Membrane inclusions as coupled harmonic oscillators: Effects due to anisotropic membrane slope relaxation

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Membrane-mediated interaction between membrane-spanning peptides or protein segments plays an important role in their function and stability. Our rigorous “coupled harmonic oscillators” representation is extended to account for the complex boundary conditions permitting anisotropic relaxation of the membrane slope along the contours of the inclusions. Using this representation and applying a highly efficient finite-difference algorithm, we have analyzed the membrane-mediated interaction triggered by deformation of the hydrophobic tails of lipid molecules to match the lipophilic exterior of the inserted peptide. We establish that anisotropic relaxation crucially affects the interaction energy, leading to a short-range attraction between two inclusions, while conventional isotropic boundary conditions result in their strong repulsion. In a multi-inclusion cluster, this attraction is further enhanced and modified due to nonpairwise interactions. The results for dimyristoyl phosphatidylcholine and glyceryl monooleate membranes are compared, and the effects of the inclusion radius are considered. The possible role of slope relaxation in the reported stabilization of linked gramicidin channels and in proteins’ functional cooperativity is outlined.

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I. INTRODUCTION

Membrane deformation accompanying the insertion of proteins affects the energetics of the inclusion, leads to a membrane-mediated interaction between the inclusions, and thus can promote aggregation and influence the stability and function of membrane proteins (see Refs. 1–7 and references therein). Recent discoveries8–10 imply that these effects can influence channel gating, which may involve movement of protein segments through the membrane accompanied by significant structural changes at the protein–membrane interface.

There is a large literature devoted to studying membrane-mediated interactions between inclusions from many different viewpoints and for a range of applications.21–24 One possible mechanism for such an interaction is hydrophobic mismatch: the difference between the membrane-spanning length of the inclusion and the hydrophobic thickness of the membrane. This produces a local membrane deformation propagating out from the inclusions to distances of about 3–4 nm. The corresponding “energy penalty” $E$ can be responsible, among other effects, for membrane-induced variation of channel lifetimes in gramicidin.19,21–24 Recently we have shown25 this mechanism has the potential to stabilize channels in clusters and to contribute to the observed increases in the lifetime of gramicidin channels modified and linked together. These observations suggest that a more thorough analysis of elastic membrane-mediated interactions is in order.

The first continuum treatments of insertion energetics21,22 raised the issue of the proper choice of boundary conditions at the membrane-inclusion interface. While there is general consensus that, near an inclusion, a membrane adjusts its interfacial thickness to match the inclusion’s hydrophobic length, there remains a controversy as to how to determine the membrane slope $s$ along the curve where the inclusion abuts the membrane surface. While Huang21 treated this contact slope as an adjustable parameter, constrained and chosen as $s \approx 0$ (termed the “null constraint” throughout this paper), Helfrich and Jakobsson22 presumed that $s$ should adjust itself to minimize the elastic energy (the “relaxed slope” $s = s_{\text{min}}$), equivalent to the condition that the inclusion applies a vanishing torque to the membrane, $M = 0$.20 The latter condition implies a much lower energy penalty for inclusion. For gramicidin A (GA) in a glyceryl monooleate (GMO) membrane at 300 K, the values are $\sim 4$ kT (relaxed) and $\sim 11$ kT (null constraint). In practice, energies derived based on the null constraint agree better with the influence of membranes on the lifetime of single GA channels.21,24 Thus recent studies have generally used this ansatz (see Refs. 17, 20, and 26 and references therein).

Why does the apparently valid requirement that the membrane slope adjusts itself to minimize the elastic energy significantly underestimate the energy penalty and, consequently, a membrane’s influence on channel lifetimes? Two answers have been proposed.

(a) The first justifies the null constraint by suggesting that other contributions, not explicit in the elastic Hamiltonian, restrict the slope,21,23,27 which naturally increases the mismatch energy penalty. Thus Harroun et al.17 introduced an additional “boundary” contribution $E_{\text{bd}}$ that effectively produces a finite torque $M \neq 0$ constraining the slope to $s \approx 0$. Their idea is that while the total energy $E_{\text{conv}}(s) + E_{\text{bd}}(s)$ should indeed be minimized over $s$, the elastic energy by itself is not at a minimum. This suggestion, while
physically sensible, leaves some important questions unaddressed.\textsuperscript{28}

(b) The nonuniform membrane model presumes free membrane relaxation and suggests that hydrophobic interaction with the peptide constrains the mobility and packing of the lipid molecules.\textsuperscript{19} Inclusions are effectively surrounded by a modified membrane, which is more rigid than its unperturbed, more distant counterpart. The contact slope is now freely variable and attains its equilibrium value \( s = s_{\text{min}} \). The perturbation \( \Delta E = E_{\text{mod}}(s) - E_{\text{conv}}(s) \), introduced due to inclusion-induced modification of the elastic constants, can be identified with the contribution \( E_{bq}(s) \) suggested in (a). However, this contribution is explicitly present in the underlying equations used in the nonuniform treatment, while in (a) it is only introduced to justify the null constraint. After its apparent success in modeling membrane influences on GA channel lifetimes, the null constraint has been applied to the study of the membrane-mediated interaction between channels.\textsuperscript{17,20} It predicts a strong attraction between two inclusions, with a significant tendency for their aggregation.\textsuperscript{17,20} When applied to GA, the consequences are a significant mutual stabilization of dimer clusters.\textsuperscript{25} The implications of the alternative approach (b), based on a relaxed slope, have not yet been studied. It is known that predictions for membrane-mediated protein interactions may be greatly altered and even reversed if the restricted slope deviates from the null constraint, \( s = 0 \).\textsuperscript{17,20} which also implies that free slope relaxation could have major effects on model predictions. Here effects due to free relaxation of the contact slope are thoroughly examined. We exploit a rigorous approach describing interacting inclusions as coupled harmonic oscillators (CHOs), which is both computationally efficient and physically transparent.\textsuperscript{25} We exploit this idea, paying special attention to the boundary conditions by introducing parameters that permit more efficient relaxation of the contact slope. In particular, we show that angular variation of the contact slope is an important consequence of the membrane-mediated interaction between inclusions.

First, we introduce the underlying equations of the smectic bilayer model for the perturbed membrane, with boundary conditions that permit variation of the membrane slope along inclusion contours. Then we describe the CHO representation of the elastic problem, reviewing its application to isolated GA inclusions and applying the technique to inclusion clusters.

For ease of analysis we start with the conventional uniform membrane model. We introduce trial functions allowing contact slope anisotropy and derive modified equations defining the CHO parameters. Then we consider a range of cluster structures and sizes, and thoroughly investigate effects due to free membrane relaxation, demonstrating that slope anisotropy greatly influences short-range membrane-mediated interactions between inclusions. Special attention is paid to the role of nonpairwise interactions.

Although illustrative, the “relaxed” treatment of the conventional model underestimates the energy of inclusion and must be modified to account for the inclusion-induced perturbation of the membrane elastic constants. Since we have not yet generalized the nonuniform model\textsuperscript{19} to describe membranes with multiple inclusions, we consider two simple treatments of a binary cluster. We show that the qualitative influence of anisotropic slope relaxation in the nonuniform approach is similar to that in the (uniform) conventional model, except that, due to local perturbation of membrane constants, the mismatch energy penalties are substantially increased.

A glossary of symbols is provided as an Appendix.

II. ELASTIC EQUATIONS AND BOUNDARY CONDITIONS

We consider a general elastic model for a membrane extending laterally in the \( x,y \) plane, with the deformation described by a two-dimensional field of “vertical” surface displacements \( u(\mathbf{r}) \) [where \( \mathbf{r}=(x,y) \) is the radius vector at the system midplane]. The elastic boundary problem can be formulated as a variational (minimum) principle for the energy functional

\[
F[u] = \int g^{(2)}(u, \nabla u, \Delta u, ...) df,
\]

where \( g^{(2)} \) is the surface density of the elastic energy, is a quadratic function of the surface displacement \( u \) and its derivatives. We consider a membrane with \( N \) cylindrical inclusions, assuming that on the contour \( \gamma_i \) of \( i \)th inclusion both \( u(\mathbf{r}) \) and \( \nabla u(\mathbf{r}) \) are fixed functions of \( \mathbf{r} \). This leads to the boundary conditions

\[
u_i(\mathbf{r}) |_{\gamma_i} = u_i(\mathbf{r}),
\]

\[
\nabla u(\mathbf{r}) |_{\gamma_i} = s_i(\mathbf{r}),
\]

where \( n \) designates the direction normal to \( \gamma_i \) at the point \( \mathbf{r} \). Note that the vertical displacement \( u_i \) along the surface where the inserted peptide is in direct contact with the membrane is typically described by the “hydrophobic matching condition” \textsuperscript{20–23}

\[
2u_0 = h_0 - l_h,
\]

where \( h_0 \) is the hydrophobic membrane thickness and \( l_h \) is the hydrophobic length of the peptide, which for GA is 2.17 nm.\textsuperscript{21–23} This leads to a special case of Eq. (2) with

\[
u_i(\mathbf{r}) |_{\gamma_i} = u_0 = \text{const}.
\]

The additional constraints at the external membrane boundary (designated as \( \gamma_a \)) are

\[
u_i(\mathbf{r}) |_{\gamma_a} = 0, \quad \nabla u(\mathbf{r}) |_{\gamma_a} = 0.
\]

The variational principle \( \delta F = 0 \) (the minimum condition on the energy functional) leads to an Euler–Lagrange equation which is generally expressed as

\[
L(u) = 0,
\]

where \( L \) is a linear differential operator; this imposes no restriction on the order of the differential equation, generally biquadratic in what follows. The elastic energy

\[
E = \min F[u]
\]
TABLE I. Selected membrane parameters (Refs. 53–56) and corresponding GA hydrophobic mismatches.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>$B_0$ (pN/nm$^2$)</th>
<th>$K_0$ (pN)</th>
<th>$h_0$ (nm)</th>
<th>$u_0$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMO</td>
<td>50.0</td>
<td>10.0</td>
<td>2.85</td>
<td>0.34</td>
</tr>
<tr>
<td>DMPC</td>
<td>50.0</td>
<td>40.0</td>
<td>2.52</td>
<td>0.165</td>
</tr>
</tbody>
</table>

is the value of $F[u]$ calculated with the solutions of Eq. (7) substituted for $u$.

A particular case of the model described by Eqs. (1)–(7), often used in applications to real membranes, is a “smectic bilayer” description. Modified to account for the nonuniformity of the membrane elastic moduli, the corresponding free energy functional is

$$F = \int \left( b(x,y)(\Delta u)^2 + a(x,y)u^2 \right) dx \, dy,$$

(9)

where

$$a(x,y) = 2B(x,y)/h_0, \quad b(x,y) = K(x,y)h_0/2;$$

$B$ and $K$ are membrane stretching and bending moduli. These, due to the effect of inclusions, can differ from the unperturbed values $B_0$ and $K_0$. Table I lists characteristic parameters for GA in GMO and in DMPC (dimyristoyl phosphatidylcholine), the two systems on which we focus. The corresponding Euler–Lagrange equation has the form of Eq. (7):

$$\Delta [b(x,y)\Delta u(x,y)] + a(x,y)u(x,y) = 0.$$

(10)

For the conventional uniform model it leads to a familiar biquadratic equation

$$b_0\Delta^2 u(x,y) + a_0u(x,y) = 0,$$

(11)

where

$$a_0 = 2B_0/h_0, \quad b_0 = K_0h_0/2.$$

In what follows, unless specifically noted, we treat the conventional model, Eq. (11). It is typically solved with the condition, Eq. (5), for contact displacement. If fluctuation of the interfacial displacements $u_i$ is allowed, then the constraint

$$u(r)|_{y_i} = u_i,$$

(12)

with $u_i \neq u_0$, is appropriate (see Ref. 25). The second condition, Eq. (3), constrains the membrane’s contact slope at the inclusion boundary: the simplest choice is to assume cylindrical symmetry and set $s_i$ constant along the boundary:

$$\nabla u(r)|_{y_i} = s_i,$$

(13)

with the slope either fixed in advance or determined by minimizing the energy (in what follows, the latter will be denoted as $s_{min}$). There is no a priori reason for cylindrical symmetry, and we shall consider a generalization, releasing this constraint to permit angular variation of $s$ and a corresponding anisotropic slope relaxation. Studies of a single inclusion, where the cylindrical symmetry assumption is natural, show that the null constraint for the contact slope $s_i$, $s_i = 0$,

(14)

is consistent with the membrane’s influence on channel lifetimes. Alternatively, we have shown that these data can be rationalized using the free boundary condition, where the contact slope is determined by energy minimization, assuming there is local perturbation of membrane elastic moduli that leads to a stiffening of the membrane in the vicinity of the inclusion, which would require solving the nonuniform model, Eq. (10).

There are two important issues: accounting for local perturbation of membrane elastic moduli and consistently treating free membrane slope relaxation. Even though the nonuniform model has not been rigorously generalized to membranes with multiple inclusions (Ref. 20 describes a preliminary attempt), studying the possible consequences of free membrane slope relaxation for membrane-mediated interactions between inclusions is significant. Consequently, we first discuss the conventional (uniform) membrane model, which demonstrates the importance of free anisotropic slope relaxation, but underestimates the energetic cost of an inclusion. This analysis seems particularly important since previous attempts at such treatments were restricted to simplified, analytically soluble inclusion geometries: the “flat wall” model and the cylindrically symmetric “Wigner–Seitz cell” model for a hexagonal array of proteins. These models preclude the possibility of anisotropy in slope relaxation, which leaves some conclusions about interinclusion interactions unsubstantiated. Treating realistic inclusion geometries requires a general two-dimensional solution to the elastic problem. The finite-difference algorithm described and tested in Ref. 20 is ideal for this purpose. First, we use this algorithm and analyze the conventional membrane model considered in Refs. 11–13, 15 and 29; this demonstrates the influence of free anisotropic slope relaxation, but underestimates the energetic cost of an inclusion.

To account heuristically for inclusion-induced local membrane rigidity and the corresponding increase in the interaction energy, we treat two simple implementations of the nonuniform model. Consider two cylindrical inclusions with their centers at $(a,0)$, $(a,0)$, and define the interinclusion distance $L = 2a(a+r)$ and radial distances centered on each inclusion, $\rho = \sqrt{(x+a)^2+y^2}$.

(a) In the “uniform patch” model the elastic constants are defined as

$$B(x,y)/B_0 = \begin{cases} \theta, & \text{for } \rho \leq \lambda_p, \\ 1, & \text{otherwise}. \end{cases}$$

(15)

(b) In the “exponential decay” model they are

$$B(x,y)/B_0 = \begin{cases} 1 + (\theta-1)\exp\left[-\frac{\rho}{\lambda_p}\right], & x \leq 0, \\ 1 + (\theta-1)\exp\left[-\frac{\rho}{\lambda_d}\right], & x \geq 0. \end{cases}$$

(16)

We choose $\lambda_p = \lambda_d = 1.5$ nm and, in both approximations, adjust $\theta$ to reproduce the mismatch energy of a single GA inclusion, determined from the lifetime of GA channels (see Ref. 19 for details).
III. MEMBRANE INCLUSIONS AS COUPLED HARMONIC OSCILLATORS: EFFECTIVE SPRING CONSTANTS

For a linear elastic problem described by Eqs. (1) and (7) with quite general boundary conditions [such as Eqs. (2), (3), etc.] the energy, Eq. (8), is rigorously represented in terms of CHO. Here we summarize this concept as applied to the membrane-inclusion problem and outline a few generalizations.

A. One inclusion

The “linear spring” (CHO) model for describing the membrane deformation energy induced by an inclusion was introduced by Ref. 23, although this result was implicit in earlier studies\(^{21,22}\) and in classical studies of elastic plates\(^{30}\) (see also Ref. 20 for a review). We briefly outline the derivation.

For one inclusion the analytic expression for the cylindrically symmetric solution to Eq. (11) for the boundary constraints of Eqs. (6), (12), and (13) is

\[
u(r) = c_1 \ker(r\lambda) + c_2 \ker(r\lambda),
\]

where “ker” and “\(\ker\)” are Kelvin functions and \(\lambda = (b/a)^{1/4}\) is the characteristic decay length.\(^{21,22}\) The constants \(c_1\) and \(c_2\) are linear functions of the boundary parameters \(u\) and \(s\):

\[
c_1 = \frac{u \ker(x_0) - s \ker(x_0)}{\Omega(x_0)}, \quad (18)
\]

\[
c_2 = \frac{s \ker(x_0) - u \ker(x_0)}{\Omega(x_0)}, \quad (19)
\]

\[\Omega(x_0) = \ker(x_0)\ker(x_0)' - \ker(x_0)\ker(x_0)'.\]

where \(x_0 = r_0/\lambda\).

It is this linearity that permits harmonic representation of inclusion energetics. When Eq. (17) is substituted into Eq. (9), the resulting energy expression is homogeneous and second order in \(u\) and \(s\):

\[E(u, s) = C_{uu} u^2 + C_{us} u s + C_{ss} s^2, \quad (20)\]

where the parameters \(C_{\alpha\beta}\), which can be expressed analytically (not shown), do not depend on either \(u\) or \(s\). Equation (20) describes the energy of two coupled harmonic oscillators with the boundary parameters \(u\) and \(s\) playing the role of coupled “displacements.” The null constraint \(s = 0\) immediately leads to a linear spring model\(^{23,24,26}\)

\[E(u, 0) = H_0(2u)^2, \quad (21)\]

where \(H_0 = C_{uu}\). Similarly, if the free energy is minimized over \(s\),

\[E(u, s_{\text{min}}) = H_{\text{min}}(2u)^2, \quad (22)\]

where

\[H_{\text{min}} = C_{uu} - (C_{us})^2/4C_{ss}.\]

B. Multiple inclusions, various boundary conditions

Although the elastic problem considered above does not have analytical solutions for an arbitrary number of inclusions, it still can be represented in terms of CHO.\(^{19,25,31}\) The elastic energy depends quadratically on the boundary parameters \(u_i, s_i\), etc.\(^{32}\) Thus the elastic energy is quite generally expressed as

\[E = \sum_{i=1}^{N} \sum_{j=1}^{N} C_{ij} \alpha_i \beta_j, \quad (23)\]

where the indices \(i\) and \(j\) enumerate the inclusions.

Additional summations are performed over the repeatedly indexed quantities \(\alpha, \beta\), which symbolically identify the complete set of boundary parameters: \(\alpha, \beta = u, s, \text{etc.}\). The coefficients \(C_{ij}\) are determined from generic solutions of Eq. (10) with boundary parameters \(\alpha_i, \beta_j\), taking values 1 or 0; they therefore do not depend on the specific numerical values of \(\alpha_i\) and \(\beta_j\).\(^{19,31}\) Equations (17)–(20) provide a general solution for one inclusion.

The CHO approach was recently used to analyze the mutual stabilization of ion channels.\(^{25}\) There we used the cylindrically symmetric, null constrained slope, Eq. (14), where the general form is a quadratic function of the boundary displacements \(u_i\).\(^{25}\) To emphasize the CHO analogy, the elastic free energy was rewritten as

\[E = \sum_i a_{ii} u_i^2 + \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} a_{ij} (u_i - u_j)^2, \quad (24)\]

where \(a_{ij}\) are “effective spring constants” related to \(C_{ij}\). Thus for a given inclusion geometry the energy of any possible boundary displacement fluctuation \(u_i\) is determined by relatively few spring constants, reducing computational complexity dramatically.\(^{25,26}\) Nondiagonal components \(a_{ij}\) describe membrane-mediated coupling between inclusions \(i\) and \(j\) due to hydrophobic length differences. The diagonal components \(a_{ii}\) describe elastic coupling for concerted displacements. As an example, consider a gramicidin cluster. Channels in their low-energy dimeric state have essentially the same hydrophobic length; their interaction (which may be responsible for aggregation)\(^{16,17,33}\) is then completely described by the diagonal constants \(a_{ii}\). In contrast, the dissociation of a single channel, with all other hydrophobic lengths fixed at \(l_h\), requires fluctuations in \(u_i - u_j\). These depend mainly on the nondiagonal constants \(a_{ij}\); the \(a_{ii}\) have a minimal influence.\(^{25}\)

The commonly used constrained boundary condition for the contact slope, Eq. (14),\(^{17,25}\) has been justified post hoc; it accounts for membrane influences on the lifetime of isolated gramicidin channels.\(^{21-23}\) However, an alternative model\(^{19}\) has emphasized the possible importance of slope relaxation, determined by free energy minimization. This aspect of membrane-mediated interactions is investigated below.

In what follows, we focus on the interaction between identical inclusions of fixed hydrophobic length, choosing \(u_i = u_{0i}\). The elastic free energy can then be written as \(E = H_{\text{min}} u_{0i}^2\) where \(H_{\text{min}}\) is an effective cumulative spring constant depending only on the inclusion geometry. The qualitative behavior of \(H_{\text{min}}\) is very sensitive to the choice of boundary conditions and on internal consistency in the description of the slope relaxation. We will see that in clusters \(s\) may be far from cylindrically symmetric, with important
consequences for membrane-mediated interactions. We compare the behavior of GA clusters in DMPC with that in GMO.

C. Anisotropy of the contact slope: The choice of trial functions

There is no obvious form for the angular dependence of the contact slope for interacting inclusions; consequently, we tested a range of trial functions. For two inclusions the simplest form is

\[ s_\phi = s + v \cos(\phi), \tag{25} \]

where \( \phi \) is the polar angle measured relative to an axis connecting the inclusion centers; \( s \) and \( v \) are variational slope parameters, to be optimized so as to minimize the free energy.\(^\text{20}\) This leads immediately to noticeable deviations from cylindrical symmetry, but it still severely constrains the functional form of the slope variation along the inclusion contour. To release this restriction, we replace \( \cos(\phi) \) by a more general expression \( t_n(a, \phi) \):

\[ t_n(a, \phi) = \frac{\chi(a, n, \phi) + b}{(1 + b)}, \quad 0 \leq |\phi| \leq \pi, \tag{26} \]

\[ \chi(a, n, \phi) = \frac{\cos(c \phi)}{1 + a(c |\phi|)^n}, \quad b = \frac{1}{\pi} \int_0^\pi \chi(\phi) d\phi, \]

so that Eq. (25) becomes

\[ s_\phi = s + vt_n(a, \phi). \tag{27} \]

Only the parameters \( a \) and \( n \) are independent. The angular scaling factor \( c \) is defined by the condition \( \partial \chi/\partial \phi |_{\phi=\pi}=0 \), requiring that the slope vary smoothly along the inclusion contour; \( b \) shifts \( t_n(a, \phi) \) so that \( t_n=0 \) corresponds to the “center of mass” of \( \chi \) and the factor \((1 + b)^{-1}\) guarantees that \( t_n(a,0)=1 \) [both properties characterizing the initial trial function, Eq. (25)]. We now set \( n=2 \), which provides sufficient functional flexibility and reduces \( t_n \) to the one-parameter family \( t_2(a, \phi) \). Table II illustrates the dependence of \( b \) and \( c \) on \( a \) for a range of \( a \). When \( a=0 \), Eq. (25) is recovered.

The general behavior of the trial functions for various \( a \) is shown in Fig. 1. While \( b \) and \( c \) vary slowly with \( a \), \( t_2(a, \phi) \) becomes even more asymmetric as \( a \) increases. In what follows we solve the elastic problem for a range of fixed \( a \) and then choose \( a=a_{\text{min}} \), minimizing the energy for this family of trial functions. In general, expanding a family of trial functions results in improvements that reduce the optimized energy. For an \( N \)-lateral distribution of inclusions (trimers, tetramers, etc.) we consider functions that account for the influence of the two nearest neighbors of each inclusion:

\[ t_N = \frac{1}{2} \left[ t_2(a, \phi + \phi_N) + t_2(a, \phi - \phi_N) \right] , \]

\[ \phi_N = \frac{N-2}{2N} \pi. \]

Here the pairwise trial functions \( t_2(a, \phi) \) are chosen of the general form, Eq. (27), used to treat the two-inclusion problem.

D. Effective spring constants and energy optimization: Relaxed anisotropic slope

We presume all inclusions have the same hydrophobic length and thus equal values of the contact displacement \( u_0 \). For illustrative simplicity we limit consideration to symmetric, equilateral arrangements. Consequently the equilibrium (minimum energy configuration) boundary parameters \((s, v)\) should be the same for all inclusions. The energy of such an inclusion cluster, Eq. (24), is then a quadratic function of the three parameters: \( u, s, \) and \( v \). This reduces the number of effective spring constants to 6, \( C_{uu}, C_{ss}, C_{uv}, C_{us}, C_{us}, C_{vv} \), as the indices \( i, j \) enumerating the individual inclusions [Eq. (23)] are now unnecessary, a result independent of the number of inclusions \( N \). The energy takes the form

\[ E = C_{uu} u^2 + C_{ss} s^2 + C_{vv} v^2 + C_{uv} uv + C_{us} us + C_{vs} vs . \tag{28} \]

Fixing the slope parameters \( s \) and \( v \) is equivalent to applying distributed torques \( M(r) \) along the inclusion

\( \) FIG. 1. Trial functions for the anisotropic slope corresponding to various choices of the parameter \( a \) [see text, Eq. (27)]: (1) \( a=0 \), solid line; (2) \( a=0.8 \), dashed line; (3) \( a=1.6 \), dotted line.
boundaries. 20 The “relaxed” boundary conditions imply that \( M(x, y) = \frac{2}{r} \) i.e., that the contact slope adjusts itself freely to minimize the elastic energy. With these conditions, the optimized slope is determined by minimizing Eq. (28):

\[
\frac{\partial E}{\partial s} = 0, \quad \frac{\partial E}{\partial \phi} = 0, \tag{29}
\]

which leads directly to the result

\[
H_{\text{min}} = \frac{C_{uv}(C_{uv}^2 - 4C_{us}C_{uu}) + C_{vv}(C_{sv}C_{uu} - C_{us}C_{uv}) + C_{ss}^2 C_{uv}}{\Delta}.
\]

The six effective spring constants \( C_{\alpha\beta} \), which determine the equilibrium energy and relaxed contact slope, can be expressed through generic solutions of the boundary problem for Eq. (7) [or Eq. (10)]. 19,25,31 They can equally well, and more conveniently, be determined for any interinclusion separation by numerically calculating the elastic energy \( E[u, s, v] \), Eq. (28), for six linearly independent sets of \( \{u, s, v\} \). 20 Substituting the resulting energy values on the left-hand side (LHS) of Eq. (28) and the corresponding \( \{u, s, v\} \) values on the RHS results in a system of six linear equations for six spring constants. The following equations illustrate an implementation of this approach and are used in what follows:

\[
\begin{align*}
C_{uu} &= \frac{E[u,0,0]}{u^2}, \\
C_{ss} &= \frac{E[0,s,0]}{s^2}, \\
C_{vv} &= \frac{E[0,0,v]}{v^2}, \\
C_{us} &= \frac{E[u,s,0] - C_{uu}u^2 - C_{ss}s^2}{us}, \\
C_{uv} &= \frac{E[u,0,v] - C_{uu}u^2 - C_{vv}v^2}{uv}, \\
C_{sv} &= \frac{E[0,s,v] - C_{ss}s^2 - C_{sv}v^2}{sv}. \tag{33}
\end{align*}
\]

These parameters completely determine \( H_{\text{min}} \), Eq. (32), and thus the equilibrium (minimized with respect to the slope parameters) elastic free energy profiles \( E_{\text{min}}(L) \), Eq. (31), with \( L \) the distance between the centers of the inclusions (the sides of the polygons).

In what follows, we compute \( E_{\text{min}}(L) \) for clusters of variable \( N \) at different separation distances \( L \), for a range of \( a \) values defining the trial function \( f_2(a, \phi) \), Eq. (27). The resulting energies are optimized over \( a \). In other words, we implement a classical Rayleigh–Ritz approach for optimizing the energy for a particular family of trial functions.

\[
\begin{align*}
\Delta &= \frac{2C_{ss}C_{uu} - C_{us}C_{uv}}{\Delta}, \\
&= \frac{2C_{st}C_{uu} - C_{sv}C_{uv}}{\Delta}. \tag{30}
\end{align*}
\]

Thus the total energy minimized over \( s \) and \( v \) can be expressed analogously to Eq. (22) as

\[
E_{\text{min}} = H_{\text{min}}u^2. \tag{31}
\]

where

\[
\begin{align*}
E_{\text{min}} &= \frac{2C_{ss}C_{uu} - C_{us}C_{uv}}{\Delta}, \\
&= \frac{2C_{st}C_{uu} - C_{sv}C_{uv}}{\Delta}. \tag{32}
\end{align*}
\]

IV. RESULTS AND DISCUSSION

A. Pairwise effects and cooperative influences

1. Isotropic approximation for the contact slope

The membrane-mediated interaction between inclusions is, in general, not additive. 11,12,17,20,29,34,35 Specific cooperativity predictions are both membrane model and perturbation type dependent. 11,20 Thus for curvature-mediated interactions it has been shown 35 that the interaction of a single pair is repulsive, while for five and more inclusions cooperativity leads to the formation of stable aggregates. In addition, some aspects of cooperativity have been addressed for the inclusion model considered here, involving both bending and compression of the membrane triggered by the hydrophobic matching condition (see, e.g., Refs. 11, 12, 17, 20, 25, 29, and 36).

We first illustrate these effects presuming the commonly used cylindrically symmetric boundary condition. As results for the constrained slope have been reported recently, 17,20,25 we focus on the consequences of the relaxed boundary condition \( s = s_{\text{min}} \). Here the pairwise interaction is repulsive. 20,36 However, an approximate treatment of a two-dimensional array of cylindrical inclusions 11 suggests that many-body interactions can lead to an attractive elastic force at small separations (see also Refs. 20 and 36). Figure 2 presents the \( L \) dependence of the total elastic free energy per inclusion for clusters of cylindrical inclusions of 1 nm radius in DMPC. It demonstrates how a similar transition occurs in clusters as the number of inclusions increases. 36 The notation \((m,k)\) indicates clusters of \( m \) inclusions at the corners of an equilateral \( m \)-gon with one \((k=1)\) or no \((k=0)\) inclusion at the cluster center. Since the parameter \( L \) is the distance between neighboring inclusions on the polygons’ sides, \( L/r_0 \) must be \( \geq 2 \). At large \( L \) the energy per inclusion approaches the mismatch energy of an isolated inclusion, as it must.

For \( N<5 \) the interaction is repulsive. For \( N=2 \) (not shown) repulsion increases rapidly as inclusions approach (also see below). As \( N \) increases, repulsion is damped until, for \( N\geq5 \), there is attraction at short distances. The attractive and repulsive regions are separated by an energy barrier, a tendency that becomes more pronounced when there is a central inclusion. This is illustrated for \( N=3 \) – 6 in Fig. 2. As
is apparent, as long as the contact slope is presumed to have cylindrical symmetry, attraction is a consequence of nonpairwise forces, and only for crowded clusters is a short-range attraction possible. However, there is no way to guarantee cylindrical symmetry, and some angular variation of the contact slope must be possible. We now consider how releasing the artificial constraint on the contact slope affects membrane-mediated interactions.

2. Anisotropic relaxation of the contact slope

Using Eqs. (31)–(33), we can study the influence of anisotropy of the relaxed contact slope for clusters of cylindrical inclusions of radius 1 nm in DMPC with the isotropic relaxed slope \( s = s_{\text{min}} \), as functions of \( L \), distance between inclusions on cluster polygon sides (see text); the asymptotic (isolated inclusion) free energy limit is 1.43 kT. Cluster structures (see text) are A: (3,1) □, (4,1) ●, (5,1) △, and (6,1) ▽ and B: (3,0) □, (4,0) ●, (5,0) △, and (6,0) ▽.

changes the interaction between inclusions for separations \( L \leq 4 \) nm. Even for a single pair, which is subject to increasingly strong repulsion at short separations presuming an isotropic boundary condition \( s = s_{\text{min}} \), anisotropy permits the appearance of an attractive region. The change from the trial functions, Eq. (25), to the more general (and more flexible) functions, Eq. (27), further intensifies the attraction at short separations.

Now contrast the results with two and four inclusions. Four inclusions are particularly interesting since, in the isotropic case, while the interaction is still everywhere repulsive, cooperativity has already had significant impact, nearly flattening the profile at short separations as is clearly illustrated in Fig. 2. We had expected that the impact of anisotropy should be less significant here than for two inclusions since with two nearest neighbors instead of one (for two inclusions) we expected the slope to vary more smoothly along the inclusions’ contours. Instead, a relaxed anisotropic
slopes have an unexpectedly strong impact, significantly reducing the elastic free energy. However, additional relaxation introduced by switching from Eq. (25) to Eq. (27) has a smaller effect than with two inclusions.

Results for DMPC and GMO are qualitatively similar. The large energy differences reflect two facts. Spring constants for the softer and thicker GMO membranes are ~1.5–2-fold smaller than those for DMPC. The equilibrium displacement \( u_0^{\text{GMO}} = 0.34 \text{ nm} \) is about twice that in DMPC. Together these factors account for the ~2.5-fold increase in elastic free energy per inclusion for GMO.

The individual spring constants are given in Table III for DMPC. The constants \( C_{uu}, C_{ss}, \) and \( C_{us} \) completely define the isotropic model. The choice of trial function describing anisotropy only affects the constants \( C_{vv}, C_{vv}, \) and \( C_{uu}, \) all functions of the anisotropy parameter \( v. \) The left-hand entries correspond to Eq. (25) and the right-hand entries to Eq. (27), in which case the parameter \( a \) was set to 1, usually the optimized value. Choosing Eq. (27) significantly softens \( C_{vv}, \) thus making anisotropic relaxation more effective than with Eq. (25).

Figure 4 illustrates the anisotropy of the optimized slope for two inclusions in DMPC. The anisotropy parameter \( v \) and the mean slope parameter \( s \) are determined by minimizing the elastic free energy, Eq. (31). Their ratio \( \rho = (v/s)_{\text{min}} \) is plotted as a function of the interinclusion separation \( L. \) For \( \rho = 0 \) the slope is isotropic, characteristic of isolated inclusions. Anisotropy is important for \( L < 4 \text{ nm} \). The transition from symmetric trial functions, Eq. (25), to asymmetric ones, Eq. (27), leads to an increased anisotropy of the optimized slope (increased \( \rho \)).

The essential features of anisotropic slope relaxation are further illustrated in Fig. 5 for two orientations: \( s_{\phi=0}, \) in the direction toward the midpoint between inclusions, and \( s_{\phi=\pi}, \) in the opposite direction. At small separations \( s_{\phi=0} \) is almost zero (the slope profile between inclusions is very smooth), but it decays steeply with varying \( L; \) \( s_{\phi=\pi} \) varies little with \( L. \) Only for \( L > 4 \text{ nm} \) does the slope become practically symmetric. The imposition of isotropic boundary conditions precludes this important relaxation pathway and leads to predictions of much larger elastic free energies per inclusion at small interinclusion separations, with their false consequences of small separation repulsion.

These results were obtained for an inclusion radius \( r_0 = 1 \text{ nm} \) characteristic of GA. One could reasonably ask how anisotropy could affect the interaction between somewhat larger inclusions, representative of the size of the peptide subunits that commonly assemble in the formation of functional physiological channels.\(^37,38\) To answer this question, we varied the inclusion radius from 1 to 2 nm. Results for a larger \( (r_0 = 2 \text{ nm}) \) inclusion in DMPC are presented in Fig. 6 (for ease of comparison, \( u_0 \) is chosen to be the same as for the GA inclusion just discussed). The trends in Figs. 3(a) and 6 are the same. Increasing \( r_0 \) by a factor of \( k \) in this range increases the energy per inclusion by a factor of \( \sim k^8, \) with \( k \) slightly less than 1. These results are in qualitative accord with the \( r \) dependence of the mismatch energy for a single inclusion,\(^23 \) \( \delta \) is also separation dependent, changing from \( \sim 0.81 \) at \( L = 2.5 \text{ nm} \) to \( \sim 0.92 \) at \( L = 6 \text{ nm} \). As the subunits forming a physiological channel assembly are quite mobile and can fluctuate relative to each other, it is more consistent to consider the membrane’s influence on a channel as perturbing the aggregation of the smaller individual peptide inclusions, rather than due to effects it may exert on a single

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**TABLE III**. Effective spring constants for two cylindrical GA inclusions in a DMPC membrane. Where appropriate, left- and right-hand entries correspond to Eqs. (25) and (27), respectively.

<table>
<thead>
<tr>
<th>( C_{uu}/L ) (nm)</th>
<th>2.5</th>
<th>3.0</th>
<th>3.5</th>
<th>4.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{aa} ) (kT/nm(^2))</td>
<td>239.2</td>
<td>257.8</td>
<td>274.4</td>
<td>290.2</td>
</tr>
<tr>
<td>( C_{ss} ) (kT)</td>
<td>242.1</td>
<td>198.2</td>
<td>188.3</td>
<td>187.3</td>
</tr>
<tr>
<td>( C_{vv} ) (kT)</td>
<td>181.14/65.1</td>
<td>137.4/44.2</td>
<td>125.0/43.7</td>
<td>121.5/43.3</td>
</tr>
<tr>
<td>( C_{uu} ) (kT/nm)</td>
<td>298.0</td>
<td>317.1</td>
<td>335.6</td>
<td>356.5</td>
</tr>
<tr>
<td>( C_{uv} ) (kT/nm)</td>
<td>(-102.5/-62.7)</td>
<td>(-95.0/-56.1)</td>
<td>(-79.8/-54.0)</td>
<td>(-56.7/-53.6)</td>
</tr>
<tr>
<td>( C_{vv} ) (kT)</td>
<td>79.1/61.3</td>
<td>33.6/21.7</td>
<td>20.7/12.8</td>
<td>15.7/9.8</td>
</tr>
</tbody>
</table>

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**FIG. 4.** \( L \) dependence of the ratio of the equilibrium slope parameters \( \rho = (v/s)_{\text{min}} \) for a DMPC membrane with symmetric [Eq. (25)], \( \Box, \) and asymmetric [Eq. (27)], \( \triangle, \) trial functions.
inclusion of very large radius. For this reason, we think that choosing \( r_0 \) in the 1–2 nm range is reasonably representative.

To this point all calculations rely on the conventional uniform membrane model, known to underestimate mismatch energy penalties. Figure 7 illustrates how local stiffening, required by the nonuniform model,\(^{19} \) may influence the pairwise inclusion interaction. We solve Eq. (10), the nonuniform Euler–Lagrange equation, with the relaxed boundary condition, Eq. (27), for both “uniform patch” and “exponential” approximations, Eqs. (15) and (16). As the characteristic decay length of elastic perturbations in membranes is short,

\[
\lambda_{\text{memb}} = \left( \frac{K h_0^2}{4 B} \right)^{1/4} \sim 1.0–1.5 \text{ nm},
\]

inclusion-induced effects are determined by membrane properties in a very narrow region; they are not sensitive to membrane behavior far from the inclusion. To demonstrate this explicitly we also treat a uniformly stiffened membrane, essentially the uniform patch model, Eq. (15), with \( \lambda_{\text{memb}} = \infty \). The stiffening parameters \( \theta \) were chosen to reproduce the elastic energy of a single GA inclusion in DMPC\(~\sim 4.6 \text{ kT}, \) required for consistency with channel lifetime measurements.\(^ {21,24} \) The \( \theta \) values were 4.75 (exponential), 4.21 (patch), and 3.75 (uniform).

All three approaches yield results qualitatively identical to those of Fig. 3(a). If the contact slope is isotropic, the inclusion interaction is always repulsive. Permitting contact slope anisotropy leads to an inclusion attraction for \( L \leq 3 \text{ nm}. \) Due to the explicit consideration of stiffening, the elastic free energies per inclusion are always greater than those of Fig. 3(a), approaching the asymptotic limit of \(~\sim 4.6 \text{ kT}, \) representative of GA in DMPC. Thus the qualitative influence of slope anisotropy is not altered by a nonuniform treatment and observations based on a uniform membrane model are quite generally representative.

\section{B. Some implications for protein function and aggregation}

The functional behavior of membrane proteins often depends on the lipid bilayer composition. It has been shown that chemical specificity is a relatively insignificant influence on protein–lipid interactions.\(^ {6,23,39–42} \) Much more important are the membranes’ general physical characteristics, such as their hydrophobic thicknesses and elastic moduli. Thus a mismatch between the proteins’ hydrophobic length and the thickness of the bilayer core results in the membrane adjusting itself to the protein by means of elastic deformation; this can affect the protein function directly\(^ {3,4} \) and also, indirectly, by inducing a redistribution or clustering of proteins in the bilayer.\(^ {1,2,5,6,44,44} \)

A recent instance where the membrane-mediated elastic interaction is important is the stabilization of linked gramicidin channels\(^ {6,25,45–50} \) (so-called “double-barreled”\(^ 6 \) or “tandem”\(^ {50} \) channels). Another factor influencing stabilization is an increase in local monomer concentration associated with the formation of the first barrel.\(^ {6,50} \) Furthermore, it is possible that in linked channels structural fluctuations within the individual GA dimers are suppressed, which also affects dimer stability.\(^ {25} \) The relative importance of these contributions has yet to be analyzed. The effect of lipid variation on interdimer separation at high GA/lipid ratios\(^ {16,17} \) and on heat capacity profiles of peptide-containing membranes\(^ {33} \) also provides indirect evidence for membrane-mediated influences in protein aggregation.

The elastic contribution to protein clustering and its influence on channel stabilization depends strongly on the choice of boundary conditions for the membrane slope at the

![Figure 6](image_url)

**FIG. 6.** Effect of slope anisotropy on the \( L \) dependence of the elastic free energy per inclusion for two cylindrical inclusions of radius \( r_0 = 2 \text{ nm} \) (asymptotic limit 2.83 kT): isotropic, \( \square \) anisotropic, Eq. (25), \( \Delta \) anisotropic, Eq. (27), \( \triangledown \).

![Figure 7](image_url)

**FIG. 7.** \( L \) dependence of the elastic free energy per inclusion for a pair of GA-like inclusions in DMPC. The “patch,” Eq. (15) (\( \bigcirc \) and \( \bullet \)), and “exponential,” Eq. (16) (\( \bigtriangleup \) and \( \blacktriangle \)), implementations of local nonuniformity are contrasted with results for a uniformly stiffened membrane (\( \bigtriangledown \) and \( \blacktriangledown \)). Open and solid symbols correspond to isotropic and anisotropic, Eq. (27), slopes, respectively. The stiffening factors \( \theta \) of Eqs. (15) and (16) are adjusted to reproduce the elastic free energy for an isolated GA inclusion in DMPC\( \sim 4.6 \text{ kT}, \) determined from the membrane’s effect on channel lifetimes; the free energies’ asymptotic limits are thus 4.6 kT.
boundary of the interacting inclusions. If the isotropic constrained condition for the contact slope, \( s = 0 \), is used, then elastic coupling alone could account for most of the stabilization observed for linked GA channel dimers, \( s \text{min} \) however, if the slope constraint were only slightly altered, becoming moderately negative, the interaction would be repulsive.17,20 Using the isotropic relaxed slope \( s = s \text{min} \) results in repulsion and destabilization of the linked channel dimers.20,23 Here only cooperative influences at high channel concentrations would lead to aggregation. However, most experiments with linked GA channels are conducted under “single-pair” conditions. As just demonstrated, accounting for the slope anisotropy and permitting the slope to adjust itself to minimize the elastic free energy results in a short-range attraction even for a single pair and can thus contribute to stabilization. This highlights the importance of a consistent approach to treating slope optimization. The relation between elastic stabilization and other contributions is still an open question. Of particular importance is modifying the nonuniform model to account for possible cooperative influences of the inclusions on elastic moduli.

Adjacent membrane proteins can be functionally coupled by the lipid bilayer. A most likely coupling mechanism is the hydrophobic matching between the membrane and protein. This results in bilayer deformation in response to conformational changes in proteins; these propagate through the membrane and thereby affect adjacent proteins. Given that about \( \sim 30\% \) of the cell surface area is occupied by bilayer-spanning segments of integral membrane proteins, bilayer-mediated protein interactions can have an important physiological role. Possible functional instances where such clustering may be significant include growth factor receptor activation and tyrosine autophosphorylation, both of which involve receptor aggregation in the cell membrane.21,23 It may also play a role in the self-assembly of proteins into ion channels. A protein’s cytoplasmic and extracellular sequences may act in much the same way as the linkers in coupled gramicidins, ensuring that the individual membrane-spanning helices are fairly close to one another. Then, due to slope anisotropy, hydrophobic mismatch can provide a driving force for association in its initial stages. At a later phase this can be further enhanced by the nonpairwise effects.

Our treatment of nonuniformity is only preliminary. Further study is needed to account for the combined influences of multiple inclusions on membrane elastic constants. But whatever the specifics, the analysis implies that the development of a consistent treatment of membrane relaxation is very important, affecting both quantitative results and qualitative aspects of membrane-mediated cooperation.

V. CONCLUSIONS

(i) The “coupled harmonic oscillator” representation combined with the finite-difference algorithm introduced previously provides a computationally efficient and physically transparent tool for studying membrane-mediated interactions.

(ii) Properly treating relaxed boundary conditions crucially influences predicted effects due to the membrane-mediated interaction between inclusions. Assuming that the relaxed slope \( s = s \text{min} \) is isotropic, the expectation is that the interaction between a single pair is everywhere repulsive. The improved treatment, permitting anisotropic slope relaxation, dramatically affects the interaction, leading to a short-range attraction. This observation holds regardless of whether the membrane, in the presence of inclusions, is modeled conventionally (uniform) or as nonuniform.

(iii) Membrane-mediated interaction due to hydrophobic matching is highly cooperative. For the isotropic relaxed slope \( s = s \text{min} \), the repulsion predicted for a single pair becomes less prominent for larger clusters and even turns into a short-range attraction for pentameric (or larger) clusters (high peptide concentration). With anisotropic slope relaxation, cooperative influences associated with increasing cluster size further augment the short-range attraction and consequently the driving force towards aggregation.

(iv) A consistent description of membrane relaxation in contact with inclusions can be important in the stabilization, aggregation, and functional coupling of membrane integral proteins.

ACKNOWLEDGMENT

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APPENDIX: GLOSSARY OF SYMBOLS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>( u )</td>
<td>monolayer thickness perturbation</td>
<td>nm</td>
</tr>
<tr>
<td>( r_0 )</td>
<td>inclusion radius</td>
<td>nm</td>
</tr>
<tr>
<td>( L )</td>
<td>distance between inclusion centers</td>
<td>nm</td>
</tr>
<tr>
<td>( u_0 )</td>
<td>monolayer perturbation at the membrane-inclusion boundary</td>
<td>nm</td>
</tr>
<tr>
<td>( h_0 )</td>
<td>unperturbed bilayer thickness</td>
<td>nm</td>
</tr>
<tr>
<td>( l_H )</td>
<td>hydrophobic length of an inclusion</td>
<td>nm</td>
</tr>
<tr>
<td>( s )</td>
<td>(isotropic) contact slope parameter</td>
<td>—</td>
</tr>
<tr>
<td>( v )</td>
<td>parameter describing contact slope anisotropy</td>
<td>—</td>
</tr>
<tr>
<td>( B(x,y) )</td>
<td>nonuniform volume-compression–expansion modulus</td>
<td>pN/nm²</td>
</tr>
<tr>
<td>( K(x,y) )</td>
<td>nonuniform bending modulus</td>
<td>pN</td>
</tr>
<tr>
<td>( B_0 ), ( K_0 )</td>
<td>unperturbed values of elastic moduli</td>
<td>—</td>
</tr>
<tr>
<td>( F[u] )</td>
<td>elastic free energy functional</td>
<td>kT</td>
</tr>
<tr>
<td>( E )</td>
<td>elastic free energy after Euler–Lagrange minimization</td>
<td>kT</td>
</tr>
<tr>
<td>( E_{\text{min}} )</td>
<td>elastic free energy, after boundary parameter minimization</td>
<td>—</td>
</tr>
<tr>
<td>( \theta )</td>
<td>nonuniform model surface stiffening parameter</td>
<td>—</td>
</tr>
<tr>
<td>( \lambda_p )</td>
<td>patch radius in nonuniform “stiffened patch” model</td>
<td>nm</td>
</tr>
<tr>
<td>( \lambda_r )</td>
<td>decay length in nonuniform “exponential” model</td>
<td>nm</td>
</tr>
<tr>
<td>( \alpha, \beta )</td>
<td>generalized boundary parameters</td>
<td>kT</td>
</tr>
<tr>
<td>( C_{\alpha\beta} )</td>
<td>coupling constants in CHO representation, Eq. (23)</td>
<td>—</td>
</tr>
<tr>
<td>( H_{\text{min}} )</td>
<td>cumulative cluster spring constant, Eq. (31)</td>
<td>kT</td>
</tr>
<tr>
<td>( s_{\phi} )</td>
<td>anisotropic slope</td>
<td>—</td>
</tr>
<tr>
<td>( \phi )</td>
<td>polar angle</td>
<td>rad</td>
</tr>
</tbody>
</table>
Membrane inclusions as coupled harmonic oscillators

\[ t_d(a, \phi) \] trial function for anisotropic slope parametrization

\[
\begin{align*}
\text{DMPC} & \quad \text{dimysteoyl phosphatidyl choline} \\
\text{GMO} & \quad \text{glyceryl monooleate} \\
\text{GA} & \quad \text{gramicidin A} \\
\text{CHO} & \quad \text{coupled harmonic oscillators}
\end{align*}
\]

\[ N \] number of inclusions in cluster

\[ E_{\text{con}}(s) \] elastic free energy, conventional (uniform model) \( \text{KT} \)

\[ E_{\text{bd}}(s) \] inclusion induced boundary term, uniform model \( \text{KT} \)

\[ E_{\text{mod}}(s) \] elastic free energy, nonuniform model \( \text{KT} \)

\[ \gamma_i \] boundary contour, inclusion \( i \)

28. As has been stressed previously (Ref. 19), if the contribution \( E_{\text{bd}} \) were important, it should be included explicitly both in the analysis of the membrane-mediated interaction between inclusions and in the energetics of channel formation, which has never been done. Also, the interactions responsible for this "boundary" contribution cannot be localized at the inclusion–membrane interface, but must affect membrane properties in the vicinity of the inclusion. It is well known that even well-defined solid interfaces are actually transition regions where the properties of both phases are strongly modified. This should be even more so for the soft contacts involving membranes. We suggest that our nonuniform model partially addresses this concern.
32. In what follows it will be apparent that, while the set of constants \( u_i \) and \( s_i \) uniquely determines the solution for isotropic boundary conditions, additional parameters are required to account for possible contact slope anisotropy.