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SUPRAMOLECULAR COMPLEXES OF CARBON NANOTUBES WITH DOXORUBICIN AND POLY (ETHYLENE GLYCOL) STUDIED USING THE MOLECULAR DOCKING AND DYNAMIC METHODS

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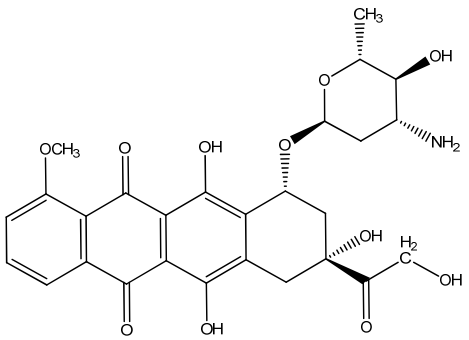
Using the molecular dynamic and docking techniques, the binary and ternary complexes of antitumour drug doxorubicin, carbon nanotubes, and poly (ethylene glycol) are studied. On this basis, we determine the hydrophilic/lipophilic behaviour, the structures and stabilities of supramolecular complexes in water and organic (octanol) solutions and give recommendations on what type of nanotubes should form the most perspective compositions for delivery of the doxorubicin. It is shown that coupling between the nanotube and solvent molecules results in a regular approximately circular alignment of both water and octanol molecules in the outer region and in the inner cavity of the nanomaterial. Both solvents influence the doxorubicin conformation. In water, the conjugated core of doxorubicin is orientated almost parallel to the nanotube surface favouring a surface complexation of the drug and tubule. In organic solvent, the core and the nanotube surface are orientated virtually perpendicular, pointing on the absence of π-π-stacking coupling. In the case of water, increase of distance between the tubule and drug results in energy increase, but in the organic solvent, the lager distance between tubule and doxorubicin the smaller is energy. The stable nanotube-doxorubicin supramolecular complexes being formed in water solutions are to be destroyed in biological tissues resulting in drug release. For nanotubes of small diameters $d < 12.5$ Å of any chirality (armchair or zigzag), the doxorubicin molecule is located on the outside of the nanotube, but for nanotubes of larger diameters it is to be located inside the tube. The tubule diameter dependence of the doxorubicin bond energy is nonmonotonic: the complexes with nanotubes having $d \sim 13.5$ Å are the most stable, the doxorubicin molecule being located inside the nanotube. The external or internal location of poly (ethylene glycol) in the complexes with nanotubes is dictated by the tube diameter too; however, the boundary diameter is different ($d \sim 11$ Å). This fact makes it possible to predict that in the ternary supramolecular complexes of (8, 8) and (14, 0) nanotubes the poly (ethylene glycol) molecule is to be located inside and the doxorubicin molecule outside the nanotube. These complexes should be the best water soluble doxorubicin carriers on the basis of the water soluble nanotubes.

Keywords: *Molecular dynamic; molecular docking; nanotube; doxorubicin*

1. Introduction

Despite the success of current treatments for several types of cancer, all known treatments have major limitations. The conventional chemotherapy or radiotherapy damage many cells, and both have significant side effects. In addition, tumour cells develop resistance to many chemotherapeutic agents, and most chemotherapeutic drugs kill dividing cancer cells and not dormant ones. The carbon nanotubes are the aromatic cylindrical molecules with inner cavity. The unique physical properties of carbon nanotubes allow for a range of novel cancer therapies including photothermal therapy, photoacoustic therapy, and radiofrequency ablation treatment of tumours [1, 2, 3, 4, 5].



During the last decade single-walled carbon nanotubes have been extensively explored as nanoscale drug carriers for potential applications in cancer treatment [6, 7, 8]. The doxorubicin ($C_{27}H_{29}NO_{11}$) is

the drug for chemotherapeutic treatment of some forms of cancer [9, 10]. Unfortunately, doxorubicin suppresses hematopoiesis and exhibits gastrointestinal toxicity [11] and cardiotoxicity [12]. This drug can be more widely and efficiently used in clinical practice if it is delivered directly to the diseased tissues. This can be provided by using nanotubes since they accumulate in diseased tissues, on the one hand, and form complexes with doxorubicin, on the other [13]. The nanotubes are almost insoluble in water, which hinders their direct use as the medicinal drug carriers. However, complexation of nanotubes with a poly (ethylene glycol) leads to water soluble nanotube derivatives. The experiments on mice have shown that the nanotubes functionalised with poly (ethylene glycol) are non-toxic [14, 15]. The circulation time of nanotubes complexes with poly (ethylene glycol) in the blood ($t_{1/2}$ equal to 22.1 hours) sharply increases as compared with the circulation time of free nanotubes (5.4 hours) [16]. This is responsible for higher transport characteristics of the nanotubes poly (ethylene glycol) complexes as compared with free nanotubes. Finally, *in vivo* experiments have confirmed that the supramolecular doxorubicin complexes with nanotubes functionalised by poly (ethylene glycol) are efficient in cancer therapy [17]. In these studies, mixtures of nanotubes of different structure (diameter and chirality) were used. The therapeutic efficiency of the composition can be enhanced by choosing a nanotube that would provide its best binding to poly (ethylene glycol) and doxorubicin.

The present study is aimed at estimating the hydrophilic/lipophilic behaviour of the nanotubes, doxorubicin, and their complexes and determining the energies of complex formation of nanotubes with doxorubicin and poly(ethylene glycol) using molecular dynamic and docking techniques. On this basis, we determine the structures and stabilities of supramolecular complexes of nanotubes with doxorubicin and poly (ethylene glycol) in water and organic solutions and give recommendations on what type of nanotubes should form the most perspective compositions for the doxorubicin delivery.

2. Simulation Methods

The doxorubicin and nanotubes are coupled through the non-covalent intermolecular forces. In the supramolecular complexes of single-walled nanotubes with poly (ethylene glycol), a chemical bonding is also intermolecular one. Therefore, to assess the structure and stability of the complexes, one should use the methods of computational chemistry adapted for the calculation of intermolecular forces. Nowadays, the molecular dynamics and docking techniques are direct approaches and the most popular to solving such problems. In both methods, a total potential energy of system is evaluated as a sum of several individual contributions, namely, the bond stretching, angle bending, torsion, stretch-bend, torsion-stretch, bend-bend, electrostatic, and van der Waals energies.

In this study, the nanotube and ligand molecules were arranged in a cell with the geometry of a rectangular parallelepiped, and the free space in this parallelepiped was filled with solvent molecules. The molecular dynamic simulations were carried out using a Tinker package [18], which previously had been applied to studies the polymer composites with nanotubes [19, 20, 21, 22]. The molecular docking studies were performed using an Autodock 3.0 computer program [23]. Both methods are essentially empirical and depend on the applied parameters scales. In our molecular dynamics simulations, we used the so-called OPLSAAL force field [24] widely used in the studies of common organic and inorganic materials, a TIP3P model [25] being applied for water. In case of molecular docking technique, we used the potential parameters specified in the form of tables for basic chemical elements and presented in Ref. 23.

In the molecular dynamics calculation, the velocity form of Brook's "Better Beeman" method [26] was used to integrate the equations of motion with a basic time step of 1.0 fs, and the Nose-Hoover thermostat algorithm [27] was used for temperature control. All the molecular dynamics simulations were implemented at 300 K. A cutoff distance of 10 Å was used for all potentials, and *NVT* (the number of particles (*N*) and the volume (*V*) of system in the ensemble are constant and the ensemble has a well-defined temperature (*T*), given by the temperature of the heat bath with which it would be in equilibrium) ensemble has been applied.

In molecular docking calculations, the nanotubes were taken to be the rigid host macromolecule with fixed geometry and position in space, and the ligand molecules [poly (ethylene glycol) and doxorubicin] were taken to be guests that executed random walks in the vicinity of the rigid nanomaterial and were considered as the nonrigid systems with large number of possible conformations. In each step of simulation, the ligand executed small random displacements along each degree of freedom: the displacement of the centre of gravity, orientation of the molecule, and rotation about nonrigid bonds with the changes in

internal dihedral angles. These displacements lead to the formation of a new configuration of the ligand and of the macromolecule ligand complex. For each configuration of complex thus generated, the energy of interaction of the ligand with the macromolecule was calculated as the sum of the interaction energies of atoms of the ligand and macromolecule. Among the vast number of possible conformations, the most stable conformations of complex were selected with the use of genetic algorithms and annealing procedure [23].

3. Hydrophilic/Lipophilic Behaviour of the Nanotube and Doxorubicin

Let us discuss the results of the molecular dynamics simulations of the hydrophilic/lipophilic behavior of carbon nanotubes and doxorubicin. To study the lipophilic interaction, we take octanol as a typical non-polar solvent. It is shown below using molecular docking that the (8, 8) tubule forms stable ternary complexes with doxorubicin and poly (ethylene glycol) in water. Correspondingly, we take the particular cases of the pure and poly (ethylene glycol) functionalised tubules (8, 8) in the molecular dynamics study of hydrophilic/lipophilic behaviour.

Figs. 1 shows a distribution of the water and octanol molecules in a neighbourhood of the (8, 8) tubule. One can see that the intermolecular coupling between the nanotube and solvent molecules results in a regular approximately circular alignment of both water and octanol in the outer region and in the inner cavity of the nanomaterial. Possibly, due to a formation of H-bonds between the H_2O molecules, the cylindrical layer-type structure of the water molecules arrangement is more pronounced in comparison with that of the organic solvent; for example, one can note some circular ordering of the H_2O molecules beyond the first layer too. The H_2O molecules in the first layer are aligned so that their OH bonds are directed to the outer and inner cylindrical surfaces of tubule, the distance between carbon cylinder and the nearest hydrogen atoms being equal to 2.0 Å approximately. Note that similar regular water distribution in region of the (16, 0) nanotube was observed previously on basic of molecular dynamics simulations with another force field parameter scale in [28].

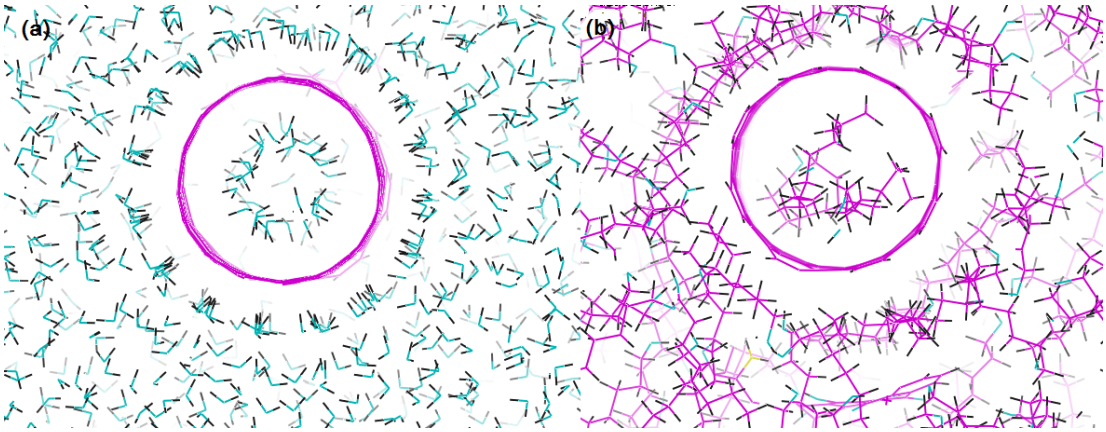


Figure 1. Distribution of solvent molecules in the region of (8, 8) nanotube: (a) water, (b) octanol

The coordination of the octanol on the outer cylindrical surface of (8, 8) nanotube is characterized by wrapping of the tubule by rather long organic molecules, manifesting the lipophilic coupling between the components. The average distance between the carbon cylinder and the nearest H atoms of octanol equals 2.5 Å is longer than in the case of water.

Figure 2 shows that water and octanol solvents influence the doxorubicin conformation. There is some change of planarity of rigid conjugated core of drug. The OCH_3, $COCH_2OH$, and $OC_6O_2NH_3$ groups vary their orientation relative to the core due to the coupling with solvent. The geometry of doxorubicin changes due to the coupling with the nanotube too, the orientation of doxorubicin relative to the tubule being sensitive to the solvent. In the case of water, the conjugating core is orientated almost parallel to the nanotube surface favouring a surface complexation of the drug and tubule. In organic solvent, the core and the nanotube surface are orientated virtually perpendicular, pointing on the absence of π-π-stacking coupling.

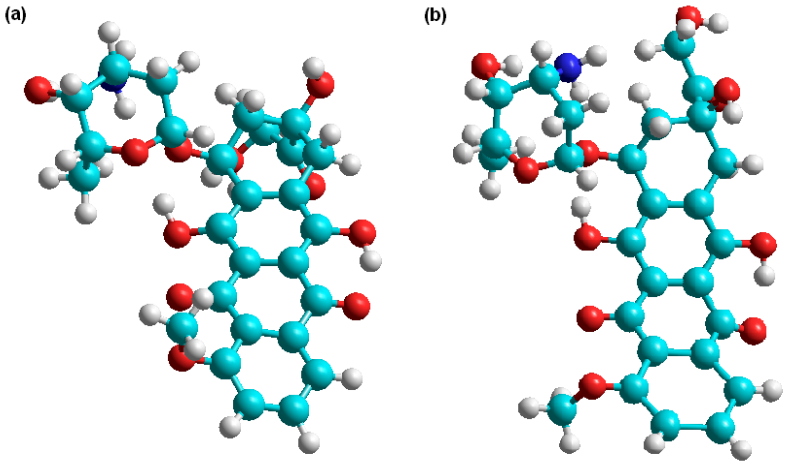


Figure 2. Conformation of the doxorubicin molecule in water (a) and octanol (b) solutions. Solvent molecules are not shown

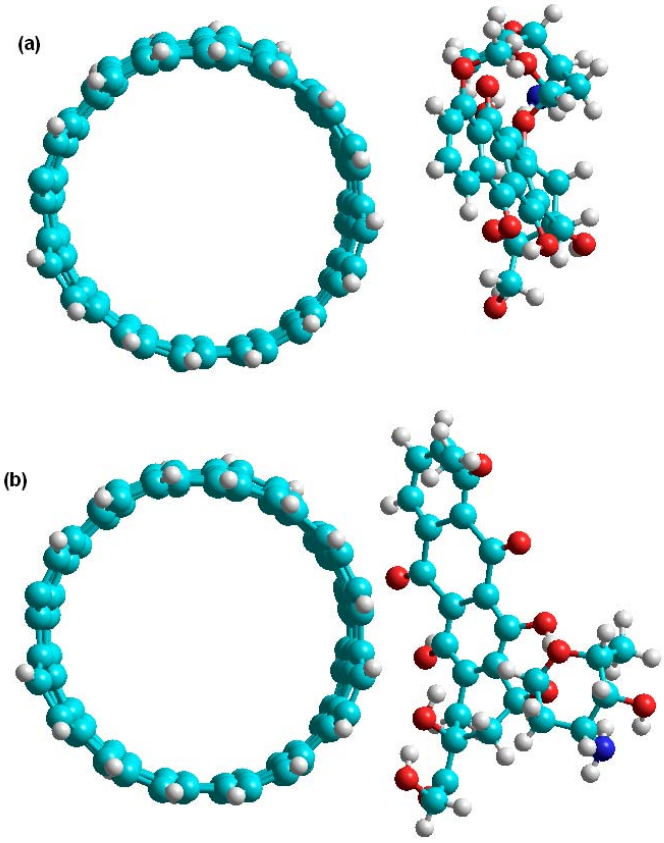


Figure 3. Conformation of the doxorubicin molecule near nanotube in solutions. In water (a), the conjugating core is orientated almost parallel to the nanotube surface. In octanol (b), the core and the nanotube surface are orientated virtually perpendicular. Solvent molecules are not shown

The differences between the solvents effects on coupling of doxorubicin and nanotube is most pronounced in the dependences of total energies on the distance between the tubule and drug (Fig. 4). In the case of water increase of this distance it results in energy increase. On the contrary, in the organic solvent, the lager distance between tubule and doxorubicin the smaller is energy. These data show that

the complex under consideration is stable in water but not in the octanol. Thus, the molecular dynamic calculations support an intuitive idea that the stable nanotube-doxorubicin supramolecular complexes being formed in water solutions are to be destroyed in biological tissues resulting in drug release.

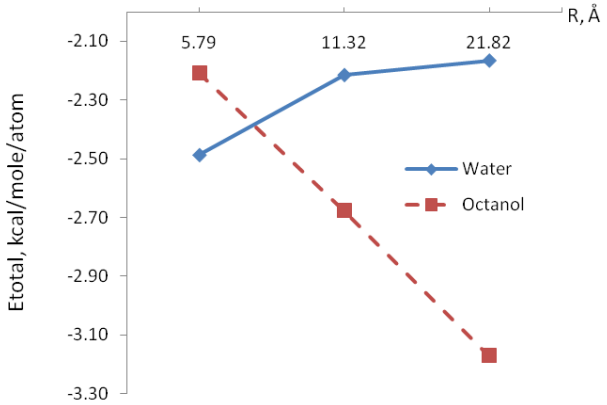


Figure 4. Total energy versus distance between doxorubicin and (8, 8) nanotube in water and octanol solution

4. Doxorubicin and Poly (Ethylene Glycol) Docking with Nanotubes

Now, let us discuss in detail the nanotube diameter and chirality dependences of the structure and stability of the nanotubes supramolecular complexes with doxorubicin and poly (ethylene glycol) in water, because it is the stabilization of appropriate complexes between doxorubicin and vehicle in this solvent that is of great importance for the drug delivery.

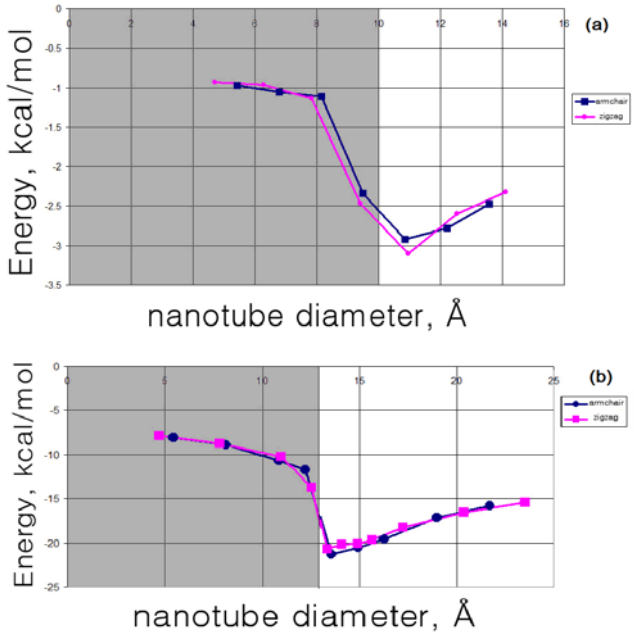


Figure 5. Formation energies of the complexes of (a) doxorubicin and (b) poly (ethylene glycol) with armchair and zigzag nanotubes

The docking results show that, for nanotubes of small diameters $d < 12.5$ Å of any chirality (armchair or zigzag); the doxorubicin molecule is located on the outside of the nanotube in energetically most favourable conformations (Fig. 5a, shaded area). For nanotubes of larger diameters, doxorubicin is to be located inside the tube. The dependence of the doxorubicin bond energy on the tube diameter is nonmonotonic: the doxorubicin complexes with (10, 10) and (19, 0) nanotubes ~ 13.5 Å in diameter are the most stable, the doxorubicin molecule being located inside the nanotube. It is clear that the bond

energy of the complex of poly (ethylene glycol) with a nanotube depends on the poly (ethylene glycol) chain length. Calculations showed that this energy is directly proportional to the number of units in the polymer. Therefore, it is convenient to recalculate this bond energy per unit (Fig. 5b). As in the case of doxorubicin, the external or internal location of poly (ethylene glycol) is dictated by the tube diameter; however, the boundary diameter is different ($d \sim 11$ Å). This fact makes it possible to obtain ternary supramolecular complexes with the poly (ethylene glycol) molecule being inside and the doxorubicin molecule being outside the nanotube. These complexes will be excellent doxorubicin carriers on the surface of the water soluble nanotubes since doxorubicin and poly(ethylene glycol) do not compete with each other for binding to the same (internal or external) tube surface (Fig. 6). This pertains to the complexes of (8, 8) and (14, 0) nanotubes.

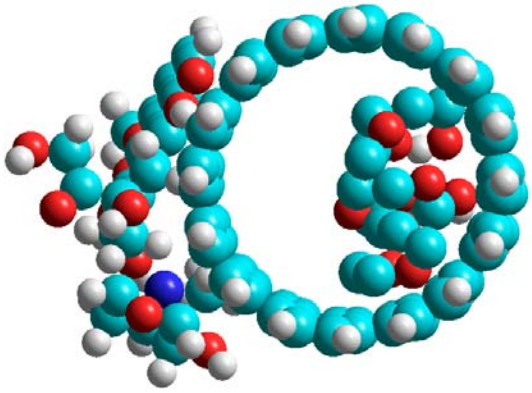


Figure 6. Supramolecular complex of the (8, 8) nanotube with doxorubicin (on the outside) and poly (ethylene glycol) (inside) according to molecular docking data

Thus, for any nanotubes of small diameters, the arrangement of doxorubicin on the outside of the nanotubes is energetically more favourable. For nanotubes of more than 12.5 Å in diameter, both doxorubicin and poly (ethylene glycol) should be located inside the nanotubes. For nanotube diameters 11 Å $\leq d \leq$ 12.5 Å, in energetically favourable complex conformations, doxorubicin is located on the nanotube surface while poly (ethylene glycol) is inside the nanotube.

5. Conclusions

Using the molecular dynamic and docking techniques, the binary and ternary complexes of antitumour drug doxorubicin, carbon nanotubes, and poly (ethylene glycol) are studied in water and organic (octanol) solutions. The hydrophilic/lipophilic behaviour, the structures and stabilities of supramolecular complexes are determined. We predict that in the ternary supramolecular complexes of the (8, 8) and (14, 0) nanotubes the poly(ethylene glycol) should be located inside and the doxorubicin molecule outside the tubules and these complexes should be the best water soluble doxorubicin carriers on the basis of the water soluble nanotubes.

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References

1. Liu, Z., Tabakman, S. M., Chen, Z., Dai, H. (2011). Preparation of carbon nanotube bioconjugated for biomedical applications. In: R. Klingeler, R. B. Sim (Eds.) *Carbon Nanotubes for Biomedical Application.* Heidelberg: Springer-Verlag.
2. Taylor, A., Turnbull, B., Seviour, C., Scroggie, S. (2011). *The Potential use of Carbon Nanotubes for Cancer Treatment,* 1–16, from http://charlieseviour.co.uk/research/Materials/master.pdf

3. Chakravarty, P., Marches, R., Zimmerman, N.S., Swafford, A.D.-E. (2008). Thermal ablation of tumor cells with antibody-functionalized single-walled carbon nanotubes. *Proc. of the Nat. Ac. Sciences,* 105, 8697–8702.
4. Kam, N. W. S., O'Connell, M., Wisdom, J. A., Dai, H. (2005). Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction. *Proc. of the Nat. Ac. Sciences,* 102, 11600–11605.
5. Iancu, C., Mocan, L. (2011). Advances in cancer therapy through the use of carbon nanotube-mediated targeted hyperthermia. *Inter. J. of Nanomedicine,* 6, 1675–1684.
6. Wu, H.-C., Chang, X., Liu, L., Zhao, F., Zhao, Y. (2010). Chemistry of carbon nanotubes in biomedical application. *J. Mater. Chem.,* 20, 1036–1052.
7. Liu, Z., Robinsonb, J.T., Tabakmanb, S.M., Yanga, K., Dai, H. (2011). Carbon materials for drug delivery and cancer therapy. *Materials Today,* 14, 316–323.
8. Ou, Z., Wu, B., Xing, D., Zhou, F., Wang, H., Tang, Y. (2009). Functional single-walled carbon nanotubes based on an integrin $\alpha_v\beta_3$ monoclonal antibody for highly efficient cancer cell targeting. *Nanotechnology,* 20, 1–7.
9. Yustein, J. T., Dang, C. V. (2007). Biology and treatment of Burkitt's lymphoma. *Curr. Opin. Hematol.,* 14, 375–381.
10. DeVita, V. T., Young, R. C., Canellos, G. P. (1975). Treatment of Ovarian Carcinoma: Possibilities for Progress. *Cancer*, 75, 98–110.
11. Morelli, D., Menard, S., Colnaghi, M. I., Balsari, A. (1996). Oral Administration of Anti-Doxorubicin Monoclonal Antibody Prevents Chemotherapy-induced Gastrointestinal Toxicity in Mice. *Cancer Res.* 56, 2082–2085.
12. Von Hoff, D. D., Layard, M. W., Basa, P. et al. (1979). Risk Factors for Doxorubicin-lnduced Congestive Heart Failure. *Ann. Int. Med.,* 91, 710–717.
13. Ali-Boucetta, H., Al-Jamal, K. T., McCarthy, D., Prato, M., Bianco, A., Kostarelos, K. (2008). Multiwalled carbon nanotube–doxorubicin supramolecular complexes for cancer therapeutics. *Chem. Commun.,* 4**,** 459–461.
14. Schipper, M. L., Nakayama-Ratchford, N., Davis, C. R. et al. (2008). A pilot toxicology study of single-walled carbon nanotubes in a small sample of mice. *Nat. Nanotechnol.,* 3, 216–221.
15. Liu, Z., Davis, C., Cai, W. et al. (2008). Circulation and long-term fate of functionalized, biocompatible single-walled carbon nanotubes in mice probed by Raman spectroscopy. *Proc. Natl. Acad. Sci. USA,* 105, 1410–1415.
16. Tabakman, S. M., Welsher, K., Liu, Z. et al. (2009). PEG Branched Polymer for Functionalization of Nanomaterials with Ultralong Blood Circulation. *J. Am. Chem. Soc.,* 131, 4783–4787.
17. Liu, Z., Fan, A. C., Rakhr, K. et al. (2009). Supramolecular Stacking of Doxorubicin on Carbon Nanotubes for In Vivo Cancer Therapy. *Angew. Chem., Int. Ed. Engl.,* 48, 7668–7672.
18. Ponder, J. W., Richards, F. M. (1987). An efficient newton-like method for molecular mechanics energy minimization of large molecules. *J. Comput. Chem.,* 8, 1016–1024.
19. Yang, M., Koutsos, V., Zaiser, M. (2005). Interactions between Polymers and Carbon Nanotubes: A Molecular Dynamics Study. *J. Phys. Chem. B,* 109, 10009–10014.
20. Hertel, T., Walkup, R. E., Avouris, P. (1998). Deformation of carbon nanotubes by surface van der Waals forces. *Phys. Rev. B,* 58, 13870–13873.
21. Rochefort, A., Salahub, D. R., Avouris, P. (1998). The Effect of Structural Distortions on the Electronic Structure of Carbon Nanotubes. *Chem. Phys. Lett.,* 297, 45–59.
22. Odegard, G. M., Gates, T. S., Wise, K. E., Park, C., Siochi, E. J. (2003). Constitutive Modeling of Nanotube-Reinforced Polymer Composites. *Compos. Sci. and Tech.,*63, 1671–1687.
23. Morris, G. M., Goodsell, D. S., Huey, R. et al. (2001). *Automated Docking of Flexible Ligands to Receptors. User's Guide*. La Jolla (CA): California Univ. Press.
24. Allinger, N. L., Yuh, Y. H., Lii, J. H. (1989). Molecular mechanics. The MM3 force field for hydrocarbons. *J. Am. Chem. Soc.,*111, 8551–8566.
25. Jorgensen, W. L., Chandrasekhar, J., Madura, J. D., et al. (1983). Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.,* 79, 926–935.
26. Brooks, B. R. (1988). *Algorithms for Molecular Dynamics at Constant Temperature and Pressure*. Bethesda, MD: National Institutes of Health.
27. Evans, D. J., Holian, B. L. (1985).The Nose–Hoover thermostat. *J. Chem. Phys*., 83, 4069–4074.
28. Walther, J. H., Jaffe, R., Halicioglu, T., Koumoutsakos, P. (2003). Molecular dynamics simulations of carbon nanotubes in water. *J. Phys. Chem. B,* 107, 1345–1352.

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