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Spicules and the Effect of Rigid Rods on Enclosing Membrane Tubes

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Membrane tubes (spicules) arise in cells, or artificial membranes, in the nonlinear deformation regime due to, e.g., the growth of microtubules, actin filaments, or sickle hemoglobin fibers towards a membrane. We calculate the axial force f exerted by the tube, and its average radius, taking into account steric interactions between the fluctuating membrane and the enclosed rod. We find a smooth crossover of the axial force between $f \sim \sqrt{\sigma}$ and $f \sim \sigma$ as the membrane tension σ increases and the tube radius shrinks. This crossover occurs around the most physiologically relevant membrane tensions. Our work may be important in (i) interpreting experiments in which axial force is related to the tube radius or membrane tension, and (ii) constructing dynamical theories for biopolymer growth in narrow tubes where these fluctuation effects control the tube radius.

There has been much recent interest in the formation of tubular membrane tethers or spicules [1–9]. These arise from the action of localized forces that act normal to the membrane and give rise to narrow membrane tubes in the nonlinear deformation regime. Such forces can arise from the polymerization of fibers, including actin [10], tubulin [11], or sickle hemoglobin [12], into stiff fibers or bundles of fibers. The polymerization rate is sometimes fast, but can be slow or stop altogether. Similar structures appear on cell membranes as filopodia or smaller tubular excursions [10,11], including the spicules of stellated or sickled red blood cells containing sickle hemoglobin (HbS) [12] or neural growth cones [13] as well as on vesicles observed *in vitro* [9]. When the tube is long (many times its radius), it is approximately cylindrical *on average* except very close to its ends [10]; see Fig. 1. Throughout we will assume that any growth of the fiber and tube is quasistatically slow [14].

In the following section, we outline a self-consistent mean-field analysis of the radial membrane fluctuations. In this the *average* radial extent of the fluctuations are controlled by the presence of the enclosed rod, an approach that is analogous to Helfrich's highly successful theory for planar membranes [15,16]. We will work in units in which $k_B T = 1$.

In order to describe our cylindrical membrane, we use the Hamiltonian $H = H_E + H_S$, with

$$H_E = \int \left[\sigma + \frac{\kappa}{2} c^2 \right] \sqrt{g} \, d\phi \, dz - fL,$$

$$H_S = \int \left[A + J[r(\phi, z) - \bar{r}] + \frac{C}{2}[r(\phi, z) - \bar{r}]^2 \right] d\phi \, dz, \tag{1}$$

where H_E is the usual Hamiltonian for membrane elasticity [16–18], containing both surface tension (σ) and rigidity (κ) controlled terms, the latter varying with the square of the local membrane curvature c. We have also included in

H_E an axial force term, f, which arises from the polymerizing fiber and controls the axial length, L, of our membrane tube. In what follows we neglect any effects that could arise from leaflet asymmetry or spontaneous curvature. H_S contains a harmonic potential (with strength $\sim C$) that confines the size of the membrane fluctuations about the average tube radius $\bar{r}$. It also contains a "radial force" (or pressure) term (with strength $\sim J$), which controls the average radius $\bar{r}$ of the membrane tube and arises from the asymmetric nature of the constraint provided by the rod. This term can also be used to include any hydrostatic or osmotic pressure differences between the inside and outside of the tube, although we neglect these in what follows. Additionally, H_S contains a term involving A, which is convenient for normalization of the steric potential. This most general harmonic potential will be used to model the steric interactions between our membrane and polymer rod by way of a mean-field approach. An analogous treatment has proven to be remarkably successful in describing the steric repulsion between flat membranes [15,16]. We pro-
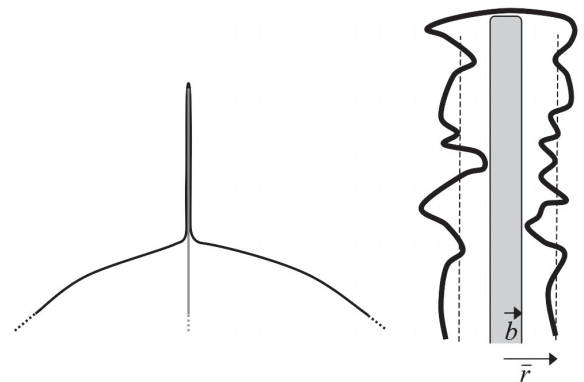

FIG. 1. Sketch of an axial slice through a membrane tube (left) and a section of tube (right) of average radius $\bar{r}$ enclosing a cylindrical rod (of fixed radius b). The radial fluctuations are of amplitude $\delta r(z, \phi)$ and have mean squared extent $\langle \delta r^2 \rangle$.

ceed from Eq. (1) by writing $r(\phi, z) = \bar{r} + \delta r(\phi, z)$ and expanding the energy H to quadratic order [19–21] in the radial perturbation $\delta r(\phi, z)$ about the average tube radius $\bar{r}$. A convenient Fourier representation of the radial fluctuations is

$$\delta r(\phi, z) = \sum_{n=-\infty}^{\infty} \sum_{m=-\infty}^{\infty} \delta r_{nm} \exp\left(im\phi + \frac{2\pi inz}{L}\right). \quad (2)$$

With $q = \frac{2\pi\bar{r}}{L}$ this yields the energy which we write as a perturbative expansion $H = H_0 + \delta H + \delta^2 H + \cdots$ with

$$H_0 = \frac{\pi\kappa L}{\bar{r}} + 2\pi\sigma L\bar{r} + 2\pi AL - fL,$$

$$\delta H = 0 \Rightarrow \langle \delta r \rangle = 0 \Rightarrow J = -\sigma + \frac{\kappa}{2\bar{r}^2}, \quad (3)$$

$$\delta^2 H = \frac{1}{2} \frac{2\pi\kappa L}{\bar{r}^3} \sum_{n=-\infty}^{\infty} \sum_{m=-\infty}^{\infty} \delta r_{nm} \delta r_{-n-m} K_{nm},$$

involving a kernel

$$K_{nm} = (n^2q^2 + m^2)^2 - \frac{n^2q^2}{2}\left(1 - \frac{\bar{r}^2}{r_0^2}\right)$$
$$- \frac{5m^2}{2}\left(1 - \frac{\bar{r}^2}{5r_0^2}\right) + 1 + \frac{C\bar{r}^3}{\kappa}, \quad (4)$$

which, in turn, involves the radial length scale $r_0 = \sqrt{\frac{\kappa}{2\sigma}}$. The first order perturbative contribution is required to vanish so that $\bar{r}$ indeed represents the true average (or ground state) membrane tube radius [19]. This condition then implies [from Eq. (3)] that the radial force is $J = -\sigma + \frac{\kappa}{2\bar{r}^2}$. The quadratic fluctuations in the radial displacement (around $\bar{r}$) contribute at order $\delta^2 H$ and depend on the strength of the harmonic potential in Eq. (1), via the parameter C. The presence of the rod sterically constrains the membrane radius, $r(\phi, z)$, to remain always greater than the rod radius b; see Fig. 1. It thus follows that the presence of the rod has an effect on both the average radius, $\bar{r}$, and the fluctuations, $\delta r(\phi, z)$. The mean squared amplitude of the fluctuations, $\langle \delta r^2 \rangle$, depends on the parameter C, as can be seen from Eq. (3). We must now determine this self-consistently. In employing a harmonic potential, controlled by the parameter C, we adopt an approximate phenomenological treatment of the steric interactions. Since this is at best a semiquantitative approach, one should be cautious in relying on the numerical prefactors that appear in our results. This limitation is common to all studies that follow the spirit of the Helfrich approach. Finally, it may help to note that the rod and the potential that we employ to mimic it have the additional role of suppressing instabilities that are known to occur on certain "rodless" cylindrical membrane tubes (such as "pearling") [22–24], for example).

The free energy of the tube $F = H_0 + \Delta F$ can be shown to involve the perturbative correction

$$\Delta F = \frac{1}{2} \sum_{n=-\infty}^{\infty} \sum_{m=-\infty}^{\infty} \log(K_{nm}). \quad (5)$$

We can now physically motivate an explicit choice for the parameter A as follows. We aim to calculate the free energy difference between the case when the rod is present and when the rod is absent and the membrane is unconstrained. In the latter case, the steric harmonic potential (of strength C) vanishes, as do terms involving C that appear in K_{nm}. Thus we choose the parameter A so that in the limit $C \to 0$ we obtain $\Delta F \to 0$ for consistency. We must then choose

$$A = -\frac{1}{4\pi L} \sum_{n=-\infty}^{\infty} \sum_{m=-\infty}^{\infty} \log(K_{nm}|_{C=0}). \quad (6)$$

After we have integrated out all radial membrane fluctuations, we therefore obtain

$$\Delta F = \frac{\pi\kappa L}{\bar{r}} + 2\pi\sigma L\bar{r} - fL + \frac{1}{2}$$
$$\times \sum_{n=-\infty}^{\infty} \sum_{m=-\infty}^{\infty} \log\left(\frac{K_{nm}}{K_{nm}|_{C=0}}\right). \quad (7)$$

Further, by converting the above summations into integrals we can write our final expression for the free energy as

$$F = \frac{\pi\kappa_{\text{eff}}L}{\bar{r}} + 2\pi\sigma L\bar{r} - fL, \quad (8)$$

in which we have defined an "effective" membrane bending modulus through which one may interpret all of the effects of the steric potential

$$\kappa_{\text{eff}} = \kappa + \frac{1}{8\pi^2} \int_0^{2\pi} d\theta \int_0^{\infty} d\rho \log[1 + C\bar{r}^3/(\kappa\beta)] \quad (9)$$

involving the variables $\rho = n^2q^2 + m^2$ and $\theta = \tan^{-1}\frac{nq}{m}$ with

$$\beta = \rho^2 - \frac{\rho}{2}\left(1 - \frac{\bar{r}^2}{r_0^2}\right) - 2\rho\cos^2\theta + 1. \quad (10)$$

We can now state quantitatively the physical condition that we wish to impose on our membrane to mimic the steric influence of the rod [25],

$$\bar{r} - \sqrt{\langle \delta r^2 \rangle} = b. \quad (11)$$

This gives the necessary self-consistency condition for the strength of the harmonic potential given that

$$\langle \delta r^2 \rangle = \frac{\bar{r}^3}{2\pi\kappa L} \sum_{n=-\infty}^{\infty} \sum_{m=-\infty}^{\infty} K_{nm}^{-1}. \quad (12)$$

By again converting the summations into integrals and changing variables, we arrive at the following consistency equation for C:

$$\left(1 - \frac{b}{\bar{r}}\right)^2 = \frac{1}{8\pi^2\kappa} \int_0^{2\pi} d\theta \int_0^{\infty} d\rho \frac{1}{\beta + C\bar{r}^3/\kappa}. \quad (13)$$

In order to calculate the average radius $\bar{r}$ for the membrane tube, we merely need to minimize F by setting $\frac{\partial F}{\partial \bar{r}} = 0$. Similarly the force f required to maintain the axial length L of our membrane tube follows from $\frac{\partial F}{\partial L} = 0$.

Wide tubes ($\bar{r}/b \gg 1$, $C\bar{r}^3/\kappa \ll 1$).—This regime corresponds to the case when the equilibrium radius of the membrane tube is typically much larger than the radius of the enclosed rod ($\bar{r}/b \gg 1$). The steric effects of the rod in this limit should therefore be weak ($C\bar{r}^3/\kappa \ll 1$), and as a consistency check, we recover the results of [1], namely, $\bar{r} = r_0 = \sqrt{\frac{\kappa}{2\sigma}}$ and $f = 2\pi\sqrt{2\sigma\kappa}$. For the present purposes we can expand about $\bar{r} = r_0$ using Eq. (13) to obtain $C = \frac{2\kappa\pi^2}{3r_0^3} \exp(-4\pi\kappa\sqrt{2})$. Stacks of membranes at large intermembrane separation have been studied before [26] and have identified a similar exponential form for the confining potential [27,28]. Substituting into Eq. (9) we obtain to leading order

$$\kappa_{\text{eff}}/\kappa = 1 + \frac{2\pi^2}{3} \exp(-4\pi\kappa\sqrt{2}). \qquad (14)$$

The rod-membrane steric interaction therefore produces a small correction to κ in this weak confinement limit ($b/r_0 \ll 1$). The energy F is given by substitution of Eq. (14) into Eq. (8). Energy minimization $\frac{\partial F}{\partial \bar{r}} = 0$ then yields the radius $\bar{r}$, to leading order

$$\bar{r}/r_0 = 1 + \frac{\pi^2}{3} \exp(-4\pi\kappa\sqrt{2}). \qquad (15)$$

The axial force can be obtained from mechanical equilibrium $\frac{\partial F}{\partial L} = 0$, giving $f = 2\pi\sqrt{2\sigma\kappa}[1 + \frac{2\pi^2}{3}\exp(-4\pi\kappa\sqrt{2})]$. Both of these results recover the well-known limiting forms [1], plus the leading order correction due to steric effects. Given a typical value of $\kappa \approx 10$ (in units of $k_B T$), we can see that the steric correction terms in $\bar{r}$ and f become very small in the present limit ($b/r_0 \ll 1$). However, as $b/r_0 \to 1$, we cross over to the narrow tube regime in which there is strong confinement of the membrane, as discussed below.

Narrow tubes ($\bar{r}/b \simeq 1$, $C\bar{r}^3/\kappa \gg 1$).—This case corresponds to when the average radius of the membrane tube is almost equal to the radius of the enclosed rod ($\bar{r}/b \simeq 1$). The steric effects of the rod in this limit should therefore be very strong, and the strength of the self-consistent, confining, harmonic potential becomes very large ($C\bar{r}^3/\kappa \gg 1$). In this case we can approximate Eq. (13) as follows:

$$\frac{(\bar{r}-b)^2}{b^2} \simeq \frac{1}{8\pi^2\kappa} \int_0^{2\pi} d\theta \int_0^\infty d\rho \frac{1}{\rho^2 + \frac{C\bar{r}^3}{\kappa}} \to \frac{1}{8}\sqrt{\frac{1}{\kappa C b^3}}$$

$$\text{as} \quad C \to \infty. \quad (16)$$

Hence in this limit $C = \frac{b}{64\kappa} \frac{1}{(\bar{r}-b)^4}$. Substituting this value of C into Eq. (9) for κ_{eff}, we obtain to leading order (as $C \to \infty$ and $\bar{r} \to b$)

$$\kappa_{\text{eff}}/\kappa = 1 + \frac{b^2}{32\kappa^2} \frac{1}{(\bar{r}-b)^2}. \qquad (17)$$

From Eq. (17) we can see that the rod-membrane steric interaction can dominate the effective rigidity in this strong confinement limit. Furthermore, substituting this dominant value of κ_{eff} into Eq. (8) we obtain (to leading order)

$$F = 2\pi\sigma Lb + 2\pi\sigma L(\bar{r}-b) - fL + \frac{\pi bL}{32\kappa} \frac{1}{(\bar{r}-b)^2}. \qquad (18)$$

A contribution to the energy that scales as the inverse squared distance, similar to the one appearing here, is well known for flat, parallel membranes at small intermembrane separation [15,26–28].

Proceeding as before, we find

$$\bar{r} = b + \left(\frac{b}{32\kappa\sigma}\right)^{1/3}, \qquad (19)$$

$$f = 2\pi\sigma b + \frac{3\pi}{2}\left(\frac{\sigma^2 b}{4\kappa}\right)^{1/3}. \qquad (20)$$

The leading order result $f \approx 2\pi\sigma b$ arises from the work done against surface tension in laying down an additional membrane area near the surface of a rod of radius b. Equation (19) and the $\sim\sigma^{2/3}$ correction in Eq. (20) are two of the primary testable predictions of this study. These results remain consistent for high enough tension or rod radius $\kappa\sigma b^2 \gg 1$. The $\sigma^{2/3}$ scaling can be traced to the inverse square law for the membrane force as a function of the membrane-rod separation [the last term in Eq. (18)].

Discussion.—Typical experimental parameter values for biological membranes might be [1,10] $\kappa \approx 10^{-19}$ J and $\sigma \approx 10^{-4}$ Jm^{-2}, which give rise to a typical tube radius of $r_0 \approx 20$ nm. Comparing this value for the membrane tube radius with typical biological fibers (or fiber bundles) with radii $b \sim 10$–100 nm, we conclude that steric effects will often be important and our results are therefore likely to be of biological significance under physiologically relevant conditions. Typical forces are also found to be in the experimentally relevant range of $f \sim 10$–100 pN. The steric corrections to $\bar{r}$ and f when the membrane is strongly confined by the presence of the enclosed rod are governed by the dimensionless parameter $\sim(\sigma\kappa b^2)^{-1/3}$, which is significant for the typical values given above. Hence these additional steric corrections may be expected to contribute a measurable correction to the expected radius $\bar{r}$ and force f. Our work may be of significant importance since measurements of the axial force are often used as an indirect way of estimating the tube radius.

Conclusions.—In this work we have studied the effect of an enclosed rod on the fluctuations of a cylindrical membrane tube, or spicule. We calculate the axial force exerted by the tube and its radius via a self-consistent harmonic potential that models the steric constraint of the rod on the

membrane fluctuations. The axial force diverges as the tube radius decreases and the membrane approaches the surface of the enclosed rod. Our approach is similar to that used for flat membranes [15,16]. However, in the present case, the fluctuating membrane tube is constrained only from inside by the presence of the rod and not from outside. This lack of symmetry gives rise to two parameters (C and J) instead of one. We believe that our results will be of importance in the context of polymerizing fibers within cell membranes [29–31]. In particular, we give the first quantitative treatment of the crossover between the "free" membrane tube, with radius $\bar{r} \gg b$, for which the axial force is small $f \sim \sqrt{\kappa\sigma}$ and the $\bar{r} \rightarrow b$ regime, in which the radius of the tube is almost constant and $f \sim \sigma$. Our analysis is also likely to be of significant utility in approaching such problems as the transport mechanism of material within the tube, e.g., monomers destined to polymerize at the tip of a growing rod. Such polymerization processes may be expected to depend intimately on the tube radius in the high tension (narrow tube) regime. In this sense, future dynamical theories for biopolymer growth in tubes (spicules) are likely to rely on an accurate treatment of the fluctuation effects that we have analyzed here.

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measurement following a halt in growth. Equilibration probably involves both relaxation of the leaflet asymmetry, arising from differential transport of material into the inner and outer monolayers of the tube, and the diffusive transport of material along the tube. The former depends on the interleaflet friction, as well as the structure and morphology of the cell membrane, and is difficult to estimate. The latter gives rise to a diffusional relaxation time that might crudely be estimated as $\tau_{\text{tube}} = (L/\bar{r})^2\tau$, where τ is the relaxation time of a "blob" of the tube of size $\bar{r}$, perhaps typically $\tau \approx 10^{-5}$ s. For rod growth rates that are up to $k \sim \mu\text{m/s}$ the quasistatic regime is then given by $L/k \gg (L/\bar{r})^2\tau$, which corresponds to $L \ll L_{\text{max}} = \bar{r}^2/(k\tau) \approx 40$ μm.

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