Nonequilibrium Fluctuations, Travelling Waves, and Instabilities in Active Membranes

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The stability of a flexible fluid membrane containing a distribution of mobile, active proteins (e.g., proton pumps) is shown to depend on the structure and functional asymmetry of the proteins. A *stable* active membrane is in a nonequilibrium steady state with height fluctuations whose statistical properties are governed by the protein activity. Disturbances are predicted to travel as waves at sufficiently long wavelength, with speed set by the normal velocity of the pumps. The *unstable* case involves a spontaneous, pump-driven undulation of the membrane, with clumping of the proteins in regions of high activity.

The functioning of active proteins in energy-dissipating processes, such as ion transport, protein translocation, and biopolymer synthesis, generates forces on the membranes of the living cell and its organelles [1,2]. As the active proteins diffuse around in the membrane, the resulting fluctuations in this force provide a nonthermal source of noise for shape fluctuations of the membrane. The membranes of a living cell are therefore nonequilibrium or *active* membranes. Although such active, nonequilibrium processes are abundant in biological membranes, physicists have focussed mainly — with considerable success [3,4] — on the statistical mechanics of membranes at thermal equilibrium. There are however reasons [5,6] to suspect that nonequilibrium processes are at work even in red-blood-cell flicker, traditionally explained as thermal equilibrium shape fluctuations [7]. The predictions [8,9] of fluctuation enhancement in active membranes and the micropipette experiments [10] on membranes laden with the photoactive proton pump bacteriorhodopsin (bR) are further motivation for our studies.

In this Letter, we consider the statistical mechanics and dynamics of a fluid membrane containing a distribution of identical, active pumps free to move in the plane of the membrane. By "pump" we mean a membrane-spanning protein which, when supplied with energy, transfers material (and thus exerts a force on the membrane) in one direction only. This ignores the complexities of some real pumps but is a good description of bR. By convention, we shall term the end towards which the force acts the *head* of the pump, and the other end its *tail*. We shall call a protein a "+ pump" ("− pump") if the vector from its tail to its head points *parallel to* (*antiparallel to*) a fixed outward normal of the membrane. Flips from + to −, prohibitively slow in any real system, are forbidden in our treatment. A membrane will be termed "balanced" ("unbalanced") if the numbers of + and − pumps are equal (unequal). The unidirectional pumping action means that a given pump knows up from down. In general, therefore, (i) a pump of a given sign will favor one sign of the local mean-curvature H of the membrane over the other (Fig. 1), and (ii) its pumping activity will depend on H. Incorporating these effects distinguishes the present work from [8,9].

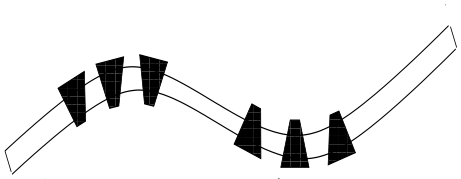

FIG. 1. Asymmetric proteins (the black wedges) imbedded in a fluid membrane are drawn to regions with curvature adapted to the protein shape.

Here is a summary of our main results: (i) An active membrane can be either linearly *stable* or linearly *unstable* against undulation and pump aggregation, depending on the structural and functional asymmetries mentioned in the previous paragraph. (ii) In the *stable* case, for plausible values of the physical parameters a "balanced" active membrane has, over an appreciable intermediate range of wavenumbers k, a height variance $\langle|h_k|^2\rangle \propto 1/k^4$, with a coefficient *independent* of the concentration of pumps. These are truly nonequilibrium fluctuations, with a strength depending on *kinetic* coefficients: $\langle|h_k|^2\rangle$ is proportional to the permeability of the membrane. This is broadly consistent with the observations of [10]. (iii) For a *stable* membrane, the longest wavelength disturbances travel as non-dispersive waves, i.e., waves with a speed independent of wavevector k. The wave speed c is set by the pump activity and independent of the membrane elasticity. (iv) In this longest wavelength regime, the height variance is much smaller, as $k \to 0$, than in the equilibrium case; specifically $\langle|h_k|^2\rangle \propto \frac{1}{k^2}$ as $k \to 0$. This result depends on the effect of nonlinearities, which also determine the final state of the system [11,12] in the unstable case.

We now construct our model and derive the results stated above. We characterize the active membrane by its mean curvature H and the areal concentrations $\nu_\pm$ of + and − pumps . In a Monge description $H \simeq -\nabla^2 h$ and $\nu_\pm = n_\pm/\sqrt{1+(\nabla h)^2}$ where $h(\mathbf{x},t)$ is the height of the membrane at time t above point $\mathbf{x}$ on a two-dimensional

reference plane, and $n_\pm(\mathbf{x},t)$ are the *projections* of the pump concentrations onto the plane. It is useful to distinguish the *protein concentration*

$$n(\mathbf{x},t) \equiv n_+(\mathbf{x},t) + n_-(\mathbf{x},t) \tag{1}$$

and the *signed protein density*

$$m(\mathbf{x},t) \equiv n_+(\mathbf{x},t) - n_-(\mathbf{x},t) \tag{2}$$

with averages m_0 and n_0 respectively. For convenience, we shall work with

$$\psi \equiv \frac{m}{n_0},\ \phi \equiv \frac{n}{n_0},\ \psi_0 \equiv m_0/n_0. \tag{3}$$

We begin with a membrane *at thermal equilibrium* at temperature $T \equiv \beta^{-1}$, where the proteins are *present* but not *activated*. The probability of a configuration $\{h(\mathbf{x}), \psi(\mathbf{x})\}$ is $\propto \exp(-\beta E)$ with a Hamiltonian [13]

$$\begin{aligned} E\,[h,\psi] \,=& \frac{1}{2}\int \mathrm{d}^2x[\kappa(\nabla^2 h)^2 + \sigma(\nabla h)^2 \\ &+A(\psi-\psi_0)^2 - 2\kappa\bar{H}\psi\nabla^2 h] \end{aligned} \tag{4}$$

to bilinear order in the variables involved. E contains a bending energy [3] with a rigidity κ, a surface tension σ, a compression energy for the signed protein density, with osmotic modulus

$$A \sim T n_0 \tag{5}$$

for $n_0 \to 0$, and a coupling $\bar{H}$ which determines the local spontaneous curvature induced [14] by the presence of a protein (Fig. 1). This last term is one of the consequences of the directionality of the pumps, and can be attributed to a head-tail size difference of magnitude

$$\ell_1 \equiv \frac{\bar{H}}{n_0}. \tag{6}$$

We expect ℓ_1 to be independent of n_0 for $n_0 \to 0$.

We now explain why (4) ignores the protein concentration field ϕ. Minimizing (4) tells us that in thermal equilibrium a membrane with a net imbalance ψ_0 of pumps will develop a spontaneous curvature

$$H_0 \equiv \left(\nabla^2 h\right)_{\min\ \mathrm{E}} = \frac{\bar{H}}{1-\frac{\kappa\bar{H}^2}{A}}\,\psi_0 \quad . \tag{7}$$

Many experiments on the physics of membranes are carried out on giant vesicles (size $\gtrsim 20\mu$m), for which the mean curvature and hence, from (7), ψ_0 are negligibly small. Accordingly, we shall work at $\psi_0 = 0$ for much of this paper. In this limit, the symmetry $h \to -h$, $\psi \to -\psi$ (equivalently $\epsilon \to -\epsilon$ for each pump of sign $\epsilon = \pm$) rules out any bilinear coupling $\psi\phi$ in (4) for $\psi_0 = 0$. Thus, in a linearized treatment of a balanced membrane, ϕ decouples from h and ψ. In addition, for $\psi_0 = 0$, the mean normal drift speed of the membrane in the active state is zero. Towards the end of this paper we shall present some important results for $\psi_0 \neq 0$.

Equation (4) implies the *equilibrium* height variance

$$\langle |h_k|^2 \rangle = \frac{T}{\sigma k^2 + \kappa_{eff} k^4} \tag{8}$$

at wavevector $\mathbf{k}$, with an effective rigidity

$$\kappa_{eff} = \kappa - \frac{(\kappa\bar{H})^2}{A} \tag{9}$$

independent of $\mathrm{sgn}(\bar{H})$. The dynamics of small fluctuations is also determined by κ_{eff}. We shall assume that $\bar{H}$ is small enough to keep $\kappa_{eff} > 0$ [16], so that the presence of the inactive but shape-asymmetric proteins merely shifts the value of the bending rigidity.

In the active state (e.g., when the bR in the experiments of [10] is illuminated with green light) even static quantities such as $\langle |h_k|^2 \rangle$ must be determined from the *dynamical* properties of the system. To this end, let us assemble the ingredients for the equations of motion, to leading orders in a gradient expansion, of a membrane with active proteins. We continue to work at $\psi_0 = 0$.

An isolated active pump with sign $\epsilon = \pm$ exerts a force ϵF_a normal to the membrane, if the local mean curvature $H = 0$. If $H \neq 0$, the symmetry $h \to -h$, $\epsilon \to -\epsilon$ permits an additional contribution $\ell_2 H F_a$ to the force, where ℓ_2, whose sign is not fixed by symmetry, is a length characterizing the sensitivity of the pumping mechanism to the bending of the membrane. The force arising from the activity of a distribution of $+$ and $-$ pumps with intrinsic concentrations $\nu_\pm$, together with the elastic force f_{el} arising from (4), give rise via permeative flow with kinetic coefficient μ_p [18] to a normal velocity

$$\begin{aligned} v_n = \mu_p[&(\nu_+ - \nu_-) + (\nu_+ + \nu_-)\ell_2 H \\ &+O(\nu_+ - \nu_-)^3]F_a - \mu_p f_{el}, \end{aligned} \tag{10}$$

where the $O(\nu_+ - \nu_-)^3$ (and higher odd order) terms arise only if the presence of a given pump affects the activity of other pumps.

Projecting (10) *normal* to the reference horizontal plane [17], defining the natural velocity scale

$$v_0 \equiv \mu_p F_a n_0 > 0, \tag{11}$$

adding the contribution v_{hyd} due to *hydrodynamic* flow [7,19] and expanding in powers of ∇h, we obtain

$$\begin{aligned} \frac{\partial h}{\partial t} &= v_0[\psi + \ell_2\phi\nabla^2 h + O(\psi^3, \psi^3(\nabla h)^2)] \\ &\quad -\mu_p \delta E/\delta h + v_{hyd} + f_h \\ &\simeq v_0(\psi + \ell_2\nabla^2 h) + v_{hyd} \\ &\quad -\mu_p(-\sigma\nabla^2 h + \kappa\nabla^4 h - \kappa\bar{H}\nabla^2\psi) + f_h. \end{aligned} \tag{12}$$

Here the Gaussian zero-mean noise f_h has variance [20,9] $2T[\mu_p + (1/4\eta q)^{-1}]$ at wavenumber q, and

$$v_{hyd} = -\int \frac{\mathrm{d}^2 q}{(2\pi)^2} e^{i\mathbf{q}.\mathbf{x}} \frac{1}{4\eta q} \frac{\delta E}{\delta h_{\mathbf{q}}(t)}, \tag{13}$$

where $h_{\mathbf{q}}(t)$ is the spatially Fourier-transformed height field and η the solvent viscosity. The signed protein density obeys the conservation law [21]

$$\begin{aligned} \frac{\partial \psi}{\partial t} &= v_0 \nabla \cdot \left(\frac{\psi^2 \nabla h}{1 + (\nabla h)^2} \right) + \Lambda \nabla^2 \delta E / \delta \psi + \nabla \cdot \mathbf{f}_\psi \\ &\simeq \Lambda A \nabla^2 \psi - \Lambda \kappa \bar{H} \nabla^4 h + \nabla \cdot \mathbf{f}_\psi, \end{aligned} \tag{14}$$

where the v_0 term comes from projecting (10) *parallel* to the reference plane, Λ is the mobility of the pumps, and $\mathbf{f}_\psi$ is a spatiotemporally white, isotropic, vector thermal noise with variance $2T\Lambda$. Since the dissipative processes that gave rise to f_h and $\mathbf{f}_\psi$ are still present, we ignore for simplicity possible additional, pumping-dependent bare noise sources. In both (12) and (14), the first equality is valid for a general energy function E including $\phi\psi$ couplings, while the second (approximate) equality applies only to a *strictly* linearized treatment at $\psi_0 = 0$ with $\phi = 1$. Equations (12) and (14) define our model.

Before using these equations, it is crucial to note that if ℓ_2 or $\bar{H}$ is sufficiently large and negative, then (12) and (14) predict that a flat membrane with a statistically uniform distribution of + and − pumps is linearly unstable, even if $\kappa_{eff} > 0$ (see eqn. (9)), with perturbations growing at a rate proportional to k^2 at small wavenumber k. To see this physically (Fig. 2), imagine a region of excess active + pumps surrounded by a statistically uniform +− mixture, in an initially flat membrane. Pumping will pull the + region ahead of its surroundings leading to mean curvature $H = -\nabla^2 h > 0$ around the + pump. This can lead to an instability in two ways: (i) If $\bar{H} < 0$, this will attract more + pumps. (ii) if $\ell_2 < 0$, the activity of the + pumps in the region is enhanced. Such physical mechanisms could well be involved in processes where cell membranes undergo large deformations. Well beyond the onset of these instabilities, nonlinearities will determine the ultimate fate of the membrane [11,12,15].

For the remainder of this paper, we assume parameter values corresponding to a *linearly stable* active membrane. The two eigenmodes for disturbances at small wavenumber k are diffusive if $v_0\ell_1$, $v_0\ell_2$ are small compared to the pump diffusivity ΛA, and propagative but highly dispersive (wavespeed $\sim k$) if v_0 is large enough.

More important is the the height variance $\langle |h_k|^2 \rangle$ at wavenumber k, which is obtained by linearizing and Fourier transforming (12) and (14) in space and time, solving for $h_{\mathbf{k},\omega}$ and $\psi_{\mathbf{k},\omega}$ at wavevector $\mathbf{k}$ and frequency ω, using the statistical properties of the noise sources f_h and $\mathbf{f}_\psi$ to obtain $\langle |h_{\mathbf{k},\omega}|^2 \rangle$, and integrating over ω.

For k much smaller than the lesser of

$$k_{max} \equiv \frac{4\eta v_0 \bar{H}}{A} \text{ and } k_D \equiv \frac{4\eta D_\psi}{\kappa}, \tag{15}$$

setting the tension $\sigma = 0$, we find from (12) and (14) that

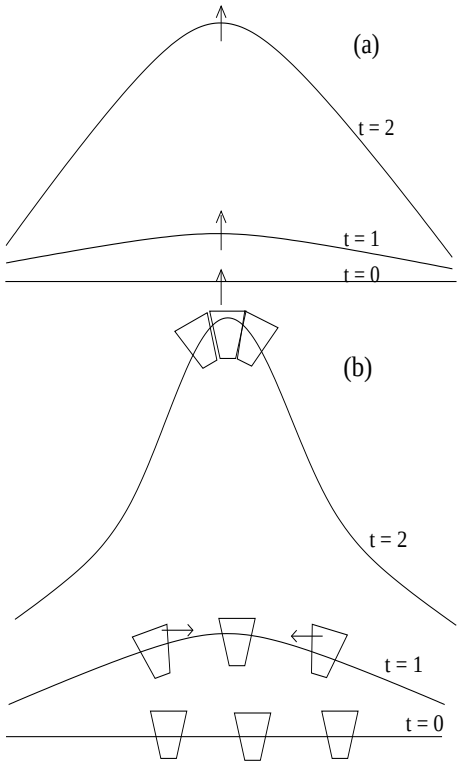

FIG. 2. Two mechanisms for instability: (a) If downward curvature enhances the upward force exerted by a single pump, and vice versa; (b) if the curvature produced by pumping attracts more pumps.

$$\langle |h_k|^2 \rangle = \frac{T}{F_a n_0 \bar{\ell} k^2 + \kappa_{eff} k^4} + \frac{\mu_p F_a}{D_\psi \bar{\ell} k^4} \tag{16}$$

where we have defined $\bar{\ell} \equiv \ell_2 + \frac{\kappa n_0}{A} \ell_1$. The thermal wandering of the membrane is thus *suppressed* by a pumping-induced tension $F_a n_0 \bar{\ell}$, but a novel *nonequilibrium* contribution to the height fluctuations now appears, mimicking a zero-tension equilibrium membrane. Note that the coupling $\bar{\ell}$ of the pumps to the curvature is crucial here: for $\bar{\ell} = 0$ we would find k^{-5} behavior as in [8]. For $n_0 \to 0$, the diffusivity $D_\psi \equiv \Lambda A$ of the pumps approaches that of an isolated pump and is hence nonzero and, from (5) and (6), the length $\bar{\ell} \simeq \ell_2 + (\kappa/T)\ell_1$. The coefficient of k^{-4} in (16) is thus independent of n_0 for small n_0.

If the tension is nonzero, the behavior in (16) is cut off for k less than

$$k_{min} \equiv \frac{\sigma A}{4\eta v_0 \kappa \bar{H}} = \frac{\sigma}{\kappa k_{max}}. \tag{17}$$

As a consequence, the excess area [22]

$$\alpha \simeq \frac{1}{2} \langle (\nabla h)^2 \rangle \simeq \frac{\mu_p F_a}{D_\psi \bar{\ell}} \ln \frac{k_{max}}{k_{min}} \tag{18}$$

can be seen to depend only logarithmically on the protein density n_0. Moreover, increasing the solvent viscosity should decrease μ_p while leaving D_ψ unaffected, and

should thus decrease α. These predictions of (18) are consistent with the experiments of [10]. However, not enough is known about the values of parameters such as μ_p, F_a, and $\bar{\ell}$ to make a detailed comparison. If instead D_ψ is decreased, $\langle |h_k|^2 \rangle$ should increase. Such predictions of the dependence of static correlations on *kinetic* quantities allow clear tests of our model and of the truly nonequilibrium nature of the fluctuations.

We turn finally to the wavelike modes mentioned at the start of the paper. A net excess of pumps of one sign ($\psi_0 \neq 0$) would yield a term $v_0 \psi_0^2 \nabla^2 h$ in the linearized (14). With (12) this would lead to propagating waves with a speed $v_0 \psi_0 = \mu_p F_a m_0$. Even for $\psi_0 = 0$, the presence of fluctuations means that $\langle \psi^2 \rangle \neq 0$. Hence, improving upon our strictly linearized treatment by letting $\psi^2 \to \langle \psi^2 \rangle$ in (14) leads to a prediction of waves with speed $c \equiv v_0 \langle \psi^2 \rangle^{1/2} = \mu_p F_a \langle m^2 \rangle^{1/2}$, and to a nonequilibrium height variance $\langle |h_k|^2 \rangle \sim (\mu_p v_0^2 T)/(v_0 \ell_2 + D_\psi) c^2 k^2$. It is important to note that these waves only appear for wavevectors $k < k_{\rm fluc} \equiv \sqrt{\frac{v_0 \langle \psi^2 \rangle}{D_\psi \ell_2}}$; for $k \gg k_{\rm fluc}$ our earlier linearized results apply. Hence, for a system with small fluctuations $\langle \psi^2 \rangle$, the linearized results (16) and (18) could well hold at the experimental wavenumbers k.

In any case, for $\psi_0 \neq 0$, a complete analysis requires, even at a linearized level, the inclusion of fluctuations in the protein density n. Replacing ψ^2 by its average, too, is simply a first step in a complete treatment of nonlinear fluctuation effects. A detailed consideration of all such effects will appear elsewhere [15].

We close by summarizing this work. Starting with a natural model of a membrane with active proteins, we show that such a membrane can be linearly stable or unstable to small perturbations in its shape and the distribution of proteins. In the stable case, we show that the k^{-4} dependence of the height fluctuations can mimic that of an equilibrium membrane, but with an enhanced temperature, consistent with the observations of [10]. Further experiments, in particular on the dependence of the height variance on the protein diffusivity, will provide more stringent tests of our predictions. We are currently in the process of studying the effects of nonlinearities on the scaling of correlation functions in the stable case and on the growth in the unstable case [11,12,15].

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