

Supporting Information for

Exploring Ion Permeation Energetics in Gramicidin A Using Polarizable Charge
Equilibration Force Fields

Sandeep Patel, Joseph E. Davis, and Brad A. Bauer

I. Simulation Protocol

Simulations were carried out using the CHARMM molecular modeling package¹ with custom modifications built upon the c34a1 version. The system geometry is hexagonal with initial dimensions of 29.7 Å x 29.7 Å x 76.8 Å and contains 20 DMPC molecules, 1070 TIP4P-FQ water molecules, 18 K⁺ ions, 18 Cl⁻ ions, and the gA dimer. The ratio of water molecules to ions is selected based on results of constant pressure and temperature ensemble (*NPT*) molecular dynamics simulations of aqueous salt solutions at 298K and 1 atmosphere. The ion concentration is thus nominally roughly 1M (0.95M), non-trivially higher than the physiological conditions under which the experimental measurements are generally performed (0.15-0.25M)². The high concentration facilitates computation of the full two-dimensional potential of mean force (to be reported in future studies) that requires sufficient sampling of ionic probability density in the bulk solution (outside of the channel pore). The effects of high ionic concentration can be corrected as demonstrated by Allen et al^{3,4}; in general, this leads to an additional stabilizing energy on the order of a few tenths of kcal/mole. The simulation super-cell is based upon the canonical form of Allen and coworkers, which is derived from early work of Woolf and Roux^{5,6}. The system is shown in **Figure 1**. The crystal structure of the gA dimer was obtained from the Protein Data Bank (PDB entry 1JNO). This structure was equilibrated using the nonpolarizable CHARMM27 force fields for protein and the CHARMM27r model for lipids (alkyl groups) to obtain a starting structure for the current simulations. The simulations were carried out in the constant pressure and temperature (*NPT*) ensemble with the temperature maintained at 298 K via a Nosé-Hoover thermostat with a piston mass of 100 kcal mol⁻¹ ps⁻². Pressure was maintained at 1 atm using the Langevin

method with a piston mass of 100 amu. Dynamics were propagated using a Verlet leapfrog integrator with a time step of 0.5 fs. To account for the conditionally convergent long-range electrostatic interactions, Particle Mesh Ewald summation⁷ was used with screening parameter $\kappa = 0.320$ and a spacing of 1 Å for the Fast Fourier Transform grid. A center of mass constraint with force constant 10 kcal/mol was applied to the gA dimer to prevent drifting inside the bilayer.

Water, protein, and lipid component interactions were treated using polarizable force fields based on the charge equilibration (electronegativity equalization) formalism; the polarization component is treated in a non-additive manner, while inter- and intra-molecular dispersion (van der Waals) interactions are modeled under the usual additivity assumption. The TIP4P-FQ fluctuating charge water model of Rick, Stuart, and Berne⁸ was employed as the solvent model; we note that the CHARMM charge equilibration (CHEQ) polarizable force field is built upon this polarizable water model that reproduces a wide range of gas- and condensed-phase water properties remarkably well. The polarizable protein model is based on the CHARMM charge equilibration force field of Patel and Brooks^{9, 10}; we do not employ any type of CMAP correction based on the work of Feig and MacKerell¹¹ as the gA peptide presents a combination of D- and L-amino acids. The CMAP (Cross-term MAP) approach allows for a dihedral cross term interaction between the phi-psi angles of a polypeptide chain. The correction is the difference between a quantum mechanical adiabatic phi-psi map and the uncorrected force field. The correction is grid-based, with intermediate points determined by cubic spline interpolation to obtain a smooth function for all phi-psi values¹². For the present, we take the approach to employ the current non-polarizable torsional potentials and apply

them without any refitting. The test of whether the model is consistent is taken to be the structural integrity of the gA channel after extended time-scale molecular dynamics simulations (both in the presence of ion and in the water-filled channel). This is in the spirit of recent work by Portella et al¹³ who employ soft positional restraints on the gA dimer coordinates for their molecular dynamics simulations; such an approach fundamentally suggests that the torsional component of the force field is superceded by an *ad hoc* restraint to maintain secondary structure (as the authors point out). The main determinant of simulation validity is that the overall structure remains faithful to experiment. In the present work, over the simulation timescales considered, the structure of the gA channel remained within 1 Å r.m.s. deviation from the crystal structure; we thus consider the simulations valid for the current purposes. We also note that the importance of protein flexibility (as it pertains to accommodating the permeant cation) has been emphasized by Mamonov et al¹⁴.

The PMF is obtained from umbrella sampling simulations in which the z -coordinate (normal) of the distance between a single K^+ ion and the center of mass of the gA dimer was taken to be the reaction coordinate. The approach of this study follows that of Allen et al^{3,4}, allowing us to retain consistency in comparing our results to that work. The system was divided into windows ranging from 0 Å (ion at the channel center) to 15 Å (ion 15 Å from the center, slightly outside the channel opening) for a total of 82 windows. We reiterate, as has been discussed in detail in previous studies, that the one-dimensional potential of mean force is rigorous within the channel due to the restrained lateral positional fluctuations imposed by the protein^{3,4}. Simulations for each window were performed for at least 3 nanoseconds, with the initial 1 nanosecond treated as

equilibration; the sampling times are consistent with those suggested by Allen et al to achieve acceptable convergence of the one-dimensional potential of mean force⁴. A harmonic potential was applied to the ion in the channel to restrain it at the desired position along the channel axis. Window spacing and force constants varied with position as finer spacing and stronger force constants were required for sampling of steeply-varying regions of the free energy surface. Specifically, the window spacing varied from 1 Å to 0.1 Å while the force constants varied from 2.5 kcal/mol to 80 kcal/mol, reflecting the steepness of the underlying free energy surface. In addition, a flat-bottomed cylindrical harmonic potential with force constant 10 kcal/mol was applied to the ion to restrain the radial (in plane) distance to the dimer center of mass to within 8 Å. This prevents the ion from moving too far from the channel for the more distant windows that are slightly outside the channel opening. Finally, to prevent other ions from entering the channel, a repulsive spherical harmonic potential with force constant 20 kcal/mol and radius 14 Å (relative to the dimer center of mass) was applied to all ions except the one in the channel.

The potential of mean force is calculated using the biased ion densities, which were reweighted to obtain the unbiased distribution (within the channel) using the Weighted Histogram Analysis Method (WHAM)¹⁵. We use the publicly available WHAM analysis program of Grossfield et al¹⁶ for the WHAM analysis.

In order to assess on a more quantitative level the effect of the reduced barrier height, we apply the approach of Allen et al³ and Mamonov et al¹⁷ to couple the 1D-PMF with Nernst-Planck diffusion theory. We use the approach for maximum single channel conductance given by Allen et al^{3, 18}

$$g_{\max} = \frac{D_{\text{effective}}}{L^2} \frac{q^2}{k_B T} \quad (1)$$

with the effective channel diffusion coefficient, $D_{\text{effective}}$, given by¹⁹:

$$D_{\text{effective}} = \left\langle D(z)^{-1} e^{+W(z)/kT} \right\rangle^{-1} \left\langle e^{-W(z)/kT} \right\rangle^{-1} \quad (2)$$

where $D(z)$ is a spatially varying diffusion coefficient and $W(z)$ is the 1-dimensional potential of mean force along the channel axis. In Equation 1, L is the channel length (taken here as in Allen et al^{3, 18} as 28Å), q is the ionic charge, T is the temperature, and “ k_B ” is Boltzmann’s constant. For the present analysis, without having computed the full two-dimensional potential of mean force, we apply the 1-D PMF, $W(z)$, of **Figure 2** (from main text) in Equation 2.

We estimate the spatially varying potassium diffusion coefficient within the channel as a constant for the present estimation. The value is taken to be one-tenth of the bulk diffusion coefficient of K^+ in a 1M KCl aqueous solution. A constant pressure and temperature (NPT)²⁰ simulation was performed in order to calculate the diffusion constant of K^+ . The system for this simulation consisted of 18 K^+ and 18 Cl^- ions in a cubic cell containing 988 TIP4P-FQ water molecules, resulting in approximately 1 M ion concentration after sufficient equilibration. Long-range electrostatic interactions were treated via Particle Mesh Ewald using a screening parameter of $\kappa = 0.33$ and Fast Fourier Transform grid spacing of less than 1 Å. The diffusion constant of K^+ was calculated from over 3 nanoseconds of equilibrated data using the Einstein relationship²⁰.

The factor of one-tenth applied for scaling the bulk diffusion coefficient value is based on the analysis of Mamonov et al¹⁷ suggesting that the Generalized Langevin Equation for the Harmonic Oscillator (GLE-HO) approach with extrapolation of the s-

dependent diffusion coefficient (Laplace transform of the memory function via Einstein relation) using small-“s” data more accurately extrapolates the channel diffusion characteristics in contrast to extrapolation from $15 < s < 35$ regime (Figure 8 in Mamonov et al¹⁷). With a bulk diffusion coefficient of 1.15×10^{-5} cm²/sec in 1M KCl solution (scaled to one-tenth over the channel length), we find that Equation 1 estimates a maximum conductance of 57pS; this overestimates the experimental value of 24 pS (picoSiemens) by a factor of 2.4. Based on the polarization response of ligands and water molecules in the pore channel, it is possible that the reduction of K⁺ diffusion coefficient will be greater than one-tenth due to the enhanced Coulombic interactions thus arising; this suggests that the actual absolute error may be lower than a factor of 2.4, and is encouraging. A more detailed analysis will be presented in a future study.

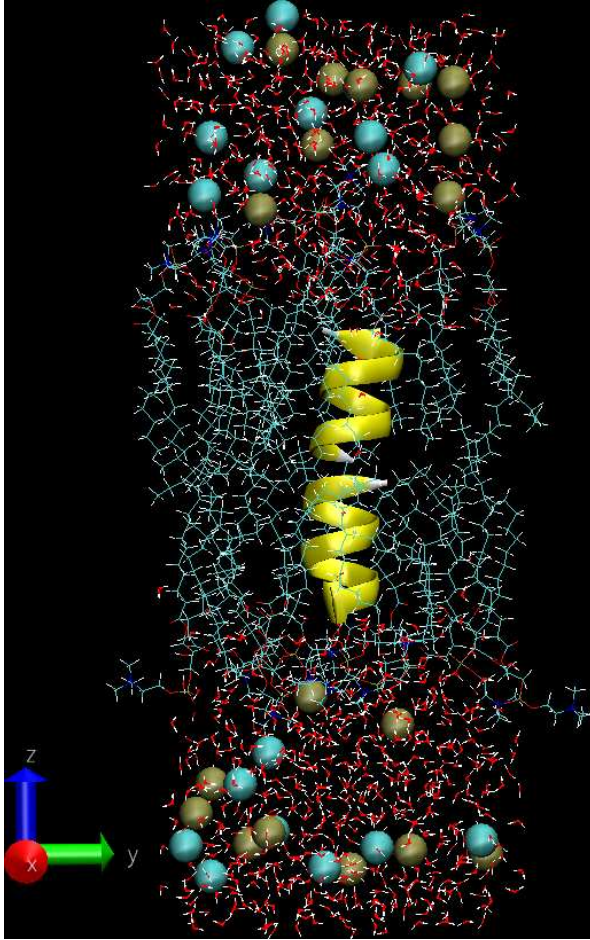


Figure 1: Snapshot of the simulation supercell used for the present calculations; the system contains the gA dimer embedded in a hexagonal DMPC bilayer solvated in TIP4P-FQ water with sufficient K^+ and Cl^- ions to provide a 1M salt concentration.

References

1. Brooks, B. R.; Brucoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M., CHARMM: A Program for Macromolecular Energy, Minimization, and Dynamics Calculations. *J. Comp. Chem.* **1983**, 4, 187-217.
2. Allen, T. W.; Bastug, T.; Kuyucak, S.; Chung, S.-H., Gramicidin A Channel as a Test Ground for Molecular Dynamics Force Fields. *Biophysical Journal* **2003**, 84, 2159-2168.
3. Allen, T. W.; Andersen, O. S.; Roux, B., Energetics of Ion conduction through the gramicidin channel. *PNAS* **2004**, 101, (1), 117-122.
4. Allen, T. W.; Andersen, O. S.; Roux, B., Ion Permeation through a Narrow Channel: Using Gramicidin A to Ascertain All-Atom Molecular Dynamics Potential of Mean Force Methodology and Biomolecular Force Fields. *Biophys. J.* **2006**, 90, 3447-3468.
5. Woolf, T. B.; Roux, B., Molecular dynamics simulation of the gramicidin channel in a phospholipid bilayer. *Proc. Nat. Acad. Sci.* **1994**, 91, 11631-11635.
6. Woolf, T. B.; Roux, B., Structure, Energetics, and Dynamics of Lipid-Protein Interactions: A Molecular Dynamics study of the Gramicidin A Channel in a DMPC Bilayer. *PROT. Struc. Funct. Gen.* **1996**, 24, 92-114.
7. Essmann, U.; Darden, T.; Lee, H.; Perera, L.; Berkowitz, M. L.; Pederson, L., A smooth particle mesh Ewald method. *J. Chem. Phys.* **1995**, 103, 8577.
8. Rick, S. W.; Stuart, S. J.; Berne, B. J., Dynamical fluctuating charge force fields: Application to liquid water. *J. Chem. Phys.* **1994**, 101, (7), 6141-6156.
9. Patel, S.; Brooks, C. L., III, CHARMM Fluctuating Charge Force Field for Proteins: I. Parameterization and Application to Bulk Organic Liquid Simulations. *J. Comp. Chem.* **2004**, 25, 1-15.
10. Patel, S.; MacKerell, A. D., Jr.; Brooks, C. L., III, CHARMM Fluctuating Charge Force Field for Proteins: II Protein/Solvent Properties from Molecular Dynamics Simulations Using a Non-Additive Electrostatic Model. *J. Comp. Chem.* **2004**, 25, 1504-1514.
11. MacKerell, A. D., Jr.; Feig, M.; Brooks, C. L., III, Extending the treatment of backbone energetics in protein force fields: Limitations of gas-phase quantum mechanics in reproducing protein conformational distributions in molecular dynamics simulations. *J. Comp. Chem.* **2004**, 25, 1400-1415.
12. Feig, M.; MacKerell A. D., Jr.; Brooks, C. L., III., Force Field Influence on the Observation of pi-Helical Protein Structures in Molecular Dynamics Simulations. *J. Phys. Chem. B* **2003**, 107, 2831-2836.
13. Portella, G.; Hub, J. S.; Vesper, M. D.; deGroot, B. L., Not Only Enthalpy: Large Entropy Contribution to Ion Permeation Barriers in Single-File Channels. *Biophysical Journal* **2008**, 95, (5), 2275-2282.
14. Mamonov, A. B.; Coalson, R. D.; Nitzan, A.; Kurnikova, M. G., The Role of the Dielectric Barrier in Narrow Biological Channels: A Novel Composite Approach to Modeling Single-Channel Currents. *Biophysical Journal* **2003**, 84, 3646-3661.
15. Kumar, S.; Bouzida, D.; Swendsen, R. H.; Kollman, P. A.; Rosenberg, J. M., The Weighted Histogram Analysis Method for free-energy calculations on biomolecules. *J. Comp. Chem.* **1992**, 13, 1011-1021.

16. Grossfield, A. *WHAM Code*, 2.0.2; 2008.
17. Mamonov, A. B.; Kurnikova, M. G.; Coalson, R. D., Diffusion constant of K⁺ inside Gramicidin A: A comparative study of four computational methods. *Biophysical Chemistry* **2006**, 124, 268-278.
18. Roux, B.; Allen, T.; Berneche, S.; Im, W., Theoretical and computational models of biological ion channels. *Quarterly Reviews of Biophysics* **2004**, 37, (1), 15-103.
19. Roux, B.; Allen, T.; Berneche, S.; Im, W., Theoretical and Computational Models of Ion Channels. *Quarterly Reviews of Biophysics* **2004**, 37, 15-103.
20. Allen, M. P.; Tildesley, D. J., *Computer Simulation of Liquids*. Clarendon Press: Oxford, 1987.