Deconstructing Permeation Energetics in a Model Potassium Channel Selectivity Filter

P.C. Jordan, G.V. Miloshevsky

Department of Chemistry, Brandeis University, Waltham, MA 02454, USA

Abstract

A computational model based on the Monte Carlo (MC) method is developed to simulate energetics and ion movement in the KcsA K^+ channel, based on the X-ray structure [1]. It extends our semi-microscopic Monte Carlo approach to the study of permeation in ion channels [2-4], making it increasingly atomistic while retaining its advantages (exact reaction field treatment, no cut-offs, few explicit bulk solvent molecules, boundary periodicity unneeded). The computational ensemble comprises four atomic level subunits (KcsA peptide, surrounding lipids, waters, ions) and continuum bulk water. Explicit features are embedded in a low dielectric continuum ($\varepsilon = 1$), sandwiched between high dielectric intracellular and extracellular bulk continua ($\varepsilon = 80$). Subunit influences can be studied individually or separately. We focus on ion and water interaction with the KcsA peptide and analyze descriptions of varying complexity, examining both electrostatic and total interaction energy for single and multiple ion occupancy of the channel. With a dehydrated filter, a single K^+ resides at the crystallographic water site, two K^+ ions reside at the crystallographic binding sites and triple ion occupancy of the filter is possible. MC simulations show that in a rigid filter ion-protein interactions are highly favorable while water-protein interactions are not. Without ions, water molecules move toward either end of the filter. Filter flexibility appears to be crucial for favorable water-filter interaction.

Introduction

Accurately modeling ion-water-channel-membrane ensembles is very intensive computationally. Simulating a channel protein, a few neighboring lipid molecules and enough water to describe the aqueous surroundings involves some 40,000 atoms [5], of which about half are extra- and intracellular bulk waters. Use of periodic boundary conditions further increases the computational load. Thus substantial theoretical effort has been expended to develop simplified models, which reliably describe aspects of permeation [2-4,6,7]; however, each suppresses significant molecular detail.

We outline a practical approach, extending semi-microscopic modeling [2-4], that still describes ion-channel interaction in great atomic detail while cutting by over half the number of atoms and sidestepping any need for periodicity. Instead of explicitly including intra- and extracellular waters, these are treated as dielectric continua, $\varepsilon \sim 80$, in a geometry for which the reaction field can be computed exactly, without electrical cut-offs or Ewald sums. MC sampling ensures that boundary reflection problems, which adversely influence molecular dynamics (MD) simulation, are not an issue.

Figure 1 is a schematic model of our KcsA ensemble. It has four domains: bulk water; explicit external water; lipid; channel protein. Bulk water is described as dielectric continua ($\varepsilon \sim 80$) and the latter three are treated explicitly. Regions between the image planes are embedded in a low ε background, $\varepsilon = 1$.

Computational Details

The total system contains four computational subunits (some with multiple replicas), each of which is free to move relative to one another. The number of subunits used in modeling (number of atoms per subunit in parentheses) is: 1 KcsA (3696); ≤ 70 DPPCs (130); ≤ 500 waters (3); ≤ 5 ions (1). In the approximation we use here, subunit structures are rigid. This constraint can be lifted as desired by decomposing a subunit. For example, the oxygen containing groups of the selectivity filter (20 carbonyls from Glu^{79} to Thr^{75} as well as the 4 COHs from Thr^{75}) could be excised from the KcsA and each treated individually. The remainder of KcsA would be then described as one unit.

Each subunit is first isolated and equilibrated (for KcsA the X-ray structure [1] or a representative MD snapshot is used). Subunits are then assembled to form a computational ensemble. Within a subunit atoms are immobilized. Since the KcsA filter is fairly inflexible at functional ionic concentrations [5,8], this approximation is not too drastic. A single MC move repositions one of the subunits and alters its interaction with all the others. For the simplest problem presented here, a single ion interacting with KcsA alone, each move involves computing 3696 ion-KcsA interaction energies as well as the effect of the associated reaction field, convergent after including 10^{th} order ion images. Moves satisfying the Metropolis criterion are accepted. The CHARMM27 force field and TIP3P water are used. Qualitative results are not water model or force field or background ε dependent. The results presented are for rigid KcsA.

Influence of the Image Plane and Bulk Water

Figure 2 compares the ion's electrical energy for several randomly generated water configurations. The image planes (see **Figure 1**) are 10 Å from the channel mouths. The cavity, mouths and 10 Å wide intracellular and extracellular water boxes are filled by the explicit water molecules. Total potential energy fluctuates greatly and is very configuration dependent.

Figure 3 compares ionic electrical energy without bulk water with the mean energy, averaged over 10 and 30 randomly generated water configurations. Filter properties depend little on whether bulk water is explicit or a dielectric continuum.

Influence of Lipid

Figure 4 illustrates the disposition of DPPC subunits about the central KcsA subunit; up to ten layers of DPPC were considered.

Figure 5 illustrates how increasing the thickness of the lipid domain affects the stability of an ion in the channel. The shape of the curves is the same regardless of the amount of lipid. Two or three layers of lipid are sufficient for convergence.

The Effect of Force Field Modification

Figure 6 contrasts electrostatic interaction energies for an axial potassium ion in an otherwise empty channel, using GROMOS, CHARMM or AMBER. While qualitatively similar, the force fields generate quantitatively quite different energy profiles.

Figure 7 compares electrostatic and total potentials for an axial K^+ using CHARMM. Structural influences (Lennard-Jones interactions) only influence behavior in the filter. The spike reflects the constriction in the filter between sites 2 and 3, and illustrates the need to implement filter flexibility.

Monte Carlo Simulations

Putative Ion Binding Sites

Figure 8 illustrates the putative stable ion binding sites identified by Monte Carlo simulations of one ion in the empty channel.

Water Structure

Figure 9 illustrates typical cavity water orientation in the absence of ions. The inner helices strongly polarize water in the mid-channel cavity with the hydrogens pointing toward the helices' C-termini, confirming results from MD simulations [9].

Ion-Channel and Water-Channel Interaction

Figure 10 illustrates that as many as five ions can reside in the cavity. They escape neither to the inner pore nor, probably because the rigid filter so impedes water mobility at site 4, to the filter. The ions are solvated both by the pore helices' C-termini and by the cavity water. Four are in contact with the carbonyl oxygens of the THR^{74}(CO) residues; the fifth remains near the cavity center.

Figure 11 shows that an ion in the inner pore is rapidly incorporated into the water filled cavity. Not surprisingly, water alignment near the filter entrance is like that in the ion-free cavity, with water hydrogens pointing toward the pore helices' C-termini.

Figure 12 illustrates the stable locations for potassium ion sin the selectivity filter of an otherwise empty KcsA channel. Without water, two ions prefer to be deep in the filter, at sites 2 and 3, and the third is near the channel mouth, between site 1 and the "external" site, reminiscent of results from MD simulations [5].

Observations and Conclusions

Replacing explicit extra- and intracellular water by continuum dielectrics has no effect on ionic behavior in the KcsA channel's selectivity filter.

Lipid has only a slight influence on ionic properties in the filter. Three layers of lipid molecules provide a quantitatively adequate model.

There are numerous stable locations for ions in the KcsA channel, especially in the mid-channel cavity.

Three ions are readily stabilized in a rigid, water-free filter.

In a rigid filter, a water molecule at site 4 appears to effectively block the lines of communication between the filter and the mid-channel cavity.

Allowing for filter flexibility is crucial for adequately modeling ion-water-filter interaction. This modification is in progress.

References

1. D. Doyle et al., *Science*, **280**:69-77 (1998)

2. V. L. Dorman et al., *Biophys. J.*, **70**:121 (1996)

3. V. Dorman et al., in Gramicidin and related ion-channel forming peptides; Novartis Foundation Symposium 225, Wiley, Chichester, **1999**, 153-169

4. S. Garofoli et al., in Ion channels-from atomic resolution to functional genomics; Novartis Foundation Symposium 245, Wiley, Chichester **2002**, 109-126

5. S. Bernèche & B. Roux, *Biophys. J.*, **78**:2900-2917 (2000)

6. S.-H. Chung et al., *Biophys. J.*, **77**:2517-2533 (1999)

7. B. Roux & R. MacKinnon, *Science*, **285**:100-102 (1999)

8. Y. Zhou et al., *Nature*, **414**:43-48 (2001)

9. L. Guidoni et al., *FEBS Lett.*, **477**:37-42 (2000)

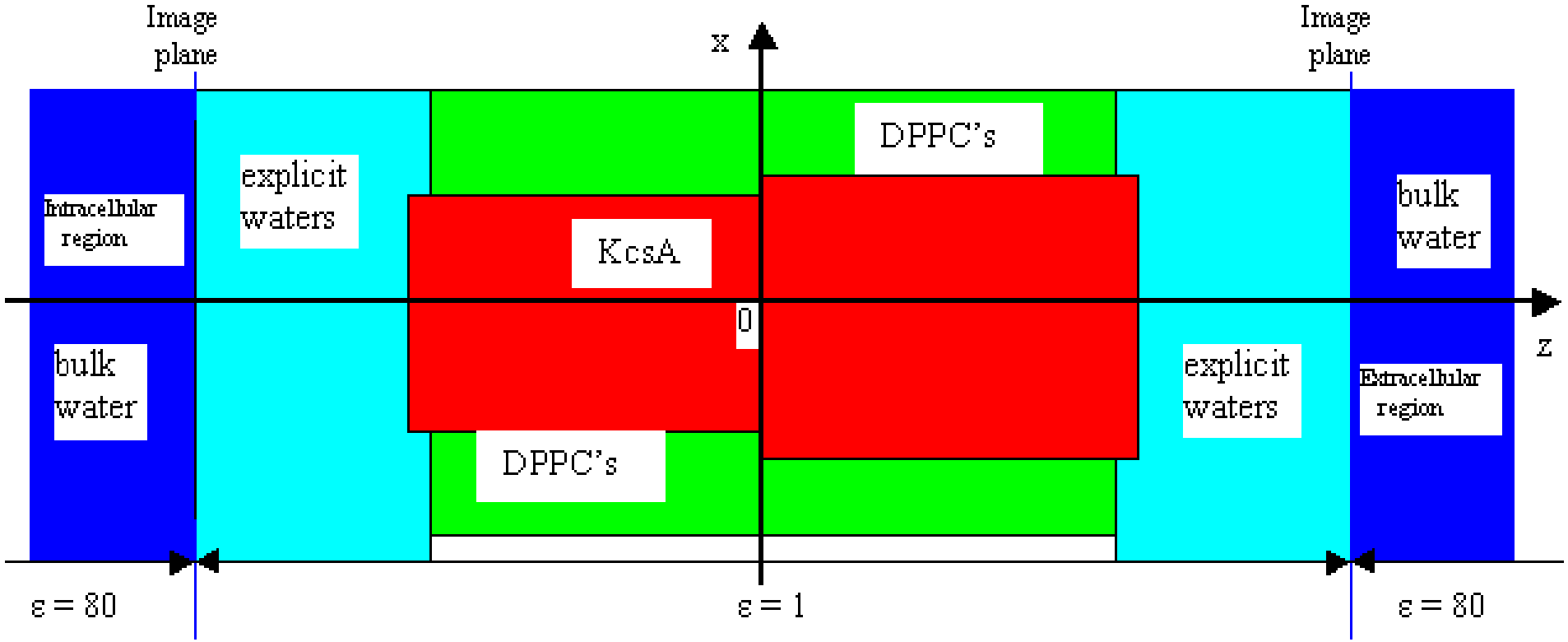

Figure 1. Schematic view of the model system. The structure of KcsA, the cavity waters, the ions and water molecules in the intracellular and extracellular mouths and water cylinders, and the DPPC molecules are treated explicitly. The KcsA is embedded in a low dielectric slab with ε=1. The intracellular and extracellular bulk regions are modeled as a continuum of high dielectric medium with ε=80.

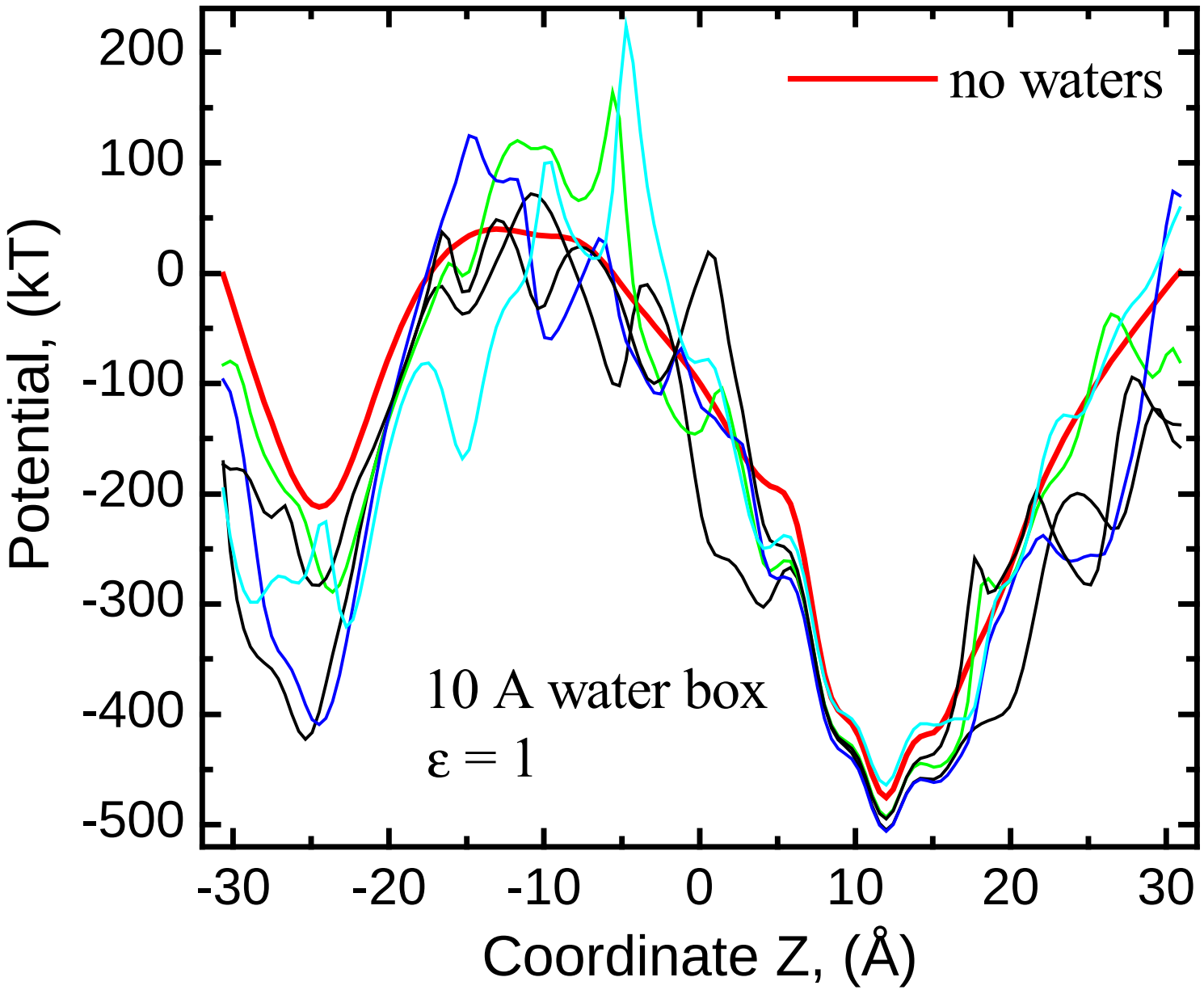

Figure 2. Ion potential energy profiles along the channel axis for several randomly generated water configurations.

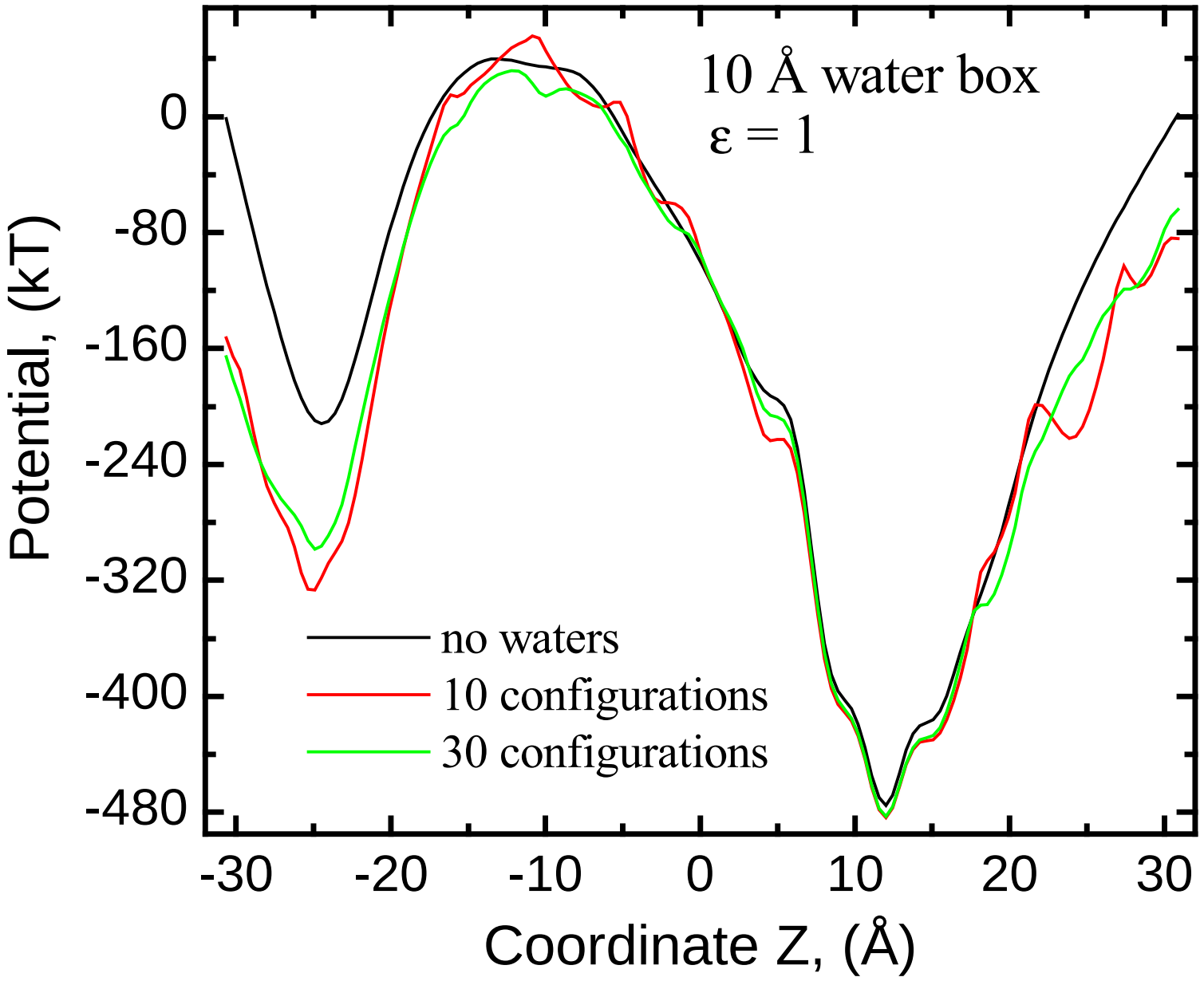

Figure 3. Average of ion potential energy along the channel axis over 10 and 30 randomly generated water configurations.

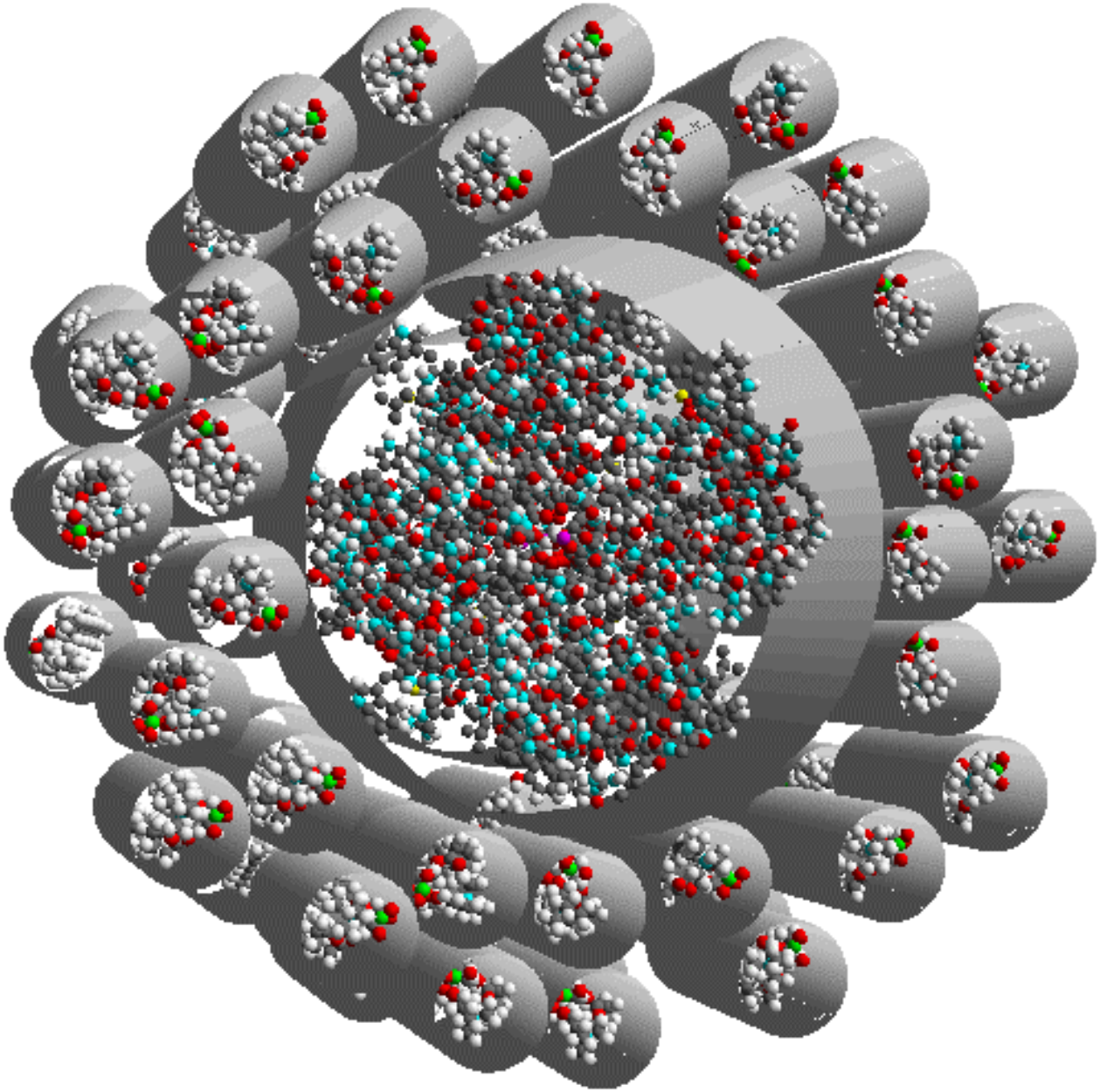

Figure 4. Illustration of explicit DPPC molecules that surround the extracellular and intracellular KcsA cylinders.

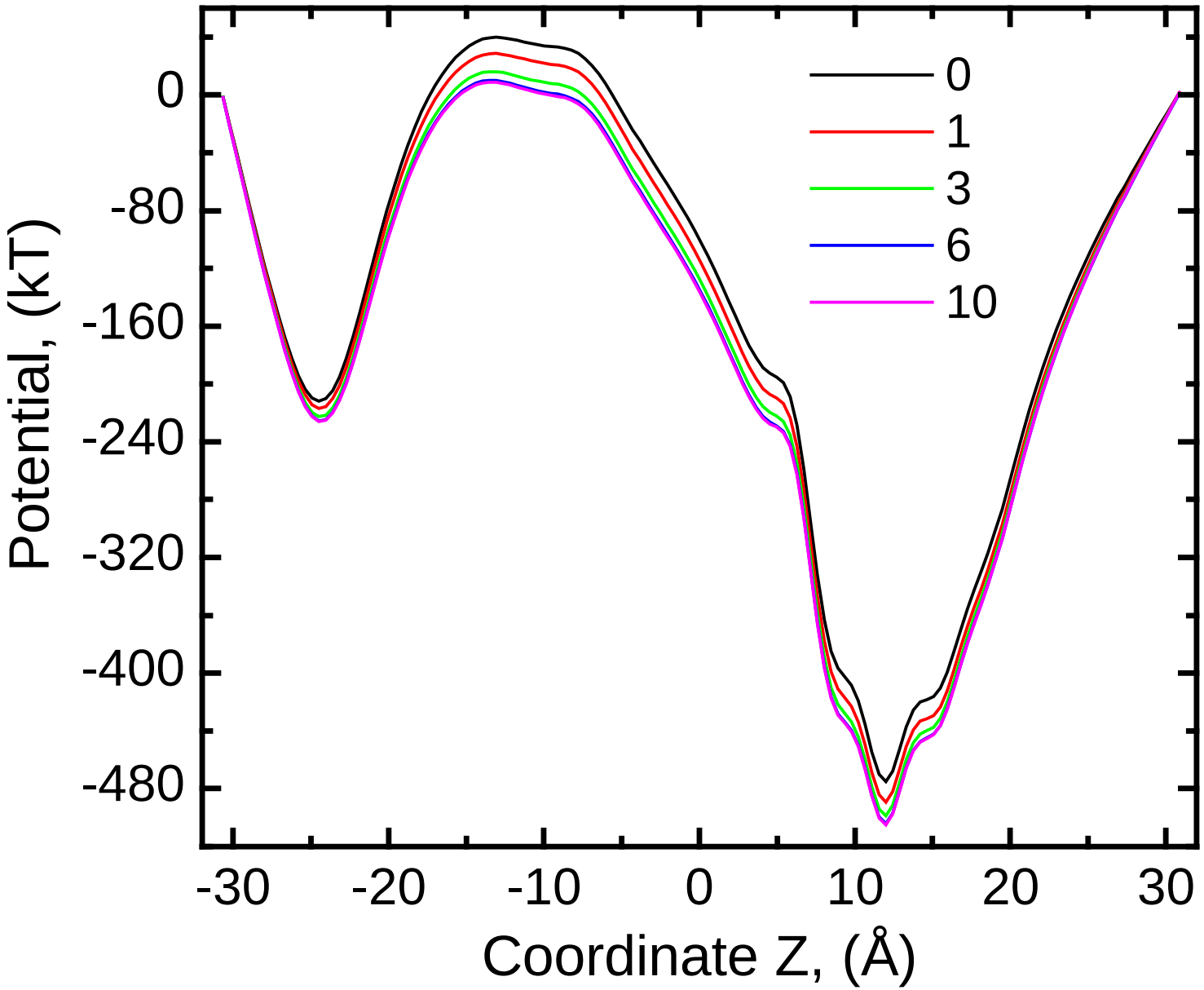

Figure 5. The effect of the thickness of the lipid domain on the potential energy along the channel axis. Legend's numbers mean the number of lipid layers around KcsA.

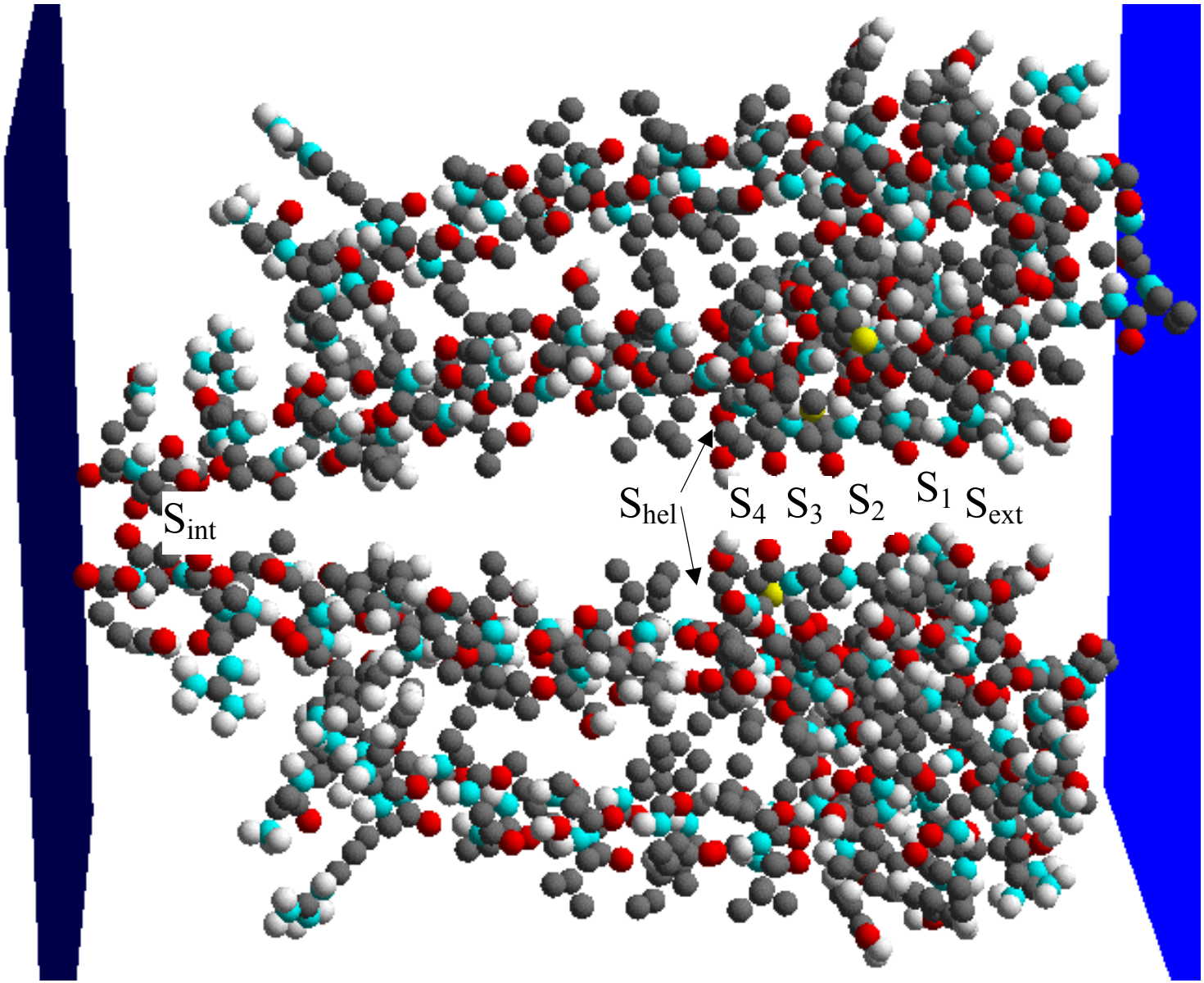

Figure 6. Detailed view of the KcsA K^+ channel structure used in our Monte Carlo simulations. The two KcsA subunits are displayed. The extracellular and intracellular image planes of the channel are on the right and left, respectively. The binding sites for K^+ ion in the KcsA found in our simulations are shown.

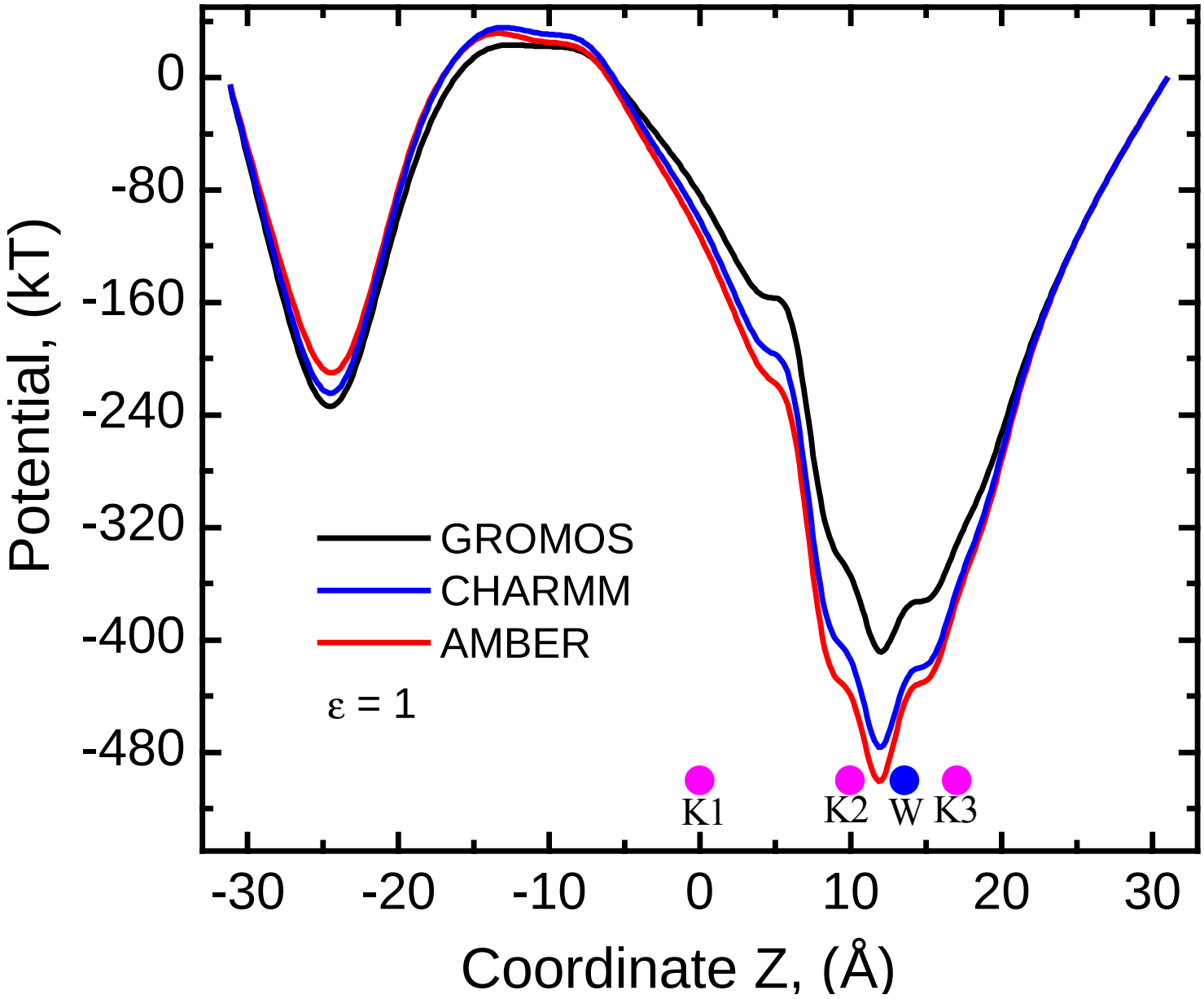

Figure 7. Electrostatic potential profiles for the KcsA channel along the z axis calculated using GROMOS, CHARMM, and AMBER force field models. The crystallographic locations of three ions and the water molecule are shown.

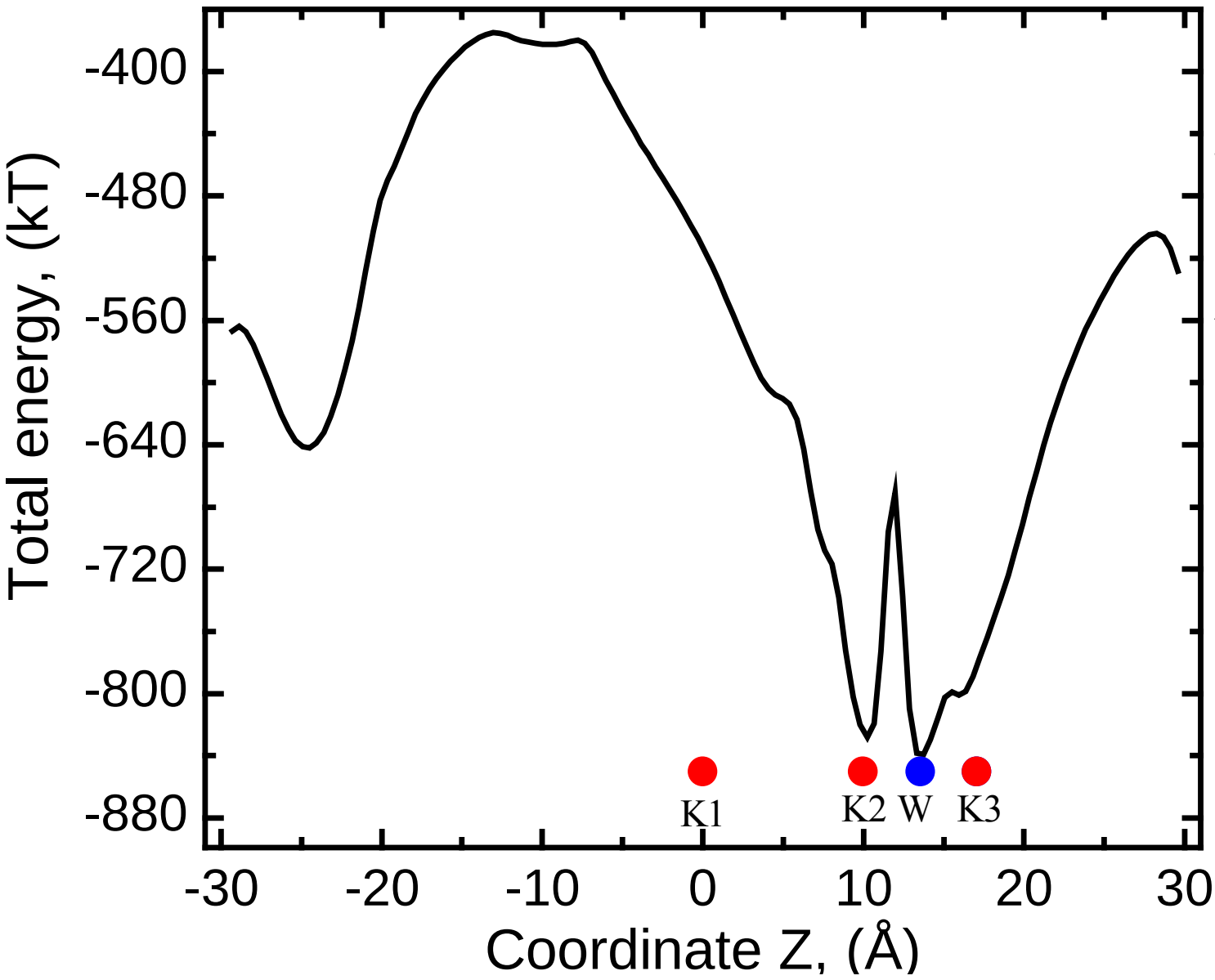

Figure 8. Total interaction energy (Coulomb + van der Waals) for the K^+ ion along the z axis calculated using CHARMM force field model.

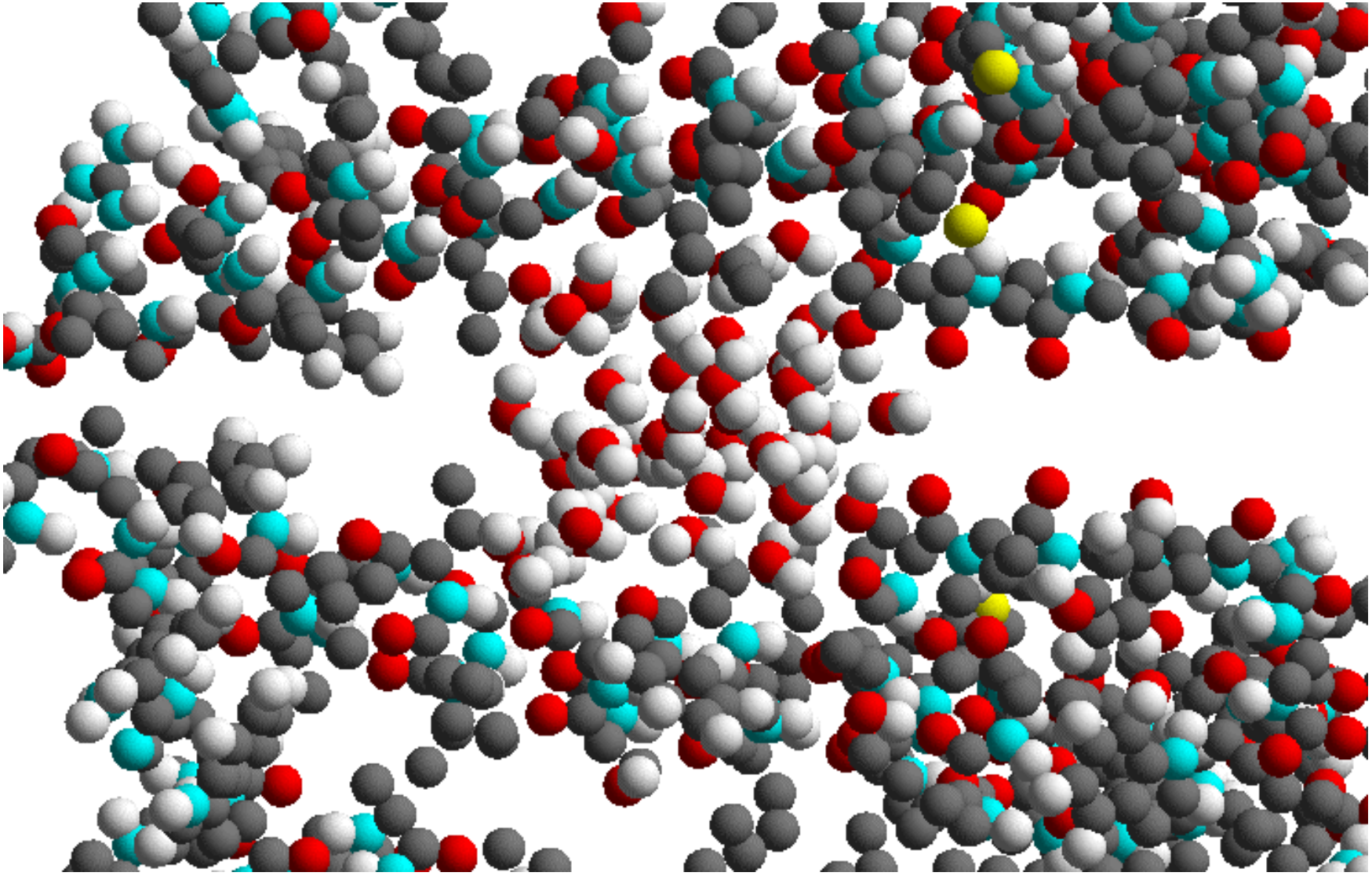

Figure 9. The ordered orientation of the water molecules in the cavity close to the entrance to the selectivity filter in the absence of ions.

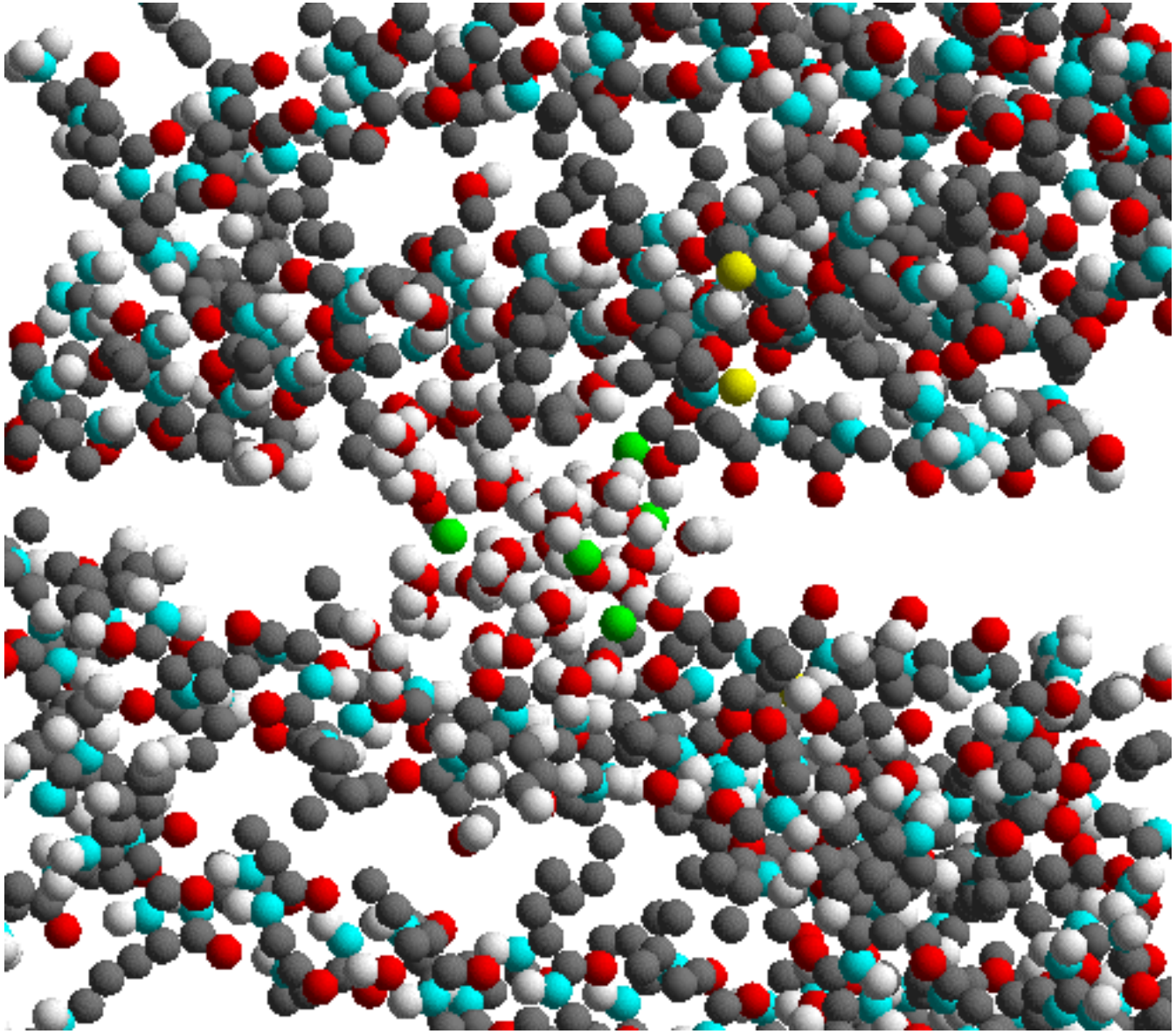

Figure 10. Multiple ion occupancy of the water-filled cavity.

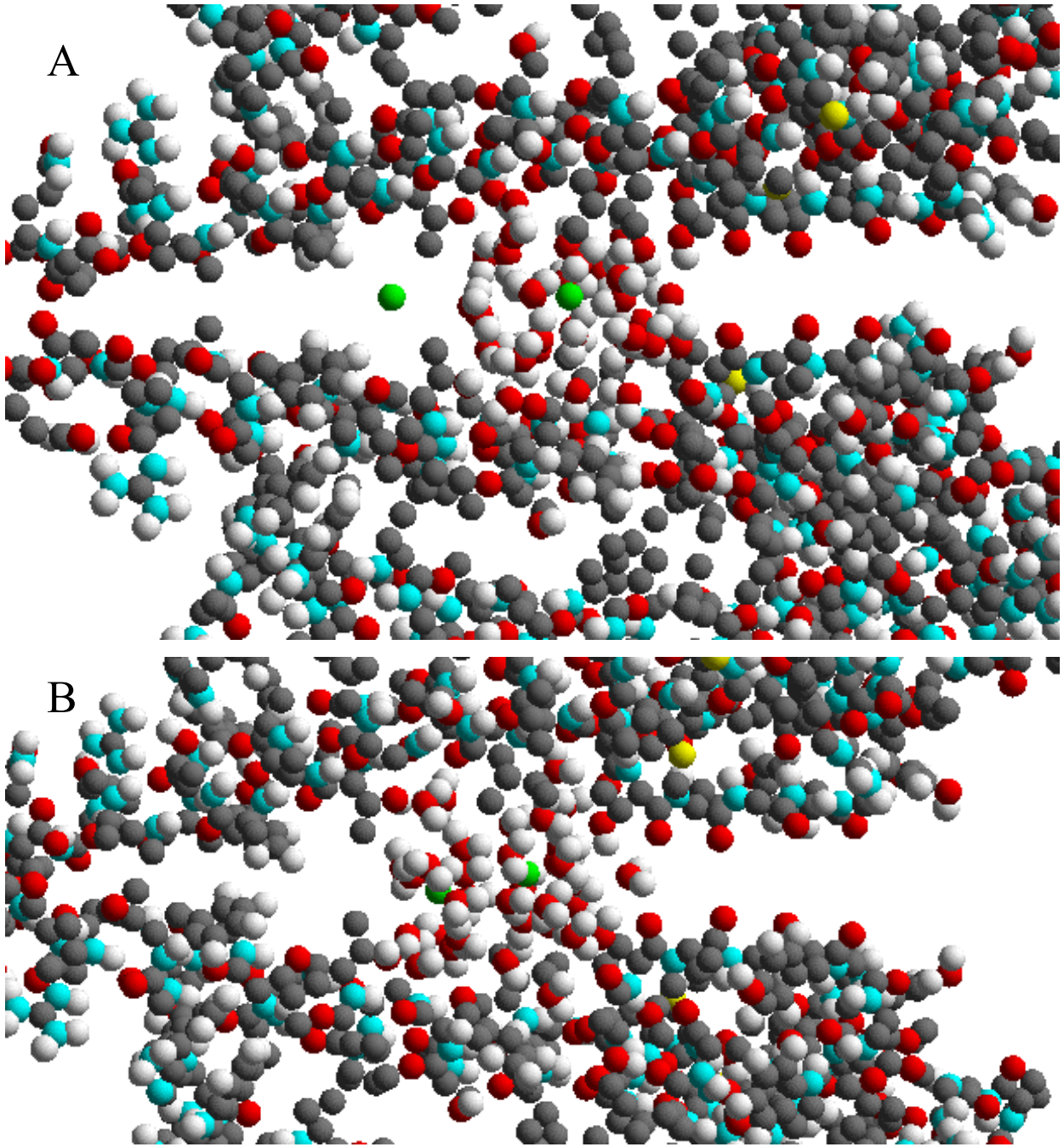

Figure 11. Mergence of the K^+ ion located in the intracellular tube (A) by the water-filled cavity (B). The K^+ ion in the center of cavity is also shown. The ordered water orientation (B) close to the entrance to the selectivity filter is occurred.

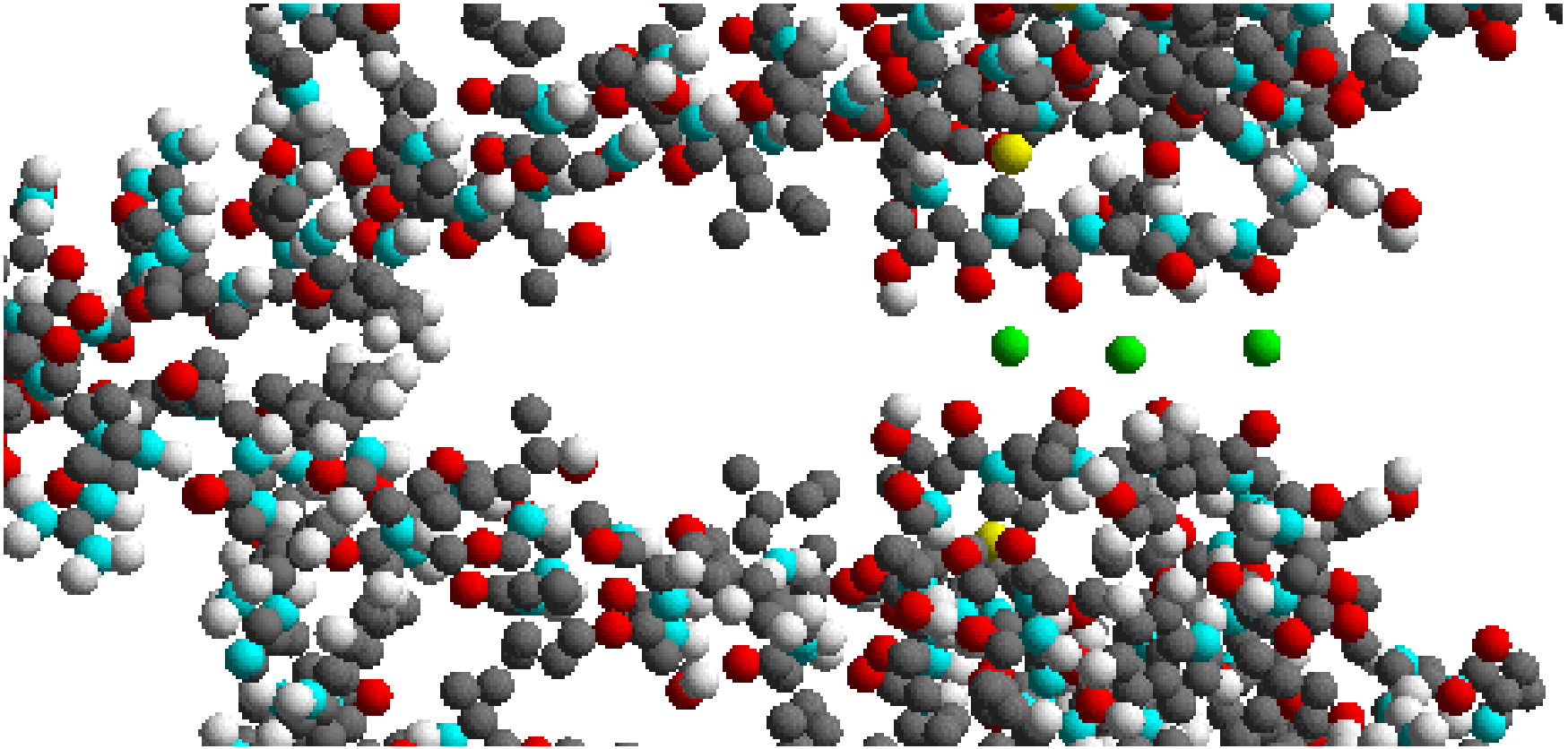

Figure 12. Stable locations of the three K^+ ions in the selectivity filter of the empty KcsA channel. First ion is at site S_3, second one is at S_2, and last that is located between sites S_1 and S_{ext}.