Membrane Undulations Driven by Force Fluctuations of Active Proteins

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We analyze the height undulations of a membrane due to fluctuations in the force generated by membrane-bound proteins that induce normal motion or bending. We compare our results to the results of experiments on red blood cells and vesicles with incorporated active proton pumps. We treat these proteins as having an intrinsic time scale for the force generation or conformational change, leading to nonthermal membrane fluctuations. We find that the active fluctuations are inversely proportional to the viscosity of the surrounding fluid. This highlights some universal features of active membrane undulations.

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Active membranes, whereby nonthermal energy contributes to the membrane height fluctuations, occur in cells [1,2] and have been produced synthetically [3]. These membranes are composed of lipid bilayers that are driven by active proteins that are either embedded in the bilayer or adsorbed to it (such as cytoskeleton proteins). These active proteins convert chemical adenosine triphosphate (ATP) or light energy into mechanical motion of the membrane. In a previous work [1], we analyzed the ATP-induced contribution to the fluctuations of the red blood cell (RBC) membrane. Here we extend this analysis to the more general case of membrane undulations induced by force fluctuations of active proteins. We then apply our results to the case of synthetic vesicles containing light-driven proton pumps, namely, bacteriorhodopsin (BR) [3]. In these systems, a lipid vesicle is prepared, with incorporated proteins [4], and the membrane fluctuations are measured through their effect on the strain-stress relation of the vesicle. It has been shown [5] that light energy absorbed by BR is converted not only to chemical energy but also to mechanical energy, which is manifested in the normal motion of the membrane. The precise origin of membrane motion induced by the light activation has not yet been determined experimentally. It could be the result of the hydrodynamic motion due to the pumping activity, as suggested in [6,7], or through conformational changes of the protein that cause bending of BR and the surrounding membrane [2], or a combination of both.

The main physical features of these active proteins are that they ''kick'' the membrane locally and independently of each other. We therefore follow here the analysis developed in [1]. The equation of motion for the amplitude h of membrane fluctuations of wave vector q (in the usual limit of large damping) is given in the familiar Langevin form

$$\dot{h}(t) + \omega_q h = \xi(t). \tag{1}$$

The first term on the left-hand side of Eq. (1) is the viscous damping of the membrane due to the surrounding fluid, and the second term is the restoring force due to the curvature stiffness of the bilayer. The natural time scale of membrane motion is $\omega_q \simeq (\kappa q^3 + \sigma q)/4\eta$, where η is the viscosity of the surrounding fluid, κ is the bending modulus of the lipid membrane, and σ is the membrane surface tension modulus.

The right-hand term $\xi(t)$ is the normalized force due to the activation of the active proteins, given by $\xi(t) = P(t)/(4\eta q)$, where $P(t)$ is the actual force per unit membrane area. Fourier transforming Eq. (1) in time we get

$$h(\omega) = \frac{\xi(\omega)}{i\omega + \omega_q} \Rightarrow \langle |h(\omega)|^2 \rangle = \frac{\langle |\xi(\omega)|^2 \rangle}{\omega^2 + \omega_q^2}. \tag{2}$$

In the present work we shall describe a system where the average force of the active protein is zero $\langle \xi \rangle = 0$, while the fluctuations have the following exponential correlation function in time [1,8]

$$\langle \xi(0)\xi(t) \rangle(q) = \left(\frac{F}{4\eta q}\right)^2 \frac{n}{2} e^{-|t|/\tau}. \tag{3}$$

This model describes active proteins that can either induce membrane motion in both directions symmetrically, or the case where the proteins are asymmetric but the membrane contains a uniform distribution of both up and down species that can diffuse freely in the liquid bilayer. In this respect our treatment is complementary to that of [6], where active proteins of finite average force were considered, which is then dominant over the fluctuating contribution.

We now describe in detail the temporal and spatial correlations of the active proteins, shown in Eq. (3). We will treat in this work two cases of active membrane-proteins. The first is of force centers that couple directly to the local membrane height h in Eq. (1), characterized by the direct-force F. This may occur when the membrane motion is induced by the cytoskeleton, such as in the RBC [1], or for ion pumps that are able to induce a direct normal force on the membrane, such as in the case of BR and Ca^{2+}-ATPase. The second case corresponds to membrane motion that is induced by the activation of conformational changes in the proteins. In this case it is natural to assume that the force F is coupled to the local membrane curvature $\nabla^2 h$ (instead of h) in Eq. (1), so that we get $F^2 \rightarrow F_r^2 (qr)^4$

in Eq. (3). The measurements and analysis of Porschke [2] show that the purple membrane that contains the BR proteins in the bacteria exhibits signs of strong bending in response to light. The light-induced conformational change of the BR protein corresponds to a spontaneous curvature of radius $r \sim 250$–350 nm [2]. Other active membrane-proteins also induce local curvature, such as the Ca^{2+}-ATPase [9].

In both cases we assume that the activity of the pointlike proteins is completely uncorrelated in space, so that the Fourier transform of the (δ-function) pressure correlations is a constant in q space $\langle P^2\rangle(q) = F^2 n$, where n is the areal density of the active proteins in the membrane. We assume here that each BR protein affects a membrane area $1/n$ around it. We neglect here any correlation between the forces induced by distant active proteins due to the flow of the surrounding fluid or the in-plane flow of the membrane. The temporal correlations of the membrane-bound protein are only characterized by its intrinsic time scale τ, and shot-noise exponential correlations [Eq. (3)]. This represents the time it takes the protein to produce the force fluctuation F.

Fourier transforming Eq. (3) in time we get

$$\langle|\xi(\omega)|^2\rangle(q) = \left(\frac{F}{4\eta q}\right)^2 \frac{n\tau}{1+(\tau\omega)^2}. \tag{4}$$

Plugging the force correlation function (4) in Eq. (2), we find

$$\langle|h(\omega, q)|^2\rangle_{\text{active}} = \left(\frac{F}{4\eta q}\right)^2 \frac{n}{\omega^2+\omega_q^2} \frac{\tau}{1+(\tau\omega)^2}. \tag{5}$$

Integrating over all frequencies, we find that the equal time (static) correlation function for height fluctuations is

$$\langle|h(q)|^2\rangle_{\text{active},d} = \left(\frac{F}{4\eta q}\right)^2 \frac{n}{2\omega_q(\omega_q+1/\tau)} \tag{6}$$

$$\langle|h(q)|^2\rangle_{\text{active},c} = \left(\frac{F_r q r^2}{4\eta}\right)^2 \frac{n}{2\omega_q(\omega_q+1/\tau)}, \tag{7}$$

where the first line is for the direct-force case and the second is for the curvature-induced motion. The different q dependence that results from Eqs. (6) and (7) are summarized in Table I, compared to the thermal case (for the thermal case, the limit $q \to \infty$ always behaves as $|h(q)|^2_{\text{thermal}} \simeq k_B T/\kappa q^4$). The results for $\sigma \neq 0$ in the limit $q \to \infty$ stands for the region of q space where $\omega_q \tau \gg 1$ but the tension σ is still dominant (i.e., $\sqrt{\sigma/\kappa} \gg q$).

There are three length scales that determine the q dependence of the static correlations. The first determines if the tension or curvature dominate, given by $q_c \sim \sqrt{\sigma/\kappa}$. The other two critical wave vectors determine wether the membrane correlations are dominated by the intrinsic time scale τ of the active proteins or by the natural motion of the membrane ω_q. It is given by $q_{c,\kappa} \sim (\eta/\kappa\tau)^{1/3}$ and $q_{c,\sigma} \sim \eta/\sigma\tau$ for the tension or curvature dominated regions, respectively.

Assuming that the active fluctuations are incoherent with respect to the thermal contribution, we predict an additive contribution to the total mean-square fluctuation amplitude [1,6]: $\langle|h(q)|^2\rangle_{\text{total}} = \langle|h(q)|^2\rangle_{\text{active}} + \langle|h(q)|^2\rangle_{\text{thermal}}$. The active temperature is therefore defined as the ratio of the active [Eqs. (6) and (7)] to the thermal fluctuations: $T_{\text{active}}(q)/T \equiv \langle|h(q)|^2\rangle_{\text{active}}/\langle|h(q)|^2\rangle_{\text{thermal}}$, and the overall effective temperature is $T_{\text{eff}}(q)/T = 1 + T_{\text{active}}(q)/T$ [1,6]. The results are summarized in Table II.

In most of the cases shown in Table I we find that the active fluctuations decay with increasing wave vector q, similar to the thermal behavior, though the power laws are somewhat different from the thermal case. The most outstanding behavior is for the tension-dominated curvature-induced motion: in this case, the fluctuations vanish for small q and approach a constant at large q (i.e., independent on q). This unusual behavior may have been observed in recent experiments on BR-containing vesicles [10]. Furthermore, our results are somewhat different from those obtained previously [6,7,11]. Comparing to these studies, we treat a uniform distribution of fluctuating force proteins, while those previous studies treated proteins that produce a constant force but allow for thermal density fluctuations of these proteins in the membrane. Note that in both our case (Table I) and the previous study [6], there are regimes where the active fluctuations have the same $1/q^4$ behavior as for thermal fluctuations.

Integrating over all wave vectors q, we get from Eq. (5) the dynamic correlation function (or power spectrum) at each point on the membrane: $\langle|h(\omega)|^2\rangle_{\text{active}}$. For the case of tensionless membranes ($\sigma = 0$) we find

$$\langle|h(\omega)|^2\rangle_{\text{active},d} \approx \left(\frac{F}{4\eta}\right)^2 \frac{n\tau}{\omega^2[1+(\omega\tau)^2]} \tag{8}$$

$$\langle|h(\omega)|^2\rangle_{\text{active},c} \approx \left(\frac{Fr^2}{4\eta}\right)^2 \frac{n\tau}{\omega^{2/3}(\kappa/4\eta)^{4/3}[1+(\omega\tau)^2]}, \tag{9}$$

TABLE I. $\langle|h(q)|^2\rangle$, where $A \equiv F^2 n$.

Force Type	q Limit	$\sigma = 0$	$\sigma \neq 0$
Direct	$q \to 0$	$(A\tau/8\eta\kappa)q^{-5}$	$(A\tau/16\eta\sigma)q^{-3}$
Direct	$q \to \infty$	$(A/2\kappa^2)q^{-8}$	$(A/8\sigma^2)q^{-4}$
Curvature	$q \to 0$	$(Ar^4\tau/8\eta\kappa)q^{-1}$	$(Ar^4\tau/16\eta\sigma)q$
Curvature	$q \to \infty$	$(Ar^4/2\kappa^2)q^{-4}$	$(Ar^4/8\sigma^2)$
Thermal	$q \to 0$	$(k_B T/\kappa)q^{-4}$	$(k_B T/2\sigma)q^{-2}$

TABLE II. $T_{\text{eff}}(q)$

Force Type	q Limit	$\sigma = 0$	$\sigma \neq 0$
Direct	$q \to 0$	$(A\tau/8\eta k_B T)q^{-1}$	$(A\tau/8\eta k_B T)q^{-1}$
Direct	$q \to \infty$	$(A/2\kappa k_B T)q^{-4}$	$(A/4\sigma k_B T)q^{-2}$
Curvature	$q \to 0$	$(Ar^4\tau/8\eta k_B T)q^3$	$(Ar^4\tau/8\eta k_B T)q^3$
Curvature	$q \to \infty$	$(Ar^4/2\kappa k_B T)$	$(Ar^4/4\sigma k_B T)q^2$

which are different from the thermal correlation function $\langle|h(\omega)|^2\rangle_{\text{thermal}} \propto 1/\omega^{5/3}$ [12] in the limit of small frequencies.

The mean-square fluctuations are given by integrating $\langle|h(q)|^2\rangle$ [Eqs. (6) and (7)] over q space. The results are summarized in Table III in the limit of large vesicle radius R.

Comparing the result for the direct force and for the thermal mean-square fluctuation amplitude (for $\sigma = 0$), we can therefore define an effective temperature of the form

$$k_B T_{\text{active},d} = \frac{F^2 n \tau R}{4\eta}. \tag{10}$$

For the largest wavelength mode $q \sim 1/R$, this definition agrees with $k_B T_{\text{eff}}(q = 1/R)$ from Table III. For the curvature-induced force in the tensionless case we find that the fluctuation amplitude is independent on the vesicle size R (Table III). For this case we can define an effective temperature

$$k_B T_{\text{active},c} = \frac{\pi F^2 r^2 n \tau^{2/3}}{12\sqrt{3}\eta^{2/3}\kappa^{1/3}}\left(\frac{r}{R}\right)^2 \simeq k_B T_{\text{active},d}\frac{r^4 q_{c,\kappa}}{R^3}. \tag{11}$$

We now compare with the results of micro-pipette experiments on BR-containing giant vesicles [3,6]. These experiments measure the integrated membrane amplitude fluctuations through the dependence of the excess membrane area α on the applied tension σ, which is given by $\alpha = \int q^2\langle|h(q)|^2\rangle d^2q$. For the thermal case the result is logarithmic: $\Delta\alpha_{\text{thermal}} \simeq (k_B T/8\pi\kappa)\log(\sigma/\sigma_0)$, and is therefore used to measure the bending modulus κ of the membrane [3,6,9] ($\sigma_0 \sim 5 \times 10^{-7}$ J/m^2 being some reference tension [3,6]). In Fig. 1 we plot the results of numerically integrating $\Delta\alpha$ for the case of thermal membrane and for membranes containing thermal and direct or curvature-force proteins. The behavior of the active membranes is seen to change at the critical tension $\sigma_\tau \simeq (\eta\sqrt{\kappa}/\tau)^{2/3}$ (Fig. 1). For the direct-force membrane we find that for small tensions ($\sigma < \sigma_\tau$) the excess area behaves as $\Delta\alpha_{\text{active},d} \simeq (F^2\tau n/\eta\sqrt{\kappa})\sqrt{1/\sigma}$. This term therefore dominates over the logarithmic contribution of the thermal fluctuations. In the limit of large tensions ($\sigma > \sigma_\tau$), the additional active contribution vanishes as $\Delta\alpha_{\text{active},d} \simeq (F^2 n)(1/\sigma)^2$, and the thermal contribution dominates (Fig. 1) so that the slope is equal to the thermal case. For the curvature force in the limit of small tensions ($\sigma < \sigma_\tau$), the excess area is $\Delta\alpha_{\text{active},c} \propto (\sigma/\sigma_0)^{3/2}$, which vanishes in this limit, so that the thermal contribution dominates (Fig. 1). In the limit of large tensions ($\sigma > \sigma_\tau$) we find $\Delta\alpha_{\text{active},c} \simeq (k_B T_{\text{active},\alpha}/8\pi\kappa)\log(\sigma/\sigma_0)$, which defines another "active" temperature:

$$k_B T_{\text{active},\alpha} \simeq (2F^2 n r^4/\kappa). \tag{12}$$

In order to quantitatively compare our results with the experimental data, we must determine the parameters describing the active proteins. The intrinsic time scale in the case of the BR photo cycle is $\tau \sim 5$ m sec [2,3,6], which is also the time scale for the protein conformational change. The normal force F generated by the active proteins is unknown: for the light-activated BR it has been estimated as $F \sim \kappa/w \sim 8$ pN [6], where the width of the lipid membrane is $w \sim 5$ nm. Alternatively, from purple-membrane bending [2] it can be estimated as $F \simeq \kappa/r \sim 0.2$ pN, using $r \simeq 350$ nm. For Ca^{2+}-ATPase [9], the bending radius is also of the order of $r \simeq 100$ nm, so the force should be similar (in both cases the active protein develops an internal angle of at most 0.1 rad). The estimated BR density in the experiments [3,6] is $nw^2 \sim 1$–0.1, and $nw^2 \sim 0.3$–0.1 in the Ca^{2+}-ATPase experiments [9]. Let us compare these numbers with the fits of our calculations to the data for vesicles containing BR (Fig. 1). For the thermal case we find $\kappa \simeq 12 k_B T$, in agreement with [3,6]. The direct-force description is seen to agree reasonably well with the data, using $F^2 n/128\eta^2 \sim 10^{-8}$ (m/sec)^2. If we use the estimated values of the density given above and the viscosity of water, then the force is $F \simeq 1$ fN, which is $\sim$100 times smaller than our lowest estimation above [13]. Using these values, the active temperature for the curvature force in the limit of large tensions becomes $k_B T_{\text{active},\alpha} \simeq (F^2 n r^4/\kappa) \sim 2 k_B T$, which is in good agreement with the slope of a linear fit through

TABLE III. Mean-square amplitude $\langle|h|^2\rangle$ for vesicle of radius R, where $A \equiv F^2 n$ and a is a small length-scale cutoff.

Force Type	$\sigma = 0$	$\sigma \neq 0$
Direct	$(A\tau/48\pi\eta\kappa)R^3$	$(A\tau/96\pi\eta\sigma)R$
Curvature	$(Ar^4\tau^{2/3}/48\sqrt{3}\eta^{2/3}\kappa^{4/3})$	$(A\tau r^4/32\pi\eta\sigma)a^{-3}$
Thermal	$(k_B T/4\pi\kappa)R^2$	$(k_B T/8\pi\sigma)\log R$

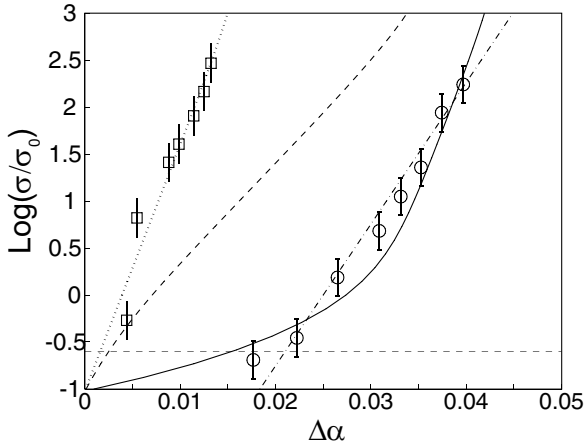

FIG. 1. Calculated excess area $\Delta\alpha$ for the thermal (dotted line), direct force (solid line), and curvature force (dashed line) as a function of the applied tension σ (using $\kappa \simeq 12 k_B T$). The experimental data is for vesicles containing BR [3]: squares—no light; circles—with BR activity. The horizontal dashed line shows the value of the crossover tension σ_τ. The dash-dotted line is a linear fit to the active data $T_{\text{active}}/T \sim 2$.

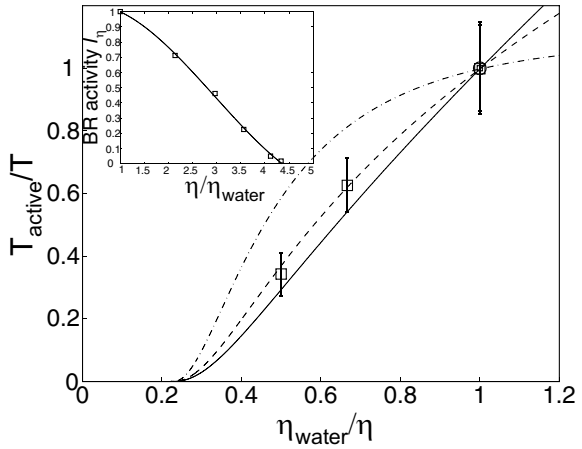

FIG. 2. Calculated active temperature $T_{\mathrm{active},d}$, $T_{\mathrm{active},c}$, and $T_{\mathrm{active},\alpha}$ for direct and curvature-induced membrane motions as a function of the viscosity (solid, dashed, and dash-dotted lines, respectively), both normalized by the values at the viscosity of water η_{water}. The experimental data: squares [3]. The glycerol inhibition of the BR activity I_η is shown in the inset (normalized to the value in water; squares-experimental data [16], solid line guide to the eye).

the light-activated data (Fig. 1). It is therefore not clear from this data which type of force (direct or curvature) is the most appropriate to describe the BR vesicles. Nevertheless, there is overall consistency and good agreement between the calculated and measured active "temperature," which both turn out to be of order $\sim k_B T$.

Throughout this Letter we found a predicted linear dependence of the active membrane fluctuations on the protein density n. This behavior has been found in recent experiments on vesicles containing Ca^{2+}-ATPase [9].

Finally, let us compare the predicted viscosity dependence of the membrane fluctuations with the experimental data. While the thermal fluctuations are independent on the viscosity, the active part depends explicitly on the viscosity (Tables I, II, and III). This dependence has been explored experimentally in RBC's, where a clear $1/\eta$ dependence was observed for the ATP-driven membrane fluctuations [14]. This behavior is well described by the direct active protein (Table III) [1]. Note that the coupling of the membrane to the cytoskeleton also modifies the elastic properties of the membrane [15]. These modifications will affect both the thermal and active membrane fluctuations, but will not change the distinct qualitative differences between them.

For the BR protein, the viscosity affects the strength of the force F. The activity of the BR protein is known to be inhibited by the presence of glycerol [16], which was used to vary the viscosity [3]. We show in the inset of Fig. 2 the BR activity I_η as a function of the viscosity [16], assuming that the viscosity increases linearly with the glycerol concentration. The force is therefore modified by the function I_η, such that $F \rightarrow F \times I_\eta$.

For the direct-force case, the viscosity dependence for both the mean-square fluctuations (Table III) and the excess area is the same: $T_{\mathrm{active},d} \propto I_\eta^2/\eta$ [Eq. (10)]. For the curvature force the viscosity dependence of the mean-square fluctuations [Table III] is $T_{\mathrm{active},c} \propto I_\eta^2/\eta^{2/3}$ [Eq. (11)], while for the excess area it is $T_{\mathrm{active},\alpha} \propto I_\eta^2$ [Eq. (12)]. We plot in Fig. 2 these active temperatures as a function of the viscosity (both are normalized to the values at the viscosity of water η_{water}). We find an excellent agreement with the experimental data [3] for both $T_{\mathrm{active},d}$ and $T_{\mathrm{active},c}$, with the data not enabling us to distinguish between them.

We therefore conclude that the height fluctuations of active membranes exhibit general properties, irrespective of the energy source. Future experiments could differentiate between the type of active protein described in this work by measuring the membrane fluctuations over as large a range in space and time as possible, while also varying the viscosity and protein density parameters. In addition, there is a need for better experimental determination of both the force F and the membrane bending induced by each protein activation.

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