Review Article: The Increasing Importance of Carbon Nanotubes and Nanostructured Conducting Polymers in Biosensors

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Abstract

The growing needs for analytical devices requiring smaller sample volumes, decreased power consumption and improved performance have been driving forces behind the rapid growth in nanomaterials research. Due to their dimensions, nanostructured materials display unique properties not traditionally observed in bulk materials. Characteristics such as increased surface area along with enhanced electrical/optical properties make them suitable for numerous applications such as nanoelectronics, photovoltaics and chemical/biological sensing. In this review we examine the potential that exists to use nanostructured materials for biosensor devices. By incorporating nanomaterials, it is possible to achieve enhanced sensitivity, an improved response time and smaller size. Here we report some of the success that has been achieved in this area. Many nanoparticle and nanofibre geometries are particularly relevant, in this paper however we specifically focus on organic nanostructures, reviewing conducting polymer nanostructures and carbon nanotubes.

Keywords: Biosensor, carbon nanotubes, conducting polymers, nanomaterials, biomolecule immobilisation.

Introduction

Investment in nanomaterials research has grown exponentially over the last number of years. This is due to the huge range of opportunities afforded by nanomaterials in areas such as clean energy (for example solar panels and hydrogen storage), environmental monitoring (sensors for harmful chemicals or biological agents), improved materials (such as stronger/lighter plastics and antimicrobial surfaces), and new products (for example nanoscale transistors). It is clear that nanotechnologies come with the potential to drive economic growth, hence in 2000 the US government set up the National Nanotechnology Initiative and since then investment has grown annually (Figure 1). The investment by NNI member agencies for 2011 is nearly $1.8 billion (http://www.nano.gov/). Currently most commercial success has resulted from the incorporation of nanomaterials into composites for reinforcement. This improves the strength of materials which can typically be used in products such as sports equipment (for example Babolat tennis rackets). Nanomaterials are also of interest for a number of other applications such as nanoelectronics (IBM and Intel both have active nanomaterials research programs). It is clear that nanotechnology will feature in many future products. Here we focus on the potential they offer for developing improved biosensors.

Nanomaterials are defined as matter with dimensions between 1 and 100 nm (Figure 2). To put this into perspective, a sheet of paper is about 100,000 nanometers thick whereas a single gold atom is about a third of a nanometer in diameter. Nanomaterials therefore are larger than individual atoms/molecules but smaller than bulk materials, and thus have characteristic properties that neither completely obey quantum- nor classical-physics. Nanoparticles can be zero-, one-, or two-dimensional. The low dimensionality of nanoparticles results in large surface-to-volume ratios, and enhanced electronic and optical properties when compared with bulk samples of the same material. They are of interest for numerous applications including sensing, where the large surface area of nanomaterials specifically facilitates interaction with an increased number of target molecules when compared to their bulk counterparts (*1-5*). Their small size is also responsible for superior electronic and optical properties which, due to quantum confinement effects, are very sensitive to minor perturbations. Thus nanomaterials can be used to facilitate label-free detection, and develop biosensors with enhanced sensitivities and improved response times. The use of

nanoparticles in biosensors is increasing due to this enhancement in sensitivity (as seen in Table 1), which is of major importance for clinical diagnostics as the concentration of targets can be very low in biological samples. A good example of this is DNA sensors which generally rely on polymerase chain reaction (PCR) for signal amplification. By developing biosensors with improved sensitivity it will eliminate the need for PCR and thus simplify DNA biosensors. This can be achieved using nanomaterials which, due to their large surface area, allow a greater number of DNA strands to be immobilised (*6*). Nanomaterials can be incorporated into many types of biosensor configurations to develop magnetic, optical, electrical or electrochemical biodevices for the detection of many biological molecules including nucleic acids, antibodies, proteins, toxins and bacteria (*7-13*)

The first biosensors were reported in the early 1960's, where a pH response for a 10 mg per cent solution of glucose was reported (*14*). Since then there have been many advances made in the field, and devices are now more sensitive and more portable. In general, a biosensor can be described as a device which has a biological sensing element connected to (or integrated with) a transducer, thus transforming a biological event into a signal which can then be interpreted. The recognition biomolecule within a biosensor is highly selective, and can be immobilised by physical adsorption, entrapment or covalent attachment (8, 15-18). The sensitivity of a biosensor is dependent on the number and accessibility of recognition biomolecules present. Nanomaterials enable the development of improved biosensors because they allow for incorporation of a greater number of recognition biomolecules which are more readily accessible to the target species, owing to greater porosity and surface area. Nanomaterials thus typically enable lower detection limits and faster response times, they can also enable label-free detection which is a major advantage (19, 20). Many types of nanomaterials are suitable for biosensor applications including metallic nanoparticles (such as gold), magnetic nanoparticles (such as iron oxide), semiconducting nanoparticles (such as quantum dots and silicon nanowires), and organic nanoparticles (such as conducting polymers, carbon nanotubes) (13, 20-25). Organic materials are more likely to be biocompatible and in this review article we will consider only organic nanomaterials, in particular we review conducting polymer nanostructures (CPs) and carbon nanotubes (CNTs).

1. Nanomaterials

1.1 Carbon Nanotubes

Carbon nanotubes (CNTs), discovered by Iijima in 1991 (26), are an allotrope of carbon comprised of graphene sheets rolled up into cylinders of sp^2 hybridized atoms. CNTs exist as single- (SWNTs), double- (DWNTs) and multi-walled (MWNTs) structures. MWNTs are essentially a number of concentric SWNTs and hence have a larger diameter (Figure 3). The diameter for SWNTs is usually less than 2nm, whereas diameters for MWNTs range between 2-100 nm, depending on the number of shells present. CNTs are typically microns long but, tubes up to 4cm in length have been reported (27). Combined with their narrow diameter, this leads to excellent material properties such as a high aspect ratio and large surface area. CNTs can be approximated to one-dimensional nanostructures, as a result (28).

The electrical properties of a CNT are determined by the tube helicity and diameter (Figure 4) (8). If a CNT is imagined as a rolled-up graphene sheet, the helicity of the tube depends on the angle at which it is rolled-up, and can be described by its chiral vector, $\mathbf{C}_h = n\mathbf{a}_1 + m\mathbf{a}_2$ (where $\mathbf{a}_1$ and $\mathbf{a}_2$ are unit vectors of the hexagonal lattice and, n and m are integers) (28). The direction of $\mathbf{C}_h$ is perpendicular to the axis of the nanotube. The chiral angle (θ), is the angle between vectors $\mathbf{C_h}$ and $\mathbf{a_1}$. The n, m and θ values for a particular CNT, determine the electronic behaviour of the tube. If n - m is a multiple of 3 the tube is metallic otherwise, the tube is semiconducting (28). This stipulates that one-third of all tubes are metallic with the remaining two-third semiconducting.

The exponential increase in CNT patents filed in recent years reflects the level of commercial interest. However, applications for CNTs are currently limited by the difficulties associated with purification and the lack of precise control over the properties (such as chirality) of CNTs produced. At the moment, production of exactly one type of CNT is limited to the number of walls on the CNT, with some SWNT batches even containing DWNTs and MWNTs, among other types of nanostructured carbon. Exact production of a single type of chiral or semi-conducting SWNT, without contaminants, is unfortunately not yet possible and considerable

batch to batch variation is also common. In addition, no clear cut strategy for purification of CNT type has been discovered to date. Therefore current CNT research is limited to working with CNT mixtures.

Early CNT research primarily focused on determining and exploiting the properties of the pristine materials. More recently however, exploration into the chemistry of CNTs, including their functionalisation, has begun to dominate the field (29, 30). The first attempts at chemical functionalisation of CNTs were in response to their poor solubility. Pristine CNTs align parallel with one another to form bundles (31), thus increasing van der Waals interactions, but also preventing their dissolution. Although pristine CNTs have been shown to form stable dispersions with the aid of surfactants (32) and biomolecules (33) or low concentration dispersions with short-term stability in amides such as N,N-dimethylformamide (DMF), N-methylpyrrolidone (NMP), and other non-hydrogen bonding Lewis bases (34) better methods for solvation are required.

CNTs are susceptible to functionalisation mediated by oxidative processes that form reactive groups at their end-caps and defect sites, or by direct modification of their sidewalls, both covalently and non-covalently. Covalent attachment involves the direct addition of functionality to CNTs via the formation of chemical bonds, whereas non-covalent attachment involves CNT-molecule interactions involving electrostatic, van der Waals and/or hydrophobic interactions. A high degree of covalent functionalisation, which alters carbon-carbon bonds from sp^2 to sp^3 structure, can however, result in a sizeable loss of electrical conductivity of the functionalised SWNTs (35).

Since the purification of CNTs is often carried out using oxidative methods that introduce carbonyl and carboxylic acid groups on the open ends of the CNTs and at defect sites along the CNT sidewalls (36-39), this has become one of the favoured routes of covalently attaching biomolecules to CNTs. The proliferation of amino functionalities on proteins, enzymes and antibodies among other biomolecules, allows for facile amide functionalisation with CNT carboxylates (Figure 5). A wide variety of biomolecules such as carbohydrates (40), oligonucleotides (41), proteins (42, 43), enzymes (44, 45) and even DNA (46-48) have been attached to CNTs in this fashion.

However, such functionalisation can be difficult to reproduce since the extent of functionalisation is dependent on the degree and type of nanotube carboxylation, which in turn varies according to CNT source.

Although rather uncontrollable, non-covalent attachment (physical adsorption) has been used effectively to attach a variety of moieties to CNTs. Unfortunately, proteins and antibodies in particular, may lose their biological activity when adsorbed on a CNT surface. This can be due to a change in conformation when binding with the CNT and/or unfavourable orientation of the active site. The interaction of biomolecules with CNTs has been of particular interest with a view to their use as biosensors (49) or improving biocompatibility (50). Non-covalent binding of streptavidin to CNTs has been achieved via covalent attachment to linkers that are adsorbed along the CNT axis (51). DNA has been shown to strongly interact with CNTs, forming uniform coatings (52). The wrapping of CNTs has recently been extended to other biopolymers including chitosan, chondroitin sulphate and hyaluronic acid (53, 54). Biomolecules of interest, including antibodies, may subsequently be anchored to these biopolymers wrapped around the CNTs (55).

1.2 Conducting Polymer Nanostructures

CPs are of interest for biosensor applications as they can be interfaced with biomolecules for effective signal transduction. CPs can be tailored to create substrates with a high surface area, controllable morphology and conductivity. These properties make them excellent transducer materials which can facilitate rapid electron transfer between immobilised biomolecules and an electrode surface (56). Like metals/semiconductors, CPs can conduct charge carriers such as holes and electrons. Unlike metals/semiconductors however they are low cost, and can be easily prepared and modified (57). In 1977 Alan Heeger, Alan, MacDiarmid and Hideki Shirakawa discovered that when polyacetylene was exposed to bromine vapours, its conductivity rose by seven orders of magnitude (58). Polyacetylene is a π-conjugated polymer meaning there are alternating single and double bonds along the polymer backbone. In a conjugated polymer the π-electrons can become delocalised and shared along the polymer chain, enabling them to conduct electricity. CPs are extremely useful as they

combine the electrical properties of a metal with the low density and cost of a polymer. Potential applications include light emitting diodes, photovoltaics, electostatic discharge coatings and printable electronics (59-62). The conductivity of a CP is however sensitive to its chemical environment and can be varied over ten orders of magnitude (ranging typically from 10^{-10}S/m to 10^{0}S/m). This change in conductivity results from a change in the bonding structure, and is accompanied by a change in the colour/spectroscopy of the material (63). Hence CPs are suitable for developing amperometric, potentiometric, conductometric, electrochemical, optical, calorimetric and piezoelectric biosensors(56).

Certain CPs, such as polyacetylene, however are unstable thus limiting them from use in practical applications. CPs such as polyaniline (PAni), polypyrrole, polythiophenes and poly-ethylene-dioxythiophene (PEDOT) have greater stability and are more commonly investigated (Figure 6). PAni, for example, switches between a non-doped insulating emeraldine base form and a doped conducting emeraldine salt form (Figure 7). Switching is reversible and accompanied by a colour change from purple to green. In the conductive form, delocalised electrons (called bipolarons) form along the polymer backbone and are responsible for charge transfer. A disruption in the conjugation of the polymer backbone results in a decrease in conductivity of the material, making it suitable for sensing applications. As PAni conductivity relies on protonation of the polymer by acid molecules, it's conductivity tends to be poor in solutions at neutral pHs (64). This can be dealt with by covalently attaching acid molecules to CP backbones, resulting in a self-doping polymer (65, 66).

The conductivity of a CP is always dependant on its oxidation state, and short term redox stability is a limitation which all unmodified CPs suffer from. CPs also tend to suffer from poor mechanical properties, for example polyprrole has been reported to have poor ductility, and is brittle (67). Therefore, although CP films can be cast onto substrates, it is not generally possible to produce robust CP films with sufficient mechanical integrity to be free-standing. The mechanical properties of CPs can however, be improved by incorporating materials such as CNTs for reinforcement (68). CPs also tend to have poor solubility in common solvents and are typically hydrophobic (69). Large CP particles tend to agglomerate resulting in poor dispersions which are difficult to process. Using the nano- (versus bulk-) form of CPs

however, tend to produce more stable homogenous dispersions. Stable aqueous colloids of PAni nanofibres have been produced without the need for surfactant stabilisation (70).

For sensing applications, the response time and sensitivity of detection is also improved for the nano- versus the bulk-form of the material (Figure 7). This is due to the increased number of reaction sites available for interaction with a target species (71). Until recently nanofibres of CPs were synthesised by solution-based methods such as electrospinning. However, this process can be complicated by the fact that most CPs are difficult to dissolve. A simpler method to synthesise nanofibres is by chemical means and Kaner *et al.* have demonstrated the synthesis of PAni nanofibres by interfacial polymerisation and also by a rapid mix process (72, 73). The BET surface area of nanofibers produced using these methods is typically in the region of 40 m^2/g (72).

Like other conductive nanomaterials, CPs are of interest as they enable simultaneous biomolecule immobilisation along with rapid electron transfer (facilitating enhanced communication with an electrode surface) (74-78) (79). However, they are cheaper to produce when compared with many other conductive nanomaterials and properties such as roughness, porosity, hydrophobicity, stability and conductivity can be controlled. Increasing the surface roughness has been shown to increase the sensitivity of CP-based biosensors (80). CPs can be incorporated into numerous biosensor configurations to enable low limits of detection, and can be tailored to detect a range of target biomolecules (Table 1). A key aspect in biosensor applications is integration of the electrical component (CP) with the biological recognition component. After immobilization it is critical that molecules maintain their activity and are accessible to the analyte so that hybridization of complimentary oligonucleotides, antigen-antibody binding, or enzyme-catalysed reactions can be monitored.

2. Biosensing

Central to much sensor research is the ability to monitor biomarkers (in particular disease biomarkers) in ‘real-time’, with high sensitivity and selectivity in real untreated samples. This demand for sensitive, rapid, ‘on-site’ biosensor techniques has taken advantage of the latest advances in nanotechnology. To improve

sensitivities, intense research has been carried out in signal amplification by nanomaterials.

2.1 CNTs

2.1.1 CNT-Electrical sensors

The oldest and most commonly used transducers in biosensors are electrochemical. Electrical detection methods are appealing because of their low cost, low power consumption, ease of miniaturization, and potential multiplexing capability (81, 82). Due to their size and electronic properties, CNTs can be used to develop highly sensitive and specific nanoscale biosensors (83-85). Challenges remain however in creating macro-sized structures that fully utilise the properties of the individual CNT nanocomponents. Three approaches to the development of electrochemical biosensors using CNTs dominate the literature (Figure 8) (86, 87). In the simplest method, CNTs are randomly deposited onto conductive surfaces in a mat configuration (CNT-mats) or packed into a micropipette for use as electrodes. This method results in an unknown configuration of CNTs which although easy to achieve may not offer optimal signals. However it allows for CNTs pre-functionalised with biomolecules to be used. Alternatively the CNTs can be coated with the biomolecule of interest post electrode fabrication. The second approach involves vertically aligned CNT forests, with one end in contact with the underlying electrode and the other end exposed in the electrolyte solution. This configuration may be achieved by growing the CNTs directly from the surface or by self assembly of shortened CNTs. Typically CNTs are functionalised after this electrode type has been assembled. A third type of nanoelectrode uses just a single CNT. If the type of CNT used could be exactly controlled (SWNT vs. MWNT, metallic vs semiconducting) this would ultimately give the best performance. However, the fabrication and manipulation challenges involved will limit its practical use.

For many important enzymes direct electron transfer with conventional electrodes is not easily achieved or is too slow for sensor applications. CNTs are comparable in size to many biomacromolecules. Their nanodimensions and high aspect ratio therefore exploit the possibility of bringing CNTs into close proximity with proteins, which is not as easily achieved by bulk substrates. This close proximity allows CNTs to communicate with the redox-active sites of biomolecules which are sometimes

obscured/ inaccessible due to the surrounding insulating protein shell. Effective electrical communication enables CNTs to act as one dimensional channels for electron transfer in proteins (88-90). This electron transfer can be further enhanced by the rapid transfer kinetics and high electrocatalytic activity of the tips of oxidised CNTs (91, 92).

Electrochemical sensors can be based on potentiometry, amperometry, voltammetry, coulometry, AC conductivity or capacitance measurements (93). Most CNT-based electrochemical biosensors detect biomolecules amperometrically. The range within which the sensor is sensitive is an important sensor parameter. Glucose sensors for example need to be sensitive in the range of a few μmol/l to 15 mmol/L since normal blood sugar levels are usually less than 6 mmol/L of glucose, while a level of 7 mmol/L or higher implies diabetes. Since the first biosensors, measuring glucose, were reported in the early 60's, it has become one of the most frequently performed routine analyses in medicine. It is thus hardly surprising that an enormous amount of literature exists on glucose biosensors and more recently CNT-based glucose biosensors, which are covered in some recent reviews (94-97). Here we will highlight some general characteristics of these biosensors and present a few pertinent examples.

An example of a CNT-mat amperometric biosensor incorporated dispersed SWNTs with the enzyme glucose oxidase into redox polymer hydrogels (63). These enzymatic redox composite films resulted in up to a 10-fold increase in the oxidation and reduction peak currents, while the glucose electro-oxidation current was increased 3-fold for glucose sensors. CNT-mat electrodes do not seem to provide significant advantages in reversibility or signal-to-noise ratio compared to the best redox protein films on conventional electrodes except in a few special cases (e.g. glucose oxidase) (98). Rubianes and Rivas (99) showed how using dispersed CNTs allowed the development of highly sensitive glucose biosensors without redox mediators, metals or anti-interferent layers. The sensitivity of the CNT-mat electrode was 43 times higher than that obtained with the control graphite composite electrode, with a clinically relevant linear range and negligible interference from ascorbic acid (AA), uric acid (UA) and acetaminophen; all common blood interferents.

Even though the nanodimensions of CNTs makes them amenable to close contact with the redox active centres of proteins and enzymes, Gooding and co-workers have demonstrated that CNTs may not fully probe the protein active site (44). In an interesting paper they describe how a self-assembled aligned shortened CNT forest probes the redox active centre of glucose oxidase, flavin adenine dinucleotide (FAD). In one experiment they conjugated glucose oxidase directly to the ends of the shortened CNTs in the aligned forest. They compared its electrochemistry to that of an array conjugated to the enzyme active centre, FAD, with subsequent enzyme reconstitution around the CNT-immobilized FAD. They found that the latter approach allowed for more efficient electron transfer to the glucose oxidase active centre. Hence, even though CNTs offer more efficient ways of communicating electrically with the active sites of biomolecules then traditional bulk substrates, there is still room for improvement when fabricating biosensors composed of them.

Cholesterol sensors must have sensitivity in the range of 2.5–10 mmol/L since a total blood cholesterol level of less than ~5 mmol/L is considered to be risk-free, whereas high cholesterol levels greater than ~6 mmol/L are considered dangerous. The layer-by-layer adsorption technique was used to immobilise cholesterol oxidase in a MWNT-mat immobilised on a gold electrode to create a biosensor for cholesterol (100). The sensor response was found to be linear in the range of 0.2–6 mmol/L. In another case, a screen-printed carbon paste electrode modified with a MWNT-mat and cholesterol oxidase could detect cholesterol directly in blood in the clinically relevant ranges (101). The authors noted how CNTs promoted the electron transfer; nearly doubled the sensitivity and improved the linearity of the electrode compared to the control. Furthermore, the CNT electrode results gave good correlation with results from clinical assays of 31 patients' blood samples.

Detecting genomic DNA sequences and identifying mutations is vital in the treatment of inheritable and infectious diseases. Electrochemical methods are aptly-suited to the detection of DNA with their high sensitivity and rapid response. CNT-based DNA sensors are well covered in recent reviews (94-97), therefore we will highlight using examples some general characteristics of these biosensors. Sensors may be fabricated by immobilising single-stranded DNA (ssDNA) onto an electrode, allowing hybridisation of the complementary DNA sequence to be detected by a current

change. An electroactive DNA intercalator is often used to amplify the signal. A 24-base pair DNA could be detected using differential pulse voltammetry and the intercalator duanomycin, at a MWNT mat electrode modified with complementary ssDNA (102). This sensor exhibited good selectivity (on the order of 4 μA/nmol/L over a linear range of 0.2–50 nmol/L) over oligonucleotide sequences having a mismatch of only a few bases. When these electrodes were decorated with Pt nanoparticles however, a superior response was obtained, again showing the amplification achieved with nanoparticles (103). The limit of detection for target DNA using the Pt nanoparticle-modified MWNTs was 1.0×10^{-11} mol/L.

By monitoring the electrochemical oxidation of guanine, DNA can be detected without an indicator. Labuda and co-workers (104) evaluated DNA biosensors from both the redox signals of the marker $[Co(phen)_3]^{3+}$ and guanine residues. They used screen printed electrodes modified with nanostructured mats of MWCNT, hydroxyapatite and montmorillonite. Based on AC voltammetric detection of guanine, a label-free DNA hybridization sensor was developed by attaching MWNTs onto a carbon paste electrode using a hybridisation assay (105). The MWNT-mat electrode exhibited large signal improvements compared to the control. Screen printed electrodes modified with MWNT, which catalysed the electrooxidation of guanine and adenine residues, were reported by Ye and Ju (106) for fast and sensitive detection of DNA and RNA. To improve the response of guanine oxidation a redox mediator can be used. For example, $Ru(bby)^{2+}_3$ allowed attomoles of oligonucleotides to be detected at ssDNA-modified MWNT forests (107). A 17-fold higher oxidation signal for DNA oxidation at CNTs compared to a glassy carbon control electrode was reported by Wang *et al.* (108). They used chronopotentiometric adsorptive-stripping in the presence of copper to measure the purine nucleobases (guanine in this case). Well defined hybridisation signals were obtained for the BRCA1 breast cancer gene with an LOD of 40 ng/mL. Gooding and co-workers have reported advantages of using bamboo type CNTs for the oxidation of DNA bases in a mat-type configuration (109). The presence of edge planes of graphene at regular intervals along the walls of the bamboo CNT were attributed to the enhancement in the oxidation signals of guanine residues.

Examples of where CNTs were used for amplified detection include enzyme-linked CNTs carrying numerous enzymes (110, 111). Wang *et al.* (112) described the use of CNTs in two ways, both for the recognition and transduction events. Target DNA strands were labelled with CNTs carrying numerous alkaline phosphatase tags. DNA target strands were captured by DNA immobilised on magnetic beads. The signal of the target analyte underwent double step amplification in both the recognition and transduction events. The CNT-alkaline phosphatase enzymatic amplification was detected using chronopotentiometric stripping at a CNT-mat electrode. The potentiometric detection of DNA was demonstrated with high sensitivity using ssDNA connected to enzyme-loaded CNTs immobilized on a glassy carbon electrode. This led to a reported detection limit of 54 aM for DNA.

Immunosensors are a good alternative to traditional immunoassays since conventional immunoassays such as enzyme-linked immunosorbent assay (ELISA) can be complex and laborious to perform. They use the high affinity reaction between an antibody, as recognition element, with its corresponding antigen, in combination with a transducer. Immunosensors can be used to monitor the presence of the antibody or antigen and either the antibody or the antigen can be immobilised or labelled depending on the assay requirements. When immobilising the antibody, it is of crucial importance that the method of immobilisation maintains the stability and activity of the antibody.

To boost the detection sensitivity of PSA (prostate specific antigen, a biomarker for prostate cancer) in serum, an amplification step was incorporated by combining SWNT forest immunosensors with HRP-MWNT-Ab2 bioconjugates. The secondary antibody (Ab2) and HRP tag were covalently linked to MWNTs at high ratios of 1:200 (89). This amplification strategy improved the detection limit 100-fold to 4 pg ml^{-1} and the sensitivity by 800-fold, compared to conventional ELISA. These results highlight the excellent promise CNTs show in ultrasensitive immunoassay research in proteomics and systems biology.

CNT-FETs

In addition to electrochemical sensors using CNTs as an electrode substrate, sensors based on transistor arrangements using CNTs have been developed (113). SWNTs are the most likely candidate for miniaturizing electronics beyond the micro

electromechanical scale currently used in electronics. They exhibit electric properties not shared by their multi-walled counterparts and certain sizes of SWNT act as semiconductors. The intrinsic bandgap in semiconducting SWNTs (typically 0.5eV, but this is diameter-dependent) allows them to be used as nanosized semiconducting channels in field-effect transistors (FETs) (114). Since FET-based biomolecular detection does not employ fluorescence, electrochemical, or magnetic tags it has been termed as 'label-free' methodology (115-117). FETs generally consist of a substrate (gate), two microelectrodes (source and drain), and a SWNT (or SWNT network) that bridges the electrodes. Usually SWNTs are grown directly via chemical vapour deposition (CVD) or cast from a dispersion onto a substrate either before or after the electrodes are patterned (118). Single-nanotube FETs require arduous screening of devices to eliminate metallic SWNTs. This need is obviated for nanotube networks cast from dispersions, where the 2 to 1 ratio of semiconducting to metallic SWNTs renders the likelihood of forming a continuous metallic pathway between source and drain unlikely. Sensing is based on the fact that the current flow in SWNT FETs is extremely sensitive to the binding of biomolecules and produces a detectable signal. A wide variety of applications for CNT FETs have been investigated, including the detection of proteins, antibody-antigen interactions, glucose, DNA and DNA hybridization. The detection limit for the sensing of proteins or protein-protein interactions has generally been in the range of 100 pM to 100 nM (98).

An SWNT-FET binding assay typically involves first immobilising a biological receptor, for example, a nucleotide, aptamer, antibody, or cofactor, thus providing recognition sites for target analytes, for example, complementary DNA strand, protein, antigen, or apo-protein. The current–voltage characteristics or conductance of the receptor-modified SWNT-FET are measured prior to analyte binding. This is generally followed by a blocking step to minimize non-specific binding of targets. Finally, the current–voltage characteristics or conductance of the SWNT-FET device is measured following exposure to the analyte (119).

50-amino-modified aptamers (oligonucleic acid or peptide molecules that bind to a specific target molecule) immobilised on a CNT-FET were used to detect immunoglobulin E (IgE) (120). The net current change increased with the IgE concentration and a detection limit for IgE of 250 pM was reported. Li *et al.* (121)

studied the detection of PSA with a FET comprised of a network of SWNTs. The authors measured the electronic interaction of an anti-PSA antibody in the act of capturing PSA. The interaction is thought to be a charge-transfer mechanism with a reported limit of detection of 14 pM, at a signal-to-noise ratio of 2. SWNT-FET biosensors can achieve pM detection limits for DNA hybridization (110) and antibody–antigen binding (98, 122).

2.1.2 CNT-Optical sensors

Individual semiconducting SWNTs exhibit photoluminescence, with discrete bands in the near-infrared region between 900 and 1600nm. Since biologically relevant samples such as blood and tissue have low absorption in this region, the sharp nanotube fluorescence spectra may be detected even in a complex biological environment. Such semiconducting SWNTs were used as near-IR fluorescent tags for cell imaging and to selectively probe cell surface receptors (123). The nanotubes were first non-covalently functionalised with amine groups using the surfactant PL-PEG-NH_2, followed by conjugation with antibodies recognising both the CD20 cell surface receptor (Rituxan) and the HER2/neu receptor on certain breast cancer cells (Herceptin). *In vitro* near-IR fluorescence imaging showed specific binding of the antibody-conjugated SWNTs to the host cells, with high specificity for the different antibodies (55:1 and 20:1 for host cells:non-host cells).

Barone *et al*. linked enzyme reactions to CNT fluorescence, creating a sensor whereby an enzymatic reaction could be followed by monitoring fluorescence (124). The authors non-covalently functionalised SWNTs with glucose oxidase (GOx) and potassium ferricyanide. The functionalisation with potassium ferricyanide quenches the SWNT fluorescence. Addition of glucose to the GOx-SWNT sensing complex resulted in the ferricyanide ions leaving the surface of the CNT yielding a recovery of the CNT fluorescence. The authors could relate the CNT near-infrared fluorescence to the glucose concentration and maintain that this type of sensor, enveloped in a small dialysis capillary, could be implanted in the body. The capillary could allow glucose to diffuse in, easily allowing sugar levels to be measured. This research demonstrates the feasibility of using CNT sensor systems in implantable biomedical sensors.

2.2 CPs

2.2.1 CP-Electrochemical sensors

An L.O.D. of 3.4 x 10^{-10} mol/L was reported for a simple electrochemical oligonucleotide (ODN) sensor made using PAni nanotubes (125). Solutions used during PAni synthesis contain polymeric acid (polymethyl vinyl ether-*alt*-maleic acid), and hence nanotubes have residual carboxylic acid functionalities which can be used to covalently graft ODN via carbodiimide chemistry. The authors report that they expect to achieve an even lower detection limit by optimizing the nanotube surface area. PAni nanowires can also be synthesised electrochemically, and subsequently modified with oligonucleotides via EDC coupling between phosphate groups and the amino groups of PAni (126). Using this method the complimentary DNA target could be detected down to a concentration of 1 x 10^{-12} mol/L. DNA-functionalised polyaniline nanofibres (100nm diameter) can also be used to specifically detect Gonorrhea. Up to 0.5 × 10^{-15} M of complementary target could be detected by differential pulse voltammetry within 60 seconds of hybridisation (127). These electrodes are found to be highly specific to distinguish the presence of *N. gonorrhoeae* from *N. meningitidis* and other Gram-negative bacteria, (such as *E. coli).* The performance of this STD sensor in clinical samples is being explored by the authors, and findings are expected to also have implications in relation to the clinical diagnosis of other sexually transmitted diseases.

CPs can also be used to detect many other targets for example, Dhand *et al.* report an electrode biosensor where cholesterol oxidase (ChOx) is covalently immobilised onto nano-structured PANI on indium tin oxide (ITO). Using this set-up good selectivity can be achieved and it is significant that interferants such as AA, UA, glucose, lactic acid, sodium pyruvate and urea were found to have a negligible effect on the sensor. ChOx/PANI/ITO electrodes retain about 85% activity after 11 weeks (when stored at 4 ^{o}C) and can be used ~ 20 times with 2–3% error range. Another example of a cholesterol sensor is where the electropolymerisation an enzyme with laponite nanoparticles in a polypyrrole matrix was shown to increase the sensitivity of detection from 5.1 (without laponite nanoparticles) to 13.2 mA M^{-1} cm^{-2} (128).

Along with good sensitivity and selectivity, nanostructured biosensors typically exhibit fast response times. For example an amperometric biosensor designed for the detection of phosphate ions has a response time of 6 seconds (129). In this example,

pyruvate oxidase (PyO) was covalently immobilised onto nano-particles (5-40nm) of poly-5,2′:5′,2″-terthiophene-3′-carboxylic acid (PTCA). The electron transfer rate constant from immobilised PyO was determined to be 0.65 s^{-1}, with a detection limit of ~0.3 μM. This biosensor can be stored and re-used for up to one month without any loss in sensitivity. A similar biosensor of PTCA nanoparticles was used to covalently immobilise glutamate oxide. Glutamate concentrations could be determined, and an LOD of 0.1μM was reported for an *in vitro* measurement (wherein the biosensor was implanted into a rat's brain).

Polypyrrole is another example of a CP which can be used in the nanoform for low L.O.D biomolecule detection. An example of this is where a pyrrole monomer and biomolecule receptor (avidin) were electropolymerised within 100 nm wide channels (130). When exposed to biotin−DNA, the conducting polymer nanowires generated a rapid change in resistance, with sensitivity as low as 1 nM. The method described offers advantages of direct incorporation of functional biological molecules into the conducting-polymer nanowire during its synthesis, site-specific positioning, built-in electrical contacts, and scalability to high-density nanoarrays. Polypyrrole nanofibres were also developed with an even lower L.O.D of 100-200 fg mL^{-1}. Nanofibres were used to detect salivary protein markers. An exceptionally low L.O.D of 10aM was reported by the authors for IL-8 mRNA (131). Advantages of this method are the low L.O.D combined with the fact that the detection method is label-free with excellent control over non-specific binding.

2.4 CP-Optical sensors

As well as electrochemical detection, nanostructured CPs can also be used for the optical detection of biomolecules. An example of this is where functionalised silica-PPy nanocomposites were used to detect anti-HSA. Flocculation of the nanocomposite dispersion occurs upon anti-HSA binding and the system can therefore be used for visual diagnostic assays (132). Human serum albumin (HSA) is of interest as a target as it was previously used to detect renal disease. In another example of a HSA biosensor, pyrrole-propylic acid nanowires can be synthesized electrochemically via a templated method and subsequently modified using EDC crosslinker to covalently bind anti-HSA. Using this as a platform, HSA can then be detected using

optical or electrical methods. Using an FET configuration nanomolar levels of HSA were reported (133).

3. Composites

By using nanostructured (versus bulk) materials, it is possible to develop biosensors which exhibit higher signal-to-background ratios, shorter response times, higher sensitivities and greater selectivity than previous biosensor configurations. Many different nanostructured geometries can be used to develop biosensors with improved sensing capabilities. However, it is interesting to also consider hybrid composites composed of two or more materials (134). Using this approach the advantageous properties of each constituent can be exploited. CNT and CPs can be combined together (and also with other materials) to produce improved biosensors (19, 135). In general the incorporation of CNTs tend to improve the sensitivity and selectivity of a biosensor (69).

Composites can be used to improve the selectivity of biosensors, for example in dopamine monitoring. Dopamine (DA) is an important neurotransmitter and abnormal levels can be used to diagnose certain nervous diseases such as Parkinsons and epilepsy. DA is easily oxidisable which can enable detection (typically levels in urine samples are monitored). However other electroactive compounds are present along with DA. In particular AA and UA can cause a problem as they oxidise at almost the same potential resulting in interference. Incorporation of CNTs and surfactant, along with CPs, have been used as ways to selectively detect DA(25). CPs can also be combined with gold and Mathiyarasu *et al.* report Poly(3,4-ethylenedioxythiophene), PEDOT-Au nanocomposite films for sensing DA and UA simultaneously (115 mV and 246 mV, for DA and UA respectively) (76). It is significant that detection can be achieved in the presence of excess AA which is present in both blood and urine, thus complicating detection. Abnormal levels of UA are symptomatic of diseases such as gout and Lesch-Nyhan syndrome. The PEDOT matrix contributes towards the peak separation (selectivity) while also promoting catalytic oxidation of the above compounds. Gold nano-particles facilitate nanomolar sensing (sensitivity). Thus, it is possible to detect nanomolar levels of DA and UA in presence of excess AA. This composite nanomaterial shows superior selectivity and sensitivity compared to the polymer film alone, and presents an interesting step forward as a major challenge is to

develop a sensitive and selective method for UA and DA detection (Ates *et al.*, 2009(25)).

Gold can also be combined with other CPs, and Prabhakar *et al.* (136) report a nucleic acid sensor whereby pathogen-specific DNA and PNA (peptide nucleic acid) probes were covalently immobilized onto a PAni-Au electrode. These nanostructured electrodes were then utilized for the detection of hybridization with a complementary sequence (*M. tuberculosis* in this case). The PNA-PANI/Au electrode exhibits a detection limit of 0.125×10^{-18} M, with the DNA-PANI/Au electrode showing 2.5×10^{-18} M. Improved specificity (1000 times) was also observed for PNA-PANI/Au. Responses were observed within 30 seconds of hybridization time. These DNA-PANI/Au and PNA-PANI/Au electrodes can be used 6−7 and 13−15 times, respectively. For increased sensitivity, reusability, and better detection limit, authors recommend the development of nanocomposites and functionalized conducting polymers. In this way it should be possible to detect other pathogens including *Salmonella typhimurium* and *Nesseria gonorrhea.*

Qu *et al.* report a nanostructured composite amperometric biosensor for choline (16), which is based on a functionalised CNT-PAni multilayer film. Carboxylic acid groups were attached to the CNTs and the films were prepared using a layer-by-layer assembly method. By linking choline oxidase (CHOD), a choline biosensor was prepared with a linear response range of 1×10^{-6} to 2×10^{-3} M, and a response time of 3s. The commonly encountered interference arising from AA and uric acid UA could be rejected successfully by the polymer. The same approach can be applied to immobilise other oxidase enzymes, such as glucose oxidase and cholesterol oxidase, for the fabrication of biosensors. This anti-interference biosensor displays a rapid response, an expanded linear response range, excellent reproducibility and good stability.

Liu *et al*. report how polyaniline-carbon nanotube multilayer films can be prepared by the layer-by-layer assembly method and used for stable low-potential detection of β-nicotinamide adenine dinucleotide (NADH) (*7*). The carbon nanotubes are modified with poly(aminobenzenesulfonic acid), and this acts as a PAni dopant, thus shifting its electroactivity to a neutral pH environment. Resulting films show good

electrocatalytic ability toward the oxidation of reduced NADH at a much lower potential than usual. The response is linear with concentration between 5×10^{-6} and 1×10^{-3} M, and the detection limit can go down to 1×10^{-6} M. Therefore the system shows good potential for developing dehydrogenase-based biosensors depending on NADH as a cofactor.

It is evident from the literature that incorporating nanoparticles into CNT-based glucose biosensors yields higher sensitivity. This is attributed to the enhanced catalytic activity and large surface area obtained by combining CNTs and nanoparticles. The most sensitive glucose biosensors however do not always operate in the most important clinical ranges as highlighted by Balasubramanian and Burghard (94). Tang *et al.* (137) have reported what can be regarded as an ideal yet practical sensor as it exhibits good sensitivity within a large clinically relevant detection range. In this case a CNT forest was grown directly on the graphite substrate followed by functionalisation with Pt nanoparticles, glucose oxidase and a thin layer of Nafion to improve stability. This system showed good reproducibility, demonstrated good correlation with independent clinical values in the analysis of glucose levels in serum and was able to deliver a signal in less than five seconds. A comparable sensor set-up was reported by Claussen *et al.* where they describe fabricating a 'CNT forest' like electrode decorated with Au-coated Pd nanocubes (138). The outer gold surface allowed for glucose oxidase functionalisation to yield a sensor with a wide working range and response time of just 6 seconds. Glucose can also be detected by a novel multilayer AU NP / MWNT / glucose oxidase membrane, developed by Liu *et al*. (139). This membrane showed excellent electrocatalytic character for glucose biosensing at a relatively low potential (-0.2 V). The resulting sensor could detect glucose up to 9.0 mM with a detection limit of 128 mM.

4. Conclusion and Outlook

A key aspect in biosensor development still remains the integration of the electrical component with the biological recognition molecule. The development of miniaturised biosensors with improved sensitivity requires immobilisation of biomolecules (including DNA, antibodies, aptamers, PNAs and enzymes) onto a surface, such that a maximum number of biomolecules per unit area can be attached,

while simultaneously being accessible to target species. Nanostructured surfaces are becoming increasingly significant in this regard as they possess high surface-to-volume ratios, providing a greater number of sites for attachment. It is important to be able to functionalise nanostructures with specific biomolecules in a controllable and reproducible fashion. Biomolecular probes must also be carefully attached to prevent reactivity loss. We have shown here that both CPs and CNTs are effective transducers which can be used for the immobilisation of biomolecules. Both materials are conductive and stable in biological systems. Future requirements include improving immobilisation efficiency, tailoring nanostructured interfaces, and integrating these optimised nanobiosensors into external circuitry. The improvement of transduction mechanisms continues to be an important focus for biosensor research, and here we have shown that CPs and CNTs show great promise as efficient transducers. Graphene is the 2D form of CNTs and is also becoming increasingly important for biosensor applications (96). Quantum effects play a significant role in the behaviour of nanomaterials and can lead to novel optical, electrical and electrochemical properties. Careful engineering of materials at the nanoscale means that their small size and novel characteristics can be exploited for practical bio-applications. Physical and chemical properties of materials, such as colour, and ability to conduct charge, are different at the nanoscale making it possible to achieve a number of improvements over more traditional bulk substrates.

The use of hybrid nanomaterials is becoming increasingly popular as it offers the opportunity to combine the advantageous properties of each individual constituent in a single composite material (140). Composites are suitable for multiplexed biosensing enabling the detection of multiple analytes using a single assay. The signal to noise ratio could be further improved using a combination of optimised nanomaterials and advanced circuitry. It is also interesting to consider the idea of personalised healthcare whereby wearable sensors are becoming increasingly important (141). Many challenges still remain in this area including the miniaturisation of integrated sensors and also issue of power supply. Thus as we have described, a wide range of nanomaterials and detection mechanisms are suitable for biosensing. The high surface area, porosity, and unique properties of nanomaterials facilitate the ultimate aim of a biosensor to achieve a significantly lower limit of biomolecule detection. The development of nanostructured biosensors is critical for further advancing the field of

medical diagnostics. Therefore, the importance of nanomaterials for biosensor development cannot be overstated.

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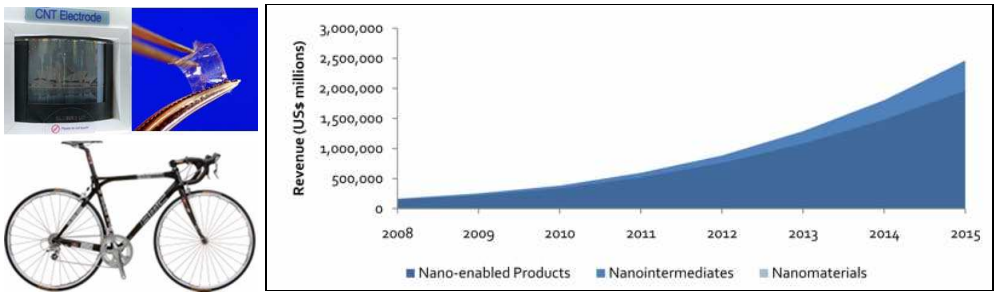

Figure 1: Nanomaterials have many potential applications. Images (left) show; a CNT-based flexible full-colour e-paper device (Samsung, 2008), a flexible transparent CNT composite(142) (image courtesy of E. Lahiff), and a CNT-reinforced BMC bike used in the 2005 Tour de France Nanotechnology is expected to generate $2.5 trillion by 2015 (right: Sourced from Lux Research Inc.)

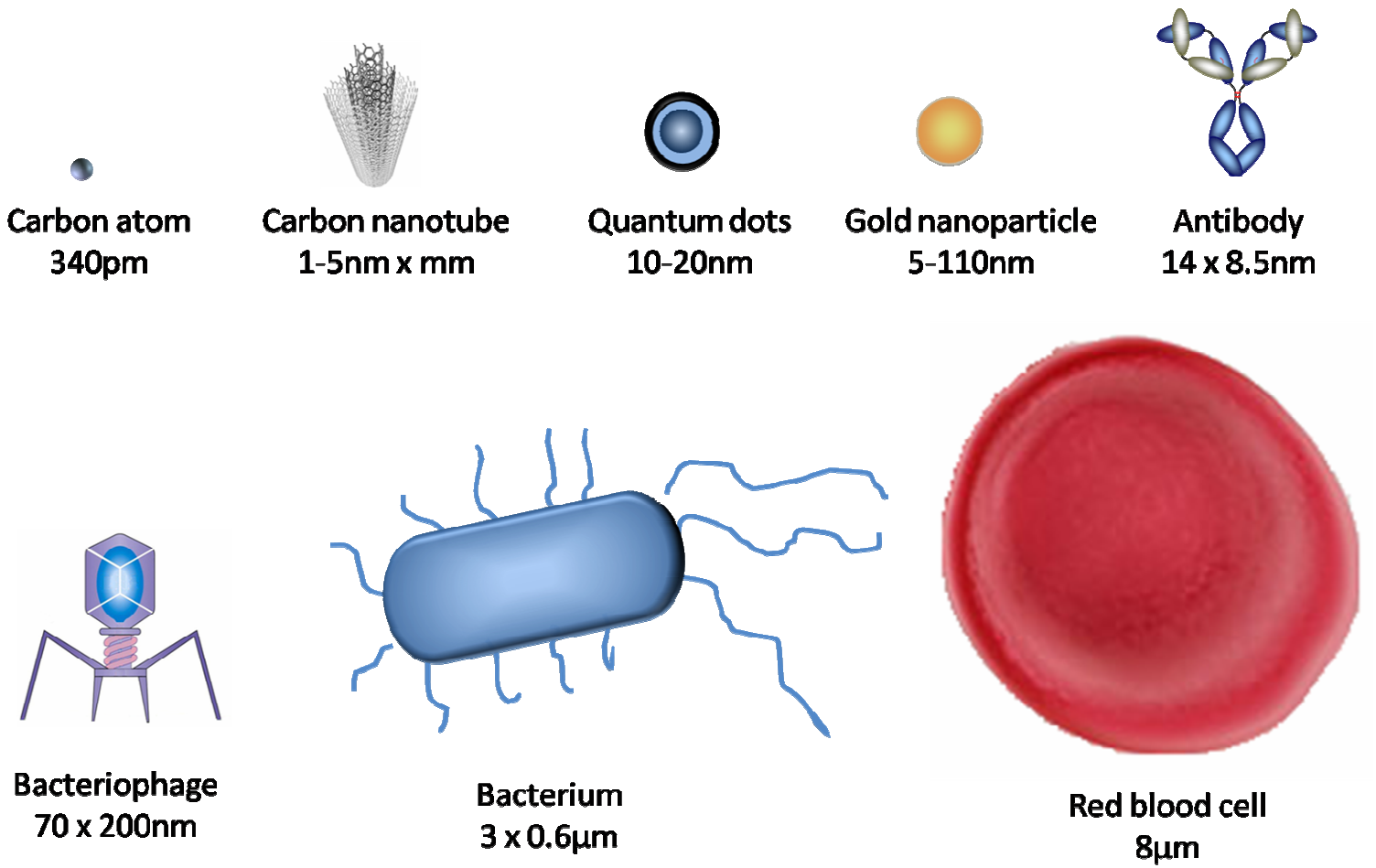

Figure 2: The relative size of some nanoparticles in comparison with biological molecules.

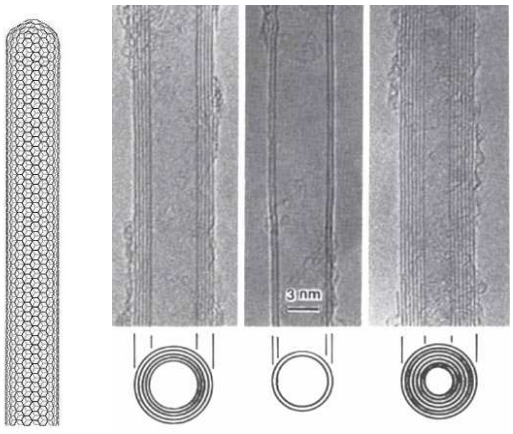

Figure 4: A SWNT can be visualised as a rolled-up sheet of graphite capped by half a C_{60} molecule (left). CNTs can also exist as DWNTs and MWNTs. TEM images reveal the number of walls present (right shows 5, 2 and 7 layers)(26). Reproduced with permission from the Nature Publishing Group.

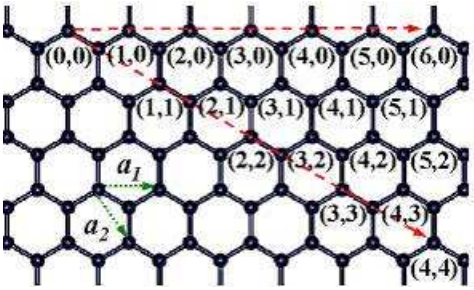

Figure 4: Nanotubes possess an armchair, zig-zag or chiral structure depending on the angle at which they are rolled up (this determines n, m and θ values).

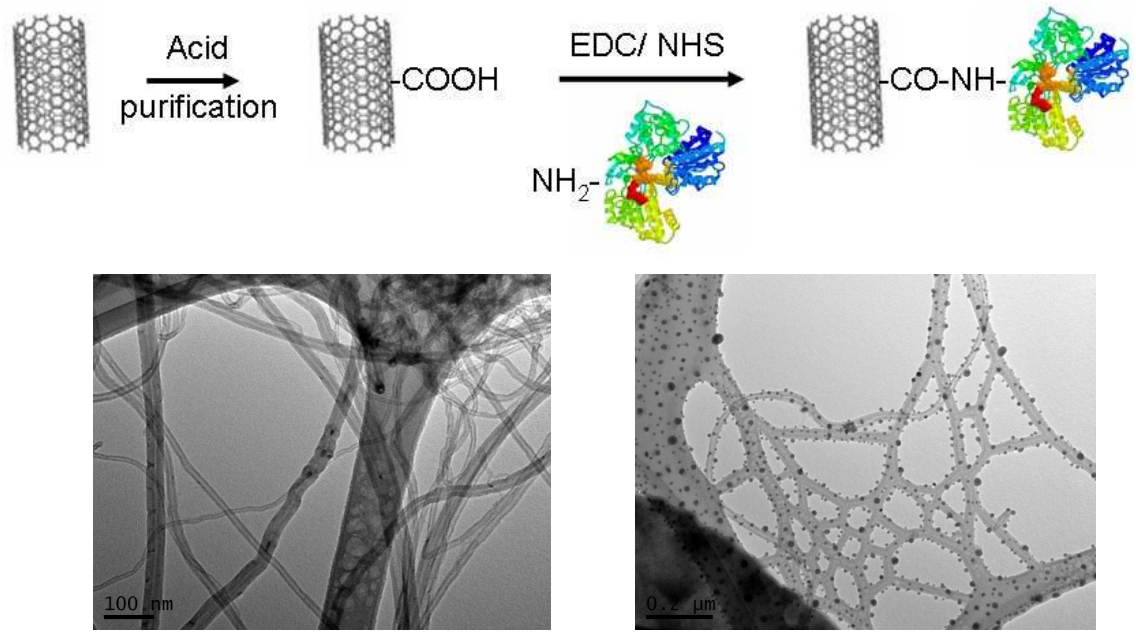

Figure 5: Biomolecules can be covalently attached to acid functionalities on CNT surfaces via EDC/NHS coupling with amide groups on a biomolecule (scheme shown top). TEM can be used to effectively image CNTs before (left) and after (right) biomolecule attachment. Dark spheres represent the iron core of HRP attached to CNT surfaces (Images courtesy of C. Lynam).

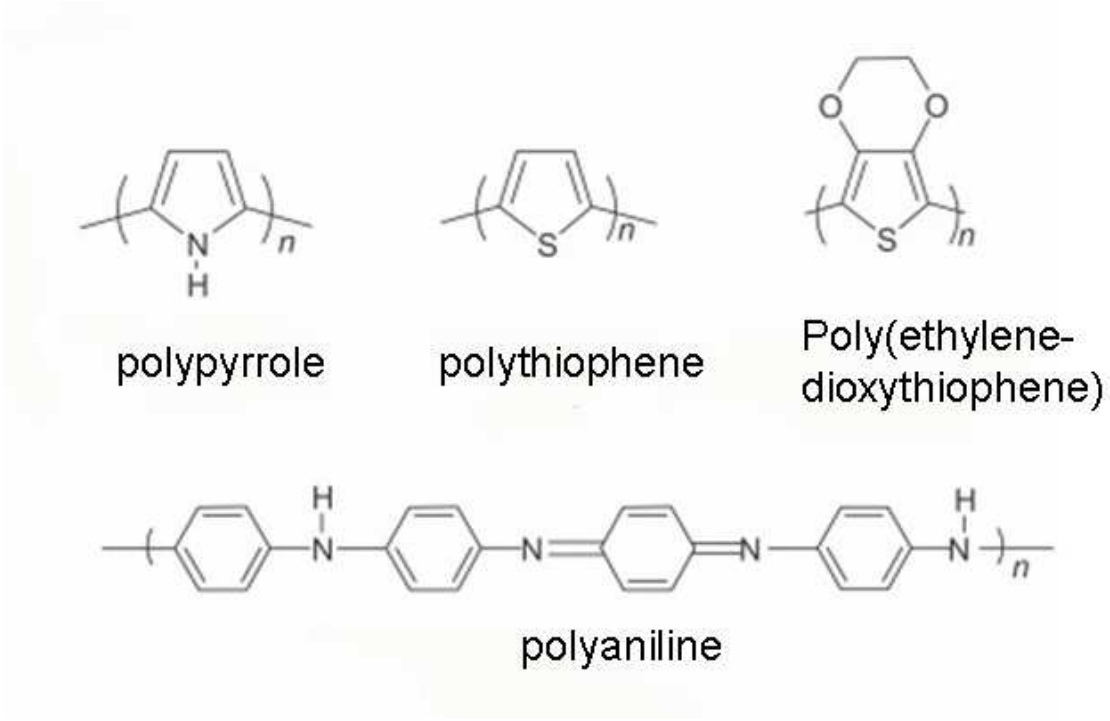

Figure 6: The chemical structure of relevant CPs. Conjugated bonds facilitate improved electron transport.

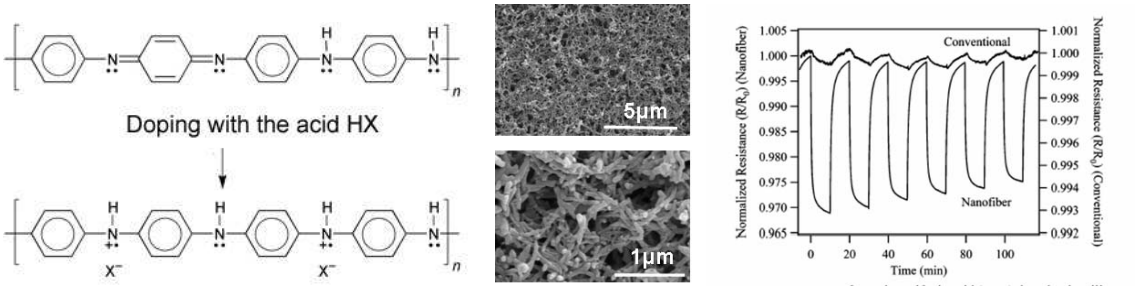

Figure 7: The chemical bonding structure of the CP PAni is sensitive to the chemical environment of the material (left). In its nanoform, the material is more response than in the bulk form (right shows sensing results, reproduced with permission from the American Chemical Society.)(143).

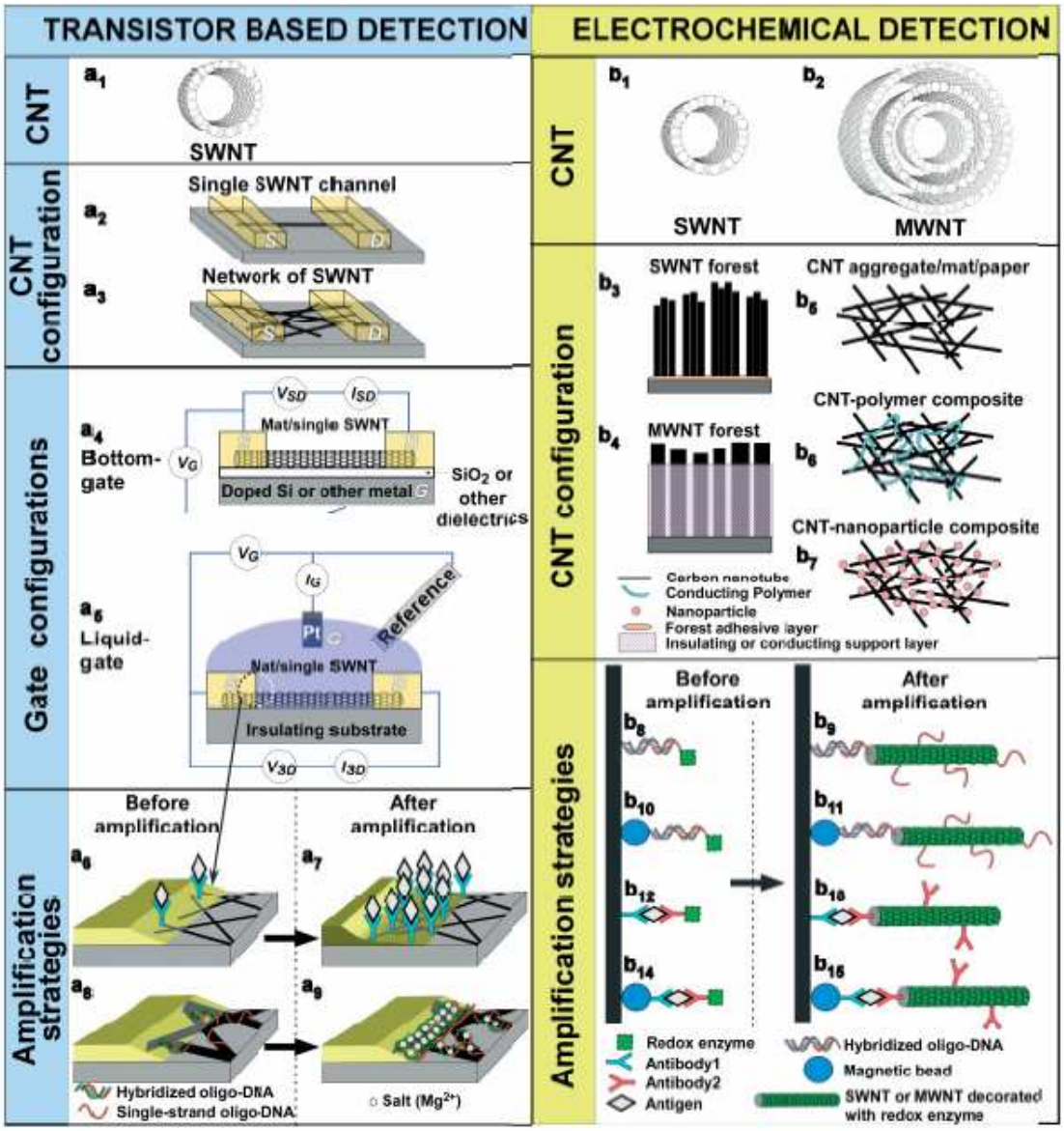

Figure 8: Schematic representation of biomolecular sensing using carbon nanotubes in various device configuration and signal amplification strategies (114). Reproduced with permission from Wiley-VCH Verlag GmbH.

Table 1: Enhanced biosensor sensitivity can be achieved by incorporating NPs.

Nanoparticle	**Detection System**	**Analyte**	**Limit of detection (L.O.D)**	**Ref**
CP (PAni)	Photometric	Cholesterol Oxidase	25mg/dL	(71)
CP (PTCA)	Amperometric	Pyruvate oxidase Glutamate oxidase	0.3μM 0.1μM	(76) (*15*)
CP (PPy)	Amperometric	Biotin-DNA	1nM	(77)
CP (PAni)	Amperometric	ODN	$3.4x10^{-10}$ mol/L (0.34 nM)	(78)
CP (PAni)	Voltammetry	Gonorrhea	$0.5x10^{-15}$ M (0.5 fM)	(77)
CP (Pani)	Capacitive	human IgG	1.87 ng mL^{-1} (1.87 ng/ml)	(144)
PAni-gold	Amperometric	Tuberculosis DNA	$0.125x10^{-18}$ M (0.125 aM)	(77)
Pani-CNTs	Electrochemical impedance spectroscopy	NADH	$1x10^{-6}$ M (1 μM)	(77)
MWNT	Amperometric	Cholesterol	0.2 mmol/l (0.2 mM)	(101)
MWNT-Pt	Differential pulse voltammetry	DNA	$1x10^{-11}$ mol/l (10 pM)	(103)
SWNT	Amperometric	PSA	4 pg/ml	(110)
SWNT	FET	Thrombin	10nM	(145)
SWNT	FET	Carcinoembryonic antigen	300fM	(129)

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