Pattern formation by electro-osmotic self-organization in flat biomembranes

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Pattern formation associated with ionic currents often occurs in biomembranes. The symmetry breaking is interpreted as resulting from an instability driven by the membrane protein segregation. The velocity of a membrane protein under electric field is determined. Two instability mechanisms are sketched in a flat geometry. Critical parameters are determined and compared with experimental data. [S1063-651X(97)13309-8]

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I. INTRODUCTION

A fundamental aspect of the growth physiology lies in the ability of a cell or a group of cells to undergo spatial differentiation. Membrane proteins, as channels and pumps, control the exchanges of molecules between external and internal media. Their densities can be periodically modulated [1] as, for example, in the acetylcholine receptors (AChR) in muscle cells where the characteristic wavelength is of the order of 10 μm. Thermodynamic models can explain such aggregations [2]. But spontaneous pattern formation is often linked to transcellular currents with typical wavelengths in the range 10 μm to 1 cm (see [3] for reviews).

The symmetry breaking may be interpreted as resulting from a dynamical instability (positive feedback effect) linked to membrane protein segregation. The central process, at the origin of the instability mechanism, pointed out by Jaffe [4] is that an electric field induces electromigration of proteins along the membrane, as observed experimentally [5]. Several authors suggested a mechanism where protein motion is induced by electric forces on the protein charges [6,7]. The very nature of the feedback depends on the intrinsic protein charges and various properties such as the transmembrane flux produced by channels and pumps. Another mechanism has been recently proposed [8] where the membrane proteins are dragged along the electro-osmotic flow induced by the motion in the Debye layer close to the membrane. Usually membrane bears negative charges and as a result proteins, even negatively charged, move in the direction of the electric field contrary to the above situation.

In this paper we study and compare, in the same framework and for a flat biomembrane, two instability mechanisms. The protein aggregation is driven either by electric force on its electric charge as in Refs. [6,7] or by electro-osmotic flow [8]. We first derive in Sec. III the general equation for the protein motion. In Secs. IV and V we give the velocity of the protein in the two limits of pure electric force or electro-osmotic flow. In Sec. VI we develop a linear analysis of electrodiffusive equations and determine the criteria and critical wave vectors. Comparison with experimental data is performed in Sec. VII.

II. THE BASIC EQUATIONS

The basic structure of a biological membrane is a lipid bilayer that is mostly an impermeable barrier to the passage of ions. Ionic exchange between intracellular and extracellular media are performed by specific proteins as pumps and channels. Chemical activity of these proteins generates membrane potential by a net transfer of charges across the membrane. In the stationary state and for small ζ potential, electric potential and ionic concentrations are constant outside the Debye layers and proteins in the membrane are at rest with a constant concentration C_{p0}. As the membrane bears charges, ions with opposite charge accumulate close to it in the Debye layer with a characteristic length $\chi^{-1}=(\varepsilon k_B T/e^2 N a \Sigma_j z_j^2 C_{j0})^{1/2}$ depending on the ionic concentrations of different species C_{j0} far from the membrane. If we assume some fluctuation of ionic charge density $\delta\rho$, electric potential $\delta\phi$, and protein concentration in the membrane δC_p, they must satisfy general linearized electrohydrodynamical equations we now briefly recall. Fluctuations of charge $\delta\rho=\Sigma_j N_a z_j e\,\delta C_j$ and electric potential $\delta\phi$ satisfy the Poisson equation

$$\Delta\,\delta\phi_{i,e}=-\delta\rho_{i,e}/\varepsilon \tag{1}$$

and electrodiffusive equation in internal (i) and external (e) media

$$\partial\delta\rho_{i,e}/\partial t=D\Delta\,\delta\rho_{i,e}-D\chi^2\delta\rho_{i,e}, \tag{2}$$

where D is the diffusion coefficient of ions we assume to be the same for all species. Boundary conditions on the membrane are now to be introduced. The first one is the continuity of electrodiffusive flux across the membrane:

$$-D\left[\left(\frac{\partial\delta\rho_{i,e}}{\partial z}\right)+\varepsilon\chi^2\left(\frac{\partial\delta\phi_{i,e}}{\partial z}\right)\right]=\delta I, \tag{3}$$

where δI is the fluctuation of the ionic flux density produced by pumps and channels. I is usually a complex nonlinear function of the membrane potential, concentrations of different ion species, pumps and channels. For simplicity, we consider only one type of mobile protein of concentration C_p (pump or channel) and neglect the membrane potential dependence of I. Then, I has the following expression:

$$I=I_0[(C_p-C_{p0})/C_{p0}] \quad \text{and} \quad \delta I=I_0\,\delta C_p/C_{p0}. \tag{4}$$

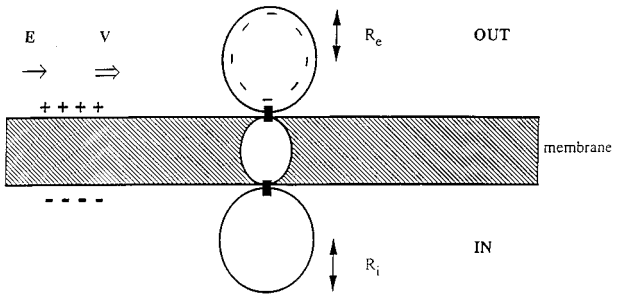

FIG. 1. Modelization of a membrane protein by three parts. For $\chi R_{i,e} \gg 1$, an electric field **E** generates two components of electric force on protein. The first one is linked to electro-osmotic flow **V** induced by the Debye layer close to the membrane: it sweeps the protuberance along the field. The other one is due to negative intrinsic charges of protuberance that induce a sphere motion in the opposite direction.

The two last boundary conditions concern the electric potential. As we shall see in the following, wave vectors of interest will satisfy $kd \ll 1$ (where $d \approx 5$ nm is the sample thickness and $k < 10^7$ m^{-1}). It implies that electric field is constant across the membrane. Then, it is straightforward to link internal and external electric fields (for a detailed discussion, see Ref. [9]),

$$\frac{\partial \delta\phi_i}{\partial z} = \frac{\partial \delta\phi_e}{\partial z}, \tag{5}$$

$$\delta\phi_i - \delta\phi_e = -\frac{\varepsilon d}{2\varepsilon_m}\left(\frac{\partial \delta\phi_i}{\partial z} + \frac{\partial \delta\phi_e}{\partial z}\right), \tag{6}$$

where ε_m is the membrane permittivity and $\phi_{i,e}$ the potential at the membrane surfaces.

The last equation is the conservation of membrane proteins:

$$\frac{\partial \delta C_p}{\partial t} + \nabla \cdot \delta \mathbf{J} = 0. \tag{7}$$

J is characterized by two terms. The first one is the diffusion term $-D_p \nabla \delta C_p$. The second one is the effect of electric field on protein motion we shall now study.

III. PROTEIN MOTION

Protein motion in the membrane results from two different electric field effects: electric force on intrinsic protein charges and electro-osmotic flow due to the charges in the Debye layers. The configuration of the mobile protein may be sketched as the following (Fig. 1): one portion included in the membrane and two protuberances in internal and external media of size $R_{i,e}$ and with charges $z_{pi,pe}$. Typical sizes $R_{i,e}$ are in the range ≈ 0 for pump H^+ bacteriorhodopsine to 10 nm for AChR or Ca^{2+} ATPase pump in the sarcoplasmic reticulum [10]. Let us first recall the electro-osmotic effect. An electric field **E**, parallel to a charged surface, generates a fluid motion **V** known as electro-osmotic flow:

$$\mathbf{V} = -\frac{\varepsilon \zeta}{\eta}(1 - e^{-\chi z})\mathbf{E}, \tag{8}$$

where η is the bulk viscosity and ζ the zeta potential or surface electric potential. Hydrodynamic velocities $\mathbf{V_{i,e}}$ on each side of the biomembrane are then coupled to electric fields through the respective zeta potentials $\zeta_{i,e}$. These potentials are characteristic of the active membrane potential and surface charges due to lipids and other proteins. The second action of an electric field on protein motion is due to their intrinsic charges characterized by electrophoretic mobilities $\mu_{i,e}$ and frictions $\beta_{i,e}^{-1}$. The determination of the protein velocity **U** results from the balance between friction forces $-\beta_m^{-1}\mathbf{U}$ on the membrane portion with electric forces $\beta_{i,e}^{-1}\mu_{i,e}\mathbf{E_{i,e}}$ and friction forces $\beta_{i,e}^{-1}(\mathbf{U} - \mathbf{V_{i,e}})$ on protuberances:

$$\mathbf{U} = \frac{\beta_i^{-1}(\mu_i \mathbf{E_i} + \mathbf{V_i}) + \beta_e^{-1}(\mu_e \mathbf{E_e} + \mathbf{V_e})}{\beta_m^{-1} + \beta_i^{-1} + \beta_e^{-1}}, \tag{9}$$

where $\mathbf{V_{i,e}}$ are the electro-osmotic flow acting on protein protuberances. Einstein relation has provided diffusion coefficient $D_p / k_B T = 1/(\beta_i^{-1} + \beta_e^{-1} + \beta_m^{-1})$.

IV. PROTEIN AGGREGATION DUE TO ELECTRIC CHARGES

A first limiting case corresponds to a localized protein charge: $\chi R_{i,e} \ll 1$. Then, electro-osmosis does not act on protein. Its motion is only due to intrinsic charges,

$$\mathbf{U} = \frac{eD_p}{k_B T}(z_{pi}\mathbf{E_i} + z_{pe}\mathbf{E_e}), \tag{10}$$

where $\mathbf{E_{i,e}}$ are the electric fields at the membrane inside the Debye layer. Equation (7) reads

$$\frac{\partial \delta C_p}{\partial t} = D_p \Delta\, \delta C_p + \frac{eD_p C_{p0}}{k_B T}(z_{pi}\Delta_s \delta\phi_i + z_{pe}\Delta_s \delta\phi_e). \tag{11}$$

Δ_s is the surface Laplacian.

As protein bears usually a negative charge, its motion is opposite to the electric field. However, some proteins like AChR move in the same sense as observed with use of fluorescently labeled ligands. Poo was the first to suggest that electro-osmosis could play a role [11].

V. THE ELECTRO-OSMOTIC LIMIT

The second limiting case corresponds to big macromolecules as AChR exploring the whole Debye layer: $\chi R_{i,e} \gg 1$. Consider for simplicity, protuberances $R_{i,e}$ as spheres uniformly charged. Then, electrophoretic mobilities $\mu_{i,e}$ are simply proportional to zeta potentials of protein $\zeta_{pi,pe}$: $\mu_{i,e} = \varepsilon \zeta_{pi,pe} / \eta$. On the other hand, hydrodynamic flows $\mathbf{V_{i,e}}$ seen by proteins are outside the Debye layer: it means $\chi z \gg 1$ in Eq. (8). Then, Eq. (9) reduces to

$$\mathbf{U} = \frac{D_p}{k_B T}[\beta_i^{-1}(\zeta_{pi} - \zeta_i)\mathbf{E_i} + \beta_e^{-1}(\zeta_{pe} - \zeta_e)\mathbf{E_e}], \tag{12}$$

which can be simplified. Indeed the ζ potential of proteins can be neglected compared to the membrane one, usually bigger than 10 mV. Evaluation of the ζ potential $\zeta_{pe} \approx e z_{pe}/4\pi R^2 \chi \varepsilon$ for acetylcholine receptors gives $\zeta_{pe} \approx -1.6$ mV for a charge number $z_{pe} \approx -16$, a characteristic size $R_e \approx 10$ nm and typical value of Debye length $\chi^{-1} \approx 1$ nm. Friction coefficients $\beta_{i,e}^{-1}$ are given by Stokes formulas for a sphere: $\beta_{i,e}^{-1} = 6\pi\eta R_{i,e}$. Assuming $R_i \approx R_e \approx R$, Eq. (12) reduces to

$$\mathbf{U} = -\frac{6\pi\varepsilon R D_p}{k_B T}(\zeta_i \mathbf{E_i} + \zeta_e \mathbf{E_e}), \tag{13}$$

where $\mathbf{E_i}$ and $\mathbf{E_e}$ are electric fields outside the Debye layer contrary to the previous case.

External ζ potential is usually negative. If we only consider external potential as in electrophoretic experiments, $\mathbf{U}$ and $\mathbf{E_e}$ are now in the same sense contrary to the electric force of Eq. (10). By analogy with Eq. (10), we can define an effective charge $z_{i,e}^{\text{eff}} = -6\varepsilon\pi R\zeta_{i,e}/e \approx +10$ of opposite sign to $z_{i,e}$. These results are in agreement with experiments on AChR [11]. Equation (7) now reads

$$\frac{\partial \delta C_p}{\partial t} = D_p \Delta \delta C_p - \frac{6\pi\varepsilon R D_p C_{p0}}{k_B T}(\zeta_i \Delta_s \delta\phi_i^* + \zeta_e \Delta_s \delta\phi_e^*). \tag{14}$$

Let us emphasize that, contrary to Eq. (11), $\delta\phi_{i,e}^*$ are the potential outside the Debye layer satisfying the simple Laplace equation.

We are now able to explain the instability mechanisms coupling the protein motion to ionic transport through the membrane. Two cases must be taken into account. First, the intrinsic charge of protein drives its motion for small protein $\chi R \ll 1$. In the other limit $\chi R \gg 1$, electro-osmotic case, proteins move under electro-osmosis flow induced by the biomembrane. These two movements are opposite but are relevant in each case with biological results. Let us note that electric potentials in Eqs. (14) and (11) are different.

VI. INSTABILITIES FOR A FLAT BIOMEMBRANE

Linear stability of the system is studied for normal mode fluctuations $\delta u_{i,e} \approx f_{i,e}(z)\exp(\omega t + ikx)$, where $u_{i,e}$ stands for ρ, C_p, and ϕ. ω is the growth rate and k the wave number. Resolution of Eqs. (1), (2), and (11) for pure charge effect or Eq. (14) for the electro-osmotic one provides functions $f_{i,e}(z)$. Dispersion relation $\omega(k)$, derived with the use of boundary conditions (3)–(6), may be simplified in the limits $\varepsilon d k/\varepsilon_m \ll 1$ and $\varepsilon d\chi/\varepsilon_m \gg 1$ and for $k/\chi \ll 1$. In these limits, the two relations have the same general expression in terms of k_0.

$$\omega^2 + \omega\left(D_p k^2 + \frac{\varepsilon d}{2\varepsilon_m} D\chi^2 k\right) + D_p \frac{\varepsilon d}{2\varepsilon_m} D\chi^2 k^2 (k - k_0) = 0, \tag{15}$$

where for the pure electric force limit,

$$k_{01} = \frac{e I_0}{\varepsilon k_B T D \chi^2}(z_{pi} - z_{pe}) \tag{16}$$

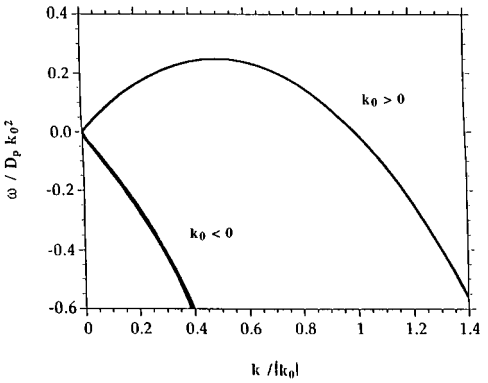

FIG. 2. Dispersion relation $\omega/D_p k_0^2$ as a function of the reduced wave vector $k/|k_0|$ shows the system instability for $k_0 > 0$ and stability for $k_0 < 0$. Conservation of the proteins number in the membrane explains the zero growth rate ω for $k = 0$. The most unstable wave number is $k_0/2$ and the corresponding growth rate is $D_p k_0^2/4$.

and for the electro-osmotic one,

$$k_{02} = \frac{6\pi R I_0}{k_B T D \chi^2}(\zeta_e - \zeta_i). \tag{17}$$

For $k_0 < 0$, the system is stable and returns to the stationary state without any oscillation (Fig. 2). The system becomes unstable for $0 < k < k_0$, i.e., for a membrane with a characteristic size bigger than k_0^{-1}. The growth rate of an homogeneous perturbation $k = 0$ is equal to zero as expected from the conservation of proteins number in the membrane.

For the first case, the instability criterion reduces to

$$I_0(z_{pi} - z_{pe}) > 0. \tag{18}$$

If we only consider the external charge, the criterion reduces to an efflux of ionic current. However, in literature [7] the established criterion is only

$$I_0 z_{pi} > 0. \tag{19}$$

In a flat biomembrane, internal and external electrical resistivities are of the same importance contrary to the cable model, as considered by Fromherz, where only the internal resistivity plays a role. Another difference is the unusual presence of the term in k and not k^2 in the dispersion relation [7]. This is an artifact due to the simplified geometry (flat membrane). Indeed a complete analysis in cylindrical geometry [12] shows that one recovers the criterion (18) and a k dependence in the limit of small wavelength $ka \gg 1$ (where a is the radius of the cell). The other limit $ka \ll 1$ leads to Eq. (19) and ak^2 dependence.

For the electro-osmotic limit, the criterion of instability reads

$$I_0(\zeta_e - \zeta_i) > 0. \tag{20}$$

For characteristic cellular parameters $\zeta_e < 0$ and $\zeta_i > 0$, a pattern occurs if the pumps or channels produce an influx of ionic current. This is the case of AChR. If we only consider

external parameters $\zeta_e<0$ and $z_{pe}<0$ (neglecting the internal one) the criteria (18) and (20) of instabilities are opposite: an influx for electro-osmotic instability and an efflux for the other case. The same remarks on geometrical effect are true for this last instability compared to results given in Ref. [8].

In the following, we only consider the unstable case: $k_0>0$. The most unstable wave number is $k=k_0/2$ and the corresponding growth rate is $\omega=D_p k_0^2/4$ (see Fig. 2). Contrary to the cylindrical case (in preparation), most unstable k is close to the marginal wave number k_0. For $k\gg k_0$, the system returns to equilibrium with the characteristic diffusion time of a protein: $\omega\approx -D_p k^2$. For $k\ll k_0$ and $\chi/k\gg 1$ and for typical protein diffusion coefficient $D_p\approx 10^{-8}$ cm^2 s^{-1}, ω varies linearly with the wave vector: $\omega\approx D_p k_0 k$. Contrary to the cylindrical case, electric characteristics of internal and external bulks do not play a role in a flat biomembrane.

VII. NUMERICAL EVALUATIONS

Let us now estimate the conditions for the appearance of self-focusing of ion pumps set forth above. First of all, it is interesting to compare the respective critical wave vectors k_0 for the two possible regimes: k_{01} for pure electric force case [Eq. (16)] and k_{02} for the electro-osmotic one [Eq. (17)]. Typical values of the zeta potential ζ and the charge number z are respectively of the order of 10 mV and 10. It leads to $k_{01}/k_{02}\approx 1$.

An estimate of the critical wave vector is rather rough due to the lack of precise values of the different parameters. The bulk resistivity is about $1/\varepsilon D\chi^2\approx 2\ \Omega$m [11]. A first estimate of k_{02} [Eq. (17)] is obtained with use of the typical intensity of ionic currents at the end of the instability $I_0\approx 0.5$ to 0.01 A m^{-2} [3]. For $I_0\approx 0.1$ A m^{-2}, the value $k_{02}\approx 10^2$ m^{-1} then obtained does not fit the size of observed patterns (10 μm to 2 cm) well. In fact, the above bulk resistivity gives a value of $D\approx 10^{-5}$ cm^{2}s^{-1}, which is certainly overestimated in cytoplasm. Indeed, it includes all species of ions and not only the one participating to the transcellular flow. In reality, the diffusion coefficients of the typical ions concerned are much lower, as measured experimentally $D_{\mathrm{Ca}}\approx 10^{-7}$ to 10^{-8} cm^2 s^{-1} or $D_H\approx 10^{-6}$ cm^2 s^{-1} [13]. For $I_0\approx 0.01$ A m^{-2} and $D\approx 10^{-7}$ cm^2 s^{-1}, $k_{02}\approx 10^3$ m^{-1}, which gives a more realistic wavelength of the order of 1 mm. Another factor that may interfere with the evaluation of k_{02} concerns the estimation of I_0, which can be very different from the value of the final pattern. Consider the extreme case of a conductance of a voltage-gated channel $\approx 10^4$ Sm^{-2}. For a typical value of membrane potential 0.1 V and $D\approx 10^{-5}$ cm^2 s^{-1}, $k_{02}\approx 10^6$ m^{-1}. These evaluations show that the range of wavelengths by such a mechanism is large and is consistent with cellular sizes (from 10 cm in *Chara corallina* to 10 μm in animal cells).

The growth rate $T\approx 1/D_p k_0^2$ can be estimated with the observed pattern wavelength. The growth rate $T\approx 10^4$ s (≈ 10 h) for $k_{02}\approx 10^4$ m^{-1}, $D_p=10^{-8}$ cm^2 s^{-1}. It is in agreement with the characteristic time of the dipolar ionic currents in Fucus. In confined geometry, growth rate can be different [12].

VIII. CONCLUSION

Growth and development of biological systems seem to be triggered by electric currents. In this paper, we have shown that the transition from a homogeneous state to a spatially ordered structure may be driven by an instability linked to electro-osmosis. This work is supported by three experimental facts. Patterns of proteins are known in biomembranes [1], the electro-osmosis role in the protein transport has been proven [11], and ionic currents are linked to out of equilibrium structures [3]. A clear example is the occurrence of bands in *Chara corallina* green algae or of a dipolar circulation of calcium ions in *Fucus*. We have characterized in the same framework instabilities driven by electro-osmotic flow and intrinsic electric charge drift. We have shown that the order of magnitude of the critical wave vector is of the same order for both instabilities, contrary to the flux criteria which are opposite. Spherical and cylindrical geometries have to be studied in order to compare more precisely with experiments on biological cells [12]. Discrimination between the two mechanisms could be obtained by the incorporation of positively charged lipids into the membrane, which would decrease its ζ potential and reduce the electro-osmotic flow. The experimental study of such instabilities on artificial biomembranes would be of great interest. Let us also note that hydrodynamic contributions that are due to electro-osmosis may also provide unexpected nonlinearities.

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