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The influences of driving forces on behaviors of Na\(^+\) and H\(_2\)O in cyclic octa-peptide nanotube: investigated by steered molecular dynamics

Tianjiao Shan\(^1,3\), Xiaoguang Zhao\(^2,2\) and Haihai Liang\(^2,1,3\)

\(^1\) Department of Pharmacology (State-Province Key Laboratories of Biomedicine-Pharmaceutics of China, Key Laboratory of Cardiovascular Research, Ministry of Education), College of Pharmacy, Harbin Medical University, Harbin, Heilongjiang 150081, People’s Republic of China
\(^2\) Northern Translational Medicine Research and Cooperation Center, Heilongjiang Academy of Medical Sciences, Harbin Medical University, Harbin, Heilongjiang 150081, People’s Republic of China
\(^3\) Authors to whom any correspondence should be addressed.

E-mail: m15004684653@163.com, zhaoxiaoguang@hrbmu.edu.cn and lianghaihai@ems.hrbumu.edu.cn

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Abstract

The behaviors of Na\(^+\) and H\(_2\)O in cyclic peptide nanotube (CPN) under different conditions are important for their applications. In this study, a series of driving forces has been applied to Na\(^+\) and H\(_2\)O constrained in the self-assembled nanotube of \{cyclo([-D-Ala-L-Ala]\(_n\))\}\(_{10}\), to understand the influence on the transport properties and behaviors of Na\(^+\) and H\(_2\)O using steered molecular dynamics (SMD). The results show that H\(_2\)O need less driving force (0.4 kcal mol\(^{-1}\) Å\(^{-1}\)) to migrate in the nanotube than that of Na\(^+\) (2.3 kcal mol\(^{-1}\) Å\(^{-1}\)). Under the same driving force, the transport speed of H\(_2\)O is about 135 times faster than that of Na\(^+\). The instantaneous velocity curves reveal that water adopts a kind of irregular hopping transport mode which does not change with the driving force, while Na\(^+\) transports in an obvious hopping mode changing with driving force in three different types. Particularly, the instantaneous velocity curves of Na\(^+\) under the driving force of 3.2–5.2 kcal mol\(^{-1}\) Å\(^{-1}\) are roughly similar to the pulse signal, which is of great significance to the treatment of human diseases and the detection of electrolytes. The transport resistance mainly comes from electrostatic interaction. Results in this work show that cyclic octa-peptide nanotubes have excellent performance sensitive to external driving forces and are good potential materials for drug design, biosensors, ion transmembrane transport and ion probe for the detection of Na\(^+\) in organisms.

1. Introduction

Cyclic peptide nanotubes (CPNs) are hollow tubular structures formed by cyclopeptides consisting of alternating D- and L-amino acids via inter-ring H-bonds [1]. CPNs have attracted extensive attention of researchers owing to their unique structures and performances [1–3] since Ghadiri MR synthesized and characterized the first cyclic peptide nanotube in 1993 [2].

CPNs are very stable and their inner and outer surface properties can be easily modified by choosing different amino acid residues with specific side chain functionalities [3–5], which provides more possibilities for their uses in the field of chemistry, biology, materials science, and medicine [6–9]. For example, Sophie et al reported a kind of cyclic peptide-polymer nanotube with anisotropic shape. Their pharmacokinetic study of rat intravenous injection showed that the nanotubes can be disassembled into small units and cleared to reduce organ accumulation, even though their renal clearance is limited and systemic circulation is elongated [10]. Darnall et al reported a cyclic octa-peptide containing a benzimidazole functionality in the ring backbones, thus proposing new biomimetic transmembrane channel materials [11].

One of the most significant potential applications of CPNs is the artificial ion channels. In 1994, Ghadiri et al firstly evaluated the properties of CPNs as artificial ion channels, and proved the conduction velocity of ions in CPNs is three times faster than that in the Gramicidin channel by measuring the conductance of Na\(^+\) and K\(^+\).
However, it is difficult to further understand the transport characteristics of CPNs in experiments because CPN is easy to aggregate, which would hinder the development of its application. Therefore, there is no doubt that the molecular dynamics simulation could be a suitable method to investigate transport mechanisms and explore the novel applications of CPNs. In 2006, Hwang et al performed SMD simulations on ion transport in peptide nanotubes, and the results showed there were energy barriers at the channel entrances and exits and energy wells in the middle of the tube [13]. Subsequently, many studies about dynamics and transport properties of ions, H$_2$O and small molecules through cyclic octa-peptide nanotube have been done [14–17]. For example, in 2010, Liu et al investigated the water chain structures in 8×cyclo-(WL)$_4$ by molecular dynamics [16]. In 2013, Song et al studied the dynamics of Na$^+$ transportation in a trans-membrane CPN of 8×(WL)$_4$/POPE and drew the curves of PMF (potential of mean force) for Na$^+$ moving through the tube based on ABF (adaptive biasing force) method, indicating that Na$^+$ has lower free energy in an α-plane region than in a mid-plane one [17].

All these works are of great significance to the application of ion channels, however, there are still numerous problems that have not been resolved, such as the lack of discussions on the relationship between driving forces and transport behaviors of ions, which is of importance to the conditions where CPNs can be used. In addition, the transport mechanism differences between H$_2$O and Na$^+$ need to be explored further. In this study, we investigated transportation in cyclic octa-peptide nanotube under a series of pulling forces, and compared their transport trajectories, velocities, modes and transport binding energies with cyclic octa-peptide nanotube. We hope these results can contribute to the basis for their application in water and sodium channel materials.

2. Materials and methods

2.1. Materials system

Among various types of synthesized CPNs, the cyclic octa-peptide nanotubes with appropriate ring tension to synthesize and prone to be self-assembled into nanotubes have been extensively researched [4, 18, 19]. So the nanotube composed of cyclo([-D-Ala-L-Ala]$_{0-4}$) with the simplest side chains was selected as the study object.

In general, there are plenty of environmental external factors, such as external electric field, osmotic pressure or the polar of lipid bilayer, all of which could provide driving forces for ions passing through CPNs. Driving forces are sensitive to these external factors. For example, DPPC bilayer membrane not only provides support or the polar of lipid bilayer, all of which could provide driving forces for ions passing through CPNs. In order to reveal the relationship between the driving forces and the ion transport behaviors in the nanotube clearly, only a single nanotube was considered in this work. The structure of this nanotube was maintained by constraining C$_\alpha$ atoms and all external factors were simplified and unified to the pulling forces exerted on the ions or water molecules in CPNs, which not only saved computation time, but also eliminated the influences outside the nanotube.

2.2. Models

First, we established the Na$^+$ transport model, which consisted of one Na$^+$, 48 H$_2$O and one CPN with 10 rings. The Na$^+$ was inserted in the center of the second ring plane, and 48 H$_2$O were randomly arranged inside or outside the nanotube, so that the nanotube was filled with H$_2$O, and Na$^+$ as well as both ends of the nanotube were surrounded by H$_2$O, see figure 1(a). Generally, the CPNs composed of eight rings should be long enough to exceed the average thickness of the biofilm (~38 Å). We added two rings to the nanotube model, and the initial position of the pulling object was from the second ring to ensure the transportation length is enough and eliminate the influence of the barrier layer at the entrance of the nanotube, for some studies have showed that there was a barrier layer at the entrance of the nanotube [13].

Next, to obtain the H$_2$O transport model, the Na$^+$ was substituted by one H$_2$O molecule which was named H$_2$O$_{pt}$ to distinguish it from other H$_2$O molecules. Na$^+$ and H$_2$O$_{pt}$ were objects that we wanted to pull through the nanotube.

2.3. Methods

The transport model was simulated by molecular mechanics (MM), equilibration molecular dynamics (MD) [22] and steered molecular dynamics (SMD) with constant force or constant rate [13]. The simulation steps were as follows: (1) Na$^+$ and H$_2$O$_{pt}$ were constrained by force constant of 1 kcal mol$^{-1}$Å$^{-2}$, and the energy of initial model was minimized to get a stable nanotube structure. In the minimized structure, the average distance between centroids of neighbouring rings was 4.8 Å, which was consistent with that of other CPNs [23]; (2) Na$^+$ and H$_2$O$_{pt}$ and all C$_\alpha$ atoms were constrained in the system of CPNs, and 200 ps equilibration MD with a time step of 1 fs at 300 K was applied to the systems using a NPT (constant-pressure, constant-temperature) ensemble to equilibrate the 48 H$_2$O molecules to avoid the poor vdW interactions; (3) Only C$_\alpha$ atoms were constrained in the system to maintain the CPNs structure. SMD with constant force was applied under the pulling force of
corresponding to the keeps. By contrary, there is no obvious platform phenomenon for H2Opf. This is 10 ns because H2Opf need less time and minor pulling force to pass through the nanotube. In addition, the

Figure 1 shows some trajectory snapshots of Na3.1. Driving force in

3. Results

3.1. Driving force influences on trajectories of Na+ and H2O_pf

Figure 1 shows some trajectory snapshots of Na+ under SMD with constant force. From figure 1, we can see that the nanotubes are filled with H2O molecules, showing that Na+ and H2O_pf are surrounded by H2O, thus they are in the H2O environment during the simulation. In addition, all external factors have been taken into account by applying the pulling forces, thus the models used in this work are reasonable although the nanotube model is in a vacuum.

We can also see that the transportation of Na+ and H2O_pf is related to the pulling forces. As the pulling force increases, the moving distance of Na+ and H2O_pf becomes longer. In figures 1(b) and (c), Na+ and H2O_pf are still in the nanotube after 10 ns. When the driving force reaches 0.4 kcal mol$^{-1}$ Å$^{-1}$ for H2O_pf and 2.3 kcal mol$^{-1}$ Å$^{-1}$ for Na+, they can reach the last ring plane, see figure 1(d). When the pulling forces are 0.6 kcal mol$^{-1}$ Å$^{-1}$ for H2O_pf and 2.4 kcal mol$^{-1}$ Å$^{-1}$ for Na+, they can transport through the nanotube, but are trapped at end of nanotube, see figure 1(e). When the pulling forces exceed 0.8 kcal mol$^{-1}$ Å$^{-1}$ for H2O_pf and 2.5 kcal mol$^{-1}$ Å$^{-1}$ for Na+, they leave the nanotube and depart far away, see figure 1(f).

In order to further understand the transportation behaviors of Na+ and H2O_pf, their trajectories along z-axis were analyzed and shown in figure 2. In figure 2, the origin of z-axis is the second ring plane (0 Å), and the coordinate of the tenth ring plane is 38.4 Å. The maximum SMD time of H2O_pf is only 800 ps, while that of Na+ is 10 ns because H2O_pf need less time and minor pulling force to pass through the nanotube. In addition, the curves of Na+ or H2O_pf rise sharply after they left the nanotube, so we cut off the z-axis at 90 Å. From figure 2, we can see that Na+ stays in the same position for a long time and forms track platforms, which are exactly corresponding to the z-axis coordinate of ring plane, and the smaller the driving force is, the longer the platform keeps. By contrary, there is no obvious platform phenomenon for H2O_pf.

Generally, the influences of driving forces on the trajectories of Na+ and H2O_pf could be classified into three types: (1) the pulling forces cannot drive Na+ or H2O_pf to the last ring of the nanotube, which is 0.2 kcal mol$^{-1}$ Å$^{-1}$ for H2O_pf or less than 2.3 kcal mol$^{-1}$ Å$^{-1}$ for Na+, that is to say, the maximum transport

Figure 1. Snapshot of Na$^+$ transporting through the CPN under a series of pulling forces. (a) initial model; (b) $t = 9000$ ps, $F = 1.2$ kcal/mol Å; (c) $t = 9500$ ps, $F = 1.6$ kcal mol$^{-1}$ Å$^{-1}$; (d) $t = 9225$ ps, $F = 2.3$ kcal mol$^{-1}$ Å$^{-1}$; (e) $t = 6000$ ps, $F = 2.4$ kcal mol$^{-1}$ Å$^{-1}$; (f) $t = 5045$ ps, $F = 2.5$ kcal mol$^{-1}$ Å$^{-1}$.
distance of Na\(^{+}\) or H\(_2\)O\(_{pf}\) is less than 38.4 Å (the red dotted line below in figure 2); (2) the driving forces cannot overcome the resistance coming from the end of nanotube, which are in range of 0.4–0.6 kcal mol\(^{-1}\) Å\(^{-1}\) for H\(_2\)O\(_{pf}\) or 2.3–2.4 kcal mol\(^{-1}\) Å\(^{-1}\) for Na\(^{+}\), and the maximum transport distance is 45.6 Å (the dotted line above in figure 2), where Na\(^{+}\) or H\(_2\)O\(_{pf}\) is trapped and cannot escape the nanotube; (3) when the pulling forces exceed 0.6 kcal mol\(^{-1}\) Å\(^{-1}\) for H\(_2\)O\(_{pf}\) or 2.4 kcal mol\(^{-1}\) Å\(^{-1}\) for Na\(^{+}\), Na\(^{+}\) or H\(_2\)O\(_{pf}\) leaves the nanotube and the transport distance is more than 90 Å.

The calculating results showed that at least 2.3–2.4 kcal mol\(^{-1}\) Å\(^{-1}\) was needed for Na\(^{+}\) to pass through the CPN, while the resistance coming from the end of nanotube was at least 2.4 kcal mol\(^{-1}\) Å\(^{-1}\). This calculating result is corresponding to Hwang et al whose computing result of barriers at the channel entrances and exits was around 2.4 kcal/mol of potential of mean force (PMF) for Na\(^{+}\) and K\(^{+}\) [22]. We think that it is not just a coincidence despite the two systems are different. Because the main difference between the two systems was the side chain which affected the outer surface properties of nanotubes, however, they had the same backbone rings composed of eight amino acid residues, which was the main factor affecting ion transport in nanotubes. Moreover, in both these two studies, the unknown or small factors (including the external factors of nanotubes) were treated approximately. Hwang et al calculated an average force (PMF) on Na\(^{+}\) by ignoring some minor influencing factors (including the side chains and membranes). In this paper, all factors were unified to pulling forces exerted on Na\(^{+}\). So we reasonably deduced that in the octa-peptide nanotube the minimum driving force of Na\(^{+}\) transporting was 2.3 kcal mol\(^{-1}\) Å\(^{-1}\) and that of H\(_2\)O\(_{pf}\) was 0.4 kcal mol\(^{-1}\) Å\(^{-1}\).

### 3.2. Driving force influences on transport speed of Na\(^{+}\) and H\(_2\)O\(_{pf}\)

Exploring the relationship between transport speed and driving force of Na\(^{+}\) and H\(_2\)O\(_{pf}\) is a matter of significance to potential applications of the CPNs. Transport times and distances of Na\(^{+}\) and H\(_2\)O\(_{pf}\) under different pulling forces were shown in table 1. In table 1, \(t_{38.4}\) is the time when Na\(^{+}\) and H\(_2\)O\(_{pf}\) arrived at the tenth ring plane (\(z\)-axis coordinate is 38.4 Å) and \(t_{\text{max}}\) is the time when Na\(^{+}\) and H\(_2\)O\(_{pf}\) moved to the maximum distance. \(S_{\text{max}}\) is the maximum distance Na\(^{+}\) and H\(_2\)O\(_{pf}\) moved. If Na\(^{+}\) and H\(_2\)O\(_{pf}\) can pass through and break free from the bondage of nanotubes to move faraway quickly, \(S_{\text{max}}\) takes 90 Å and \(t_{\text{max}}\) takes the time when Na\(^{+}\) and H\(_2\)O\(_{pf}\) reach 90 Å.

Using data in table 1, we calculated the transport average speed of Na\(^{+}\) or H\(_2\)O\(_{pf}\) inside the nanotube according to equation (1).

\[
V = S / (t)
\]

Where \(V\) is the average speed inside the nanotube, \(S\) is the transport distance. \(S\) takes 38.4 Å and \(t\) takes \(t_{38.4}\) if \(S \geq 38.4\) Å, or it takes \(S_{\text{max}}\) and \(t\) takes \(t_{\text{max}}\) if \(S < 38.4\) Å. The relationship between the driving force of Na\(^{+}\) and H\(_2\)O\(_{pf}\) and transport average speed are shown in figure 3. From figure 3, we can see the influences of driving forces on transport speed of Na\(^{+}\) and H\(_2\)O\(_{pf}\) more clearly. In general, as the driving force increases, the transport speed increases. But according to the rising trend, the curves of transport average speed versus driving force can be divided into three stages. In the first stage, the impact of driving force on speed is different between Na\(^{+}\) and H\(_2\)O\(_{pf}\). For H\(_2\)O\(_{pf}\) with the driving force increasing from 0.2 kcal mol\(^{-1}\) Å\(^{-1}\) to 0.4 kcal mol\(^{-1}\) Å\(^{-1}\), the speed
increases rapidly. While for Na\(^+\), driving force increases from 0.4 kcal mol\(^{-1}\) Å\(^{-1}\) to 3.2 kcal mol\(^{-1}\) Å\(^{-1}\), and the speed curve is linear with a slight increase. In the second stage, the driving forces are from 0.4 kcal mol\(^{-1}\) Å\(^{-1}\) to 1.0 kcal mol\(^{-1}\) Å\(^{-1}\) for H\(_2\)O\(_{pf}\) and 3.6 kcal mol\(^{-1}\) Å\(^{-1}\) to 4.8 kcal mol\(^{-1}\) Å\(^{-1}\) for Na\(^+\), while the speeds have no significant increase. This shows the forces make no effect on the transport speed. Especially, there are even several data points lower than those at the lower forces, such as the transport velocity for water at 0.6 kcal mol\(^{-1}\) Å\(^{-1}\) is lower than that at 0.4 kcal mol\(^{-1}\) Å\(^{-1}\). This error may attribute to the initial atomic velocities randomly generated according to the Boltzmann distribution. In the third stage, the driving forces are from 1.2 kcal mol\(^{-1}\) Å\(^{-1}\) to 2.3 kcal mol\(^{-1}\) Å\(^{-1}\) for H\(_2\)O\(_{pf}\) and 4.8 kcal mol\(^{-1}\) Å\(^{-1}\) to 7.0 kcal mol\(^{-1}\) Å\(^{-1}\) for Na\(^+\), speeds of Na\(^+\) and H\(_2\)O\(_{pf}\) increase rapidly.

We also compared the transport speed of Na\(^+\) and H\(_2\)O\(_{pf}\) under the pulling force of 2.3 kcal mol\(^{-1}\) Å\(^{-1}\), because under the driving force, Na\(^+\) could arrive at the end of nanotube and H\(_2\)O\(_{pf}\) could move at a suitable speed. The speed of Na\(^+\) is 4.16 Å ns\(^{-1}\), which is corresponding to the results of Ghadiri et al\[12\] and Hwang.

### Table 1. Transport time and distance of H\(_2\)O and Na\(^+\) through CPN under a series of pulling forces.

| F (kcal mol\(^{-1}\) Å\(^{-1}\)) | t\(_{38.4}\) (ps) | t\(_{\text{max}}\) (ps) | S\(_{\text{max}}\) (Å) | F (kcal mol\(^{-1}\) Å\(^{-1}\)) | t\(_{38.4}\) (ps) | t\(_{\text{max}}\) (ps) | S\(_{\text{max}}\) (Å) |
|---|---|---|---|---|---|---|---|
| 0.4 | — | 6000 | 4.8 | 0.2 | — | 800 | 13.4 |
| 0.8 | — | 9000 | 9.6 | 0.4 | 350 | 380 | 43.2 |
| 1.2 | — | 9000 | 19.2 | 0.6 | 380 | 400 | 45.6 |
| 1.6 | — | 9500 | 28.8 | 0.8 | 328 | 320 | >90 |
| 2.0 | — | 9800 | 33.6 | 1.0 | 332 | 320 | >90 |
| 2.3 | 9225 | 9700 | 45.6 | 1.2 | 242 | 260 | >90 |
| 2.4 | 5430 | 6000 | 45.6 | 1.6 | 140 | 130 | >90 |
| 2.5 | 4425 | 5045 | >90 | 2.3 | 68 | 65 | >90 |
| 2.8 | 4560 | 4900 | >90 | 5.2 | 1765 | 2100 | >90 |
| 3.2 | 1765 | 2100 | >90 | 4.0 | 760 | 875 | >90 |
| 3.6 | 620 | 700 | >90 | 4.4 | 550 | 590 | >90 |
| 4.0 | 760 | 875 | >90 | 4.8 | 575 | 630 | >90 |
| 4.4 | 550 | 590 | >90 | 5.2 | 290 | 320 | >90 |
| 4.8 | 575 | 630 | >90 | 5.6 | 146 | 200 | >90 |
| 5.2 | 320 | 200 | >90 | 6.0 | 150 | 160 | >90 |
| 5.6 | 146 | 200 | >90 | 7.0 | 60 | 75 | >90 |

**Figure 3.** The pulling force of Na\(^+\) or H\(_2\)O\(_{pf}\) versus transport average speed. As the driving force increases, the transport speed increases.
et al [22], but that of H₂O₂ is 564.71 Å ns⁻¹, which is about 135 times faster than Na⁺. This suggests that CPNs are more facility to water molecules transport.

### 3.3. Driving force influences on transport mode of Na⁺ and H₂Oᵦf

In order to insight the transport mode of Na⁺ and H₂Oᵦf further, we analyzed their instantaneous velocity along z-axis, which is shown in figures 4 and 5. From the curves, we find that transport mode is also closely related to driving forces. For instance, when the driving force is less than 2.8 kcal mol⁻¹ Å⁻¹, the curves of Na⁺ in figure 4 have a similar form, that is, the velocity of Na⁺ in each ring plane vibrates near zero, then increases suddenly, and then decreases rapidly to zero. The high speed of Na⁺ appears as a sharp peak, appearing only between two adjacent rings and remaining for a very short time, which represents a typical hopping transport mode. This can also be seen from the z-axis trajectories of Na⁺ (figure 2), we can see Na⁺ adopts a significant hopping transport mode, because it fluctuates at track platform corresponding to the z-axis coordinate of ring plane, then jumps to another platform quickly. The leap distances are just the distances between adjacent rings (4.8 Å). With the increase of driving force, the peaks become wider and higher, and Na⁺ still passes the adjacent rings in a hopping mode when the driving force is within 3.2–5.2 kcal mol⁻¹ Å⁻¹. The whole curves like square pulse signal. When the driving force reaches 5.6 kcal mol⁻¹ Å⁻¹, the curve shapes change into two continuous peaks, and hopping transport mode tends to disappear.

Although H₂Oᵦf also had a hopping mode, it was not as obvious and regular as that of Na⁺. Occasionally, H₂Oᵦf would pass through two ring planes with a constant speed at one time. For example, under the pulling force of 1.6 kcal mol⁻¹ Å⁻¹, at the red circle marked in figures 2 and 5, the time is from 77 ps to 84 ps, and H₂Oᵦf moves from the fifth ring to the seventh ring at a similar instantaneous velocity without staying on the sixth ring plane. Similarly, under the pulling force of 1.0 kcal mol⁻¹ Å, at the green circle mark of figures 2 and 5, the time is from 150 ps to 180 ps, and H₂Oᵦf moves from the sixth ring to the eighth ring without staying on the seventh ring.

Moreover, the transport mode of H₂Oᵦf had a few changes with the pulling force and differed to that of Na⁺. From figure 5, we can see the instantaneous velocity peaks of H₂Oᵦf are mostly positive and distribute irregularly. The whole curves seem like electroencephalogram. Instantaneous velocities of Na⁺ under pulling force of 1.6 / 3.2 / 5.6 kcal mol⁻¹ Å⁻¹ and that of H₂Oᵦf under the driving force of 2.3 kcal mol⁻¹ Å⁻¹ are shown in figure 6. From figure 6, we can clearly see the transport mode of H₂Oᵦf is different from those three modes of Na⁺.

### 3.4. Transport binding energies of Na⁺ and H₂Oᵦf with cyclic octa-peptide nanotube

In order to insight the interactions of Na⁺ and H₂Oᵦf with CPN, we compared the binding energy of Na⁺ or H₂Oᵦf with cyclic peptide lumen when they were pulled through the nanotube along z-axis. At constant rate, the time for particles to pass through each ring was equal, so we could easily get the relationship between binding energy and position. It took about 4560 ps for Na⁺ and H₂Oᵦf to escape from the end of nanotubes (45.6 Å), and the simulation time was set to 6000 ps. The total energy, electrostatic energy and vDW energy of Na⁺ and H₂Oᵦf with CPN were calculated and shown in figure 7(a).

From the analysis results in figure 7(a), it can be seen that the curves of total energy and electrostatic energy of Na⁺ or H₂Oᵦf are almost the same, indicating that the interaction between nanotube and Na⁺ or H₂Oᵦf mainly comes from electrostatic interaction, and the energy in the ring plane is lower than that between rings, while the energy at the end of nanotubes is particularly low, which is also corresponding to Hwang’s [22]. In addition, the electrostatic energy of Na⁺ (−20 to −40 kcal mol⁻¹) is much stronger than that of H₂Oᵦf (0 to −5 kcal mol⁻¹), which indicates that nanotubes are negatively charged and have stronger electrostatic attraction with positively charged Na⁺. Especially at the end of the nanotube, the electrostatic binding energy was much stronger and formed end barriers because the free negative charged C=O groups gave more powerful electrostatic attractions to Na⁺ and trapped it at the end of nanotube. However, H₂Oᵦf interacted with nanotube only with a weak instantaneous dipole charge, resulting in weak electrostatic binding, so the transport resistance was very small, which is why H₂Oᵦf need less pulling force than Na⁺.

It can be seen from figure 7(b) that the electrostatic binding energies coming from SMD with constant force has little effect on the energies either for Na⁺ or H₂Oᵦf. So it is easy to explain why Na⁺ exhibits hopping transport mode. When the driving force was less than the resistance provided by the binding energies, Na⁺ could jump to the next plane only when enough energy was accumulated.

### 4. Discussion

The most significant application of CPNs is their potentiality to deliver drugs or ions across cell membranes to treat human diseases. This application is becoming possible, because more and more research results of internal
and external structural modification have been achieved. The structural modification of outer surface allows CPNs to bind to various specific membrane receptors of specific cells, and the structural modification of inner surface extends the types of drugs they can transport [27–31], thus laying the foundation for the application of targeted drug delivery systems [31, 32]. For example, Brendel et al reported a barrel-shaped peptide nanotube (length: 260 nm) self-assembled by polymer/cyclic peptide conjugate with a hydrophobic core (diameter: 16 nm) and a hydrophilic shell, which was similar to those of viruses, and was able to perforate the lysosomal

![Figure 4. Instantaneous velocities of Na⁺ along z-axis in cyclic octa-peptide nanotube. Na⁺ adopts an obvious hopping transport mode and the curves of instantaneous velocities are similar to pulse signals.](image-url)
Figure 5. Instantaneous velocities of $\text{H}_2\text{O}_{\text{pf}}$ along z-axis in cyclic octa-peptide nanotube. The curves seem like electroencephalogram and the peaks are mostly positive and distribute irregularly. In red circle, $\text{H}_2\text{O}_{\text{pf}}$ moved from the fifth ring to the seventh ring without staying on the sixth ring plane under the pulling force of $1.6 \text{ kcal mol}^{-1} \text{ Å}^{-1}$. In green circle, $\text{H}_2\text{O}_{\text{pf}}$ moved from the sixth ring to the eighth ring without staying on the seventh ring under the pulling force of $1.0 \text{ kcal mol}^{-1} \text{ Å}^{-1}$.

Figure 6. Instantaneous velocities of $\text{Na}^+$ and $\text{H}_2\text{O}_{\text{pf}}$ along z-axis in cyclic octa-peptide nanotube. That of $\text{Na}^+$ is under pulling force of $1.6/3.2/5.6 \text{ kcal mol}^{-1} \text{ Å}^{-1}$ and that of $\text{H}_2\text{O}_{\text{pf}}$ is under the pulling force of $2.3 \text{ kcal mol}^{-1} \text{ Å}^{-1}$.
membrane in cells and release small molecules with similar sizes to conventional drugs into the cytosol to highlight their potential as drug delivery materials [33].

The purpose of this study is to reveal the relationship between the transport mode and speed of Na$^+$ and H$_2$O in CPNs and the driving force, so as to provide a reference for the application of CPNs under appropriate conditions. In this work, we found that H$_2$O need minor driving force to transport through cyclic octa-peptide nanotube than Na$^+$. And under the same driving force of 2.3 kcal mol$^{-1}$ Å$^{-1}$, the transport speed of H$_2$O was about 135 times faster than that of Na$^+$, which would be helpful for some applications requiring elimination of excessive H$_2$O in body. For instance, the edema is common in many human diseases. Any insult to the brain are typically accompanied with cytotoxic (intracellular) edema, including trauma, infarction, neoplasm, abscess, or conditions such as hypoxia or toxic or metabolic perturbation [34, 35]. If CPNs are used as adjuvant drugs to treat these diseases, there will be synergistic effects through drainage of excess water. For example, Liu et al proved that 5-fluorouracil combined with CPNs had synergistic antitumor effect through animal experiments [36].

Another noteworthy issue in this work is the transport mode of Na$^+$ changing with the driving force. When Na$^+$ transported in CPNs, it stayed at every ring plane for a long time, and then jumped to another ring plane quickly. In each ring plane, the instantaneous velocity fluctuated near the zero point for a long time, and reached the peak value between rings with a short duration. This phenomenon showed that Na$^+$ transporting in CPNs could transmit pulse signals, especially when the driving force was 3.2–5.6 kcal mol$^{-1}$ Å$^{-1}$. These pulse signals are very useful in treatments of human diseases because many therapies are based on the bioelectricity of human bodies [37, 38]. The low frequency pulse current has various significant uses in the physiology and the therapeutics, especially in stimulating the nervous tissue and alleviating pains [39]. In addition, the pulse signals are also useful in detecting the concentration of electrolyte in human bodies [37, 40–42].

### 5. Conclusions

Using steered molecular dynamics (SMD), we investigated the transport trajectories, critical driving forces, velocities, modes and binding energies of Na$^+$ or H$_2$O with the cyclic octa-peptide nanotube composed of cyclo[(D-Ala-L-Ala)$_4$]. From the above results and discussions, we conclude that free negatively charged C = O groups at the end of nanotubes make the end of nanotubes have potential barriers. The driving force of Na$^+$ or H$_2$O to pass through nanotube must exceed the critical driving force of 0.6 kcal mol$^{-1}$ Å$^{-1}$ for H$_2$O and 2.4 kcal mol$^{-1}$ Å$^{-1}$ for Na$^+$. Under the driving force of 2.3 kcal mol$^{-1}$ Å$^{-1}$, the transport speed of H$_2$O is about

![Figure 7. Binding energies of Na$^+$ and H$_2$O with cyclic octa-peptide nanotube lumen. (a) Binding energies come from SMD with constant rate, showing total energies are almost contributed by electrostatic energies. (b) The electrostatic binding energies come from SMD with constant force, showing driving force has little effect on the binding energy of either Na$^+$ or H$_2$O.](image-url)
135 times faster than that of Na\(^+\), which would be useful in applications of eliminating H\(_2\)O molecules. H\(_2\)O adopts a kind of irregular hopping transport mode, which can not be influenced by driving force. Na\(^+\) adopts an obvious hopping transport mode, and it has three types with the change of pulling force. It’s worth mentioning that under the driving force of 3.2~5.2 kcal mol\(^{-1}\) Å\(^{-1}\), the instantaneous velocity curves are similar to the pulse signal, having potential applications in treatments of human diseases or detecting the concentration of electrolyte in human body. The transport resistance mainly comes from electrostatic interaction, which can not be influenced by driving force. We hope that this work can provide predictable evidence for the applications of CPNs in new drug design, biosensor or human disease treatment in the foreseeable future.

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Conflicts of interest

The authors declare no conflict of interest.

ORCID iDs

Tianjiao Shan https://orcid.org/0000-0001-7804-989X

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