G-Quadruplex DNA Channels

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# Free-Energy Landscapes of Ion Movement through a G-Quadruplex DNA Channel**

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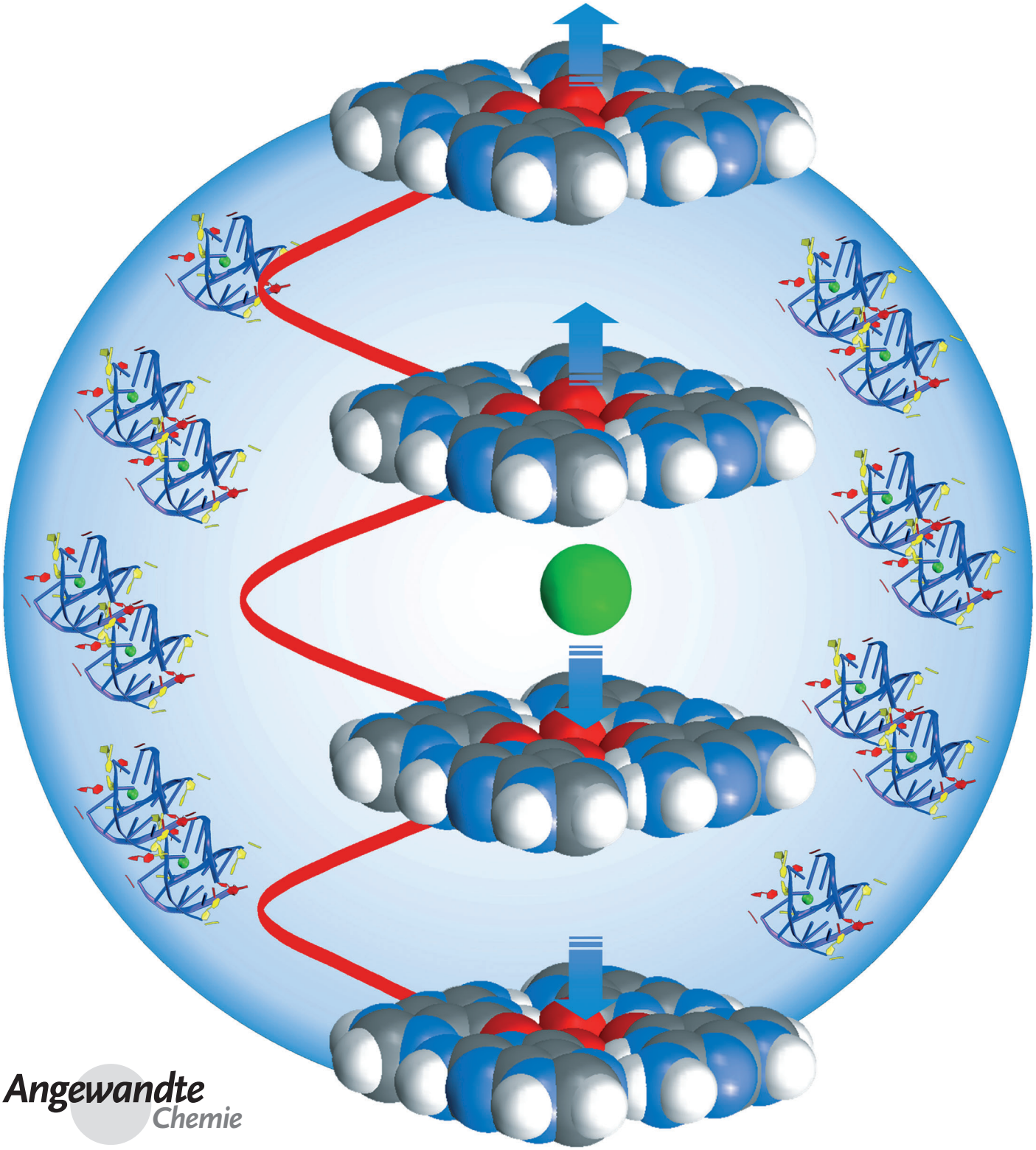

The folding of guanine(G)-rich DNA and RNA sequences into high-ordered G-quadruplex structures is known to depend critically on the presence of monovalent ions such as $Na^+$, $K^+$, and $NH_4^+$.[1] Although G-quadruplex DNA and RNA structures can display a diverse range of topologies, they all contain a very similar central core structure consisting of multiple G-quartets that are stacked on top of one another to form a channel-like pore. The major force holding the stacked G-quartets together comes from the ions that reside inside the channel. Most often these are monovalent ions ($Na^+$, $K^+$, $Rb^+$, $NH_4^+$, $Tl^+$), but occasionally they can be divalent ions ($Sr^{2+}$, $Ba^{2+}$, $Ca^{2+}$) as well. Inside the G-quadruplex channel, each ion is fully dehydrated and coordinated to the pore-lining guanine carbonyl O6 atoms. This special feature of the G-quadruplex structure is reminiscent of the selectivity filter found in $K^+$ ion channel proteins as first noted by Feigon and co-workers.[2] Because of this structural similarity, the stacked G-quartet motif was considered to be a candidate in the design of artificial ion channels.[3] While the modes of ion binding inside G-quadruplex channels have been established by many X-ray crystallographic and NMR studies, much less is known about the energetic and dynamic properties of ion translocation through a G-quadruplex DNA channel. In this regard, detailed information about the movement of $NH_4^+$ ions through G-quadruplex channels can be obtained from $^{15}N$ NMR studies as originally demonstrated by Feigon, Hud, and co-workers[4] and more recently by Plavec and co-workers.[5] However, other ions such as $Na^+$, $K^+$, $Rb^+$, $Tl^+$, and $Ca^{2+}$ are much more difficult to study and, as a result, only a few scattered NMR studies have been reported.[6] All these previous studies have shown that the ion movement in G-quadruplex DNA generally occurs on a timescale between milliseconds and seconds. As such long timescales of ion movement are associated with steep features in the free-energy landscape, it is rather difficult to achieve satisfactory sampling in the configuration space by classic brute-force molecular dynamics (MD) simulations, although we should note that conventional MD simulations have been used previously to generate useful insight into various aspects of G-quadruplex DNA.[7] While extensive computational studies can be found in the literature dealing with ion transport through ion channels such as $K^+$ ion channels,[8] cyclic peptide nanotubes,[9] and carbon nanotubes,[10] to the best of our knowledge, modern free-energy methods have not been used to explicitly evaluate the energetic properties of ion movement through any G-quadruplex DNA channel. In this work, we employ the adaptive biasing force (ABF) method[11] in unconstrained MD simulations and obtain, for the first time, the three-dimensional free-energy landscapes for ion transport ($Na^+$, $K^+$, and $NH_4^+$) through a G-quadruplex DNA channel.

Figures 1a and b show the parallel-stranded $[d(TG_4T)]_4$ G-quadruplex DNA structure used in this study; computational and structural details are given in the Supporting Information. To investigate the free-energy profiles for ion movement through this G-quadruplex channel, we divide the ion movement into two regions. One region is within the channel pore between the two exit/entrance points as marked in Figure 1c. The other is from the channel exit/entrance points to the bulk solution. In the discussion that follows, we examine these two regions separately.

Inside the narrow channel pore, as the ion movement is confined, it is natural to define the reaction coordinate ($\xi$) for ion movement to be along the channel axis (the $z$ axis; see Figure 1a). The average forces on the targeted ion were calculated using the ABF method as implemented in the NAMD program[12] and the CHARMM27 force field.[13] All computational details are given in the Supporting Information. Figure 1c shows the potential of mean force (PMF) profiles for the movement of $Na^+$, $K^+$, and $NH_4^+$ ions inside the channel. Quite remarkably, while both $K^+$ and $NH_4^+$ ions experience a free-energy barrier of roughly 13–15 kcal mol$^{-1}$ for moving between adjacent channel binding sites, the corresponding barrier for $Na^+$ movement is significantly smaller, about 4–5 kcal mol$^{-1}$. This computed free-energy barrier for $NH_4^+$ movement within the $[d(TG_4T)]_4$ channel is in reasonably good agreement with those reported by Plavec and co-workers[5d] for $NH_4^+$ movement within a unimolecular G-quadruplex formed by $d[G_4(T_4G_4)_3]$, approximately 12–15 kcal mol$^{-1}$. Although there has been no direct experimental data in the literature with respect to the free-energy barriers for $Na^+$ and $K^+$ movement within a G-quadruplex channel, the computed results for $K^+$ and $Na^+$ are understandable on the basis of their ionic radii ($Na^+$: 0.95 Å; $K^+$: 1.33 Å; $NH_4^+$: 1.43 Å). Therefore, while large $K^+$ and $NH_4^+$ ions must "squeeze" through a G-quartet to reach the adjacent channel binding site, $Na^+$ can be considered to "diffuse" nearly continuously through the channel. This result is also consistent with the observation made by Plavec and co-workers[5a] that the movement of $NH_4^+$ ions inside the $[d(G_4T_4G_4)]_2$ G-quadruplex channel is considerably faster for the $NH_4^+/Na^+$ mixed form than for the pure $NH_4^+$ form.

It is important to point out that, while it is always assumed in the literature that ions move in and out the narrow G-quadruplex channel through the entrance points at the two ends, there has never been experimental proof that ions would not "leak" out from the side wall of the G-quadruplex channel. To test this hypothesis, we obtained the PMF profiles for the "sideways" ion movement within the $[d(TG_4T)]_4$ channel. As clearly seen in Figure 1d, for the sideways movement within the channel, $Na^+$, $K^+$, and $NH_4^+$ ions would encounter a free-energy barrier of 50–60 kcal mol$^{-1}$, which is significantly larger than those along the channel axis. It is also interesting to note that all three ions experience essentially the same barrier for sideways movement within the channel. Thus if the channel ions were to "leak" through the channel wall, all three ions should exhibit the same dynamics, which is

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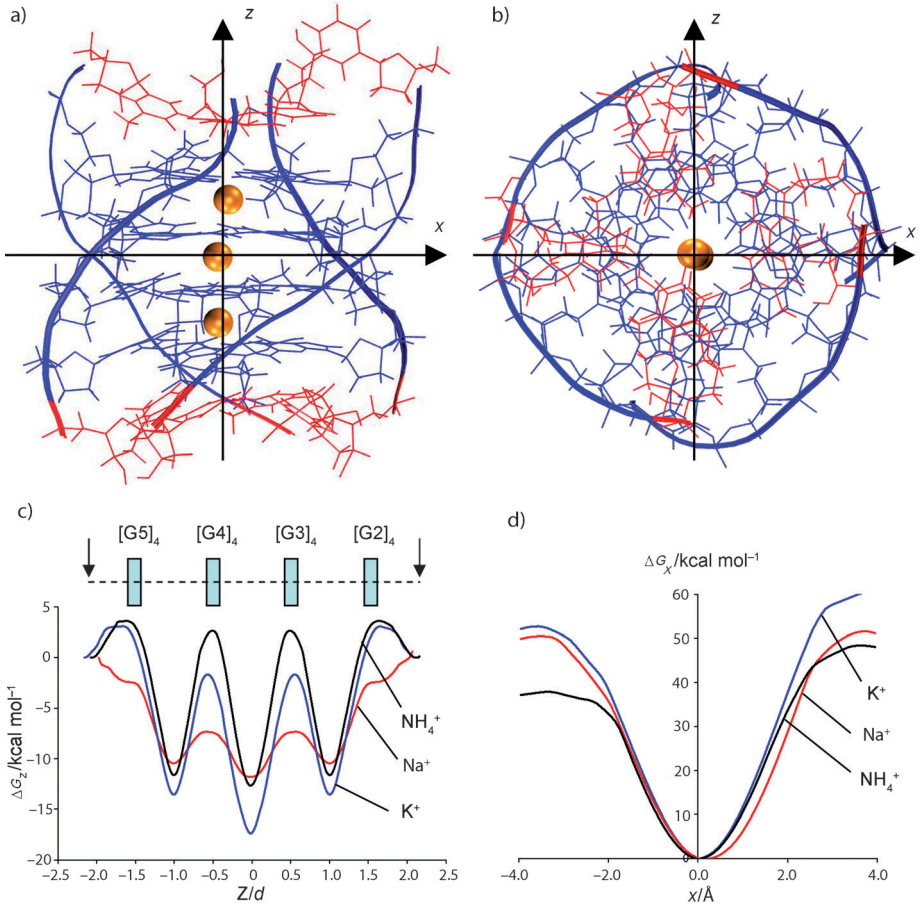


***Figure 1.*** a) Side and b) top views of the $K^+$ form of the $[d(TG_4T)]_4$ G-quadruplex structure. c) PMF profiles for ion movement inside the $[d(TG_4T)]_4$ G-quadruplex channels. The arrows indicate the exit/entrance points of the channel. For easy comparison, a normalized reaction coordinate ($Z/d$) is used in the diagram where $d$ is the averaged separation between two adjacent channel binding sites ($d = 2.71$, 2.95, and 3.09 Å for the $Na^+$, $K^+$, and $NH_4^+$ forms of the G-quadruplex, respectively). d) Typical one-dimensional PMF profiles for the "sideways" ion movement inside the channel ($Z = 0$ and along the $x$ axis). See the Supporting Information for a complete set of 2D PMF profiles.

obviously in contradiction with experimental observations. Thus, our computational results provide a definite proof that channel ions ($Na^+$, $K^+$, and $NH_4^+$) do not exit the channel through the channel wall.

Now we turn our attention to the second region of ion movement to investigate how an ion, after reaching the channel exit/entrance point, would move into the bulk solution. In this region, as there is no natural confinement for ion movement, we decided to perform a two-dimensional (2D, $x$–$y$) PMF mapping for a series of $z$ positions. In particular, for each $z$ position, we performed ABF calculations for eight directions within the $x$–$y$ plane and then reconstructed a 2D PMF topographic profile. As an example, Figure 2 shows a typical 2D PMF profile for $K^+$ movement from the 5′-end exit/entrance point toward the bulk solution. A complete set of 2D PMF profiles are given in the Supporting Information. In general, the free-energy landscapes in this region are quite flat and have a barrier of only about 4 kcal mol$^{-1}$ for $Na^+$, $K^+$, and $NH_4^+$ movement. Now combining the computational results obtained for both regions (Figure 1c and Figure 2), we conclude that the free-energy barrier for ion movement from the channel site to the bulk solution is approximately 20 and 14 kcal mol$^{-1}$ for $K^+/NH_4^+$ and $Na^+$, respectively. Recently, Šket and Plavec[5f] reported that the exchange rate constant for $NH_4^+$ movement between channel sites and bulk solution in $[d(TG_4T)]_4$ is approximately 40 s$^{-1}$ at 25 °C, corresponding to a free-energy barrier of 15 kcal mol$^{-1}$ if the Eyring equation is used. For the $d(G_3T_4G_4)_2$ G-quadruplex, the same authors performed variable-temperature experiments and obtained a free-energy barrier of approximately 16 kcal mol$^{-1}$ at 27 °C for the movement of an $NH_4^+$ ion from one of the channel sites to the bulk solution.[5c] It should be noted that the experimental determination of exchange rate constants (thus a free-energy barrier) for $NH_4^+$ movement from a channel site to the bulk solution is sometimes complicated by the fact that proton exchange between $NH_4^+$ and solvent (water) can be facile. Nonetheless, our computational results appear to be consistent with the limited experimental data. For $Na^+$, Wu and co-workers[6c] reported that the residence time of channel $Na^+$ ions in [d-

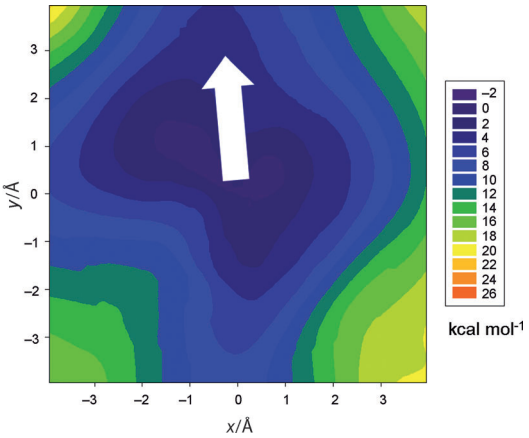


***Figure 2.*** A typical 2D PMF landscape for $K^+$ movement in the XY plane at the exit/entrance points. The arrow indicates the least resistant pathway for ion movement away from the channel axis ($X = 0$ and $Y = 0$) toward bulk solution. In this case, the Z position was chosen to be approximately 2.3 Å above the 5′-end G-quartet, $[G2]_4$ as defined in Figure 1c.

$(TG_4T)]_4$ G-quadruplex is more than 0.5 ms at 25 °C. Using magnetic relaxation dispersion (MRD) experiments, Snoussi and Halle[6e] determined the residence time of $Na^+$ ions inside the $[d(G_3T_4G_3)]_2$ G-quadruplex channel to be 0.6–1.0 ms at 27 °C. On the basis of the Eyring equation, again, our computed free-energy barrier of 14 kcal mol$^{-1}$ for $Na^+$ is then in qualitative agreement with the aforementioned experimental residence times determined for channel $Na^+$ ions in G-quadruplexes.

As mentioned earlier, the ions residing inside a G-quadruplex DNA channel are expected to be fully dehydrated. In our ABF-MD simulations, we can monitor the hydration state of the targeted ion as it moves along the reaction coordinate, the $z$ axis. Figure 3 depicts the hydration profile for the $NH_4^+$ ion moving in a range of $-12 \le z \le +12$ Å along the $z$ axis of the $[d(TG_4T)]_4$ channel. Similar results were obtained for $Na^+$ and $K^+$ ions in $[d(TG_4T)]_4$ and are presented in the Supporting Information. As seen from Figure 3, the $NH_4^+$ ion is almost completely dehydrated inside the channel (between the end G-quartets, $[G5]_4$ and $[G2]_4$). However, we do observe that occasionally water molecules enter into the channel to be within the first coordination shell of $NH_4^+$, resulting in a fractional number of coordination water molecules around the $NH_4^+$ ion. Interestingly, Plavec and co-workers[5g] did recently find NMR evidence for water molecules to reside inside the $[d(G_3CT_4G_3C)]_2$ G-quadruplex channel. As the $NH_4^+$ ion exits the channel, it quickly becomes hydrated. It is particularly interesting to note the situations occurring in the vicinity of the channel exit/entry points ($6.0 \ge z \ge 5.0$ Å and $-5 \ge z \ge -6.0$ Å). In each of these interfacial regions, the $NH_4^+$ ion is still coordinated to the four carbonyl O6 atoms from the end G-quartet on one side; it starts to receive on average approximately three water molecules on the opposite side. Similar situations were previously seen in the crystal structure of the $K^+$ form of $[d(G_4T_4G_4)]_2$ G-quadruplex[14] as well as in a $^{23}Na$ NMR study of the $Na^+$ form of the same sequence.[6d] Beyond these interfacial regions, the $NH_4^+$ ion becomes nearly fully hydrated, approaching the hydration state of a $NH_4^+$ ion in bulk solution.

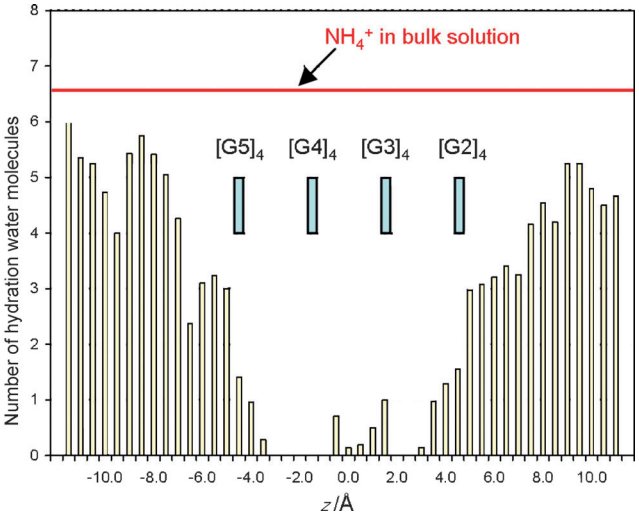


***Figure 3.*** Averaged number of hydration water molecules around the targeted $NH_4^+$ ion as it moves along the $[d(TG_4T)]_4$ G-quadruplex channel axis. The positions of the four G-quartets are also shown for easy comparison. In our MD simulations, the average number of water molecules within the first coordination shell of an $NH_4^+$ ion in bulk aqueous solution is 6.5 ($r_{cutoff} = 3.68$ Å). See the Supporting Information for more details.

Now that we have obtained the energetic properties for ion movement through a G-quadruplex DNA channel, it is worth comparing them with those found in $K^+$ ion channels (e.g., KcsA). As mentioned earlier, the ion coordination geometries are strikingly similar at the binding sites in these two systems. Yet, our computational results suggest much higher free-energy barriers, 14–20 kcal mol$^{-1}$, for an ion to move through a G-quadruplex channel than that seen in the KcsA channels, 2–3 kcal mol$^{-1}$.[8b] This implies that different mechanisms for ion translocation are operative in the two systems. While the exact mechanism for ion transport through a G-quadruplex channel is still being actively investigated in our laboratory, the high free-energy barriers found in this system are most likely due to the fact that the G-quadruplex channel pore is much more rigid than that in the KcsA selectivity filter. As the flow of $K^+$ ions through a G-quadruplex channel has never been directly measured, the computational results reported in this study provide some key insights into the process. Meanwhile we should point out that, as the computed free-energy barrier for $Na^+$ movement within the G-quadruplex channel is only 4–5 kcal mol$^{-1}$, it may still be possible to utilize a G-quadruplex DNA as an artificial $Na^+$ ion channel. Of course, the challenge is how to reduce the free-energy barriers at the channel exit/entrance points.

In summary, we have obtained a complete picture of the free-energy landscapes for the movement of $Na^+$, $K^+$, and $NH_4^+$ ions through a G-quadruplex DNA channel. This is the first time that the energetic and dynamic aspects of ion movement in G-quadruplex DNA have been investigated quantitatively by MD simulations. The computed results are in qualitative agreement with the very limited experimental data available in the literature. We believe that this general ABF-MD approach can be applied to other G-quadruplex structures. As G-quadruplex structures exhibit a very diverse range of topologies, it would be interesting to see whether the energetics of ion movement is somehow related to structures. Research along this line is underway in our laboratory.

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