

Release of an Encapsulated Peptide from Carbon Nanotubes Driven by Electric Fields: A Molecular Dynamics Study

Qu Chen,^{*,†} Lijun Liang,[†] Zhisen Zhang, and Qi Wang^{*}Cite This: *ACS Omega* 2021, 6, 27485–27490

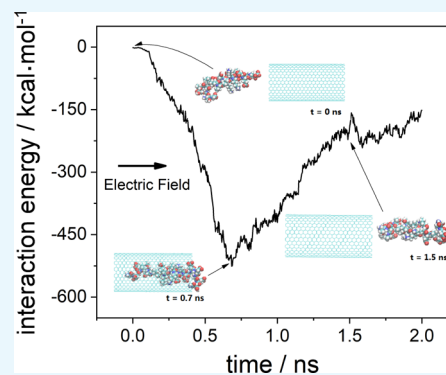
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ABSTRACT: Targeted drug delivery into cells has been of tremendous scientific interest, and carbon nanotubes (CNTs) can be deemed as a promising material for the loading and unloading of drugs. One of the major challenges is the release of drugs from CNTs, which have a great potential well to trap molecules. By performing molecular dynamics simulations, this work attempts to study the releasing process of encapsulated protein/peptide molecules from CNTs in the presence of uniform electric fields. Zadaxin serves as a model for protein/peptide drugs. External electric fields can assist the peptide in overcoming the potential well during its release. It is found that successful release of the peptide depends on the pore width, the pore length, and the net charges on the peptide. The peptide is less likely to be released either from CNTs with a smaller pore diameter due to a deeper potential well of the tubes or from CNTs with a longer pore length due to a broader and deeper potential well. Peptides with more net charges are ideal for the releasing process driven by electric fields. This work can provide insights into the design of an optimal tube size for effective release of a given protein/peptide.



1. INTRODUCTION

The open-ended single-walled carbon nanotube (CNT), due to its ultrahigh surface area, can find its use in medical diagnosis and drug delivery.¹ The potential application of this carbon-based material to biotechnology is principally twofold. The first involves the efficient separation of biomolecules (e.g., peptide/protein). Successful selection mainly depends on the size^{2–4} and properties^{5–7} of the nanostructure. CNTs can also be functionalized covalently and noncovalently, acting as “goalkeepers” to select targeted molecules.^{8,9} Numerous experimental research studies have shown that nanoporous materials have been extensively utilized in protein purification,^{10,11} biosensor design,^{12,13} and membrane chromatography.¹⁴ However, high throughput is one of the key factors in increasing the sensitivity and efficiency of the separation process.¹⁵ In the case of peptide/protein separation, it is reported that the diffusion flux of protein in nanotubes is 5 orders of magnitude smaller than that in bulk solutions.⁵ Strong interaction between the protein and nanotube surface could be the reason of this slow transport. In order to increase the transport rate, external pressure or electric fields can be applied. However, external pressure may cause the nanotube membranes to fold, making this method infeasible. On the other hand, an external electric field is more likely to accelerate the protein transport without altering the configuration of the tubes because proteins are naturally equipped with charges whereas CNTs are neutral. It is noted that an electric field is also of tremendous use in separating proteins with an opposite

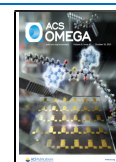
charge. Therefore, it is very important to understand the interaction between protein and CNTs during the transport process driven by an electric field.

The second application of the carbon-based material is associated with the targeted drug delivery. Many experimental research studies have demonstrated that CNT membranes have exhibited great chemical, mechanical, and thermodynamic stability in the transport process of molecules ranging from fluids to biomolecules.^{16–18} Wu et al. effectively employed electro-osmosis and electrophoresis to transfer nicotine through functionalized CNT membranes in the programmable transdermal drug delivery.¹⁹ Liu et al. used direct electrical measurement of ion transport to detect the electrophoretic transport of single-stranded DNA through one CNT devised to connect two fluid reservoirs.²⁰ Yu et al. applied transmembrane electric potentials to explore the effects of pore diameter and solution pH on the rate and selectivity of protein transport through the gold nanotube membranes.²¹ Consequently, understanding the transport process of biomolecule through nanochannels requires the knowledge of interaction energy

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profile between protein and CNTs. An appropriate pore width and length are also crucial in efficiently delivering protein through the tubes. However, it is still a challenge for experiments to analyze the details of delivery process at a molecular level, such as intermolecular forces and molecule behaviors. Computer simulation is a powerful tool to aid experimentalists and engineers in studying the mechanisms of various chemical and biological phenomena, especially in mesoscale systems, which exhibit remarkably different properties from bulk systems.^{22,23}

In our previous studies, using molecular dynamics (MD) simulations, we reported that Zadaxin, a peptide drug, can spontaneously enter a CNT and oscillate around the center of the tube.²⁴ In other words, CNTs can trap peptide/protein due to the van der Waals (vdW) interaction between them. This work has been further confirmed by many related studies.^{25–27} In light of these studies, the release of encapsulated peptide from CNTs seems substantial and has spurred much discussion in the references. Dai et al. used quantum mechanical molecular simulation to theoretically predict that neutral CNTs housed in an outer CNT can be ejected by positively charging the outer tube uniformly.²⁸ Sun et al. have successfully pumped charged lysozymes across electrochemically oxidized multi-walled CNT membrane by an electric field while larger bovine serum albumin is rejected.²⁹ Even though there are other methods, such as the displacement method,³⁰ heating method,³¹ domino effect,³² and water boiling,³³ to release the encapsulated molecules, applying an electric field is a dominant technique to release the encapsulated peptide/protein.

In this work, we employed MD simulations to systematically study the interaction between CNTs and peptide drugs during the whole transport process in an electric field. We mainly emphasized on exploring the impact of tube length, tube diameter, and charge on a peptide on the releasing process. This paper is organized as follows. Section 2 presents and discusses the simulation results on the trajectories of the peptide and the interaction energy between the peptide and CNTs, and Section 3 summarizes our findings. The simulation details are outlined in Section 4.

2. RESULTS AND DISCUSSION

2.1. Impact of Tube Width. In the first set of simulations, an external electric field with a strength of 200 mV/nm was applied in the axial direction, driving the peptide Zadaxin into CNTs. The armchair CNTs used in this set of simulations were (14, 14), (16, 16), (18, 18), and (20, 20) with the same length of 4.90 nm. Each simulation was performed for 2 ns. Figure 1 shows the normalized center-of-mass (CoM) separation between the peptide and CNTs (d/d_0) with respect to simulation time, where d is the CoM separation and d_0 is the initial CoM separation. When d/d_0 equals 0, the CoM of Zadaxin is exactly located at the center of the CNT. We also measured the electric current with respect to time, where the magnitude of electric current equals the quantity of charges moving past a cross section per second. Once the peptide was inside the CNTs, it would block the flow of charges through the tubes, hence causing the electric current to be zero. However, once the peptide was released from the CNTs, the charges could freely move through the tubes, and an electric signal could therefore be detected.

As is shown in Figure 1, the peptide Zadaxin failed to move through the smaller (14,14) and (16,16) CNTs in the presence

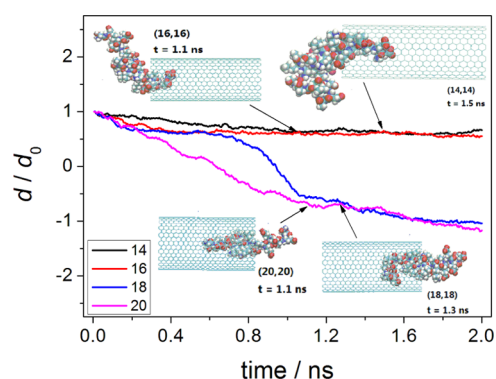


Figure 1. Normalized CoM distance, d/d_0 , between the peptide and indicated CNTs as a function of simulation time, where d_0 is the initial CoM. In the inset snapshots, water molecules are not shown for clarity.

of the electric field and was stuck at one end of the tubes even though a small segment of the peptide entered the tubes (the ratio d/d_0 decreased slightly). This can be clearly confirmed by the inset snapshots in Figure 1 and nearly zero electric current in Figure 2. On the contrary, driven by the electric field, the

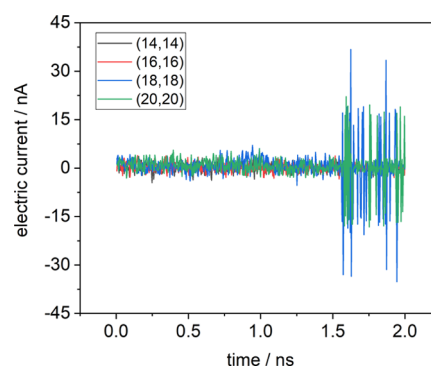


Figure 2. Electric current with respect to simulation time in the indicated CNTs.

peptide Zadaxin was able to move through the larger (18, 18) and (20, 20) CNTs, as can be also illustrated by the inset snapshots in Figure 1 and the electric current in Figure 2. For (18, 18) and (20, 20) CNTs, the electric current suddenly became strong almost after 1.6 ns, when the peptide was completely released from the tubes.

In an early work, Liu and Wang reported that the diameter of (14, 14) CNT is the critical size for the peptide Zadaxin to spontaneously enter and be completely encapsulated.³⁴ However, the spontaneous insertion is generally an equilibrium process which takes a long time [e.g., almost 8 ns for Zadaxin entering a (20, 20) CNT in our previous work²⁴ and where the peptide makes stepwise conformational changes to maximize its affinity to the CNT walls.²⁶ In our study, the insertion of the peptide in the presence of an electric field was a much faster non-equilibrium process. There was not enough time for the peptide to adjust its conformation to fit the size of the tubes. As a result, the peptide was likely to be stuck at the entrance of a smaller tube. It is suggested that we should select CNTs with much larger diameters to allow for the insertion of a peptide in the presence of an electric field.

To further explore the interactions between the peptide and the CNTs from insertion and release, we computed the vdW

potential energy between Zadaxin and (18, 18)/(20, 20) CNTs as a function of simulation time as shown in Figure 3.

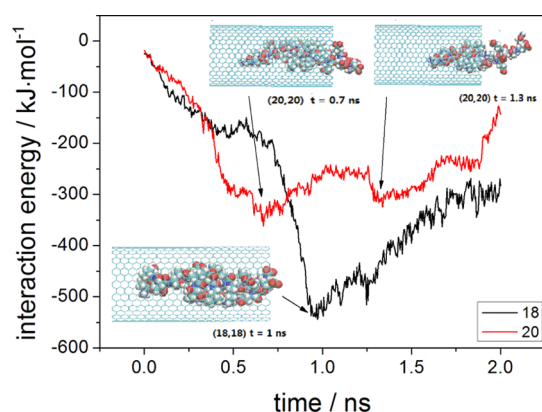


Figure 3. vdW interaction energy between the peptide and the (18, 18)/(20, 20) CNTs as a function of simulation time. Water molecules are not shown for clarity in the inset snapshots.

The inset snapshots were taken at the indicated time. It should be noted that the CNTs are neutral, so there is no electrostatic interaction between the peptide and the CNTs. After the insertion of the peptide, the electric field further drove the peptide to overcome a potential well until its release from the tube. For the (18, 18) CNT, the peptide experienced a potential well of -550 to -450 kJ/mol during the period of 1.0–1.2 ns; for the (20, 20) CNT, the peptide experienced a potential well of -350 to -300 kJ/mol during the period of 0.7–1.3 ns. It was obvious that the peptide experienced a deeper potential well in the (18, 18) CNT than in (20, 20), indicating that the vdW interactions between the peptide and the (18, 18) CNT were greater than those between the peptide and the (20, 20) CNT. It is worth mentioning that, in this non-equilibrium process, it is not very accurate to quantify the interaction energy, but the qualitative comparison is almost valid. Simulation results show that the smaller the tube diameter is, the more difficult it will be for the encapsulated peptide to be released from the CNTs.

It is worth noting that in many literature, CNTs were embedded in a lipid bilayer to help translocate biomolecules through the membrane in both experiments^{35,36} and simulations.^{18,27,37} In the release process, the major difference between this work and the abovementioned studies consisting of a biological membrane is the influence of the lipid membrane, which could play an essential role. For example, while Sahoo et al. did not observe any of the bioactive molecules (e.g., dendrimers, asiRNA, ssDNA, and ubiquitin protein) being ejected from the tube within 100 ns²⁷ Chen et al. found that the drug molecule Pregabalin could be spontaneously released from the tubes due to the influence of the DPPC lipid.¹⁸ However, in our system, the external electric field acts as a dominating factor in the releasing process, regardless of the absence or presence of cell membranes. The electric field strength is almost 1 order greater than the one on the cell membrane, so even in a more sophisticated system with a lipid membrane, a similar phenomenon could also be observed.³⁷

2.2. Impact of Tube Length. To understand the impact of tube length on the release of the peptide, we doubled the length of the CNTs studied in the previous section. Therefore, a (20, 20) CNT with a length of 9.8 nm was used in this set of

simulations. In the presence of electric fields with the same strength (200 mV/nm), the peptide Zadaxin was driven into and released from the longer tube, a simulation process that was performed for 4 ns. The normalized CoM separation between the peptide and the CNT as a function of simulation time is shown in Figure 4, and the interaction energy between

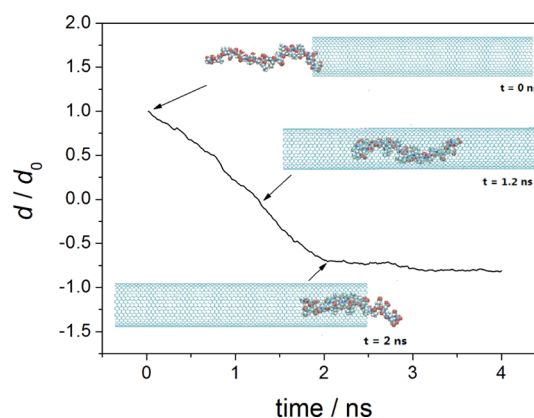


Figure 4. Normalized CoM distance, d/d_0 , between the peptide and the (20, 20) CNT with a length of 9.8 nm as a function of simulation time, where d_0 is the initial CoM. Water molecules are not shown for clarity in the inset snapshots.

the peptide and the CNT with respect to time is shown in Figure 5. At the indicated time, the inset snapshots were taken,

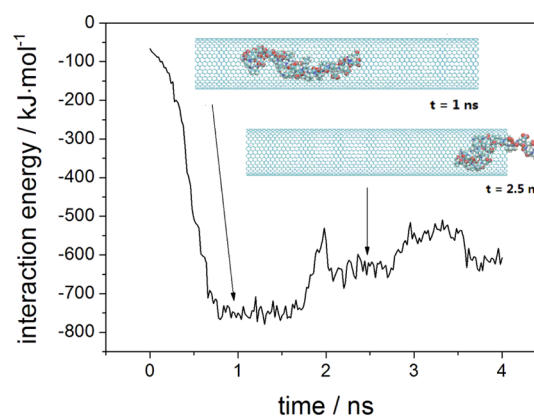


Figure 5. vdW interaction energy between the peptide and the (20, 20) CNT with a length of 9.8 nm as a function of simulation time. Water molecules are not shown for clarity in the inset snapshots.

where water molecules are not shown for clarity. Between 1 and 1.5 ns, the peptide was completely inserted into the nanotubes. At 2 ns, the peptide moved near the end of the tube; however, from 2 to 4 ns, the peptide was unable to be released by the external electric field. We conducted three similar simulations to confirm that the result was reproducible and that our simulation time is sufficiently long.

From the interaction energy perspective, it is clearly shown in Figure 5 that Zadaxin experienced a much broader and deeper potential well in the longer (20, 20) CNT after insertion. The interaction energy between the peptide and the CNT in the potential well approximated -750 kJ/mol during the period of 0.8–1.6 ns. Therefore, the interaction in the longer (20, 20) CNT was much greater than that in the shorter (20, 20) CNT. Driven by the electric field, the peptide reached

the other end and part of it moved outside the tube, when the interaction energy increased slightly to nearly -650 kJ/mol. However, it was difficult to release the whole peptide from the tube because the strength of the electric field was not great enough to overcome the potential well which could be ascribed to the vdW interaction. Simulation results suggest that the longer a CNT is, the less likely the encapsulated peptide is to be released from the tube by an external electric field.

2.3. Impact of Charge on the Peptide. Because different pH conditions in the solution influence the quantity of net charges on a peptide, it is of great necessity to understand the impact of net charges of peptides on the releasing process. Normally, Zadaxin has five negative charges under the human physiological conditions, where the pH ranges between 7.35 and 7.45. However, the quantity of negative charges on the peptide can be altered from zero to six by changing between COO^-/COOH or between $\text{NH}_2/\text{NH}_3^+$. In this work, we chose Zadaxin with two, three, and four negative charges, which can be typically achieved in the environments with pH values of roughly 5.2, 6.0, and 6.7, respectively. The third set of simulations were performed in the presence of electric fields of 200 mV/nm, when Zadaxin with different net charges was driven into the (18, 18) CNTs with a length of 4.9 nm. The normalized CoM separation between Zadaxin and the CNTs with respect to simulation time is plotted in Figure 6, and the

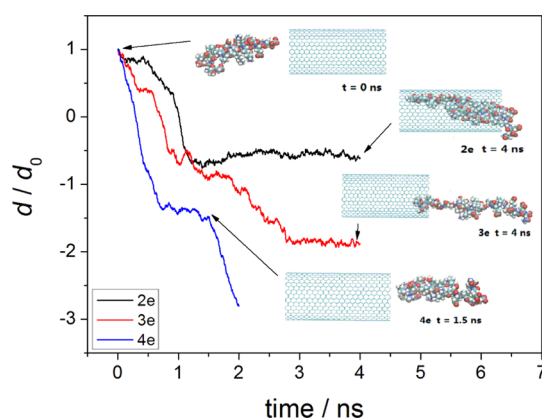


Figure 6. Normalized CoM distance, d/d_0 , between the peptide with different net charges and the (18, 18) CNT with a length of 4.9 nm as a function of simulation time, where d_0 is the initial CoM. Water molecules are not shown for clarity in the inset snapshots.

vdW interaction energy between the peptide and the CNTs as a function to time is plotted in Figure 7. Inset snapshots were taken to illustrate the configurations of the system at a particular time.

It is apparent that the peptide with fewer net charges is not favorable for the releasing process in the presence of an electric field. On the contrary, the more net charges a peptide has, the greater external force will be applied to the peptide once the field strength is constant. For the peptide with two negative net charges, Figure 6 shows that the value d/d_0 became almost invariable after 1.3 ns, indicating that it was impossible for the peptide to be released from the CNT. The interaction energy, on the other hand, kept dropping as shown in Figure 7, which meant that the vdW interaction between the peptide and the CNT grew stronger. To explain the energy changes, more snapshots were taken at $t = 1.0, 2.0, 3.0,$ and 3.8 ns. In Figure 8, the encapsulated Zadaxin experienced continuous conforma-

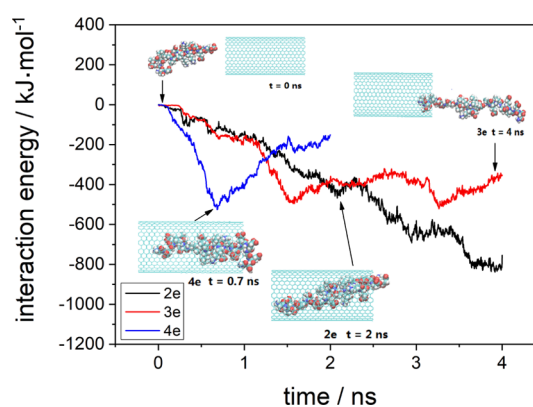


Figure 7. vdW interaction energy between the peptide with different net charges and the (18, 18) CNT with a length of 4.9 nm as a function of simulation time. Water molecules are not shown for clarity in the inset snapshots.

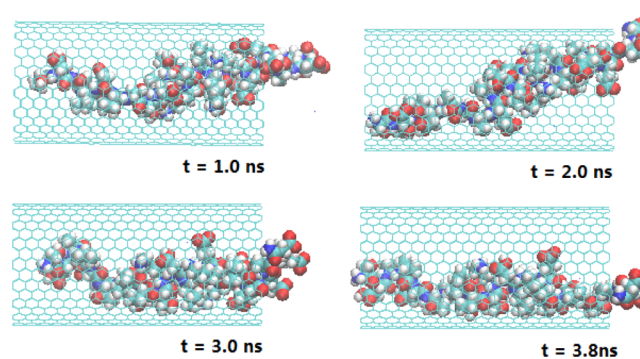


Figure 8. Snapshots of Zadaxin with two negative net charges encapsulated in the (18, 18) CNT with a length of 4.9 nm. Water molecules are not shown for clarity in the inset snapshots.

tional changes under the influence of the electric field, when the peptide came closer to the tube walls leading to greater interaction between them. For the peptide with three negative net charges, the peptide was almost released from the CNT at around 1.6 ns when a small fragment of the peptide was still inside the nanotube. The external electric field failed to assist the peptide in overcoming the potential well and hence kept stretching the peptide structure. This is the reason why the value d/d_0 continuously decreased until it equaled about 1.8 at 2.7 ns, as shown in Figure 6. For the peptide with four negative net charges, a successful release was observed. At 1.5 ns, the peptide Zadaxin was entirely released from the nanotube. Therefore, Zadaxin with four negative net charges is the basic requirement for a successful release under the influence of an external electric field with a field strength of 200 mV/nm.

It should be noted that CNTs and aqueous solution both contribute to the releasing process of peptides, but the vdW interaction between peptides and CNTs plays a major role.²⁴ Also, it might be a concern that the peptide, after its release, will be under conformational changes in the presence of external electric fields and hence could affect the function of the peptide. This problem could be addressed by the equilibration of the peptide in bulk aqueous solution without electric fields.

3. CONCLUSIONS

This work aims to understand the factors influencing the releasing process of the peptide Zadaxin from CNTs driven by

electric fields using MD simulations. The pore width, pore length, and net charges on the peptide play a determinant role in an effective release of the peptide. In the presence of external electric fields, the encapsulated peptide can overcome the interaction energy well. CNTs with a smaller tube diameter have a deeper potential well to trap the peptide, and CNTs with a greater tube length have a deeper and broader potential well to trap the peptide. Therefore, a successful release could be possible with a larger and shorter tube. Also, it is more likely for a peptide with more net charges to be released from CNTs in the presence of electric fields. A typical example is Zadaxin with four net negative charges being the basic requirement for an effective release from (18, 18) CNTs. For the design of a drug delivery device, it is important to maintain a balance between its trapping effect and requirements for drug release.

4. MODELS AND METHODS

In this work, (14, 14), (16, 16), (18, 18), and (20, 20) open-ended single-walled armchair CNTs were selected with diameters of 1.90, 2.18, 2.45, and 2.72 nm, respectively. For (20, 20) CNTs, three lengths of 4.90, 7.35, and 9.80 nm were selected. Zadaxin, an acetylated polypeptide, is considered as a model for protein/peptide drugs. It has a molecular weight of 3108 Da and consists of 28 residues of amino acids with the following sequence: Ac-Ser-Asp-Ala-Ala-Val-Asp-Thr-Ser-Ser-Glu-Ile-Thr-Thr-Lys-Asp-Leu-Lys-Glu-Lys-Lys-Glu-Val-Val-Glu-Glu-Ala-Glu-Asn-OH. The structure of this peptide was constructed with HyperChem³⁸ and was relaxed in vacuum for 0.6 ns using the MD technique, followed by further relaxation with MD for 1 ns in a 1.0 g/mL dense water solution until it reached an equilibration state. For the initial configuration, the peptide was aligned with CNTs and placed along the axial direction of the tubes. The peptide and CNTs were separated by a certain distance of 0.48 nm. The complex was then solvated in a cubic water box, the size of which was designed to avoid the interaction among the repeated boxes with periodic boundary conditions. Because Zadaxin is naturally equipped with negative charges, equivalent counter sodium ions were added to neutralize the system.

All the MD simulations were performed in Gromacs 4.5.2.^{39,40} The Charmm27 all-atom force field⁴¹ was used for the peptide, the simple point charge⁴² model for water molecules, and the parameters taken from graphite⁴³ for the CNTs. The Lennard–Jones parameters for the cross-interactions were obtained from the Lorentz–Berthelot combining rules.⁴⁴ The vdW interactions were truncated at 12 Å. The particle mesh Ewald summation⁴⁵ was employed to calculate the full-system periodic electrostatic interactions using a cutoff of 12 Å for the separation of the direct and reciprocal space summation. During the simulations in the isothermal–isobaric (NpT) ensemble, the temperature and pressure were set to 310 K and 101.3 kPa controlled by the Nosé–Hoover thermostat and the Parrinello–Rahman barostat, respectively. The external electric field with a strength of 200 mV/nm was acted on both sides of the simulation box along the axial direction. The electric field strength should be neither too small to release the peptide from CNTs nor too large to acquire many simulation details.³⁷ Periodic boundary conditions were applied in three dimensions for the entire system. A time step of 2 fs was used and atom coordinates were saved every 1 ps during the simulation. MD was performed on the system for 2 ps to relax the system, followed by a production run of 10–20 ns. The trajectories were visualized

using VMD software.⁴⁶ In subsequent data analysis, the vdW interactions between the peptide and the CNT were computed using the following equation:

$$E_{\text{int}}(t) = E_{\text{protein-CNT}}(t) - E_{\text{protein}}(t) - E_{\text{CNT}}(t) \quad (1)$$

where $E_{\text{int}}(t)$ stands for the time-dependent vdW interaction energy; $E_{\text{protein-CNT}}(t)$, $E_{\text{protein}}(t)$, and $E_{\text{CNT}}(t)$ represent the time-dependent potential energy of the Zadaxin–CNT complex, Zadaxin, and the CNT, respectively.

AUTHOR INFORMATION

Corresponding Authors

Qu Chen – School of Biological and Chemical Engineering, Zhejiang University of Science and Technology, Hangzhou 310023, People's Republic of China; orcid.org/0000-0002-4390-0303; Email: chenqu@vip.126.com; Fax: +86-571-85070390

Qi Wang – Department of Chemistry, Zhejiang University, Hangzhou 310027, People's Republic of China; orcid.org/0000-0002-2246-1401; Email: qiwang@zju.edu.cn; Fax: +86-571-87951895

Authors

Lijun Liang – College of Life Information Science and Instrument Engineering, Hangzhou Dianzi University, Hangzhou 310023, People's Republic of China; orcid.org/0000-0003-3753-1107

Zhisen Zhang – Research Institute for Soft Matter and Biomimetics, Department of Physics, Xiamen University, Xiamen 361005, People's Republic of China; orcid.org/0000-0002-8638-0993

Complete contact information is available at: <https://pubs.acs.org/10.1021/acsomega.1c04436>

Author Contributions

[†]Q.C. and L.L. contributed equally to this work.

Notes

The authors declare no competing financial interest.

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