Membrane Tension Lowering Induced by Protein Activity

M. D. El Alaoui Faris,[1] D. Lacoste,[2] J. Pécréaux,[1,*] J.-F. Joanny,[1] J. Prost,[1,3] and P. Bassereau[1]

[1]*Institut Curie, Centre de Recherche; CNRS, UMR 168; Université Pierre et Marie Curie, Paris, F-75248 France*
[2]*ESPCI, Laboratoire de Physico-Chimie Théorique; CNRS, UMR 7083, 75231 Paris Cedex 05, France*
[3]*ESPCI; ParisTech, 10 rue Vauquelin, 75231 Paris Cedex 05, France*

(Received 20 June 2008; published 21 January 2009)

Using videomicroscopy we present measurements of the fluctuation spectrum of giant vesicles containing bacteriorhodopsin pumps. When the pumps are activated, we observe a significant increase of the fluctuations in the low wave vector region, which we interpret as due to a lowering of the effective tension of the membrane.

DOI: 10.1103/PhysRevLett.102.038102 PACS numbers: 87.16.dj, 05.40.−a, 05.70.Ln

Membranes are self-assembled bilayers of surfactants or phospholipids, which form flexible surfaces. The mechanical properties of membranes are essentially controlled by parameters, such as the membrane tension, bending modulus, and spontaneous curvature [1]. These parameters completely characterize the membrane fluctuation spectrum at thermal equilibrium, but they are not sufficient to characterize real biological membranes, such as cell plasma membranes, which are nonequilibrium systems [2,3]. Biological membranes are in general active in the sense that they are constantly maintained out of equilibrium either by active proteins inside the membrane that are often ATP-consuming enzymes or by an energy flow, for example, a lipid flux. Because of the complexity of active biological systems *in vivo*, recent studies have focused on *in vitro* biomimetic systems. An example of an active system is a giant unilamellar vesicle (GUV) which is rendered active by the inclusion of light-activated bacteriorhodopsin (BR) pumps. The bacteriorhodopsin pumps transfer protons unidirectionally across the membrane as a consequence of their conformational changes, when they are excited by the light of a specific wavelength. Experiments on giant unilamellar vesicles containing bacteriorhodopsin pumps or Ca^{2+} ATPase pumps have shown that the nonequilibrium forces arising from ion pumps embedded in the membrane are able to significantly enhance the membrane fluctuations [4–7]. In these experiments, the membrane tension has been fixed by means of micropipet aspiration and the corresponding excess area measured. The slope of such a curve defines an effective temperature, measuring the enhancement of the membrane fluctuations by the protein activity. The amplification of the fluctuations due to activity was originally predicted in Ref. [8].

In this Letter, we report the first experimental measurements, using videomicroscopy, of an active fluctuation spectrum of GUVs containing bacteriorhodopsin pumps. The details of the technique and of the analysis are given in Ref. [9]. These measurements are complementary to previous experiments using micropipets since they probe a range of wave vectors not accessible otherwise. Micropipet experiments provide information on the active fluctuations integrated over all wave vectors which require an independent calibration of the membrane tension. On the contrary, videomicroscopy gives a direct measurement of the fluctuation spectrum from which the membrane tension can be extracted [9]. In this Letter, we analyze theoretically the active fluctuation spectrum using the model of Ref. [10], which is an extension of the hydrodynamic model of Ref. [5], to quasispherical vesicles, taking into account the active noise of the pumps and localized active forces.

Let us first discuss the passive behavior of giant vesicles. For the sake of simplicity we use notations appropriate to quasiplanar membranes. We consider a quasiplanar membrane surface in the Monge gauge, in which it is defined by its height $u(\mathbf{x})$ at position $\mathbf{x}$. Two density fields are defined on the surface, $\psi^{\uparrow}$ and $\psi^{\downarrow}$, corresponding to the surface densities of proteins oriented with the two possible orientations [5]. The membrane free energy is a function of the height field $u(\mathbf{x})$ and of the imbalance of protein densities $\psi(\mathbf{x}) = \psi^{\uparrow}(\mathbf{x}) - \psi^{\downarrow}(\mathbf{x})$. We expand the free energy to quadratic order in these variables:

$$F[u, \psi] = \frac{1}{2}\int d^2\mathbf{x}[\kappa(\nabla^2 u)^2 + \sigma(\nabla u)^2 + \chi\psi^2 - 2\Xi\psi\nabla^2 u], \quad (1)$$

where κ is the bending modulus, σ the surface tension, χ a susceptibility coefficient, and Ξ a coefficient characterizing the coupling between the membrane curvature and the average orientation of the proteins. In the following, we neglect the curvature induced coupling; this is justified for bacteriorhodopsin pumps since the incorporation of proteins does not lead to any measurable renormalization of the bending modulus [5]. Note that for dilute proteins in the membrane, $\chi = k_B T/n_0$, where n_0 is the average density of proteins, of the order of 10^{16} m^{-2}. In our experiments, only the vesicle contour at the equator corresponding to a slice of the vesicle in the plane $y = 0$ is recorded and is used to calculate the fluctuation spectrum. The measured quantity is

$$\langle |u(q_x, y=0)|^2 \rangle = \frac{1}{2\pi} \int_{-\infty}^{+\infty} \langle |u(q_x, q_y)|^2 \rangle dq_y. \quad (2)$$

The passive equilibrium value of this fluctuation spectrum is calculated using the equipartition theorem from Eqs. (1) and (2):

$$\langle |u(q_x, y=0)|^2 \rangle = \frac{k_B T}{2\sigma} \left[\frac{1}{q_x} - \frac{1}{\sqrt{\frac{\sigma}{\kappa} + q_x^2}} \right] = g(q_x, \sigma, \kappa). \quad (3)$$

Fluctuation spectra are often analyzed in terms of power laws defined by $g(q_x, \sigma, \kappa) \sim q_x^{\nu}$. Two regimes can be distinguished depending on the position of the wave vector q_x compared to the crossover wave vector $q_c = \sqrt{\sigma/\kappa}$. For $q \ll q_c$, membrane tension dominates and $\nu = -1$, whereas for $q \gg q_c$ bending elasticity dominates and $\nu = -3$. Our experiments lead to $\nu \simeq -2$, in the crossover region between these two regimes.

We now discuss active membrane fluctuations. In Ref. [5], a hydrodynamic theory was developed to calculate the nonequilibrium fluctuations of an active membrane containing ion pumps. This work has stimulated substantial theoretical interest, focused mainly on the general question of the proper description of nonequilibrium effects associated to protein conformation changes [11,12]. More recent developments of a similar hydrodynamic approach have led to a general theoretical description of active gels [13–15]. In Ref. [10], the active force distribution is modeled as a superposition of dipoles located along the membrane normal $\mathbf{n}$ (and not along z as in previous models), which generalizes Ref. [5]. Experiments do not probe this force distribution directly but rather its first two moments. The first moment represents the active contribution to the membrane tension and is denoted by $\sigma_{\rm dip}$ and the second moment Q represents the modification of the membrane bending moments by the activity of the force dipoles [10]. Both active contributions to the tension and to the membrane curvature are also present in Ref. [16] where the activity is due the myosin molecular motors in the cortical layer bound to the cell membrane.

The fluctuation spectrum of an active membrane in a quasispherical geometry can be calculated using an expansion around a spherical shape with radius R_0 to first order in the deviations from the sphere using spherical harmonics, with $\mathbf{R} = \mathbf{R_0} + \mathbf{n} R_0 \sum_{l,m} u_{lm} \mathcal{Y}_{lm}$. The fluctuation spectrum of a quasispherical vesicle is characterized by the amplitude of the spherical modes $\langle |u_{lm}|^2 \rangle$ which are function of l only for symmetry reasons. In Ref. [9], the fluctuation spectrum of a quasispherical vesicle is compared to the corresponding spectrum for a planar membrane, and this comparison showed that for modes $l > 5$, the fluctuation spectrum of a quasispherical vesicle is very well approximated by that of a planar membrane at a wave vector q, $\langle |u(q)|^2 \rangle$; at a wave vector $q = l/R_0$, the two spectra are related by $\langle |u(q)|^2 \rangle = R_0^4 \langle |u_{lm}|^2 \rangle$. For the sake of simplicity, we discuss in the following the effect of the activity on the fluctuation spectrum only at the level of planar membranes. We have also simplified the calculation of Ref. [10], by neglecting the shot noise, which is the intrinsic noise of the pumps [8]. This approximation is reasonable because the main effect of the shot noise is to modify the fluctuation spectrum in the high wave vector region [10] which is not the region where we observe a large modification of the spectrum due to activity in our experiments. We denote here F_2 as the constant entering in $Q = F_2 \psi$ [10]. This generalizes the notation of Ref. [5], where this quantity represented the quadrupole moment of the force dipole. With these three approximations, we obtain the following spectrum

$$\langle |u(q_x, q_y)|^2 \rangle = \frac{k_B T}{\kappa q^4 + \tilde{\sigma} q^2} \left\{ 1 + \frac{F_2^2}{4\chi} \frac{q^2}{\kappa q^2 + 4\eta D q + \tilde{\sigma}} \right\}, \quad (4)$$

where D is the diffusion coefficient of the proteins which is of the order of 10^{-12} m^2 s^{-1} [17] and η is the viscosity of water. Since the tension of the vesicles is at least of the order of 10^{-8} N/m, we find that the term $4\eta D q$ can be neglected with respect to $\tilde{\sigma}$ in the range of wave vectors relevant to the experiments. The effective tension $\tilde{\sigma}$ is defined as $\tilde{\sigma} = \sigma + \sigma_{\rm dip}$, where σ is the passive contribution to the tension and $\sigma_{\rm dip}$ the active part due to BR proteins. After integration as in Eq. (3), we find

$$\langle |u(q_x, y=0)|^2 \rangle = g(q_x, \tilde{\sigma}, \kappa) + \frac{F_2^2 n_0}{16\kappa^2} \frac{1}{(q_x^2 + \frac{\tilde{\sigma}}{\kappa})^{3/2}}, \quad (5)$$

in which both terms on the right-hand side contain active contributions when $\sigma_{\rm dip}$ or F_2 do not vanish. Note that Eq. (4) is very similar to the active spectrum given in Ref. [5] with the correspondence $F_2 = 2w\mathcal{P}_a$ except for one important difference: the tension contains an active contribution but not the bending modulus, whereas in

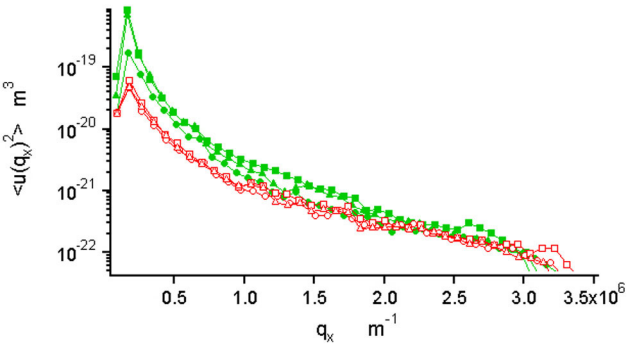

FIG. 1 (color online). Fluctuation spectrum of a single GUV containing BR in a buffer with 1 mM sodium azide. The red (empty) symbols correspond to three consecutive recorded passive spectra, and the green (filled) ones to three consecutive active spectra.

Ref. [5] it is the opposite. Because of this difference the active spectrum of Ref. [5] does not fit our experiments (it would predict a large effect of activity at high wave vectors, whereas the effect is observed here mainly at small wave vectors). Another consequence of this difference is that the active spectrum of Eq. (5) cannot in general be described in terms of an active temperature even in the low wave vector limit [18].

Experiments have been performed with GUVs made of egg phosphatidylcholine at a molar fraction of 240 lipids per protein, using the BR reconstitution protocol developed in Ref. [19]. We have used a polyethylene glycol passivated substrate and checked that vesicles fluctuate without adhering [9]. In Fig. 1, we show the measured fluctuation spectrum for the same GUV containing BR in active and passive states. To ensure reproducibility of the data, three consecutive passive and then three active spectra were recorded. This figure confirms that activity leads to an enhancement of the fluctuations as expected from previous experimental studies [5,6]. It is important to point out that to observe this effect 1 mM of azide must be present in the solution. In the absence of this compound no enhancement of the fluctuation spectrum was observed [20]. This observation is consistent with several studies which suggest that azide enhances the proton transfer in BR [21]. A large enhancement of the active as compared to passive fluctuations is observed at low wave vectors, which we attribute to a lowering of the membrane tension due to the activity.

We first fit the passive spectrum using Eq. (3). This leads to $\sigma = 3.9 \times 10^{-7} \pm 3 \times 10^{-8}$ N/m and an apparent bending modulus $\kappa = 5.6 \times 10^{-19} \pm 4.4 \times 10^{-20}$ J. The value of the bending modulus deduced from such a fit is overestimated. Several artifacts perturb the measurements at high wave vectors: the pixel noise due to the discrete detection of the images, the integration time of the camera, and the effect of gravity [9]. Taking into account these corrections at high q becomes very difficult in the active case; we thus have chosen a simple fitting procedure, in which the corrections due to the integration time and to the gravity are neglected for both passive and active spectra. This procedure is justified by the observation that both spectra are superimposed at high q. The determination of the bending modulus κ is therefore delicate with our video-microscopy technique, while the measurement of the tension is reliable. With the value of the bending modulus kept fixed, we fit the three active spectra of Fig. 1 using Eq. (5). One fit which is shown in Fig. 2 leads to $\tilde{\sigma} = 5.3 \times 10^{-8}$ N/m and $F_2 = 3.9 \times 10^{-28}$ J m. Thus the active contribution to the tension $\sigma_{\mathrm{dip}} = \tilde{\sigma} - \sigma$ is negative and of the order of -3.3×10^{-7} N/m for this particular vesicle (see Table I for a summary of the results of the fits for the GUV of Fig. 1). The lowering of the tension is systematic in all our experiments. The fact that $\sigma_{\mathrm{dip}} \simeq -3.3 \times 10^{-7}$ N/m is very different from the estimate of 1.6×10^{-3} N/m based on Refs. [5,10] suggests that the modeling of the force distribution of these references is not appropriate to our experiments. In Table I, we have also shown the exponent ν, which is the apparent power law exponent of the spectrum. When the vesicle is active, the exponent gets closer to -3, which is the expected value in the bending dominated regime. This is consistent with a lowering of the crossover wave vector $q_c = \sqrt{\tilde{\sigma}/\kappa}$, and thus with a lowering of the tension since in this model κ is not affected by activity. We have ignored any dependence of the protein activity on the local membrane curvature, an assumption justified by the observation that the main effect is a correction to the tension and not to the bending modulus. The value of the parameter F_2 deduced from our fit is of the same order of magnitude as that estimated in Ref. [5] based on micropipet experiments since the factor 2–3 increase in effective temperature measured in this reference leads to $F_2 \simeq 9 \times 10^{-28}$ J m. To summarize, Eq. (4) successfully describes both the micropipet and the fluctuation spectrum measurements.

We now discuss in more detail the lowering of the membrane tension due to activity. We have checked that this effect is compatible with the constraints of constant surface and volume of the vesicle, which must be imposed if permeation is negligible [5]. To do so, we estimate the excess area $\alpha = \int q^3 dq \langle |u(q)|^2 \rangle / 4/\pi$ for passive and active vesicles based on the above fluctuation spectra, by

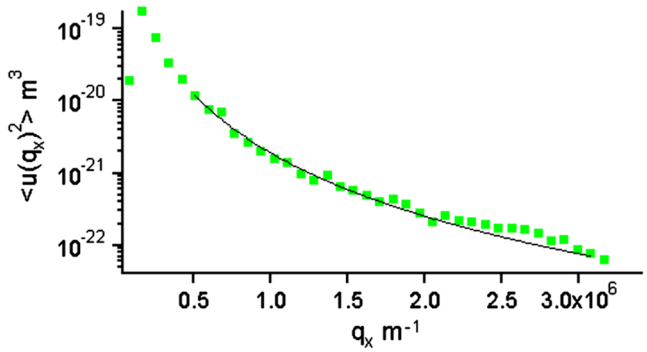

FIG. 2 (color online). Fit of one active spectra of Fig. 1 using Eq. (5). The parameter apparent κ was fixed to 5.6×10^{-19} J obtained by fitting the passive spectrum. This fit gives $\tilde{\sigma} = 5.3 \times 10^{-8}$ N/m and $F_2 = 3.9 \times 10^{-28}$ J m.

TABLE I. Fitting parameters using the fluctuation spectrum of Eq. (5). The passive spectrum is fitted with the condition $F_2 = 0$. Then three consecutive active spectra are fitted with the same vesicle using the value of $\kappa_{ap} = 5.6 \times 10^{-19} \pm 4.4 \times 10^{-20}$ J determined from the fit of the passive spectrum. From this, the membrane tension σ, F_2 and the exponent ν are deduced.

Spectrum	σ ($\times 10^{-7}$ N/m)	F_2 ($\times 10^{-28}$ J m)	ν
Passive	3.9 ± 0.3	0 (imposed)	-2.15 ± 0.04
Active 1	0.53 ± 0.2	3.9 ± 1.1	-2.7 ± 0.04
Active 2	0.79 ± 0.06	9.6 ± 1.1	-2.78 ± 0.06
Active 3	0.35 ± 0.1	7.2 ± 0.6	-2.87 ± 0.07

taking the lower bound of integration to be $q_{\min} = 2\pi/R_0$, with $R_0 = 10\ \mu$m the radius of the vesicles; this lower bound corresponds roughly to the lowest measured value in Fig. 1. In the accessible range of q values, the excess area density $q^3\langle|u(q)|^2\rangle/4/\pi$ of the active vesicles is always larger than the excess area density of the passive vesicles. At higher q wave vectors, it should be the opposite, but the q range where this would happen may be outside the accessible range.

Another important question is the sign of the effect, which cannot be fixed by symmetry arguments [4]. Indeed, both orientations of the force dipoles [5,11], inwards or outwards with respect to the membrane surface, are possible. The present experiments suggest that here the force dipoles must have an inward orientation (corresponding to contractile forces [14]) to produce a lowering of the tension. Another possibility would be to consider lateral dipoles. Here, we propose that the lowering of the tension is a consequence of electrostatic effects. This interpretation is not incompatible with the fact that BR conformational changes also play an important role. We have checked experimentally that a pH gradient builds up in our GUVs when they are activated, which suggests that a significant voltage drop exists across the membrane. Furthermore in membranes containing BR, the activity of the BR can be suppressed by applying an external voltage drop across the membrane [22]. In view of these observations, we propose that Maxwell stresses created by ion transport lead to a renormalization of the tension as shown in a simple model developed by two of us [23]. In this model, the electrostatic correction to the tension is proportional to the square of the electric current going across the membrane. Maxwell stresses are large because of the large mismatch in dielectric constants at the membrane, and they tend to reduce the membrane area, thus producing a negative electrostatic contribution to the tension. A lowering of the membrane tension due to the application of normal electric fields very generally leads to instabilities of the membrane [24,25].

To summarize, we have presented what are to our knowledge the first measurements, using videomicroscopy, of active fluctuation spectra of GUVs containing BR proteins. The experiments show that activity enhances the fluctuations. The effect is strong in the low wave vector region, which we interpret as a lowering of the tension due to activity. This effect could not have been detected in micropipet experiments which are done at constant membrane tension. A possible candidate for explaining the lowering of the tension would be that it is caused by Maxwell stresses due to the transport of ions across the membrane. Further experiments (using, for instance, patch-clamp techniques with the same GUV or other GUVs containing ion channels rather than pumps) are required to confirm these suggestions.

We acknowledge many stimulating discussions with J. Tittor, G. Menon, S. Ramaswamy, N. Gov, and M. Lomholt. We thank D. Oesterhelt for kindly providing us with BR. We also acknowledge support from the European Network of Excellence SoftComp (NMP3-CT-2004-502235) and the Indo-French Center CEFIPRA (Grant No. 3504-2).

*Also at MPI for Molecular Cell Biology and Genetics.

[1] U. Seifert, Adv. Phys. **46**, 13 (1997).
[2] B. Alberts, *Molecular Biology of the Cell* (Garland, New York, 2002).
[3] S. Ramaswamy and M. Rao, C. R. Acad. Sci. Paris, t. 2, Ser. IV, 817 (2001).
[4] S. Ramaswamy, J. Toner, and J. Prost, Phys. Rev. Lett. **84**, 3494 (2000).
[5] J. B. Manneville, P. Bassereau, S. Ramaswamy, and J. Prost, Phys. Rev. E **64**, 021908 (2001).
[6] J. B. Manneville, P. Bassereau, D. Lévy, and J. Prost, Phys. Rev. Lett. **82**, 4356 (1999).
[7] P. Girard, J. Prost, and P. Bassereau, Phys. Rev. Lett. **94**, 088102 (2005).
[8] J. Prost and R. Bruinsma, Europhys. Lett. **33**, 321 (1996).
[9] J. Pécréaux, H.-G. Döbereiner, J. Prost, J.-F. Joanny, and P. Bassereau, Eur. Phys. J. E **13**, 277 (2004).
[10] M. A. Lomholt, Phys. Rev. E **73**, 061913 (2006); **73**, 061914 (2006).
[11] S. Sankararaman, G. Menon, and P. B. S. Kumar, Phys. Rev. E **66**, 031914 (2002).
[12] N. Gov, Phys. Rev. Lett. **93**, 268104 (2004); H.-Y. Chen, *ibid.* **92**, 168101 (2004); D. Lacoste and A. W. C. Lau, Europhys. Lett. **70**, 418 (2005).
[13] R. A. Simha and S. Ramaswamy, Phys. Rev. Lett. **89**, 058101 (2002).
[14] S. Ramaswamy and M. Rao, New J. Phys. **9**, 423 (2007).
[15] K. Kruse, J. Joanny, F. Jülicher, J. Prost, and K. Sekimoto, Eur. Phys. J. E **16**, 5 (2005).
[16] G. Salbreux, J. Joanny, J. Prost, and P. Pullarkat, Phys. Biol. **4**, 268 (2007).
[17] N. Kahya, E. Pecheur, W. de Boeij, D. Wiersma, and D. Hoekstra, Biophys. J. **81**, 1464 (2001).
[18] B. Różycki, T. R. Weikl, and R. Lipowsky, Phys. Rev. E **73**, 061908 (2006).
[19] P. Girard, J. Pécréaux, G. Lenoir, P. Falson, J. Rigaud, and P. Bassereau, Biophys. J. **87**, 419 (2004).
[20] A. Giahi, M. E. A. Faris, P. Bassereau, and T. Salditt, Eur. Phys. J. E **23**, 431 (2007).
[21] H. Steinhoff, M. Pfeiffer, T. Rink, O. Burlon, M. Kurz, J. Riesle, E. Heuberger, K. Gerwert, and D. Oesterhelt, Biophys. J. **76**, 2702 (1999).
[22] S. Geibel, T. Friedrich, P. Ormos, P. G. Nagel, and E. Bamberg, Biophys. J. **81**, 2059 (2001).
[23] D. Lacoste, M. C. Lagomarsino, and J. F. Joanny, Europhys. Lett. **77**, 18 006 (2007); D. Lacoste, G. I. Menon, M. Bazant, and J. F. Joanny, arXiv:0806.4321.
[24] R. Dimova, K. A. Riske, S. Aranda, N. Bezlyepkina, R. Knorr, and R. Lipowsky, Soft Matter **3**, 817 (2007).
[25] P. L. H. T. Ambjörnsson and M. A. Lomholt, Phys. Rev. E **75**, 051916 (2007).