Physical model for the gating mechanism of ionic channels

Cheol-Min Ghim[1] and Jeong-Man Park[2]
[1]*School of Physics, Seoul National University, Seoul 151-747, Korea*
[2]*Department of Physics, The Catholic University of Korea, Puchon 420-743, Korea*
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We propose a physical model for the gating mechanism of ionic channels. First, we investigate the fluctuation-mediated interactions between two proteins imbedded in a cellular membrane and find that the interaction depends on their orientational configuration as well as the distance between them. The orientational dependence of interactions arises from the fact that the noncircular cross-sectional shapes of individual proteins constrain fluctuations of the membrane differently according to their orientational configuration. Then, we apply these interactions to ionic channels composed of four, five, and six proteins. As the gating stimulus creates the changes in the structural shape of proteins composing ionic channels, the orientational configuration of the ionic channels changes due to the free energy minimization, and ionic channels are open or closed according to the conformation thereof.

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Ionic channels are elementary excitable elements composed of protein molecules in the cell membranes of nerve, muscle, and other tissues. They are of great medical and biological importance because ionic channels are responsible for information processing in the nervous system, for coordination of muscle contraction, and they are intimately involved in the transport and secretion of biomolecules in all cells, tissues, and organs of the body [1,2].

Ionic channels are membrane protein complexes. Since membranes, or phospholipid bilayers, build a hydrophobic, low dielectric barrier to hydrophilic and charged molecules, there is a need to use specialized cellular devices that can transport biomolecules in and out through membranes. Ionic channels provide a high conducting, hydrophilic pathway across the hydrophobic barrier of the membrane to biomolecules. The biomolecules that are usually carried in physiological conditions are free ions in solution, typically sodium, potassium, calcium, and chlorine. Ionic channels are formed of glycoproteins that transverse the membrane. They are formed by aggregation of subunits, each a protein by itself, into a cylindrical configuration that allows a pore, forming a kind of tube. Very few ionic channels are open all the time and most have very complex opening and closing mechanisms. In all cases where the channels open and close, the conformation of the channels is changed, sometimes passively and sometimes with energy expenditure.

The conformation change between the closed and open states is called gating, as in opening and closing a gate. Sometimes channels change some physical aspects inside the pore leaving no space for ion flux, and sometimes the change involves a different distribution of charges inside the pore that constrains the passage of charged particles. Ionic channels can be classified according to which chemical or physical modulator controls their gating activity: the voltage-gated channel, the ligand-gated channel, and the mechanosensitive channel. The voltage-gated channels are controlled by the transmembrane potential, opening or closing when charges aggregate and a certain potential threshold is crossed. The ligand-gated channels are controlled by a ligand binding to a receptor in the channels. Receptors consist of sections of a protein, which allow very specific molecules to bind causing it to change conformation. The mechanosensitive channels are controlled by phosphorylation and dephosphorylation of the protein. A phosphorylated protein has more energy and can therefore have a different configuration in shape than that of a dephosphorylated protein causing the opening or closing of the channels.

Through intensive biological works, the first steps towards getting insight into the structure of the pores [3], ion channels [4,5], and gap junctions [6] have been indeed set forward. In Ref. [3], Unwin carried out the crystallographic analysis of electron micrographs of arrays of Torpedo receptors showing that one pore is a pentametric channel composed of five subunits. In Refs. [4,5], MacKinnon and coworkers showed that the KcsA K^+ channel is composed of four subunits and addressed the response of the channel to the changing ionic environment. With these high-resolution structural data, considerable efforts are taken to the massive numerical simulation studies (for a recent review, see Ref. [7]), which show a draft of how channels work at atomistic level. In this paper, we propose one possible gating kinetics of ionic channels, modeling the structure of channel proteins as geometric objects. From the known structures of a few ionic channels, we find the channels are composed of four, five, or six integral membrane proteins. Modeling these integral membrane proteins as cylindrical geometric objects imbedded within the lipid bilayer membrane, we calculate the fluctuation-mediated interaction between membrane proteins using the field theoretic approach [8–11]. Applying this kind of interaction to the tetrametric, pentametric, and hexametric arrangements of ionic channels, we find the channels are open or closed depending on the structural shape of proteins.

We start from investigating the fluctuation-mediated interactions between external objects immersed in a fluctuating medium. When external objects are immersed in a fluctuating medium, they modify the fluctuations in their vicinity and experience induced interactions. The overall strength of the interactions are proportional to the driving energy of fluctuations (k_BT for thermal fluctuations) and their ranges

are related to those of the correlations of the fluctuations. These interactions are mostly independent of the structural details of the objects but are sensitive to the geometry of the objects and their mutual arrangements in the medium. The fluctuating membrane composed of a lipid bilayer has no in-plane shear modulus, and hence the only in-plane deformation energetically relevant is the global compression or stretching of the membrane, which is described by the surface tension. However, being allowed to deform out of plane, the membranes have bending (or curvature) modes, which can be described in terms of the local curvature tensor. Beyond these low-lying modes, general deformation of the free membrane involves volume changes which cost higher energy, but contribute little to the thermodynamics of the membrane. From symmetry considerations, the Hamiltonian of the membrane must be only a functional of the position vector field $\mathbf{R}(\mathbf{x})=(\mathbf{x},\phi(\mathbf{x}))$, invariant under displacements and rotations in the embedding space, and reparametrization invariant [12]. Furthermore, a bilayer that is in equilibrium with amphiphiles in solution can easily adjust its area per molecule by exchanging molecules with this reservoir [13]. That is, the surface tension is no longer relevant and it is therefore the curvature energy that entirely determines the thermodynamic properties of the membrane [14]. Expanding in terms of the curvature and keeping only the relevant terms by the power counting, the most general form of the Hamiltonian $\mathcal{H}_\kappa$ has the following form [15]:

$$\mathcal{H}_\kappa=\int d^2\mathbf{x}\sqrt{g}\left(\frac{1}{2}\kappa(M-M_0)^2+\eta G\right), \tag{1}$$

where g is the determinant of the metric tensor $g_{ab}=\partial_a\mathbf{R}\cdot\partial_b\mathbf{R}$, $M\equiv g^{ab}K_{ab}$ is the local mean curvature, $G\equiv\det K_a^b$ is the Gaussian curvature, and K_{ab} is the curvature tensor of the membrane defined by $\mathbf{N}\cdot D_aD_b\mathbf{R}(\mathbf{x})$ where $\mathbf{N}$ is the unit normal to the membrane and D_a is a covariant derivative. The spontaneous curvature M_0 arises when the two layers of the membrane do not have the same area per molecule or the same number of molecules. The energy cost of deviating from the spontaneous curvature is described by the bending (or curvature) modulus κ. The parameter η, known as the saddle-splay modulus, measures the energy cost of saddlelike deformation. Since the topological changes of the membrane are not considered in our model system, we will drop the Gaussian curvature energy due to the Gauss-Bonnet theorem. Thus, the Hamiltonian of the membrane is given by the bending energy only.

Membrane proteins have bending stiffness quite different from that of the fluctuating membrane. Consequently, the fluctuations of the membrane in the region occupied by inclusions are modified due to the existence of proteins. As a result, the modified fluctuations of the membrane induce the interactions between proteins. To understand how proteins modify the fluctuations and how the modified fluctuations induce the interactions, we need to specify proteins' characteristics and the coupling between proteins' characteristics and that of the membrane. Since the origin of the modification of the fluctuations is the difference in the bending stiffnesses of proteins and the membrane, we take the bending stiffness difference as the characteristics of proteins relative to the membrane, that is, each inclusion is characterized by the deviation of the bending stiffness from the membrane bending stiffness. Using the symmetric-traceless tensor, we characterize the bending stiffness difference in terms of the multipole moments,

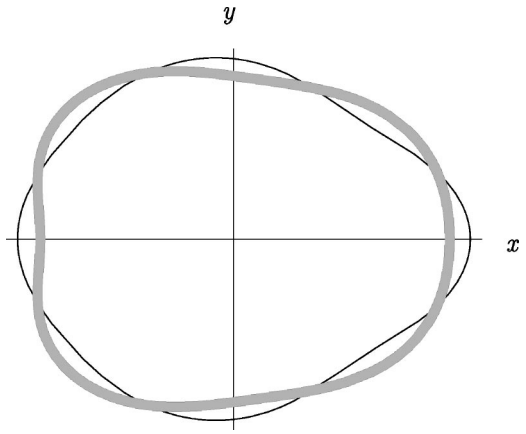

FIG. 1. Characters of inclusions for the given radius function, $r=\sqrt{1+0.1\cos 3\theta+\Delta\cos 4\theta}$. The sharper shape (thin black) is for $\Delta=+0.1$, and the rounder one (thick gray) for $\Delta=-0.1$.

$$Q_n{}^{a_1a_2\cdots a_n}(\mathbf{x})=\frac{1}{\mathcal{A}}\int_{\mathcal{D}}d^2\mathbf{x}'\;P_n{}^{a_1a_2\cdots a_n}(\mathbf{x}')[\kappa(\mathbf{x}')-\kappa_0], \tag{2}$$

where $\mathcal{D}$ and $\mathcal{A}=\int_{\mathcal{D}}d^2\mathbf{x}'$ denote the domain of integration and the area occupied by protein with the area center at $\mathbf{x}$, respectively. $P_n{}^{a_1a_2\cdots a_n}$ is the spherical tensor which is the nth rank symmetric-traceless tensor constructed with the unit vector $\mathbf{x}'/|\mathbf{x}'|$ [16]. Depending on the bending stiffness $\kappa(\mathbf{x})$ of the protein and the cross-sectional geometry $\mathcal{D}$, proteins have different multipolar distributions. For simplicity, we assume that the bending rigidity of proteins, κ_{p}, is constant over $\mathcal{D}$, and the cross-sectional geometry $\mathcal{D}$ is the wedge shape with the symmetry line having a characteristic direction, the locus of which is given by $r^2(\theta)=\mathcal{A}(1+2\Delta'\cos 3\theta+2\Delta\cos 4\theta)$ in polar coordinates (Fig. 1). Then the only nonvanishing multipole moments $Q_n^{a_1a_2\cdots a_n}$ defined above are Q_0, Q_3^{abc}, and Q_4^{abcd}, and these can be expressed as the multiplication of the moment strength and the spherical tensor $P_n{}^{a_1a_2\cdots a_n}$ constructed with the unit vector along the characteristic direction of the protein; $Q_0=\mathcal{A}\kappa_{\mathrm{p}}$, $Q_3^{abc}=\mathcal{A}\kappa_{\mathrm{p}}P_3^{abc}(\hat{\mathbf{n}})$, $Q_4^{abc}=\mathcal{A}\kappa_{\mathrm{p}}P_4^{abcd}(\hat{\mathbf{n}})$. Taking these multipole moments of the bending stiffness difference as characteristics of proteins, we determine the coupling between proteins and the membrane. For a membrane that respects an up-down symmetry, the most general form of the coupling with relevant terms by the power counting is written as

$$\mathcal{H}_{\mathrm{int}}=\int d^2\mathbf{x}\frac{1}{2}K_{ab}(\mathbf{x})\Omega^{abcd}(\mathbf{x})K_{cd}(\mathbf{x}), \tag{3}$$

where $K_{ab}(\mathbf{x})$ is the local curvature tensor of the membrane and the coupling tensor Ω^{abcd} is the fourth-rank tensor com-

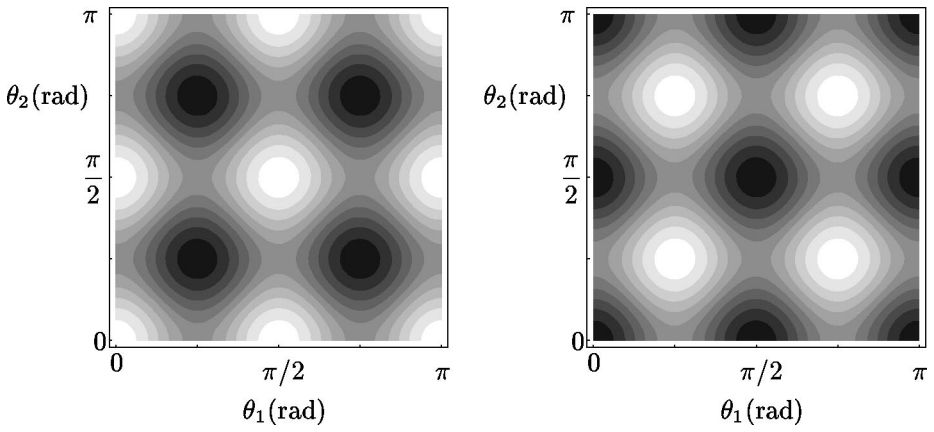

FIG. 2. Evolution of the free energy minima for corresponding values of Δ. The left figure is for $\Delta=0.1$, and the right one for $\Delta=-0.1$.

posed of all possible constructions from the multipole moments with the coupling constants,

$$\Omega^{abcd}=\lambda_4 Q_4^{abcd}+\lambda_0\delta^{ab}\delta^{cd}+\lambda_0'\delta^{ac}\delta^{bd}. \tag{4}$$

The various coupling constants (λ's) describe the strength of the coupling between the multipole moments and the curvature. For simplicity, we assume all these couplings are the same, that is, $\lambda_4=\lambda_0=\lambda_0'\equiv\lambda$.

With the coupling determined above, the free energy of the system is given by

$$e^{-\beta(\mathcal{F}-\mathcal{F}_0)}=\frac{\int \mathcal{D}\phi\, e^{-\beta(\mathcal{H}_\kappa+\mathcal{H}_{\rm int})}}{\int \mathcal{D}\phi\, e^{-\beta\mathcal{H}_\kappa}}\equiv\langle e^{-\beta\mathcal{H}_{\rm int}}\rangle_0, \tag{5}$$

where $\langle\cdots\rangle_0$ denotes the ensemble average over the membrane Hamiltonian only. Using the cumulant expansion and dropping the distance-independent terms, we find the free energy expression for two identical proteins located at **x** and **y**,

$$\mathcal{F}=-\frac{\beta}{4}\Omega^{abcd}(\mathbf{x})\Omega^{ijkl}(\mathbf{y})\langle K_{ab}(\mathbf{x})K_{ij}(\mathbf{y})\rangle_0\langle K_{cd}(\mathbf{x})K_{kl}(\mathbf{y})\rangle_0, \tag{6}$$

where we used Wick's theorem and the symmetry under exchange of the pair of indices $(ij)\leftrightarrow(kl)$. The two-point correlation function of the curvature tensor can be derived from the two-point correlation function of the height fluctuation field. Using the two-point correlation function of the height fluctuation field

$$\langle\phi(\mathbf{x})\phi(\mathbf{y})\rangle_0=\frac{1}{\beta\kappa}(\nabla^4)^{-1}\delta(\mathbf{x}-\mathbf{y})=\frac{1}{16\pi\beta\kappa}R^2\ln(R/\Lambda)^2 \tag{7}$$

for a given distance between two inclusions $R=|\mathbf{x}-\mathbf{y}|$, the two-point correlation function of the curvature tensor is given as

$$\langle K_{ab}(\mathbf{x})K_{cd}(\mathbf{y})\rangle_0=\langle\partial_a\partial_b\phi(\mathbf{x})\partial_c\partial_d\phi(\mathbf{y})\rangle_0=\frac{S_{abcd}(\mathbf{x}-\mathbf{y})}{4\pi\beta\kappa|\mathbf{x}-\mathbf{y}|^2}, \tag{8}$$

with

$$\begin{aligned}S_{abcd}=&8e_ae_be_ce_d-2(e_ae_b\delta_{cd}+e_ae_c\delta_{bd}+e_ae_d\delta_{bc}\\&+e_be_c\delta_{ad}+e_be_d\delta_{ac}+e_ce_d\delta_{ab})+(\delta_{ab}\delta_{cd}+\delta_{ac}\delta_{bd}\\&+\delta_{ad}\delta_{bc}),\end{aligned} \tag{9}$$

where e_a is a component of the unit vector along the $(\mathbf{x}-\mathbf{y})$ direction and can be written as $\mathbf{e}=(\cos\theta,\sin\theta)$. Combining Eq. (8) with Eq. (6), we obtain

$$\begin{aligned}\mathcal{F}&=-\frac{\beta}{4}\Omega^{abcd}(\mathbf{x})\Omega^{ijkl}(\mathbf{y})\frac{S_{abij}(\mathbf{x}-\mathbf{y})S_{cdkl}(\mathbf{x}-\mathbf{y})}{16\pi^2\beta^2\kappa^2|\mathbf{x}-\mathbf{y}|^4}\\&=-\left(\frac{\lambda\kappa_p\mathcal{A}}{\pi\kappa_0}\right)^2\frac{k_BT}{R^4}\left\{1+\frac{3}{32}\Delta\cos[4(\theta_1+\theta_2)]\right.\\&\quad\left.+\frac{1}{64}\Delta^2(\cos4\theta_1+\cos4\theta_2)\right\},\end{aligned} \tag{10}$$

where θ_i is the angle that the characteristic axis of cross section of each protein makes with the distance vector **R**.

This result tells us that the interaction is attractive and falls off as $1/R^4$ which has been obtained by other researchers [17–19]. This long-ranged attractive interaction is responsible for the aggregation of proteins in the fluctuating membrane. In addition to the long-range nature of the interaction, we also find the orientational dependence of the interaction. Depending on the parameter Δ which measures the relative strength of the monopole moment to the hexadecapole moments of the bending stiffness difference, the free energy is minimized at the different configurations of the two inclusions (see Fig. 2). For positive Δ which corresponds to the sharp-edged shape inclusions, the free energy is minimized at the points with $\theta_1=n\pi/2$ and $\theta_2=m\pi/2$ independently. For negative Δ, which corresponds to the dull-wedge shaped inclusions, the free energy is minimized at the points with $\theta_1=(2n+1)\pi/4$, and $\theta_2=(2m+1)\pi/4$ independently.

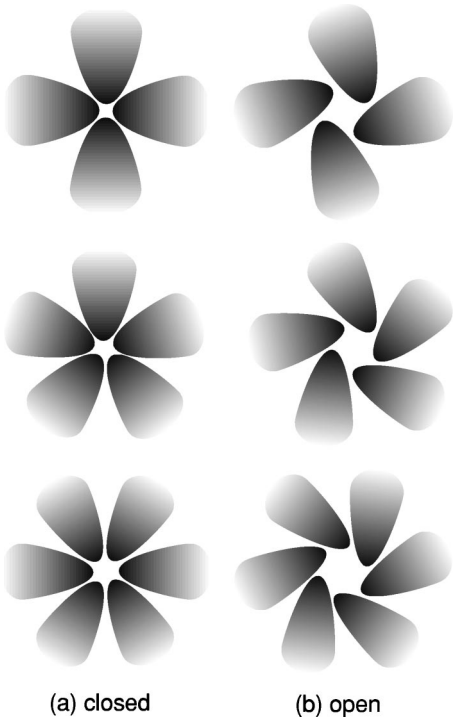

FIG. 3. Conformational gating of protein aggregations for tetrametric, pentametric, and hexametric configurations. As Δ, the relative strength of the monopole to hexadecapole moments, grows across zero, orientational relaxation with steric constraints renders the channel from closed state to open state.

Next, we apply the orientational interaction to the complexes of aggregated proteins which model ionic channels. Assuming that four, five, or six proteins are located at the vertices of square, pentagon, or hexagon, respectively, separated by a distance comparable to the linear size of individual protein, we derive the free energies of ionic channels with four, five, and six proteins. The orientation-dependent part of the free energies are given as

$$\mathcal{F}_4=\frac{1}{8}\{84\Delta\cos(4\delta\theta)-9\Delta^2\cos(8\delta\theta)\},$$

$$\mathcal{F}_5=\frac{1}{100}\{458\Delta\cos(4\delta\theta)-57\Delta^2\cos(8\delta\theta)\},$$

$$\mathcal{F}_6=\frac{1}{288}\{1812\Delta\cos(4\delta\theta)-329\Delta^2\cos(8\delta\theta)\}, \quad (11)$$

where, from symmetry consideration, we used the single variable $\delta\theta$ which measures the deviation angle from the closed state. Minimizing these free energies with respect to $\delta\theta$, we find that the closed state with $\delta\theta=0$ is the stable equilibrium configuration for negative Δ, and the open state with $\delta\theta=\pi/4$ is the stable equilibrium configuration for positive Δ (see Fig. 3). In this calculation, we have dropped the terms of higher order than quadratic in two-body interactions, and all the many-body interactions more than two-body interactions, since three-body interactions are comparable to the third-order two-body interactions, four-body interactions are comparable to the fourth-order two-body interactions, etc., in our calculation and since the third-order two-body interactions are smaller than the quadratic term by $(\beta\kappa)^{-1}$ which is of order 10^{-2} for the cell membranes at room temperature.

We have derived the fluctuation-induced interactions between the two identical inclusions. In addition to the long-ranged distance dependence $1/R^4$, we have found the orientational dependence that determines the configuration of two inclusions according to the relative strength of the monopole and the hexadecapole moments. As the parameter Δ, which measures the relative strength of the moments, changes from negative to positive, the configuration of two inclusions changes from one state to the other for the given distance between inclusions. The change in Δ can be induced from many origins based on the changes in the environment. One possible origin of the change in Δ is due to the aggregation of the ions near proteins. As the extra ions come close to inclusions, the lateral pressure profile of the membrane changes and thereby the cross-sectional geometry and the bending stiffness of the inclusions change [20]. Consequently, according to the change of the cross-sectional geometry and the bending stiffness, the relative strength of the monopole to the hexadecapole moments change. This implies that the channel switching between open and closed states depends on the ion concentration. Moreover, there is a threshold value (corresponding to $\Delta=0$) when the switchings start to occur. This result is consistent with the results found by MacKinnon and co-workers [4,5]. Using high-resolution structure determination of the KcsA K^+ channel, they found that the change in the K^+ ion concentration near the channel induces the conformational changes of the channel and these changes are crucial to the gating mechanism of this channel.

This mechanism might also shed light on the gating kinetics of the voltage-gated ionic channels. The voltage-gated ionic channels are open or closed depending on the potential difference created by the aggregation of ions near the channels. Since the action potential is generated and propagates by the current of ions, the activated potential may correspond to the high concentration of ions near channels. Another possible origin of the change in Δ is the binding of a certain biomolecule to the channel proteins. The best example is the structural shape change of hemoglobin by oxygenation [21]. The hemoglobin molecule consists of two α and two β subunits, each cradling a heme group in an internal pocket and capable of binding an O_2 molecule. As O_2 molecules bind to the heme group, a major quarternary conformational change develops. One pair of $\alpha\beta$ subunits rotates 15° with respect to the other $\alpha\beta$ subunit locking into a new stable ''oxy'' position from a ''deoxy'' position. This deoxy-oxy conformational change might be viewed as an analog of the closed-open gating transition of the ligand-gated ionic channels.

In summary, we proposed a physical model of the gating mechanism of ionic channels. Although we cannot provide an understanding of the aggregation of ions near channels or the binding of ligands to the channel proteins by external stimulus, once the membrane potential is created by the aggregation of ions or the ligand is bound to the channel protein we know the channel proteins might undergo a conformational shape change. According to this conformational

shape change, the relative strength of the monopole to the hexadecapole moments, Δ, changes. As the parameter Δ changes from a negative to a positive value, the state of the ionic channel changes from closed to open and vice versa.

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