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Effect of stochastic gating on the flux through a membrane channel: A steady-state approach

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Abstract

This paper deals with the effect of stochastic gating on the flux of solute molecules through a membrane channel. According to the conventional approach, this flux is given by the product of the flux through the open channel and the probability of finding the channel in the open state. Recently we derived an expression for the flux through a stochastically gated channel (Berezhkovskii and Bezrukov 2017) that showed that the conventional approach may underestimate the flux at fast gating by orders of magnitude. The present work proposes a novel approach to the problem: While our initial derivation of the expression for the flux focuses on the molecule propagator in the channel, here we treat the problem by considering the steady-state flux through an ensemble of identical stochastically gated channels. We show now that the effect of gating on the flux is independent of the gate position, i.e., whether the gate is located at the channel entrance or exit.

1. Introduction

Stochastic gating of membrane channels – spontaneous transitions of channel-forming proteins between their open and closed states – is known for about a half-century. To the best of our knowledge, first experimental demonstrations of voltage-dependent gating in single channels date back to 1969, when Bean and co-authors studied membrane activity of the so-called “Excitability Inducing Material” reconstituted in planar lipid bilayers (Bean et al. 1969). This publication was followed by the work of Ehrenstein and colleagues (Ehrenstein et al. 1970) who showed that the channel open probability can be described by the Boltzmann distribution with the voltage sensitivity characterized by “the interaction strength of 3.3 electron charges”. The concept of stochastic gating is much older; at the very least, it can be traced down to 1952, to the groundbreaking work of Hodgkin and Huxley (Hodgkin and Huxley 1952), where the authors discuss a hypothetical “particle which blocks the flow of sodium ions when it reaches a certain position in the membrane.” Since then stochastic gating was recognized to be the very means by which biological channels respond not only to transmembrane voltage, but also to the changes in other environmental parameters such as ligand concentrations, osmotic pressure, temperature, membrane tension, and membrane lipid composition (Alberts et al. 2008). According to the conventional approach, the flux

through a channel undergoing gating is equal to the product of the flux through the permanently open channel and the probability of finding it in the open state (Hille 2001).

Recently, we found that in the case of fast gating the conventional approach fails as it does not account for important effects (Berezhkovskii and Bezrukov 2017). We showed that when the characteristic time of gating is much smaller than that of molecule diffusion through the channel, the flux is significantly larger than that predicted by the conventional approach. Specifically, the flux increase can reach orders of magnitude if the channel open probability is small. Analysis in Ref. (Berezhkovskii and Bezrukov 2017) is based on the consideration of how gating at the channel entrance – the channel opening that faces the incoming flux – changes the probability for the molecule entering the channel to traverse it and escape through the channel exit. Such a consideration may create an impression that the effect is direction-dependent that would violate detailed balance.

In the present paper we demonstrate that the effect is independent of whether the gate is at the channel entrance or exit, and hence direction-independent. To this end we consider the case of the gate located at the channel exit and derive an expression for the flux, which turns out to be identical to that when the gate is at the channel entrance.

Our derivation in Ref. (Berezhkovskii and Bezrukov 2017) focused on the molecule propagator in the channel. Here we derive the result using a different formalism, namely, by considering the steady-state flux through an ensemble of identical stochastically gated channels. Thus, this paper complements our previous work by providing an alternative approach to the problem.

The outline of the paper is as follows. In the next Section 2 we formulate the problem and provide a summary of the results obtained in Ref. (Berezhkovskii and Bezrukov 2017). The derivation of the expression for the flux in the case of the gate located at the channel exit is given in Section 3. Some concluding remarks are made in the final Section 4.

2. Preliminaries

In this paper we consider transport through membrane channels driven by the concentration difference on the two sides of the membrane, assuming that $c_L = c$ and $c_R = 0$, as shown in Fig. 1. We begin with transport of non-interacting point particles in the absence of gating. In this case the flux, J^{ng} , is given by the product of the of the influx, $k_H c$, and the translocation probability, P_{tr}^{ng} , where the superscript “ng” indicates that the case of no-gating is considered. The Hill rate constant, k_H , determining the influx, describes trapping of point particles freely diffusing in the bulk by an absorbing circular disk on the otherwise reflecting flat wall. This rate constant is proportional to the product of the radius a of the channel opening on the left side of the membrane and the molecule diffusivity in the bulk, D_b . Thus, we have

$$J^{ng} = k_H c P_{tr}^{ng}, \quad k_H = 4aD_b. \quad (2.1)$$

The particle translocation probability through such a channel is given by (Berezhkovskii et al. 2002)

$$P_{tr}^{ng} = \frac{1}{2 + \nu}, \quad (2.2)$$

The term ν in the denominator is determined by the geometric parameters a and L of the channel, as well as the particle diffusivities in the channel and in the bulk, D_{ch} and D_b , respectively,

$$\nu = \frac{4LD_b}{\pi a D_{ch}} = \frac{\kappa L}{D_{ch}}, \quad \kappa = \frac{4D_b}{\pi a} = \frac{k_H}{\pi a^2}. \quad (2.3)$$

Here we have introduced the parameter κ which is used in our further analysis. Finally, we note that, in reality, solute molecules are not point particles. Assuming that these molecules are of spherical shape, we interpret the radius a as an effective radius which is the difference between the channel and molecule radii.

We model stochastic gating by assuming that the gate located at the channel end jumps between its open and closed states as shown in Fig. 2. These transitions are assumed to be Markovian and described by the rate constants α and β (see Fig. 2), which are the inverse of the mean channel lifetimes in the open and closed states, respectively. Conventional (*conv*) approach (Hille 2001) suggests that the flux of solute molecules through a stochastically gated (*sg*) channel, denoted by J_{conv}^{sg} , is the flux through the open channel multiplied by the equilibrium probability of finding the channel open, P_{op} ,

$$J_{conv}^{sg} = P_{op} J^{ng} = P_{op} k_H c P_{tr}^{ng}. \quad (2.4)$$

For Markovian transitions between open and closed states of the channel, the equilibrium probability P_{op} is $P_{op} = \beta / (\alpha + \beta)$. Substituting this into Eq. (2.4), we arrive at the conventional expression for the flux through a stochastically gated channel.

$$J_{conv}^{sg} = \frac{k_H c P_{tr}^{ng} \beta}{\alpha + \beta}. \quad (2.5)$$

The conventional approach, being based on general arguments rather than a rigorous derivation, has a restricted range of applicability. It underestimates the flux when the characteristic gating time, $1/(\alpha + \beta)$, is comparable to or shorter than the characteristic time of solute passage through the channel, L^2/D_{ch} . The first derivation of the flux through a stochastically gated channel, which rigorously accounts for the interplay between molecule intra-channel dynamics and gating, is presented in a recent publication (Berezhkovskii and Bezrukov 2017) for the case of the gate located at the channel entrance (Fig. 2A). The

analysis in this reference shows that the reason why the conventional expression fails is that fast gating affects the translocation probability of the molecules entering the channel. When P_{tr}^{ng} in Eq. (2.4) is replaced by its counterpart in the presence of stochastic gating, P_{tr}^{sg} , the flux at arbitrary gating rate, denoted by J^{sg} , is given by

$$J^{sg} = P_{op} k_H c P_{tr}^{sg}, \quad (2.6)$$

The expression for the translocation probability P_{tr}^{sg} derived in Ref. (Berezhkovskii and Bezrukov 2017) is

$$P_{tr}^{sg} = \frac{\alpha + \beta}{\alpha F_1(\lambda|\nu) + \beta(2 + \nu)}, \quad (2.7)$$

with function $F_1(\lambda|\nu)$ of the form

$$F_1(\lambda|\nu) = \frac{2\lambda\nu + (\lambda^2 + \nu^2)\tanh\lambda}{\lambda(\nu + \lambda\tanh\lambda)}, \quad \lambda = L\sqrt{\frac{\alpha + \beta}{D_{ch}}}. \quad (2.8)$$

Substituting $P_{op} = \beta/\alpha + \beta$ into Eq. (2.6), we arrive at the final expression for the flux,

$$J^{sg} = \frac{k_H c \beta}{\alpha F_1(\lambda|\nu) + \beta(2 + \nu)}. \quad (2.9)$$

The ratio of this flux to its conventional counterpart is

$$\frac{J^{sg}}{J_{conv}^{sg}} = \frac{P_{tr}^{sg}}{P_{tr}^{ng}} = \frac{(\alpha + \beta)(2 + \nu)}{\alpha F_1(\lambda|\nu) + \beta(2 + \nu)}. \quad (2.10)$$

This flux ratio shows that J_{conv}^{sg} provides a good approximation for J^{sg} at slow gating ($\lambda \rightarrow 0$), where $F_1(\lambda|\nu)|_{\lambda=0} = 2 + \nu$ and

$$P_{tr}^{sg}|_{\lambda=0} = 1/(2 + \nu) = P_{tr}^{ng}. \quad (2.11)$$

At fast gating ($\lambda \rightarrow \infty$), J^{sg} is greater than J_{conv}^{sg} because in this limiting case $F_1(\lambda|\nu)|_{\lambda=\infty} = 1$ and

$$P_{tr}^{sg} \Big|_{\lambda \rightarrow \infty} = (\alpha + \beta) / [\alpha + \beta(2 + \nu)] > 1/(2 + \nu) = P_{tr}^{ng}. \quad (2.12)$$

One can see that the flux J^g significantly exceeds its conventional counterpart, J_{conv}^{sg} , when $\alpha \gg \beta$. Under such conditions, the probability P_{tr}^{sg} is close to unity, i.e., the channel is predominantly closed.

Based on general arguments, one expects that transport through a stochastically gated channel is independent of whether the gate is located at the channel entrance or the exit. This is obvious in the conventional approach, where the flux is given by Eq. (2.4). In Ref. (Berezhkovskii and Bezrukov 2017) we derived the expression for the flux in Eq. (2.9) assuming that the gate was located at the channel entrance (Fig. 2A). Here we show that the result for J^g in Eq. (2.9) remains unchanged when the gate is located at the exit from the channel (Fig. 2B). While the derivation in Ref. (Berezhkovskii and Bezrukov 2017) focused on the translocation probability, P_{tr}^{sg} , below we use a different formalism and derive J^g in Eq. (2.9) in the framework of the steady-state picture of the flux through an ensemble of identical channels.

3. Derivation

Consider an ensemble of identical copies of the stochastically gated channel shown in Fig. 2B. To describe transport of diffusing point particles through this ensemble of channels, we introduce one-dimensional steady-state concentrations of the particles in the open (*op*) and closed (*cl*) channels by averaging over the ensemble. These concentrations, denoted by $c_{op}(x)$ and $c_{cl}(x)$ where x is the distance from the channel entrance, $0 < x < L$, are prorated by multiplying the actual concentrations by the equilibrium probabilities of finding the channel in its open and closed states, respectively. Inside the channel, the concentrations $c_{op}(x)$ and $c_{cl}(x)$ satisfy

$$D_{ch} \frac{d^2 c_{op}}{dx^2} - \alpha c_{op} + \beta c_{cl} = 0, \quad (3.1)$$

$$D_{ch} \frac{d^2 c_{cl}}{dx^2} + \alpha c_{op} - \beta c_{cl} = 0, \quad (3.2)$$

subject to the following boundary conditions at the channel ends

$$-D_{ch} \frac{dc_{op}(x)}{dx} \Big|_{x=0} = P_{op} k_H^c - \kappa c_{op}(0), \quad (3.3)$$

$$-D_{ch} \frac{dc_{cl}(x)}{dx} \Big|_{x=0} = P_{cl} k_H^c - \kappa c_{cl}(0), \quad (3.4)$$

$$-D_{ch} \frac{dc_{op}(x)}{dx} \Big|_{x=L} = \kappa c_{op}(L), \quad \frac{dc_{cl}(x)}{dx} \Big|_{x=L} = 0, \quad (3.5)$$

where P_{cl} in Eq. (3.4) is the probability of finding the channel closed, $P_{cl} = 1 - P_{op}$.

To find the concentrations, we introduce auxiliary functions $u(x)$ and $v(x)$,

$$u(x) = c_{op}(x) + c_{cl}(x), \quad (3.6)$$

$$v(x) = \alpha c_{op}(x) - \beta c_{cl}(x). \quad (3.7)$$

These functions satisfy

$$\frac{d^2 u(x)}{dx^2} = 0 \quad (3.8)$$

and

$$\frac{d^2 v(x)}{dx^2} - q^2 v = 0, \quad q^2 = \frac{\alpha + \beta}{D_{ch}}. \quad (3.9)$$

Solutions to these equations are

$$u(x) = A + Bx \quad (3.10)$$

and

$$v(x) = C e^{-qx} + D e^{qx}, \quad (3.11)$$

where factors A , B , C , and D can be determined from the four boundary conditions at the channel ends, Eqs. (3.3) - (3.5).

The concentrations $c_{op}(x)$ and $c_{cl}(x)$ are related to the functions $u(x)$ and $v(x)$ by the relations

$$c_{op}(x) = \frac{\beta u(x) + v(x)}{\alpha + \beta}, \quad (3.12)$$

$$c_{cl}(x) = \frac{\alpha u(x) - v(x)}{\alpha + \beta}. \quad (3.13)$$

Function $v(x)$ vanishes, as both rate constants α and β tend to infinity. As a result, at fast gating Eqs. (3.12) and (3.13) simplify and reduce to

$$c_{op}(x) = P_{op}u(x), \quad c_{cl}(x) = P_{cl}u(x). \quad (3.14)$$

Thus, in this limiting case, the actual (not prorated) steady-state concentrations in the open and closed states are equal to each other and given by $u(x)$, Eq. (3.10), which is a linear function of x , as can be expected.

We use these expressions to find the steady-state flux through the channel

$$J^{sg} = -D_{ch} \left(\frac{dc_{op}(x)}{dx} + \frac{dc_{cl}(x)}{dx} \right) = -D_{ch} \frac{du}{dx} = -D_{ch}B. \quad (3.15)$$

Factor B can be found from the boundary conditions in Eqs. (3.3) - (3.5). The result is

$$B = -\pi a^2 c \beta q K^{-1} (\cosh \lambda + \Lambda q \sinh \lambda), \quad (3.16)$$

where

$$\Lambda = D_{ch}/\kappa \quad (3.17)$$

and

$$K = q[2\alpha \Lambda + \beta(L + 2 \Lambda)] \cosh \lambda + [\alpha(1 + \Lambda^2 q^2) + \beta \Lambda (L + 2 \Lambda) q^2] \sinh \lambda. \quad (3.18)$$

Substituting the above result for factor B into Eq. (3.15), we arrive at the expression for the flux which reduces to that in Eq. (2.9). Indeed, using the relations in Eqs. (2.3), (3.16), and (3.17) one can write the flux in Eq. (3.15) as

$$J^{sg} = k_H cb K^{-1} \Lambda q (\cosh \lambda + \Lambda q \sinh \lambda). \quad (3.19)$$

Then, one can show that the ratio $K[\Lambda q (\cosh \lambda + \Lambda q \sinh \lambda)]$ is identical to the denominator in Eq. (2.9),

$$K[\Lambda q (\cosh \lambda + \Lambda q \sinh \lambda)]^{-1} = \alpha F_1(\lambda|\nu) + \beta(2 + \nu). \quad (3.20)$$

Thus, we have demonstrated that the effect of gating on the flux through the membrane channel is independent of whether the gate is at the channel entrance or exit.

4. Concluding remarks

One of the consequences of the interplay between stochastic gating and the dynamics of the molecules inside the channel is that the gating differently affects transport of molecules with different intra-channel diffusivity. The strength of the effect depends on the relation between the characteristic time of molecule diffusion through the channel and that of the gating. For slowly moving molecules the gating of a given rate can be fast enough to greatly increase their flux against the conventional estimate, Eq. (2.4), while for the molecules with higher diffusivity the same gating can be effectively slow, so that the conventional estimate holds true. This feature was explored in our recent publication (Berezhkovskii and Bezrukov 2018) which proposes the new gating-based mechanism of channel selectivity.

We suggest that this selectivity mechanism can play an important functional role in nature. It is well known that metabolite-specific channels are different from the highly ion-selective channels of neurophysiology by their structural organization. They are mostly channels with the beta-barrel scaffolds which have larger cross-sections of their water-filled pores to accommodate metabolites. In addition to metabolites, these channels also transport ions such as those of potassium, sodium, and chloride. Importantly, due to their smaller size and reduced interactions with the channel walls, these ions translocate much faster than metabolites (Bezrukov 2000). Therefore, large channels are able to jeopardize one of the major functions of biological membrane, namely, to serve as a barrier that keeps contents of cellular organelles compartmentalized. The effect of the stochastic gating on the channel-facilitated transport described in Refs. (Berezhkovskii and Bezrukov 2017, 2018) and the present study constitutes a mechanism by which the unfavorable shunting action of these large channels can be minimized. Indeed, it was shown (Berezhkovskii and Bezrukov 2018) that at a reasonable choice of parameters the selective improvement of transport of slower moving metabolites relatively to that of small ions can reach orders of magnitude.

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Figure 1. Permanently open membrane channel of radius a and length L in the presence of translocating molecules with concentration c at the left side of the membrane and zero at the opposite side.

Figure 2.

Gated channels with different positions of the gate. **A:** The gate is located at the channel entrance, i.e., at the side of the incoming flux. **B:** The gate is located at the channel exit.