Tube formation and spontaneous budding in a fluid charged membrane

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We determine the equations governing the equilibrium shape of an axially symmetric charged fluid membrane under the action of an external field. We consider that the charges are free to diffuse along the membrane, and we neglect the effect of screening counterions. By numerically integrating these equations for a membrane spread across a hole by a constant tension, we show that there exists a threshold electric field above which an infinitely long tube can be pulled. The threshold field for pulling a tube decreases as the surface charge density of the membrane increases, reaching zero at a finite critical value. Above this critical density of charge, the membrane spontaneously buds, in the absence of externally applied fields, because of the electrostatic repulsion of its charges.

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I. INTRODUCTION

Lipid bilayers are self-assembled bidimensional fluid sheets formed by two contacting monolayers of amphiphilic molecules having opposite orientations, such that the hydrophilic heads of the molecules are located at the outer parts of the structure, while the hydrophobic tails are shielded from contact with water. Biological membranes are constituted by fluid bilayers containing a large number of embedded proteins and intercalated particles. This complexity is further increased by the fact that the bilayer itself consists of a mixture of different lipids. Many of these lipids are electrically charged, such as phosphatidylserine, phosphatidylglycerol, phosphatidic acid, or phosphatydil-inositol. Therefore, electrostatic interactions play an important role in the interactions of a cell with its environment, as in cellular adhesion, in the interactions with proteins, in fusion processes, and in the interactions between peptides and bacterial membranes.

Many theoretical studies have addressed the effect of surface charges on the curvature elasticity of membranes, under different geometries and boundary conditions [1,2]. By supposing that the charges are fixed and in the presence of added salt, it was found that, in the Debye-Hückel approximation, the electric contribution to the free energy tends to rigidify the membrane [3]. When the membrane is highly charged or the salt concentration is low, the Debye-Hückel approximation fails and, at the mean-field level, the full nonlinear Poisson-Boltzmann equation has to be solved. This has been done in some particular cases analytically [4] or by numerical calculations [5], always showing a rigidification of the membrane. In the previous models, the surface charges were considered fixed: however, lipid membranes are fluid and therefore the electric charges are free to diffuse. In fact, it is known that the shape changes of membranes are accompanied by significant variations of the charge distribution and of the transmembrane potential [6]. By considering an infinite flat membrane having free electric charges, in the presence of added salt and in the Debye-Hückel approximation, Kumaran [7] found that, for small deformations around the flat state, the coupling between the curvature of the membrane and the charge distribution can lead to instabilities. This could explain the fact that vesicles can be formed at equilibrium—even if their bending energy is large compared to the thermal energy—when a mixture of lipids with charges of opposite signs are used [8,9].

In all these studies, the corrections to the elasticity moduli of the membrane are determined from the change in the electrostatic energy due to the curvature around some reference state. However, biological membranes often form highly distorted structures—e.g., in the form of tubular networks [10,11]. It is therefore essential to study the effect of the presence of electric charges on large distortions imposed on a membrane. In this paper we address the problem of determining the equilibrium shape of an axially symmetric membrane possessing electric charges free to diffuse in the presence of an external electric field, without any assumption on the amplitude of the deformation. However, for the sake of simplicity, we do not consider the presence of screening counterions; this is a somewhat artificial situation for a biological system: in fact, even for uncharged membranes, the unbalanced electric stress created by ionic currents can give rise to destabilizing out-of-equilibrium undulations [12]. Nevertheless, neglecting the effect of counterions is an interesting simple limit of electrostatic interactions that is a reasonable approximation as long as the Debye screening length is sufficiently large with respect to the induced deformations. We shall also suppose that the membrane is infinite and spread across a hole by means of a constant tension. The case in which the tension of the membrane is constant is in fact particularly relevant for biological situations, since almost all cells are able to adjust the amount of lipids in their membrane in order to maintain its lateral tension at some specific set point.

Our paper is organized as follows. In Sec. II we present our model of the charged membrane in an external electric field and we formulate the equations that determine its equilibrium shape. The resulting shape equations are summarized in Sec. III, together with the approach used to numerically

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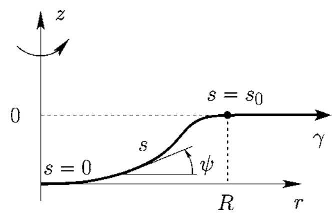

FIG. 1. Geometry of the axisymmetric membrane. The membrane is held on the planar frame $z=0$ having a hole of radius R centered on the symmetry axis z. The contour of the membrane is parametrized by its tilt angle ψ, its radial coordinate r, and its height z as a function of the arclength s. The membrane is stretched by a tension γ parallel to the frame.

solve them. In Sec. IV we determine the excess electrostatic free energy of the deformed membrane and we discuss the conservation of the total charge of the membrane. In Sec. V we present numerical results for the equilibrium shape of the membrane under the action of an external uniform electric field. In particular, we present diagrams for the elongation of the membrane as a function of the applied field, showing that above a critical field a membrane tube is infinitely pulled. In Sec. VI we show that, above a critical density of charged lipids, the membrane spontaneously buds and we present the corresponding stability diagram. In Sec. VII we discuss these results and we give a simple analytical estimate of the budding instability threshold. Our results are summarized in the final section VIII.

II. THEORY

We consider an *infinite* fluid lipidic membrane flat, on the average, in its ground state. We describe the deformation free energy of the membrane by means of the Helfrich bending energy [13]

$$\mathcal{F}_b = \frac{1}{2}\kappa \int (c_1 + c_2)^2 dS, \tag{1}$$

were κ is the bending rigidity, c_1 and c_2 are the principal curvatures of the membrane, and S its area. We suppose that the two sheets of the membrane are symmetric, so that no spontaneous curvature is present; moreover, since we assume that the membrane has a fixed topology, we disregard the Gaussian curvature free energy, which according to the Gauss-Bonnet theorem is a constant [14]. For an axisymmetric shape, the bending free energy can be written as [15]

$$\mathcal{F}_b = \frac{1}{2}\kappa \int_0^{s_0} 2\pi r \left(\dot{\psi} + \frac{\sin\psi}{r}\right)^2 ds, \tag{2}$$

where (see Fig. 1) ψ is the angle that the tangent to the contour makes with respect to the radial direction r and the overdot denotes the derivative with respect to the arclength s along the contour (with $s=0$ on the symmetry axis z).

We assume that the membrane is stretched on the planar frame $z=0$—having a hole of radius R centered on the symmetry axis z—by means of a radial force per unit length γ; the excess free energy required to increase the surface of the free membrane against the lateral tension γ is then

$$\mathcal{F}_t = \gamma \int_0^{s0} 2\pi r ds. \tag{3}$$

To complete our model, we assume that a fraction of the lipids forming the membrane bears a fixed electric charge. Indicating with $N(s)$ the number of charged lipids per unit surface and with q their charge, the corresponding electrostatic free energy can be written as

$$\mathcal{F}_e = \int_0^{\infty} 2\pi r q N(s) V_e(r,z) ds + \frac{1}{2}\int_0^{\infty} 2\pi r q N(s) V(r,z) ds, \tag{4}$$

where the first term accounts for the electrostatic interaction between the charged lipids and the external electric potential $V_e(r,z)$, while the second describes the electrostatic self-energy of the charged lipids, which create the electric potential $V(r,z)$. We assume that the latter is not screened. This is the limiting case corresponding to the absence of salt in the solution and to a finite-size membrane immersed in an infinite volume of water, such that, because of the entropic gain, the counterions of the charged lipids diffuse infinitely away from the membrane. Moreover, for the sake of simplicity, we neglect the entropy of the charged lipids. We note that, while the bending [Eq. (2)] and the tension [Eq. (3)] free energies only depend on the shape of the free portion of the membrane ($0 \leq s \leq s_0$), the electrostatic contribution (4) depends also on the reservoir membrane adhering to the frame, which for simplicity we assume to extend up to infinity.

A. Equilibrium shape of the membrane

The equilibrium shape of the membrane minimizes the total free energy

$$\mathcal{F} = \mathcal{F}_b + \mathcal{F}_t + \mathcal{F}_e. \tag{5}$$

To find the corresponding equilibrium equations, we consider the modified functional

$$\mathcal{F}^* = \mathcal{F} + \mathcal{F}_L, \tag{6}$$

where $\mathcal{F}_L$ contains Lagrange multiplier fields $L(s)$, $M(s)$, and $B(\mathbf{r})$:

$$\mathcal{F}_L = \int_0^{s_0} 2\pi L(s)(\dot{r} - \cos\psi) ds + \int_0^{s_0} 2\pi M(s)(\dot{z} - \sin\psi) ds + \int B(\mathbf{r})[\nabla^2 V(\mathbf{r}) + \epsilon^{-1} q N(s)\delta_S(\mathbf{r} - \mathbf{r}_S)] d\mathbf{r}. \tag{7}$$

In the last integral, the vector $\mathbf{r}$ locates the position of the points in the three-dimensional space, ϵ is the dielectric constant of the medium, which we suppose uniform, and the function $\delta_S(\mathbf{r}-\mathbf{r}_S)$ is a surface Dirac function centered on the points $\mathbf{r}_S$ of the surface of the membrane, such that, for any function $f(\mathbf{r})$,

$$\int f(\mathbf{r})\delta_S(\mathbf{r}-\mathbf{r}_S)d\mathbf{r} = \int f(\mathbf{r}_S)dS. \tag{8}$$

The functional (7) allows one to enforce the conditions that s be the contour arclength—i.e., that $\dot{r}=\cos\psi$ and $\dot{z}=\sin\psi$—and that the electrostatic potential $V(\mathbf{r})$ generated by the charged lipids obey the Poisson equation

$$\nabla^2 V(\mathbf{r}) = -\epsilon^{-1} q n(s)\delta_S(\mathbf{r}-\mathbf{r}_S). \tag{9}$$

Note that, for a finite membrane, one should also impose the condition that the total electric charge be conserved: as we shall see in Sec. IV, for an infinite membrane this condition is automatically satisfied.

The equilibrium shape of the membrane can be found by extremizing the modified functional (6) with respect to the variables $r(s)$, $z(s)$, $\psi(s)$, $N(s)$, and $V(r,z)$, treated as independent. A final point is to be remarked: since we modeled the membrane as a charged sheet, the electrostatic potential $V(r,z)$ will have a discontinuous normal derivative across to it. At first, we shall neglect this discontinuity: later on, we will take it into account by a suitable regularisation of the equations.

In the following, we normalize all energies with respect to the bending rigidity κ and all lengths with respect to the correlation length

$$\xi = \sqrt{\frac{\kappa}{\gamma}}. \tag{10}$$

Furthermore, we introduce the normalized electrostatic coupling constant

$$\Delta = \frac{q^2}{\epsilon\kappa\xi}, \tag{11}$$

which measures the electrostatic interaction energy between two lipids at a distance of the order of ξ in units of the bending rigidity and the normalized charge density, electric potentials, and Lagrange fields

$$n = \xi^2 N, \quad v_e = \frac{q}{\kappa}V_e, \quad v = \frac{q}{\kappa}V, \tag{12}$$

$$\lambda = \frac{\xi}{\kappa}L, \quad \mu = \frac{\xi}{\kappa}M, \quad \beta = \frac{\xi}{q}B. \tag{13}$$

In terms of these normalized variables, with the variations

$$\psi(s) = \psi_0(s) + \delta\psi(s), \tag{14a}$$

$$r(s) = r_0(s) + \delta r(s), \tag{14b}$$

$$z(s) = z_0(s) + \delta z(s), \tag{14c}$$

$$n(s) = n_0(s) + \delta n(s), \tag{14d}$$

$$v(\mathbf{r}) = v_0(\mathbf{r}) + \delta v(\mathbf{r}), \tag{14e}$$

$$s_0 = s_{00} + \delta s_0, \tag{14f}$$

one finds for the variation of the functional (6) the formal expression

$$\begin{aligned}\delta\mathcal{F}^* = 2\pi\int_0^{s_0}\Bigg\{&\left[\frac{\sin 2\psi}{2r} - \dot{\psi}\cos\psi - r\ddot{\psi} + \lambda\sin\psi - \mu\cos\psi\right]\delta\psi + \Bigg[n\left(v_e + r\frac{\partial v_e}{\partial r} + \frac{1}{2}v + \frac{1}{2}r\frac{\partial v}{\partial r} + \Delta\beta + \Delta r\frac{\partial\beta}{\partial r}\right)\\ &+ \frac{1}{2}\left(\dot{\psi}^2 - \frac{\sin^2\psi}{r^2}\right) + 1 - \dot{\lambda}\Bigg]\delta r + \left[n\left(r\frac{\partial v_e}{\partial z} + \frac{1}{2}r\frac{\partial v}{\partial z} + \Delta r\frac{\partial\beta}{\partial z}\right) - \dot{\mu}\right]\delta z\Bigg\}ds + 2\pi\int_0^{\infty} r\left[v_e + \frac{1}{2}v + \Delta\beta\right]\delta n ds\\ &+ \int\left[\nabla^2\beta + \frac{1}{2}n\delta_S(\mathbf{r}-\mathbf{r}_S)\right]\delta v d\mathbf{r} + 2\pi\Bigg\{r(s_0)\left[\dot{\psi}(s_0) + \frac{\sin\psi(s_0)}{r(s_0)}\right]\delta\psi(s_0) + \lambda(s_0)\delta r(s_0) + \mu(s_0)\delta z(s_0) - \lambda(0)\delta r(0)\\ &- \mu(0)\delta z(0)\Bigg\} + 2\pi\Bigg\{\frac{1}{2}r(s_0)\left[\dot{\psi}(s_0) + \frac{\sin\psi(s_0)}{r(s_0)}\right]^2 + r(s_0) + \lambda(s_0)[\dot{r}(s_0) - \cos\psi(s_0)] + \mu(s_0)[\dot{z}(s_0) - \sin\psi(s_0)]\Bigg\}\delta s_0.\end{aligned} \tag{15}$$

Setting this variation to zero for arbitrary variations δv in the volume gives the equation for the β Lagrange field:

$$\nabla^2\beta + \frac{1}{2}n\delta_S(\mathbf{r}-\mathbf{r}_S) = 0. \tag{16}$$

Comparing this equation with the Poisson equation satisfied by the electric potential [see Eq. (9)], which in our normalized units becomes

$$\nabla^2 v + \Delta n\delta_S(\mathbf{r}-\mathbf{r}_S) = 0, \tag{17}$$

we see that the β field, apart from an inessential constant, has to be proportional to v:

$$\beta(\mathbf{r}) = \frac{1}{2\Delta}v(\mathbf{r}). \tag{18}$$

With this condition, setting Eq. (15) to zero for arbitrary variations δn of the normalized density of lipids gives the

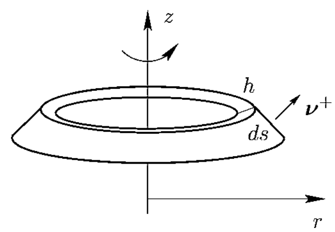

FIG. 2. Volume element for the regularization of the electrostatic force.

boundary condition for the electrostatic potential $v(s)$ on the membrane:

$$v_e(s) + v(s) = 0. \tag{19}$$

This condition means that, at equilibrium, the membrane is an equipotential surface; i.e., it behaves as an electric conductor: indeed, since the membrane is fluid, the charged lipids are free to diffuse along the membrane.

Using Eqs. (18) and (19), the contributions to Eq. (15) depending on δr and δz become, respectively,

$$\delta\mathcal{F}_r^* = 2\pi\int_0^{s_0}\left[\frac{1}{2}\left(\dot{\psi}^2 - \frac{\sin^2\psi}{r^2}\right) + 1 - \dot{\lambda} - nre_r\right]\delta r ds,$$

$$\delta\mathcal{F}_z^* = -2\pi\int_0^{s_0}[\dot{\mu} + nre_z]\delta z ds, \tag{20}$$

where e_r and e_z are, respectively, the r and z components of the normalized total electric field

$$\mathbf{e} = -\boldsymbol{\nabla}(v + v_e) \tag{21}$$

on the membrane. However, the latter is discontinuous on the membrane, because of the charge density localized on it. Therefore, as they stand, Eqs. (20) are meaningless. To make them well defined, we have to consider the charge confined to the surface as the limit of a volume charge density spread on a finite thickness h around the ideal bidimensional membrane. By Maxwell's equations, the electromagnetic force acting on the volume charge density ρ in the volume Ω delimited by the surface $\partial\Omega$ is equal to the flux of the Maxwell stress tensor through $\partial\Omega$ [16]; for static fields and with our normalizations for lengths and electric field,

$$\int_\Omega \bar{\rho}\mathbf{e}d\mathbf{r} = \frac{1}{\Delta}\oint_{\partial\Omega}\left[\mathbf{e}\otimes\mathbf{e} - \frac{1}{2}e^2\mathbf{1}\right]\cdot\boldsymbol{\nu}dS, \tag{22}$$

where $\bar{\rho}=\rho\xi^3/q$ is the normalized charge density, $\otimes$ indicates dyadic product, $\mathbf{1}$ is the identity tensor, e^2 is the square modulus of the normalized electric field $\mathbf{e}$, and $\boldsymbol{\nu}$ is the outward normal to the surface. Taking as volume an annulus at a distance r from the rotational symmetry axis z, of thickness h orthogonal to the contour length ds (see Fig. 2), such that $d\mathbf{r}=2\pi rhds$, Eq. (22) gives

$$2\pi rh\bar{\rho}\mathbf{e}ds = \frac{\pi r}{\Delta}(e_+^2 - e_-^2)\boldsymbol{\nu}_+ ds, \tag{23}$$

since in the limit $h\to 0$ only survive the two contributions coming from the outer and inner lateral surfaces, on which the normal is

$$\boldsymbol{\nu}_\pm = \mp\sin\psi\hat{\mathbf{r}} \pm \cos\psi\hat{\mathbf{z}} \tag{24}$$

[$\hat{\mathbf{r}}$ and $\hat{\mathbf{z}}$ being the unit vector in the radial and z directions, respectively (see Fig. 2)], $dS=2\pi rds$, and the electric field is $\mathbf{e}_\pm=e_\pm\boldsymbol{\nu}_\pm$—i.e., normal to the membrane—which is equipotential. In the limit $h\to 0$, the quantity $h\bar{\rho}$ becomes the normalized surface density of charged lipids:

$$h\bar{\rho}\to n. \tag{25}$$

Therefore, inserting Eqs. (23)–(25) in Eqs. (20) gives the required regularized variations

$$\delta\mathcal{F}_r^* = 2\pi\int_0^{s_0}\left[\frac{1}{2}\left(\dot{\psi}^2 - \frac{\sin^2\psi}{r^2}\right) + 1 - \dot{\lambda} + \frac{r\sin\psi}{2\Delta}(e_+^2 - e_-^2)\right]\delta r ds,$$

$$\delta\mathcal{F}_z^* = -2\pi\int_0^{s_0}\left[\dot{\mu} + \frac{r\cos\psi}{2\Delta}(e_+^2 - e_-^2)\right]\delta z ds. \tag{26}$$

Setting now to zero Eqs. (26) with respect to arbitrary variations δr and δz and setting to zero Eq. (15) with respect to arbitrary variations $\delta\psi$ leads to the differential equations governing the equilibrium shape of the charged membrane:

$$\ddot{\psi} = \frac{\sin 2\psi}{2r^2} - \frac{\dot{\psi}\cos\psi}{r} + \frac{\lambda\sin\psi}{r} - \frac{\mu\cos\psi}{r}, \tag{27a}$$

$$\dot{\lambda} = 1 + \frac{1}{2}\left(\dot{\psi}^2 - \frac{\sin^2\psi}{r^2}\right) + \frac{r\sin\psi}{2\Delta}(e_+^2 - e_-^2), \tag{27b}$$

$$\dot{\mu} = -\frac{r\cos\psi}{2\Delta}(e_+^2 - e_-^2). \tag{27c}$$

To these equations we must add the constraint that s be the arclength of the contour, a condition that supplements two other differential equations for r and z:

$$\dot{r} = \cos\psi, \tag{27d}$$

$$\dot{z} = \sin\psi. \tag{27e}$$

The condition that the membrane be held on the frame means that, for any arbitrary variation δs_0 of the contour length,

$$r(s_0 + \delta s_0) = R, \tag{28a}$$

$$z(s_0 + \delta s_0) = 0, \tag{28b}$$

$$\psi(s_0 + \delta s_0) = 0. \tag{28c}$$

Taking the variations of these conditions and using Eqs. (27d) and (27e) leads, to first order, to

$$\delta r(s_0) = -\delta s_0, \tag{29a}$$

$$\delta z(s_0) = 0, \tag{29b}$$

$$\delta\psi(s_0) = -\dot{\psi}(s_0)\delta s_0. \tag{29c}$$

Substituting conditions (27d) and (29c) into Eq. (15) and setting the latter to zero for arbitrary δs_0 leads to the boundary condition

$$\lambda(s_0) = R\left[1 - \frac{1}{2}\dot{\psi}^2(s_0)\right]. \tag{30a}$$

Since the membrane on the z axis ($s=0$) is free to translate along z, $\delta z(0)$ is arbitrary; then, setting Eq. (15) to zero for arbitrary $\delta z(0)$ gives the boundary condition

$$\mu(0) = 0. \tag{30b}$$

The other boundary conditions enforcing that the membrane is held on the frame and that $s=0$ corresponds to the axis of revolution of the membrane are

$$r(s_0) = R, \tag{30c}$$

$$z(s_0) = 0, \tag{30d}$$

$$\psi(s_0) = 0, \tag{30e}$$

$$r(0) = 0. \tag{30f}$$

Finally, to guarantee that the bending energy (2) remains finite, the membrane must not have a cusp on the axis of revolution—i.e.,

$$\psi(0) = 0. \tag{30g}$$

If the electric fields $e_\pm$ just above and below the membrane are known, Eq. (27) together with the boundary conditions (30) uniquely determine the possible equilibrium shapes of the membrane. In fact, Eq. (27) constitutes a set of six first-order differential equations in the unknowns $(\psi, \dot{\psi}, \lambda, \mu, r, z)$, which has to obey to the seven boundary conditions (30); however, in the latter, the total contour length s_0 is an extra unknown parameter, therefore effectively increasing by 1 the number of free equations.

B. Electrostatic potential created by the membrane

To determine the total electric fields $e_\pm$ we have to solve in all space the Poisson equation (17), together with the boundary condition (19), which expresses the fact that at equilibrium the membrane is equipotential. To this aim, we suppose that the membrane carries, for $r\to\infty$ (for which $z=0$ by hypothesis), a given normalized density of lipids n_0:

$$n_0 = \lim_{r\to\infty} n(r). \tag{31}$$

This is also the uniform density of lipids of the membrane in its flat state—i.e., in the absence of external electric fields. Since the normal derivative of the normalized electric potential $v(\mathbf{r})$ created by the lipids is discontinuous through the membrane, we set $v=v_+$ in the half-space starting from the membrane and moving in the direction $\boldsymbol{\nu}_+$ in Fig. 2 and $v=v_-$ in the remaining half-space; moreover, we set

$$v_+ = -\frac{1}{2}\Delta n_0 z + u_+, \tag{32a}$$

$$v_- = \frac{1}{2}\Delta n_0 z + u_-, \tag{32b}$$

where the first terms in the right-hand side of Eqs. (32) correspond to the normalized electric potential created by the flat membrane uniformly charged with the density n_0. The potentials $u_\pm$ obey the Laplace equation

$$\nabla^2 u_\pm = 0, \tag{33}$$

tend to zero at infinity (where the total potential created by the lipids tend to the potential created by the unperturbed flat membrane), and obey the boundary conditions on the membrane,

$$u_+(s) = \frac{1}{2}\Delta n_0 z(s) - v_e(s), \tag{34a}$$

$$u_-(s) = -\frac{1}{2}\Delta n_0 z(s) - v_e(s), \tag{34b}$$

because of Eq. (19).

To solve the Laplace equation $\nabla^2\Phi=0$ with Dirichlet boundary conditions in the volume Ω bounded by the surface $\partial\Omega$ of outward normal $\boldsymbol{\nu}$, we transform it into an integral equation with the help of the second Green identity [17]

$$\oint_{\partial\Omega} G(\mathbf{r}-\mathbf{r}')\frac{\partial\Phi(\mathbf{r})}{\partial\nu}dS = \oint_{\partial\Omega}\Phi(\mathbf{r})\frac{\partial G(\mathbf{r}-\mathbf{r}')}{\partial\nu}dS - \frac{1}{2}\Phi(\mathbf{r}'), \tag{35}$$

where $G(\mathbf{r})=-1/(4\pi|\mathbf{r}|)$ is the Green's function of the Laplace equation in three dimensions, $\nabla^2 G=\delta(\mathbf{r})$, and $\mathbf{r}'$ is a point on the surface $\partial\Omega$. For our axisymmetric case, expressing Eq. (35) in cylindrical coordinates (r,z,ϕ) and integrating over the polar angle ϕ yields the integral equations for the potentials $u_\pm$ on the membrane:

$$\int_0^\infty \left[\mathcal{A}(r,r',z-z')\left.\frac{\partial u_\pm}{\partial\nu}\right|_{(r,z)} - \mathcal{B}(r,r',z-z',\dot{r},\dot{z})u_\pm(r,z)\right]ds = \mp\frac{\pi}{2}u_\pm(r',z'), \tag{36}$$

where [see Eq. (24)]

$$\boldsymbol{\nu} \equiv \boldsymbol{\nu}_+ = -\sin\psi\hat{\mathbf{r}} + \cos\psi\hat{\mathbf{z}}; \tag{37}$$

$r=r(s)$, $z=z(s)$ is the contour of the membrane in terms of the arclength s, (r',z') is a point of the contour of the membrane, and the kernels $\mathcal{A}$ and $\mathcal{B}$ are given by

$$\mathcal{A}(r,r',z) = \frac{r}{\sqrt{(r+r')^2+z^2}}K\left(\frac{4rr'}{(r+r')^2+z^2}\right), \tag{38a}$$

$$\mathcal{B}(r,r',z,\dot{r},\dot{z}) = \frac{1}{\sqrt{(r+r')^2+z^2}}\left\{\frac{\dot{z}}{2}\left[K\left(\frac{4rr'}{(r+r')^2+z^2}\right) - E\left(\frac{4rr'}{(r+r')^2+z^2}\right)\right] + \frac{r[\dot{z}(r-r')-\dot{r}z]}{(r-r')^2+z^2}E\left(\frac{4rr'}{(r+r')^2+z^2}\right)\right\}, \tag{38b}$$

$K(m)$ and $E(m)$ being complete elliptic integrals of the first and second kind, respectively, defined as

$$K(m) = \int_0^{\pi/2} \frac{d\phi}{\sqrt{1-m\sin^2\phi}}, \tag{39}$$

$$E(m) = \int_0^{\pi/2} \sqrt{1-m\sin^2\phi}\,d\phi. \tag{40}$$

Applying the Gauss theorem to the Poisson equation (17) and using expressions (32) for the normalized electric potential on the two sides of the membrane, the normalized charge density n of the membrane becomes

$$n = n_0\cos\psi + p, \tag{41}$$

with the excess normalized charge density

$$p = \frac{1}{\Delta}\left(\frac{\partial u_-}{\partial \nu} - \frac{\partial u_+}{\partial \nu}\right). \tag{42}$$

From the boundary conditions (34) and the integral equation (36), the excess normalized charge density p is a solution of the integral equation

$$\int_0^\infty \mathcal{A}(r(s),r',z(s)-z')p(s)ds = -\int_0^\infty n_0 z(s)\mathcal{B}(r(s),r',z(s)-z',\dot{r}(s),\dot{z}(s))ds - \frac{\pi}{\Delta}v_e(r',z'). \tag{43}$$

To compute the electrostatic pression acting on the membrane, we furthermore introduce the quantity

$$m = -\frac{1}{\Delta}\left(\frac{\partial u_-}{\partial \nu} + \frac{\partial u_+}{\partial \nu}\right), \tag{44}$$

which, according to Eqs. (34) and (36), is given by the solution of the integral equation

$$\int_0^\infty \mathcal{A}(r(s),r',z(s)-z')m(s)ds = \int_0^\infty \frac{2v_e(r(s),z(s))}{\Delta}\mathcal{B}(r(s),r',z(s)-z',\dot{r}(s),\dot{z}(s))ds + \frac{\pi}{2}n_0 z'. \tag{45}$$

Finally, according to Eqs. (21), (19), (32), (42), and (44), the normalized electrostatic pression acting on the membrane is given by

$$\frac{e_+^2 - e_-^2}{\Delta} = (n_0\cos\psi + p)\left(\Delta m + 2\sin\psi\frac{\partial v_e}{\partial r} - 2\cos\psi\frac{\partial v_e}{\partial z}\right). \tag{46}$$

III. SHAPE EQUATIONS OF THE CHARGED MEMBRANE

In summary, the equilibrium shape of the charged membrane is determined by the following set of coupled differential equations:

$$\ddot{\psi} = \frac{\sin 2\psi}{2r^2} - \frac{\dot{\psi}\cos\psi}{r} + \frac{\lambda\sin\psi}{r} - \frac{\mu\cos\psi}{r}, \tag{47a}$$

$$\dot{\lambda} = 1 + \frac{1}{2}\left(\dot{\psi}^2 - \frac{\sin^2\psi}{r^2}\right) + \frac{1}{2}r\sin\psi(n_0\cos\psi + p) \times\left(\Delta m + 2\sin\psi\frac{\partial v_e}{\partial r} - 2\cos\psi\frac{\partial v_e}{\partial z}\right), \tag{47b}$$

$$\dot{\mu} = -\frac{1}{2}r\cos\psi(n_0\cos\psi + p) \times\left(\Delta m + 2\sin\psi\frac{\partial v_e}{\partial r} - 2\cos\psi\frac{\partial v_e}{\partial z}\right), \tag{47c}$$

$$\dot{r} = \cos\psi, \tag{47d}$$

$$\dot{z} = \sin\psi, \tag{47e}$$

accompanied by the boundary conditions

$$r(0) = 0, \tag{48a}$$

$$\psi(0) = 0, \tag{48b}$$

$$\mu(0) = 0, \tag{48c}$$

$$r(s_0) = R, \tag{48d}$$

$$z(s_0) = 0, \tag{48e}$$

$$\psi(s_0) = 0, \tag{48f}$$

$$\lambda(s_0) = R\left[1 - \frac{1}{2}\dot{\psi}^2(s_0)\right]. \tag{48g}$$

The normalized charge densities p and m appearing in the differential equations (47) are in turn a solution of the integral equations

$$\int_0^\infty \mathcal{A}(r(s),r',z(s)-z')p(s)ds = -\int_0^\infty n_0 z(s)\mathcal{B}(r(s),r',z(s)-z',\dot{r}(s),\dot{z}(s))ds - \frac{\pi}{\Delta}v_e(r',z'), \tag{49a}$$

$$\int_0^\infty \mathcal{A}(r(s),r',z(s)-z')m(s)ds = \int_0^\infty \frac{2v_e(r(s),z(s))}{\Delta}\mathcal{B}(r(s),r',z(s)-z',\dot{r}(s),\dot{z}(s))ds + \frac{\pi}{2}n_0 z', \tag{49b}$$

with the kernels $\mathcal{A}$ and $\mathcal{B}$ defined by Eqs. (38). The normalized charge density of the membrane is given by Eq. (41).

To numerically solve this set of coupled integro-differential equations, we use an iterative scheme. We start from an initial guess for the normalized charge densities $p(s)$ and $m(s)$ and we solve the two point boundary value problem (47) and (48) using a finite-difference scheme with deferred correction and Newton iteration [18]. To cope with the unknown right boundary $s=s_0$, we actually make the change of variable $s=s_0 t$, with the new independent variable t ranging from 0 to 1; the unknown contour length s_0 is then determined by supplying the extra differential equation $ds_0/dt=0$ [19]: we thus obtain a total of seven coupled first-order differential equations with seven boundary conditions. Once this shape of the membrane is determined, we solve the integral equations (49) by means of a collocation method [19] to obtain new normalized charge densities $p(s)$ and $m(s)$. We then iterate the process until we obtain convergence.

Note that the integral equations (49) contain integrable singularities in their kernels $\mathcal{A}$ and $\mathcal{B}$. To cope with them, we extrapolate the profile of the membranes $r(s)$ and $z(s)$ between each two points s_1 and s_2 of the discretized profile by means of a third-order polynomial in the arclength s, such that the extrapolated profile and its derivative are continuous at the discretisation points; then, using an adaptive Gaussian quadrature rule, we compute the first three moments $\mathcal{A}_n(s_1,s_2)$ and $\mathcal{B}_n(s_1,s_2)$ $(n=0,1,2)$ of the kernels on each interval of the discretization:

$$\mathcal{A}_n(s_1,s_2) = \int_{s_1}^{s_2} \mathcal{A}(s)s^n ds, \tag{50a}$$

$$\mathcal{B}_n(s_1,s_2) = \int_{s_1}^{s_2} \mathcal{B}(s)s^n ds, \tag{50b}$$

which are finite. A repeated application of a Gaussian three-point quadrature rule [19], with the kernels as weight functions, then converts the integral equations to well-behaved systems of linear equations for the densities of charge at the discretization points.

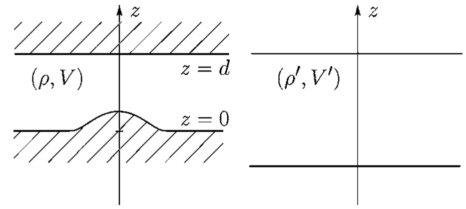

FIG. 3. Geometry for the application of the reciprocity theorem to the calculation of the variation of the electric free energy stored above the membrane. The (ρ,V) system consists in a condenser whose lower plate has the shape of the membrane and is kept at zero potential and whose upper plate is a plane at a finite height d. The (ρ',V') system is a planar condenser whose upper plate is at the height d and whose lower plate is below the membrane.

IV. ELECTROSTATIC FREE ENERGY

For an infinite membrane, the electrostatic free energy (4) is infinite. However, its variation $\mathcal{F}_e'$ with respect to the flat, uniformly charged, state is finite. We shall compute it in the case in which the external applied electric field is a uniform field $E_0\hat{\mathbf{z}}$ parallel to the z axis. To this aim, we make use of the reciprocity theorem for electrostatic fields [20], according to which, given two charge distributions $\rho(\mathbf{r})$ and $\rho'(\mathbf{r})$ that create the electrostatic potentials $V(\mathbf{r})$ and $V'(\mathbf{r})$, respectively,

$$\int \rho(\mathbf{r})V'(\mathbf{r})d\mathbf{r} = \int \rho'(\mathbf{r})V(\mathbf{r})d\mathbf{r}, \tag{51}$$

where, throughout all this section, all variables are not normalized if not otherwise explicitly stated.

Since the membrane at equilibrium is equipotential, we can think of it as a thin conducting sheet carrying a surface charge density σ_+ (σ_-) on the upper (lower) side. Let us then begin to apply the reciprocity theorem to the system depicted in Fig. 3. As $V(\mathbf{r})$ we take the potential inside a condenser whose lower plate is a *massive* conductor whose surface is shaped as the membrane and whose upper plate, also *massive*, is at a finite height d. The lower plate is kept at zero potential. Inside the condenser there is a uniform dielectric with dielectric constant ϵ. The condenser is charged and submitted to the uniform external electric field $E_0\hat{\mathbf{z}}$. We write the potential inside the condenser as

$$V(\mathbf{r}) = -\frac{\sigma_0}{2\epsilon}z - E_0 z + U(r,z), \tag{52}$$

where $U(r,z=d)=0$ and $U(r,z)\to 0$ for $r\to\infty$. Note that, for $d\to\infty$, $U(r,z)$ coincides with the (denormalized) potential u_+ defined in Eq. (32a). Far from the z axis, the surface charge density σ_+ of the lower plate tends to the value

$$\sigma_\infty = \frac{\sigma_0}{2} + \epsilon E_0. \tag{53}$$

This corresponds to the uniform surface charge density of the lower plate when the latter is flat. We write the surface charge density of the upper plate as

$$\sigma_d(r) = -\sigma_\infty - \delta\sigma_d(r). \tag{54}$$

Finally, we call $-V_0$ the potential of the upper plate,

$$V(r,z=d) = -V_0, \tag{55}$$

such that V_0 is the potential difference across the condenser.

As $V'(\mathbf{r})$ we take the potential inside a planar condenser whose upper plate, at $z=d$, is charged with the uniform surface charge density $-\sigma_\infty$ and whose lower plate, situated below the lower level of the membrane, is charged with the uniform surface charge density σ_∞—i.e.,

$$V'(\mathbf{r}) = -\frac{\sigma_\infty}{\epsilon}z. \tag{56}$$

Applying the reciprocity theorem then yields

$$\int_{S_d} \sigma_\infty V_0 dS = -\frac{\sigma_\infty}{\epsilon}\int_S \sigma_+ z dS + \int_{S_d} (\sigma_\infty + \delta\sigma_d) V_0 dS, \tag{57}$$

where S is the surface of the membrane and S_d is the planar surface $z=d$. Keeping the potential difference V_0 constant, the variation of the electric free energy of the condenser formed by the membrane and the upper plate, due to a deformation of the membrane, is given by

$$\mathcal{F}_+ = -\frac{1}{2}V_0 \delta Q_d, \tag{58}$$

where

$$\delta Q_d = \int_{S_d} \delta\sigma_d dS \tag{59}$$

is the variation of the charge of the condenser. Using Eq. (57) we then have

$$\mathcal{F}_+ = -\left(\frac{\sigma_0}{4\epsilon} + \frac{E_0}{2}\right)\int_S \sigma_+ z dS. \tag{60}$$

As $d \to \infty$, Eq. (60) gives the finite variation of the electric free energy stored above the membrane. Note that since $\mathcal{F}_+$ remains finite as $d \to \infty$, while V_0 diverges, according to Eq. (58) the total charge stored in the upper sheet is conserved. In a similar way, we can compute the variation of the free energy stored below the membrane:

$$\mathcal{F}_- = \left(\frac{\sigma_0}{4\epsilon} - \frac{E_0}{2}\right)\int_S \sigma_- z dS. \tag{61}$$

Again, also the total charge stored in the lower sheet is conserved: for an infinite membrane, the total charge for a deformation associated with a finite energy is automatically conserved.

Finally, reintroducing our normalizations for the energies, the lengths, and the fields, the total electric free energy variation is given by

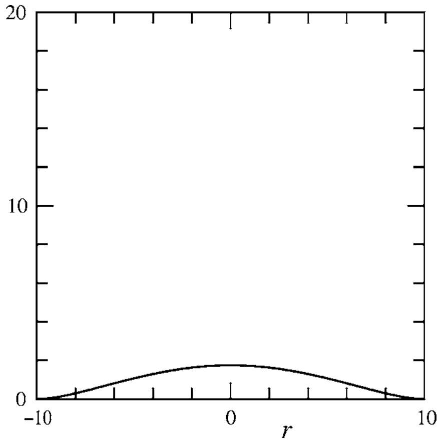

FIG. 4. Equilibrium shape of the charged membrane, for $\Delta = 8 \times 10^{-3}$, $n_0=1$, and $R=10$, in a uniform electric field of normalized amplitude $\eta = 2.8 \times 10^{-2}$.

$$\mathcal{F}'_e = -\int_0^{s_0} \left[\eta\left(n_0 \cos\psi + \frac{1}{2}p\right) + \frac{1}{4}n_0 \Delta m\right] 2\pi r z ds, \tag{62}$$

where

$$\eta = \frac{q\xi}{\kappa}E_0 \tag{63}$$

is the normalized external electric field, associated with the external normalized potential

$$v_e = -\eta z. \tag{64}$$

V. EQUILIBRIUM SHAPES UNDER A UNIFORM ELECTRIC FIELD

In the following, we consider the equilibrium shape of the membrane when it is submitted to a uniform electric field parallel to the z axis, of normalized amplitude η [see Eqs. (63) and (64)].

We recall that the normalized parameters characterizing the charged membrane are the normalized strength of the electrostatic interaction Δ [see Eq. (11)] and the normalized surface density of charged lipids n_0 [see Eq. (31)]. To estimate the electrostatic coupling parameter Δ, we take as bending modulus of the membrane the typical value $\kappa \simeq 10^{-19}$ J $\simeq 25 k_B T$ at room temperature [21]; with a typical tension of $\gamma \simeq 5 \times 10^{-5}$ J m^{-2} [22], the correlation length (10) is of the order of $\xi \simeq 45$ nm; then, for monovalent charged lipids in water (relative dielectric constant $\epsilon_r \simeq 80$), $\Delta \simeq 8 \times 10^{-3}$. Figure 4 shows the resulting equilibrium shape of the membrane in an external electric field of normalized amplitude $\eta = 2.8 \times 10^{-2}$ (corresponding to $E_0 \simeq 0.4$ V μm^{-1} with our typical parameters), with a normalized frame radius $R = 10$ and a normalized density of charged lipids $n_0 = 1$ (for charged and uncharged lipids having a surface of the order of 1 nm^2, this corresponds to having $\simeq 0.05\%$ charged lipids on the membrane): the membrane is uniformly bent across the frame in the direction of the applied field. The corresponding

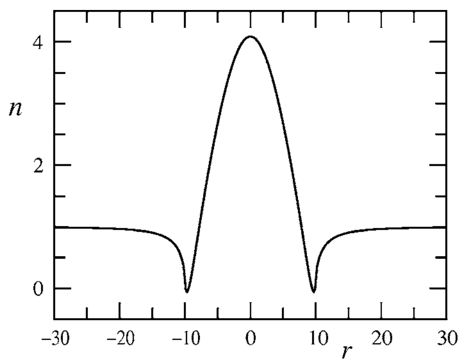

FIG. 5. Normalized surface density n of the charged lipids on the membrane at equilibrium, as a function of the radial position r of the membrane, corresponding to Fig. 4.

normalized surface density of the charged lipids is shown in Fig. 5: the central part of the membrane presents an excess of charged lipids, because of a conducting tip effect; to fulfill the conservation of the electric charge, this excess of charge is compensated, around the rim of the frame, by a sharp dip (note that in Fig. 5, n gets slightly negative). In the flat part attached to the frame, the density of lipids quickly recovers its constant value for a flat membrane.

In Fig. 6 we present the behavior of the maximum extension z_0 of the membrane (that corresponds to the point on the rotation axis $r=0$) as a function of the applied electric field η, for a normalized frame radius $R=10$, a normalized electrostatic coupling $\Delta \simeq 8\times10^{-3}$, and various normalized surface densities n_0 of the charged lipids. At sufficiently low fields, the maximum extension z_0 is a linear function of the applied electric field η. As the field increases, the maximum elongation z_0 grows faster than linear, up to a critical field η_c, corresponding to an infinite slope $\partial z_0/\partial\eta$, above which no stationary state exists anymore: the membrane is infinitely pulled by the applied electric field.

Figure 7 shows the slope $\partial z_0/\partial\eta$ of the low-field linear regime as a function of the surface density n_0: the slope increases monotonically with increasing n_0 and diverges at a critical finite charge density n_{0c}. Correspondingly, as shown by the solid line in Fig. 8, the critical field η_c, above which

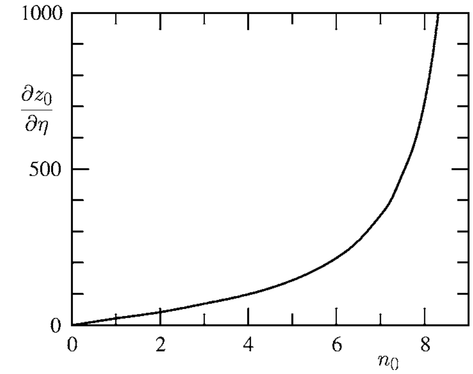

FIG. 7. Slope $\partial z_0/\partial\eta$ of the low-field linear regime of the elongation curve $z_0(\eta)$ as a function of the normalized density of charged lipids n_0, for $\Delta=8\times10^{-3}$ and $R=10$.

the membrane can be infinitely pulled, monotonically decreases, reaching zero at the same critical charge density n_{0c}.

VI. BUDDING INSTABILITY

For a given radius of the frame R and a given electric coupling Δ, the critical charge density n_{0c}, which corresponds to an infinite slope $\partial z_0/\partial\eta$ and a zero critical field η_c, defines the limit of a budding instability of the membrane. For $n>n_{0c}$ and without any applied external field, the flat state becomes unstable: the electrostatic repulsion of the charged lipids of the membrane cannot be anymore counteracted by the tension and the curvature energy. Any fluctuation from the flat state spontaneously grows indefinitely, pulling a membrane tube of lateral size $\simeq\xi$ out of the frame. The stability diagram in the (n_0,R) plane, for $\Delta=8\times10^{-3}$, is shown in Fig. 9. Note that the critical charge density n_{0c} is a monotonic decreasing function of the radius R of the frame, which tends to zero as $R\to\infty$.

VII. DISCUSSION

The behavior of the maximum elongation z_0 as a function of the applied electric field η shown in Fig. 6 resembles the behavior of the force versus length curves when pulling a

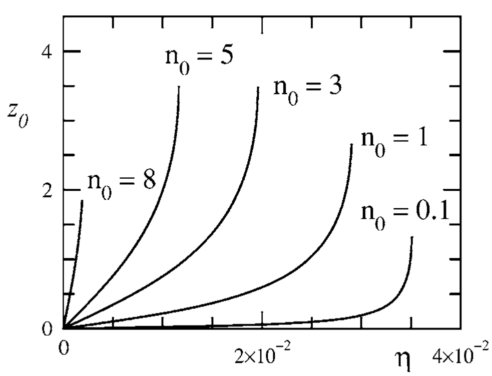

FIG. 6. Maximum elongation of the membrane z_0 (on the rotation axis $r=0$) as a function of the applied electric field η for $\Delta=8\times10^{-3}$, $R=10$, and different surface densities of charged lipids (n_0=0.1, 1, 3, 5, and 8).

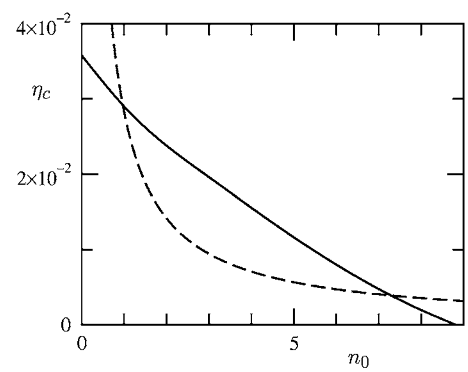

FIG. 8. Critical field η_c as a function of the charge density n_0 for $\Delta=8\times10^{-3}$, $R=10$. Solid line: numerical results. Dashed line: estimation of the critical field using the pointlike pulling force approximation of Eq. (67).

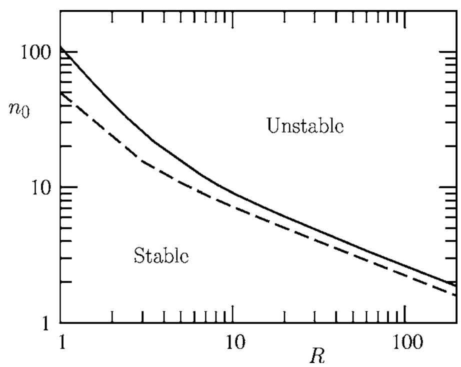

FIG. 9. Stability diagram in the (R,n_0) plane for $\Delta=8\times10^{-3}$. Solid line: numerical results. Dashed line: analytical estimation according to Eq. (74).

membrane attached to a frame with a pointlike force [22,23]. In our case, however, since we cannot control the height of the membrane and determine the corresponding equilibrium force, we are not able to access the negative-slope unstable parts shown in Fig. 1(b) of Ref. [22]. When the applied electric field η exceeds the critical field η_c, our iterative numerical algorithm builds successive nonequilibrium membrane shapes whose total excess free energy with respect to the flat state steadily lowers, while a tube of lateral size of the order of ξ develops. Eventually, the shape self-intersects, causing the algorithm that solves the integral equations giving the density of charge of the membrane to compute an inconsistent answer. This, in turn, determines the failure of the successive iterations, which give more and more diverging and self-intersecting shapes. However, this behavior suggests that, above the critical field η_c, a tether of lateral size ξ is infinitely pulled, as happens with a sufficiently strong pointlike force.

A very simple estimation of the critical field η_c can be obtained by computing the total electric force f that acts on the free part of the membrane when the latter is flat and the surface charge density is constant:

$$f=\frac{\pi R^2 n_0\kappa\eta}{\xi}, \tag{65}$$

where R is the normalized radius of the frame. For a pointlike pulling force, the threshold force f_0 for the formation of a tube is well approximated by the asymptotic result [22]

$$f_0=\frac{2\pi\sqrt{2}\kappa}{\xi}. \tag{66}$$

With these approximations, the critical field η_c for pulling a tether becomes

$$\eta_c=\frac{2\sqrt{2}}{n_0R^2}. \tag{67}$$

As is shown by the dashed line in Fig. 8, this approximation gives only a rather crude estimate of the order of magnitude of the actual critical field: in particular, the latter tends to a finite limit as $n_0\to 0$, instead of diverging, and goes to zero at the finite critical surface density of charged lipids n_{0c}.

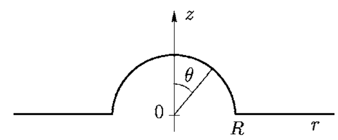

FIG. 10. Spherical cap shape for the estimation of the stability threshold for the membrane budding.

Even more interestingly, we have found that the charged membrane becomes unstable, in the absence of any applied external field, when the surface density of charged lipids, n_0 exceeds a threshold value n_{0c}. To estimate this threshold, let us consider a shape of the membrane in the form of a half sphere of radius R equal to the radius of the frame, as shown in Fig. 10. Neglecting the unphysical discontinuity of the tangent plane at the rim of the frame, the curvature free energy of the half-sphere is

$$\mathcal{F}_b=4\pi\kappa \tag{68}$$

[note that in Eqs. (68)–(73) all variables are not normalized]. Because of the tension γ, the excess surface πR^2 with respect to the flat state gives rise to the excess tension energy

$$\mathcal{F}_t=\gamma\pi R^2. \tag{69}$$

At electric equilibrium, the membrane behaves as a thin conducting sheet carrying, far from the z axis, the total surface charge density σ_0. The electric potential V above the membrane can be easily written, as it corresponds to the superposition of the potential created by a flat sheet of surface charge density σ_0 and the potential of an electric dipole directed along z and located at the center of the sphere:

$$V=\frac{\sigma_0 z}{2\epsilon}\left[\frac{R^3}{(r^2+z^2)^{3/2}}-1\right]. \tag{70}$$

By the Gauss theorem, the corresponding surface charge density of the upper sheet is

$$\sigma_+=\frac{3\sigma_0\cos\theta}{2}, \tag{71}$$

on the spherical cap, where θ is the polar angle with respect to the z axis, and

$$\sigma_+=\frac{\sigma_0}{2}\left[1-\left(\frac{R}{r}\right)^3\right], \tag{72}$$

on the flat part $z=0$, $r\geqslant R$. According to Eq. (60), the corresponding excess electrostatic energy associated with the electrostatic pression exerted by these charges is

$$\mathcal{F}_e=-\frac{\pi R^3\sigma_0^2}{4\epsilon}. \tag{73}$$

Because of the (smaller) electrostatic pression acting inward by the charges σ_- on the lower sheet, the actual gain of electrostatic energy is smaller. However, by dimensional considerations, the charges σ_- only affect the numerical prefactor in Eq. (73). The membrane becomes unstable when the total excess free energy $\mathcal{F}_b+\mathcal{F}_t+\mathcal{F}_e$ becomes negative—i.e., lower than the free energy of the flat membrane. Reintroduc-

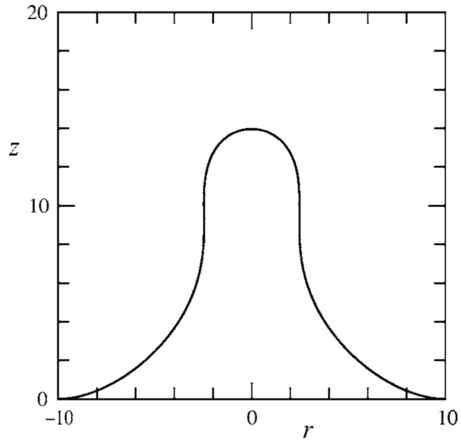

FIG. 11. Nonequilibrium shape obtained by our iteration algorithm after 55 iterations for $n_0=9.2$, $\Delta=8\times10^{-3}$, and $R=10$ in the absence of any external field. The total free energy of this configuration is lower than that of the flat state. The initial condition corresponds to the equilibrium shape for $n_0=8$, $\Delta=8\times10^{-3}$, and normalized electric field $\eta=10^{-3}$.

ing our normalized units, the normalized critical density of charged lipids becomes

$$n_{0c}=2\sqrt{\frac{4+R^2}{\Delta R^3}}. \tag{74}$$

As is shown in Fig. 9, this simple analytical expression gives a reasonable good approximation of the actual instability threshold, particularly for large radii R.

When the density of charged lipids exceeds the critical value n_{0c}, our iterative algorithm, starting from an initial condition slightly perturbed from the flat state, builds a succession of nonequilibrium membrane shapes having a negatively diverging free energy, accompanied by the formation of a tube of lateral size of the order of ξ. An example of a shape obtained during this iteration process, just before that the algorithm fails because of a self-intersecting shape, is shown in Fig. 11. This behavior again suggests that, above the stability threshold n_{0c}, the membrane tends to spontaneously develop a tether of lateral size of the order of ξ.

VIII. CONCLUSIONS

To conclude, we have determined the integro-differential equations governing the equilibrium shape of a charged axisymmetric membrane stretched by a tension γ and submitted to an arbitrary axisymmetric external electric field. For the sake of simplicity, we have neglected the entropy of the charged lipids and the presence of counterions in the solution. By a numerical integration of these equations, we have determined the equilibrium configurations in the case of an infinite membrane, supported by a planar frame having a hole of radius R and submitted to a uniform electric field parallel to the symmetry axis. For low applied fields, the maximum extension z_0 of the membrane—on the symmetry axis—is a linear function of the applied field. At higher fields, the effective spring constant of the membrane becomes weaker. Above a critical field η_c, the tension and curvature restoring forces cannot counteract anymore the external pulling force: our results suggest that above η_c an infinite tether of lateral size of the order of the correlation length ξ is pulled. This behavior resembles the pulling of a tube induced by a pointlike force [22].

The critical field η_c, above which a tether is pulled, decreases monotonically as the density of charged lipids n_0 increases, reaching zero at a critical density n_{0c}. Correspondingly, the low-field slope of the response $\partial z_0/\partial\eta$ monotonically increases and diverges at n_{0c}. For $n>n_{0c}$ the membrane spontaneously buds in the absence of any applied external field. Again, our results suggest that a tether of lateral size of the order of ξ forms. This instability can be qualitatively understood by making a balance between the electrostatic repulsion of the charged lipids and the tension and curvature restoring forces acting on the membrane. This result is consistent with the theoretical finding that, in the presence of salt, the coupling between the charge density and the curvature of a charged membrane could lead to instabilities for sufficiently highly charged membranes [5,7]. In particular, a linear stability analysis for weakly charged membranes showed that, for an infinite flat membrane, the threshold density of charged lipids should go to zero as the Debye length diverges [7]: this is consistent with our zero threshold for an infinite membrane in the absence of counterions.

To make a direct contact with experiments, the entropy of the charged lipids and the presence of the screening counterions in solution should be taken into account, along with the finite size of the membrane. Qualitatively, it is expected that the last two effects correspond to an effective size R of our holding frame. We thus expect that the budding instability survives in the presence of the electrostatic screening; this is also confirmed numerically by the fact that cutting the electrostatic interactions at a distance large with respect to R does not qualitatively alter the results. As for the entropy of the charged lipids, since it reacts against the modulation of the charge density (avoiding, by the way, the unphysical change of sign of n observed around the rim of the frame in Fig. 5), one expects that it should increase the threshold for the budding instability: in fact, it is known that fixed electric charges rigidify the membrane.

ACKNOWLEDGMENTS

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[1] R. Lipowsky and E. Sackmann, *Structure and Dynamics of Membranes, Handbook of Biological Physics*, Vol. 1B (Elsevier, Amsterdam, 1995).
[2] A. Fogden and B. W. Ninham, Adv. Colloid Interface Sci. **83**, 85 (1999).
[3] D. Bensimon, F. David, S. Leibler, and A. Pumir, J. Phys. (Paris) **51**, 689 (1990).
[4] A. Fogden, J. Daicic, and A. Kidane, J. Phys. II **7**, 229 (1997).
[5] V. Kumaran, Phys. Rev. E **64**, 051922 (2001).
[6] A. Voight and E. Donath, in *Biophysics of the Cell Surface*, edited by R. Glaser and D. Gingell (Springer-Verlag, Berlin, 1990).
[7] V. Kumaran, Phys. Rev. Lett. **85**, 4996 (2000).
[8] E. W. Kaler, A. K. Murthy, B. E. Rodriguez, and J. A. N. Zasadzinski, Science **245**, 1371 (1989).
[9] H. Hoffmann, C. Thunig, P. Schmiedel, and U. Munkert, Langmuir **10**, 3982 (1994).
[10] C. Lee and L. B. Chen, Cell **54**, 37 (1988).
[11] H. H. Mollenhauer and D. J. Morré, Histochem. Cell Biol. **109**, 533 (1998).
[12] P. Sens and H. Isambert, Phys. Rev. Lett. **88**, 128102 (2002).
[13] W. Helfrich, Z. Naturforsch. C **28**, 693 (1973).
[14] J.-B. Fournier and P. G. Dommersnes, Europhys. Lett. **39**, 681 (1997).
[15] U. Seifert, K. Berndl, and R. Lipowsky, Phys. Rev. A **44**, 1182 (1991).
[16] L. Landau and E. Lifchitz, *Théorie des Champs* (Mir, Moscou, 1989), pp. 109–111.
[17] C. Pozrikidis, *A Practical Guide to Boundary-Element Methods with the Software Library BEM-LIB* (Chapman & Hall/CRC Press, London, 2002).
[18] U. Ascher, R. M. M. Mattheij, and R. D. Russell, *Numerical Solution of Boundary Value Problems for Ordinary Differential Equations* (Prentice-Hall, Englewood Cliffs, NJ, 1988).
[19] W. H. Press, S. A. Teukolsky, W. T. Vetterling, and B. P. Flannery, *Numerical Recipes in C* (Cambridge University Press, Cambridge, England, 1992).
[20] K. A. Milton, J. Norton, J. Schwinger, Wu-yang Tsai, and L. L. DeRaad Jr., *Classical Electrodynamics* (Westview Press, Philadelphia, 1998).
[21] C.-H. Lee, W.-C. Lin, and J. Wang, Phys. Rev. E **64**, 020901(R) (2001).
[22] I. Derényi, F. Jülicher, and J. Prost, Phys. Rev. Lett. **88**, 238101 (2002).
[23] T. R. Powers, G. Huber, and R. E. Goldstein, Phys. Rev. E **65**, 041901 (2002).