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## Derivation of a new free energy for biological membranes

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**Abstract** A new free energy for thin biomembranes depending on chemical composition, degree of order and membranal-bending deformations is derived in this paper. This is a result of constitutive and geometric assumptions at the three-dimensional level. The enforcement of a new symmetry group introduced in (Deseri et al., in preparation) and a 3D–2D dimension reduction procedure are among the ingredients of our methodology. Finally, the identification of the lower order term of the energy (i.e. the membranal contribution) on the basis of a bottom-up approach is performed; this relies upon standard statistical mechanics calculations. The main result is an expression of the biomembrane free energy density, whose local and non-local counterparts are weighted by different powers of the bilayer thickness. The resulting energy exhibits three striking aspects:

- (i) the local (purely membranal) energy counterpart turns out to be completely determined through the bottom-up approach mentioned above, which is based on experimentally available information on the nature of the constituents;
- (ii) the non-local energy terms, that spontaneously arise from the 3D–2D dimension reduction procedure, account for both bending and non-local membranal effects;
- (iii) the non-local energy contributions turn out to be uniquely determined by the knowledge of the membranal energy term, which in essence represents the only needed constitutive information of the model. It is worth noting that the coupling among the fields appearing as independent variables of the model is not heuristically forced, but it is rather consistently delivered through the adopted procedure.

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## 1 Introduction

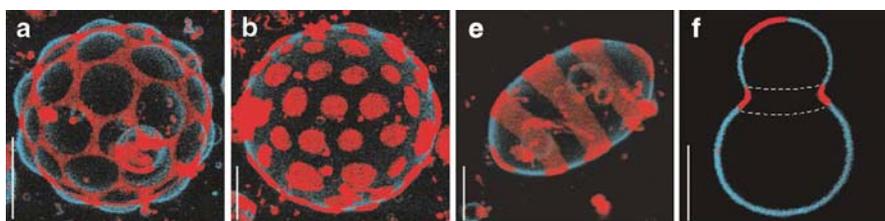
In this work, we present a novel derivation of an energetics for biomembranes, such as liposomes, i.e. non-reacting mixtures of different kinds of phospholipid molecules and cholesterol. The lipids are amphiphilic, namely hydrophilic and hydrophobic at the same time; for certain values of temperature and concentration (called CMC, critical micellar concentration) these molecules spontaneously self-organize in aqueous environment into a shell-like structure called lipid bilayer. Multilayers are also possible (see e.g. [17]). In both cases the structure of the membrane is formed by two or more mono-layers of lipids, respectively. They are arranged so that their hydrophobic tails face one another while their hydrophilic heads face the aqueous solution on either side of the membrane. In liposomes, the lipid bilayer (which has a thickness of few nanometers) is shaped into closed, hollow shells with diameter that ranges from 50 nm to tens of micrometers. Larger liposomes are often referred to as giant unilamellar vesicles (GUVs).

The growing interest on liposomes is due to the fact that there is nowadays a strong experimental evidence that GUVs exhibit a surprisingly rich phase transitions behavior, under externally controlled temperature and osmotic pressure (e.g. the experimental works [6,48,51–54] as well as the the book [10] and the encyclopedia volumes [38] where the issue of phase transitions in biomembranes is extensively discussed).

New and advanced high-resolution fluorescence imaging techniques (e.g. [6] in particular) have indeed highlighted the coexistence of regions (or phases) characterized by different curvature, chemical composition and degree of order of the phospholipid molecules (see Fig. 1). For the sake of argument the concept of order is here referred to the single molecule, although in reality clusters of them are observed to be in the given phase. Depending on the temperature of the environment and on the surrounding conditions, each phospholipid molecule admits an ordered state, where the hydrophobic tails appear straightened and taller, and a disordered state, where the tails appear curly and shortened. Lipid membranes are also fluid-in-plane, which means that molecules are free to move on the membrane surface; for these reasons, the two phases are often referred to as liquid ordered ( $L_o$ ) and liquid disordered ( $L_d$ ) (e.g. [6,36]).

The above mentioned experimental results lead to think that there may exist a strong interplay among membrane shape, order and chemical composition during loading; this paper represents an attempt toward a better comprehension of the correlation among these three aspects.

Liposomes do not merely capture attention because of their fascinating phase transitions; they represent prototypical structures of cell membranes, basic bricks of each living organism. The chemo-mechanical behavior of the cell membrane is strictly related to the control of biological functions: for this reason, the study of the phase-transition behavior of liposomes represents a step toward a better comprehension of the cell functionality. Furthermore, in recent years, liposomes have been widely adopted in the most advanced applications of pharmaceuticals (for example, the targeted delivery of drugs), of diagnostic, of bio-engineering (for instance to build biosensors, where membranes are combined with optoelectronic devices), of food industry, of cosmetics (see [38], Chap. 10 for an overview on these topics). Another interesting feature of cell membranes is, in some cases, the presence of lipid rafts (see [15] among many others); these are domains enriched of a particular type of lipids, called glycosphingolipid. Such rafts may be induced in artificial biomembranes, such as GUVs, and they have the key property that they are detergent-resistant. Lipid rafts basically occur when, for example, fully saturated chains of sphingomyelin and glycosphingolipids bonding between neighboring active



**Fig. 1** Images experimentally obtained by Baumgart et al. [6], showing how phase separation is strictly related to the equilibrium shapes of GUVs in water solution. In red and blue (in the online version) respectively, liquid-disordered and liquid-ordered phases. Scale bars 5  $\mu\text{m}$  (images by courtesy of Tobias Baumgart)

functional glycosyl groups of the latter species; previous experimental papers (see [15]) showed spectroscopic evidence for an ordered “raft” phase coexisting with a disordered one. Henceforth, an energetics predicting the  $L_o$ – $L_d$  transition is consistent with the presence of such rafts.

For all these applications, as well as for more basic researches related to cell functionality, the knowledge of the chemo-mechanical behavior of the lipid membrane is essential.

The existing literature on phospholipid bilayers is very large. Beyond scientific articles, where these are treated within the most different frameworks (from chemistry to differential geometry), there are books as well as encyclopedia volumes entirely devoted to this topic (see for example [10, 18, 38] among many others). This is very complex, because it represents a unique *playground* (to quote Sackmann [38, Chap. 1]) where the theory of non-reacting mixtures and the theory of phase transitions are strictly related to the theory of thin, fluid shells. Due to this intrinsic complexity, a computationally manageable theory, capable to embracing in a satisfactory way all the different features going from the chemistry of the constituents to their mechanical strains and stresses, is hard to find and yet to formulate.

Beyond a considerably large part of the scientific literature on phospholipid membranes, where mechanics is neglected and attention is paid to the correlations between chemical composition and phase transitions, the studies on the purely mechanical behavior of lipid bilayers are generally traced back to the works of Canham [8], Fung [19], Fung and Tong [20] and Helfrich [26]. There a quadratic expression for the curvature elasticity of the bilayer was determined. These works opened the field of biological membranes, traditionally tackled by biochemists and biophysicists, to scientists interested at mechanical problems of elastic surfaces.

The purely mechanical, quadratic bending energy of the lipid bilayer (generally referred to as the Helfrich energy), for the last 35 years or so, was a real source of inspiration for scientists interested at problems of equilibrium of closed fluid membranes under various boundary conditions. In several articles appeared during this time-period, the attention was restricted to purely mechanical problems, such as the stretching, the adhesion and more in general the shaping of lipid bilayers under suitable boundary conditions (see e.g. [7, 9, 44, 50] among many others). In all of these articles, the Helfrich energy is adopted and the lipid bilayer is always described as a homogeneous, elastic, intrinsically 2D body.

The issue of phase transitions of the order–disorder type has been modelled via improvements and/or slight modifications of Helfrich’s energy, hence always conceiving the membrane as an intrinsically 2D body. In some of these cases (see for example [5, 32, 33]) the authors investigated the possibility of the occurrence of buds in non-homogeneous membranes endowed by different and prescribed bending stiffnesses in different phases, whose ratio is also prescribed. In other cases, the Helfrich energy was supplemented with further energy contributions, heuristically chosen in order to produce simple couplings among geometrical parameters (such as the membrane mean or Gaussian curvature) and suitable order parameters (e.g. [2, 11, 34, 38] among several others).

Beyond Helfrich’s energy there are other models where the attention is also focused on purely mechanical aspects. Indeed, further forms for the energy of lipid bilayers were proposed. For instance, besides the pioneering work of Keller and Merchant [35] about the evaluation of the flexural rigidity of a liquid surface, Steigmann [46] proposed a general expression of the constitutive dependence for 2D fluid films endowed of curvature elasticity, on the basis of the Murdoch and Cohen [39] theory of elastic surfaces; in order to study specific boundary value problems Baesu et al. [4] proposed a general form for the 2D stretching-bending energy density of the fluid membrane. Healey [25] found a Helfrich-like energy whose moduli have a suitable dependence on the areal stretch of the surface yielding the type of phase transitions observed in close phospholipid vesicles [6].

Common factor of all the cited models is that the bilayer is always described as a 2D body and hence thickness is never explicitly considered. In the present work, lipid membranes are recognized to be thin but still 3D bodies. In particular, our motivation comes from the fact that lipids are close to be straight in the ordered  $L_o$  phase, whereas they appear curly and shorter in the disordered phase  $L_d$ . Indeed, the membrane thickness is higher in ordered zones than in disordered areas. In other words, in lipid membranes the thickness *does* matter; this may be taken as the order parameter field for such structures.

As it is well known from asymptotic theories of non-linear elastic shells (see e.g. [37] among many others), thickness has the fundamental role to weight the membranal and the bending contributions of the energy density. In particular, ignoring the membranal term (as many formulations do) neglects an energy contribution to the overall energy which is two orders of magnitude more important than the bending term.

The result of our model is represented by an expression of the energy density of the lipid bilayer in which chemical composition and order degree turn out to be coupled with the membranal and bending strains. This is a result of a dimension reduction procedure. In particular, after making constitutive hypotheses at the 3D level

(that include the enforcement of a new symmetry group introduced in [12]), we derive the Helmholtz energy density per unit (reference mid-surface) area of the bilayer via asymptotic expansion of the bulk energy with respect to a reference thickness. No heuristic coupling among the unknown fields is forced: the only recipes of the model are assumptions of constitutive and geometrical nature.

The resulting energy density confirms a precise hierarchy between local and non-local energy terms. Many are the new and key results of the procedure worked out in the present paper:

- the local energy term is purely membranal and it appears completely determined on the basis of experimentally available information on the nature of the constituents;
- the non-local energy terms account for both bending effects and for a gradient term, penalizing the spatial variations of a field related to the order–disorder transition;<sup>1</sup>
- strikingly, the gradient term spontaneously arises from the dimension reduction procedure, rather than being heuristically introduced in the model;
- even more striking is the fact that the bending counterpart of the resulting energy is completely determined by the knowledge of the local–purely membranal energy density; this implies that, in essence, such membranal contribution is the only needed constitutive information of the model. Furthermore, such information can be strictly related to the chemical composition of the membrane.

## 2 Assumptions of the model

On the grounds of the experimental evidences discussed in Sect. 1, we formulate the following assumptions:

- (a) for GUVs, the small ratio between thickness and diameter (few nanometers vs. micrometers) fully justifies the assumption of thin shells;
- (b) we ignore effects leading to a spontaneous or natural curvature of the bilayer and thus we assume that the natural configuration of the membrane is flat;
- (c) we assume that the membrane kinematics is restricted to the class of normal preserving deformations; this means that in our model lipid molecules remain orthogonal to the bilayer mid-surface; nevertheless, we do not impose restrictions on the membrane thickness, which plays a crucial role in the  $L_o$ – $L_d$  transition;
- (d) we assume, according with assumption (b) of flat natural configuration, that the chemical composition of the membrane is homogeneous moving along the bilayer mid-surface normal. A non-homogeneous chemical composition (as well as differences of pH or of electric charge) between the upper and lower monolayers may induce a non-zero spontaneous curvature of the bilayer (e.g. [31]); such instance will be taken in consideration in a forthcoming generalization of the present model.

## 3 Dimension reduction procedure

### 3.1 Preliminary notions

The summation convention is assumed, unless otherwise specified. Greek indices take their values in the set  $\{1, 2\}$  and Latin indices take their values in the set  $\{1, 2, 3\}$ . Let  $(\mathbf{E}_1, \mathbf{E}_2, \mathbf{E}_3)$  be an orthonormal basis for the Euclidean point space  $\mathbb{R}^3$  and let a reference placement of a body  $\mathcal{B}$  to occupy the open, bounded region  $\mathcal{B}_0$  of  $\mathbb{R}^3$ . Let  $\mathbf{f}$  be a smooth deformation of  $\mathcal{B}_0$  into the current placement  $\mathcal{B} = \mathbf{f}(\mathcal{B}_0)$ . Points of  $\mathcal{B}_0$  will be denoted by  $\mathbf{X} = X_i \mathbf{E}_i$  and their images in the current configuration  $\mathcal{B}$  will be denoted by  $\mathbf{x} = \mathbf{f}(\mathbf{X})$ .

Let  $\mathcal{S}$  a smooth, open and oriented surface endowed at each point of a unit normal vector  $\mathbf{m}$ , let  $\mathcal{T}_{\mathcal{S}} := \mathbf{m}^\perp$  the tangent plane to  $\mathcal{S}$ . The perpendicular projector of  $\mathbb{R}^3$  on  $\mathcal{T}_{\mathcal{S}}$  is defined as

$$\mathbb{P}_m := \mathbf{I} - \mathbf{m} \otimes \mathbf{m}$$

where  $\mathbf{I}$  is the identity operator in the space  $\text{Lin}$  of linear transformations from  $\mathbb{R}^3$  in  $\mathbb{R}^3$ . Given a vector field  $\mathbf{c}$  defined on  $\mathcal{B}_0$ , we denote the material gradient with respect to points  $\mathbf{X} \in \mathcal{B}_0$  by  $\nabla \mathbf{c}$  and the material divergence by  $\text{Div } \mathbf{c} := \text{tr}(\nabla \mathbf{c}) = \nabla \mathbf{c} \cdot \mathbf{I}$ . Analogously, given a vector field  $\mathbf{b}$  defined on  $\mathcal{B}$ , we denote the spatial gradient with respect to points  $\mathbf{x} \in \mathcal{B}$  by  $\text{grad } \mathbf{b}$  and the spatial divergence by  $\text{div } \mathbf{b} := \text{tr}(\text{grad } \mathbf{b}) = \text{grad } \mathbf{b} \cdot \mathbf{I}$ .

<sup>1</sup> The non-local energy reduces to the Helfrich's one whenever the gradient term is negligible with respect to the bending one and the elastic moduli do not significantly change with areal stretch and concentration.

Let now  $\Omega$  and  $\omega$  be smooth, open and oriented surfaces with unit normal  $\mathbf{m}$  and  $\mathbf{n}$ , respectively, both surfaces, respectively, endowed of non-zero intersections with  $\mathcal{B}_0$  and  $\mathcal{B}$ . The material and spatial surface gradients of the vector fields  $\mathbf{c}$  and  $\mathbf{b}$  are defined by

$$\nabla_{\Omega}\mathbf{c} := (\nabla\mathbf{c})\mathbb{P}_{\mathbf{m}}, \quad \text{grad}_{\omega}\mathbf{b} := (\text{grad}\mathbf{b})\mathbb{P}_{\mathbf{n}},$$

respectively. It is straightforward to check that

$$\begin{aligned} \nabla\mathbf{c} &= \nabla\mathbf{c}(\mathbb{P}_{\mathbf{m}} + \mathbf{m} \otimes \mathbf{m}) = \nabla_{\Omega}\mathbf{c} + \frac{\partial\mathbf{c}}{\partial\mathbf{m}} \otimes \mathbf{m}, \\ \text{grad}\mathbf{b} &= \text{grad}\mathbf{b}(\mathbb{P}_{\mathbf{n}} + \mathbf{n} \otimes \mathbf{n}) = \text{grad}_{\omega}\mathbf{b} + \frac{\partial\mathbf{b}}{\partial\mathbf{n}} \otimes \mathbf{n} \end{aligned}$$

where the normal derivatives of  $\mathbf{c}$  and  $\mathbf{b}$  are defined by

$$\frac{\partial\mathbf{c}}{\partial\mathbf{m}} := (\nabla\mathbf{c})\mathbf{m}, \quad \frac{\partial\mathbf{b}}{\partial\mathbf{n}} := (\text{grad}\mathbf{b})\mathbf{n}.$$

Using a smooth extension of the unit normal field  $\mathbf{n}$  to a neighborhood of  $\omega$ , we can introduce the curvature tensor of the surface as

$$\mathbf{L} := -\text{grad}_{\omega}\mathbf{n} = -(\text{grad}\mathbf{n})\mathbb{P}_{\mathbf{n}}.$$

It can be proved (e.g. [23]) that the curvature tensor  $\mathbf{L}$  is symmetric and it satisfies

$$\mathbf{L}\mathbf{n} = \mathbf{0}.$$

The mean curvature and the Gaussian curvature of the surface  $\omega$  are, respectively, defined by

$$H := \frac{1}{2}\text{tr}\mathbf{L}, \quad K := \det\mathbf{L}.$$

According to the Cayley–Hamilton theorem for symmetric rank-2 tensors, the curvature tensor  $\mathbf{L}$  satisfies the following useful identity

$$\mathbf{L}^2 - (\text{tr}\mathbf{L})\mathbf{L} + (\det\mathbf{L})\mathbb{P}_{\mathbf{n}} = \mathbf{0} \implies \mathbf{L}^2 - 2H\mathbf{L} + K\mathbb{P}_{\mathbf{n}} = \mathbf{0}.$$

For tangential tensors, i.e. the ones for which at every point there exists a vector  $\mathbf{m}$  such that  $\mathbf{A} \equiv \mathbb{P}_{\mathbf{m}}\mathbf{A}\mathbb{P}_{\mathbf{m}}$ , we may use the following identification for their determinants:  $\det\mathbf{A} \equiv \det[A_{\alpha\beta}]$ .

### 3.2 Geometry, chemistry and energetics in the bulk

Accordingly with assumption (b) of our model, we describe the natural configuration of the membrane as the 3D cylindrical domain in  $\mathbb{R}^3$  defined as follows

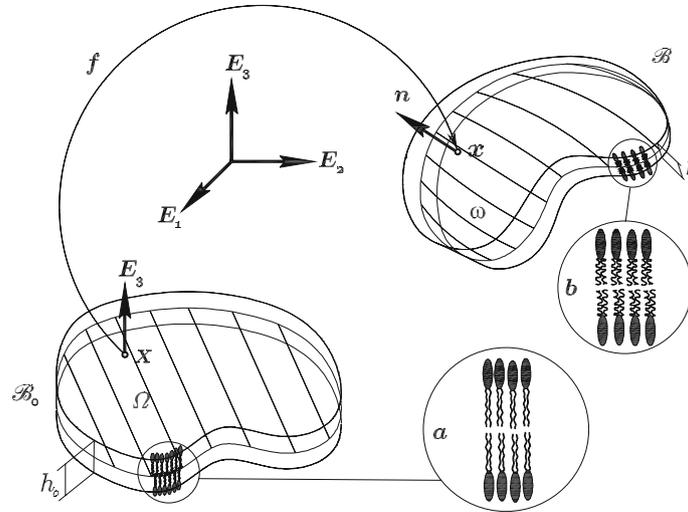
$$\mathcal{B}_0 := \{\mathbf{X} = (\mathbf{X}, X_3) \in \Omega \times (-h_0/2, h_0/2)\} \quad (1)$$

being  $\Omega$  an open, bounded and simply connected region in  $\mathbb{R}^2$  and  $h_0$  the constant membrane thickness. Points of the membrane mid-surface  $\Omega$  have been denoted by  $\mathbf{X} = X_{\alpha}\mathbf{E}_{\alpha}$ . Let  $\mathbf{f}$  be a smooth deformation of the body from its reference configuration  $\mathcal{B}_0$  to its current configuration  $\mathcal{B} := \mathbf{f}(\mathcal{B}_0) \subset \mathbb{R}^3$  and let the 3D deformation gradient be  $\mathbf{F} := \nabla\mathbf{f}$ . We will assume that the body is made of a non-reacting mixture of  $C$  constituent molecules, differing from each other for the chemical structure and composition. At each point of the current configuration of the body  $\mathcal{B}$  it is possible to define the molar fractions

$$\hat{\chi}_k^{3D}(\mathbf{x}) := \frac{dN_k(\mathbf{x})}{dN_{\text{tot}}(\mathbf{x})}, \quad k = 1, \dots, C$$

where  $dN_k(\mathbf{x})$  and  $dN_{\text{tot}}(\mathbf{x}) = \sum_{k=1}^C dN_k(\mathbf{x})$  represent a measure of the number of molecules of the  $k$ -th constituent and a measure of the total number of molecules contained in a neighbor of the point  $\mathbf{x} = \mathbf{f}(\mathbf{X})$  (in the current configuration), respectively. The material description of the molar concentration fields reads as

$$\chi_k^{3D} := \hat{\chi}_k^{3D} \circ \mathbf{f}.$$



**Fig. 2** A schematic representation of the lipid bilayer in its natural, reference configuration  $\mathcal{B}_0$  and in the current configuration  $\mathcal{B}$ . According to the assumptions of our model, the lipid molecules are in the ordered phase in the reference configuration; here the hydrocarbon tails of the lipid molecules are straight (a) and the thickness is constant and equal to  $h_0$ . In the current configuration, the lipid molecules have in general non-constant tail-length (b) hence  $h$  is non-constant; we assume that the lipid molecules remain perpendicular to the current mid-surface  $\omega$  during the deformation  $f$

The normalization condition

$$\sum_{k=1}^C \chi_k^{3D} = 1 \quad (2)$$

imposes that only  $(C - 1)$  molar fractions are linearly independent fields. The constitutive behavior of the membrane is given in terms of the frame indifferent Helmholtz energy density per unit of reference volume

$$W^{3D} = \tilde{W}^{3D} \left( \chi_1^{3D}, \dots, \chi_{(C-1)}^{3D}, \|\nabla \chi_1^{3D}\|, \dots, \|\nabla \chi_{(C-1)}^{3D}\|, \mathbf{C}; T \right) \quad (3)$$

where each  $\|\nabla \chi_k^{3D}\| := (\nabla \chi_k^{3D} \cdot \nabla \chi_k^{3D})^{1/2}$  represents the norm of the gradient of the molar concentration field, where  $\mathbf{C} := \mathbf{F}^T \mathbf{F}$  represents the Cauchy–Green strain tensor and  $T$  represents the absolute temperature. Since we are dealing with equilibrium problems, we will consider  $T$  as a given constant all over the body, hence for simplicity of notation the constitutive dependence on  $T$  will be omitted. For the sake of simplicity, we will adopt the following notation for the bulk energy density of the membrane

$$W^{3D} = \tilde{W}^{3D} \left( \chi_k^{3D}, \|\nabla \chi_k^{3D}\|, \mathbf{C} \right), \quad (4)$$

where we here mean that  $W^{3D}$  depends on all the  $(C - 1)$  components of the fields  $\chi_k^{3D}$  and on  $\|\nabla \chi_k^{3D}\|$ .

Assumption (c) of our model is mathematically traduced by imposing an ansatz on the liposome kinematics. Accordingly with the requirement that possible membrane deformations are normal preserving, but not thickness preserving, the current configuration of the membrane admits the following representation

$$\mathcal{B} := f(\mathcal{B}_0) = \left\{ \mathbf{x} = f(\mathbf{X}) = \mathbf{p}(\mathbf{X}) + X_3 \frac{h(\mathbf{X})}{h_0} \mathbf{n}(\mathbf{X}) \mid \mathbf{X} \in \mathcal{B}_0 \right\} \quad (5)$$

where the map  $\mathbf{p} : \Omega \mapsto \mathbb{R}^3$  represents the deformation of the membrane mid-surface, whose image in the current configuration is

$$\omega := \{ \mathbf{x} \in \mathbb{R}^3 \mid \mathbf{x} = \mathbf{p}(\mathbf{X}), \mathbf{X} \in \Omega \},$$

where  $\mathbf{n}$  represents the material description of the outward unit normal  $\mathbf{n} : \omega \mapsto \mathbb{R}^3$  to the surface  $\omega$ , that is

$$\mathbf{n} := \mathbf{n} \circ \mathbf{p}, \quad \mathbf{n} : \Omega \mapsto \mathbb{R}^3$$

and where  $h(\mathbf{X})$  represents the material description of the thickness of the current configuration  $\hat{h}(\mathbf{x})$ , that is  $h = \hat{h} \circ \mathbf{p}$ .<sup>2</sup>

From now on, any dependence on the coordinates  $(\mathbf{X}, X_3)$  will be stressed just when necessary; in particular, we recall that the scalar and vector fields  $h, \mathbf{p}$  and  $\mathbf{n}$  do not depend on  $X_3$ . For the sake of simplicity, let us set

$$\phi := h/h_0,$$

so that the 3D gradient of the deformation, which now may be written in terms of  $\phi$  since  $\mathbf{f} = \mathbf{p} + X_3\phi\mathbf{n}$ , reads as follows

$$\mathbf{F} = \nabla \mathbf{f} = \nabla \mathbf{p} + \phi \mathbf{n} \otimes \mathbf{E}_3 + X_3 [\mathbf{n} \otimes \nabla \phi + \phi \nabla \mathbf{n}].$$

On setting for simplicity  $\mathbb{P}_3 = \mathbb{P}_{\mathbf{E}_3}$  and recalling that  $\mathbf{I} = \mathbb{P}_3 + \mathbf{E}_3 \otimes \mathbf{E}_3$ , the gradient  $\mathbf{F}$  can be recast as follows

$$\begin{aligned} \mathbf{F} &= \mathbf{F}(\mathbb{P}_3 + \mathbf{E}_3 \otimes \mathbf{E}_3) = \nabla_{\Omega} \mathbf{p} + \phi \mathbf{E}_3 + X_3 [\mathbf{n} \otimes \nabla_{\Omega} \phi - \phi \mathbf{L}(\nabla_{\Omega} \mathbf{p})] \\ &= \mathbf{F} + \phi \mathbf{n} \otimes \mathbf{E}_3 + X_3 [\mathbf{n} \otimes \nabla_{\Omega} \phi - \phi \mathbf{L}\mathbf{F}] \end{aligned} \quad (6)$$

where  $\mathbf{F} := \nabla_{\Omega} \mathbf{p}$  represents the surface gradient of deformation of fibers lying on the reference mid-surface  $\Omega$  and where  $\mathbf{L}$  is the material description of the curvature tensor  $\hat{\mathbf{L}}$  of the current mid-surface  $\omega$ , namely

$$\mathbf{L} := \hat{\mathbf{L}} \circ \mathbf{p}, \quad \hat{\mathbf{L}} := -(\text{grad } \mathbf{n})\mathbb{P}_{\mathbf{n}} = -\text{grad}_{\omega} \mathbf{n}.$$

We remark that since

$$\mathbf{F}\mathbf{E}_3 = \mathbf{0}, \quad \mathbf{F}^T \mathbf{n} = \mathbf{0}, \quad \mathbf{L} = \mathbf{L}^T, \quad \mathbf{L}\mathbf{n} = \mathbf{0},$$

the following useful identities hold

$$\mathbf{F} \equiv \mathbb{P}_{\mathbf{n}} \mathbf{F} \mathbb{P}_3, \quad \mathbf{L} \equiv \mathbb{P}_{\mathbf{n}} \mathbf{L} \mathbb{P}_{\mathbf{n}}.$$

The right Cauchy–Green tensor  $\mathbf{C} = \mathbf{F}^T \mathbf{F}$  arising from the explicit expression of the deformation gradient (6) can be put in the form

$$\mathbf{C}(\mathbf{X}, X_3) = \mathbf{C}_0(\mathbf{X}) + X_3 \mathbf{C}_1(\mathbf{X}) + X_3^2 \mathbf{C}_2(\mathbf{X}) \quad (7)$$

where the three symmetric tensors  $\mathbf{C}_j(\mathbf{X})$  ( $j = 1, 2, 3$ ) are

$$\begin{aligned} \mathbf{C}_0 &:= \mathbf{F}^T \mathbf{F} + \phi^2 \mathbf{E}_3 \otimes \mathbf{E}_3 = \mathbf{G} + \phi^2 \mathbf{E}_3 \otimes \mathbf{E}_3; \\ \mathbf{C}_1 &:= -2\phi \mathbf{F}^T \mathbf{L}\mathbf{F} + \phi(\nabla_{\Omega} \phi \otimes \mathbf{E}_3 + \mathbf{E}_3 \otimes \nabla_{\Omega} \phi); \\ \mathbf{C}_2 &:= \nabla_{\Omega} \phi \otimes \nabla_{\Omega} \phi + \phi^2 \mathbf{F}^T \mathbf{L}^2 \mathbf{F}, \end{aligned}$$

where the tensor

$$\mathbf{G} := \mathbf{F}^T \mathbf{F}$$

evidently represents the Cauchy–Green tensor of the mid-surface  $\Omega$ . For later use, let us also calculate the Cauchy–Green tensor relative to fibers laying on planes parallel to the reference mid-surface  $\Omega$ , namely the tensor

$$\mathbf{C}(\mathbf{X}, X_3) := [\mathbf{F}(\mathbf{X}, X_3)\mathbb{P}_3]^T [\mathbf{F}(\mathbf{X}, X_3)\mathbb{P}_3] = \mathbb{P}_3 \mathbf{C}(\mathbf{X}, X_3) \mathbb{P}_3$$

which can be expanded as follows

$$\mathbf{C}(\mathbf{X}, X_3) = \mathbf{C}_0(\mathbf{X}) + X_3 \mathbf{C}_1(\mathbf{X}) + X_3^2 \mathbf{C}_2(\mathbf{X}) \quad (8)$$

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<sup>2</sup> For simplicity of notation we will set for any arbitrary scalar, vector or tensor function  $\xi$

$$\xi(\mathbf{X}, 0) := \xi(\mathbf{X}).$$

where the three symmetric tensors  $\mathbf{C}_j(\mathbf{X})$  ( $j = 1, 2, 3$ ) are

$$\begin{aligned}\mathbf{C}_0 &:= \mathbf{F}^T \mathbf{F} = \mathbf{G}; \\ \mathbf{C}_1 &:= -2\phi \mathbf{F}^T \mathbf{L} \mathbf{F}; \\ \mathbf{C}_2 &:= \nabla_\Omega \phi \otimes \nabla_\Omega \phi + \phi^2 \mathbf{F}^T \mathbf{L}^2 \mathbf{F}.\end{aligned}$$

Furthermore it may be easily checked that

$$C_{33} = \mathbf{C} \mathbf{E}_3 \cdot \mathbf{E}_3 = \phi^2. \quad (9)$$

### 3.3 In-plane-fluidity in the bulk

As already mentioned in Sect. 1, within this model we focus attention on the so-called liquid phases  $L_o$  and  $L_d$ ; in such phases the membrane behaves like an in-plane-fluid, which roughly means that lipid molecules can move freely on the membrane surface. From the mechanical point of view, in-plane-fluidity means that the membrane does not offer resistance to shears contained in planes orthogonal to  $\mathbf{E}_3$ . The derivation of a suitable symmetry group which describes the peculiarity of in-plane-fluidity at the level of the bulk material was obtained in [12], where an expression of the reduced bulk energy density starting from a frame invariant bulk energy density was given. According to [12], this material property can be imposed by requiring invariance of the constitutive response under the group of transverse unimodular transformations with axis  $\mathbf{E}_3$ , namely the set

$$\text{Unim}\{\mathbf{E}_3\} := \{\mathbf{H} = H_{\alpha\beta} \mathbf{E}_\alpha \otimes \mathbf{E}_\beta + \mathbf{E}_3 \otimes \mathbf{E}_3 \text{ with } \det \mathbf{H} = 1\},$$

that is deformations which leave unaltered the area of planes parallel to the reference mid-surface  $\Omega$ . Invariance of the energy density (4) under the symmetry transformations group is imposed by requiring that

$$\tilde{W}^{3D}(\cdot, \cdot, (\mathbf{F}\mathbf{H})^T (\mathbf{F}\mathbf{H})) = \tilde{W}^{3D}(\cdot, \cdot, \mathbf{F}^T \mathbf{F}), \quad \forall \mathbf{H} \in \text{Unim}\{\mathbf{E}_3\},$$

where bullets represent scalars which are unaffected by the symmetry transformation. As it is shown in [12], invariance under the group of transverse unimodular transformations implies the following reduced form of the bulk energy density (4):

$$W^{3D} = \tilde{W}^{3D}(\chi_k^{3D}, \|\nabla \chi_k^{3D}\|, \det \mathbf{C}, \det \mathbf{C}, C_{33}) \quad (10)$$

where  $\mathbf{C}$  is the rank-2 tensor defined by

$$\mathbf{C} := \mathbb{P}_3 \mathbf{C} \mathbb{P}_3 = C_{\alpha\beta} \mathbf{E}_\alpha \otimes \mathbf{E}_\beta.$$

For the sake of simplicity in the forthcoming calculations, we set

$$\delta := \det \mathbf{C}, \quad \Delta := \det \mathbf{C}. \quad (11)$$

### 3.4 Asymptotic expansion of the stored energy

The Helmholtz energy stored in the body can be calculated by integration of the bulk energy density over the reference configuration  $\mathcal{B}_0$

$$E := \int_{\mathcal{B}_0} W^{3D}(\mathbf{X}, X_3) \, dA \, dX_3, \quad (12)$$

being  $dA$  the area measure on the reference mid-surface  $\Omega$ . We now make the following change of variables from  $\mathcal{B}_0$  to a rescaled domain  $\mathcal{B}_1$  which is independent of  $h_0$  via the re-parametrization

$$\mathbf{r} : \mathbf{Z} = (\mathbf{Z}, Z_3) \in \mathcal{B}_1 \longrightarrow \mathbf{X} = (\mathbf{X}, X_3) = (\mathbf{Z}, h_0 Z_3) \in \mathcal{B}_0.$$

The rescaled domain  $\mathcal{B}_1$  is defined by

$$\mathcal{B}_1 := \left\{ \mathbf{Z} = (\mathbf{Z}, Z_3) \in \Omega \times \left( -\frac{1}{2}, \frac{1}{2} \right) \right\} \quad (13)$$

and since the Jacobian of the re-parametrization  $\mathbf{r}$  equals  $h_0$ , then  $dV_0 = dAdX_3 = dAh_0dZ_3 = h_0dV_1$ . After the change of variables the integral (12) takes the form

$$E = \int_{\mathcal{B}_1} h_0 W^{3D}(\mathbf{Z}, h_0 Z_3) dA dZ_3.$$

On the grounds of the assumption (a) of small bilayer membrane thickness, it makes sense to consider an asymptotic expansion of  $E$  with respect to the constant reference thickness  $h_0$  (the dependence on  $h_0$  is here stressed on purpose)

$$E(h_0) = [E(h_0)]_{h_0=0} + h_0 \left[ \frac{d(h_0)}{dh_0} \right]_{h_0=0} + \frac{h_0^2}{2} \left[ \frac{d^2 E(h_0)}{dh_0^2} \right]_{h_0=0} + o(h_0^2). \quad (14)$$

In the purely mechanical context, an analog procedure was performed in [27–29] and more recently in [47]. Within our model we will neglect terms of order higher than the second and we will show that the considered ones account for local membranal effects, for non-local membranal effects related to the order/disorder phase transition and for bending effects. Since  $\mathcal{B}_1$  is independent of  $h_0$ , we can carry on the asymptotic expansion of  $E$  in powers of  $h_0$  by expanding its integrand around  $h_0 = 0$ ; this yields

$$E = \int_{\mathcal{B}_1} h_0 \left\{ [W^{3D}]_{h_0=0} + h_0 \left[ \frac{dW^{3D}}{dh_0} \right]_{h_0=0} + \frac{h_0^2}{2} \left[ \frac{d^2 W^{3D}}{dh_0^2} \right]_{h_0=0} \right\} dA dZ_3. \quad (15)$$

In order to derive the expression of the surface energy density of a thin lipid bilayer which satisfies the assumptions (a)–(d) of our model, we will now consider the special form of the bulk energy (4) for lipid bilayers endowed of in-plane-fluidity, with the expressions of the tensors  $\mathbf{C}$  and  $\mathbf{C}$  given by Eqs. (7) and (8).

As first thing, in agreement with the assumption (d) of our model, we impose invariance of the molar fraction fields  $\chi_k^{3D}$  with respect to the coordinate  $X_3$  by setting

$$\chi_k^{3D}(\mathbf{X}, X_3) \equiv \chi_k^{3D}(\mathbf{X}, 0) =: \chi_k(\mathbf{X}) \quad \forall \mathbf{X} \in \mathcal{B}_0.$$

By the expressions (7)–(9) we also know that the determinants  $\Delta = \det \mathbf{C}$  and  $\delta = \det \mathbf{C}$  depend on  $h_0$ , while  $C_{33} = \phi^2$  thus it does not depend on  $h_0$ . On stressing the dependence on  $h_0$ , it thus makes sense to set for simplicity

$$w = \bar{w}(\delta(h_0), \Delta(h_0)) := \tilde{W}^{3D}(\chi_k, \|\nabla \chi_k\|, \delta(h_0), \Delta(h_0), C_{33}). \quad (16)$$

The asymptotic expansion of  $w$  with respect to  $h_0$  yields

$$\bar{w}(\delta(h_0), \Delta(h_0)) = \bar{w}(\delta(0), \Delta(0)) + h_0 \dot{\bar{w}}(\delta(0), \Delta(0)) + \frac{h_0^2}{2} \ddot{\bar{w}}(\delta(0), \Delta(0)) + o(h_0^2), \quad (17)$$

where derivation with respect to  $h_0$  has been denoted by the dot. We record the following relations:

$$\begin{aligned} \dot{w} &= w_\delta \dot{\delta} + w_\Delta \dot{\Delta} \\ \ddot{w} &= w_{\delta\delta} \ddot{\delta} + w_{\Delta\Delta} \ddot{\Delta} + w_{\delta\delta} \dot{\delta}^2 + w_{\Delta\Delta} \dot{\Delta}^2 + 2w_{\delta\Delta} \dot{\delta} \dot{\Delta}. \end{aligned}$$

Detailed calculations of the derivatives of  $\delta$  and  $\Delta$  with respect to  $h_0$  are worked out in Appendix; the resulting expressions, calculated at  $h_0 = 0$  are

$$\begin{aligned} \Delta(0) &= \phi^2 J^2 \\ \dot{\Delta}(0) &= -4Z_3 \phi^3 JH \\ \ddot{\Delta}(0) &= 4Z_3^2 J^2 \phi^4 K + 8Z_3^2 \phi^4 H^2 \end{aligned}$$

$$\begin{aligned}\delta(0) &= J^2 \\ \dot{\delta}(0) &= -4Z_3 J^2 \phi H \\ \ddot{\delta}(0) &= Z_3^2 \left( 8\phi^2 J^2 H^2 + 4\phi^2 J^2 K + 2J^2 \|\text{grad}_\omega \hat{\phi}\|_m^2 \right); \end{aligned}$$

here

$$J = (\det \mathbf{G})^{1/2}$$

represents the areal stretch measured at a place of the reference mid-surface  $\Omega$ , where  $H$  and  $K$  are, respectively, the material descriptions of the mean and Gaussian curvatures in a (corresponding) place of the current mid-surface  $\omega$ .<sup>3</sup>

The asymptotic expansion of the energy (17) yields an explicit dependence on the coordinate  $Z_3$ , hence it is now possible to obtain the surface energy density of the membrane by integration over the thickness of the rescaled domain  $\mathcal{B}_1$ ; linear terms in  $Z_3$ , which factor  $\dot{w}$ , cancel during integration, and after straightforward calculations we finally get that the stored Helmholtz energy can be expressed as

$$E = \int_{\Omega} \tilde{\varphi}(\chi_k, \nabla \chi_k, J, \phi, H, K, \|\text{grad}_\omega \hat{\phi}\|_m) \, dA$$

where  $\varphi$  represents the surface Helmholtz energy, per unit reference area, given by

$$\varphi = \varphi^{\text{loc}} + h_0^2 \varphi^{\text{nloc}}. \quad (18)$$

The local energy density  $\varphi^{\text{loc}}$  is defined by

$$\varphi^{\text{loc}} := \check{\varphi}^{\text{loc}}(\chi_k, \nabla \chi_k, J, \phi) := h_0 \left[ \tilde{W}^{3D}(\chi_k, \nabla \chi_k, \delta, \Delta, C_{33}) \right]_{\delta=J^2, \Delta=\phi^2 J^2, C_{33}=\phi^2} \quad (19)$$

and where the non-local energy density  $\varphi^{\text{nloc}}$  is defined by

$$\varphi^{\text{nloc}} := \check{\varphi}^{\text{nloc}}(\chi_k, \nabla \chi_k, J, \phi, H, K, \|\text{grad}_\omega \hat{\phi}\|_m^2) := \kappa_1 H^2 + \kappa_2 K + \beta \|\text{grad}_\omega \hat{\phi}\|_m^2 \quad (20)$$

with the bending moduli  $\kappa_{1,2}$  and the non-local modulus  $\beta$  completely determined in terms of the bulk energy  $W^{3D}$  by the relations

$$\begin{aligned}\kappa_1 &= \check{\kappa}_1(\chi_k, \nabla \chi_k, J, \phi) := (\phi^2 J^2 w_1 + \phi^4 w_2 + 2J^4 \phi^2 w_3 + 2\phi^6 J^2 w_4 + 4J^3 \phi^4 w_5) / 3, \\ \kappa_2 &= \check{\kappa}_2(\chi_k, \nabla \chi_k, J, \phi) := (\phi^2 J^2 w_1 + J^2 \phi^4 w_2) / 6 \\ \beta &= \check{\beta}(\chi_k, \nabla \chi_k, J, \phi) := w_1 J^2 / 12, \end{aligned} \quad (21)$$

and where, conclusively, we have set

$$\begin{aligned}w_1 &:= h_0 \left[ \tilde{W}_\delta^{3D}(\chi_k, \nabla \chi_k, \delta, \Delta, C_{33}) \right]_{\delta=J^2, \Delta=\phi^2 J^2, C_{33}=\phi^2}, \\ w_2 &:= h_0 \left[ \tilde{W}_\Delta^{3D}(\chi_k, \nabla \chi_k, \delta, \Delta, C_{33}) \right]_{\delta=J^2, \Delta=\phi^2 J^2, C_{33}=\phi^2}, \\ w_3 &:= h_0 \left[ \tilde{W}_{\delta\delta}^{3D}(\chi_k, \nabla \chi_k, \delta, \Delta, C_{33}) \right]_{\delta=J^2, \Delta=\phi^2 J^2, C_{33}=\phi^2}, \\ w_4 &:= h_0 \left[ \tilde{W}_{\Delta\Delta}^{3D}(\chi_k, \nabla \chi_k, \delta, \Delta, C_{33}) \right]_{\delta=J^2, \Delta=\phi^2 J^2, C_{33}=\phi^2}, \\ w_5 &:= h_0 \left[ \tilde{W}_{\Delta\delta}^{3D}(\chi_k, \nabla \chi_k, \delta, \Delta, C_{33}) \right]_{\delta=J^2, \Delta=\phi^2 J^2, C_{33}=\phi^2}. \end{aligned} \quad (22)$$

The expression of the surface energy density  $\varphi$ , consistently derived from the bulk energy (10), represents the main target of our model. Its structure establishes a precise hierarchy between local terms, which factor  $h_0$ , and non-local terms, which factor  $h_0^3$ . Worth noting, the bending/non-local moduli constitutively depend on local fields, which express the chemical composition, the areal stretch at the level of the bilayer mid-surface and the bilayer thickness variations.

<sup>3</sup> Here  $\hat{\phi}$  is the spatial description of  $\phi$ , in the sense that  $\phi = \hat{\phi} \circ p$ . The term  $\|\cdot\|_m^2$  represents the material description of  $\|\cdot\|^2$ .

### 3.5 Quasi-incompressibility

The expressions of the moduli given in general form in Eqs. (21), (22) can be appreciably simplified on the basis of a further assumption on the bulk behavior of the bilayer.

Indeed, besides their full generality, Eqs. (21), (22) may result of no practical use. Nevertheless, there are strong experimental evidences that lipid molecules behave keeping their volume constant; this in turn implies that the mid-surface areal stretch  $J$  and the thickness extension (or contraction)  $\phi$  in a thin biomembrane are not independent fields. In order to provide an experimental justification of these further assumptions on the bilayer behavior, let us review some well known discussions on the topic from the biophysical literature on biomembranes.

According to the encyclopedia article on biological membranes by Lipowsky and Sackmann [38], “. . .the hydrocarbon film within the membrane is essentially incompressible, stretching of the bilayer area implies a thinning of the bilayer thickness. . .”.

According to Safran [45], the assumption of quasi-incompressibility is proper since “. . .in addition to being characterized by the area per amphiphile, the . . . membrane is also characterized by its thickness  $[h]$ , which can also change under deformations of the film . . . we assume that the equation of state of the flat membrane determines the thickness as a function of the area per molecule. (A simple example is the case of an incompressible molecule where the product of  $[J\phi]$  is constrained to equal the molecular volume so that  $[J\phi = 1]$ ). We thus take the flat membrane to be characterized only by the area per molecule. . .”, where in square brackets we adopt our notation for the same entities used by the Authors.

About molecular volume incompressibility, Goldstein and Leibler [21], “. . .adopt the viewpoint that these lyotropic liquid crystals may be treated in analogy with conventional models of binary liquid mixtures, namely that the two components of the system, lipid and water, are assumed to be characterized by invariant molecular volumes  $v_l$  and  $v_w$ . . . the bilayer thickness  $[h]$  and effective area per head group  $\Sigma$  are simply related by  $h\Sigma = v_l$ ”.

For these reasons we impose that in thin biomembranes the fields  $J$  and  $\phi$  are related at each point by the relation

$$J\phi = 1. \quad (23)$$

It is easy to check that the relation (23) can be seen as an quasi-incompressibility constraint for a thin membrane; indeed, according to the constrained kinematics deriving from the ansatz (5), the volume variation  $\det \mathbf{F} = \sqrt{\Delta}$  is given by

$$\det \mathbf{F} = \phi J - 2X_3\phi^2 H + X_3^2 J\phi^3 K;$$

under the constraint (23) the volume variation at each point of the membrane becomes

$$\det \mathbf{F} = 1 - 2X_3\phi^2 H + X_3^2\phi^2 K$$

which shows that the exact incompressibility constraint is actually satisfied for points  $\mathbf{X} = (\mathbf{X}, 0)$  belonging to the bilayer mid-surface, whereas it is not satisfied in general for points with  $X_3 \neq 0$ . In general, it results that

$$\det \mathbf{F} = 1 + O(h_0),$$

and for this reason, in the limit of thin bilayers where  $h_0 \rightarrow 0$ , we define the requirement (23) as a quasi-incompressibility constraint.

It should be remarked that relation (23) also plays a crucial role in the definition of a suitable coarse-grained order parameter for the order–disorder phase transition in the biomembrane: such transition is indeed detectable both by the straightening/shortening of the hydrocarbon tails, and by the variation of the head-group area, roughly speaking the measure of the transversal cross section of a single lipid molecule.

In light of the discussion above, we restrict our attention to bulk energy densities of the form

$$W^{3D} = \tilde{W}^{3D}(\chi_k^{3D}, \|\nabla\chi_k^{3D}\|, \delta), \quad (24)$$

with  $\delta$  defined by (11). Obviously, this type of energy is a special case of (10).

This energy may capture the main features of a thin biomembrane undergoing the  $L_o$ – $L_d$  order–disorder phase transition; indeed, for a thin body, it results that  $\Delta \approx 1$  and that  $C_{33}$  is deducible by  $\delta$  evaluated at any

arbitrary point of the mid-surface; the latter is indeed the areal stretch  $J$  measured at any point right on such a surface.

It should be remarked that in Eq. (24),  $\delta(\mathbf{X})$  actually depends on all the coordinates  $(\mathbf{X}, X_3)$ . For this reason, the expression of the energy density (24) leads to think about this model as a thin, continuous layer of fluid elastic surfaces, whose energy density (beyond chemical composition) solely feels local variations of area on planes perpendicular to  $\mathbf{E}_3$  and whose kinematics is restricted by the ansatz (5).

It is now straightforward to check that the expressions of the moduli (21) and (22) undergo a strong simplification, since in this special case it results that

$$w_2 = w_4 = w_5 = 0,$$

hence the bending and non-local moduli are completely determined as functions of derivatives of the bulk energy with respect to  $\delta$ . In particular, letting  $\hat{J}$  the spatial description of  $J$ , that is  $J = \hat{J} \circ \mathbf{p}$ , it results that

$$\hat{\phi} \hat{J} = 1 \implies \text{grad}_\omega \hat{\phi} = -\hat{J}^{-2} \text{grad}_\omega \hat{J} \implies \|\text{grad}_\omega \hat{\phi}\|_m^2 = J^{-4} \|\text{grad}_\omega \hat{J}\|_m^2$$

hence we get that the Helmholtz energy density per reference area unit admits the following simplified expressions for the local counterpart of the energy density

$$\varphi^{\text{loc}} := \check{\varphi}^{\text{loc}}(\chi_k, \nabla \chi_k, J) := h_0 \left[ \tilde{\tilde{W}}^{\text{3D}}(\chi_k, \nabla \chi_k, \delta) \right]_{\delta=J^2} \quad (25)$$

and for the non-local counterpart of the energy density

$$\varphi^{\text{nloc}} := \check{\varphi}^{\text{nloc}}(\chi_k, \nabla \chi_k, J, H, K, \|\text{grad}_\omega \hat{J}\|_m^2) := \kappa_1 H^2 + \kappa_2 K + \alpha \|\text{grad}_\omega \hat{J}\|_m^2 \quad (26)$$

where again

$$\begin{aligned} \kappa_1 &= \check{\kappa}_1(\chi_k, \nabla \chi_k, J) := (w_1 + 2J^2 w_3)/3, \\ \kappa_2 &= \check{\kappa}_2(\chi_k, \nabla \chi_k, J) := w_1/6, \\ \alpha &= \check{\alpha}(\chi_k, \nabla \chi_k, J) := w_1/(12J^2), \end{aligned} \quad (27)$$

with

$$\begin{aligned} w_1 &:= h_0 \left[ \tilde{\tilde{W}}_\delta^{\text{3D}}(\chi_k, \nabla \chi_k, \delta) \right]_{\delta=J^2}, \\ w_3 &:= h_0 \left[ \tilde{\tilde{W}}_{\delta\delta}^{\text{3D}}(\chi_k, \nabla \chi_k, \delta) \right]_{\delta=J^2}. \end{aligned}$$

The final expression of the moduli may be evaluated after substituting  $\varphi^{\text{loc}}$  in place of  $W^{\text{3D}}$ , which gives

$$\begin{aligned} \kappa_1 &= \check{\kappa}_1(\chi_k, \nabla \chi_k, J) := \frac{1}{6} \frac{\partial^2 \check{\varphi}^{\text{loc}}(\chi_k, \nabla \chi_k, J)}{\partial J^2}, \\ \kappa_2 &= \check{\kappa}_2(\chi_k, \nabla \chi_k, J) := \frac{1}{12J} \frac{\partial \check{\varphi}^{\text{loc}}(\chi_k, \nabla \chi_k, J)}{\partial J}, \\ \alpha &= \check{\alpha}(\chi_k, \nabla \chi_k, J) := \frac{1}{24J^3} \frac{\partial \check{\varphi}^{\text{loc}}(\chi_k, \nabla \chi_k, J)}{\partial J}. \end{aligned} \quad (28)$$

The final expression of the surface Helmholtz energy density per reference unit area for a quasi-incompressible lipid bilayer is represented by

$$\varphi = \varphi^{\text{loc}} + h_0^2 \left[ \frac{1}{6} \frac{\partial^2 \varphi^{\text{loc}}}{\partial J^2} H^2 + \frac{1}{12J} \frac{\partial \varphi^{\text{loc}}}{\partial J} K + \frac{1}{24J^3} \frac{\partial \varphi^{\text{loc}}}{\partial J} \|\text{grad}_\omega \hat{J}\|_m^2 \right] \quad (29)$$

This expression of the surface Helmholtz energy of the lipid membrane presents several novel features with respect to the existing literature on the argument, in particular:

- (i) local and non-local effects are weighted by different powers of the bilayer reference thickness  $h_0$ , in particular local effects are two orders of magnitude more “important” than non-local ones; this should address attention to the fact that ignoring membranal effects may be inappropriate when studying the equilibrium of liposomes, although this is customarily done in all articles where the only Helfrich energy density [26]

$$w = \kappa_1 H^2 + \kappa_2 K \quad (30)$$

with  $\kappa_\alpha$  given constants is considered; as matter of fact, it would be easy to check that the Helfrich energy can be simply recovered by ignoring chemical and membranal effects;

- (ii) the gradient term which penalizes spatial variations of the thickness (that is, in our model, of the mid-surface areal stretch) and the relative modulus  $\alpha$  are not heuristically introduced, rather these are consistently derived from the procedure of dimension reduction on the grounds of the assumptions of our model; this provides a tool in order to relate the amplitude of the boundary layer between the ordered–disordered phases and the surface tension;
- (iii) the fact that the interplay between  $K$  and  $(J^{-2} \|\text{grad}_\omega \hat{J}\|_m^2)$  is penalized in Eq. (29) may not be surprising; in other words, such energy penalizes local changes in the gradient of the areal stretch  $J$  (the determinant of the metric tensor, which is associated to the first fundamental form of the surface) and changes in Gaussian curvature; as it is well known, Gauss Egregium theorem assures that the Gaussian curvature  $K$  changes only if the first fundamental form does; further, Brioschi’s formula gives the explicit expression of  $K$  in terms of the first fundamental form. No other proposed energetics for biomembranes include the term  $(K + J^{-2} \|\text{grad}_\omega \hat{J}\|_m^2/2)$ , which is in general non-zero;
- (iv) the bending moduli  $\kappa_1$  and  $\kappa_2$  are not heuristically set equal to different constants for each phase domain (what is customarily done in the literature on this topic, as discussed in Sect. 1), rather these moduli are known functions of the local energy term, which in turn is completely determined on the basis of experimental data on the mixture constituents.

#### 4 Special forms of the energy density

Let us consider the special case of quasi-incompressible membranes whose energy density is given by Eq. (29). The dimension reduction procedure carried out in the previous section has shown that, within our model, the non-local moduli  $\kappa_1$ ,  $\kappa_2$  and  $\alpha$  can be calculated from Eq. (28) as functions of the local energy density  $\varphi^{\text{loc}}$ . In essence, by Eq. (29), this energy represents the only needed constitutive information of our approach.

Researchers involved in laboratory analysis of GUVs’ properties generally make use of binary mixtures of ternary liposomes (e.g. [6, 51–53]). For this reason in this work we confine attention to the two cases of binary saturated lipid/unsaturated lipid and lipid/cholesterol mixtures. This choice does not obviously represent a limit for our theory, which is fully general and virtually applies to more complex cases.

In establishing the expression of the surface Helmholtz energy  $\varphi^{\text{loc}}$  of both kinds of mixtures, we here consider the membrane as a surface made of a compound of two non-reacting components. Because of the normalization condition (2), there is one only independent molar fraction field  $\chi$ .

Within this intrinsically 2D approach, the order–disorder phase transition is entrusted with the mid-surface areal stretch  $J$ , univocally related to the bilayer current thickness  $h$  by the relation  $hJ = h_0$ . Accordingly, we arbitrarily assume that the reference configuration of the surface is in the ordered phase  $L_o$ , which corresponds to  $J = 1$  (that is  $h = h_0$ ).

The existing literature on non-reacting mixtures offers nowadays a huge variety of phenomenological models providing suitable expressions for the Helmholtz energy  $\varphi^{\text{loc}}$  in different cases. Most of these formulas are based on a bottom-up approach: this allows for finding the (effective) local part of the free energy density starting from mean field approaches employed in statistical mechanics (see e.g. [42, Chap. 3], and references cited therein); this part of the energy accounts for short-to-medium range interactions among lipids and/or cholesterol.

A family of energies for  $\varphi^{\text{loc}}$  may be considered, in which the temperature of the environment  $T$  may be assumed as the parameter for such family. This may be provided by the models cited above, among which we recognize a common structure for the energy density of liquid, binary mixtures undergoing order–disorder phase transitions. This is essentially represented by the following form

$$\hat{\varphi}^{\text{loc}}(\chi, \|\nabla \chi\|, J; T) = [\hat{\varphi}_{\text{chem}}^{\text{loc}}(\chi; T) + \gamma(\chi) \|\nabla \chi\|^2] + \hat{\varphi}_{\text{elast}}^{\text{loc}}(\chi, J; T), \quad (31)$$

where the term  $\varphi_{\text{chem}}^{\text{loc}}$  represents the classical enthalpic/entropic energy density for mixtures,  $\varphi_{\text{elast}}^{\text{loc}}$  represents the membranal elastic energy density and the term  $\gamma \|\nabla \chi\|^2$  represents the free energy cost due to concentration gradients.

Without entering the details of the derivation, for the particular cases of saturated lipid/unsaturated lipid mixtures ( $\ell\ell$ ) and lipid/cholesterol mixtures ( $\ell c$ ), accordingly to the work by Komura et al. [36], we take the expressions for the local counterparts of the local energy density derived in (31) and listed in the sequel:

( $\ell\ell$ ) mixtures: here, the purely chemical counterpart of the energy density (31) has the form

$$\varphi_{\text{chem}}^{\text{loc}} = \rho_0 \left[ \psi^0 + \psi_{\text{mix}}^{id} + \psi^e \right].$$

Here  $\rho_0$  represents the molar density per unit of area of the reference configuration, while

$$\psi^0 = \mu_1^0 \chi + \mu_2^0 (1 - \chi)$$

represents the formation energy, being  $\mu_i^0$  the standard chemical potentials of each component and

$$\psi_{\text{mix}}^{id} = RT [\chi \ln \chi + (1 - \chi) \ln(1 - \chi)]$$

represents the classical ideal energy of mixing, with  $R$  a material constant. The so-called excess energy  $\psi^e$  represents a correction to the ideal energy of mixing deriving from different interactions between like and unlike particles in the aggregate. A typical form of such contribution is represented by the Bragg–Williams energy (see e.g. [42, Sect. 3.3] and references cited therein), i.e.:

$$\psi^e = w \chi (1 - \chi)$$

where  $w$  represents the interaction parameter.<sup>4</sup> For the elastic counterpart of the energy density, a Landau expansion with respect to the order parameter  $J$  is assumed by slightly adapting the expression proposed in [36]. In particular, we take the following expression for such free energy:

$$\varphi_{\text{elast}}^{\text{loc}} = \frac{a_2}{2} [T^*(\chi) - T](J - 1)^2 + \frac{a_3}{3} (J - 1)^3 + \frac{a_4}{4} (J - 1)^4$$

where the constants  $a_2 > 0$ ,  $a_3 < 0$ ,  $a_4 > 0$  are experimentally determined (see [36]) and where  $T^*(\chi)$  represents a reference temperature for the order–disorder transition, depending on the pointwise value of the chemical concentration. In agreement with [36], we adopt for  $T^*(\chi)$  a simple linear interpolation between the reference temperatures of both constituents of the mixture, that is

$$T^*(\chi) = \chi T_s^* + (1 - \chi) T_u^*$$

where  $T_s^*$  and  $T_u^*$ , respectively, represent reference temperatures related to the  $L_o$ – $L_d$  transition in pure saturated and pure unsaturated mixtures, respectively. The proposed expression of  $\varphi_{\text{elast}}^{\text{loc}}$  presents a temperature-modulated non-convexity in  $J$ ; this is essential in order to capture the  $L_o$ – $L_d$  transition;

( $\ell c$ ) mixtures: here, the purely chemical counterpart of the energy density has the form

$$\varphi_{\text{chem}}^{\text{loc}} = \rho_0 \left[ \psi^0 + \psi_{\text{mix}}^{\text{FH}} + \psi^e \right].$$

where the term  $\psi^0$  has the same expression given for ( $\ell\ell$ ) mixtures; the mixing energy is derived on the basis of the Flory–Huggins polymer solution theory (see e.g. [42, Sect. 3.5], and references cited therein) and it has the form

$$\psi_{\text{mix}}^{\text{FH}} = RT [\chi_c \ln 2\chi_c + (1 - 2\chi_c) \ln(1 - 2\chi_c)]$$

where  $\chi_c$  represents the mole fraction of cholesterol in the mixture. We refer the interested readers to the article [36] and to the book by Hill [30] for further details on the Flory–Huggins theory and its applications in lipid–cholesterol mixtures. The excess contribution  $\psi^e$  here accounts for the different roles played by cholesterol in ( $\ell c$ ) mixtures toward the order–disorder phase transition; Komura et al. [36], in order to

<sup>4</sup> In classical models  $w$  is understood not to depend upon the measure of the local stretch, although it should. Nevertheless, as a first approximation, here we assume  $w$  as a constant.

phenomenologically describe the experimental evidence that at small concentrations cholesterol favors the disordered  $L_d$  phase, while at higher concentrations it favors the ordered  $L_o$  phase, introduced the following term

$$\psi^c = \frac{1}{J}(\Gamma_1\chi_c - \Gamma_2\chi_c^2),$$

where  $\Gamma_i$  are experimentally determinable constants. The elastic counterpart of the energy has here the same form already discussed for  $(\ell\ell)$  mixtures, but here  $T^*$  is simply the reference temperature of the pure lipid system.

This completes the determination of the local energy density for the two considered cases of binary model membranes. In both cases of  $(\ell\ell)$  and  $(\ell c)$  mixtures, the concentration dependent penalization moduli  $\gamma$  is a constant depending on the mixture constituents and interactions (see for example [45] for more detailed expositions on the argument). Once the local energy term has been determined, Eq. (28) permits the calculation of the bending and non-local moduli  $\kappa_{1,2}$  and  $\alpha$ ; at this point the construction of the energetics (29) of the biological membrane is finally complete.

Let us now calculate for example the expression of the bending and non-local moduli in the case of  $(\ell\ell)$  mixtures. After straightforward calculations, we get that

$$\begin{aligned}\kappa_1^{(\ell\ell)} &= \frac{a_2}{6}[T^*(\chi) - T] + \frac{a_3}{3}(J - 1) + \frac{a_4}{2}(J - 1)^2, \\ \kappa_2^{(\ell\ell)} &= \frac{a_2(J - 1)}{12J}[T^*(\chi) - T] + \frac{a_3(J - 1)^2}{12J} + \frac{a_4(J - 1)^3}{12J}, \\ \alpha^{(\ell\ell)} &= \frac{a_2(J - 1)}{24J^3}[T^*(\chi) - T] + \frac{a_3(J - 1)^2}{24J^3} + \frac{a_4(J - 1)^3}{24J^3}.\end{aligned}\tag{32}$$

It is worth noting that our procedure naturally yields coupling among the independent fields  $\chi$ ,  $J$ ,  $H$  and  $K$  which substantially agree with some heuristic models proposed in the biophysical literature on phase transitions in lipid bilayers.

Indeed, expanding the energy (29) with moduli given by Eq. (32), we see that for the particular case of lipid-lipid mixtures, the following coupling among  $\chi$ ,  $J$ ,  $H$  and  $K$  arises:

$$\varphi_{\text{coupl}}(\chi, J, H, K) = c_0\chi H^2 + g_0(J)H^2 + g_1(J)\chi K + g_2(J)K\tag{33}$$

where  $c_0$  is a constant and  $g_i(J)$  ( $i = 0, 1, 2$ ) are known polynomials of  $J$ . In particular, Ayton et al. [2] adopt in their expression of the energy a coupling term between chemical composition and mean curvature of the form

$$w_{\text{Ayton}}(\chi, H) \approx \chi H^2,$$

which is recovered from (33) by setting  $g_i = 0$ . Chen et al. [11] adopt a coupling term among the chemical composition and the Gaussian curvature of the form

$$w_{\text{Chen}}(\chi, K) \approx \chi K,$$

which is recovered from (33) by setting  $c_0 = g_0 = g_2 = 0$  and tacitly ignoring the dependence on  $J$ . In the previous models, no dependence upon  $T$  is accounted for.

Returning to the discussion on our model, the expressions of the bending moduli derived in (32) for lipid-lipid mixtures offer the possibility of understanding how, even in the special case of null membranal tractions (i.e.  $\partial\varphi^{\text{loc}}/\partial J = 0$ ), the order-disorder transition may have influence on the bending stiffness, in particular on  $\kappa_1$  (since as we have seen, in this case  $\kappa_2 = \alpha = 0$ ). Indeed, it is possible to show [14] that for temperatures

$$T < T^* - \frac{2a_3^2}{9a_2a_4}$$

the energy density  $\varphi^{\text{loc}}$  reaches its absolute minimum at  $J = 1$ , that is in the ordered phase  $L_o$ , where the mean curvature modulus attains the value

$$\kappa_1^{\ell\ell}(L_o) = \frac{a_2}{6}[T^*(\chi) - T].$$

Furthermore, for values of the external temperature

$$T > T^* - \frac{2a_3^2}{9a_2a_4}$$

the absolute minimum is attained at a value of the areal stretch  $J_d > 1$ , that is in the disordered phase  $L_d$ , where

$$\kappa_1^{\ell\ell}(L_d) = \frac{(a_3^2 - 4a_4a_2\tau) - a_3\sqrt{a_3^2 - 4a_4a_2\tau}}{12a_4};$$

for the sake of brevity we have set  $\tau = T^* - T$ . If the externally controlled temperature equals the transition temperature

$$T_{\text{tr}} = T^* - \frac{2a_3^2}{9a_2a_4}$$

then a coexistence of two phases endowed of different bending moduli is expected.

Naively, by picturing an isometric bending of an initially flat square membrane under opposite couples applied along two opposite edges, the non-homogeneity of the bending modulus  $\kappa_1$  will naturally determine the occurrence of phase domains with sensibly different curvatures.

We expect that this kind of behavior can explain the phase separation phenomena in GUVs, where things are indeed more complex because these are curved, closed surfaces, subject to internal pressure. In [14], we will discuss in greater detail the applications of the energetics derived in the present paper and its generalization to the case of non-negligible spontaneous curvature.

As a concluding remark, it is worth noting that since we assumed that the natural configuration of the membrane is flat, we expect that the derived expression of the energy (29) attains a minimum at  $J = 1$ ,  $H = 0$  and  $K = 0$ . As matter of fact, the moduli evaluated at  $J = 1$  read

$$\kappa_1^{(\ell\ell)} = \frac{a_2}{6}[T^*(\chi) - T], \quad \kappa_2^{(\ell\ell)} = \alpha^{(\ell\ell)} = 0$$

where since the reference configuration is here assumed in the ordered phase  $L_o$  ( $J=1$ ) then  $T^* - T > 0$  and thus  $\kappa_1 > 0$ . The energy density (29) evaluated at  $J = 1$  becomes

$$\varphi = \frac{a_2}{6}[T^*(\chi) - T]H^2$$

which indeed has a minimum at  $H = 0$ , hence the flat configuration with  $H = K = 0$  is in particular an absolute minimizer for the surface energy; nevertheless, the fact that variations of  $K$  do not appear in the expression of  $\varphi$  evaluated at  $J = 1$  means that as long as the membrane is not kept in a regime of membranal tension (that is, as long as  $\partial\varphi^{\text{loc}}/\partial J = 0$ ) then all configurations endowed of constant, zero mean curvature are natural configurations for the membrane, i.e. absolute minimizers of the surface energy density (29).

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## Appendix

In this section, we work out the main calculations needed in the article. As first thing let us calculate the expression of the det  $\mathbf{F}$  with  $\mathbf{F}$  deriving from the ansatz (5). As we have seen the current mid-surface of the bilayer is defined as

$$\omega := \{\mathbf{x} = \mathbf{p}(\mathbf{X}), \quad \mathbf{X} \in \Omega \subset \mathbb{R}^2\}.$$

Let  $\mathcal{T}_\omega$  be the tangent plane at a given point of  $\omega$ , and let the local natural basis  $(\mathbf{a}_1, \mathbf{a}_2)$  with  $\mathbf{a}_\alpha \in \mathcal{T}_\omega$  be defined as usual by

$$\mathbf{a}_\alpha := \mathbf{F}\mathbf{E}_\alpha = (\nabla_{\Omega}\mathbf{p})\mathbf{E}_\alpha = \frac{\partial \mathbf{p}}{\partial X_\alpha} =: \mathbf{p},_\alpha$$

and let the reciprocal basis  $(\mathbf{a}^1, \mathbf{a}^2)$  with  $\mathbf{a}^\alpha \in \mathcal{T}_\omega$  be univocally determined by the relation

$$\mathbf{a}_\alpha \cdot \mathbf{a}^\beta = \delta_\alpha^\beta.$$

Hatted vectors here denote spatial descriptions of the material, non-hatted vectors, that is

$$\mathbf{a}_\alpha := \hat{\mathbf{a}}_\alpha \circ \mathbf{p}, \quad \mathbf{a}^\alpha := \hat{\mathbf{a}}^\alpha \circ \mathbf{p}.$$

The material description of the outward unit normal to the current mid-surface  $\omega$  is defined by

$$\mathbf{n} := \frac{\mathbf{a}_1 \times \mathbf{a}_2}{|\mathbf{a}_1 \times \mathbf{a}_2|} = \frac{\mathbf{a}^1 \times \mathbf{a}^2}{|\mathbf{a}^1 \times \mathbf{a}^2|}.$$

The local variation of volume is measured by the determinant of  $\mathbf{F}$ , defined as

$$\det \mathbf{F} := (\mathbf{F}\mathbf{E}_1 \times \mathbf{F}\mathbf{E}_2) \cdot \mathbf{F}\mathbf{E}_3.$$

On the basis of the expression of the 3D deformation gradient deduced in (6), letting the ratio  $h/h_0$  explicit we have that

$$\begin{aligned} \mathbf{F}\mathbf{E}_1 &= \mathbf{a}_1 - X_3 \frac{h}{h_0} \mathbf{L}\mathbf{a}_1 + X_3 \frac{1}{h_0} h_{,1} \mathbf{n}, \\ \mathbf{F}\mathbf{E}_2 &= \mathbf{a}_2 - X_3 \frac{h}{h_0} \mathbf{L}\mathbf{a}_2 + X_3 \frac{1}{h_0} h_{,2} \mathbf{n}, \\ \mathbf{F}\mathbf{E}_3 &= \frac{h}{h_0} \mathbf{n}, \end{aligned}$$

where  $h_{,\alpha} = (\nabla_{\Omega}h) \cdot \mathbf{E}_\alpha$ . Since by definition

$$J = |\mathbf{a}_1 \times \mathbf{a}_2| = [\det(\mathbf{F}^T \mathbf{F})]^{1/2}$$

where  $J$  represents the areal stretch of points of the mid-surface  $\Omega$  under the deformation  $\mathbf{p}$ , after substitution we obtain

$$\begin{aligned} \det \mathbf{F} &= \frac{h}{h_0} (\mathbf{a}_1 \times \mathbf{a}_2) \cdot \mathbf{n} - X_3 \frac{h^2}{h_0^2} (\mathbf{a}_1 \times \mathbf{L}\mathbf{a}_2 + \mathbf{L}\mathbf{a}_1 \times \mathbf{a}_2) \cdot \mathbf{n} \\ &\quad + X_3^2 \frac{h^3}{h_0^3} (\mathbf{L}\mathbf{a}_1 \times \mathbf{L}\mathbf{a}_2) \cdot \mathbf{n}. \end{aligned}$$

At this point, from the identity

$$\mathbf{n} \times \mathbf{a}_\alpha = \varepsilon_{\alpha\beta} \mathbf{a}^\beta$$

with  $\varepsilon_{\alpha\beta}$  equal to  $(1, -1)$ , respectively, if  $\alpha\beta = (12, 21)$  and equal to 0 otherwise, we get that

$$(\mathbf{a}_1 \times \mathbf{L}\mathbf{a}_2) \cdot \mathbf{n} + (\mathbf{L}\mathbf{a}_1 \times \mathbf{a}_2) \cdot \mathbf{n} = \mathbf{L} \cdot (\mathbf{a}^\alpha \otimes \mathbf{a}_\alpha) \equiv \mathbf{L} \cdot \mathbb{P}_n = 2H,$$

where  $H = \hat{H} \circ \mathbf{p}$  represents the material description of the local mean curvature of the current mid-surface  $\omega$ . Furthermore, reminding that  $\mathbf{L} \equiv \mathbb{P}_n \mathbf{L} \mathbb{P}_n$  and since  $\forall \mathbf{v} \in \mathcal{T}_\omega$  then  $\mathbf{L}\mathbf{v} \perp \mathbf{n}$ , we can express the Gaussian curvature as

$$K = \det \mathbf{L} := \frac{|\mathbf{L}\mathbf{a}_1 \times \mathbf{L}\mathbf{a}_2|}{|\mathbf{a}_1 \times \mathbf{a}_2|} = \frac{(\mathbf{L}\mathbf{a}_1 \times \mathbf{L}\mathbf{a}_2) \cdot \mathbf{n}}{(\mathbf{a}_1 \times \mathbf{a}_2) \cdot \mathbf{n}} = \frac{(\mathbf{L}\mathbf{a}_1 \times \mathbf{L}\mathbf{a}_2) \cdot \mathbf{n}}{J}$$

being  $K = \hat{K} \circ \mathbf{p}$  the Gaussian curvature of the surface  $\omega$ , expressed in material form. Hence we finally get

$$\det \mathbf{F} = \frac{h}{h_0} \left[ J - 2X_3 \frac{h}{h_0} H + X_3^2 \frac{h^2}{h_0^2} J K \right].$$

Let us now calculate the derivatives of  $\delta$  and  $\Delta$  with respect to  $h_0$ . As first thing we set  $X_3 = h_0 Z_3$  and secondly, for the sake of brevity in the following calculations, let us set

$$a = \phi J, \quad b = -2Z_3 \phi^2 H, \quad c = Z_3^2 J \phi^3 K$$

so that

$$\det \mathbf{F} = a + b h_0 + c h_0^2.$$

It then results:

1.  $\Delta = \det \mathbf{C} = (\det \mathbf{F})^2 = (a + b h_0 + c h_0^2)^2$ , thus  $\Delta(0) = a^2 = \phi^2 J^2$ ;
2.  $\dot{\Delta} = 2(ab + 2h_0 ac + b^2 h_0 + 3bch_0^2 + 2c^2 h_0^3)$ , thus  $\dot{\Delta}(0) = 2ab = -4Z_3 \phi^3 J H$ ;
3.  $\ddot{\Delta} = 2(2ac + b^2 + 6bch_0 + 6c^2 h_0^2)$ , thus  $\ddot{\Delta}(0) = 4ac + 2b^2 = 4Z_3^2 J^2 \phi^4 K + 8Z_3^2 \phi^4 H^2$ ;
4.  $\dot{\mathbf{C}} = Z_3 \mathbf{C}_1 + 2h_0 Z_3^2 \mathbf{C}_2$ , thus  $\dot{\mathbf{C}}(0) = -2Z_3 \phi \mathbf{F}^T \mathbf{L} \mathbf{F}$ ;
5.  $\ddot{\mathbf{C}} = 2Z_3^2 \mathbf{C}_2$ , thus  $\ddot{\mathbf{C}}(0) = 2Z_3^2 (\nabla_{\Omega} \phi \otimes \nabla_{\Omega} \phi + \phi^2 \mathbf{F}^T \mathbf{L}^2 \mathbf{F})$ ;
6.  $\delta(0) = \det \mathbf{G} = J^2$ ;
7.  $\dot{\delta} = (\det \mathbf{C}) \mathbf{C}^{-1} \cdot \dot{\mathbf{C}}$ , thus  $\dot{\delta}(0) = (\det \mathbf{G}) \mathbf{G}^{-1} \cdot (-2Z_3 \phi \mathbf{F}^T \mathbf{L} \mathbf{F}) = -4Z_3 J^2 \phi H$ ;
8.  $\ddot{\delta} = (\det \mathbf{C}) [(\mathbf{C}^{-1} \cdot \dot{\mathbf{C}})^2 - (\mathbf{C}^{-1} \dot{\mathbf{C}} \cdot \mathbf{C}^{-1} \dot{\mathbf{C}}) + \mathbf{C}^{-1} \cdot \ddot{\mathbf{C}}]$ , where we used  $\mathbf{C}^{-1} \mathbf{C} = \mathbb{P}_3$ ; at this point, since
  - i.  $(\mathbf{C}^{-1} \cdot \dot{\mathbf{C}})^2|_{h_0=0} = 16Z_3^2 \phi^2 H^2$ ;
  - ii.  $(\mathbf{C}^{-1} \dot{\mathbf{C}} \cdot \mathbf{C}^{-1} \dot{\mathbf{C}})|_{h_0=0} = 4Z_3^2 \phi^2 (4H^2 - 2K)$ ;
  - iii.  $(\mathbf{C}^{-1} \cdot \ddot{\mathbf{C}})|_{h_0=0} = 2Z_3^2 (\phi^2 (4H^2 - 2K) + \|\text{grad}_{\omega} \hat{\phi}\|_m^2)$ , where  $\phi = \hat{\phi} \circ \mathbf{p}$ .

We then deduce that

$$\ddot{\delta}(0) = Z_3^2 \left( 8\phi^2 J^2 H^2 + 4\phi^2 J^2 K + 2J^2 \|\text{grad}_{\omega} \hat{\phi}\|_m^2 \right).$$

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