J. Nano- Electron. Phys.
3 (2011) No1, P. 776-782

PACS numbers: 87.14.gk, 61.48.De

A NANO-BIOSENSOR FOR DNA SEQUENCE DETECTION USING ABSORPTION SPECTRA OF SWNT-DNA COMPOSITE

J. Bansal, I. Singh, N. Mathur, A. Aneja, P.K. Bhatnagar, P.C. Mathur

University of Delhi South Campus,
Benito Juarez Road, New Delhi-21, INDIA
E-mail: jyoti_2681986@yahoo.com

A biosensor based on Single Walled Carbon Nanotube (SWNT)-Poly $(GT)_n$ ssDNA hybrid has been developed for medical diagnostics. The absorption spectrum of this assay is determined with the help of a Shimadzu UV-VIS-NIR spectrophotometer. Two distinct bands each containing three peaks corresponding to first and second van Hove singularities in the density of states of the nanotubes were observed in the absorption spectrum. When a single-stranded DNA (ssDNA) having a sequence complementary to probic DNA is added to the ssDNA-SWNT conjugates, hybridization takes place, which causes the red shift of absorption spectrum of nanotubes. On the other hand, when the DNA is noncomplementary, no shift in the absorption spectrum occurs since hybridization between the DNA and probe does not take place. The red shifting of the spectrum is considered to be due to change in the dielectric environment around nanotubes.

Keywords: *BIOSENSOR, DNA, SWNT, ABSORPTION, EXCITON BINDING ENERGY, CHIRALITY.*

(Received 04 February 2011, in final form 14 October 2011)

1. INTRODUCTION

Medical Diagnostics based on DNA sequence detection has recently become very popular because it is a low cost, fast and reliable technique requiring a very small amount of DNA without requiring polymerase chain reaction (PCR). The basic principle of DNA sequence detection is that the ssDNA to be tested must first be immobilized on a suitable target with the help of chemical adsorption [1, 2], covalent bonding [3], electrostatic attraction [4, 5] or co-polymerization [6, 7] and the immobilized ssDNA must produce a signal, capable of being detected and analyzed. This signal should change significantly when ssDNA to be tested is hybridized with a complementary ssDNA. The testing method should be such that extensive sample pretreatment and derivatization are not needed; that is the quantity of sample required for testing is ultra low (~ 10^{-8} - 10^{-7} moles) so that the detection method is robust and inexpensive. Also, the sensor should be portable and of small dimensions. However, the most important feature of a DNA sensor is its ability to discriminate between complementary (cDNA) and non-complementary DNA (nDNA) samples. The most commonly used analytical signals for DNA hybridization are electrochemical [8] and piezoelectric [9]. Optical detection, particularly based on absorption from solution-based system is advantageous due to high sensitivity and selectivity.

In the present work, we have developed a simple technique for DNA sequence detection using optical absorption properties of single walled carbon

nanotubes (SWNTs). The optical absorption in SWNTs is dominated by the transitions between van Hove singularities located symmetrically on the opposite sides of the Fermi level. The absorption spectrum of semiconducting SWNTs is in the near infrared (NIR) region and therefore this biosensor can be conveniently used in biological systems because absorption of blood and tissues in NIR region is very low. Yet, other advantages of SWNTs based biosensors are that labeling of DNA strands with dyes is not required as is the case with Fluorescence resonance energy transfer (FRET) based biosensors [10,11]. Besides this the SWNTs are resistant to photo bleaching and can therefore have a higher operating life as compared to other biosensors. The main obstacle in using SWNTs in biosensors [12] and nanoprobes [13] is their poor solubility in any organic or aqueous solvent. Further, their properties are strongly dominated by their physical characteristics such as diameter, length and most importantly chirality (n, m).

The interaction mechanism of DNA-SWNT hybrid is the following: Both SWNT and ssDNA are hydrophobic in nature (only one surface of ssDNA is hydrophobic while the other surface is hydrophilic [14]). As a result of their hydrophobic nature, the ssDNA segment is very quickly adsorbed and wrapped (on a time scale of few hundred pico seconds) [14] on the SWNT surface, which is individually dispersed by the adsorbing DNA molecules. However, it is known that only ssDNA sequences of repeats of alternating G and T i.e. poly $(GT)_n$ with $n \geq 10$ can efficiently disperse SWNTs [15].

The principle of DNA –SWNT sensor is the following: A ssDNA (probic DNA) is tagged to the poly $(GT)_n$ sequence and the resulting DNA sequence i.e. poly $(GT)_n$ –probic DNA is used to disperse SWNTs in the aqueous solution. The probic DNA binds itself non-covalently to the surface of SWNTs through π stacking [16]. As the experimental ssDNA (having sequence complementary to that of probic DNA – cDNA) is added to this solution, it hybridizes itself with the probic DNA. The hybridization of cDNA results in a decrease of surface area of SWNTs exposed to the aqueous dispersing medium and hence decreases the effective dielectric constant of the SWNT environment. The exciton binding energy of SWNTs is very sensitive to the dielectric constant of the ambient in which SWNTs are kept. The exciton binding energy increases with the decrease in the dielectric constant of the local environment [17] due to increase of electrostatic interaction between quantum mechanically confined electrons and holes. This results in decrease in optical band gap (E_{ii}) of SWNTs due to which absorption peaks of SWNTs get red shifted in accordance with the relation [18],

$$E_{ii} = E_{ii}^{gr} + E_{ii}^{ee} - E_{ii}^{eh}$$

where E_{ii}^{gr} is the self-energy of an electron due to the repulsive interaction between electrons; E_{ii}^{ee} represents the energy of repulsive interaction between electrons; and E_{ii}^{eh} denotes the binding energy of an exciton.

We have utilized this change in optical transition energy due to DNA wrapping in determining a known DNA sequence corresponding to antigen Escherichia coli. We have studied the absorption spectrum of SWNTs by dispersing them with probic DNA in the presence of cDNA and nDNA separately. Using SWNT of different chiralities we have found that the red shifting of the absorption peaks is highly sensitive to the chirality of the SWNT sample.

2. MATERIALS AND METHODS

The as grown SWNT samples and ssDNA primers were commercially procured from Sigma Aldrich, USA. The procured SWNT samples have diameter ranging from 0.7 nm to 1.2 nm and length upto 20 μm. The sequences of procured ssDNA samples are as shown in table 1.

Table 1 *– Oligonucleotides used in the experiment*

S. No.	Sample	Sequence
1	ssDNA probe	5'-$(GT)_{15}$–(GTAAATGGTGTTAGGGTTGC)-3' (50 mer)
2	cDNA	5'-(GCAACCCTAACACCATTTAC)-3' (20 mer)
3	nDNA	5'-(CGTTGGGATTGTGGTAAATG)-3' (20 mer)

For preparing ssDNA-SWNT hybrid, an aqueous solution containing probic ssDNA with concentration 1 mg/ml was prepared in TRIS buffer (pH 7.4). To this solution, SWNTs were added keeping their concentration as 1 mg/ml and the resultant solution was ultrasonicated for about 2 hrs. to obtain a homogeneous suspension of SWNTs in the solution. This solution was then centrifuged for 2 hrs at 15,000 g in a microcentrifuge to remove nondispersible SWNTs and other accompanying impurities. The UV-VIS-NIR absorption spectrum of this solution was studied using Shimadzu Spectrophotometer Model Solid Spec 3700. To this solution, cDNA and nDNA primers were added separately and their effect on the absorption spectrum of the SWNTs was studied after annealing the solutions at 50 °C for about 30 min. The annealing improves hybridization of DNA and the temperature and duration of annealing were optimized after many trials and errors.

3. RESULTS & DISCUSSION

The absorption spectrum of the SWNTs dispersed in poly $(GT)_n$ probic ssDNA primer solution is shown in Fig. 1. We have used this absorption spectrum to determine the band gap energies between van Hove singularities of the nanotubes present in the given sample. Two dominant sets of three peaks corresponding to the transitions between first (λ_{11}) and second (λ_{22}) van Hove singularities are observed in the spectrum. The first set of peaks corresponding to first valance band level (V_1) and first conduction band level (C_1) transitions are observed at 976, 1178 and 1388 nm. Another three peaks corresponding to second-order valence band level (V_2) and second order conduction band level (C_2) transitions are observed at 540, 570 and 800 nm. The three V_1-C_1 and V_2-C_2 peaks correspond to three different semiconducting nanotubes having different diameters in the solution.

To determine the chiralities and diameters of these semiconducting nanotubes, we have used the experimental data on optical transitions studied by Weisman and Bachilo [19] on 33 semiconducting SWNT samples having different diameters and chiralities. Using the experimental values and data of Weisman and Bachilo for λ_{11} and λ_{22}, we have obtained the value of diameter and chirality of our samples which are shown in Table 2.

The effect of hybridization of cDNA on the probic DNA is shown in Fig. 2. A red shift of 20 nm in absorption peak of (10, 0) tube is observed from 1178 to 1198 nm). The reason for this red shift is attributed to the

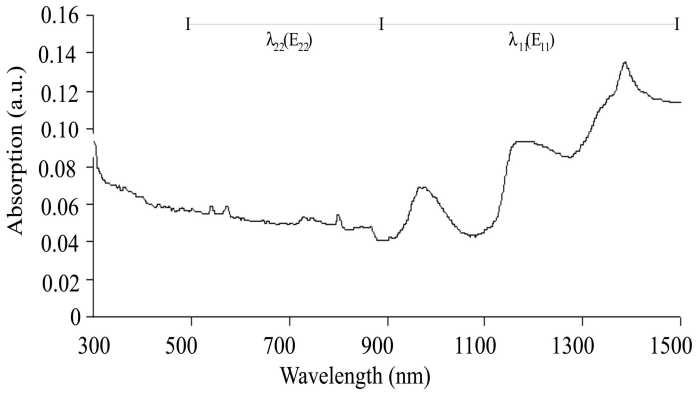

Fig. 1 *– Absorption spectrum of SWNTs dispersed in poly $(GT)_n$ – probic DNA aqueous solution*

Table 2 *– Estimation of diameter and chirality of nanotubes corresponding to observed absorption peaks using absorption spectrum*

S. No.	λ_{11} (nm)	λ_{22} (nm)	Diameter (nm)	Chirality (n,m)	Chiral Angle $\theta = \tan^{-1}(\sqrt{3}\, m/(2n+u))$	Type(2n+m) mod3
1	976	570	0.757	(6,5)	26	II
2	1178	540	0.794	(10,0)	0	II
3	1388	800	1.170	(9,8)	28	II

denser coverage of SWNT surface by DNA oligonucleotides. Due to DNA hybridization on the surface of nanotubes, the surface gets more covered and its contact with water gets reduced. This results in decrease in dielectric constant of the local environment of SWNTs, which increases the exciton binding energy in SWNTs due to enhancement of coloumbic interactions. The increase in exciton binding energy is the main cause of red shifting of absorption peak of (10, 0) nanotube. Therefore, this shift in absorption can be considered as a measure to analyze DNA hybridization on the SWNT surface.

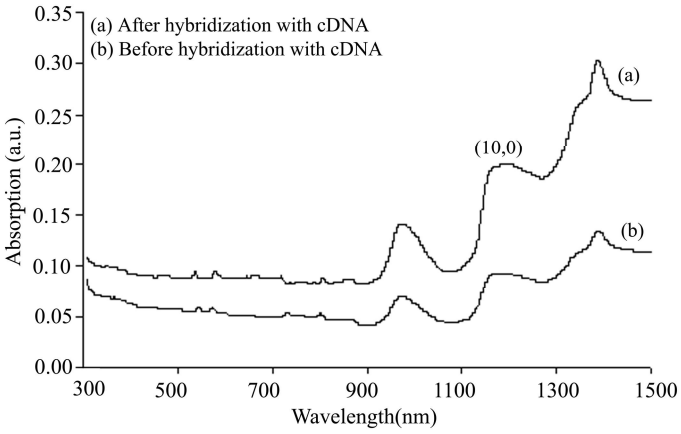

Fig. 2 *– Absorption spectra of probic DNA-SWNT hybrid with and without cDNA*

We have also studied the effect of addition of nDNA on the absorption spectrum of SWNTs (fig. 3). In this case a negligible red shift of 2 nm is observed in the absorption peak of (10, 0) nanotube. Therefore, this DNA-SWNT hybrid system can be effectively used to detect any particular DNA sequence.

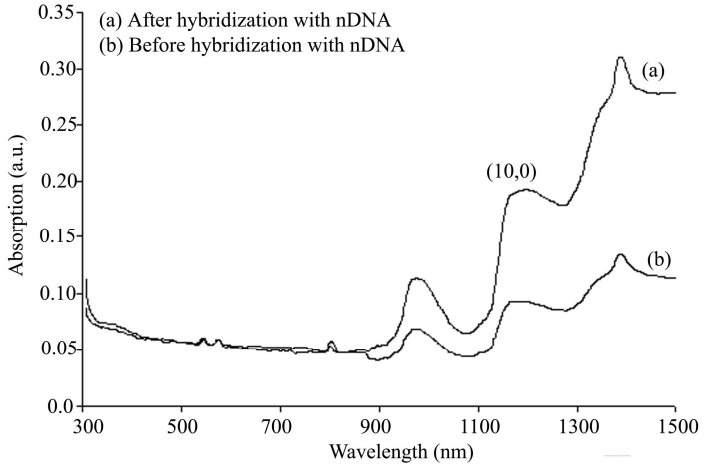

Fig. 3 *– Absorption spectra of probic DNA-SWNT hybrid with and without nDNA*

It is further observed that the effect of cDNA hybridization on SWNT surface is not the same for all the three observed nanotubes. While for (10, 0) tube a red shift of 20 nm is observed, the shift reduces to 4 nm for (6, 5) nanotube and for (9, 8) tube very minor shift of 1 nm is observed (as shown in table 3). This discrepancy in the red shifting of absorption peaks is due to the different conformation of SWNTs [20]. The exciton binding energy of SWNTs is strongly dominated by the effective mass, which in turn is a function of chirality of the nanotubes.

Table 3 *– Shifting of first order absorption peaks of SWNTs due to cDNA hybridization*

S.No.	Before Hybridization		After Hybridization		$\Delta E_{11} = E_{11} - E'_{11}$ (eV)	Chirality (n, m)	Angle (θ°)	Diameter (nm)
	λ_{11} (nm)	E_{11} (eV)	λ'_{11} (nm)	E'_{11} (eV)				
1	976	1.27	980	1.2653	0.0047	(6,5)	26	0.757
2	1178	1.052	1198	1.035	0.017	(10,0)	0	0.794
3	1388	0.8933	1389	0.8927	0.0005	(9,8)	28	1.17

The cDNA hybridization also results in shifting of second order absorption peaks of SWNTs. The shifting pattern of λ_{22} peaks is quite interesting. While for (6, 5) and (9, 8) nanotubes a red shift of 2 nm is observed, for (10, 0) nanotube a blue shift of 6nm is observed as shown in

Table 4. The observed behavior of peak shifting can be related to the chiral angles of the nanotubes. Ohno et al. [20] have studied the effect of chirality & diameter of the nanotubes on the change in optical band gap E_{11} and E_{22} (corresponding to λ_{11} and λ_{22}) due to change in dielectric constant of local environment of the SWNTs for type I and type II SWNTs separately. They have found that for family I SWNTs that ΔE_{11} increases with increasing chiral angles while for family II SWNTs the trend is opposite i.e. ΔE_{11} decreases with increasing chiral angles. It is further shown by these authors that for family II tubes for lower chiral angles (till ~ 15 °) E_{22} peaks gets blue shifted with decrease in dielectric constant and for higher angles a red shift of E_{22} peaks is observed. Our results (family II) are in agreement with Ohno et al. [20] results. In our case, for (10, 0) nanotube the chiral angle is 0° and therefore a large shifting of 20nm is observed in E_{11} peak while for other two tubes the estimated chiral angles are 26° and 28° that's why a lesser peak shift is observed. Further, for E_{22} peaks, the absorption peak of (10, 0) tube (chiral angle 0°) gets blue shifted by 6 nm and for other two tubes (26° and 28° angle) peaks are red shifted (Table 4).

Table 4 – *Shifting of second order absorption peaks of SWNTs due to cDNA hybridization*

S.No.	Before Hybridization		After Hybridization		$\Delta E_{22} = E_{22} - E'_{22}$ (eV)	Chirality (n, m)	Angle $(\theta°)$	Diameter (nm)
	λ_{22} (nm)	E_{22} (eV)	λ'_{22} (nm)	E'_{22} (eV)				
1	570	2.1754	572	2.1678	0.0076	(6, 5)	26	0.757
2	540	2.296	534	2.322	-0.026	(10, 0)	0	0.794
3	800	1.55	802	1.546	0.0097	(9, 8)	28	1.17

A possible explanation for these observations was given by J. Jiang et al. [21]. These authors said that dependence of chiral angle on the exciton binding energy in SWNTs comes via an effective mass m^* so that binding energy is proportional to $(m^*)^{\alpha - 1}$ where α is an empirical parameter.

4. CONCLUSION

It is concluded that DNA-SWNT hybrid system can be used as an efficient bio-sensing probe to detect a known antigen. The sensitivity of the sensor is strongly dominated by the chirality of SWNTs. While the high chiral angled tubes do not give any significant signal for cDNA hybridization, the low chiral angled SWNTs have proved themselves a promising candidate for DNA sensing.

REFERENCES

1. P.W. Barone, S. Baik, D.A. Heller, M.S. Strano, *Nat. Mater.* **4**, 86 (2005).
2. A. Star, E. Tu, J. Niemann, J.C.P. Gabriel, C.S. Joiner, C. Valcke, *PNAS* **103(4)**, 921 (2006).

3. H. Gao, Y. Kong, *Annu. Rev. Mater. Res.* **34**, 123 (2004).
4. S. Han, D. Ralin, J. Wang, X. Li, F. Zhou, *Langmuir* **19**, 8943 (2003).
5. J.M. Jaklevic, H.R. Garmer, G.A. Miller, *Annu. Rev. Biomed. Eng.* **01**, 649 (1999).
6. A.V. Vasiliskov, E.N. Timofeev, S.A. Surzhikov, A.L. Drobyshev, V.V. Shick, A.D. Mirzabekov, *Biotechniques* **27**, 592 (1999).
7. Hornes et al: *Oligonucleotide linked magnetic Particles and uses thereof*. US Patent 5, 512, 439, 1996.
8. J. Li, H.T. Ng, A. Cassell, W. Fan, H. Chen, Q. Ye, J. Koehne, J. Han, M. Meyyappan, *Nano Lett.* **3(5)**, 597 (2003).
9. M. Minunni, S. Tombelli, E. Mariotti, M. Mascini, *Fresenius J. Anal. Chem.* **369**, 589 (2001).
10. N. Mathur, A. Aneja, P.K. Bhatnagar, P.C. Mathur, *J. of Sensors* **2008**, 1 (2008).
11. A. Aneja, N. Mathur, P.K. Bhatnagar, P.C. Mathur, *J. Biol. Phys.* **34**, 487 (2008).
12. H. Cai, X. Cao, Y. Jiang, P. He, Y. Fang, *Anal. Bioanal. Chem.* **375**, 287 (2003).
13. H.J. Dai, J.H. Hafner, A.G. Rinzler, D.T. Colbert, R.E. Smalley, *Nature* **384**, 147 (1996).
14. X. Zhao, K. Johnson (http://puccini.che.pitt.edu/~karlj/Papers/DNA-SWNT.pdf).
15. M. Zheng, A. Jagota, M.S. Strano, A.P. Santos, P. Barone, G. Chou, B.A. Diner, M.S. Dresselhaus, R.S. Mclean, G.B. Onoa, G.G. Samsonidze, E.D. Semke, M. Usrey, D.J. Walls, *Science* **302**, 1545 (2003).
16. M. Zheng, A. Jagota, E.D. Semke, B.A. Diner, R.S. Mclean, S.R. Lusting, R.E. Richardson, N.G. Tassi, *Nat. Mater.* **2**, 338 (2003).
17. E.S. Jeng, A.E. Moll, A.C. Roy, J.B. Gastala, M.S. Strano, *Nano Lett.* **6**, 371 (2006).
18. Y. Ohno, S. Maruyama (http://www.intechopen.com/articles/show/title/).
19. R.B. Weisman, S.M. Bachilo, *Nano Lett.* **3**, 1235 (2003).
20. Y. Ohno, S. Iwasaki, Y. Murakami, S. Kishimoto, S. Maruyama, T. Mizutani, *Phys. Rev. B* **73**, 235427 (2006).
21. J. Jiang, R. Saito, G.G. Samsonidze, A. Jorio, S.G. Chou, G. Dresselhaus, M.S. Dresselhaus, *Phys. Rev. B* **75**, 035407 (2007).