INFLUENCE OF OLIGONUCLOTIDE AND PEPTIDE INTERACTIONS ON ELECTRONIC PROPERTIES OF SINGLE WALLED CARBON NANOTUBES

by
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INFLUENCE OF OLIGONUCLOTIDE AND PEPTIDE INTERACTIONS ON ELECTRONIC PROPERTIES OF SINGLE WALLED CARBON NANOTUBES

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ABSTRACT

INFLUENCE OF OLIGONUCLEOTIDE AND PEPTIDE INTERACTIONS ON ELECTRONIC PROPERTIES OF SINGLE WALLED CARBON NANOTUBES

Carbon nanotubes have been a point of attention since their discovery by Iijima in 1991. However, the biggest problem ahead for the applications of carbon nanotubes is their low solubility properties. Detergents are considered as surfactants which can be used to gain solubility for carbon nanotubes. On the other hand, DNA is a more significant solubilizing ligand which can be enrolled in cooperative binding interactions with SWCNTs. Raman spectroscopy is a powerful tool that can be used to study molecular systems, such as oligonucleotide/SWCNT structures. The changes in the electronic properties of SWCNTs due to the interactions between oligonucleotides can be detected by Raman spectroscopy.

In this study, the solubilization difficulties of SWCNTs are reduced by DNA interaction and covalent attachment to SWCNTs. The changes in the electronic properties of SWCNTs after the non-covalent interactions between oligonucleotides and SWCNTs and the covalent attachment of oligonucleotides to SWCNT are investigated by Raman spectroscopy. SWCNTs are interacted with short (10 bases) and long (25 bases) oligonucleotides. Also, to investigate the selectivity of SWCNT and oligonucleotide interactions, the non-covalent interactions between selected peptides and SWCNTs were studied by Raman spectroscopy. The pristine, carboxyl functionalized and covalently oligonucleotide attached SWCNTs are characterized by IR and X-ray photoelectron spectroscopy.

ÖZET

TEK KATMANLI KARBON NANOTÜPLERİN OLİGONÜKLEOTİD VE PEPTİD İLE ETKİLEŞİMLERİNİN ELEKTRONİK ÖZELLİKLERİ ÜZERİNDEKİ ETKİSİ

Karbon nanotüpler, Iijima tarafından 1991'deki keşfinden bu yana nanoteknoloji alanında ilgi odağı olmuşlardır. Bununla birlikte, karbon nanotüp uygulamalarının önündeki en büyük engel zayıf çözünürlük özellikleridir. Deterjanlar, karbon nanotüplere çözünürlük kazandıran sürfaktanlar olarak düşünülmüşlerdir. Fakat, TKKNT'ler ile kooperatif bağlanma ve etkileşimlere giren DNA, kayda değer bir çözünürlük sağlayan ligand olarak kullanılabilmektedir. Buna ek olarak, Raman spektroskopisi oligonükleotid/TKKNT yapıları gibi birçok moleküler sistemin tetkik edilebileceği güçlü bir yöntemdir. TKKNT'lerin elektronik özelliklerindeki değişimler Raman spektroskopisi ile tespit edilebilmektedir.

Bu çalışmada, TKKNT'lerin çözünürlüğünde karşılaşılan zorluklar DNA ile girdiği kovalent olmayan etkileşimler ve DNA'ya kovalent olarak bağlanması ile giderilmektedir. TKKNT'lerin kısa (10 bazlık) ve uzun (25 bazlık) polinükleotidler ile kovalent olmayan etkileşimler ve kovalent bağlanma sonrasında elektronik yapılarındada meydana gelen değişiklikler Raman spektroskopisi ile incelenmiştir. Bunun yanında, TKKNT'ler ile oligonükleotidlerinkovalent olmayan etkileşiminin seçiciliğini incelemek için peptid ve TKKNT'lerin kovalent olmayan etkileşimleri de Raman spektroskopisi ile incelenmiştir. Saf, karboksillenmiş ve kovalent olarak oligonükleotid bağlanmış TKKNT'lerin karakterizasyonu IR ve X-ışını fotoelektron spektroskopisi ile yapılmıştır.

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a1, a2	Unit vectors of the hexagonal lattice
d	Diameter of SWCNT
GPa	Gigapascal
h	Hour
m,n	The number of unit vectors
min	Minute
nm	Nanometer
rpm	Rate per minute
ω	Raman shift of RBM band
1D	One dimensional
3D	Three dimensional
AgNP	Silver Nanoparticle
ATR	Attenuated total reflectance
AuNP	Gold Nanoparticle
CNT	Carbon nanotube
DFTB	Density-functional tight-binding method
DNA	Deoxyribonucleic acid
DWCNT	Double walled carbon nanotube
FTIR	Fourier transform infrared spectroscopy
IR	Infrared
MD	Molecular dynamics modeling
MNP	Magnetic nanoparticle
MWCNT	Multi walled carbon nanotube
NIR	Short near-infrared
QD	Quantum dots
RBM	Radial breathing mode
SWCNT	Single walled carbon nanotube
XPS	X-ray photoelectron spectroscopy

1. INTRODUCTION

Nanotechnology can be defined as "the design, characterization, production and application of materials, devices and systems by controlling shape and size at nanoscale" [1]. The range from 1 to 100 nm is interpreted as nanoscale by International Standards Organization.

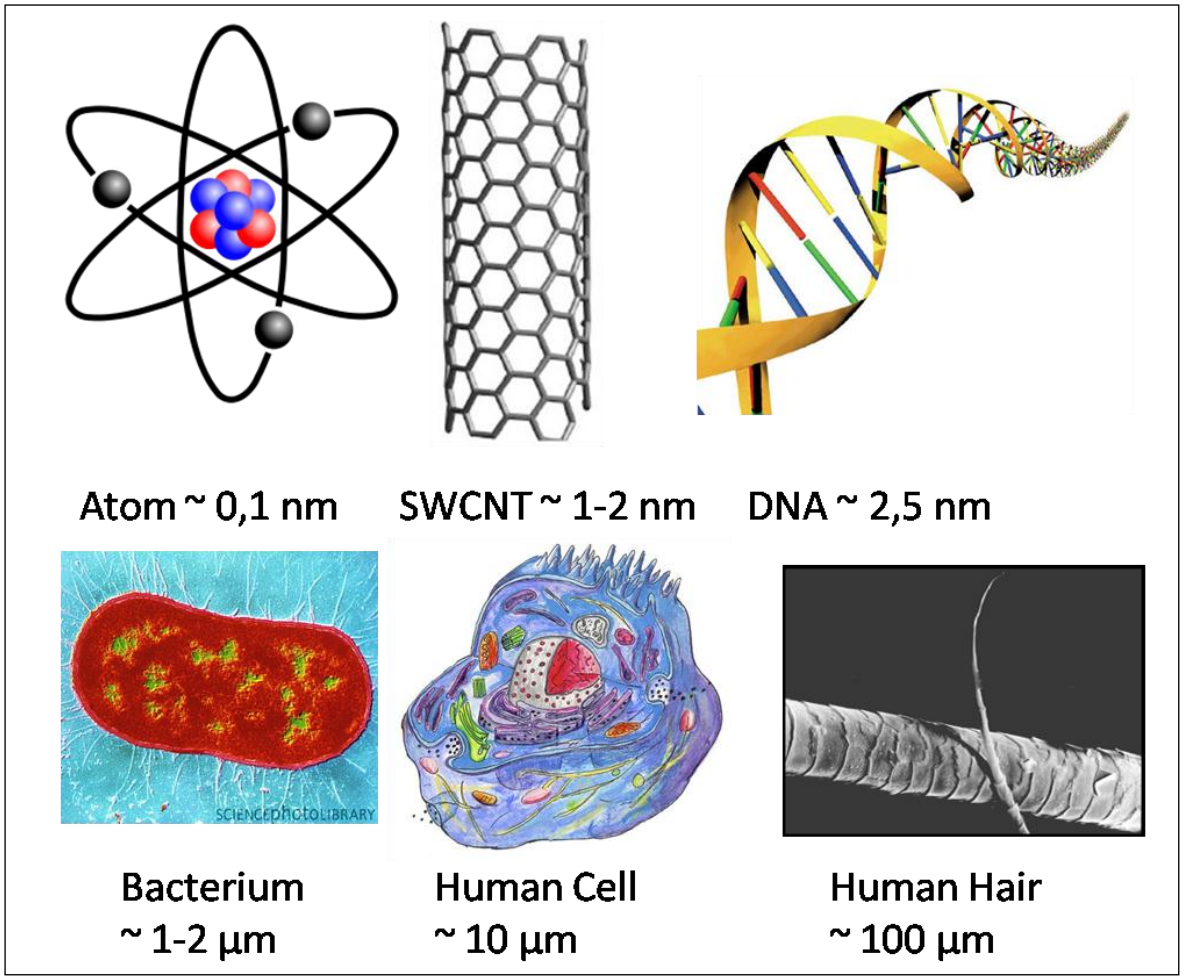

Figure 1.1. The overview of the structures from nano to macroscale

1.1. HISTORY of NANOTECHNOLOGY

The nanotechnology concept was first named by the physicist Richard Feynman's lecture at an American Physical Society meeting on 1959 [2]. Feynman reflected on the idea of direct manipulation of individual atoms and expressed the new era of science with the notable words "There's Plenty of Room at the Bottom". The aim of Feynman was to participate in a new discipline of physics, which can be considered as limitless.

1.1.1. The Rise of Nanotechnology

Indeed, it was believed that the rising of nanotechnology reaches back to the ancient ages. As an example, by the production of carbon nanoparticles in water, Indian ink was supplied around 2700 b.c. in China. Also, silver and copper nanoparticles were used in Europe in the coating pots to give glitter [3]. Moreover, it was discovered that iron carbide nanowires and carbon nanotubes were used in the production of medieval Damascus swords shown in Figure 1.2. The exceptional hardness of these swords was explained by the presence of carbon nanotubes in its structure [4]. The perspective of the medieval nanotechnology leads the modern world to advance new instruments which can inspect at nanoscale and experience absolutely new compositions that were not known to exist [5].

Figure 1.2. Damascus Sword

1.1.2. The Evolution of Nanotechnology

In this era of nanotechnology, it was aimed to construct nanoparticles that are independently achieving useful functions such as a magnetic nanoparticle storing a single bit of data by defining the direction of its magnetization. To construct these kinds of nanoparticles, the physical and chemical properties of each kind of material at nanoscale should be considered. Magnetic core shell particles coated with biological substances according to the purpose of use such as thiol attached gold nanoparticles acting as a transistor and examples of these nanoparticles can be given which are waiting for the solution to be controlled to self-assembly into their arrays [5].

1.2. BIONANOTECHNOLOGY

Apart from the biotechnology, which can be defined as the direct use of organisms to assemble profitable products, bionanotechnology can be described as the implementation of biological molecules and systems to nanotechnology. Polypeptides, nucleic acids, polysaccharides and oligosaccharides are given examples of biological molecules that are applied to nanotechnological systems [6].

Bionanotechnology combines the capability to engineer and adjust the atomic-level specifications to biotechnological applications. Bionanomachines are presumed to execute 3D molecular tasks with their embedded devices for individual control [6].

2. THEORETICAL BACKGROUND

2.1. BOTTOM-UP/TOP-DOWN APPROACH

The main approaches in nanotechnology to construct a device at nanoscale are bottom-up and top-down approaches. Bottom-up approach handles the process as firstly building the nanoparticles and then assembling them to produce the target material or device. The main aims of bottom-up approach are single molecule components to self-assemble and keep the positional assembly. Molecular self-assembly and molecular recognition are the techniques applied in the bottom-up approach.

However, in the top-down approach, it is started with a block of material and then a structure is constructed out of that block of material. Traditional microfabrication methods, which include cutting, milling and shaping materials with externally directed instruments are used in the top-down method. Photolithography can be named as an example of micropatterning methods, which can be classified as a top-down approach [5]. Figure 2.1 shows an example of the combination of both approaches.

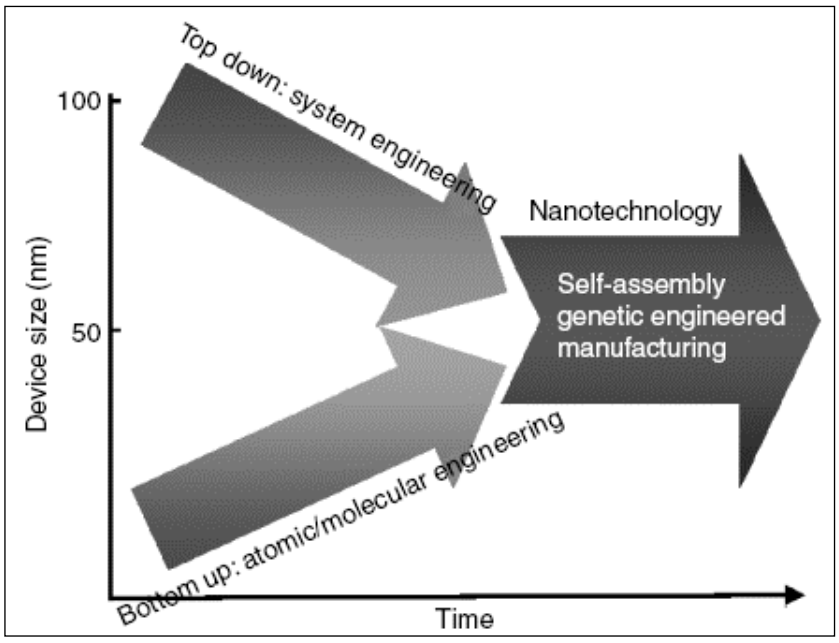

Figure 2.1. Top-down bottom-up approach [7]

2.2. NANOPARTICLES

2.2.1. Noble Metal Nanoparticles

Au and Ag nanoparticles (NPs) can be classified as noble metal nanoparticles. Their plasmonic properties have drawn attention on these NPs. Their ability to absorb and scatter light is used for imaging since the absorbed light is efficiently transmitted to heat. Au and Ag NPs can be utilized to chemical, biological, medical applications with their ability to enhance Raman scattering [8-11]. Due to the individual optical, electrical and magnetic properties, Ag NPs found variety of utilizations in optics, electronics and catalysis. Nowadays, Ag NPs are means of antibacterial and antifungal applications. Besides this, Au NPs are biocompatible agents and different from Ag NPs, they are used in biomedical applications such as photo thermal therapy [12], gene therapy [13] and drug carrier systems [14].

2.2.2. Magnetic Nanoparticles

Elemental and the chemically functionalized states of iron, nickel and cobalt can be given as examples of magnetic nanoparticles (MNPs). MNPs can be easily functionalized and directed by external magnetic field. Thus, they are convenient agents for magnetic resonance imaging, catalysis, data storage and environmental remediation [15-17].

2.2.3. Quantum Dots

Quantum dots (QD) take place in computing (i.e. solid-state quantum computation), biology (i.e. organic dyes, cellular imaging), light emitting devices (i.e. light emitting diodes) and photodetector devices as well. QDs have a size in a range of 1 to 10 nm and they are preferable agents in especially cellular imaging with their extraordinary photochemical and photophysical properties [18-21].

2.2.4. Carbon Nanotubes

Carbon nanotubes (CNTs) were discovered by Iijima in 1991 and they have been a point of focus since their discovery. Benzene-like hexagonal rings of carbon atoms generate the tube form with the cylindrical shape and sp2 hybridization. The structure of a single-walled carbon nanotube (SWCNT) can be described as a rectangle cut from a graphene sheet and rolled to join the atoms in a hexagonal arrangement. This rolling act can be done with different angles and the rolling angle of the nanotube defines the chirality character of the CNT and causes changes in the electronic properties. The axis angle of the SWCNT, which is known as n-shift, has an important role on identifying the tube properties as well as the tube diameter and length [5].

CNTs can be assorted based on their structures as single-walled (SWCNT), double-walled (DWCNT) and multi-walled carbon nanotubes (MWCNT). While the diameter of the CNTs is measured with nanometers, the length of the tube can reach to micron size. Thus, they have a high aspect ratio. In addition to this categorization, CNTs can be classified as metallic or semiconducting tubes according to their conductivity [22].

Because of the peculiar combining of their dimension, structure and topology, carbon nanotubes possess excellent structural, mechanical and electronic properties. According to the accomplished studies, carbon nanotubes were listed as extremely strong, highly conductive, thermally and chemically stable [23]. As a result of these individual properties, carbon nanotubes were presented for various practices such as from scanning probes [24], hydrogen storage [25] and biosensors [26].

The standard procedure to identify the chirality of a nanotube is based on the unit vectors (na1 + ma2) of the hexagonal lattice. The chirality of the nanotube is shown as (m, n). The tubes with the (n, n) chirality are named as “armchair” and (n, 0) chirality is named as “zigzag”. The end caps of the tubes can be either open or closed [5].

2.3. ELECTRONIC PROPERTIES of SWCNTs

Carbon nanotubes can be characterized as a semi-metal due to its properties in the middle between semiconductors and metals. To understand the electronic properties of the carbon nanotubes, a simple equation shown in (2.1) is valid since the chirality of the tube must be known [5]. If,

$$n - m = 3i \ (i = integer) \tag{2.1}$$

then, the nanotube has a metallic character. If the subtraction is not equal to 3i, then the tube has a semiconductor character. Also, armchair carbon nanotubes (n, n) are considered to have a metallic character [5]. Figure 2.2 shows the a1 and a2 vectors and the chirality possibilities up to the rolling of the graphene sheet.

As a result of carbon nanotubes' distinctive composition, various new and original properties such as high strength and performance in electrical and thermal conductors name them as one of the recent topics in physics and also materials science as well [27].

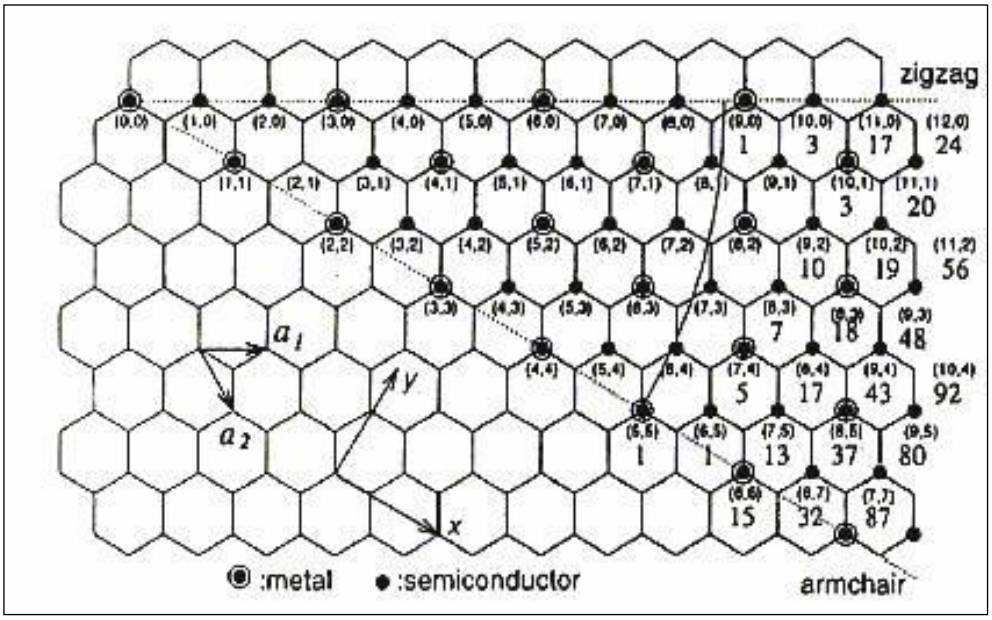

Figure 2.2. Chirality of carbon nanotubes [28]

2.3.1. Density and Strength

The densities of carbon-based materials are explained in Lange's Handbook of Chemistry as 2.267 g/cm^3 for graphite and 3.5153 g/cm^3 for diamond. The density of carbon nanotubes vary from 1.3 to 1.4 g/cm^3 which is substantially less than other carbon based material forms. The reasons for the low density of carbon nanotubes can be explained by the less dense packaging of carbon atoms in nanotubes than diamond and graphite. Also, the variety in carbon nanotube's density is related with the rolling of the graphene sheet, which affects the chirality of the SWCNT. To comprehend the strength of carbon nanotubes, Young's modulus, which is a scale of the strength of a substance, can be examined. The force per unit area was calculated as 13 GPa for wood, 128 GPa for copper and around 1000 GPa for SWCNTs. Thus, SWCNTs can be named as one of the strongest and most flexible molecular material according to Young's modulus [29].

2.3.2. Electrical and Thermal Conductivity

Among the carbon-based materials, graphite is a good conductor because of the overlapping of the valence and conducting bands, which provides electrons mobility. However, when the graphite sheet is rolled and turned into a SWCNT, the parallelism between the bands disappears. Thus, SWCNTs are not as good conductors as graphites. Besides this, SWCNTs have high thermal conductivity, which means that they are able to conduct energy as heat [29].

The important challenge with carbon nanotubes is the large-scale applications. The tendency of aggregation into bundles when dispersed in liquids is one of the most severe problems of carbon nanotube applications. Also, adjusting the length for targeted applications and manipulating the nanotubes into the necessary positions are other challenges for the applications of carbon nanotubes [30].

2.4. SOLUBILIZATION of SWCNTs

Due to the low solubility properties of carbon nanotubes in both aqueous and organic solvents, chemical modification is thought to be a solution of managing carbon nanotubes without altering their specific properties listed above. Strong acids are used to shorten the tubes as an approach to increase the solubility of carbon nanotubes [31]. However, this approach has a negative impact on the high aspect ratio, which can be considered as an appealing property of carbon nanotubes. Thus, chemical functionalization is an alternative technique for increasing the solubility and manageability without altering unique properties of carbon nanotubes [22, 32].

2.4.1. Detergents as Surfactants

Pyrene salts and tensides, such as sodium dodecyl sulfate, sodium dodecyl benzene sulfonate, or sodium cholate were detergents used to gain solubility to carbon nanotubes and produce stable nanotube suspensions [33-37].

For the nanotube suspension to retain its stability, excess amount of detergent molecules are needed. Though, CNTs tend to reform bundles when excess amount of detergent molecules are removed from the suspension due to the fast off-rate for the complexes with the nanotubes. However, the free surfactant molecules affect all of the applications, characterization procedures and even prohibit the functionalization of CNTs [32].

2.4.2. Oligonucleotides as Solubilizing Ligands

To suppress the challenge of fast off-rate, a ligand, which can enroll in cooperative binding interactions with SWCNTs such as biomacromolecules, which can enroll in multivalent interactions with big binding energies for their complexes can be preferable. Through all other biomacromolecules, deoxyribonucleic acid (DNA) comes forward by resulting in significant yields of solubilized tubes. Although its natural function as a transporter of genetic data, synthetic DNA serves as an effective surfactant for solubilization of SWCNTs. Oligonucleotides both can be covalently attached to [38-41] and non-covalently interact with SWCNTs [33, 42]. Properties of oligonucleotide-SWCNT dispersions such as

unbroken composition of SWCNTs even after the formation of oligonucleotide-SWCNT complex, high thermal stability in aqueous solutions and stability for almost one year at room temperature make the use of DNA preferable and advantageous.

Based on the simulation done in order to understand the molecular contacts between DNA and SWCNTs include the aromatic nucleobases of the oligonucleotides and the surface of the SWCNTs [43]. A helical wrapping around the nanotube surface is expected for oligonucleotides [44]. After the wrapping, the negatively charged backbone of the oligonucleotide exposed to water. Rebundling of the SWCNTs was inhibited by the effective repulsion of the negative charges of the phosphodiesters. Unlike the detergents, excess amount of oligonucleotides can be expelled from the suspension [32].

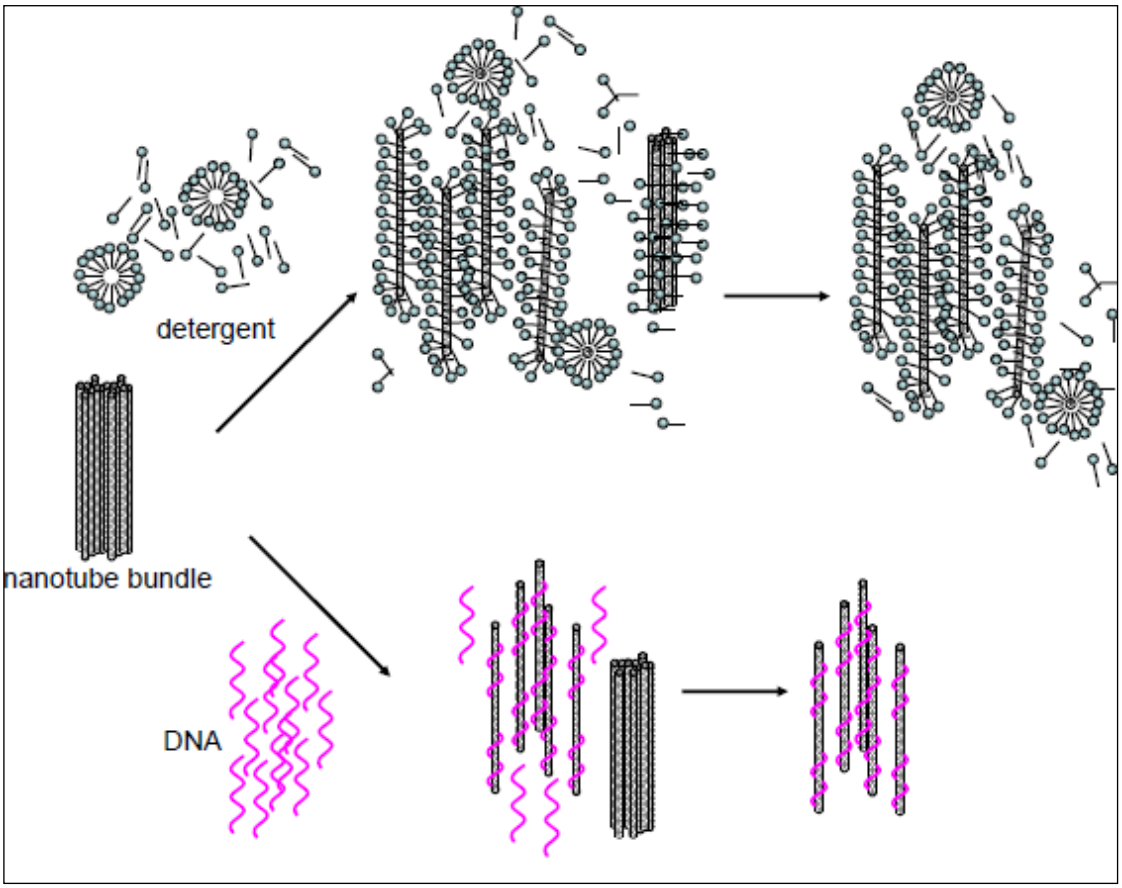

Figure 2.3. Solubilization of a nanotube bundle by using either detergents or deoxyribonucleic acid [32]

2.5. NON-COVALENT and COVALENT FUNTIONALIZATION of SWCNTs with OLIGONUCLEOTIDES

There are a number of theoretical and experimental studies for a better understanding of the interactions of SWCNTs with oligonucleotides. Various parameters such as base-length and sequence of oligonucleotides, metallic/semiconducting properties and chirality of SWCNTs, etc. were considered. It was first proposed by Lustig et al. that oligonucleotides wrap helically onto the surface of SWCNTs [45]. Zheng et al. later explained that the non-covalent interactions between oligonucleotides and SWCNTs occur through π stacking interactions of sidewalls of SWCNTs and aromatic nucleotide bases [46]. It was also suggested that the sequence and base composition of the oligonucleotides had an influence on these interactions.

According to Zheng et al., oligonucleotides are suitable agents for dispersing SWCNTs in aqueous solutions. In addition, the dispersed SWCNTs remain stable for months. Another study to understand the effect of oligonucleotide sequence on the dispersion of SWCNTs was reported by Martin et al. They studied the interaction of a decamer (10 based) oligonucleotide with SWCNTs and found that several parameters such as π stacking, dispersion interactions, oligonucleotides' configurational stress and solution effects had influence on the interaction between oligonucleotide and SWCNT [47]. Karachevtsev et al. indicated that the purine bases do not generate a whole roll around the nanotube surface [48]. Thus, the pyrimidine bases wrap around the nanotube with a higher affinity when compared to purine bases. Stepanian et al. indicated that exploring surface stacking properties of CNTs and polynucleotides (polyC) are promising studies for improving bionanosensor applications [49]. Wang and Bu studied the interactions between SWCNT and cytosine, and reported that the non-covalent interactions exist between polyC and SWCNT [50].

2.5.1. Non-Covalent Functionalization of SWCNTs

Polymers, biomolecules, surfactants and polyaromatic compounds are the molecules variedly used for non-covalent interactions with CNTs [51]. The molecules used with high efficiency in the non-covalent interactions with CNTs can be listed as: polyvinyl

pyrrolidone, polystyrene sulfonate, poly(1-vinyl pyrrolidone-co-vinyl acetate), dextran, dextran sulfate, poly(1-vinyl pyrrolidone-coacrylicacid), poly(1-vynil pyrrolidone-co-dimethylaminoethyl methacrylate), bovine serum albumin, starch and DNA [36, 42, 52].

Van der Waals and π–stacking forces are the primary forces for the non-covalent interactions between these molecules and CNTs. The generation of supramolecular structure between CNTs and DNA as the recognition efficiency of DNA is remarkable, has drawn attention these days. According to Zheng et al, the well-defined chemical being of DNA wrapped CNT is seen in aqueous solutions because of the powerful non-covalent interactions between DNA and CNTs [53]. For either covalent attachment or non-covalent interactions, DNA proposes the superiority of specified base length and sequence, high dispersion capability, well developed chemistries for additional functionalization when compared to polymers listed above [42].

2.5.2. Covalent Functionalization of SWCNTs

Nitric acid (HNO_3) is a preferable method for the surface modification of carbon nanotubes. The oxygen containing functional groups attached on CNTs surface improve the solubility in both aqueous and organic solvents. These functional groups decrease the capability of Van der Waals interactions between other CNTs in the solution and enhance the separation of CNT bundles into particular tubes. Carboxylation process by means of HNO_3 stimulates the carbon nanotube's cap disclose, yet the pristine CNTs' electronic and mechanical properties maintain. As the chemical modification happens at the disclosed caps of the nanotube, critical defects on the nanotubes' surface are not observed during the carboxylation process with HNO_3 [54].

2.6. APPLICATIONS of DNA WRAPPED SWCNTs

The supramolecular structures between DNA and CNT have been an attention of study and an offering for utilizations such as biological transporters and biosensors [55], fibers for artificial muscles [56] and bioelectrodes for fuel cells [57]. Moreover, the purification and separation of carbon nanotubes are other techniques which DNA wrapped CNTs are used with high efficiency.

2.6.1. Chemical Biosensors

The first utilization of DNA wrapped CNTs as biosensors were reported by Hu et al [58]. The detection of various types of molecules such as glucose [59-60], peroxide [61], dopamine [62-63], pesticides [64], vapors [65], proteins [66] and ions [67-68] can be carried out by DNA wrapped CNT biosensors. For example, an electrochemical biosensor detecting dopamine was generated by laying DNA wrapped SWCNTs over glass substrates which was found to have sensational selectivity and sensitivity. Figure 2.4 shows an example of a peroxide biosensor derived from oligonucleotide- SWCNT complexes.

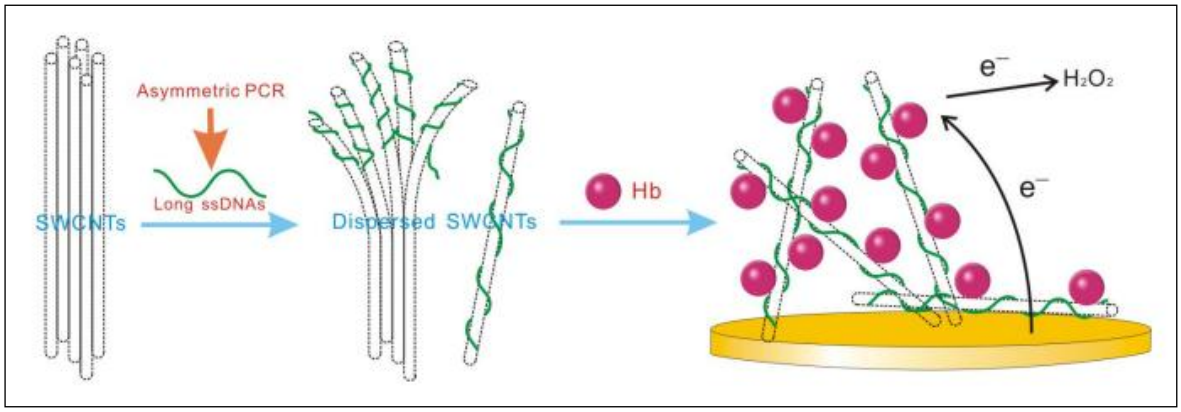

Figure 2.4. Schematic illustration of the peroxide biosensor prepared with oligonucleotide-SWCNTs complexes [61]

2.6.2. DNA Hybridization Biosensors

Hybridization of an oligonucleotide wrapped CNT and the complementary oligonucleotide was studied to explore if the hybridization occurs between the oligonucleotides on the nanotube surface or the hybridization of the DNA strands leads the displacement of the nanotube. According to the studies of Cheng and Zhang, the hybridized DNA separates as a layer and the solubility of the CNT decreases immediately and precipitation of CNT occurs [69]. In addition to these studies, it was evidenced that the hybridization event occurs in solution rather than on the nanotube surface [70-71]. On the other hand, another study claimed that the hybridization between a target oligonucleotide and complementary oligonucleotide sequence wrapped around the SWCNT takes place on the nanotube surface

[72]. However, in place of executing the hybridization on the nanotube surface, Hwang et al used a DNA complex covering both an oligonucleotide and a double stranded DNA to interact with SWCNT and the biosensor was able to detect a specific DNA sequence within a complex genome [73]. Still, the many studies carried out to utilize a DNA hybridized CNT biosensor showed that the carrier transport properties of SWCNTs are not always reliable after the hybridization process.

2.6.3. Biological Transporter

Because of the high biostability properties of DNA wrapped CNT complexes, they were considered as cellular transporters. As an example of a cellular transporter, it was reported that DNA probes interacted with SWCNTs are shielded from nuclease digestion and blocking of DNA binding proteins [74].

On the contrary, in another study it was explained that by the means of energy dependent endocytosis mechanism, DNA wrapped SWCNTs are effective in the transportation of oligonucleotides into living cells [75-76]. It was also expressed that short near-infrared (NIR) pulses have an effect on detaching DNA cargoes from SWCNTs and the detached oligonucleotides are transferred into the cell nucleus [77]. In an alternative study, particular cancer cells were destructed with continuous NIR which causes cell death because of the extensive local heating of the SWCNTs. These studies clarifies the ability of DNA wrapped SWCNTs for the use of cancer therapy and drug delivery [22].

2.6.4. Purification and Separation

Because of the low solubility properties of CNTs in both aqueous and organic solvents, oligonucleotide wrapped and chemically attached CNT dispersions are less demanding to purification and separation. DNA wrapped carbon nanotubes can be separated up to their conductivity properties, diameter, chirality and length by chromatographic techniques. According to the study by Zheng et al, ion exchange chromatography is an alternative way to separate the DNA-CNT complexes into various conductivity and length [46]. However, during the separation up to length of the tubes, the complexes with both metallic and semiconducting properties were collected together [78-80]. According to the ion exchange

chromatography technique, early fractions contain smaller diametered and metallic carbon nanotubes and late fractions contain larger diametered semiconducting carbon nanotubes [46]. Also, another chromatographic technique is size exclusion chromatography which can separate DNA wrapped carbon nanotubes by length [81-83]. Furthermore, to disunite the DNA-CNT complexes by diameter, length, conductivity and chirality, an alliance of size exclusion chromatography and afterwards ion exchange chromatography is used as a powerful technique [84]. In addition to these techniques, flow-field flow fractionation [82], ultracentrifugation in aqueous density gradients [85], dielectrophoresis [86] and agarose gel electrophoresis [87] can be preferred for the separation of carbon nanotubes by length, diameter and conductivity properties.

2.7. CHARACTERIZATION of SWCNTs with RAMAN SPECTROSCOPY

Raman spectroscopy is a very effective technique for characterization of carbon-based structures such as graphene, fullerenes and CNTs [88]. Information about the particular 1D properties of SWCNTs such as electronic structure can be studied with Raman Spectroscopy. In Raman spectra of SWCNTs, the radial breathing mode (RBM), which is only observed with SWCNTs, acts as a unique phonon mode, and it is the evidence of carbon nanotube in a sample. The RBM band reveals important information about nanotube's diameter through the changes in its frequency and electronic structure through the changes in its intensity [89]. For SWCNTs in a diameter between 0.7 and 2.0 nm, the RBM band is seen in the frequencies between 150 and 300 cm^{-1}. Because of the phonon wave vector confinement along the SWCNT circular direction and symmetry breaking effects combining with SWCNT curve, the G band of SWCNT is made of several peaks compared to the graphite's Raman Spectrum G band. The frequency of G band can be utilized to characterize the diameter of the SWCNT, to detect the difference between metallic and semiconducting SWCNTs with the help of the differences in Raman line shapes and to investigate the charge transfer resulting from a doped SWCNT and to work on the Raman scattering geometries and processes. The G band in the spectrum of SWCNTs is seen between 1570 cm^{-1} (G^+, in correlation with carbon atom vibrations along the nanotube axis) and 1590 cm^{-1} (G^-, in correlation with the circular vibrational movement of SWCNT). The D and G' bands both appear as rather narrow peaks compared to graphite and change with the effect of chirality and the diameter of SWCNTs. These properties

depend on the electronic and phonon structure of the SWCNT. Most of the SWCNT electronic and phonon structure studies based on G' band as it is less sensitive to nanotube defects [89]. The band area ratios of D to G of SWCNTs are essential structural parameters in both non-covalent interactions and covalent bonding of a molecule to SWCNT [90]. Raman spectra of pristine SWCNT and the specific bands can be seen in Figure 2.5.

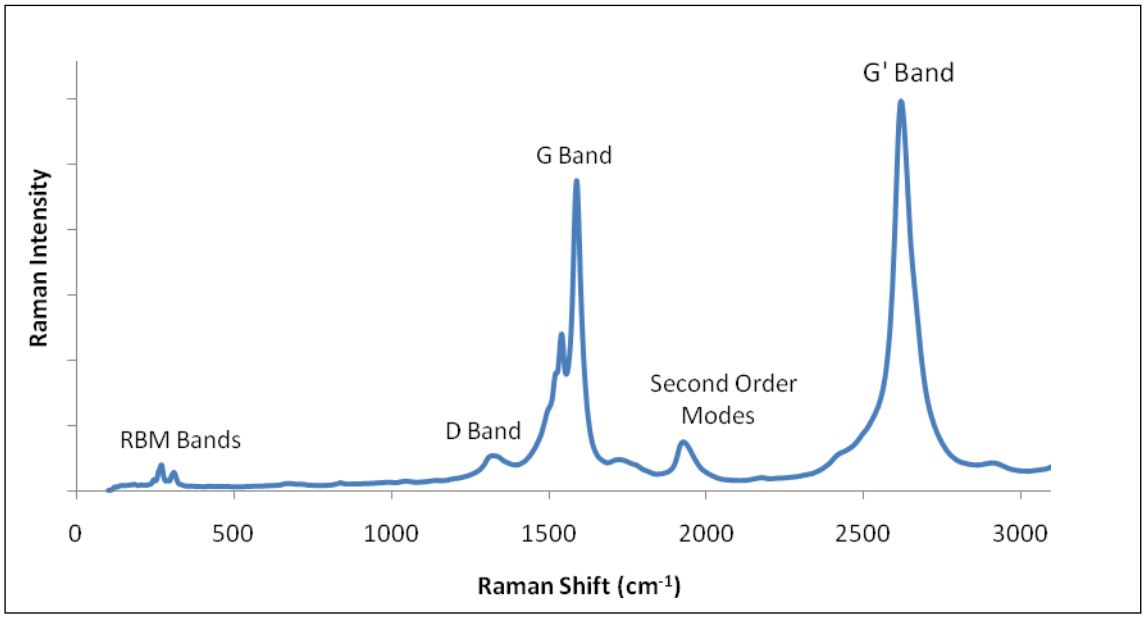

Figure 2.5. Raman Spectrum of pristine SWCNT

The aim of this study was to investigate the changes in the electronic properties of SWCNTs after the covalent attachment and non-covalent interactions with oligonucleotides by Raman Spectroscopy. As a point of focus since their discovery, the major challenge of carbon nanotubes was the solubilization properties. Oligonucleotides were chosen as solubilizing agents for this study due to their favorable properties compared to other biomacromolecules and detergents.

As a novel idea, different from literature, the base length and sequence effects of oligonucleotides in covalent attachment and non-covalent interactions were investigated apart from different modeling techniques by Raman Spectroscopy.

3. MATERIALS

3.1. REAGENTS

0.7-0.9 nm diametered (6,5) chirality SWCNT was purchased from Sigma-Aldrich (Germany). HNO_3 (98%), N-hydroxisuccinimide (98%) (NHS) and N-ethyldiisopropilamin was purchased from Sigma-Aldrich (Germany) and acetonitrile was purchased from Merck (Germany) for the carboxylation and activation processes of pristine SWCNTs. The oligonucleotides were purchased from Alpha DNA (Canada) were given in Table 3.1 and Table 3.2. The peptides were purchased from CPC Scientific (U.S.A.) were given in Table 3.3.

3.2. OLIGONUCLEOTIDES

The oligonucleotides were purchased from Alpha DNA (Canada) as 200 nmol and prepared as 100 μM with ddH_2O. The sequence and base length properties of given oligonucleotides in Table 3.1 were used in non-covalent interactions between oligonucleotides and SWCNTs. The sequence and base length properties of given oligonucleotides in Table 3.2 were used in covalent attachment of oligonucleotides to SWCNTs. The sequences are given as from 5’ end to 3’ end.

3.3. PEPTIDES

The peptides were purchased from CPC Scientific (U.S.A.) and prepared as 10^{-3} M with ddH_2O. The amino acid sequence of given peptides in Table 3.3 were used in non-covalent interactions between oligonucleotides and SWCNTs.

Table 3.1. The properties of oligonucleotides used in non-covalent interactions with SWCNTs

Label	Sequence	Base Number	Brand
polyA-10	AAAAAAAAAA	10	Alpha DNA
polyA-25	AAAAAAAAAAAAAAAAAAAAAAAAA	25	Alpha DNA
polyT-10	TTTTTTTTTT	10	Alpha DNA
polyT-25	TTTTTTTTTTTTTTTTTTTTTTTTT	25	Alpha DNA
polyG-10	GGGGGGGGGG	10	Alpha DNA
polyG-25	GGGGGGGGGGGGGGGGGGGGGGGGG	25	Alpha DNA
polyC-10	CCCCCCCCCC	10	Alpha DNA
polyC-25	CCCCCCCCCCCCCCCCCCCCCCCCC	25	Alpha DNA

Table 3.2. The properties of oligonucleotides used in covalent attachment to SWCNTs

Label	Sequence	Base Number	Brand
polyA-10	NH_2-$(CH_2)_6$-AAAAAAAAAA	10	Alpha DNA
polyA-25	NH_2-$(CH_2)_6$-AAAAAAAAAAAAAAAAAAAAAAAAA	25	Alpha DNA
polyT-10	NH_2-$(CH_2)_6$-TTTTTTTTTT	10	Alpha DNA
polyT-25	NH_2-$(CH_2)_6$-TTTTTTTTTTTTTTTTTTTTTTTTT	25	Alpha DNA
polyG-10	NH_2-$(CH_2)_6$-GGGGGGGGGG	10	Alpha DNA
polyG-25	NH_2-$(CH_2)_6$-GGGGGGGGGGGGGGGGGGGGGGGGG	25	Alpha DNA
polyC-10	NH_2-$(CH_2)_6$-CCCCCCCCCC	10	Alpha DNA
polyC-25	NH_2-$(CH_2)_6$-CCCCCCCCCCCCCCCCCCCCCCCCC	25	Alpha DNA

Table 3.3. The properties of peptides used in non-covalent interactions with SWCNTs

Label	Sequence	Brand
M1	Asp-Gly-Asn-Leu-Ala-Asn-Cys	CPC Scientific
M3	Cys-Cys-Phe-Phe	CPC Scientific
M4	Cys-Cys-Ala-Leu-Asn-Asn	CPC Scientific
M7	Cys-Cys-Ala-Leu-Asn-Asn-Phe-Phe	CPC Scientific

4. METHODS

4.1. NON-COVALENT INTERACTIONS of OLIGONUCLEOTIDES with SWCNTs

A 4 mg of 0.7-0.9 nm diameter SWCNTs (6,5) (Sigma-Aldrich, Germany) were added into 4 mL double distilled water (ddH_2O). The mixture was sonicated for 15 minutes in 0°C water bath. A 10 µL of 100 mM each oligonucleotide were added into 500 µL portions of SWCNT/ddH_2O mixture in eppendorfs.

To investigate the effect of oligonucleotide sequence on wrapping, each oligonucleotide listed above was interacted with SWCNTs in ddH_2O at 0 °C. It was aimed to slow down molecular vibrations of oligonucleotides by cooling to 0 °C. The oligonucleotides and SWCNTs were sonicated for a short time (30 mins) and a longer time (6 hours) at 0 °C to observe the influence of time on their interactions. The oligonucleotide/SWCNT dispersions were centrifuged at 14000 rpm for 90 minutes to separate the oligonucleotide/SWCNT structures from the uninteracted SWCNTs. Since the oligonucleotides wrap around SWCNTs and increase the solubility, oligonucleotide/SWCNT adducts remain in water (Figure 4.1, Figure 4.2). The supernatant was separated from the precipitate and a 2 µL of the supernatant was dropped on CaF_2 surface and dried at room temperature for Raman spectroscopy measurements.

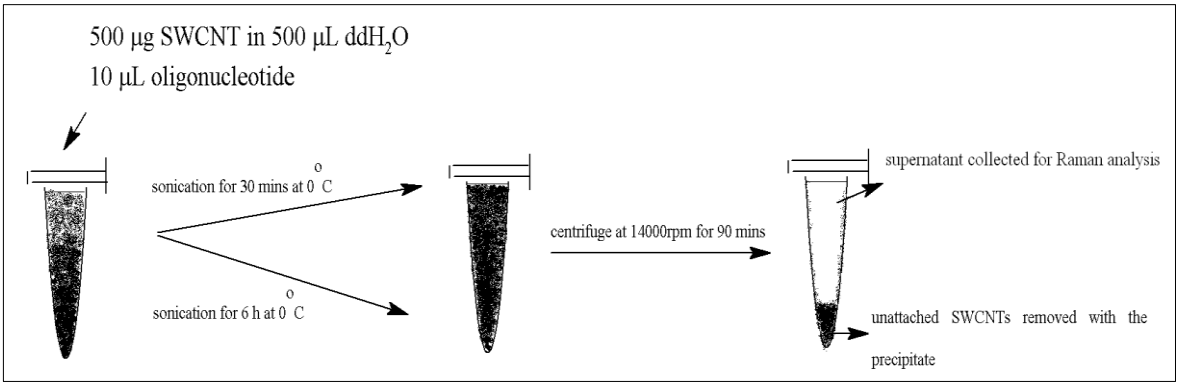

Figure 4.1. The schematic illustration of the design of non-covalent interaction investigations

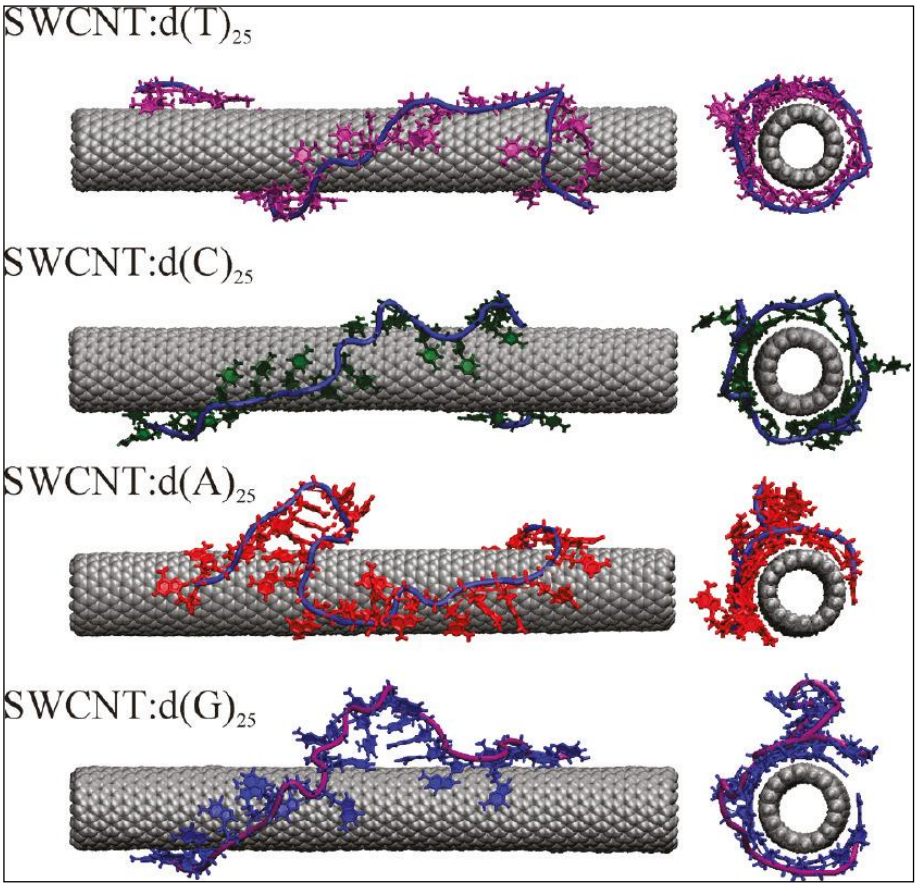

Figure 4.2. Snapshots of hybrids formed by nanotube (16,0) with oligonucleotides d(T)25, d(C)25, d(A)25, and d(G) after 50 ns simulation [48]

4.2. NON-COVALENT INTERACTIONS of PEPTIDES with SWCNTs

A 4 mg of 0.7-0.9 nm diameter SWCNTs (6,5) (Sigma-Aldrich, Germany) were added into 4 mL double distilled water (ddH_2O). The mixture was sonicated for 15 minutes in 0°C water bath. A 10 µL of 10^{-3} M each peptide were added into 500 µL portions of SWCNT/ddH_2O mixture in eppendorfs.

To investigate the non-covalent interactions between peptides and SWCNTs, each peptide listed above was interacted with SWCNTs in ddH_2O at 0 °C. The peptides and SWCNTs were sonicated for a short time (30 min) and a longer time (6 hours) at 0 °C to observe the influence of time on their interactions. The peptide/SWCNT dispersions were centrifuged at 14000 rpm for 90 minutes to separate the peptide/SWCNT structures from the uninteracted SWCNTs. To compare the dispersion properties of SWCNTs interacted with

peptides to the dispersion properties of SWCNTs interacted with oligonucleotides, the separated supernatant was investigated with Raman Spectroscopy. 2 μL of the supernatant was dropped on CaF_2 surface and dried at room temperature for Raman spectroscopy measurements.

4.3. COVALENT ATTACHMENT of OLIGONUCLEOTIDES to SWCNTs

In many studies, SWCNT was refluxed with HNO_3 for carboxylation process but the reflux time and acid concentration change depending on the applied process. These differences affect the carboxylation percentage of the SWCNT. Casabo et al. used 2.6 M HNO_3 and reflux time of 3-6 hours for carboxylation [91]. In another study, 1 M HNO_3 were used along with 12 hours of reflux time [92]. In our study, a 5 mg of SWCNT (Sigma-Aldrich, Germany) were refluxed in 5 mL of 4 M HNO_3 (Sigma-Aldrich, Germany) for 4 hours. After the reflux, the SWCNTs were washed with ddH_2O and pH was adjusted below 5 for the activation process to prevent the hydrolysis of SWCNT–NHS esters [92]. For the activation process, a 32.4 μg of N-hydroxisuccinimide (%98) (NHS) (Sigma-Aldrich, Germany), 9 mL of acetonitrile (Merck, Germany) and 150 μL of N-ethyldiisopropilamin (Sigma-Aldrich, Germany) were added into the SWCNTs dispersed in water and sonicated for 6 hours. The SWCNT dispersion was centrifuged at 14000 rpm for 90 minutes and the precipitate was dispersed in ddH_2O. A 25 μL of 100 mM each oligonucleotide was added to 500 μL portions of SWCNT/ddH_2O mixture in eppendorfs vials and incubated overnight. The schematic illustration of carboxylic acid functionalization and activation of SWCNT is given in Figure 4.3.

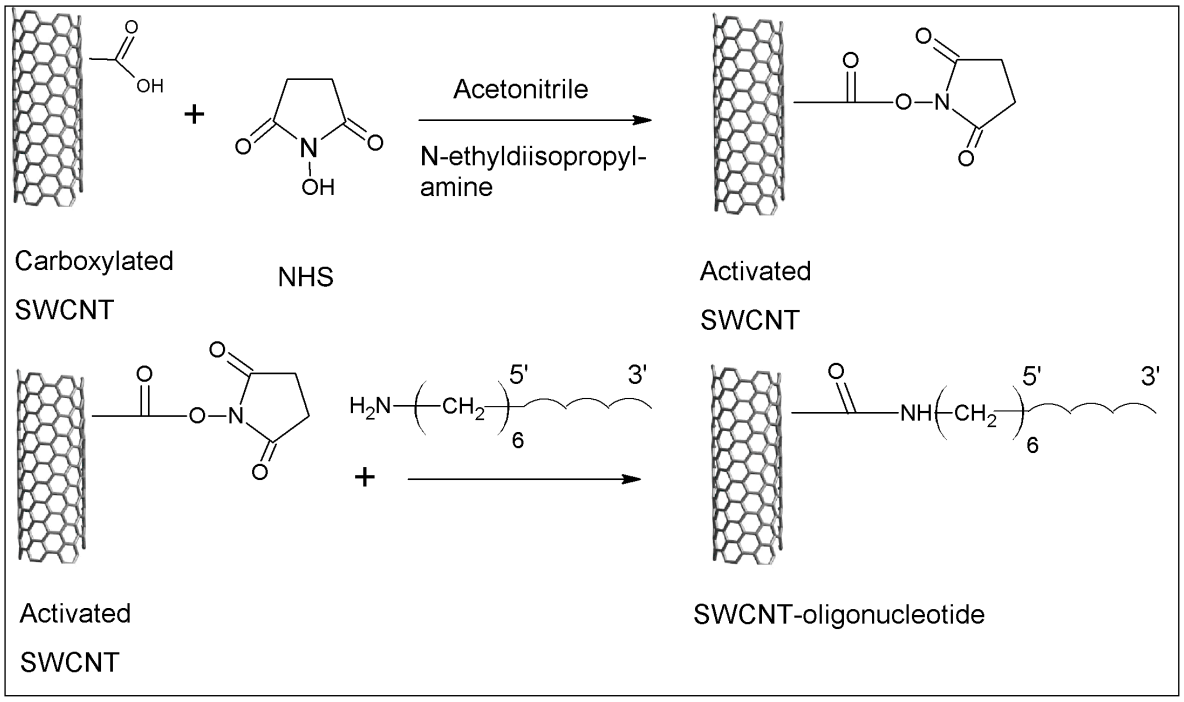

Figure 4.3. The scheme of covalent attachment of SWCNT-oligonucleotide

Figure 4.4 shows the RBM band comparison of pristine and carboxylated SWCNTs. During carboxylation process, as RBM bands give significant information about the tube's diameter, the defects in SWCNT's nanotube form causes the RBM bands disappear in the Raman spectra. As seen in Figure 4.4, after the carboxylation process with 2.6 M HNO_3, RBM bands are not disappeared. When compared to pristine SWCNT, a structural modification can be seen in carboxylated SWCNT's RBM bands due to the carboxylation of SWCNTs with diverse diameter properties (0.7-0.9).

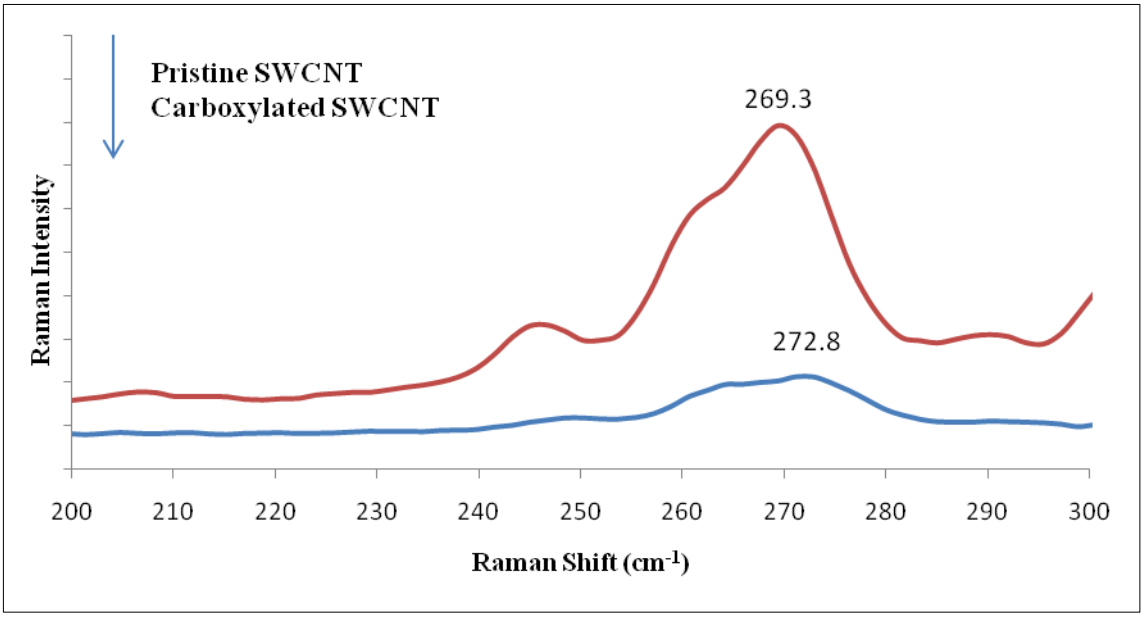

Figure 4.4. The Raman spectra comparison of carboxylated SWCNT (4 hours of reflux in 2.6 M HNO_3) and pristine SWCNT

4.4. RAMAN ISTRUMENTATION

A Raman Microscopy System (InVia Reflex, Renishaw, UK) was used with a diode laser at 514 nm and a 50X objective. The system was automatically calibrated against a silicon wafer peak at 520 cm^{-1}. The power of the laser beam at the sample position was 3 mW. The exposure time for each measurement was 10 seconds. The spectrum was processed with WIRE 3.2 software provided by Renishaw. Ten spectra were acquired from each droplet area, and averaged. The experiments for each oligonucleotide were repeated for at least three times for both non-covalent interaction and covalent attachment.

4.5. IR MEASUREMENTS

4.5.1. Comparison of Pristine SWCNT – Carboxylated SWCNT IR Spectra

FTIR analysis was done by Thermo Scientific Smart iTR in ATR mode with a diamond plate and ZnSe lens. Figure 4.5 shows the comparison of FTIR-ATR spectra of pristine SWCNT and carboxylated SWCNT. The broad peak at around 3200 cm^{-1} corresponds to –

OH vibrations coming from carboxyl groups. The peak at 1720 cm^{-1} indicates to C=O stretching vibrations of carboxyl and carbonyl groups. The peak at 1550 cm^{-1} shows the conjugation C=O and C=C groups. The peak at 1320 cm^{-1} belongs to COH groups. The peaks around 1040 cm^{-1} and 1260 cm^{-1} corresponds to C=O stretching vibrations.

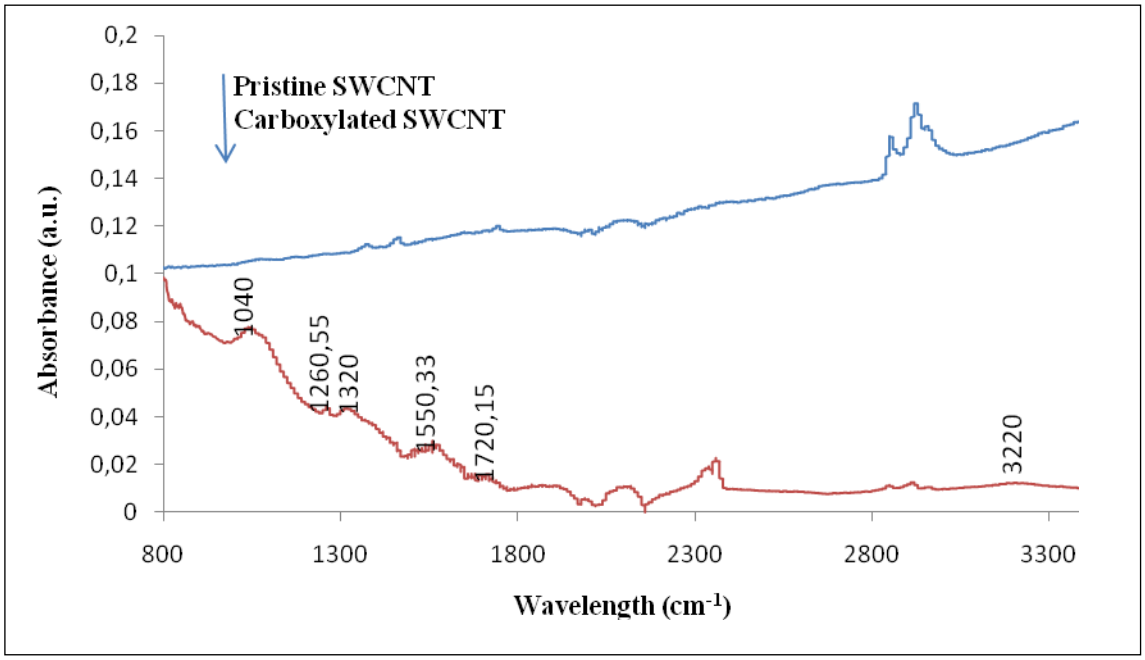

Figure 4.5. The IR spectra comparison of pristine and carboxylated SWCNT

4.5.2. IR Spectrum of Oligonucleotide Attached SWCNT

Figure 4.6 shows the FTIR-ATR spectrum of oligonucleotide attached SWCNT. The broad peak at around 3200 cm^{-1} shows the –OH stretching of water. The peak groups between 2800 cm^{-1} and 3000 cm^{-1} indicates the –CH stretching bonds which belong to the carbohydrate groups of oligonucleotides. The amide bond is seen as a peak between 1600-1700 cm^{-1} and in our case the peak at 1610 cm^{-1} corresponds to the amide bond. It is an evidence of the covalent bond between the carboxyl functions of SWCNT and oligonucleotide. The peaks between 1300 and 1500 cm^{-1} relate to the different vibrations and stretching of oligonucleotide bases. The peak at 1054 cm^{-1} shows the C=O stretch of oligonucleotides.

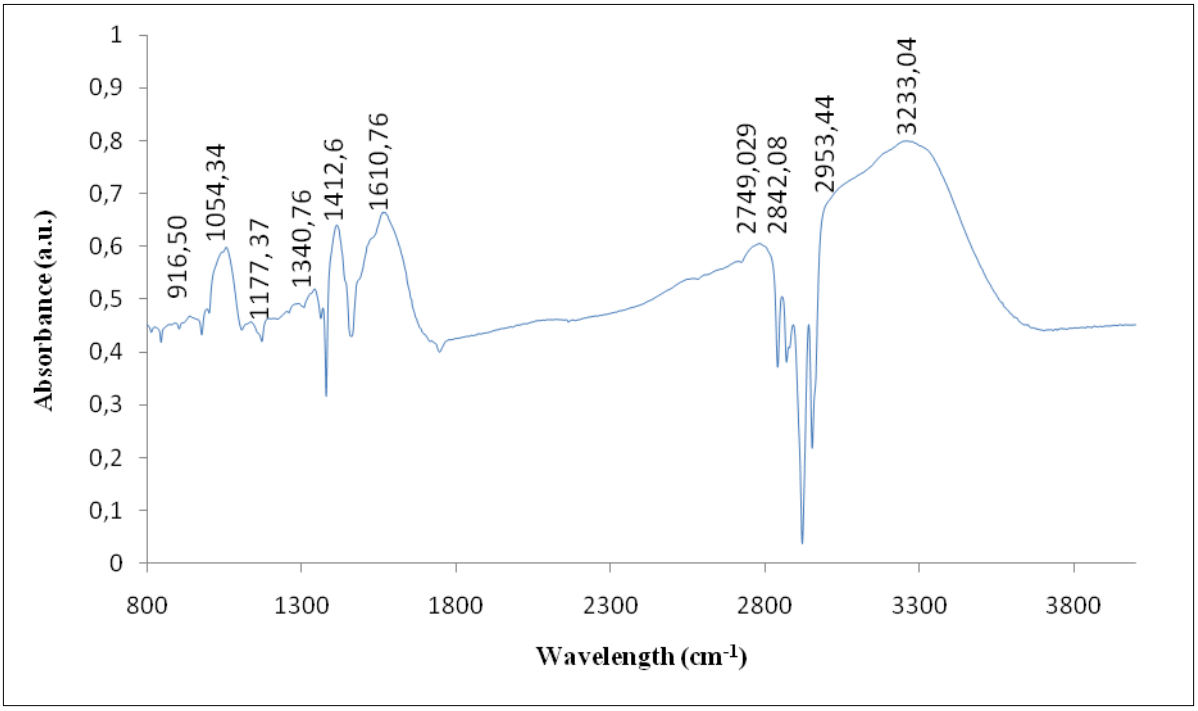

Figure 4.6. The IR Spectrum of oligonucleotide attached SWCNT

4.6. XPS MEASUREMENTS

The chemical attachment of oligonucleotides to the SWCNTs was verified with XPS analysis. AXIS NOVA photoelectron spectrometer (Kratos Analytical, Manchester, UK) equipped with monochromatic AlKα (*hv* = 1486.6 eV) anode was used in the studies. A typical analysis results are presented in Table 4.1. As seen, after the attachment of the oligonucleotides, 4.3 % of nitrogen appears in the SWCNT composition. Figure 4.7-4.9 shows the XPS spectra of pristine SWCNT (YT1), carboxylated SWCNT (YT2) and polyG-25 oligonucleotide attached to SWCNT (YT6) respectively.

Table 4.1. XPS elemental analysis percentages of pristine SWCNT, carboxylated SWCNT and SWCNT-oligonucleotide (25 based polyG)

	O1s	C1s	N1s
Pristine SWCNT (%)	60.4	13.2	-
Carboxylated SWCNT (%)	61.7	13.3	-
SWCNT-oligonucleotide (%)	50.2	19.5	4.3

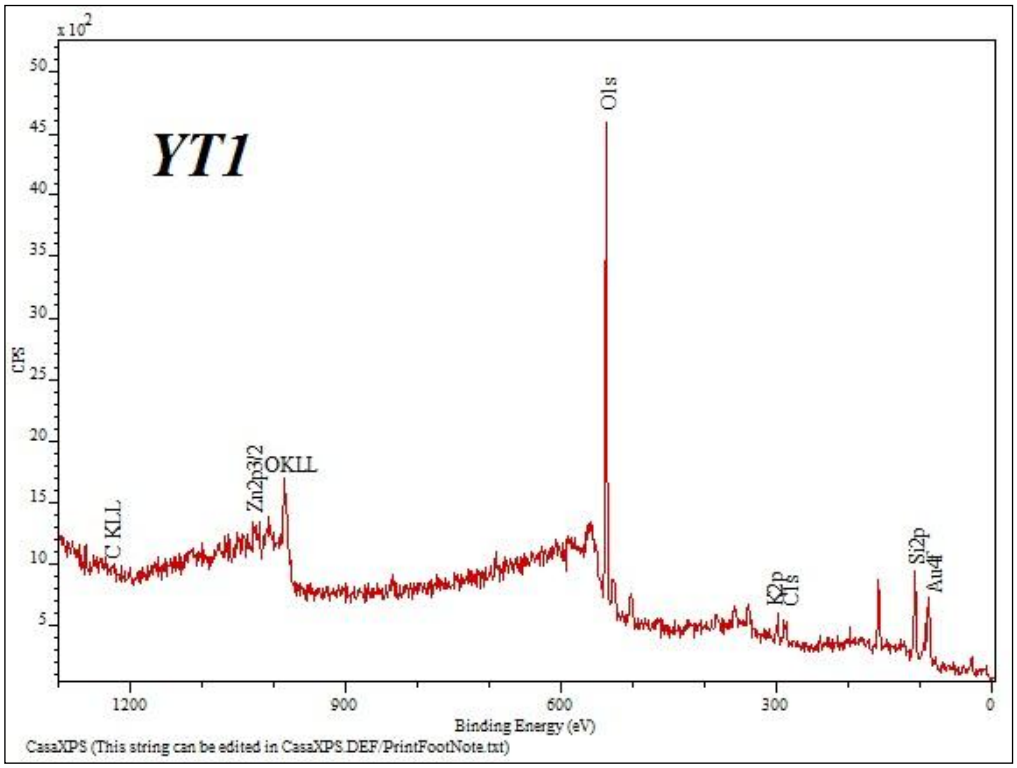

Figure 4.7. XPS spectrum of pristine SWCNT

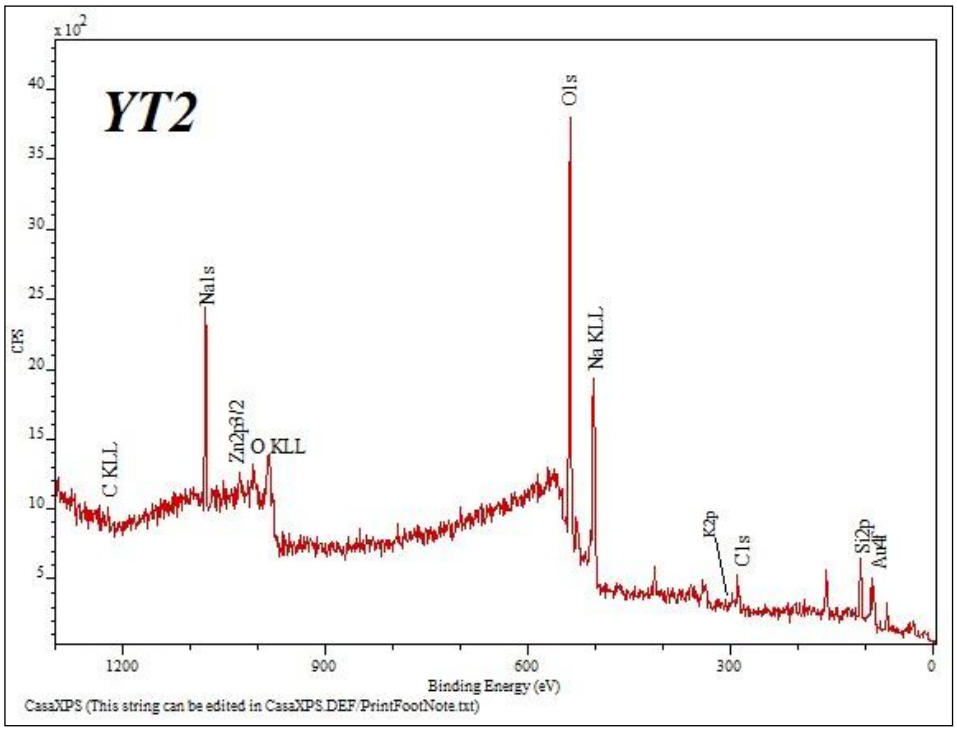

Figure 4.8. XPS spectrum of carboxylated SWCNT

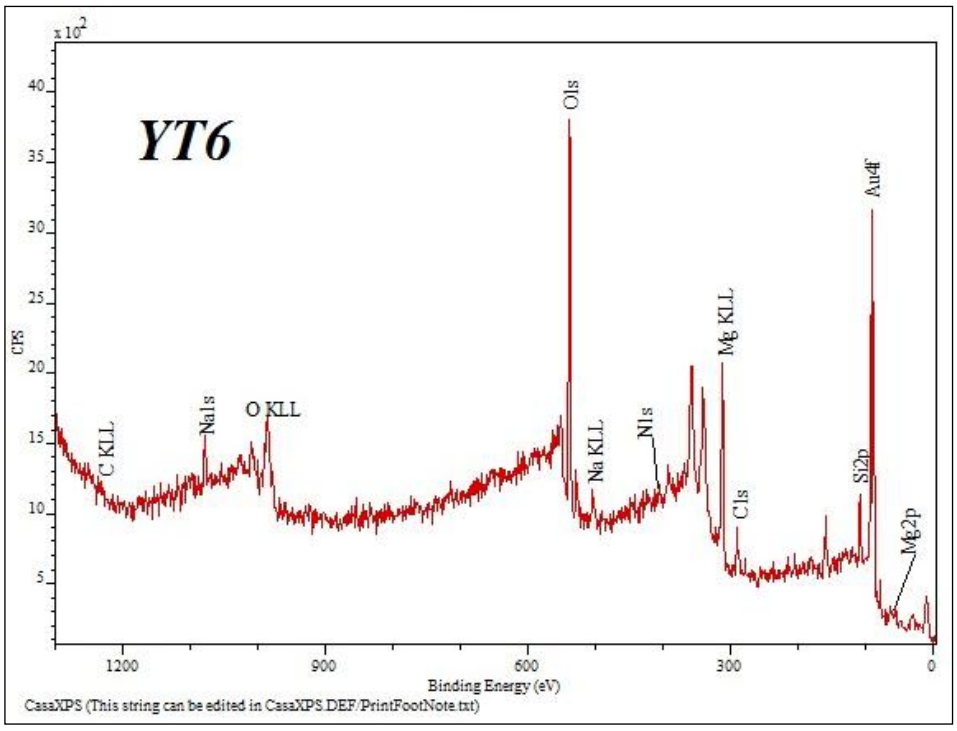

Figure 4.9. XPS spectrum of polyG-25 attached to SWCNT

5. RESULTS AND DISCUSSION

5.1. CHARACTERIZATION of SWCNTs

The presence of three RBM bands on the Raman spectra at 244.9, 260.7 and 269.3 cm^{-1} indicates the existence of SWCNTs with three different diameters in the purchased sample. The diameters of SWCNTs in the sample are determined using the equation (5.1) [95]. In Figure 5.1, the main bands detected in RBM region which correspond to specific diameters of pristine SWCNT are given. The calculated diameters are given on Table 5.1.

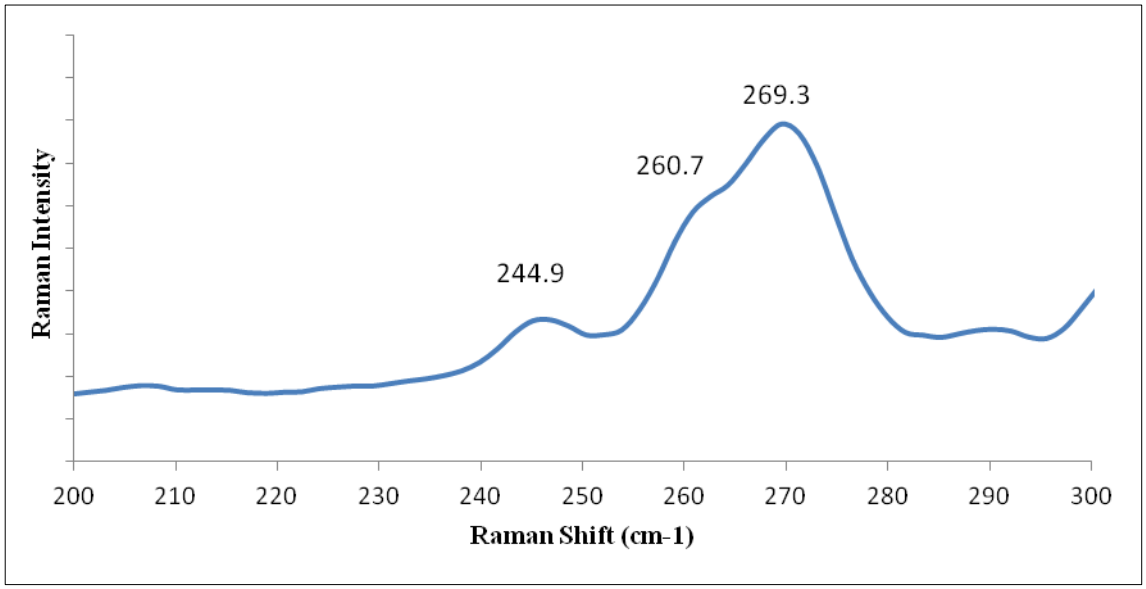

Figure 5.1. The RBM band region of Raman spectrum of pristine SWCNT

The broader nature of the bands on the RBM region of the spectrum suggests that there are several sizes of SWCNTs in the sample in the range of 0.7-1.0 nm.

$$(\omega(cm^{-1}) = \frac{223.5}{d(nm)} + 12.5) \quad (5.1)$$

Table 5.1. Diameter distribution of pristine SWCNT determined from RBM band frequency

$\omega_{RBM}(cm^{-1})$	$d\ (nm)$
244.9	0.96
260.7	0.90
269.3	0.87

5.2. RAMAN INVESTIGATIONS

5.2.1. Non-Covalent Interactions between Oligonucleotides and SWCNTs

Oligonucleotide wrapping around SWCNTs causes an increase in the surface pressure of SWCNTs, which causes an upshift in the RBM band [93]. The upshift in RBM bands suggests that once the oligonucleotide achieves the wrapping, the similar wrapping intensity exerted onto the nanotube surface. Therefore, the degree of the shift to higher wavenumber along with the intensity of the bands gives information about the degree of the interactions. Figure 5.1-5.4 shows the RBM region of the oligonucleotide wrapped SWCNTs. Figure 5.1 shows the RBM bands after the interaction of polyA-10 and polyA-25 and Figure 5.2 shows the RBM bands after the interaction of polyT-10 and polyT-25 with SWCNTs for 30 min and 6 h sonication at 0°C along with the pristine SWCNTs. The RBM region of the spectra for polyA-10 and polyA-25 is given on Figure 5.2. The low intensity of the band features indicates the poor interaction of the oligonucleotides with the SWCNTs, which results with poor dispersion in the aqueous media. When the aqueous solution of SWCNT-oligonucleotide adducts was centrifuged, a very small amount of the adducts was remained in the solutions. Therefore, the intensity of the bands under the same experimental conditions was not high enough to observe. As it is seen, the polyA-10 and polyA-25 sonicated for 30 mins and 6 h at 0 ^{o}C, respectively, have rather poor Raman spectra. The polyA-10 and polyA-25 sonicated for 6 h and 30 mins at 0 ^{o}C, respectively, have better spectra. When the oligonucleotide length is 10-base long, it wraps around the SWCNTs with a better efficiency, as the incubation time gets longer. However, when the length gets longer, the efficiency gets worse with the incubation time. This suggests that increased time results with the un-wrapping due to the self-stacking.

Figure 5.3 shows the RBM bands for the polyT case. It seems that polyT-10 does not wrap the SWCNTs very effectively. In 30 mins, the wrapping takes place and increasing the time to 6 h does reduce the interaction efficiency. However, when the oligonucleotide size increased to 25, the wrapping becomes very effective in 30 mins. Similar to the polyA-25 case, the interaction efficiency gets worse, which indicates the self-staking. The increment in the intensity of the RBM band demonstrates that the interaction of short oligonucleotide with SWCNTs improves with time. However, for the longer oligonucleotides, the decrement in the intensity of the RBM band shows that interaction efficiency decreases with time. The increments and decrements in the intensity of the RBM band are related with the concentration of SWCNTs in the dispersion.

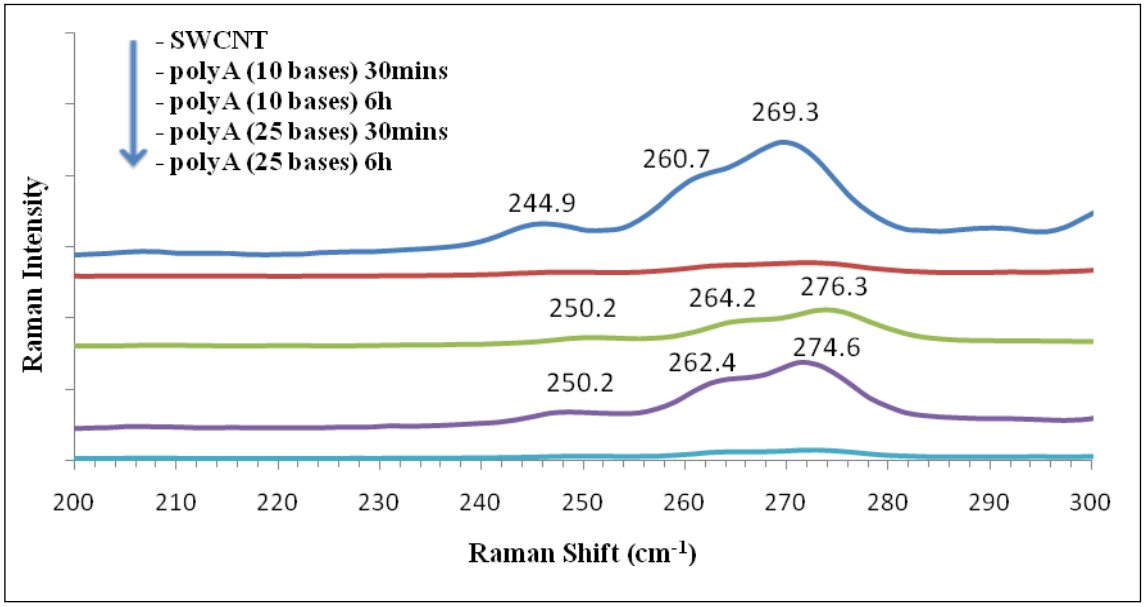

Figure 5.2. RBM region of Raman spectra of oligonucleotide wrapped SWCNTs at 30 minutes and 6 hours at 0 °C; 10 and 25 based polyA-SWCNT

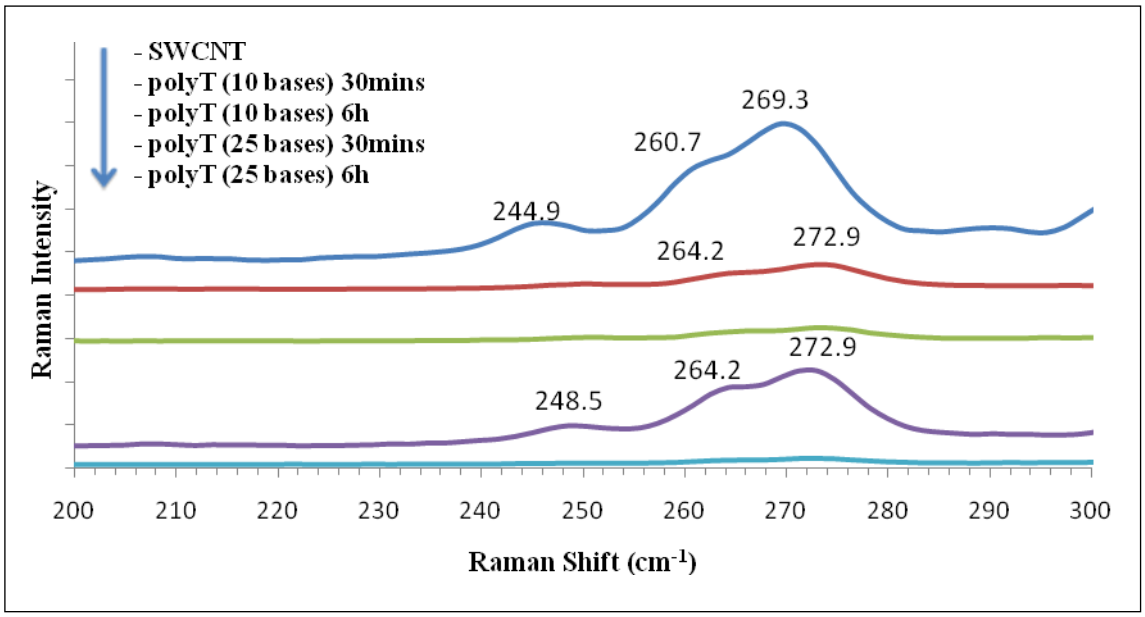

Figure 5.3. RBM region of Raman spectra of oligonucleotide wrapped SWCNTs at 30 minutes and 6 hours at 0 °C; 10 and 25 based polyT-SWCNT

Figure 5.4 and Figure 5.5 shows the RBM bands upon interaction of polyG and polyC with the SWCNTs. Figure 5.4 shows the case for polyG. As seen, the polyG-10 sequence is not wrapping the SWCNTs in 30 mins. However, increasing the time to 6 h makes a large impact wrapping the SWCNTs. Increasing the length of the polyG sequence to 25 increases the interaction to some degree but increasing the incubation time to 6 h reduces their interaction with the SWCNTs dramatically. Figure 5.5 shows the case for polyC. As seen, in all cases polyC effectively interacts with the SWCNTs. However, increasing the incubation time for polyC-10 further increases the interaction. Although the cases for the polyC-10 and polyC-25 are similar, increasing the incubation time slightly increases the interaction of polyC-25 with the SWCNTs.

In a recent report, the interaction of the polynucleotides with 25-base long was simulated using molecular dynamics modeling (MD) [48]. That study concluded that the order of the interaction energies were polyT-25 > polyC-25 > polyA-25 ≈ polyG-25. The lower affinity of polyA and polyG was explained with the self-stacking of the bases in a long oligonucleotide. However, although our results fairly overlap with their results with polyA, polyG and polyC, there is significant deviation with polyT, which was found to have best interaction with the SWCNTs. The upshift is present in all conditions when the RBM

bands are compared to pristine SWCNT. This means that polyC interacts with SWCNT regardless of base length and sonication time. Karachevtsev et al. explained this with MD pointing that 25 based polyC was the fastest of the four bases to make a pitch around the tube (20 ns). Also in 10 ns, polyC was stacked with the SWCNT surface with 18 bases. At the same time, polyT was stacked with 13, polyA and polyG was stacked with only 8 bases. In addition to these, the self-stacking rate of polyC was considerably low when compared to other bases. Enyahsin et al. studied the salvation of SWCNTs with 12 base long poly oligonucleotides in aqueous solutions using their density-functional tight-binding method (DFTB) and found that polyC and polyT oligonucleotides have higher affinity for the SWCNTs [44].

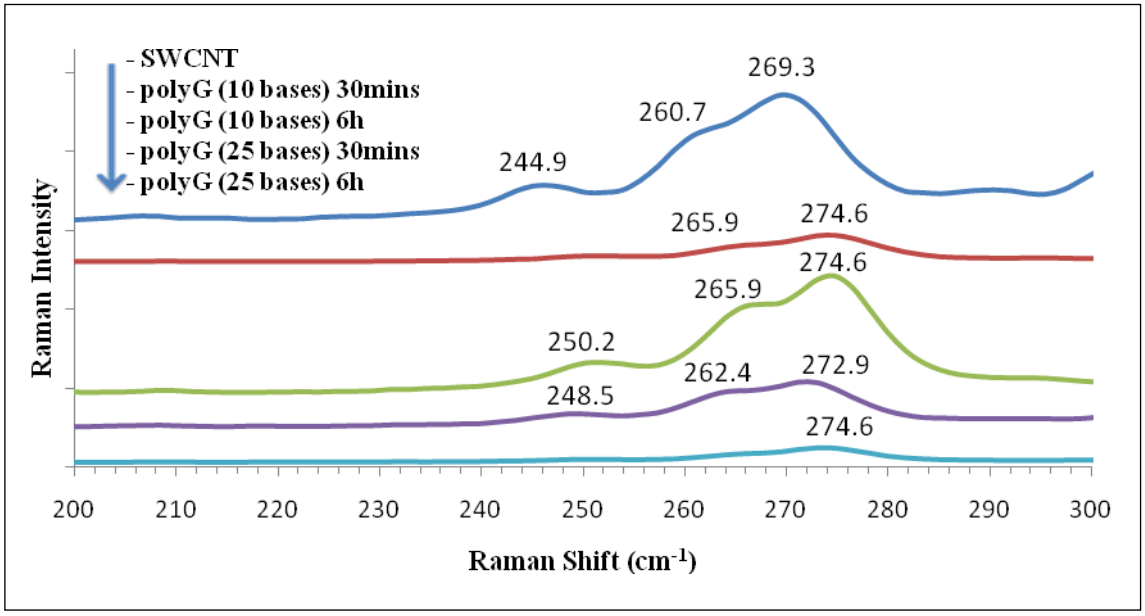

Figure 5.4. RBM region of Raman spectra of oligonucleotide wrapped SWCNTs at 30 minutes and 6 hours at 0 °C; 10 and 25 based polyG-SWCNT

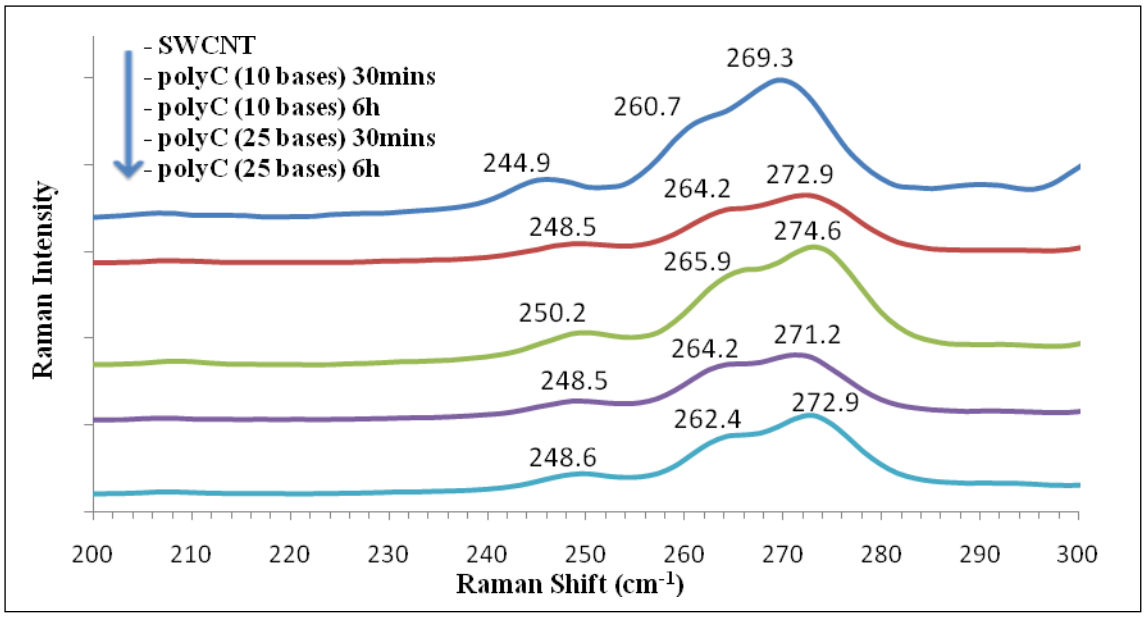

Figure 5.5. RBM region of Raman spectra of oligonucleotide wrapped SWCNTs at 30 minutes and 6 hours at 0 °C; 10 and 25 based polyC-SWCNT

5.2.2. Non-Covalent Interactions between Peptides and SWCNTs

To investigate the selectivity of SWCNT and oligonucleotide interactions, SWCNTs were interacted with M1, M3, M4 and M7 peptides and the interactions were observed by Raman Spectroscopy. The peptides were chosen according to their solubilization properties in water. After the sonication of peptides and SWCNTs for 30 mins in 0 ºC water bath, the supernatant separated from uninteracted SWCNTs were investigated by Raman Spectroscopy. However, no interacted SWCNTs with peptides were observed in Raman Spectroscopy analyses. This results shows that short time sonication does not offer convenient interaction conditions for peptide and SWCNTs. In Figure 5.10, the changes in the RBM bands after the interaction of SWCNTs with peptides after 6 h of sonication in 0 ºC water bath can be seen. The investigation for the interactions between peptides and SWCNTs were repeated 3 times. Only in one of the investigations, SWCNTs were detected in the dried droplets with diluted concentration. However, it is possible that while taking samples from supernatant, trace amount of the unattached SWCNTs could be present in the dispersion. Thus, the Raman Spectra collected from peptide-SWCNT dispersion is thought to come from unattached SWCNTs. Regardless of the slight upshift (274.6 to 276.3) seen in SWCNT/M1 interaction, the upshift observed in the RBM bands

after the non-covalent interactions between oligonucleotides and SWCNTs is not present in the non-covalent interactions between peptides and SWCNTs. Thus, it can be declared as the peptides do not wrap around SWCNTs and affect the electronic structure of SWCNTs.

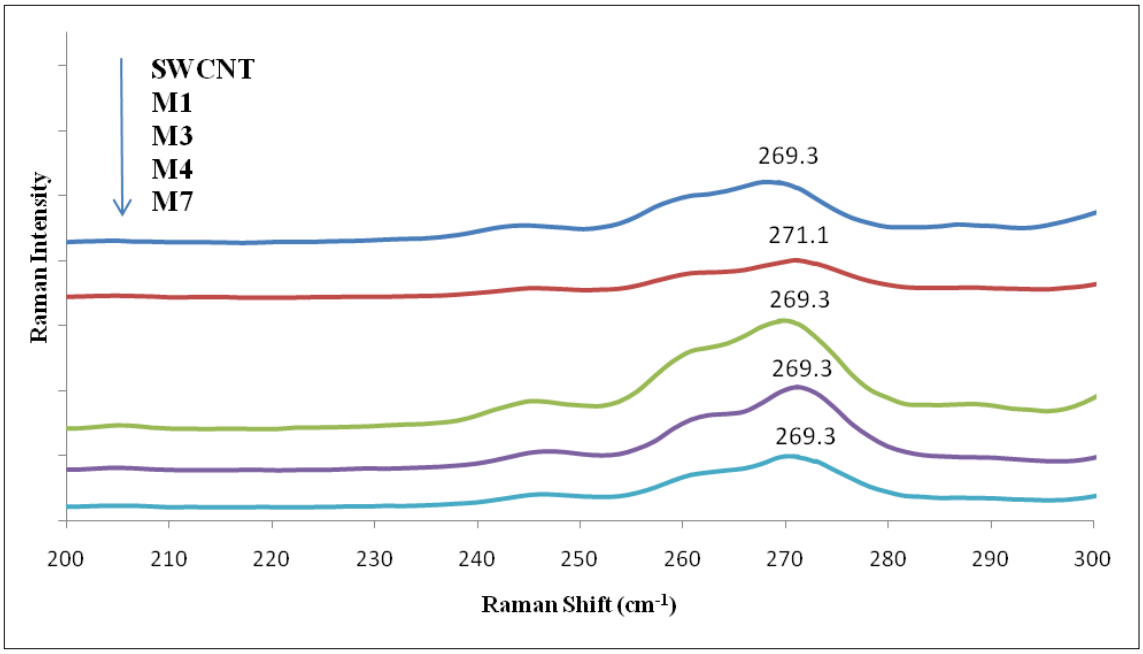

Figure 5.6. RBM region of Raman spectra of SWCNTs interacted with M1, M3, M4 and M7 for 6 hours at 0 °C

5.2.3. Covalent Attachment of Oligonucleotides to SWCNTs

The influence of the chemical attachment of the same oligonucleotides on the electronic structure of the SWCNTs was investigated. Figure 5.7 and Figure 5.8 shows the comparison of the RBM bands of pristine SWCNT, carboxylated SWCNT and SWCNT covalently attached oligonucleotides with different lengths. The carboxylation process causes an upshift of the RBM bands compared to RBM band of pristine SWCNTs. After initial upshift with the oxidation of the SWCNTs, there is only small upshift observed (from 272.8 to 274.5) with the attachment of the oligonucleotides. This indicates that the attachment of the oligonucleotides has an influence on the SWCNT structure. However, since the upshift is smaller than the upshift observed with non-covalent interactions, it is not clear at this moment that this change is due to the chemical attachment of the oligonucleotides. On the other hand, it is still possible that the chemically attached

oligonucleotides may wrap around the SWCNTs or cross-wrap each other. Since the oligonucleotides are chemically immobilized onto the CNT wall, the wrapping may not be as effective as they are free. In the latter scenario, the expected upshift in the longer oligonucleotides is greater. However, as seen on Figure 5.8, the upshift values are very similar to the values observed with the shorter oligonucleotides. It should be also noted that we do not have the information of the attachment locations of the oligonucleotides on the SWCNT wall. When the oxidation locations are the ends and the defects of the SWCNT, it is possible that the oligonucleotides can bind to any of these locations. Besides we do not currently know the number of oligonucleotides bound to SWCNTs either. We should note that, after covalent attachment process, the SWCNT dispersions were centrifuged at 14000 rpm for 90 minutes to remove the unattached oligonucleotides that are present in the supernatant phase.

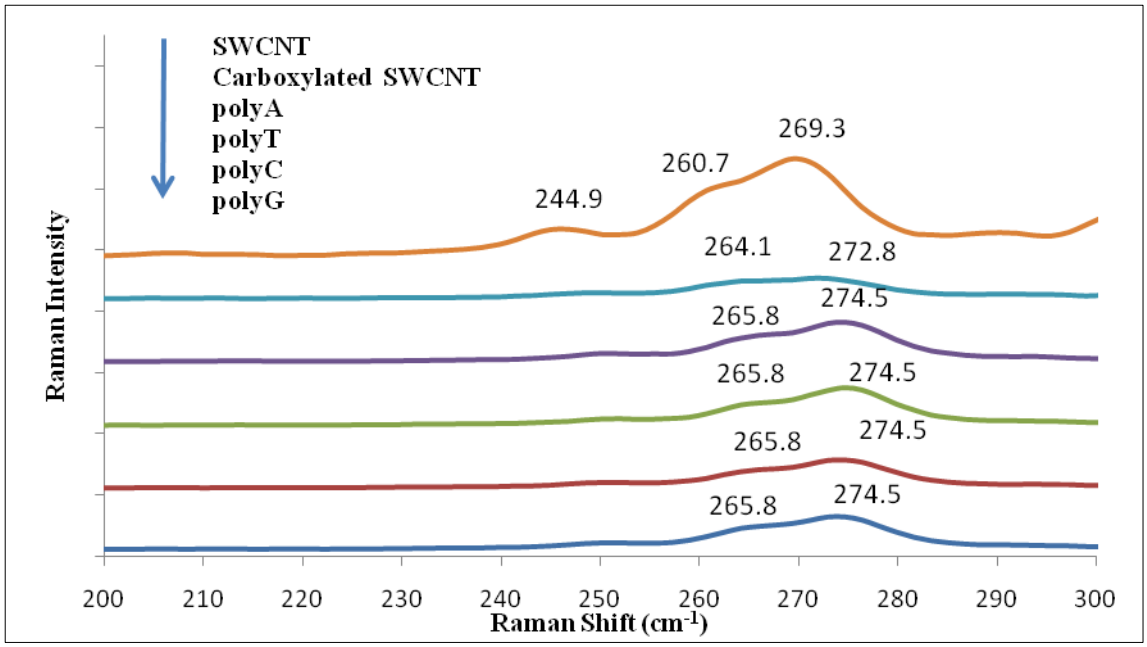

Figure 5.7. RBM region of Raman spectra of pristine SWCNT, carboxylated SWCNT and SWCNTs after chemical attachment of 10 base long oligonucleotides (1 day, 23 ºC)

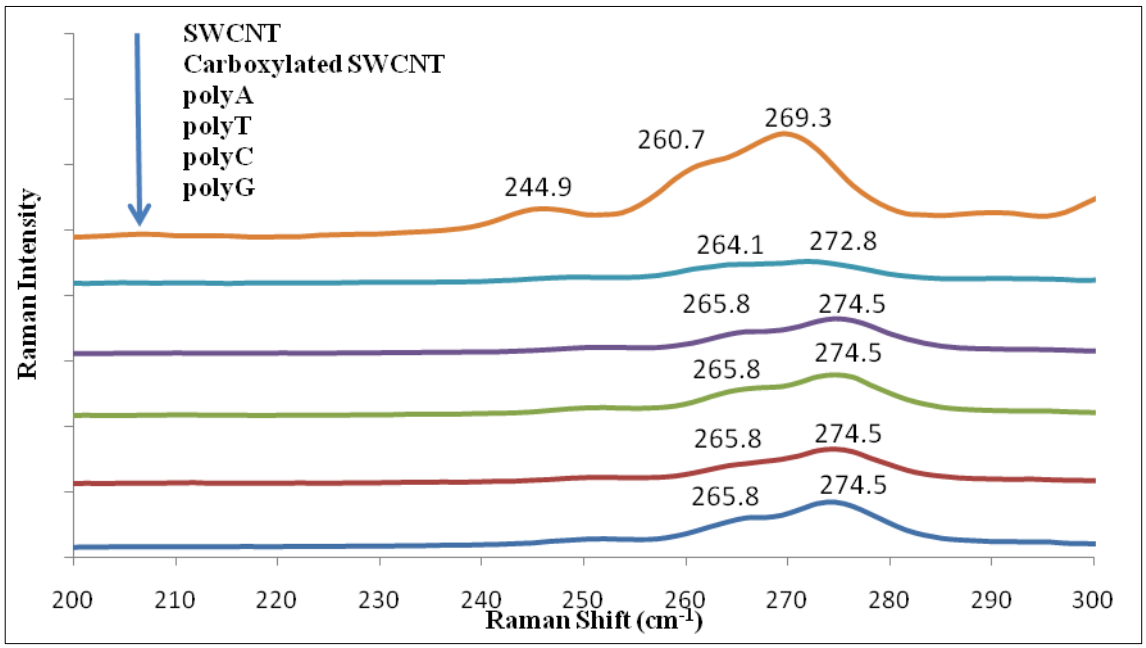

Figure 5.8. RBM region of Raman spectra of pristine SWCNT, carboxylated SWCNT and SWCNTs after chemical attachment of 25 base long oligonucleotides (1 day, 23 ºC)

Further, D and G bands were analyzed for the possible changes in the electronic structure of the SWCNTs with the chemical modification. The change in the D/G band area ratio between pristine SWCNT and carboxylated SWCNT gives information about the structural integrity of the SWCNTs. The increase of the D/G band ratio shows the graphene sheet symmetry distortion while this ratio increases with carboxylation SWCNT and its dispersion in water [90]. Figure 5.9 shows the change in the D/G band ratios after carboxylation and chemical attachment of the oligonucleotides. A small decrease in D/G ratio is observed with the attachment of oligonucleotides. This is possibly due to the increased dispersion of SWCNT-oligonucleotide structures in water [94]. However, when the D/G ratios of modified SWCNTs are compared, it seems that the ratio is higher in the case of shorter oligonucleotides. In addition, the solutions of SWCNT-oligonucleotide are very stable. The solutions were kept at 4°C for 30 days and any precipitation was not observed in the solutions (Figure 5.10).

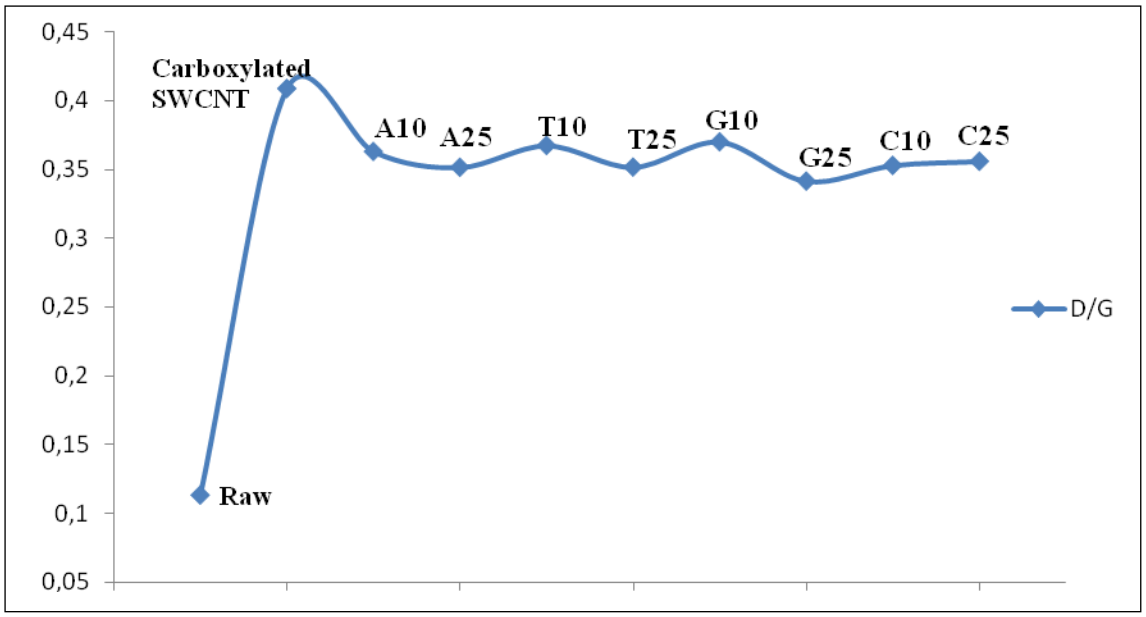

Figure 5.9. The D/G band ratios of pristine SWCNT, carboxylated SWCNT and different base and sequenced oligonucleotides covalently attached to SWCNTs

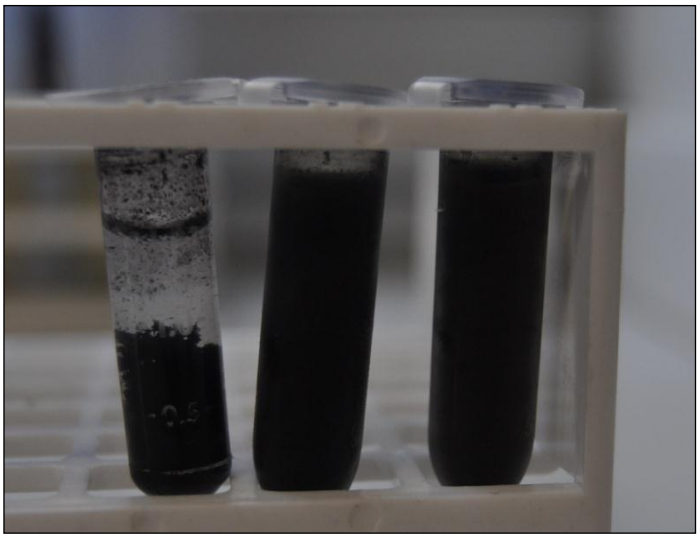

Figure 5.10. The images of pristine SWCNT, polyC-10 and polyC-25 attached SWCNT

6. CONCLUSION

The interaction of SWCNTs with short (10-base) length polyA, polyT and polyG increases as the time of incubation increases from 30 minutes to 6 hours. However for the interaction of SWCNT with long (25-base) length polyA, polyT and polyG, the RBM band intensity decreases with the incubation time with the exception of polyC. In addition, when the intensity of each RBM band on the spectrum is examined, it is seen that the intensity ratios before and after their interaction with the oligonucleotides does not change very much. This indicates that the oligonucleotides do not recognize the SWCNT diameter and wraps all sizes unselectively. This is much clearly seen in the case of polyC, which shows a significant affinity against the all sizes of the SWCNTs present in the sample regardless of the incubation time.

The non-covalent interactions between peptides and SWCNTs were also studied to investigate the selectivity of SWCNT and oligonucleotide interactions. The upshift seen in non-covalent interactions between oligonucleotides and SWCNTs cannot be observed in the interactions between peptides and SWCNT. Thus, it is clear that peptides do not wrap around SWCNTs as oligonucleotides but they seem they interact through points on the peptide and nanotube surfaces.

In the case of covalent attachment, an initial upshift is observed after the oxidation process; however, a small upshift is observed after the chemical attachment of the oligonucleotides, which was much smaller than the upshift observed with non-covalent attachment. This could be due to the partial wrapping around the SWCNT or cross wrapping of chemically attached oligonucleotides SWCNTs.

In conclusion, both non-covalent interactions of oligonucleotides and their covalent attachment seem to have an impact on electronic structure of the SWCNTs. However, this does not seem to influence electronic properties in a large extent in the studied system. Covalent attachment investigations result in a highly stable dispersion of oligonucleotide/SWCNT structure.

The possible effects on the unique properties of SWCNTs such as electrical conductivity are the subject to further investigations. The changes in the electrical conductivity of SWCNTs after the covalent attachment of short and long oligonucleotides can be investigated with the STM mode of Atomic Force Microscopy. The combinations of various sequences of nucleotide bases can also be designed for both covalent attachment and non-covalent interactions for further electrical conductivity investigations.

In this study, we have used SWCNTs with an average 0.9 nm diameter, which is comparable to the diameter of ssDNA (about 1.1 nm). The size relationship between the oligonucleotides and SWCNTs could be another parameter affecting the strength of the interaction. Therefore, the size relationship in their interaction can be investigated further. The non-covalent interaction investigations were designed for short and long sonication times at 0 °C. The interactions can be studied with increasing temperature by heating the dispersion during the sonication process until boiling temperature since the ssDNA with 10 and 25-base lengths do not degrade at boiling temperature of water. The effect of pH in the non-covalent interactions between oligonucleotides and peptides can also be investigated to explore if the interaction tendency changes at different pH values. The pH study can be extended to peptides for comparative evaluation of the interactions of oligonucleotides and peptides with SWCNTs.

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