We are IntechOpen, the world’s leading publisher of Open Access books
Built by scientists, for scientists

4,000 Open access books available
116,000 International authors and editors
120M Downloads

154 Countries delivered to
TOP 1% Our authors are among the most cited scientists
12.2% Contributors from top 500 universities

WEB OF SCIENCE™
Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com
Dispersions Based on Carbon Nanotubes – Biomolecules Conjugates

Ignác Capek
Slovak Academy of Sciences, Polymer Institute, Institute of Measurement Science, Dúbravská cesta, Bratislava, Trenčín University, Fac. Ind. Technol., Púchov, Slovakia

1. Introduction

The discovery of carbon nanotubes (CNTs) [1] followed by their large-scale production have paved the way to wide CNT integration into modern nanotechnology by taking advantage of their excellent mechanical properties and high electrical and thermal conductivities [2]. Carbon nanotubes have emerged as new class nanomaterials that are receiving considerable interest because of their unique structure, high chemical stability and high surface-to-volume ratio. Composite nanomaterials based on integration of CNTs and some other materials to possess properties of the individual components with a synergistic effect have gained growing interest [3].

The use of carbon nanotubes as “building blocks” in nano-/microelectronic devices could revolutionize the electronic industry in the same way that the microchips have revolutionized the computer industry. However, it has been a long-standing big challenge to efficiently integrate the carbon nanotube “building blocks” into multicomponent/multifunctional structures or devices. It has been shown that carbon nanotubes could promote electron transfer with various redox active proteins, including glucose oxidase, cytochrome c, and horseradish peroxidase. Li and co-workers have demonstrated that carbon nanotubes can promote electron transfer with certain proteins and enzymes, and the electrochemical behavior with cytochrome c [4].

The CNT is nearly inert and therefore its functionalization is needed to increase its reactivity and to form with other components conjugates or hybrids. Chemical functionalization of CNTs usually destroys the sp² structure of CNTs, therefore, damages the intrinsic properties of them. Thus, non-covalent modification of CNTs is of great significance. Surfactants can disperse CNTs in water, however, which needs relatively higher amount and cannot be used for possible biological and chemical application [5].

Pioneering studies have reported on the use of single-walled carbon nanotubes (SWCNTs) as atomic force microscopy (AFM) imaging tips of biomacromolecules, such as antibodies, DNA, proteins, viruses... [6]

The integration of biomaterials (e.g., DNA, proteins/enzymes, or antigens/antibodies) with CNTs provides new hybrid systems that combine the conductive or semiconductive properties of CNTs with the recognition or catalytic properties of the biomaterials. This may
yield new bioelectronic systems (biosensors, field-effect-transistors,...) or templated nanocircuitry. Functionalyzed CNTs are able to cross cell membranes and accumulate in the cytoplasm, and even reach the nucleus, without being cytotoxic (in concentrations up to 10 mM) [7]. CNTs could act as carriers that transport and deliver other bioactive components into cells. In fact, the effective delivery of biomolecules into cells has been used for their immunization and enhanced generation of antibodies [8].

New materials for the intracellular transport of biological cargos such as DNA, proteins, and drug molecules have been actively sought to effectively breach the cell-membrane barriers for delivery and enabling functionality of extracellular agents. Single-walled carbon nanotubes (SWNT) have been recently shown to shuttle various molecular cargos inside living cells including proteins, short peptides, and nucleic acids [9, 10]. The internalized nanotubes were found to be biocompatible and nontoxic at the cellular level. The utilization of the intrinsic physical properties of SWNTs allows the realization of a new class of biotransporters and opens up new possibilities in drug delivery and near infrared (NIR) radiation therapy.

Carbon nanotubes as well as other nanotube structures, such as self-assembled lipid microtubes or peptide nanotubes, have been explored for possible applications in nanobiotechnology [11]. Also, biomedical applications of biomaterial-functionalized CNTs are envisaged. At the present time, several fundamental issues remain to be addressed for the use of carbon nanotubes as potential biological transporters. One such issue is the entry mechanism that regulates the cellular internalization of SWNTs and their cargos.

The attractive properties of CNT make them promising candidates for DNA hybridization detection [12]. Wang et al. [13] demonstrated the use of CNT loaded alkaline phosphatase through cross linking for dramatically amplifying enzyme-based bioaffinity electrical sensing of proteins and DNA. Khairoutdinov et al. [14] and Panhuis and co-workers [15] reported the approach to covalent attachment of ruthenium complex to carboxylated single-wall carbon-nanotubes (SWNT) and to amino functionalized multiwall carbon nanotubes, respectively.

A brief summary of the most recent research development in the field of carbon nanotube/biomolecules is reported. Even this brief account has revealed the versatility of carbon nanotubes for making nanoconjugates with excellent physical and chemical properties. Within the present chapter we focus on decoration of CNTs with DNA to produce unique and smart nanocomposites. Continued research and development in this field favours the possibility of producing biosensors and/or smart nanostructures based on carbon nanotube/DNA conjugates.

2. Carbon nanotubes

Carbon nanotubes (CNTs) are anisotropic structures with sp² bonding properties. A carbon nanotube consists of either one cylindrical graphite sheet (single-walled carbon nanotube, SWCNT) or several nested cylinders (multi-walled carbon nanotube, MWCNT). Carbon nanotubes are macromolecules with radii of as small as a few nanometers, which can be grown up to 20 cm in length. Multi-walled nanotubes can reach diameters of up to 100 nm. Single-walled nanotubes possess the simplest geometry, and have been observed with diameters ranging from 0.4 to 3 nm [16]. The rolling-up of the hexagonal lattice can be performed in different ways. The sheet can be rolled-up along one of the symmetry axes, producing, either a zigzag or an armchair tube.
Single-walled carbon nanotubes (SWCNTs) exhibit excellent optical properties such as Raman, fluorescence and absorption spectra. Due to their small size and sensitive optical characteristics, SWCNTs have been shown to be ideal candidates for optical nano-biomarkers and/or nano-biosensors [17]. SWCNTs individually dispersed in aqueous medium show strong fluorescence in the near infrared region and sharp absorption peak distributions caused by van Hove singularities [18]. Absorption spectrum gives a variety of information including electronic structures such as metallic, semiconducting and chiralities [19].

It is well known that individually dispersed carbon nanotubes exhibit a series of sharp absorption peaks in the visible and near infrared regions due to quasi one-dimensionality. The absorption peaks become broad due to the disturbance and superposition of electronic structures by the aggregation of nanotubes. Some materials such as surfactants (sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB),...), DNA, and proteins are known to individually disperse SWCNTs without disturbing the electronic properties of nanotubes [20]. There can also be a slight shift in the wavelength for the different dispersing medium. This could be due to the hypsochromic shift which was also observed in Ref. [20].

The change in dielectric environments around nanotubes, caused by the different surface coverage of wrapping agents, can lead to a shift in wavelength.

It has been stated that nonionic surfactants cause suspension of nanotubes by coating them [20]. The presence of nonionic surfactant could lead to complex micelle formation with CNT in both aqueous and nonaqueous systems. It was shown that the high-molecular weight Pluronic surfactants enhance dispersal of SWCNTs. It is possible that such surfactant-nanotube interactions alter the nanotubes' surface properties, thus modulating their interaction with other additives. The cylindrical micelles were formed in aqueous dispersions of sodium dodecyl sulfate (SDS)-SWNT [21]. For all samples, the UV-vis spectra exhibited the sharp van Hove transitions anticipated from individualized nanotubes. For the dispersion of SWNTs the SDS molecules were considered to form noninteracting core-shell cylindrical micelles with a single nanotube acting as the core.

It was reported that the behavior of a surfactant in dispersing the carbon nanotubes is similar to that in the case of solid particles [22]. Since surfactant effects depend strongly on the medium’s chemistry, aqueous and organic polymeric systems of carbon nanotubes should, in principle, obey different colloidal processes. However, a common factor is that surfactants having long tail groups and more unsaturated carbon-carbon bonds greatly contribute to stabilizing the carbon nanotube dispersions and reducing the size of CNT agglomerates. In a water-soluble polymer, e.g. poly(ethylene glycol), cationic surfactants show some advantage, owing to their preferential attraction to negatively charged CNT surfaces.

To covalently bond (bio)molecules to the CNTs, it first requires the formation of functional groups on the CNTs. The carboxylic group is often the best choice because it can undergo a variety of reactions and is easily formed on CNTs via oxidizing treatments. It is reported that the presence of carboxylic group at the nanotube ends and at defects on the sidewalls has advantages to perform acid base chemistry and to introduce on the nanotube amide, ester linkage, and so on [23]. The control of reactants and/or reaction conditions may control the locations and density of the functional groups on the CNTs, which can be used to control the locations and density of the attached biomolecules. For example, concentrated acids are known to introduce acidic groups to the sidewalls and ends of CNTs [24].

The SEM images of as-synthesized MWCNTs showed that MWCNTs are held together into bundles via Van der Waals forces [25]. The f-MWCNTs are discrete and shorter than that of as-synthesized MWCNTs due to the acid treatments. The TEM image of as-synthesized...
MWCNTs illustrated that the nanotubes are entangled and randomly oriented. The outer surface of MWCNTs is smooth. The diameter and length of MWCNTs are ~ 30-80 nm and 10-20 μm, respectively. After the acid treatment, the MWCNTs are dispersed and most of the nanotubes are shortened (length of MWCNTs ~ 1-5 μm). The TEM image of amino f-MWCNTs indicated that the nanotubes surface is rough compared with the nanotubes without functionalization treatment.

Carbon nanotubes can be functionalized with various biomolecules without their covalent coupling [26]. Open-ended carbon nanotubes provide internal cavities (1-2 nm in diameter) that are capable of accommodating organic molecules and biomolecules of respective sizes. Functionalized carbon nanotubes are able to cross cell membranes and accumulate in the cytoplasm, and even reach the nucleus, without being cytotoxic (in concentrations up to 10 mM) [27].

Thus, carbon nanotubes could act as carriers that transport and deliver other bioactive components into cells. In fact, the effective delivery of biomolecules into cells has been used for their immunization and enhanced generation of antibodies [28]. Pioneering studies have reported on the use of single-walled carbon nanotubes as atomic force microscopy (AFM) imaging tips of biomacromolecules, such as antibodies, DNA... [29].

Carbon nanotubes (CNTs) possess a hollow core and large specific surface area suitable for storing guest molecules [30]. It was demonstrated that CNTs could promote electron transfer reactions with enzymes [31] and enhance the electrochemical activities of many biomolecules, which could allow them to be used as mediators in biosensor systems [32]. The attractive structural, electrical and mechanical properties of CNT make them promising candidates for electrochemical biosensors [33]. Most CNT-sensing work has focused on the ability of surface-confined CNT to accelerate the electron-transfer reactions in connection to amperometric enzyme electrodes. CNT have been recently used as transducers for enhanced electrical detection of DNA hybridization [34].

As the leading nanodevice candidate, SWNTs have shown great potential applications ranging from molecular electronics to ultrasensitive biosensors [35]. Single-stranded DNA (ssDNA) has recently been demonstrated to interact noncovalently with SWNTs, and forms stable complexes with individual SWNTs by wrapping around them by means of π-π stacking between nucleotide bases and SWNT sidewalls [36]. Double-stranded DNA (dsDNA) has also been proposed to interact with SWNTs, but its affinity is significantly weaker than that of ssDNA [37]. Also, scatter examples of noncovalent interactions of SWNTs with organic dyes or dye-labeled biomolecules have now been reported [38] and SWNTs can act collectively as fluorescence quenchers for dyes [39].

3. CNTs/DNA nanoconjugates

Deoxyribonucleic acid (DNA) is a naturally occurring polymer that plays a central role in biology, and now it has gained increasing attention in various biotechnology fields such as biosensor, bioimplant, and so forth [40]. It has been shown that single-stranded DNA (ssDNA) exhibits sequence-dependent effects of non-covalent binding to the surface of SWCNTs through π-stacking whereas double-stranded DNA (dsDNA) can hardly do [36]. Such novel properties enable SWCNTs to be widely applied in nanobiosystems. SWCNTs dispersed by ssDNA were used as molecular tags for Southern blotting assay [17]. They were also utilized in electrochemical analysis of dopamine [41] and cellular uptake observations [9].

Arrayed carbon nanotubes (CNTs) represent an ideal scaffold for the generation of ordered nanostructures featuring biomolecular components. These structures, exhibiting high...
electrical conductivity [42], also constitute a useful base material for nanoscale biosensors [43]. Most of the hybrid CNT-DNA structures reported to date used unordered CNTs that were functionalized by nonspecific and random adsorption of biomolecular components [44]. Taft et al. have described a rational strategy that permits discrete regions of arrayed CNTs to be functionalized simultaneously and specifically with DNA oligonucleotides [45]. These authors have exploited the different chemical properties of two regions on single CNTs and orthogonal chemical coupling strategies to derivatize CNTs within highly ordered arrays with multiple DNA sequences. The bifunctional chemical structure of CNTs was suggested to facilitate the selective attachment of multiple DNA sequences using two distinct DNA-CNT linking strategies [45]. In one strategy, by accessing the free carboxyl groups of CNTs, single-stranded, amine-terminated DNA oligonucleotides are attached to the CNT array using amide-coupling chemistry in aqueous/organic solvent mixtures. A second and orthogonal modification strategy involves the attachment of oligonucleotides to the sidewalls of the CNTs through hydrophobic (pyrene unit) interactions. This dual functionalization then allowed to use differential hybridization to deliver two gold nanoparticles with distinct dimensions to discrete regions of an individual CNT. Single-stranded DNA attachment was performed in series, while the hybridization of complementary nanoparticle-labeled strands was performed in parallel.

The results clearly show that the specificity of DNA duplex formation permits each Au-DNA conjugate to be selectively directed toward a target site on the CNT. These experiments illustrate that individual CNTs can be functionalized with special selectivity and can be used to differentiate between two DNA sequences. In addition, they represent an augur for using DNA to controllably produce assemblies of hybrid nanostructures. Delivering different payloads to specific areas of functionalized nanotubes may facilitate the production of new nanomaterials.

The ability of DNA-CNTs conjugates to hybridize reversibly with high specificity to complementary DNA sequences [46] suggests that such conjugates are very promising for gene therapy, conducting and semiconducting substrates medical apparatus and so on. However, these devices have inherent limitations in terms of precision, specificity, and interconnection, which obstruct their integration in large scale devices and complex circuits. Among the solutions to solve the problem, self-assembly is the most promising alternative. A variety of techniques have been developed for DNA hybridization detection, including fluorescence imaging [47], electrochemical [48], micro-gravimetric [49], bioluminescence [50], chemiluminescence [51] and electrogeneated chemiluminescence (ECL) techniques [52]. ECL technique has many distinct advantages over fluorescence technique because it does not involve a light source and avoids the attendant problems of scattered light and impurities luminescent.

Single-walled carbon nanotubes (SWNTs) self-assembly have been a rapidly evolving research area targeted at integrating nanoscale building blocks into functioning devices. To this end, various researchers have shown that DNA molecules can serve especially well to create highly definable supramolecular networks that can be used to advantage for the programmed self-assembly of objects with nanometer precision [53]. It has been demonstrated that DNA hybridization can induce the self-assembly of gold nanoparticles [54]. So far, DNA has been used to guide the assembly of gold nanoparticles into discrete structures with defined numbers of particles [55] as well as one-dimensional (1D) [56] or two-dimensional (2D) [53] arrays. To enrich the family of objects that can be used for DNA-directed self-assembly, researchers have tried to covalently conjugate DNA to CNTs and have attempted to use DNA hybridization to drive the self-assembly of CNTs [43].
4. CNT/DNA-based nanosensors

Nanostructures, such as nanowires, nanotubes and nanoparticles, offer new and sometimes unique opportunities that can be exploited for sensing [57, 58]. Specifically, the modification of transducers with carbon nanotubes has recently attracted considerable attention in the field of electro-analytical chemistry. The high surface area and the useful mechanical properties of CNTs combined with their electronic conductivity and ability to promote electron transfer reactions provide new exciting nanoelectrodes for the catalysis of biomolecules and inorganic compounds [59]. For the design of a genosensor the crucial step is the immobilization of single stranded DNA probes onto the electrode surface with sufficient stability, activity and well controlled packing density [60].

Carbon nanotubes [61, 62] show great potential for use as highly sensitive electronic biosensors. Single-walled carbon nanotubes arguably are the ultimate biosensor in this class for a number of reasons: SWNTs have the smallest diameter (~ 1 nm), directly comparable to the size of single biomolecules and to the electrostatic screening length in physiological solutions [63]. Furthermore, the low charge carrier density of SWNTs [64] is directly comparable to the surface charge density of proteins, which intuitively makes SWNTs well suited for electronic detection that relies on electrostatic interactions with analyte (bio)molecules. Finally, in SWNTs all the atoms are in direct contact with the environment, allowing optimal interaction with nearby biomolecules. Although an appreciable amount of biosensing studies has been conducted using carbon nanotube transistors, the physical mechanism that underlies sensing is still under debate [61]. Some mechanisms of biosensing are electrostatic gating [65], changes in gate coupling [66], carrier mobility changes [63] and Schottky barrier effects [67].

Tama et al. have developed a DNA sensor based on multi-walled carbon nanotubes (MWCNTs) [68]. The functionalized MWCNTs act as linkers to immobilize the probe DNA strands on the sensor surface for direct and label-free detection of influenza virus. The DNA-based sensor, a member of the biosensor family, is considered a promising tool in pre-diagnostics, and in the prevention and control of infectious diseases in real-time and on site analysis [69]. These sensors have numerous potential applications including the diagnosis of genetic diseases, the detection of infectious agents, and identification in forensic and environmental cases [70]. There are various types of DNA sensors which have developed over the years. Methods used for DNA sequence detection in those sensors have been reported to be based on radiochemical, enzymatic, fluorescent, electrochemical, optical, and acoustic wave techniques [71]. Some disadvantages of the optical sensors, however, include the requirement of a separate labeling process and an equipment to stimulate the transducer; they are also highly complex, and thus, entail higher cost in order to conduct an analysis [72].

The DNA sequence attachment on the surface of the sensor is a key to high sensitivity, long life-span, and short response time. In the immobilization technique, it is necessary that the binding chemistry is stable during subsequent assay steps; the sequence of the DNA probe should not change the chemical structure, and the bio-recognition molecules have to be attached with an appropriate orientation. Nowadays, various methods are used to immobilize the DNA strands on the sensor surface, such as the covalent bonds to the functionalized support [73], electrochemical [74], physical absorption [75] and monolayer self-assembling [76]. Among these methods, the covalent bond induced immobilization provides advantages over other methods in terms of simplicity, efficiency, ordered binding, and low cost. In this method, various mediators can be used to attach the DNA sequences on
the sensor surface such as carbon nanotubes (CNTs), aminopropyltriethoxy Silane (APTES), alkanethiols and so on. The covalent immobilization of the carbon nanotubes (CNTs) is usually performed by reacting amino-terminated DNA with the carboxylic acid groups of the CNTs, or directly reacting with the amino group of the oxidized CNTs. Several groups have reported using the covalent binding of the CNTs to immobilize the DNA sequences. Krishna et al. reported the synthesis of functionally engineered single-walled carbon nanotubes (SWNTs) – peptide nucleic acid (PNA) conjugates especially for nanoelectronic applications [77]. Jung et al. demonstrated that the DNA strands can be covalently attached to immobilized SWNT multilayer films [78]. They showed that the SWCNTs multilayer films were constructed via consecutive condensation reactions creating stacks of functionalized SWCNTs layers linked together by dianiline derivatives.

The developer, a multistep route to the formation of covalently linked adducts of single-wall carbon nanotubes (SWNT) and deoxyribonucleic acid (DNA) sequence was reported by Baker et al. [46]. In their report, the DNA molecules covalently linked to SWNTs are accessible to hybridization and strongly favored hybridization with molecules having complementary sequences compared with non-complementary sequences [78]. Recently, Zhang et al. [79] synthesized a type of compound, MWCNTs—CONH(CH2)n—SH, via carboxylation, and investigated a thickness-tunable multilayer film DNA biosensor built layer-by-layer (LBL) covalent attachment of gold nanoparticles (AuNPs) and multi-walled carbon nanotubes on an Au electrode [46]. Nanoparticles-based materials offer excellent prospects for DNA detection because of its unique physical and chemical properties. It is another efficient way to improve the sensitivity of DNA-probe. Many new protocols are based on colloidal gold tags, semiconductor quantum dot tracers and polymeric carrier. The power and scope of such nanoparticles can be greatly enhanced by coupling them with biological recognition reactions and electrical process [80]. Magnetite nanoparticles have both the properties of nanoparticles and magnetism. It can collect DNA by magnetic field and trigger DNA detection easily [81]. Palecek and Fojta [82] reported that non-specific adsorption, which is the important error in DNA detection could be remarkably suppressed by hybridization and transduction at the surface of magnetic beads.

5. Electrochemical nanosensors

Electrochemical methods of hybridization detection present a good alternative in comparison with well-developed fluorescent detection. Over the past decade, a significant progress has been made towards the development of the electrochemical DNA sensors. Considerable advantages have been ascribed to these devices owing to their potential for obtaining specific information in a faster, simpler, and less expensive way. These sensors rely on the conventional hybridization signal of the DNA sequences into useful electrical signal. The modification of electrochemical sensors with carbon nanotubes (CNTs) has attracted considerable attention in the field of DNA sensing technology due to its attractive electronic, chemical, and mechanical performances. Thus, many different schemes for electrochemical DNA sensing based on CNTs have been reported [83]. In the Niu et al.’s work, carboxyl-functionalized multi-walled carbon nanotubes (MWCNTs-COOH) and redox intercalators were utilized in the fabrication of DNA electrochemical biosensor [84]. The presence of carboxyl groups on carbon nanotubes is necessary for the covalently bonding of the oligonucleotides. Oligonucleotide probes with an amino group at the 5’-phosphate end can
form covalent bonds with the carboxyl groups in MWCNTs–COOH with the aid of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). A complex of rutin (R) \( \text{C}_{54}\text{H}_{58}\text{MnO}_{32} \) (abbreviated by MnR) was synthesized and used as the indicator for the detection of hybridization between the probe DNA and the target sequence. Interaction between MnR and double-stranded salmon sperm DNA was studied using voltammetry and fluorescence spectroscopy. Using MnR as a novel electroactive indicator, ssDNA fragment could be selectively detected on the new electrochemical DNA biosensor with a detection limit of \( 3.81 \times 10^{-11} \text{M} \) and a linear range from \( 1.60 \times 10^{-9} \text{M} \) to \( 4.80 \times 10^{-8} \text{M} \) \[84\].

In recent years, microfabricated interdigitated array microelectrodes have received great attention in the areas of biosensing \[85\]. The use of interdigitated microelectrodes is perhaps the most successful of all the recently introduced simple and rapid methods in biosensing for detection of various biological species \[86\]. Special configurations of the interdigitated microelectrodes have also been developed for improvement of their sensing performance \[87\]. The development of electrochemical DNA sensors with high potential for miniaturization and integration has become a subject of intense research, with the hope to make sophisticated and challenging molecular diagnostics available for low-cost routine clinical practice. Newly reported research on SWNT - Field-Effect Transistors (FET) based protein \[88\], and DNA \[89\] sensors has indicated that the sensing mechanism differs significantly when applied to different analyte molecules despite the commonality among the devices themselves.

The development of sequence-selective DNA sensors for diagnosis of genetic or pathogenic disease has attracted increasing interest. Most DNA detection methods rely on optical, piezoelectric, or electrochemical transductions. However, these sensors may have significant device-to-device variations and their fabrication requires high production costs. Recently, field-effect transistors based on single-walled carbon nanotube networks have been fabricated \[90\] and their electrical properties depend on the percolation paths of SWNTs in conduction channels, where device variations are expected to be small.

Label-free electrical detection of DNA and biomolecules using SWNT network FETs (SNFETs) has been successfully achieved \[91\], with typical detection limits on the order of ca. 1 nM of DNA. Dong et al. have reported that the detection sensitivity of SNFETs for DNA can be further improved to ca. 100 fM by using a “nanoparticle enhancement” approach, in which the target DNAs are hybridized with probe DNAs on the device, and reporter DNAs labeled with Au nanoparticles (AuNPs) flank a segment of the target DNA sequence \[92\]. It was noted that the enhancement of DNA detection by incorporating nanoparticles (e.g., CdS and Au) has been reported by using electrochemical approaches \[93\]. On the other hand, enhancing the sensitivity of SNFETs from 1 nM to 1 pM by adding a bivalent salt (MgCl\(_2\)) during the hybridization process has also been reported \[89\]. In some of these approaches, adsorption of target DNA on a SiO\(_2\) surface via divalent coordination (DNA–Mg\(^{2+}\)–SiO\(_2\)) \[94\] rather than specific binding cannot be ruled out and may confound the sensing results. Dong et al. block the SiO\(_2\) surface by octyltrichlorosilane (OTS) treatment to reduce possible non-specific binding (NSB) of DNA to SiO\(_2\). In addition, blocking of vacant SWNT surfaces by using polyethylene glycol (PEG; molecular weight 400 kg mol\(^{-1}\)) has also been performed to reduce the NSB of DNA to SWNTs.

Li et al. have demonstrated the electrochemical properties of DNA–SWNTs sensor and the interaction between DNA and Riboflavin (VB\(_2\)) (Figure 1) \[95\]. First, the self-assembled monolayers of SWNTs on an amide platinum electrode were made by covalent linking of CO–NH bonds. Then, DNA was attached to SWNTs film via carboxyl-amine coupling.
Electrochemical experiments demonstrated that DNA still retained the bioactivity and the ability to interact with other biomolecules. Furthermore, the self-assembled biosensor could easily detect VB$_2$ in a wide range of linearity and exhibited low detection limit for VB$_2$. The new biosensor design was based on the incorporation of the biomolecule into amino modified Pt electrode, and exhibited improved analytical performance with respect to previous methods. The well-known capabilities of Pt electrode could retain the biological activities of DNA upon adsorption, and the electrocatalytic abilities of carbon nanotubes allow the electrooxidation of biochemical molecules with interest. The coupling of Pt electrode and carbon nanotubes led to robust biosensors with enhanced analytical characteristics that are useful for many applications [95].

Tang et al. have developed fully electronic DNA sensors based on carbon nanotube field effect devices, which are readily scalable to high density sensor arrays and amenable to integration with “lab-on-a-chip” microanalysis systems [96]. The generality of the sensors was demonstrated with synthetic oligonucleotides consisting of random generated sequences and also two different oligo lengths (15mer and 30mer). The random sequenced 15mer thiolated ssDNA probe (p15), its completely complementary target ssDNA (CM15), and its randomly generated mismatched target ssDNA (MM15) were used. SWNT serves as the transducer which translates and amplifies DNA hybridization on Au into a directly detectable electrical signal. Compared to optical and other electrochemical methods, the essentially two-terminal SWNT DNA sensors involve much simpler chemistry and easier setup. It is highly desirable to fully utilize the surface and electrical properties of SWNT for biosensing in general, where chemical schemes for SWNT-biomolecule conjugation are in critical need that (1) preserve pristine nanotube property, (2) maintain biomolecule functionality, and (3) facilitate efficient SWNT-biomolecule charge transfer. The sensing mechanism suggested by Star et al. attributes the electrical conductance change to the electron doping by DNA hybridization on the SWNT sidewall [89]. It is well accepted
that SWNT-FETs operate as unconventional Schottky barrier (SB) transistors, in which switching occurs primarily by modulation of the contact resistance rather than the channel conductance [97]. Results suggest that the strong binding between the directly absorbed ssDNA molecules and the sidewalls of SWNTs largely inhibits further hybridization.

It is proposed that the modulation of the Schottky barrier at the metal-tube contact by efficient hybridization on Au electrodes is the dominate sensing mechanism [96]. Furthermore, the DNA hybridization kinetics observed real-time in these sensing experiments, consistent with that on gold surface, also strongly suggests that the electrical signal originates from hybridization events on the gold contact. Quartz crystal microbalance and XPS data conclude that the ssDNA probes wrapped on SWNTs played little role in hybridization; instead they blocked the NSB of analyte ssDNA oligos complementary or mismatched alike. The slight response to mismatched target DNAs is believed to be a combined result of the sequence-dependent DNA-SWNT affinity and the disruption of probe packing due to photoresist residues on the sensor surface. Nonspecific interactions of oligonucleotides with carbon nanotubes could enhance the polymerase chain reaction (PCR), due to the local increase in the reaction components on the surface of CNTs [98]. DNA could also enter into the carbon nanotube cavities [99].

Tama et al. have developed a DNA sensor based on multi-walled carbon nanotubes (MWCNTs) [68]. The functionalized MWCNTs act as linkers to immobilize the probe DNA strands on the sensor surface for direct and label-free detection of influenza virus. These developed sensors comprise a highly sensitive, low-cost, and rapid method and therefore, they have potential application in controlling this disease. To detect influenza virus DNA strands, the DNA sensor was soaked into a solution containing the target DNA sequences. Upon hybridization, double-stranded DNA molecules were formed on the sensor surface. In the case of a perfect match between the target DNA and the immobilized DNA sequences, a concentration-dependent change in surface conductance was detected (Fig. 2 [68]). The output signal of our sensor was linearly proportional to the target DNA concentration in a range between 1 and 10 nM. The conductance remained unchanged when non-matching DNA strands were used (Fig. 2 [68]).

![Fig. 2. The curve of the DNA sequence hybridization.](www.intechopen.com)
Here, the detection limit of the sensor was about 0.5 nM concentration of the influenza virus sample. This is lower than that of the electrochemical transducer using square wave voltammetry and fluorescence of Vincent Noel (25 nM) [100], or the one using the electrochemical impedance spectroscopy of Hui Peng (0.98 nM) [101]. The sensitivity of the DNA sensor was 0.06 mV/nM. The conductance modulation of these sensors can be explained based on the mechanism which has been well studied in literature [65]. The mechanism for electrical detection of the DNA hybridization in semiconducting carbon nanotube network devices in this work was likely due to the modification of junction barrier energy, whereas the conductance change forming the metal-nanotube's contact by efficient hybridization on the Pt electrodes was the dominant sensing mechanism.

Fig. 3. The FTIR spectra of the MWCNTs and the DNA probe sequences-MWCNTs bonds.

Fig. 4. The Raman spectra of the MWCNTs and the MWCNTs/DNA film.
As observed in the FTIR spectra (Fig. 3) for MWCNTs, there were peaks at 1463 cm\(^{-1}\), which correspond to the CH\(_2\) of the MWCNTs [79]. During interaction with the DNA strands, a shift in the CH\(_2\) stretch could be seen centered around 1474 cm\(^{-1}\), and also at 1248 cm\(^{-1}\) corresponding to the C–O vibration [102]. Fig. 3 also shows the existence of DNA/CNTs interaction at around 750 cm\(^{-1}\), 892 cm\(^{-1}\), corresponding to the asymmetric stretching mode of the phosphate group and the DNA backbone of the DNA sequence, respectively [79]. The change in the wave number of mode for CH\(_2\)–O–P–O was indicated at 1251 cm\(^{-1}\) [102].

Fig. 4 shows the Raman spectra of the MWCNTs and the MWCNTs/DNA film. The main features of these samples in the Raman spectra are the G band at 1580 cm\(^{-1}\) and the D band at 1350 cm\(^{-1}\). A downshift of the tangential G band and D band were observed in the Raman spectra of the DNA–MWCNTs, which corresponded to peaks at 1576 cm\(^{-1}\) and 1343 cm\(^{-1}\), respectively. This is attributed to the results of the charge transfer between the oxygen groups on the CNT surface and the DNA matrix [103].

6. CNTs-DNA hybrids

The formation of ssDNA–SWCNT hybrids and their purification is schematically illustrated in Fig. 5 [104].

The ssDNA–SWCNT hybrids show well-resolved absorption spectra (Fig. 6 [104]). The reaction was carried out with the complementary DNA (cDNA) and noncomplementary DNA (ncDNA), respectively. The absorption spectra exhibit sharp peaks composed of the first van Hove transition of metallic SWCNTs \((M_{11} \approx 400–600 \text{ nm})\), the first \((S_{11} \approx 800–1400 \text{ nm})\) and second \((S_{22} \approx 550–800 \text{ nm})\) van Hove transition of semiconducting SWCNTs. A red shift was observed after hybridization with cDNA whereas there was no shift in the absorption spectra after the reaction with ncDNA. Also, semiconducting species showed clear red shift whereas the change in the spectra was negligible for metallic nanotubes. The reaction with ncDNA did not result in any shift in the fluorescence spectra [105]. However, a
blue shift was observed after hybridization with cDNA. Jeng et al. also observed a blue shift in the fluorescence spectra after hybridization with cDNA [106]. As shown by AFM, the average height was increased from 2.02 nm to 2.81 nm after the reaction, indicating that DNA hybridization was achieved between ssDNA and cDNA.

![Absorption spectrum of ssDNA-SWCNT hybrids.](image)

**Fig. 6.** Absorption spectrum of ssDNA-SWCNT hybrids.

### 7. CNTs-enzyme based nanoconjugates

CNTs are attractive materials for application to biosensors due to the low-potential detection of hydrogen peroxide and β-Nicotinamide adenine dinucleotide and the minimal surface passivation during the electrochemical oxidation of NADH [107]. The electrocatalytic behavior of CNTs can be attributed to the ends of CNTs [108]. These characteristics of CNTs are very useful for the development of novel enzyme-based sensors where rapid electron transfer at the electrode is required. Many enzymes, over 100 oxidases and 200 dehydrogenases, catalyze specifically the reactions of important analytes to generate the electrochemically detectable products hydrogen peroxide and NADH. The importance of electrochemical biosensors lies in the fact that they combine the specificity of biological systems with the advantages of electrochemical transduction. CNTs have been widely used for application in enzyme-based biosensors.

The electrocatalytic oxidation of NAD(P)H \([\text{NAD(P)}H=1,4\text{-dihydronicotinamide adenine dinucleotide (phosphate)}]\) cofactors and the reduction/oxidation of \(\text{H}_2\text{O}_2\) stimulated by CNTs are particularly important, since these electrocatalytic reactions may be easily coupled to enzymatic transformations [109]. For example, two kinds of amperometric biosensors have been prepared for analyzing ethanol and glucose by the encapsulation of alcohol dehydrogenase or glucose oxidase, respectively, in a carbon nanotube/Teflon composite material [109]. These sensors operate in diffusion mode, yielding NADH or \(\text{H}_2\text{O}_2\) in the presence of NAD\(^+\) and ethanol or \(\text{O}_2\) and glucose (GOx), respectively. The biocatalytically generated NADH or \(\text{H}_2\text{O}_2\) were then detected electrochemically by the catalytic electrodes. Enzymes generating \(\text{H}_2\text{O}_2\) were covalently linked to carbon nanotubes or encapsulated into polymer coatings associated with carbon nanotubes. For example, GOx was covalently
coupled to carboxylic groups at the ends of short CNTs using carbodiimide coupling [110] or cross-linked with a CNT/Pt-nanoparticle hybridlayer [111] yielding amperometric glucose sensors based on H₂O₂ detection [26]. Coaxial nanowires consisting of a concentric layer of polypyrrole uniformly coated onto aligned carbon nanotubes have provided a template for making glucose sensors with a large amount of electrochemically entrapped GOx in the ultrathin polypyrrole film. Similarly, amperometric detection of organophosphorus pesticides and nerve agents was performed using a screen-printed biosensor based on co-immobilized acetylcholine esterase, choline oxidase, and CNTs [112].

8. CNTs/protein conjugates

Proteins can be non-specifically bound to the external sides of the carbon nanotube walls [113]. Proteins adsorb individually, strongly, and noncovalently along the nanotubes. For example, it has been shown that open single-walled carbon nanotubes can accommodate small proteins such as cytochrome c inside their internal cavities, leading to the stable immobilization of the proteins in bioactive conformations [114]. Ortiz-Acevedo et al. used a novel class of cyclic peptides containing alternating L- and D-amino acids (AAs), called reversible cyclic peptides (RCPs), for the diameter-selective solubilization of HiPco single-walled carbon nanotubes [115]. In L-/D-peptides, all side chains reside on one face of the backbone, encouraging a ringlike conformation with the side chains on the ring exterior. In addition, present cyclic peptides have N- and C-termini that are derivatized to contain thiol groups, allowing reversible peptide cyclization through a disulfide bond. These results suggest that peptides with different N-to-C-terminal lengths wrap around SWNTs having sufficiently small diameters, promoting selective enrichment of small-diameter CNTs dispersed in solution. By controlling the length of the reversible cyclic peptides, the authors demonstrated limited diameter-selective solubilization of single-walled carbon nanotubes, which may prove useful in SWNT purification. In addition, RCPs covalently closed around SWNTs do not dissociate from the SWNTs unless the disulfide bond is reduced. RCPs thus provide a platform to which other functional groups could be attached without disturbing the covalent structure of SWNTs.

The attachment of BSA protein and DNA to the amino f-MWCNTs was verified by comparing the FTIR spectrum of as-prepared amino f-MWCNTs and amino f-MWCNTs-BSA/DNA samples [25]. The biomolecules contain both amine and carboxylic groups. In the present experiment, the carboxylic groups of biomolecules (such as BSA protein and DNA) react with the free amine groups of the amino f-MWCNTs. As a result, the carboxylic bonds in biomolecules have been converted into amide bonds (-NH-C=O). The interaction between amino f-MWCNTs and biomolecules (protein and DNA) is noticed by the shift of the amide bond (C=O) peak (1 650 to 1 642 and 1 650 to 1 645 cm⁻¹ for amino f-MWCNTs-BSA and amino f-MWCNTs-DNA samples, respectively) in the FTIR spectrum. Furthermore, TEM studies confirmed the success of the attachment of BSA protein molecules and DNA to amino f-MWCNTs. The BSA protein molecules densely decorate the side walls of the MWCNTs. The location of the BSA protein is representative of where the amine groups were present.

Kam et al. have presented the investigation of the cellular uptake mechanism and pathway for carbon nanotubes [116]. The authors showed that intracellular transportation of proteins
and DNA by SWNTs is general, thus further confirming the transporter ability of these materials. They presented evidence that shows clathrin-dependent endocytosis as the pathway for the uptake of various SWNT conjugates with proteins and DNA. They also discussed the differences between the nanotube materials and the experimental procedures used in our work and by Pantarotto et al. who suggested an energy-independent nonendocytotic uptake of nanotubes.

9. Conclusion

One of the most exciting classes of nanomaterials is represented by the carbon nanotubes. Carbon nanotubes have emerged as new class nanomaterials that are receiving considerable interest because of their unique structure, high chemical stability and high surface-to-volume ratio. Composite nanomaterials based on integration of CNTs and some other materials to possess properties of the individual components with a synergistic effect have gained growing interest. The use of carbon nanotubes as “building blocks” in nano- /microelectronic devices could revolutionize the electronic industry in the same way that the microchips have revolutionized the computer industry. Significant enhancement of optical, mechanical, electrical, structural, and magnetic properties are commonly found through the use of novel nanomaterials. Composite materials based on integration of carbon nanotubes and some organic and bioorganic materials to possess properties of the individual components with a synergistic effect have gained growing interest. The integration of carbon nanotubes (CNTs) with these materials has led to the development of new hybrid nanomaterials and sensors. The integration of carbon nanotubes with biological systems to form functional hybrid assemblies is, however, a new and relatively unexplored area. Carbon nanotubes have been explored for possible applications in nanobiotechnology. At the present time, several fundamental issues remain to be addressed for the use of carbon nanotubes as potential biological transporters.

Single-walled carbon nanotubes (SWCNTs) exhibit excellent optical properties such as Raman, fluorescence and absorption spectra. Due to their small size and sensitive optical characteristics, SWCNTs have been shown to be ideal candidates for optical nanobiomarkers and/or nano-biosensors. SWCNTs individually dispersed in aqueous medium show strong fluorescence in the near infrared region and sharp absorption peak distributions caused by van Hove singularities. Absorption spectrum gives a variety of information including electronic structures such as metallic, semiconducting and chiralities. Ionic and nonionic surfactants and some biomolecules such as DNA and protein cause suspension of nanotubes by coating them. These materials, individually disperse SWCNTs without disturbing the electronic properties of nanotubes. The presence of nonionic surfactant leads to complex micelle formation with CNT in both aqueous and nonaqueous systems. It is possible that such surfactant-nanotube interactions alter the nanotubes’ surface properties, thus modulating their interaction with other additives. The cylindrical micelles were formed in aqueous dispersions of sodium dodecyl sulfate (SDS)-SWNT. For all dispersions, the UV-vis spectra exhibit the sharp van Hove transitions anticipated from individualized nanotubes. For the dispersion of SWNTs the SDS molecules were considered to form noninteracting core-shell cylindrical micelles with a single nanotube acting as the core.

To covalently bond molecules to the CNTs, it first requires the formation of functional groups on the CNTs. The control of reactants and/or reaction conditions may control the
locations and density of the functional groups on the CNTs, which can be used to control the locations and density of the attached biomolecules. The edges of carbon nanotubes are more reactive than their sidewalls, thus allowing the attachment of functional groups to the nanotube ends. Concentrated acids are known to introduce acidic groups to the sidewalls and ends of CNTs. The FTIR spectra of as-synthesized MWCNTs and after functionalization showed the presence of carboxylic and amino groups. Arrayed carbon nanotubes (CNTs) represent an ideal scaffold for the generation of ordered nanostructures featuring biomolecular components. These structures, exhibiting high electrical conductivity, also constitute a useful base material for nanoscale biosensors. Most of the hybrid CNT-DNA structures reported to date used unordered CNTs that were functionalized by nonspecific and random adsorption of biomolecular components.

It has been shown that single-stranded DNA (ssDNA) exhibits sequence-dependent effects of non-covalent binding to the surface of SWCNTs through π-stacking whereas double-stranded DNA (dsDNA) can hardly do. Such novel properties enable SWCNTs to be widely applied in nanobiosystems. SWCNTs dispersed by ssDNA were used as molecular tags for Southern blotting assay. Single-stranded DNA (ssDNA) has recently been demonstrated to interact noncovalently with SWNTs, and forms stable complexes with individual SWNTs by wrapping around them by means of π–π stacking between nucleotide bases and SWNT sidewalls. Double-stranded DNA (dsDNA) has also been proposed to interact with SWNTs, but its affinity is significantly weaker than that of ssDNA. The ssDNA–SWCNT hybrids show well-resolved absorption spectra. The absorption spectra exhibit sharp peaks composed of the first van Hove transition of metallic SWCNTs \( M_{11} \approx 400–600 \text{ nm} \), the first \( S_{11} \approx 800–1400 \text{ nm} \) and second \( S_{22} \approx 550–800 \text{ nm} \) van Hove transition of semiconducting SWCNTs. A red shift was observed after hybridization with cDNA whereas there was no shift in the absorption spectra after the reaction with ncDNA.

Hybrid nanoscale materials are well established in various processes such as nucleic acid detachment, protein separation, and immobilization of enzymes. Those nanostructures can be used as the building blocks for electronics and nanodevices because uniform bioorganic coatings with the small and monodisperse domain sizes are crucial to optimize conductivity and absorption and to detect changes in conductivity and absorption induced by analyte adsorption on the CNT surfaces. DNA, protein and enzymes are useful as an engineering material for the construction of smart objects at the nanometer scale because of its ability to selforganize into desired structures through the specific selfassembling or hybridization of complementary sequences. Particularly, color changes induced by the association of nanometer-sized metal nanoparticles with CNTs conjugates provide a basis of a simple yet highly selective method for detecting specific biological reactions between anchored ligand molecules and receptor molecules in the milieu. Arrayed carbon nanotubes/DNA represent an ideal scaffold for the generation of ordered nanostructures. These structures, exhibiting high electrical conductivity, also constitute a useful base material for nanoscale biosensors.

The ability of DNA-CNTs conjugates to hybridize reversibly with high specificity to complementary DNA sequences suggests that such conjugates are very promising genetherapy, conducting and semiconducting substrates medical apparatus and so on. A variety of techniques have been developed for DNA hybridization detection, including fluorescence imaging, electrochemical, micro-gravimmetrical, bioluminescence, chemiluminescence and electrogenerated chemiluminescence (ECL) techniques. Among these methods, the covalent attachment immobilization provides advantages in terms of simplicity, efficiency, ordered binding, and low cost.
The DNA molecules covalently linked to SWNTs are accessible to hybridization and strongly favored hybridization with molecules having complementary sequences compared with non-complementary sequences. Nanoparticles-based materials offer excellent prospects for DNA detection because of its unique physical and chemical properties. It is another efficient way to improve the sensitivity of DNA-probe. Many new protocols are based on colloidal gold tags, semiconductor quantum dot tracers and polymeric carrier. The power and scope of such nanoparticles can be greatly enhanced by coupling them with biological recognition reactions and electrical process. Magnetite nanoparticles have both the properties of nanoparticles and magnetism. It can collect DNA by magnetic field and trigger DNA detection easily. It was reported that non-specific adsorption, which is the important error in DNA detection could be remarkably suppressed by hybridization and transduction at the surface of magnetic beads.

During the past decade, considerable progress has been made towards the development of the electrochemical DNA sensors. Considerable advantages have been ascribed to these devices owing to their potential for obtaining specific information in a faster, simpler, and less expensive way. These sensors rely on the conventional hybridization signal of the DNA sequences into useful electrical signal. The modification of electrochemical sensors with carbon nanotubes (CNTs) has attracted considerable attention in the field of DNA sensing technology due to its attractive electronic, chemical, and mechanical performances. Thus, many different schemes for electrochemical DNA sensing based on CNTs have been reported. Field-effect transistors based on single-walled carbon nanotube networks have been fabricated and their electrical properties depend on the percolation paths of SWNTs in conduction channels, where device variations are expected to be small. Label-free electrical detection of DNA and biomolecules using SWNT network FETs (SNFETs) has been successfully achieved, with typical detection limits on the order of ca. 1 nM of DNA. This sensing mechanism attributes the electrical conductance change to the electron doping by DNA hybridization on the SWNT sidewall. It is well accepted that SWNT-FETs operate as unconventional Schottky barrier (SB) transistors, in which switching occurs primarily by modulation of the contact resistance rather than the channel conductance.

Proteins can be bound to the external sides of the carbon nanotube walls. Proteins adsorb individually, strongly, and noncovalently along the nanotubes. For example, it has been shown that open single-walled carbon nanotubes can accommodate small proteins inside their internal cavities, leading to the stable immobilization of the proteins in bioactive conformations.

The CNTs are very useful for the development of novel enzyme-based sensors where rapid electron transfer at the electrode is required. Many enzymes, over 100 oxidases and 200 dehydrogenases, catalyze specifically the reactions of important analytes to generate the electrochemically detectable products hydrogen peroxide and NADH. The importance of electrochemical biosensors lies in the fact that they combine the specificity of biological systems with the advantages of electrochemical transduction. CNTs have been widely used for application in enzyme-based biosensors.

10. Acknowledgements

This research is supported by the VEGA project No. 2/0037/10 and 2/0160/10.
11. Nomenclature

1D    one-dimensional  
2D    two-dimensional  
AFM    atomic force microscopy  
APTES    aminopropyltriethoxy Silane  
AuNPs    Au nanoparticles  
AuNPs    gold nanoparticles  
BSA    bovine serum albumin  
cDNA    complementary DNA  
CNT    carbon nanotube  
CTAB    cetyltrimethylammonium bromide  
DNA    deoxyribonucleic acid  
dsDNA    double-stranded DNA  
ECL    electrogenerated chemiluminescence  
EDC    1-(3-dimethylaminopropyl)-3-ethylcarbo diimide hydrochloride  
FET    field effect transistor  
LBL    layer-by-layer  
MWCNT    multi-walled carbon nanotube  
NAD    β-nicotinamide adenine dinucleotide  
NIR    near IR  
NSB    nonspecific binding  
OTS    octyltrichlorosilane  
PCR    polymerase chain reaction  
PEG    polyethylene glycol  
PNA    peptide nucleic acid  
R    complex of rutin  
RCPs    reversible cyclic peptides  
SB    schottky barrier  
SDS    sodium dodecyl sulfate  
SNFETs    biomolecules using SWNT network FETs  
ssDNA    single-stranded DNA  
SWCNT    single-walled carbon nanotube  
SWNT    single-wall carbon nanotubes  
TEM    transmission electron microscopy

12. References

Dispersions Based on Carbon Nanotubes – Biomolecules Conjugates

Carbon Nanotubes are among the strongest, toughest, and most stiff materials found on earth. Moreover, they have remarkable electrical and thermal properties, which make them suitable for many applications including nanocomposites, electronics, and chemical detection devices. This book is the effort of many scientists and researchers all over the world to bring an anthology of recent developments in the field of nanotechnology and more specifically CNTs. In this book you will find:

- Recent developments in the growth of CNTs
- Methods to modify the surfaces of CNTs and decorate their surfaces for specific applications
- Applications of CNTs in biocomposites such as in orthopedic bone cement
- Application of CNTs as chemical sensors
- CNTs for fuelcells
- Health related issues when using CNTs

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:
