\lambda^\beta(W_{DH}-W_{DD})+\tilde{b}^{\alpha\beta}[W_{HK}-W_{DK}+\frac{1}{2}(W_{HD}-W_{DD})]. \tag{162}$$

- The terms with coefficient $(\Delta_s u)^2$, obtained using (231), are given by

$$\frac{1}{4}(W_{HH}+W_{DD}-2W_{HD}). \tag{163}$$

The second variation of the energy functional is required to be positive, $\ddot{E}>0$, for a given configuration in equilibrium to be stable with respect to the admissible variations. The variations are permissible if they satisfy the kinematic constraints on areal and volume incompressibility as mentioned in (60) and (61).

4. Conclusion

The stability analysis presented here extends the theory of conventionally studied isotropic biomembranes to those which possess orthotropic symmetry in the presence of BAR-like proteins. The study opens new avenues to investigate stability and shape transformations of membranes interacting with BAR-like proteins. One specific example where the developed framework can be used is for studying spontaneous tubulation of lipid membranes in the presence of BAR proteins [32]. Although the derived theory currently does not account for diffusion of BAR proteins, this assumption shall be relaxed in future theoretical studies by incorporating a constitutive law for the evolution of protein orientation on the surface.

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ORCID iD

Ashutosh Agrawal https://orcid.org/0000-0002-9923-8603

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Appendix A

Here, we derive the tangential variation of the *shape equation*

$$G = (W_D \lambda^\alpha \lambda^\beta)_{;\beta\alpha} - \frac{1}{2}\Delta_s(W_D) + 2HDW_D + \frac{1}{2}\Delta_s W_H + (W_K)_{;\beta\alpha}\tilde{b}^{\beta\alpha} + W_H(2H^2 - K) + 2H(KW_K - W) - 2H\sigma - p. \qquad [(164)]$$

Variation of the first term can be written as

$$\overline{(W_D \lambda^\alpha \lambda^\beta)_{;\beta\alpha}}^{\cdot} = \overline{(W_D)_{;\beta\alpha}\lambda^\alpha\lambda^\beta}^{\cdot} + 2\overline{(W_D)_{,\beta}(\lambda^\alpha\lambda^\beta)_{;\alpha}}^{\cdot} + \overline{(W_D)(\lambda^\alpha\lambda^\beta)_{;\beta\alpha}}^{\cdot}. \qquad [(165)]$$

Using the relation that for any scalar field f, defined on the surface,

$$f_{;\alpha\beta} = f_{,\alpha\beta} - f_{,\lambda}\Gamma^\lambda_{\alpha\beta}, \qquad [(166)]$$

we obtain

$$\overline{(W_D)_{;\beta\alpha}\lambda^\alpha\lambda^\beta}^{\cdot} = (\dot{W}_D)_{,\beta\alpha}\lambda^\alpha\lambda^\beta - (\dot{W}_D)_{,\lambda}\Gamma^\lambda_{\beta\alpha} - (W_D)_{,\lambda}\dot{\Gamma}^\lambda_{\beta\alpha}\lambda^\alpha\lambda^\beta + 2(W_D)_{;\beta\alpha}\dot{\lambda}^\alpha\lambda^\beta. \qquad [(167)]$$

On combining the first two terms above to form a second-order covariant derive, we obtain

$$\overline{(W_D)_{;\beta\alpha}\lambda^\alpha\lambda^\beta}^{\cdot} = (\dot{W}_D)_{;\beta\alpha}\lambda^\alpha\lambda^\beta - (W_D)_{,\lambda}\dot{\Gamma}^\lambda_{\beta\alpha} + 2(W_D)_{;\beta\alpha}\dot{\lambda}^\alpha\lambda^\beta. \qquad [(168)]$$

A.1. First term

Using (77), the variation of the first term in the above equation can be written as

$$(\dot{W}_D)_{;\beta\alpha}\lambda^\alpha\lambda^\beta = (u^\gamma(W_D)_{,\gamma} - u^\gamma\frac{\partial W_D}{\partial\theta^\gamma} - u^\gamma W_{DD} b_{\phi\psi}(\lambda^\phi\lambda^\psi)_{;\gamma})_{;\beta\alpha}\lambda^\alpha\lambda^\beta. \qquad [(169)]$$

We expand the first term in the right-hand side above to obtain

$$[u^{\gamma}(W_D)_{,\gamma}]_{;\beta\alpha}\lambda^{\alpha}\lambda^{\beta}=2u^{\gamma}_{;\beta}(W_D)_{;\gamma\alpha}\lambda^{\alpha}\lambda^{\beta}+u^{\gamma}_{;\beta\alpha}(W_D)_{,\gamma}\lambda^{\alpha}\lambda^{\beta}+u^{\gamma}[(W_D)_{,\beta}]_{;\gamma\alpha}\lambda^{\alpha}\lambda^{\beta}, \tag{(170)}$$

and further rewrite it as

$$=2u^{\gamma}_{;\beta}(W_D)_{;\gamma\alpha}\lambda^{\alpha}\lambda^{\beta}+\{u^{\gamma}_{;\beta\alpha}(W_D)_{,\gamma}+u^{\gamma}[(W_D)_{,\beta}]_{;\gamma\alpha}-u^{\gamma}[(W_D)_{,\beta}]_{;\alpha\gamma}+u^{\gamma}[(W_D)_{,\beta}]_{;\alpha\gamma}\}\lambda^{\alpha}\lambda^{\beta}. \tag{(171)}$$

As $(W_D)_{,\beta}$ is a surface vector, we obtain

$$[u^{\gamma}(W_D)_{,\gamma}]_{;\beta\alpha}\lambda^{\alpha}\lambda^{\beta}=2u^{\gamma}_{;\beta}(W_D)_{;\gamma\alpha}\lambda^{\alpha}\lambda^{\beta}+u^{\gamma}_{;\beta\alpha}(W_D)_{,\gamma}\lambda^{\alpha}\lambda^{\beta}+u^{\gamma}(W_D)_{,\phi}a^{\phi\eta}R_{\beta\eta\alpha\gamma}\lambda^{\alpha}\lambda^{\beta}+u^{\gamma}[(W_D)_{,\beta}]_{;\alpha\gamma}\lambda^{\alpha}\lambda^{\beta}. \tag{(172)}$$

Using (87), which relates the Riemann tensor to the metric components and the intrinsic curvature, we obtain

$$\begin{aligned}R_{\beta\eta\alpha\gamma}a^{\phi\eta}\lambda^{\alpha}\lambda^{\beta}(W_D)_{,\phi}&=K(a_{\beta\alpha}a_{\eta\gamma}-a_{\beta\gamma}a_{\eta\alpha})a^{\phi\eta}\lambda^{\alpha}\lambda^{\beta}(W_D)_{,\phi}\\&=K[\lambda^{\beta}\lambda_{\beta}(W_D)_{,\gamma}-\lambda^{\alpha}\lambda_{\gamma}(W_D)_{,\alpha}].\end{aligned} \tag{(173)}$$

Hence,

$$[u^{\gamma}(W_D)_{,\gamma}]_{;\beta\alpha}\lambda^{\alpha}\lambda^{\beta}=2u^{\gamma}_{;\beta}(W_D)_{;\gamma\alpha}\lambda^{\alpha}\lambda^{\beta}+u^{\gamma}_{;\beta\alpha}(W_D)_{,\gamma}\lambda^{\alpha}\lambda^{\beta}+u^{\gamma}[(W_D)_{,\beta}]_{;\alpha\gamma}\lambda^{\alpha}\lambda^{\beta}+u^{\gamma}K[(W_D)_{,\gamma}-\lambda^{\alpha}\lambda_{\gamma}(W_D)_{,\alpha}]. \tag{(174)}$$

We expand the second term in (168) using (73) to write

$$\begin{aligned}(W_D)_{,\gamma}\dot{\Gamma}^{\gamma}_{\beta\alpha}\lambda^{\alpha}\lambda^{\beta}&=\frac{1}{2}(W_D)_{,\lambda}\lambda^{\alpha}\lambda^{\beta}[a^{\gamma\eta}(u_{\eta;\alpha\beta}+u_{\eta;\beta\alpha}+u_{\alpha;\eta\beta}-u_{\alpha;\beta\eta}+u_{\beta;\eta\alpha}-u_{\beta;\alpha\eta})]\\&=(W_D)_{,\gamma}\lambda^{\alpha}\lambda^{\beta}[u^{\gamma}_{;\alpha\beta}+a^{\gamma\eta}(u_{\beta;\eta\alpha}-u_{\beta;\alpha\eta})]\\&=(W_D)_{,\gamma}\lambda^{\alpha}\lambda^{\beta}(u^{\gamma}_{;\alpha\beta}+a^{\gamma\eta}R_{\beta\phi\alpha\eta}u^{\phi}).\end{aligned} \tag{(175)}$$

Again, using (87), we obtain

$$u^{\phi}a^{\gamma\eta}R_{\beta\phi\alpha\eta}=K(u^{\gamma}a_{\beta\alpha}-\delta^{\gamma}_{\beta}u_{\alpha}), \tag{(176)}$$

which can be used to obtain the variation in (175) and arranged as

$$\begin{aligned}(W_D)_{,\gamma}\dot{\Gamma}^{\gamma}_{\beta\alpha}\lambda^{\alpha}\lambda^{\beta}&=(W_D)_{,\gamma}(u^{\gamma}_{;\beta\alpha}\lambda^{\alpha}\lambda^{\beta}+Ku^{\gamma}-Ku_{\alpha}\lambda^{\alpha}\lambda^{\gamma})\\&=u^{\gamma}_{;\beta\alpha}(W_D)_{,\gamma}\lambda^{\alpha}\lambda^{\beta}+u^{\gamma}K[(W_D)_{,\gamma}-\lambda^{\alpha}\lambda_{\gamma}(W_D)_{,\alpha}].\end{aligned} \tag{(177)}$$

The variation listed as the third term in (168) can be obtained by substituting the variation of the contravariant component of the protein alignment as mentioned in (119) to yield

$$2(W_D)_{;\beta\alpha}\dot{\lambda}^{\alpha}\lambda^{\beta}=-2u^{\alpha}_{;\gamma}(W_D)_{;\beta\alpha}\lambda^{\gamma}\lambda^{\beta}=-2u^{\gamma}_{;\beta}(W_D)_{;\gamma\alpha}\lambda^{\alpha}\lambda^{\beta}. \tag{(178)}$$

Thus, from (167) and by substituting variations obtained in (169), (177), and (178) we obtain

$$\overline{(W_D)_{;\beta\alpha}\lambda^{\alpha}\lambda^{\beta}}^{\,\cdot}=u^{\gamma}[(W_D)_{;\beta\alpha}]_{;\gamma}\lambda^{\alpha}\lambda^{\beta}-[u^{\gamma}\frac{\partial W_D}{\partial\theta^{\gamma}}+u^{\gamma}W_{DD}b_{\phi\psi}(\lambda^{\phi}\lambda^{\psi})_{;\gamma}]_{;\beta\alpha}\lambda^{\alpha}\lambda^{\beta}. \tag{(179)}$$

A.2. Second term

Variation of the second term in (165) is given by

$$\overline{2(W_D)_{,\beta}(\lambda^\alpha\lambda^\beta)_{;\alpha}}^{\cdot} = 2(\dot{W}_D)_{,\beta}(\lambda^\alpha\lambda^\beta)_{;\alpha} + 2(W_D)_{,\beta}(\lambda^\beta)_{;\alpha}\dot{\lambda}^\alpha + 2(W_D)_{,\beta}(\lambda^\alpha)_{;\alpha}\dot{\lambda}^\beta + 2(W_D)_{,\beta}\lambda^\alpha\overline{(\lambda^\beta)_{;\alpha}}^{\cdot} + 2(W_D)_{,\beta}\overline{(\lambda^\alpha)_{;\alpha}}^{\cdot}\lambda^\beta. \quad [(180)]$$

Using (81) and the relations from (86), we obtain that

$$\overline{(\lambda^\alpha)_{;\alpha}}^{\cdot} = \lambda^\gamma u^\phi(b^\alpha_\phi b_{\gamma\alpha} - b_{\phi\gamma}b^\alpha_\alpha) - \lambda^\gamma_{;\alpha}u^\alpha_{;\gamma} = -u^\gamma\lambda_\gamma K - \lambda^\gamma_{;\alpha}u^\alpha_{;\gamma}. \quad [(181)]$$

Thus,

$$\overline{2(W_D)_{,\beta}(\lambda^\alpha\lambda^\beta)_{;\alpha}}^{\cdot} = 2[u^\gamma(W_D)_{,\gamma} - u^\gamma\frac{\partial W_D}{\partial\theta^\gamma} - u^\gamma W_{DD}b_{\phi\psi}(\lambda^\phi\lambda^\psi)_{;\gamma}]_{;\beta}(\lambda^\alpha\lambda^\beta)_{;\alpha} + 2(W_D)_{,\beta}\{\underbrace{-u^\alpha_{;\gamma}\lambda^\gamma\lambda^\beta_{;\alpha} - u^\beta_{;\gamma}\lambda^\gamma\lambda^\alpha_{;\alpha}} + \lambda^\alpha[u^\phi\lambda^\gamma(b^\beta_\phi b_{\gamma\alpha} - b_{\phi\gamma}b^\beta_\alpha) - \underbrace{u^\beta_{;\gamma}\lambda^\gamma_{;\alpha}}] + \lambda^\beta[u^\phi\lambda^\gamma(b^\alpha_\phi b_{\gamma\alpha} - b_{\phi\gamma}b^\alpha_\alpha) - \underbrace{u^\alpha_{;\gamma}\lambda^\gamma_{;\alpha}}]\}. \quad [(182)]$$

Expanding the first term and combining the underbraced terms, we obtain

$$\overline{2(W_D)_{,\beta}(\lambda^\alpha\lambda^\beta)_{;\alpha}}^{\cdot} = [2u^\gamma_{;\beta}(W_D)_{,\gamma} + 2u^\gamma(W_D)_{;\beta\gamma}](\lambda^\alpha\lambda^\beta)_{;\alpha} - 2[u^\gamma\frac{\partial W_D}{\partial\theta^\gamma} - u^\gamma W_{DD}b_{\phi\psi}(\lambda^\phi\lambda^\psi)_{;\gamma}]_{;\beta}(\lambda^\alpha\lambda^\beta)_{;\alpha} + 2(W_D)_{,\beta}[-\underbrace{u^\alpha_{;\gamma}(\lambda^\gamma\lambda^\beta)_{;\alpha}} - u^\beta_{;\gamma}(\lambda^\gamma\lambda^\alpha)_{;\alpha} + \underbrace{u^\phi\lambda^\alpha\lambda^\gamma(b^\beta_\phi b_{\gamma\alpha} - b_{\phi\gamma}b^\beta_\alpha) + u^\phi\lambda^\beta\lambda^\gamma(b^\alpha_\phi b_{\gamma\alpha} - b_{\phi\gamma}b^\alpha_\alpha)}]. \quad [(183)]$$

Here, we can write the above underbraced term as

$$2(W_D)_{,\beta}u^\alpha_{;\gamma}(\lambda^\gamma\lambda^\beta)_{;\alpha} = 2(W_D)_{,\beta}u^\gamma_{;\alpha}(\lambda^\alpha\lambda^\beta)_{;\gamma} = 2(W_D)_{,\beta}[u^\gamma(\lambda^\alpha\lambda^\beta)_{;\gamma}]_{;\alpha} - 2u^\gamma(W_D)_{,\beta}(\lambda^\alpha\lambda^\beta)_{;\gamma\alpha}. \quad [(184)]$$

On using (85) and (87), we can write

$$(\lambda^\alpha\lambda^\beta)_{;\gamma\alpha} - (\lambda^\alpha\lambda^\beta)_{;\alpha\gamma} = \lambda^\alpha(\lambda^\beta_{;\gamma\alpha} - \lambda^\beta_{;\alpha\gamma}) + \lambda^\beta(\lambda^\alpha_{;\gamma\alpha} - \lambda^\alpha_{;\alpha\gamma}) = \lambda^\alpha\lambda^\eta R^\beta_{\eta\alpha\gamma} + K\lambda^\beta\lambda_\gamma, \quad [(185)]$$

Combining the above equations, we obtain

$$2(W_D)_{,\beta}u^\alpha_{;\gamma}(\lambda^\gamma\lambda^\beta)_{;\alpha} = 2(W_D)_{,\beta}(u^\gamma(\lambda^\alpha\lambda^\beta)_{;\gamma})_{;\alpha} - 2u^\gamma(W_D)_{,\beta}(\lambda^\alpha\lambda^\beta)_{;\alpha\gamma} - 2u^\gamma(W_D)_{,\beta}[\lambda^\alpha\lambda^\eta R^\beta_{\eta\alpha\gamma} + K\lambda^\beta\lambda_\gamma]. \quad [(186)]$$

Again using (85) and (87) for the remaining underbraced terms in (182), we obtain

$$2u^\phi(W_D)_{,\beta}[\lambda^\alpha\lambda^\gamma(b^\beta_\phi b_{\gamma\alpha} - b_{\phi\gamma}b^\beta_\alpha) + \lambda^\beta\lambda^\gamma(b^\alpha_\phi b_{\gamma\alpha} - b_{\phi\gamma}b^\alpha_\alpha)] = 2u^\phi(W_D)_{,\beta}[-R^\beta_{\gamma\alpha\phi}\lambda^\alpha\lambda^\gamma - K\lambda^\beta\lambda_\phi]. \quad [(187)]$$

We can see that the last two terms of (186) cancel with the terms of (187) when substituted into (183) to yield

$$\overline{2(W_D)_{,\beta}(\lambda^\alpha\lambda^\beta)_{;\alpha}}^{\cdot} = 2u^\gamma[(W_D)_{,\beta}(\lambda^\beta\lambda^\alpha)_{;\alpha}]_{;\gamma} - 2(W_D)_{,\beta}[u^\gamma(\lambda^\beta\lambda^\alpha)_{;\gamma}]_{;\alpha} - 2[u^\gamma\frac{\partial W_D}{\partial\theta^\gamma} + u^\gamma W_{DD}b_{\phi\psi}(\lambda^\phi\lambda^\psi)_{;\gamma}]_{,\beta}(\lambda^\alpha\lambda^\beta)_{;\alpha}. \quad [(188)]$$

A.3. Third Term

Variation of the last term in right-hand side of (165) is given by

$$\begin{aligned}\overline{(W_D)(\lambda^\alpha\lambda^\beta)_{;\beta\alpha}}^{\cdot} &= (\dot{W}_D)(\lambda^\alpha\lambda^\beta)_{;\beta\alpha} + W_D\overline{(\lambda^\alpha\lambda^\beta)_{;\beta\alpha}}^{\cdot} \\ &= (\dot{W}_D)(\lambda^\alpha\lambda^\beta)_{;\beta\alpha} + (W_D)\overline{(\lambda^\alpha_{;\beta\alpha}\lambda^\beta + \lambda^\alpha_{;\beta}\lambda^\beta_{;\alpha} + \lambda^\alpha_{;\alpha}\lambda^\beta_{;\beta} + \lambda^\alpha\lambda^\beta_{;\beta\alpha})}^{\cdot}.\end{aligned} \quad [(189)]$$

From (87), we obtain

$$\overline{\lambda^\alpha_{;\beta\alpha}}^{\cdot} = \overline{\lambda^\alpha_{;\alpha\beta}}^{\cdot} + \dot{K}\lambda_\beta + K\dot{\lambda}_\beta. \quad [(190)]$$

As $\lambda^\alpha_{;\alpha}$ is a scalar, we can write

$$\overline{\lambda^\alpha_{;\alpha\beta}}^{\cdot} = (\overline{\lambda^\alpha_{;\alpha}}^{\cdot})_{;\beta}. \quad [(191)]$$

Hence, using (92) we compute

$$\begin{aligned}\overline{\lambda^\alpha_{;\alpha\beta}}^{\cdot} &= [-u^\gamma\lambda_\gamma K - (u^\gamma\lambda^\alpha_{;\gamma})_{;\alpha} + u^\gamma\lambda^\alpha_{;\gamma\alpha}]_{;\beta}, \\ &= [-(u^\gamma\lambda^\alpha_{;\gamma})_{;\alpha} + u^\gamma\lambda^\alpha_{;\alpha\gamma}]_{;\beta}.\end{aligned} \quad [(192)]$$

As for any scalar $(\lambda^\alpha_{;\alpha})_{;\gamma\beta} = (\lambda^\alpha_{;\alpha})_{;\beta\gamma}$, we obtain

$$\overline{\lambda^\alpha_{;\alpha\beta}}^{\cdot} = u^\gamma(\lambda^\alpha_{;\alpha\beta})_{;\gamma} + u^\gamma_{;\beta}\lambda^\alpha_{;\alpha\gamma} - (u^\gamma\lambda^\alpha_{;\gamma})_{;\alpha\beta}. \quad [(193)]$$

Using the above relation, the variation of $\lambda^\alpha_{;\beta\alpha}$ can be obtained using (190) as

$$\overline{\lambda^\alpha_{;\beta\alpha}}^{\cdot} = \underbrace{u^\gamma(\lambda^\alpha_{;\alpha\beta})_{;\gamma} + u^\gamma_{;\beta}\lambda^\alpha_{;\alpha\gamma}} - (u^\gamma\lambda^\alpha_{;\gamma})_{;\alpha\beta} + \underbrace{u^\gamma K_{,\gamma}\lambda_\beta + u^\gamma_{;\beta}K\lambda_\gamma}. \quad [(194)]$$

Combining the underbraced terms in the above equation, we obtain

$$\overline{\lambda^\alpha_{;\beta\alpha}}^{\cdot} = u^\gamma(\lambda^\alpha_{;\beta\alpha})_{;\gamma} - u^\gamma K\lambda_{\beta;\gamma} + u^\gamma_{;\beta}\lambda^\alpha_{;\gamma\alpha} - (u^\gamma\lambda^\alpha_{;\gamma})_{;\alpha\beta}. \quad [(195)]$$

Using the above equation along with (74) yields

$$\overline{\lambda^\alpha_{;\beta\alpha}\lambda^\beta}^{\cdot} = u^\gamma(\lambda^\alpha_{;\beta\alpha})_{;\gamma}\lambda^\beta - u^\beta_{;\gamma}\lambda^\gamma\lambda^\alpha_{;\beta\alpha} - u^\gamma K\lambda_{\beta;\gamma}\lambda^\beta + u^\gamma_{;\beta}\lambda^\alpha_{;\gamma\alpha}\lambda^\beta - (u^\gamma\lambda^\alpha_{;\gamma})_{;\alpha\beta}\lambda^\beta. \quad [(196)]$$

We note here that because $\boldsymbol{\lambda}$ is a unit vector,

$$\begin{aligned}&(\lambda^\beta\lambda_\beta)_{;\gamma} = 0, \\ &\Rightarrow \lambda^\beta_{;\gamma}\lambda_\beta = -\lambda^\beta\lambda_{\beta;\gamma}.\end{aligned} \quad [(197)]$$

By raising and lowering of indices, we obtain

$$\lambda^\beta_{;\gamma}\lambda_\beta = \lambda^\beta\lambda_{\beta;\gamma}, \quad [(198)]$$

which yields the relation

$$\lambda^{\beta}_{;\gamma}\lambda_{\beta} = \lambda^{\beta}\lambda_{\beta;\gamma} = 0, \tag{[(199)]}$$

and, hence,

$$\begin{aligned}\lambda^{\alpha}_{;\beta\alpha}\lambda^{\beta}_{;\gamma} &= \lambda^{\alpha}_{;\alpha\beta}\lambda^{\beta}_{;\gamma} + K\lambda_{\beta}\lambda^{\beta}_{;\gamma} \\ &= \lambda^{\alpha}_{;\alpha\beta}\lambda^{\beta}_{;\gamma}.\end{aligned} \tag{[(200)]}$$

Substituting this into (196), we obtain that

$$\overline{\dot{\lambda^{\alpha}_{;\beta\alpha}\lambda^{\beta}}} = u^{\gamma}(\lambda^{\alpha}_{;\beta\alpha}\lambda^{\beta})_{;\gamma} - u^{\gamma}\lambda^{\alpha}_{;\beta\alpha}\lambda^{\beta}_{;\gamma} - (u^{\gamma}\lambda^{\alpha}_{;\gamma})_{;\alpha\beta}\lambda^{\beta}. \tag{[(201)]}$$

Similarly, using (193) and (74), we compute

$$\begin{aligned}\overline{\dot{\lambda^{\beta}_{;\beta\alpha}\lambda^{\alpha}}} &= \dot{\overline{\lambda^{\beta}_{;\beta\alpha}}}\lambda^{\alpha} + \lambda^{\beta}_{;\beta\alpha}\dot{\lambda}^{\alpha} \\ &= u^{\gamma}(\lambda^{\beta}_{;\beta\alpha})_{;\gamma}\lambda^{\alpha} + \underbrace{u^{\gamma}_{;\alpha}\lambda^{\beta}_{;\beta\gamma}\lambda^{\alpha}} - (u^{\gamma}\lambda^{\beta}_{;\gamma})_{;\beta\alpha}\lambda^{\alpha} - \underbrace{u^{\alpha}_{;\gamma}\lambda^{\beta}_{;\beta\alpha}\lambda^{\gamma}},\end{aligned} \tag{[(202)]}$$

which can be rearranged after canceling out the underbraced terms above to obtain

$$\overline{\dot{\lambda^{\beta}_{;\beta\alpha}\lambda^{\alpha}}} = u^{\gamma}(\lambda^{\beta}_{;\beta\alpha}\lambda^{\alpha})_{;\gamma} - u^{\gamma}\lambda^{\beta}_{;\beta\alpha}\lambda^{\alpha}_{;\gamma} - (u^{\gamma}\lambda^{\beta}_{;\gamma})_{;\beta\alpha}\lambda^{\alpha}. \tag{[(203)]}$$

Next, using (81), (85), and (86), we can evaluate the variation

$$\begin{aligned}\overline{\dot{\lambda^{\alpha}_{;\beta}\lambda^{\beta}_{;\alpha}}} &= 2\lambda^{\alpha}_{;\beta}\dot{\overline{\lambda^{\beta}_{;\alpha}}} = 2\lambda^{\alpha}_{;\beta}(\lambda^{\gamma}u^{\phi}R^{\beta}_{\gamma\phi\alpha} - \lambda^{\gamma}_{;\alpha}u^{\beta}_{;\gamma}) \\ &= 2u^{\phi}\lambda^{\alpha}_{;\beta}\lambda^{\gamma}R^{\beta}_{\gamma\phi\alpha} - 2\lambda^{\beta}_{;\alpha}(u^{\gamma}\lambda^{\alpha}_{;\gamma})_{;\beta} + 2u^{\gamma}\lambda^{\alpha}_{;\gamma\beta}\lambda^{\beta}_{;\alpha}.\end{aligned} \tag{[(204)]}$$

We can rewrite the dummy indices in the first term above and use the chain rule of differentiation for the last term using (87) to obtain

$$\overline{\dot{\lambda^{\alpha}_{;\beta}\lambda^{\beta}_{;\alpha}}} = 2u^{\gamma}\lambda^{\beta}_{;\alpha}\lambda^{\eta}R^{\alpha}_{\eta\gamma\beta} - 2\lambda^{\beta}_{;\alpha}(u^{\gamma}\lambda^{\alpha}_{;\gamma})_{;\beta} + 2u^{\gamma}\lambda^{\alpha}_{;\beta\gamma}\lambda^{\beta}_{;\alpha} + 2u^{\gamma}\lambda^{\beta}_{;\alpha}\lambda^{\eta}R^{\alpha}_{\eta\beta\gamma}. \tag{[(205)]}$$

As $R^{\alpha}_{\eta\gamma\beta} = -R^{\alpha}_{\eta\beta\gamma}$, we obtain

$$\overline{\dot{\lambda^{\alpha}_{;\beta}\lambda^{\beta}_{;\alpha}}} = -2\lambda^{\beta}_{;\alpha}(u^{\gamma}\lambda^{\alpha}_{;\gamma})_{;\beta} + u^{\gamma}(\lambda^{\beta}_{;\alpha}\lambda^{\alpha}_{;\beta})_{;\gamma}. \tag{[(206)]}$$

Using (181) and (87), we write the following variation as

$$\begin{aligned}\overline{\dot{\lambda^{\alpha}_{;\alpha}\lambda^{\beta}_{;\beta}}} &= 2\dot{\overline{\lambda^{\alpha}_{;\alpha}}}\lambda^{\beta}_{;\beta} = 2\lambda^{\beta}_{;\beta}(-u^{\gamma}\lambda_{\gamma}K - \lambda^{\gamma}_{;\alpha}u^{\alpha}_{;\gamma}) \\ &= \underbrace{-2u^{\gamma}\lambda^{\beta}_{;\beta}\lambda_{\gamma}K} - 2\lambda^{\beta}_{;\beta}(u^{\gamma}\lambda^{\alpha}_{;\gamma})_{;\alpha} + 2u^{\gamma}\lambda^{\alpha}_{;\alpha\gamma}\lambda^{\beta}_{;\beta} + \underbrace{2u^{\gamma}\lambda_{\gamma}\lambda^{\beta}_{;\beta}K},\end{aligned} \tag{[(207)]}$$

which, after canceling the underbraced terms, can be written as

$$\overline{\dot{\lambda^{\alpha}_{;\alpha}\lambda^{\beta}_{;\beta}}} = -2\lambda^{\beta}_{;\beta}(u^{\gamma}\lambda^{\alpha}_{;\gamma})_{;\alpha} + u^{\gamma}(\lambda^{\alpha}_{;\alpha}\lambda^{\beta}_{;\beta})_{;\gamma}. \tag{[(208)]}$$

Thus, summing the variations in (201), (203), (206), and (208), we obtain

$$\begin{aligned}\overline{\dot{(\lambda^{\alpha}\lambda^{\beta})_{;\beta\alpha}}} &= u^{\gamma}[(\lambda^{\alpha}\lambda^{\beta})_{;\beta\alpha}]_{;\gamma} - u^{\gamma}\lambda^{\alpha}_{;\gamma}\lambda^{\beta}_{;\alpha\beta} - (u^{\gamma}\lambda^{\alpha}_{;\gamma})_{;\alpha\beta}\lambda^{\beta} - \lambda^{\beta}_{;\alpha}(u^{\gamma}\lambda^{\alpha}_{;\gamma})_{;\beta} \\ &\quad - \lambda^{\beta}_{;\beta}(u^{\gamma}\lambda^{\alpha}_{;\gamma})_{;\alpha} - u^{\gamma}\lambda^{\beta}_{;\gamma}\lambda^{\alpha}_{;\alpha\beta} - \underbrace{(u^{\gamma}\lambda^{\beta}_{;\gamma})_{;\beta\alpha}\lambda^{\alpha}} - \lambda^{\alpha}_{;\beta}(u^{\gamma}\lambda^{\beta}_{;\gamma})_{;\alpha} - \lambda^{\alpha}_{;\alpha}(u^{\gamma}\lambda^{\beta}_{;\gamma})_{;\beta}.\end{aligned} \tag{[(209)]}$$

For the underbraced term above, we use (87) and (199) to compute

$$(u^\gamma \lambda^\beta_{;\gamma})_{;\beta\alpha}\lambda^\alpha = (u^\gamma \lambda^\beta_{;\gamma})_{;\alpha\beta}\lambda^\alpha - u^\gamma \lambda_{\alpha;\gamma}\lambda^\alpha K = (u^\gamma \lambda^\beta_{;\gamma})_{;\alpha\beta}\lambda^\alpha. \qquad [(210)]$$

Combining the following terms in (209), we obtain

$$-u^\gamma \lambda^\alpha_{;\gamma}\lambda^\beta_{;\alpha\beta} - (u^\gamma \lambda^\alpha_{;\gamma})_{;\alpha\beta}\lambda^\beta - (u^\gamma \lambda^\alpha_{;\gamma})_{;\beta}\lambda^\beta_{;\alpha} - (u^\gamma \lambda^\alpha_{;\gamma})_{;\alpha}\lambda^\beta_{;\beta} = -(u^\gamma \lambda^\alpha_{;\gamma}\lambda^\beta)_{;\alpha\beta}, \qquad [(211)]$$

and, similarly, combining the remaining terms after using (210), we obtain

$$-u^\gamma \lambda^\beta_{;\gamma}\lambda^\alpha_{;\alpha\beta} - (u^\gamma \lambda^\beta_{;\gamma})_{;\beta\alpha}\lambda^\alpha - \lambda^\alpha_{;\beta}(u^\gamma \lambda^\beta_{;\gamma})_{;\alpha} - \lambda^\alpha_{;\alpha}(u^\gamma \lambda^\beta_{;\gamma})_{;\beta} = -(u^\gamma \lambda^\beta_{;\gamma}\lambda^\alpha)_{;\alpha\beta}. \qquad [(212)]$$

Substituting the above two equations into (209), we obtain

$$\dot{\overline{(\lambda^\alpha \lambda^\beta)_{;\beta\alpha}}} = u^\gamma[(\lambda^\alpha\lambda^\beta)_{;\beta\alpha}]_{;\gamma} - [u^\gamma(\lambda^\alpha\lambda^\beta)_{;\gamma}]_{;\alpha\beta}. \qquad [(213)]$$

Using the above equation along with (77) and substituting into (189), we obtain

$$\dot{\overline{(W_D)(\lambda^\alpha \lambda^\beta)_{;\beta\alpha}}} = u^\gamma[W_D(\lambda^\alpha\lambda^\beta)_{;\beta\alpha}]_{;\gamma} - [u^\gamma \frac{\partial W_D}{\partial \theta^\gamma} + u^\gamma W_{DD} b_{\phi\psi}(\lambda^\phi\lambda^\psi)_{;\gamma}](\lambda^\alpha\lambda^\beta)_{;\beta\alpha}. \qquad [(214)]$$

Using the variations obtained in (168), (179), (188), and (214), and substituting them in (165), we obtain the tangential variation of the first term of G as

$$\begin{aligned} \dot{\overline{(W_D\lambda^\beta \lambda^\alpha)_{;\beta\alpha}}} = {} & u^\gamma[(W_D\lambda^\alpha\lambda^\beta)_{;\beta\alpha}]_{;\gamma} - [(u^\gamma \frac{\partial W_D}{\partial \theta^\gamma} + u^\gamma W_{DD} b_{\phi\psi}(\lambda^\phi\lambda^\psi)_{;\gamma})\lambda^\alpha\lambda^\beta]_{;\beta\alpha} - u^\gamma (W_D)_{;\beta\alpha}(\lambda^\alpha\lambda^\beta)_{;\gamma} \\ & - 2(W_D)_{,\beta}[u^\gamma(\lambda^\beta\lambda^\alpha)_{;\gamma}]_{;\alpha} - (W_D)[u^\gamma(\lambda^\alpha\lambda^\beta)_{;\gamma}]_{;\alpha\beta}. \end{aligned} \qquad [(215)]$$

We simplify it further by combining the last three terms in the above equation and rewrite it as

$$\begin{aligned} \dot{\overline{(W_D\lambda^\beta \lambda^\alpha)_{;\beta\alpha}}} = {} & u^\gamma[(W_D\lambda^\alpha\lambda^\beta)_{;\beta\alpha}]_{;\gamma} - [u^\gamma W_D(\lambda^\alpha\lambda^\beta)_{;\gamma}]_{;\alpha\beta} \\ & - [(u^\gamma \frac{\partial W_D}{\partial \theta^\gamma} + u^\gamma W_{DD} b_{\phi\psi}(\lambda^\phi\lambda^\psi)_{;\gamma})\lambda^\alpha\lambda^\beta]_{;\beta\alpha}. \end{aligned} \qquad [(216)]$$

Appendix B

In this section, we combine all the derived variations arising from the tangential variation of G. Combining (105), (107), (109), (110), (114), (115), (116), and (117), we obtain

$$\begin{aligned} \dot{G}_t = {} & u^\gamma G_{;\gamma} - [u^\gamma \frac{\partial W_D}{\partial \theta^\gamma}\lambda^\alpha\lambda^\beta]_{;\beta\alpha} + \frac{1}{2}\Delta_s(u^\gamma \frac{\partial W_D}{\partial \theta^\gamma}) - 2u^\gamma HD\frac{\partial W_D}{\partial \theta^\gamma} \\ & - \frac{1}{2}\Delta_s(u^\gamma \frac{\partial W_H}{\partial \theta^\gamma}) - \tilde{b}^{\alpha\beta}(u^\gamma \frac{\partial W_K}{\partial \theta^\gamma})_{;\alpha\beta} - u^\gamma(2H^2 - K)\frac{\partial W_H}{\partial \theta^\gamma} \\ & - 2u^\gamma H(K\frac{\partial W_K}{\partial \theta^\gamma} - \frac{\partial W}{\partial \theta^\gamma}) + 2u^\gamma \sigma_{,\gamma}H - [u^\gamma W_D(\lambda^\alpha\lambda^\beta)_{;\gamma}]_{;\beta\alpha} \\ & - [u^\gamma W_{DD} b_{\phi\psi}(\lambda^\phi\lambda^\psi)_{;\gamma}\lambda^\alpha\lambda^\beta]_{;\alpha\beta} + \frac{1}{2}\Delta_s[u^\gamma W_{DD}(\lambda^\alpha\lambda^\beta)_{;\gamma}b_{\alpha\beta}] \\ & - \frac{1}{2}\Delta_s[u^\gamma W_{HD} b_{\alpha\beta}(\lambda^\alpha\lambda^\beta)_{;\gamma}] - \tilde{b}^{\alpha\beta}[u^\gamma W_{KD} b_{\theta\phi}(\lambda^\theta\lambda^\phi)_{;\gamma}]_{;\alpha\beta} \\ & + u^\gamma W_{HD} b_{\alpha\beta}(\lambda^\alpha\lambda^\beta)_{;\gamma}K - 2u^\gamma H(\lambda^\alpha\lambda^\beta)_{;\gamma}b_{\alpha\beta}(DW_{DD} + KW_{KD} + HW_{HD}). \end{aligned} \qquad [(217)]$$

As can be seen, the tangential variation of scalar G is not convected with the tangential variation to the surface unlike some other fields such as the mean and the Gaussian curvatures. This is because of two reasons.

- The heterogeneity is decoupled from the changes in the position of the surface. This also led to the non-convected terms in the tangential variations for heterogeneous surface as shown in [23].
- The orientation of the protein $\boldsymbol{\lambda}$ is also decoupled from the surface map for the tangential variations to the surface.

Hence, on suppressing the dependence of W on $\boldsymbol{\lambda}$ and removing heterogeneity, we obtain the classical variation $\dot{G}_t = u^\gamma G_{,\gamma}$ (see [19]).

Appendix C

To derive the normal variation $(W_K)_{,\lambda}\dot{\Gamma}^\lambda_{\alpha\beta}\tilde{b}^{\alpha\beta}$ in (147), we use the normal variation of the Christoffel symbols in (121) and compute

$$\dot{\Gamma}^\lambda_{\alpha\beta}\tilde{b}^{\alpha\beta} = -a^{\lambda\theta}[(ub_{\alpha\theta})_{;\beta} + (ub_{\beta\theta})_{;\alpha} - (ub_{\alpha\beta})_{;\theta}]\tilde{b}^{\alpha\beta}. \quad [(218)]$$

As $\tilde{b}^{\alpha\beta}$ are the contravariant adjugate of curvature tensor and is divergence free, we obtain

$$\tilde{b}^{\alpha\beta}b_{\beta\theta} = \delta^\alpha_\theta K, \quad [(219)]$$

and

$$\tilde{b}^{\alpha\beta}_{;\beta} = 0. \quad [(220)]$$

Using these relations and substituting into (218), we obtain

$$\begin{aligned}\dot{\Gamma}^\lambda_{\alpha\beta}\tilde{b}^{\alpha\beta} &= -a^{\lambda\theta}[(uK)_{,\theta} + (uK)_{,\theta} - 2u_{,\theta}K - uK_{,\theta}],\\ &= -uK_{,\theta}a^{\lambda\theta}.\end{aligned} \quad [(221)]$$

Using the above relations, we then obtain

$$(W_K)_{,\lambda}\dot{\Gamma}^\lambda_{\alpha\beta}\tilde{b}^{\alpha\beta} = -u(W_K)_{,\lambda}K_{,\theta}a^{\lambda\theta}. \quad [(222)]$$

In addition, from the definition of $\tilde{b}^{\alpha\beta}$, we have

$$\tilde{b}^{\alpha\beta} = \varepsilon^{\alpha\lambda}\varepsilon^{\beta\gamma}b_{\lambda\gamma}, \quad [(223)]$$

where $\varepsilon^{\alpha\lambda} = \epsilon^{\alpha\lambda}/\sqrt{a}$ with $\epsilon^{\alpha\lambda}$ being the surface permutation tensor ($\epsilon^{12} = -\epsilon^{21} = 1$, $\epsilon^{11} = \epsilon^{22} = 0$). The variation of this term yields

$$\dot{\tilde{b}}^{\alpha\beta} = -\epsilon^{\alpha\lambda}\epsilon^{\beta\gamma}\frac{\dot{a}}{a^2}b_{\lambda\gamma} + \varepsilon^{\alpha\lambda}\varepsilon^{\beta\gamma}\dot{b}_{\lambda\gamma}. \quad [(224)]$$

Using the variation in areal stretch ratio (J) owing to the normal perturbations

$$\frac{\dot{J}}{J} = \frac{\dot{a}}{2a} = -2uH. \quad [(225)]$$

and the variation of the components of the curvature tensor from (119), we obtain

$$\dot{\tilde{b}}^{\alpha\beta} = 4uH\tilde{b}^{\alpha\beta} + \varepsilon^{\alpha\lambda}\varepsilon^{\beta\gamma}(u_{;\lambda\gamma} - ub^\eta_\lambda b_{\eta\gamma}). \quad [(226)]$$

Appendix D

To obtain the normal variation

$$\dot{\overline{2HDW_D}} = 2(\dot{H}DW_D + H\dot{D}W_D + HD\dot{W}_D), \quad [(227)]$$

we use (120), (127), and rearrange the terms to obtain

$$\overline{2HDW_D}^{\cdot} = (\Delta_s u)[(D-H)W_D + (HD)(W_{DH} - W_{DD})] + u_{;\alpha\beta}[(2HW_D + 2HDW_{DD})\lambda^\alpha\lambda^\beta + 2HDW_{DK}\tilde{b}^{\alpha\beta}] + u[2(2H^2-K)(DW_D + HDW_{DH}) + 4H^2DW_D + 4H^2D^2W_{DD} + 4H^2DKW_{DK}]. \quad [(228)]$$

Similarly, for the rest of the terms, we obtain

$$\begin{aligned}
&\overline{W_H(2H^2-K) + 2H(KW_K - W)}^{\cdot} \\
&= (\Delta_s u)[\frac{1}{2}W_{HH}(2H^2-K) + HW_H + HKW_{HK} + (KW_K - W) - \frac{1}{2}W_{HD}(2H^2-K) - H(KW_{KD} - W_D)] \\
&+ u_{;\alpha\beta}\{\tilde{b}^{\alpha\beta}[W_{HK}(2H^2-K) - W_H + 2HKW_{KK}] + \lambda^\alpha\lambda^\beta[W_{HD}(2H^2-K) + 2H(KW_{KD} - W_D)]\} \\
&+ u\{W_{HH}(2H^2-K)^2 + W_{KK}(2HK)^2 + 4HK(2H^2-K)W_{HK} + 4HW_H(H^2-K) + 2(2H^2-K)(KW_K - W) \\
&+ 2HD[W_{HD}(2H^2-K) + 2H(KW_{KD} - W_D)]\},
\end{aligned} \quad [(229)]$$

and

$$2\sigma\dot{H} = \sigma[\Delta_s u + 2u(2H^2-K)]. \quad [(230)]$$

The normal variation of the terms in the shape equation G $(21)_2$ can therefore be obtained by combining the individual variations from (138), (142), (143), (144), (149), (228), (229), and (230) as

$$\begin{aligned}
\dot{G}_n = {} & (\overline{W_D\lambda^\beta\lambda^\alpha}^{\cdot})_{;\beta\alpha} + uW_DK(2H - b_{\beta\gamma}\lambda^\beta\lambda^\gamma) + u_{;\alpha\beta}\tilde{b}^{\alpha\beta}W_D \\
& - (W_D\lambda^\alpha\lambda^\gamma)_{,\beta}[(ub^\beta_\gamma)_{;\alpha} + u_{,\gamma}b^\beta_\alpha - u_{,\theta}b_{\alpha\gamma}a^{\beta\theta}] - 2[W_D\lambda^\beta\lambda^\gamma(2uH)_{,\gamma}]_{;\beta} \\
& + \frac{1}{2}(\Delta_s\dot{W}_H - \Delta_s\dot{W}_D) + (W_H - W_D)_{,\gamma}[(ub^{\beta\gamma})_{;\beta} - (uH)_{;\eta}a^{\gamma\eta}] \\
& + u(W_H - W_D)_{;\alpha\beta}b^{\alpha\beta} + (\dot{W}_K)_{;\alpha\beta}\tilde{b}^{\alpha\beta} - u(W_K)_{,\lambda}K_{,\eta}a^{\lambda\eta} \\
& + 4uH(W_K)_{;\beta\alpha}\tilde{b}^{\beta\alpha} + \varepsilon^{\alpha\lambda}\varepsilon^{\beta\gamma}(u_{;\lambda\gamma} - ub^\eta_\lambda b_{\eta\gamma})(W_K)_{;\beta\alpha} \\
& + (\Delta_s u)[DW_D + HD(W_{DH} - W_{DD}) + \frac{1}{2}(W_{HH} - W_{HD})(2H^2-K) \\
& + HW_H + HK(W_{HK} - W_{KD}) + (KW_K - W) - \sigma] \\
& + (u_{;\alpha\beta}\lambda^\alpha\lambda^\beta)[2HDW_{DD} + W_{HD}(2H^2-K) + 2HKW_{KD}] \\
& + u_{;\alpha\beta}\tilde{b}^{\alpha\beta}[2HDW_{KD} + (2H^2-K)W_{HK} - W_H + 2HKW_{KK}] \\
& + u[-2KDW_D + 4(2H^2-K)HDW_{HD} + 4H^2DW_D + 4H^2D^2W_{DD} \\
& + 4H^2DKW_{DK} + W_{HH}(2H^2-K)^2 + W_{KK}(2HK)^2 \\
& + 4HK(2H^2-K)W_{HK} + 4H(H^2-K)W_H + 2(2H^2-K)(KW_K - W) \\
& + 4H^2DKW_{KD} - 2\sigma(2H^2-K)].
\end{aligned} \quad [(231)]$$