How Nature Modulates Inherent Fluctuations for Biological Self-Organization – The Case of Membrane Fusion

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Abstract. Biological systems in nano-scale, due to the weak electrostatic interactions and structural connectivity therein, are flexible so that they undergo conformational transition subject to thermal fluctuations and external noises. In the presence of barriers, nature utilizes the fluctuations to give rise to self-organization, typically accompanied by conformational transitions. In two opposing membranes with like-charges, the cooperative coupling between the undulation and charge fluctuations give rise to a dynamic instability to spontaneous growth of the in-phase membrane undulation, and thus a great reduction of the energy barrier to fusion. The multivalent counter-ions, the Ca^{2+} for example, enhance the necessary charge density fluctuation leading to surface charge inversion and over-condensation.

Key words: membrane fusion, conformational transitions, thermal fluctuations, self-organization, charge fluctuations, dynamic instability, charge inversion, over-condensation

Among various levels of biological worlds, we will focus upon the mesoscopic scale over length larger than 1 nm. This is precisely the level of concern to biologists, where cells and their constituent biopolymers like proteins and DNAs, membranes, and other subcellular structures are the main entities on the scene. Consider a cell membrane associated with bound proteins and immersed in a fluctuating, aqueous environment. What we observe here is complex and diverse conformations, yet accompanied with high orders for biological functions, the so called self-organization; lipid self-assembles to form a bilayer and functions as a barrier to permeation of polar molecules and ions, while the proteins fold in the bilayer to form channels for specific ions to cross the barrier.

Then what are the basic physical features of this biological self-organization? First, the biological entities in this mesoscopic level, polymers and membranes, are typical soft condensed matter. Structurally interconnected with monomers, they have collective excitations of energy as low as the order of thermal energy. Such a low energy is also ubiquitous since the relevant interaction in the scale is the weak

electrostatic interaction such as hydrogen-bonding, hydrophobic/hydrophilic, the Van der Waals, and the screened Coulomb interactions mediated by water with the dielectric constant as high as 80. Because of these features, the biological soft matter tends to be susceptible to ambient thermal agitation and external noises. In the vitro situations where it can approach to equilibrium state, it minimizes the free energy by easily changing its shape (conformation) at body temperature. The conformational transitions such as above-mentioned lipid self-assembly, membrane-protein folding and the transition of an ion channel are the examples.

Due to macromolecular nature, and viscous hydrodynamic background, their dynamics is usually dissipative, slow and stochastic. In order to understand the process of self-organization we often study dynamics of barrier crossing where the above-mentioned condensed matter nature will reveal. We recently have been investigating theoretically a number of biological dynamics problems, such as polymer and ion transport through membranes [1, 2], membrane-protein folding [3], and membrane fusion [4]. In all the cases, we have found that, for efficient barrier crossing, the biological systems can modulate conformational transitions, thanks to their fluctuations and flexibility. Below we describe how it can happen for the case of membrane crossing over a fusion barrier.

The membrane fusion, one of most ubiquitous way for transferring molecules from one cell to another, occurs continuously in critical biological processes such as neuro-transmission, infection of enveloped viruses, and fertilization [5]. Also, membrane fusion is an essential step of targeted drug delivery and gene therapy. In physiological situations, two membranes about to fuse are immersed in an ionic solution, and are typically negative-charged, so that, with electrical double layers formed, they are mutually repulsive.

One mechanism for reducing the electrostatic repulsion is development of the negative correlation in distribution of adjacent charges. For example, as recently have been investigated [6], two negatively-charged polymers such as DNA can attract each other due to multivalent cations in solution. They become condensed and distributed on the polymers in the manner to nullify repulsion and even induce attraction. In the case of membrane, however, the story is quite different, due to its different dimensionality (approximately, polymer is one-dimensional, while membrane is two-dimensional). When the two membranes separated at a long distance, they interact via the Van-der Waals attraction. They form a state of adhesion at a shorter distance where this force balances the electrostatic repulsion from the electrical double layers. In bringing the two planar membranes in closer proximity, the energy at least ten times larger than the electrostatic repulsion energy should be spent to take intervening water molecules out [7]. Therefore it has been an enigma how the two apposing membranes can surmount the huge energy barrier for the sake of fusion, which appears to be impossible in view of simple energetics. One universal experimental fact to explain is that vesicles of various types start to fuse above a threshold concentration of calcium ions of millimolar range [8–10].

It is known that the initial stage of membrane fusion is composed of two major kinetic steps: membranes first adhere to the state of inter-membrane potential minimum, called adhesion, often accompanied by fusion protein bridging. And then the membranes merge together due to a certain instability process [7]. In order to study what happens after adhesion, we set out a mesoscopic theory [4, 11] that highlights coupling of two aspects of thermal fluctuation in membranes. One is fluctuating distribution of mobile membrane-bound charges inclusive of the counter-ions condensed on membrane surface, noted above. The other one is undulation of the flexible membrane surfaces, which are excited by thermal agitation. We outline as below our theoretical model [4].

Suppose that two equally and uniformly charged, and flat membranes are brought to adhesion *with their average distance fixed*, and then ask what happens for merging. The free energy of the membranes consists of energy costs for bending and charge non-uniformity that are induced, and for confining two membranes to the fixed adhesion distance, and intra- and inter-membrane Coulomb interactions screened by the ionic environment [4]. The free energy is given in terms of four variables, two surface undulations $h_1(\vec{x})$, $h_2(\vec{x})$ and the surface charge densities $\sigma_1(\vec{x})$, $\sigma_2(\vec{x})$, measured over the reference plane of the membranes described by two dimensional coordinate $\vec{x}$. We now ask how these variables change in a process of approaching the equilibrium, that is, the state of free energy minimum. The charge densities change following two, stochastic diffusion equations of the Cahn-Hilliard type, while the undulations evolve in hydrodynamic interaction with one another through the viscous background fluid. Their coupling occurs in such a manner that the membranes thermally undulate in response to variation of the charge distributions, which, in turn, rely on the membrane configurations. The coupled equations of motion for the Fourier transforms of the four variables, taken to their linear order, are written as $\dot{X}_q = -\Gamma_q X_q(t) + R_q(t)$ where $X_q = (h_1(\vec{q}), h_2(\vec{q}), \delta\sigma_1(\vec{q}), \delta\sigma_2(\vec{q}))$ and R_q is the associated thermal noise which satisfies the fluctuation-dissipation theorem. Here $\delta\sigma_i(\vec{q}) = \sigma_i(\vec{q}) - \bar{\sigma}$ and $\vec{q}$ is the Fourier variable conjugate to $\vec{x}$. The dynamical matrix Γ usually should have positive eigenvalues if it is to describe dissipative, decay dynamics.

For a small range of the wave-number q around $q_m \approx 0.07 \text{ nm}^{-1}$, however, we find that Γ_q can have a negative eigenvalue, γ , in physiological conditions detailed in [4]. It occurs provided that the membranes are overcharged so that $\bar{\sigma}$, the average value of effective surface charge, exceed a critical positive value, $|\bar{\sigma}| > \sigma^*$, as is discussed below. The mode with this negative eigenvalue grows exponentially in time. This eigenmode takes the form of $(u, u, w, -w)$, meaning that the membranes are unstable with respect to growths of in-phase undulation and out-of phase charge coherence with the wavelength $\lambda = \frac{2\pi}{q_m} \approx 90 \text{ nm}$ between the apposed membranes at an adhesion distance.

The Figure 1 illustrates the underlying physics behind the dynamic instability. Initially the two membranes about to fuse are uniformly charged and flat (1-a). As time goes on, the surface charges on a membrane redistribute in correlation with

Figure 1. The process of the dynamic instability (a→c→d) at adhesion. Thick parallel lines are the average centerlines of two apposed membranes. The circled signs represent the effective surface charges. $d = 5$ nm, $\lambda = 90$ nm.

those on the other to reduce the electrostatic interaction (b). Since the membranes are flexible, this out-of phase charge correlation is assisted by in-phase undulation (c, d), to reduce further the electrostatic interaction (by bringing the unlike charges closer and like charges more separated on more curved surfaces).

Now let us discuss the mechanism of the overcharging, $|\bar{\sigma}| > \sigma^*$, that leads to such an instability. We consider the membrane is initially charged with $\sigma_0 = -0.3$ e/nm² in a solution of monovalent ions (such as NaCl) at the concentration of 0.1M and added divalent cations (Ca²⁺). Given these values, we determine the average surface charge densities of the condensed counter-ions and free ions, using the condition of two state equilibria, $\mu_Z^{\text{cond}} = \mu_Z^{\text{free}}$ for each valency $Z = 1$ and 2. The results of calculations are shown in Figure 2. In the absence of divalent cations, the monovalent cations themselves cannot reverse the net effective surface charge density. When the concentration of Ca²⁺ ions is increase to 3 mM, there occurs the charge inversion ($\bar{\sigma} = 0$), leading to over-charging above 4 mM ($\bar{\sigma} > \sigma^*$). These results are consistent with the experimental observations [8–10] that show fusion is facilitated by the addition of Ca²⁺ at milli-molar ranges. Using the analysis as above, we find charge over-condensation of trivalent cations at the concentration as small as μM range (Figure 3), which is in accord with a recent theoretical result [12] and with fusion experiments using La³⁺ and Tb³⁺ [8]. The discontinuity in change of the effective surface charge density shown in Figure 3 is the outcome of a first-order phase transition between the condensed trivalent ion and free ions [11]. This strong effect of multi-valency can be explained as follows. As the bulk concentration of multi-valent cations increases, counter-ion condensation and thus charge fluctuation enhances, yielding a pronounced correlation-induced attraction between multivalent cations and membranes. This attraction further in-

Figure 2. Absolute value of the average effective surface charge density $\bar{\sigma}$ (solid line) and the critical density σ^* (dashed line) as a function of calcium concentration. We consider two apposed membranes with the bare charge density $-\sigma_0 = -0.30 \text{ e/nm}^2$ embedded in a solution of 0.1M NaCl. In the absence of Ca^{2+} , $\bar{\sigma} = -0.14 \text{ e/nm}^2$, whose magnitude is below σ^* . At the concentration $[\text{Ca}^{2+}] = 3 \text{ mM}$, the condensed counter-ions completely neutralize the negative bare charges (downward cusp in the figure), leading to the charge inversion and, upon addition of 4 mM Ca^{2+} , $\bar{\sigma}$ increases to $+0.22 \text{ e/nm}^2$ just above σ^* .

Figure 3. Effective surface charge density as a function of Lithium (trivalent counter-ions) concentration in the bulk. The bare membrane charge density is given by $-\sigma_0 = -0.5 \text{ e/nm}^2$ and the thickness of the condensed counter-ion layer is again assumed to be $l = 10 \text{ \AA}$. The discontinuous changes of net charge take place when trivalent counter-ions of micro-molar concentrations are added.

duces additional counter-ion condensation even after net-charge neutralization and thus gives rise to the over-condensation with a large $\bar{\sigma}$, which is positive.

To conclude, we find that, for concentration of millimolar range, divalent cations, typically calcium ions in solution, induce an unusually strong correlated-charge distribution on each membrane, leading to sign inversion of surface charge density to positive. When the charge fluctuation is cooperatively coupled to the undulation, the two approaching membranes can spontaneously develop sub-micron wavelength ripples in phase and inter-membrane charge correlation out of phase, which grow

within the apposed regions, thereby greatly enhancing electrostatic attraction and reducing repulsion to overcome fusion barrier. This dynamic instability presents a novel example of how nature can modulate diverse fluctuations in soft biological systems to give rise to self-organization, the fusion barrier crossing in this case.

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References

1. Sung, W. and Park, P.J.: Polymer Translocation Through a Pore in a Membrane, *Phys. Rev. Lett.* **77** (1996), 783–786.
2. Lee, K. and Sung, W.: A Stochastic Model of Conductance Transitions in Voltage-Gated Ion Channels, *J. Biol. Phys.* **28** (2002), 279–287.
3. Park, P.J. and Sung, W.: Statistical Mechanics of Membrane Protein Conformation: A Homopolymer Model, *Phys. Rev. Lett.* **80** (1998), 5687–5690.
4. Kim, Y.W. and Sung, W.: Effects of Charge Fluctuation on Two-Membrane Instability and Fusion, *Phys. Rev. Lett.* **91** (2003), 118101.
5. Wilshut, J. and Hoeksra, D. (eds.): *Membrane Fusion*, Marcel Dekker, New York, 1991.
6. Butler, J.C., Angelini, T., Tang, J.X. and Wong, G.C.L.: Ion Multivalence and Like-Charge Polyelectrolyte Attraction, *Phys. Rev. Lett.* **91** (2003), 028301.
7. Israelachvili, J.N.: *Intermolecular and Surface Forces*, Academic Press, San Diego, 1992.
8. Arnold, K.: in Lipowsky, R. and Sackmann, E. (eds.), *Structure and Dynamics of Membranes*, Vol. 2, Elsevier, Amsterdam, 1995.
9. Papahadjopoulos, D., *et al.*: in Ohki, S., *et al.* (ed.), *Molecular Mechanisms of Membrane Fusion*, Plenum Press, New York, 1988.
10. Ohki, S.: in Ohki, S. (ed.), *Cell and Model Membrane Interactions*, Plenum Press, New York, 1991.
11. Kim, Y.W. and Sung, W.: Charge Inversion on Membrane Induced by Multivalent-Counterion Fluctuations, *J. Phys. Condens. Matter* **17** (2005), S2943.
12. Lau, A.W.C., Lukatsky, D.B., Pincus, P. and Safran, S.A.: Charge Fluctuations and Counterion Condensation, *Phys. Rev. E* **65** (2002), 051502.
13. Albert, B., *et al.*: *Molecular Biology of the Cell*, Garland, New York, 1994.