

## Simulation of Water in a Small Pore: Effect of Electric Field and Density II: Immobilized Molecules

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A Monte Carlo simulation of water in a small pore has been modified to trace individual molecules. The locations, orientations, and local density of those that move less than 1 Å during the simulation are shown to depend heavily on the locations of other molecules that are fixed on the sides of the pores. The electric field produces secondary effects, while density, which produces major effects on the average water properties (Green, M. E.; Lu, J. *J. Phys. Chem. B* 1997, 101, 6512.) is secondary in determining the properties of individual water molecules; in particular, the location of those molecules so immobile as to form an effective block of the pore is little affected by field or density. The electric field does have an effect on the orientation of the immobilized molecules large enough to observe clearly, but it is not a large effect. In this set of simulations, the number of moves per molecule was extended to ensure that the effect was real, and not an artifact of certain molecules starting slowly. Principal results included approximate 4-fold symmetry of the immobilization, corresponding to the location of water molecules fixed to the wall, and with fixed orientation; these molecules were attached to ensure that the pore was not entirely hydrophobic. If the pore were in a protein, one should expect instead of hydrogen bonding water molecules, hydrogen bonding amino acid side chains. Nevertheless, the effect would be expected to be similar, albeit with different symmetry. The role of density was also limited, as the symmetry of the location of the fixed molecules, and their orientation, turned out not to be heavily dependent on density. Instead, the density effects seem to be strongest on the mobile molecules, as can be seen by comparing these results with those from Green and Lu.<sup>1</sup> It appears that the water molecules could well block a pore of appropriate dimensions; a rearrangement of hydrogen bonds, possibly under the influence of an electric field, could in turn unblock it.

### I. Introduction

Water in pores has been the subject of numerous studies, experimental and theoretical. We carried out Monte Carlo simulations on one such system, in which we varied the density and the electric field that the water molecules experienced.<sup>1</sup> In it we determined the electrical potential consequent on each of four distributions of charges, as well as the absence of fixed charges. We also determined the average distributions of the molecules with respect to the walls, and the radial distribution functions of the molecules. Among other results, we saw that the molecules clustered at certain distances to the wall, and were largely missing at other distances. In this paper, we follow that result by examining the motions of individual molecules. Certain of the molecules, having reached their positions with the system at “equilibrium” (that is, at its final energy, with all sections at their final density, and in a condition beyond which no further average changes are observed), fail to move again. While the time scale of the simulation (to the extent that a Monte Carlo simulation has a time scale) would still be too short for direct comparison with, for example, physiological experiments, it is still quite long enough for the molecules to make appreciable moves. If molecules do not move, they can be considered to be immobilized for a period that is at least long with respect to the normal motions of molecules. We can call these molecules

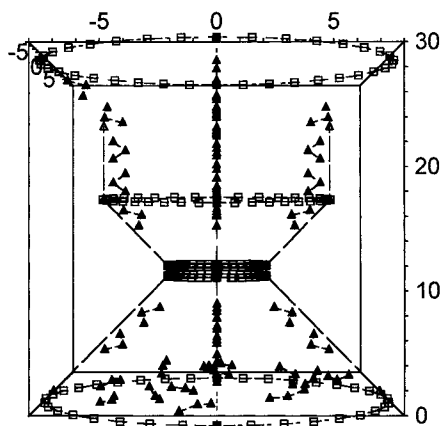
“frozen”, with the understanding that the “freeze” is on the time scale of the simulation.

The question is of interest with respect to pores in proteins, particularly ion channels. It has been shown by Bezrukov and Kasianowicz<sup>2</sup> that changing the pH to allow a number of the residues to become charged, for example, can considerably constrict an alamethicin pore. The charged residues are able, in their interpretation, which seems to us very reasonable, to hold water immobile and thus to leave a smaller opening for large molecules to pass through the channel. If a similar phenomenon occurred in a smaller pore, for example a voltage gated ion channel of the type found in neurons, it would block the channel completely. The existence of large fields in pores has been found experimentally<sup>3–5</sup> and theoretically,<sup>6,7</sup> by a number of workers, and the subject has been summarized in our previous paper.<sup>1</sup> The effect of these fields on water has also been discussed, often in the same papers.<sup>3,6,8–10</sup> The results suggest electrostriction, ordering, and increase of density. Since the most important results have been introduced in our previous paper we will not consider them here again in detail. However, it is clear that it will repay the effort to study the behavior of individual molecules in the simulation, and that is the purpose of this paper.

### II. Model and Methods

The model and methods are essentially the same as those used for the preceding paper,<sup>1</sup> so will be described extremely briefly here.

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**Figure 1.** The outline of the pore, showing the positions of the water molecules (filled triangles) fixed along the walls: in Figures 2, 4, and 6 the pore boundary shown is the same dashed line. The water molecules are fixed to the wall in pairs so as to form quadrupoles. Molecules within the space defined by these molecules are mobile in the simulation. Those are the molecules shown in Figures 2, 4, and 6, in which the fixed molecules shown here are omitted; these molecules are immobilized as part of the model, not by the potential energy or hydrogen bonding, and are omitted elsewhere to avoid confusion with molecules that are allowed to move during the simulation, but do not. In Figure 6, the wall molecules are raised 2 Å in  $z$ , placing some outside the boundary. The top 6 Å in Figure 1 are the reservoir, not shown in the other figures, as new molecules are added in this region.

**1. Pore Model.** The pore model is partially shown in Figure 1, in which a reservoir holding a fixed number of water molecules is attached to the cylinder at the upper end; the number in the reservoir can be chosen. This is equivalent to Figure 1 of Green and Lu.<sup>1</sup> However, the water molecules fixed to the side of the pore define the space within which the other molecules may move. The circles show the dimensions of the pore at points of change of slope of the walls. The upper reservoir is truncated in the subsequent figures, as the water molecules of interest are those in the other sections, where the number of molecules is allowed to vary. Note that the wall molecules have been fixed to pair their dipoles, so as not to contribute a large field; they are not allowed to rotate or translate during the simulation. The model has a cylinder below the reservoir, and a tapered region down to the double circles, which are separated by 1 Å. Below this, the pore widens again. The angle is approximately  $27.6^\circ$  with the vertical axis in all tapered sections.

**2. Calculation.** (a) This has been described in detail in the previous paper;<sup>1</sup> briefly, the field and potential were determined on a 2 Å lattice. The dielectric coefficient of the wall was taken to be 4, as is approximately reasonable for a protein.<sup>7,11</sup> Surfaces below and above the pore had dielectric coefficients set to 80. The induced charge on each 1 Å times 1 Å segment (0.5 Å times 0.5 Å in certain critical regions) of the boundary was determined, and thus the contribution of the dielectric boundary could be added to those of the water molecules, and of the fixed charges, if present. Since the complete calculation, including details of the boundary charge determination, has already been described in print,<sup>12,13</sup> we will not consider it further here. See Green and Lu<sup>1</sup> for typical fields and potentials resulting from the simulation.

(b) *Simulation:* The simulation was essentially identical to that previously described,<sup>1</sup> save that (i) Only certain densities in the constant density reservoir were used: with normal density equivalent to 26 molecules, values of 22, 24, 26, 28, and 30 were tested. This sufficed to show that density was not a crucial variable for the particular information sought here. “Low” and

“high” densities shown in several figures correspond to 22 and 30 molecules in the reservoir, respectively. (ii) Only two charge configurations were used, one of the highest, and the other zero. The reason will be clear when the differences are examined. (iii) The total number of moves per molecule was extended from 6000 to 12000. Equilibration requires 4000 moves/molecule,<sup>1</sup> so there are now 8000 moves for the molecules to move or not move, rather than 2000. This helps to ensure that the molecules have been sufficiently tested as to the stability of their configuration. (iv) Each run was repeated six times, and then six times again; all averages shown are based on 12 repeats. Although in each case we are tracing individual molecules, it is necessary in the end to use average properties to make significant assertions concerning the results, and a large number of runs are needed to obtain adequate statistics. Comparison of two pairs of six runs each allowed reasonable error estimates, and helped to ensure that apparent trends could be reproduced in replicate sets of simulations; individual runs would not have sufficed for error estimates.

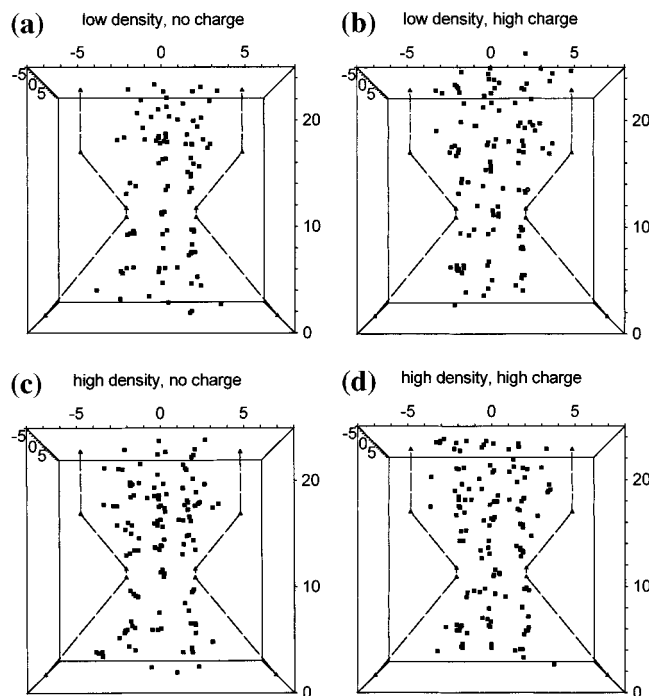
Statistics for the energy were discussed quantitatively in Green and Lu.<sup>1</sup> The error estimates will be considered with the results here.

(c) *Water Model.* The same PSPC (polarizable SPC) water model was used as in the previous work.<sup>1</sup> There was a minor change; in the previous simulations<sup>1</sup> a cutoff below 2.5 Å was included, as well as a small increase in repulsive potential in the 2.5–2.8 Å range. At its maximum, at 2.5 Å, this increase reached approximately  $3k_B T$  ( $k_B$  = Boltzmann’s constant,  $T$  = temperature). Here, the 2.5 Å cutoff was kept, but the added repulsion leading up to it was removed (on further consideration, it probably should not have been included). The effect was very slight, but the  $g(r)$  figures shown in our earlier work would have had higher initial peaks had the full PSPC model been used. Other than that, there appears to have been no effect of the change of potential.

(d) *Charge and Density.* (i) *Charge.* The two charge states studied included one with zero charge. The other (“high charge”) had three positive charges, 0.5 Å inside the wall, at  $z = 17.4$  Å, two at 13.5 Å, two negative charges at 7.2 Å, and three at 4.5 Å. This is one of the charge states studied previously.<sup>1</sup> (ii) *Density:* The reservoir is a tapered section from  $z = 17.5$  Å to the top of the system, at 23.2 Å. Molecules starting in this section were allowed to travel into the sections below (except for the fixed molecules, of course). After each round of one move per molecule, the density in this section was tested, and if changed, restored. Normal water density corresponds to 26 molecules in this section, and number densities of 22 (15% below normal), 24, 26, 28, and 30 molecules (15% above normal) were used in this work.

(e) *Initial Placement of Mobile Water Molecules.* The molecules were initially placed on an ice lattice within the simulation volume, which in turn was shrunk 10% in volume to approximate the volume of liquid water. The number of molecules in the upper reservoir was set independently.

(f) *Molecules Fixed along the Walls.* Molecules were fixed to the wall and not allowed to rotate, as shown in Figure 1. This is one of the two cases used in the earlier work (in the other, the molecules were allowed to rotate, in which case they lined up and produced an addition to the electric field that was appreciable<sup>1</sup>). The molecules were paired to form a quadrupole, to minimize their contribution to the field. They were necessary as the pore would otherwise have been completely hydrophobic, and thus inappropriate for comparison with proteins, for example (in proteins, the hydrophobic surfaces tend to be buried in the



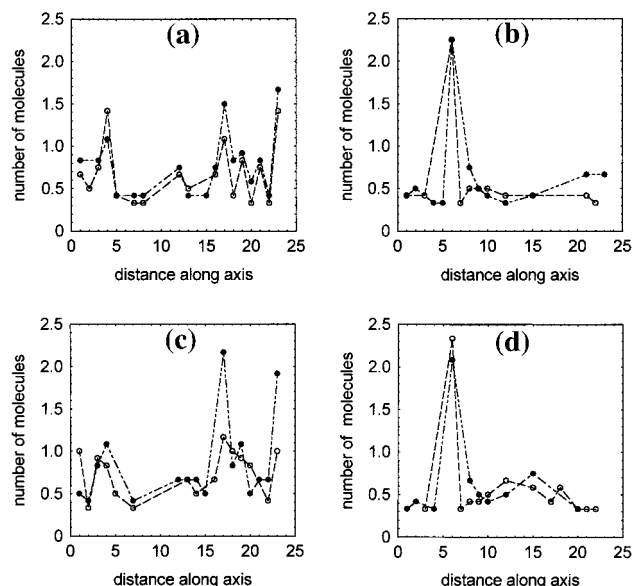
**Figure 2.** View of the interior of the pore: the dashed line is the same as that of Figure 1. The filled squares represent the position of the mobile molecules that moved less than 1 Å during the simulation after completion of the 4000 moves/molecule required for equilibration. Each molecule shown has remained (nearly) immobile in one of six runs. The conditions for the sets of runs are indicated on the figures: (a) low density, no charge, (b) low density, high charge, (c) high density, no charge, and (d) high density, high charge. The similarities are clearly stronger than the differences. Observe in particular the nonrandom arrangement of the molecules in the tapered sections: molecules are arranged roughly in vertical rows, with space between. Figure 4 shows this as well. The order is lost in the upper (cylindrical) portion of the pore.

interior of the protein). Two layers, totaling twelve molecules, were placed on the bottom surface of the pore, to allow a buffer between the dielectric 80 region at that boundary, and the interior. The remainder were those paired along the walls. Note the 4-fold symmetry of the placement. The wall molecules were not counted in any of the results; they are, in effect, part of the pore structure.

### III. Results

The new element in this work is the tracking of individual molecules. We therefore consider only the results derived from this tracking, and refer back to the earlier work for all other results. The main points considered are: location of the immobile molecules as a function of distance along the axis ( $z$  coordinate), location of these molecules with respect to the center of the pore ( $x, y$  coordinates), and orientation of these molecules.

**1. Distribution of Locations of the Fixed Molecules.** Figure 2 shows the locations of molecules at two densities (approximately 15% larger, and 15% smaller, than normal, in the reservoir), and both charge cases, looking into the pore from the side. It is possible to see the distribution of both the peripheral and the central fixed molecules, and to observe the relative absence of molecules between, especially in the tapered parts of the pore. The order is partly lost as the pore widens to the cylindrical section. The distribution with respect to the pore axis must be considered separately for the molecules along the periphery, and for those at the center. The effect of the fixed



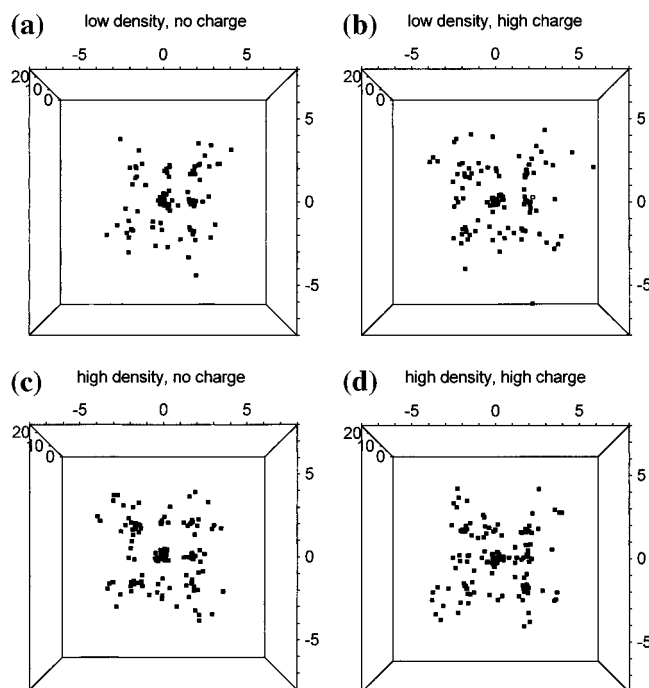
**Figure 3.** Positions of the immobilized molecules: the molecules do not become immobilized in random positions. Furthermore, there is a difference in probability of immobilization depending on whether the molecule is in the center of the pore or near the wall. (Figure 2 showed the small fraction between, especially in the tapered sections.) The abscissa is the position along the  $z$  axis, and corresponds to the positions in Figure 1. The ordinate is the number of molecules immobilized in a 1 Å slice at that position, averaged over six runs. Under all conditions of density and charge, there is a maximum probability of immobilization in the lower tapered region for molecules near the periphery (Figure 3a,c); similarly, there is a peak in the probability for molecules near the axis for locations near the upper tapered region/cylinder boundary. In each part of Figure 3, squares are high density, circles low density. Parts of figure: (a) molecules near axis, high charge (there is also a peak near the wider part of the lower tapered section in these curves); (b) molecules near periphery, high charge; (c) molecules near axis, zero charge; (d) molecules near periphery, zero charge. The geometry and, presumably, fixed water as shown in Figure 1, are more important than charge or density.

molecules and the pore geometry is larger than the differences due to charge, while density has even less influence. If molecules are immobilized with differing probability in different parts of the pore, it should be informative to take 1 Å slices. Since there should be a difference between molecules hydrogen bonded directly to the fixed molecules, and those in the central column, within 2 Å of the pore axis, these cases are treated separately. Otherwise, locations of the potential minima holding the water molecules would be lost, as minima along the axis would average with nonminima on the periphery, and vice versa.

For both densities and charges, there is a maximum along the axis (Figure 3, parts a and c) below the constriction (at approximately 4 Å on the abscissa), and at the lower edge of the cylinder (the tapered section/cylinder boundary), at approximately 17 Å. The abscissa of Figure 3 corresponds to the ordinate of Figure 1. The maximum at the tapered section/cylinder boundary is more prominent at high density, but this may reflect mainly the larger number of molecules, especially in Figure 3a, high charge. We do not believe that this difference is nearly as important as the fact that highly reproducible peaks exist under all conditions of density and charge, and are consequences apparently of geometry and locations of the fixed water molecules along the periphery to which the axis molecules may hydrogen bond at the lower peak, albeit not directly at the upper peak.

Along the periphery, there is obviously one dominant peak (Figure 3, parts b and d). This appears just below the constric-





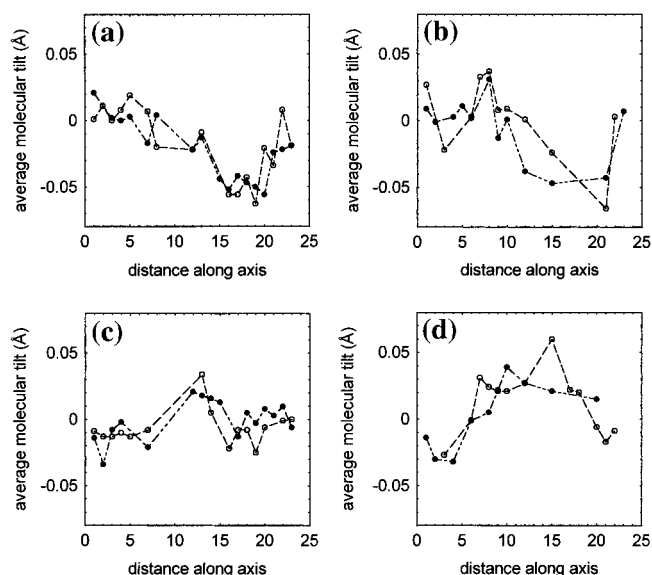
**Figure 4.** These are the same molecules shown in Figure 2. The entire figure is rotated 90° perpendicular to the axis, so that we are now looking down the pore from what was the upper end of the channel in Figures 1 and 2. Parts (a)–(d) are in the same order as in Figure 2. It is immediately evident that the cylindrical symmetry of the pore wall is not reproduced by the positions of the immobilized molecules. Instead, their positions are distorted into nearly the 4-fold symmetry of the fixed molecules and charges.

tion. Again, this is fairly independent of charge and density. These molecules should hydrogen bond directly to the fixed molecules along the wall, and may also bond to the molecules in the central molecule peak about 2 Å below. An apparent peak at 23 Å just below the reservoir may not be significant. The reservoir itself is treated differently in the simulation from the remainder of the pore (that is, where water molecules are added to keep the density constant). Results on its border cannot be considered as significant as the remainder of the volume.

The most important lesson of the data in Figure 3 is the existence of certain locations that, as a consequence of geometry and ability of neighboring molecules to hydrogen bond, can act consistently as traps for water molecules. Presumably, in a protein, side-chain motion or reorientation could have a major effect on where water molecules could block a pore, or whether they would at all; however, this carries us beyond our present computation.

In Figure 4 we view the distribution of molecules in a radial direction. The data are the same as those in Figure 2, but the figures are rotated 90° to allow a view down the pore, with the axis perpendicular to the plane of the paper. It is even clearer here that the fixed molecules determine the symmetry of the distribution; the immobilized water is far closer to 4-fold symmetry than to the cylindrical symmetry of the pore wall. The charges are also placed with the same 4-fold symmetry as the fixed molecules, and may reinforce the effect. The separation of the peripheral molecules from those near the axis, at the center of the figures, is also clearer in this view than in Figure 2.

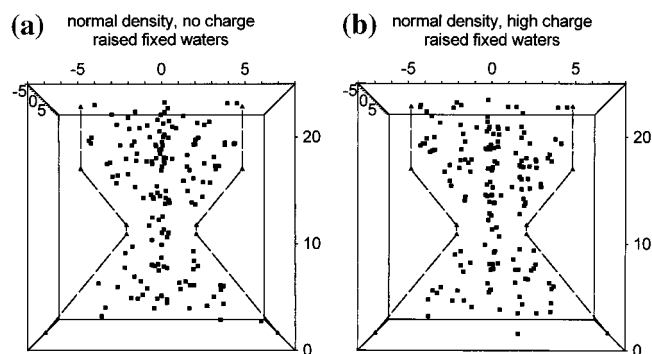
**2. Distribution of Orientations of the Immobilized Molecules.** The orientations of the molecules are shown in Figure 5a,b when charge is present, and in Figure 5c,d when it is not. Orientation is defined as the mean  $z$ -position of the H atoms, minus that of the O atom. Molecules within 2 Å of the axis



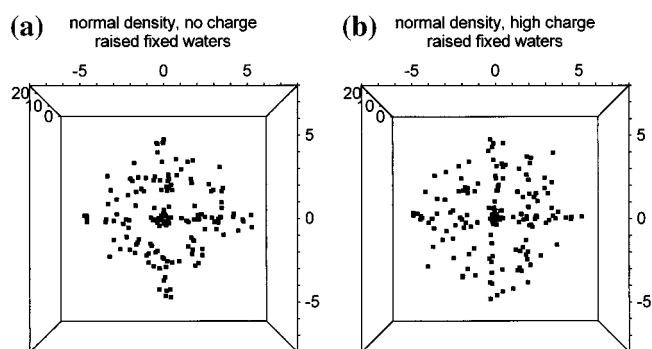
**Figure 5.** The “tilt” ( $z$  component of the orientation of molecular dipoles) of the molecules: “tilt” is defined as the average  $z$  position of the two H atoms minus the  $z$  position of the oxygen. The axes are in Å, with the abscissa representing the position along the  $z$  axis, exactly as in Figure 2. In all parts, open circles are for low density, filled circles high density. (a) high charge, center molecules (within 2 Å of the axis), (b) high charge, periphery, (c) no charge, center molecules, (d) no charge, periphery. The region between 16 and 23 Å corresponds to the cylinder, as shown in Figure 1, and this is the region where charge produces reproducible tilt, in the direction to be expected from the charges.

again are significantly different from those along the periphery. The same two densities as before are shown; again, the others are quite similar. Here we observe a direct effect of field, with the mean  $z$ -position of the oxygen being approximately  $0.07 \pm 0.02$  Å separated from the mean  $z$ -position of the H atoms, in the presence of the field (error estimate from scatter of replicate runs), in the cylindrical part of the pore, where the field is high. Without the field, there is much less difference, reaching to  $\pm 0.03$  Å in the tapered region (except for one point in Figure 5d). In the lower region, the polarity is reversed, and so is the orientation, to 0.03 Å in the opposite direction. We could, in principle, estimate the direct energy of the field–dipole interaction if we assumed that the orientation was entirely a thermal matter, by using the Langevin equation. As the energy is not entirely thermal, with at least some of the orientation due to the interaction with the walls, one could only get a net energy, of uncertain importance. It is not possible to sort the energy into wall and field components. However, as the interaction with the wall appears to be dominant, the interaction with the field must be at least fairly large to produce even as much orientation as is observed. Thermal effects are likely to be third in importance.

**3. Effect of Interaction with the Wall Molecules.** To further test the effect of the wall molecules, a set of runs with normal density, but with wall molecules other than those at the base displaced upward by 2 Å, was performed. The molecules in some cases have now become wall dipoles. The results are shown in Figure 6, looking into the pore, and Figure 7, looking down the pore. It is apparent that the orientation of the immobilized molecules has been rotated by approximately 45° with respect to its orientation with the water in the previous positions, at least with the charges present. The effect of difference in density again seems small. Charge does have an effect, however. The difference between the cases shown in Figure 7 is apparent also in the other two sets (charged and



**Figure 6.** The effect of moving the fixed water molecules. The fixed water molecules are raised 2 Å in the  $z$  direction in this figure, with respect to their position in all previous figures. This places some of the molecules outside the pore boundary, making them unavailable for hydrogen bonding. There is a drastic rearrangement of the immobilized molecules as a consequence. However, there remains a space between center and periphery. The parts are (a) normal density, no charge, and (b) normal density, high charge.



**Figure 7.** These figures bear the same relation to Figure 6 as Figure 4 does to Figure 2: i.e., the pore is rotated 90° so that the view is down the pore, viewed from above. Note the essentially 45° rotation (compared to Figure 4) of the pattern of molecules in the no-charge case, and the high-charge case has water molecules so distributed as to make the near 4-fold symmetry almost disappear. It is apparent that water molecules are immobilized in large part by the interactions with the molecules fixed in or near the walls.

uncharged) of six runs with this fixed water configuration (not shown). There remains some order to the molecules even in this view; however, it is clearly different than when the fixed molecules are in the original positions with the data in Figures 2–5.

#### IV. Discussion and Conclusions

Water molecules in a pore are held in place in certain locations, principally by dipoles arrayed along the wall. The presence of a large electric field, approaching  $10^9$  V m<sup>-1</sup>, serves largely to alter the orientation of the molecules, and not greatly at that. The model includes molecules arrayed along the pore wall, and kept entirely fixed; it is to be expected that, were these real dipolar components of a protein, a large field would move these, or at least change their orientation. In doing so, they should likewise move the location of the water molecules immobilized or at least greatly slowed in their motion in the pore. It is likely that a small change in orientation of these wall dipoles could create a larger change in the location of the immobilized molecules, as hydrogen bonding appears to be a significant part of the reason for the immobilization of these

molecules. Assuming the molecule's positions to be also dependent on hydrogen bonds with each other, small changes in the orientation of those molecules hydrogen bonded to the wall would be amplified in the next layer, and probably in the pore center.

It is something of a surprise that the immobilized molecules are so little dependent on the density, as average energies and intermolecular distances show appreciable dependence.<sup>1</sup> The main effects of density appear to be on the more mobile molecules. If the trapped molecules are held primarily by the fixed wall water molecules, and their hydrogen bonds, then this can be understood, as the fixed wall molecules were kept constant when the reservoir densities were changed. Whether this is responsible for the fact that the densities in the lower regions of the pore, while proportional to the reservoir density, have a slope much less than one,<sup>1</sup> is not clear. It is plausible.

If we apply these ideas to an ion channel, it seems possible that we should modify our earlier gating model,<sup>12,13</sup> in which high field alone kept molecules “frozen”. Based on the present analysis of the simulations, the high field is likely to have much of its effect mediated through rearrangement of the dipoles of the protein, which in turn hold “frozen” those water molecules needed to block the channel in the closed state. We have already suggested how the large field could change in response to membrane depolarization (which is a smaller field change, of the order of only about  $10^7$  V m<sup>-1</sup>),<sup>14</sup> via proton tunneling. The direct effect of the field on the orientation of “frozen” water molecules in this calculation is sufficient to suggest that the effect on dipoles held in the wall (amino acid side chains) must be appreciable. A rearrangement of these dipoles of the order of 2 Å would be sufficient to “unfreeze” water, thus making it possible for sodium or potassium ions to pass the gate, which may be at the intracellular end of the channel, in their hydrated state. This is likely to be at least as significant as the direct influence of the field on the water molecules. Of course, further work would be needed to test this modification of the original model.

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