1. Introduction

Molecular recognition plays a central role in many biomolecular processes from enzyme reactions to signal transduction. An accurate description of protein–ligand interactions is, therefore, essential for understanding the function of biomolecules. Design of new drugs that modulate the behavior of biomolecules by selectively binding to them would also benefit from such an undertaking. The computational methods used for this purpose range from microscopic molecular dynamics (MD) simulations to mesoscopic Brownian dynamics and phenomenological docking algorithms, with many approximate methods in between. At present, no single method can describe fully and accurately the ligand-binding process, which therefore necessitates a more eclectic approach to this problem.

Classical MD simulations provide the most accurate and yet feasible method for description of protein–ligand binding. However, they are computationally very intensive, and a straightforward use of MD in searching for a ligand-binding site would be a very time-consuming task. Thus at present, their application to protein–ligand binding is mostly limited to those cases where the structure of the bound complex is already known from experiments. Of course, most of the time such information is not available, and in precisely such cases we would like to be able to predict the configuration of the bound complex given the structures of the protein and the ligand. With the computational power currently available, this would only be feasible by using a coarse-grained approach. Indeed many docking algorithms have been developed in the last 2–3 decades—especially in the context of drug discovery programs—that enable fast prediction of ligand binding conformations. However, despite the steady progress made over the years in improving the docking methods, they are still far from providing a satisfactory solution to the protein–ligand binding problem.

In this initial study, we choose the gA channel and organic cations for the protein–ligand system because of their simplicity and the wealth of information available. The structure of gA is very well-known from NMR studies—it is formed from the dimer of right-handed $\beta$-6.3 helices with 15 residues, which forms a cylindrical hole in the membrane with a radius of 2 Å. The gA channel is typically occupied by about 8 water molecules in single file. There are plenty of experimental data on
permeation properties of the gA channel. In summary, gA conducts monovalent cations near diffusion rates, binds divalent cations, and rejects all anions. There are two well-defined binding sites at the pore mouths, which are defined by the carbonyl groups of three Leu residues. Unlike other residues in gA, these carbonyls point outward and do not make any H-bonds with neighboring residues. The binding sites in gA have been probed in various experimental studies by using both cations and organic cations.

While there have been many MD studies of cation binding in gA, there appears to be only one such study for organic cations. In this study the PMF’s of methylammonium (MA), ethylammonium (EA), formamidinium (FMI), and guanidinium (GNI) were determined along the central axis of gA, using the umbrella sampling method. The calculated well depths at the binding sites were quite small (~1 kcal/mol), which may have resulted from using insufficient sampling—each umbrella window was simulated for 10 ps whereas more than 100 ps is deemed necessary in current MD simulations of cations to ensure the convergence of the PMF. Because the organic cations have more complex structures, they presumably require even longer simulations. In the present PMF calculations, we simulate each umbrella window for 1 ns, which should provide sufficient sampling. To develop a feeling for how the ligand size affects the binding results, we include in this study two larger cations, tetramethylammonium (TMA) and tetraethylammonium (TEA), in addition to the smaller MA, EA, FMI, and GNI. TMA and especially TEA are well-known blockers of ion channels, so understanding their mechanism of binding could be of interest for other channels. After comparing the docking and MD simulation results, we present detailed studies of the ligand configurations in the binding site of gA for each organic cation.

Blocking of ion channels by drugs/toxins bound to the pore mouth has many applications in pharmacology, physiology, and medicine. A molecular-level understanding of drug/toxin interactions with ion channels is, therefore, of great interest in these fields. The methods developed in this work are especially geared for such applications, and will be employed in future computational studies of drugs targeting ion channels.

2. Model Systems and Methods

2.1. Gramicidin A Channel. The simulation system for the gA channel is taken from recent computational studies where the effect of finite system size on ionic free energies was investigated and the convergence of the PMF of cations was studied. In ref 40, the 1JNO structure of the gA dimer was embedded in a lipid bilayer with a varying number of dimeristoylphosphatidylecholine (DMPC) molecules and hydrated with an increasing layer of water molecules. Comparison of free energy calculations for different system sizes indicated that a minimal system should consist of 16 DMPC molecules per layer and 5–6 layers of water between the lipid bilayer and the simulation box. Here we adopt this optimal, well-equilibrated gA system to study the binding of organic cations. Details of how the system was constructed and equilibrated can be found in refs 40 and 41. A snapshot of the simulation system with a methylammonium molecule in the lower binding site of the gA channel is shown in Figure 1.

Figure 1. A snapshot of the simulation system with a methylammonium molecule in the lower binding site of the gA channel. Lipid molecules are excluded for clarity.

2.2. Organic Cations. In this study we consider six organic cations as ligands: methylammonium, ethylammonium, formamidinium, guanidinium, tetramethylammonium, and tetraethylammonium. Three-dimensional (3D) structures of these molecules are generated with the CORINA (COoRdINAtes) software, which converts the 2D information contained in a connection table or a linear string to a 3D structure by using rules and databases. Accuracy and performance of CORINA among the competing 3D molecular structure generators is well established. The initial structures obtained from CORINA are then employed in the Gaussian98 program to optimize the structure and to calculate the partial charges on the atoms. The ab initio calculations are carried out by using the 6-31G(d) basis set at the MP2 level. The differences between the initial and optimized coordinates are found to be much less than 1%. The final structures of the organic cations are shown in Figure 2. The partial charges obtained from Mulliken analysis are listed in Table 1. For convenience, we have included the charges for the united atom groups (e.g., CH3, NH3) as well. It is seen that only in the case of guanidinium does the full ionic charge reside in the central atom. In all other cases, the charge +e is well spread among the various groups. We note that similar ab initio calculations performed in an aqueous phase with use of a continuum model yielded somewhat lower partial charges for TEA compared to the gas-phase results presented here. Nevertheless, the lowest energy structure predicted by CORINA for TEA is the correct quasiplanar conformation with D2d symmetry in agreement with the aqueous phase calculations.

2.3. Docking Calculations. We use AutoDock 3 to find the initial configurations for the organic cation-gA channel complexes that are employed in subsequent MD simulations. Because of its free license, good accuracy, and versatility, AutoDock has become the most popular docking program in recent years. AutoDock calculates the ligand–protein interaction energies over a grid that encompasses the binding site or active region of the protein. The scoring function used in the
energy calculations consists of electrostatic, Lennard-Jones, hydrogen bond, solvation, and torsional entropy terms. AutoDock uses a Lamarckian genetic algorithm in searching for the free energy minimum of the protein–ligand complex. In the current version, the protein is treated as rigid while the ligand is allowed torsional flexibility. Most of the binding configurations predicted by AutoDock are found to be very similar, so the one with the lowest free energy is adopted in MD simulations as the starting structure.

2.4. Molecular Dynamics Simulations. MD simulations are carried out by using the NAMD code, version 2.5, with the PARAM27 version of the CHARMM force field, which provides a complete set of parameters for all the atoms in the gA simulation system. The VMD suit of software is used in construction of the simulation systems and analysis of the result. MD simulations are performed by using an NpT ensemble with periodic boundary conditions. During the simulations, pressure is kept at 1 atm by using the Langevin piston method with a damping coefficient of 5 ps$^{-1}$. Similarly, temperature is maintained at 298 K through Langevin damping with a coefficient of 5 ps$^{-1}$. Electrostatic interactions are computed with the particle-mesh Ewald algorithm. The list of nonbonded interactions is truncated at 13.5 Å, and a switching cutoff distance of 10 Å is used for the Lennard-Jones interactions. A time step of 2 fs is employed for all simulations. Trajectory data are written at 1 ps intervals during both equilibration and production runs. Throughout the simulations, the center of mass (CM) of the gA channel is restrained by applying a harmonic force with $k = 100$ kcal/mol/Å$^2$.

To generate the initial protein–ligand complexes we make use of a previous gA simulation system with K$^+$ and Cl$^-$ ions in the bulk. In each case, one of the K$^+$ ions in the vicinity of the pore mouth is replaced with the corresponding organic cation and the overlapping water molecules are removed from the system. This initial system is subjected to 5000 steps of energy minimization followed by 100 ps MD simulations while the organic cation is restrained with a 10 kcal/mol/Å$^2$ harmonic force. Next the organic cation is steered to the binding position predicted by AutoDock by applying a harmonic force with $k = 20$ kcal/mol/Å$^2$. In this position, the organic cation is first equilibrated for 50 ps while it is restrained by a weak restraint (0.5 kcal/mol/Å$^2$). This restraint is then removed from the organic cation and the system is equilibrated for a further 1 ns. After ensuring that the system is properly equilibrated—that is, the organic cation has remained in the binding pocket and no distortions have occurred in the gA dimer—MD simulations lasting 1 ns are carried out for analysis of the binding configuration. A substantial distortion of the gA dimer is observed only in the case of formamidinium, for which AutoDock predicts an extreme off-axis binding site where the van der Waals radii of the gA and cation atoms almost overlap. In this case, the cation is pulled toward the axis so as to avoid distortion of the gA dimer.

2.5. Potential of Mean Force. The PMF’s of the organic cations along the reaction path defined by the channel axis are calculated by using umbrella sampling together with the weighted histogram analysis method (WHAM). As the method was explained in some detail in our earlier work, we give a brief account here. An umbrella potential is applied to the CM of the organic cation, and the CM position is sampled at equal intervals along the channel axis during MD simulations of the system. The biased CM distributions obtained from the production runs are then unbiased and combined with WHAM. In all cases, the CM coordinates of the organic cation are measured with respect to the CM of gA. We employ umbrella potentials with a force constant of 10 kcal/mol/Å$^2$ at 0.5 Å intervals. The initial system is taken from the 1 ns free MD simulations of the organic cations in their respective binding sites. The ligand is then pulled in either direction along the reaction path at 0.5 Å steps. The system is equilibrated for 1 ns in each umbrella window to ensure adequate equilibration of the organic cation. The PMF is calculated from a 1 ns production run for each window, where the CM coordinates of the organic cation are collected at every time step. The validity of the 1D-PMF approximation in the bulk region has been previously checked by comparing the PMF results with independent free energy perturbation calculation of the binding energy, which show complete agreement. Because the organic cations are much heavier than the cations used in the previous study, this is even less of a problem in the present case. We have nevertheless repeated the PMF calculation for TEA—whose binding site is furthest from gA—by including umbrella potentials in the radial directions to the channel axis. The 1D and 3D PMF’s are found to agree within 1 kT, which is the accuracy limit of the free energy calculations (we use the unit of kT for PMF, which is given by 1 kcal/mol = 1.7 kT at 298 K).

We use the PMF results to estimate the binding constants of the organic cations. To a good approximation, the binding constant can be evaluated by integrating the PMF, $W(z)$, along the reaction coordinate

$$K = \pi R^2 \int_{z_1}^{z_2} e^{-W(z)/kT} dz$$

where $R$ is the effective radius of a cylinder in the binding site that captures the organic cation. It is determined by sampling the radial position of the organic cation with respect to the channel axis during MD simulations in the binding pocket, and calculating from this sample the average radial position of the cation, $\rho$, and its standard deviation, $\Delta \rho$. The effective radius is then taken as $R = \rho + \Delta \rho$. Because of the exponential dependence of the integral in eq 1, the majority of the contribution comes from the binding pocket, which justifies this approximate determination of $R$. We note that a more accurate calculation of the binding constant requires a 3-dimensional integral of the Boltzmann factor in eq 1, and hence determination of the PMF in a 3-dimensional grid. This would require about 2 orders of magnitude more computational resources compared to the present calculation, which is clearly beyond our means.
The integration limits \( z_1 \) and \( z_2 \) in eq 1 should be chosen in the bulk region where \( W \) vanishes. Here we exploit the symmetry of the \( gA \) and choose \( z_1 \) inside \( gA \) where the PMF steeply rises, and therefore any contribution to the integral is negligible. The above expression is then multiplied by two to take into account the two binding sites in \( gA \).

3. Results and Discussion

Here we first compare the AutoDock predictions with the MD results to assess its suitability in generating initial protein–ligand configurations that can be profitably employed in MD studies of docking. This is followed by a detailed analysis of binding configurations for each organic cation. For this purpose we use the PMF results in Figures 3–5 and the 1 ns production data from free MD simulations of the organic cations in the binding pockets. The results are compared to experimental data where available.

3.1. Comparison of AutoDock and MD Results. We characterize the binding position of the organic cation with the \( z \) and \( \rho \) coordinates of its CM measured with respect to the CM of \( gA \). In Table 2, we compare the AutoDock predictions for these coordinates with those obtained from MD simulations. The MD results are obtained from the average of 1 ns simulations of the organic cations in the binding pocket. Except in the case of FMI, AutoDock predictions are quite close to the MD results differing at most by a few angstroms. As already mentioned in the Methods section, FMI is assigned an extreme off-axis position by AutoDock. This configuration could not be used in MD simulations as a starting point, and FMI had to be pulled toward the axis to prevent distortion of the \( gA \) dimer. MD simulation results in Table 1 show that FMI binds to \( gA \) in a slightly off-axis position, similar to the other organic cations.

![Figure 3. PMF’s for binding of methylammonium (top) and ethylammonium (bottom) to the \( gA \) channel. The zero level of the PMF is indicated by the dashed line. It is determined from the average of the PMF after it has leveled off in the bulk region.](image)

![Figure 4. PMF’s for binding of formamidinium (top) and guanidinium (bottom) to the \( gA \) channel.](image)
We also observe a definite trend in the binding positions determined from MD with respect to the molecular size. The smallest MA occupies an innermost binding site, and the larger size molecules bind progressively further away from the center of gA. With the exception of FMI, AutoDock is seen to reproduce this trend.

Further information on the binding of the organic cations to gA is extracted from their PMF’s presented in Figures 3–5. We have listed the binding positions obtained from the minimum of the PMF’s in the z column of Table 2. They are seen to agree well with the average z positions determined from the free MD simulations. The binding energies of the organic cations are determined from the PMF’s and listed in the last column of Table 2. The AutoDock predictions, listed in the previous column, are typically larger than those obtained from the PMF’s. Although it is difficult to surmise a definite trend from such data, we find that the average and standard deviation of this angle are $32(\pm 13)^\circ$. Thus the MA axis is not parallel to the gA axis but slightly tilted away from it.

From studies of cation binding to gA, we know that the carbonyl groups of Leu26, Leu28, and Leu30 residues, which do not make H-bonds with the other residues in gA, play a significant role in the binding process. Here the Leu carbonyl oxygens play a similar role in coordinating the hydrogen atoms of the NH$_3$ and CH$_3$ groups. We note that because the H atoms in either group have a net positive charge, N–H (or C–H) does not have to be oriented along the C=O bond for the interaction to be effective. For the same reason, the H–O interaction is stronger compared to that of an H-bond. A typical snapshot of MA in the binding pocket demonstrates this point (Figure 6). One H atom from NH$_3$ is coordinated by the Leu26 oxygen and two H atoms from CH$_3$ are coordinated by the Leu28 and Leu30 oxygens (distance about 2 Å). This picture also helps to explain why the MA axis is tilted—it reduces the H–O distances and hence maximizes the H–O interactions. While this snapshot shows the dominant binding configuration of MA, in reality it is far from being static. To make the dynamic nature of the MA binding to gA clear, we show in Figure 7 the H–O distances between the amine H atoms and the Leu26 oxygen. It is seen that the hydrogen bond is retained most of the time albeit with different H atoms in NH$_3$. It is broken on a few occasions, which coincide with the fluctuations of MA in the z direction. Then the amine H atoms form alternate hydrogen bonds with the other residues in gA, e.g., Leu28. More interestingly though, the H atom making the hydrogen bond in NH$_3$ is frequently switched around, demonstrating that the MA molecule makes random 120° rotational jumps around the N–C axis. Due to the symmetry of MA around the N–C axis, there is no energy penalty associated with such rotations. Thus the entropic contributions from such rotations help to increase the absolute binding free energy.

The binding constant $K$ of MA can be estimated from eq 1 by using the PMF result in Figure 3 and numerically integrating the Boltzmann factor. For the effective radius, we use $R = \rho + \Delta \rho$, and take the $\rho$ and $\Delta \rho$ values from Table 2. The resulting binding constant for each organic cation is listed in the last column of Table 2. For MA, the calculated value of $K = 4.1$
M$^{-1}$ is in good agreement with the experimental value of 4.4 M$^{-1}$. MA is known to permeate the gA channel at a rate slightly less than those of the alkali cations. However, our PMF calculations inside the pore region reveal a steeply rising free energy profile just as in the case of K$^+$ and Na$^+$ ions. As argued in ref 41 this barrier is likely to result from the lack of polarization interaction in the current generation of force fields. So a proper description of the permeation properties of cations in the gA channel has to wait for the new polarizable force fields that are under construction.

3.3. Ethylammonium. In EA the NH$_3$ group has the same partial charges as in MA but the remaining 0.5e charge is distributed among the CH$_2$ and CH$_3$ groups, which reduces the effectiveness of the H–O interactions associated with these groups. This is reflected in the smaller binding energy of EA compared to MA (Table 1 and Figure 3). A plot of the $z$ coordinates of the N and the middle C atoms shows that most of the time they occupy similar positions to the corresponding N and C atoms in MA. There are some small differences, e.g., compared to that of MA, the binding pocket for the N atom is slightly further outside ($z = 11.5$ Å) and a little more off-axis ($\rho = 0.9$ Å). Also the N–C axis is slightly more tilted (the angle between the N–C and gA axes is 38(±18)$^\circ$). During the 1 ns simulation, EA is observed to make one excursion outside the binding pocket where the N–C–C plane takes a parallel position to the membrane at $z = 13.5$ Å for about 150 ps. In the following we will consider the dominant binding configuration, where NH$_3$ remains in the binding pocket. In this configuration, the H–O interactions among the H atoms of EA and the Leu oxygens of gA are quite similar to those of MA, e.g., the Leu26 oxygen coordinates an H atom from NH$_3$ and the Leu28 and Leu30 oxygens coordinate the two H atoms in CH$_2$ (see Figure 6). One significant difference is that rotation of the EA around the N–C axis is suppressed because it would require breaking of the H–O bond for one of the H atoms in CH$_2$. Thus unlike in MA, rotation costs energy in EA due to its asymmetric structure. Entropic cost of this rotational immobilization of EA is also responsible for the reduction in its binding free energy compared to MA.

Comparing the PMF of EA with that of MA (Figure 3), we see that its binding free energy is much smaller and it steeply rises even before entering the pore region of gA. The binding energy of EA is also smaller than those of the alkali cations. Finally the calculated binding constant of EA is about 20 times smaller compared to that of MA (Table 2). All these are consistent with the experimental observations that find no block effect associated with EA, indicating that EA neither enters the pore of the gA channel nor competes with other cations to bind to the pore mouth long enough to have an observable effect. Considering the similarity of the binding configurations of EA and MA, a slight redistribution of charge away from the binding pocket in EA is seen to have quite a drastic effect on its binding properties.

3.4. Formamidinium. The charge distribution in FMI is seen to be very different compared to those of MA and EA (Table 1). The majority of charge resides on the central C atom and the NH$_2$ groups have much smaller charges compared to NH$_3$ in MA and EA. This results in weakening of the H–O interactions among the NH$_2$ groups and Leu oxygens and changes the character of binding. Instead of coordinating the H atoms, the Leu oxygens actually coordinate the central C atom, which is similar to the binding of alkali cations. Inspection of the $z$ coordinates of the atoms in the 1 ns production data shows

![Figure 6](image6.png)

Figure 6. Snapshots of MA (left) and EA (right) in the binding pocket of gA depicting the main H–O interactions of the H atoms in MA (and EA) with the Leu oxygens in gA. The positions of the oxygens are as follows: Leu26, lower-left; Leu28, lower-right; and Leu30, upper-right.

![Figure 7](image7.png)

Figure 7. Hydrogen bond formation between the NH atoms of MA and the Leu26 oxygen in gA. During the 1 ns period, all three H atoms are observed to make H-bonds with the Leu26 oxygen.
that in the main binding configuration, the C atom is at $z = 12.5 \, \text{Å}$, one of the NH$_2$ groups points toward the pore and the other away from it. This binding location is about 1 Å further away compared to that of the K$^+$ ion, which is due to the larger size of FMI. Analysis of the C–O distances between the C atom and the Leu oxygens reveals a coordination distance of between 3 and 3.5 Å for Leu26 and 28, and 3–5 Å for Leu30. These are very similar to those found for the K$^+$ ion, except they are slightly larger due to the size constraint in FMI. This explains the smaller binding free energy of FMI compared to that of the K$^+$ ion. We remark that H–O interactions play a relatively minor role in binding of FMI. Only the H(C) atom makes a stable H-bond with the Leu oxygens—mostly with Leu26 as shown in Figure 8. However, the average H–O distance is 2.7 Å in this case, which is considerably larger than the usual 2 Å found in the other H–O interactions, making this a weak interaction. One of the H atoms in the NH$_2$ group in the binding pocket also makes sporadic H-bonds with the Leu oxygens. FMI is observed to make one inward and one outward fluctuation from this binding position. In the outward case (lasting 250 ps), EA takes a different configuration where the C atom moves further away and both NH$_2$ groups point toward the pore. This is very similar to the main binding configuration found in GNI (see below).

The calculated binding constant of FMI (0.6 M$^{-1}$) is much smaller than the experimental value of 23 M$^{-1}$. To reproduce this value, the binding free energy in Table 2 needs to be roughly doubled. Because the interactions of other cations with gA are rather well described in the pore mouth, we do not anticipate that there could be such a large discrepancy in binding of FMI to the pore mouth. An alternative explanation of this discrepancy is that there is a central binding site with a lower binding free energy, which leads to the larger binding constant observed in experiments. This is also supported by the experimental observation that FMI is unique among the cations in stabilizing the gA channel, i.e., keeping the two monomers forming the channel together. This determines that FMI must bind to gA with a much stronger free energy at the dimer junction (channel center).

3.5. Guanidinium. GNI has a similar charge distribution to FMI except it is even more centralized—the whole charge resides in the central C atom. In contrast to other organic cations, there are no extra partial charges on the H atoms that could strengthen the H–O interactions with the Leu oxygens. Despite this, GNI exhibits the least amount of fluctuation in the z direction and its (absolute) binding free energy is larger than that of FMI (Table 1). Inspection of the z coordinates of the C and N atoms and the angles C–N axes make with the gA axis shows that GNI has a well-defined configuration in the binding site, which persists throughout the 1 ns simulation period. In this configuration, two of the NH$_2$ groups insert to the pore and the remaining one points away. One H atom from each of the inserted NH$_2$ groups makes an H-bond with Leu26 and Leu28 oxygens, respectively (see Figure 8). Otherwise the central C atom is coordinated by the Leu oxygens in a manner similar to that for FMI. Thus the presence of extra H-bonds in GNI helps to stabilize its binding configuration and leads to a deeper binding well compared to that of FMI, which lacks such H-bonds. Because of its symmetric structure, any pair of the NH$_2$ groups in GNI could insert to the channel. Thus, in principle, one should observe 120° rotations of GNI around an axis perpendicular to its plane—similar to the rotations seen in MA (cf. Figure 7). The fact that no such rotations occur during the 1 ns simulation period indicates that the activation barrier is much higher in the case of GNI. Experiments show that GNI causes voltage-relieved flicker block of K$^+$ currents in the gA channel. The calculated binding free energy for GNI (−5.9 kT) is slightly deeper than that of K$^+$ (−4.5 kT), which is consistent with this observation.

3.6. Tetramethylammonium. In TMA the charge is distributed over the four CH$_3$ groups, which weakens the electrostatic interactions. This is compensated to a degree by the fact that the H atoms in the CH$_3$ groups carry a net partial charge (Table 1), which strengthens the H–O interactions with the Leu oxygens. In addition, thanks to its nonplanar structure, TMA is quite compact, allowing it to enter the binding pocket of gA and make H-bonds with the Leu oxygens. Analysis of the z coordinates of the C atoms shows that, at any given time, one of the C atoms is in the binding pocket (z ≈ 11–12 Å) with the N–C axis making an ca. 20° angle with the gA axis. The other three C atoms are slightly outside (z ≈ 13–4 Å) and their N–C axes point away from the membrane plane by a small angle (0–30°). A typical snapshot of TMA in the binding pocket is shown in Figure 9. We remark that, unlike in GNI, this is a very dynamic configuration. TMA makes sudden rotational jumps around its central axis, and the C atom that is inserted in the pocket is frequently swapped around with the other C atoms. Because of this dynamic nature, the H-bonds the CH$_3$ groups make with the Leu oxygens also change frequently both in nature and in number. For example, the number of H-bonds varies between 3 and 6. This makes it difficult to give a simple analysis of the situation as in the other cases. An important point to make here is that, although TMA is further away from EA, thanks to the entropic contributions, its binding free energy is deeper than that of EA.

There appears to be only one experimental study of blocking effects of TMA in the gA channel, which was carried out with...
H$^+$ ions. In this study, TMA was actually found to facilitate H$^+$ conductance when the current was directed away from the TMA side, and had no effect when the current was toward the TMA side. This peculiar effect can presumably be explained by the fact that H$^+$ conducts by hopping over the H network of water and other molecules, which is beyond this study. Our results suggest that TMA would directly compete with alkali water and other molecules, which is beyond this study. Our predictions (e.g., binding constants of organic cations), which are based on the available experimental data. We have also made specific predictions (e.g., binding constants of organic cations), which can be tested in future experiments. The success of the combined AutoDock and MD simulations in these relatively simple systems should encourage application of this methodology to more sophisticated and medically relevant systems such as drug and toxin binding to biological ion channels.

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Binding of Organic Cations to Gramicidin A Channel


