
14 An Update on Active Membranes

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14.1 INTRODUCTION

Bilayer membranes formed from phospholipid molecules are an essential component of cellular membranes. While the properties of equilibrium membranes are well understood using the Helfrich Hamiltonian constructed from the two elastic moduli, the surface tension and the curvature modulus (for a review, see, for instance, Seifert, 1997), the nonequilibrium behavior of real biological membranes of living cells has not reached an equal level of understanding. In this chapter, we are interested in active membranes, which share one of the important characteristics of membranes of living cells: the sustained uptake and dissipation of energy.

Active membranes can be thought of as composite systems, which contain some active elements in addition to a membrane. These active elements exert either directly or in an effective way, non-conservative forces on the membrane surface. These forces are generalized in the sense that they may not be necessarily mechanical in origin: they can be produced by a chemical reaction such as actin polymerization, mechanical stresses, or by electric fields for instance. A common feature in active membranes is that some kind of energy input has to be brought from a source that is exterior to the membrane itself. This energy input (brought by adenosine triphosphate (ATP) hydrolysis, or light for instance) is precisely what drives the membrane out of equilibrium. As a result, the fluctuations of an active membrane are different from the thermal fluctuations of passive, equilibrium membranes. Classical models of active membranes have been reviewed in Ramaswamy and Rao (2001), so that, in this chapter, we can focus on more recent developments in the field. Over the years, active membranes have served as a test ground for theoretical models of more complex active systems, such as active fluids or active gels (Kruse et al., 2005) and there has been a strong feedback between experiments and theoretical models. These days, there are many branches of research on active fluids, which focus, for instance, on the collective motions of active particles, or on specific rheological properties. As a proof of this tendency, we would like to recommend three recent

reviews on active matter systems, written by researchers who originally made key contributions in the field of active membranes (Ramaswamy, 2010; Menon, 2010; Marchetti et al., 2013).

Nerve cells are a particularly interesting example of active membranes because the chemical energy fed into the system is used to transmit information, in the form of electrical signals (action potentials) (Hille, 2001). According to the standard Hodgkin–Huxley model, this propagation is achieved by the voltage-dependent opening and closing of ion channels, but the specificity of the membrane physical properties does not play any role. In view of this, the Hodgkin–Huxley model has been challenged in particular by the group of T. Heimburg, who suggested that some lipid phase transition occurs in the membrane in the course of the action potential. This phase transition makes the membrane more conductive to ions and is thus relevant even in the absence of ion channels in the membrane (Heimburg, 2010). At this time of writing, these ideas are not completely accepted by the community of biologists and electrophysiologists, and the role of the membrane composition in the action potential is still not established. In this chapter, we focus mainly on active membranes, defined as membranes that contain active inclusions in the form of ion channels or pumps. These elements are called active because they are able to transport ions from one side of the membrane to the other in a selective way when a source of energy (light, ATP, etc.) is provided. The ion transport itself is called active because it requires an energy source, which can be the hydrolysis of ATP or light for instance. This energy source is required to induce protein conformational changes and consequently ion transfer, in contrast to passive transport, which does not require energy input.

The main purpose of this chapter is to review a number of recent experimental and theoretical reports in the field of active membranes. The chapter is organized as follows: in Section 14.2, we present some experiments on active membranes, while in Section 14.3, we review a number of different theoretical works on active membranes.

14.2 EXPERIMENTS ON ACTIVE MEMBRANES

14.2.1 RECONSTITUTED SYSTEMS CONTAINING ACTIVABLE ION PUMPS

Biomimetic active membranes have been experimentally prepared and studied mainly at the Curie Institute (Paris), in the form of giant unilamellar vesicles (GUV) containing ion pumps such as bacteriorhodopsin (BR) (Figure 14.1), or calcium ATPase (Manneville et al., 1999, 2001; Girard et al., 2005; El Alaoui Faris et al., 2009). BR is a light-activable proton pump, which can switch from a passive to active state when receiving light of the appropriate wavelength. It can then transfer protons through a lipid bilayer. This system is particularly interesting as it allows to compare the active and passive behaviors of the same system. Calcium ATPase is a calcium pump that uses the energy of ATP hydrolysis for performing a conformational change and allows for instance to build up Ca^{2+} gradients across the membrane of muscle cells. In this case, experiments in the presence or in the absence of ATP are compared. Not many other *in vitro* active membrane experiments have been performed so far, probably because it requires to reconstitute ion channels or ion pumps in

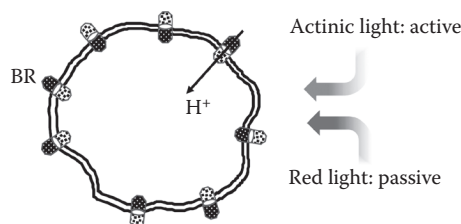


FIGURE 14.1 (See color insert.) Example of active membranes. GUV contains light-activable proton pumps. (BR, bacteriorhodopsin).

model membrane systems and to keep them functional. Different methods have been established for reconstituting transmembrane proteins in GUVs (Kahya et al., 2001; Girard et al., 2004b; Doeven et al., 2005; Aimon et al., 2011) that preserve protein activity, but sample preparation still remains a milestone of such experiments. They are usually based on the reconstitution of the proteins in small liposomes as a preliminary step, followed by gentle electroformation of the partially dried film formed by the proteoliposomes. Alternative methods may have to be found so that the purification and the reconstitution steps could be avoided in the future. Indeed, GUVs have now been obtained directly from purified native membranes (Méléard et al., 2009) or from detachment of blebs from plasma membranes of cells (plasma membrane spheres [PMS] or giant plasma membrane vesicle [GPM]) (Sezgin et al., 2012).

Vesicles containing active pumps have been studied using the microaspiration technique (Manneville et al., 1999, 2001; Girard et al., 2005) developed by E. Evans (Evans and Rawicz, 1990) (Figure 14.2a). In thermal equilibrium, the following relation exists between the excess area (measured from the length of the aspirated tongue in the pipette) Δa and the applied tension (deduced from the aspiration pressure)

$$\Delta a = \frac{A_0 - A_p}{A_0} = \frac{k_B T}{8\pi k} \ln\left(\frac{s}{s_0}\right), \tag{14.1}$$

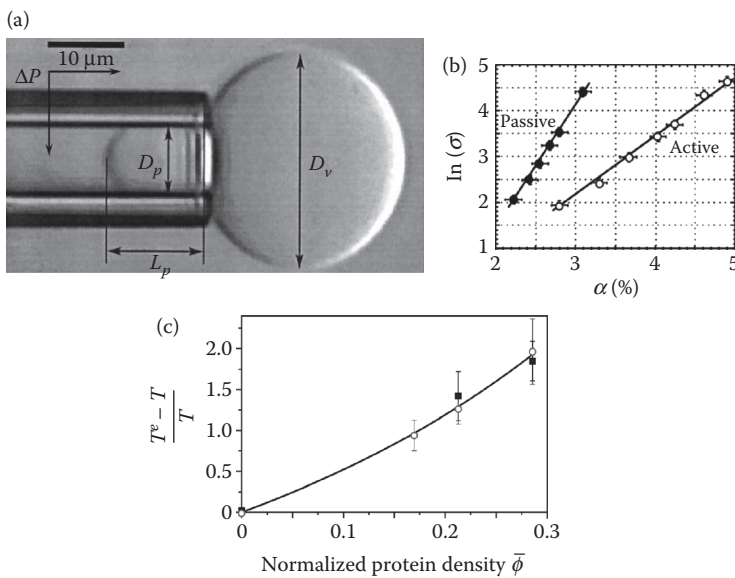


FIGURE 14.2 Micropipette aspiration experiments on active GUVs. (a) Differential interference contrast (DIC) image of a GUV of diameter D_v aspirated in a micropipette of diameter D_p . The membrane tension can be deduced from the difference of pressure ΔP , D_v and, D_p , the corresponding excess area from the length of the tongue in the micropipette L_p . (b) When the BR is activated by actinic light (empty symbols), the variation of the logarithm of the membrane tension with excess area remains linear as compared to the same GUV in a passive state illuminated with red light. However, the slope is lowered by a factor on the order of 2. This has been interpreted as an effective temperature larger than the thermal temperature. (From Manneville, J.-B. et al. 1999. Activity of transmembrane proteins induces magnification of shape fluctuations of lipid membranes. *Phys. Rev. Lett.* 82 (21), 4356–4359. Copyright 1999 by The American Physical Society.) (c) Relative evolution of the effective temperature T^e of an ATP-activated GUV containing Ca^{2+} -ATPase as a function of the normalized protein density in the membrane. (Reprinted with permission from Girard, P., J. Prost, and P. Bassereau. 2005. Passive or active fluctuations in membranes containing proteins. *Phys. Rev. Lett.* 94, 088102. Copyright 2005 by The American Physical Society.)

where A_0 is the optically measured area at the minimal tension σ_0 allowing to grab the vesicle, and A_p is the projected area for a tension σ . For passive GUVs, this relation—for tensions typically below 10^{-5} N/m—allows to measure the bending rigidity of the membrane κ . Experiments have been performed on the same vesicle in the passive and active state for BR, and on a statistically relevant number of GUVs in the absence and in the presence of ATP for the Ca-ATPase. As a result, a relative excess area/stress relation, formally identical to Equation 14.1, has been found in the low-tension regime, but with a renormalized prefactor. The effect of protein activity has been described by assigning an effective temperature T^e to the active membrane, but it could also have been interpreted as an effective bending rigidity of the membrane. In both cases, an amplification of the GUV fluctuations has been observed when the proteins are activated, corresponding to a reduction of the slope of the log (tension) versus excess area plots. The effect of protein activity is rather strong and an effective temperature of twice the room temperature has been found for BR (Figure 14.2b) (Manneville et al., 1999, 2001), and is even much stronger, of the order of 1000 K, for Ca-ATPase (Girard et al., 2005). The nonequilibrium nature of the effect was further demonstrated by showing that this effective temperature depends on the viscosity of the bulk medium (it decreases as the viscosity of the bulk medium increases), an effect that does not occur in membranes at thermodynamical equilibrium. The amplification of fluctuations is directly related to the density of active proteins in the membrane (Figure 14.2c) (Girard et al., 2005).

Videomicroscopy has been used more recently (El Alaoui Faris et al., 2009) as a different technique to analyze the fluctuation spectrum of BR membranes without applying external tension to the vesicle. With this technique, the contour of freely floating GUVs was detected using phase contrast microscopy with a resolution <1 pixel (Pécéréaux et al., 2004) (Figure 14.3a), allowing to compute the mean square displacement of the membrane from its average position. For passive membranes, it has been possible to fit the fluctuation spectrum over a range of wave vectors, while accounting for the integration time of the camera and the specific geometry of microscopy experiments, which probes only a planar section of the GUV (for technical details, see, e.g., Pécéréaux et al., 2004). Owing to the complexity of the required mathematical modeling, the same level of analysis has not been reached for active membranes so far. Nevertheless, for BR membranes, a significant increase of the fluctuation amplitudes at low wave vectors has been detected when the pumps were light activated (Figure 14.3b) (El Alaoui Faris et al., 2009). This effect has been interpreted as an active reduction of the tension from $\sigma_0 = 4 \times 10^{-7}$ N m $^{-1}$ in the passive case (red light) to $\sigma_a = 5.3 \times 10^{-8}$ N m $^{-1}$ for the active membrane (green light) (Figure 14.3b). In general, BR activation led to a 3–7-fold reduction of membrane tension. A tension reduction has not been detected by pipette aspiration, as the pipette sets the tension in this case. Recently, a more advanced analysis of the flickering data (Méléard et al., 2011) has been developed, by which the full probability distribution of the configurational fluctuations is taken into account. In principle, this method allows for a more precise measurement of the bending modulus of lipid membranes and it has been used by the MEMPHYS group (Denmark) to analyze active GUVs containing sodium pumps, triggered by ATP (Bouvrais, 2011). In these experiments, an active reduction of the bending modulus has been measured, when the membrane can be considered in a “pseudo-equilibrium” state, that is, the variance of the amplitudes of fluctuations still scales with the fourth power of the mode number in the bending-dominated regime. Another promising technique uses multiple optical tweezers, to drive membrane oscillations on GUVs and to measure the corresponding membrane response (Brown et al., 2011). More complex fluctuations spectra can be expected with active membranes. However, no extensive analysis has been possible so far, allowing to verify or falsify fluctuations spectra predicted by different theoretical models.

14.2.2 RED BLOOD CELLS

Historically, investigations on cell membrane fluctuations have started with red blood cells (RBCs), as their flickering can be easily observed under a microscope. Brochard and Lennon (1975) were

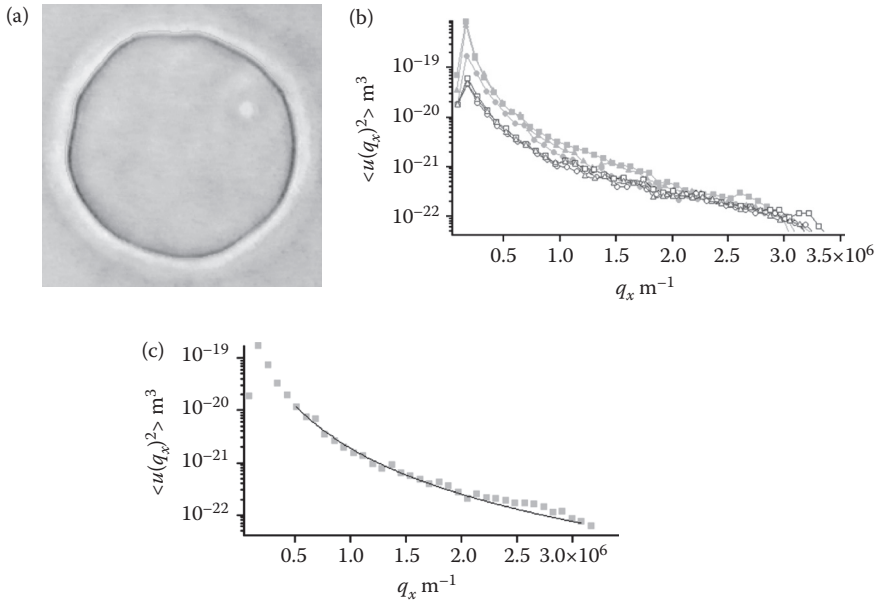


FIGURE 14.3 (See color insert.) Fluctuation spectrum of an active membrane. (a) Flickering spectroscopy: the contour of a GUV (typical diameter of the order of 20–40 μm) obtained with phase-contrast microscopy is detected by image analysis and the fluctuations around the average membrane position. The fluctuation spectrum $\langle u^2(q_x) \rangle$ is calculated from the Fourier analysis of the mean square displacement of the membrane. (b) Effect of BR activation on the fluctuation spectrum. A few measures have been made on the same GUV corresponding to the active (green dots) and passive BR (red dots). The BR activity induces an amplification of the amplitudes of the modes mainly at small q vectors. (c) Fit of one active spectrum of Figure 14.3b, based on the hydrodynamic model developed in Lomhold (2006b). The apparent bending rigidity was fixed to 5.6×10^{-19} J, obtained by fitting the passive spectrum. This fit gives an effective membrane tension $\sigma_a \sim 5.3 \times 10^{-8}$ N/m and F_2 , related to the second moment of the active force, $F_2 = 3.9 \times 10^{28}$ J m. (Reprinted with permission from El Alaoui Faris, M. D. et al. 2009. Membrane tension lowering induced by protein activity. *Phys. Rev. Lett.* 102 (3), 038102. Copyright 2009 by The American Physical Society.)

among the first to quantitatively describe the RBC-flickering phenomenon. Their model describes membrane fluctuations as thermally excited undulations, mainly governed by the bending elasticity of the membrane. This seminal work paved the way to many theoretical and experimental studies aiming to describe these fluctuations. Since reliable methods for GUV preparation were not yet available, different techniques were developed to analyze these fluctuations; among them, micropipette aspiration (Waugh and Evans, 1979) or flickering spectroscopy is described above (Zilker et al., 1992; Peterson et al., 1992; Strey et al., 1995).

A debate opened rapidly on the role of active metabolic cell forces in these fluctuations after it has been observed that the amplitude of the fluctuations decreases either upon ATP depletion (Levin and Korenstein, 1991; Tuvia et al., 1998), or when the viscosity of the surrounding medium was increased (Tuvia et al., 1997) (Figure 14.4A), suggesting that flickering is not purely of thermal origin. ATP hydrolysis is important to control membrane–spectrin cytoskeleton interactions because the phosphorylation of the 4.1R protein, controlling this connection, is ATP dependent. A clear consequence of ATP depletion shows up, for instance, during the retraction of a tether, mechanically pulled from RBC (Borghi and Brochard-Wyart, 2007).

Two recent experimental papers using different techniques have also shown that the fluctuations of RBC membranes correspond to a nonequilibrium situation (Park et al., 2010) and (Betz et al., 2009). Betz et al. have recorded the mechanics and dynamics of the edge of the RBC membrane,

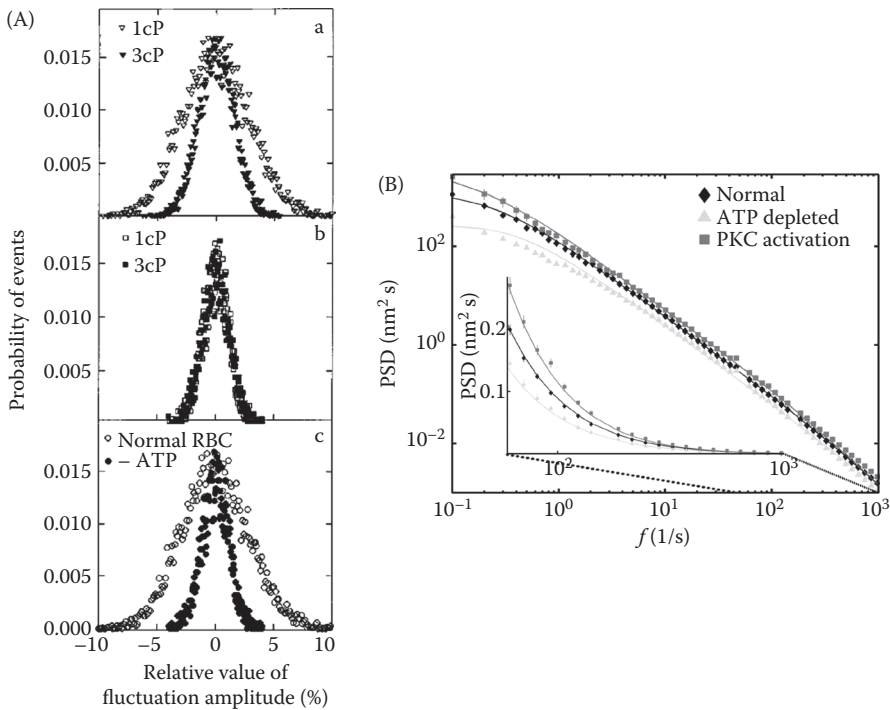


FIGURE 14.4 RBC flickering. (A) Amplitude distribution of RBC membrane fluctuations. (a) Effect of external medium viscosity (1 (▽) and 3 cP (▼)) on normal RBCs. (b) Effect of external medium viscosity (1 (◻) and 3 cP (◼)) on ATP-depleted RBCs. (c) Effect of ATP depletion, with medium viscosity of 1 cP (normal (○)) and ATP depleted (●). (From Tuvia, S. et al. 1997. Cell membrane fluctuations are regulated by medium macroviscosity: Evidence for a metabolic force. *Proc. Natl. Acad. Sci. USA* 94, 5045–5049. Copyright 1997, National Academy of Sciences, USA.) (B) Mean PSD of normal RBCs (diamonds), ATP depleted (triangles), and PKC activated (squares). (Inset: Semilog plot of the same data for the high-frequency range.) (From Betz, T. et al. 2009. ATP-dependent mechanics of red blood cells. *Proc. Natl. Acad. Sci. USA* 106, 15320–15325. Copyright 2009, National Academy of Sciences, USA.)

spanning a frequency range of more than four orders of magnitude at subnanometer resolution (Betz et al., 2009). This has been accomplished by adapting an interferometric particle detection method, based on very weak optical tweezers and a quadrant photodiode for recording the signal. They have analyzed the power spectral density (PSD) of RBC, that is, the time-wise Fourier transform of real space correlations. With this method, Betz and coworkers have been able to precisely measure the bending rigidity and the surface tension, including the internal effective viscosity of RBC. Results were consistent with the previously published data. In particular, they confirmed the previous reports by Tuvia et al. (1998) of decreased static fluctuation amplitudes under ATP-depletion conditions. However, no effect on the fluctuation amplitudes due to changes of the external viscosity was detected. This is in good agreement with a recent theoretical model of Auth et al. (2007) but contradicts (Tuvia et al., 1997). Nevertheless, their dynamic analysis shows a clear deviation at frequencies lower than 10 Hz between the PSD measured for normal cells and those ATP depleted, whereas at higher frequencies, the PSDs are well fitted by thermal models. At low frequencies, the amplitude of the fluctuations is higher in the presence of ATP (Figure 14.4B). Similar to the observations on *in vitro* active membranes (see above), these results can be interpreted in terms of a reduced effective membrane tension. So far, it has not been possible to quantitatively relate in a model this reduction of the tension to the expected modifications in the mechanics of the membrane–cytoskeleton complex. Finally, protein kinase C (PKC)

activation—leading to phosphorylation of the 4.1R protein and thus to a weakening of spectrin–membrane interaction—results in enhanced fluctuations as compared to normal cells. The ATP dependence described by Betz et al. is fully consistent with the results presented by Park et al. (2010). In this paper, the fluctuations of the entire cell were recorded with dynamic full-field laser interferometry (not only those of the cell edge), also revealing larger amplitudes in the presence of ATP. Park et al. measured a non-Gaussian parameter, defined by the second and fourth moments of the membrane height displacement. This parameter is expected to be equal to two for thermal fluctuations and is larger if nonequilibrium processes are contributing, which is the case for normal RBC. The authors concluded that ATP facilitates nonequilibrium dynamic fluctuations in the RBC membrane, enhanced at the scale of spectrin mesh size.

However, the debate is still not over. Recently, Boss et al., using digital holographic microscopy and fluctuation mode analysis, claimed that the mean fluctuation amplitudes of the eigenmodes of RBC membranes are in agreement with the thermal equilibrium theory and that ATP controls the mechanical properties of the cell membrane, but not its fluctuations (Boss et al., 2012). Interestingly, the authors qualitatively find the same results as in Betz et al. (2009) after 4 h of ATP depletion, but conclude differently. This demonstrates the necessity for a stringent test to discriminate whether, or not, flickering of RBC corresponds to a nonequilibrium phenomenon. One way to address this point unambiguously would be to test whether the fluctuation–dissipation theorem is violated due to the presence of active processes, by carrying out a measurement of “spontaneous” membrane fluctuations and comparing it to a measurement of the response function.

14.2.3 NONEQUILIBRIUM MEMBRANES IN THE PRESENCE OF LIPID FLUXES

In cells, membranes exchange lipids and proteins with their surroundings. Budding and fusion of small vesicles with the membrane of cell compartments participate in intracellular trafficking. In some cases, such as in activated dendritic cells or mastocytes, membrane instabilities have been observed following a massive fusion of small vesicles. Thus, studying the consequences of nonequilibrium addition of lipids to membranes is a relevant problem for cell biology. An *in vitro* assay was designed, where fusion of positively charged small liposomes with negatively charged GUVs was induced by electrostatic interaction (Solon et al., 2006). Interestingly, this leads to a high lipid add-on rate that creates instabilities in the membrane (Figure 14.5a through c). Such instabilities have been predicted from theory, following a formalism similar to that of active membranes (Rao and Sarasij, 2001; Ramaswamy and Rao, 2001; Girard et al., 2004a). Using flickering spectroscopy, extremely long correlation times have been measured for the fluctuations (typically on the order of 1 s for the 10th mode), which can only be explained by a negative effective tension on the membrane induced by lipid fluxes. Eventually, the membrane is no longer stable. The GUV shape changes and dense lipid aggregates, called “catons,” appear on the GUV membrane, recovering a spherical shape (Figure 14.5d). This transition is analogous to a collapse of the bilayer.

14.3 THEORETICAL MODELS

Below, we present two different classes of active membrane models. We call the first class “hydrodynamic models,” because these models rely on symmetry arguments to postulate a specific form for the active forces. These arguments are very general, but they hold only in the hydrodynamic limit, corresponding to long times and large length scales. In such models of active membranes, electrostatic effects associated with the ion transport in the ion pumps are not described explicitly. To address this possibly serious limitation for vesicles containing ionic pumps and channels, a second class of models has been developed. These models are called “electrokinetic models,” because they describe effects that couple the hydrodynamics of the fluid phase to the electrostatics associated with ion transport.

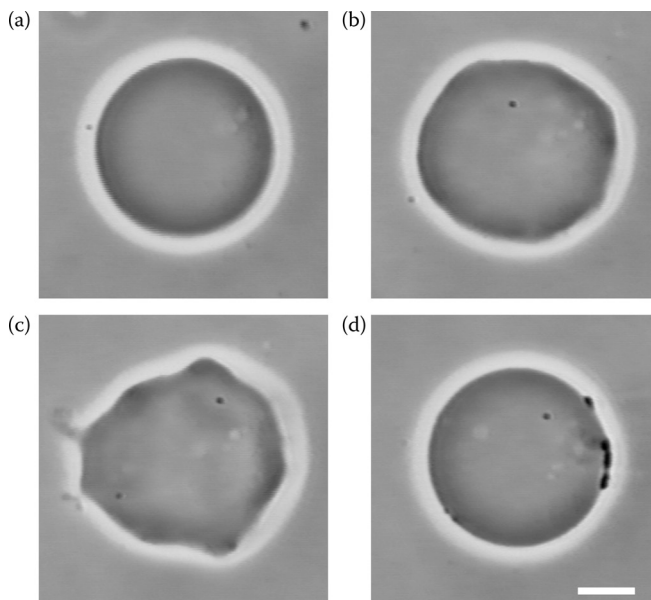


FIGURE 14.5 Effect of nonequilibrium lipid addition on the stability of a GUV. The fusion of suboptic vesicles with a GUV by electrostatic attraction induces membrane instabilities and destabilization. (a) $t = 0$, the vesicle is optically tense. (b) $t = 10$ s, the amplitude of the fluctuations increases rapidly. (c) $t = 15$ s, formation of thin tubular instabilities, and (d) $t = 45$ s, the vesicle recovers its initial spherical shape and tension but with dense lipid aggregates (cations) (bar, $10 \mu\text{m}$). (Reprinted with permission from Solon, J. et al. 2006. Negative tension induced by membrane fusion. *Phys. Rev. Lett.* 97, 098103. Copyright 2006 by The American Physical Society.)

14.3.1 HYDRODYNAMIC MODELS

In 1996, Prost and Bruinsma proposed in a landmark paper the first simple hydrodynamic model of active membranes (Prost and Bruinsma, 1996), in which the activity of the membrane originates from embedded ion channels. These ion channels switch stochastically between open and closed states, which produce a collective noise (Salman et al., 1996; Hille, 2001) called shot noise in analogy to fluctuations of the electron current transported in wires. This model is able to predict conditions for which the active noise can dominate over thermal (equilibrium) noise. Note that this can happen despite the diffusion of the ion channels in the membrane, which tends to diminish this effect. Prost and Bruinsma derived the fluctuation spectrum of tensionless membranes and found—in addition to the equilibrium part, which goes as $k_B T / \kappa q^4$ —an additive nonequilibrium correction, proportional to the square of the noise amplitude divided by q^5 . Owing to this difference in the two contributions, the nonequilibrium correction to the spectrum cannot be expressed by a multiplicative factor (usually called effective temperature) with respect to the equilibrium part. This feature is not only found in generic models of active membranes, it is in fact present in a large class of nonequilibrium systems.

With the first experimental realization of active membranes, that is, reconstituting BR in GUV (Manneville et al., 1999, 2001), also came a new idea on how to describe the coupling of protein activity to membranes. For BR specifically, a coupling via curvature rather than via shot noise was proposed. The introduction of this new type of coupling in the model leads to significantly new physics as compared to the Prost and Bruinsma model (Prost and Bruinsma, 1996), because it implies a feedback of membrane curvature onto the distribution of active proteins, an effect that was absent in the Prost and Bruinsma model. As a result of this feedback, active membranes are characterized by a different fluctuation spectrum and new types of instabilities of the membrane are possible. A linear stability analysis shows that instabilities can occur if (i) local curvature enhances

the force produced by a single pump and (ii) the curvature produced by pumping attracts more pumps (Ramaswamy et al., 2000).

Below, we sketch the basic assumptions of the Ramaswamy, Toner, Prost (RTP) model, for details and for a more precise discussion of the results, we refer to Ramaswamy et al. (2000), Manneville et al. (2001), and Sankararaman et al. (2002). Let us denote the fluid velocity as \mathbf{v} and let $h(\mathbf{r}, t)$ be the membrane displacement in the quasi-planar approximation (Monge's gauge). The membrane is subjected to random forces, which can be either thermal \mathbf{f}_{th} or active \mathbf{f}_a and \mathbf{f}_p . The difference between the latter two quantities is that \mathbf{f}_p describes the active noise acting on the membrane due to the dissipation of energy in the permeation process, while \mathbf{f}_a describes the active forces arising from changes in the conformation of BR proteins. The equation of motion of the membrane is coupled to the fluid velocity through the boundary conditions (BCs) at the membrane. The Navier–Stokes equation for a fluid of viscosity η

$$h\Delta\mathbf{v} = -P + \mathbf{f}_{th} + dP\hat{z} + \mathbf{f}_a, \tag{14.2}$$

where P denotes the pressure in the bulk fluid, and δP represents the corresponding pressure difference across the membrane. Note that in addition to δP , an osmotic pressure difference should be present, which results from the transfer of ions from one side to the other. However, on a closer analysis, this term leads to a negligible contribution in the fluctuations spectrum; so, we omit it here for simplicity (Manneville et al., 2001). The equation above is complemented by the incompressibility condition $\nabla \cdot \mathbf{v} = 0$. The BC at the membrane is $\mathbf{v}_\perp(\mathbf{r}, t) = 0$ for the component parallel to the membrane and

$$\frac{\partial h(\mathbf{r}, t)}{\partial t} - v_z(\mathbf{r}, t) = \lambda(dP\hat{z} + \mathbf{f}_a + \mathbf{f}_p), \tag{14.3}$$

for the component normal to the membrane plane (along the z axis), where λ is a permeation coefficient. The pressure difference δP is equal to the restoring force due to the membrane so that

$$dP = -\frac{\partial F}{\partial h}, \tag{14.4}$$

in terms of the membrane free energy F . For instance, for a tensionless membrane with a curvature–concentration coupling to a concentration field ψ and a curvature coupling coefficient Ξ , we obtain $dP = -kq^4 h(\mathbf{q}, t) - \Xi \mathbf{y}(\mathbf{q}, t) q^2 \hat{z}$. In experiments with BR pumps, the concentration field ψ typically represents the difference in density of pumps in their two possible orientations with respect to the membrane normal. Equation 14.3 expresses Darcy's law, which implies that the total pressure difference across the membrane produces a relative velocity of the fluid with respect to the membrane. The activity enters into this equation and also into the Stokes equation where it leads to an active contribution to the stress tensor. Using Fourier's transforms, it is possible—in this simple planar geometry—to eliminate the pressure and to obtain an effective equation of motion for the membrane including active terms. This equation can be solved to obtain the fluctuation spectrum of the active membrane. This active spectrum depends on dynamic parameters such as the viscosity of the surrounding fluid η or the permeation coefficient λ , because it is essentially nonequilibrium in origin. In contrast to that, the spectrum of an equilibrium membrane cannot depend on such parameters, as imposed by the fluctuation–dissipation theorem.

In general, the form of the distribution of active forces is unknown, but it must obey some important constraints. For instance, there is no external force on the combined system pump + membrane + solvent. Thus, in a description of this combined system, the space integral of the force density field due to the pumps must be zero. The pump exerts a force on the membrane and solvent, which in turn exerts an equal and oppositely directed force on the pump. Therefore, the force

density field of the pumps needs to have a zero monopole moment. In general, it will have higher multipole moments, the lowest being the dipole moment (Manneville et al., 2001). By summing over the contributions of all these dipoles, one can write the active force as

$$\mathbf{f}_a = \hat{z} \int d\mathbf{r} \mathbf{F}_a(\mathbf{r}) \mathbf{d}(\mathbf{r} - \mathbf{R}) (\mathbf{d}(z - w) - \mathbf{d}(z - w')), \quad (14.5)$$

where \mathbf{R} denotes the position of the active inclusion in the membrane while w and w' denote the location of the ends of the dipoles along the z axis. Equivalently, one can also describe the effect of these active forces as an active contribution to the stress tensor (Ramaswamy and Rao, 2001).

This model (Manneville et al., 2001) triggered substantial theoretical interest in the problem, mainly focusing on the proper description of nonequilibrium effects associated with protein conformational changes (Sankararaman et al., 2002; Chen, 2004; Gov, 2004; Lacoste and Lau, 2005; Lomholt, 2006a,b). An important distinction between two possible active forces, the so-called “direct forces” and “curvature forces,” depending on whether active proteins couple or not to the membrane curvature, has been introduced in Gov (2004). In a subsequent work by this author, this method has been used to characterize the membrane fluctuations caused by diffusing active proteins that couple to membrane curvature (Gov and Gopinathan, 2006), a topic of general interest in the field. In Sankararaman et al. (2002), a particularly careful and complete hydrodynamic model of active membranes also inspired by the experiments (Manneville et al., 2001) was presented. This study is based on the assumption that the activity arises only from curvature coupling in a way similar to the “curvature forces” mentioned above; this means, in particular, that active permeation also considered in Manneville et al. (2001) is excluded. In Chen (2004) and Chen and Mikhailov (2010), active membrane models were constructed based on the idea that active inclusions may switch between several internal states. In this case, the membrane fluctuations are nonequilibrium fluctuations because the transitions between the various internal states of the proteins do not obey detailed balance.

To distinguish active from passive fluctuations, it was shown in Lacoste and Lau (2005) that dynamic measurements are better than static measurements, because static measurements may not be able to distinguish between the shot noise and the active noise generated by curvature coupling, while time-resolved measurements of unequal time correlation functions are able to do so. In Lomholt (2006b), the question of the specific form of the distribution of active forces has been further investigated. In this reference, it was shown how to transform an arbitrary active force distribution into an excess current of momentum in the membrane equation of motion. In the simplest possible case, this transformation leads to the force dipole distribution discussed above. In the more general case, the quantities of interest for membrane fluctuations are contained in the first two moments of this active force distribution. This model has been used to interpret the measurements of the fluctuation spectrum in El Alaoui Faris et al. (2009), and it was shown to be compatible with both the micropipette (Manneville et al., 2001) and the videomicroscopy experiment. Incidentally, another advantage of the general framework developed in Lomholt (2006b) is that nonplanar membranes can be described. Indeed, this has been applied in Lomholt (2006a) for quasi-spherical vesicles, a geometry which is appropriate for experiments with GUVs.

In the above models, active forces were introduced into membrane dynamical equations disregarding the interplay between tension and excess area of the membrane (Sankararaman et al., 2002; Gov, 2004; Chen, 2004; Lacoste and Lau, 2005; Lomholt, 2006a,b). The previous work on passive composite membrane systems teaches us, however, that the distribution of membrane excess area is generically affected by coupling of the membrane to an external system, and as a result, the tension is also affected by this coupling. This was shown, in particular, in a passive composite membrane model for a membrane coupled to a cytoskeleton (Fournier et al., 2004). In this work, a change in the value of the membrane tension near the wave vector corresponding to the mesh size of the cytoskeleton was predicted; such a variation is compatible with the available data on the fluctuations of RBCs, although more precise data are needed to fully confirm this prediction.

The interplay between tension and excess area of the membrane can be important in active membranes as well. The question of an active contribution to the tension has been investigated in Loubet et al. (2012), using a model where the activity is described by nonthermal noise of various origins (direct force, curvature force, or permeation force). In this study, there is no direct active contribution to the bending modulus, and no additional confining potential in contrast to Gov et al. (2003). A central result of the paper is the derivation of the active membrane tension, done in the same way as in the passive case, namely, by a Lagrange multiplier associated with the conservation of the total membrane area. It was found that the active correction to the tension is always positive. However, the experiments of El Alaoui Faris et al. (2009) reported an increase in the large wavelength fluctuations, which was interpreted as a lowering of the tension. Together with the increase of effective temperature reported in the micropipette experiment (Manneville et al., 2001), this implies an active fluctuation spectrum that is consistently larger than the passive fluctuation spectrum for all accessible wave numbers. If this is so for all wave numbers (even the nonobservable ones), these results would mean that the excess area is not conserved in these experiments. One possibility to interpret the apparent nonconserved excess area, suggested in Loubet et al. (2012), would be that the area that proteins take up increases when they become active, as a result of the conformational change of the protein.

14.3.2 ELECTROKINETIC MODELS

In this section, we review a number of studies inspired by the same active membrane experiments, but with a different focus. In Lacoste et al. (2007, 2009), Ziebert et al. (2010), and Ziebert and Lacoste (2010, 2011), a comprehensive model was developed that accounts for many features of a lipid membrane driven by a normal electric field. The model describes not only membranes containing ionic pumps or channels, but also includes the more general deformation of a lipid membrane in an electric field (Dimova et al., 2009; Vlahovska et al., 2009), a complex process that is important for many biotechnological applications. The main assumptions of the model are that (i) the membrane has a small homogeneous but nonzero ohmic conductivity for ions, (ii) the membrane is surrounded by a simple electrolyte and is impermeable for water, (iii) the membrane is quasi-planar and the electric field is normal to this surface, (iv) the hydrodynamics of the surrounding fluid phase is described at the level of low Reynolds numbers, (v) the membrane thickness is effectively zero, and, finally, (vi) flexoelectric effects or any effect involving a modification of the orientational order of the lipids due to the electric field is neglected. Incidentally, flexoelectric effects describe the dependence on the membrane spontaneous curvature with respect to the voltage across the membrane. A more realistic description of the liquid crystalline order of the lipids and their coupling to the electric field in a nonconductive membrane is given in Bingham et al. (2010). Note that assumption (i) may not be sufficient to describe, for instance, voltage-gated channels, which typically respond nonlinearly to voltage; assumption (ii) could be relaxed by including additional permeation mechanisms.

Figure 14.6 shows a sketch of the studied planar geometry: we consider a steady current, driven by a direct current (DC) voltage drop V across two electrodes at a fixed distance L . The membrane is quasi-planar, located at $z = 0$ and is embedded in an electrolyte of monovalent ions. It contains channels for both ion species but is itself neutral, that is, it does not carry fixed charges. The channels or pumps are assumed to be homogeneously distributed in the membrane and enter calculations only via the conductance G . Since the membrane is quasi-planar, it can be described using the Monge representation. Our method is essentially perturbative with respect to the flat reference state of the membrane. The electric field, assumed to be perfectly aligned in z -direction, is perpendicular to the membrane surface. Further, we assume a quasi-static approach (Ambjörnsson et al., 2007; Lacoste et al., 2009), in which membrane fluctuations are considered to be much slower than the characteristic diffusion time of ions along the Debye length. The effect of the electric field is described as a small perturbation with respect to this base state.

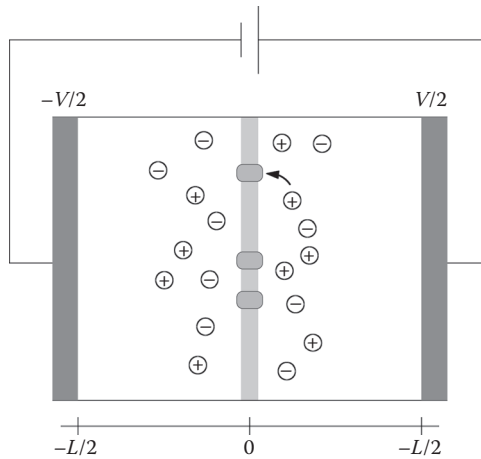


FIGURE 14.6 Electrokinetic model. The initially flat bilayer membrane, represented by the plane $z=0$, is embedded in a symmetric electrolyte. Membrane fluctuations around this base state are not represented. A voltage V is applied far from the membrane on electrodes separated by a distance L . The membrane contains ion channels, which give rise to a conductance G . (Reprinted from *A Planar Lipid Bilayer in an Electric Field: Membrane Instability, Flow Field and Electrical Impedance*, Ziebert, F. and D. Lacoste, Copyright 2011, with permission from Elsevier.)

When an electric field is applied across a lipid membrane, the main physical effect is a large accumulation of ions on both sides of the membrane as a result of capacitive effects. These capacitive effects are significant because of the large dielectric mismatch between the dielectric constant of water and that of the lipid membrane, which results in large electric fields across the membrane.

The model takes the conductance of the membrane, which can be significantly enhanced by ion channels, into account, but for most situations, this conductance is small, so that the system is rather well described by a leaky capacitor with only a small leakage current. In our framework, we have obtained an analytical solution of the electrokinetic equations in this quasi-planar geometry using a linear Debye–Hückel approximation in Lacoste et al. (2007, 2009), Ziebert et al. (2010), and Ziebert and Lacoste (2011) and a nonlinear Poisson–Boltzmann approach in Ziebert and Lacoste (2010). The solution of the electrostatic problem is coupled to the hydrodynamic equations in the surrounding fluid, through the force balance equation that is enforced at the membrane surface. Remarkably, this approach is able to correctly describe the capacitive effects of the membrane and of the structure within the Debye layers, while conserving the simplicity of the “zero-thickness approximation” on which most of the literature on lipid membranes is based. This is accomplished by the use of an effective Robin-type BC to describe the electrostatic BC at the membrane.

From the force balance equation, we have obtained electrostatic corrections to the elastic moduli of the membrane. It is useful to decompose the corrections for the tension $\Delta\Sigma$ and for the bending modulus ΔK into two parts, $\Delta\Sigma = \Delta\Sigma_\kappa + \Delta\Sigma_m$ and $\Delta K = \Delta K_\kappa + \Delta K_m$, where the moduli with index κ represent the contribution coming from the Debye layers located near the membrane on both sides, while the moduli with index m represent the contribution due to the field that is inside the membrane. In Ziebert et al. (2010), we have discussed the dependence of these moduli on various parameters such as the amount of salt, the conductivity of the membrane, or the amplitude of the electric field. In these models, the dependence on the corrections is always quadratic in the applied external field. Linear terms are allowed only if flexoelectric couplings or asymmetric charged membranes are considered. The dependence as a function of the salt concentration is more complex and is illustrated in Figure 14.7a for a nonconductive membrane and in Figure 14.7b, for a conductive membrane with conductivity $G = 0.1 \text{ S m}^{-2}$.

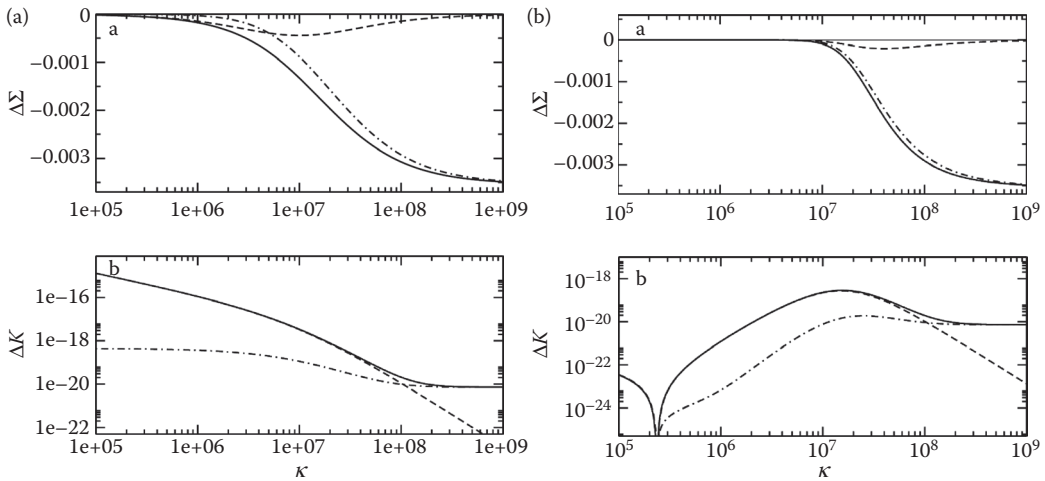


FIGURE 14.7 Electrostatic corrections to surface tension $\Delta\Sigma$ (panels a), (in N m^{-1}) and to bending rigidity ΔK (panels b), (in J) as a function of the inverse of the Debye length κ (in m^{-1}) for an applied voltage difference $V = 1 \text{ V}$ and a system size $L = 1 \text{ mm}$. The various curves illustrate the different contributions to the elastic moduli. The dashed lines are the contributions due to the Debye layers in the fluid surrounding the membrane and the dash-dotted lines are the contributions due to the field inside the membrane. Solid lines: sum of both corrections. (a) The membrane is nonconductive to ions, $G = 0$. (b) The membrane is a slightly conductive case to ions, with a conductivity $G = 0.1 \text{ S m}^{-2}$. (From Ziebert, F. and D. Lacoste. 2010. A Poisson–Boltzmann approach for a lipid membrane in an electric field. *New J. Phys.* 12, 095002. With permission.)

Using this framework, we have tested results known from the previous work using particular limits. For instance, we have exactly recovered the results obtained in Ambjörnsson et al. (2007) for the tension correction and essentially recovered the bending modulus, which was calculated in this reference for a nonconductive equilibrium membrane of finite thickness using the same geometry. In the limit of high salt, we have also recovered the tension correction obtained in the conductive membrane model of Sens and Isambert (2002). It is important to point out that additional nonequilibrium processes can be included in our model rather easily, because they are based on a calculation of the force balance at the membrane. Another advantage of the force balance formulation is that one can also describe the ionic currents flowing through the membrane, which in turn create fluid flows near the membrane. In Lacoste et al. (2009) and Ziebert et al. (2010), in particular, we have shown that these flows are analogous to the electroosmosis flows, generally studied in microfluidic devices. Other nonequilibrium effects that can be included as well are for instance ion channel stochasticity or active pumping. From our calculations of the electrostatic corrections of the elastic moduli of the membrane in various conditions (amount of salt, conductance of the membrane, etc.), we have also analyzed conditions for which an instability can occur in the membrane. The analysis of such an instability could be useful to describe, for instance, the initial stages in the process of vesicle formation from thin lipid films (electroformation).

We have specifically studied nonlinear electrostatic effects using the Poisson–Boltzmann approach in Ziebert and Lacoste (2010), while we had relied mainly on the linear Debye–Hückel approximation in all of our other work. This extension has been motivated by an experiment with a floating lipid bilayer placed in an electric field (Lecuyer et al., 2006), where the corrections to the elastic moduli have been measured using neutron reflectivity. However, the electric field was too large in this experiment to apply the linear regime. A benefit of this study is to realize that the linear model underestimates the surface charge at the membrane surface, and that it is particularly limited in its application when the amount of salt is lowered. As a result, the threshold for the appearance

of a membrane instability is much lower in the nonlinear model as compared to the linear model. Finally, we have extended the model to time-dependent alternate current (AC) electric fields, and discussed the role of system size, membrane conductivity, and the diffusion coefficients of the ions (Ziebert and Lacoste, 2011).

We hope that these results, and future extensions thereof, will be useful for specifically understanding the role of ionic selectivity in biological membranes, and more generally, the effect of applying electric fields to membranes, a complex process that has important applications for biotechnological applications such as electroformation and electroporation.

14.4 CONCLUSION

At the current stage of active membranes studies, there is an obvious lack of experiments, in particular on *in vitro* systems. Many theoretical models have been developed exploring different ways of including nonequilibrium activity of proteins or the effect of transmembrane potentials, or ion transfer through lipid membranes. Even though more advanced and comprehensive models can be envisioned in the future, many basic predictions of the existing models have not been tested, yet. In particular, only the effect of some ion pumps has been considered, but not the effect of ion channels. This is despite the facts that they were the first to be described theoretically (Prost and Bruinsma, 1996) and their effect is expected to be much more impressive due to the typically 100–1000-fold increased transfer rates of ions as compared to pumps. To do this, biochemistry and reconstitution pitfalls must be overcome. In addition, the effect of protein activity has been mainly described by effective quantities in experimental reports so far. No direct fit of the fluctuation spectra using theoretical predictions has been performed, which would allow a more stringent test of the models. Along the same line, experimental tests of electrokinetic models are also very limited, probably due to technical challenges when implementing such experiments. For passive membranes, the repulsion of entropic origin due to the presence of a wall or another membrane, first identified by W. Helfrich (1978), has been well documented. This interaction is expected to be even strengthened and the scaling law should change in the case of active membranes (Prost et al., 1998; Granek and Pierrat, 1999). This effect could have interesting biological consequences for cell–cell or cell–surface interactions. Unfortunately, no experiment has been performed yet to check these predictions. In addition, most experiments are performed on GUVs using either optical microscopy methods that can be spatially limited or micromanipulation techniques providing indirect information on the fluctuations. New technical developments based, for instance, on scattering techniques could allow measurements of fluctuations on a wider wave vector range.

Recent theoretical developments have addressed specific dynamical aspects of active membranes. For instance, Chen et al. (Chen and Mikhailov, 2010) constructed an active membrane model in which inclusions undergo a chemical cycle between several internal states, and in which variations of the local curvature of the membrane modify the transition rates between the conformational states of the protein. In such a model, interesting dynamical effects occur such as hydrodynamic flows, instabilities, and traveling waves.

The diffusion of proteins in membranes—whether active or passive—is a central question in the field that should be tackled more systematically both experimentally and theoretically. This includes, in particular, studies of self-propelled active membrane inclusions, also called nanoswimmers in Huang and Mikhailov (2012). Finally, one important challenge for theoreticians is to develop an active membrane model that could capture the action potential of neurons. In conclusion, even though the ultimate objective of the pioneering work on membranes in the 1970s was to model cell membranes of live cells, more than 40 years later, it appears that still more work is needed to establish a comprehensive model including the effects of active proteins and of the connected cytoskeleton.

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