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Unveiling the Active Nature of Living-Membrane Fluctuations and Mechanics

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Keywords

active membranes, biological membranes, membrane fluctuations, active materials, nonequilibrium physics, fluctuation-dissipation relation

Abstract

Soft-condensed matter physics has provided, in the last decades, many of the relevant concepts and methods allowing to successfully describe living cells and biological tissues. This recent quantitative physical description of biological systems has profoundly advanced our understanding of life, which is shifting from a descriptive to a predictive level. Similar to other active materials investigated in condensed matter physics, biological materials still pose great challenges to modern physics as they form a specific class of non-equilibrium system. Actively driven membranes have been studied for more than two decades, taking advantage of rapid progress in membrane physics and in the experimental development of reconstituted active membranes. The physical description of activity within *living* biological membranes remains however a key challenge, which animates a dynamic research community, bringing together physicists and biologists. Here, we first review the past two decades of experimental and theoretical advances, that enabled the characterization of mechanical properties and non-equilibrium fluctuations in active membranes. We distinguish, in particular, active processes originating from membrane proteins or from external interactions, such as cytoskeletal forces. Then, we focus in a specific chapter, on the emblematic case of red blood cell flickering, the active origin of which has been debated for decades, until recently. We finally close this review by discussing future challenges in this ever more interdisciplinary field.

Contents

1. Introduction

The quantitative understanding of active materials far from thermodynamic equilibrium remains one of the most fascinating topics in modern physics (1, 2, 3, 4, 5, 6, 7). From a physics point of view, biological materials belong to the general class of soft-condensed matter, the physics of which is rooted in statistical mechanics. As living cells and biological tissues continuously burn chemical energy to generate forces and dynamically rearrange their internal structure, their description requires to go beyond equilibrium mechanics as known from classical condensed matter physics. A special case of active materials are membranes, such as phospholipid bilayers, that are simplified as lamella-like 2D materials, which have been successfully modeled by the physics of liquid crystals (8). Although these 2D liquids are almost incompressible materials, they can easily deform out-of-plane, driven by both thermal agitation but also active, non-equilibrium forces. As a result, sustained fluctuations of biological and biomimetic membranes are commonly observed. While the passive properties of such biomembranes are quite well understood, the physics of active membranes is still under active investigation. Our limited knowledge stands in stark contrast to the importance of membrane activity in living cells, where they represent a key element for their quantitative description.

Brownian motion and RBC flicker: reversed histories

Just like Brownian motion, the dynamic membrane movement of red blood cells has been already described in the 19th century (9). Interestingly, the interpretations offered for these two phenomena evolved in precisely opposite directions. Brownian motion was initially thought to be a consequence of animated, living organisms and the detailed investigation by Brown suggested soon that these microscopy fluctuations are a purely passive phenomenon (10). In contrast, the red blood cell membrane fluctuations, also called flickering, were initially interpreted as a purely passive, thermal motion (9), and it was demonstrated only recently that active metabolic contributions play an important role for this process. Although flickering amplitude was shown to depend on ion transport (11), it was considered as passive (12) for most of the past 130 years. This notion was further supported by the first quantitative measurement of flickering by Brochard and Lennon, who could in 1975 explain the observed spectrum by a passive model (13). This analysis was the starting point of a - still ongoing - detailed biophysical characterization of RBC mechanics.

Only about 20 years ago, the dogma of passive RBC flickering was challenged by mea-

suring ATP and viscosity-dependent flickering amplitudes (14). These observations can be seen as the starting point of a rich scientific debate about metabolic contributions to the flickering, where a series of contradicting results were published (15, 16, 17, 18, 19, 20). Recently, a direct violation of equilibrium thermodynamics was reported, demonstrating the contribution of active processes to flickering (21). Re-analyzing the different perspectives and experiments appears as an instructive lesson, demonstrating that two opposite hypothesis may be able to explain many experimental findings for a long time, and showing how a controversial scientific debate eventually converges to a generally accepted model.

A lesson in the history of science: tracing the evolution of a consensus

The quantitative description of active membranes has become a research field on its own with the seminal paper of Prost and Bruinsma (22), where the first theoretical description of membrane fluctuations driven by an active process was introduced. This initial model introduces ion channels are active force sources, where the energy is provided by a chemical potential due to a concentration gradient across the membrane. It was soon developed further to describe more complex scenarios, such as fluctuations close to walls (23), coupling between curvature and protein density (24, 25), diffusion of active channels (26), rotational activity (27), active forces generated by membrane bound ion pumps (28, 29, 30) and cytoskeletal forces acting on the membrane (31, 32). The first experimental observation of activity in membranes was reported in micropipette aspiration assays on vesicles containing bacterio-rhodopsin (BR), a light driven proton pump (33). In this review we recall the mechanics of passive membranes before we discuss the main sources of mechanical activity in membranes. Because of its importance, the origin of RBC flickering is discussed in a focus section. As this review is mainly concerned with active membranes in a mechanical sense, we will not discuss here active processes changing the membrane organization or chemical composition.

2. The mechanics of passive membranes

The mechanical properties of phospholipid bilayer membranes has been intensively studied, with many excellent resources summarizing this rich field of physics (35, 36). The commonly used coarse-grained model introduced by Canham and Helfrich (37) is generally sufficient to describe membrane mechanics. Helfrich suggested to view the membrane as a 2D fluid (Fig. 1a), which allows to construct a Hamiltonian based on the local energy required for bending and stretching the bilayer. While the bending energy is simply dependent on the curvature of the membrane, the concept of membrane tension requires some explanation. For an incompressible membrane, where the lipids are at equilibrium, the free energy shall not change with respect to variation in area and it has a vanishing tension by definition. However, in fluctuating membranes, the measured membrane area is typically the apparent membrane area A, which is smaller than the absolute membrane area $\mathcal{A}$, since fluctuations store effectively some membrane surface (see Fig 1a). Pulling laterally on the membrane increases the apparent membrane area by pulling out the fluctuations. This reduces entropy, effectively leading to a force that resists area change, and that is measured via a membrane tension. When all fluctuations have been pulled out, an additional tension term of enthalpic origin will measure the cost of changing the absolute bilayer area. In most realistic situations, the total membrane area remains unchanged (incompressible 2D fluid), and hence the tension is dominated by the entropic contribution. From a more mathematical point of view, membrane tension is generally introduced as a Lagrange multiplier that

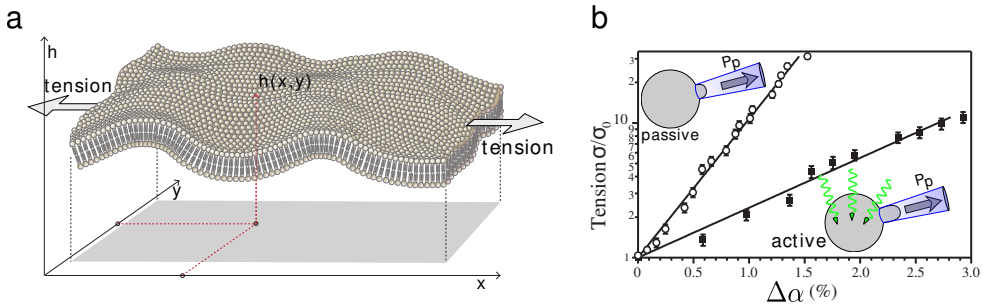

Figure 1

a) Sketch illustrating the Helfrich approach to model membrane mechanics. The complex phospholipid bilayer is typically described in the Monge gauge where to each point in the projected xy plane a height h(x,y) is assigned. Tension is pulling out the spontaneous fluctuations of the membrane, thus enhancing the apparent surface area, which is called excess area. b) The apparent surface area change upon controlled tension application can be measured by a micropipette assay. When irradiating included light activated bacterio-rhodopsin channels, an increased excess area can be measured. This was the first experimental proof for actively driven membrane fluctuations (33), that was later investigated in more detail (34) (Data replotted from (34), with permission).

Bending modulus κ: Material property of the membrane defining the energy required bending at curvature C

Membrane tension σ: Force per length that defines the energy required to change the membrane area

adjusts to conserve the absolute membrane area (38). The Canham-Helfrich Hamiltonian is hence generally written :

$$\mathcal{F} = \frac{1}{2}\kappa \int_{\mathcal{A}} \left[C(\mathbf{r}) - C_0\right]^2 d\mathcal{A} + \sigma \int_{\mathcal{A}} d\mathcal{A} \qquad 1.$$

Here $\mathcal{F}$ is the free energy, κ the bending modulus, C and C_0 are the local and spontaneous membrane curvatures, σ the membrane tension and $\mathcal{A}$ the area of the membrane. It is important to note here that κ is a material property of the membrane that depends on its molecular composition while σ describes the energy required to change the apparent membrane area (39, 38). A convenient description is based on the Monge representation, where the position of a quasi-flat membrane is defined by the local height $h(x, y)$, measured from a plane parametrized with the coordinates (x, y) (Fig. 1a). In the approximation of small membrane deflections $|\nabla h| \ll 1$, the area element becomes at leading order $d\mathcal{A} \sim \left(1 + \frac{1}{2}\left(\nabla h\right)^2\right) dxdy$ and the Helfrich Hamiltonian takes a simpler expression, where the area integral is now on the apparent area A :

$$F = \frac{1}{2} \int_A dxdy[\kappa(\nabla^2 h)^2 + \sigma(\nabla h)^2] \qquad 2.$$

Typical values for the bending modulus κ of biological and reconstituted membranes range from 5 to 50 $k_{\mathrm{B}}T$, while the tension depends largely on the actual mechanical situation of the membrane. Hence, below the critical tension of $\sigma_c \approx 3 \times 10^{-3}$ N/m at which the membrane ruptures, any value of the tension is possible, even vanishing or negative tension(38, 40, 41, 21). In the case of negative tension, the membrane may buckle, and hence fall back to a vanishing tension. The bending modulus being of the same order as the thermal energy $k_{\mathrm{B}}T$ results in significant deformations at ambient temperature. Equation (2) can be expressed as function of the individual wave-modes using a spatial

Fourier transformation of $F = \frac{1}{2}\int qdq\left(\kappa q^4 + \sigma q^2\right) h\left(q\right) h^*\left(q\right)$. At equilibrium, a energy $\frac{1}{2}k_{\mathrm{B}}T$ is associated to each Fourier mode q according to the equipartition theorem, yielding the static fluctuation spectrum (42) :

$$\left\langle |h_q|^2 \right\rangle = \frac{k_{\mathrm{B}}T}{\kappa q^4 + \sigma q^2} \qquad 3.$$

Relaxation time τ_q: Timescale required to relax a membrane fluctuation excited at a specific mode q.

To describe membrane dynamics, the Navier-Stokes equation needs to be solved by balancing the membrane forces with the tangential and normal fluid stress at the membrane (43). Since the system of interest here is in the low Reynolds number regime, inertial terms are generally negligible, and the fluid dynamics reduces to the linear Stokes equation where each excited mode decays with a relaxation time $\tau_q = \frac{4\eta q}{\kappa q^4 + \sigma q^2}$.

The dynamic equation of motion for each mode q can be expressed as an overdamped Langevin equation:

$$\frac{dh_q}{dt} = \tau_q^{-1} h_q(t) + \zeta(t) \qquad 4.$$

where $\zeta\left(t\right)$ represents the thermal white noise obeying the fluctuation dissipation theorem for an equilibrium membrane. Based on this Langevin approach, the average membrane dynamics can be expressed in terms of an auto-correlation function (ACF), which is more easily accessible experimentally:

$$\langle h_q(t) h_q(0) \rangle = \frac{k_B T}{\kappa q^4 + \sigma q^2} \times e^{-\frac{t}{\tau_q}} \qquad 5.$$

Projected area A: The apparent, or projected membrane area is typically the one detected by video microscopy. Rapid, short wave-length membrane fluctuations are beyond the microscope resolution, but accumulate a significant total area.

Besides the ACF, the membrane dynamics can be probed by the power spectral density PSD $\left\langle h\left(\omega\right)^2 \right\rangle$, defined as the Fourier transform of the ACF. Since experimentally, fluctuations are measured generally at a single membrane point, we need to sum over all wave-modes to obtain the PSD at one point in the membrane:

$$\left\langle h(\omega)^2 \right\rangle = \frac{4\eta k_{\mathrm{B}}T}{\pi} \int_{q_{\min}}^{q_{\max}} \frac{dq}{(\kappa q^3 + \sigma q)^2 + (4\eta\omega)^2} \qquad 6.$$

where $q_{\max} = \pi/a$ and $q_{\min} = \pi/\sqrt{A}$ are respectively the microscopic and macroscopic mode cutoffs , with a the smallest accessible length-scale, either a molecular lipid size or the experimental resolution of the detection method. The integral in q can be solved in the special cases of tension and bending dominated regimes where simple powerlaws in the form of $\left\langle h(\omega)^2 \right\rangle_\sigma = \frac{k_B T}{2\sigma\omega}$ and $\left\langle h(\omega)^2 \right\rangle_\kappa = \frac{k_B T}{6\pi(2\eta^2\kappa)^{1/3}\omega^{5/3}}$ emerge. These powerlaws are in perfect agreement with experiments (44), see also (13). Typically, only the projected area A of the membrane is experimentally accessible. This leads to the notion of excess area defined as the relative difference between total and projected membrane areas that can be analytically described (42):

$$\alpha = \frac{\mathcal{A} - A}{\mathcal{A}} \sim \frac{1}{2A}\int_A dxdy(\nabla h)^2 = \frac{k_{\mathrm{B}}T}{8\pi\kappa} \ln(\frac{\kappa q_{\min}^2}{\sigma}). \qquad 7.$$

An appealing experimental way to prove this dependence of excess area was developed by Evans and coworkers (45): A micropipette applies a well defined suction pressure to a patch of membrane of a giant unilamellar vesicle (GUV), hence controlling membrane tension, easily inferred using Laplace's law. The apparent area change $\Delta\alpha = \alpha_0 - \alpha$ of the vesicle is measured (46, 47, 48), where α_0 is the excess area at the minimal tension σ_0

Laplace law: Connects membrane tension, pressure differences across the membrane and membrane curvature. Hence it allows to calculate tension knowing the applied pressure.

sufficient to suck up the liposome at the start of the experiment. In the entropic limit, where the thermal fluctuations are pulled out of the membrane, theory predicts :

$$\Delta\alpha = \frac{k_B T}{8\pi\kappa} \ln(\frac{\sigma}{\sigma_0}). \quad 8.$$

The classical Helfrich approach allows defining the mechanics of a membrane with only two parameters, the bending modulus κ and the surface tension σ, complemented by volume and area constraints. Any shape change in a closed membrane leads to a variation of apparent area to volume ratio. While the volume remains constant, the apparent area must vary in a self-consistent way, thus fixing membrane tension according to Eq. 7.

3. Sources of membrane activity

3.1. Active membranes driven by ion pumps and channels

Membrane activity: Active forces on the membrane increase its fluctuations amplitude.

Ion channels: Protein structures that can allow selected ions to pass the membrane along (electro-)chemical potential gradients. Channels can be in open and closed configuration.

Ion pumps: Protein structures that use metabolic energy to transport well defined ion species across membrane and against gradients.

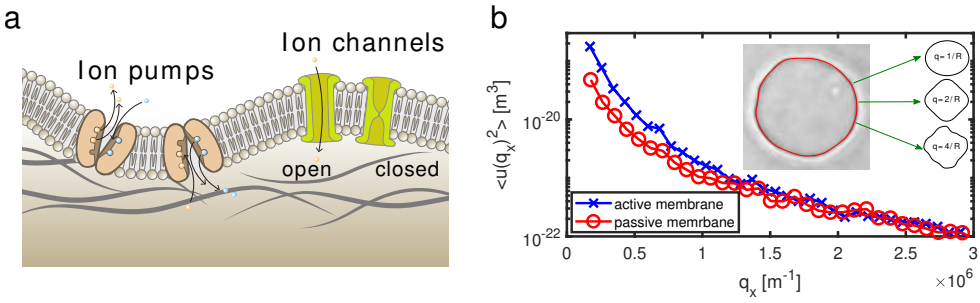

Figure 2

a) Sketch illustrating transmembrane proteins known to actively excite membrane fluctuations. Ion pumps use the energy provided by ATP hydrolysis to transport ions against a concentration gradient across the membrane. The force applied on the ions results in an opposing force of the same magnitude on the membrane. Similar force kicks are experienced by ion channels that open and allow the transport of ions across the membrane along a concentration gradient. Here the force is driven by the chemical potential difference across the membrane. b) Direct observation of fluctuations mode dependent active fluctuations in the case of ion pump (bacterio-rhodopsin) driven membranes. As predicted theoretically, the main contribution of activity is on the large wavelength (low q modes). The inset illustrates the mode decomposition of the video microscopy acquired GUV shape fluctuations. Data replotted from (49))

Ion pumps and channels are key components of biological membranes. Pumps allow to generate ion concentration gradients across the membrane, to control the osmotic pressure jump and the electrochemical potential. In contrast, ion channels allow the passive flow of ions across the membrane, for example to balance chemical or electrical potentials. In both cases, the transport of ions across the membrane comes along with mechanical momentum. To obey the 3rd law of Newton, this momentum should be balanced by a localized force on the membrane. A local ion concentration change leads to a local osmotic pressure jump, entraining water molecules across the membrane, and hence implying a reaction force from the surrounding fluid. The first paper recognizing that such processes can lead to active fluctuations of the membrane was published in 1996 by Prost and Bruinsma (22). This work can be considered as the starting point of the field of active membranes. In this

pioneering model, ion channel activity is described as a shot-noise process via a two-state variable $S_k(t)$ switching stochastically between 0 and 1. In agreement with experimental results, single ion-channel gating is supposed to have an exponentially decaying time correlation $g(t) = \langle S_k(t)S_k(0)\rangle = g(0)\exp\left(-\frac{t}{\tau_a}\right)$ defining an characteristic active timescale τ_a.

A typical pressure force $f \sim k_B T/w$ on the membrane of thickness w results from the gating of a single ion channel located at the position r_k leading to active fluctuations that add up to the membrane Langevin equation introduced in equation 4:

$$\frac{dh_q}{dt} = \tau_q^{-1} h_q(t) + \zeta(t) + f\lambda_p \sum_k S_k(t) e^{iqr_k(t)} \qquad 9.$$

Here, the relaxation time $\tau_q^{-1} = \frac{\kappa q^3}{4\eta} + \lambda_p \kappa q^4$ takes into account explicitly the membrane permeability λ_p, which is key to the existence of a pressure force in reaction to the passage of an ion across the membrane. Interestingly the model of Prost and Bruinsma already predicted that non-equilibrium fluctuations dominate at large wavelength, a common experimental observation over the last 20 years.

Changes in excess area: The first experimental proof that active fluctuations have a measurable impact on membrane mechanics was given in (33), three years after their first theoretical prediction (22). The experimental system is based on micropipette aspiration, where the excess area is measured as a function of membrane tension (Fig. 1b). Active forces are applied to the GUV membrane by (BR) a well characterized light-activated ion pump optimally operating when illuminated with wavelength around 566nm (50). As shown in Fig. 1b the excess area change $\Delta\alpha$ at comparable tension is strikingly increased in the active case, suggesting that more membrane area is stored in the fluctuations when compared to the passive case. An important result is that for both the passive and active situations, a logarithmic dependence of the excess area on the tension is found experimentally, in agreement with the equilibrium prediction (Eq. 8). This means that the equilibrium model is able to fit the active situation, with only a change in the prefactor. This prefactor depends on the temperature T and the bending modulus κ. The authors show independently that the bending modulus is not affected, and define hence an active temperature $T_{\text{eff}} > T$, which is an elegant way to quantify the activity in the view of an additional random thermal energy source driving the fluctuations. However, since the underlying equation is derived at thermodynamic equilibrium the concept of effective temperature is problematic. Detailed studies show that the active fluctuations are excited in a mode, and frequency dependent way, and can thus not be simply cast into an effective temperature (40). As we will show later, detailed dynamic measurements also show the limits of the concept of effective temperature. While the seminal paper of Prost & Bruinsma started the field of active membrane fluctuations, it ignored several possible feedbacks. Granek and Pierrat first calculated the activity-enhanced diffusion of pumps and channels in the membrane (26). In a further and important conceptual step, the theory by Ramaswamy, Toner and Prost (RTP) addresses properly the feedback of membrane deformations on activity, by explicitly coupling membrane curvature with the density of active membrane proteins, which are generally asymmetrically shaped (51, 24). The shape-density coupling used here extended previous ideas to the non-equilibrium situation (52). This resulting self-consistent framework leads to a rich self-organization behavior, exhibiting spontaneous traveling waves and local shape instabilities in the membrane. Formally, these non-equilibrium features emerge

Active timescale τ_a: Correlation time of the active process. This is the average cycling time of active force production.

Effective temperature T_{eff}: Assumes that the active process has same statistics as thermal forces, and will hence only rescale the temperature. This concept breaks down if the impact of activity on the membrane fluctuations is inherently time and length-scale dependent.

by extending the Helfrich Hamiltonian as:

$$F = \frac{1}{2}\int_A dxdy[\kappa(\nabla^2 h)^2 + \sigma(\nabla h)^2 + \chi_s \psi^2 - 2\Xi\psi\nabla^2 h] \qquad 10.$$

where $\psi(\mathbf{r},t) = n^+(\mathbf{r},t) - n^-(\mathbf{r},t)$ is a signed protein density, which measures the local difference between proteins with preference to positive curvature relative to proteins with preference to negative curvature, χ_s denotes the susceptibility for the imbalance between curvature-positive and negative proteins, and Ξ quantifies the coupling between the protein density and membrane curvature (24). This improved Hamiltonian was then used to reanalyze the experimental data of the increased excess area, in a model where the activity is injected in the membrane Langevin equation as force dipoles mediated by the signed protein density. The improved model allows to replace the phenomenologically introduced effective temperature by an analytical term that depends on the active force amplitude and the mechanical characteristics of the membrane (25). A more simplified model by Gov ignores the hydrodynamic coupling between the channels and treats the active forces either as force kicks (force monopoles) or "curvature forces", the latter corresponding to active fluctuations of the membrane spontaneous curvature (28). The curvature-force activity, and to some extent the active kicks, are shown to reproduce the experimental dependence of excess area on tension for light-activated vesicles, but also the dependence of fluctuations on viscosity, which was put forward as sign of activity in red-blood cells by (14). Since the RTP model was developed to explain static micropipette aspiration measurements, the dynamical random nature of the active forces was not considered. This was overcome by a series of papers deriving dynamic fluctuation spectra, including the shot noise characteristics expected for uncorrelated active processes (53, 29, 30). To describe spherical vesicles, Lomholt extended the RTP model to spherical geometry (53), where membrane tension naturally arises as Lagrange multiplier for membrane area (38), while the tension due to activity-enhanced fluctuations was considered only more recently (40). As the membrane is pushing against the surrounding fluid, it ultimately results into frictional forces applied on the membrane over length-scales larger than the deformation. Hence, the fluid reaction force cannot, in general, be considered as point-like, and the spatial integral of forces over the system {protein + membrane + fluid} should vanish by force balance, implying a force density field for active proteins with zero monopole moment. Hence, unless extrinsic agents - not considered so far - may enter the force balance (such as cytoskeletal forces), the first active contribution of active membrane proteins should be of the form of force dipoles (54, 28, 29). A dipolar contribution is therefore expected from a permeation force (53), and in the absence of dipolar contribution, higher multipole moments may still contribute to the active membrane fluctuations, such as 'curvature forces', which can actually be considered as quadrupoles (53, 40).

Force monopoles: Force monopoles result from forces applied on the membrane through processes in the ambient medium, such as from the cytoskeleton or optical tweezers

Force dipoles: If active forces are generated by membrane proteins, force balance requires a zero monopole moment, and the first expected contribution is a local force dipole

Flickering analysis: While the first quantitative experiments on active membranes tested the static property of excess area, theory predicts well-defined correlation times and mode dependent active amplitudes. The required measurements need to analyze the dynamic membrane fluctuations or flickering, and the first quantitative flickering analysis was done by Brochard and Lennon (13) on RBCs, that were further refined by video microscopy analysis. Several experimental approaches for the fluctuation analysis of membrane were developed in the past decades (reviewed recently in (55)). Initially, shape analysis using video microscopy was experimentally introduced for passive vesicles (56) and soon extended

to RBCs (57). Later, the contour detection methods were also used to analyze shape fluctuations of active membranes that are driven by light induced BR (Fig. 2b). The theoretical basis to understand the fluctuations of spherical vesicles was developed by (43) and extended to tensed vesicles by Milner and Safran (58). Interestingly, although the theoretical model predicts an increase in tension, the mode-dependent increase of fluctuations leads to the conclusion that the membrane tension is effectively reduced in the active case (49). Besides this surprise, the contour analysis could show that long wavelength modes are more affected by activity than the shorter ones (Fig. 2b), which directly confirmed one of the main prediction of the initial paper introducing active membranes more than 10 years earlier (22). A big strength of the contour analysis is access to both, the spatial modes and the temporal relaxation times, because both carry information about the mechanics and the energy injected in the system by activity. This was exploited more recently to analyze active membranes driven by the pump Na+, K+ ATPase (59), where slight effects of the activity on the bending modulus were reported. Strikingly, in this later study, two relaxation timescales were identified. Besides the expected mode dependent relaxation, an additional slow relaxation timescale of about 0.5 sec was measured, which is consistent with the cycling time of the pumps used in these experiments. In a more theoretical approach the flickering has also been simulated (60), which confirmed that membrane mechanics can be varied by the addition of active forces on the membrane.

Complementing these video microscopy driven approaches, time resolved membrane fluctuation spectroscopy (TRMFS) was used to very precisely measure the out-of-plane fluctuations of single membrane points (44, 61). Here the self organized pore α-Hemolysin was added to GUVs, inducing activity by making the membrane permeable to small molecules and thus letting the gradient of glucose and sucrose equilibrate.

3.2. Activity driven by the cytoskeleton and dynamic attachments

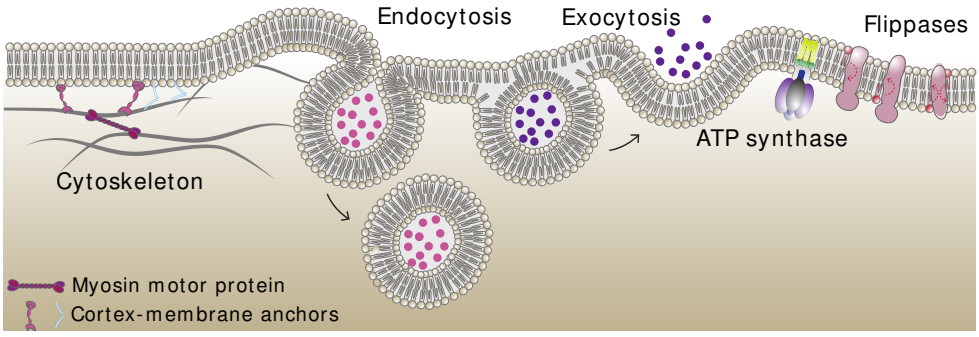

Figure 3

The active forces can also be generated by the dynamic attachment and detachment for underlying structures, such as the cytoskeleton or a substrate. If this turnover consumes a source of energy, it is possible to generate active non-equilibrium forces. Furthermore the contractile forces of the cytoskeleton can couple to the membrane via adhesion molecules, and thereby excite active membrane fluctuations. Additional processes are endo and excocytosis, phospholipid flipping mediated via flippases and floppases, and artificial active processes such as micro-swimmers located in or at the membrane.

While channels and pumps are embedded directly in the membrane, membrane activity can also result from forces applied through processes in the ambient medium. A prominent, and substantially studied example of external active forces is provided by a highly dynamic, and often contractile **cytoskeleton** (62), lying right beneath the membrane. Most biological cells ensure mechanical support to their plasma membrane via a thin and cross-linked network of cytoskeletal proteins. In RBCs, the cytoskeleton consists primarily of a network of spectrin filaments (63), while in more complex eukaryotic cells a thin meshwork of actin filaments, called the actin cortex is mechanically anchored to the membrane (64). The actin cytoskeleton is inherently active, continuously stirred internally by molecular motors from the myosin family, and constantly turning over by polymerization and depolymerization. A dynamic interaction between the cytoskeleton and the membrane is also expected to happen at thermal equilibrium (65). But it can also be driven by metabolic changes in the binding activity of proteins regulating this attachment, such as in red blood cells (66). In eukaryotic cells, a highly dynamic coupling between the actin cytoskeleton and the bilayer is associated to phosphorylation of the attachment proteins. In this situation the conversion of chemical energy to mechanical work is expected to drive membrane fluctuations of non-equilibrium nature. Motor proteins, principally myosins, can also interact directly with the membrane via specific lipids, and may hence pull on it by power strokes applied on the actin filaments(Fig. 3). Such cytoskeletal forces may lead to both normal and tangential active deformations of the membrane. The theoretical description of such active membrane-cytoskeleton composite systems has been worked out from several points of view, with an initial focus on the RBC membrane. Several models have been introduced that explicitly take into account the active interaction between the membrane and the spectrin cytoskeleton (31, 67, 68). In these models the activity is typically introduced as uncorrelated direct force centers, that are assumed to be driven by detachments of a tensed spectrin cytoskeleton from the membrane. In an alternative approach (21), the mechanical coupling between the membrane and the spectrin cytoskeleton is considered explicitly, in particular their relative tangential motion, allowed by the bilayer tangential fluid character. Since metabolic events identified in the spectrin network, or in its anchoring proteins, were systematically associated with a decreased mechanical strength of the membrane, it is supposed that any phosphorylation event leads to a local decrease of the network shear modulus, which is the only parameter necessary to characterize the spectrin network mechanics at a coarse-grained level. As a consequence, the shear modulus is supposed to fluctuate around a mean value that decreases with the fraction $\langle n_a \rangle$ of active sites $\langle \mu \rangle = \mu_0(1 - \langle n_a \rangle)$. This metabolic activity is shown to lead to active stretching fluctuation modes in a tensed network, which are mechanically coupled to bending modes of deformations for a curved elastic membrane. The metabolic activity in the spectrin cytoskeleton is finally predicted to drive active membrane shape fluctuations only in prestressed regions of non-zero mean curvature. Interestingly, by considering explicitly the volume and area constraints, an excess area of only a few percents can generate a negative bilayer tension. This can compensate and maintain a positive prestress in the spectrin network, leading ultimately to a tension for the composite membrane close to zero, as it was assumed in the seminal work of Brochard et Lennon (13). The model developed in (21) is derived in quasi-spherical geometry, but it ignores the discrete lattice structure of the spectrin network, which was shown to couple fluctuation modes of wavelength shorter and larger than the network meshsize (69, 70). It should be noted that the actual major source of active forces in the RBC membrane has not been experimentally pinpointed. The activity in the

Actin cortex: Dense network of the biopolymer actin that mechanically supports the membrane. Active forces from the actin cortex can couple to the membrane.

Spectrin network: Main cytoskeletal network supporting the RBC membrane. Regular attachment sites lead to an hexagonal symmetry. Multiple sites of phosphorylation have been identified in the network and attachment protein complexes.

spectrin cytoskelton is a potential source, but the aforementioned channels and pumps, and also other transmembrane proteins such as flippases, may be alternative sources of active flickering. This possibility was also considered in (21) using stochastic simulations of the membrane, where the shape of the RBC is closely mimicked. For other cell types, it is experimentally well established that the actin cortex continuously generates significant active forces on the plasma membrane (71, 72). This highly complex interaction between the actin cytoskeleton and the two-dimensional fluid membrane is still not fully understood, although a series of models have been proposed (73, 74, 75, 76). Typically, these models introduce an additional energy term in the Helfrich Hamiltonian and couple the cytoskeleton to the membrane via binding proteins, actin nucleators and molecular motors. Such feedback then leads to instabilities like local protrusions, waves and oscillations, all being common shape changes and patterns widely observed in biological cells.

RBC active flickering: Although the extent of active fluctuations has been recently measured in the red blood cell, the actual proteins mediating this activity remain unknown.

Adhesion is a well-studied subject in membrane physics, but most of the work has been done in the context of thermodynamic equilibrium (77), notably using reconstituted liposomes with specific adhesion to surfaces (78). For active membranes, the sole presence of a close wall was early proposed theoretically to enhance active fluctuations (23). In cells, adhesion is typically a non-equilibrium process requiring metabolic energy to dynamically renew broken adhesive bonds. In the context of so called active stickers, the dynamic and energy consuming turnover of membrane adhesion was studied (79). Dynamical attachment can also generate active membrane shape changes within cells, as illustrated by the nucleation of blebs, which correspond to the detachment and bulging of a membrane patch from the actin cortex (80, 81, 82). These studies suggest that by modulating the degree of membrane attachment to the cytoskeleton, to substrates or to other membranes, active membrane fluctuations play an important role in several biological processes.

3.3. Additional processes potentially driving membrane activity.

Flippase, Floppase, Scramblases: Classes of membrane proteins that transport lipids across the membrane either by ATP consumption (Flippases, Floppases) or by equilibrating gradients.

Although the active forces generated by ion pumps and channels, as well as cytoskeletal interactions remain the best studied origins of activity, a series of other processes have been proposed and experimentally observed.

Out-of-equilibrium membrane fluctuations have been proposed to result from local jumps in the spontaneous membrane curvature via **flipping of phospholipids** from one leaflet to the other(Fig. 3). Such processes are highly relevant for the membrane organization (83), since the asymmetry of the different membrane compartments is vital for the physical properties of the membrane and controls local phase transition and small domains.

Flipping phospholipids against a gradient requires chemical energy in form of ATP and is accomplished by a protein class called flippases and floppases (84). Furthermore, scramblases are independent of an external energy source and allow to equilibrate the composition gradients of well defined lipids by a bidirectional switching processes (84). The mechanical forces are here induced by a local change of spontaneous curvature since either a whole lipid is moved from one leaflet to the other or by exchanging lipids with different properties. The rapid and local change of spontaneous curvature will effectively lead to a local deformation of the membrane, thus generating an active force (28, 30, 40). Although such forces have been already integrated into theoretical descriptions, so far only static shape changes of GUVs have been studied experimentally (85, 86).

The biological membranes are continuously reorganizing by the addition and removal of small vesicles from the membrane. This process of **endo- and exocytosis** (see Fig.3)

requires well defined membrane deformation and fission/fusion, both process require protein generated forces that actively interact with the membrane, thus driving it out of equilibrium (87, 88, 89). Such protein driven local curvatures are not only a key step for endocytosis, but can be used by cells to regulate the total membrane area, and hence to adjust membrane tension (90). In other *in vitro* experiments where membrane was added to GUVs in a processes mimicking exocytosis it was shown that a rapid increase of the membrane area leads to shape changes via buckling instabilities, extruding the extra membrane area in tubes and dense membrane compartments (91).

F1F0 ATP-synthase: Transmembrane protein that uses the proton gradient across the membrane to physically rotate a protein domain. This rotation brings ADP and phosphate in close proximity thus allowing for the generation of ATP.

More recently **rotating motors** embedded in the bilayer have been shown to drive non-equilibrium behavior. The transmembrane protein F1F0-ATP synthase, responsible for the synthesis of ATP (92) embedded in the membrane generates a mechanical forces acting on the membrane (93). Theoretically the important feature of rotational motors is to generate hydrodynamic interactions that are predicted to lead to self-organized arrangement of the motors (27). Although this model made clear predictions for the active membrane, it turned out that the RTP model originally developed for pumps and channels was sufficient to explain the data. Besides these biological active forces, membrane activity can also be induced by artificial force generating processes. Typical examples are active particles that are embedded in the membrane or in the surrounding fluid. Hydrodynamic flows can generate forces on the membrane and thus lead to active fluctuations, especially when active swimmers are moving in close vicinity to the membrane. So far, mainly theoretical studies and simulations have been published in this respect (94).

A very attractive approach to systematically study active forces on the membrane is by using optical tweezers to drive membrane fluctuations. Here, random forces can be applied, but also controlled pulling forces are used (95). An representative example was developed recently by multiplexed optical tweezers that can excite individual bending modes, and thus directly test the mechanical properties of GUVs in a mode-dependent way (96). Finally, recent effort has been made to model how electrical fields will produce active forces on conductive membranes (97, 98, 99).

4. The emblematic case of RBC flickering

When reviewing the past 20 years of research on active membranes, it appears clearly that the experimental and theoretical descriptions of RBC membrane flickering has been both a source of inspiration, but also a subject of fierce debates on the physical nature of these fluctuations. Already 1890 (9) Browicz described the spontaneous flicker of RBCs. He observed this motion even when the cells were stored for days, or taken from a dead body, and concluded hence that they are not a sign of life, but rather passive Brownian motion. This view was challenged more than 60 years later when a correlation between the activity of ion pumps and the flickering was reported (11), suggesting a metabolic origin of the flickering. But this idea based on active processes was torn down soon after, as as flickering was still observable in ATP-depleted cells and in RBC ghosts, where any possible metabolic energy source was removed (12). Since at this time no quantitative measurement methods were available, it was not possible to determine possible changes in the fluctuation amplitude upon the experimental conditions. Such quantitative access was first introduced by the seminal work of Brochard and Lennon that could provide detailed spectra of the flickering which enabled them to show that a purely thermal model was able to explain the data (13). Based on these experiments, it was generally accepted that the RBC flickering is a thermal

process. In the following 20 years thermal analysis of flickering was systematically used to determine the mechanical properties of cells, relying on the fluctuation-dissipation theorem (FDT) that connects, in the linear regime, the observable fluctuations to the dissipative mechanical properties of a system at equilibrium (100). However, up to this point the passive origin was only supported by the success of fitting a a passive theory to the data. It is important to point out that these findings do not exclude active forces as the source of flickering, especially if these active forces are random and uncorrelated in nature. When comparing to the measurements of excess area, and mode analysis, it appears legitimate to describe these active membranes by passive models. However, using these passive approaches on active data leads to wrong estimates for mechanical properties of the membrane.

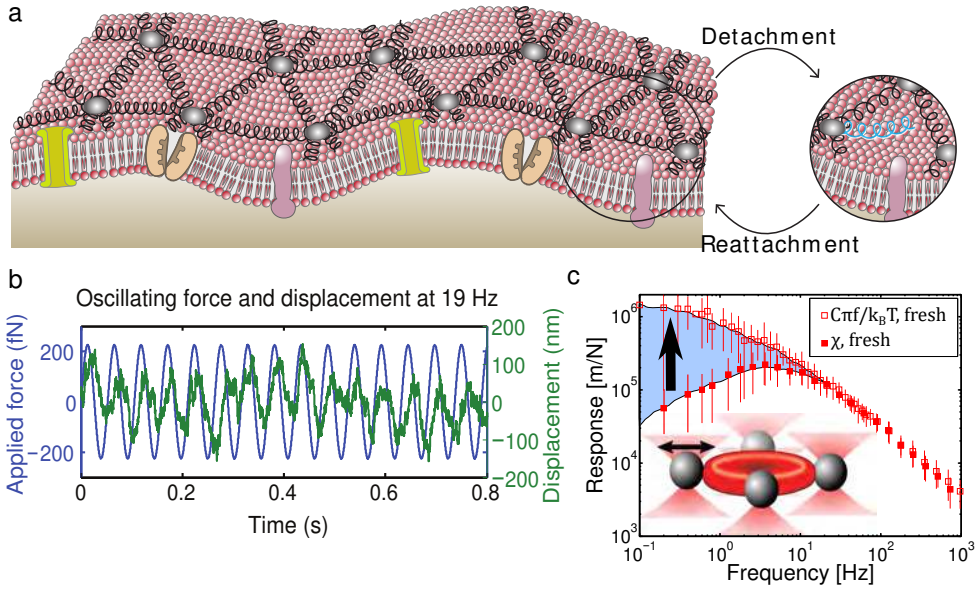

Figure 4

(a) Schematic view of the red blood cell membrane with the organized spectrin network, ion pumps and channels. (b) Active rheology of RBCs. To determine the mechanical response function, an optical tweezer is used to apply an oscillating forces (blue) on the RBC membrane, while the displacement is measured (green). (c) To test whether the behavior is consistent with equilibrium statistical mechanics, the fluctuation dissipation theorem is used to determine the expected response function (open squares), which is plotted with the directly measured dissipative response (filled squares). While the two curves are equal at high frequencies (>10Hz), they diverge for slow timescales. This is a direct evidence that the fluctuations at timescales slower 100ms are largely driven by an active process. Data replotted from (21) with authors permission.

The first quantitative challenge for a passive explanation of membrane flickering came from the Korenstein group, that suggested that flickering amplitude decreased when depleting ATP and strongly depends on medium viscosity (14). The experimental finding that RBC flickering depends on ATP was heavily discussed as some authors could not reproduce this dependence, and reported that the fluctuation amplitude remained unchanged (15, 19). In contrast, a large number of recent experimental work have now confirmed the ATP-

dependence of membrane fluctuations (18, 101, 67, 17, 16, 102, 103), including authors that had initially not been able to confirm the flickering decrease upon ATP depletion (20). A possible reason why the very detailed investigation of Evans (15) could not confirm the ATP-dependence is that exclusively discocyte cells were used. Since ATP-depleted red cells typically undergo drastic shape changes, it is possible that only the few RBCs still containing ATP were chosen in this study, and hence no fluctuation decrease was found. This is supported by detailed fluctuations mode analysis (101), where a significant decrease in flickering was observed only when the ATP depletion drugs were applied for four hours. An important finding of this mode analysis is the highly non-linear dependence of flickering on ATP levels, hinting for secondary effects of ATP depletion, for example on the mechanical properties of the cell membrane. Indeed the metabolic remodeling of the RBC cytoskeleton is essential to the maintenance of its elastic properties and a simple explanation for the ATP depend flickering decrease can be found in the stiffening of the membrane. Hence, after settling the discussion about ATP-dependence of flickering amplitude, a new debate about the possible interpretations of the data was started. The activity critical view was further powered by difficulties to reproduce the viscosity dependence of membrane fluctuations that were initially used as leading argument for active driving (14). Since in an equilibrium system, changes in dynamic variables, such as the viscosity, should not have any incidence on thermodynamic averages, such as the membrane fluctuations amplitude, this finding was interpreted as a support of the active membrane hypothesis. Yet the viscosity dependence could not be confirmed in later studies (18). Furthermore, a simple explanation for the initially found viscosity dependence might be that the sampling time was not sufficiently increased in this initial study (14, 21).

Fluctuation Dissipation Theorem: Fundamental relation between thermally excited fluctuations and energy dissipation. Has to be fulfilled in any system at equilibrium to ensure the laws of thermodynamics.

Response function: Connects the force applied on a system to its mechanical response, which is commonly a deformation or a movement.

To further address the question whether metabolic forces drive flickering, a series of experiments were published supporting the idea that RBC flickering is at least partially an active processes. Park et al analyzed the probability distribution of the membrane position, looking for non-gaussian behavior that require non-equilibrium contributions if considering a linear force displacement model (104). Indeed, such non-gaussian features were found and modeled in a theory including activity (67). However, even these findings don't constitute a definitive proof of activity, as they rely on the assumption that the membrane deformations are in the linear regime, which is not evident. At this point the signs were pointing towards an importance of active forces in membrane flickering, although it remained feasible to explain flickering without such activity (18, 105, 16). However, it is possible to show that pure thermal agitation is not sufficient to explain the flickering if fundamental logical consequences derived using equilibrium physics, such as the fluctuation dissipation theorem, are inconsistent with the observations.

A common approach is to experimentally test the fluctuation dissipation theorem (106, 107), by performing independent measurements of the spontaneous fluctuations and the mechanical response of the system. The first experimental approach using a double optical trap was limited to frequencies higher than 10 Hz (108). Recently, it was shown that the fluctuations and dissipation function deviate for frequencies below 10 Hz (21), indicating that one or several active processes contribute to membrane fluctuations on long timescales. Formally, the FDT connects the dissipation and thermal fluctuations of a system as

$$\left\langle h^2\left(\omega\right)\right\rangle = \frac{2k_BT}{\omega}\chi''\left(\omega\right) \qquad 11.$$

where $\chi(\omega) = \tilde{x}(\omega)/\tilde{f}(\omega)$ is the complex, frequency-dependent mechanical response function. The membrane response function is measured by applying a sinusoidal force f at a

driving frequency ω_d on a bead attached to the membrane, while recording its displacement x reflecting the membrane response (Fig. 4b). In Fourier space, the complex part of the response function $\chi''(\omega)$ describes energy dissipation. By comparing the PSD $\langle h^2(\omega)\rangle$ of the free fluctuations (thermal and active) with the dissipative response function a frequency dependent divergence becomes evident (Fig. 4c). For frequencies lower than 10 Hz, the response function derived from the free fluctuations using the FDT is larger that the directly measured. In contrast, for higher frequencies, the prediction and the direct measurement are consistent. This frequency dependence is important, because the experiments not only show that an active force is increasing the membrane fluctuations in this low frequency regime, but also that a simple interpretation of this activity as higher effective temperature is not reasonable, because the concept of temperature is independent of frequency (Fig. 4c).

The extensive discussion of the origin of the RBC membrane flickering is a beautiful example how initially conflicting experimental results slowly converged to a generally accepted picture. Up to now no other independent experimental confirmation of the FDT violation was reported, and the studies will undoubtedly be pursued, in particular on the precise molecular origin for active fluctuations: metabolic activity of the spectrin cytoskeleton or ion pumps/channel gating activities (Fig. 4a). The question of the biological role of active fluctuations in the RBC membrane also remains a prevailing question in the field.

5. Activity induced changes of membrane mechanics: prospect and critique

A very important question in the field of active membranes are potential mechanical changes induced by the activity. In the past two decades of experimental measurements, many signs of such modifications of the passive mechanical properties of the membrane by the activity have been reported (18, 104, 109, 102). From a theoretical perspective, most of the work has focused on the RBC membrane, where the presence of the spectrin cytoskeleton is predicted to confine the membrane fluctuations (31, 110). This will create a shear elasticity that can renormalize the tension and, to a lesser extent, the bending modulus of the composite membrane (111, 41, 69). The magnitude of these effects is expected to depend on the level of ATP in the cell, which controls the degree of phosphorylation of the various cytoskeletal proteins (112, 109, 113). However the reported mechanical changes upon ATP-depletion need to be interpreted with caution, as membrane mechanical parameters such as the bending modulus and tension have not been measured independently, but are generally derived by applying passive fluctuation models to the experimental data. The common pitfall is to use a passive model on active membranes. This will systematically lead to incorrect estimations of the mechanics, and this might be the reason why the reported values for the bending modulus of RBCs varies by an order of magnitude depending on the reports. However, even when an active model is used, the validity of the extracted mechanical parameters is not ensured. The resulting values are largely dependent on the model chosen, and without additional investigation on the validity of the model the interpretation might be corrupted. A possible way to overcome this problem is to have additional experimental access to the passive mechanics by direct mechanical probing.

An typical example is the determination of the membrane tension, which is not an intrinsic property of the membrane, but depends on the fluctuations amplitude and area/volume constraints. In GUVs, a decrease of membrane tension upon membrane activation was initially reported (49) and this finding was corroborated by comparative measurements in living and ATP-depleted red blood cells (18, 102). Furthermore, when using electrical fields

to actively excite membranes, a decrease of tension is also reported (60), as well as in membranes actively driven by protein adsorption (114). From a theoretical perspective, the increase of fluctuations should however lead to a decrease of the apparent area (Eq. 7), and as a consequence to an increase of membrane tension (40). Yet most aforementioned reports of tension decrease in active membranes rely on a thermal interpretation, for which the tension is likely to be underestimated. Since the membrane tension is a key and finely tuned mechanical parameter in eukaryotic cells (90), which controls several processes such as endo- and exocytosis (115) or mechanosensation (116), it will be essential to address carefully this question in the following years.

Physiological relevance of active membrane fluctuations

Non-equilibrium fluctuations are necessary byproducts of protein activity, but the potential physiological roles of enhanced membrane fluctuations remains an open question. As fluctuations are ubiquitous at micrometer scales, cells may have evolved to take advantage of active noise, in order to facilitate or regulate essential cellular functions. To date, it remains difficult, both experimentally and theoretically, to discriminate the potential roles played by active fluctuations, from the main purpose of the active process itself. A number of putative biological functions have however been suggested: activity-driven fluctuations are predicted to increase the mixing of lipids and the diffusion of proteins within the membrane (26), enhanced membrane fluctuations presumably help large trans-membrane proteins to overcome steric barriers created by cytoskeletal components (117). Furthermore, by allowing the membrane to explore larger regions, increased fluctuations may assist the specific binding to a substrate or another membrane, and they have been shown recently to favor lateral interactions of adhesion proteins such as cadherins (78). But they could also make the binding simply more dynamic by increasing simultaneously membrane attachment and detachment rates. In contrast, active fluctuations may help suppressing nonspecific interactions by creating an effective entropic repulsive force when an object gets close to the membrane(23). Finally, active membrane fluctuations are expected to affect directly the membrane tension, and may regulate further mechanical properties such as the bending modulus or spontaneous curvature, all being important parameters regulating various cellular functions like cell motility to endo- and exocytosis and mechanosensing.

6. Future challenges

6.1. Understanding the active mechanics of composite membranes

Most biological membranes are mechanically stabilized by a cytoskeletal structure that provides mechanical rigidity, but also exerts forces on the membrane. The best studied examples are the actin cortex of animal cells, the spectrin network of RBCs and the lamin network supporting the double lipid bilayer of the nuclear envelope. More recently, further cytoskeletal structures interacting directly with the membrane have been characterized experimentally, such as septins in eukaryotic cells (118), or FtsZ filaments (119), both involved notably in cell division. The continuous active turnover and force generation of these cytoskeletal proteins constitutes a major source of mechanical activity in biological membranes(120). In particular, the direct rearrangement of the actin network is involved in large scale morphological changes such as blebs (80), lamellipodia (121) or filopodia (122), but also at smaller scales, in active processes such as endocytosis and exocytosis (123) as

well as membrane ruffles. One major challenge for physicists is to integrate in a consistent theoretical framework these various actin cytoskeleton structures, and to properly describe their coupling to the membrane. Apart from a few exceptions (124), the prevailing approach so far has been to focus on the cytoskeleton, by generally treating the membrane effectively or by neglecting its mechanical contribution in the description. The actomyosin cortex was hence recently considered in the framework of active gels (125) as a 2D active material (126, 127), but the membrane is essentially absent from these descriptions. These decoupled approaches are attractive starting points, but they don't encompass important processes involving the direct coupling between the membrane and the cytoskeleton. On the contrary, a number of theoretical approaches consider such coupling explicitly, but from the point of view of the membrane, generally by including additional terms in the Helfrich Hamiltonian to model the cytoskeleton mechanics (128, 32, 73, 74, 129, 75, 21). From an experimental perspective, pioneering reconstituted experimental model systems have been developed in the past years to investigate the active membrane-cytoskeleton coupling (124, 130, 131, 132, 133, 134, 135, 136), allowing to generate well-controlled and reproducible experiments, that more easily amenable to theoretical descriptions. However these artificial systems are still subjects to several limitations compared to biological cells, such as the unavoidable degradation of ATP, or the lack of significant cytoskeletal turnover. Yet, these recent advances and the rapid progress expected in the next years will undoubtedly trigger new theoretical investigations on active composite membranes, thereby contributing to better understand the various facets of active membranes in biological cells.

6.2. Designing multiscale simulations of active membranes

The physics of active membranes involves various molecular, hydrodynamic and cellular processes spanning multiple length- and timescales. This inherent multiscale feature of active membranes poses several challenges for its physical characterization and modeling. To describe an active membrane at the full-scale of a cell, taking into account its non-linear geometry, the surrounding fluid hydrodynamics and thermal agitation, while keeping some important molecular aspect of the active process in the description, makes the use of numerical simulations largely indispensable. To date, most of the effort has concentrated on the simulation of RBC membranes, using different simulation techniques. In a first approach close to analytical formulations, the membrane Langevin equations are solved numerically directly in Fourier space to avoid the explicit simulation of surrounding fluid (137, 138, 139, 68). Despite its relative simplicity, this description is limited to quasi-flat (periodic) membranes, as a result of the Fourier decomposition, and did not spread widely in the community. In alternative approaches, the fluid hydrodynamics is modeled, either by explicitly solving Stokes flow using immersed boundary methods (140, 141), boundary integral methods (142) or by coarse-graining hydrodynamics through the framework of dissipative particle dynamics (DPD) (143). Boundary integral methods have been used by different groups to model RBC deformations in Stokes flow (142, 144), but these models ignored Brownian motion and the active nature of the membrane. Immersed boundary methods are not stochastic by nature either, but in recent developments a source of noise can be added in the hydrodynamics (145), allowing theoretically to study thermal and active membrane fluctuations. DPD techniques are stochastic by nature and avoid the need to discretize space to simulate hydrodynamics, but they require special care to map pair-wise soft potentials and continuous hydrodynamics. They have been applied successfully

to various soft-matter physics problems, and were used recently to model both thermal and active fluctuations in full-scale simulations of the RBC (146, 147, 21). Combination of these powerful numerical methods with active-gel descriptions of the actin cytoskeleton could help characterizing the composite active membranes beyond RBCs.

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LITERATURE CITED

1. Ramaswamy S. 2010. *Annual Review of Condensed Matter Physics* 1:323–345
2. Menon GI. 2010. Active Matter. In *Rheology of Complex Fluids*. Springer New York, 193–218
3. Saintillan D. 2018. *Annual Review of Fluid Mechanics* 50:563–592
4. Gross P, Kumar KV, Grill SW. 2017. *Annual Review of Biophysics* 46:337–356
5. Ramaswamy S. 2017. *Journal of Statistical Mechanics: Theory and Experiment* 2017:054002
6. Marchetti MC, Joanny JF, Ramaswamy S, Liverpool TB, Prost J, et al. 2013. *Reviews of Modern Physics* 85:1143–1189
7. Ahmed W, Betz T. 2015. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research* 1853:3083–3094
8. Gennes PGd, Prost J. 1995. The Physics of Liquid Crystals. Clarendon Press, Oxford, UK
9. Browicz. 1890. *Zbl. Med. Wiss* 28:625
10. Brown R. 1828. *The Philosophical Magazine* 4:161–173
11. Blowers R, Clarkson E, Maizels M. 1951. *J Physiol* 113:228–39
12. Parpart A, Hoffman J. 1956. *J Cell Comp Physiol* 47:295–303
13. Brochard F, Lennon J. 1975. *Journal de Physique* 36:1035–1047
14. Tuvia S, Almagor A, Bitler A, Levin S, Korenstein R, Yedgar S. 1997. *Proceedings of the National Academy of Sciences* 94:5045–5049
15. Evans J, Gratzer W, Mohandas N, Parker K, Sleep J. 2008. *Biophys J* 94:4134–44
16. Evans AA, Bhaduri B, Popescu G, Levine AJ. 2017. *Proceedings of the National Academy of Sciences* 114:2865–2870
17. Park Y, Best CA, Badizadegan K, Dasari RR, Feld MS, et al. 2010. *Proceedings of the National Academy of Sciences* 107:6731–6736
18. Betz T, Lenz M, Joanny JF, Sykes C. 2009. *Proceedings of the National Academy of Sciences* 106:15320–15325
19. Szekely D, Yau TW, Kuchel PW. 2009. *European Biophysics Journal* 38:923–939
20. Puckeridge M, Chapman BE, Conigrave AD, Kuchel PW. 2014. *European Biophysics Journal* 43:169–177

21. Turlier H, Fedosov DA, Audoly B, Auth T, Gov NS, et al. 2016. *Nature Physics* 12:513–519
22. Prost J, Bruinsma R. 1996. *Europhysics Letters (EPL)* 33:321–326
23. Prost J, Manneville JB, Bruinsma R. 1998. *The European Physical Journal B* 1:465–480
24. Ramaswamy S, Toner J, Prost J. 2000. *Physical Review Letters* 84:3494–3497
25. Manneville JB, Bassereau P, Ramaswamy S, Prost J. 2001. *Physical Review E* 64
26. Granek R, Pierrat S. 1999. *Physical Review Letters* 83:872–875
27. Lenz P, Joanny J, Julicher F, Prost J. 2003. *Phys Rev Lett* 91:108104
28. Gov N. 2004. *Physical Review Letters* 93:268104
29. Lacoste D, Lau AWC. 2005. *Europhysics Letters (EPL)* 70:418–424
30. Lin LCL, Gov N, Brown FLH. 2006. *The Journal of Chemical Physics* 124:074903
31. Gov N, Safran S. 2005. *Biophysical Journal* 88:1859–1874
32. Auth T, Safran SA, Gov NS. 2007. *New Journal of Physics* 9:430–430
33. Manneville JB, Bassereau P, Levy D, Prost J. 1999. *Physical Review Letters* 82:4356–4359
34. Girard P, Prost J, Bassereau P. 2005. *Physical Review Letters* 94:088102
35. Seifert U. 1997. *Advances in Physics* 46:13–137
36. Nelson D, Piran T, Weinberg S. 2004. Statistical Mechanics of Membranes and Surfaces. WORLD SCIENTIFIC, Singapur
37. Helfrich W. 1973. *Zeitschrift fur Naturforschung C* 28:693–703
38. Seifert U. 1995. *Zeitschrift fur Physik B Condensed Matter* 97:299–309
39. Deuling H, Helfrich W. 1976. *Journal de Physique* 37:1335–1345
40. Loubet B, Seifert U, Lomholt MA. 2012. *Physical Review E* 85:031913
41. Fournier JB, Lacoste D, Raphael E. 2004. *Physical Review Letters* 92:018102
42. Helfrich W, Servuss RM. 1984. *Il Nuovo Cimento D* 3:137–151
43. Schneider M, Jenkins J, Webb W. 1984. *Biophysical Journal* 45:891–899
44. Betz T, Sykes C. 2012. *Soft Matter* 8:5317–5326
45. Evans E, Needham D. 1987. *The Journal of Physical Chemistry* 91:4219–4228
46. Evans E, Rawicz W. 1990. *Physical Review Letters* 64:2094–2097
47. Evans E, Rawicz W. 1997. *Physical Review Letters* 79:2379–2382
48. Rawicz W, Olbrich K, McIntosh T, Needham D, Evans E. 2000. *Biophysical Journal* 79:328–339
49. Faris MDEA, Lacoste D, Pecreaux J, Joanny JF, Prost J, Bassereau P. 2009. *Physical Review Letters* 102:038102
50. Lanyi JK. 2004. *Annual Review of Physiology* 66:665–688
51. Ramaswamy S, Toner J, Prost J. 1999. *Pramana* 53:237–242
52. Andelman D, Kawakatsu T, Kawasaki K. 1992. *EPL* 19:57–62
53. Lomholt MA. 2006. *Physical Review E* 73:061914
54. Chen HY. 2004. *Physical Review Letters* 92:168101
55. Monzel C, Sengupta K. 2016. *Journal of Physics D: Applied Physics* 49:243002
56. Engelhardt H, Duwe H, Sackmann E. 1985. *Journal de Physique Lettres* 46:395–400
57. Duwe HP, Zeman K, Sackmann E. 1989. Bending undulations of lipid bilayers and the red blood cell membrane: A comparative study. In *Progress in Colloid & Polymer Science*. Steinkopff, 6–10
58. Milner ST, Safran SA. 1987. *Physical Review A* 36:4371–4379
59. Bouvrais H, Cornelius F, Ipsen JH, Mouritsen OG. 2012. *Proceedings of the National Academy of Sciences* 109:18442–18446
60. Loubet B, Lomholt MA, Khandelia H. 2013. *The Journal of Chemical Physics* 139:164902
61. Lemiere J, Guevorkian K, Campillo C, Sykes C, Betz T. 2013. *Soft Matter* 9:3181
62. Bezanilla M, Gladfelter AS, Kovar DR, Lee WL. 2015. *The Journal of Cell Biology* 209:329–337
63. Lux SE. 2015. *Blood* 127:187–199
64. Salbreux G, Charras G, Paluch E. 2012. *Trends in Cell Biology* 22:536–545

65. Sackmann E, Smith AS. 2014. *Soft Matter* 10:1644
66. Manno S, Takakuwa Y, Mohandas N. 2004. *Journal of Biological Chemistry* 280:7581–7587
67. Ben-Isaac E, Park Y, Popescu G, Brown FLH, Gov NS, Shokef Y. 2011. *Physical Review Letters* 106:238103
68. Zhang R, Brown FLH. 2008. *The Journal of Chemical Physics* 129:065101
69. Dubus C, Fournier JB. 2006. *Europhysics Letters (EPL)* 75:181–187
70. Okamoto R, Komura S, Fournier JB. 2017. *Physical Review E* 96:012416
71. Biswas A, Alex A, Sinha B. 2017. *Biophysical Journal* 113:1768–1781
72. Hui KL, Wang C, Grooman B, Wayt J, Upadhyaya A. 2012. *Biophysical Journal* 102:1524–1533
73. Kabaso D, Shlomovitz R, Schloen K, Stradal T, Gov NS. 2011. *PLoS Computational Biology* 7:e1001127
74. Gov NS, Gopinathan A. 2006. *Biophysical Journal* 90:454–469
75. Shlomovitz R, Gov NS. 2008. *Physical Review E* 78:041911
76. Srivastava P, Shlomovitz R, Gov NS, Rao M. 2013. *Physical Review Letters* 110:168104
77. Seifert U. 1994. *Physical Review E* 49:3124–3127
78. Fenz SF, Bihr T, Schmidt D, Merkel R, Seifert U, et al. 2017. *Nature Physics* 13:906–913
79. Rozycki B, Lipowsky R, Weikl TR. 2006. *Physical Review Letters* 96:048101
80. Charras GT, Coughlin M, Mitchison TJ, Mahadevan L. 2008. *Biophysical Journal* 94:1836–1853
81. Peukes J, Betz T. 2014. *Biophysical Journal* 107:1810–1820
82. Goudarzi M, Tarbashevich K, Mildner K, Begemann I, Garcia J, et al. 2017. *Developmental cell* 43:577–587
83. Montigny C, Lyons J, Champeil P, Nissen P, Lenoir G. 2016. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* 1861:767–783
84. Hankins H, Baldridge R, Xu P, Graham T. 2015. *Traffic* 16:35–47
85. Papadopulos A, Vehring S, Lopez-Montero I, Kutschenko L, Stockl M, et al. 2007. *Journal of Biological Chemistry* 282:15559–15568
86. Ramachandran S, Kumar PBS, Laradji M. 2008. *The Journal of Chemical Physics* 129:125104
87. Rao M, Sarasij RC. 2001. *Physical Review Letters* 87:128101
88. Ramaswamy S, Rao M. 2001. *Comptes Rendus de l'Academie des Sciences - Series IV - Physics-Astrophysics* 2:817–839
89. Sens P. 2004. *Physical Review Letters* 93:108103
90. Sinha B, Koster D, Ruez R, Gonnord P, Bastiani M, et al. 2011. *Cell* 144:402–413
91. Solon J, Pecreaux J, Girard P, Faure MC, Prost J, Bassereau P. 2006. *Physical Review Letters* 97:098103
92. Junge W, Nelson N. 2015. *Annu Rev Biochem* 84:631–57
93. Almendro-Vedia V, Natale P, Mell M, Bonneau S, Monroy F, et al. 2017. *Proc Natl Acad Sci U S A* 114:11291–11296
94. Paoluzzi M, Leonardo RD, Marchetti MC, Angelani L. 2016. *Scientific Reports* 6:34146
95. Bar-Ziv R, Moses E, Nelson P. 1998. *Biophysical Journal* 75:294–320
96. Brown A, Kotar J, Cicuta P. 2011. *Phys Rev E Stat Nonlin Soft Matter Phys* 84:021930
97. Ziebert F, Bazant MZ, Lacoste D. 2010. *Physical Review E* 81:031912
98. Lacoste D, Lagomarsino MC, Joanny JF. 2007. *Europhysics Letters (EPL)* 77:18006
99. Ambjornsson T, Lomholt MA, Hansen PL. 2007. *Physical Review E* 75:051916
100. Callen HB, Welton TA. 1951. *Physical Review* 83:34–40
101. Boss D, Hoffmann A, Rappaz B, Depeursinge C, Magistretti PJ, et al. 2012. *PLoS ONE* 7:e40667
102. Rodriguez-Garcia R, Lopez-Montero I, Mell M, Egea G, Gov N, Monroy F. 2016. *Biophys J* 111:1101
103. Monzel C, Schmidt D, Kleusch C, Kirchenbuchler D, Seifert U, et al. 2015. *Nature Commu-*

nications 6:9162
104. Park Y, Best CA, Auth T, Gov NS, Safran SA, et al. 2010. *Proceedings of the National Academy of Sciences* 107:1289–1294
105. Gogler M, Betz T, Kas JA. 2007. *Optics Letters* 32:1893
106. Mizuno D, Tardin C, Schmidt CF, MacKintosh FC. 2007. *Science* 315:370–373
107. Martin P, Hudspeth AJ, Julicher F. 2001. *Proceedings of the National Academy of Sciences* 98:14380–14385
108. Yoon YZ, Kotar J, Brown AT, Cicuta P. 2011. *Soft Matter* 7:2042–2051
109. Manno S, Takakuwa Y, Nagao K, Mohandas N. 1995. *Journal of Biological Chemistry* 270:5659–5665
110. Seifert U, Langer SA. 1994. *Biophysical Chemistry* 49:13–22
111. Gov NS. 2007. *Physical Review E* 75:011921
112. Subrahmanyam G, Bertics PJ, Anderson RA. 1991. *Proceedings of the National Academy of Sciences* 88:5222–5226
113. Sheetz MP. 1977. *The Journal of Cell Biology* 73:638–646
114. Rangamani P, Mandadap K, Oster G. 2014. *Biophys J* 107:751–762
115. Gauthier NC, Fardin MA, Roca-Cusachs P, Sheetz MP. 2011. *Proceedings of the National Academy of Sciences* 108:14467–14472
116. Charras GT, Williams BA, Sims SM, Horton MA. 2004. *Biophysical Journal* 87:2870–2884
117. Lin LCL, Brown FL. 2004. *Biophysical Journal* 86:764–780
118. Mostowy S, Cossart P. 2012. *Nature Reviews Molecular Cell Biology* 13:183–194
119. Loose M, Mitchison TJ. 2013. *Nature Cell Biology* 16:38–46
120. Rao M, Mayor S. 2014. *Current Opinion in Cell Biology* 29:126–132
121. Small J, Stradal T, Vignal E, Rottner K. 2002. *Trends in Cell Biology* 12:112–120
122. Mattila PK, Lappalainen P. 2008. *Nature Reviews Molecular Cell Biology* 9:446–454
123. Dmitrieff S, Nedelec F. 2015. *PLOS Computational Biology* 11:e1004538
124. Koster DV, Husain K, Iljazi E, Bhat A, Bieling P, et al. 2016. *Proceedings of the National Academy of Sciences* 113:E1645–E1654
125. Prost J, Julicher F, Joanny JF. 2015. *Nature Physics* 11:111–117
126. Turlier H, Audoly B, Prost J, Joanny JF. 2014. *Biophysical Journal* 106:114–123
127. Salbreux G, Julicher F. 2017. *Physical Review E* 96:032404
128. Shlomovitz R, Gov N. 2009. *Phys Biol* 6:046017
129. Maitra A, Srivastava P, Rao M, Ramaswamy S. 2014. *Physical Review Letters* 112:258101
130. Caorsi V, Lemiere J, Campillo C, Bussonnier M, Manzi J, et al. 2016. *Soft Matter* 12:6223–6231
131. Guevorkian K, Manzi J, Pontani LL, Brochard-Wyart F, Sykes C. 2015. *Biophysical Journal* 109:2471–2479
132. Carvalho K, Tsai FC, Lees E, Voituriez R, Koenderink GH, Sykes C. 2013. *Proceedings of the National Academy of Sciences* 110:16456–16461
133. Carvalho K, Lemiere J, Faqir F, Manzi J, Blanchoin L, et al. 2013. *Philosophical Transactions of the Royal Society B: Biological Sciences* 368:20130005–20130005
134. Enas AS, Kinneret K. 2013. *Frontiers in Immunology* 4:01001
135. Linsmeier I, Banerjee S, Oakes PW, Jung W, Kim T, Murrell MP. 2016. *Nature Communications* 7:12615
136. Loiseau E, Schneider JAM, Keber FC, Pelzl C, Massiera G, et al. 2016. *Science Advances* 2:e1500465–e1500465
137. Lin LCL, Brown FLH. 2004. *Physical Review Letters* 93:256001
138. Brannigan G, Lin LCL, Brown FLH. 2005. *European Biophysics Journal* 35:104–124
139. Lin LCL, Brown FLH. 2006. *Journal of Chemical Theory and Computation* 2:472–483
140. Peskin CS. 2002. The immersed boundary method. In *Acta Numerica 2002*, ed. A Iserles. Cambridge University Press, 479–518
141. Eggleton CD, Popel AS. 1998. *Physics of Fluids* 10:1834–1845

142. Pozrikidis C. 2005. *Annals of Biomedical Engineering* 33:165–178
143. Hoogerbrugge PJ, Koelman JMVA. 1992. *Europhysics Letters (EPL)* 19:155–160
144. Peng Z, Asaro RJ, Zhu Q. 2011. *Journal of Fluid Mechanics* 686:299–337
145. Atzberger PJ, Kramer PR, Peskin CS. 2007. *Journal of Computational Physics* 224:1255–1292
146. Fedosov DA, Caswell B, Karniadakis GE. 2010. *Biophysical Journal* 98:2215–2225
147. Peng Z, Li X, Pivkin IV, Dao M, Karniadakis GE, Suresh S. 2013. *Proceedings of the National Academy of Sciences* 110:13356–13361