Macromol. Symp. **2014**, 335, 51–57 DOI: 10.1002/masy.201200121

Controlled Aggregation of Biopolymer-Wrapped Carbon Nanotubes in Aqueous Suspension, Induced by Cationic Porphyrin

*E. S. Zarudnev, V. A. Karachevtsev**

Summary: Unusual aggregation of biopolymer-wrapped single-walled carbon nanotubes (SWCNTs) induced by cationic porphyrin *meso*-tetrakis(4-N-methyl-pyridyl)porphine (TMPyP4) in aqueous suspension has been studied by absorption spectroscopy and molecular dynamics simulation. After the addition of a small dose of TMPyP4 (10^{-6} M) into poly(rC):SWCNTs aqueous suspension, nanotubes begin to aggregate, but the aggregates are stable without precipitation during a few days. Spectral observations indicate that the porphyrin core stacked with π-systems of the biopolymer:SWCNT hybrid and all nanotube species take part in this aggregate formation without any selection. Molecular dynamic simulation demonstrates that the stable complex consisting of two nanotubes can be formed when TMPyP4 molecules bound nanotubes both owing to π-π stacking of the porphyrin core with the surface of one nanotube and with the oligonucleotide adsorbed onto another nanotube. TMPyP4 molecules not only couple the biopolymer:nanotube hybrids, but also they stitch polymer's strands wrapping around each nanotube resulting in continuous floatation of aggregates in water. It was shown that the aggregate is stable even at 373 K. At this temperature additional contacts between two neighbor's polymers appear which strengthen this aggregate too. The controlled aggregation of biopolymer-wrapped carbon nanotubes can be employed at the fabrication of multifunctional supramolecular assemblies, photovoltaic cells and in biosensing.

Keywords: biopolymers; carbon nanotube aggregation; cationic porphyrin; molecular dynamics; van der Waals interaction

Introduction

Owing to their unique quasi-one-dimensional structure SWCNTs have great potential in electronics, optics, mechanics, and biosensing. To integrate the carbon nanotube into multicomponent structures or devices, an approach based on self-assemblies from individual carbon nanotubes can be very efficient.[1] For this purpose DNA which wraps tightly around the nanotube in water[2] can be applied as a very useful interface polymer which has an excellent molecular recognition capability and which exploits widely to develop different nanostructures.[3] The controlled aggregation of individual nanotubes with a possibility of the formation of high density of DNA-wrapped individual nanotubes in aqueous suspension is the first stage in the route of the integration of these quasi one-dimensional materials into technology.

Controlled aggregation of individual nanotubes can be realized by the hybridization of a free long ssDNA chain simultaneously with two complementary polymers adsorbed to different nanotubes.[4,5] Proteins or enzymes which bind effectively to DNA in water can also cause an aggregation of nanotubes covered with DNA.[6]

B. I. Verkin Institute for Low Temperature Physics and Engineering, Kharkov 61103, Ukraine
E-mail: karachevtsev@ilt.kharkov.ua

Small molecules which are actively employed in DNA biochemistry and which have several contact points/groups interacting with this polymer can also induce such aggregation.[7] Among them the water soluble cationic porphyrins which are effectively bound with DNA are the ideal candidate for this purpose because there are π-system and several positive charged groups in the structure of this molecule.[8] Note that the porphyrin associates with the nanotube surface effectively and an existence of positive charges in the porphyrin structure increases significantly this binding.[9] It was shown that the electronic excitations transfer effectively from the excited porphyrin states to the SWCNTs directly or via ssDNA as an electron mediator.[10]

Recently we have showed that molecules of cationic porphyrin TMPyP4 generate the aggregation of poly(rC)-wrapped carbon nanotubes in aqueous suspension.[11] These aggregates is of an unusual behavior, their suspension remained stable during a few days without quick precipitation as distinct from conventional aggregates of carbon nanotubes. Analysis of structures of aggregates obtained by molecular dynamics showed that TMPyP4 molecules connect the polymer-nanotube hybrids using four charged groups placed around the porphyrin core as well as the π-π stacking interaction with π-system of the nanotube and nitrogen bases. On the other hand they strengthen the polymer coverage of nanotubes because porphyrin adsorbed between neighbor pitches of the wrapped polymer "stitching" them together.

In this work we continue a characterization of the unusual aggregation of poly(rC)-wrapped nanotubes induced by cationic porphyrin employing absorption spectroscopy and molecular dynamics. The main purposes of this work are analysis of aggregate structures obtained in detail, elucidation of the temperature influence on the aggregate stability and, in addition, we try to verify the possible selection among nanotube species under the aggregate formation.

Experimental Part

Samples Preparation

SWCNTs produced by CoMoCat method[12] (SouthWest NanoTechnologies, USA) were used without further purification. Semiconducting tubes with (6,5) chirality (SWeNT® SG 65) prevailed in the starting material.

Aqueous solution of potassium salts of polycytidylic acid poly(rC) (Sigma-Aldrich, USA) dissolved in 0.005 $\mathrm{mol \cdot L^{-1}}$ $\mathrm{Na^+}$ cacodylate buffer (pH7) (Serva, Germany) with 0.005 $\mathrm{mol \cdot L^{-1}}$ NaCl was used to prepare an aqueous suspension of SWCNTs.[13] A stable aqueous suspension of SWCNTs was prepared by sonication of nanotube bundles with poly(rC) for 60 min (1 W, 44 kHz). Then the solution was centrifuged at 70000 g for 40 minutes. After ultracentrifugation the supernatant was decanted and dialyzed (dialysis tubing with a molecular weight cutoff of 13–14 kDa) against the buffer solution for 36 h to remove free polynucleotides which were not adsorbed on SWCNTs.

Aggregation of poly(rC)-wrapped SWCNTs in aqueous suspension was studied after adding tetra-*p*-tosylate salt of *meso*-tetrakis(4-N-methyl-pyridyl)porphine (TMPyP4) (Sigma-Aldrich, USA) in aqueous suspension. The porphyrin concentration was determined spectrophotometrically in water, using the extinction coefficient of $\varepsilon_{424} = 226000\ \mathrm{M^{-1}\ cm^{-1}}$ at the Soret band maximum.[8] In titration experiments, the minimal dose of [TMPyP4] was 50 μM (1 μL) which was added into the nanotube suspension (400 μL).

Absorption Spectroscopy

Absorption spectra in the range of 300–1100 nm were recorded with the double-channels spectrophotometer (Hitachi M 356, Japan) with photomultiplier detection and with NIR spectrometer in the range 1100–1400 nm with the signal detection using a thermocooled InGaAs photodiode. Accuracy of measurements of the transmission coefficient was not worse than 0.2%. All investigations were carried out at room temperature.

Molecular Dynamics Simulation

The formation of poly(rC):SWCNT hybrid was simulated by the molecular dynamics method. The program package NAMD[14] was employed with Charmm27 force field parameter set.[15] The box of $50 \times 75 \times 135$ Å dimensions were used with $r(C)_{30}$: SWCNT embedded in water (15000 H_2O molecules). SWCNTs were selected as a zigzag (10,0) carbon nanotube. Its length and diameter were 9.7 nm and 0.78 nm, respectively. The length of oligonucleotides was 30 nucleotides. Before the optimization procedure, we chose backbone the torsion angles of poly(rC) in such a manner that nucleic bases were located close to the carbon nanotube surface and the sugar-phosphate backbone was directed into water, forming 45° angle between the direction of the polymer and the nanotube axes. This angle was determined experimentally in circular dichroism measurements.[16] For neutralization of the charge on the sugar-phosphate backbone, 30 Na^+ ions were added close to each oligonucleotide. The distance between stacked bases and the tube surface was about 3.4–3.5 Å. Modeling temperature and pressure in the periodic box were 300 K (or 373 K) and 1 bar, respectively. Interaction energy was calculated by NAMD Energy Plugin (Version 1.3) which was implemented in VMD program package.[17] The electrostatic interaction was not taken into account in calculations of the interaction energy between poly(rC) and SWCNT because atoms of the nanotube were not charged. In such a case, vdW interaction determined the energy of the oligonucleotides binding to the nanotube.

Results and Discussion

Spectroscopic Study of Poly(rC)-Wrapped SWCNTs Aggregates in Aqueous Suspension, Induced by Cationic Porphyrin

Addition of cationic porphyrin TMPyP4 into poly(rC):SWCNT aqueous suspension leads to the decrease of the total absorption of nanotubes because of the light scattering which appeares as a result of the aggregate formation (Figure 1). As follows from Figure 1, the absorption is weakened with the porphyrin concentrations increase while in the 400–450 nm range an additional band appears, which is assigned to the Soret band of TMPyP4.

To analyze the electronic absorption of nanotubes in detail, we subtracted the background intensity which enhances to short wavelengths and is caused by the broadband π-plasmon absorption and possible residual carbonaceous impurities.[18] The nanotube absorption spectrum in the 900–1100 nm range is assigned to the E_{11}^{S} absorption transitions of semiconducting nanotubes while bands in the 450–700 nm spectral range attributed to the E_{22}^{S} transitions. The nanotube absorption spectrum in the E_{11}^{S} transition range was fitted by a sum of 7 Lorentzians which are shown in Figure 2a. The most intensive band at 998 nm is assigned to the E_{11}^{S} transition of (6,5) semiconducting nanotubes, which dominates in the pristine material. Peak positions of the neighbor bands and associated chirality of carbon nanotubes were determined too the value of which is presented in Figure 2a.[19] We controlled the dependence of the intensity of different

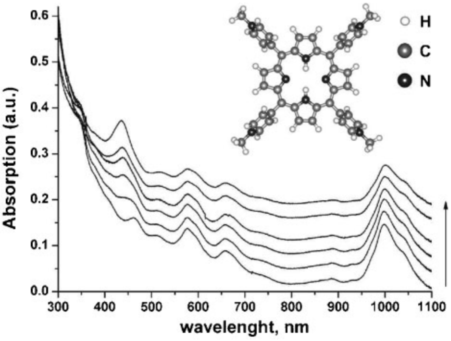

Figure 1.
Absorption spectra of poly(rC):SWCNTs water suspension at different TMPyP4 concentrations (0, $8.8 \cdot 10^{-7}$, $1.9 \cdot 10^{-6}$, $3.9 \cdot 10^{-6}$, $7.9 \cdot 10^{-6}$, $1.6 \cdot 10^{-5}$ M) in the 300–1100 nm range. Arrows indicate the change of the band intensity with the porphyrin concentration increases. The structure of TMPyP4 molecule was shown in insert.

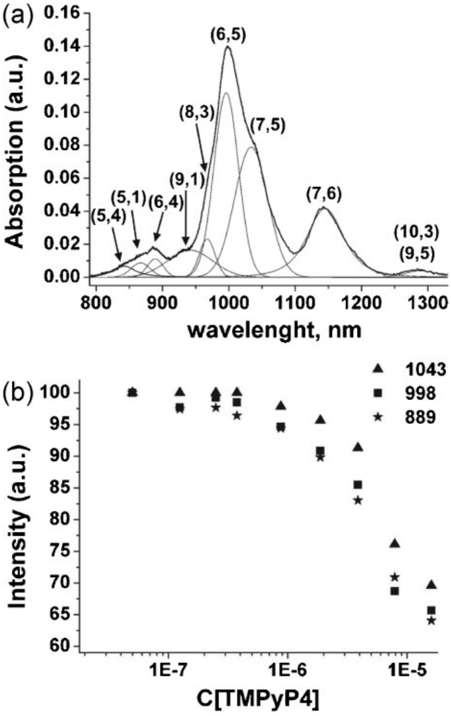

Figure 2.

Absorption spectrum of poly(rC):SWCNTs water suspension in the range 800–1300 nm (thin curves show Lorentzians used for fitting to the experimental spectrum) (a), and intensity dependences of three bands (peak positions are indicated in insert) on the TMPyP4 concentration (b).

bands on porphyrin concentrations to elucidate the possible influence of tube chirality which can appear due to the different adsorption energies of TMPyP4 on the different nanotube species. Figure 2b demonstrates that the (6,5) nanotube band intensity decreases by 35% at the TMPyP4 concentration about 10^{-5} M. The similar intensity decrease was observed for (7,5) and (8,3) nanotubes. It means that the nanotube selection under the aggregate formation is not occurred. Note that the red-shift of bands takes place up to 4–5 meV at the TMPyP4 concentration about 10^{-5} M. A small value of the spectral shift indicates that the nanotubes do not aggregate in usual bundles the formation of which is accompanied with a more significant red shift (more than 70 meV[20]). The small red shift of nanotube bands can be explained by the partial sliding down of the polymer from the nanotube surface as a result of the interaction with TMPyP4 or because of the close location of other nanotubes, which is accompanied with the partial polymer displacement. As a result, water molecules gain access to the nanotube surface, changing its dielectric surrounding, and this leads to the red shift of the nanotube electronic transition.[4,6]

Knowledge about the TMPyP4 interaction with the poly(rC):SWCNT hybrid and its role in the aggregate formation can be obtained from the analysis of the intensity and spectral position of the Soret band at different TMPyP4 concentrations. It is well known that the Soret band is assigned to the π-π transition of porhyrin core electrons[21] and it is sensitive to the environment.[22] The Soret band was separated from the total absorption spectrum at each porphyrin concentration (Figure 3) by the deconvolution procedure. It was found that at the lowest TMPyP4 concentration ($\sim10^{-7}$) this band is located at 439.6 nm.

It means that the spectral shift of the Soret band is $\sim$18 nm, relatively to this band position of the porphyrin monomer (421.8 nm) (Figure 3). However, this band is blue-shifted with TMPyP4 concentration

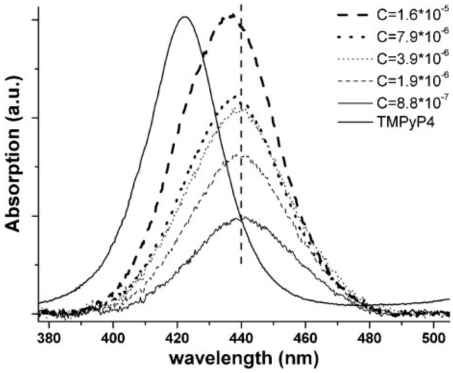

Figure 3.

Spectra of the Soret band of TMPyP4, obtained at different porphyrin concentrations (indicated in the insert) in suspension with SWCNTs. Spectra were separated from total absorption spectra for various porphyrin concentrations, using a spectral deconvolution.

rises, so the Soret band has a peak at 435.8 nm at $1.6 \cdot 10^{-5}$ TMPyP4 concentration. We believe that at low concentrations TMPyP4 is adsorbed on the nanotube surface with a relatively high binding energy (the maximal red shift). Then at higher concentrations, when free spaces on SWCNTs are filled in, TMPyP4 binds to the nitrogen base of poly(rC) (a lower binding energy than with the nanotube). Thus, the occupation of the second binding site on poly(rC) results in the blue shift of the Soret band at higher TMPyP4 concentrations. This statement is confirmed with the determination of the peak position of the TMPyP4 Soret band in different environments, the value of which is presented in Table 1. So, the Soret band of water solution of poly(rC):TMPyP4 complex is located at 431.9 nm (~10 nm red shift in relation to free TMPyP4) and at 435 nm for TMPyP4 adsorbed to SWCNTs directly in the dried state (~13 nm red shift).

Molecular Dynamics Simulation

We simulated the aggregation of $r(C)_{30}$–wrapped nanotubes induced by 4 molecules of TMPyP4 arranged in the start configuration presented in Figure 4. After simulation during 30 ns (at 300 K) the stable conformation of aggregate was got, though there is some divergence of axes of nanotubes (Figure 4). Molecular dynamics simulation demonstrates that in the formation of such unique complexes TMPyP4 molecules perform two functions: first, porphyrins couple the polymer:nanotube hybrids, second, they strengthen the polymer coverage of nanotubes because the porphyrin is adsorbed between neighbor pitches of the polymer wrapped, “stitching” them together that results in the aggregate continuous floatation in the aqueous medium. Such an effective aggregation process is caused with different types of TMPyP4 binding with the nanotube and polymer: through the π-π-stacking interaction of the porphyrin core with the nitrogen base (the binding energy is ~15 kcal/mol) or with the nanotube surface (the binding energy is ~30 kcal/mol) free from polymer as well as with the electrostatic interaction between the cationic group of N-methylpyridyl ring and the negatively charged phosphate group of $r(C)_{35}$ (the binding energy is ~60 kcal/mol). Therefore, it is reasonable to assume that, initially at low concentrations, TMPyP4 is adsorbed on the nanotube surface free from the polymer with a relatively high binding energy (the maximal spectral red shift). Then at

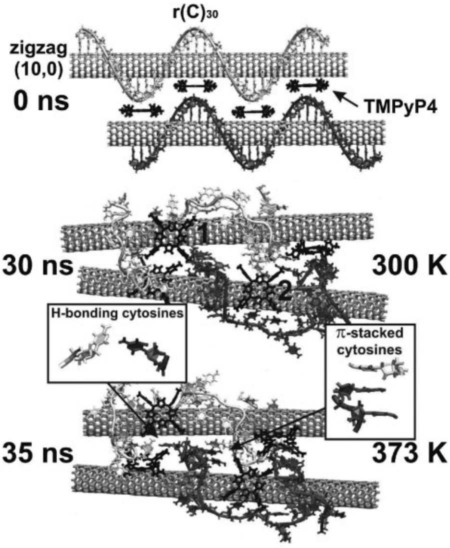

Figure 4.
Snapshot of two parallel $(rC)_{30}$-wrapped SWNCTs with four TMPyP4 molecules at starting position (0 ns) and during different simulation times: after 30 ns (300 K), after 35 ns (373 K). π-stacked cytosines and H-bonding cytosines of two oligonucleotides are shown in the insert. Two TMPyP4 molecules located between negatively charged $(PO_4)^-$ groups of each oligonucleotide are marked with **1** and **2.**

Table 1.
Peak position (nm) and width (Δ, nm) (at half of height) of the Soret band of TMPyP4, obtained in different environments (in aqueous suspensions and film).

Sample	Peak position, nm	Δ, nm
TMPyP4	421.8	26
poly(rC):TMPyP4	431.9	29
SWCNT:TMPyP4 (film)	434.5	34
SWCNT:poly(rC):TMPyP4 ($8.8 \cdot 10^{-7}$)	439.6	35
SWCNT:poly(rC):TMPyP4 ($1.6 \cdot 10^{-5}$)	435.8	37

higher concentrations, when free spaces on SWCNTs are filled in, TMPyP4 binds with cytosines (the binding energy is lower than that with the nanotube). Thus, the activation of the second binding site on poly(rC) results in the blue shift of the Soret band at higher TMPyP4 concentrations.

At increasing temperature of the system to 373 K, further simulation during next 5 ns (total simulation was 35 ns) showed that the aggregate did not dissociate. The oligomers remained on the tube, although their conformation on the tube was changed. So, heating of the system is accompanied with the compact arrangement of oligomer on the nanotube and with the formation of U-like structures the appearance of which is caused the higher level of the oligomer flexibility. At heating the oligomer binding to other nanotube surface increases and partial displacement of the oligomer from one nanotube to another because of nanotubes close location is observed.

At higher temperature the new contacts between $r(C)_{30}$ and neighbor SWCNTs are created that appear due to the partial displacement of oligomer partly from one nanotube toward another tube (Figure 4): it is the π-π-stacking of cytosine of one oligomer with neighbor nanotube (~12 kcal/mol), there is a formation of stacking dimer (or even trimer (Figure 4)) cytosines of two oligomers (~8 kcal/mol) or an appearance of H-bonding dimer (~3 kcal/mol) (Figure 4). Although, in some parts the oligomer is shifted from the basic tube to nearby SWCNT, nevertheless, porphyrins located between two pitches of the oligomer restrain their sliding off from the nanotube.

Conclusion

Doping of poly(rC)-wrapped SWCNTs aqueous suspension with cationic porphyrin TMPyP4 in a small doze (1 μM) leads to nanotube aggregation a visible manifestation of which can be observed at concentrations above 10 μM. These aggregates have an unusual feature because their stable suspension remains during a few days without quick precipitation that differs from the conventional aggregates of carbon nanotube. In the absorption spectrum the Soret band of TMPyP4 demonstrates the red shift at doping with respect to the free porphyrin. This shift has the maximal value at the lowest porphyrin concentration (~18 nm) and is followed with the gradual decrease of this value at increasing concentrations. In aggregates the nanotube absorption intensity is weakened and their band positions are red-shifted with the porphyrin concentration increasing but the value of this shift is essentially smaller than that observed earlier at the formation of the usual bundles.[20] The absorption intensity decrease in different bands assigned to different nanotube species is similar. It means that the nanotube selection is not occurred under the aggregate formation.

Molecular dynamics simulation demonstrates that in the formation of such unique complexes TMPyP4 molecules perform two functions: first, porphyrins couple polymer-nanotube hybrids owing to π-π-stacking with cytosine and through charged groups with phosphate groups of the polymer, second, they strengthen the polymer coverage of nanotubes because porphyrin adsorbed between neighbor pitches of wrapped polymer "stitches" them together that results in the aggregate floatation in water. It was shown that nanotube aggregate is stable even at 373 K. At this temperature additional contacts between two neighboring polymers appear which strengthen this aggregate too.

This controlled aggregation of DNA-wrapped carbon nanotubes gives new possibility in preparing of isolated nanotubes of high density in water environment which can be applied in near-IR photophysics, for developing light-energy harvesting and photovoltaic materials.

Acknowledgment: We are grateful to Dr. A.M. Plokhotnichenko for helpful discussion and V.S. Leontiev for sample preparation and characterization. The authors acknowledge the Computational

Centers at B. Verkin Institute for Low Temperature Physics and Engineering National Academy of Sciences of Ukraine for providing computer time.

[1] M. Kwak, J. Gao, D. K. Prusty, A. J. Musser, V. A. Markov, N. Tombros, M. C. A. Stuart, W. R. Browne, E. J. Boekema, G. Brinke, H. T. Jonkman, B. J. van Wees, M. A. Loi, A. Herrmann, *Angew. Chem. Int. Ed.* **2011**, *50*, 3206.

[2] M. Zheng, A. Jagota, E. Semke, B. Diner, R. Mclean, S. Lustig, R. Richardson, N. Tassi, *Nature Mater.* **2003**, *2*, 338.

[3] A. Star, E. Tu, J. Niemann, J. P. Gabriel, C. S. Joiner, C. Valcke, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 921.

[4] V. A. Karachevtsev, G. O. Gladchenko, M. V. Karachevtsev, A. Yu. Glamazda, V. S. Leontiev, O. S. Lytvyn, U. Dettlaff-Weglikowska, *Mol. Cryst. Liq. Cryst.* **2008**, *497*, 339.

[5] V. A. Karachevtsev, M. V. Karachevtsev, V. S. Leontiev, O. S. Lytvyn, A. T. Bogorosh, *Fullerenes, Nanotubes, Carbon Nanostruct.* **2010**, *18*, 531.

[6] P. W. Barone, M. S. Strano, *Angew. Chem. Int. Ed.* **2006**, *45*, 8138.

[7] R. J. Chen, Y. Zhang, *J. Phys. Chem. B* **2006**, *110*, 54.

[8] R. F. Pasternack, C. Bustamante, P. J. Collngs, A. Giannetto, E. J. Gibbs, *J. Am. Chem. Soc.* **1993**, *115*, 5393.

[9] V. A. Karachevtsev, E. S. Zarudnev, S. G. Stepanian, A. Yu. Glamazda, M. V. Karachevtsev, L. Adamowicz, *J. Phys. Chem. C* **2010**, *114*, 16215.

[10] F. D'Souza, S. K. Das, M. E. Zandler, A. S. D. Sandanayaka, O. Ito, *J. Am. Chem. Soc.* **2011**, *133*, 19922.

[11] E. S. Zarudnev, A. M. Plokhotnichenko, V. S. Leontiev, I. A. Levitsky, V. A. Karachevtsev, *J. Mater. Chem.* **2012**, *22*, 10795.

[12] W. E. Alvarez, F. Pompeo, J. E. Herrera, L. Balzano, D. E. Resasco, *Chem. Mater.* **2002**, *14*, 1853.

[13] V. A. Karachevtsev, A. M. Plokhotnichenko, M. V. Karachevtsev, V. S. Leontiev, *Carbon* **2010**, *48*, 3682.

[14] C. Phillips, R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R. D. Skeel, L. Kale, K. Schulten, *J. Comput. Chem.* **2005**, *26*, 1781.

[15] N. Foloppe, A. D. MacKerell, Jr., *J. Comput. Chem.* **2000**, *21*, 86.

[16] J. Rajendra, A. Rodger, *Chem. Eur. J.*, **2005**, *11*, 4841.

[17] W. Humphrey, A. Dalke, K. Schulten, *J. Molec. Graphics* **1996**, *14*, 33.

[18] A. V. Naumov, S. Ghosh, D. A. Tsyboulski, S. M. Bachilo, R. B. Weisman, *ACS Nano* **2011**, *5*, 1639.

[19] C. Fantini, V. Jorio, A. P. Santos, V. S. T. Peressinotto, M. A. Pimenta, *Chem. Phys. Lett.* **2007**, *439*, 138.

[20] S. K. Doorn, *J. Nanosci. Nanotechnol.* **2005**, *5*, 1023.

[21] M. Gouterman, in: "*The Porphyrins*" (Ed., D. Dolphin, Academic Press, New York **1978**.

[22] P. Kubat, K. Lang, P. Janda, O. Frank, I. Matulkov, J. Sykora, S. Civis, M. Hof, L. Kavan, *J. Nanosci. Nanotechnol.* **2009**, *9*, 5795.