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Revisiting the curvature-mediated interactions between proteins in biological membranes

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Proteins embedded in soft biological membranes experience a long-range force mediated by elastic curvature deformations. The classical linearized Helfrich–Canham Hamiltonian based derivations reveal the nature of the force between a pair of proteins to be repulsive in the zero-temperature limit and the interaction potential is inversely proportional to the fourth power of the distance separating the inclusions. Such a result is the starting point to understand many-body interactions between proteins in biological membranes and the study of their clustering or, more broadly, self-organization. A key observation regarding this widely quoted result is that any two (mechanically rigid) proteins will experience an identical force. In other words, there is no specificity in the currently employed continuum models that purport to explain protein interactions. In this work we argue that each protein has a unique mechanical signature based on its interaction with the surrounding lipid bilayer membrane and cannot be treated as a non-specific rigid object. We modify the classical Helfrich–Canham theory of curvature elasticity to incorporate protein–membrane specificity, discuss the estimation of the new model parameters *via* atomistic simulations and re-evaluate the curvature-mediated force between proteins. We find that the incorporation of protein-specificity can reduce the interaction force by several orders of magnitude. Our result may provide at least one plausible reason behind why in some computational and experimental studies, a net attractive force between proteins is in evidence.

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1 Introduction

Cell membranes are often regarded as passive or inert spectators to various biological processes that are governed by proteins. There is however compelling evidence from a multitude of works that appear to suggest that the mechanical deformation of membranes controls the long-range forces between proteins.^{1–26} Most proteins are often thought to be elastically “rigid”. Accordingly an embedded protein in a soft biological membrane causes significant mechanical deformation in its vicinity which is “felt” by the neighboring proteins. Thus, the membrane plays an essential role in communicating the force between proteins over (comparatively speaking) fairly long distances. Protein clustering, or in general, spatial organization of proteins, is one such consequence of this mechanical interaction;^{4,10,27–30} cooperative behavior of mechanosensitive ion channels is yet another consequence.^{31–36}

The two key mechanisms that lead to membrane-mediated forces between proteins are: (i) membrane thickness deformation

due to hydrophobic mismatch, and (ii) curvature deformation. Extensive work has been carried out on both topics and the present work is focussed on revisiting the currently accepted knowledge-base for curvature-mediated force between proteins. Interested readers may refer to the following articles for further details on the hydrophobic mismatch problem.^{18–26} In particular, two recent articles by Deserno *et al.* and Deserno provide a very thoughtful review of this subject.^{16,17}

Both membranes and proteins are microscopically quite complex. However, the mechanical behavior of membranes can be fairly well described by the (thermodynamically based) phenomenological theory of elasticity. This so-called Helfrich–Canham curvature elasticity theory can be expressed mathematically as:^{37–41}

$$U_{\text{HC}} = \int_{\text{U}} 2\kappa_{\text{b}}(H - H_0)^2 + \kappa_{\text{g}}(K - K_0). \quad (1)$$

Here κ_{b} and κ_{g} are the bending moduli that, respectively, parametrize the energy change due to changes in the mean (H) and Gaussian (K) curvatures. The corresponding spontaneous curvatures are denoted by H_0 and K_0 .[†] The elastic energy scale is set by the bending modulus and surface tension. Their typical

[†] We have ignored surface tension in this expression and throughout the paper as it is not central to the main objective of this work.

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values are such that membranes are usually hard to stretch but bend (curve) quite easily.^{42,43} Furthermore, the embedded proteins are (nearly always) regarded as mechanically rigid.

Despite the apparent simplicity of the Helfrich–Canham Hamiltonian in eqn (1), the elastic solution of even a single rigid protein embedded in a membrane is difficult although obtainable under some simplifying conditions.⁴ An exact solution to the interaction force between two inclusions is unknown even in the linearized case let alone for the fully nonlinear problem. Predicated in eqn (1), numerous works have attempted to elucidate the force between two membrane proteins using a variety of methods and approximations.^{1–3,5,11–15} A fairly recent and comprehensive review of the literature as well as the subtleties of this topic may be found in two articles by Deserno and Deserno *et al.*^{16,17} In particular, we would like to here cite some very clever expositions by Deserno and co-workers where, using a covariant differential geometric formulation,^{44,45} they have even been able to obtain information on the effect of nonlinearity on the protein interactions in certain asymptotic limits.

The currently prevalent viewpoint, starting from the initial work by Goulian *et al.*² and from the perspective of the linearized version of eqn (1), is that two rigid proteins repel each other with an interaction potential inversely proportional to the fourth power of the distance (R) separating the inclusions: $\sim \frac{1}{R^4}$. Aside from corrections to the pre-factor of this repulsive interaction, the qualitative nature of this result has been derived and verified extensively in the literature.^{1–3,5,11,12} The interaction force or potential between two inclusions can be used as a starting point for numerical calculations for self-assembly and related problems as for example exploited by researchers such as Fournier, Oster and others.^{3,4,10}

Based on both numerical (atomistic) simulations and at least in one case, some experimental observations,⁴⁶ it is not clear whether we can even fully believe the sign of the force between proteins. While most works appear to confirm a repulsive ground-state force, some authors have argued that it is much less repulsive than what is believed or even suggested that it may be attractive – see, for example, a detailed discussion in the following review articles.^{16,17} How might proteins cluster together if the force is purely repulsive? One possible reason is that curvature-mediated interaction cannot be added pairwise.^{4,47} When multi-body interactions are appropriately accounted for, screening of the repulsive force occurs and the effective repulsive force may be reduced. Together with the weak thermal-fluctuation induced attraction,^{48,49} self-organization can occur.²⁷

While this topic is likely to remain an active area of research for the near future and perhaps can only be elucidated by a strong combination of experiments and detailed atomistic simulations, in this work we attempt to modify the continuum model to improve one aspect that is pertinent to the problem.

‡ Throughout this work, we will refer to only the ground-state *i.e.* zero-temperature limit of the force between proteins. Thermal fluctuations may induce a Casimir-like attractive force^{48,49} also as described in some recent works.²⁷ This aspect is not central to the main point of our work however we will revisit it in Section 6.

A key assumption in the various continuum derivations of the repulsive force presented in the literature so far is that the proteins are mechanically rigid and inert entities that do not interact chemically with the surrounding membrane. In other words, one particular mechanically rigid protein is no different than another one. Indeed, the solutions to this problem that currently exist in the literature lack specificity and any two proteins (no matter how different they may be) interact with the same force as long as the bending modulus of the membrane is the same. We believe that treating proteins merely as rigid objects is flawed and that each protein has a unique mechanical signature based on its coupling with the surrounding lipid membrane. In this work, we present a theoretical framework – essentially a modified version of the classical Helfrich–Canham theory – that introduces some specificity to protein–membrane interactions and thus provides an approach to compute a specific force between two proteins. In particular, we show that the phenomenological parameters of our new model can be obtained readily through atomistic simulations. Our results appear to provide a strong plausibility basis for the possibility of a dramatic reduction in the repulsive force between proteins.

The outline of this paper is as follows: we discuss the central idea in Section 2 and present a modified version of Helfrich–Canham theory that adds protein–membrane specificity. Using the developed theory, we present the solution to the interaction force between two proteins in Section 3. After a brief discussion, in Section 4, of how atomistic simulations may be used to obtain the phenomenological parameters of our theory, we present numerical results in Section 5. Our results appear to provide a different perspective on how the ground-state force between proteins may be modulated by membranes and this, along with other insights, is discussed in Section 6 where we also conclude this work.

2 Central idea and theoretical formulation

Let $\mathbb{U} \subset \mathbb{R}^2$ be an open bounded domain in the xy -plane. Consider a thin fluid membrane occupying $\mathbb{U} \times (-h/2, h/2) \subset \mathbb{R}^3$, where h is the thickness of the membrane. If the thickness h is much less than the lateral area of the membrane, then the membrane may be idealized as a two-dimensional body; and the thermodynamic state is described by the out-of-plane displacement $w: \mathbb{U} \rightarrow \mathbb{R}$. At the outset we work in the Monge gauge and linearized setting. As introduced in the previous section, the Helfrich–Canham elastic energy of the membrane in the absence of lateral tension is given by:[§]

$$U_{\text{HC}}[w] = \int_{\mathbb{U}} \left[\frac{\kappa_{\text{b}}}{2} |\Delta w|^2 + \kappa_{\text{g}} \det(\nabla \nabla w) \right]. \quad (2)$$

We note here that $2H \sim \Delta w$ and $K \sim \det(\nabla \nabla w)$. Also, for the physically relevant case of rigid inclusions, the energy term related to the Gaussian modulus is ignored for simplicity in

§ We have ignored the spontaneous curvature here for our derivation. However, it can be added easily if necessary.

the remainder of the work.⁴ Future generalization of the work to the finite elastic modulus must account for this.¶

We propose that at the molecular scale, the insertion of a protein in the membrane involves several chemical and physical processes. First, the insertion of a protein breaks the original in-plane lipid–lipid interactions and protein–water molecule (ambient medium) bonds and forms new bonds between protein and lipids. This causes the structural rearrangement of lipids around the protein. In other words, we expect each protein to have a unique interfacial coupling to the surrounding lipid bilayer. To account for protein specificity, we phenomenologically incorporate this interfacial coupling into the classical Helfrich model. To achieve this, we introduce a jump of displacement and rotation angles in the continuum model. Let the jump of a quantity (\cdot) across $\partial\Omega$ ($\partial\Omega$ is the boundary between protein and lipids as shown in Fig. 1) be denoted by $[[(\cdot)]] := (\cdot)|_{\partial\Omega^+} - (\cdot)|_{\partial\Omega^-}$. We remark that the jump in the rotation angle has also been discussed previously (in a different context) by Baumgart *et al.*⁵⁰ So the jump in the displacement and the rotation angle can be denoted as $[[w]]$ and $[[\mathbf{n} \cdot \nabla w]]$, respectively. Here \mathbf{n} is the outward normal to the domain $\mathbb{U} \setminus \Omega$. We further propose that these jumps cost energy and the energy due to these jumps can be approximated as follows:

$$U_{\text{jump}}[w] = \int_{\partial\Omega} \left[\frac{1}{2} k_1 [[w]]^2 + \frac{1}{2} k_2 [[\mathbf{n} \cdot \nabla w]]^2 \right] \quad (3)$$

where k_1 and k_2 are phenomenological parameters like the bending stiffness κ_b , and can be obtained from experiments or atomistic simulations. Summing up the effects of lipid protein interaction, we propose the total elastic energy of the protein–bilipid membrane system as follows:

$$U_e[w] = \int_{\mathbb{U}} \left[\frac{\kappa_b^{(x)}}{2} |\Delta w|^2 \right] + \int_{\partial\Omega} \left[\frac{1}{2} k_1 [[w]]^2 + \frac{1}{2} k_2 [[\mathbf{n} \cdot \nabla w]]^2 \right] \quad (4)$$

where $\kappa_b^{(0)}$ is the bending modulus of the membrane and $\kappa_b^{(1)}$ is the bending modulus of the protein. In short, in the proposed energetic model, the newly introduced interfacial parameters, (k_1, k_2) , phenomenologically account for the protein–lipid membrane interaction and specificity. In some sense, the interfacial jumps represent a more sophisticated way of incorporating a constant interface energy.¶

¶ We remark here that, as clearly highlighted in past papers *e.g.* Baumgart *et al.*,⁵⁰ even if there is no change in the topology, the Gaussian curvature cannot be ignored if boundaries are present. However, there is indeed no exception to this insight – when the proteins are rigid, then the Gaussian curvature energy becomes irrelevant. Had we considered proteins of a finite bending modulus, then we would have to consider the contribution of the Gaussian curvature also. We have avoided this by making (a reasonable) assumption that most proteins are mechanically rigid compared to the surrounding lipid bilayer. However our derivation will be the full general case and we will only make the simplifying assumption to obtain results. The issue of neglecting the Gaussian curvature in the case of rigid proteins was discussed in detail in the paper of Kim *et al.*⁴

¶ We emphasize here that a finite thickness layer of material with some intermediate properties could be used however then what thickness should be chosen? Our current approach draws a connectivity with interfacial or surface energy and assumes a zero thickness interface. The requisite physics is then embodied in the boundary conditions. Having said all this, there are more ways than one to achieve the same result, introduction of protein–lipid specificity *via* some type of interfacial coupling.

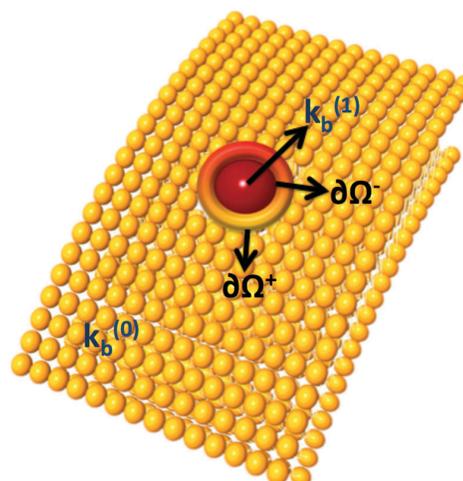


Fig. 1 The thin interface between the peptide and the lipid is shown as a gradient of red and yellow. When a protein is inserted in a membrane, there is a structural rearrangement of lipids around the peptide. Old interactions between some lipids are lost and new interactions between the lipids surrounding the peptide and the peptide are formed. This leads to the creation of a thin interface which has the hybrid properties of lipids and proteins. $\kappa_b^{(0)}$ and $\kappa_b^{(1)}$ are the corresponding bending modulus of the lipid membrane and protein. The interface between the membrane and the protein is represented as $\partial\Omega$.

Moreover, the classic Helfrich–Canham model that does not allow the discontinuity of displacement w and rotation angle $\mathbf{n} \cdot \nabla w$ can be regarded as the asymptotic limit of the generalized model (4) at $k_1, k_2 \rightarrow +\infty$.

To mimic a uniformly bent homogeneous membrane we impose the boundary conditions:

$$w = 0, \quad \mathbf{n} \cdot \nabla w = H^{\text{ext}} \mathbf{x} \cdot \mathbf{n} \quad \text{on } \partial\mathbb{U}, \quad (5)$$

where H^{ext} can be regarded as the macroscopic curvature of the membrane and \mathbf{x} is the position vector on the outer boundary of the membrane. By the principle of minimum free energy, the equilibrium state of the membrane is determined by the variational principle:

$$\min\{U_e[w]; w \text{ satisfies (5)}\}. \quad (6)$$

We now calculate the Euler–Lagrange equations and boundary conditions associated with the above variational principle (6). Let w satisfying (5) be a minimizer. Then for any perturbation $w \rightarrow w \pm \varepsilon w_1$ we have ($0 < \varepsilon \ll 1$)

$$U_e[w \pm \varepsilon w_1] \geq U_e[w], \quad (7)$$

which implies

$$\frac{d}{d\varepsilon} U_e[w + \varepsilon w_1] \Big|_{\varepsilon=0} = 0. \quad (8)$$

Upon integration by parts, the above equation can be rewritten as

$$\int_{\mathbb{U}} w_1 \Delta \left[\kappa_b^{(x)} (\Delta w) \right] + T_1^- + T_1^+ + T_2^- + T_2^+ = 0, \quad (9)$$

where T_1^-, T_2^- (T_1^+, T_2^+) are integrals over the interior interface $\partial\Omega^-$ (exterior interface $\partial\Omega^+$) and given by

$$\begin{aligned} T_1^- &= \int_{\partial\Omega^-} \left\{ -\mathbf{n} \cdot \nabla \left[\kappa_b^{(x)}(\Delta w) \right] - k_1 \llbracket w \rrbracket \right\} w_1, \\ T_2^- &= \int_{\partial\Omega^-} (\mathbf{n} \cdot \nabla w_1) \left[\kappa_b^{(x)}(\Delta w) - k_2 \llbracket \mathbf{n} \cdot \nabla w \rrbracket \right], \\ T_1^+ &= \int_{\partial\Omega^+} \left\{ \mathbf{n} \cdot \nabla \left[\kappa_b^{(x)}(\Delta w) \right] + k_1 \llbracket w \rrbracket \right\} w_1, \\ T_2^+ &= \int_{\partial\Omega^+} -(\mathbf{n} \cdot \nabla w_1) \left[\kappa_b^{(x)}(\Delta w) - k_2 \llbracket \mathbf{n} \cdot \nabla w \rrbracket \right]. \end{aligned} \quad (10)$$

Since w_1 and $\mathbf{n} \cdot \nabla w_1$ in $\partial\Omega^-$ and $\partial\Omega^+$ can be specified independently, eqn (9)–(10) imply the following Euler–Lagrange equations and boundary conditions associated with (6):

$$\begin{cases} \Delta[\kappa_b^{(x)}(\Delta w)] = 0 & \text{on } \mathbb{U} \setminus \partial\Omega, \\ \mathbf{n} \cdot \nabla \left[\kappa_b^{(x)}(\Delta w) \right] + k_1 \llbracket w \rrbracket = 0 & \text{on } \partial\Omega^-, \\ \kappa_b^{(x)}(\Delta w) - k_2 \llbracket \mathbf{n} \cdot \nabla w \rrbracket = 0 & \text{on } \partial\Omega^-, \\ \mathbf{n} \cdot \nabla \left[\kappa_b^{(x)}(\Delta w) \right] + k_1 \llbracket w \rrbracket = 0 & \text{on } \partial\Omega^+, \\ \kappa_b^{(x)}(\Delta w) - k_2 \llbracket \mathbf{n} \cdot \nabla w \rrbracket = 0 & \text{on } \partial\Omega^+. \end{cases} \quad (11)$$

In the case of rigid proteins, we will study the asymptotic behavior as $\kappa_b^{(1)} \rightarrow +\infty$. In this limit, using (11) the boundary value problem implied by (6) can be written as:

$$\begin{cases} w = h + \beta x + \beta' y & \text{on } \Omega, \\ \Delta\Delta w = 0 & \text{on } \mathbb{U} \setminus \Omega, \\ \kappa_b^{(0)} \mathbf{n} \cdot (\nabla\Delta w) + k_1 \llbracket w \rrbracket = 0 & \text{on } \partial\Omega^+, \\ \kappa_b^{(0)} \Delta w - k_2 \llbracket \mathbf{n} \cdot \nabla w \rrbracket = 0 & \text{on } \partial\Omega^+, \\ w = 0, \quad \mathbf{n} \cdot \nabla w = H^{\text{ext}} \mathbf{x} \cdot \mathbf{n} & \text{on } \partial\mathbb{U}, \end{cases} \quad (12)$$

where the first equation follows from the rigid motion of the protein, $h \in \mathbb{R}$ describes the translation, and β (resp. β') is the (infinitesimal) tilt angle around the y (resp. x)-axis.

3 Interaction energy and force between two proteins embedded in a membrane

As discussed earlier, the insertion of a protein causes the structural rearrangement of lipids around the protein. The effects of this thin re-arranged layer of lipids may be captured by penalizing the jump of the displacement and the rotation angle in a continuum model. The total elastic energy of the membrane with two proteins (as shown in Fig. 2) is given

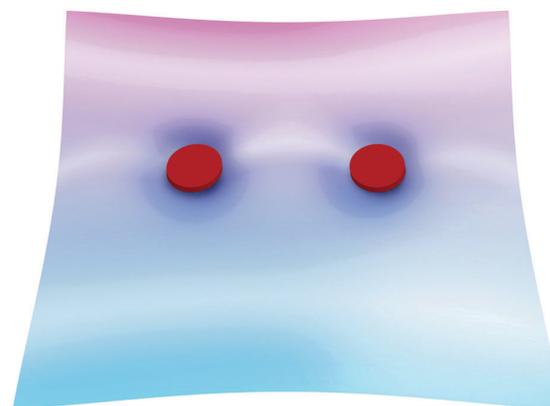


Fig. 2 The mid-plane of a membrane–protein system, in which two proteins are embedded in a membrane. Note that the deformation lines around the two proteins are identical and as the distance from the membrane tends to infinity, the membrane becomes flat, which is one of our boundary conditions.

by (cf. (4))

$$\begin{aligned} U_e[w] &= \int_{\mathbb{R}^2 \setminus (\Omega_1 \cup \Omega_2)} \frac{\kappa_b^{(0)}}{2} |\Delta w|^2 \\ &+ \sum_{p=1,2} \int_{\partial\Omega_p} \left[\frac{1}{2} k_1 \llbracket w \rrbracket^2 + \frac{1}{2} k_2 \llbracket \mathbf{n} \cdot \nabla w \rrbracket^2 \right]. \end{aligned} \quad (13)$$

where p labels the first or second protein, and for simplicity, Ω_p ($p = 1, 2$) are assumed to be circular of radius a . By symmetry, we expect that two proteins would tilt in the same direction. On account of the spontaneous curvature of proteins, we specify the boundary displacement as (cf. (12)₁)

$$\begin{cases} w = h_p + a\beta_p \cos \phi_p \\ \mathbf{n} \cdot \nabla w = \alpha_p \beta_p \cos \phi_p \end{cases} \quad \text{on } \partial\Omega_p^-, \quad (p = 1, 2). \quad (14)$$

where ϕ_p denotes the polar angle with respect to the centre of inclusion p (see Fig. 3), h_p describes the out-of-plane translation, α_p is the given contact angle between the protein and the membrane that arises from the spontaneous curvature, and β_p is the tilt angle of the protein to be determined. We are interested

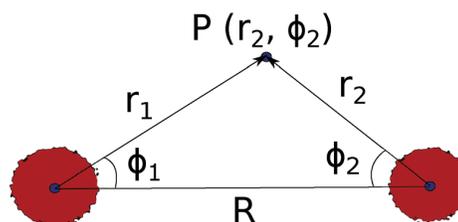


Fig. 3 As outlined in the work of Weikl *et al.*,¹ we convert the polar coordinates of first inclusion (r_1, ϕ_1) in terms of that of the second inclusion (r_2, ϕ_2) ; the relationship between the two polar coordinates is depicted in this figure. Here R is the center to center distance between the two proteins.

in the interaction energy and force between the two proteins and how they depend on separation distance R and contact angles α_p ($p = 1, 2$). To this end, we consider the variational problem:

$$U_e^* = \min\{U_e[w]; w \text{ satisfies (14) and } |\nabla\nabla w| \rightarrow 0 \text{ as } |\mathbf{x}| \rightarrow \infty\}. \quad (15)$$

Using (12) and (14), we obtain the associated boundary value problem for the membrane with two interacting proteins (Fig. 2) as:

$$\begin{cases} \Delta\Delta w = 0 & \text{on } \mathbb{R}^2 \setminus (\Omega_1 \cup \Omega_2), \\ \kappa_b^{(0)} \mathbf{n} \cdot (\nabla\Delta w) + k_1 \left(w - (h_p + a\beta_p \cos\phi_p) \right) = 0 & \text{on } \partial\Omega_p^+, \\ \kappa_b^{(0)} \Delta w - k_2 \left(\mathbf{n} \cdot \nabla w - \alpha_p - \beta_p \cos\phi_p \right) = 0 & \text{on } \partial\Omega_p^+, \\ |\nabla\nabla w(\mathbf{x})| \rightarrow 0 & \text{as } |\mathbf{x}| \rightarrow +\infty. \end{cases} \quad (16)$$

Explicit series solutions to (16) can be achieved by the method of multipole expansions. Following the solution strategy laid out by Weigl *et al.*,¹ we first find the general form of solution for a single inclusion, and denote by w_1 (resp. w_2) the solution induced by the first (resp. second) inclusion. For brevity, we establish two polar coordinate systems (r_p, ϕ_p) as illustrated in Fig. 3. By the separation of variables, we can write the general solution to $\Delta^2 w_p = 0$ in $\mathbb{R}^2 \setminus \Omega_p$ ($p = 1, 2$) as:

$$\begin{aligned} w_p(r_p, \phi_p) &= c_{p0} \ln \frac{r_p}{a} + c_{p1} r_p \cos\phi_p \\ &+ c_{p2} r_p \ln r_p \cos\phi_p + c_{p3} \cos 2\phi_p \\ &+ \frac{c_{p4} \cos(\phi_p)}{r_p} + \frac{c_{p5} \cos(2\phi_p)}{r_p^2} + \dots \end{aligned} \quad (17)$$

We then consider the superposition of solutions induced by two proteins:

$$w = w_2(r_2, \phi_2) + w_1(r_1, \phi_1) \quad (18)$$

as a trial solution to (16). Upon applying the variational principle (15) or the boundary conditions (16)_{2,3}, we can determine unknown constants c_{pi} in (17) and obtain physical quantities including the interaction energy and force between proteins.

For explicit approximate solutions, we truncate the expansion (17) at $O(1/r_p^2)$ and evaluate the total elastic energy of the system in terms of unknown coefficients c_{pi} in (17), tilt angles β_p and displacements h_p in (14). For asymptotically flat membrane $\mathbf{n} \cdot \nabla w \rightarrow 0$ for $r_p \rightarrow +\infty$, we infer that $c_{p2} = 0$ for $p = 1, 2$ and that $c_{11} + c_{21} = 0$. Also, it is easy to check that $r_1 \cos\phi_1 - r_2 \cos\phi_2 = R$ is constant on the entire plane \mathbb{R}^2 . Therefore, without the loss of generality, we set $c_{p1} = 0$ for $p = 1, 2$. It will be useful to rewrite w_1 in terms of r_2 and ϕ_2 for $r_2 < R$, which can be achieved by Taylor expansions in terms

of the small parameter $\varepsilon = r_2/R$ (see Fig. 3):

$$r_1 = \sqrt{R^2 + r_2^2 - 2Rr_2 \cos\phi_2} \quad (19)$$

and

$$\cos\phi_1 = \frac{R - r_2 \cos\phi_2}{\sqrt{R^2 + r_2^2 - 2Rr_2 \cos\phi_2}} \quad (20)$$

Therefore, using (17), (20)–(19) we can write w_1 as a function of (r_2, ϕ_2) as

$$\begin{aligned} w_1(r_2, \phi_2) &\approx c_{10} \left(\log\left(\frac{R}{a}\right) + \frac{r_2^3 \cos(\phi_2)}{R^3} - \frac{r_2^2 \cos(2\phi_2)}{2R^2} - \frac{r_2 \cos(\phi_2)}{R} \right) \\ &+ c_{14} \left(\frac{r_2^2 \cos(2\phi_2)}{R^3} + \frac{r_2 \cos(\phi_2)}{R^2} + \frac{1}{R} \right) \\ &+ c_{13} \left(-\frac{4r_2^2 \cos(\phi_2)}{R^3} - \frac{r_2^2(1 - \cos(2\phi_2))}{R^2} + 1 \right) + \frac{c_{15}}{R^2}. \end{aligned} \quad (21)$$

Moreover, we recall that

$$w_2(r_2, \phi_2) = c_{20} \log\left(\frac{r_2}{a}\right) + c_{23} \cos(2\phi_2) + \frac{c_{24} \cos(\phi_2)}{r_2} + \frac{c_{25} \cos(2\phi_2)}{r_2^2}. \quad (22)$$

We now proceed to calculate the energy of the trial solution (18). By the divergence theorem, the bending energy of the membrane can be written as

$$\frac{\kappa_b^{(0)}}{2} \int_{\mathbb{R}^2 \setminus (\Omega_1 \cup \Omega_2)} |\Delta w|^2 dA = S + G,$$

where

$$\begin{aligned} S &= \frac{\kappa_b^{(0)}}{2} \int_{\mathbb{R}^2 \setminus \Omega_1} |\Delta w_1|^2 dA + \frac{\kappa_b^{(0)}}{2} \int_{\mathbb{R}^2 \setminus \Omega_2} |\Delta w_2|^2 dA, \\ G &= -\frac{\kappa_b^{(0)}}{2} \int_{\Omega_2} |\Delta w_1|^2 dA - \frac{\kappa_b^{(0)}}{2} \int_{\Omega_1} |\Delta w_2|^2 dA \\ &+ \frac{\kappa_b^{(0)}}{2} \int_{\mathbb{R}^2 \setminus (\Omega_1 \cup \Omega_2)} 2\Delta w_1 \Delta w_2 dA. \end{aligned} \quad (23)$$

Physically, we recognize that S is the self-energy of the membrane (independent of R) and G is the membrane mediated interaction energy of the two proteins (function of R). Since

$$\Delta w_p = -\frac{4c_{p3} \cos(2\phi_p)}{r_p^2} \quad \text{on } \mathbb{R}^2 \setminus \Omega_p, \quad (24)$$

integrating (23)₁ we obtain

$$S = \frac{4\pi c_{13}^2 \kappa_b^{(0)}}{a^2} + \frac{4\pi c_{23}^2 \kappa_b^{(0)}}{a^2}. \quad (25)$$

The first two integrals of G in (23)₂ can be directly evaluated. Using (21), we find that

$$\begin{cases} \Delta w_1 \approx -4c_{13}/R^2 & \text{on } \Omega_2, \\ \frac{\partial}{\partial r_2} \Delta w_1 \approx \frac{8c_{13}(r_2 - R \cos\phi_2)}{R^4} & \text{on } \Omega_2, \end{cases} \quad (26)$$

and hence,

$$\frac{\kappa_b^{(0)}}{2} \int_{\Omega_2} |\Delta w_1|^2 dA \approx \frac{8\pi a^2 c_{13}^2 \kappa_b^{(0)}}{R^4}. \quad (27)$$

By the divergence theorem, we rewrite the third integral of G as:

$$\begin{aligned} \int_{\mathbb{R}^2/(\Omega_1 \cup \Omega_2)} \Delta w_1 \Delta w_2 dA &= - \int_{\partial\Omega_2} [w_{1,ii} w_{2,j} n_j - w_{1,ij} w_{2j} n_j] ds \\ &\quad - \int_{\partial\Omega_1} [w_{1,ii} w_{2,j} n_j - w_{1,ij} w_{2j} n_j] ds \\ &= \frac{8\pi c_{20} c_{13}}{R^2} + \frac{8\pi c_{10} c_{23}}{R^2}, \end{aligned} \quad (28)$$

where n_j is the outward normal on $\partial\Omega_p$ and the last equality follows from (21) and (26) and the symmetry. In conclusion, we have

$$G = -\frac{8\pi a^2 c_{13}^2 \kappa_b^{(0)}}{R^4} - \frac{8\pi a^2 c_{23}^2 \kappa_b^{(0)}}{R^4} + \frac{8\pi c_{20} c_{13} \kappa_b^{(0)}}{R^2} + \frac{8\pi c_{10} c_{23} \kappa_b^{(0)}}{R^2}. \quad (29)$$

The energy (3) due to the jumps in rotation angles and displacements has contributions from the first or second inclusion, which, using (14), can be written as

$$\begin{aligned} U_{\text{jmp}}^{(p)} &= \int_{\partial\Omega_p} \left[\frac{1}{2} k_2 (\mathbf{n} \cdot \nabla w - \alpha_p - \beta_p \cos \phi_p)^2 \right. \\ &\quad \left. + \frac{1}{2} k_1 (w - h_p - a\beta_p \cos \phi_p)^2 \right]. \end{aligned} \quad (30)$$

First focusing on the second inclusion, by (16)₃ we have

$$\begin{aligned} \int_{\partial\Omega_2} \frac{1}{2} k_2 (\mathbf{n} \cdot \nabla w - \alpha_2 - \beta_2 \cos \phi_2)^2 \\ = \frac{a\kappa_b^{(0)2}}{2k_2} \int_0^{2\pi} |\Delta w|_{r_2-a}^2 d\phi_2 \\ \approx \frac{a\kappa_b^{(0)2}}{2k_2} \int_0^{2\pi} \left| \frac{4c_{23} \cos(2\phi_2)}{a^2} - \frac{4c_{13}}{R^2} \right|^2 d\phi_2, \end{aligned} \quad (31)$$

where the last equality follows from (24) and (26)₁. Moreover, by (16)₂ we find that

$$\begin{aligned} \int_{\partial\Omega_2} \frac{1}{2} k_1 (w - h_2 - a\beta_2 \cos \phi_2)^2 &= \frac{a\kappa_b^{(0)2}}{2k_1} \int_0^{2\pi} \left| \frac{\partial \Delta w}{\partial r_2} \right|_{r_2-a}^2 d\phi_2 \\ &\approx \frac{a\kappa_b^{(0)2}}{2k_1} \int_0^{2\pi} \left| \frac{8c_{23} \cos(2\phi_2)}{a^3} + \frac{8c_{13}(r_2 - R \cos \phi_2)}{R^4} \right|^2 d\phi_2, \\ &\approx \frac{a\kappa_b^{(0)2}}{2k_1} \int_0^{2\pi} \left| \frac{8c_{23} \cos(2\phi_2)}{a^3} \right|^2 d\phi_2, \end{aligned} \quad (32)$$

where the last equality follows from (24) and (26)₂. The contribution $U_{\text{jmp}}^{(1)}$ from the first inclusion can be evaluated in a similar manner.

In summary, by adding up self-energy (25), interaction energy (29) and jump energy (31) and (32), we obtain the total energy (13) of the system:

$$U_e = S + G + U_{\text{jmp}}^{(1)} + U_{\text{jmp}}^{(2)}. \quad (33)$$

From the right-hand sides of (25), (29), (31) and (32) we observe that the total energy is an algebraic function of twelve unknown parameters $c_{10}, c_{20}, c_{13}, c_{23}, c_{14}, c_{24}, c_{15}, c_{25}, \beta_1, \beta_2, h_1$ and h_2 . One can determine these unknowns by applying the boundary conditions (16)_{3,4}. Alternatively, we can come back to the original variational principle (15) and minimize the total energy (33) $U_e = U_e(c_{pi}, \beta_p, h_p)$ against these unknown parameters. The necessary condition for a minimizer is given by (cf. (43))

$$\frac{\partial U_e}{\partial c_{pi}} = 0, \quad \frac{\partial U_e}{\partial \beta_p} = 0, \quad \frac{\partial U_e}{\partial h_p} = 0. \quad (34)$$

Upon solving the above equations (i.e. (34)) for all unknowns, we can obtain the total energy U_e , the interaction force between proteins and its dependence on R . The details of these calculations are presented in A1 and the solution is represented graphically in the Results section. The analytical expression for interaction energy with jumps is presented in A2.

4 Atomistic determination of model parameters

There is a rather simple way to estimate the newly introduced phenomenological parameters (k_1, k_2) in the definition of energy (3). Insertion of proteins in the membrane ought to change the apparent bending modulus of the membrane. Mechanically, proteins are usually regarded as rigid inclusions. In most theoretical models, such a notion immediately suggests that a membrane will stiffen due to the presence of these rigid inclusions (provided that the inclusions are anchored in the membrane and do not diffuse). However, this leads to some rather interesting paradoxes. A conventional Helfrich–Hamiltonian based approach yields the result that the apparent bending modulus of a membrane

in the presence of anchored rigid proteins is $\frac{\kappa_b^{(0)}}{1-f}$, where $\kappa_b^{(0)}$ is the bending modulus of a pure membrane and f is the area fraction of the protein. This naive result suggests that all proteins (which are essentially rigid compared with the membrane) will stiffen the membrane in an identical manner. In other words, there is no protein specificity. Experiments suggest otherwise; experiments performed on proteins like Alamethicin, Magnanin, HIV Fusion Peptide, RESA and many others show that different proteins affect the bending modulus of a membrane in a different way.^{52–56} We now derive the expression for the effective bending modulus of a protein–membrane system using our modified theoretical formulation.⁵¹

We consider a representative area of a protein–membrane system which, for simplicity, is selected as a single circular rigid inclusion (protein) of radius a embedded in a circular membrane of radius b . The mid-plane profile w of the membrane is determined using the variational problem (6); the associated boundary value problem is given by (11) with $\mathbb{U} = \{(x,y): r < b\}$. In the limit that the inclusion is rigid, *i.e.*, $\kappa_b^{(1)} \rightarrow +\infty$, the boundary value problem can be rewritten as (12). By symmetry we infer that the solution to (12) can be written as $w = w(r)$. Using (12)_{1,2} we can write the solution as

$$w(r) = \begin{cases} A_0 & \text{if } r < a, \\ B_0 + B_1 \ln r + B_2 r^2 + B_3 r^2 \ln r & \text{if } a < r < b. \end{cases} \quad (35)$$

By (12)_{3,4,5}, we have

$$\begin{aligned} \llbracket w \rrbracket = 0, \quad B_3 = 0, \quad \kappa_b^{(0)} 4B_2 - k_2 \left(\frac{B_1}{a} + 2B_2 a \right) = 0, \\ \frac{B_1}{b^2} + 2B_2 = H_{\text{ext}}. \end{aligned} \quad (36)$$

Solving the above set of equations we obtain

$$\begin{aligned} B_1 &= \frac{ab^2 H_{\text{ext}} (2\kappa_b^{(0)} - ak_2)}{(b^2 - a^2)k_2 + 2a\kappa_b^{(0)}}, \\ B_2 &= \frac{ab^2 H_{\text{ext}} (ak_2 - 2\kappa_b^{(0)})}{(b^2 - a^2)k_2 + 2a\kappa_b^{(0)}}, \\ \llbracket \mathbf{n} \cdot \nabla w \rrbracket|_{\partial\Omega} &= \frac{2b^2 H_{\text{ext}} \kappa_b^{(0)}}{2a\kappa_b^{(0)} + k_2(b^2 - a^2)}. \end{aligned} \quad (37)$$

Inserting (35)–(37) into (4), we obtain the total elastic energy of the system:

$$U_e[w] = \frac{2\pi b^4 k_2 \kappa_b^{(0)} H_{\text{ext}}^2}{2a\kappa_b^{(0)} + k_2(b^2 - a^2)}. \quad (38)$$

In the homogenization framework, the lipid–protein system is replaced by an equivalent homogeneous membrane with an effective bending modulus κ_b^{eff} . With the same boundary conditions as in the last of (12), the homogeneous membrane admits the solution $w = \frac{1}{2} H_{\text{ext}} (r^2 - b^2)$ and its total elastic energy is given by $\frac{1}{2} \pi b^2 \kappa_b^{\text{eff}} (2H_{\text{ext}})^2$. Equating this energy to that of the lipid–protein system, *i.e.* (38), we identify the effective bending modulus of the protein–membrane system as:

$$\kappa_b^{\text{eff}} = \frac{\kappa_b^{(0)}}{(1-f) + \frac{2f\kappa_b^{(0)}}{k_2 a}}. \quad (39)$$

We remark that for $k_2 \rightarrow \infty$, we recover the classical Helfrich solution which (as already mentioned) provides no protein specificity and predicts that all rigid proteins will stiffen the membrane in an identical manner. The parameter k_2 may now be found through either fitting the measured bending modulus result with either experiments or atomistic simulations.

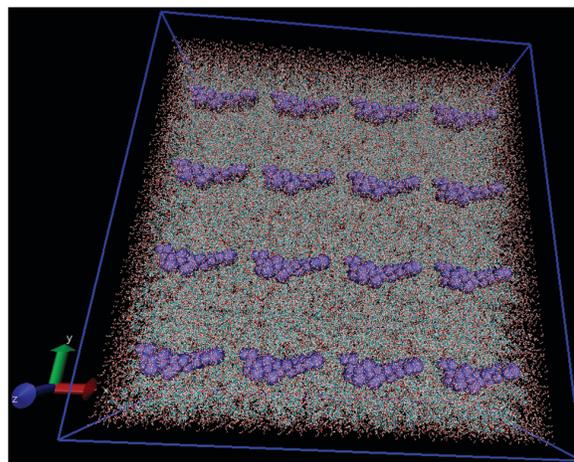


Fig. 4 16 HIV Fusion peptides embedded in a lipid membrane containing 1600 lipids. Atomistic simulations were performed on this system and the bending modulus was evaluated using lipid tilt and splay.^{57–59} The bending modulus thus obtained was matched with theoretical results⁵¹ to obtain the value of parameter k_2 .

We used published data on the experimentally determined bending modulus of the following protein membrane systems: HIV Fusion Peptide – DOPC,^{52–54} Alamethicin – DOPC⁵⁵ and Magnanin – POPC.⁵⁶ We fit our theoretical model to the experimental data to obtain k_2 values for different membrane protein systems.⁵¹ Alternatively, the value of k_2 can also be extracted from atomistic simulations (see (Fig. 4)), performed for several areas of fractions of protein. The methodology of calculating the bending modulus of a membrane from atomistics is detailed in ref. 57–59. This method makes use of the fact that the bending modulus of a membrane and its lipid tilt and splay are closely related. Once k_2 is obtained, we can now obtain the curvature mediated force of interaction between two proteins using simulations ref. 27. The theoretical expression for force involves both k_1 and k_2 . The value of k_2 is substituted in the expression for force and k_1 is obtained by matching it with simulation values.

5 Results and discussion

In what follows, we non-dimensionalize our results to comprehensively study the effect of k_1 and k_2 on U_e , by introducing dimensionless quantities ψ and σ :

$$\psi = \frac{\kappa_b^{(0)}}{ak_2}, \quad \sigma = \frac{\kappa_b^{(0)}}{a^2 k_1} \quad (40)$$

Furthermore, the force of interaction between proteins is $F = -\frac{\partial U_e}{\partial R}$ and we present the variation of F with inclusion separation R for different values of k_1 and k_2 . The contact angles α_1 and α_2 are taken to be 0.5 as in the work of Weikl *et al.*¹

From Fig. 5, we observe that F_H (force derived from the conventional Helfrich Hamiltonian) is really an upper bound to the repulsive force between the two transmembrane

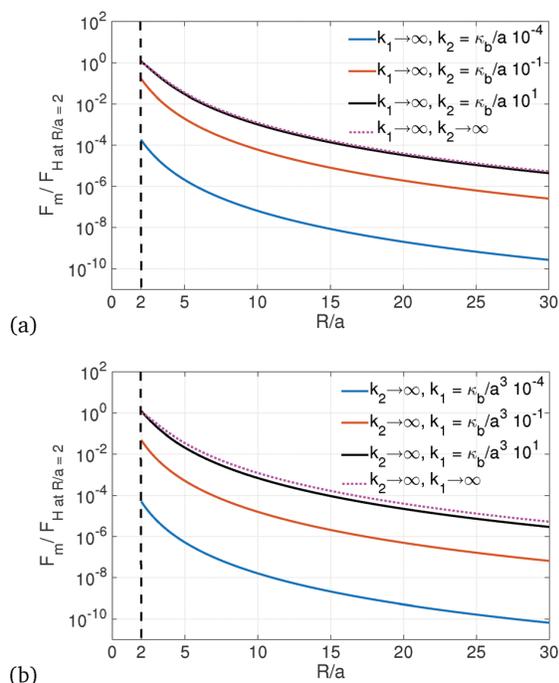


Fig. 5 Variation of membrane mediated transmembrane protein-protein interaction force F (normalized) as a function of normalized inter-protein distance R for different values of k_1 and k_2 . The classical Helfrich case is represented by dotted pink lines. The results indicate the decrease in F , as the distance between proteins increases. (a) Shows the variation of F when $k_1 \rightarrow \infty$. Among all values of k_2 , the maximum repulsive force is observed for the Helfrich case (i.e. when $k_2 \rightarrow \infty$). The force decreases significantly when the value of k_2 becomes very small. (b) Shows the variation of F when $k_2 \rightarrow \infty$. Here again, among all values of k_1 , the maximum repulsive force is observed for the Helfrich case (i.e. when $k_1 \rightarrow \infty$). The force also decreases dramatically, when the value of k_1 becomes very small.

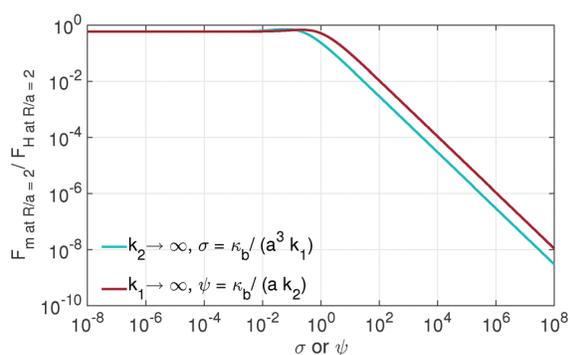


Fig. 6 The plot compares normalized F as a function of σ and ψ for a constant value of inter-protein distance ($R/a = 2$). σ and ψ are inversely proportional to k_1 and k_2 . F_m (force derived from our model) is comparable to F_H (force derived from the classical Helfrich Hamiltonian) at large values of k_1 and k_2 . When we decrease k_1 and k_2 the F_m is still comparable to F_H for a certain range, after which F_m starts decreasing exponentially and ultimately becomes zero when either k_1 or k_2 tends to zero.

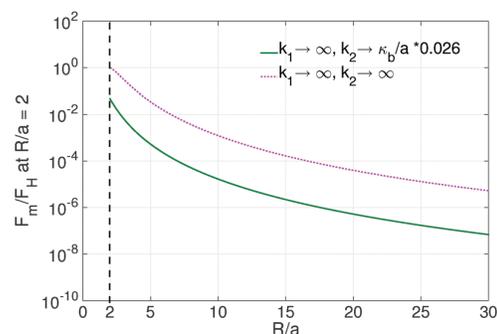


Fig. 7 The pink curve shows the modified force of interaction between two HIV peptides as a function of interprotein distance R/a normalized with the classical Helfrich force at $R/a = 2$. The green curve represents the classical force of interaction between the peptides normalized with classical Helfrich force at $R/a = 2$.

proteins and corresponds to the case when k_1 and k_2 are infinitely large. For finite values of k_1 and k_2 , this repulsive interaction is always weaker than what the classical Helfrich model predicts. Also, for all k_1 and k_2 , the repulsive force is inversely proportional to the interprotein distance. Fig. 6 presents the variation of F_m with σ and ψ which are inversely proportional to k_1 and k_2 . As fully expected, F_m is comparable to F_H at large values of k_1 and k_2 . We applied our modified theory to calculate the force of interaction between two HIV fusion peptides. As evident from Fig. 7 the modified theory predicts a force that is almost two orders of magnitude less repulsive than the classical Helfrich force.

We take the limit $k_1 \rightarrow \infty, k_2 \rightarrow \infty$ in the expression of total energy, U_e , to obtain the classical Helfrich energy:

$$U_e|_{k_1 \rightarrow \infty, k_2 \rightarrow \infty} = \frac{12\pi(\alpha_1^2 + \alpha_2^2)a^4\kappa_b^{(0)}}{R^4} - \frac{64\pi\alpha_1\alpha_2a^6\kappa_b^{(0)}}{R^6} + \frac{24\pi a^4(\alpha_1^2a^4 + \alpha_2^2a^4)\kappa_b^{(0)}}{R^8} \quad (41)$$

We also obtain a very important result when k_1 and k_2 tend to zero:

$$U_e|_{k_1 \rightarrow 0} = 0; \quad U_e|_{k_2 \rightarrow 0} = 0 \quad (42)$$

This means that, depending on the specific nature of the protein-bilayer coupling which may result in values of k_1 and k_2 that are close to zero, the repulsive interaction between two membrane proteins can be close to negligible also.

In various computational and experimental studies, a net attractive force between proteins^{27,60-62} and colloids⁴⁶ is in evidence. At zero temperature, the classical Helfrich theory predicts strong repulsive membrane mediated interaction between proteins which is inversely proportional to the fourth power of the distance separating the inclusions. At finite temperature there are fluctuation mediated interactions or Casimir forces that result in weak attraction between proteins. However, these attractive forces are not strong enough to overcome the strong repulsive forces predicted by the classical Helfrich theory. Hence, the existing theories struggle to

explain the aggregation and clustering of proteins caused by membrane mediated interactions.

Our theory takes into account the protein membrane specificity into curvature mediated force of interaction and introduces two material parameters for the membrane protein interface, *i.e.* k_1 and k_2 into the theoretical formulation. According to our modified theory, the membrane mediated force of interaction between proteins can be even 10 orders of magnitude less repulsive than the classical result as evident in Fig. 5. The force can even be zero if for a particular membrane protein system the parameters k_1 and k_2 are zero. Hence, the ground state force of interaction calculated using our modified theory when combined with force due to thermal fluctuations can result in a net attractive force, which is observed for many membrane-protein systems. The inclusion of thermal fluctuations within the framework of our proposed model will be pursued in future studies.

6 Concluding remarks

The mechanical behavior of membrane protein systems can be described by the phenomenological theory of elasticity. However, the conventional theory fails to adequately explain phenomena such as softening of the lipid membrane due to rigid proteins and aggregation and clustering of membrane proteins. Keeping the limitations of the classical theory in mind, we proposed a modified approach by taking into account the membrane protein interface and hence introducing a specificity of the protein and the membrane into the classical model. The specificity in the system is established through introducing new material parameters which can be determined through atomistics or experiments just like the bending modulus. Our modified theory predicts that the force of interaction between two transmembrane proteins can be several orders of magnitude lower than the force predicted by the classical models and in the extreme case can even vanish. For the specific case of HIV peptides, we show that the classical model overestimates the repulsive force by two orders of magnitude. The weak repulsive force for some protein membrane systems (as predicted by the modified theory) when combined with Casimir attractive forces can result in a net attractive force, which causes protein aggregation and clustering.

Appendix

A1 Solution of boundary conditions involving jump conditions

For the explicit approximate solution represented in (18), (21) and (22) we need to evaluate the twelve variables, c_{10} , c_{20} , c_{13} , c_{23} , c_{14} , c_{24} , c_{15} , c_{25} , β_1 , β_2 , h_1 and h_2 . Enforcing (16)_{3,4} with respect to the leading Fourier modes, we obtain the 12

equations as follows:

$$\left\{ \begin{aligned} \frac{8c_{23}\kappa_b^{(0)}}{a^3} + \frac{a^2c_{14}k_1}{R^3} - \frac{a^2c_{10}k_1}{2R^2} + \frac{a^2c_{13}k_1}{R^2} + \frac{c_{25}k_1}{a^2} + c_{23}k_1 &= 0 \\ \frac{a^3c_{10}k_1}{R^3} - \frac{4a^3c_{13}k_1}{R^3} + \frac{ac_{14}k_1}{R^2} - \frac{ac_{10}k_1}{R} + \frac{c_{24}k_1}{a} - a\beta_2k_1 &= 0 \\ -\frac{a^2c_{13}k_1}{R^2} + c_{10}k_1 \log\left(\frac{R}{a}\right) + \frac{c_{15}k_1}{R^2} + \frac{c_{14}k_1}{R} + c_{13}k_1 - h_2k_1 &= 0 \\ \frac{8c_{13}\kappa_b^{(0)}}{a^3} + \frac{a^2c_{24}k_1}{R^3} - \frac{a^2c_{20}k_1}{2R^2} + \frac{a^2c_{23}k_1}{R^2} + \frac{c_{15}k_1}{a^2} + c_{13}k_1 &= 0 \\ \frac{a^3c_{20}k_1}{R^3} - \frac{4a^3c_{23}k_1}{R^3} + \frac{ac_{24}k_1}{R^2} - \frac{ac_{20}k_1}{R} + \frac{c_{14}k_1}{a} - a\beta_1k_1 &= 0 \\ -\frac{a^2c_{23}k_1}{R^2} + c_{20}k_1 \log\left(\frac{R}{a}\right) + \frac{c_{25}k_1}{R^2} + \frac{c_{24}k_1}{R} + c_{23}k_1 - h_1k_1 &= 0 \\ \frac{2c_{25}k_2}{a^3} - \frac{4c_{23}\kappa_b^{(0)}}{a^2} - \frac{2ac_{14}k_2}{R^3} + \frac{ac_{10}k_2}{R^2} - \frac{2ac_{13}k_2}{R^2} &= 0 \\ -\frac{3a^2c_{10}k_2}{R^3} + \frac{12a^2c_{13}k_2}{R^3} + \frac{c_{24}k_2}{a^2} - \frac{c_{14}k_2}{R^2} + \frac{c_{10}k_2}{R} + \beta_2k_2 &= 0 \\ \frac{2ac_{13}k_2}{R^2} - \frac{c_{20}k_2}{a} - \frac{4c_{13}\kappa_b^{(0)}}{R^2} + \alpha_2k_2 &= 0 \\ \frac{2c_{15}k_2}{a^3} - \frac{4c_{13}\kappa_b^{(0)}}{a^2} - \frac{2ac_{24}k_2}{R^3} + \frac{ac_{20}k_2}{R^2} - \frac{2ac_{23}k_2}{R^2} &= 0 \\ -\frac{3a^2c_{20}k_2}{R^3} + \frac{12a^2c_{23}k_2}{R^3} + \frac{c_{14}k_2}{a^2} - \frac{c_{24}k_2}{R^2} + \frac{c_{20}k_2}{R} + \beta_1k_2 &= 0 \\ \frac{2ac_{23}k_2}{R^2} - \frac{c_{10}k_2}{a} - \frac{4c_{23}\kappa_b^{(0)}}{R^2} + \alpha_1k_2 &= 0. \end{aligned} \right. \quad (43)$$

Solving the above set of equations we get:

$$\left\{ \begin{aligned} c_{10} &= \frac{2a^7(ax_1k_1k_2 - 2\alpha_1k_1\kappa_b^{(0)})}{R^4(a^3k_1k_2 + 2a^2k_1\kappa_b^{(0)} + 8k_2\kappa_b^{(0)})} + \alpha_1a \\ c_{20} &= \frac{2a^7(ax_2k_1k_2 - 2\alpha_2k_1\kappa_b^{(0)})}{R^4(a^3k_1k_2 + 2a^2k_1\kappa_b^{(0)} + 8k_2\kappa_b^{(0)})} + \alpha_2a \\ c_{13} &= \frac{a^6\alpha_2k_1k_2}{R^2(a^3k_1k_2 + 2a^2k_1\kappa_b^{(0)} + 8k_2\kappa_b^{(0)})} \\ &\quad - \frac{2a^{11}\alpha_1k_1^2k_2^2}{R^4(a^3k_1k_2 + 2a^2k_1\kappa_b^{(0)} + 8k_2\kappa_b^{(0)})^2} \\ c_{23} &= \frac{a^6\alpha_1k_1k_2}{R^2(a^3k_1k_2 + 2a^2k_1\kappa_b^{(0)} + 8k_2\kappa_b^{(0)})} \\ &\quad - \frac{2a^{11}\alpha_2k_1^2k_2^2}{R^4(a^3k_1k_2 + 2a^2k_1\kappa_b^{(0)} + 8k_2\kappa_b^{(0)})^2}. \end{aligned} \right. \quad (44)$$

A2 The interaction energy of proteins with jumps

The interaction energy of proteins embedded in a membrane (with jumps) can be written in the following way:

$$U_e = \frac{A(k_1, k_2)}{R^4} + \frac{B(k_1, k_2)}{R^6} + \frac{C(k_1, k_2)}{R^8}. \quad (45)$$

where

$$A(k_1, k_2) = \frac{12\pi a^7 (\alpha_1^2 + \alpha_2^2) k_1 k_2 k_b^{(0)}}{a^3 k_1 k_2 + 2a^2 k_1 k_b^{(0)} + 8k_2 k_b^{(0)}},$$

$$B(k_1, k_2) = -\frac{64\pi a^{12} \alpha_1 \alpha_2 k_1^2 k_2^2 k_b^{(0)}}{(a^3 k_1 k_2 + 2a^2 k_1 k_b^{(0)} + 8k_2 k_b^{(0)})^2},$$

$$C(k_1, k_2) = 8\pi a^{13} k_1^2 k_2 k_b^{(0)} (3\alpha_1^2 a^4 k_1 k_2^2 + 3\alpha_2^2 a^4 k_1 k_2^2 - 4\alpha_1^2 a^2 k_1 k_b^{(0)2} - 4\alpha_2^2 a^2 k_1 k_b^{(0)2} + 8\alpha_1^2 a k_2^2 k_b^{(0)} + 8\alpha_2^2 a k_2^2 k_b^{(0)} - 16\alpha_1^2 k_2 k_b^{(0)2} - 16\alpha_2^2 k_2 k_b^{(0)2}) / (a^3 k_1 k_2 + 2a^2 k_1 k_b^{(0)} + 8k_2 k_b^{(0)})^3.$$

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