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Many-body forces between membrane inclusions: A new pattern-formation mechanism

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Abstract

The elastic interaction of membrane inclusions provides a unique physical realization of multibody forces. We derive an expression for the multibody curvature interaction between membrane proteins, and show that this interaction cannot be approximated by a pairwise additive theory. However, the interaction can be decomposed into a repulsive pairwise part and a non-pairwise part of similar magnitude which is anisotropic and can be attractive. This non-pairwise interaction can create stable spatial patterns which arise from an interplay between thermal fluctuations and competing pairwise repulsive and non-pairwise attractive interactions. We present numerical simulations which show how aggregates can assemble into ordered patterns from a random initial distribution at room temperature.

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Introduction

Whenever the interaction of two bodies is affected by the presence of other nearby bodies, the principle of pairwise superposition may not be adequate to describe the net interaction energy of the many-body system. A well-known example of this is the van der Waals interaction between molecules. The contribution of this non-additivity to the overall energy is usually small, less than 20%^[1], and so in many cases one can treat non-pairwise interactions as a perturbation to the dominant pairwise interactions. Here we investigate the interaction between membrane inclusions which is strongly non-additive. The non-pairwise contributions are too large to be treated as a perturbation. Moreover, they are anisotropic, which makes the interaction energy strongly dependent on the relative orientations of the inclusions, giving rise to ordered patterns of aggregates. This form of the interaction permits an explicit separation into pairwise and non-pairwise terms. This illuminates the nature of these multibody interactions, and lends itself to analytical treatment.

Recent studies have focused on a particular kind of interaction between membrane proteins (here referred to as inclusions) that is mediated by the elasticity of the membrane^[2-5]. An embedded inclusion creates a deformation field in the surrounding membrane that influences neighboring inclusions. The nature of this deformation field is dictated by the inclusion's elasticity, its shape, and by the membrane's elastic properties. In an earlier paper^[6], we examined the deformation field generated by inclusions embedded in a membrane whose elastic energy is determined by its curvature. Previous work revealed a long range interaction between two inclusions with potential energy proportional to $1/r^4$ ^[5]. In the absence of thermal fluctuations ($T \equiv$ absolute temperature = 0), this interaction is repulsive. Our previous work examined the zero temperature limit, and agreed with prior studies when the system consists of only two inclusions^[6]. However, we found that the interaction between $N > 2$ inclusions was strongly non-pairwise additive. Therefore, simple pairwise theories of inclusion aggregation due to membrane curvature are misleading and cannot accurately describe large assemblies of inclusions. This led to the surprising result that aggregates relax to stable equilibria even though the pairwise component of the interaction is repulsive. Here we explore further the properties of this interaction and generalize the result to demonstrate that patterned arrays can arise from random initial distributions under the influence of non-pairwise forces and thermal fluctuations.

The multibody interaction energy

Our derivation of the interaction energy begins by representing a membrane, as a surface \mathbf{S} , parameterized by the height, h , above the (x, y) -plane, denoted as \mathbf{s} in fig.1. Denote points in \mathbf{S} by \mathbf{X} , and points in \mathbf{s} by $\mathbf{x} = (x_1, x_2)$. Unit vectors in the coordinate directions are denoted $(\mathbf{1}, \mathbf{2}, \mathbf{3})$. We will restrict our attention to a membrane system consisting of rigid inclusions whose contact curve with the membrane, \mathbf{C} , is a unit circle,

and the tangent planes of \mathbf{S} along \mathbf{C} have a uniform contact angle, γ . Lifting these restrictions introduces additional degrees of freedom with interesting consequences, which we will explore in a subsequent publication.

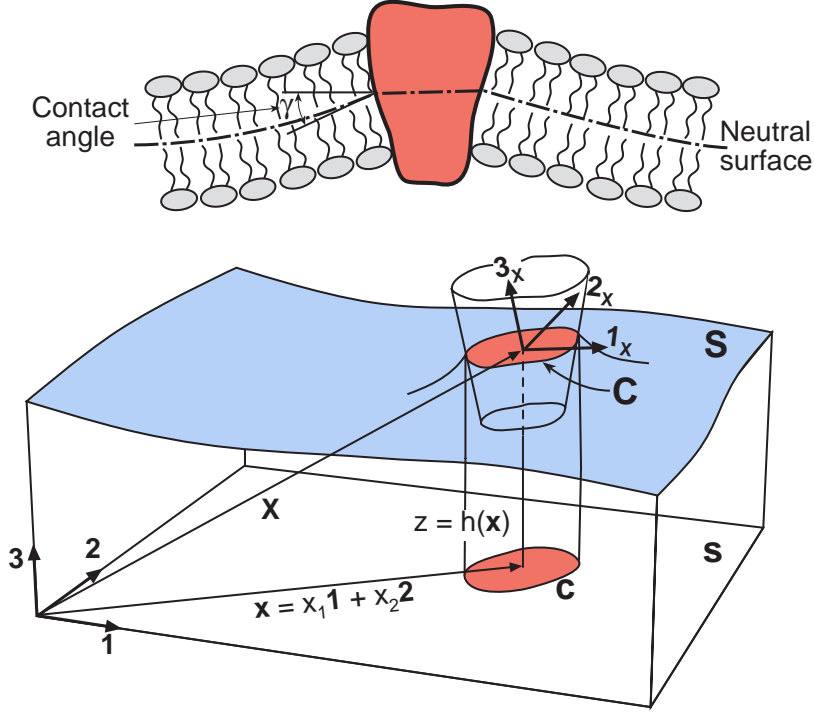


Figure 1 *a)* A cross-section of a bilayer in which a rigid protein is embedded.
b) The membrane surface, \mathbf{S} , cuts the protein along the curve, \mathbf{C} .

The explicit expression of the total interaction energy between N inclusions derived in [6] is:

$$E_m \approx 4\pi B \gamma^2 \sum_{j=1}^N \left| \sum_{i \neq j} \frac{1}{(z_i - z_j)^2} \right|^2. \quad (1)$$

An abbreviated derivation of this expression is given in the **Appendix**. Here, B is the bending modulus and the position of an inclusion, \mathbf{x} , is conveniently represented in complex notation $z = x_1 + ix_2$. The multibody nature of the interaction is evident from the form of (1).

The failure of linear superposition has important consequences for how the inclusions distribute themselves over the surface, \mathbf{S} . Here we shall study how this interaction

energy can stabilize large aggregates of inclusions even though isolated pairs of constituent bodies interact repulsively^[6]. It is not immediately obvious how such aggregation patterns could be absolutely stable, since the pairwise terms have the repulsive $1/r^4$ dependence. However, there are components of this interaction which are not found by pairwise superposition; we will call them *non-pairwise* terms. Fortunately, the energy (1) lends itself to a simple decomposition into a pairwise additive terms and non-pairwise terms. This provides a concrete setting from which to analyze this many-body system. The non-pairwise terms appear as cross-terms in the expansion of (1); in the language of waves, they can be thought of as ‘interference’ terms which introduce an anisotropic angular dependence to the interaction between inclusions. Since these terms can be of opposite sign and of similar magnitude to the pairwise repulsive terms, they can effectively cancel, or overpower, the pairwise repulsive components, analogous to ‘destructive interference’ of waves. In^[6] we show that a configuration of five inclusions positioned at the vertices of a regular pentagon is the smallest stable aggregate. In this configuration the non-pairwise components exactly cancel the repulsive pairwise components. The anisotropic property of the interaction makes this interaction sensitive to the domain boundaries, and therefore plays an important role in the determining the domain shape.

So far, we have neglected the effect of thermal fluctuations (i.e. $T = 0$). When $T > 0$, thermal fluctuations induce normal motions of the membrane and diffusion of the inclusions in the plane of the membrane. The higher the temperature, the stronger is the entropic dispersion tending to randomize the distribution of inclusions. In the next section we show that a stable pattern of inclusions can emerge from an initially disordered aggregate at room temperature.

Numerical results

We describe the motion of an array of inclusions using the Smoluchowski equation (i.e. the overdamped Langevin equation), where thermal fluctuations are introduced by adding random terms to the equations of motion^[9]. The equations of motion are generated by the gradient flow of the mean curvature energy, $E_m(\mathbf{X})$, given by (1). Let $\mathbf{X} \equiv (\text{Re } z_1, \dots, \text{Re } z_N, \text{Im } z_1, \dots, \text{Im } z_N)$ be a $2N$ -dimensional vector specifying the N inclusion positions. The positions of the inclusions are described by the system of stochastic differential equations:

$$\zeta \frac{d\mathbf{X}}{dt} = -\nabla E_m(\mathbf{X}) + \sqrt{2k_B T \zeta} \xi(t) \quad (2)$$

where $\zeta = 4 \times 10^{-5}$ pN-s/nm is the friction coefficient for a protein in a lipid bilayer; ζ is related to the diffusion coefficient by the Einstein relation: $D = k_B T / \zeta \approx 10^5$ nm²/s. The deterministic force is given by the gradient of the interaction energy, $-\nabla E_m$. $\sqrt{2k_B T \zeta} \xi(t)$ is the Brownian force, where $T = 300^\circ\text{K}$, and $\xi(t)$ is a random force with the Gaussian

white-noise property: $\langle \xi(t) \rangle = 0$, $\langle \xi(0)\xi(t) \rangle = \delta(t)$ ^[9]. Each simulation begins from a Poisson distributed initial configuration. In these simulations we have not included thermal fluctuations of the membrane normal to the surface. Goulian, *et al.* have shown that the entropic force arising from such fluctuations opposes the diffusive spreading ^[5]; however, its magnitude is small relative to the pairwise repulsion, and so we have neglected it here.

In ^[6] we proved that a system of five inclusions arranged in a regular pentagon is a zero energy equilibrium state, and is the smallest stable protein aggregate. Figure 2 shows the initial and final configurations of a system of five inclusions which are initially distributed randomly. The system evolves into a pentagonal array. In an infinite domain, diffusive expansion continues until the inclusions are so far apart that their interactions are swamped by thermal fluctuations and the pentagonal geometry eventually evaporates. The characteristic distance at which this occurs is when the interaction energy is of the same magnitude as $k_B T$. In this example, this distance is about 50 nm. If we solve the equations on a finite domain with pinned boundary conditions, then each inclusion sees an energy barrier as it approaches a boundary. This partially arrests the diffusive expansion and stabilizes the pentagonal array. The domain in fig. 2 is a 200 nm circle, pinned at the boundary; the plot shows a 20 nm square window on that domain.

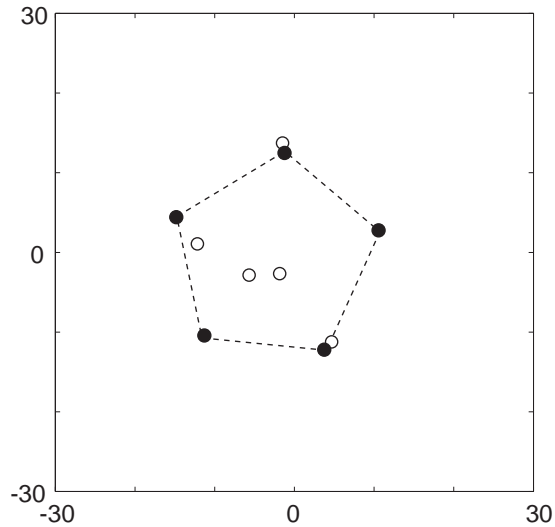


Figure 2 A simulation illustrating that an initially random configuration of five inclusions evolves into a pentagonal aggregate. The figure shows a 20 nm diameter subdomain of a 200 nm circular field. Open circles: initial distribution, solid circles: final distribution. If the initial conditions were a regular pentagon, it would remain a regular pentagon, even in the presence of thermal fluctuations. The diffusion coefficient of the inclusions is $D = 10^5 \text{ nm}^2/\text{s}$, the bending modulus of the membrane is $B = 200 \text{ pN nm}$, and the contact angle of the inclusions is $\gamma = 1$ (for $\pi/4$).

Figure 3 shows the evolution of a system of sixteen inclusions. The initial configuration was Poisson distributed within a square subdomain of 45 nm^2 . The evolution of the system from its initial to final states was not monotonic, but displayed a rich and complex behavior which we have not been able to quantify. Therefore, we limit our observations to a specific initial condition with two clusters, one with five and the other with six inclusions. Each inclusion in a cluster is within 15 nm of its nearest neighbor. In repeated runs, the two clusters each form local pentagons during the transient intermediate state, with the six-membered group ‘expelling’ one inclusion. The system of inclusions eventually forms a symmetric two-layer structure. The interior layer forms a regular pentagon while the outer layer rounds up to a circularly symmetric distribution. This configuration continues to expand slowly, but the overall pattern of a central pentagon with a symmetric outer layer persists even to 1 millisecond (figs. 3c, d). In an unbounded domain the inclusions would eventually drift apart until the interaction energy falls below $k_B T$. With a pinned boundary that repels inclusions the pattern stabilizes to one close to that shown in fig. 3d.

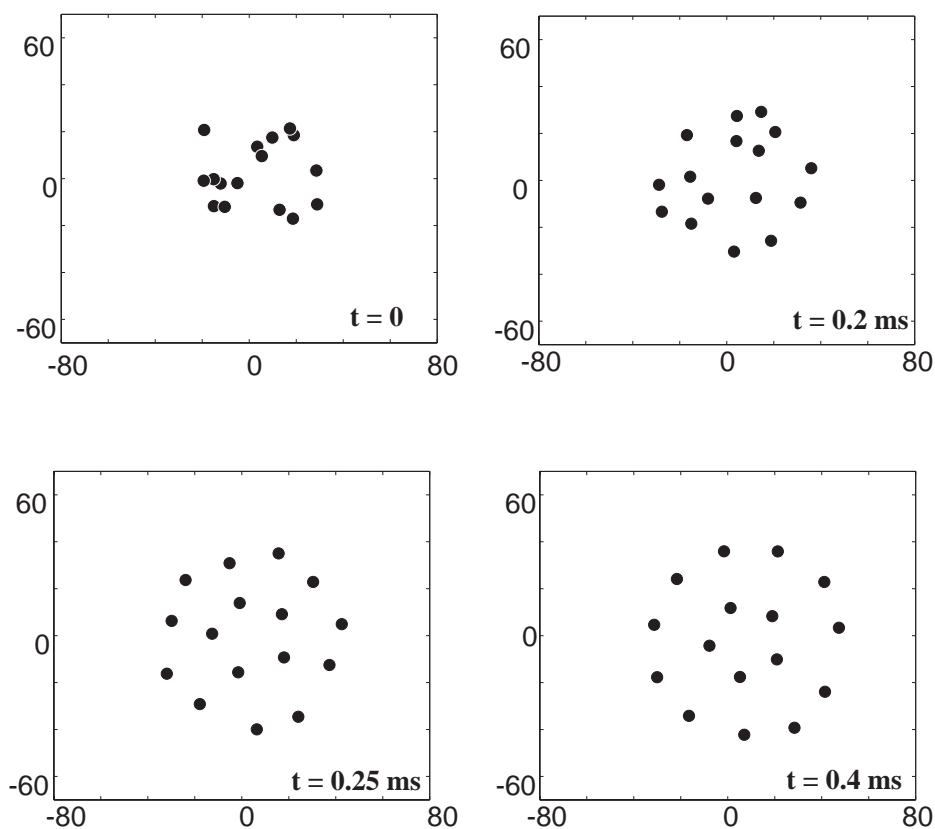


Figure 3 *a)* A system of 16 inclusions, with the initial configuration of two localized clusters of five and 6 inclusions. *b)* An intermediate state where the two clusters have organized into pentagonal units. *c)* At 0.25 ms. *d)* At 0.40 ms. This pattern persists through 1 millisecond.

Discussion

There are many ways in which an ordered structure can arise within an initially unstructured or disordered system. Perhaps the most studied mechanism is the Turing instability that arises in nonlinear reaction-diffusion systems^[10, 11]. Here we examined the evolution of an initially disordered many-body system of membrane inclusions under the influence of thermal fluctuations and a multibody curvature interaction. The interaction can be decomposed analytically to a pairwise component which is always repulsive, and a non-pairwise component which can be either repulsive or attractive. Thus the non-pairwise forces can enhance or cancel the repulsive forces, and so spatial patterns can arise from competing interactions. The non-pairwise component depends on the angles between inclusions making the overall interaction anisotropic. This generates a rich class of stable symmetric configurations. We found the simplest such configuration to have pentagonal symmetry, which forms the smallest stable array; it may be one of many ground states. Exploiting this fact, we show that a randomly distributed initial condition of five inclusions self-assembles into a regular pentagon which then gradually expands uniformly. This shape persists until it reaches a diameter where the interaction energy is of the same order of magnitude as $k_B T$ whereupon the pattern dissipates.

For a larger ensemble of 16 inclusions, simulations demonstrate the formation of large-scale regular patterns. Local clusters of five or more inclusions always tend towards local pentagons. From our earlier investigations^[6], we would expect these pentagons to be stable both in shape and size at zero temperature. Thus a system of local clusters of five inclusions would, if the clusters are far enough apart, form isolated islands of pentagons. However, with thermal fluctuations, local structures can diffuse towards, and coalesce with, neighboring structures giving rise to networks where pentagons share common sides (see fig. 3c). This interplay of thermal fluctuations and non-pairwise forces generates rich patterns of structural phases. Initially disordered arrangements of inclusions eventually evolve into ordered patterns which persists for long times at room temperature. At high enough temperatures a melting transition transforms the system back into a disordered state. In this work, we only dealt with temperatures in the biological range. In subsequent work, we will examine more closely the phase behavior of these systems in wider temperature ranges.

Appendix

In this Appendix we give a condensed recapitulation of the derivation of the energy function, E_m given in equation (1).

Referring to fig. 1, in the small deformation limit, $|\nabla h(\mathbf{x})| \ll 1$, the local mean curvature of \mathbf{S} at $\mathbf{X} = \mathbf{x} + z\mathbf{3}$ is:

$$\kappa(\mathbf{x}) \equiv \frac{1}{2} \nabla^2 h(\mathbf{x}) \quad (\text{A.1})$$

The elastic bending energy for a membrane is given by ^[7]

$$E_m(\mathbf{X}) = \frac{B}{2} \int_{\mathbf{s}} (\nabla^2 h(\mathbf{x}))^2 d\mathbf{x} = 2B \int_{\mathbf{s}} \kappa^2 d\mathbf{x} \quad (\text{A.2})$$

where B is the bending modulus. We neglect the *total* Gaussian curvature term since it plays no role in the interaction between rigid inclusions; however, we will see that the *local* Gaussian curvature plays an important role. Minimization of the energy (1) leads to a boundary value problem (BVP) for $h(\mathbf{x})$, depending on the physical constraints applied along the boundary, $\partial\mathbf{s}$. In this BVP, $h(\mathbf{x})$ satisfies the biharmonic equation, $\nabla^4 h(\mathbf{x}) = 0$, $\mathbf{x} \in \mathbf{s}$. In addition, there are boundary integral constraints which contain the balance of net vertical forces and horizontal torques on the inclusions.

The biharmonic equation for $h(\mathbf{x})$ implies that the mean curvature, $\kappa(\mathbf{x})$, is harmonic. Now consider solutions with nonzero mean curvature in $r > 1$. From (A.2), it is evident that the energy E_m is finite if κ is square integrable on \mathbf{s} . A mean curvature field, κ , which is square integrable (i.e. finite energy) and harmonic has a multipole expansion whose leading term is a quadrupole:

$$\kappa = \frac{1}{r^2} (a_2 \cos 2\theta + b_2 \sin 2\theta) + \frac{1}{r^3} (a_3 \cos 3\theta + b_3 \sin 3\theta) + \dots \quad (\text{A.3})$$

The mean curvature energy associated with the quadrupole term is

$$E_m = \pi B (a_2^2 + b_2^2) \quad (\text{A.4})$$

A basic feature of the mean curvature (A.3) is the rapid $1/r^2$ decay. In the many body system, we expect that the mean curvature and associated energy density are similarly concentrated about the inclusions. In the large expanse of the bilayer far from the inclusions, the mean curvature is negligible and, to leading order, the displacement field is approximated by a harmonic background field $\phi(\mathbf{x})$. In a region small compared to the inter-inclusion distance, the local behavior of $\phi(\mathbf{x})$ is dominated by quadratic terms. Specifically, choose the unit vectors $[\mathbf{1}, \mathbf{2}, \mathbf{3}]$ so $\mathbf{1}, \mathbf{2}$ span the tangent plane of \mathbf{S} at a point. The local behavior of $\phi(\mathbf{x})$ is given by

$$\phi \approx \frac{\alpha}{2}(x_1^2 - x_2^2) + \beta x_1 x_2 \quad (\text{A.6})$$

where $\alpha \equiv \partial_{11}\phi(\mathbf{0}) = -\partial_{22}\phi(\mathbf{0})$, $\beta \equiv \partial_{21}\phi(\mathbf{0})$.

Now consider how the local structure of the displacement field is modified when an inclusion is introduced at the origin, $\mathbf{0}$. It can be shown that the quadrupole coefficients a_2 and b_2 in (1) are identified with 2α and 2β respectively through asymptotic matching. Therefore, the energy cost of introducing a inclusion at $\mathbf{0}$ is $4\pi B(\alpha^2 + \beta^2)$, or

$$E_m \approx -4\pi B \text{Det } \kappa(\mathbf{0}) \quad (\text{A.7})$$

where $\kappa \equiv \begin{pmatrix} \partial_{11}\phi & \partial_{12}\phi \\ \partial_{21}\phi & \partial_{22}\phi \end{pmatrix}$ is the extrinsic curvature tensor of the neutral surface $z = \phi(\mathbf{x})$, in

absence of an inclusion at $\mathbf{0}$. $\text{Det } \kappa(\mathbf{0})$ approximates the Gaussian curvature of the surface $z = \phi(\mathbf{x})$ at $\mathbf{x} = \mathbf{0}$ ^[8]. Thus, *the energy cost of introducing a inclusion at an point of the bilayer is proportional to the pre-existing Gaussian curvature at that point.*

Suppose the background field arises from a single inclusion at position \mathbf{x}' . This background field is approximated by the single inclusion solution:

$$\phi = -\gamma \ln|\mathbf{x} - \mathbf{x}'| \quad (\text{A.8})$$

The energy cost of inserting an inclusion into the membrane at the origin is computed as follows. The formula for α , β suggests a complex variables notation. If we represent position \mathbf{x}' by the complex number $z' = x_1' + ix_2'$, then the components α and β of the curvature tensor are contained in the complex curvature *scalar*:

$$\eta \equiv \alpha - i\beta = \gamma \frac{x_1 z_2 - x_2 z_1 - 2ix_1 x_2}{(x_1 + x_2)^2} = \frac{\gamma \bar{z}}{|z|^4} = \frac{\gamma}{z^2} \quad (\text{A.9})$$

Using this notation, the Gaussian curvature at the origin can be written

$\text{Det } \kappa(0) = \alpha^2 + \beta^2 = |\eta|^2$, and the energy cost of inserting a inclusion at the origin is

$E_m = 4\pi B |\eta|^2 = \frac{4\pi B \gamma^2}{|z|^4}$. If the inclusion at z' is the only other inclusion present, the total energy of the two inclusion system is

$$E_m = \frac{8\pi B \gamma^2}{r^4}, \quad r \equiv |z| \quad (\text{A.10})$$

This circularly symmetric, repulsive, $1/r^4$, two body energy is the same as reported by Arando-Espinoza et. al. [2].

The extension to $n \geq 3$ inclusions reveals the non-pairwise character of the interaction. Suppose the background field $\phi(x)$ is due to $N-1$ inclusions at positions represented by complex numbers z_2, \dots, z_N . The curvature scalar at the origin due to these inclusions is

approximated by the superposition of single inclusion curvature scalars, $\eta = \sum_{i=2}^N \frac{\gamma}{z_i^2}$,

and the total energy is approximately

$$E_m \approx 4\pi B \gamma^2 \sum_{j=1}^N \left| \sum_{i \neq j} \frac{1}{(z_i - z_j)^2} \right|^2. \quad (\text{A.11})$$

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