

# "Remote Knock-on", as a New Permeation-enhancement Mechanism In Ion Channels

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## Abstract

We discuss a novel mechanism of enhanced conduction in biological ion channels different from conventional knock-on. It results from charge fluctuations near the channel mouth, amplified by the presence of the protein of the channel walls with low permittivity. We use Brownian dynamics simulations to show that, as in conventional "knock-on" permeation, these interactions can strongly enhance the channel current; but unlike the conventional mechanism the enhancement occurs without the bath ion entering the channel. The transition between these two mechanisms is clearly demonstrated, emphasizing their distinction. A simple model accurately reproduces the observed phenomena. We point out that electrolyte plus protein of low relative permittivity are universal in living systems, so that long-range ion-ion correlations of the kind considered must be common.

## Model

The model considered is a single-file ion channel with cylindrical pathway filled with water and dielectric walls. The length of the channel is  $L$ ,  $R$  is the channel radius, protein permittivity is  $\epsilon_p = 2$  and water permittivity is  $\epsilon_w = 80$ .

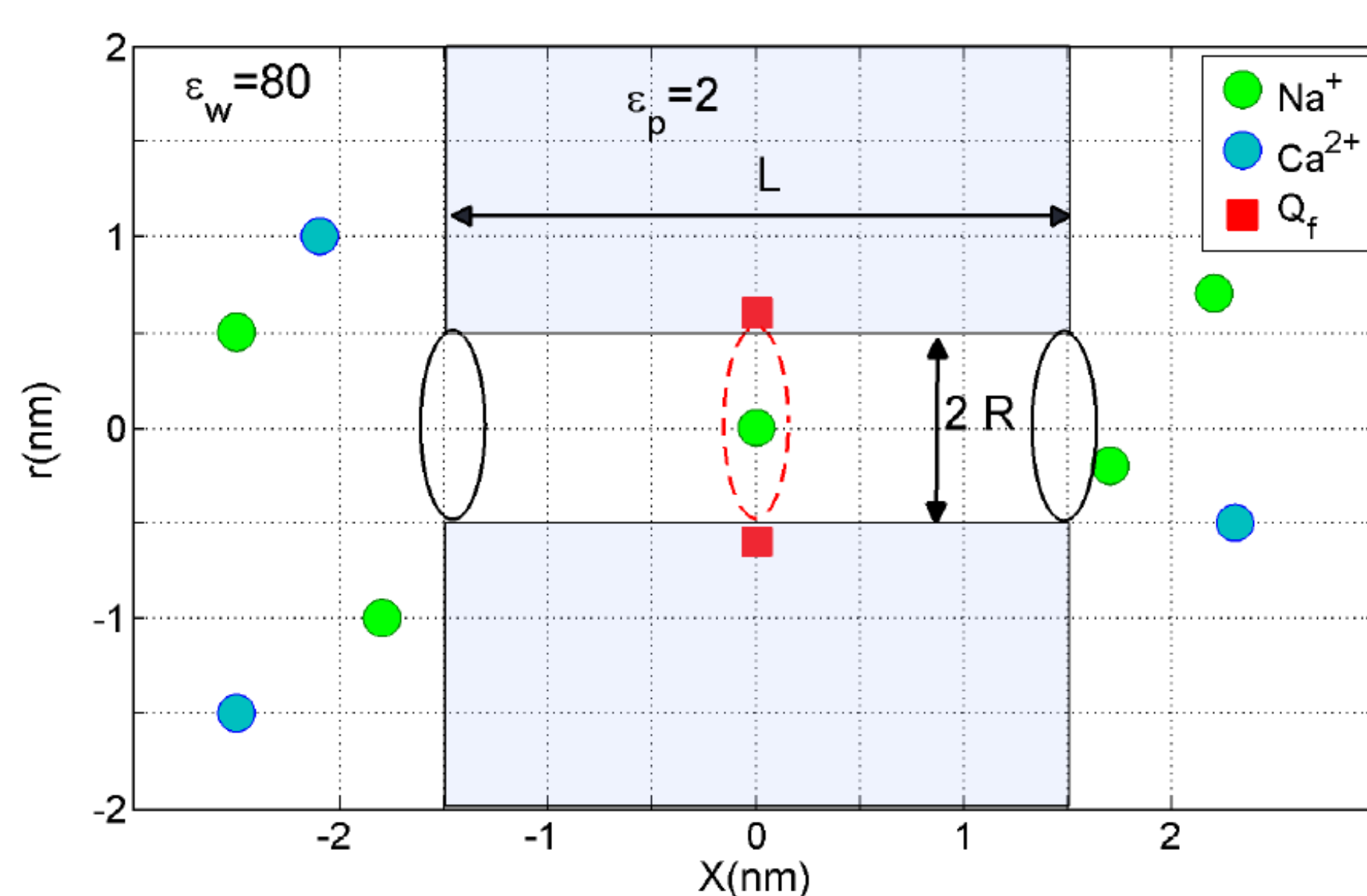


Figure 1. Computational domain for a simple model of the single-site Gramicidin-A like channel. There is a centrally-placed, uniformly-charged, rigid ring of negative charge  $Q_f$  embedded in the wall at  $R_f = R$ . The left-hand bath, modeling the extracellular space, contains non-zero concentrations of ions.

## Standard Reaction Rate

First we consider ion transition through the channel using standard rate theory [1]. If  $P_L$  and  $P_R$  are the populations of the left and right channel mouths,  $P_0$  is the channel population,  $k_{1,2}$  are entrance and exit rates to the right, while  $k_{-1,-2}$  are entrance and exit rates to the left (see figure). Under assumption of singly occupied channel ( $Q_f \leq -1.2e$ ) we have

$$\begin{aligned} \frac{dP_L}{dt} &= j_{ar}^L - k_{ref} P_L - k_1 P_L (1 - P_0) + k_{-1} P_0, \\ \frac{dP_0}{dt} &= k_1 P_L (1 - P_0) - k_{-1} P_0 + k_2 P_R (1 - P_0) - k_2 P_0, \\ \frac{dP_R}{dt} &= k_2 P_0 - k_{-2} P_R (1 - P_0) + j_{ar}^R - k_{dif} P_R. \end{aligned}$$

Under additional simplifying assumptions of  $P_R \approx 0$  and  $k_2$  small as compared to  $k_1$  we have

$$P_0 = \frac{k_1 j_{ar}^L}{k_1 j_{ar}^L + (k_2 + k_{-1}) k_{ref}} \text{ and } J = k_2 P_0.$$

We now show that unlike in standard RR theory current through the channel can be enhanced due long range ion-ion interaction.

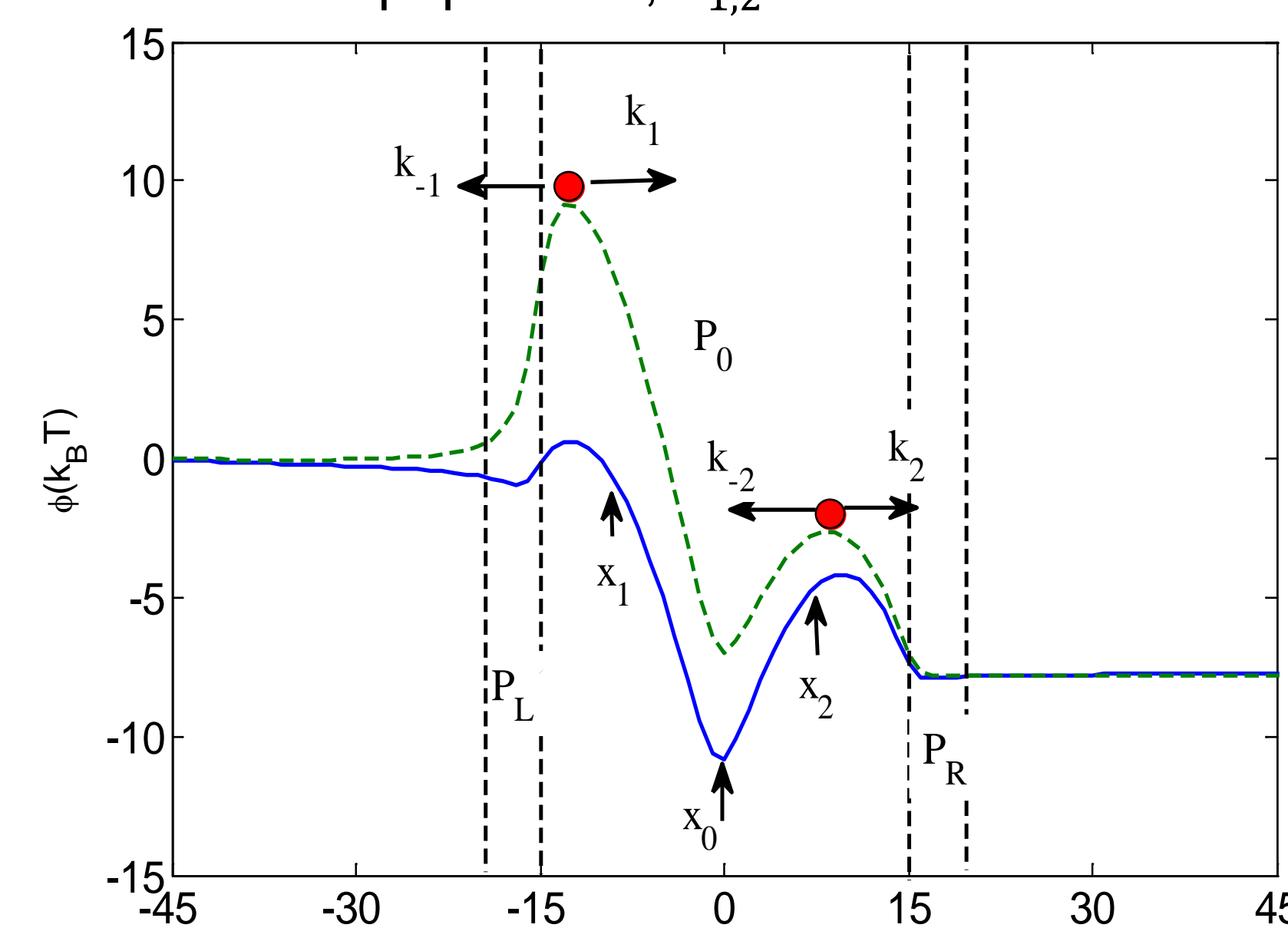


Figure 2. Potential energy profiles  $\phi(x)$  for a channel with a single binding site centred on  $x = 0$ . The full curve shows the unperturbed profile (state 1). The dashed curve shows the modified potential (state 2) due to the presence of a second ion at the channel's left mouth.

## Remote knock-on theory

Consider Langevin equation for the motion of the ion in the channel interacting with the ions outside the channel. Because of the Debye screening we can consider only ions within Debye screening length from the channel entrance. For physiological concentrations there are only two states 1 or 0 ions at the entrance at a time. The channel potentials in these two states are shown in Fig. 2.

$$m\gamma \frac{d\tilde{x}}{dt} = -\frac{d\tilde{\phi}(\tilde{x}, \tilde{t})}{d\tilde{x}} + \sqrt{2m\gamma k_B T} \tilde{\xi}(\tilde{t}); \quad \phi_i(\mathbf{x}) = \phi(\mathbf{x}) + \sum_j \phi_j(\mathbf{x}, \mathbf{x}_j)$$

The diffusion of the ion stochastically modulate potential is described using Chapman-Kolmogorov equation [2] for the probability density  $p_n(x, t|y, t')$  for the ion to exit the channel at the right at time  $t$  starting at  $t' = 0$  at the bottom of the potential barrier.

$$\begin{aligned} \partial_t p_n(x, t|y, t') &= -A_n(y, t') \partial_y p_n(x, t|y, t') - \frac{1}{2} B_n(y, t') \partial_y^2 p_n(x, t|y, t') \\ &+ \sum_m W(m|n, t') [p_m(x, t|y, t') - p_n(x, t|z, t')] \end{aligned}$$

Introducing mean first exit time from the channel  $T_i(y)$  in the form

$$T_i(y) = \int_0^\infty \int_{x_0}^{x_2} p_i(x, t|y, 0) dx dt$$

we obtain equations for  $T_i(y)$  in the form

$$-\frac{1}{m\gamma} U_{1,x} T_{1,x} + \frac{k_B T}{m\gamma} T_{1,xx} - \omega_{21} T_1 + \omega_{21} T_2 = -1; \quad -\frac{1}{m\gamma} U_{2,x} T_{2,x} + \frac{k_B T}{m\gamma} T_{2,xx} - \omega_{12} T_2 + \omega_{12} T_1 = -1$$

The solution of these equations provides required escape rate  $k_2 = 1/T_1(0)$ . The obtained theoretical results will now be compared to the results of Brownian Dynamics simulations.

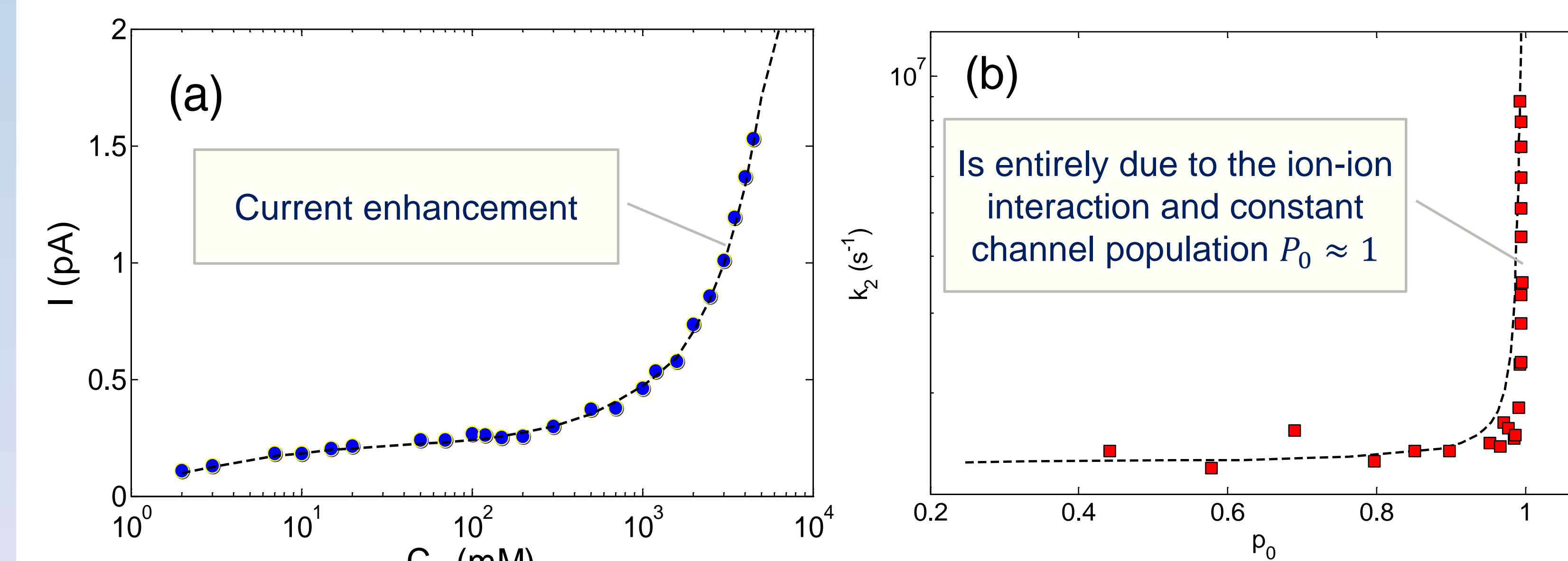
## Brownian Dynamics

Langevin equation was solved numerically using Brownian dynamics simulations. The BD simulation involves solving Poisson equation for each time step of ions in Langevin equation. The Poisson equation coupled to the Langevin equation above

$$\nabla[\epsilon_0 \epsilon \nabla(\phi_i(\mathbf{x}))] = -\sum_{i=1}^N z_i e \delta(\mathbf{x}_i) - e \rho_{fs}(\mathbf{x})$$

was solved using a two-dimensional Poisson solver [3] and stored in a look-up table for all locations of that ion, thus enabling the field for an arbitrary configuration of point charges to be calculated by linear superposition.

## Current enhancement



Some numerical results, obtained with diffusion coefficient  $D = 1.17 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$  in a channel of effective radius  $R = 2 \text{ \AA}$ , length  $L = 30 \text{ \AA}$ , fixed charge  $Q_f = -0.81e$ , and an external potential 200mV are shown by symbols in the figures.

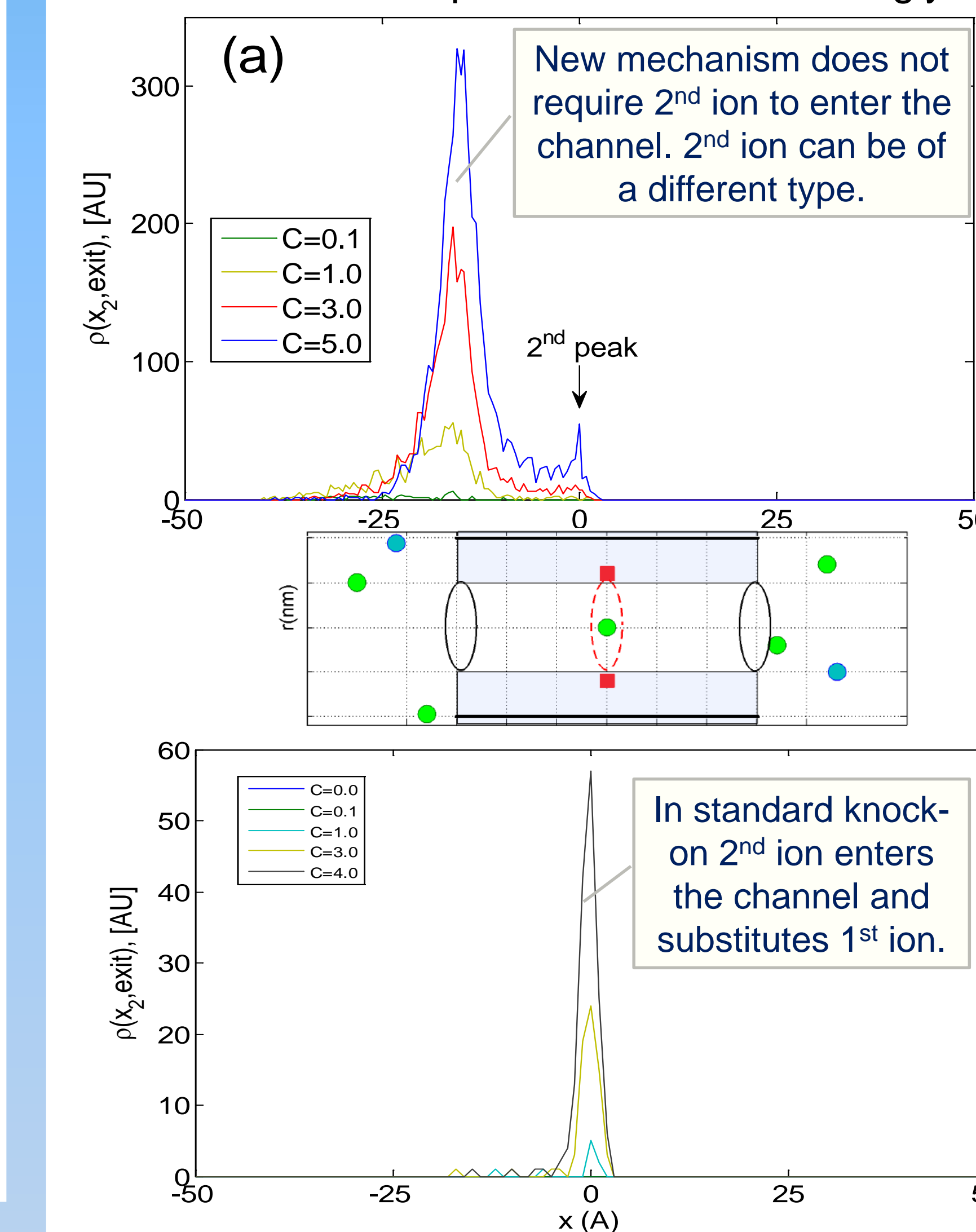
For small enough  $C_L$  the arrival rate of ions to the channel is small and the channel potential remains unperturbed most of the time, the escape rate remains constant, and the current is determined by the variation of the binding site population  $\rho_0$ , which follows the Michaelis-Menten dependence on concentration  $C_L$ . The rapid rise in channel current that occurs with further increase of  $CL$  (and correspondingly of the modulation rate  $\omega_{12} \propto \pi RCLD$ ) is clearly seen in the figures. It occurs for a constant population of the binding site  $\rho_0 \approx 1$ . The current enhancement is then due entirely to the interaction of the ion at the binding site with an ion outside the channel, amplified by focusing of the electrostatic field in the channel, leading to the almost vertical dependence of the escape rate  $k_2$  on  $\rho_0$  shown in left figure.

## New mechanism is different from standard knock-on

To clearly distinguish new mechanism from the standard knock-on mechanism, proposed by Hodgkin and Keynes [4], we note that the latter assumes that the second ion enters the channel, lowers the exit potential barrier for the first ion, the first ion leaves the system, while the second remains in the channel. I.e. effectively the second ion pushes the first one out and substitutes it in the channel. A few features are specific for the standard knock-on mechanism: (i) it involves step when both ions are in the channel; (ii) the second ion remains in the channel when the first leaves it; and (iii) for selective conduction both ions must be of the same type.

To measure the location of the 2<sup>nd</sup> ion during transition we have introduced a new quantity – conditional pdf  $\rho(x) = P(x_2 = x | x_1 > x_{ex})$  that gives the probability to find a 2<sup>nd</sup> ion in the system at the location  $x$  at time instant  $t$  when the 1<sup>st</sup> ion is exiting the system, i.e. is located near the top of the exit potential barrier  $x_{ex}$ .

A clearly resolved peak in the conditional pdf  $\rho(x)$  at the channel entrance can be seen at in the Fig. 3(a). The peak appears at  $CL \approx 0.5M$  and is increasing as a function of concentration, indicating that the exit over the potential barrier is strongly correlated with the presence of the 2<sup>nd</sup> ion at the



channel mouth. The profiles of  $\rho(x)$ , which reflect the ions distribution at the time related to the exit of the ion from the channel, present a maximum in the region comprised between 2–3 Å outside the channel mouth.

Furthermore, a transition to standard knock-on mechanism is also clearly captured by measurements of the  $\rho(x)$ . It can be seen from the figure that a second peak in conditional escape pdf appears at the binding site (at the channel center with  $x = 0$ ) for concentrations above 3M.

It also seems possible that the new mechanism is closely related to the "loosely coupled knock on" process of enhanced selectivity and permeation recently observed by Corry in MD simulations of the NavAb sodium channel [5].

Figure 3. Conditional pdf showing location of the 2<sup>nd</sup> ion during escape for the new mechanism (top) 2<sup>nd</sup> ion is at the entrance and does not enter the channel. For standard knock-on 2<sup>nd</sup> ion is at the center.

## Discussion & Conclusion

- We show using semi-analytic analysis of the archetypal model and BD simulations that a mechanism of enhanced conduction may exist in ion channels that is different from the standard knock-on. The observed ion current enhancement does not require the second ion to enter the channel and allows the interacting ions to be of a different type.
- In the wider context, we note that electrolyte plus protein of low relative permittivity are always present in living systems. As here, this combination can yield long-range correlations of ionic motion comparable with thermal fluctuations in their effect on ion permeation through the complex potential landscapes that arise in molecular biology [6,7]. It seems likely that such charge fluctuations are used by Nature to organize and direct ionic motion in many different contexts.

## References

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