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Self-Assembly of Membrane Junctions

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ABSTRACT We present a mechanism for the aggregation of mobile intermembrane junctions, such as the connexon dyad of gap junctions. The model demonstrates that intermembrane repulsion provides a powerful self-assembly pressure. If the membrane repulsion is strong enough to prevent membrane adhesion, then the self-assembly pressure is of effective *infinite range*.

INTRODUCTION

In this paper, we analyze a new mechanism to explain the self-assembly of mobile intermembrane junctions that is based on the resistance of membranes against bending. A variety of membrane proteins are known to aggregate spontaneously into domains that can comprise tens of thousands of units. This self-assembly, which must be caused by attractive interactions, is of direct importance for the design of novel biomaterials based on the collective activity of large numbers of membrane proteins (e.g., functionalized interfaces (Blankenburg, 1989; Uzgiris, 1983) and optical switches (Birge, 1990)). Of particular interest are junctions[1] connecting two membranes, such as the connexon dyads of gap junctions and mobile cellular-adhesion molecules (CAMs). Connexon dyads, which are gated junctions consisting of two joined hexameric transmembrane channels, one in each membrane (Lowenstein, 1981) (see Fig. 1), aggregate spontaneously to form large domains (Unwin, 1980, 1984; Caspar, 1988; Tibbitts, 1990; Sosinsky, 1990). The junction-junction interaction in the aggregated state was examined by correlation analysis (Braun, 1984; Abney, 1987; Sosinsky, 1990), and only short range *repulsive* forces were found. This means there must exist some *long range* attraction that can act like an effective assembly pressure on the domain, but that does not contribute to the pair-correlation function of nearby proteins in the aggregated phase. Braun et al. (BAO) (Braun, 1984; Abney, 1987) proposed that the required attraction between dyads is not a direct attractive force but instead a membrane-mediated coupling. In particular, they suggested that junction *attraction* could be caused by *repulsion* between the two membranes, which are held together by the dyads (see Fig. 2). With the junctions assembled in a single patch, the remainder of the two membranes can disjoin, lowering the over all repulsive energy. The membrane-membrane repulsion could either be electrostatic repulsion between charged surfactant molecules constituting the membrane bilayers or nonspecific steric repulsion between glycoproteins at the membrane surfaces (Peracchia, 1985). Note that the BAO mechanism is dependent on the fact that junctions couple *two* membranes, so junction aggregation is assumed to have a different origin from protein aggregation in single membranes.[2]

In this paper we show that if we include the *bending rigidity* (Canham, 1970; Helfrich, 1973) of the membranes, a powerful long range self-assembly pressure results. The bending rigidity has been found to be a controlling factor in many studies of the physics of membranes (see, for instance, Gruner, 1989). Intuitively, it seems clear that two repulsive, stiff membranes joined by dyads would tend to "spread out" the attraction between the junctions provided by the BAO mechanism. We will show in the next section that for large membrane-bending rigidities, a strong surface pressure is present that will lead to aggregation without affecting the pair-correlation functions at length-scales of the order of the junction radius. On the other hand, membranes with weak bending rigidity are known to be subject to strong thermal fluctuations. In the subsequent section, we will first consider two membranes joined by a single junction and argue that, as a result of membrane collisions induced by these thermal fluctuations (Helfrich, 1978, 1984), the average separation between membranes grows linearly with lateral distance from the junction. We then argue that in the case of many junctions, this repulsion results in an *infinite-range* attraction between junctions: growing like $\log(R)$, with R the nearest-neighbor distance. As a result, the ideal gas pressure for a gas of junctions is modified: Boltzmann's constant is effectively reduced and might well be negative,

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[1] Throughout, we use the term "junction" to refer to a single point at which two membranes are joined. Thus, each connexon dyad or cellular adhesion molecule is a junction. The term "gap junction" conventionally applies to an aggregated collection of dyads.

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[2] Aggregation in single membranes has been attributed to demixing (Sperotto, 1991) and to membrane fluctuations (Goulian, 1993).

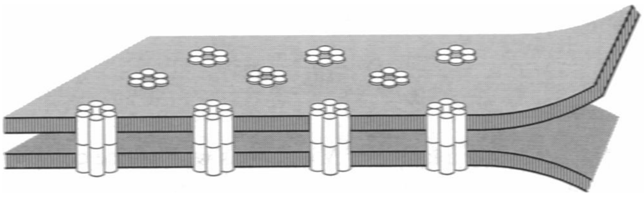

FIGURE 1 Cross section showing two membranes joined by a lattice of dyads. Each dyad consists of two hexameric transmembrane channels, one in each membrane.

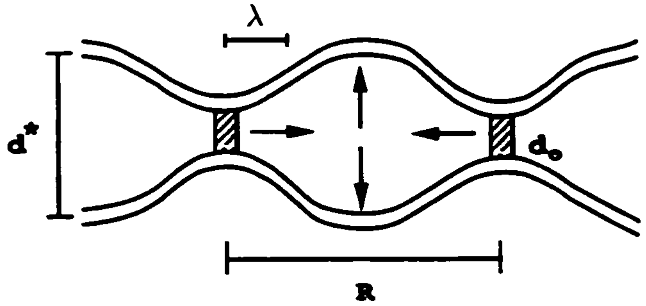

FIGURE 2 Two membrane junctions in the van der Waals regime. After a distance of order λ, the separation between membranes has returned to the optimal value d^*. The arrows indicate the repulsion between membranes and the resulting junction-junction attraction.

which would imply that junctions assemble into a condensed phase at all concentrations.

In either case, we can conclude that the generalized BAO mechanism provides a self-assembly force that will act as a global self-assembly pressure on inter-membrane junctions. Our treatment focuses on the dilute phase of junctions and we determine when the self-assembly pressure is sufficient to render this phase unstable towards aggregation. As a result, we can make no conclusions concerning the nature of the dense phase (i.e., whether the junctions are liquid or crystalline); at the small inter-junctional distances within the dense phase, short ranged electrostatic, steric, and structural forces between junctions, which we neglect, become important in determining the degree of order of the junctions.

For a dilute phase, the distance between junctions is sufficiently large (compared with the membrane thickness) that we can work with the continuum formulation for membranes (Canham, 1970; Helfrich, 1973). This model has been quite successful in explaining the long distance physics of fluid membranes (see, e.g., Nelson, 1989). Therefore, we parametrize the two membranes by heights, $u_1(r)$, $u_2(r)$, above a reference plane (the x-y plane) and with an effective free energy

$$\mathcal{F} = \int d^2r \left[\frac{\kappa}{2} (\nabla^2 u_1)^2 + \frac{\kappa}{2} (\nabla^2 u_2)^2 + V(u_2 - u_1) \right]. \quad (1.1)$$

Here, κ is the bending rigidity, which we take to be the same for the two membranes. We have assumed that the membranes are not under tension. A tension could be included; however, a strong tension would suppress thermal fluctuations and preclude the possibility of the Helfrich regime discussed below.[3] The potential energy per unit area, $V(u)$, describes the nonspecific part of the interaction between the two membranes (Israelachvili, 1992) (including steric repulsion by membrane proteins). We first decompose the two height profiles into a relative spacing u and a center of mass displacement v:

$$\mathcal{F} = \int d^2r \left[\frac{\kappa}{2} (\nabla^2 u)^2 + V(u) + \frac{\kappa}{2} (\nabla^2 v)^2 \right] \quad (1.2)$$

$$u \equiv \frac{u_2 - u_1}{\sqrt{2}}, \quad v \equiv \frac{u_2 + u_1}{\sqrt{2}}.$$

Because v is insensitive to the potential $V(u)$ and to the constraints imposed by the junctions, $\mathcal{F}$ is minimized by v = constant. Below, we will only focus on u.

To include the protein-controlled binding between the two membranes, we assume that there are N junctions spread out over an area A of the membranes with a mean spacing R between junctions of order A/N. The junctions are located at sites r_i, $i = 1, \ldots, N$, and form permanent bonds, forcing the two membranes to approach within a distance of order $d_o \sim 10$ Å. The radius of the junctions is assumed to have a similar magnitude. We also assume that the surfactant molecules near the junction are aligned with the junction direction so that the membrane tangent planes are parallel at the junctions. These conditions imply the boundary conditions

$$u(r_i) = d_o; \qquad \vec{\nabla} u(r_i) = 0.$$

Outside the patch of junctions, we assume that the two membranes are unconstrained and free to separate. Surfactant molecules are free to enter or leave the junction patch so the surfactant area of which A forms the basal projection is not a conserved quantity. To demonstrate self-assembly, we must show that a highly diluted collection of junctions is unstable towards aggregation.

We will distinguish two regimes for $V(u)$: (i) the "van der Waals regime" and (ii) the "Helfrich regime." For case (i), we will assume that the bending rigidity is quite large compared with k_BT. In the presence of strong electrostatic screening, there then must be a minimum in $V(u)$ at $u = d^*$ such that for $u \gg d^*$, the attractive van der Waals force is the dominant intermembrane force, whereas for $u \ll d^*$, steric repulsion forces between membrane proteins will dominate (Fig. 3 a). Typically, d^* will be of order 30 Å. For case (ii), we will assume that the bending rigidity is of order k_BT. In this case, thermal fluctuations produce membrane-membrane collisions (Helfrich, 1978, 1984) that can remove the minimum in the effective potential and produce a $1/u^2$

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[3] More precisely, thermal fluctuations would be suppressed for length scales greater than $\sqrt{\kappa/\mu}$, with μ the surface tension. For a fairly high tension of $\mu \sim 0.01$ erg/cm^2 and $\kappa \sim 10k_BT$, $\sqrt{\kappa/\mu} \sim 600$ Å.

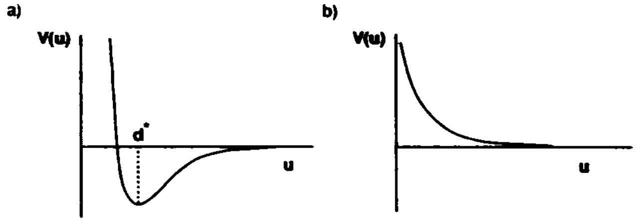

FIGURE 3 Intermembrane potentials exhibiting (*a*) a minimum at finite membrane separation (van der Waals regime) and (*b*) no minimum (Helfrich regime). Note in *b* that the purely repulsive potential is a combination of the bare intermembrane potential and the effective entropic potential arising from the Helfrich mechanism (Helfrich, 1978, 1984).

repulsion for large u (Fig. 3 *b*). We will start with the van der Waals regime.

VAN DER WAALS REGIME

For small deviations from the minimum energy configuration $u = d^*$, we can expand $V(u)$:

$$V(u) \approx V(d^*) + \tfrac{1}{2}V''(d^*)(u - d^*)^2 + \cdots$$

Substituting this expansion in (1.2), we find

$$\mathscr{F} = \int d^2r\left[\frac{\kappa}{2}(\nabla^2 u)^2 + \frac{V''(d^*)}{2}(u - d^*)2 + \cdots\right]. \tag{2.1}$$

Within this harmonic approximation, $\mathscr{F}$ is minimized if u satisfies

$$\nabla^4 u + \frac{u - d^*}{\lambda^4} = 0$$
$$\lambda = \left(\frac{\kappa}{V''(d^*)}\right)^{1/4} \approx d^*\left(\frac{\kappa}{H}\right)^{1/4}. \tag{2.2}$$

We have made a crude estimate of $V''(d^*)$ by assuming the only relevant length scale at the minimum of $V(d^*)$ is d^* and taking the order of magnitude of $V(d^*)$ to be the van der Waals expression H/d^{*2}, where $H \sim k_BT$ is the Hamaker constant (Israelachvili, 1992). Physically, λ plays the role of a "healing length": if we deform the intermembrane spacing away from d^* at some point, then the membranes will return to the equilibrium spacing after a distance of order λ (Fig. 2).

If we consider an isolated junction at the origin then the solution to (2.2) satisfying the boundary conditions (1.3) (and decaying to d^* at infinity) is

$$u = d^* - \frac{4}{\pi}(d_o - d^*)\mathrm{kei}\left(\frac{r}{\lambda}\right),$$

where kei is a Kelvin function (Abramowitz, 1972). Substituting u into (1.2) we find the elastic free energy cost for inserting an isolated junction:

$$\mathscr{F}_1 = 4\kappa\frac{(d_o - d^*)^2}{\lambda^2}. \tag{2.3}$$

Now consider a lattice of N junctions with lattice constant R. The appropriate solution to (2.2) is in this case

$$u(r) = d^* + B\sum_{r_i}\mathrm{kei}\left(\frac{|\mathbf{r} - \mathbf{r}_i|}{\lambda}\right).$$

The first boundary condition in (1.3) will be satisfied if we set

$$B = \frac{d_o - d^*}{\sum_{r_i}\mathrm{kei}(r_i/\lambda)}.$$

The second boundary condition in (1.3) will be automatically satisfied by the symmetry of the lattice (strictly speaking, this will not be true for the boundary sites of the lattice, but the error will be small for large N).The free energy for a lattice of N junctions is then

$$\mathscr{F}_N = N\frac{\pi\kappa(d_o - d^*)^2}{\lambda^2\sum_{r_i}\mathrm{kei}(r_i/\lambda)}.$$

In the low density limit, $R \gg \lambda$, we have

$$\mathscr{F}_N \underset{R\gg\lambda}{\approx} N\mathscr{F}_1\left[1 - 12\sqrt{\frac{2\lambda}{\pi R}}e^{-R/\sqrt{2\lambda}}\sin\left(\frac{R}{\sqrt{2\lambda}} + \frac{\pi}{8}\right)\right].$$

Thus, for dilute systems there is an attractive interaction that is exponentially screened. This is not surprising because, for widely separated junctions, the separation between membranes is of order d^* over most of the inter-junctional area. Presumably, the van der Waals interaction between junctions is the dominant force in this case. For the higher densities $R \ll \lambda$, relevant for aggregated patches, we can approximate the lattice sums by integrals. In this limit,

$$\mathscr{F}_N \underset{R\ll\lambda}{\approx} \frac{N}{4}\mathscr{F}_1\frac{R^2}{\lambda_2}.$$

Thus, we have a negative surface pressure Π:

$$\Pi = -\frac{1}{4}\frac{\mathscr{F}_1}{\lambda^2}. \tag{2.4}$$

If we view the combined system of junctions plus membrane as a two-dimensional many-body system of "particles" occupying an area $A = NR^2$, then after including the entropy for the free particles, the low-density free energy becomes:

$$\mathscr{F} = -\Pi A - Nk_BT\log(A/N).$$

The area A will thus decrease (i.e., the junctions will aggregate) until $\mathscr{F}$ reaches a minimum at $-\Pi A = Nk_BT$ or until short ranged forces become important. Using (2.2), (2.3), and (2.4), the two conditions for self-assembly become

$$\frac{(d_o - d^*)^2}{d^{*4}}R^2 > \frac{k_BT}{H} \qquad R > d^*\left(\frac{\kappa}{H}\right)^{1/4}.$$

The second condition ($R < \lambda$) is easily satisfied for large bending energies, R a typical spacing of the junction (10 nm), and $d^* \sim 3$ nm. The first condition ($\Pi A > Nk_BT$) will be obeyed provided d^* is large compared with d_o; self-assembly will take place if the range of the repulsive potential exceeds the height of the junction.

HELFRICH REGIME

When the bending energy κ is small enough, thermal fluctuations of the membrane shape are important. Thermal wandering will cause "collisions" when the two membranes approach locally to within a distance set by the short distance repulsion. The number of such collisions per unit area is $1/\xi_d^2$ where the "patch size" ξ_d is given by $\xi_d \approx d\sqrt{\kappa/k_BT}$ and d is the mean separation between membranes. Helfrich showed that, as a result of these collisions, there is an effective interaction energy per area

$$V \approx C\frac{(k_BT)^2}{2\kappa}\frac{1}{d^2},$$

where C is a numerical factor that is estimated to be of order unity (Helfrich, 1978, 1984). For small κ, this entropic repulsion can overwhelm the van der Waals attraction, and the membranes will favor infinite separation. In this regime, we take into account thermal fluctuations by constructing a coarse grained effective free energy, which should be valid at long distances:

$$\mathcal{F} = \int d^2r\left[\frac{\kappa}{2}(\nabla^2u)^2 + \frac{C(k_BT)^2}{2\kappa}\frac{1}{u^2}\right]. \tag{3.1}$$

When considering distances large compared with d_o, it is useful to think of each junction as forcing a collision between membranes. However, a junction-induced collision is not identical to a real collision. In the latter case, the membranes approach each other until there is an energetic cost of order k_BT. For junctions, the membranes are forced to approach to within a distance d_o and, thus, such "collisions" can be more energetically costly. Furthermore, a junction-induced collision is really a constraint imposed on the membrane, which will result in many more real collisions near it. Real collisions, however, are statistically independent; the presence of one such collision does not imply that there will be others nearby.

We will separate the region around a junction into a "core," of size δ, where the separation d is of order d_o, and a "corona." The former we assume to be dominated by the repulsive bare potential and the latter to be dominated by thermal fluctuations. The free energy (3.1) applies in the corona. To estimate the size of the core we minimize the free energy, which gives

$$\delta \sim \left(\frac{\kappa d_o}{V'(d_o)}\right)^{1/4}.$$

With the estimates $V(d_o) \sim 1$ erg/cm^2, $d_o \sim 10$ Å, and $\kappa \sim 10^{-14}$ erg, we find δ is of order d_o.

We start with the case of an isolated junction connecting two membranes. If we assume that d_o and δ are unimportant far away from the core, then at long distances there is only *one* relevant length scale: the size of the membranes L. This implies the patch size ξ_d (d is the mean separation between membranes) must be of order L and, hence, the mean profile of $u(r)$ must be linear in r. (This "self-similar" corona is shown schematically in Fig. 4.) To demonstrate this, we minimize $\mathcal{F}$, which requires

$$\nabla^4u - C\frac{k_BT^2}{\kappa^2}\frac{1}{u^3} = 0. \tag{3.2}$$

This, indeed, has a linear solution (i.e., a cone)

$$u(r)_{\text{cone}} = C^{1/4}\sqrt{\frac{k_BT}{\kappa}}\,r. \tag{3.3}$$

Substituting $u_{\text{cone}}(r)$ into (3.1) gives

$$\mathcal{F}_{\text{corona}} \approx k_BT\sqrt{C}\log(L/\delta).$$

The free energy of a lattice of junctions, therefore, is estimated to be

$$\mathcal{F} \approx Nk_BT\sqrt{C}\log(R/\delta) + \cdots,$$

where $\cdots$ denotes terms independent of R (e.g., the free energy of the core).

For a membrane with free boundaries, $u_{\text{cone}}(r)$ cannot be the exact solution of interest because it does not satisfy the proper boundary conditions at the outer edge of the membrane ($r = L$) (Landau, 1986). Also, we have not shown that the solution matches the appropriate $u(\delta)$ at the boundary of the core. Nevertheless, $u_{\text{cone}}(r)$ does have the linear profile that we have previously argued should be satisfied in the mean, and thus provides a reasonable estimate of the free energy of the corona.

We can derive the behavior $\mathcal{F} \sim \log(R)$ more carefully by a variational approach. We focus on a Wigner-Seitz unit cell of the lattice of junctions (or for a random array, a Voronoi polyhedron) (Ashcroft, 1976), which we approximate by a circle of radius R. At $r = R$, u must satisfy

$$\left.\frac{\partial u}{\partial r}\right|_{r=R} = 0, \qquad \left.\frac{\partial^3 u}{\partial r^3}\right|_{r=R} = 0. \tag{3.4}$$

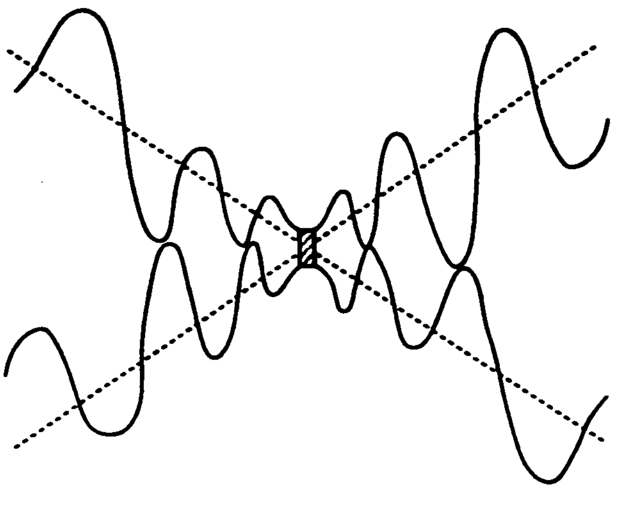

FIGURE 4 A schematic depiction of the corona and core regions in the Helfrich regime. Far from the core, the corona should not depend on any fixed length-scale and, hence, will be self-similar, with a linear profile on average.

As discussed previously, at long distances the only relevant length scale is R and we can take for the boundary condition at the origin

$$u\,|_{r=0} \approx 0. \tag{3.5}$$

We use the trial function

$$u_{\mathrm{trial}}(r) = bR\sqrt{\frac{k_{\mathrm{B}}T}{\kappa}}\left(\frac{\sin \pi r}{2R}\right)^{\alpha}, \tag{3.6}$$

with b a variational parameter. Substituting u_{trial} in (3.1), we find that $\mathscr{F}$ is minimized for $\alpha = 1$ and $b = 1$. Note that in this case u_{trial} goes over into the cone solution (3.3) for small r. In the large-R limit, the minimal value of the free energy for the class of trial functions (3.6) is given by

$$\mathscr{F}_{\mathrm{trial}} \approx Nk_{\mathrm{B}}T\sqrt{2\pi C}\,\log(R/\delta). \tag{3.7}$$

If we view again the combined system of junctions plus membrane as a two-dimensional, many-body system of "particles" occupying an area $A = NR^2$, then, after including the ideal gas entropy for the particles, we find a surface pressure

$$\Pi = \frac{NT}{A}k_{\mathrm{B}}\left(1 - \sqrt{\frac{\pi C}{2}}\right).$$

Thus, in the Helfrich regime the effective value of Boltzmann's constant is reduced. The final result is quite sensitive to the precise numerical coefficient appearing in (3.7), which we cannot reliably compute with our approximations. However, if $\pi C/2 > 1$, then the junctions are always confined to either solid or liquid aggregates; there will be no unbound two-dimensional gas phase.

By working with model membrane systems, one can test a number of the concepts discussed in this paper. The junctions could either be connexon dyads or cellular adhesion molecules. If the membranes are composed of charged surfactants, then the range of the short distance electrostatic repulsion between membranes can be controlled by changing the salt concentration in the ambient solution. If the surfactants are also chosen so that the bending rigidity is much larger than $k_{\mathrm{B}}T$ (the van der Waals regime), then one can tune the salt concentration so that the optimal separation between membranes, d^*, is equal to the separation at the location of the junctions, d_0. In this case, there should be no interaction between junctions and aggregation should not occur. However, for lower salt concentrations, where $d^* > d_0$, the junctions should aggregate. The phase behavior of the junctions can be measured by microscopy or, if a lamellar phase of membranes is used, by scattering.

The bending rigidity of the membrane can be varied by adding cosurfactant, which can lower the bending rigidity, κ, to of order $k_{\mathrm{B}}T$ (the Helfrich regime). In this regime, it might be possible to observe directly the linear increase in membrane separation with lateral distance by microscopy. As discussed above, if the constant C is sufficiently large, then the junctions will aggregate even in this limit of weak bending rigidity. If aggregation fails to occur, then it would be interesting to measure the "renormalized Boltzmann's constant." However, this would require a means of measuring the surface pressure of a dilute collection of junctions.

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