



## Characterizing DNA Assisted Dispersion & DNA-SWNTS Hybrids Using Photoluminescence

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### ARTICLE INFO

#### Article history:

Received: 12 May 2015;

Received in revised form:

2 June 2015;

Accepted: 8 June 2015;

#### Keywords

Single Wall Carbon Nanotube (SWNTs), 1-Dimension (1-D) Carbon Nanotubes (CNTs), Single stranded DNA (ssDNA), Photoluminescence (PL).

### ABSTRACT

Inherent dominating Coulombic interaction of the man-made 1-D carbon nanotubes is influenced by the biotic nanomaterial namely Deoxyribonucleic acid (DNA) that staunchly binds to the SWNTs surface and thus modifies the electronic structure of Single Wall Carbon Nanotube (SWNTs), as noticed by the optical process of Photoluminescence (PL) using Spectrofluorophotometer. This modification finds several applications in various disciplines & one of its applications of DNA sequence detection in field of biotechnology is observed in present work.

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### Introduction

Novel properties of 1-Dimension nanostructure when unify with another nanomaterial result in idiosyncratic characteristics and properties which can be utilized in variety of applications. Carbon Nanotubes (CNTs) are 1-D man made material that are hydrophobic in nature and poorly soluble in the aqueous and non-aqueous solution. Based on geometry it can be either metallic or semiconducting. CNTs evince stupendous electronic, mechanical and optical properties which can be controlled by transmuting its dimensions and chirality. But, the entanglement of CNTs due to strong van der waal interaction impedes them from utilizing their potential. To utilize their potential several techniques have been proposed to separate them with minimal deterioration[1-3].

Plenty of methods have been developed for disunion of the clustered CNTs. Like Physical approach where ultra-sonication and high shear stress is applied onto the bundled CNTs. This consumes more time, shatters CNTs and results in poor stability of dispersed CNTs. Chemical (covalent) approach involves functionalization with various moieties covalently to modify the surface properties to enhance the solubility but its major snag is the disruption of  $\pi$ -conjugated system of CNT surface, defect creation and thus resulting into CNT destruction. On the other hand chemical (non-covalent) approach is simpler, cheap and non-destructive in which surfactant or dispersant is physically adsorbed onto the surface of cluster CNTs to separate them [4-7].

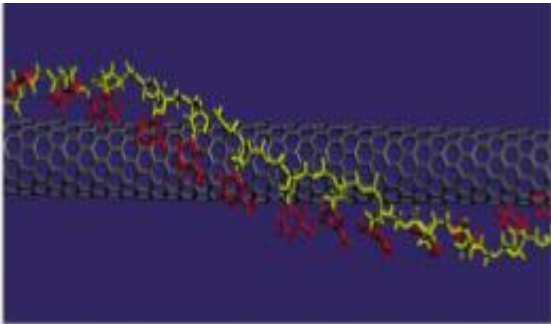
In the previous research work, combination of physical & chemical approach was utilized to disperse CNTs using various surfactants and effective results were observed [4]. Also in past, several biocompatible dispersants such as proteins, amino acids, carbohydrates, amylase and starch have been tested for segregation of bundled CNTs. Zheng and coworkers are the first to unveil that single stranded Deoxyribonucleic Acid (ssDNA) is a natural effective biological dispersant for CNTs in contrast to chemical surfactants [8].

DNA is an organic nanomaterial found in almost all living organisms that exists in double helical structure, where each strand is made of sugar & phosphate and consists of any or all of the four bases (Adenine, Guanine, Cytosine and Thymine). The base pairs of ssDNA are hydrophobic in nature and get adsorb onto the surface of single wall carbon nanotubes by attractive  $\pi$ - $\pi$  stacking interaction while sugar & phosphate group being hydrophilic in nature extends into the solution. Although, CNTs are poorly soluble and unstable in both aqueous and non-aqueous medium but ssDNA-CNT hybrids are chemically compatible and soluble in the aqueous solution. Binding of ssDNA to CNT is so ardent that removal of free DNA by either nuclease digestion or ion exchange column chromatography does not cause flocculation of CNTs even for months & this hybrid is rigid like rod [9-11].

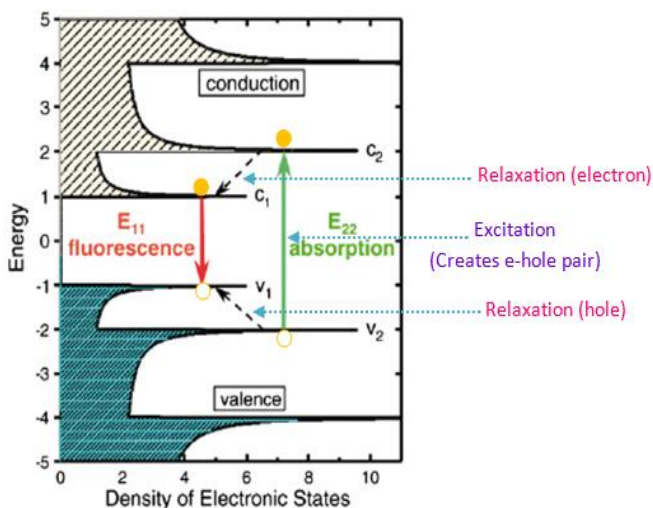
ssDNA can bind unto the CNT surface with linearly extended structure or helically wrapped around CNT with right or left handed turn as shown in Figure 1. Molecular dynamics, a computational technique discloses that soon after adsorption, ssDNA wrapped helically in few nanoseconds due to electrostatic and torsional interactions within the sugar-phosphate backbone from 3' end to 5' end. All of four bases undergoes the stacking interaction but stacking energy varies among bases as  $G > A > T > C$ . This is due to the fact that stacking energy increases with increase in interacting base pair surface area where G exhibits the largest surface area. Driving forces for ssDNA adsorption and helical wrapping arises due to ssDNA backbone but not from the specific base sequence. Thus, the general ssDNA (any sequence) wraps in the same way as ssDNA wraps here. Several experiments ensures that base-sequence determines the physical and chemical properties of the hybrid [11-13]

The negative charge on phosphate group of CNT-ssDNA hybrid results in negative charge density on surface of carbon nanotube. Metallic CNT-ssDNA hybrid carry less surface charge in comparison to semiconducting CNT-ssDNA because opposite

image charge is created in metallic tube. Difference in charge density helps to separate the metallic and semiconducting carbon nanotubes by ion-exchange liquid chromatography which is extremely difficult task in general [9, 14].



**Figure 1. Adsorption of ssDNA on SWNTs surface proposed by Zheng et al. [8]**



**Figure 2. Electronic structure of semiconducting SWNT [20]**

This unique biotic-abiotic nanostructure found applications in almost all disciplinary and interdisciplinary areas like biology, chemistry, biotechnology, biomedicine, bioelectronics, electronics where they are used:-for drug delivery, as biosensor for DNA sequencing & detecting harmful virus like (H1N1), as electrochemical sensor for enhancing the understanding of bio-interfacial phenomenon, for development of hybrid biomaterials, diagnosis the pathogenic and genetic disease, in biofuel cell to enhance the produced power, in Field effect transistor (FET) to enhance the chemical sensing, etc [15-19].

The density of states as a function of energy for semiconducting SWNTs is depicted in Figure 2 that contains van Hove singularity resulting from quantum confinement. When the electromagnetic radiation of suitable frequency or wavelength that matches the energy of an allowed transition between valence and conduction band falls on the SWNT, it gets absorbed and the e-hole pair is created.

The generated e-hole pair then recombines by giving its energy in the form of radiation, this process is referred as Photoluminescence (PL). In SWNTs the e-hole pairs are created by E22-absorption ( $v_2 \rightarrow c_2$ ), then electron relaxes to  $c_1$  and hole relaxes to  $v_1$ . Finally e-hole pair recombines through E11 transition (fundamental band gap of semiconducting SWNT) by giving rise to an electromagnetic radiation (PL) in NIR region (900-1600nm). The created e-hole pair with coulombic

attraction (bound e-hole) is referred as exciton. The coulombic attraction energy for SWNT ranges from 300-400 meV with an exciton size of few nm (2-5nm). During exciton life (before recombination that varies from 10-100ps), it diffuses along the SWNT surface (over distances of the order of 100-300 nm) and their motion can be easily influenced by perturbations of the chemical environment that makes SWNTs highly appreciable for sensing applications [20-24].

In the present work, SWNTs characterization and DNA hybridization detection are performed optically. For effective and efficient disunion of SWNTs agglomerates single stranded DNA (ssDNA) of specific sequence of particular length is taken, because dispersion is sequence and length sensitive so poly(GT)<sub>20</sub> is used for the required dispersion [24-26].

The ssDNA which is to be detected is referred as target DNA (t-ssDNA) and is attached to the poly(GT)<sub>20</sub>. Combined assembly of poly(GT)<sub>20</sub> and target ssDNA is referred as p-ssDNA.

After disunion of SWNTs, photoluminescence (PL) of the SWNT-p-ssDNA hybrid is taken which falls in near-infrared region (NIR). This PL of SWNTs were not affected by the presence of DNA because the DNA exhibits its PL in visible region. But, as complimentary single stranded DNA (c-ssDNA) it readily binds to the pDNA through hydrogen bonding between base pairs and shift in the PL was observed.

In contrast, no shift was found in PL under addition of non-complimentary single stranded DNA (nc-ssDNA) as it doesn't bind to pDNA and settle down at the bottom of solution. The shift in PL reflects the occurrence of DNA hybridization which confirms the utilization of discussed technique in bio-sensing application for detecting specific sequence of DNA or disease. Peaks at different wavelength in the PL evince the dispersion of SWNTs and dispersed SWNTs are characterised through the assistance of Weisman and Bachilo Table [27, 28].

So far several techniques have been utilized for DNA sequence detection, all of them demands ample quantity of DNA that requires Polymerase Chain Reaction (PCR) like in "Gel electrophoresis", "Frequency Resonance Energy Transfer (FRET)" which further demands the labelling with fluorescent dyes, "electrochemical techniques", etc.

But the present research has forced the medical practitioners to utilize another technique which is optical in nature and requires little amount of DNA (few nano-moles). It is faster, reliable and relatively cheaper. Also, CNT based DNA detection is free from photo-bleaching as it does not require any dye for labelling [29-32].

Optical Characterization specifically based on PL is advantageous due to high selectivity and sensitivity [25, 26].

## Materials and Methodology

### Reagents

Single Wall Carbon Nanotubes (SWNTs) semiconducting type, different types of single stranded DNA (ssDNA), TRIS buffer (biological buffer), deionized water (18MΩ-cm), etc were utilized in the experiment.

SWNTs were commercially acquired from cheap tubes (Brattleboro, VT, USA) whose diameter ranges from 1 to 2 nm whereas length varies from 5-30um whereas different types of single stranded DNA (ssDNA) are procured from sigma-Aldrich (St. Louis, MO, USA). The sequence of ssDNAs used in experiment are shown in the Table 1.

**Table 1. DNA samples used in experiment**

S No.	Sample	Sample Characteristic	DNA Samples Sequence
1.	poly (GT)	Single stranded DNA (ssDNA) (for dispersion of SWNTs)	5'-(GTGTGTGTGTGTGTGTGTGTGT)-3' (20 mer)
2.	t-ssDNA	Disease DNA which is to be detected	5'-(TAATGTGGTCGCGAT)-3' (15 mer)
3.	p-ssDNA	Combination of poly(GT) and t-ssDNA	5'(GT) <sub>20</sub> -(TAATGTGGTCGCGAT)-3' (35 mer)
4.	c-ssDNA	DNA complimentary to t-ssDNA	3'-(ATTACACCAGCGCTA)-5' (15 mer)
5.	nc-ssDNA	DNA not complimentary to t-ssDNA	3'-(GATTCATAAGCTCTC)-5' (15 mer)

### Sample Preparation and Apparatus

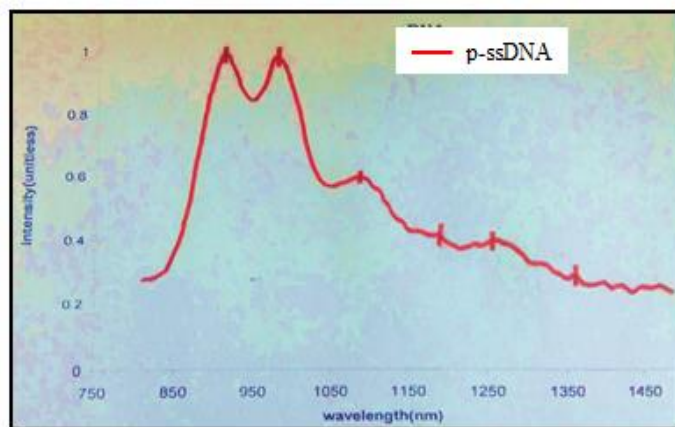
An aqueous solution of probic ssDNA was prepared by adding 5-nanomole of p-ssDNA in 2 ml of TRIS buffer (pH 7.4). Then, Bundled SWNTs (0.5mg) were added to the made solution. The resultant solution is then sonicated by probe type sonicator (Sonics Vibra Cell 20KHz $\pm$ 50Hz) at 2.25W of power for 20-30 mins to obtain the homogenous suspension of SWNTs in the solution. During sonication the solution (which was present in test tube) was kept in the ice beaker to prevent the degradation of DNA. After sonication, the solution was centrifuged for 30 min at the 10,000 rpm in a micro centrifuge to settle down the undispersed SWNTs and other accompanying impurities. The Photoluminescence of dispersed SWNTs was studied. To this dispersed solution c-ssDNA and nc-ssDNA were added separately and the resulting samples were annealed in order to improve the hybridization at 50°C for 30mins & their effects on PL were observed. Optimised process parameters were chosen in order to have effective results.

### Apparatus

Quartz cuvette, micro pipette, test-tubes, beaker, Electronic Weighing Balance (least count 0.1mg), Probe sonicator, Centrifuge machine, Spectrofluorophotometer (PTI Quanta Master 50).

### Result and Discussion

The base pairs of ssDNA being hydrophobic in nature get adsorbed non-covalently through aromatic interaction and wrapped helically on the surface of SWNTs (hydrophobic) immediately i.e. few picoseconds. Due to electrostatic and torsional interactions within the sugar-phosphate backbone from 3' end to 5' end and thus form highly stable hybrid (SWNT-ssDNA) that helps in dispersion of SWNTs. The dispersion depends on the length and type of base pairs of ssDNA. poly (GT)<sub>20</sub> sequence effectively disperses the bundled SWNTs and probe sonication of the resultant hybrid further assists the SWNTs dispersion as explained by zheng et al. Dispersion of bundled SWNTs was confirmed by PL itself because the PL exists for individual SWNTs only because for bundled nanotubes PL gets quenched due to non-radiative relaxation of excited carriers.

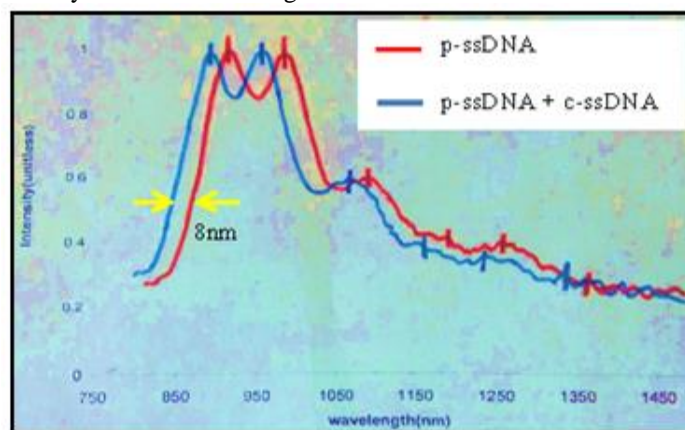
**Figure 3. PL of dispersed SWNTs (only p-ssDNA)**

Distinct peak marked in the PL corresponds to the SWNTs of different diameters and chirality which were calculated by the Weisman and Bachilo Table shown here as Table 2.

**Table 2. Characterising the dispersed SWNTs in presence of p-ssDNA**

Emitted wavelength (nm)	Diameter (nm)	Chirality (n, m)	Chiral angle (degree)
915	0.757	(9,1)	5.21
1090	0.916	(9,4)	17.48
1196	1.014	(11,3)	11.74
1368	1.068	(11,4)	14.92

As the c-ssDNA is added into the complex hybrid (SWNT - p-ssDNA) it readily binds to the t-ssDNA (as DNA binds to its complimentary pair only) and thus form the double stranded DNA which is adsorbed at the SWNT's surface. This leads to the decrease in the effective surface area of SWNTs that were exposed to the water. The dielectric constant of SWNTs being dependent on its ambience medium gets effectively reduced relatively in comparison when t-ssDNA was present alone. PL of such hybrid is shown in Figure 4.

**Figure 4. PL of dispersed SWNTs (p-ssDNA + c-ssDNA)**

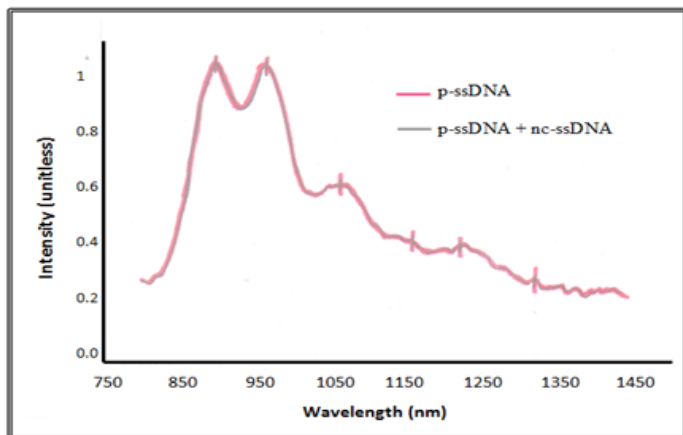
PL in presence of c-ssDNA is exactly the replica of one when only t-ssDNA was present with the slight shift towards the lower wavelengths (higher energies) and its characterization is shown in Table 3.

**Table 3. Characterising the dispersed SWNTs in presence of p-ssDNA & c-ssDNA**

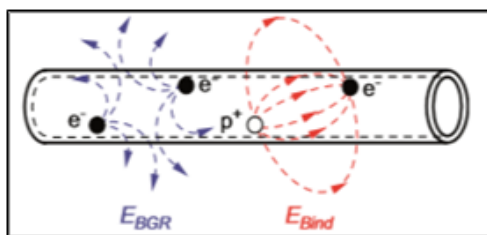
Emitted Wavelength (nm)	Diameter (nm)	Chirality (n,m)	Chiral angle (degree)
910	0.757	(9,1)	5.21
1083	0.916	(9,4)	17.48
1184	1.014	(11,3)	11.74
1362	1.068	(11,4)	14.92

As the nc-ssDNA is added into the complex hybrid (SWNT - p-ssDNA) it settles down in the solution without binding to t-ssDNA (as DNA binds to its complimentary pair only). Thus only p-ssDNA remains adsorbed at the SWNT's surface. The effective surface area of SWNTs exposed to the water remains almost same. Hence, the dielectric constant remains the same as when t-ssDNA was present alone. Resulting PL therefore remains the same (negligible shift) as is shown in figure 5 where both PL's are overlapping.

In all the cases considered above, neither  $(n-m) = 0$  nor it is divisible by 3 (criteria for metallic nanotube) thus ensuring that the SWNTs used were actually semiconducting.

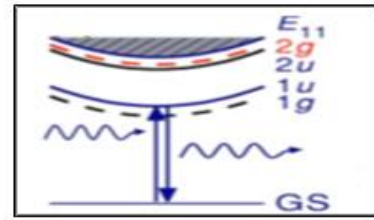
**Figure 5. PL of dispersed SWNTs (p-ssDNA + c-ssDNA)**

The Coulombic interaction in the 1-D structure like carbon nanotube plays very crucial role due to the substantial quantum confinement of the electrons and holes. This Coulombic interaction involves: 1.) electron-electron interaction and 2.) electron-hole interaction (excitonic effect) which influences the optical properties of SWNTs (Figure 6) by varying the optical energy band gap.

**Figure 6: Coulombic interaction in SWNT (Walsh et.al) [33]**

Electron-electron interaction results in the increase in the optical energy band gap (free particle band gap) i.e. it leads to the blue shift in emission and the process is referred as Band Gap Renormalization(BGR). At the same time the electron-hole interaction gives rise to the bound states (excitonic energy states) within the band gap (below band gap) which results in

effective reduction of optical band gap that results in red shift in emission as shown in Figure 7. These interaction energies are labelled as  $E_{BGR}$  (e-e interaction) and  $E_{Bind}$  (e-hole interaction).

**Figure 7: Electron-hole interaction results in excitonic states within the bandgap (Maultzsch et.al) [34]**

Since, these interaction energies enter into the Hamiltonian with opposite polarity ( $E_{BGR}$  as positive &  $E_{Bind}$  as negative) thus opposing the effect of each other thereby determines the effective optical band gap.

Mathematically, the effective optical band gap including these effects is given equation (1) by Walsh, et al [33].

$$E_{\text{optical}} = E_{\text{SP}} + E_{\text{BGR}} + E_{\text{Bind}} \dots \dots \dots (1)$$

where  $E_{\text{SP}}$  is the single particle energy band gap.

The exciton binding energy ( $E_{\text{Bind}}$ ) is related to dielectric constant as  $E_{\text{Bind}} \propto \epsilon^{-x}$  where  $\epsilon$  is the effective dielectric constant of SWNT,  $x$  is the scaling factor varies from 1-1.4. (The value of  $x$  depends on the dielectric screening which has not been considered here for simplification thus taken as unity). Presence of c-ssDNA effectively decreases the dielectric constant which in turns increases  $E_{\text{Bind}}$ . Increase in  $E_{\text{Bind}}$  tends to decrease the effective optical band gap. Simultaneously, binding and adsorption of c-ssDNA intensify net negative charge on the SWNTs surface due the (-ve) phosphate group of the c-ssDNA strand which increases the electron-electron interaction that tends to increase the effective optical band gap.

It can be concluded that electron-electron interaction dominates in contrast to electron-hole interaction in presence of c-ssDNA that cause blue shift in PL whereas neither of two interactions were affected in the presence of nc-ssDNA thus no shift is observed in its PL.

## Conclusion

Hydrophobic base pairs of ssDNA adsorb unto the surface of SWNTs by attractive  $\pi$ - $\pi$  stacking interaction and in few nanoseconds ssDNA wrapped helically due to electrostatic and torsional interactions within the sugar-phosphate backbone from 3' end to 5' end forming a stale ssDNA-CNT hybrid. Binding of ssDNA disperses the SWNTs which was further intensified by probe sonication. PL revealed that bundled SWNTs were effectively dispersed by (poly(GT)<sub>20</sub>) sequence of ssDNA due to highest stacking energy of G among all the base pairs. Resulting dispersed SWNTs were characterized using Weisman & Bachilo table. The addition of c-ssDNA modifies the electronic band structure of SWNT and causes the notable blue shift (~6-8nm shift) in the formerly existing PL due to dominating electron-electron interaction, ensuring that variation in electrostatic interaction can never be avoided at the atomic level in 1-D material like SWNT whereas negligible (no) shift in PL was observed when nc-ssDNA was added because it did not bind to the t-ssDNA confirming that shift in PL is sequence selective. Few nano-moles of DNA were used in the experiment.

Observation certifies that the shift in PL is sensitive to selective DNA sequence, which is basic demand for DNA detection. Also PL demands very small amount of ssDNA (few nano-mole) and inherently faster. Selectivity, comparably low

cost and quick responsivity are some undistinguishing features that make PL a promising technique for DNA detection.

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