Stabilization of ion channels due to membrane-mediated elastic interaction

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Recent work shows that linked gramicidin channels may have much longer lifetimes than single channels. We establish that the stabilization of the individual channels can be caused by membrane-mediated elastic interactions between such inclusions. In linear elastic theory, interaction can be rigorously described in terms of coupled harmonic oscillators. We determine the ''effective spring constants'' for various assemblies using the smectic bilayer model. We consider a range of aggregates; in clusters, channel lifetimes may increase by several orders of magnitude, an effect that is especially pronounced for a channel with many near neighbors. © *2003 American Institute of Physics.* [DOI: 10.1063/1.1572460]

I. INTRODUCTION

Membrane-spanning peptides and other ''defects'' in lipid bilayer membranes cause local membrane deformations; these extend laterally for 2–3 nm and contribute significantly to both the energetics of the inclusion[1–5] and the membrane-mediated interaction between peptides.[6–13] An ideal system for studying this is the ion channel gramicidin A (GA), a transmembrane dimer (D) formed by head-to-head monomeric association.[14] Usually the hydrophobic length of the assembled GA dimer is less than the hydrophobic thickness of hosting membranes; thus, formation of a GA channel is expected to cause local thinning and compression of the membrane. Measurements of the dependence of GA channel lifetimes on the hydrophobic thickness of different groups of bilayers indirectly supports this prediction.[15–17] Recently, thinning of dimyristoylphosphatidylcholine (DMPC) bilayers associated with the insertion of GA channels was measured directly by x-ray diffraction.[10]

Changes in gramicidin channel lifetimes accurately report the energetics of membrane deformation associated with an inclusion.[1–4] Membrane deformation imposes elastic forces on a channel, stressing the hydrogen bonding between the GA monomers (M). For isolated channels, increased deformation facilitates monomer separation and shortens channel lifetimes. Recently, interest has shifted to studying collective effects in channel kinetics. This is a reflection of more general interest in the influence of cooperativity on the functioning of physiological ion channels.[18–26] For this purpose, specially designed GA monomers have been synthesized, covalently coupled at their water termini by various macromolecular water-soluble linkers.[27–32] These typically exhibit noticeably increased channel lifetimes.

We show that this mutual stabilization may be rationalized in terms of membrane-mediated interaction between the peptides. The physics is straightforward. The energy barrier for the $D \Leftrightarrow 2M$ reaction controls the lifetime of the GA dimers; it is determined by the complex relative movements of the monomers involving fluctuations in the length of the channel assembly. Membrane deformation created by the neighboring channels influences the magnitude of these fluctuations. By effectively thinning the membrane immediately surrounding an individual channel, its neighbors decrease the elastic force tending to separate its monomers, thus stabilizing the dimer.[33] A similar mechanism was proposed in general terms to describe aggregates of membrane ''pinning sites,'' such as specific ''lock and key'' molecules holding membranes together or a membrane to an adhesive surface.[8] In our approach, the forces stabilizing the channel are explicitly modeled, which permits a quantitative treatment of channel lifetimes.

We first formulate the problem in terms of the smectic bilayer model.[1–3,6,13,34] The elastic coupling between insertions is then expressed using a rigorous representation of the system of membrane inclusions in terms of coupled harmonic oscillators.[13,35] This permits description of the membrane-mediated interactions in a physically transparent fashion, in terms of ''effective spring constants.'' In addition to conceptual simplicity, this approach also leads to significant computational efficiency in the treatment of channel aggregates.

The issue of membrane-mediated interaction between inclusions is not a new one. It has been intensively studied during the last decade from various perspectives and for different models of membranes and insertions (see Refs. 6–12, 36–38, and references therein). The special significance of our approach is that it provides a natural unified treatment of both the lateral interaction forces between the channels, which have been the focus of most previous studies, and the normal forces affecting the fluctuations in length of an individual channel, which are directly related to the stability of that channel. It is this unification that allows us to describe quantitatively cooperative influences on channel stability. In what follows, we consider some representative clusters, analyze the behavior of the spring constants, show how these determine the energy barrier for the $2M \Leftrightarrow D$ transition, and demonstrate that, under experimentally realizable conditions clustering can lead to orders of magnitude increases in GA lifetimes.

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II. MODEL AND EQUATIONS

In the simple model for GA insertion, the elastic free energy arises from the vertical displacement (u_0) of lipid molecules in immediate contact with a (cylindrical) insertion of radius r_0. This is required to better match the hydrophobic tails of lipid molecules to the hydrophilic exterior of the inserted peptide. It is defined by the "hydrophobic matching condition:"[1,2,11]

$$2u_0 = h_0 - l_h, \tag{1}$$

where h_0 and l_h are the unperturbed hydrophobic membrane thickness and the hydrophobic length of the peptide, respectively. Being perturbed at the interface, the membrane profile $u(x,y)$ adjusts itself gradually to minimize the elastic energy.

These phenomena are traditionally described in terms of "smectic bilayer" theory.[1–3,6,34,39] Neglecting the relatively insignificant surface tension contribution[2,40] leads to the elastic free-energy expression

$$F = \int [b\ (\nabla u)^2 + a\ u^2] d^2\mathbf{r}, \tag{2}$$

where $a = 2B/h_0$ and $b = h_0 K/2$, B and K are stretching and bending moduli respectively, and integration is over the membrane surface. The corresponding Euler–Lagrange equation

$$b\nabla^2 u(x,y) + a u(x,y) = 0, \tag{3}$$

can be solved numerically with the appropriate boundary conditions.

For finite-size clusters far from the membrane boundaries, both $u(r)$ and ∇u vanish at the external boundaries L_∞:

$$u(L_\infty) = \nabla u(L_\infty) = 0. \tag{4}$$

Equilibrium displacement of the ith insertion along the contour L_i is defined by the matching condition, Eq. (1). To study many-body effects, we permit the interfacial displacements, u_i, to fluctuate relative to their equilibrium values u_0, generally assuming that

$$u(L_i) = u_i, \tag{5}$$

where $u_i \neq u_0$. There are several ways to choose the second boundary condition at L_i (see Refs. 12 and 13 for details). Typically, one fixes the contact slope of the membrane at the boundary of an inclusion:

$$\nabla u(L_i) = s_i, \tag{6}$$

where s_i is usually considered constant, either fixed or determined by energy minimization,[1–3] although this can be generalized to allow an azimuthal variation of the contact slope along the boundaries L_i.[35,41]

Studies of a single inclusion show that the so called "constrained boundary condition" for the contact slope s_i,

$$s_i = 0, \tag{7}$$

is consistent with the influence of the membrane on channel lifetimes.[1,3,42] As lifetimes are the focus of this article, we use this boundary condition in our calculations. With these conditions Eq. (3) is solved numerically using a finite difference approach.[12,41] When tested against analytic results for a single inclusion,[41] agreement in energy was better than 2% for the grid size used here, 0.05 nm.

III. EFFECTIVE SPRING CONSTANTS FOR INTERACTING INCLUSIONS

In the linear elastic theory used here and in most related studies,[43] channel interaction and cooperativity can be rigorously characterized in terms of effective spring constants.[13,35] The fact that the elastic energy due to a single insertion is a quadratic function of the boundary parameters u_0 and s was first derived in Ref. 3 from an analytical solution of the cylindrically symmetric problem (see also Refs. 4 and 5). Later, it was noticed that the quadratic form for the energy in terms of the boundary parameters is a general consequence of the linearity of Eq. (3) and its analogs (accounting, for instance, for a possible spatial variation of the membrane elastic moduli) and of the boundary conditions, Eqs. (5)–(7).[13,35]

We briefly outline the derivation for a particular case, assuming in Eq. (6) that s_i is constant (i.e., neglecting any possible azimuthal dependence of the slope along the contour of the ith inclusion). Following the discussion in Refs. 13 and 35, we introduce the "superfinite" elements, $\phi_i^u(r)$ and $\phi_i^s(r)$, solutions of Eq. (3) satisfying the boundary conditions Eq. (4) and the following conditions on the contour γ_k for the kth insertion:

$$\phi_i^u(\mathbf{r})\big|_{\gamma_k} = \delta_{ik}, \quad \nabla\phi_i^u(\mathbf{r})\big|_{\gamma_k} = 0, \tag{8}$$

$$\phi_i^s(\mathbf{r})\big|_{\gamma_k} = 0, \quad \nabla\phi_i^s(\mathbf{r})\big|_{\gamma_k} = \delta_{ik}, \tag{9}$$

where δ_{ik} is the Kronecker symbol. Then, as the problem is linear, the solution of Eqs. (3)–(6) can be expressed in terms of the boundary parameters u_i and s_i

$$u(r) = \sum_{i=1}^{N} [u_i \phi_i^u(r) + s_i \phi_i^s(r)]. \tag{10}$$

Substituting this into Eq. (2) leads directly to an expression for the elastic energy as a biquadratic function of the boundary parameters u_i and s_i.

Quite generally, given that more than two boundary parameters can be fixed (for instance, accounting for possible azimuthal variation of the slope,)[41] the energy can be expressed as

$$F = \sum_{i=1}^{N} \sum_{j=i+1}^{N} c_{ij}^{\alpha\beta} \alpha_i \beta_j,$$

where an additional summation is performed over the repeated indexes α, β; these are symbolic names for the boundary parameters, α, $\beta = u$, s, etc. The coefficients $c_{ij}^{\alpha\beta}$ are expressed via generic functions ϕ_i^α and ϕ_k^β satisfying the boundary conditions with α_i, β_i taking values 1 or 0 [similar to Eqs. (8) and (9)] and thus do not depend on the actual numerical values of the boundary parameters.[13,35]

With the restricted boundary condition Eq. (7), this general result is reduced to a quadratic function of the boundary displacements u_i. To emphasize the analogy with coupled harmonic oscillators, we express it as

$$F=\sum_{i}^{N} a_{ii}u_i^2+\sum_{i=1}^{N-1}\sum_{j=i+1}^{N} a_{ij}(u_i-u_j)^2, \tag{11}$$

where a_{ij} are effective spring constants.[3,12,13,35,44] Diagonal components a_{ii} describe the elastic "self-energy" due to the deformation of the membrane surrounding the ith insertion; nondiagonal components a_{ij} describe the coupling between insertions i and j propagated via the membrane deformation. Equation (11) indicates that for any fixed inclusion configuration, the energies of all possible fluctuations in the displacements u_i are defined by a relatively few spring constants, which reduces computational complexity dramatically.

To demonstrate this, consider two inclusions. To study the statistical properties of such a pair, one would have to solve Eqs. (3)–(7) numerically for all conceivable combinations of u_1 and u_2 at all interinsertion distances, d. The preferred alternative is to use the expression

$$F=a_{11}(d)(u_1^2+u_2^2)+a_{12}(d)(u_1-u_2)^2 \tag{12}$$

derived from Eq. (11) with the symmetry condition $a_{11}=a_{22}$. To find the elastic constants at each separation, the elastic problem need be solved for only two linearly independent sets (u_1, u_2). The system of two equations for a_{11} and a_{12} is derived by substituting these sets of values on the right-hand side of Eq. (12), and the corresponding values of energy Eq. (2) on the left-hand side. With these constants in hand, Eq. (12) describes the energies of all possible fluctuations. The gains are obvious. This approach can be easily generalized for $N>2$, where the benefits are even greater.

IV. PROPERTIES OF THE EFFECTIVE SPRING CONSTANTS

To investigate the interaction between inclusions, we treat an illustrative set of clusters. The maximum number of spring constants, a_{ij}, is $N(N+1)/2$. The more symmetric the N-mer cluster, the fewer a_{ij} that are independent. Three specific cases are sufficient to demonstrate the general tendencies: Two inclusions, three symmetric collinear inclusions, and seven inclusions forming a regular, centered, and symmetric hexagon.

We now particularize to the case of gramicidinlike cylindrical inclusions of radius $r_0=1$ nm in DMPC membranes:[11] $h_0=2.53$ nm, $B=50$ pN nm^{-2}, $K=40$ pN, and $u_0=0.165$ nm. We vary the distance of closest approach between neighboring insertion surfaces (d) from 0.5 to 4.0 nm. At yet larger d effective elastic interinclusion coupling is found to be negligible; they are essentially isolated. Figure 1 illustrates the d dependence of the a_{ij} in representative cases:

(a) two inclusions, two independent a_{ij}, $a_{11}(\equiv a_{22})$ and a_{12};
(b) three symmetric collinear inclusions, four independent a_{ij}, $a_{11}(\equiv a_{33})$, $a_{12}(\equiv a_{23})$, a_{22}, and a_{13}, with practically no coupling between the cluster edges as a_{13} is always small; and
(c) seven inclusions forming a regular, centered, symmetric hexagon, equivalent to the "crowded" cluster limit of dense hexagonal packing in a two-dimensional array, with six independent a_{ij}, a_{11}, a_{12}, a_{13}, a_{14}, a_{77} and a_{17} (with 7 the central insertion); here second, and third-nearest-neighbor inclusions are effectively decoupled since a_{13} and a_{14} are always negligible (not shown).

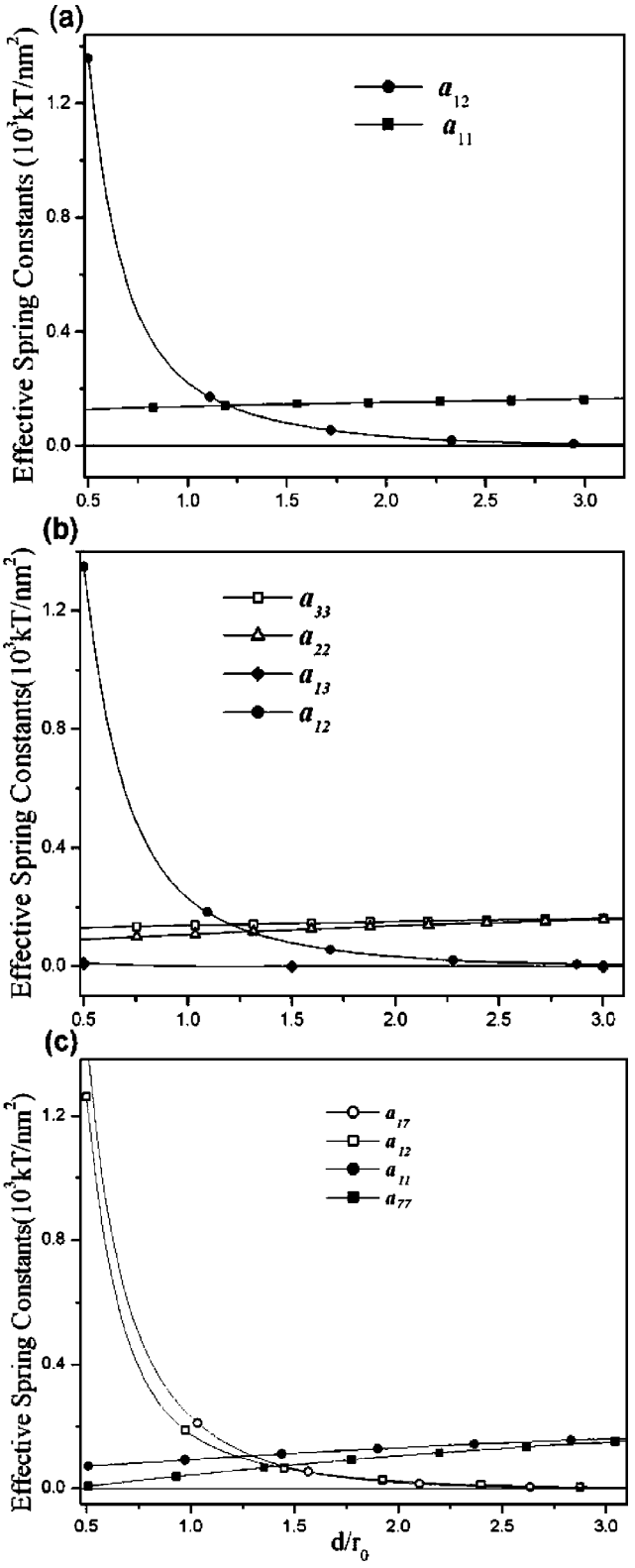

FIG. 1. Effective spring constants for three representative clusters: (a) two inclusions, (b) three symmetric collinear inclusions, and (c) seven inclusions forming a regular symmetric centered hexagon.

Regardless of cluster type, the a_{ij} exhibit some common features:

(1) For $d \gtrsim 3$ nm, diagonal constants a_{ii} approach those for independent inclusions; nondiagonal constants vanish. Second- (and higher) neighbor interaction is always insignificant. As noted herein, at such large separations the inclusions may be considered isolated.
(2) The a_{ii} decrease at small d, roughly proportionally to the number of nearest neighbors. For two inclusions ~ 0.5 nm apart a_{11} is $\sim 15\%$ less than for the isolated insertion [Fig. 1(a)], at this separation a_{77} (six neighbors) is nearly zero [Fig. 1(c)].
(3) Nondiagonal a_{ij} increase as d decreases. Nearest-neighbor coupling is well fit by the expression $a_{i,i+1} \sim \alpha \exp(2.5/\lambda)\exp[-(x+2)/\lambda]$, with $\alpha \sim 1.25-1.38$ and $\lambda \sim 0.3-0.35$; here $x+2$ is the scaled center-to-center interinclusion distance (in units of r_0). The coupling strength is only weakly ($<10\%$) dependent on the ''packing'' of the insertions, implying that the nondiagonal contributions are also effectively additive.
(4) Both the softening of the diagonal constants a_{ii} and the increase in the a_{ij} reflects the fact that in clusters inclusions are better adjusted to the collectively deformed membrane than the isolated insertion was to the original (unperturbed) membrane. Put differently, for channels, collective deformation of the membrane decreases the elastic force trying to separate the monomers and return the membrane to its original state. In fact, the deformed state of the membrane effectively becomes a new reference state for the elastic ''springs'' acting on the channels.
(5) In sum, clustered inclusions tend to stabilize one other, which, for channels, should lead to increased lifetimes.

V. CHANNEL LIFETIMES

Given the effective elastic constants for a particular inclusion arrangement, it becomes possible to study the elastically coupled fluctuations of channel forming monomers which contribute to possible cooperativity in channel lifetimes τ_i for GA clusters.

A detailed description of τ_i requires considering multiple phenomena including the complex internal dynamics of GA dimers and their coupling with the membrane, diffusion and recombination of the monomers, etc. We discuss a simplified picture emphasizing how elastic coupling stabilizes ion channels. We assume all channels but one (whose stability is being studied) are locked in the conductive dimer state, and determine how they influence the stability of the selected channel relative to an isolated channel. This simplification is reasonable because V_{intr}, the ''intrinsic barrier'' to separation due to the six intermonomer hydrogen bonds, is very deep (~ 40 kT) and steep.[45] Thus, a dimer rarely strays from the potential minimum ($u=u_0$).

The total cluster energy includes both intrinsic and elastic components

$$W(\mathbf{u}_N) = F(\mathbf{u}_N) + \sum_{i=1}^{N} V_{\text{intr}}(u_i), \tag{13}$$

where $\mathbf{u}_N = \{u_1, u_2, \ldots, u_N\}$. We assume that the short-range intrinsic contribution is unaffected by the surrounding channels. Using a molecular model for the GA dimer, we have shown this assumption to be reasonable for $d \gtrsim 0.5$ nm.[46] At yet smaller separations, short-range (quadrupole type) electrostatic and van der Waals attractive forces between the dimers become dominant. These effects have been studied for three known GA structures:[46] 1GRM,[47] 1JNO,[48] and 1MAG.[49] Depending on the structure employed, for $d \lesssim 0.5$ nm, the attractive interaction energy between a pair of rigid dimers in favorable orientations can be quite large, a substantial fraction of the total hydrogen bonding stabilization in the $2M \Leftrightarrow D$ reaction;[46] it can thus be an important component of the driving force in the formation of double-barreled GA channels.[32] However, at such small dimer separations interaction is highly dependent on the structure and relative orientation of the individual channels. Tryptophan–tryptophan interactions may play a specific role, conceivably limiting direct interchannel contact.[32,50,51] Thus, we restrict our analysis to larger d, $\gtrsim 0.5$ nm, where these short-range interdimer effects are not significant and cooperative effects between the channels can be reasonably described in terms of membrane-mediated interaction.[6–8,10,11,32,36,37,52]

We now consider gramicidin rupture using the Arrhenius description of activation. From modeling and experiments with modified GA channels[4,53] the intermonomer hydrogen bonds break easily when the monomer separation δ (relative to the ''dimeric'' state) reaches a ''transition state'' distance ~ 0.16 nm. Following Ref. 4, we use this critical separation distance in estimating channel lifetimes.

The relative probability of separation (i.e., attaining the transition state) for the preselected channel (i) in the cluster is

$$\frac{P^i_{\text{cluster}}(d)}{P_{\text{isolated}}} \sim \exp[-(\Delta W^i(d) - \Delta W^i(\infty))/kT], \tag{14}$$

where $\Delta W^i(d)$ is the transition barrier for channel i, the energy difference between the configuration with $u_i = u_0 - \delta/2$ (the transition state), and $u_i = u_0$ (the ground dimer state). The other $N-1$ channels are ''locked'' in their dimer states ($u_j = u_0$ for $j \neq i$). The limit $d=\infty$ corresponds to an isolated channel. The intrinsic barriers cancel in Eq. (14), and the relative probability is determined by the elastic interaction between the channels. Since τ is inversely proportional to P, the lifetime ratio is:

$$\log(\tau^i_{\text{cluster}}/\tau_{\text{isolated}}) = (\Delta F^i(d) - \Delta F^i(\infty))/2.3, \tag{15}$$

where $\Delta F^i(d)$ is the transition elastic barrier, scaled in units of kT at 300 K. Our results are presented in Figs. 2(a)–2(c) for different clusters and channels.

Clustering increases the channel lifetimes, and is more pronounced for channels with more nearest neighbors. Thus, for our choice of membrane (DMPC), the lifetime increase is $\sim$fivefold for two channels at $d=1.0$ nm; for the middle channel of the trimer the effect is more than tenfold and it reaches several orders of magnitude for the channel at the hexagon center.

The Arrhenius approach is based on the implicit assumption of a ''reaction coordinate'' and an associated transition

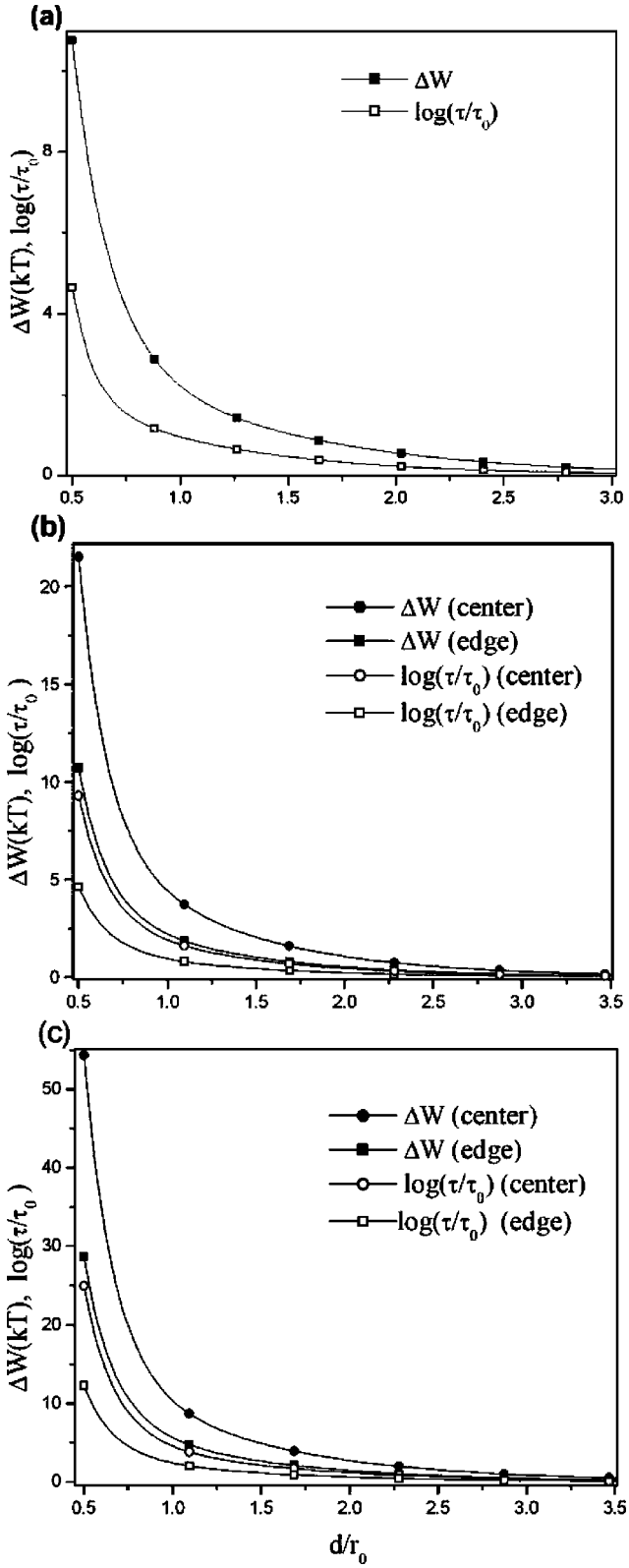

FIG. 2. d dependence of the energy barrier and lifetime ratio for three representative channel clusters: (a) two insertions, (b) three symmetric collinear insertions, and (c) seven insertions forming a regular symmetric centered hexagon.

state. However, sufficient energy does not guarantee reaction. ''Transmission coefficients'' can be substantially less than unity,[54] a point recently stressed in the context of ion channel transport.[55] As our concern is not with absolute reaction rates but with relative reactivity due to the environmental changes we should not expect major changes in transmission due to modification of the surroundings of the channel.

The behavior illustrated in Fig. 2 is only representative. The lifetime ratio, Eq. (15), depends exponentially on free-energy changes in clustering, which are very sensitive to membrane specifics, especially the mismatch. A mismatch change of only 20% (assuming no other changes) would double the lifetime ratio. Thus, it follows that the significant stabilization of linked GA channels, resulting in up to 100-fold increases in channel lifetimes,[27–31] can be reasonably rationalized as due to membrane-mediated elastic interaction. A recent report, in accord with our results, indicates that in the presence of polyelectrolytes with multiple binding sites (polylysine chains) GA-analogs form oligomeric clusters (domains) enhancing the stability of the channel.[56]

These lifetime estimates are for channels separated by $d=1$ nm. The strongly interacting (double-barreled) assemblies observed experimentally are believed be in even closer, possibly immediate, contact.[32] This can further stabilize the channels[46] by directly hindering some of the relative monomer motions associated with the rupture pathway, e.g., separation, screw movement, tilt and lateral displacement.[45,57] The balance between these factors in promoting channel stabilization, and the dynamics of the transition between the double-barreled and semiseparated states, are both important questions for the future.

Further analysis is needed to explain finer details of the experiments, to reproduce opening–closing kinetics, and to extract from the experimental data information about clustering of the linked channels. The influence of the linker on lateral diffusion of monomers may also affect channel stabilization.[30] Ongoing experimental studies on how membrane thickness and elastic moduli alter channel lifetimes[32] would stimulate further progress in the theoretical understanding of these phenomena.

VI. SUMMARY

In conclusion, we reformulated the elastic problem for interacting membrane inclusions, explicitly accounting for possible variability in channel length. These degrees of freedom permit a natural description of the forces affecting the relative motion of GA monomers in the dimer, thus influencing the stability of the channel. Membrane-mediated cooperative behavior is rigorously expressed in terms of coupled harmonic oscillators. We described an efficient numeric algorithm determining the corresponding effective spring constants for any particular inclusion arrangement, thus allowing straightforward study of the stability of individual channels in a cluster. We found that membrane-mediated interaction can significantly increase channel lifetimes, an effect which becomes more pronounced as channel concentration increases.

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[42] For a single ion channel this results in a ''linear spring model'' describing membrane deformation due to insertion as in C. Nielsen, M. Goulian, O. S. Andersen, Biophys. J. **74**, 1966 (1998).
[43] In contrast, a nonlinear elastic approach has been used in R. Menes and S. Satran, Phys. Rev. E **56**, 1891 (1997), where the focus was on the significant membrane thinning due to pinning sites. It should be stressed that linearity means that Euler–Lagrange equations [such as Eq. (3)] are of the form $\mathbf{L}(\mathbf{u})=\mathbf{0}$, where $\mathbf{L}$ is a linear differential operator, a requirement that imposes no restrictions on the order of the differential equations, typically biharmonic for the problems of interest here.
[44] We omitted the normal coefficient of 1/2 in Eq. (11), a convention corresponding to the definition of the effective spring constant for an individual insertion [Eq. (21) of C. Nielsen, M. Goulian, and O. S. Andersen, Biophys. J. **74**, 1966 (1988)]; it has no physical influence. Equation (11) also holds if the contact slope is not constrained to 0 but adjusts spontaneously to minimize the energy. It is also applicable if interaction with the insertion locally stiffens the membrane, an alternative model that accounts for membrane influences on individual channel lifetimes. This nonuniform treatment has not yet been extended to account for membrane-mediated interactions. Therefore, we use a conventional model, with the ''constrained'' boundary condition.
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