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Investigation of polarization effects in the gramicidin A channel from 
*ab initio* molecular dynamics simulations

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Polarization is an important component of molecular interactions and is expected to play a particularly significant role in inhomogeneous environments such as pores and interfaces. Here we investigate the effects of polarization in the gramicidin A ion channel by performing quantum mechanics/molecular mechanics molecular dynamics (MD) simulations and comparing the results with those obtained from classical MD simulations with non-polarizable force fields. We consider the dipole moments of backbone carbonyl groups and channel water molecules as well as a number of structural quantities of interest. The *ab initio* results show that the dipole moments of the carbonyl groups and water molecules are highly sensitive to the hydrogen bonds (H-bonds) they participate in. In the absence of a K⁺ ion, water molecules in the channel are quite mobile, making the H-bond network highly dynamic. A central K⁺ ion acts as an anchor for the channel waters, stabilizing the H-bond network and thereby increasing their average dipole moments. In contrast, the K⁺ ion has little effect on the dipole moments of the neighboring carbonyl groups. The weakness of the ion-peptide interactions helps to explain the near diffusion-rate conductance of K⁺ ions through the channel.

We also address the sampling issue in relatively short *ab initio* MD simulations. Results obtained from a continuous 20 ps *ab initio* MD simulation are compared with those generated by sampling ten windows from a much longer classical MD simulation and running each window for 2 ps with *ab initio* MD. Both methods yield similar results for a number of quantities of interest, indicating that fluctuations are fast enough to justify the short *ab initio* MD simulations.

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I. INTRODUCTION

Molecular dynamics (MD) simulations have become an indispensable tool for studying properties of condensed matter systems ranging from materials1,2 to carbon nanotubes,3 and biomolecules.4,5 For historical and practical reasons, the force fields (i.e., potential functions) employed in most MD codes consist of non-polarizable pair-wise potentials. As the polarization interaction cannot be completely ignored, its effects are included in an average way by increasing the partial charges on atoms, which imparts an effective induced dipole moment on groups of atoms. This approximation appears to work quite well in homogeneous or bulk-like systems where the force fields are optimized, but problems have been found in inhomogeneous systems such as pores,6–10 cavities,11–13 and interfaces.14–16 For a proper description of such systems, it is imperative that the polarization interaction is included in the classical force fields. Despite ongoing efforts,16–20 a properly optimized polarizable force field with broad applicability is yet to be developed (see, e.g., Ref. 21 and the accompanying special issue on polarization).

Due to its many-body aspect, it is much more difficult to incorporate the polarization interaction in classical force fields. Experimental data have usually been sufficient to constrain the model parameters for nonpolarizable force fields, but this strategy has not worked as well in the case of polarizable force fields. Therefore, complementary information from *ab initio* studies of polarization effects would be welcome, and could be very helpful in the construction of polarizable force fields. A pertinent example is provided by the *ab initio* calculations of the potential of mean force (PMF) for dissociation of Na–Cl and Ca–Cl ion pairs.22, 23 There are no experimental data for constraining the ion–ion interactions. Therefore, the corresponding Lennard-Jones parameters are parameterized using *ad hoc* combination rules, resulting in substantial variations among various force fields.24 The *ab initio* PMFs and the associated dipole moment and hydration number results22, 23 provide an alternative method for parameterizing the ion–ion interactions, which would be especially useful in the construction of polarizable force fields.

It is worthwhile to stress the key role played by water as the medium in the above example, and the importance of its correct modeling. The experimental dipole moment of the water molecule is 1.86 D in the gas phase25 and 2.9 ± 0.6 D in the bulk phase.26 The non-polarizable TIP3P water model has a fixed value of 2.35 D,27 while *ab initio* calculations yield a value of 3.0 D for bulk water,28–31 and rather large variations from this value depending on the environment. For example, the dipole moment of water gets smaller in the hydration shell of monovalent ions while it is boosted in the hydration shell of divalent ions (e.g., 2.8 D for K⁺ and 3.4 D for Ca²⁺).30–33 These results highlight the important role played by the H-bond network in polarization of water molecules.34–36 Its disruption even by a monovalent ion...
leads to loss of polarization for hydration waters, and only the electric field of a divalent ion is strong enough to compensate for it.

In narrow pores, the above picture for bulk water is expected to undergo two significant changes. First, breaking of the H-bond network results in loss of polarization for pore waters. Second, the presence of an ion polarizes pore waters more strongly because their dipoles are aligned along the pore axis—hence, they cooperate rather than compete as they do in a bulk environment. This intuitive picture was encouraged by ab initio calculations of water dipole moments in the gramicidin A (gA) channel and a carbon nanotube (CNT).37 In both cases, pore waters have a single-file configuration, and their dipole moments are significantly reduced compared to bulk. But introduction of a K+ ion at the center of the pore induces further polarization of the water chain, increasing their dipole moments to near bulk values.

The gA channel provides a simple yet realistic system for studying the polarization effects in narrow pores. Despite the disruption of the hydration shell to a single-file configuration, monovalent cations can permeate across gA near diffusion rates thanks to the carbonyl groups on the β-helix peptide.38 PMF calculations for a K+ ion in the gA channel with non-polarizable force fields have resulted in a large (∼20 kT) free energy barrier at the channel center,6–8 which is not compatible with the observed diffusion rates. As stressed above, and demonstrated in a preliminary calculation,39 resolution of this problem requires inclusion of polarization in the force fields. Recognizing the importance of the gA channel for development and benchmarking of the polarizable force fields, here we perform detailed quantum mechanics/molecular mechanics (QM/MM) MD simulations of gA with and without an ion at the channel center, and contrast the results with those obtained from classical MD simulations of gA with a non-polarizable force field. Our detailed ab initio simulations confirm that the dipole moments of the channel waters are boosted by the presence of a central ion. Substantial variations occur in water dipole moments depending on the environment, and therefore, polarization should be taken into account in the description of processes where the environment changes.

II. METHODS

A. Classical MD simulations

In a previous classical MD study of gA, we determined the PMF of a K+ ion along the channel axis from umbrella sampling simulations.40 The last frame of the umbrella sampling run in that study, where the K+ ion is at the center of the channel, is taken as the starting configuration for this study. The system consists of the gA dimer (32 amino acids) embedded in a bilayer of 32 dimyristoylphosphatidylcholine (DMPC) molecules, and hydrated with 1532 water molecules and 4 KCl ion pairs. One of the K+ ions is at the center of the channel and the rest of the ions are in the bulk. The dimensions of the periodic cell are 38 Å, 32 Å, and 72 Å in the x, y, and z directions, respectively. We will refer to this as the “ion” system.

The central K+ ion in the initial configuration is restrained by a harmonic potential with $k = 10$ kcal/mol Å$^2$, and the system is simulated for 1 ns with classical MD. Ten frames are chosen from this set for the purpose of addressing sampling issues in ab initio MD simulations. The frames are chosen such that they are at least 70 ps apart to ensure statistical independence and the average K+–O distances with the carbonyl oxygens in the hydration shell (i.e., the Val1, Ala3, Val1′, and Ala3′ residues) are maintained. Note that residues in both monomers are numbered from 1 to 16 to make the symmetry of the structure explicit. The two sets are distinguished by using primes for one set. A second system is generated by exchanging the central K+ ion in the initial configuration with a bulk water molecule. We will refer to this as the “water” system. This system is equilibrated for 1 ns, and a frame with nine water molecules in the pore is chosen for ab initio MD simulations. Both the “ion” and “water” systems are run for a further 2 ns to collect production data, and all classical results are calculated from these data.

The classical MD simulations are performed using the NAMD software,41 with the CHARMM force field.42 The VMD software is used in system preparation and data analysis.43 Simulations are performed in the NpT ensemble using Langevin damping to maintain the temperature at 300 K and Langevin piston pressure control to keep the pressure at 1 atm, using a damping constant of 5 ps$^{-1}$ in both cases. Electrostatic interactions are computed using the particle-mesh Ewald method. For atom pairs between 10 Å and 12 Å apart, a switching function is employed to bring the Lennard-Jones interactions to zero smoothly at the cut off distance of 12 Å. A time step of 2 fs is used in all classical MD simulations. Production data are recorded every 500 fs.

B. QM/MM MD simulations

Because the simulation system is too large for a purely quantum treatment, we use a quantum mechanical/molecular mechanical method to perform the ab initio MD simulations.44–46 In a QM/MM simulation, the system is divided into two parts consisting of the quantum and classical regions. In the classical region, the atomic motion is modeled using a classical force field as described in Sec. II A. In the quantum region, the electronic motion is determined using the density functional theory (DFT),37 and the nuclei propagate in the corresponding ab initio potential according to Newton’s equation of motion.38 The coupling between the quantum mechanical (QM) and molecular mechanical (MM) regions is handled following the prescription in Ref. 49, which provides a smooth transition between the two regions. Here the electron spill out problem is avoided by saturating the Coulomb potential of the classical atoms near the QM region. The QM/MM boundary is chosen such that it cuts only through the $C_{α}$–C bonds of the peptide chain and $C_{α}$–$C_{β}$ bonds to remove side chains. The resulting dangling bonds in the QM region are capped by H atoms. The remaining bonded interactions that involve the QM and MM atoms are described by the classical force field. The AMBER PARM99 force field has been used for description of the MM part, except for the
lipid molecules, which are described using the general AMBER force field (GAFF).52

The quantum problem is solved using Car-Parrinello MD (CPMD) as implemented in the CPMD code.53 All quantum atoms are described using norm-conserving pseudopotentials—Martins-Troullier pseudopotentials 54 for K+, O, N, and C, and a von Barth-Car pseudopotential 55 for H. For the K+ ion, the 3s and 3p semicore states are treated explicitly as valence states. We use the Becke-Lee-Yang-Parr (BLYP) exchange-correlation functional,56,57 which provides a satisfactory description of polarization effects in aqueous systems.28,29,58–60 The dispersion correction to DFT is not included in the present work because in tests for a K+ ion in water it has been found to have negligible effect on dipole moments (see Table S1 in the supplementary material61). Atomic positions are calculated using a timestep of 4 a.u. (∼0.1 fs), and the electrons are given a fictitious mass of 400 a.u. The plane wave basis set, used to represent the electronic structure, is truncated at 70 Ry. For each configuration, the wavefunction is optimized using the method of preconditioned conjugate gradients and is considered to be converged once the largest component of the gradient is less than 10−5. This is followed by 2 ps of equilibration, and then data collection. Throughout the equilibration and data collection phases, the temperature is maintained at 300 K using a Nosé-Hoover thermostat chain for the ions with a frequency of 1200 cm−1.62,63 Snapshots of the trajectory are saved every 1 fs for analysis.

We consider two QM regions to facilitate the ab initio calculations. To address the sampling issues, which require lengthy simulations, we use a small QM region consisting of the central K+ ion, the two neighboring water molecules (denoted by W1 and W1′), and the gA residues that are involved in the ion’s coordination (Fig. 1). The gA residues included are Val1, Gly2, Ala3, Leu4, Val1′, Gly2′, Ala3′, Leu4′, and the formyl moieties (the gA residues in the upper monomer are numbered from 1 to 16, and the lower ones from 1′ to 16′). Since the carbonyl groups of Leu4 and Leu4′ are not involved in coordinating the ion, the boundary is cut across the Cα–C bonds of Leu4 and Leu4′. The side chains of all residues point away from the channel, therefore they are excluded from the QM region. The resulting system contains 59 atoms in the QM region in addition to the 8 H atoms used in capping the dangling bonds. The Kohn-Sham orbitals are calculated on a 3D grid of size 26.5 × 27 × 25 Å3, which is large enough to avoid any spill-out of the electronic density over the course of the simulations. Ab initio MD simulations are performed for each of the ten frames obtained from the classical MD simulations. In each case the system is equilibrated for 2 ps, followed by 2 ps of data collection. For one frame the ab initio simulations are continued for a further 18 ps to obtain a continuous 20 ps of data. For comparison, an analogous set of simulations is also run for a system in which the central K+ ion is replaced by a water molecule (the “water” system).

The small QM region considered above focuses on the ion and its immediate environment, which is not sufficient to understand the role polarization plays in the channel dynamics. For a proper investigation of the polarization effects, one needs to include all the water–water and water–peptide interactions in the QM region, in addition to the ion–water and ion–peptide interactions. This is because the ion’s unhindered permeation across the channel is due to the interaction of the ion–water chain with the entire peptide backbone. For this purpose, we consider a larger QM region that covers the whole length of the gA channel and includes a central K+ ion and eight pore waters (see Fig. 1). Again the side chains and parts of the etanomyl groups, which point away from the channel, are excluded from the QM region. The number of atoms in the QM region has increased to 223 and 30 hydrogen atoms are needed to cap the dangling bonds. The grid size used in the calculation of the Kohn-Sham orbitals is 31.3 × 27 × 59.5 Å3, which again, is large enough to avoid any spill-out of the electronic density. Such a large set of quantum atoms is computationally very expensive, and therefore, only 10 ps of data are collected for analysis following 3 ps of equilibration. For comparison, an identical calculation is performed for the “water” system where the K+ ion is replaced with a water molecule. In this case, however, a water molecule leaves the channel during the simulation, permitting only 5.6 ps of production data to be collected, following 1 ps of equilibration. Justification for the shorter simulation time and equilibration period is provided while discussing the results.

The effects of polarization are investigated by calculating the dipole moments of water molecules and carbonyl groups in the QM region. For this purpose we use the maximally localized Wannier functions (MLWFs) analysis.64 The MLWF orbitals are obtained from the DFT calculations via a unitary transformation and their spread is minimized iteratively. Using the centers of the
MLWFs and the charges associated with them, dipole moments can be calculated from the classical formula,

$$\mu = \sum_n Z_n e \mathbf{R}_n - \sum_i z_i e \mathbf{r}_i.$$  

(1)

Here, the first sum is over the nuclei at positions $\mathbf{R}_n$ with charges $Z_n e$, and the second sum is over the MLWF centers at positions $\mathbf{r}_i$ with charges $-z_i e$. For water, $Z_O = 6$, $Z_H = 1$, and there are four MLWF centers each with $z_i = 2$. Two of the MLWF centers correspond to the covalent O–H bonds and the other two correspond to the lone-pair orbitals. For carbonyls, $Z_O = 6$, $Z_C = 4$, and six MLWF centers are used. Two of them form the C–O double bond each with $z_i = 2$, the other two represent the lone-pairs of the oxygen atom with $z_i = 2$, and the remaining two form the C–N and C–C$_a$ bonds each with $z_i = 1$. Note that both water molecules and carbonyl groups are charge neutral, and hence their dipole moments do not depend on the choice of the origin. The MLWF analysis has been used in numerous ab initio calculations of dipole moments in the past. 22, 23, 28–33 Also, this method has been successful in reproducing the dipole moments of water clusters $(n = 1, \ldots, 5)$, where experimental data are available. 65, 66

III. RESULTS AND DISCUSSION

A. QM/MM MD simulations in a small QM box: Addressing the sampling issue

Sampling is a major issue in ab initio MD simulations yet it is rarely addressed in the literature. Typically, one runs an ab initio MD simulation for as long as is feasible and assumes that the system has been sampled sufficiently to draw valid conclusions from the collected data. An alternative method is to begin with many independent snapshots of the system from classical MD simulations and perform short ab initio MD simulations on each of them. Here the assumption is that the system is equilibrated after each short simulation. A simple way to test these assumptions is to compare the ab initio MD results obtained using these two methods.

In Fig. 2, we compare a number of structural quantities calculated from two sets of data: one continuous 20 ps simulation vs. ten independent 2 ps simulations. All plots in Fig. 2 are for microscopic quantities, which will be further discussed in Secs. III B 1–III B 4 using the larger QM system. In particular, Fig. 2 gives (a) the average dipole moments of the carbonyl groups coordinating the K$^+$ ion (Val1, Val1’, Ala3, and Ala3’) and all the QM water molecules, (b) the average angle of these four carbonyl groups relative to the channel-axis, (c) the average distances between the ion and each of its coordinating oxygen atoms (i.e., Val1, Val1’, Ala3, Ala3’, W1, and W1’), and (d) the average C–O–K$^+$ angles associated with these four carbonyl groups. For the majority of these quantities, both data collection methods give similar results. The difference between the average values is much smaller than the corresponding $\sigma$s, and the $\sigma$ values, which measure the spread of the distributions, are themselves very similar. This point can be made more quantitative by inspecting the distributions of the calculated dipole moments. Data provided in the supplementary material61 show that there is a very good overlap between the dipole distributions obtained from one long and ten short simulations. Furthermore, both distributions are approximately Gaussian. These observations indicate that sufficient sampling is achieved for the quantities considered from a single 20 ps ab initio simulation and sampling from multiple starting configurations is not necessary.

Ab initio simulations of the large QM system is computationally very demanding, and running this system for 20 ps is beyond our present capacity. We have, therefore, used the 20 ps data from the small QM system to investigate whether converged results can be achieved in shorter simulations. For each of the quantities given in Fig. 2, we break the 20 ps simulation into two 10 ps blocks and calculate the corresponding 10 ps block averages. These averages, together with the averages calculated from the entire 20 ps of data, are provided in

![Fig. 2](image-url)  

Comparison of the results obtained from the continuous 20 ps simulations (black bars on the right) with those obtained from ten 2 ps simulations (red bars on the left) for various structural quantities. (a) Dipole moments of the coordinating water molecules and carbonyl groups. (b) Angle between the channel axis and the coordinating carbonyl groups. (c) Distance between the K$^+$ ion and its coordinating oxygen atoms. (d) C–O–K$^+$ angle made by the K$^+$ ion and the carbonyl groups coordinating the ion.
the supplementary material (Fig. S3). The most important observation for our purposes is that variations in the average dipole moments between the two 10 ps sets of data are much smaller than the fluctuations, thus, 10 ps appears to be sufficient for reliable calculations of dipole moments. For the “water” system, other quantities are also converged within statistical accuracy using only 10 ps of data. For the “ion” system, quantities that depend on the ion’s position are affected by its motion.

Thus, converged results can be achieved using 10 ps of data with some provisions. First, when an ion is present, one must track its motion and use the position data in interpreting quantities that involve its position. Second, dipole moments of water molecules are highly dependent on the set of H-bonds they form. Water molecules in the channel tend to donate a H-bond to an adjacent water molecule, but breaks in the water chain can occur, which has occurred in two of the short 2 ps “water” simulations. Thus, attention needs to be paid to the H-bond network of the water molecules, and dipole moments of water molecules should be understood in this context. We will elaborate on this idea in Sec. III B when we examine the H-bond networks in the large QM systems.

B. QM/MM MD simulations in a large QM box: Effects of polarization in gA

Here we present the results obtained from the QM/MM simulations using the large QM region and compare them to their classical counterparts calculated using the CHARMM force field. We begin by examining the effects of polarization on the average dipole moments of the channel water molecules and the carbonyl groups of gA. Due to the helical structure of the channel, which is lined by carbonyl groups, there is a rich network of H-bonds inside the channel formed by the channel waters and the backbone carbonyl groups. We pay special attention to this H-bond network as it is highly responsible for the particular dipole moments calculated for the water chain and the carbonyl groups. We then compare a number of structural quantities calculated from the QM/MM and classical MD simulations, which provide further understanding of the effects of polarization in the gA channel.

We first examine the motion of the ion and identify any stable positions it takes. Figure 3 gives the distance between the ion and the carbonyl oxygen atoms of the central valine and alanine residues as a function of time, taken from the 10 ps simulations using the large QM region. The column “ion” refers to the “ion” system and the column “water” refers to the “water” system. Results are calculated approximately every 100 fs—each average value is determined from 100 data points in the “ion” simulations and 54 data points in the “water” simulations. To ensure that no charge transfer occurs between the coordinating carbonyl groups and the ion, we compare the distances between the carbonyl-oxygen atoms and their lone pairs calculated from the “ion” and “water” simulations, which are found to be very similar (see Table S2 in the supplementary material). The most striking feature of the results in Table I is that the water dipole moments are considerably enhanced in the presence of the ion. However, this cannot be a simple electrostatic effect because the waters next to the ion (W1 and W1′) have smaller dipole moments than the next neighbors (W2, W3, and W2′, W3′). A similar behavior is also observed for an ion in bulk water—dipole moments of hydration waters in the first shell are considerably enhanced in the presence of the ion. We will elaborate on this idea in Sec. III B when we examine the H-bond networks in the large QM systems.

1. Dipole moments

The average dipole moments of each water molecule in the channel and each carbonyl group in the backbone are presented in Table I and Fig. 4, respectively. Dipole moments are calculated approximately every 100 fs—each average value is determined from 100 data points in the “ion” simulations and 54 data points in the “water” simulations. To ensure that no charge transfer occurs between the coordinating carbonyl groups and the ion, we compare the distances between the carbonyl-oxygen atoms and their lone pairs calculated from the “ion” and “water” simulations, which are found to be very similar (see Table S2 in the supplementary material). The most striking feature of the results in Table I is that the water dipole moments are considerably enhanced in the presence of the ion. However, this cannot be a simple electrostatic effect because the waters next to the ion (W1 and W1′) have smaller dipole moments than the next neighbors (W2, W3, and W2′, W3′). A similar behavior is also observed for an ion in bulk water—dipole moments of hydration waters in the first shell are considerably enhanced in the presence of the ion.
Val1, Val1 $\pm$ 0.17 D. This observation holds even for the carbonyls of moments of W1 dipole moments. In the “water” system, however, the dipole averages for the “water” system are 2.32 the right and left monomers, respectively, while the same averages are lower than those in the second shell.31 This is explained from the “ion” and “water” simulations. The

**FIG. 4.** Dipole moments of the carbonyl groups in the gA channel obtained from the “ion” and “water” simulations. The x-axis denotes the residue number of the carbonyl group. Standard deviations are not shown to avoid cluttering but they remain around the average value of 0.26 D.

are lower than those in the second shell.31 This is explained in terms of breaking of the H-bond network by the ion, and a similar explanation will be given here for the gA channel below.

Comparing the dipole moments of waters between the right (W1–W3) and left (W1′–W3′) sides of the dimer, we find only slight differences in the “ion” system, which are well within the fluctuations (Table I). This confirms that the ion’s position does not have an appreciable effect on the water dipole moments. In the “water” system, however, the dipole moments of W1′–W4′ are consistently larger than those of W1–W4. Although the gA channel is symmetric about its center, this symmetry is broken upon the introduction of the water chain because the water dipole moments are aligned in one direction. As will be elaborated below, the central water molecule donates a H-bond to W1′ (and not W1), thus creating a more favorable H-bond network on that side.

The average dipole moments of carbonyl groups exhibit large variations from residue to residue, covering a range of 1.8–2.8 D (Fig. 4). The zig-zag pattern, which is explained by the H-bond network, becomes especially apparent in the “ion” system because it preserves the symmetry of the channel, while this symmetry is broken in the “water” system. An important observation here is that the presence of the ion does not increase the dipole moments of carbonyls on average. The average dipole values obtained from Fig. 4 for the “ion” system are 2.32 $\pm$ 0.20 D and 2.29 $\pm$ 0.31 D for the right and left monomers, respectively, while the same averages for the “water” system are 2.32 $\pm$ 0.18 D and 2.33 $\pm$ 0.17 D. This observation holds even for the carbonyls of Val1, Val1’, Ala3, and Ala3’, which are in the coordination shell of the ion. Where the dipole moment of one carbonyl increases, the corresponding carbonyl on the other monomer decreases by about the same amount. This indicates that the strength of the ion-carbonyl interactions are comparable to those of water–carbonyl, and the presence of the ion causes a minimal perturbation to the gA peptide. Similarity of the ion–peptide and water–peptide interactions ensures that the movement of the ion–water column across the channel is not impeded by the presence of the ion. This rationalizes the experimental observation that K+ ions are conducted through the gA channel at near diffusion rates.

Both water and carbonyl dipoles in the gA channel exhibit substantial variations, which are not accounted for in non-polarizable models. For example, the CHARMM force field uses the rigid TIP3P model for water molecules with a fixed dipole moment of 2.35 D, and a tightly bonded carbonyl group whose dipole moment is more or less fixed at 3.01 D. Comparing these values with the ab initio results in Table I and Fig. 4, it is seen that CHARMM underestimates the water dipole moments and overestimates the carbonyl dipole moments by similar amounts. It is possible that the combined result of this underestimation/overestimation may balance out the forces and lead to an accurate description of H-bond networks in the channel. We will examine whether or not this is the case after taking a closer look at the nature of the H-bonds predicted by the ab initio simulations.

### 2. H-bond network

We now turn to an in-depth look at the H-bond network in the gA channel. As mentioned above, this is motivated by the desire to understand the variations in the dipole moments of water molecules and carbonyls in the channel, and also to see if a non-polarizable model can describe the predicted H-bond network. Ideally, each water molecule in the chain donates two H-bonds; one to an adjacent water molecule and another to a carbonyl-oxygen atom. In this way, each water molecule accepts a H-bond with its oxygen atom, except for W1 and W1′ in the “ion” system where the oxygen atoms interact electrostatically with the ion. Due to the abundance of carbonyl groups lining the interior of the channel, each water molecule has a number of H-bond configurations it can take. This makes the H-bond network of the water chain very dynamic, allowing H-bonds to be broken and re-formed elsewhere even during the short ab initio simulations used here. The breaking and re-forming of a H-bond is extremely fast, occurring on a sub-picosecond timescale. All of the H-bond results given below are obtained from the “ion” simulations, except where stated otherwise. Conclusions about how H-bonds affect dipole moments of water molecules and carbonyl groups also apply to the “water” simulations.

We begin with an analysis of all of the H-bonds donated by the channel water molecules. Results for the “ion” system are given in Table II. H-bonds are characterized by the distance between the oxygen atoms, $d$, and the O–H–O angle, $\phi$. For a H-bond between a water molecule and a carbonyl, we also give the C–O–H angle, $\theta$, where C–O belongs to the carbonyl and H to the water molecule, and $\omega = \cos(180^\circ − \theta)$. The donated H-atom pulls the electron cloud of the carbonyl-oxygen towards itself, and this effect will increase the carbonyl dipole moment most when $\theta = 180^\circ$ (perfect alignment), while $\theta = 90^\circ$ will result in a minimal change. Thus, a simple measure of the effectiveness of a H-bond in boosting carbonyl dipole moments is given by the quantity $\alpha$, which varies from 1 to 0. Finally, the last column in Table II gives the length of time, $T$, during which the H-bond is intact. The overall polarizing effect of a given H-bond on its accepting
TABLE II. Analysis of the H-bonds donated by the channel water molecules during the “ion” simulations. The third and fourth columns give the average O–O distance and the average O–H–O angle in a H-bond, with cutoffs of 3.2 Å and 130°, respectively. The fifth and sixth columns give the average C–O–H angle for a carbonyl-water H-bond and \( \alpha \) = \( \cos(180° - \theta) \). The duration of each H-bond (T) is specified in the last column (maximum value 10 ps). Only those H-bonds with \( T > 1 \) ps are shown. Standard deviations are given in parentheses.

<table>
<thead>
<tr>
<th>Donor Acceptor</th>
<th>( d ) (Å)</th>
<th>( \phi ) (°)</th>
<th>( \theta ) (°)</th>
<th>( \alpha )</th>
<th>T (ps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1 W2</td>
<td>2.78 (0.11)</td>
<td>165 (6)</td>
<td>-</td>
<td>0.29</td>
<td>10.0</td>
</tr>
<tr>
<td>W1 Ala5</td>
<td>2.95 (0.10)</td>
<td>163 (8)</td>
<td>107 (6)</td>
<td>0.29</td>
<td>6.6</td>
</tr>
<tr>
<td>W2 W3</td>
<td>2.71 (0.09)</td>
<td>166 (6)</td>
<td>-</td>
<td>0.29</td>
<td>10.0</td>
</tr>
<tr>
<td>W2 Trp9</td>
<td>2.91 (0.10)</td>
<td>158 (9)</td>
<td>119 (7)</td>
<td>0.48</td>
<td>8.2</td>
</tr>
<tr>
<td>W3 W4</td>
<td>2.78 (0.10)</td>
<td>164 (7)</td>
<td>-</td>
<td>0.29</td>
<td>10.0</td>
</tr>
<tr>
<td>W3 Trp11</td>
<td>2.90 (0.10)</td>
<td>163 (8)</td>
<td>119 (8)</td>
<td>0.48</td>
<td>8.8</td>
</tr>
<tr>
<td>W4 Trp15</td>
<td>2.88 (0.11)</td>
<td>160 (8)</td>
<td>127 (7)</td>
<td>0.60</td>
<td>9.0</td>
</tr>
<tr>
<td>W1′ W2′</td>
<td>2.78 (0.11)</td>
<td>165 (7)</td>
<td>-</td>
<td>0.29</td>
<td>8.8</td>
</tr>
<tr>
<td>W1′ Ala5′</td>
<td>2.95 (0.09)</td>
<td>164 (7)</td>
<td>106 (6)</td>
<td>0.28</td>
<td>5.4</td>
</tr>
<tr>
<td>W1′ Val7′</td>
<td>2.95 (0.12)</td>
<td>160 (8)</td>
<td>117 (8)</td>
<td>0.47</td>
<td>2.9</td>
</tr>
<tr>
<td>W2′ W3′</td>
<td>2.78 (0.11)</td>
<td>165 (7)</td>
<td>-</td>
<td>0.29</td>
<td>9.5</td>
</tr>
<tr>
<td>W2′ Trp9′</td>
<td>2.94 (0.11)</td>
<td>161 (8)</td>
<td>113 (7)</td>
<td>0.39</td>
<td>8.3</td>
</tr>
<tr>
<td>W3′ W4′</td>
<td>2.81 (0.10)</td>
<td>162 (8)</td>
<td>-</td>
<td>0.29</td>
<td>9.4</td>
</tr>
<tr>
<td>W3′ Trp11′</td>
<td>2.89 (0.11)</td>
<td>164 (8)</td>
<td>115 (8)</td>
<td>0.42</td>
<td>4.3</td>
</tr>
<tr>
<td>W3′ Trp13′</td>
<td>2.94 (0.11)</td>
<td>157 (10)</td>
<td>114 (10)</td>
<td>0.41</td>
<td>2.7</td>
</tr>
<tr>
<td>W4′ Trp15′</td>
<td>2.97 (0.11)</td>
<td>159 (8)</td>
<td>114 (7)</td>
<td>0.41</td>
<td>3.0</td>
</tr>
</tbody>
</table>

The table shows the analysis of the H-bonds donated by the channel water molecules during the “ion” simulations. The second row is broken—W0 donates to W1′ and the H-bond direction on the right side (W1–W4) is reversed compared to the “ion” system. This is seen to have an adverse effect on the quality of the H-bonds on the right side. Second, the water chain is much more dynamic and more prone to breaking and making new H-bonds. This results in more carbonyls contributing as H-bond partners and generally a less stable H-bond network. For example, in the “ion” system, the water–water H-bonds persist on average for 96% of the time, while the same quantity in the “water” system is 75%. Also, the quality of the H-bonds as measured by the bond distances (d) and angles (\( \phi \)) are relatively lower in the “water” system, pointing to weaker H-bond interactions. Contrasting the “ion” and “water” simulation results shows that a central K+ ion acts as an anchor to the water chain, suppressing the fluctuations of the water molecules and thus helping to maintain a robust water chain linked with high-quality H-bonds on both sides of the ion.

In addition to the H-bonds made by the channel waters, a network of H-bonds also exists within the backbone itself, where the amine groups of a number of residues donate H-bonds to opposing carbonyl-oxygen atoms (a complete list is given in Table IV). These H-bonds remain, more or less, intact for the duration of all simulations of the gA channel. They are responsible for stabilizing the helical structure of the monomers and binding of the two monomers to form the gA channel in lipid bilayers. Breaking of the inter-monomer H-bonds depicted in Table IV signals the dissociation of the gA dimer and degeneration of the channel. Because nitrogen is less electronegative than oxygen, H-bonds between carbonyl and amine groups are generally weaker than those between carbonyl and water. Nonetheless, the amine-carbonyl H-bonds in the backbone contribute significantly to the dipole moments of the carbonyl groups. To characterize the backbone H-bonds, we show in Fig. 5 the N–O distances and the corresponding N–H–O angles. Comparing the H-bond distances and angles made by the amine groups (Fig. 5) to those made by the water molecules (Table II), we see that they are quite similar on the whole with slightly larger distances and smaller angles in the case of amine H-bonds.

### 3. Rationalization of dipole moment variations in terms of the H-bond network

Variations in the average dipole moments of the channel waters can be explained in terms of their H-bond interactions. In this regard, we note that accepting a H-bond has a greater polarizing effect on a water molecule than donating one. From...
the present simulations, we find that the dipole moment of the accepting water is boosted by $\sim 0.5$ D and that of the donating water is boosted by $\sim 0.2$ D. Similar results are obtained in the presence of a K$^+$ ion. Thus, when the water chain is broken, the water molecule left with an unpartnered oxygen atom experiences a drastic reduction in dipole moment. We first consider the “ion” system. As noted in the discussion of Table I, the first neighbors of the ion (W1 and W1$'$) have lower dipole moments than the second and third neighbors. We attribute this to the breaking of the H-bond pattern by the ion leading to a non-optimal configuration for W1 and W1$'$ interactions. More specifically: (i) For both waters, the dipole vector makes an angle of about 35° with the ion–oxygen vector, which reduces the polarizing power of the ion, (ii) the electric field of the water molecules in one monomer is in the opposite direction to the ion’s field in the other monomer and hence reduces the ion’s effectiveness, and (iii) The H-bonds W1 and W1$'$ make with the carbonyls are of lower quality compared to other water–carbonyl H-bonds (from Table II) and from Table III. The carbonyls of all residues except for the leucine residues (10, 12, 14, 10', 12', and 14') form ideal H-bonds with classical water molecules, which have much smaller dipole moments (2.35 D). Thus, the drop in their dipole moments is presumably a simulation artefact caused by the boundary. The slightly larger dipole moment of W4 compared to W4' can be explained again by the fact that W4 makes a higher quality H-bond with a carbonyl than does W4'.

Turning to the “water” system, the smaller values of the dipole moments of channel waters compared to the “ion” system can be readily explained in terms of the shorter duration and lower quality of the H-bonds in which they participate (Table III). Variations in the dipole moments in Table III similarly correlate well with these quantities. For example, W1 and W2, which have the smallest dipole moments, are involved in H-bonds as acceptors for the shortest durations. Conversely, W2', which has the largest dipole moment, participates in a H-bond as an acceptor 95% of the time with the highest quality (see Table III). We stress that, unlike the “ion” system which is quite stable, the “water” system is highly dynamic, and therefore, one expects substantial variations to occur in the H-bond patterns obtained from different ab initio simulations. Nevertheless, we expect the main feature of our findings—namely, smaller water dipole moments in the “water” system compared to the “ion” system—to remain intact because it arises precisely from the dynamic nature of the water chain.

Finally, we consider the variations in the dipole moments of the carbonyl groups in the “ion” system (Fig. 4), which is easier to interpret than the “water” system because the system is symmetric about the center and the H-bond network is more stable. Inspection of Table IV shows that the carbonyls of all residues accept a H-bond from an amine except for the outer leucine residues (10, 12, 14, 10', 12', and 14'). These leucine carbonyls form H-bonds only with the bulk waters in the MM region, and therefore, will not be discussed further here. The dominant feature of the carbonyl dipole moments in Fig. 4 is the zig-zag pattern they follow. That is, the odd-numbered residues have larger carbonyl dipole moments compared to their even-numbered neighbors. In particular, all the odd-numbered residues from 5 to 15 (except 7 and 13) and from 5' to 15' have dipole moments larger than 2.5 D, while all the even-numbered residues from 2 to 8 and 2' to
8′ (except 8) have dipole moments smaller than 2.2 D. Inspection of Table II shows that the carbonyls of all the odd-numbered residues mentioned above make H-bonds with the channel waters, which boost their dipole moments. The even-numbered residues, and Val7 and Trp13 have lower dipole moments because they do not accept H-bonds from waters. The carbonyls of residues 1, 3, 1′, and 3′ coordinate the ion, and as a result their dipole moments are also boosted but to a lower degree compared to those carbonyls making H-bonds with waters.

Further variations in the carbonyl dipole moments can be understood from the quality of the H-bonds they form with the backbone amines (Fig. 5). For example, the carbonyl of Val8 forms the strongest H-bond and thus has the largest dipole moment in the even residue set. In contrast, the carbonyl of Val1 has the lowest dipole moment among the residues coordinating the ion because it forms the weakest H-bond. To stress the importance of the effect of the H-bond formation on polarization, we note that despite being a poor coordinator of the ion (Fig. 3), the carbonyl of Val1′ has a larger dipole moment than that of Val1 because it forms a much stronger H-bond. Large variations in the dipole moments of opposing residues (e.g., Val8 and Val8′) can be similarly understood. The carbonyls of Val8 and Leu4 have substantially larger dipole moments than those of Val8′ and Leu4′ because they form relatively stronger H-bonds.

4. Comparison with classical MD

Here we compare the ab initio results with those obtained from the classical MD simulations using the CHARMM force field. In view of the substantial variations observed in the dipole moments of waters and carbonyls, an outstanding question is whether the H-bond pattern found in ab initio simulations can be replicated in classical MD with fixed dipole moments. Using the first nanosecond of the production data from the MD simulations of the “ion” system, we analyze the water–water and water–carbonyl H-bonds. We exclude the peripheral water molecules (W4 and W4′) because they are frequently exchanged with bulk waters. Results are given in Table V. Comparing Table II, we find that there is a rather good agreement between the classical and ab initio results. Because of the longer duration of the classical MD simulations, water molecules have a chance to sample more carbonyl groups but otherwise the H-bond pattern found is consistent with the ab initio case. More quantitatively, the water–water H-bond distances are well reproduced with the angles slightly over-estimated. The only notable discrepancy occurs in the water–carbonyl H-bonds, where the distances are consistently larger by 0.1–0.2 Å from the ab initio results. This signals that the carbonyl dipole moments are likely to be overestimated in CHARMM. In the gA channel, this is not expected to cause major problems in description of ion permeation because carbonyls run parallel to the channel axis and should not interact strongly with the ion (see below, however). In contrast, in the selectivity filter of potassium channels, carbonyls point to the channel axis and directly coordinate a K+ ion. Therefore, overestimation of carbonyl strengths is more likely to cause problems in the description of ion permeation in potassium channels.

We next discuss a number of structural quantities involving the ion and compare the results of the QM/MM simulations with the corresponding MD simulations. Table VI gives the average K+–O distances between the ion and the coordinating carbonyls and water molecules, and the average C–O–K+ angles involving these carbonyls. In classical MD, four oxygens (Ala3, Val1′, W1, and W1′) coordinate the ion, whereas five oxygens (Val1, Ala3, Ala3′, W1, and W1′) are involved in the coordination of the ion in QM/MM. In compensation, the K+–O distances for waters and Ala3 are smaller in MD, indicating a tighter coordination by these oxygens. In particular, the strong interaction between the ion and the Ala3 oxygen is presumably caused by the strength of the carbonyl dipoles in CHARMM, which forces the ion to choose one of the carbonyls in its vicinity. To provide further evidence for this, we give in Table VII the average angles between the channel axis and the carbonyl groups involved in the coordination of the ion for both the “ion” and “water” systems. It is seen that the perturbation caused by the introduction of the ion is within

<table>
<thead>
<tr>
<th>Donor</th>
<th>Acceptor</th>
<th>d (Å)</th>
<th>φ (°)</th>
<th>T (ps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1</td>
<td>W2</td>
<td>2.75 (0.09)</td>
<td>159 (9)</td>
<td>960</td>
</tr>
<tr>
<td>W1</td>
<td>Ala5</td>
<td>2.80 (0.11)</td>
<td>158 (9)</td>
<td>156</td>
</tr>
<tr>
<td>W1</td>
<td>Val7</td>
<td>2.82 (0.12)</td>
<td>160 (9)</td>
<td>615</td>
</tr>
<tr>
<td>W2</td>
<td>W3</td>
<td>2.77 (0.09)</td>
<td>157 (9)</td>
<td>843</td>
</tr>
<tr>
<td>W2</td>
<td>Trp9</td>
<td>2.85 (0.12)</td>
<td>156 (10)</td>
<td>501</td>
</tr>
<tr>
<td>W2</td>
<td>Trp11</td>
<td>2.86 (0.13)</td>
<td>155 (10)</td>
<td>167</td>
</tr>
<tr>
<td>W3</td>
<td>Trp11</td>
<td>2.85 (0.12)</td>
<td>156 (10)</td>
<td>192</td>
</tr>
<tr>
<td>W3</td>
<td>Trp13</td>
<td>2.81 (0.12)</td>
<td>156 (10)</td>
<td>715</td>
</tr>
<tr>
<td>W3</td>
<td>Trp15</td>
<td>2.88 (0.14)</td>
<td>155 (10)</td>
<td>402</td>
</tr>
<tr>
<td>W1′</td>
<td>W2′</td>
<td>2.80 (0.11)</td>
<td>156 (9)</td>
<td>927</td>
</tr>
<tr>
<td>W1′</td>
<td>Ala5′</td>
<td>2.78 (0.11)</td>
<td>159 (9)</td>
<td>638</td>
</tr>
<tr>
<td>W1′</td>
<td>Val7′</td>
<td>2.81 (0.13)</td>
<td>157 (9)</td>
<td>151</td>
</tr>
<tr>
<td>W2′</td>
<td>W3′</td>
<td>2.80 (0.10)</td>
<td>156 (9)</td>
<td>867</td>
</tr>
<tr>
<td>W2′</td>
<td>Trp9′</td>
<td>2.84 (0.12)</td>
<td>159 (9)</td>
<td>632</td>
</tr>
<tr>
<td>W2′</td>
<td>Trp11′</td>
<td>2.82 (0.13)</td>
<td>158 (9)</td>
<td>103</td>
</tr>
<tr>
<td>W3′</td>
<td>Trp11′</td>
<td>2.86 (0.13)</td>
<td>156 (10)</td>
<td>296</td>
</tr>
<tr>
<td>W3′</td>
<td>Trp13′</td>
<td>2.86 (0.13)</td>
<td>157 (10)</td>
<td>562</td>
</tr>
</tbody>
</table>

TABLE VI. Average distance between the K+ ion and the oxygen atoms in its coordination shell (left). Average C–O–K+ angles for the carbonyls coordinating the ion. Standard deviations are given in parentheses.

<table>
<thead>
<tr>
<th>d(K+,O) (Å)</th>
<th>(C–O,K+) (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QM/MM</td>
<td>MD</td>
</tr>
<tr>
<td>Val1</td>
<td>2.85 (0.13)</td>
</tr>
<tr>
<td>Ala3</td>
<td>2.96 (0.24)</td>
</tr>
<tr>
<td>Val1′</td>
<td>3.57 (0.41)</td>
</tr>
<tr>
<td>Ala3′</td>
<td>2.85 (0.14)</td>
</tr>
<tr>
<td>W1</td>
<td>2.71 (0.10)</td>
</tr>
<tr>
<td>W1′</td>
<td>2.71 (0.10)</td>
</tr>
</tbody>
</table>
the fluctuations in the QM/MM simulations, which is consistent with the results obtained from the NMR experiments.\(^67,68\) In contrast, a sizable increase occurs in the Ala3 angle in the case of MD, where the carbonyl oxygen swings towards the ion. No such change occurs in other carbonyl angles, confirming the strong coupling between the ion and the Ala3 carbonyl.

### IV. CONCLUSIONS

In summary, we have performed a number of \textit{ab initio}\footnote{\textsuperscript{1}} MD simulations of the gA channel with and without an ion present. Using small QM systems we have shown that converged results are achieved using a single long \textit{ab initio} simulation, rendering the use of multiple starting configurations for sampling purposes unnecessary. Using large QM systems we have calculated the average dipole moments of the channel water molecules and the backbone carbonyl groups. Dipole moments of both water molecules and carbonyl groups are seen to be highly dependent on the particular H-bonds they participate in. An in-depth investigation of the full H-bond network inside the channel explains the overall pattern of the dipole moments, as well as the asymmetries observed in their values. A central ion induces comparable dipole moments on its surrounding carbonyl groups as a water molecule in its place, indicating that the strength of these interactions are comparable. This rationalizes the experimental observation that the gA channel conducts K\(^{+}\) ions near the diffusion rate.

Analysis of the water dipole moments and the H-bond networks inside the channel explains the overall pattern of the dipole moments of both water molecules and carbonyl groups. Dipole moments, as well as the asymmetries observed in their values. A central ion induces comparable dipole moments on its surrounding carbonyl groups as a water molecule in its place, indicating that the strength of these interactions are comparable. This rationalizes the experimental observation that the gA channel conducts K\(^{+}\) ions near the diffusion rate.

ACKNOWLEDGMENTS

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61 See supplementary material at http://dx.doi.org/10.1063/1.4768247 for further discussion of sampling issues.