Steered molecular dynamics simulation study on dynamic self-assembly of single-stranded DNA with double-walled carbon nanotube and graphene

Chang-Li Cheng* and Guang-Jiu Zhao*

Received 29th December 2011, Accepted 13th February 2012
DOI: 10.1039/c2nr12112c

In the present work, we explored the diameter selectivity of dynamic self-assembly for the single-strand DNA (ssDNA) encapsulation in double-walled nanotubes (DWNTs) via molecular dynamics simulation method. Moreover, the pulling out process was carried out by steered molecular dynamics simulations. Considering π–π stacking and solvent accessibility together, base–CNT binding should be strongest on a graphene sheet and weakest on the inner CNT surface. When pulling the ssDNA out of the single-walled carbon nanotube (SWNT), the force exhibits characteristic fluctuations around a plateau about 300 pN. Each fluctuation force pulse to pull ssDNA corresponds to the exit of one base. In addition, the solvents used for the system are also of significant interest. Water does play an important role in encapsulation process but doesn’t in the pulling out process.

Introduction

During the past few years, the merging of biotechnology and materials science has been paid extraordinary attention. In particular, the carbon nanotube (CNT) with nucleic acids has been extensively studied due to applications in bioengineering and clinical medicine. 1–4 CNT were used to deliver plasmid DNA into cells, 5–7 functionalised carbon nanotubes (f-CNT) are soluble in water and can penetrate into the cells without toxic effects, ammonium-functionalised CNTs were able to form supramolecular complexes with nucleic acids via electrostatic interactions and enhance the efficiency of DNA transfer to cells, whilst single-stranded DNA (ssDNA)-functionalized single-walled CNT (SWNT) can recognize DNA by hybridizing with complementary strands. 8 Moreover, in order to measure the structure and behavior of these single molecules, Weiss and coworkers have presented an excellent overview of the strikingly diverse classes of measurements that can be used to quantify single-molecule properties, including those of single macromolecules and single molecular self-assemblies, and discuss the quantitative insights they provide. 9

In addition to the sidewall or tip functionalization, CNTs can be encapsulated by DNA. Zheng et al. reported DNA-assisted dispersion and separation of CNTs and suggested ssDNA could bind to outer surfaces of CNTs through π–π stacking, resulting in helical wrapping to the surface. 10–14 Johnson and co-workers demonstrated that short sequences (such as those with less than 20 bases) behave differently from much longer 40–60mer oligonucleotides, which have a strong preference for helically wrapped DNA–CNT. Short DNA segments are unlikely to wrap a CNT due to the strong deformation energy required for the DNA to bend. 15

Furthermore, the hollow interior of CNTs can also be stuffed with DNA. Molecular dynamics simulations of a CNT interacting with a DNA oligonucleotide demonstrated that a DNA oligonucleotide could be spontaneously encapsulated inside a CNT in water owing to van der Waals (vdW) attractions between the nanotube and DNA. 16 CNT encapsulation by DNA reduces DNA conformational entropy, S, and then increases the free energy of the whole system. However, the combining effect of vdW attractions between the oligonucleotide and the CNT, combined with hydrophobic force arising from water–water, DNA–water, and CNT–water interactions, may produce a larger decrease in the total potential energy, U, and a net reduction in F. The complementary process of encapsulation, i.e. pulling a piece of ssDNA out of a single-walled CNT, has also been researched. Very recently, Lulevich et al. used an atomic force microscope to pull a single-stranded DNA oligomer from a carbon nanotube pore. 17 DNA extraction from CNT pores occurs at a nearly constant force, which means that there is no frictional force between the ssDNA and the CNT pore walls. Li et al. compared the extraction of ssDNA from the nanotube to pulling a string of carts on the smooth ground with a hillside. 18 Each cart climbing the hillside corresponds to a force fluctuation around the plateau. Similarly, each fluctuating pulse of force to pull ssDNA corresponds to the exit of one base.

CNT arrays can also serve as surfaces for DNA segments self-assembly. Zhao found that short DNA segments can concatenate to each other to form a longer DNA strand when they are placed in the grooves of nanotube bundles. 19 DNA–CNT interaction
and the resulting hybrid structure are dependent on both DNA sequence and CNT structure. MD simulations have been performed for DNA interaction with SWNTs of different diameters to explore the effect of nanotube size. The reduction $\Delta U$ of the total potential energy upon DNA encapsulation was found to increase with CNT diameter, which implied that the encapsulation of oligonucleotide should be easier for larger SWNTs. As CNT curvature increases, base–CNT contacts are lost and the $\pi$–$\pi$ stacking interaction is weakened. Thus, base–CNT binding should be greatest on a graphene sheet. Molecular dynamics (MD) and ab initio studies predict the base–CNT binding free energy values of about $-10$ kcal mol$^{-1}$ that follow the trend $\Delta F_{\text{bind}}^g < \Delta F_{\text{bind}}^a < \Delta F_{\text{bind}}^m < \Delta F_{\text{bind}}^c$. G has the lowest (most negative) $\Delta F_{\text{bind}}$ and thus has the highest affinity for CNT. This trend is understandable according to the geometries. The purines, G and A, which contain two aromatic rings, have a stronger interaction with CNT than the pyrimidines, C and T, which contain only a single ring.

The study of DNA–CNT encapsulation has revealed the possibility of using DNA as a template/carrier to deliver various metallic, ceramic, or semiconductor nanoparticles that normally cannot be encapsulated into CNT. It is possible to sequence DNA by passing the molecule through CNT or a small hole in a sheet of graphene (GRA). Theoretical description of the interface and the interactions between CNT/GRA and DNA provides the foundation for analysis and simulation of bio-nano-systems and assembled nanoscale devices in service. More researches are focused on ssDNA and SWCN interactions. As far as we know, there is currently only one work about ssDNA and triple-walled CNT interactions. Furthermore, they only simulate encapsulation of oligonucleotide into inter-layer CNT. Single-stranded DNA between two concentric SWNTs has not been researched. In the present work, the encapsulation and pulling-out process of a piece of ssDNA from double-walled CNT/GRA was simulated by molecular dynamics method. In addition, CNT size effect was investigated. The particularity of double-walled carbon nanotubes (DWNT) must be emphasized here. Their resistance to chemicals is significantly improved than double-walled CNT. In the case of SWNT, covalent functionalization will break some C=C double bonds, leaving “holes” in the structure of nanotube and thus modifying its mechanical and electrical properties. Moreover, only outer wall is modified for the DWNT.

### Methods

For simplicity, an ssDNA oligonucleotide with four bases (ATCG) was used to model DNA fragments. The designed DNA-CNT/GRA system consists of model ssDNA and a two-walled CNT or two parallel graphene sheets. Details of various systems simulated in this study are shown in Table 1. CNT length is 3 nm and graphene layer is single. In the initial configuration, ssDNA was placed at the mouth of CNT/GRA. Classical MD package NAMD was used to simulate dynamics of designed CNT-oligonucleotide. Depending upon the nanotube diameter, the molecular complexes were solvated in a periodic box with TIP3P water as solvent. Sodium ions were added as counter-ions to compensate for negative net charges on oligonucleotide. The CHARMM 27 force field was applied to model atomic interactions in ssDNA oligonucleotides. The carbon atoms of nanotubes were treated as uncharged Lennard-Jones particles. The positions of all CNT atoms were constrained with a harmonic potential during simulations. In this work, all of the simulations were performed in the isothermal isobaric (NpT) ensemble unless otherwise stated. A temperature of 400 K was maintained using Langevin dynamics, and the Langevin piston Nose–Hoover method was employed to maintain a pressure at 101.3 kPa. A time step of 2 fs was used with atom coordinates saved every 2 ps. The particle mesh Ewald (PME) summation was used to calculate the full-system periodic electrostatic interactions. The cutoff distance for van der Waals interaction was 1.2 nm. All visualizations were made using VMD.

### Results and discussion

Fig. 1 shows simulation snapshots of the investigated ssDNA–CNT systems. As shown in mwcn1, two of the four DNA bases remain outside the nanotube after 14 ns. The diameter of inner

<table>
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<th>System name</th>
<th>Inner CNT</th>
<th>Outer CNT</th>
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<td>DNA-gra</td>
<td>$x = 3$ nm, $y = 2$ nm</td>
<td></td>
</tr>
<tr>
<td>DNA-2gra</td>
<td>$x = 3$ nm, $y = 2$ nm, $d = 1.5$ nm</td>
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and outer layers are 0.81 nm and 2.17 nm, respectively. The space left for DNA is 0.68 nm in the radial direction. In SWCN simulation by Gao, the diameter (1.08 nm) of the (8,8) nanotube is the critical size for inserting a single strand of DNA into a CNT. Since the DNA encapsulation inside the nanotube is highly sensitive to the initial position of the oligonucleotide relative to CNT, we repeated molecular dynamics simulations with different initial configurations and the result was the same. The near-constant center of mass (COM) distance d (data not shown) between the oligonucleotide and the carbon nanotube also indicated that the system has reached equilibrium. There are two possible explanations to unsuccessful insertion. One is that the diameter is too small and the oligonucleotide has to be severely deformed in order to enter the nanotube. The other is that the repelling of water molecules to the outside of CNTs is energetically costly and provides an effective resistance for DNA insertion. The effective resistance against encapsulation has overcompensate the reduction in vdW interaction energies so that the DNA oligonucleotide could not be encapsulated. To clarify which one is true, we removed the water and run simulation in vacuo. The result is the same with and without water. So the former is the right explanation. Water didn’t play an important role here.

In contrast to the system mwcn1, the smoothly spontaneous insertion of the oligonucleotide into the CNT can be observed in the system mwcn2 within 3 ns. Fig. 2(a) shows initial configuration of system mwcn2b. The difference from mwcn2 is that DNA rotates around z axis by 180°. The vdW energy between nanotubes and DNA of the system mwcn2 smoothly decreases with time, until the DNA is completely inside the tube, up to 800 ps (Fig. 2(d)). At the same time, in system mwcn2b, the vdW energy decreases dramatically at the beginning, then becoming steady and almost unchanged for an adequate length of time. After that, the ssDNA continues to move into the tube with simulation time up to 2 ns. Then the cytosine (Fig. 2(b), yellow color) hardly continued entering but just wandered near the entrance of the tubes at the last 5ns. The oligonucleotide was stuck in the tube entrance so that its position at 7 ns was very close to that at 2 ns (Fig. 2(c)). Conversely, in the mwcn2 system the oligonucleotide moves leisurely into the tube. It is easy to understand this phenomenon because the inner layer blocks the way in. It indicates that the DNA encapsulation inside the nanotube is highly sensitive to the initial position of the oligonucleotide, relative to the CNT.

As can be seen from Fig. 3(a), in system mwcn2, three bases (A, C, T) stacked to outer nanotube and one base (G) stacked to inner nanotube after encapsulation. The space left for DNA is 1.22nm in the radial direction and it’s divided into three roughly equal parts. In the case of system mwcn3, two bases (A and G) stacked to the outer wall, while other two stacked to the inner wall. As for mwcn4, all bases align parallel to the inner wall. The vdW interaction energy between DNA and the inner layer is about −65 kcal mol⁻¹, far more than that between DNA and the outer layer, which has the value of −2 kcal mol⁻¹. It indicates that the interaction between DNA and the outer layer decreased as the inter-wall space increased. Having the same inter-wall space, the larger the diameter of inner layer, the less influence outer layer has on DNA dynamics. To demonstrate this, we repeated the simulation with the outer/inner layer of mwcn4 removed and other conditions remain the same. Our results show that DNA stacks to the inner wall just like before and cannot insert from the bare outer wall, though the reduction of the total potential energy upon DNA encapsulation increases with CNT diameter. This can be explained by solvent effects. The base is most accessible to water molecules when bound to the outer wall of CNT of high curvature. Base–water interactions would be minimized for bases bound to the CNT inner wall. Thus, considering π–π stacking and solvent accessibility together, base–CNT binding should be greatest on a graphene sheet and weakest on the inner CNT surface. The solvent effect can be demonstrated by Fig. 3(b). DNA can encapsulate inside the nanotube in vacuo and can’t be absorbed in aqueous solution.

In system DNA-gra, ssDNA was deposited on the graphene and all bases aligned parallel to the surface after 7 ns. Table 2 gives base–carbon interaction energy of various systems. All values are about 10 kcal mol⁻¹ and follow the trend G > A > T > C, in accordance with both molecular dynamics (MD) and ab initio methods by others. Furthermore, the interaction energy increases with CNT diameter and base–graphene binding is greatest. The first principle calculations by Antony and Grimme indicate that the noncovalent interactions of stacked nucleobase and graphene are in the range of 20 to 25 kcal mol⁻¹.

![Fig. 2](image_url) System mwcn2b. (a) Initial configuration. (b) Configuration after 2 ns. (c) Final configuration after 7 ns. (d) van der Waals energy between nanotubes and DNA as a function of simulation time.

![Fig. 3](image_url) (a) Top view of oligonucleotide-nanotube system simulated after 3 ns. (b) Left, (30,30) in vacuo; Right, (30,30) in water.
as the same as ours when the contribution of sugars and phosphates are included. In the case of system DNA-2gra, DNA climbs the ladder and inserts into two graphene sheets.

Now we consider the pulling out process. The last configuration of the DNA–CNT complex in the MD simulation was used as the initial position in the steered molecular dynamics (SMD) simulation, and then the DNA was pulled out of the tube via the constant velocity pulling. SMD simulations were performed under the microcanonical NVE ensemble. Fig. 4 shows pulling an ssDNA out of a DWNT for the case of mwcn3. At first, ssDNA slides within the DWNT having tiny friction force. With all the nucleotides sliding in the SWNT, the vdW energy holds at a low and constant level, which makes the pulling force fluctuate about 0 pN. After 10 ps, the ssDNA starts to exit from the DWNT. The force immediately jumps to 250 pN and fluctuates around a plateau of 300 pN afterwards. One counts 4 pulses of force fluctuation, exactly resembling the number of bases. At around a plateau of 300 pN afterwards, one counts 4 pulses of force fluctuation, exactly resembling the number of bases. At last, the ssDNA is extracted from the nanotube and the force drops back down to about 0 pN. The physics that leads to the appearance of the force plateau region is the combination of the frictionless nanotube pore walls and an unfavorable DNA solvation energy, which generates constant force kinetics. With part of the nucleotides out of the DWNT, the vdW energy between DNA and nanotube increases sawtoothedly. As a result, the drawing force, which is determined by the gradient of the vdW energy with respect to the sliding distance of ssDNA in SWCNT, fluctuates around a plateau. Our results are in accordance with simulations of pulling ssDNA out of SWCNT in vacuo by Li and colleagues.\(^{18}\) We can also see that the work done on the system by pulling is about 81 kcal mol\(^{-1}\). However, DWNT–ssDNA vdW energy gains 86 kcal mol\(^{-1}\) during this process. We find the deficit is covered by the decrease of DNA internal energy, ssDNA–water and DWNT–water interaction energy.

**Conclusion**

In summary, the size effect on the spontaneous encapsulation of the DNA in CNT/GRA was investigated through the MD simulations, and pulling out process was explored by SMD simulations. Considering \(\pi–\pi\) stacking and solvent accessibility together, base–CNT binding should be strongest on a graphene sheet and weakest on the inner CNT surface. The interaction between DNA and outer layer decreases as the diameter of outer nanotube increases. Having the same outer-wall space, the larger the diameter of inner layer, the smaller the influence of the outer layer on DNA dynamics. When pulling the ssDNA out of the DWCNT, the force exhibits characteristic fluctuations around a plateau about 300 pN. Each fluctuation pulse of force to pull ssDNA corresponds to the exit of one base. A combination of the frictionless nanotube pore walls and an unfavorable DNA solvation energy produces these constant force profiles.

**Acknowledgements**

This work was supported by NSFC (Nos 20903094 and 20833008), NKBRSF (2009CB220010), and 863 project (2009AAA01A130). G.-J. Zhao also thanks the financial support of DICP and CAS.

**References**


"Table 2 Base–carbon interaction energy for the DNA bases adenine (A), cytosine (C), guanine (G), and thymine (T). All values in kcal mol\(^{-1}\)"

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<td>10.8</td>
<td>11.4</td>
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**Fig. 4** Pulling an ssDNA out of a DWCNT in the mwcn3 system. (a) Black squares: force variations at the pulling rate of 15 m s\(^{-1}\), original results smoothed by FFT filter of half-width 20 ps; blue squares: work done on the system by pulling. (b) DWCNT–ssDNA vdW energy vs. ssDNA slide curve.
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