

Fluctuation Spectrum of Fluid Membranes Coupled to an Elastic Meshwork: Jump of the Effective Surface Tension at the Mesh Size

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We identify a class of composite membranes: fluid bilayers coupled to an elastic meshwork that are such that the meshwork’s energy is a function $F_{el}[A_\xi]$ not of the real microscopic membrane area A , but of a *smoothed* membrane’s area A_ξ , which corresponds to the area of the membrane coarse grained at the mesh size ξ . We show that the meshwork modifies the membrane tension σ both below and above the scale ξ , inducing a steep crossover of amplitude $\Delta\sigma = dF_{el}/dA_\xi$. The predictions of our model account for the fluctuation spectrum of red blood cell membranes coupled to their cytoskeleton. Our results indicate that the cytoskeleton might be under extensional stress, which would provide a means to regulate available membrane areas. We also predict an observable tension jump for membranes decorated with polymer “brushes.”

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Random surfaces [1] play an important role in many areas of physics, spanning from biophysics [2] and chemical physics [3] to high-energy physics [4]. In the last decades, there has been considerable interest in the properties of fluid bilayer membranes composed of amphiphilic molecules in water. It is well known [5] that their fluctuations are dominated by the membrane bending rigidity, κ , which leads to a spectrum proportional to $(\kappa q^4 + \sigma q^2)^{-1}$ [6–8]. Here, σ is an effective surface tension, i.e., an adjustable thermodynamic parameter arising from the constraint of constant surface area, which is usually several orders of magnitude smaller than the surface tensions of ordinary liquid interfaces [9,10]. Additional complexity arises if the membrane interacts with other systems (e.g., a rigid substrate, another membrane, a network of polymers or filaments) [11–13], or contains inclusions (passive or active) [14,15].

In this paper, we identify a class of composite membranes, i.e., of membranes coupled to an external system (like the cytoskeleton of red blood cells [16]), for which the coupling energy can be described, in a first approximation, by a function of the membrane area *coarse grained* at a characteristic length scale ξ (the mesh size in the cytoskeleton case). For such systems, we show that the effective membrane tension should exhibit a steep crossover of amplitude $\Delta\sigma$ at the scale ξ . In other words, the fluctuation spectrum is proportional to $(\kappa q^4 + \sigma^< q^2)^{-1}$ for $q \lesssim \xi^{-1}$ and to $(\kappa q^4 + \sigma^> q^2)^{-1}$ for $q \gtrsim \xi^{-1}$. As we shall see, $\Delta\sigma = \sigma^< - \sigma^>$ is directly related to the coupling between the membrane and the external system. Understanding the scale dependence of the effective surface tension, and, in particular, its value at short length scales, is important in a number of phenomena, e.g., membrane adhesion [17,18], cell fusion [19,20], and other microscopic biological mechanisms, such

as endocytosis [21]. It should also help interpret experiments on composite membranes [22–24], which attempt to determine the value of κ by fitting the fluctuation spectrum.

We shall consider two different systems for which our model predicts a crossover in surface tension: (i) membranes decorated with polymer “brushes” [25], and (ii) red blood cell membranes interacting with their attached cytoskeleton [16]. Polymer decorated membranes are useful in providing a sterical stabilization for liposome drug carriers [26,27]; we expect that better understanding their membrane tension should yield new insight in their stability and mechanical properties. Here, we shall mainly focus, however, on the composite membrane of the red blood cell (or erythrocyte). The cytoskeleton of red blood cells is a roughly triangular flexible protein meshwork, composed of spectrins attached to the bilayer by association with integral membrane proteins [16]. The resulting mechanical strength enables red blood cells to undergo large extensional deformations as they are forced through narrow capillaries. In the last two decades, the red blood cell membrane has been thoroughly studied and a rich description of its attached cytoskeleton has been obtained [28–30]. Very recently, the spectrum of the short wavelength membrane fluctuations of red blood cells [22] has been successfully fitted by including the effect of confinement from the cytoskeleton [24]; however, this approach leads to an unexplained, rather abrupt change in the value of the membrane tension. We shall show that our model explains quantitatively this change, provided that the cytoskeleton is under (extensional) stress, which is an important issue currently debated in cell biology [31,32]. Specific implications concerning red blood cells as well as possible experiments on polymer decorated membranes will be discussed at the end of the paper.

Let us introduce our model by considering a membrane (embedded in the Euclidean three-dimensional space) connected to an elastic meshwork. The meshwork is composed of N Hookean springs (spring constant λ , free length ℓ_0) and is attached to the membrane through its nodes. The shape of the membrane is specified by giving the position $\mathbf{R}(s^1, s^2)$ of a membrane element as a function of two intrinsic coordinates. The total free energy of the system may be written as $F[\mathbf{R}] = F_{\text{memb}}[\mathbf{R}] + F_{\text{el}}[\mathbf{R}]$, where $F_{\text{memb}}[\mathbf{R}]$ is the membrane free energy and

$$F_{\text{el}}[\mathbf{R}] = N\frac{1}{2}\lambda(\xi[\mathbf{R}] - \ell_0)^2 \quad (1)$$

is the meshwork elastic energy. Since the meshwork cannot follow the wiggles of the membranes of wavelength shorter than the mesh size (see Fig. 1), the spring elongation $\xi[\mathbf{R}]$ actually depends on the membrane conformation. To simplify, we have assumed that $\xi[\mathbf{R}]$ is identical for all the springs, which is reasonable since the membrane is fluid. The mesh size, i.e., the spring length, can thus be expressed as

$$\xi^2[\mathbf{R}] = g \frac{A_\xi[\mathbf{R}]}{N}, \quad (2)$$

where $A_\xi[\mathbf{R}]$ is the area of the membrane coarse grained at the mesh size, and g is a constant depending on the meshwork topology (e.g., $g = 2\sqrt{3}$ for triangular meshwork). Determining $\xi[\mathbf{R}]$ is the two-dimensional analogous of measuring the length of a fractal coast (e.g., the coast of Brittany [33]) with a straight ruler and determining the ruler length that allows one to go through the whole coast in N steps.

Solving Eq. (2) for ξ in the general case of an arbitrary membrane shape is a rather difficult task. In order to simplify the problem, let us consider the case of a quasi-spherical membrane of fixed volume V and fixed area A . If V is expressed as $V = \frac{4}{3}\pi R_0^3$, the area can then be written in the form $A = \bar{A} + \delta A$ where $\bar{A} = 4\pi R_0^2 \gg \delta A$ is called the projected area. We now consider a very

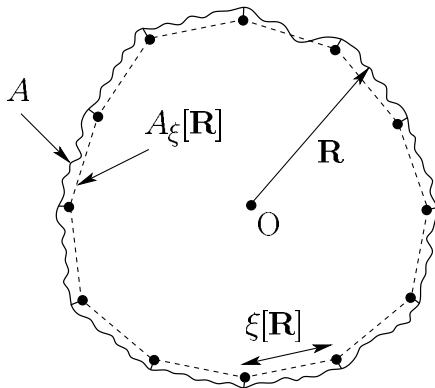


FIG. 1. Section of a fluctuating vesicle (thick line) to which is attached a meshwork of springs (dashed lines), with common length ξ . The actual membrane area is A and the mesh area is A_ξ , which corresponds to the membrane area coarse grained at ξ .

small, almost planar portion $\bar{a} = \epsilon\bar{A}$ of the projected area that still contains a rather large number $n = \epsilon N$ of springs. We represent the local membrane shape by its fluctuation modes $u_{\mathbf{q}}$ above the local projected area. Locally, Eq. (2) can be expressed as

$$\frac{n}{g}\xi^2[u] = a_\xi[u] = \bar{a} + \sum_{q \lesssim \xi^{-1}[u]} \frac{1}{2}q^2|u_{\mathbf{q}}|^2 + \mathcal{O}(u_{\mathbf{q}}^4), \quad (3)$$

where $a_\xi \approx \epsilon A_\xi$ is the local coarse-grained area. Consistently with the second-order expansion, the upper limit $\xi^{-1}[u]$ in the above sum can be replaced by its zeroth order approximation:

$$\xi_0^{-1} = \sqrt{\frac{n}{g\bar{a}}} = \sqrt{\frac{N}{g\bar{A}}}. \quad (4)$$

The elastic energy $f_{\text{el}} = \frac{1}{2}n\lambda(\xi[u] - \ell_0)^2 \approx \epsilon F_{\text{el}}$ of the small portion under consideration is then given by

$$f_{\text{el}}[u] = f_{\text{el}}^{(0)} + \Delta\sigma \sum_{q \lesssim \xi_0^{-1}} \frac{1}{2}q^2|u_{\mathbf{q}}|^2 + \mathcal{O}(u_{\mathbf{q}}^4), \quad (5)$$

where

$$\Delta\sigma = \frac{1}{2}g\lambda\left(1 - \frac{\ell_0}{\xi_0}\right). \quad (6)$$

To proceed further, we introduce the standard form of the local membrane elastic energy ($f_{\text{memb}} \approx \epsilon F_{\text{memb}}$), which is given, to quadratic order, by [3]

$$f_{\text{memb}}[u] = \sum_{\mathbf{q}} \frac{1}{2}(\gamma + \sigma q^2 + \kappa q^4)|u_{\mathbf{q}}|^2. \quad (7)$$

Here γ is a confining potential, σ an effective tension yet to be determined (a Legendre transform multiplier constraining the total membrane area), and κ is the bending rigidity [5]. From the total energy $f_{\text{el}}[u] + f_{\text{memb}}[u]$, we deduce, using equipartition, the fluctuation spectrum:

$$\begin{aligned} \langle |u_{\mathbf{q}}|^2 \rangle &= \frac{\epsilon k_{\text{B}} T}{\gamma + (\sigma + \Delta\sigma)q^2 + \kappa q^4}, & \text{for } q \lesssim \xi_0^{-1}, \\ \langle |u_{\mathbf{q}}|^2 \rangle &= \frac{\epsilon k_{\text{B}} T}{\gamma + \sigma q^2 + \kappa q^4}, & \text{for } q \gtrsim \xi_0^{-1}. \end{aligned} \quad (8)$$

The adjustable parameter σ is determined from the requirement $\sum_{\mathbf{q}} \frac{1}{2}q^2 \langle |u_{\mathbf{q}}|^2 \rangle = \epsilon \delta A$. It follows that the decomposition of the low wave vector tension, $\sigma^<$, and of the high wave vector tension, $\sigma^>$, in terms of σ and $\Delta\sigma$ ($\sigma^< = \sigma + \Delta\sigma$ and $\sigma^> = \sigma$) is quite arbitrary: only the values of $\sigma^<$ and $\sigma^>$ really matter. Note that the position ξ_0^{-1} of the crossover enters only as a rough characteristic scale in our model; a more refined calculation would be required to determine the wave vector width of the crossover. We have also omitted the corrections to the bending rigidity originating from the meshwork, which may also be scale dependent.

The results obtained above are based on the fact that the meshwork energy is actually a function of $A_\xi[\mathbf{R}]$ through Eqs. (1) and (2). The same conclusions concerning the effective tension therefore hold for any composite membrane for which the coupling may be approximated by a function $F_{el}(A_\xi)$ of the membrane area coarse grained at a characteristic scale ξ . The general expression giving the tension “jump” occurring at $\xi_0 \approx \xi$ is simply

$$\Delta\sigma = \left. \frac{\partial F_{el}}{\partial A_\xi} \right|_{A_\xi=\bar{A}}. \quad (9)$$

Before making predictions concerning other systems, such as polymer decorated membranes, let us discuss in more detail the cytoskeleton case.

Inspired by the recent work of Gov *et al.* [24], we have reanalyzed the data of Zilker *et al.* [22] on the fluctuation spectrum of normal red blood cells in the wavelength range $300 < \lambda < 6000$ nm. By fitting these data, the authors of Ref. [24] have demonstrated the necessity to introduce a confining potential, arising from the presence of the cytoskeleton. They acknowledge, however, that the data at very short scales are better fitted by a value of the membrane tension much smaller than the one obtained at large scales. Since the cytoskeleton is well described by a meshwork of elastic springs [16,32], we believe that the theory put forward in this paper provides an explanation to this abrupt change in surface tension. Fluctuation spectra are usually fitted via an effective wave vector-dependent bending rigidity κ_q defined by

$$\langle |u_{\mathbf{q}}|^2 \rangle = \frac{k_B T}{\kappa_q q^4}. \quad (10)$$

According to Eq. (8), this quantity should roughly exhibit a discontinuity at the mesh wave vector $q_0 = \xi_0^{-1}$. This is indeed the case, as evidenced by the solid line fit in Fig. 2. The position of the discontinuity being fairly marked at $\xi_0^{-1} \approx 125 \text{ nm}^{-1}$, and the value $\kappa \approx 2 \times 10^{-20} \text{ Nm}$ being already well documented [6,23,24], we have fitted the data to Eq. (8) for the parameters γ , $\sigma^<$, and $\sigma^>$ only, obtaining $\gamma = (5.2 \pm 0.6) \times 10^6 \text{ Jm}^{-4}$, $\sigma^< = (1.5 \pm 0.2) \times 10^{-6} \text{ Jm}^{-2}$, and $\sigma^> = (-0.8 \pm 1.2) \times 10^{-7} \text{ Jm}^{-2}$. According to our model, $\Delta\sigma = \sigma^< - \sigma^> \approx 1.6 \times 10^{-6} \text{ Jm}^{-2}$ should be given by Eq. (6). Indeed, assuming $\xi_0 > \ell_0$ (and yet $\xi \approx \ell_0$) we deduce $\lambda \approx 10^{-6} \text{ Jm}^{-2}$, which agrees well with the measured cytoskeleton elastic constants [34]. From the positive sign of $\Delta\sigma$, our analysis predicts that the cytoskeleton is under extensional stress: this important issue would require more experiments to test. Finding $\sigma^>$ essentially zero, or even negative, is also quite remarkable. It suggests that one important role of the cytoskeleton might be to regulate, via its elastic stress, the membrane tension at length scales shorter than the cytoskeleton mesh, in order to produce either large fluctuations, or even a short-scale buckling. This new type of area regulation (see Ref. [35]) might prove useful to allow for large deformations.

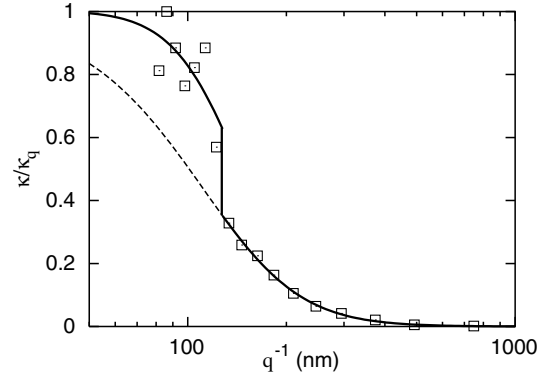


FIG. 2. Fit (solid line) of the measured fluctuation spectrum of a normal red blood cell (squares) from the data of Zilker *et al.* [22]. The dashed line extrapolates the large length-scale fit. The discontinuity and the corresponding tension “jump” occurs at a length scale ≈ 125 nm comparable to the cytoskeleton mesh.

Another system to which our model can be applied is a membrane bearing an anchored polymer brush. For a fixed number p of polymers in good solvent conditions, we have [25]

$$\frac{F_{el}}{pk_B T} \approx Nb^{5/3}(1 - 2\chi)^{1/3}\Sigma^{5/6}, \quad (11)$$

where N denotes now the index of polymerization, b the monomer length, χ the Flory parameter, and Σ the grafting density. As illustrated in Fig. 3, the relevant area defining the grafting density is not the total area A , but the area A_ξ coarse grained at the blob scale. Hence,

$$\Sigma \approx \frac{p}{A_\xi} \approx \frac{1}{\xi^2}. \quad (12)$$

Expressing as in Eq. (5) the brush energy in terms of the local coarse-grained area, or equivalently, using Eq. (9), we obtain

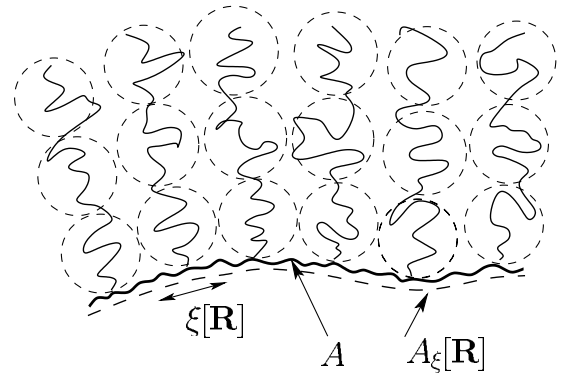


FIG. 3. Sketch of a polymer “brush” anchored on a fluctuating membrane. The actual grafting density is the number of polymers divided by the membrane area A_ξ (dashed line) coarse grained at the blob size ξ .

$$\Delta\sigma \simeq -Nk_B T b^{5/3} (1 - 2\chi)^{1/3} \left(\frac{p}{A}\right)^{11/6}. \quad (13)$$

This actually corresponds to the brush osmotic pressure integrated over the brush height: $\Delta\sigma \simeq -\Pi_{\text{osm}} H \simeq -k_B T (p/\bar{A})^{3/2} \times bN(b^2 p/\bar{A})^{1/3}$. Note that $\Delta\sigma < 0$, i.e., the brush reduces the large-scale tension with respect to the short-scale one. It actually draws some of the membrane area stored at length scales shorter than the blob size in order to lower its grafting density. Let us estimate $\Delta\sigma$ in typical experiments [12]: with $N = 100$, $b = 1$ nm, and a (projected) area per chain of 100 nm², we obtain $|\Delta\sigma| \approx 10^{-4}$ J m⁻², which compares with ordinary membrane tensions. At length scales larger than the brush height H , the brush elasticity also yields a renormalization of κ [36]. Note that for distortions wave vectors in the interval $H^{-1} < q < \xi^{-1}$, the brush will actually heal over $\sim q^{-1}$, implying that the crossover will probably be spread over this interval [37]. Our result agrees with a recent direct calculation of Bickel *et al.* [38], showing that membranes bearing polymers in the “mushroom” regime should possess an excess tension $\simeq 2k_B T \Sigma$ at scales shorter than the gyration radius R_g . Indeed, at the grafting density $\Sigma \simeq 1/R_g^2$ which crosses over to the brush regime, this gives $\Delta\sigma \simeq -\Pi_{\text{osm}} H$, with $\Pi_{\text{osm}} \simeq k_B T/R_g^3$ and $H \simeq R_g$. A possible way to test our prediction Eq. (13) would be to measure, on a given vesicle decorated with a polymer brush, the variation of the large-scale membrane tension induced either by a modification of the solvent quality or by a chemical alteration of the index of polymerization.

To conclude, the steep crossover of the effective surface tension evidenced in this paper arises from a competition between the free energy of the membrane, which requires a certain amount of excess area to be stored in the short wavelength modes, and the free energy of the coupled elastic system, which is lowered if either less or more excess area (depending on the system) is transferred to the large-scale modes. Very generally, a wave vector-dependent membrane tension should therefore occur whenever an external system coupled to the membrane produces a scale-dependent area transfer.

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