International Journal of Computational Materials
Science and Engineering
Vol. 4, No. 3 (2015) 1550014 (9 pages)

DOI: 10.1142/S2047684115500141

Imperial College Press
www.icpress.co.uk

Functionalized single-walled carbon nanotube (5, 0) as a carrier for isoniazid — A tuberculosis drug

M. Rajarajeswari*, K. Iyakutti†,‖, I. Lakshmi‡,
R. Rajeswarapalanichamy§ and Y. Kawazoe¶

*School of Physics, Madurai Kamaraj University
Madurai 625021, Tamil Nadu, India

†Department of Physics and Nanotechnology, SRM University
Katankulathur, Chennai 603203, Tamil Nadu, India

‡Department of Computer Science, Stella Maris College
Chennai 600086, Tamilnadu, India

§Department of Physics, N.M.S.S.V.N. College
Madurai 625019, Tamilnadu, India

¶New Industry Creation Hatchery Center, Tohoku University
Aramaki, Sendai 980-8579, Japan
‖iyakutti@gmail.com

Received 6 January 2014
Revised 25 May 2015
Accepted 26 May 2015
Published 26 June 2015

Nanostructures functionalized with amino acid are able to penetrate the cell wall. In this first principle study, we have demonstrated that the amino acid alanine functionalized carbon nanotubes (CNTs) (5, 0) can be a drug carrier for the tuberculosis drug isoniazid. Isoniazid is binding with both the non-covalently and covalently functionalized CNTs through the π–π stacking and NH$\cdots\pi$ interactions. The planar structure of isoniazid and hydrophobic nature of CNT promote the π–π stacking interactions. The amine group present in the isoniazid enables the NH$\cdots\pi$ interaction with the delocalized π electron cloud of CNT.

Keywords: Carbon nanotube; functionalization; drug carrier; isoniazid; density functional theory.

1. Introduction

The unique structure and size of carbon nanotubes (CNTs), especially large surface area to volume ratio and the high chemical stability make them as a suitable material for the medical applications [Yang *et al.*, 2007a]. From the extensive research during the past decades, now nanotubes are explored as a possible drug carrier for infectious

*Corresponding author.

diseases, cancer treatment, etc. [Wu *et al.*, 2005; Prato *et al.*, 2008; Chen *et al.*, 2008; Tan *et al.*, 2014; Wu *et al.*, 2014; Peci *et al.*, 2015]. These ideal drug carriers act as a transporter of drugs and deliver the load to the specific site in the body [Hilder and Hill, 2009]. After the delivery of the drug, nanotubes can be cleared from the systemic blood circulation through the renal excretion route without any side effects [Sato *et al.*, 2005; Singh *et al.*, 2006]. Thus the nanotubes are biocompatible and can be used for clinical purposes.

Functionalization is a crucial step in the development of nanotube-based targeted drug delivery [Marchesan *et al.*, 2015]. Functionalized CNTs are able to enter the cells like nanoneedles and pass through the cell membrane without causing cell death [Pantarotto *et al.*, 2004]. Carboxylic acid functionalized nanotubes are utilized as a drug carrier for the intercellular drug delivery of protein cargo [Kam and Dai, 2005]. Multifunctionalized CNTs covalently linked with the amphotericin B is taken up by mammalian cells without specific toxic effect [Wu *et al.*, 2005]. Therapeutic functional groups like antibiotics can be directly attached to the nanotubes for the treatment of infectious diseases [Prato *et al.*, 2008]. Biotin functionalized CNT conjugates are designed for tumor targeting chemotherapy and the cancer specific receptor-mediated endocytosis is observed with the efficient drug delivery [Chen *et al.*, 2008].

Tuberculosis or tubercle bacillus (TB) is one of the common and deadly infectious diseases caused by mycobacterium tuberculosis. Two million deaths are reported every year due to the Mycobacterium tuberculosis [Gelperina *et al.*, 2005]. The unusual structure and chemical composition of the mycobacterial cell wall complicates the effective treatment. Treatment of TB needs continuous and frequent multiple drug dosing which can be achieved by slow and sustained release of antimicrobial agent. Isoniazid and rifampicin are the most common therapeutic agents used for tuberculosis. Nanostructures are expected to be promising drug delivery agents for the potential treatment of tuberculosis. There are some reports available for the nanoparticle [Gelperina *et al.*, 2005; Saraogi *et al.*, 2011] and nanotubes [Gallo *et al.*, 2007; Saikia and Deka, 2010] based drug delivery for tuberculosis.

Gallo *et al.* [2007] reported the uptake of isoniazid by the 1, 3-dipolar cycloaddition of azomethine ylides functionalized CNT $(5, 5)$ and fullerene. Structural properties and relativities of the covalently attached three antitubercular drugs isoniazid, 2-methylheptylisonicotinate and pyrazinamide with the azomethine ylide functionalized CNT $(5, 5)$ are investigated by Saika and Deka [2010]. Also in their recent density functional study, they have shown that non-covalent functionalization of isoniazid (INH) is preferred over covalent attachment on pristine and B-doped $(5, 5)$ and $(9, 0)$ single wall CNTs [Saika and Deka, 2013].

In general, bio-functionalization of nanotubes makes them mono dispersive and biocompatible which reduce the toxic effects [Firme III and Bandaru, 2010]. Liu *et al.* [2007] have shown that non-covalently or covalently prefunctionalized CNTs with phospholipids can adsorb the cancer chemotherapy drug doxorubicin. Cancer

drug bound with the CNT through the π-stacking interaction and their release can be controlled by varying the pH. This study introduced the concept of adding different functional groups on the nanotube surface i.e. functionalization partitioning of CNTs. Yang *et al.* [2007b] reported that bucky amino acid enables the peptide transport into cells. Hydrophobic surface of the fullerene core assisted the delivery of peptide into the interior of the targeted cells. Thus the amino acid functionalized nanomaterials will be biocompatible and they can penetrate through the cell walls. This gives an idea to model the amino acid functionalized CNT as a drug carrier.

In our previous studies, we have investigated the functionalization of CNTs $(5, 0)$ with amino acids alanine [Rajarajeswari *et al.*, 2012] and valine [Rajarajeswari *et al.*, 2011]. Both the non-covalent and covalent functionalization of CNT with amino acids is possible. Non-covalent functionalization leave the CNT unchanged but the covalent functionalization often impairs the CNT at the adsorption site. We utilized the alanine functionalized CNT $(5, 0)$ (both covalent and non-covalent) as a drug carrier for the tuberculosis drug isoniazid. Here, we have demonstrated the non-covalent binding of isoniazid with the pristine and alanine functionalized CNTs. Hydrophobic surface of the nanotube facilitates the non-covalent binding of the planar molecule isoniazid.

2. Computational Details

The total energy calculations are performed using density functional theory with a plane wave basis set as implemented in Vienna *ab initio* simulation package (VASP) [Kresse and Furthmuller, 1996]. Projector augmented wave method is used to model the electron-ion interactions [Blochl, 1994, Kresse and Joubert, 1999]. Local density approximation is employed to describe the exchange correlation [Perdew and Zunger, 1981]. Cut-off energy for the plane wave expansion of valence electrons is chosen as 500 eV. Brillouin zone integration is performed for the k-points meshes generated by the Monkhorst–Pack scheme $1 \times 1 \times 5$ with Gamma centered grid [Monkhorst and Pack, 1976]. Residual minimization/direct inversion in the iterative subspace algorithm is used for electronic minimization. Super cell approach is employed to study the interaction. Lateral separation of 24Å between the centers (CNT) is taken to make the periodic images non-interactive.

Isoniazid ($C_6H_7N_3O$, INH, isonicotinic acid hydrazine) is one of the first-line drugs used for antituberculosis medication for prevention and treatment. It is a planar molecule having one six member ring with an amine group protruding out of the plane. Initially nanotube $(5, 0)$ and isoniazid are optimized within the super cell separately. Then the isoniazid is allowed to interact with pristine CNT. The favorable adsorption position of isoniazid on the CNT surface is found out by translating the isoniazid toward the CNT and away from the CNT and by rotating the isoniazid above the CNT surface (Fig. 1). We have got four local minima for the

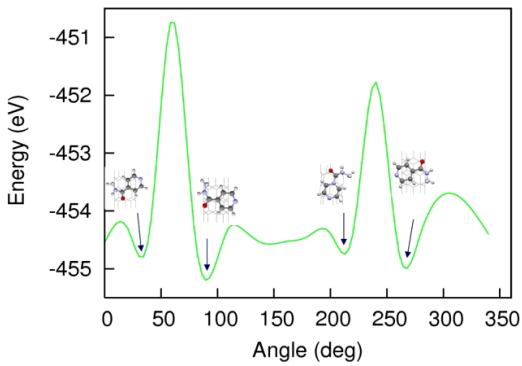

Fig. 1. Rotational energy scan for isoniazid adsorption on CNT $(5, 0)$.

different rotation angles, indicated by arrows with the respective structure. Finally, we have performed a full optimization for the lowest energy configuration in which all atoms are free to relax.

3. Results and Discussion

3.1. *Structure and binding energies*

We have investigated the binding of isoniazid with pristine and amino acid functionalized CNTs. On the pristine CNT surface, isoniazid got adsorbed at the distance of 2.92Å(Table 1), without any bending or wrapping. The small equilibrium distance between the nanotube and isoniazid makes the amine group of isoniazid to be close with the CNT surface. Thus the amine group makes hydrogen bonds with the carbon atoms of CNT. Binding of isoniazid is facilitated by the π-electron network of CNT and also enhanced by the amine group. Binding energy of isoniazid indicates that it is physisorbed through the combination of π–π stacking and NH$\cdots\pi$ interactions.

A potential drug carrier should be able to penetrate the cellular barrier to deliver the drug to the specific site. Though the isoniazid can bind with the pristine CNT, regrettably pristine CNTs are insoluble in many liquids. This makes impossible to visualize the proposed application. On the other hand, functionalization of CNTs increases its usability and biocompatibility [Marchesan *et al.*, 2015]. Yang *et al.* [2007], shows that amino acid functionalized fullerene is having ability to penetrate

Table 1. Equilibrium distances between the CNT and isoniazid, charge transfer to isoniazid and binding energy of isoniazid.

System	Distance (Å)	Charge transfer (e)	Binding energy (eV)
CNT $(5, 0) + \mathrm{I}$	2.92	−0.064	0.37
CNT $(5, 0)\mathrm{A} + \mathrm{I}$	3.18	0.059	0.34
CNT $(5, 0)\mathrm{AR} + \mathrm{I}$	3.31	−0.008	0.09

the cellular barriers to deliver the cargo. Thus modeling a functionalized nanotube as a drug carrier will represent a closer situation of the experimental investigation.

In our earlier studies on amino acid functionalization of nanotube $(5,0)$, nanotube is non-covalently functionalized with the amino acid alanine and covalently functionalized with alanine radical [Rajarajeswari *et al.*, 2012]. In the non-covalent functionalization, alanine is allowed to interact with the CNT through the functional groups CH_3, NH_2, COOH and OH in different orientations. For the covalent functionalization, alanine radical is formed by removing hydrogen atoms from the functional groups and then the alanine is allowed to interact with the CNT in different sites. After structural optimization, we found the stable conformers with respect to their binding energies. Both in the non-covalent and covalent binding of alanine with CNT $(5,0)$, amine group of alanine established the strong interaction with the CNT. Hence, we have taken the alanine functionalized CNT $(5,0)$ through the amine group for this study.

Isoniazid is allowed to get adsorbed on the CNT surface, in the optimized structure of CNT $(5,0)+$alanine complex (CNT $(5,0)\mathrm{A}+\mathrm{I}$) and CNT $(5,0)+$alanine

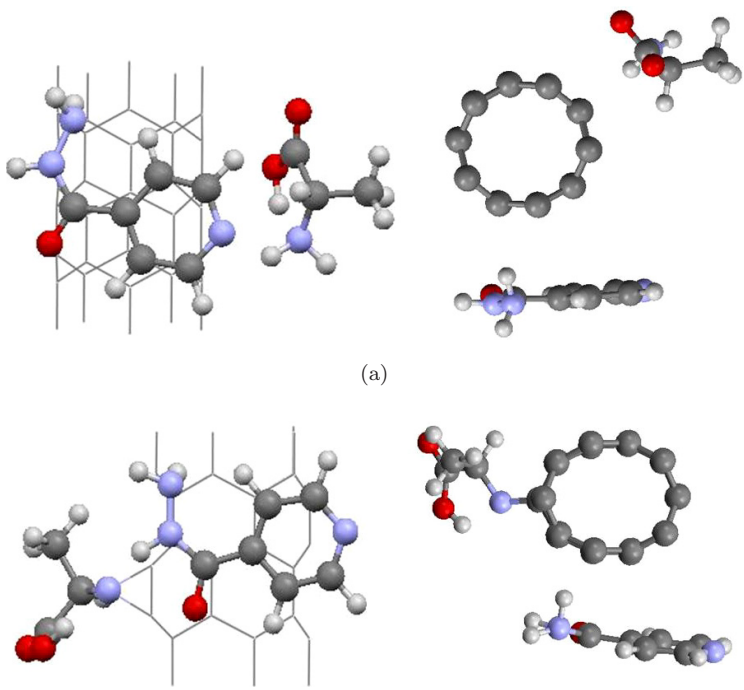

Fig. 2. (a) Adsorption of isoniazid on the non-covalent alanine functionalized CNT $(5,0)$ (CNT $(5,0)\mathrm{A}+\mathrm{I}$). (b) Adsorption of isoniazid on the covalent alanine radical functionalized CNT $(5,0)$ (CNT $(5,0)\mathrm{AR}+\mathrm{I}$) (side and top views).

radical complex (CNT $(5,0)$AR + I). Then the functionalized CNT + isoniazid complexes are allowed to relax and the optimized structures are presented in Fig. 2. Distance separation of the isoniazid from the CNT surface, charge transfer values and binding energies of the complexes are presented in Table 1. In the optimized complex CNT $(5,0)$A + I, isoniazid is adsorbed at 3.18 Å from the CNT (Fig. 2(a)). Amine group of isoniazid is at a distance of 2.4 Å from the surface of the nanotube and it forms hydrogen bonds with the CNT. Bader analysis [Henkelman *et al.*, 2006] shows a charge transfer of 0.059e from the CNT to isoniazid. Binding energies (Table 1) show the possible adsorption of isoniazid on the amino acid functionalized CNT.

In the case of CNT $(5,0)$AR + I complex (Fig. 2(b)), isoniazid is adsorbed at 3.31 Å with 0.09 eV binding energy. Though the isoniazid is adsorbed parallel to the CNT surface, the hexagon ring is moved away from the CNT surface and the lower half of isoniazid having amine group and oxygen atom moved close to the CNT (2.73 Å). As a result, the amine group has formed hydrogen bonds with the carbon atoms of CNT. Also the nano tube got elongated in the radial direction. Though the adsorption of isoniazid is possible with the covalently functionalized CNT, the binding energy of isoniazid is lower compared to the non-covalently functionalized CNT. Due to the strong covalent interaction of alanine with CNT, the binding strength of isoniazid with CNT is low.

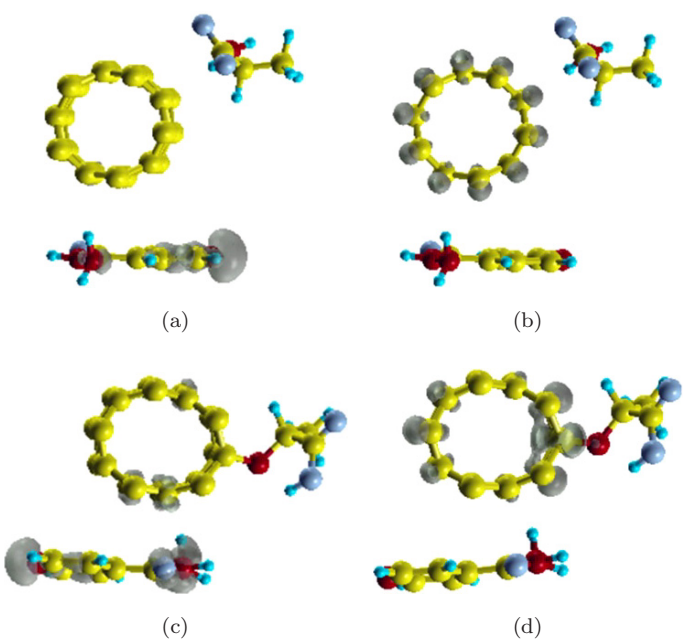

Fig. 3. (a) HOMO and (b) LUMO of CNT $(5,0)$A + I (c) HOMO and (d) LUMO of CNT $(5,0)$AR + I.

3.2. *Charge density analysis*

To know more about the interaction between the amino acid functionalized nanotube and isoniazid, we have analyzed the electronic charge density distribution (isosurface) in the molecular orbitals [Kokalj, 2003]. Figure 3 depicts the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of CNT $(5,0)\mathrm{A}+\mathrm{I}$ and CNT $(5,0)\mathrm{AR}+\mathrm{I}$ complexes. In the HOMO of CNT $(5,0)\mathrm{A}+\mathrm{I}$ complex (Fig. 3(a)), charge is localized on the electronegative atoms oxygen and nitrogen present in the isoniazid. On the other hand, in LUMO, charge is homogeneously distributed over the nanotube (Fig. 3(b)). The HOMO of isoniazid and LUMO of nanotube confirms the partial charge transfer which stabilize the CNT $(5,0)\mathrm{A}+\mathrm{I}$ complex and manifest the physisorption through electrostatic interaction. In the LUMO of CNT $(5,0)\mathrm{AR}+\mathrm{I}$ complex (Fig. 3(d)), charge is accumulated only on the carbon atoms of the nanotube which are elongated in the radial direction. Covalent bonding of alanine with nanotube rearranges the LUMO levels of the CNT and also it impacts the binding strength of the isoniazid in the CNT $(5,0)\mathrm{AR}+\mathrm{I}$ complex.

4. Conclusion

We have demonstrated the possibility of using CNT $(5,0)$ as a nanocarrier for the TB drug delivery. The pristine and amino acid functionalized nanotubes has manifested as drug carrier. Non-covalent adsorption of isoniazid on the pristine CNT $(5,0)$ is possible. Also, isoniazid can get adsorbed on the non-covalently amino acid functionalized CNT $(5,0)$ and the binding strength (0.37 eV) of isoniazid is comparable with the pristine CNT case (0.34 eV). But in the case of covalently functionalized CNT $(5,0)$, binding energy of the isoniazid is relatively low but stable. The covalent interaction of alanine with CNT has reduced the strength of non-covalent adsorption of isoniazid on CNT. Molecular orbital analysis confirms that the electronegative atoms of isoniazid mold the non-covalent adsorption through the $\mathrm{NH}\cdots\pi$ interaction in addition with the π–π stacking interaction. Compared to the other cases, non-covalently amino acid functionalized CNT can serve as a potential drug carrier for isoniazid. Targeted delivery of antimicrobial agent can be achieved by the receptor-mediated drug delivery [Majumdar and Basu, 1991; Feixas *et al.*, 2014]. It is one of the most viable solutions because the large surface area of CNT gives the privileges for multiple functionalizations.

Acknowledgments

The University Grants Commission is acknowledged for supporting this project with a senior research fellowship, under University with Potential for Excellence. One of the authors K.I. acknowledges the financial support of the Council of Scientific and Industrial Research under the Emeritus Scientist Scheme. The authors would

like to express their sincere thanks to the crew of the Center for Computational Materials Science at Institute for Materials Research, Tohoku University, Japan for their continuous support in using the SRI6000 supercomputing facilities.

References

Blochl, P. E. [1994] "Projector augmented-wave method," *Phys. Rev. B* **50**, 17953.

Chen, J., Chen, S., Zhao, X., Kuznetsova, L. V., Wong, S. S. and Ojima, I. [2008] "Functionalized single-walled carbon nanotubes as rationally designed vehicles for tumor-targeted drug delivery," *J. Am. Chem. Soc.* **130**, 16778–16785.

Feixas, F., Lindert, S., Sinko, W. and McCammon, J. A. [2014] "Exploring the role of receptor flexibility in structure-based drug discovery," *Biophys. Chem.* **186**, 31–45.

Firme III, C. P. and Bandaru, P. R. [2010] "Toxicity issues in the application of carbon nanotubes to biological systems," *Nanomed. Nanotechnol. Biol. Med.* **6**, 245–256.

Gallo, M., Favila, A. and Glossman-Mitnik, D. [2007] "DFT studies of functionalized carbon nanotubes and fullerenes as nanovectors for drug delivery of antitubercular compounds," *Chem. Phys. Lett.* **447**, 105–109.

Gelperina, S., Kisich, K., Iseman, M. D. and Heifets, L. [2005] "The potential advantages of nanoparticle drug delivery systems in chemotherapy of tuberculosis," *Am. J. Respir. Crit. Care. Med.* **172**, 1487–1490.

Henkelman, G., Arnaldsson, A. and Jonsson, H. [2006] "A fast and robust algorithm for Bader decomposition of charge density," *Comput. Mater. Sci.* **36**, 354–360.

Hilder, T. A and Hill, J. M. [2009] "Modeling the loading and unloading of drugs into nanotubes," *Small* **5**, 300-308.

Kam, N. W. S. and Dai, H. [2005] "Carbon nanotubes as intracellular protein transporters: Generality and biological functionality," *J. Am. Chem. Soc.* **127**, 6021–6026.

Kokalj, A. [2003] "Computer graphics and graphical user interfaces as tools in simulations of matter at the atomic scale," *Comput. Mater. Sci.* **28**, 155–168.

Kresse, G. and Furthmuller, J. [1996] "Efficient iterative schemes for *ab initio* total-energy calculations using a plane-wave basis set," *Phys. Rev. B* **54**, 11169.

Kresse, G. and Joubert, D. [1999] "From ultrasoft pseudopotentials to the projector augmented-wave method," *Phys. Rev. B* **59**(3), 1758.

Liu, Z., Sun, X., Nakayama-Ratchford, N. and Dai, H. [2007] "Supramolecular chemistry on water-soluble carbon nanotubes for drug loading and delivery," *ACS Nano* **1**, 50–56.

Majumdar, S. and Basu, S. K. [1991] "Killing of intracellular mycobacterium tuberculosis by receptor-mediated drug delivery," *Antimicrob. Agents. Chemother.* **35**, 135–140.

Marchesan, S., Kostarelos, K., Bianco, A. and Prato, M. [2015] "The winding road for carbon nanotubes in nanomedicine," *Mater. Today* **18**, 12-19.

Monkhorst, H. J. and Pack, J. D. [1976] "Special points for Brillouin-zone integrations," *Phys. Rev. B* **13**, 5188.

Pantarotto, D., Singh, R., mccarthy, D., Erhardt, M., Briand, J.-P., Prato, M., Kostarelos, K. and Bianco, A. [2004] "Functionalized carbon nanotubes for plasmid DNA gene delivery," *Angew. Chem.* **116**, 5354–5358.

Peci, T., John S. Dennis, T. and Baxendale, M. [2015] "Iron-filled multiwalled carbon nanotubes surface-functionalized with paramagnetic Gd (III): A candidate dual-functioning MRI contrast agent and magnetic hyperthermia structure," *Carbon* **87**, 226–232.

Perdew, J. P. and Zunger, A. [1981] "Self-interaction correction to density-functional approximations for many-electron systems," *Phys. Rev. B* **23**, 5048.

Prato, M., Kostarelos, K. and Bianco, A [2008] "Functionalized carbon nanotubes in drug design and discovery," *Acc. Chem. Res.* **41**, 60–68.

Rajarajeswari, M., Iyakutti, K. and Kawazoe, Y. [2011] "Interaction of valine and valine radicals with single-walled carbon nanotube $(5, 0)$," *Chem. Phys. Lett.* **511**, 299–303.

Rajarajeswari, M., Iyakutti, K. and Kawazoe, Y. [2012] "Noncovalent and covalent functionalization of a $(5, 0)$ single-walled carbon nanotube with alanine and alanine radicals," *J. Mol. Model.* **18**, 771–781.

Saikia, N. and Deka, R. C. [2010] "Theoretical study on pyrazinamide adsorption onto covalently functionalized $(5, 5)$ metallic single-walled carbon nanotube," *Chem. Phys. Lett.* **500**, 65–70.

Saikia, N. and Deka, R. C. [2013] "Density functional study on the adsorption of the drug isoniazid onto pristine and B-doped single wall carbon nanotubes," *J. Mol. Model.* **19**, 215–226.

Saraogi, G. K., Sharma, B., Joshi, B., Gupta, P., Gupta, U. D., Jain, N. K. and Agrawal, G. P. [2011] "Mannosylated gelatine nanoparticles bearing isoniazid for effective management of tuberculosis," *J. Drug. Target.* **19**, 219–227.

Sato, Y., Yokoyama, A., Shibata, K., Akimoto, Y., Ogino, S., Nodasaka, Y., Kohgo, T., Tamura, K., Akasaka, T., Uo, M., Motomiya, K., Jeyadevan, B., Ishiquro, M., Hatakeyama, R., Watari, F. and Tohji, K. [2005] "Influence of length on cytotoxicity of multi-walled carbon nanotubes against human acute monocytic leukemia cell line THP-1 *in vitro* and subcutaneous tissue of rats *in vivo*," *Mol. Biosyst.* **1**, 176–182.

Singh, R., Pantarotto, D., Lacerda, L., Pastorin, G., Klumpp, C., Prato, M., Bianco, A. and Kostarelos, K. [2006] "Tissue biodistribution and blood clearance rates of intravenously administered carbon nanotube radiotracers," *Proc. Natl. Acad. Sci. USA* **103**, 3357–3362.

Tan, J. M., Karthivashan, G., Arulselvan, P., Fakurazi, S. and Hussein, M. Z. [2014] "Characterization and *in vitro* studies of the anticancer effect of oxidized carbon nanotubes functionalized with betulinic acid," *Drug. Des. Deve. Ther.* **8**, 2333–2343.

Wu, W., Wieckowski, S., Pastorin, G., Benincasa, M., Klumpp, C., Briand, J.-P., Gennaro, R., Prato, M. and Bianco, A. [2005] "Targeted delivery of amphotericin B to cells by using functionalized carbon nanotubes," *Angew. Chem.* **117**, 6516–6520.

Wu, P., Li, S. and Zhang, H. [2014] "Design real-time reversal of tumor multidrug resistance cleverly with shortened carbon nanotubes," *Drug. Des. Devel. Ther.* **8**, 2431–2438.

Yang, J., Wang, K., Driver, J., Yuan, J. and Barron, A. [2007] "The use of fullerene substituted phenylalanine amino acid as a passport for peptides through cell membranes," *Org. Biomol. Chem.* **5**, 260–266.

Yang, W., Thordarson, P., Gooding, J. J., Ringer, S. P. and Braet, F. [2007] "Carbon nanotubes for biological and biomedical applications," *Nanotechnology* **18**, 412001.