Statistical theory of mixed-valence selectivity in biological ion channels

W.A.T. Gibby*, M.L. Barabash*, C. Guardiani*, D.G. Luchinsky*† and P.V.E. McClintock*

*Department of Physics, Lancaster University, Lancaster, UK, LA1 4YB
Email: w.gibby@lancaster.ac.uk
†SGT Inc., Ames Research Center, Moffet field, CA, 94035, USA
Email: d.luchinsky@lancaster.ac.uk

Abstract—In this article we apply our multi-component statistical and linear response theory to the process of mixed-valence ionic transport in a biological sodium ion channel. We analyse the free energy spectra, and statistical fluctuations (which are proportional to the conductivity) to investigate conditions and optimal parameters required for selectivity.

I. INTRODUCTION

Understanding, predicting, and controlling how ions permeate nanoscale pores is increasingly important in the fields of nano- and biophysics. Ionic transport governs a large and growing number of applications including, but not limited to, DNA sensing [1], water treatment [2] and natural gas purification [3]. In general the pores are designed to fulfil two key criteria: (i) their ability to select amongst ionic species; and (ii) ability to conduct ions at a high rate. Fortunately, biological ion channels which are abundant in nature, have been perfectly optimised by evolution to perform these tasks. Ion channels are holes through proteins, formed from thousands of atoms, and even minor mutations in structure can greatly influence their function and can be associated with disease [4]. Although it is clear that structure is a direct determinant of function, the mechanism through which this occurs has remained unclear and represents a major scientific challenge that we now address.

We will consider mixed sodium $\text{Na}^+$ and calcium $\text{Ca}^{++}$ transport and selectivity through the selectivity filter of a typical biological $\text{Na}^+$ channel with 2 binding sites. In doing so, we will apply our multi-species equilibrium statistical theory [5]–[8], calculating the linear response conductivity from the fluctuations, using generalised Einstein relations. The development of theory in this area has already led to several important theoretical insights. These include the creation of a continuous statistical theory and its relationship with molecular dynamics [9], the analysis of selectivity through density functional theory [10], [11], and multi-species transport in narrow charged nanopores [12]–[15].

II. THEORETICAL APPROACH

A. Defining the system

We consider two bulk reservoirs with aqueous solutions containing ionic species $i \in 1, \ldots, S$, diffusively and thermally coupled via a pore of volume $V_c$ (see Fig. 1 taken from [7]). Under standard conditions the thermal de Broglie wavelength is smaller than system dimensions, and thus the system is classical and described by canonical statistics. The pore itself can be considered as a one-dimensional lattice with $M$ binding sites, and individual site occupancy is characterised by the number $n_{im}$. We further assume that the steric properties of the site prohibit multiple ion occupancy and so $n_{im} \in 0, 1$ for all $m \in 1, \ldots, M$ sites. As a result the total occupancy which is given by the double sum \( \sum_i \sum_m n_{im} \) represents a state of the system. If the sites are assumed to be identical then each state $n_j$, is defined by the total number of ions of each species in the pore and its total set is given by \( \{n_j\} \).

Within the bulk the electrochemical potential can be calculated using Widom’s insertion [18]. It contains contributions from the natural logarithm of the mole fraction $x_i^b$, the electric potential $\phi^b$ and the excess chemical potential $\mu_i^b$. This latter term provides the non-polar (surface tension and excluded volume) and electrostatic (Born and Debye-Hückel) contributions
to the solvation free energy [11], [19], [20]. Meanwhile we can establish two scales of interaction in the confined pore. It is known that pores replete with charge (including total pore charge $Q_f = n_fq$ where $q$ is the proton charge) must reproduce strong electrostatic correlations $E$ [13], dependent on its geometry and total enclosed charge. However on the scale of the binding sites, the ions must also interact locally through short-range interactions $\tilde{\mu}_i^f$ including solvation and bonding [21].

### B. Energy spectrum

The total Gibbs free energy of each state can be expressed by writing the total energy and separating the degrees of freedom from the bulk and pore phases. Thus it reads as,

$$G(\{n_j\}; n_f) = E(\{n_j\}; n_f) - \sum_i n_i \Delta \tilde{\mu}_i$$

$$+ \sum_i kT \ln (n_i)! + kT \ln \left( M - \sum_i n_i \right)!,$$

(1)

where: $k$ and $T$ are the Boltzmann constant and system temperature respectively and $\Delta \tilde{\mu}_i$ is the summation of the difference in excess chemical potential between bulk and pore $\Delta \tilde{\mu}_i = \tilde{\mu}_i^b - \tilde{\mu}_i^f$ plus the natural logarithm of the mole fraction. The final term represents the entropy of mixing for both the ions $n_i$ and empty sites $(n_{w} = M - \sum_i n_i)$. We note that these energetic contributions are in exact agreement with the density functionals that are introduced to investigate ionic transport through quantum dots [23].

Here, we shall consider the form ($E = U_c(n_f + \sum_i z_in_i)^2$), taken from [13], were $U_c$ represents the capacitative charging energy, which is dependent on pore geometry and it takes the value of $\sim 6kT$ in Na$^+$ channels. It is of interest to note that this interaction is identical to that used in electron transport through quantum dots [23].

In (A) and (B) of Fig. 2 we display the free energy profiles for the full configuration of states. We consider $\Delta \tilde{\mu}_{Ca} = 5kT$ and $\Delta \tilde{\mu}_{Na} = 4kT$ and $-20kT$ for (A) and (B) respectively. Solid blue and red lines represent pure Ca$^{++}$ and Na$^+$ states, whereas the mixed species states are presented in green. Each curve is parabolic in nature due to its quadratic dependence on $n_f$, and minimises when the total charge is neutralised i.e. equal to zero. Due to the difference in valence both $E$ and $\Delta \tilde{\mu}_i$ govern the selectivity because these parameters translate the curves vertically and horizontally. To observe this let us consider the differences between (A) and (B). When $\Delta \tilde{\mu}_{Na}$ is positive and comparable to $\Delta \tilde{\mu}_{Ca}$, the Ca$^{++}$ levels are vertical translated when $n_f$ is small due to the large electrostatic barrier; and hence unfavoured. Consequently the pure Na$^+$ crossings occur at a lower energy (highlighted for the 0-1 transition by the purple circle at $n_f \sim -0.1$). As this parameter is made strongly negative it imposes a blocking barrier for Na$^+$ which ensures that the pure Ca$^{++}$ crossings now occur at a lower energy and shifted value of $n_f$.

### C. Statistical fluctuations and conductivity

The statistical fluctuations can be computed from the grand potential ($\Omega = -kT \ln Z$) which reads as follows,

$$\Omega = -kT \ln \left( \sum_{\{n_j\}} \frac{1}{n_{w}!} \prod_i \frac{1}{n_i!} \right) \exp \left( \sum_i \Delta \tilde{\mu}_i^b - E(n_j; n_f) \right),$$

(2)

which includes the average number of ions in the pore of species $i$ at constant system temperature and volume $V$,

$$\langle n_i \rangle = -\frac{\partial \Omega}{\partial \tilde{\mu}_i^f} \bigg|_{T,V},$$

(3)

together with the variance and covariance [20], [24],

$$\langle (\Delta n_i)^2 \rangle = \frac{\partial^2 \Omega}{\partial \tilde{\mu}_i^f \partial \tilde{\mu}_j^f}, \quad \langle n_i n_j \rangle - \langle n_i \rangle \langle n_j \rangle = \frac{\partial \langle n_i \rangle}{\partial \tilde{\mu}_j^f},$$

(4)

respectively. Here, $\eta^c$ is the chemical potential in the pore which, at equilibrium, is equal to its bulk counterpart.

In multicomponent mixtures, non-equilibrium thermodynamics states that the Fickian diffusivity $D_{ij}$ is related to the flux density $j_i$ [15] via,

$$j_i = -\frac{S}{z_i} q \left( D_{ii} \nabla c_i + \sum_{j \neq i} D_{ij} \nabla c_j \right) - \sum_{i=1}^S \frac{q}{z_i} \nabla \phi. \quad (5)$$

Here, $c$ is the concentration in the pore ($c_i = \langle n_i \rangle / V_c$). Cross diffusivities $D_{ij}$ represent the ability for one species type to conduct in the presence of the concentration gradient for the other ion, and are equal to zero in independent solutions. We have shown in earlier work [5], [6], and it follows closely from [25], that the conductivity $\sigma$ can be calculated under linear response conditions. If we assume that the kinetic properties of the ions are comparable such that $D_{ii} \sim D_{jj} \sim D_{ij}$, then the total conductivity through the pore at linear response
$\sigma = \sum_{i=1}^{S} \sigma_i$, is proportional to the variance in total particle number i.e.

$$\sigma \propto \left( \sum_i S \frac{\partial \langle n_i \rangle}{\partial \eta_i^c} + \sum_{j \neq i} S \frac{\partial \langle n_j \rangle}{\partial \eta_i^c} \right). \tag{6}$$

III. ANALYSIS

To investigate the selective properties of the pore we should also analyse both the mean number of ions (occupancy) of Na$^+$ (blue) and Ca$^{++}$ (grey) in Fig. 3, and the variance of total particle number in Fig. 4 (which is proportional to the conductivity Eqn. (6)), vs. $\Delta \mu_{Na}$ and $n_f$, with $\Delta \mu_{Ca} = 5kT$.

Occupancy forms a staircase, where plateaus correspond to integer values of ions in the pore. As $n_f$ becomes more negative the total number of ions residing in the pore increases because of the increased electrostatic attraction to the pore. As we have already seen the selectivity is defined by both $E$ and $\Delta \mu$. To observe this let us consider $n_f = -2$ because the electrostatic interaction can then be neutralised with either 1 Ca$^{++}$ ion or 2 Na$^+$ ions. Consequently when $\Delta \mu_{Na} \lesssim \Delta \mu_{Ca}/2$ the pore favours Ca$^{++}$. Non-selectivity occurs when each of the species states are energetically indistinguishable and so the vertical steps for each species converge. An example of this occurs when $\Delta \mu_{Na} \sim -4kT$ and $n_f \sim 0.8$.

As shown in Fig. 4, the variance in particle number forms a complicated structure with two sets of peaks ($T_1$ and $T_2$) corresponding to 0-1 and 1-2 transitions, that are separated by a region of negligible conductivity. Peaks form at the midpoints of the vertical occupancy steps where there is a change in total number of ions in the pore, i.e. a step in the total occupancy. This result is intuitively clear, because these points correspond to degeneracies in the system, when adding or removing an ion does not cost any energy. Away from these points there is a corresponding energy barrier impeding conduction. As a result we can establish two conditions on that must be satisfied for optimal conductivity: (i) the pore must be degenerate i.e. $\Delta G \sim 0$ and (ii) both states must correspond to the favoured occupancy’s of the pore i.e. lowest free energy (see purple circles in Fig. 2). Since this occurs between neighbouring states it also corresponds directly to knock-on conduction. Collectively these phenomena are known as ionic Coulomb blockade a classical analogy \cite{26}–\cite{28} of the process observed in electron transport \cite{23}.

The structure of the peaks differs because $T_2$ allows for conduction involving mixed states. As a result, it can produce Na$^+$ conduction between the pure (1Na$^+$ and 2Na$^+$) and mixed (1Ca$^{++}$ and 1Ca$^{++}$1Na$^+$) states and pure Ca$^{++}$ conduction. Mixed state conduction between these states has been qualitatively observed in simulations of Na$^+$ channels. In \cite{29}, the system was initiated with both Na$^+$ and Ca$^{++}$ present in the pore, and Na$^+$ was shown to bypass the resident Ca$^{++}$ ion, in a similar manner to \cite{30}. Finally we must note that the branch parallel to $n_f$ appears to suggest an anomalous conduction event. This branch occurs when $-1.4 \lesssim n_f \lesssim -2.1$ and $\Delta \mu_{Na} \sim -2.8kT$, and corresponds to the degeneracy between the 1Ca$^{++}$ and 2Na$^+$ states. As a result conduction does not occur via the knock-on mechanism. It’s importance and implications will be further explored in future work.

These properties can be combined to generate a phase diagram, further helping to elucidate the picture, Fig. 5. The coloured blocks correspond to the occupancy and are labelled, meanwhile the purple regions denote instability because the pore can reside in multiple states of different numbers of particles. As a result there is a quasi-phase-boundary separating different locations of non-zero conductivity. The blue and orange lines correspond to the midpoint of the Na$^+$ and Ca$^{++}$ occupancy step, and the black line corresponds to the maximal conductivity. It is clear that the pore switches from Na$^+$ to Ca$^{++}$ conduction when $\Delta \mu_{Na} \lesssim -6kT$, and that mixed state conduction occurs when the mixed state is the
favoured 2 ion state which occurs when $-2.1 \lesssim n_f \lesssim -2.7$.

IV. CONCLUSION

In conclusion, we have investigated the mixed-valence transport properties of narrow ion channels, through analysis of a multi-component statistical and linear response theory. In particular we have focused on Na$^+$ vs. Ca$^{2+}$ selectivity in a typical biological Na$^+$ channel. Strong selectivity is observed in both the variance in total particle number and the mean number of ions in the pore. This is determined by both the electrostatic interaction because the ions have a differing valence, and also the local interactions. We can identify two conditions required for optimal selective transport: (i) the pore must be degenerate i.e. $\Delta G \sim 0$ and (ii) both states must correspond to the energetically favoured occupancy of the pore i.e. lowest free energy. Therefore, it is possible to predict the pore changes required to shift the selectivity and/or the conduction mechanism.

In future we will further analyse the far-from-equilibrium regime through the development of a master equation model. Finally we expect that the proposed formalism should also be applicable to other artificial nano-pores or biological channels.

REFERENCES


