

# Kinetic model of selectivity and conductivity of the KcsA filter

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**Abstract**—We introduce a self-consistent multi-species kinetic theory based on the structure of the narrow voltage-gated potassium channel. Transition rates depend on a complete energy spectrum with contributions including the dehydration amongst species, interaction with the dipolar charge of the filter and, bulk solution properties. It displays high selectivity between species coexisting with fast conductivity, and Coulomb blockade phenomena, and it fits well to data.

## I. INTRODUCTION

Narrow biological ion channels allow the permeation of ions at near to the diffusion rate, whilst being highly selective. Paradoxically, potassium ( $K^+$ ) channels allow conduction at close to  $10^8 s^{-1}$ , coexisting with high selectivity amongst mono-valent species [1].

The general permeation properties through the filter are preserved among all voltage-gated  $K^+$  channels due to the conserved amino acid structure of the filter [2]. Experimentally it is known that conduction occurs via the single-file concerted motions of ions separated by a water molecule, such that we have transitions between 2 and 3 ions [3], [4]. The filter charge  $Q_f = n_f q$  plays an important role in the permeation process. It is attributed to the dipolar interactions with the oxygen atoms in the permeation pathway, and these 20 atoms form 5 binding sites within the filter S1-S4 and 1 site in the entrance to the extracellular bulk S0.

Kinetic theory has been widely studied and applied to channels [5]–[8], with some success in comparing predictions with experimental data of  $K^+$  channels [9], [10] and explaining properties such as rectification. Yet kinetic models are often criticised for inconsistencies in the definition of the transition rates and the choice of energy barriers [11].

Here we introduce a novel self-consistent kinetic model of the KcsA filter that resolves its selectivity *vs* conductivity paradox. The model is based on a first principles statistical analysis of the energy of the filter that incorporates the effects of site-binding, dehydration, excluded volume etc by including the excess chemical potential difference between the bulk and the channel  $\Delta\bar{\mu}_i^b = \bar{\mu}_i^b - \bar{\mu}_i^c$  [12]. The self-consistent transition rates are included into the model using Grand Canonical Monte Carlo theory [13]. We validate the theory by comparing model predictions with experimental data, including mixed species solutions and mutants [9], [14].

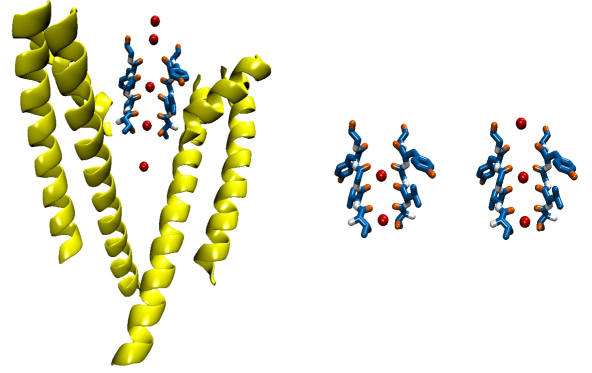


Fig. 1. **Left:** reduced image of closed conformation high- $K^+$  KcsA (pdb 1K4C), rendered with VMD software [15]. The yellow ribbons represent two of the amino acid chains, whilst the narrow selectivity filter has its residues highlighted. The 4/5 binding sites are displayed with a S0S2S4 occupation. **Right:** Schematic of the transition S2S4 $\leftrightarrow$ S0S2S4 (note that we may also have an additional two ion state S1S3).

In the work that follows, with SI units:  $q, k, N_0, T, \epsilon_0$  and  $\epsilon_w$ , respectively represent the proton charge, Boltzmann's and Avogadro's constants, system temperature, and water and vacuum dielectric permittivities ( $\epsilon_w = 80$ ) and constants. We use the following bulk diffusion coefficients:  $D_K^b = 1.96 \times 10^{-9} m^2 s^{-1}$  and  $D_{Na}^b = 1.33 \times 10^{-9} m^2 s^{-1}$ , and ionic radii:  $R_K = 1.4 \text{ \AA}$  and  $R_{Na} = 1 \text{ \AA}$ . The filter has the following geometry, radius  $1.5 \text{ \AA}$ , length  $12 \text{ \AA}$ , and 5 binding sites.

## II. MIXED-SPECIES KINETIC THEORY

We consider a filter diffusively and thermally coupled to intra-cellular ( $L$ ), and extra-cellular ( $R$ ) bulk reservoirs, between which the membrane potential ( $\phi_m$ ) acts from left to right. Anions cannot enter the filter and so we only account for their presence via their contributions to the energy spectrum.

We introduce indistinguishable sites, with the following reduced state space describing the number and species of occupying ions  $\{n_j\}$ ,

$$\{K^+ K^+\}, \{K^+ K^+ K^+\}, \{Na^+ K^+ K^+\}. \quad (1)$$

To simplify notation the subscript 0 will denote the ground state, whilst either excited state will be distinguished by its species:  $K^+$  or  $Na^+$ , denoted by  $i$ . Transitions are described via a set neighbouring states master-equations,

$$\begin{pmatrix} \dot{P}_0 \\ \dot{P}_K \\ \dot{P}_{Na} \end{pmatrix} = \begin{pmatrix} -\Gamma_{01}^K - \Gamma_{01}^{Na} & \Gamma_{10}^K & \Gamma_{10}^{Na} \\ \Gamma_{01}^K & -\Gamma_{10}^K & 0 \\ \Gamma_{01}^{Na} & 0 & -\Gamma_{10}^{Na} \end{pmatrix} \cdot \begin{pmatrix} \dot{P}_0 \\ \dot{P}_K \\ \dot{P}_{Na} \end{pmatrix}, \quad (2)$$

where we have introduced the notation:  $\Gamma^i = \Gamma^{L,i} + \Gamma^{R,i}$ . The general solution can be found using standard linear algebra methods when we consider probability conservation, taking a simplified form by considering the binding factor  $\mathcal{B}$  [13] defined via the ratio of probabilities to the ground state,

$$P(\{n_j\}) = \frac{\mathcal{B}(\{n_j\})}{\sum_{\{n_j\}} \mathcal{B}(\{n_j\})}, \quad \mathcal{B}(\{n_j\}) = \frac{P(\{n_j\})}{P_0}. \quad (3)$$

To proceed in analysing the theory we first need to derive our rates, and we follow [13], [16], [17], and use our statistical theory. This approach starts by considering transitions between the mouth, which is in quasi-equilibrium with the bulk solution and the channel. We establish the local balance relation between each bulk  $b$  and the channel, and normalise such that rates sum to 1, leaving sigmoidal rates as functions of the full transition energy barrier:  $\Delta G(\{n_j\}; n_f; b)$ . Each rate is a function of the energy barrier from each bulk and so we allow the condition of equilibrium between both bulks to be broken. This ensures we have a non-equilibrium steady-state with non-zero current and failure to establish the Boltzmann ratio.

$$\begin{aligned} \Gamma_{10}^{b,i} &= \tau_i \times D_i^b / L^2 \frac{\exp[(\Delta G(\{n_j\}; n_f; b)) / kT]}{1 + \exp[(\Delta G(\{n_j\}; n_f; b)) / kT]}, \\ \Gamma_{01}^{b,i} &= \tau_i \times D_i^b / L^2 \frac{1}{1 + \exp[(\Delta G(\{n_j\}; n_f; b)) / kT]} \end{aligned} \quad (4)$$

The prefactor  $\tau_i \times D_i^b / L^2$ , is added under the condition that  $0 < \tau_i \leq 1$ . These rates converge to a Kramer's exponential with a large energy barrier, and  $1/2\tau_i \times D_i^b / L^2$  at the barrier-less condition ( $\Delta G \approx 0$ ) because the net flow is zero [18].

The free energy barrier was derived and defined earlier in [12], and here we present only a brief discussion.

$$\begin{aligned} \Delta G(\{n_j\}; n_f; b) &= \Delta \mathcal{E}(\{n_j\}; Q_f) - \Delta \phi^b - kT \ln(x_i^b) \\ &+ kT \ln[(n_i + 1) / n_w] - \Delta \bar{\mu}_i^b. \end{aligned} \quad (5)$$

It represents the energy required for a transition between the bulk and channel to occur. It consists of an electrostatic component  $\Delta \mathcal{E}(\{n_j\}; Q_f)$  between ions and  $Q_f$  [12], [19], bulk parameters: mole fraction  $\sim c_i^b / c_w^b$  (where  $c$  is the concentration of either species or water molecule) and influence from the membrane potential  $\Delta \phi^b$  in transition to a site at  $\eta$ . We include a permutation factor due to binding statistics where  $n_i$  and  $n_w$  are the initial numbers of ions and water

molecules in the filter, and the excess chemical potential difference between bulk and channel  $\Delta \bar{\mu}_i^b$ .

This final term provides a distinction between species because it depends strongly on the ionic concentration, charge and radii. These contribute via a large number of terms including: dehydration at either filter entrance, site bonding, ion-ion interactions in the bulk and volume exclusion [12], [20], [21]. Our term takes the following form,

$$\Delta \bar{\mu}_i^b = \Delta \tilde{\mu}_i^b - \frac{q^2 \kappa}{8\pi \epsilon_w \epsilon_0 (1 + \kappa R_i)}, \quad \kappa = \sqrt{\frac{N_0 q^2 \sum_i 2z_i^2 c_i^b}{kT \epsilon_0 \epsilon_w}} \quad (6)$$

where the term:  $\Delta \tilde{\mu}_i^b$  includes dehydration, site-bonding and volume exclusion, and will be used as a fitting parameter. The final term is calculated explicitly and given by the Debye-Hückel ion-ion interaction, screened by a solvent and includes the presence of anions. The difference of  $\Delta \bar{\mu}_i^b$  between two species gives the thermodynamic selectivity  $\Delta \Delta \mu^b$ , and for  $K^+$  and  $Na^+$  this is around  $\sim 8kT$  [22], [23], strongly disfavoring  $Na^+$ .

In the next section we introduce equations for  $K^+$  and  $Na^+$  current and consider selectivity.

### III. SELECTIVITY *vs* CONDUCTIVITY OF THE KCSA FILTER

The current can be computed in the standard way as the balance of probability fluxes over each barrier, subject to Kirchoff's law where  $I_i = I_i^L = I_i^R$ . It is given below for the left barrier,

$$I_i = q \cdot \left( \Gamma_{01}^{L,i} \cdot P_0 - \Gamma_{10}^{L,i} \cdot P_i \right). \quad (7)$$

After inserting the solutions to the master equations (2), it can be simplified to,

$$I_K = q \cdot \frac{D_K^c}{L^2} \cdot \left( \Gamma_{01}^{L,K} - \Gamma_{01}^{R,K} \right) \cdot \frac{1}{2 \frac{D_K^c}{L^2} + \Gamma_{10}^{Na} \frac{\Gamma_{01}^{Na}}{\Gamma_{10}^{Na}}} \quad (8)$$

$$I_{Na} = q \cdot \frac{D_{Na}^c}{L^2} \cdot \left( \Gamma_{01}^{L,Na} - \Gamma_{01}^{R,Na} \right) \cdot \frac{1}{2 \frac{D_{Na}^c}{L^2} + \Gamma_{10}^{Na} \frac{\Gamma_{01}^{K}}{\Gamma_{10}^{K}}} \quad (9)$$

This system is easily reducible back to two states describing  $K^+$ , if we take the  $Na^+$  concentration to be zero. The occupancy of the filter can be calculated from the ensemble average,

$$\langle n \rangle = 2 \times P_0 + 3 \times (P_K + P_{Na}) \quad (10)$$

In Fig: 2 we display current and occupancy of the filter for both species *vs*  $Q_f$ .  $K^+$  is energetically favoured and so its conductivity and contribution to the occupancy is much larger. Hence the resonant transition occurs at the midpoint of the step which corresponds to a degeneracy in the energy spectrum i.e. the barrier-less knock-on condition  $\Delta G_K = 0$ , because here the ground and  $K^+$  excited state probabilities are non-zero and equal to each other. The  $Na^+$  peak is shifted because it is trying to enter a stable fully-occupied filter and hence requires

a  $K^+$  ion to exit; hence it peaks at the minimum in energy to remove a  $K^+$  and add a  $Na^+$ . This phenomena is known as Coulomb blockade (CB) [19], [24] because outside of the  $K^+$  peak the filter is in a blocked state with minimal conduction.

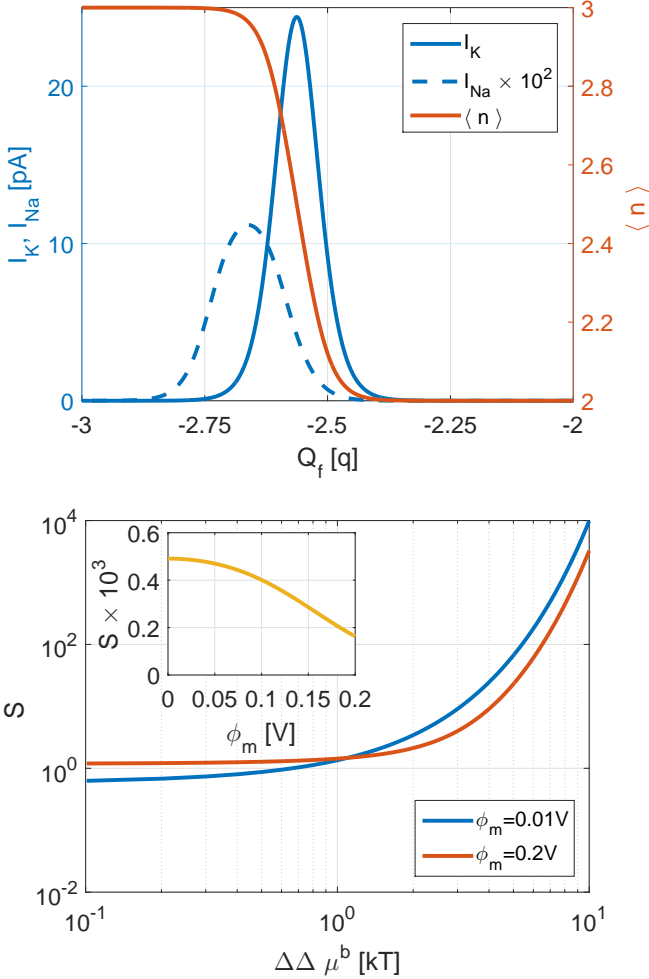


Fig. 2. **Top:**  $I_K$  and  $I_{Na} \times 10^2$  vs  $Q_f$ , with:  $\tau_i = 0.5$ ,  $\phi_m = 50\text{mV}$ ,  $\eta = 0.5$ ,  $\Delta\tilde{\mu}_K^b = 4kT$  and  $x_i = 0.1/55$ . Each species provides a peak although  $Na^+$  is shifted and  $\ll I_K$ . **Bottom** Selectivity between species decreases vs  $\Delta\Delta\mu^b$  with parameters:  $\tau_i = 0.5$ ,  $Q_f = -2.5q$ ,  $\eta = 0.5$ ,  $\Delta\tilde{\mu}_K^b = 4kT$  and  $x_i = 0.1/55$ . For  $\Delta\Delta\mu^b \lesssim 1kT$  the filter disfavours  $K^+$  and this is due to the binding statistics, for values  $\gtrsim 1.1kT$  we see high selectivity. The inset plot shows selectivity decreasing vs  $\phi_m$  at  $\Delta\Delta\mu^b = 8kT$ , culminating with  $S \sim 160$  at  $\phi_m = 0.2V$ .

Selectivity is defined via the ratio:  $S = I_K/I_{Na}$ , and is plotted in the bottom figure of Fig: 2 and its inset. As expected selectivity strongly depends on the difference  $\Delta\Delta\mu^b$ , but decreases with membrane potential. We also observe that, due to the binding factor when  $\Delta\Delta\mu^b \lesssim 1.1kT$ , it results in selectivity favouring  $Na^+$ . The membrane voltage provides the driving force governing conduction in symmetrical solutions, and we observe later that at large voltages the current saturates. Thus when  $\phi_m \sim 0.2V$   $K^+$  conduction has, or is approaching, saturation at its diffusion limit, and it becomes independent of voltage; meanwhile the energy barrier for  $Na^+$  conduction is lowered allowing conduction. Thus at these large voltages we

find a non-negligible  $Na^+$  current which continues to increase with even larger  $\phi_m$  until it reaches its diffusion limit. This non-negligible current is observed experimentally and known as *punch-through* [25].

#### IV. EXPERIMENTAL COMPARISONS

There is a vast experiential literature about the behaviour of  $K^+$  channels and in this paper we will compare the theoretical current with current-voltage ( $I - V$ ) curves under differing concentrations, mixed solutions and the effect of filter mutations, from the Shaker channel [9], KcsA [14]. Unless stated, only  $K^+$  is present in the solution, and hence the state space reduces to:  $\{K^+K^+\}$ ,  $\{K^+K^+K^+\}$ , and we take  $Q_f = -2.5q$ .

In Fig: 3 we display the results of comparisons with the Shaker channel, noting that the diamonds indicate symmetrical solutions and crosses the asymmetrical solutions, with concentrations given in the legend. The asymmetrical solutions consisted of  $K^+$  in the extracellular solution with concentration 0.095M, and either 0.105M  $K^+$  or  $Na^+$  in the intracellular solution.

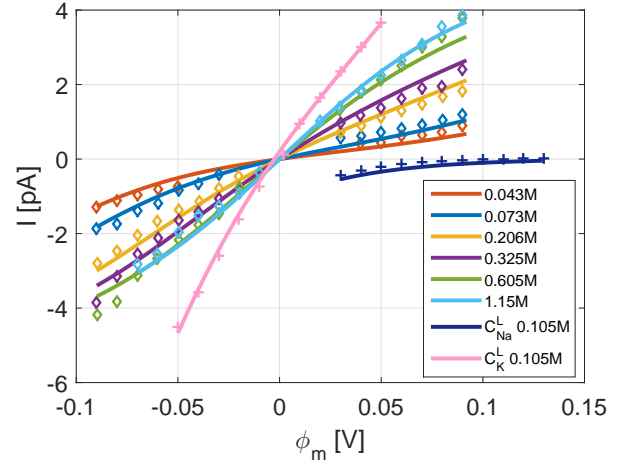


Fig. 3. Theoretical  $I - V$  curves are compared with the experimental values for the Shaker  $K^+$  channel [9]. Diamonds indicating symmetrical solutions, are fitted with the parameters:  $\tau_K = 0.0486$ ,  $\eta = 0.617$  and  $\Delta\tilde{\mu}_K^b = 3.92kT$ . The asymmetrical solutions denoted by crosses are fitted with:  $\tau_i = 0.102$ ,  $\eta = 0.629$ , and  $\Delta\tilde{\mu}_i^b = 4.14kT$ .

The fitting parameters were very similar, although we note that the diffusion rate doubled when we had asymmetrical solutions. Importantly, however it was much less than the bulk value in agreement with simulations [26]. The remaining fitting parameters are consistent with both experiments for,  $\eta \sim 0.6$  and  $\Delta\tilde{\mu}_K^b \sim 4kT$ . This indicates a slight asymmetry in the channel and a low energy barrier for entry. The theory fits better at low concentrations possibly due to the break-down of the Debye-Hückel interaction term, which works best for dilute solutions.

In the final comparison we compare our theory to wild-type (WT) KcsA and its mutant (MUT), created by replacing the

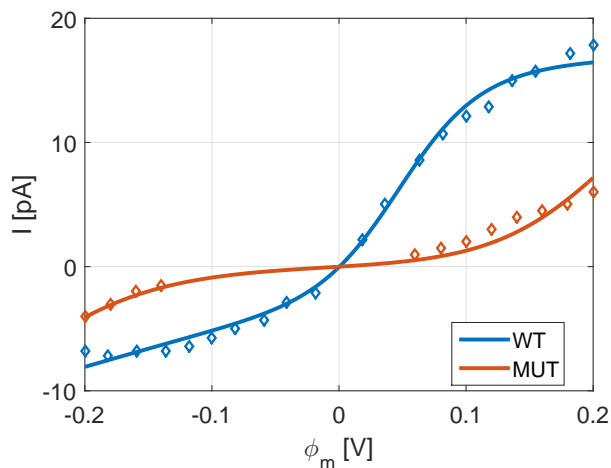


Fig. 4. Theoretical  $I - V$  curves are compared with the experimental values for WT (blue) and MUT (orange) [14]. Resulting in:  $\tau_K = 0.161$ ,  $\Delta\tilde{\mu}_{WT}^b = 4.1kT$ ,  $\Delta\tilde{\mu}_{MUT}^b = 0.979kT$ ,  $\eta_{WT} = 0.175$  and  $\eta_{MUT} = 0.446$ .

amino-acid threonine with cysteine at the S4 site (for full mutagenesis details refer to [14]), strongly affecting conduction. To model this mutation we allow for differences in symmetry and binding, and hence vary  $\Delta\tilde{\mu}_K^b$  and  $\eta$ , between the WT and MUT. In Fig: 4 we display the results, and there is a strong difference in the symmetry and  $\Delta\tilde{\mu}_K^b$ . In WT KcsA the energy barrier for entry was much smaller because  $\Delta\tilde{\mu}_K^b$  was much larger than the mutant, and tuned to produce faster conduction. The difference in the  $\eta$  parameters were large suggesting the site mutation caused an asymmetry from the original WT.

## V. CONCLUSION

We have introduced a self-consistent kinetic model describing transitions of state in the selectivity filter of narrow channels. It demonstrates large selectivity amongst monovalent species coexisting with high conductivity, and CB effects. Predictions of the model are in good agreement with experimental data and we have considered the effect of a filter mutation. It can be extended by considering site-specific binding energies to increase the number of states, and with further comparison to experimental data.

We fully expect the theory to be applicable to other narrow channels such as voltage-gated sodium channels e.g. NaCh-Bac, and to artificial nano-pores.

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## REFERENCES

[1] B. Hille, *Ion Channels Of Excitable Membranes*, 3rd ed. Sunderland, MA: Sinauer Associates, 2001.  
 [2] Q. Kuang, P. Purhonen, and H. Hebert, "Structure of potassium channels," *Cell. Mol. Life Sci.*, vol. 72, no. 19, pp. 3677–3693, 2015.

[3] A. L. Hodgkin and R. D. Keynes, "The potassium permeability of a giant nerve fibre," *J. Physiol.*, vol. 128, no. 1, pp. 61–88, 1955.  
 [4] J. H. H. Morais-Cabral, Y. Zhou, and R. MacKinnon, "Energetic optimization of ion conduction rate by the  $K^+$  selectivity filter," *Nature*, vol. 414, no. 6859, pp. 37–42, 2001.  
 [5] P. Lauger, "Ion transport through pores: A rate-theory analysis," *Biochim. Biophys. Acta (BBA) – Biomembranes*, vol. 311, no. 3, pp. 423–441, 1973.  
 [6] E. von Kitzing, "A novel model for saturation of ion conductivity in transmembrane channels," in *Membrane Proteins: Structures, Interactions and Models: Proc. 25th Jerusalem Symposium on Quantum Chemistry and Biochemistry, Jerusalem, May 18-21, 1992*, A. Pullman, J. Jortner, and B. Pullman, Eds. Dordrecht: Kluwer, 1992, pp. 297–314.  
 [7] I. S. Tolokh, S. Goldman, and C. Gray, "Unified modeling of conductance kinetics for low-and high-conductance potassium ion channels," *Phys. Rev. E*, vol. 74, no. 1, p. 011902, 2006.  
 [8] P. H. Nelson, "A permeation theory for single-file ion channels: One- and two-step models," *J. Chem. Phys.*, vol. 134, pp. 165 102–165 114, 2011.  
 [9] L. Heginbotham and R. MacKinnon, "Conduction properties of the cloned shaker  $k^+$  channel," *Biophys. J.*, vol. 65, no. 5, pp. 2089–2096, 1993.  
 [10] D. Meuser, H. Splitt, R. Wagner, and H. Schrempf, "Exploring the open pore of the potassium channel from streptomyces lividans," *FEBS letters*, vol. 462, no. 3, pp. 447–452, 1999.  
 [11] K. Cooper, P. Gates, and R. Eisenberg, "Diffusion theory and discrete rate constants in ion permeation," *J. Membr. Biol.*, vol. 106, no. 2, pp. 95–105, 1988.  
 [12] D. G. Luchinsky, W. A. T. Gibby, I. Kaufman, D. A. Timucin, and P. V. E. McClintock, "Statistical theory of selectivity and conductivity in biological channels," *arXiv[physics.bio-ph]*, p. 1604.05758v3, 2016.  
 [13] B. Roux, "Statistical Mechanical Equilibrium Theory of Selective Ion Channels," *Biophys. J.*, vol. 77, no. 1, pp. 139–153, 1999.  
 [14] M. Zhou and R. MacKinnon, "A mutant kcsa  $k^+$  channel with altered conduction properties and selectivity filter ion distribution," *J. mol. biol.*, vol. 338, no. 4, pp. 839–846, 2004.  
 [15] W. Humphrey, A. Dalke, and K. Schulten, "VMD – Visual Molecular Dynamics," *J. Mol. Graph.*, vol. 14, pp. 33–38, 1996.  
 [16] W. Im, S. Seefeld, and B. Roux, "A grand canonical Monte Carlo-Brownian dynamics algorithm for simulating ion channels," *Biophys. J.*, vol. 79, no. 2, pp. 788–801, 2000.  
 [17] B. Roux, T. Allen, S. Berneche, and W. Im, "Theoretical and computational models of biological ion channels," *Quart. Rev. Biophys.*, vol. 37, no. 1, pp. 15–103, 2004.  
 [18] C. W. Gardiner, *Handbook of Stochastic Methods: for Physics, Chemistry and the Natural Sciences*. Berlin: Springer, 2002.  
 [19] I. K. Kaufman, P. V. E. McClintock, and R. S. Eisenberg, "Coulomb blockade model of permeation and selectivity in biological ion channels," *New J. Phys.*, vol. 17, no. 8, p. 083021, 2015.  
 [20] D. A. McQuarrie, *Statistical Mechanics*, 1st ed. University Science Books, Jun. 1976.  
 [21] D. Krauss, B. Eisenberg, and D. Gillespie, "Selectivity sequences in a model calcium channel: role of electrostatic field strength," *Eur. Biophys. J.*, vol. 40, pp. 775–782, 2011.  
 [22] S. Y. Noskov and B. Roux, "Importance of hydration and dynamics on the selectivity of the KcsA and NaK channels," *J. Gen. Physiol.*, vol. 129, no. 2, pp. 135–143, 2007.  
 [23] S. W. Lockless, "Determinants of cation transport selectivity: Equilibrium binding and transport kinetics," *J. Gen. Physiol.*, vol. 146, no. 1, pp. 3–13, 2015.  
 [24] C. W. J. Beenakker, "Theory of Coulomb-blockade oscillations in the conductance of a quantum dot," *Phys. Rev. B*, vol. 44, no. 4, pp. 1646–1656, 1991.  
 [25] C. M. Nimigean and C. Miller, " $Na^+$  block and permeation in a  $k^+$  channel of known structure," *J. Gen. Physiol.*, vol. 120, no. 3, pp. 323–335, 2002.  
 [26] D. P. Tieleman, P. C. Biggin, G. R. Smith, and M. S. P. Sansom, "Simulation approaches to ion channel structure-function relationships," *Quart. Rev. Biophys.*, vol. 34, no. 4, pp. 473–561, 2001.