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# Stochastic Dynamics of Remote Knock-On Permeation in Biological Ion Channels

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**Abstract**—Brownian dynamics simulations provide evidence for a remote knock-on mechanism facilitating the permeation of a biological ion channel by an ion that is initially trapped at the selectivity filter (SF). Unlike the case of conventional direct knock-on, the second ion that instigates permeation does not need to enter the channel. Nor does it necessarily take the place of the permeating ion at the SF, and it can even be of a different ionic species. The study is based on the simultaneous, self-consistent, solution of the coupled Poisson and Langevin equations for a simple generic model, taking account of all the charges present. The new permeation mechanism involves electrostatic amplification attributable to the permittivity mismatch between water and protein: the arrival of the instigating ion at the channel entrance reduces the exit barrier for the ion trapped at the SF, facilitating escape.

## I. INTRODUCTION

### A. Knock-on permeation of ion channels

The concept of knock-on permeation was introduced by Hodgkin and Keynes [1] to account for their experimental measurements of conduction of  $K^+$  across the membranes of giant axons from the common cuttlefish *Sepia officinalis*, in an era before the channel structure was known. They concluded that the mechanism involved interactions between the  $K^+$  ions and that there were typically 2–3 ions within the channel. They inferred that there was single-file movement in which an arriving ion knocked into the outermost ion on one side of the channel, causing the trapped ion furthest from it to exit from the other side of the channel, and they built a mechanical model to simulate the process. Later, after the structure had been revealed [2], Yeslevskyy and Kharkyanen showed [3] that the knock-on mechanism could involve more than 2 ions in concerted motion through the selectivity filter. Furthermore, they also demonstrated that the optimal conductivity occurs almost as a barrier-less process, thereby helping to account for the long-standing conundrum of how channels could conduct at such a high rate while still being highly selective for particular ions. Nelson subsequently considered 1-step and 2-step knock-on models for  $K^+$  [4]; Corry and Thomas have examined what they call “loosely coupled knock on conduction” in a  $Na^+$  channel [5]. Armstrong and Neyton have also investigated the knock-on process in a  $Ca^{2+}$  channel [6], where it involves just a single ion trapped at the selectivity filter. We have recently

shown that the conduction bands seen in the calcium-sodium family of ion channels [7] involve this almost barrier-less knock-on conduction process [8].

In the course of recent Brownian dynamics (BD) investigations of a simple model channel, we have obtained results that illuminate the permeation process by demonstrating the existence of an additional knock-on mechanism. In particular, as we show below, the ion inside the channel can be knocked-on without the instigating ion from the bath necessarily entering the channel at all. Furthermore, the instigating ion does not immediately take the place of the ion that it knocks on. In what follows we summarise these results; fuller details of the simulations will be presented elsewhere [9].

### B. Simple model of an ion channel

We base our investigations on the generic ion channel model [10], [11] illustrated in Figure 1(a). It consists of a cylindrical hole with protein walls, embedded in the cellular membrane. There is a narrow cylinder of fixed charge around the centre ( $x = 0$ ), representing the negatively-charged protein residues that form the selectivity filter, whose net charge  $Q_f$  provides a binding site for the positive permeating ions. In the present case we set  $Q_f = -0.81e$ , corresponding to the L0 peak in the  $Na^+$  current as seen in BD simulations [8]. We take explicit account of Debye screening for ions in the bath. Only those that come within a screening length (typically  $\lambda_D \sim 5\text{\AA}$ ) of the end of the channel can influence ions within it. This influence can be of great importance because the mismatch of the dielectric constants of the water ( $\epsilon_w = 80$ ) and protein ( $\epsilon_p = 2$ ) causes the channel to act as an electrostatic amplifier of the Coulomb force between ions inside/outside the channel [12]. The dielectric mismatch also creates strong electrostatic forces favoring 1-D axial motion of ions within the channel. Summation of the contributions from the self-potential, fixed charge and externally applied potential difference results in the net potential shown in Figure 1(b). Note that, in addition to the strong binding site in the middle ( $x = 0$ ), there are two additional shallow potential minima forming weak binding sites at the ends of the channel ( $x = \pm 15\text{\AA}$ ). We regard the left-hand bath as the extracellular electrolyte of finite concentration, and the right-hand bath as the intracellular medium where, for convenience, we take the ionic concentration to be zero. We consider permeation events from the bath (channel entrance on the left) into the cell (exit to the right).

### C. Effect of ion-ion interactions

The electrostatic amplification effect means that an ion in the bath can strongly influence the permeation process without entering the channel, provided that it comes within  $\lambda_D$  of the entrance. When there is an ion trapped at the selectivity filter, the proximity of a second ion in the bath outside the channel markedly modifies the trapping potential, as shown by the dashed line in Figure 2 for the extreme case where the ion in the bath has arrived in the weak binding site at the left-hand entrance of the channel. The potential barrier impeding

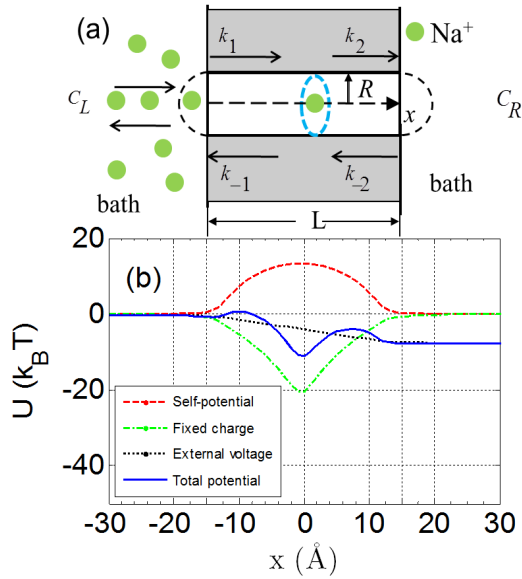


Fig. 1. (a) Simplified model of an ion channel, with the left-hand bath representing the extra-cellular electrolyte and the right-hand bath the interior of the cell. (b) Contributions to the potential energy. At  $x = 0$ , from top downwards, the curves represent: the self-potential; the externally applied potential; the total potential; and the potential resulting from the fixed charge at the selectivity filter. The vertical dashed lines are to indicate the positions of the ends of the channel at  $\pm 15 \text{ \AA}$ .

escape of the permeating ion is significantly reduced, and it is a reasonable inference that escape will usually occur in this configuration. Thus the random arrivals of bath ions at the left entrance constitutes a form of multiplicative shot noise. The escape rate can be calculated [13] using fluctuating barrier theory [14].

#### D. Brownian dynamics simulations

The Brownian dynamics (BD) simulations involved numerical solution of the 1-D over-damped Langevin equation:

$$x' = -Du_x + \sqrt{2D}\xi(t) \quad (1)$$

where  $x$  is the ion's position,  $D$  the diffusion coefficient,  $u$  the self-consistent potential in units of  $k_B T/e$ , and  $\xi(t)$  is normalized white noise. Self-consistent numerical solution of Poisson's equation

$$-\nabla(\varepsilon\nabla u) = \frac{\sum_i e z_i n_i}{\varepsilon_0} \quad (2)$$

was used to find the electrostatic field  $u_x$ . Here  $\varepsilon_0$  and  $\varepsilon$  are the dielectric permittivities of vacuum and the medium (water or protein) respectively,  $e$  is the elementary charge,  $z_i$  is the charge number, and  $n_i$  is the number density of ions. Numerical solution of (1) was implemented with the Euler forward scheme.

To avoid having to simulate ionic dynamics in the bulk electrolyte in the bath, which is demanding in terms of computational resources, we inject ions near the channel entrance at

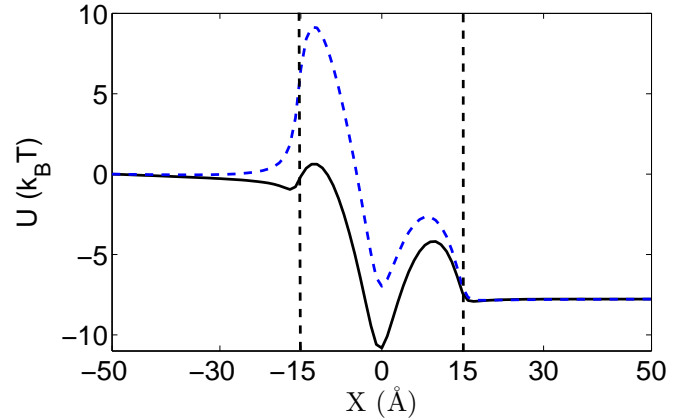


Fig. 2. (Color online) Potential energy along the channel axis, for 200mV applied voltage. The full black line is the potential of a single  $\text{Na}^+$  ion moving along the channel axis. The dashed line is the potential acting on a  $\text{Na}^+$  ion moving along the channel axis when there is one  $\text{Na}^+$  ion at the mouth of the channel. The vertical dash-dotted lines indicate the positions of the ends of the channel.  $\omega_{21}$  is the average inverse time during which the potential remains deep (full curve) before a  $\text{Na}^+$  ion arrives at the left entrance, making the potential shallower (dashed);  $\omega_{12}$  is the average inverse time during which the potential remains shallow, before the  $\text{Na}^+$  ion diffuses away from the channel entrance, thereby returning the potential to its original deep configuration.

the Smoluchowski diffusion rate:  $j_{arr} = 2\pi DR_a C$  [15], [16], i.e. using the rate at which they would naturally be arriving there. They are released on the axis at the point  $x = -22.5 \text{ \AA}$ . Technical details of the BD simulations are given in [17].

## II. RESULTS AND DISCUSSION

After their simulated release near the channel entrance, the majority of the ions diffused away, back into the bath. Sometimes, however, an ion would enter the channel and then continue fluctuating for a while near the main binding site. Eventually, it would exit the channel by thermal activation over the exit barrier. The permeating current, corresponding to the charge passing per unit time, can be calculated. Comparison of the results with predictions based on a modified form of reaction rate theory (RRT) [9] show good agreement over a wide range of bath concentrations  $C_L$  and externally applied potential differences  $V_{app}$  across the channel, as shown in Fig. 3. The grey surface shows how the current  $I$  varies with  $C_L$  and  $V_{app}$ , as calculated by RRT. In the absence of ion-ion interactions we would expect  $I \propto C_L$ , and this is indeed the case for small  $C_L$ . As  $C_L$  increases, however,  $I(C_L)$  rises much more rapidly due the rising importance of ion-ion interactions and the associated onset of stochastic amplification, facilitating permeation by aiding escape of the ion trapped at the SF. The blue data points, representing the BD results, leave the surface and rise even faster; the reason for this discrepancy lies in a very small shift in the average position of the instigating ion near the mouth, revealed by measurements of the conditional probability distribution at the moment of escape (see below).

Our interest is focused mainly on the mechanism through which the permeation event occurs and, in particular, we wish

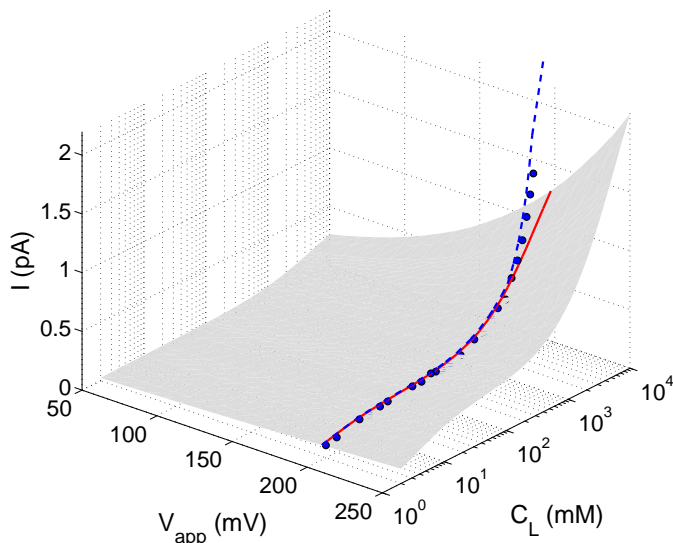


Fig. 3. The permeating current  $I$  as a function of the left-bath concentration  $C_L$  and applied voltage  $V_{app}$ , calculated by modified RRT (the grey surface). The results of BD simulations (blue circles) are shown with  $V_{app} = 200\text{mV}$  for comparison; the red line is a smooth curve drawn through their projection onto the surface, as a guide to the eye.

to know whether any form of knock-on is involved. Because these processes are stochastic, we work in terms of probability distributions. Fig. 4(a) plots the steady state occupancy as a function of position. There is a narrow peak at  $x = 0$  corresponding to individual ions trapped at the selectivity filter, and a broader distribution outside the channel entrance corresponding to the cloud of ions being released in its vicinity (the distribution would be flat if we were relying on the natural Smoluchowski rate to bring ions to the channel). The slight cusp at  $-22.5\text{\AA}$  is an artefact due to the release of the ions occurring at that point.

We are now in a position to ask the question: where exactly is the second ion (whose arrival somewhere near the entrance has facilitated permeation) at the moment when the permeating ion has reached the top of the barrier impeding its escape? Fig. 4(b) provides the answer by plotting the corresponding conditional probability distribution. It is immediately evident that the most probable position of the second ion is *at the entrance*. For the highest concentrations, however, there is also a small but clearly-resolved peak in the conditional distribution at  $x = 0$ .

It appears therefore that the permeation process usually involves the following stages. First, an ion gets attracted into the channel and trapped by the negative charge at the selectivity filter. The probability of escape remains extremely small because the ion is in a deep potential well (full curve of Fig. 2). Secondly, some time later, a second ion arrives in the vicinity of the channel entrance, and its presence lowers the escape barrier substantially (Fig. 2 dashed curve). Thirdly, thermally-activated escape of the permeating ion takes place over the reduced barrier, either almost immediately, or on a

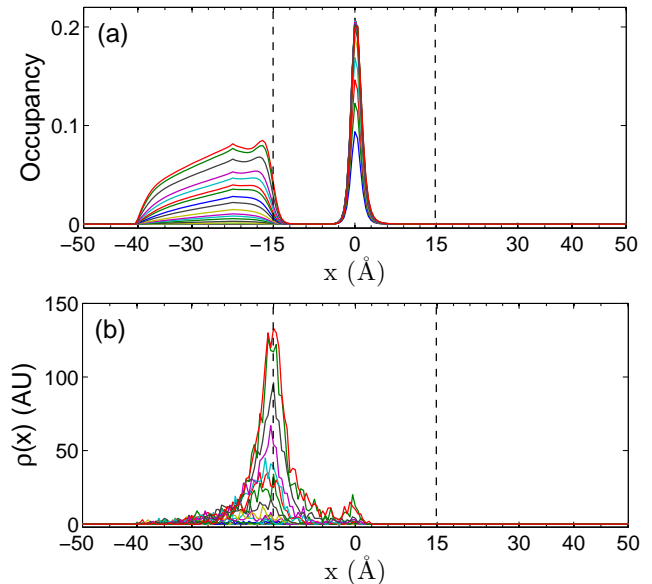


Fig. 4. (Color online) (a) Occupancy plotted as a function of position  $x$ , for different values of the left-bath concentration  $C_L$ , in the range (bottom to top) of  $0.002 - 4.5\text{M}$ . (b) Conditional probability distribution for the second ion at the moment of escape by the first ion, when it passes over the exit barrier (see Figure 2, for the same concentrations). The positions of the ends of the channel are indicated by vertical dashed lines.

later occasion when a bath ion again comes near the entrance. Fourthly, but only on rare occasions under present conditions, the second ion may take the place of the permeating ion as it exits, which is what is indicated by the small central peak at  $x = 0$  in Fig. 4(b).

It seems to us that this relatively improbable fourth process – in which the ion from the bath immediately takes the place of the permeating ion, effectively knocking it over the exit barrier – corresponds to the conventional knock-on mechanism. Under the conditions of the present BD simulations (in particular,  $Q_f = -0.81e$ ), however, it is clearly far more likely that exit takes place while the instigating bath ion is still at the channel entrance, its main role being to reduce the height of the exit barrier. What happens next? The fact that the conditional distribution for the instigating bath ion is centred on the end of the channel suggests that, following the escape of the permeating ion, the instigating ion has a roughly 50% chance of diffusing away from the channel without entering it; but there is also an approximately 50% chance that it will surmount the low barrier at  $x = -15\text{\AA}$  and slide down the potential (full curve in Fig. 2) to the right, moving inwards to become trapped at the selectivity filter ready for the next permeation event.

This remote form of knock-on permeation carries a number of implications, one of the most interesting being that the ion from the bath that instigates the barrier-crossing need not necessarily be of the same species as the permeating ion. Thus the escape of e.g. a permeating  $\text{K}^+$  ion can in principle be stimulated by the presence and Brownian motion of e.g. a

$\text{Na}^+$  ion at the channel entrance. The presence of the  $\text{Na}^+$  ion has a dual effect. First, it lowers the barrier impeding the escape of the  $\text{K}^+$  ion (Fig. 2, dashed curve). Secondly, electrostatic amplification [12] of the thermal fluctuations of the  $\text{Na}^+$  ion adds to the stochastic force responsible for the thermally activated escape of the  $\text{K}^+$  ion.

We infer that these results are quite general. Although the notion of knock-on was introduced in relation to  $\text{K}^+$  channels in which there are usually two ions trapped at the selectivity filter, rather than the one in our generic model, we infer that a very similar mechanism will operate. Again, because of the stochastic amplification effect, knock-on can occur remotely, without the instigating ion from the bath entering the channel. Again, although we have been considering singly-charged ions, the same scenario may be expected to apply to permeation by e.g.  $\text{Ca}^{2+}$  ions but with the potentials (Fig. 2) scaled appropriately. Further work will be needed to establish the effect of altering  $Q_f$ , but it seems likely that there will be a sequence of changes in the relative likelihoods of conventional knock-on permeation and the remote form of knock-on discussed here, depending on whether or not the conditions are met for barrier-less permeation by the coordinated motion of two or more ions.

### III. CONCLUSION

Based on Brownian dynamics simulations, we conclude that an understanding of the knock-on permeation of biological ion channels must take proper account of two important factors: (a) that the process is inherently stochastic; and (b) that there is no need for the bath ion to enter the channel in order to exert a strong influence on the energetics of an ion trapped at the selectivity filter. Taken together, these factors mean that the classical “billiard ball” picture of direct knock-on is incomplete. It appears that a remote form of knock-on is also possible, without the instigating ion entering the channel. We speculate that the relative probabilities of the direct and remote knock-on processes will vary with the fixed charge  $Q_f$  at the selectivity filter.

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