Fluctuations and instability of a biological membrane induced by interaction with macromolecules

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This paper studies the dynamics and fluctuations of a phospholipidic membrane in the presence of a diffusion field of foreign molecules, such as polymers, proteins, etc., which have the ability to adsorb on, and to desorb from, the membrane. We develop a model that includes, besides hydrodynamics, molecular diffusion in the surrounding fluid and lateral diffusion (i.e., diffusion along the membrane) as well as the kinetics of attachment and detachment to and from the membrane. This model is exploited here for the case of a free membrane which is globally at equilibrium while the nonequilibrium part will be presented in the future. We show that if the coupling between the membrane and the molecules is strong enough, the flat membrane can suffer a morphological instability. The numerical calculation in the nonlinear regime reveals budding, and an initial stage of spontaneous vesicle emission. When the condition of stability is satisfied we show how kinetic fluctuations lead to a rich variety of dynamical behaviors expressing the dominant dissipation mechanisms. We show that in the limit of well separated length scales related to the physical mechanisms that enter into play, the width w of the membrane fluctuations exhibits various dynamical scalings with universal scaling exponents. It is shown that the usual behavior with time $w \sim t^{1/3}$ is altered in various time and length scales of interest. For example, we find that $w \sim t^{1/4}$, $w \sim t^{1/2}$, and $w \sim [t \ln(t)]^{1/2}$, depending on which physical mechanism limits the membrane fluctuations on the time scale under consideration. The experimental study of the fluctuation spectrum can be viewed as a precious tool in order to have access to the underlying microscopic physical mechanisms.

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I. INTRODUCTION

Phospholipidic membranes play a reference role in various scientific communities ranging from engineering to biology. On the one hand, phospholipidic membranes constitute a basic system in many biological functions. To name but a few examples, they are vital barriers for living cells, they play the role of a reservoir for generating vesicles in the Golgi apparatus, and so on. On the other hand, they are currently the focus of many biotechnological applications, one of the most promising being their usage as drug carriers [1]. On the fundamental level, phospholipid membranes are extensively studied as a biomimetic system in order to focus on certain aspects, with the hope of identifying some generic features that continue to play an essential role in a more complex situation.

Since the seminal work by Helfrich [2], the field of membrane and vesicles has known a considerable interest both theoretically and experimentally. A major achievement is the study of equilibrium shapes of pure phospholipidic membranes [3]. The phase diagram of the equilibrium shapes as well as the fluctuations of pure membranes are fairly understood. Little is known, however, in the presence of inclusions, though some key ingredients are beginning to emerge. Inclusions play an important role in many circumstances. They are known to control the membrane elasticity [4]. In some cases inclusions may even destroy the membrane internal integrity leading to spontaneous vesicle emission [5,6]. In the biological world, inclusions are known to induce crenation of red blood cells [7], or even alter the cell functionality [8]. For example, it is known that clathrin and its adapter can bind to liposomes and cause budding in the absence of ATP or other source of energy [9]. It is thus highly desirable to study on a simple ground what are the first important effects related to inclusions, and analyze their far reaching consequences.

Leibler [10] analyzed the effect of inclusion on the membrane shape stability. He showed that the planar membrane may become unstable due to the coupling between the membrane elasticity and the inclusion. In that model the inclusion was bound to remain within the membrane. In many real situations the chemical potential of the aqueous solution is fixed and a permanent exchange between the membrane and the surrounding solution occurs on reasonable time scales. The first goal of this paper is to analyze the effect of exchange of foreign molecules between a membrane and the surrounding solution. The exchange is accounted for by introducing a consistent kinetic law expressing how mass current from the membrane toward the bulk, and vice versa, is related to the lack of local thermodynamical equilibrium. One of the most important results to emerge is that the amplitude of fluctuations of the membrane, before the entire configurations have been explored, assumes unusual laws as a function of time, which could affect the interpretation of the experimental results. The second result concerns the nonlinear behavior in the unstable regime, where budding takes place. Recently we have given a brief account of the problem of membrane fluctuations [11]. Here we present an extensive study of the fluctuations. In addition, we analyze the behavior of the membrane in the regime where it is unstable. We perform a nonlinear analysis of the membrane dynamics in the unstable regime.

For a pure membrane, fluctuations from a planar configuration are governed by the Boltzmann distribution

$$P\{h(\mathbf{r},t)\} \sim \exp\left(-\frac{\kappa}{2}\int (\nabla^2 h)^2 d\mathbf{r}/k_B T\right) \quad (1)$$

where κ is the membrane rigidity and h is the membrane profile at position $\mathbf{r}$ and time t. This distribution yields trivially the static spectrum $\langle h_{\mathbf{q}} h_{-\mathbf{q}}\rangle = k_B T/\kappa q^4$, where $q = 2n\pi/L$ and the fluctuation width $w_{eq} \equiv (\langle h^2\rangle)^{1/2}$, via the relation

$$w_{eq}^2 \sim \frac{k_B T}{\kappa} L^2, \quad (2)$$

where L is the lateral size of the membrane. The equilibrium width is reached after a certain time (to be specified below). In the kinetic sense, any deviation from the planar configuration with a lateral size $1/q$ relaxes over a time scale which is fixed by the hydrodynamic dissipation. The restoring force is the Helfrich one, $\sim \kappa q^4 h$, which counterbalances the viscous tension $\eta v/\ell$ where v is a typical velocity, given here by the membrane velocity $\partial h/\partial t \sim \omega h$ (ω is the relaxation frequency), while ℓ is the typical length scale of the perturbation $\sim 1/q$. Equating both forces yields the classical dispersion law for membrane fluctuations

$$\omega \sim (\kappa/\eta) q^3. \quad (3)$$

If the time scale of observation is large in comparison with the saturation time inferred from the above relation, $\tau_s \sim \eta L^3/\kappa$, then the membrane fluctuation width is given by (2). In the opposite limit, the width is a function of time. The temporal behavior can be exploited in order to have access to the physical dissipation mechanisms. For a pure membrane it can be shown (see later) that the dynamical width is given by

$$w^2 \sim (\kappa/\eta)^{2/3} (t)^{2/3}. \quad (4)$$

It will be shown here that the width obeys different power law behaviors when the length scales associated with the various dissipation mechanisms are disparate (otherwise mixing of length scales destroys power law behavior). Let us quote now two examples. If lateral diffusion due to a local deviation of the membrane curvature is the dominant dissipation effect then

$$w^2 \sim \kappa \Lambda^2 D_s t \quad (5)$$

at short time, and

$$w^2 \sim \kappa \Lambda^2 D_s t \ln(t) \quad (6)$$

at later time. Here D_s is the diffusion coefficient on the membrane. The analysis of the dynamical width provides precious information on the kinetic (e.g., D_s, desorption time, etc.) processes.

We shall see that if the coupling between the membrane and the inclusions is high enough the planar membrane becomes unstable. Once the instability threshold is reached nonlinear effects become important. We shall see that instability leads to a vigorous membrane deformation leading to overhangs, and thus to vesicle emission.

This paper is organized as follows. In Sec. II we introduce the energetics of the membrane, and determine the static correlation function. In Sec. III, we introduce the dynamical equations that govern the membrane shape evolution. We then study the stability of a nearly flat membrane. Section IV is devoted to the thermal fluctuations of the membrane. We study the dynamical correlation function and the thermal fluctuations of the membrane as a function of time. We discuss the results of the membrane fluctuation in Sec. V. In Sec. VI we present the nonlinear regime of the unstable membrane and our conclusions are summarized in Sec. VII.

II. ENERGETIC ASPECTS

In this section we discuss in more detail (as compared to [11]) the formulation of the free energy and the coupling to the molecule concentration. We shall also derive in this paper the static correlation function before dealing with kinetics. We consider a fluid membrane that is impermeable. The membrane is embedded in a three-dimensional viscous fluid. The membrane may be closed (vesicle) or unfolded (floating membrane) and the characteristic size of the membrane is either the radius of the vesicle R or its length L. The curvature energy of the membrane is given by the Helfrich energy [12]

$$F_1 = \int \frac{\kappa}{2}(H - H_0)^2 dA \quad (7)$$

where $\kappa \simeq 20 k_B T$ is the bending rigidity modulus, H is twice the local mean curvature (which is just the curvature for one dimensional deformations to be considered here), and H_0 is a spontaneous curvature in the case of intrinsically asymmetric monolayers.

At a large distance from the membrane (i.e., large compared with the size of the membrane modulation) a reservoir of molecules maintains the concentration at a given value ψ_{eq}. The molecules are characterized by their typical size Λ ranging from a few angstroms to a few nanometers. The molecules diffuse through the surrounding fluid as well as on the membrane. They are assumed to experience reversible adhesion onto the membrane with a lifetime τ. We shall account for the coupling between the molecules and the membrane bending by introducing a concentration-dependent local spontaneous curvature [10].

We assume that the molecules can adsorb on the membrane and then induce a local spontaneous curvature which is proportional to the surface concentration of molecules ϕ. The local spontaneous curvature arises from local asymmetry in the two halves of the bilayer since molecules adsorb only on one monolayer. Dimensionally, the coupling parameter is a length. Since the only length entering into this process is the characteristic size of the molecules we expect the coupling parameter, denoted as Λ, to be of the same order. The coupling term between curvature and surface concentration is written as

$$F_2 = -\int \kappa \Lambda \phi H \, dA. \quad (8)$$

In fact the molecules induce a positive (negative) curvature if Λ is positive (negative) so the characteristic size of the molecules is rather $|\Lambda|$ and the sign of Λ is related to the sign of

the curvature that the molecules induce. Because of inhomogeneous bending the concentration of adsorbed molecules deviates from its equilibrium value ϕ_{eq}. The resulting energetical cost is accounted for by the classical Ginsburg-Landau energy

$$F_3 = \int \left(\frac{\alpha}{2}(\phi - \phi_{eq})^2 + \frac{\beta}{2}(\nabla_\parallel \phi)^2 \right) dA \tag{9}$$

where ϕ_{eq} is the equilibrium concentration on the flat membrane and $\nabla_\parallel \phi$ is the gradient of the surface concentration on the membrane. $\nabla_\parallel$, which is understood to be evaluated on a curved geometry, will be approximated by the ordinary (x,y) gradient in the analytical analysis of fluctuations. When the membrane is unstable the full nonlinear term will be retained. We take positive values for α and β in order to exclude spontaneous aggregation of the adsorbed molecules.

The incompressibility constraint for the local area of the membrane is ensured by a local Lagrange multiplier Σ, which takes the form of an effective surface tension contribution

$$F_4 = \int \Sigma \, dA. \tag{10}$$

The total free energy of the membrane is given by the sum of the various terms introduced above, $F = F_1 + F_2 + F_3 + F_4$.

Let us consider a finite size membrane with a length L. We study the linear stability and the fluctuations of this flat membrane for which the position is given by $z = h(x,y)$. We assume that the molecules diffuse (in the upper half space) from $z = z_\infty$ where the reservoir maintains a constant concentration ψ_∞. The flat membrane and the quiescent fluid serve as a reference state. The bulk diffusion field ψ is constant in the upper fluid $\psi = \psi_{eq} = \psi_\infty$. The bulk and the membrane equilibrium concentrations are related by a relation that will be written below. It is legitimate to take the reference state to be the flat membrane only if one imposes that the spontaneous curvature $H_0 = -\Lambda \phi_{eq}$, and this is what we shall assume in the following. So we have to fix ψ_∞ to ensure that $H_0 = \Lambda \phi_{eq}$. The total energy of the membrane then reads

$$F = \int \left(\frac{\kappa}{2}[H^2 - 2\Lambda(\phi - \phi_{eq})H] + \frac{\alpha}{2}(\phi - \phi_{eq})^2 + \frac{\beta}{2}(\nabla_\parallel \phi)^2 + \Sigma \right) dA. \tag{11}$$

The quantity H is given by $H = \nabla^2 h$ and the surface element $dA = [1 + (\nabla h)^2/2] dx\, dy$. In this limit, the free energy F for the entire membrane with adsorbed molecules reduces to a second-order functional of the membrane shape $h(x,y)$ and the adsorbed molecule concentration $\phi(x,y)$,

$$F = \frac{1}{2} \int \{\kappa[(\nabla^2 h)^2 - 2\Lambda(\phi - \phi_{eq})(\nabla^2 h)] + \Sigma(\nabla h)^2 + \alpha(\phi - \phi_{eq})^2 + \beta(\nabla \phi)^2\} dx\, dy \tag{12}$$

The functional derivative of the energy with respect to the height h and the membrane concentration ϕ yields

$$\frac{\delta F}{\delta h} = -\Sigma \nabla^2 h + \kappa \nabla^4 h - \kappa \Lambda \nabla^2 \phi, \tag{13}$$

$$\frac{\delta F}{\delta \phi} = \alpha(\phi - \phi_{eq}) - \beta \nabla^2 \phi - \kappa \Lambda \nabla^2 h. \tag{14}$$

Obviously $h = 0$ and $\phi = \phi_{eq}$ is a solution of the problem $\delta F / \delta h = 0$ and $\delta F / \delta \phi = 0$.

For small deviations from the planar state, we consider a plane wave in the y direction, $h(x,y) \equiv h_q e^{iqy}$ and $\phi \equiv \phi_{eq} + \phi_q e^{iqy}$. The Fourier-transformed free energy is thus

$$F = \frac{1}{2}(h_q, \phi_q) \mathbf{E}(q) \begin{pmatrix} h_q \\ \phi_q \end{pmatrix}^*, \tag{15}$$

where we have defined

$$\mathbf{E}(q) \equiv \begin{pmatrix} \kappa q^4 + \Sigma q^2 & \kappa \Lambda q^2 \\ \kappa \Lambda q^2 & \alpha + \beta q^2 \end{pmatrix}. \tag{16}$$

We can easily check that for uncoupled variables, that is, for $\Lambda = 0$, we find the well known result for a pure elastic membrane with surface tension,

$$F_0 = \frac{1}{2} h_q (\kappa q^4 + \Sigma q^2) h_q^*. \tag{17}$$

The static correlation functions $\langle X_q X_q^* \rangle$ with $X = h$ or ϕ can be obtained from the inverse of the energy matrix $\mathbf{E}$ thanks to the equipartition of energy, which leads to

$$\mathbf{G}_0(q) \equiv \left\langle \begin{pmatrix} h_q \\ \phi_q \end{pmatrix} (h_q, \phi_q)^* \right\rangle = \frac{k_B T}{L^2} \mathbf{E}^{-1}(q) = \frac{k_B T}{L^2[(\kappa q^4 + \Sigma q^2)(\alpha + \beta q^2) - \kappa^2 \Lambda^2 q^4]} \begin{pmatrix} \alpha + \beta q^2 & -\kappa \Lambda q^2 \\ -\kappa \Lambda q^2 & \kappa q^4 + \Sigma q^2 \end{pmatrix}, \tag{18}$$

where L denotes the linear dimension of the rectangular patch of membrane under consideration.

The above inversion of the matrix $\mathbf{E}(q)$ is possible only if the determinant of this matrix is different from zero for all wave vectors. If the determinant is equal to zero for some wave vectors, the flat configuration of the membrane with homogeneous membrane concentration is no longer a minimum of the energy for all wave vectors. By increasing Λ, the

membrane becomes "thermodynamically" unstable. This occurs for a critical size Λ_c and critical wave number q_c which are given by

$$q_c = \left(\frac{\Sigma\alpha}{\beta\kappa}\right)^{1/4}, \quad \Lambda_c = \sqrt{\frac{\alpha}{\kappa}} + \sqrt{\frac{\Sigma\beta}{\kappa^2}}. \tag{19}$$

The membrane is stable only if $|\Lambda|$ is smaller than the critical value Λ_c. If we assume that the surface tension is equal to zero, we find the result of Leibler [10] $q_c=0$ and $\Lambda_c=\sqrt{\alpha/\kappa}$. The unstable regime, which requires a highly nonlinear study, is discussed in Sec. VI.

For $0 \leqslant |\Lambda| < \Lambda_c$, the membrane is stable and the static correlation function for the height displacement is given by

$$\langle |h_q|^2 \rangle = \frac{k_B T(\alpha + \beta q^2)}{L^2[(\kappa q^4 + \Sigma q^2)(\alpha + \beta q^2) - \kappa^2 \Lambda^2 q^4]}. \tag{20}$$

For $\Lambda=0$, we find the result for a pure membrane $\langle |h_q|^2 \rangle = k_B T/L^2(\kappa q^4 + \Sigma q^2)$. This result is a good approximation when $|\Lambda| \ll \Lambda_c$ but is no longer valid for $|\Lambda|$ of the order of Λ_c (but always smaller).

Until now we have kept the surface tension Σ in our equations. The order of magnitude of this surface tension for a closed membrane can be found by studying the thermal fluctuations of quasispherical vesicles or by micropipette techniques. Using thermal fluctuations, Méléard *et al.*[13] found $\Sigma = 1.3 \times 10^{-5}$ mN/m which is much smaller than the surface tension of the oil-water interface (7.5×10^{-2} N/m) but not vanishingly small. In a forthcoming work we study the dynamics of vesicles where Σ plays a decisive role. For an extended and free membrane, on which we focus our attention in this paper, the effective surface tension vanishes, so that the Lagrange multiplier Σ does not enter into play. From now on we specialize our study to the case $\Sigma=0$.

III. DYNAMICAL EQUATIONS

A. Hydrodynamics

For the evaluation of the dynamical correlation functions, we consider the hydrodynamics of the surrounding aqueous solution as well as mass diffusion. The physical properties of the membrane and its undulation energy will enter the boundary conditions and the force balance at the membrane. In comparison to what was reported in our previous brief work [11], we give here a detailed discussion of how the final equations are obtained, as well as a more involved discussion of the main results.

We assume that the membrane is embedded in a Newtonian incompressible fluid with a viscosity η. The hydrodynamic equations take the form

$$\eta \nabla^2 \mathbf{v} = \nabla p, \tag{21}$$

$$\nabla \cdot \mathbf{v} = 0, \tag{22}$$

where $\mathbf{v}$ is the velocity field of the fluid and p the hydrostatic pressure. This approximation, neglecting the inertial terms in the Navier-Stokes equation, is valid for low Reynolds numbers. Typically, a characteristic length for displacement of the membrane $L' \simeq 10$ μm, and a characteristic velocity $U \simeq 10$ μm s^{-1} and kinematic viscosity $\nu' \simeq 10^{-2}$ cm^2 s^{-1} for the surrounding solution so that $\mathrm{Re} = UL'/\nu' \simeq 10^{-4}$. The Stokes approximation is valid beyond any doubt.

We assume that the membrane experiences a fluctuation with a wave vector q. This entails that we seek solutions of the form $h(y,t) = h_q e^{iqy+\omega t}$, where ω is called the growth or attenuation rate and is *a priori* a complex number. We assume a translational invariance in the x direction, that is to say, we consider that the membrane lateral extent is large in comparison with the modulation wavelengths of interest. Moreover, to linear order all thermodynamic fields vary with the same q but with different amplitudes which are self-consistently determined as we shall see.

The velocity and the pressure fields take the forms $\mathbf{v} = [v_{y,q}(z)\mathbf{e}_y + v_{z,q}(z)\mathbf{e}_z] e^{iqy+\omega t}$ and $p = p_q(z) e^{iqy+\omega t}$. The general solution for the hydrodynamic equations (21) and (22) satisfying the condition of vanishing velocity at infinity is given by

$$\begin{aligned} v^+_{z,q}(z) &= (A + Bqz) e^{-qz}, \\ p^+_q(z) &= 2\eta q B e^{-qz}, \\ v^-_{z,q}(z) &= (C + Dqz) e^{+qz}, \\ p^-_q(z) &= 2\eta q D e^{+qz}, \end{aligned} \tag{23}$$

where the superscripts $\pm$ apply to $z>0$ and $z<0$, respectively. The incompressibility condition (22) yields $v^{\pm}_{y,q} = i\partial_z v^{\pm}_{z,q}(z)/q$. The constants A, B, C, and D are to be determined from boundary conditions at the membrane, which may, for small displacements, be evaluated at $z=0$. Continuity of the normal velocity v_z leads to $A=C$, while the same condition on the tangential part provides us with the relation $B-A=C+D$. The assumption of impermeability of the membrane to the flow imposes the condition that $\partial h/\partial t = v_z(0)$ that sets $A=C=\omega h_q$. Furthermore, the forces have to balance in the tangential and normal directions at the membrane, leading to

$$-T^+_{zy} + T^-_{zy} = 0, \tag{24}$$

$$-T^+_{zz} + T^-_{zz} = -\frac{\delta F}{\delta h}, \tag{25}$$

where we have introduced the liquid stress tensor $T^{\pm}_{ij} \equiv -p\delta_{ij} + \eta(\partial_i v_j + \partial_j v_i)$, evaluated at the membrane. The tangential balance provides us with a relation between the four constants: $A-B=C+D$. The two previous relations between A, B, C, and D fix $B=A$ and $D=-C$. Using Eq. (13), the normal stress balance provides a relation between h_q and ϕ_q,

$$\omega h_q = -\frac{\kappa q^3}{4\eta} h_q - \frac{\kappa \Lambda q}{4\eta} \phi_q. \tag{26}$$

In order to close the set of equations, one needs to specify the diffusion field.

B. Bulk and membrane diffusion of molecules

We concentrate now on the diffusion problem. We assume that the molecules can diffuse freely in the bulk surrounding the vesicle with the bulk diffusion coefficient D. We have to solve the classical diffusion equation with a concentration ψ_∞ at $z=z_\infty$, in the presence of advection,

$$\frac{\partial \psi}{\partial t} + \mathbf{v} \cdot \boldsymbol{\nabla} \psi = D \Delta \psi \tag{27}$$

where ψ is the concentration in the bulk.

The adsorbed molecules have the ability to diffuse on the membrane and possibly desorb into the surrounding fluid. When the concentration of the adsorbed molecules is different from ϕ_{eq}, the chemical potential of these molecules is different from the equilibrium one, μ_{eq}. The variation of the chemical potential of the adsorbed molecules is related to the free energy by

$$\Delta \mu = \frac{\delta F}{\delta \phi} \tag{28}$$

where $\delta F / \delta \phi$ is given by Eq. (14). The chemical potential difference induces a lateral current of molecules which is given by

$$\mathbf{j} = - \frac{D_s}{\alpha} \boldsymbol{\nabla}_\parallel (\Delta \mu) \tag{29}$$

where D_s designates the surface diffusion coefficient of the adsorbed molecules.

The equation for the time evolution of the membrane concentration takes the form

$$\frac{\partial \phi}{\partial t} + \mathbf{u} \cdot \boldsymbol{\nabla}_\parallel \phi = - \boldsymbol{\nabla}_\parallel \cdot \mathbf{j} + J_n \tag{30}$$

where $\mathbf{u}$ is the tangential component of the velocity of a fluid element moving with the membrane. J_n is the net mass current of the molecules at the membrane. This coupling term between membrane and bulk molecules is classically written as:

$$J_n = \mathbf{n} \cdot D \, \boldsymbol{\nabla} \, \psi \tag{31}$$

where $\mathbf{n}$ is a unit vector which is directed from the membrane onto the fluid. This source term $D \boldsymbol{\nabla} \psi \cdot \mathbf{n}$ is the total number of molecules per unit surface and time, representing the exchange between the bulk and the membrane.

In order to close the system of equations we need to specify the constitutive equation that relates the bulk and the membrane concentrations. At equilibrium the net current across the membrane is obviously zero, $J_n=0$. A lack of local equilibrium induces mass current. The question is how to relate that mass current to a deviation from equilibrium. The mass current is simply proportional to departure from equilibrium between the bulk and the membrane. For a flat membrane the current is written as

$$J_n \sim \frac{D}{\xi} \psi - \frac{\phi}{\tau}. \tag{32}$$

ξ is the characteristic "diffusion" length of the molecules in the bulk and τ is the residence time of the molecules on the membrane. The quantity $\sqrt{D_S \tau}$ is the diffusion length of the molecules on the membrane before they desorb. This quantity represents the typical distance of residence of a molecule in the membrane before it gets a chance to regain the solution. At equilibrium the mass current vanishes, entailing that the bulk and membrane concentrations are related by

$$\phi_{eq} = \frac{\tau D}{\xi} \psi_{eq}. \tag{33}$$

In reality the chemical potential of the membrane is affected not only by the concentration field, but also by the membrane configuration as dictated by the membrane free energy, leading to a chemical potential given by Eq. (28). Thus the full mass current must, for a nonplanar membrane, have the following form to ensure the proper thermal equilibrium:

$$J_n = \frac{D}{\xi} (\psi - \psi_{eq}) - \frac{1}{\tau \alpha} \frac{\delta F}{\delta \phi} = \frac{D}{\xi} \psi - \frac{1}{\alpha \tau} (\alpha \phi - \beta \nabla^2 \phi - \kappa \Lambda \nabla^2 h). \tag{34}$$

It can be shown from kinetic theory that at long time we recover Eq. (20), obtained directly from thermodynamical considerations.

The kinetic equation expresses the permanent exchange between molecules on the membrane and in the bulk. We have now a closed system of differential equations which determines the velocity $\mathbf{v}$ (as we have already seen) and the concentrations in the bulk and on the membrane (ψ, ϕ).

For the sake of simplicity, we confine ourselves to the situation where the concentration reaches its equilibrium value at a distance that is large in comparison to all fluctuation wavelengths of interest. That is, we assume that $q z_\infty \gg 1$. In this limit, the solution of the bulk diffusion equation (27) with the boundary condition $\psi(z_\infty) = \psi_{eq}$ reads

$$\psi = \psi_{eq} + \psi_q e^{iqy + \omega t} e^{-Qz} \tag{35}$$

where $Q = \sqrt{q^2 + \omega / D}$; we must take the solution with a positive real part to satisfy boundary conditions. The identification of the two expressions (31) and (34) for the current J_n fixes ψ_q as a function of h_q and ϕ_q:

$$\frac{D}{\xi} \psi_q = \frac{1}{1 + Q\xi} \left[\left(1 + \frac{\beta}{\alpha} q^2 \right) \frac{\phi_q}{\tau} + \frac{\kappa \Lambda q^2}{\alpha \tau} h_q \right]. \tag{36}$$

Inserting this equality (36) into the surface diffusion equation (30), we obtain

$$\omega \phi_q = - \left(D_s q^2 + \frac{Q\xi}{\tau (1 + Q\xi)} \right) \left[\left(1 + \frac{\beta}{\alpha} q^2 \right) \phi_q + \frac{\kappa \Lambda q^2}{\alpha} h_q \right]. \tag{37}$$

C. The dispersion relation

By using Eqs. (26) and (37), we obtain the dispersion relation in an implicit form:

$$\omega^2 + \left[\frac{\kappa q^3}{4\eta} + \left(D_s q^2 + \frac{Q(\omega,q)\xi}{\tau(1+Q(\omega,q)\xi)} \right)\left(1 + \frac{\beta}{\alpha} q^2 \right) \right] \omega + \frac{\kappa q^3}{4\eta} \left(D_s q^2 + \frac{Q(\omega,q)\xi}{\tau(1+Q(\omega,q)\xi)} \right)\left(1 + \frac{\beta}{\alpha} q^2 - \frac{\kappa \Lambda^2}{\alpha} \right) = 0. \tag{38}$$

This equation does not have simple analytical solutions, but it can easily be analyzed for extreme values of the wave number. At small q Eq. (38) admits two solutions $\omega \sim q^2$ and $\omega \sim q^3$. The first one is precisely $\omega \simeq -Dq^2$ which is the classical diffusion mode. The membrane is stable and the relaxation of the membrane is governed by bulk diffusion. The second solution is

$$\omega \simeq -\frac{\kappa q^3}{4\eta}\left(1 - \frac{\kappa \Lambda^2}{\alpha} \right). \tag{39}$$

For $\Lambda = 0$, we recognize the "classical" bending mode of a free membrane damped by bulk viscosity [14]. The membrane is then stable and its relaxation is due to the dissipation in the fluid. It is seen that this mode becomes unstable for $\Lambda > \Lambda_c \equiv \sqrt{\alpha/\kappa}$. The physical mechanism for this instability is the following. A fluctuation which tends to curve the membrane will result in an accumulation of the molecules on the crests of the waves (if Λ is positive). This accumulation will further result in a force that tends to increase the amplitude of the perturbation due to the coupling between the surface concentration and curvature. Acting against this effect is the Landau-Ginzburg restoring term F_3 [Eq. (9)] which favors a uniform concentration and the bending elasticity F_1 [Eq. (7)] which tends to reduce the curvature.

In the limit of large q, the dispersion relation yields two relevant branches which are the continuations of the two above-mentioned branches for small wave vectors. These two branches possess an imaginary part. Their real parts read

$$\omega_r \simeq -D_s \frac{\beta}{\alpha} q^4, \tag{40}$$

$$\omega_r \simeq -\frac{\kappa q^3}{4\eta}. \tag{41}$$

The latter relation is, as we have already mentioned, the dispersion relation of an elastic membrane in a viscous fluid. The former relation expresses the relaxation due to the lateral gradient of concentration of the adsorbed molecules. The real part of ω is negative signaling a stability (it is an oscillatory damping since $\omega_i \neq 0$). For the second solution (41), the imaginary part tends to zero (and is always much smaller than the real part) when $q \to \infty$, while the imaginary part of the first solution (40) tends to a constant value given by

$$\omega_i \simeq -\frac{1}{\xi\tau}\left(\frac{\beta D}{\alpha D_s} \right)^{1/2}. \tag{42}$$

This oscillation expresses the competing effect between membrane diffusion, bulk diffusion, and desorption. For example, if a concentration inhomogeneity takes place on the membrane (just by transporting molecules along the membrane), then there are two channels for relaxation: (i) desorption and (ii) membrane diffusion. If desorption takes place on a faster time scale then, ahead of the protruberance, the bulk concentration increases, causing thereby a diffusion short circuit through the bulk and a membrane enrichment elsewhere. This induces a slowing down of the protruberance. If all the effects operate on comparable time scales, the relaxation of the protruberance may operate in an oscillating fashion.

IV. THERMAL FLUCTUATIONS

It has been shown that a planar membrane may become unstable if the coupling constant Λ exceeds a certain value Λ_c. In that case nonlinear effects become decisive, and must be included if one wishes to study the subsequent development of the instability and/or to ascertain the long time behavior. This will be addressed in Sec. VII. In the opposite limit, that is, when the membrane is stable, still lateral diffusion and desorption may significantly affect the fluctuation spectrum. Analysis of these fluctuations provides a tool for identification of microscopic processes that are responsible for membrane kinetics in a given range of length and time scales. The goal of this section is to analyze these fluctuations.

The full fluctuation spectrum can be analyzed in principle, but we shall confine ourselves to a limit where a quasistatic approximation of the diffusion field in the bulk can be made. This approximation corresponds to the situation where the characteristic diffusion time in the fluid is much smaller than the characteristic time of the membrane relaxation. In that case bulk diffusion is legitimately approximated by its quasistatic form $\Delta\psi = 0$. Formally this amounts to setting $Q = q$ in the dispersion relation. This simplification provides an easy access to some of the important physics of the system such as the dynamics of the thermal fluctuations of the membrane. Since we consider in this section the situation where the flat membrane is deterministically stable, we must keep in mind that $|\Lambda| < \Lambda_c$.

In principle we could directly use the dispersion relation, but since it is of second order in ω (this is due to the fact that the concentration field has been integrated out), we find it more convenient to leave explicitly the two degrees of freedom h_q and ϕ_q. That is to say, we consider the two coupled equations (26) and (37) with $Q(\omega,q) = q$. We then write this system as

$$\omega \mathbf{X}_q = \mathbf{A}_q \mathbf{X}_q \tag{43}$$

with

$$\mathbf{A}_q \equiv -\begin{pmatrix} \frac{\kappa q^3}{4\eta} & \frac{\kappa\Lambda q}{4\eta} \\ \left(D_s q^2 + \frac{q\xi}{\tau(1+q\xi)}\right)\left(\frac{\kappa\Lambda q^2}{\alpha}\right) & \left(D_s q^2 + \frac{q\xi}{\tau(1+q\xi)}\right)\left(1+\frac{\beta}{\alpha}q^2\right) \end{pmatrix} \tag{44}$$

and

$$\mathbf{X}_q \equiv \begin{pmatrix} h_q \\ \phi_q \end{pmatrix}. \tag{45}$$

The eigenvalues of $\mathbf{A}_q$ are given by

$$\omega_1 = -\frac{1}{2\tau}\left\{\frac{q^3}{q_1^3} + \left(\frac{q^2}{q_2^2} + \frac{q}{q+q_0}\right)\left(1+\frac{q^2}{q_3^2}\right) - \left[\left(\frac{q^2}{q_2^2} + \frac{q}{q+q_0}\right) \times \left(1+\frac{q^2}{q_3^2}\right) - \frac{q^3}{q_1^3}\right]^2 + \frac{4\kappa\Lambda^2}{\alpha}\frac{q^3}{q_1^3}\left(\frac{q^2}{q_2^2} + \frac{q}{q+q_0}\right)\right\}, \tag{46}$$

$$\omega_2 = -\frac{1}{2\tau}\left\{\frac{q^3}{q_1^3} + \left(\frac{q^2}{q_2^2} + \frac{q}{q+q_0}\right)\left(1+\frac{q^2}{q_3^2}\right) + \left[\left(\frac{q^2}{q_2^2} + \frac{q}{q+q_0}\right) \times \left(1+\frac{q^2}{q_3^2}\right) - \frac{q^3}{q_1^3}\right]^2 + \frac{4\kappa\Lambda^2}{\alpha}\frac{q^3}{q_1^3}\left(\frac{q^2}{q_2^2} + \frac{q}{q+q_0}\right)\right\}, \tag{47}$$

where we have introduced several crossover wave vectors

$$q_0 \equiv \frac{1}{\xi}, \quad q_1 \equiv \left(\frac{4\eta}{\kappa\tau}\right)^{1/3}, \quad q_2 \equiv \frac{1}{\sqrt{D_s\tau}}, \quad q_3 \equiv \sqrt{\frac{\alpha}{\beta}}. \tag{48}$$

The constant $\kappa\Lambda^2/\alpha$ that appears in the dispersion relation is dimensionless and is always smaller than unity, due to the requirement of the flat membrane stability. Due to the number of length scales involved, there are many different scenarios for the relaxation rate.

The slowest mechanism is the limiting factor for dissipation, and thus is responsible for the fluctuation amplitude of the membrane. For example, in the absence of hydrodynamics, the relaxation frequency due to bulk diffusion is given by $\omega=-Dq^2$, while that due to membrane diffusion is given by $\omega=-D_s(\beta/\alpha)q^4$ (where the ratio β/α has the dimension of a length squared). If q is large it is clear that the slowest mode is bulk diffusion. If on the contrary q is small, then membrane diffusion is the slowest mode (its ω is small—since it behaves like q^4—and thus the typical time is large). The crossover length is simply $\sqrt{\beta/\alpha}$ (below this length bulk diffusion dominates, whereas above it membrane diffusion takes over). The crossover length fixes (from the dispersion relation) a crossover frequency (or time) that separates between two regimes: for large time bulk diffusion dominates, while for short time membrane diffusion wins (membrane diffusion is inefficient at large scales, and thus at large time). Below we will make a systematic analysis.

Instead of presenting an exhaustive discussion of all possible cases, we will focus on one possible generic scenario. We consider that the crossover wave vectors satisfy the inequalities $q_0 \ll q_1 \ll q_2 \ll q_3$. This assumption will be commented on in Sec. V. Under this condition the two branches take the following forms:

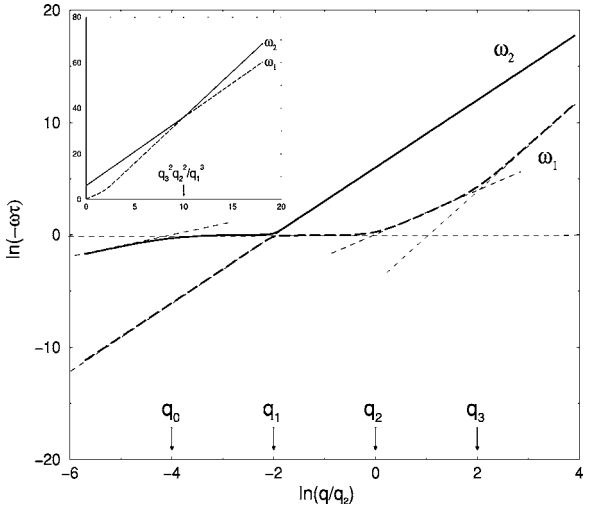

FIG. 1. Dispersion relations $\omega\tau$ as a function of q/q_2 for $\kappa\Lambda^2/\alpha=0.1$. We have assume that $q_{i+1}/q_i=10^2$. The dashed lines indicate the asymptotic behaviors. We then check the different power laws: $\omega\sim -q^n$ with $n=0$, 1, 2, 3, and 4.

$$\omega_1 \simeq -\begin{cases} \frac{\kappa}{4\eta}\left(1-\frac{\kappa\Lambda^2}{\alpha}\right)q^3, & q \ll q_1, \\ \frac{1}{\tau}, & q_1 \ll q \ll q_2, \\ D_s q^2, & q_2 \ll q \ll q_3, \\ D_s\frac{\beta}{\alpha}q^4, & q_3 \ll q \ll q_3^2 q_2^2/q_1^3, \\ \frac{\kappa}{4\eta}q^3, & q_3^2 q_2^2/q_1^3 \ll q, \end{cases}$$

$$\omega_2 \simeq -\begin{cases} \frac{\xi}{\tau}q, & q \ll q_0, \\ \frac{1}{\tau}, & q_0 \ll q \ll q_1, \\ \frac{\kappa}{4\eta}q^3, & q_1 \ll q \ll q_3^2 q_2^2/q_1^3, \\ D_s\frac{\beta}{\alpha}q^4, & q_3^2 q_2^2/q_1^3 \ll q. \end{cases} \tag{49}$$

For several wave-vector domains, we obtain the classical mode of a membrane damped by viscosity: $\omega\simeq-\kappa q^3/4\eta$. For $q \ll q_1$, the expression of the damping rate of that mode contains a factor $(1-\kappa\Lambda^2/\alpha)$ which stems from the coupling between curvature and diffusing molecules. We have seen that this factor expresses an instability if $(|\Lambda|>\Lambda_c)$. The branch $\omega\simeq-1/\tau$ represents a pure desorption mode of the molecules. Another mode is $\omega\simeq-q\xi/\tau$ and corresponds to the exchange between bulk molecules and molecules adsorbed on the membrane. Finally two modes of diffusion appear. These two modes are $\omega\simeq-D_s q^2$ and $\omega\simeq-D_s\beta q^4/\alpha$. The former corresponds to the usual lateral diffusion dispersion relation while the latter refers to diffusion for large wave vectors.

We have computed numerically the full dispersion relation and checked the asymptotic behavior of the two branches. For example, if we assume that $q_{i+1}/q_i=10^2$ for $i=0$, 1, and 2, we obtain the modes shown in Fig. 1 where we have drawn $\omega\tau$ as a function of q/q_2.

Experimentally, a quantity of practical interest is the height correlation function of the membrane, and this is why we would like to express the above results in these terms.

A. Height correlation function

The static correlation functions give only informations about equilibrium values. To obtain values such as the adsorption time or the diffusion coefficients, we have to study the dynamical correlation functions. The rigidity constant κ can of course be found by using the static correlation function. However, even for this equilibrium quantity, dynamical correlation functions are often used [13] with the aim of having more accurate values.

As shown in Appendix A, the dynamical correlation function for the height displacement can be expressed in the form

$$\langle h_q(t)h_q^*(0)\rangle = \frac{k_{\mathrm{B}}T}{L^2\kappa q^4(\omega_1\tau-\omega_2\tau)(1-\kappa\Lambda^2/\alpha+q^2/q_3^2)} \times\left\{\left[\left(1+\frac{q^2}{q_3^2}\right)\left(\omega_1\tau+\frac{q^3}{q_1^3}\right)-\frac{\kappa\Lambda^2}{\alpha}\frac{q^3}{q_1^3}\right]e^{\omega_2 t} -\left[\left(1+\frac{q^2}{q_3^2}\right)\left(\omega_2\tau+\frac{q^3}{q_1^3}\right)-\frac{\kappa\Lambda^2}{\alpha}\frac{q^3}{q_1^3}\right]e^{\omega_1 t}\right\}. \tag{50}$$

The amplitudes A_1^h and A_2^h of the contributions of the two modes whose inverse relaxation times are ω_1 and ω_2 are given by

$$A_1^h = -\frac{k_BT}{L^2\kappa q^4(\omega_1\tau-\omega_2\tau)(1-\kappa\Lambda^2/\alpha+q^2/q_3^2)} \times\left[\left(1+\frac{q^2}{q_3^2}\right)\left(\omega_2\tau+\frac{q^3}{q_1^3}\right)-\frac{\kappa\Lambda^2}{\alpha}\frac{q^3}{q_1^3}\right], \tag{51}$$

$$A_2^h = \frac{k_BT}{L^2\kappa q^4(\omega_1\tau-\omega_2\tau)(1-\kappa\Lambda^2/\alpha+q^2/q_3^2)} \times\left[\left(1+\frac{q^2}{q_3^2}\right)\left(\omega_1\tau+\frac{q^3}{q_1^3}\right)-\frac{\kappa\Lambda^2}{\alpha}\frac{q^3}{q_1^3}\right]. \tag{52}$$

For the different domains of wave vectors introduced in the preceding section, these amplitudes assume simpler forms:

$$A_1^h \simeq \frac{k_BT}{L^2}\begin{cases}\frac{1}{\kappa q^4(1-\kappa\Lambda^2/\alpha)}, & q \ll q_1,\\ \frac{1}{\kappa q^4(1-\kappa\Lambda^2/\alpha)}\frac{\kappa\Lambda^2}{\alpha}, & q_1 \ll q \ll q_3,\\ \frac{\alpha}{\kappa\beta q^6}\frac{\kappa\Lambda^2}{\alpha}, & q_3 \ll q \ll q_2^2q_2^2/q_1^3,\\ \frac{\kappa\alpha^3}{16\eta^2\beta^3D_s^2q^8}\frac{\kappa\Lambda^2}{\alpha}, & q_3^2q_2^2/q_1^3 \ll q,\end{cases} \tag{53}$$

$$A_2^h \simeq \frac{k_BT}{L^2}\begin{cases}\frac{\kappa\tau^2}{16\eta^2\xi^2(1-\kappa\Lambda^2/\alpha)}\frac{\kappa\Lambda^2}{\alpha}, & q \ll q_0,\\ \frac{\kappa\tau^2q^2}{16\eta^2(1-\kappa\Lambda^2/\alpha)}\frac{\kappa\Lambda^2}{\alpha}, & q_0 \ll q \ll q_1,\\ \frac{1}{\kappa q^4(1-\kappa\Lambda^2/\alpha)}, & q_1 \ll q \ll q_3,\\ \frac{1}{\kappa q^4}, & q_3 \ll q.\end{cases} \tag{54}$$

The classical amplitude of the height of a pure membrane is $k_BT/L^2\kappa q^4$ (see A_2^h for $q \gg q_3$). New expressions with unusual exponents such as 0 and 2 for small wave vectors appear, indicating thus another mechanism of relaxation. These new regimes are governed by the exchange of molecules between the bulk and the membrane, and this is relevant in the regime where $q \ll q_0$. In the interval $q_0 \ll q \ll q_1$ molecule desorption is the mechanism that fixes the amplitude. The asymptotic behaviors have been checked numerically by plotting $A^hL^2q_2^4\kappa/k_BT$ as a function of q/q_2. We always assume that $q_{i+1}/q_i=10^2$ for $i=0$, 1, and 2. We can see on Fig. 2 that A_1^h is larger than A_2^h for wave vectors smaller than q_1. For wave vectors larger than q_3, A_2^h is much larger than A_1^h. This means that the damping rate that governs relaxation is ω_1 for $q<q_1$ and ω_2 for $q>q_3$. The two amplitudes have the same order of magnitude for wave vectors between q_1 and q_3 but A_2^h is always larger than A_1^h. Relaxation will then be dominated by the slowest mode whose rate is ω_2 but for q near q_1, the two damping rates have nearly the same order of magnitude so that the height correlation function should exhibit a biexponential behavior.

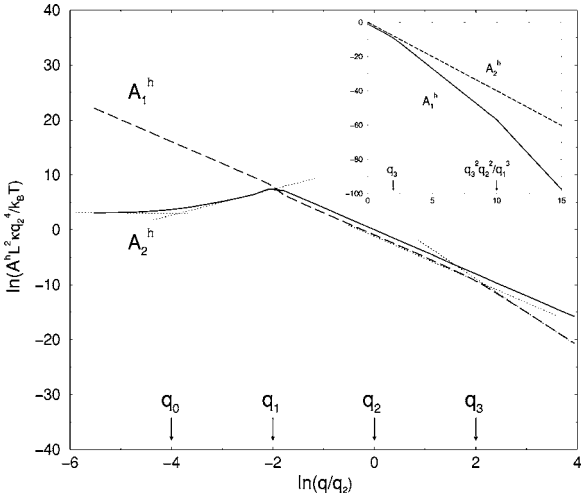

FIG. 2. Amplitudes of the height dynamical correlation $A^h\kappa L^2q_2^4/k_{\mathrm{B}}T$ as a function of q/q_2 for a membrane with adsorbed molecules for $\kappa\Lambda^2/\alpha=0.1$. We have assume that $q_{i+1}/q_i=10^2$. The dashed lines indicate the asymptotic behavior. We then check the different power laws : $A^h \sim q^p$ with $p=-6$, -4, 0, and 2.

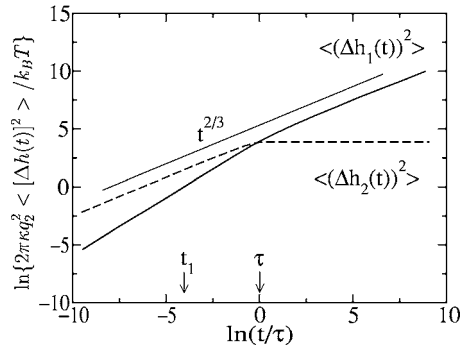

FIG. 3. Amplitudes of the dynamical correlation functions for a membrane with adsorbed molecules in units of q_2 and $k_{\mathrm{B}}T/\kappa q_2^2$ for $\kappa\Lambda^2/\alpha=0.1$. We have assume that $q_{i+1}/q_i=10^2$ for $i=0$, 1, and 2. The dashed lines indicate the asymptotic behavior. We then check different power laws : $t^{2/3}$ and $t\ln t$.

B. Kinetics of fluctuations

From the height correlation function we can extract the mean-square displacement (MSD) of the membrane as a function of time.

The MSD is expressed as (Appendix B)

$$\langle[\Delta h(t)]^2\rangle \equiv \langle[h(t)-h(0)]^2\rangle = \frac{2L^2}{4\pi^2}\int_{2\pi/L}^{2\pi/a}[\langle h_q(0)h_q^*(0)\rangle - \langle h_q(t)h_q^*(0)\rangle]2\pi q\, dq \tag{55}$$

where a is a microscopic length, typically the length of the phospholipid, which defines a macroscopic cutoff for the wave vectors. We set

$$\langle[\Delta h_1(t)]^2\rangle \equiv -\int_{2\pi/L}^{2\pi/a}\frac{k_BT\, dq}{\pi\kappa q^3(\omega_1\tau-\omega_2\tau)(1-\kappa\Lambda^2/\alpha+q^2/q_3^2)} \times\left[\left(1+\frac{q^2}{q_3^2}\right)\left(\omega_2\tau+\frac{q^3}{q_1^3}\right)-\frac{\kappa\Lambda^2}{\alpha}\frac{q^3}{q_1^3}\right](1-e^{\omega_1 t}), \tag{56}$$

$$\langle[\Delta h_2(t)]^2\rangle \equiv \int_{2\pi/L}^{2\pi/a}\frac{k_BT\, dq}{\pi\kappa q^3(\omega_1\tau-\omega_2\tau)(1-\kappa\Lambda^2/\alpha+q^2/q_3^2)} \times\left[\left(1+\frac{q^2}{q_3^2}\right)\left(\omega_1\tau+\frac{q^3}{q_1^3}\right)-\frac{\kappa\Lambda^2}{\alpha}\frac{q^3}{q_1^3}\right](1-e^{\omega_2 t}) \tag{57}$$

with

$$\langle[\Delta h(t)]^2\rangle = \langle[\Delta h_1(t)]^2\rangle + \langle[\Delta h_2(t)]^2\rangle, \tag{58}$$

and we introduce two characteristic times

$$t_0 \equiv \left(\frac{4\eta}{\kappa}\right)^4\left(\frac{\beta D_s}{\alpha}\right)^3 = \frac{q_1^{12}}{q_2^6 q_3^6}\tau, \quad t_1 \equiv \frac{\beta}{\alpha D_s} = \frac{q_2^2}{q_3^2}\tau. \tag{59}$$

We then obtain the following analytical expressions for the different temporal domains:

$$\langle[\Delta h_1(t)]^2\rangle \simeq \frac{k_BT}{2\pi\kappa}\begin{cases}\frac{1}{3}\frac{\kappa\Lambda^2}{\alpha}D_s t, & t\ll t_0,\\ -\frac{1}{4}\frac{\kappa\Lambda^2}{\alpha}D_s t\ln\left(\frac{D_s\alpha}{\beta}t\right), & t_0\ll t\ll t_1,\\ -\frac{1}{2}\frac{\kappa\Lambda^2}{\alpha}\frac{D_s t}{1-\kappa\Lambda^2/\alpha}\ln\left(\frac{t}{\tau}\right), & t_1\ll t\ll\tau,\\ -\frac{2}{3}\Gamma\left(-\frac{2}{3}\right)\frac{1}{1-\kappa\Lambda^2/\alpha}\left[\frac{\kappa}{4\eta}\left(1-\frac{\kappa\Lambda^2}{\alpha}\right)\right]^{2/3}t^{2/3}, & \tau\ll t,\end{cases} \tag{60}$$

where $\Gamma(x)$ is the Gamma function, and

$$\langle[\Delta h_2(t)]^2\rangle \simeq \frac{k_BT}{2\pi\kappa}\begin{cases}-\frac{1}{2}\Gamma\left(-\frac{1}{2}\right)\left(\frac{D_s\beta}{\alpha}\right)^{1/2}t^{1/2}, & t\ll t_0,\\ -\frac{2}{3}\Gamma\left(-\frac{2}{3}\right)\frac{1}{1-\kappa\Lambda^2/\alpha}\left(\frac{\kappa}{4\eta}\right)^{2/3}t^{2/3}, & t_0\ll t\ll\tau,\\ \left(\frac{\kappa\tau}{4\eta}\right)^{2/3}, & \tau\ll t.\end{cases} \tag{61}$$

The Gamma function satisfies the relation $\Gamma(x+1)=x\Gamma(x)$ and we have the tabulated values $\Gamma(1/2)=\pi^{1/2}$ and $\Gamma(1/3)\simeq 2.678\,94$. We have checked numerically the asymptotic expressions with $q_{i+1}/q_i=10^2$.

For large enough time (i.e., $t\gg\tau$), we obtain the classical exponent 2/3 found in the case of a pure membrane [14] where $\langle[\Delta h_1(t)]^2\rangle$ is the leading amplitude, whereas $\langle[\Delta h_2(t)]^2\rangle$ exhibits a plateau with time. The exponent 2/3 is due to the elasticity of the membrane and the viscosity of the fluid. Note that for small time (i.e., $t\ll t_0$), we obtain either 1 or 1/2. The exponent 1 is related to a pure diffusive phenomenon of adsorbed molecules. It seems that for most available data the time t_0 is so small (molecular time scales) that pure lateral diffusion is ineffective in fixing the fluctuation width. We obtain also two temporal intervals for which the amplitude of the roughness is proportional to $t\ln t$ which is related to membrane-bulk exchange. This kind of behavior occurs also for membranes with active pumps [15]. We observe on Fig. 3 a crossover between Δh_1 and Δh_2 at time τ. For the chosen parameters the behavior with the exponent 2/3 seems to prevail, but in the range where t is close to τ a regime with an amplitude behavior as $t\ln t$ may take over, or at least it will be difficult to disregard that contribution in comparison to the traditional one, since both effects are of comparable orders of magnitudes.

In fact there is an upper temporal limit which is given by the microscopic cutoff wave vector $2\pi/L$. For time larger than $\tau_s=4\eta L^3/\kappa(2\pi)^3$, $\langle[\Delta h(t)]^2\rangle$ tends to a limit which is a function of the width of the membrane L. This limit takes the form

$$\langle(\Delta h)^2\rangle = \frac{2L^2}{4\pi^2}\int_{2\pi/L}^{2\pi/a} 2\pi q\, dq\langle|h_q|^2\rangle$$
$$= \frac{k_BT}{\pi\kappa}\int_{2\pi/L}^{2\pi/a}\frac{\alpha+\beta q^2}{\alpha-\kappa\Lambda^2+\beta q^2}\frac{dq}{q^3}$$
$$= \frac{k_BT}{\pi\kappa}\int_{2\pi/L}^{2\pi/a} dq\left[\frac{\alpha}{\alpha-\kappa\Lambda^2}\frac{1}{q^3} + \frac{\beta\kappa\Lambda^2}{2(\alpha-\kappa\Lambda^2)^2}\left(\frac{2\beta q}{\alpha-\kappa\Lambda^2+\beta q^2}-\frac{2}{q}\right)\right]$$
$$= \frac{k_BT}{2\pi\kappa}\left[\frac{\alpha(L^2-a^2)}{4\pi^2(\alpha-\kappa\Lambda^2)} + \frac{\beta\kappa\Lambda^2}{(\alpha-\kappa\Lambda^2)^2}\ln\left(\frac{4\pi^2\beta+(\alpha-\kappa\Lambda^2)a^2}{4\pi^2\beta+(\alpha-\kappa\Lambda^2)L^2}\right)\right]. \quad (62)$$

Note, however, that if the membrane is entirely free to move in the fluid, the absolute transversal MSD, in contrast to the one measured relative to the base surface $z=0$, will not saturate. There will indeed be a crossover at $t\sim\tau_s$ to a linear time dependence that is controlled by the diffusion of the center of mass.

As a^2 is small as compared to L^2, the second term which involves the ln contribution is much smaller than the first one. We then obtain the equilibrium fluctuation width of a pure membrane with an effective rigidity constant given by $\kappa_{eff}=\kappa(1-\kappa\Lambda^2/\alpha)$. The constant α (see below) is given by $\alpha=k_BT/\phi_{eq}$. We then have

$$\kappa_{eff}(\phi_{eq}) = \kappa\left(1-\frac{\kappa\Lambda^2}{k_BT}\phi_{eq}\right) = \kappa\left(1-\frac{\kappa\Lambda^2 D\tau}{k_BT\xi}\psi_{eq}\right). \quad (63)$$

This means that the effective rigidity is always smaller than the rigidity of the pure membrane and does not depend on the sign of the curvature that molecules induce on the membrane.

V. DISCUSSION OF THE FLUCTUATION PROBLEM

In this section, we would like to specify the orders of magnitudes of different parameters entering the model in order to decide which effect would be important in a given realistic situation. These questions were not addressed in our previous work [11].

We take for the density of adsorbed molecules 1 over 10^4 lipid molecules, which implies a density of the molecules on the surface $\phi_{eq}\simeq 10^{10}$ cm^{-2}. Experimental estimation of the concentration of bacteriorhodopsin on membranes leads to a value $\phi_{eq}\sim 10^{12}$ cm^{-2}, or equivalently one protein for nearly 10^3 lipid molecules [16,17]. The term $\alpha(\phi-\phi_{eq})^2/2$ in the energy expression (12) is in fact related to the entropy of mixing $k_BT[\phi\ln(\phi\lambda^2)-\phi]$ where λ is the de Broglie thermal wave length $\lambda\equiv(h/k_BTm)^{1/2}$ with m the mass of an adsorbed molecule, and h is the Planck constant. This entropic term gives normally the chemical potential $\mu=k_BT\ln(\phi\lambda^2)$. We then have at equilibrium $\mu_{eq}=k_BT\ln(\phi_{eq}\lambda^2)$ and by assuming a small perturbation $\phi=\phi_{eq}+\delta\phi$ we have

$$\mu = k_BT\ln\left[\phi_{eq}\left(1+\frac{\delta\phi}{\phi_{eq}}\right)\right] = \mu_{eq}+\frac{k_BT}{\phi_{eq}}\delta\phi$$
$$= \mu_{eq}+\frac{k_BT}{\phi_{eq}}(\phi-\phi_{eq}). \quad (64)$$

We thus obtain

$$\Delta\mu = \mu-\mu_{eq} = \frac{k_BT}{\phi_{eq}}(\phi-\phi_{eq}). \quad (65)$$

This is exactly what we have written with $\Delta\mu=\delta F/\delta\phi$ where $\delta F/\delta\phi=\alpha(\phi-\phi_{eq})$ by keeping only the first term in Eq. (14). Comparing the two expressions, we obtain $\alpha=k_BT/\phi_{eq}$. The parameter α depends on the mean concentration of adsorbed molecules [10]. The thermal energy is $k_BT\simeq 1/40$ eV$\simeq 10^{-1}$ eV, so that $\alpha\simeq 10^{-23}$ g cm^4 s^{-2}. The parameter β is related to α by the relation $\beta\equiv\alpha\xi'^2$ where ξ' is the correlation length for composition fluctuations, which is of the order of 10 nm [18]. We then have $\beta\simeq 10^{-35}$ g cm^6 s^{-2}. A typical value for the bulk diffusion coefficient is at most $D\simeq 10^{-6}$ cm^2 s^{-1}. We take for the rigidity constant $\kappa\simeq 20k_BT$.

The surface diffusivity is given by

$$D_s\sim \nu a'^2\exp(-E_{dif}/k_BT) \quad (66)$$

where ν is the vibration frequency of adsorbed molecules, a' is the microscopic displacement of adsorbed molecules, and E_{dif} is the activation energy that the molecules have to cross in order to move. The typical microscopic displacement is $a'\simeq 10^{-9}$ m; the vibration frequency of molecules is between 10^{13} Hz for small molecules (atoms) and 10^{10} Hz for large molecules (proteins). Cole *et al.*[19] studied the diffusion mobility of Golgi proteins in membranes of living cells and found that the diffusion coefficients ranged from 3×10^{-9} to 5×10^{-9} cm^2 s^{-1}. We find from this that the energy barrier is about $E_{dif}\simeq 0.3$ eV. For definiteness we take $D_s\simeq 10^{-9}$ cm^2 s^{-1}, but we have to keep in mind that values which are at least two orders of magnitude smaller are quite plausible, and a more precise evaluation must focus on the specific situation under consideration.

Adsorption-desorption, like diffusion, is a thermally activated process so the adsorption time is of the form

$$\tau\sim \nu^{-1}\exp(E_{ad}/k_BT) \quad (67)$$

where E_{ad} is the adsorption energy of molecules on the membrane. The adsorption energy is larger than the diffusion energy E_{dif}. If we set $E_{ad}\simeq 0.45$ eV for large molecules ($\nu\simeq 10^{10}$ Hz) we obtain $\tau\simeq 10^{-2}$ s. However data on Golgi apparatus [20] indicate that several proteins (such as Arf1 and coatomer [20]) have a residence time of about 30 s. It must be noted, however, that these proteins show temperature-insensitive exponential decay of the kinetic release, and thus cannot be modeled in terms of simple thermodynamics. If one uses for the residence time about 30 s and for the diffusion constant $D_s\simeq 3\ 10^{-9}$ cm^2 s^{-1}, one obtains for the diffusion length $\xi\sim 3$–4 μm. For definiteness we shall take 10 μm. It must be noted that the above data are indicative.

Using the above values we obtain $\Lambda_c \simeq 10$ nm and $\psi_{eq} \simeq 10^{-5}$ mol ℓ^{-1}. This equilibrium bulk concentration is consistent with values available for proteins in a Golgi apparatus [21] We have seen that the membrane is unstable when the characteristic size of these molecules is larger than Λ_c. The typical value 10 nm of the critical size is of the order of magnitude of protein size for proteins like COP which plays a role in the budding process within the Golgi apparatus.

Since $\alpha = k_B T/\phi_{eq}$, $\Lambda_c = \sqrt{k_B T/\phi_{eq}\kappa}$. The critical size is then a decreasing function of the molecule concentration ϕ_{eq}, which is quite intuitive. However, it could seem strange that the critical size of the adsorbed molecules decreases when the rigidity κ increases. The reason is that the coupling factor between curvature and surface concentration is not Λ but $\kappa\Lambda$ so that $\kappa\Lambda_c = \sqrt{k_B T\kappa/\phi_{eq}}$. We then obtain that the more flexible is the membrane (i.e., κ small) the more easily is its instability achieved. Once the instability takes place highly nonlinear effects must be taken into account and this task can, in general, only be achieved numerically. For example, what is the ultimate stage of the dynamics? Would budding take place? Would the spacing between buds reach a certain value, or would budding continue in a coarsening process? This task is discussed in the next section.

If the condition of stability is satisfied, the membrane still fluctuates and reveals various dissipation mechanisms. We have seen that depending on length and time scales some dissipation will prevail over others provided that the associated mechanisms operate on quite distinct length scales.

Using the values given above we can evaluate the order of magnitude of the characteristic wave vectors. Typically, $q_0 \simeq 10^5$ m^{-1}, $q_1 \simeq 10^6$ m^{-1}, $q_2 \simeq 10^7$ m^{-1}, and $q_3 \simeq 10^8$ m^{-1}. The wave-vector hierarchy assumed, $q_0 < q_1 < q_2 < q_3$, is thus satisfied, albeit the length scales are not strongly separated.

We assumed here the quasi-steady-state approximation for the diffusion field. This means $Dq^2 \gg \omega$ for all q. A close inspection of the dispersion relation reveals that this approximation is valid as long as the bulk diffusion coefficient is not too small (not much smaller than 10^{-7} cm^2 s^{-1}). This is not always satisfied and there will be a need in the future to extend the analysis to the non-quasi-steady-state limit.

VI. BUDDING INSTABILITY

In this section we shall provide a brief outlook on the nonlinear dynamics of an infinite one-dimensional membrane. A thorough analysis, including the two-dimensional problem, will be performed in a forthcoming work. One of the major questions concerns whether or not, after the instability obtained by a linear stability analysis, we shall achieve a new steady state characterized, for example, by a regular modulation of the membrane shape. Another possibility is that the instability leads to a perpetual increase of the deformation, until, for example, overhangs take place and the membrane self-intersects (and thus vesicle emission may start). We can also imagine that the amplitude increases indefinitely with time without leading to overhangs. These are the most exciting unexplored area on which we have obtained some preliminary results.

First, we must note that budding of membranes is known in 3D, and this is due to the fact that there are surfaces (such as cathenoids) which have a zero total mean curvature [3], so that forming a bud costs no energy. In the present study it is important not to confuse a budding due to the Helfrich curvature energy with budding due to coupling with macromolecules. In addition, one effect may induce the other, and it is highly desirable to focus only on the possible budding due to coupling to macromolecules. One natural way to prevent budding due to curvature energy is to impose one-dimensional deformation of the membrane. The second important simplification concerns the dissipation mechanisms. Once a membrane moves it induces hydrodynamic flow, and *stricto sensu* full Stokes equations must be solved with a free boundary problem, as done for example in Ref. [22], if one wants to have complete information. However, in order to know whether or not there are steady-state solutions within the model there is no need to take hydrodynamic flow into account. Indeed, steady-state solutions do not depend on kinetics, and therefore it is sufficient to consider a simple dynamics (like a local dynamics as treated in [23]). If, however, it turns out that there is no steady-state solution, then inclusion of flow should not affect this general result, but only the temporal increase of the deformation will be affected. Thus within a simplified version of dynamics we may obtain some general information. Our aim is to present a general view of what might happen in this problem, rather than focusing on all details. We find that if the coupling between macromolecules and the membrane is weak, then there are steady-state solutions. Above a certain magnitude of the coupling constant, steady-state solutions cease to exist: budding takes place, which is a precursor to vesicle emission. It is well known in fluid dynamics, for example, that the viscosity does not preclude droplet emission. Thus taking into account hydrodynamics should not affect the overall qualitative picture. More than that, if viscosity prevents vesicle emission, it should mean either (i) that it restabilizes the membrane toward a planar state, and this is not possible, since we have established from linear stability analysis by including the flow, that the membrane is unstable (if the coupling constant lies beyond a threshold), or (ii) that it acts against vesicle emission by stopping the budding evolution, which would mean it drives the system toward a steady state. This fact is absurd, since steady-state solutions are independent of the flow (even more, if there is a steady-state profile of the membrane the dissipation due to viscosity should suppress the flow after a certain time).

Further assumptions are adopted. We assume that there is no adsorption or desorption of molecules at the membrane, which is not crucial for the emergence of morphological instability. In the following, we shall also set α, β, and κ to unity and $\Sigma = 0$ and just keep Λ to control the strength of the instability. This corresponds to changing units of measure.

A. Numerical method

In order to obtain a powerful geometrical description of the membrane, which also allows for overhangs, we perform the numerical calculations in intrinsic coordinates. The mem-

brane curve is now given by $\theta(s)$, where θ is the local angle of the normal with respect to some fixed direction and s the arclength along the curve. The mean curvature simply reads $H=\partial_s\theta(s)$.

Our first task is to rewrite the dynamical equations in intrinsic coordinates. We then discretize the membrane curve and the concentration field and solve the resulting system of ordinary differential equations (ODEs) using common ODE solvers. In order to have good numerical accuracy, we keep the relative distance between discretization points constant. This is achieved through a suitable dynamical reparametrization of the membrane curve.

As discussed in the previous subsection, our aim is to capture the essential features (existence or nonexistence of steady solutions, their overall behavior, and so on), and thus there is no need to solve for the full hydrodynamical problem, which is more involved and time consuming. Rather, we shall adopt a simplistic picture based on a local dynamics [23]. Including full hydrodynamics as in [22] is feasible and will be the subject of a future work. In a local description, the velocity $\mathbf{v}$ of a membrane point at position $\mathbf{r}$ can be evaluated from

$$\mathbf{v} = -\frac{\delta F}{\delta \mathbf{r}}, \tag{68}$$

where F is the free energy of the membrane as given by Eq. (11). Performing the functional derivative in intrinsic coordinates, we find

$$v_n = \partial_{ss}H + \frac{1}{2}H^3 - \Lambda\partial_{ss}\phi \tag{69}$$

for the normal component of the velocity.

For the tangential velocity we have to distinguish between two contributions. The first,

$$v_{t,p} = -\Lambda H\partial_s\phi, \tag{70}$$

is physical in origin and results from Eq. (68). It describes the advection of proteins along the membrane.

The second is an artificial *gauge* velocity,

$$v_{t,g} = \frac{s}{L}\int_0^L Hv_n ds' - \int_0^s Hv_n ds', \tag{71}$$

and stems from the condition that the distance between discretization points remains constant. It can be derived from differential geometry [24].

The evolution of the local angle is solely determined by the gauge velocity and the normal velocity [24]:

$$\frac{\partial\theta}{\partial t} = v_{t,g}H - \partial_s v_n. \tag{72}$$

Finally, we need the equation for the time evolution of the protein concentration for the chosen reparametrization of the membrane curve. It takes the form

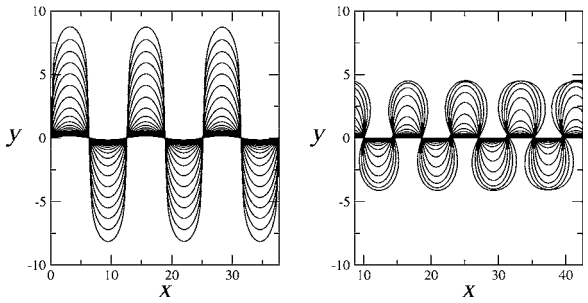

FIG. 4. Snapshots of the membrane at different times. In the left panel, $\Lambda=1.2$ and the membrane develops a fingerlike morphology. In the right panel, $\Lambda=1.4$ and budding is observed. Both pictures show part of a larger system that was simulated using white-noise initial conditions and periodic boundary conditions along the x axis.

$$\frac{\partial\phi}{\partial t} = \partial_{ss}(\phi - \Lambda H - \partial_{ss}\phi) - (v_{t,p} - v_{t,g})\partial_s\phi. \tag{73}$$

B. Simulation results

In order to test our implementation, we checked the long-wave approximation of the growth rate,

$$\omega \approx (\Lambda^2 - 1)q^4, \tag{74}$$

and found good quantitative agreement. For small q ($q \approx 0.01$), the deviation of the critical Λ is less than 0.1%.

Once the dynamics are obtained in terms of the angle θ as a function of the arclength, it is a simple matter to deduce the profile $Y(X)$ by means of the following relations: $dX = ds\cos(\theta)$ and $dY = -ds\sin(\theta)$, if θ is the angle between the vertical axis Y and the normal to the membrane. The nonlinear evolution, which is shown in Fig. 4, is characterized by the development of fingers for values of Λ that are slightly above the threshold (roughly between 1.0 and 1.2) and by the development of buds with a marked pinch off for higher values of Λ. That the pattern is periodic is obtained naturally, and since the appropriate unstable mode for an initially flat membrane is of Fourier type, one generically expects a periodic pattern to take place. This is what happens indeed. The instability under question is intrinsic and could take place without forcing by a noise term. The initial condition was random, but the equations which are solved are deterministic. If we start from any other initial condition (like a sine or cosine for example), then the ultimate pattern and dynamics are the same.

Some remarks are in order. First the appearance of budding is not due to the Helfrich contribution (as is known a cathenoid has a zero mean curvature, and in 3D one can form buds). Because the present model is purely one dimensional, a pure curvature effect is clearly penalized. The present instability is due to the coupling between the membrane curvature and the inclusions. Second, we have seen no tendency whatsoever toward a slowing down of the instability when Λ is large enough. Rather, the overhangs continue to increase in the course of time, until a self-intersection of the membrane occurs. We believe that the ultimate stage of the dynamics is a vesicle emission out of the initial planar membrane. Once

the vesicle is emitted the instability repeats itself. It would be interesting to see whether or not the point where the pinch off occurs constitutes a favorable site for new vesiculization. This question is currently under study. Another task for future investigation would be to analyze the frequency of vesicle emission as a function of various pertinent parameters. It would also be interesting to identify if there is a simple, and relevant, ingredient to be included in the model in order to suppress the perpetual increase of the membrane deformation. Finally, hydrodynamical dissipation must be included, instead of having a local dissipation. Hydrodynamics involve nonlocal interactions, and should act on the time scale for the instability, but we believe that the instability should manifest itself whether hydrodynamics are present or not within the model.

VII. CONCLUSIONS

We have analyzed the coupling of the membrane to a diffusion field. We have first focused on the situation where the system is in global equilibrium. When the membrane is stable we have analyzed the effect of the kinetics of the various processes on the membrane fluctuations. We have shown that when the length scales associated with each mechanism are quite distinct, the fluctuations obey scaling laws with time. Diffusion, desorption, and adsorption drastically affect these laws, leading to nontrivial behaviors such as $t\ln(t)$. It has been shown that although it seems, generically, that the law of fluctuations for the total height should reflect the dissipation through hydrodynamics, in the time interval around the desorption time, lateral diffusion caused by a local curvature fluctuation may significantly alter the total height dynamics, or even dominate it. Thus on that time scale experimental data must be analyzed with care in the light of the present investigation.

A simple coupling between the membrane and adsorbed molecules shows that the rigidity is reduced by increasing concentration. However, there are many experimental situations which indicate either an increase [25] or a decrease [26] depending on the type of molecules that are inserted (and probably depending on the strength of the interinclusion interaction). The following scenario may arise: it is likely that with some substances, there is not just a simple passive adsorption, but rather that the molecules are intercalated in the membranes in such a way that the microscopic energies that fix the membrane entity may change, increasing thereby the effective molecular cohesion, and thus the effective rigidity of the membrane. Microscopic studies are necessary in order to shed light on the precise interaction, and thus in order to resolve this dilemma.

Finally, when the condition of membrane instability is met, we have included nonlinear effects in order to ascertain the subsequent development of the membrane morphology. The membrane develops a budding with a regular spacing, and the dynamics show a clear tendency toward vesiculization. This result is interesting inasmuch as this type of evolution could not be inferred *a priori* (a large manifold of possibilities could be expected in the nonlinear regime). Our analysis is based on a very simplistic model which must be improved further before possible application to real systems. However, that the simplicity of the model gives rise to a rich scenario in the nonlinear regime points probably to the fact that vesicle emission is a robust feature when inclusions are sufficiently coupled to a membrane. Thus the vesiculization process itself should depend on generic but simple prototypes.

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APPENDIX A

Having obtained the matrix of kinetic coefficients, we can now calculate the dynamical correlation functions. The system (43) can be written as $\partial_t \mathbf{X}_q = \mathbf{A}_q \mathbf{X}_q$. This equation is solved by $(h_q, \phi_q)(t) = \exp(\mathbf{A}_q t)(h_q, \phi_q)(0)$. The first step is to perform diagonalization, from which one obtains the relaxation times of the various modes as the inverse values of the eigenvalues ω_i of $\mathbf{A}_q$. The time-dependent correlation functions can be obtained from the corresponding eigenvectors $\mathbf{X}_i$. The matrix $\mathbf{U}_q = [\mathbf{X}_1 \mathbf{X}_2]$ diagonalizes $\mathbf{A}_q$. The dynamical correlation matrix $\mathbf{G}(t)$ is defined by

$$\mathbf{G}(t) \equiv \left\langle \begin{pmatrix} h_q \\ \phi_q \end{pmatrix}(t)(h_q, \phi_q)^*(0) \right\rangle = e^{\mathbf{A}_q t}\mathbf{G}_0 = \mathbf{U}_q \exp\left(\begin{bmatrix} \omega_1 & 0 \\ 0 & \omega_2 \end{bmatrix} t \right) \mathbf{U}_q^{-1}\mathbf{G}_0. \tag{A1}$$

In order to obtain the static correlation functions for $t \to 0$, we have used the fluctuation-dissipation theorem $\mathbf{G}(t=0) = \mathbf{G_0} = (k_B T/L^2)\mathbf{E}^{-1}$.

APPENDIX B

Here we derive Eq. (55) for the mean square displacement. The stochastic field $h(y,t)$ is expressed through its Fourier transform

$$h(y,t) = \frac{1}{L^2}\sum_q h_q(t) e^{iqy}. \tag{B1}$$

This leads to

$$\langle [h(y,t) - h(y,0)]^2 \rangle = \frac{1}{L^4}\sum_q [\langle h_q(t) h_{-q}(t) \rangle + \langle h_q(0) h_{-q}(0) \rangle - 2\langle h_q(t) h_{-q}(0) \rangle], \tag{B2}$$

where we have used $\langle h_q(t) h_{-q'}(t') \rangle \propto \delta_{q,-q'}$. The equal time correlation functions $\langle h_q(t) h_{-q'}(t) \rangle$ are independent of time since $h_q(t)$ is a stationary stochastic process, and are equal to $\langle |h_q|^2 \rangle$. Transforming the sum on q to an integral, we immediately obtain Eq. (55).

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