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A New Theoretical Framework for Designing Ion Transport Pathways

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ABSTRACT

The rapid transport of specific ions through matter is critical to energy storage, membrane separations, and health. However, commercial materials resist ion transport, lack specificity, or both, making ion transport costly and ineffective. Inspiration for new material designs can be taken from biology, where membrane transport proteins exert exquisite control over the specificity and rate of ion transport. The challenge in understanding and designing transport pathways is that ions often exchange their hydrating waters for direct contacts with atoms in the transport pathway. Despite intense study over decades, no theory exists to explain local ion binding and transport mechanisms and experiments cannot differentiate reliably between ions and water in binding sites. Here, we developed a new approach, based on quantum methods and extension of the quasi-chemical free energy theory, to understand and design pathways through materials for rapid transport of specific ions. Understanding ion transport mechanisms will significantly advance our nation's ability to develop cost-effective materials for energy sustainability and therapeutics for health.

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EXECUTIVE SUMMARY

Experiments cannot resolve ion transport mechanisms currently. Standard molecular models fix atomic charges, an incorrect assumption leading to inaccurate results. Thus, ion transport mechanisms remain unresolved despite their critical importance to developing cost-effective materials for energy sustainability, including separation membranes, and battery electrolytes. The approach pursued here involves developing a new theoretical framework to enable the use of accurate quantum mechanical studies of ion transport mechanisms in systems occupied by multiple ions. The goals are to develop a new modeling capability and apply it to a model ion transport system well-characterized experimentally. The chosen model system is the celebrated potassium (K) ion channel protein, a well-characterized model molecule for low-resistance and highly selective ion transport. Despite a Nobel Prize and intense study over the last two decades, the fundamental question about how this model protein conducts specific ions rapidly remains unresolved. In particular, debate rages about whether ions occupy all four neighboring binding sites, or whether waters insert between ions to screen repulsive ion-ion interactions. We hypothesize that a structure-based free energy approach we developed earlier to study ion solvation in liquids can be advanced to treat ion binding to macromolecules where multiple ions may occupy multiple binding sites during transport. The theory, called quasi-chemical theory, separates local and distant solvation environments, permitting application of accurate, but expensive quantum-based density function methods to the local solvation environment around ions, where charge transfer and polarizability may be prominent. The key results of the project are that (1) quasi-chemical analysis of local ion exchange reactions permits quantum mechanical treatment of ion transport configurations; (2) K channel protein conducts through a hard knock-on mechanisms where ions occupy adjacent binding sites without intervening waters that transport with the ions. The impact of the work is broad. Since the theoretical framework is general, this new modeling capability enables accurate computational studies of ion transport generally, for any ion through any molecule or material. We conducted additional studies of ions interacting with a variety of ligands found in other transport materials and molecules, and another transport protein to illustrate the broad application of our new theoretical framework. Finally, this project supported early career development of a Sandia postdoc, and a graduate student at the University of Texas at Austin through an Academic Alliance project, and produced many peer-reviewed publications and generated many invited presentations.

ACRONYMS AND TERMS

Acronym/Term	Definition
DFT	density functional theory
NMA	N-methyl acetamide
NMR	nuclear magnetic resonance spectroscopy
PCM	polarizable continuum solvation model
PDB ID	protein data bank identification number
QCT	quasi-chemical free energy theory
QM	quantum mechanics
2D-IR	two-dimensional infrared spectroscopy

1. PROBLEM

The rapid transport of specific ions through matter is critical to energy storage, membrane separations, and health. However, commercially-available synthetic pathways resist ion transport, lack specificity, or both, making ion transport costly and ineffective. Designs for new pathways can be taken from biology — proteins in cellular membranes demonstrate the desired rapid (low-resistance), selective ion transport function. Inspiration from biology recently led to a breakthrough in Sandia's patented membranes for low-cost carbon capture.[1] To carry that success over to charged molecules requires an understanding of how molecular structure correlates with function. In the case of membrane transport proteins, this molecular-scale understanding is lacking, and thus not available for inspiring breakthroughs in material designs for ion transport.

Inspiration lies in the celebrated potassium (K)-channel protein, a model molecule for low-resistance and highly-selective ion transport. K-channels are ideal for developing new capabilities because they are well-characterized. K-channels catalyze ion conduction across cellular membranes at rates (10^8 ions/s) and selectivity (1000:1 K/Na) orders of magnitude higher than commercial membranes. This function is crucial to electrical signaling, hence life. Despite a Nobel Prize and intense study over the last 20+ years (e.g., X-ray crystallography, 2D-IR, NMR, molecular simulations), the fundamental question about how this model protein conducts specific ions rapidly remains unresolved.[2,3,4] In particular, debate rages about whether ions occupy all four neighboring binding sites, and conduction occurs with a so-called hard knock-on mechanism, or whether waters insert between ions to screen repulsive ion-ion interactions and conduction occurs with a soft knock-on mechanism.

The challenge in understanding/designing transport pathways is that ions often exchange some, or all, of their hydrating waters for direct contacts with atoms in the transport pathway. No theory exists to explain local ion binding and transport mechanisms because every established free energy theory treats local and distant solvent as a single entity.[5,6] Experiments cannot differentiate reliably between ions and water in binding sites. Predictions of ion binding and conduction mechanisms by molecular simulations are currently unreliable due to limitations in force fields that describe atomic interactions. In particular, nearly every prior study has used force fields with fixed charges despite well-documented evidence that ions polarize nearby functional groups.[7,8] A new approach is needed.

We hypothesize that a structure-based free energy approach we developed earlier to study ion solvation in liquids can be advanced to treat ion binding to macromolecules,[9] where multiple ions may occupy multiple sites during transport. The theory, nicknamed QCT or quasi-chemical theory, links free energy directly to local ion solvation structure. That link will enable us to decipher the mechanisms of rapid transport and specific ion binding. Further, the separation of local solvation environment enables the application of accurate, but expensive, quantum-based density functional (DFT) methods to the region near the ion, where polarizability is prominent. Quantum methods treat electronic degrees of freedom explicitly, permitting polarization to occur naturally.

Here, we extend QCT to treat multiple ions and binding sites, then test it on K-channels. Outcomes include a new method for designing ion transport pathways generally, and resolution of a hotly debated scientific question about ion transport mechanism in a protein critical to health and inspirational for new material designs. The key lessons learned are that reduced models and ion exchange reactions enable local application of quantum mechanics to study ion transport mechanisms, and ions occupy adjacent sites in K channels without intervening waters due to screening by atoms of the protein.

1.1. Objectives and Approach

Two main objectives were pursued in this work: (1) Establish the theoretical framework and validate the computational models; (2) Apply the theoretical framework and resolve the question of K channel conduction mechanism. We chose to focus the free energy theory on ion exchange reactions evaluated with density functionals (DFT) and extensive basis sets validated by comparing to experimental data on gas-phase ion clustering free energies, aqueous ion solvation structures, and absolute hydration free energies. Following an initial brute force molecular dynamics simulation study with a polarizable force field of the bacterial K channel called KcsA, we developed a reduced model of KcsA from the X-ray crystallographic data. Then we applied the theoretical framework and carried out DFT calculations of ion and water occupancy in the four consecutive binding sites of the protein selectivity filter, which determine ion conduction rates and ion selectivity. Additionally, we applied the framework to a variety of ions and ligands and a channel protein relevant to optogenetics to establish the broad applicability of the method to understanding and designing any ion transport pathway.

1.2. Project Metrics

Detailed results that meet the defined objectives and descriptions of the approach can be found in these publications. A list of presentations is also given, including those that occur in the future. This project received recognition through an award for Outstanding Publication by the Biophysical Society and invited award talk to Dr. Rempe (2022). Other recognition came from the organizing and chairing of several international conferences about ions, including the 54th Gordon Research Conference on Water and Aqueous Solutions (chaired by Rempe), the CINT Annual User Meeting Symposium on Ions (chaired by Rempe and Stevens), and the Telluride Science Workshop on Ions (chaired by Rempe in 2023 and 2025).

1. Stevens, M.J.; Rempe, S.L.B. 2024. Ion transport mechanism in potassium ion channels: A DFT perspective on hard knocks, soft knocks, and ion selectivity. *J. Phys. Chem.* (invited Perspective, in prep).
2. Allen, C; Stevens, MJ; Ren,* P; and Rempe,* SB. 2024. A density functional analysis of ion occupancy in a reduced model of the potassium ion channel protein, KcsA. (in prep).
3. Stevens, M.J. and Rempe, S.B. 2024. Binding of sulfates and water to monovalent cations. *J. Phys. Chem. B* (invited for Rich Saykally special issue; in review).
4. Prignano, L.A.; Stevens, M.J.; Vanegas, J.M.; Rempe, S.B.*; Dempski, R.E.*. 2024. Metadynamics simulations reveal mechanisms of Na^+ and Ca^{2+} transport in two open states of the channelrhodopsin chimera, C1C2. *Plos One* 19:e0309553.
5. Stevens, M.J.; Rempe, S.L.B. 2024. Insight into the K channel's selectivity from binding of K^+, Na^+ and water to N-methylacetamide. *Faraday Discuss.* 249:195-209 (invited).
6. Advincula, X.R.;…; Rempe, S.L.B.; … 2024. Ice interfaces: General discussion. *Faraday Discuss.* 249:133-161 (invited).
7. Backus, E.H.G.;…; Rempe, S.L.B.; … 2024. Soft matter-water interfaces: General discussion. *Faraday Discuss.* 249:485-520 (invited).
8. Advincula, X.R.;…; Rempe, S.L.B.; … 2024. Dynamics and nano-rheology of interfacial water: General discussion. *Faraday Discuss.* 249:243-266 (invited).
9. Stevens, M.J.; Rempe, S.L.B. 2023. Binding of Li^+ to negatively charged and neutral ligands. *J. Phys. Chem. Lett.* 14:10200-10207.

10. Stevens, M.J.; Rempe, S.L.B. 2023. Binding of carboxylate and water to monovalent cations. *Phys. Chem. Chem. Phys.* (Judith Herzfeld Festschrift) 25:29881-29893 (invited).
11. Stevens, M.J.; and S.L.B. Rempe. 2022. Carboxylate binding to two ions. *Phys. Chem. Chem. Phys.* 24:22198-22205.
12. Gomez, D.T.; Pratt, L.R.; Asthagiri, D.N.; and S.B. Rempe. 2022. Hydrated anions: From clusters to bulk solution with quasi-chemical theory. *Acc. Chem. Res.* 55:2201-2212 (invited).
13. Jing, Z.; Rackers, JA; Pratt, LR; Liu, C; Rempe, SB* and P. Ren*. 2021. Thermodynamics of ion binding in potassium channels. *Chem. Sci.* 12:8920.

14. 2025 NATO Adv. Study Instit. Sequel, Aqueous systems: The frontier & beyond, Kalamata (invited, Rempe)
15. 2025 CECAM on Membranes and Membrane Proteins, Pisa (invited, Rempe; invited, Stevens)
16. 2025 BPS Optogenetics Symp, Los Angeles (invited, Rempe)
17. 2024 CINT Annual User Meeting, Symposium on Ions, Santa Fe (invited, Ren)
18. 2024 ACS Fall Meeting, Denver (poster, Allen)
19. 2024 GRC Water & Aq Solutions, Holderness (invited, Ren)
20. 2024 Satellite on Exp & comp molec bio, Florence (invited, Rempe)
21. 2024 Chemistry Dept., Czech Acad Sci, Prague (invited, Rempe)
22. 2024 CECAM AI cell biology, Pisa (invited, Rempe; invited, Stevens)
23. 2023 Kings College, London (invited, Rempe)
24. 2023 University of Dundee, Scotland (invited, Rempe)
25. 2023 Faraday Disc on water at interfaces, London (invited, Rempe)
26. 2023 Telluride workshops (2), Telluride (invited, Rempe)
27. 2023 Chemistry & MCD Biology, Yale, New Haven (invited, Rempe)
28. 2023 CECAM Biomolec exascale simulation, Pisa (invited, Rempe)
29. 2022 ACS SWRM, Baton Rouge (invited, Rempe)
30. 2022 U Paris Descarte Chemistry, Paris (invited, Rempe)
31. 2022 CECAM (Mike Klein 80th b'day), Rome (invited, Rempe)
32. 2021 PacifiChem Clusters, Web
33. 2021 PacifiChem H-bonds, Web
34. 2021 Texas Tech Health Sci Biophys Seminar, Web (invited, Rempe)

1.3 Summary of K Channel Occupancy

Following extensive validation of the theoretical framework and quantum mechanical computational model on ions in aqueous solution, and application of the new computational approach to ions interacting with a variety of ligands important to ion transport (see publications listed in **Section 1.2**), we applied our new computational approach to the K channel problem with the goal of understanding its ion transport mechanism by resolving the question of ion and water occupancy. A challenge of treating the K channel is its occupancy by multiple dehydrated ions and possibly individual waters in multiple consecutive binding sites, labeled S1-S4 (Figure 1). The direct interaction of ions with ligands demands treatment of complex electronic effects, preferably with quantum mechanical methods. The focus here on multiple ions in multiple binding sites with a local quantum mechanical analysis of ion binding free energy is what differentiates this work from prior work, which treated only a single ion in a single binding site.[9]

The system size for the KcsA potassium ion channel in a lipid bilayer and aqueous solution is computationally prohibitive for a quantum mechanical analysis. In a preliminary step, we studied KcsA with a polarizable force field and molecular dynamics simulations (Jiang et al. *Chem. Sci.* 2021; Figure 1A). We found that the free energy barriers for K ion transport were too high to represent the rapid transport found experimentally, meaning that the polarizable force fields needs refinement. This finding motivates a quantum mechanical analysis to treat the complex electrostatic interactions.

1.3.1 Methods

To combat the large system size and treat the atomic interactions accurately, quasi-chemical theory (QCT) was extended to treat ion exchange reactions in the context of the multiple binding sites of KcsA. In QCT, the most important direct site of interaction (selectivity filter and ions) is represented explicitly with quantum mechanical accuracy and the less important long-range interactions (bulk water, protein, lipid bilayer) are treated implicitly using a polarizable continuum model (PCM). QCT allows the total system, including protein, bulk water, and lipid bilayer (~62000 atoms), to be reduced to a small model system that is local to the ions and includes the selectivity filter (~175 atoms). The reduced model system size allows quantum mechanical calculations to be performed on the protein selectivity filter using (GAUSSIAN16) electronic structure software. The selectivity filter from the KcsA crystal structure (PDB ID: 1K4C) was used as a starting point for quantum mechanical calculations with the ωb97X-D functional and the mixed basis set of 6-311++G(2df,2pd) for potassium and 6-31G* for carbon, oxygen, nitrogen, and hydrogen (Figure 1D). Structures were optimized in gas phase and 1-heptanol while constrained to emulate physical conditions (fixed α-carbons). Thermal corrections to the electronic energy were obtained from frequency calculations to provide the free energy at room temperature (300 K). PCM corrections were obtained from the difference between optimized structure energies in PCM and gas phases.

A thermodynamic cycle is made for each occupational state and is used to calculate binding free energy of ion and water binding in the selectivity filter. Using a series of thermodynamic cycles, it is possible to calculate the relative binding free energy between a series of occupational states and determine preferential ion binding locations for single, double, and triple occupancies. For occupancies when water occupies a selectivity filter binding site, a wide range of starting structures were generated and minimized using XTB software and GFN2-xTB semi-empirical methods with the α-carbons and potassium ions constrained. The lowest energy structures were taken as starting structures for quantum mechanical calculations

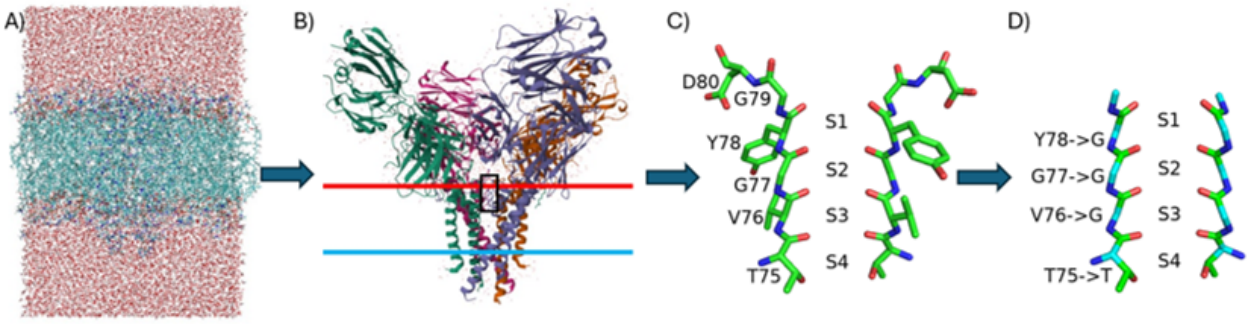

Figure 1. Model development from (A) full KcsA protein in lipid bilayer and surrounded by aqueous solution, to (B) KcsA protein, to (C) KcsA selectivity filter with ion binding sites S1-S4, to (D) reduced model for quantum calculations in new theoretical framework. Amino acids: D, aspartate; G, glycine; Y, tyrosine; V, valine; T, threonine.

1.3.2 Results

Optimizations of double occupancy find that not only is double occupancy favored, but also that adjacent double occupancy by K is preferred over other double occupancies and single occupancy (Figure 2). The most preferred double occupancy has a change in free energy ΔΔG of ~20 kcal/mol for sites 2 and 3 (K23), while the most preferred single occupancy has ΔΔG of ~50 kcal/mol for K3. This is a 30 kcal/mol preference and clearly shows that two K^+ can occupy adjacent sites in the selectivity filter. This result clearly shows that adjacent K^+ in the selectivity filter are not repelled and prevented, but that the electrostatic environment actually has a lower free energy as the combination of the polarized, negatively charged carbonyl O atoms and the K^+ provide the alternating + and – charge structure that is electrostatically favored. The adjacent double occupancy is consistent with the calculation of the carbonyl binding to K^+ in *N*-methyl acetamide (NMA), which also found that 3 unconstrained layers of NMA with 2 K^+ between have lower change in binding free energy ΔG than a single K^+ between 2 NMA layers (see Stevens and Rempe, *Faraday Discuss.* 2024, listed in **Section 1.2**).

Geometry optimization with one of the two ions in binding site 4 optimizes to different occupancies with the consistent movement of the ion from binding site 4 to binding site 3, e.g. K24 optimizes to K23. This ion movement from site 4 to 3 is conserved across the environment from gas to 1-heptanol PCM. The optimization from starting occupancy of K34 notably has ion movement in gas phase to K23 with the ion in binding site 4 pushing the adjacent ion from site 3 to 2. In all cases, regardless of phase, where ion movement from site 4 to 3 occurs the side chain of the threonine which comprises the lower bound of binding site 4 shows flexibility which pushes the side chain towards the interior of the filter, aiding the ion movement from site 4 to 3 as binding site 4 compresses. To test whether the behavior is dependent on threonine, the selectivity filter was mutated from the reduced form GGGT to GGGGG. The two carbonyl oxygens from the two mutated glycine replace the carbonyl oxygen and side chain hydroxyl group of the threonine. When

similar calculations were conducted on this mutated reduced model, no movement of the site 4 ions nor compression of site 4 were observed. As such the site compression and a partition of ion movement can be attributed to the threonine comprising binding site 4.

Given that adjacent occupancy of two selectivity filter sites is preferred, it is not surprising to find that 3 and 4 (all) adjacent occupancies are also favored. The relative binding free energies show increased occupancy with K^+ is thermodynamically favorable, including to quadruple or full occupancy. The binding free energies referenced in this discussion are in 1-heptanol PCM unless otherwise noted. For triple occupancy, binding sites 1, 2, and 4 (K124) are the most stable configuration by ~2 kcal/mole with binding sites 2, 3, and 4 (K234) being the second most favored. These states (K124 and K234) are nearly equally stable. The magnitude of preference between binding site occupations diminishes with each ion added. Triple ion occupancy is thermodynamically favorable compared to double occupancy by ~10kcal/mole, while quadruple ion occupancy is thermodynamically favorable compared to triple occupancy by ~6.5 kcal/mole, making K1234 the most stable state sampled. The stability with increasing number of layers is consistent with the structure of the selectivity filter being alternating layers of negatively polarized O atoms and K^+. Such a structure should be stable for any number of layers.

The intermediate states consistent with the soft knock-on mechanism (K1W2K3W4 and W1K2W3K4) are less favorable by ~9 kcal/mole compared to other quadruple occupancies with adjacent K occupancies (i.e. K123W4, K1234). The hard knock-on mechanism consists of a combination of quadruple and triple ion occupancies, which have been identified as favorable over lower occupancies. The favorability of K1W2K3W4 over W1K2W3K4 is explained by the preference of water in BS4 where hydrogen bonds can be formed over BS1, BS2, and BS3.

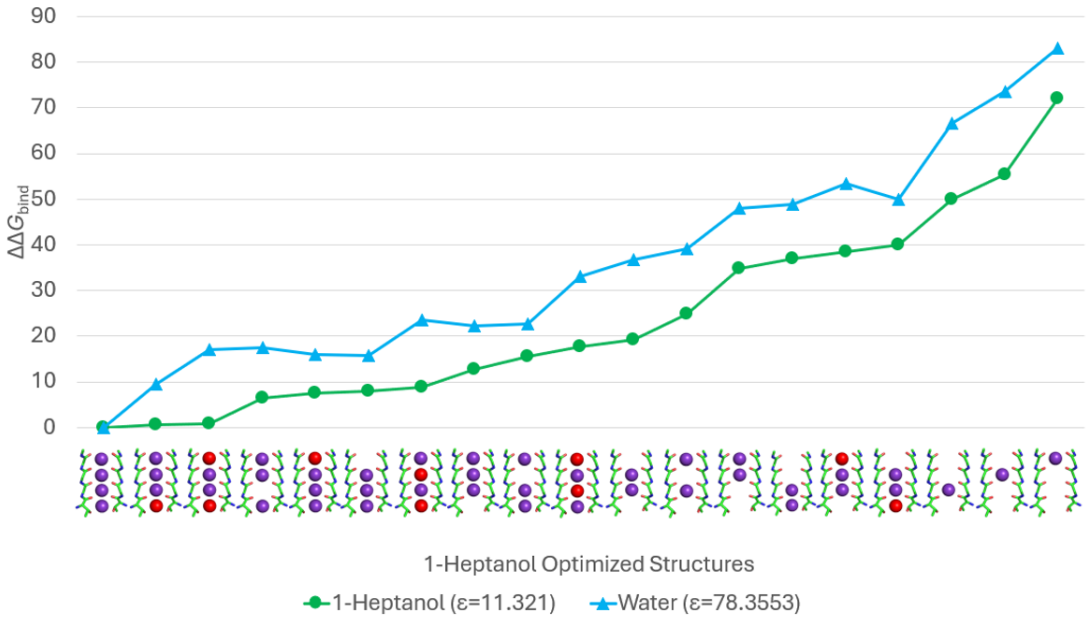

Figure 2. Ion and water occupancy determined from quantum mechanical (DFT) calculations carried out in new theoretical framework on the reduced model (Figure 1D) surrounded by implicit solvent.

1.3.3 Future Work

The next steps to be taken in include introducing Na^+ occupancies, including explicit water outside the selectivity filter, calculating ensemble average from multiple starting points. By adding Na^+ to the system and calculating binding free energies for Na^+ occupancies in addition to K^+ and water, the selectivity mechanism may be investigated. By including explicit water outside the selectivity filter, the accuracy of the model may be increased by including interacting waters near binding sites S1 and S4. By taking several low energy frames from molecular dynamics simulation as starting points for quantum calculations, ensemble averages can be calculated.

1.4 Conclusions

The current practice for studying ion transport mechanisms is to carry-out molecular dynamics simulations with fixed charge force fields, known to give inaccurate results. We developed an alternative approach by extending the quasi-chemical free energy theory to enable the study of ion transport mechanisms, involving multiple ions and multiple binding sites, accurately with quantum mechanical methods. This resulted in a new capability with broad application to understanding and designing ion transport pathways important to developing new materials for energy sustainability, including batteries and separation membranes. A take-home message from the first applications of this new capability is that taking into account the complexity of ions and the atoms in direct contact with them yields different ion transport mechanisms than obtained earlier from fixed-charge force fields.

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