All-atom molecular dynamics simulations of an artificial sodium channel in a lipid bilayer: the effect of water solvation/desolvation of the sodium ion

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All-atom molecular dynamics is used to investigate the transport of Na⁺ across a 1,2-dioleoyl-sn-glycero-3-phosphocholine lipid bilayer facilitated by a diazacrown hydraphile. Specifically, the free energy of Na⁺ passing through the bilayer is calculated using the adaptive biasing force method to study the free energy associated with the increase in Na⁺ transport in the presence of the hydraphile molecule. The results show that water interaction greatly influences Na⁺ transport through the lipid bilayer as water is pulled through the bilayer with Na⁺ forming a water channel. The hydraphile causes a reduction in the free energy barrier for the transport of Na⁺ through the head group part of the lipid bilayer since it complexes the Na⁺ reducing the necessity for water to be complexed and, therefore, dragged through with Na⁺, an energetically unfavorable process. The free energy associated with Na⁺ being desolvated within the bilayer is significantly decreased in the presence of the hydraphile molecule; the hydraphile increases the number of solvation states of Na⁺ that can be adopted, and this increase in the number of available configurations provides an entropic explanation for the success of the hydraphile.

Keywords: ion transport; free energy calculations; ion solvation; ion complexation; molecular dynamics

Introduction

Hydraphiles, a class of synthetic molecules, have been found to possess antibacterial properties (Leevy et al., 2002; McLaughlin & Eisenberg, 1975). Hydraphiles facilitate the transport of Na⁺ across lipid membranes, thereby, causing a disruption within a bacterial cell (Atkins, Patel, Cusumano, & Gokel, 2010; Gokel & Negin, 2012; Smith et al., 2011; Wang, Yammitz, & Gokel, 2007). A multitude of different hydraphiles have been synthesized, and the facilitated transport rate of sodium ion across the lipid bilayers has been found to be highly dependent on the size and type of groups that comprise the hydraphile (Gokel, 2000; Gokel & Carasel, 2007; Gokel & Mukhopadhyay, 2001; Shabany, Pajewski, Abel, Mukhopadhyay, & Gokel, 2001; Weber et al., 2006).

Figure 1 shows the structure of a hydraphile found to be among the most effective sodium facilitators (Gokel & Daschbach, 2008), and it is the subject for the atomistic simulation reported in this study. The hydraphile consists of three diaza-18-crown-6 rings separated by a hydrocarbon chain and has a separator containing 12 carbon atoms (12C). Phenyl groups at the ends of the hydraphile (sidearm) serve to anchor the molecule in the lipid bilayer and the diaza-18-crown-6 has four oxygen atoms and two nitrogen atoms where one nitrogen atom is attached to a phenyl group and the other to a 12C hydrocarbon chain. It has been shown experimentally that when the phenyl group is replaced with other groups or when the length of the spacer is modified, the Na⁺ transport rate is reduced. Furthermore, when the central crown is replaced by a hydrocarbon chain and when no sidearm group is used, the transport rate is decreased to nearly zero (Gokel, 2000). The objective of this study was to investigate why the hydraphile shown in Figure 1 is particularly effective. The mechanism and the free energy profile associated with Na⁺ transfer across a lipid bilayer with hydraphile facilitation are investigated. Srinivas, Lopez, and Klein (2004) reported coarse-grained (CG) simulations of the hydraphile/1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) system using a CG force field based on the Shinoda force field (Shinoda, DeVane, & Klein, 2010). In their study, water molecules are modeled as an assembly of three water molecules, but it is reasonable to expect that hydrogen bonding should play a key role in the mechanism of ion transport. These hydrogen-bonding interactions should manifest themselves as cooperative effects where each water molecule interacts with other water molecules; for example, one water molecule entering the lipid bilayer.
can interact with a second water molecule dragging it into the bilayer. The cooperative effects of water can be better represented by a water model that treats each hydrogen and oxygen atom of the water molecule explicitly (albeit with fixed point charges, bond distances, and angles) compared to a CG model that represents a cluster of three water molecules as a single bead. Atomistic simulation enables the study of the effect of the explicit water molecules in the transport process. A number of simulation studies have investigated the free energy of unassisted ion transport through lipid bilayers (Khavrutskii, Gorfe, Lu, & McCammon, 2009; Tepper & Voth, 2006; Wilson & Pohorille, 1996) and have shown the importance of the solvation of the ions by water molecules as they pass through the lipid bilayer. Transport in biological ion channels has been effectively studied by simulation (Shi, Izvekov, & Voth, 2006). In the case of gramicidin, for example, simulations have shown that water molecules move in a single-file water column across the channel, assisting the transport of cations across the lipid bilayer (Baştuğ & Kuyucak, 2007; De, Boda, & Ali, 2010; Li, Andersen, & Roux, 2010; Wang & Fried, 2007).

Simulation studies of the complexation of crown ethers with \( \text{Na}^+ \) (De et al., 2010) have been reported, but to the best of our knowledge, there have been no simulation studies of the complexation of \( \text{Na}^+ \) by diaza-18-crown-6. This study investigates this complexation using both quantum chemistry and atomistic molecular simulations to better understand how complexation relates to the facilitated transport of \( \text{Na}^+ \) through lipid bilayers. There are two ways by which the hydraphile could facilitate the transport of \( \text{Na}^+ \) across lipid bilayers. For example, it could act as an ion channel (Capone et al., 2012) where the hydraphile lies across the bilayer providing a favorable environment for \( \text{Na}^+ \) to move across the lipid bilayer. Alternatively, the hydraphile may act as a transporter (Vanni, Campomanes, Marcia, & Rothlisberger, 2012; Zdravkovic, Zhao, Lev, Cuervo, & Noskov, 2012), where the molecule is more dynamic within the bilayer and can capture \( \text{Na}^+ \) and move it across the lipid bilayer releasing it at the other side.

The work by Gokel indicates that the hydraphile acts as an ion channel rather than an ion transporter (Gokel, Leevy, & Weber, 2004). This is because the \( \text{Na}^+ \) release rate, which is of the order of \( 10^7 \text{ s}^{-1} \), is too high for a transporter mechanism. According to the current proposed model, the hydraphile spans the lipid bilayer and \( \text{Na}^+ \) cations relay between crowns with very little movement of the crowns themselves. This is based upon the following observations: (1) The transfer of \( \text{Na}^+ \) is dependent on spacer length (Murray & Gokel, 1998); (2) In experiments, where the hydraphile side arm group is changed for fluorescent groups, the groups are found to be located within the mid-polar region, the phospholipid head group region (Abel et al., 1999); (3) One of the most active hydraphiles are more conformationally restricted with four crowns – two of which are in the center (Shabany et al., 2000). It has been suggested that the hydraphile acts as a channel by assisting with the reorganization of water, which in turn helps to solvate the \( \text{Na}^+ \) (Murray, Shabany, & Gokel, 2000). Similar observations were made in our recent study of chloride channels (Burkhardt, Skelton, & Fried, 2013). One goal of this study is to evaluate Gokel’s model for demonstrating the importance of hydraphile in the transportation of \( \text{Na}^+ \) across a lipid bilayer and provide insight into the mechanism of \( \text{Na}^+ \) transportation by the hydraphile. The study tries to answer the question of whether the hydraphile acts as channel or transporter.

**Methodology**

**Force field**

The CHARMM force field was used in this study. CHARMM36 provides a standard lipid force field (Feller, Gawrisch, & MacKerell, 2002; Klauda et al., 2010). The TIP3P (Jorgensen, Chandrasekhar, Madura,
Impey, & Klein, 1983) site model was used for water. In relation to the parameterization of the CHARMM force field, the hydrphile contains non-standard groups. Fortunately, it contains chemical groups that are very similar to those contained in lipid and protein molecules and, therefore, the force field parameters can be obtained from existing lipid and protein force fields as discussed in the Supplementary Material.

Quantum mechanical calculations
To test the validity of the force field, density functional theory (DFT), and Møller Plesset (MP2) (Riley, Platts, Řezáč, Hobza, & Hill, 2012) calculations were performed for fragments of the hydrphile using the 6–311 + G(d,p) basis set and Gaussian 09 (Frisch et al., 2009). DFT calculations using the functionals, B3LYP (Becke, 1993) and oB97XD, were used for geometry optimization; single-point energies of the B3LYP geometry-optimized structure were obtained using MP2 calculations. The oB97XD functional was used due to its ability to treat dispersion interactions (Chai & Head-Gordon, 2008a, 2008b; Granatier, Pitoňák, & Hobza, 2012). Figure 2 shows the diaza-crown used in the DFT calculations. The nitrogen atoms in the diaza-18-crown were terminated by a methyl group instead of a phenyl or hydrocarbon chain as in the case of the hydrphile. Force field predictions were compared with QM results for the isolated diaza-crown. The geometry-optimized configurations for different diaza-crown, water, and Na$^+$ interactions are shown in Figure 2 (Figure 2(a); diaza-crown/water; Figure 2(b); diaza-crown/Na$^+$ and Figure 2(c)) water/Na$^+$ configurations. The interaction energies ($E_{int}$) were calculated as:

$$E_{int} = E_{mo1} - E_{mo12} - E_{mo1} - E_{mo12}$$

where mo1 and mo2 are components of the complex. For example, in the water–Na$^+$ case, mo1 represents water and mo2 represents Na$^+$ in water–Na$^+$ calculations.

Classical molecular dynamics
The NAMD molecular dynamics program was used in this study (Phillips et al., 2005). The simulation cell contained the lipid bilayer, water, and hydrphile molecule as shown in Figure 3. The simulation contains 120 DMPC molecules with 9989 TIP3P water molecules. The system was set up by placing the lipid molecules in a bilayer arrangement (tails together) and adding the water to the two head surfaces. Particle mesh Ewald was used to treat the electrostatics interactions where the cutoff for such interactions was 10 Å. Isothermal-isobaric (NPT) dynamics at 310 K and 1 atm was performed for approximately 10 ns. The Nosé-Hoover, Langevin temperature control and piston pressure control methods were used. The averaged cell dimensions from the last 5 ns of NPT dynamics (approximately $60 \times 60 \times 120$ Å) were used as the starting point for effective NVT (canonical ensemble) dynamics. The timestep for the simulation was 1 fs. A ‘vacuum’ layer was incorporated in the simulation model by increasing the z-axis by 40 Å; this ensures there is no mixing between the top and bottom water. A small charge imbalance was adopted for free energy calculations. A Na$^+$ and a Cl$^-$ were placed in one side of the bilayer, and the other side was pure water. An additional simulation was set up containing the hydrphile in water and the system is shown in Figure 2S. In this system, Na$^+$ was placed in the center of the central diazacrown and a Cl$^-$ was placed into the water.

Free energy calculations
The free energy of Na$^+$-diaza-crown interaction was calculated using a potential of mean force (PMF) approach, utilizing the adaptive bias force method (ABF) (Darve, Rodríguez-Gómez, & Pohorille, 2008; Hénin, Fiorin, Chipot, & Klein, 2010), implemented in NAMD (Phillips et al., 2005). A reaction coordinate ($\xi$) of interest is chosen and an extra force is added, following the expression:

$$F_{ABF} = \nabla_x A = -\langle F_{\xi} \rangle_{\xi} \nabla_{\xi} \xi$$

where $A$ is the current estimate of the free energy, and $F_{\xi}(\xi)$ is the current average force. $F_{ABF}$ encourages the system to sample a chosen range of reaction coordinates that may not be sampled in conventional MD. The average force along this variable is obtained and integrated to give the PMF over the range of distances. Two different types of ABF calculations were performed, each with different ABF parameters. The ABF of Na$^+$ transport through DMPC was calculated with a bin size of .01 Å and a force constant of 1000 kcal/Å. It was ensured that each bin was sampled 10,000 times. For the coordination number ABF calculations, the sampling is highly computationally demanding. For this reason, the bin size was made much larger than for the 2D cases, that is, each bin was .5 Å and .5 coordination units for the distance and coordination number dimensions, respectively. It was ensured that in each bin there were 100,000 samples. For ABF simulations, the convergence was tested by calculating the ABF for a lower number of samples and increasing the number of samples until the result did not change (Neale, Bennett, Tieleman, & Pomès, 2011; Paloncýová, Berka, & Otyepka, 2012).
Results and discussion

Evaluation of the force field using QM calculations

Table 1 shows the interaction energies between the molecule pairs identified in Figure 2 using B3LYP-optimized structures. Also included was a cluster that included the Na⁺ with six water molecules; the interaction energy shown is the interaction energy per water molecule. Table 1 also contains energies calculated using the CHARMM36 force field, where the energy was calculated based on the B3LYP-optimized structure and the optimized structure using the CHARMM36 force field. Figure 4 shows a bar graph showing the interaction energies of the molecular pairs (shown in Figure 2).

Since the water may assist in the release of the Na⁺ from the diazacrown, it is important that the force field interaction quantitatively follows the QM results for interactions between water and Na⁺ in relation to the interaction between Na⁺ and the diazacrown and the diazacrown and water. The ratio between all these interactions should be similar. The interaction energies obtained from QM and the CHARMM FF calculations show good agreement. In all cases, the interaction energy of diazacrown – Na⁺ is the greatest followed by the Na⁺ – water interaction energy and the diazacrown – water interaction energy. The diazacrown – Na⁺ interaction energy is the greatest because Na⁺ interaction occurs between for four oxygen atoms and two nitrogen atoms.

As shown in Table 1 and Figure 4, there are some discrepancies between the QM and force field results; however, these discrepancies are small relative to the large differences in the three interaction energies. The force field energy (before and after optimization) for the diazacrown – water interaction falls between the highest (ωB97XD, −16.97 kcal/mol) and lowest values (B3LYP, −11.17 kcal/mol). The force field energy for the diazacrown – Na⁺ is close to the energy obtained from the ωB97XD functional calculations but is ~10 kcal/mol greater (12% difference) than the MP2 energy. Overall, the force field energies show the same trends as the QM

![Table 1](image)

| Interaction                  | B3LYP (kcal/mol) | ωB97XD (kcal/mol) | MP2 (B3LYP geometry) (kcal/mol) | FF (B3LYP geometry) (kcal/mol) | FF (after optimization) (kcal/mol) |
|-----------------------------|------------------|------------------|---------------------------------|---------------------------------|-----------------------------------|
| Crown-water                 | −11.17           | −16.97           | −15.4                           | −14.0                           | −12.65                            |
| Crown-Na⁺                   | −84.6            | −88.63           | −81.5                           | −90.8                           | −91.6                             |
| Na⁺-water                   | −25.78           | −24.74           | −24.58                          | −29.25                          | −29.73                            |
| Na⁺-6 water                 | −17.82           | −18.27           | −                              | −21.38                          |                                    |

Figure 2. Illustration of (a) diaza-crown/water (b) diaza-crown/Na⁺ and (c) water/Na⁺ configurations (from B3LYP DFT). DH-N is DH(water)-N(crown) and DH-O is DH(water)-O(crown). (Color scheme: Sodium (Na⁺) = yellow, oxygen (O) = red, carbon (C) = Cyan, Nitrogen (N) = blue, Hydrogen (H) = white).
calculations for each interaction and, therefore, should provide the correct ion/water-release behavior in the MD simulations.

**PMF of water/Na⁺ in bulk water**

A PMF of the distance of the Na⁺ from the center of mass (COM) of central diazacrown heavy atoms (excluding hydrogen atoms) within the water/hydraphile system (Figure 2S) was calculated and is shown in Figure 5. The PMF levels off from 8 to 10 Å as the Na⁺ enters the bulk water, where there is no free energy change. Close to 0 Å, when the Na⁺ is bound to the central diazacrown, there is a free energy minimum indicating that it is favorable for Na⁺ to be positioned within the central diazacrown compared Na⁺ being in bulk water. The diazacrown, therefore, provides a favorable environment for the Na⁺ to be positioned.

**PMF of Na⁺ transport through DMPC**

Figure 6 shows Na⁺ as it is being driven through the lipid membrane (lipid molecules are rendered invisible) from the top of the picture to the bottom in the absence (Figure 6(a)) and in the presence (Figure 6(b)) of the hydraphile. A water channel is formed as water is pulled through the bilayer along with Na⁺ due to the strong electrostatic interactions between Na⁺ and oxygen atoms of water regardless of whether Na⁺ is pulled through with the hydraphile or not.

Figure 7 shows the PMF as a function of the z-distance through half of the lipid bilayer in the presence and absence of the hydraphile. In both cases, the lipid bilayer starts at approximately 7 Å on the x-axis. Values of less than 7 Å on the x-axis, therefore, correspond to Na⁺ being pulled into the lipid bilayer, and values of greater than 7 Å correspond to Na⁺ moving into the water phase. Only half of the bilayer is shown because the transfer of Na⁺ from the opposite water bath to the halfway point in the bilayer will be opposite but equivalent. In both the absence and presence of the hydraphile,
there is a free energy penalty for the highly hydrophilic Na⁺ to move through the hydrophobic bilayer; the water molecules being dragged through the bilayer should make this process even more unfavorable. The two profiles are quite different; from 10 to 2 Å, there is no free energy change in the presence of the hydraphile but a steady increase in free energy in its absence. From 0 to −10 Å, the free energy changes at a similar rate in the presence and absence of the hydraphile. The reason for this difference is that in the presence of the hydraphile, the coordination of Na⁺ with the diazacrown oxygen and nitrogen atoms stabilizes the position of Na⁺ by providing polar groups to replace the interactions with water. In the absence of the hydraphile, however, there is no such stabilization. This effect is only observed for the first 10 Å because this is the headgroup region of the lipid bilayer where there are polar interactions with Na⁺ for further stabilization of the Na⁺ position. Overall the barrier to transport is smaller in the presence of the hydraphile (~35 kcal/mol) than in its absence (~26 kcal/mol).

Quantifying the effect of water solvation

Since the effect of water coordination to Na⁺ is of crucial importance to the transport of Na⁺, it would be useful to have a way to control the number of water molecules that are coordinated to the Na⁺ and to calculate the free energy of solvation for Na⁺. For this reason, the coordination number of the Na⁺ and water oxygen atoms was used as a reaction coordinate for a PMF calculation. This was defined as

$$C_{\text{Ow}, \text{Na}^+} = \sum_{j=1}^{n(\text{Na}^+)} \sum_{i=1}^{n(\text{Ow})} \frac{1 - |x_i - x_j|/d_o}{1 - |x_i - x_j|/d_o}$$

where the sum is over all water oxygen atoms. The sum over the Na⁺ ions only takes into account one value since there is only one Na⁺; |x_i - x_j| is the distance of the Na⁺ and a particular water oxygen atom, d_o is a cutoff distance (set to 3.5 Å).

Figure 8 shows the PMF profile as a function of C_{\text{Ow}, \text{Na}^+} in the presence and absence of the hydraphile. The starting configuration for both cases is where the Na⁺ was in the lipid bilayer as shown in Figure 8. Also shown on Figure 8 are the coordination states (configurations) that relate to the C_{\text{Ow}, \text{Na}^+} values. When C_{\text{Ow}, \text{Na}^+} is in the range between 0 and .5, there are no close interactions (O-Na⁺ distances less than 3.5 Å). From 1 to 1.5, there is one dipole–ion interaction interaction; from 2 to 2.5, there are two dipole–ion interactions; from 3 to 3.5, there are three dipole–ion interactions, and from 4 to 4.5, four dipole–ion interactions occur.
There are significant differences between the two curves. The free energy penalty of desolvating the Na\(^+\) is reduced when it is complexed to the diazacrown. It takes ~60 kcal/mol in the absence of the hydraphile to remove every water molecule but only 30 kcal/mol in the presence of the hydraphile. This indicates that it is easier to desolvate the Na\(^+\) when it is complexed to the hydraphile than when it is not. It can be noticed that the curve is more ‘stepped’ in the presence of the hydraphile and contains regions where the PMF does not change as the \(C_{ow;Na^+}\) value changes; there is no free energy change from 2.5 to 3.3, from 1.2 to 2.0 and from .2 to .7. There is almost a steady increase in free energy with decreasing \(C_{ow;Na^+}\) in the absence of the hydraphile, however. For both cases, there is an abrupt increase in free energy for \(C_{ow;Na^+} < .02\).

The plot in Figure 8 gives information about the desolvation of Na\(^+\) within the lipid bilayer but provides no information about desolvation driving transport through the bilayer. To fully investigate this, a 2D-ABF, where the two reaction coordinates are \(z\)-distance through the headgroup region of the bilayer and the coordination number is necessary. Figure 9a shows the 2D-PMF in the absence of the hydraphile, and Figure 9(b) shows the 2D-PMF in the presence of the hydraphile.

There are significant differences between the 2D-ABF in the absence and presence of the hydraphile. In the presence of the hydraphile, the ABF is almost a flat plane that increases slightly in both distance as well as coordination number dimensions. There are some fluctuations in the ABF in the coordination number dimension that relate to the steps shown in Figure 8.

**Figure 8.** The PMF as a function of \(C_{ow;Na^+}\) in the presence and absence of the hydraphile. Also showing coordination states that relate to \(C_{ow;Na^+}\) values. The annotated numbers are the ranges of \(C_{ow;Na^+}\) that relate to the coordination states.

**Figure 9.** Two dimensional PMF, where the reaction coordinates are the \(C_{ow;Na^+}\) and the distance through the bilayer. (a) in the absence and (b) in the presence of the hydraphile.

In the absence of the hydraphile, the desolvation free energy barrier is greater than the barrier to move Na\(^+\) through the bilayer (with Na\(^+\) fully solvated); therefore, it is unlikely that desolvation will occur in the absence...
of the hydraphile. The consequences of these differences are as follows: In the presence of the hydraphile, it is likely that Na⁺ can be transported across the bilayer in a range of solvation states. In the absence of the hydraphile, however, Na⁺ can only adopt a limited number of solvation states as it is transferred through the bilayer. This points to an entropic reason for the success of the hydraphile as a sodium transporter; the more configurations, at similar energies, that can be adopted as Na⁺ moves through the bilayer, the greater the opportunity there is for transport. For example, if there were many more pathways (each pathway has a different $C_{ow,Na⁺}$ value) for Na⁺ movement through the bilayer, of similar free energy changes, the rate of transfer would be many times faster.

One reason for the reduction in the free energy of solvation in the presence of the hydraphile is that the oxygen and nitrogen atoms of the diazacrown replace the coordination of Na⁺. The configuration in Figure 10 (inset) shows that there are 4 Na⁺/O interactions and one Na⁺/N interactions causing the reduction in Na⁺/water coordination number to be less unfavorable.

To better understand the complexation of the Na⁺, the PMF was calculated as a function of the coordination number of the diazacrown oxygen and nitrogen atoms with Na⁺ ($C_{O,N-Na⁺}$) and is shown in Figure 10. The PMF changes very little between $C_{O,N-Na⁺}$ values of 0 to 5 indicating there is no real preference for the Na⁺ to interact with the diazacrown atoms instead of water oxygen atoms. It should be noted that from $C_{O,N-Na⁺}$ values of 0 to 4, the main binding atoms to Na⁺ are the diazacrown oxygen atoms, and from $C_{O,N-Na⁺}$ values of 4 to 4.6, there is some binding of one of the diazacrown nitrogen atoms. The PMF, however, steeply increases from $C_{O,N-Na⁺}$ values of 5 to 6 indicating that the maximum coordination number from one diazacrown is 5.

The configuration at a coordination number of 5 is a configuration where there is coordination of Na⁺ to four oxygen atoms and one nitrogen atom. Binding of Na⁺ to water molecules complete the coordination of Na⁺, which should have a coordination number of approximately 6.

**Radial distribution function**

Figure 11 shows radial distribution functions (RDF) calculated for interactions between Na⁺ with water oxygen atoms and diazacrown nitrogen and oxygen atoms as the Na⁺ moves through the lipid bilayer, complexed to the diazacrown. The RDFs provide greater insight into the complexation of Na⁺. It can be noticed that the Na⁺-O(water) and Na⁺-O(diazacrown) peaks appear at the same distance indicating a similar strength of interaction for Na⁺ with both types of oxygen atom. There is a difference in peak height and area, however, since there are four diazacrown oxygen atoms but, usually, only two water oxygen atoms that bind to Na⁺. The Na⁺-(diazacrown) peak is at a much greater distance, and this result agrees with the result of the PMF as a function of the coordination number of terminal diazacrown oxygen and nitrogen atoms with Na⁺ shown in Figure 9; that is, the diazacrown oxygen atom to Na⁺ interaction is more preferable than that of the diazacrown nitrogen atom to Na⁺ interaction. We are currently extending our QM work to address whether this is a realistic result.

**Implication of results**

It would useful to analyze how our results compare with experimental results and the current view of how the hydraphile works as an ion channel/transporter. Results did not show any indications of the hydraphile assisting

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**Figure 10.** The PMF as a function of the coordination number of terminal crown oxygen and nitrogen atoms with Na⁺. The figure in the inset shows the starting configuration where the ion is within the crown.

**Figure 11.** The RDF calculated for interactions between Na⁺ with water oxygen atoms and diazacrown nitrogen and oxygen atoms as the Na⁺ moves through the lipid bilayer, complexed to the crown.
in the formation of a water channel. Attempts were made to test this by calculating the PMF of a water molecule as it passed through the bilayer (starting in close proximity to the hydraphile), but results showed that the water molecule showed no particular preference to remain associated with the hydraphile molecule as it passed through the bilayer. The water reorganization probably involves a collection of water molecules rather than a single one, and this effect is more difficult to probe using PMF methods. Furthermore, the discrepancies, between the force field and quantum results, in the interaction energies between water hydrogen atoms and diazacrown nitrogen and oxygen atoms could be responsible for an improper description of water reorganization. Moreover, the quantum results indicated that polarization effects may be important (readers may refer to the Supplemental Material) but were not taken into consideration in this study; it has been shown that these can be implicitly included into classical simulations (Allen, Andersen, & Roux, 2006; Jämbeck & Lyubartsev, 2013). The aforementioned factors outlined should be investigated further in future studies.

The results here indicate that, unless a water channel is formed, it is not reasonable for Na⁺ to leave the coordination of the terminal diazacrown so that it can travel to the central diazacrown. It would be more likely that the terminal diazacrown would be transported with Na⁺ toward the central diazacrown before the Na⁺ swaps from the terminal to the central diazacrown. This would require a conformational change of the hydraphile itself, causing the hydraphile to no longer span the lipid bilayer as in the current proposed model; it would indicate that the hydraphile is a transporter rather than a channel. Since we do not want to discount the wealth of experimental information available, we cannot conclusively state that the whole transport process has been described in this study. We can, however, state that we have provided insight into the initial process of the transport, that is, we have shown that the diazacrown helps to pull Na⁺ into the headgroup region and hold it there with no free energy penalty; perhaps this is long enough for water molecules to be reorganized and a channel to form.

Conclusions
The results of this study have helped to highlight the factors that affect how Na⁺ is transported through a DMPC lipid bilayer by a synthetic sodium channel, the hydraphile. Quantum chemical calculations (DFT and MP2 calculations) of a fragment of the hydraphile were performed and compared with calculations performed using the CHARMM force field. Some differences were shown in interaction energies between diazacrown oxygen and nitrogen atoms with Na⁺; however, it was deemed that the force field would capture the correct behavior and was sufficiently accurate to use in classical molecular dynamics simulations of Na⁺ interacting with the hydraphile in the lipid bilayer.

PMF calculations of the transport of Na⁺ through the bilayer showed that in the presence of the hydraphile, there was no change in the free energy as the ion is moved through the head region of the bilayer but changes at a similar rate to the unassisted Na⁺ through the tail region. This results in an overall reduction in the barrier to transport in the presence of the hydraphile.

To further investigate the effect of water solvation of the Na⁺ in the presence and absence of the hydraphile, the coordination number of water oxygen atoms (C_{Na-Ow}) and Na⁺ with water was chosen as a reaction coordinate. The PMF showed that the free energy of desolvation was far greater in the absence of the hydraphile than when it was present. A 2D-PMF was computed where the reaction coordinates were the C_{Na-Ow} and distance through the headgroup region of the bilayer; quite different profiles were shown. In the absence of the hydraphile, the free energy surface only increased in the distance dimension. This meant that there was very little increase in the free energy with a decrease in C_{Na-Ow} and, therefore, there are many different solvation states that can be adopted during the transport of the Na⁺. In the absence of the hydraphile, there is a large increase in free energy with a decrease in C_{Na-Ow} and the result of this is that there are limited paths that can be taken for the transport of Na⁺—those involving a high level of water coordination.

The results of this work have demonstrated the importance of hydraphile in the transport of Na⁺ across a lipid bilayer. It has shown how the hydraphile can complex the Na⁺, thereby affecting the solvation of the Na⁺. This study has provided molecular insight into the mechanism of Na⁺ transportation by the hydraphile and opens the way for further studies to understand how modifications of the hydraphile will affect transport rates.

Supplementary material
The supplementary material for this paper is available online at http://dx.doi.org/10.1080/07391102.2015.1044473.

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