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ABSTRACT

Biosensors are important tools in biomedical research. Moreover, they are becoming an essential part of modern healthcare. In the future, biosensor development will become even more crucial due to the demand for personalized-medicine, point-of care devices and cheaper diagnostic tools. Substantial advances in sensor technology are often fueled by the advent of new materials. Therefore, nanomaterials have motivated a large body of research and such materials have been implemented into biosensor devices. Among these new materials carbon nanotubes (CNTs) are especially promising building blocks for biosensors due to their unique electronic and optical properties. Carbon nanotubes are rolled-up cylinders of carbon monolayers (graphene). They can be chemically modified in such a way that biologically relevant molecules can be detected with high sensitivity and selectivity. In this review article we will discuss how carbon nanotubes can be used to create biosensors. We review the latest advancements of optical carbon nanotube based biosensors with a special focus on near-infrared (NIR)-fluorescence, Raman-scattering and fluorescence quenching.

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Contents

1. Introduction	0
2. Carbon nanotubes	0
2.1. Structure and synthesis	0
2.2. Photophysics of carbon nanotubes	0
2.3. Surface functionalization	0
2.4. Sensing mechanism	0
2.5. Advantages and disadvantages of carbon nanotube based optical sensors	0
3. DNA detection	0
3.1. DNA hybridization	0
3.2. Single nucleotide polymorphism (SNPs)	0
3.3. Conformational polymorphism	0
3.4. DNA-sequence detection	0
4. Small molecule detection	0
4.1. Adenosine 5'-triphosphate (ATP)	0
4.2. Reactive oxygen/nitrogen species detection	0
4.3. H ₂ O ₂ sensing	0
4.4. NO sensing	0
5. Protein and biomarker detection	0
6. Glucose detection	0
7. Glycoprofiling	0
8. Conclusions and outlook	0
Acknowledgment	0
References	0

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1. Introduction

Biosensors have an important impact on basic scientific research and healthcare. In this area, scientific breakthroughs are often facilitated by new sensing technologies that enable investigation of unstudied biological phenomena. In healthcare, new diagnostic tools are needed to improve clinical treatments. Moreover, many challenges of the healthcare-system can only be solved by new sensor technology such as point-of care sensor devices. Therefore, biosensors will become even more important in the future. Biosensor development is catalyzed by the discovery of new materials, and in the last decade, a great amount of work has been dedicated to develop nanomaterial-based biosensors [1–9].

Materials like nanoparticles [10], quantum dots [1], nanowires [2], graphene [3], graphene quantum dots [4] or carbon nanotubes [5–9] have shown their potential in the fabrication of highly sensitive and selective biosensors. An intriguing example for new approaches, which were impossible without these new materials is carbon-nanotube endoscopes, which have been used to probe the interior of living cells [11]. In the future, these approaches could be useful to transduce electrochemical information from inside the cell to the outside.

This example illustrates how the nanoscale size of a sensing element might improve sensor performance or enhance spatial resolution. In many cases, a biological application would only be possible if the sensing element has the same size (i.e. nm) or a smaller size than the biological structures. Single cells or even organelles could only be probed without major perturbations if the sensor had such small dimensions.

Low-dimensional materials do not only provide a new length scale. These materials have electrons and phonons that are confined in fewer than three dimensions leading to unique physical and chemical properties that can be used for sensing applications. Using such properties it was possible to detect complementary nucleotide sequences with extremely high sensitivities in the femtomolar-range [12] or the cancer marker PSA (prostate specific antigen) in the attomolar-range [13].

Among the different kinds of nanomaterials carbon nanotubes are especially promising building blocks for biosensor design. The electrical detection of chemical solvent vapors [14] was one of the first examples of highly sensitive carbon nanotube-based sensors. More recently, an electrical sensor was developed that could identify fruit ripeness by measuring ethylene gas concentrations [15]. In the biomedical field, pioneering work on optical sensors for glucose detection [16] or DNA polymorphisms [17] has been conducted. In some cases carbon nanotubes have also been used to enhance the sensitivity of classical biosensors. For example, carbon fiber microelectrodes are used to measure neurotransmitter concentrations by cyclic voltammetry (CV). Recently, carbon nanotube coated carbon fiber microelectrodes showed enhanced sensitivity and selectivity [18].

All the aforementioned examples show that there is a plethora of distinct applications for biosensors, and that nanomaterials like carbon nanotubes can add or create beneficial properties. But what are the most important design principles and what are the most challenging problems for biosensor design? To understand this fundamental question the definition of a (bio)sensor is a good starting point.

A sensor is a device that detects a chemical, physical or biological quantity and transduces it into a signal. Biosensors are sensors that detect such quantities in a biological environment. In most of the cases biosensors are used to detect a certain biologically relevant analyte. Every sensor has to be composed of at least two units: 1) A recognition unit that provides a selective interaction with the analyte and 2) a transduction unit that converts the recognition event into a signal that can be directly observed.

This definition immediately serves as a guide for biosensor design. A sensor is usually built by combining a recognition unit such as antibodies, aptamers, DNA-sequences or lectins and a signal transduction mechanism/material. The analytes can be detected by a molecular recognition event but also by other physico-chemical properties. One

prominent example is the electrochemical dopamine detection with carbon fiber electrodes by utilizing its redox chemistry. By using such electrodes, basal and transient levels of dopamine in the brain were measured [19].

In combination with the signal transduction mechanism the recognition element is the reason for high sensitivity of the sensor. However, especially in biological environments the selectivity of the sensor is of similar or even higher importance. For example, detection of rare biomolecular species in blood is hindered by non-specific protein adsorption to nanoparticle sensors [20]. Useful methods to circumvent non-specific protein adsorption involve surface passivation, which renders the surface inert to unspecific adsorption of molecules [21].

To date, there are several excellent reviews on electrical, electrochemical and field-effect transistor (FET) based carbon nanotube biosensors [5,22–29]. In this review article we will focus on the emerging area of optical carbon nanotube based biosensors [6,9]. First, we will introduce the basic physical and chemical properties of carbon nanotubes that are important for biosensor design. Then we will review recent advances. The different sections are classified into important biomolecule subgroups. Finally, we will summarize the current status of these sensors and identify the most challenging technical and biological problems for the future.

2. Carbon nanotubes

Until the 1980s only three carbon allotropes were known: graphite, amorphous carbon and diamond. Since then, the discovery, characterization and isolation of three additional carbon allotropes (fullerenes [30], carbon nanotubes [31] and graphene [32]) have fueled the advancement of a major field of science.

Carbon nanotubes have attracted a lot of basic research efforts because of their unique physical and chemical properties. The interest in carbon nanotubes has even increased due to possible commercial applications [33]. In addition to biosensors, other biomedical applications of carbon nanotubes include drug delivery, nanoinjectors, phototherapy, imaging or artificial muscles [6,34–38].

In this section, we will discuss the basic physical and chemical properties of carbon nanotubes. Understanding these properties is the key to successful biosensor design. We will start with a discussion on the structure and synthesis of carbon nanotubes. Then we will discuss the structure-related optical properties and photophysics. After that we will present an overview on chemical strategies for carbon nanotube functionalization, which is essential for rendering them selective and biocompatible. Finally, we will summarize signal transduction mechanisms and give an overview on advantages and disadvantages of optical carbon nanotube based biosensors compared to other approaches.

2.1. Structure and synthesis

Carbon nanotubes are composed of sp²-hybridized carbon atoms and can be rationalized as rolled-up cylinders of graphene sheets (Fig. 1). If the tube is composed of only one cylinder it is called a single-walled carbon nanotube (SWCNT). If there are multiple cylinders stacked in each other these structures are called multi-walled carbon nanotubes (MWCNTs). SWCNTs have diameters of 0.4 to 2 nm and lengths up to several cm [39–41]. Although they are only built of carbon atoms their physical and chemical properties can differ depending on their structure. It is a common convention to use the chiral index (n,m) to assign the structure. These two integer values can describe all possible ways of rolling-up SWCNTs. In Fig. 1b the definition of the chirality index is depicted: The carbon nanotube can be rolled up along the vector $\mathbf{c} = n\mathbf{a}_1 + m\mathbf{a}_2$ by superimposing the first and the last carbon atom of $\mathbf{c}$ on the graphene lattice. The vector $\mathbf{c}$ is a linear combination of the lattice basic vectors $\mathbf{a}_1$ and $\mathbf{a}_2$ of the graphene layer. All possible geometries can be therefore described by the (n,m)

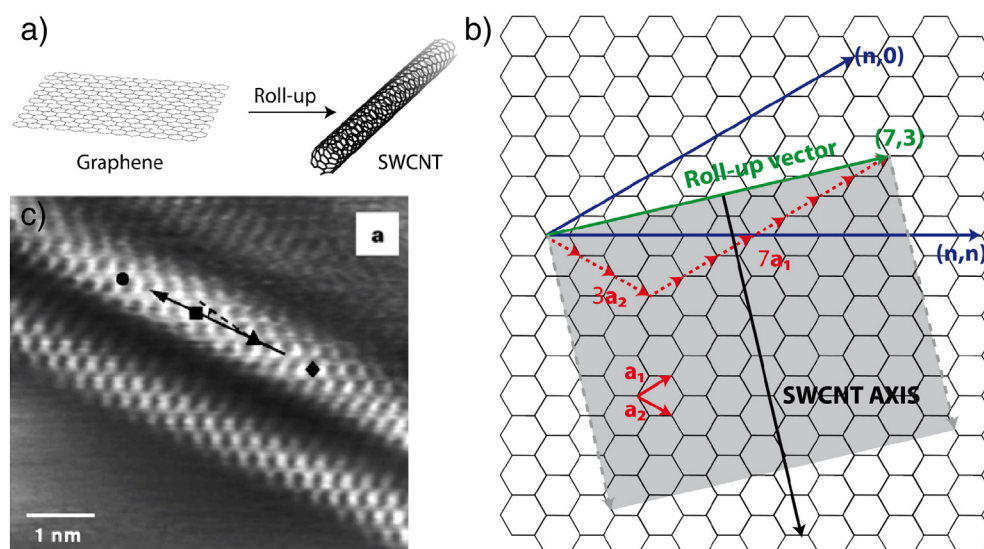


Fig. 1. Structure and nomenclature of single-walled carbon nanotubes (SWCNTs): a) The structure of single-walled carbon nanotubes (SWCNTs) can be rationalized by rolling up a monolayer of graphite (graphene). b) Every SWCNT can be generated by rolling up the graphene sheet along the roll-up vector $\mathbf{c} = n\mathbf{a}_1 + m\mathbf{a}_2$ and superimposing the first and the last carbon atom. The tube axis is perpendicular to the roll-up vector $\mathbf{c}$. In green the roll-up vector $\mathbf{c} = 7\mathbf{a}_1 + 3\mathbf{a}_2$ of a (7,3) SWCNT is depicted. The graphene lattice base vectors $\mathbf{a}_1$ and $\mathbf{a}_2$ are shown in red. c) Scanning tunneling microscopy image of a semiconducting SWCNT. The image in c) is reproduced with permission from [183].

index. In this context, the term chirality has a different meaning than the same term in organic and inorganic chemistry.

The diameter d of a given (n,m) SWCNT can be calculated by the formula:

$$d = \frac{|\mathbf{c}|}{\pi} = \frac{a_0}{\pi} \sqrt{n^2 + nm + m^2}$$

with a_0 being the graphene lattice constant (0.246 nm). Hence, the (n,m) index can be directly related to the structure and diameter of a carbon nanotube. Moreover, this index can be used to classify them into carbon nanotube families. Depending on the n,m values SWCNTs can exhibit metallic, semimetallic or semiconducting properties (Table 1). These properties are directly related to the density of electronic states and to the band gap between the conduction and valence band, which determines whether a carbon nanotube is fluorescent or not (see Section 2.2).

Carbon nanotubes can be synthesized mainly by three methods: chemical vapor deposition (CVD), carbon arc-discharge and laser ablation [39–41] and are commercially available in kilogram quantities. Although great efforts have been conducted towards selective synthesis of specific carbon nanotubes, most methods produce samples with mixed chiralities, diameters and impurities. Nevertheless, process conditions during synthesis can result in chirality enriched samples (e.g. (6,5)-SWCNTs fabricated by the CoMoCAT process [41]). Single chirality SWCNT-samples can be generated by purification of mixed chirality samples via density-gradient centrifugation [42,43], ion-exchange [44] or gel chromatography [45–47]. Recently, Liu et al. demonstrated chirality-controlled synthesis of SWCNTs using purified nanotubes as seeds [48]. In the future, similar approaches might lead to large-scale single-chirality synthesis of carbon nanotubes.

Table 1
Chirality dependent classification of SWCNTs.

Type of carbon nanotube	Definition	Electronic structure
Metallic	$n - m = 0$	No band gap
Semimetallic	$n - m = 3x, x \in \mathbb{N}, x > 0$	Small band gap (≈ 1 –100 meV)
Semiconducting	All other (n,m) , $n, m \in \mathbb{N}$	Large band gap (≈ 0.5 –1 eV)

2.2. Photophysics of carbon nanotubes

SWCNTs of different chiralities have different electronic properties (see Table 1). These electronic properties are correlated with respective differences in optical properties. To understand optical carbon nanotube sensors it is therefore necessary to introduce this relationship between electronic structure and photophysical behavior. Semiconducting single-walled carbon nanotubes (SWCNTs) are fluorescent in the near-infrared (NIR, 900–1600 nm) due to their electronic band-gap between valence and conduction band [49]. The density of electronic states (i.e. the normalized number of possible electron states) of semiconducting carbon nanotubes is shown in Fig. 2a. Due to quantum confinement van-Hove singularities appear in the density of electronic states function. Light can be absorbed if the wavelength matches the energy of an allowed transition between valence and conduction band. In particular, fluorescence takes place by transitions from the conduction to the valence band. The most important transitions are the E_{22} -absorption ($v_2 \rightarrow c_2$) and the E_{11} fluorescence emission ($c_1 \rightarrow v_1$). The diagram in Fig. 2a resembles Jablonski-diagrams, which are typically used to illustrate how electrons are excited in organic fluorophores during photoluminescence events. However, the nature of the excited state in semiconducting SWCNTs is excitonic rather than electronic. An exciton is an electron/hole pair with a certain coulombic interaction. In carbon nanotubes this coulombic interaction energy is in the order of 400 meV [50] with an exciton size of around 2 nm for (6,5)-SWCNTs [51]. During their lifetime, excitons diffuse over distances on the order of 100–300 nm [52,53]. As excitons move along the carbon nanotube surface they can be influenced by perturbations of the chemical environment, which makes them highly interesting for sensor applications. Typical lifetimes of excitons in carbon nanotubes range from 10 to 100 ps, which means that this type of photoluminescence falls under the definition of fluorescence [54].

The band gap of semiconducting SWCNTs depends on the chiral index (n,m) . This relationship between chiral index/diameter and band gap is usually presented in Kataura plots and analytical equations are available [55]. Because of this relationship, fluorescence spectroscopy in combination with Raman spectroscopy was used to assign the different chiralities (n,m) to the corresponding spectral peaks [56]. This method is now a standard tool for SWCNT-sample characterization. An example of how carbon nanotube color changes with chiral index

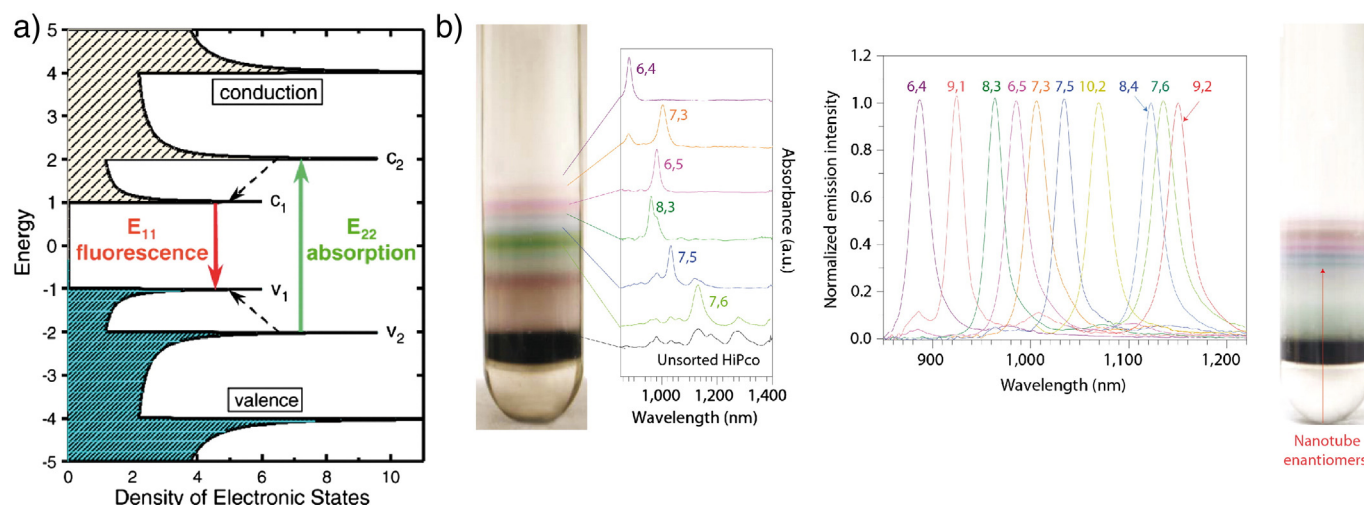


Fig. 2. Electronic structure and fluorescence spectra of semiconducting single-walled carbon nanotubes: a) Density of electronic states of a semiconducting SWCNT. The van-Hove-singularities arise due to quantum confinement. The most important transitions that are the reason for near-infrared fluorescence are depicted in red (E_{11}) and green (E_{22}). b) Sample with separated SWCNT of different chiralities and corresponding absorption and fluorescence spectra. Reproduced with permission from [42] and [56].

(n,m) is shown in Fig. 2b [42]. In this case, density gradient centrifugation was used to isolate different chiralities. The colors arise due to the absorption (E_{22}) of light in the visible range. The E_{11} transitions are shown in the respective NIR fluorescence spectra. This chirality dependence of the optical transitions holds great promise for sensor multiplexing.

An additional and very useful optical property of SWCNTs is their resonance enhanced Raman signatures [41]. The different vibrational modes of the carbon nanotube lattice give rise to several intense bands like radial breathing modes (RBM), G and G'-bands. What makes Raman spectroscopy of carbon nanotubes so powerful is the extremely high Raman scattering cross-section of SWCNTs, as well as the resonance enhancement of SWCNT Raman signals near their optical transitions [41].

2.3. Surface functionalization

The surface of carbon nanotubes is functionalized for mainly two purposes: First, to solubilize/isolate them and second to render them biocompatible or functional for a specific application. When an analyte approaches a carbon nanotube based sensor it interacts with the molecules surrounding the carbon nanotube. This direct chemical environment of a nanoscaled object is called the corona. It determines important sensor properties like toxicity or selectivity [57].

Surface chemistry of carbon nanotubes is a very broad field. We will give a short overview and refer the reader to more extensive reviews on the specific topic of carbon nanotube surface chemistry [58–60]. Surface functionalization is one of the most important steps for biosensors in general but it is especially true for carbon nanotube based sensors. In general, carbon nanotubes aggregate due to hydrophobic interactions and are not soluble in aqueous solvents and likewise in biological environments. Hence, functionalization of the carbon nanotube surface is necessary to disperse the carbon nanotubes. Many basic studies on physical and chemical properties used surfactants like sodium dodecyl sulfate (SDS) or sodium cholate (SC) to isolate and disperse carbon nanotubes [61]. However, such surfactants need a critical micelle concentration and are only stable in the presence of an excess of surfactant monomer, which implies that such surfactants are not suited for in vivo biosensor applications. In vitro measurements are possible with excess monomers but might affect the analyte or molecular recognition element e.g. due to denaturation of proteins under these conditions.

Therefore surface chemistry is not only necessary to disperse the carbon nanotube and render the sensor selective. Additionally, it affects how the sensor material interacts with its environment especially under physiologically relevant conditions [20]. Toxicity of carbon nanotubes is not completely understood so far and might be a risk for future in vivo applications. There are several excellent reviews on this topic [62–64]. So far toxicological assessment is very difficult because the used carbon nanomaterials are very distinct with respect to their structure, chemical modification and how the material is employed in toxicological assays. However, there is increasing evidence that surface functionalization decreases toxicity and long and bundled carbon nanotubes increase toxicity [65].

Carbon nanotubes can be modified by covalent- or non-covalent surface functionalization. Table 2 shows an overview of different functionalization strategies and review articles from the literature that can be consulted for details. Covalent functionalization has the advantage that it is supposed to be more stable and can be easily used for site-directed immobilization of biological recognition units. It can take place at more reactive regions of the carbon nanotube like ends or defect sites or directly at the sidewalls. However, covalent modification of the carbon nanotubes introduces a new sigma-bond and changes the

Table 2
Common carbon nanotube functionalization methods.

Method	Examples	
Covalent modification	A comprehensive overview can be found in [58–60,185]	t2.4
Oxidation	Oxygen-containing groups are introduced via reactions in oxidizing acids (e.g. H_2SO_4/H_2O_2), with O_3 or plasma-treatment	t2.5
Halogenation	Reaction with fluorine gas or with oxidizing acids like peroxytrifluoroacetic acid	t2.6
Radical reactions	Aryldiazonium salt reactions, silylation + UV light	t2.7
Cycloaddition	Diels–Alder addition, 1,3-cycloaddition of azomethine ylides	t2.8
Nucleophile addition	Reactions with organolithium or organomagnesium compounds	t2.9
Electrophile addition	Alkylhalide addition, Friedel–Crafts acylation	t2.10
Non-covalent modification	A comprehensive overview can be found in [58,59,186,187]	t2.11
Polymer-wrapping	DNA, chitosan, polyvinyl alcohol (PVA), poly(acrylic acid) (PAA)	t2.12
Lipid-grafting	Encapsulation by amphoteric molecules like modified phospholipids	t2.13
Grafting of aromatic compounds	Grafting of compounds with aromatic moieties e.g. pyrene-groups	t2.14

Table 3

Overview of the most important sensing mechanisms of carbon nanotube based biosensors.

Transduction mechanism	Explanation	Example
Electrical/FET	Binding of the analyte changes the dielectric environment/charge carriers of the carbon nanotube and transduces this event via several discussed mechanisms [188].	Monitoring the exocytosis of biomolecules from living cells on a carbon nanotube network [189]
Electrochemical	Redox-reaction (electron transfer) of the analyte on the carbon nanotube surface.	Dopamine detection with carbon nanotube coated carbon fibers [190]
Fluorescence	Change of the fluorescence spectrum due to the interaction between analyte and SWCNT/wrapping (intensity or wavelength shift).	Selective real-time detection of nitric oxide (NO) in living cells [74]. SWCNTs on gold surfaces as a molecular ruler and to investigate exocytosis [191].
Quenching	Proximity quenching of other (organic) fluorophores.	Detection of complementary DNA-sequences [192].
Raman scattering	Carbon nanotubes are used as a tag that exhibits extremely high Raman scattering.	Disease marker detection from human serum [69]

carbon hybridization from sp^2 to sp^3 , which can change electronical and optical properties. For example, covalent modification of semiconducting SWCNTs can change light absorption and reduce band gap fluorescence [66,67].

Non-covalent functionalization does not change the covalent backbone of the carbon nanotube and preserves desired electronical and optical properties. Binding between the molecules and the carbon surface is mediated by hydrophobic interactions or π - π stacking. Several approaches are based on biomolecules like DNA, which might increase the biocompatibility (see Table 2) [68].

Sensitivity and specificity of sensors are governed by surface chemistry. Most SWCNT-biosensors make use of molecular recognition elements that are borrowed from nature such as antibodies or lectins in order to achieve specificity and selectivity [69,70]. These recognition elements can be covalently attached to the SWCNT-surface e.g. by using His-tagged antibodies that bind to a nitrilotriacetic acid (NTA)- Ni^{2+} complex on the SWCNT surface [71]. Other strategies involve direct wrapping of SWCNTs by artificial aptamers that specifically bind the analyte [72].

Although most sensors are based on high affinity interactions between recognition elements and analytes ($K_d < 1 \mu M$) new concepts like artificial noses underline the importance of low affinity interactions [73]. In such a sensor, not a single key-lock interaction is detected but an interaction (fingerprint) pattern of the analyte with distinguished sensor arrays.

Using known and available biological recognition units limits sensor design. A more general approach towards specificity is based on finding random polymers that provide specificity and selectivity. By pinning a polymer to a carbon nanotube, the conformation and the arrangement of functional groups is changed, thus changing the affinity to certain analytes. In a high-throughput screening such an approach was used to find a DNA-sequence (AT₁₅) that enables specific NO detection with fluorescent SWCNTs [74].

In summary, sensors are affected by the crowded environment of biological systems. The intrinsic properties of carbon nanotubes must be combined with chemical design of the biomolecular corona around and on the carbon nanotubes to render the sensor both sensitive and selective [57].

2.4. Sensing mechanism

The transduction of the molecular recognition event (sensing mechanism) is a central feature of every sensor. It determines and limits possible applications. For example, sensors with optical transduction mechanisms are better suited for in-vivo sensing because there is no need for a physical connection between molecular recognition element and the rest of the sensor device. Table 3 shows an overview over the most common signal transduction mechanisms in carbon nanotube based biosensors.

Most of the optical sensors that are described in this review article are based on the band-gap fluorescence of semiconducting SWCNTs. However, the mechanism governing fluorescence based SWCNT sensors is still a field of extensive research and the dominating mechanism might differ for every combination of analyte and surface chemistry. Table 4 gives an overview on several mechanisms that modulate the fluorescence of SWCNTs. The classification that is shown in Fig. 4 is not explicit in terms of the mechanism/terminology because exciton modulation is a mutual feature of several mechanisms. We used this classification to point to the various routes/molecules that modulate NIR fluorescence of SWCNT. In turn, these mechanisms are the basis of sensors if specificity and sensitivity can be tailored. It is noteworthy that not all of these mechanisms were so far used for sensors. So far many studies aimed to increase the fluorescence intensity of SWCNT by adding reducing agents or applying well-defined oxidation procedures [75–77]. Increasing the fluorescence intensity and understanding

Table 4

Mechanisms of fluorescence modulation in carbon nanotubes.

Sensing mechanism	Explanation	Example
Solvatochromism	The dielectric environment around the carbon nanotube affects polarizability and thus absorption and emission spectra. An analyte could change the dielectric environment directly, by replacing solvent molecules or by changing the conformation of the polymer-wrapping.	–Solvatochromic shifts of different SWCNT samples in various solvents [193,194].
Charge-transfer	Analyte orbitals overlap with SWCNT/wrapping orbitals. Electron transfer leads to a change of ground states or excited states, which affects the fluorescence spectrum via altered rates of exciton quenching.	–Quenching of SWCNT-fluorescence by NO [195], ferricyanide [16] or redox active dyes [150].
Doping and Redox-reactions	The analyte changes the number of defects of the carbon lattice and thus exciton decay routes.	–Brightening of SWCNT-fluorescence by reducing agents like dithiothreitol [77]. –Reversible quenching by protonation [52]. –Removal of oxygen by enzymatic oxidation increases fluorescence [76]. –Oxygen-doping shifts and modulates the NIR-emission spectrum [75].

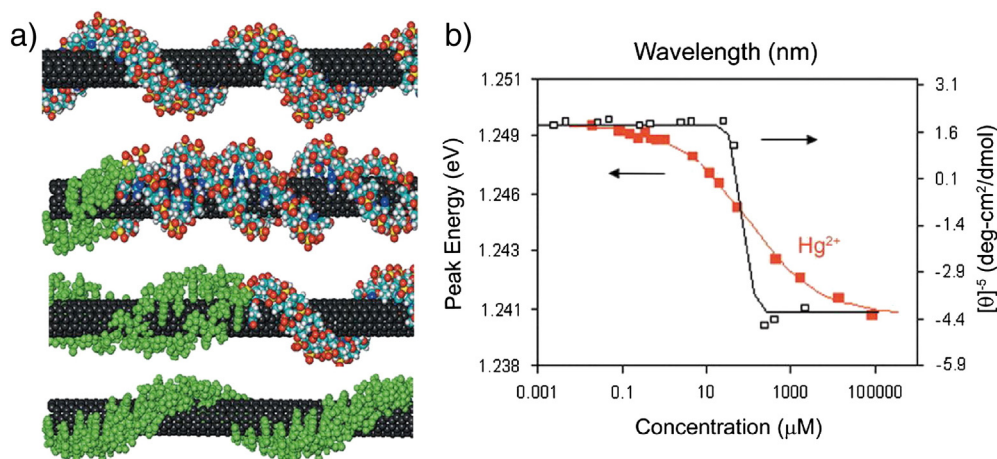


Fig. 3. SWCNT-DNA hybrid for detection of conformational polymorphism: a) Illustration of DNA undergoing a transition from the B (top) to Z (bottom) conformation on a carbon nanotube. b) DNA-SWCNT emission energy plotted versus Hg²⁺ concentration (red curve) and the ellipticity of the 285 nm peak obtained via circular dichroism measurements upon addition of mercuric chloride to the same oligonucleotide (black curve). Reproduced with permission from [17].

the underlying mechanisms are important goals by themselves but also pave the way for rational sensor design.

2.5. Advantages and disadvantages of carbon nanotube based optical sensors

Most of the advantages and disadvantages of a sensor material have to be discussed in the context of the signal transduction mechanism. Nevertheless, some general properties of carbon nanotubes are supposed to be beneficial for biosensor design. One general hope is that the one dimensional character of carbon nanotubes allows for ultrasensitive detection of analytes because all atoms are surface atoms and minor perturbations of the chemical environment might change electrical or optical properties dramatically. However, this argument has to be validated for every sensor. An obvious advantage of carbon nanotubes is the diversity of different chiralities. For semiconducting SWCNTs these different (n,m) structures can be used for optical multiplexing because every (n,m) SWCNT could be used as a single color [78]. This diversity of structures is also a drawback because it means that so far ensembles of different chiralities, length and impurities are used, which might decrease sensitivity and selectivity. The toxicity of carbon nanotubes is affected mainly

by its surface functionalization and length and is still under investigation [62–64]. For every application a separate toxicity assessment might be necessary and could delay or compromise in vivo applications. The lower fluorescence quantum yield (QY) is so far a drawback of SWCNTs compared to fluorophores with extremely high QY including quantum dots [79]. In organic solvents a bulk QY of up to 20% was reported by using an oxygen excluding surfactant. However, bulk measurements in aqueous solutions showed much smaller QY of <1% [80]. In contrast, QY of single SWCNTs in aqueous solutions of up to 30% was recently reported [77,81]. In the latter study by Lee et al. the QY was increased by adding a reducing agent [77]. It can be envisioned that by improved synthesis methods the QY of SWCNTs can be further increased.

SWCNT-fluorescence based sensors have several advantages. The most obvious advantage is that the NIR emission range of the SWCNTs coincides with the tissue transparency window [82–84]. In vivo applications can benefit from this property. Moreover, the SWCNT-fluorescence exhibits no blinking, no bleaching and a large Stokes shift [49,56,85]. These beneficial photophysical properties make fluorescent SWCNTs ideal candidates for long term sensing applications. In this context, regeneration/reversibility of the sensor response becomes an important issue. So far, most fluorescent SWCNT sensors were used in vitro and in

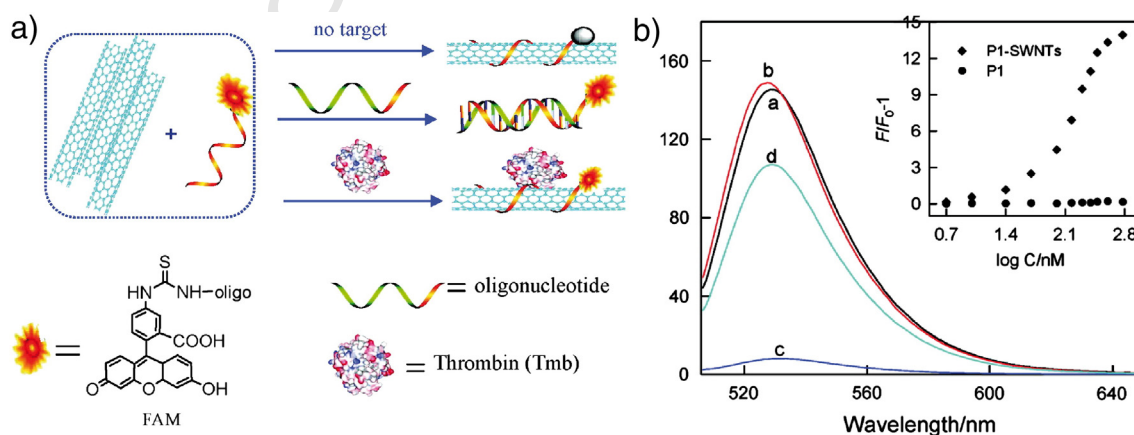


Fig. 4. DNA-sequence detection by carbon nanotube proximity quenching: a) Schematic of the detection mechanism. A fluorophore labeled DNA sequence wraps the carbon nanotube. If the complementary DNA sequence is present in solution it binds to the strand and pushes the fluorophore away from the quenching carbon nanotube surface and fluorescence is recovered. Using a thrombin aptamer this mechanism can be used also for thrombin detection. b) Fluorescence spectra of the labeled DNA sequence (a), with complementary sequence (b), with SWCNTs but without complementary sequence (c) and with SWCNTs + complementary sequence. The inset shows the concentration dependent calibration curve of the fluorescence quenching by the complementary DNA-sequence. Reproduced with permission from [98].

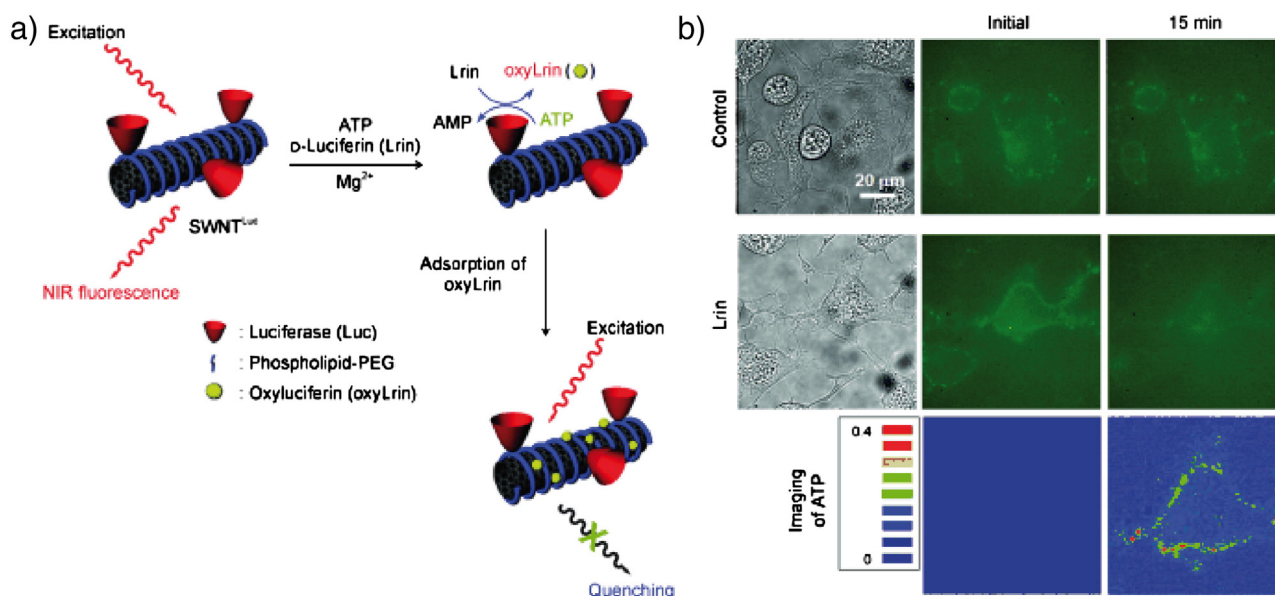


Fig. 5. SWCNT/luciferase hybrid for cellular ATP detection: a) SWCNT-based sensor that is able to selectively recognize ATP against other interfering molecules such as AMP, AMP, CTP and GTP, which allows to spatiotemporally detect ATP in living cells. The oxidized production of D-luciferin, oxyluciferin, quenches the NIR fluorescence of SWCNT. b) NIR fluorescence images of HeLa cells containing SWCNT^{Luc} sensor (2 µg/mL) with and without addition of Lrin. The NIR fluorescence in HeLa cells is quenched after addition of Lrin (240 µM), which is correlated with the ATP concentration.

Reproduced with permission from [184].

solution, which makes regeneration more difficult [16,17]. Nevertheless, other sensors were immobilized onto surfaces, which enabled washing and regeneration [71,74]. Additionally, the affinities between analyte and recognition element were not too high to remove the analyte by washing or dilution. Some sensors for reactive oxygen species were even able to resolve single-molecule adsorption and desorption events, thus proving reversibility and regeneration on the smallest possible scale [74,86]. For future in vivo applications, additional general processes such as unspecific protein adsorption (biofouling), degradation or endocytosis have to be taken into account because they can compromise sensor regeneration.

3. DNA detection

The ability to detect changes in DNA sequence and structure may prove useful for applications in personalized medicine [87]. For example, single nucleotide polymorphisms (SNPs) are one of the most common types of genetic mutation, and can lead to a variety of autoimmune diseases [88], or the development of cancer [89,90], among others. It has been demonstrated that DNA-wrapped SWCNTs can directly detect SNPs by examining hybridization kinetics between the SWCNT-bound sequence, and a complementary sequence in solution. Because of the nanoscale dimensions of the SWCNT sensors, this could enable the screening of high frequency single-nucleotide polymorphisms in a high throughput manner.

3.1. DNA hybridization

Some early SWCNT-based optical sensors demonstrated the ability to detect DNA hybridization [91,92]. In this study, the exposure of single-stranded DNA-wrapped SWCNTs to a complementary 24-mer oligonucleotide resulted in a hypsochromic shift of 2 meV, allowing the detection of a complementary DNA strand down to concentrations of 6 nM. A model, based on the local dielectric environment, demonstrated that this shift could be attributed to an increased coverage of DNA on the SWCNT surface. The attribution of this shift to DNA hybridization was further validated by a FRET assay, in which a SWCNT-bound DNA strand and complement were conjugated with donor and acceptor fluorophores, respectively; upon addition of the complementary strand,

a decrease in the donor emission was observed in conjunction with an increase in the acceptor emission. As may be expected, while hybridization kinetics of the free (non-SWCNT bound) DNA strands were very fast – occurring in less than 10 min – the kinetics of the SWCNT-bound DNA strand were considerably slower, reaching completion only after 13 h at 25 °C. A detection limit of 6 nM was observed, which is comparable with electrical microarray techniques [93], but higher than more recently techniques that utilize surface initiated enzymatic polymerization [94].

3.2. Single nucleotide polymorphism (SNPs)

Because the nanotube emission undergoes solvatochromic shifts upon DNA hybridization, it has been possible to utilize DNA-wrapped SWCNT as optical sensors for detecting single nucleotide polymorphisms [95]. In this study, SWCNT was suspended in a ssDNA sequence, and exposed to either a complementary DNA sequence, or a sequence containing a SNP. Using this system, the authors demonstrated the ability to detect a SNP in a complementary strand by comparing the wavelength shift, upon hybridization, of the SNP to that of the cDNA. Here, it was found that the final, equilibrium wavelength shifts were influenced by the presence of a SNP. In addition, while the equilibrium binding constants were the same for both complementary and SNP strands, the hybridization kinetics were faster for cDNA. As with previous hybridization studies, the kinetics of hybridization for SWCNT-immobilized DNA were very slow, and in order to detect SNPs in this study, it was necessary to allow incubation times of up to 24 h. Therefore, current SWCNT-based methods for the detection of SNPs are much slower than molecular beacon based approaches, in which incubation times of 30 min are sufficient [96]. It may be possible to decrease the incubation time required for SWCNT-based SNP sensors through further design of the SWCNT–DNA interface.

3.3. Conformational polymorphism

Heller and co-workers were able to use the wavelength shift of SWCNTs to detect the Hg^{2+} -induced transition of DNA between B and Z conformations (Fig. 3) [17]. In this work, GT_{15} -suspended SWCNTs underwent a red-shift of up to 15 meV upon exposure to Hg^{2+} ions.

In contrast to the previous hybridization work, in which the presence of SWCNT hindered the hybridization kinetics, plots of polymorphic transition of free-DNA and SWCNT-bound DNA, as evaluated by circular dichroism and shift in emission energy, respectively, showed nearly identical inflection points, indicating little difference in the energy required for the polymorphic transition on, or off, the nanotube surface. Using this platform, it was possible to measure conformational changes in complex environments, including whole blood, tissue, and within mammalian cells.

3.4. DNA-sequence detection

Carbon nanotubes can quench organic fluorophores in solution [97]. This mechanism is distance dependent and can be used for biosensing applications. This principle is heavily used for graphene biosensors [3] but there are several examples with carbon nanotubes as well. Yang et al. used this approach to detect complementary DNA sequences and proteins from solution [98]. They prepared dye-labeled oligonucleotide sequences that could interact with complementary sequences or as aptamers with the protein thrombin (Fig. 4). The dye-labeled DNA-sequence was adsorbed onto the SWCNT-surface. If a complementary DNA-sequence was present in the sample competitive binding took place. The fluorescence of the dye was recovered because this binding event released the primary DNA sequence or at least changed its proximity to the quenching carbon nanotube species. This approach was used to detect oligonucleotide-sequences in solution with detection limits in the nanomolar range.

4. Small molecule detection

4.1. Adenosine 5'-triphosphate (ATP)

Adenosine 5'-triphosphate (ATP) is used as a universal energy storage molecule in all organisms. Therefore, the ability to spatially detect ATP is useful for understanding a variety of cellular processes, ranging from ion-channel regulation [99] to intercellular signaling cascades [100]. To date, several approaches for ATP detection have been described, either based on electrochemical [101] or spectroscopic methods including bioluminescence [102–106], fluorescence [107–114],

color [115,116], and Raman spectroscopy [117,118]. Bioluminescence based on ATP-dependent luciferase–luciferin reaction [102,119] has been the most commonly used method since 1947, when McElroy [120] was the first to apply this reaction to determine ATP level.

Kim et al. were able to utilize SWCNT fluorescence for the detection of ATP by conjugating luciferase enzyme to polymer-coated nanotubes (Fig. 5a). Upon exposing the luciferase–SWCNT constructs to ATP in the presence of D-luciferin, the SWCNT-conjugated luciferase converts ATP to AMP, while D-luciferin is simultaneously oxidized to oxyluciferin. The oxyluciferin then quenches the nanotube fluorescence, providing an indirect indicator of ATP concentration. Using this platform, a detection limit of 240 nM of ATP was achieved. While commercial assay kits can achieve a lower detection limit (0.01 nM from Roche, 1 nM from BioVision), SWCNT-based sensors offer the benefit of high photostability, as well as low autofluorescence. Further, because the luciferase enzyme imparts selectivity to the sensor, the construct retained selectivity to ATP in the presence of potentially interfering molecules, including AMP, ADP, CTP, and GTP. Using this platform, it was possible to spatially resolve ATP concentrations within living cells by monitoring the extent of fluorescence quenching from spatially distributed SWCNT-luciferase constructs which had been uptaken by HeLa cells (Fig. 5b).

4.2. Reactive oxygen/nitrogen species detection

SWCNT fluorescence-based sensing is particularly advantageous for the detection of reactive oxygen/nitrogen species (ROS/RNS). Unlike other molecules, ROS and RNS products can be localized to specific organelles in the cell in response to a cellular event; due to their high reactivity towards O₂ and other cellular chemicals, the ROS/RNS rapidly decays as a function of distance from the production center. As a result, even if local concentration at the center of production is very high, the average concentration in the cellular media could potentially be negligible. Unlike conventional fluorophores, which are highly diffusive, SWCNTs can easily be immobilized to a substrate. Therefore, along with their high sensitivity at single-molecule level [121–124], SWCNTs are particularly advantageous in tracking local RNS/ROS burst events that are not tractable using conventional fluorophores.

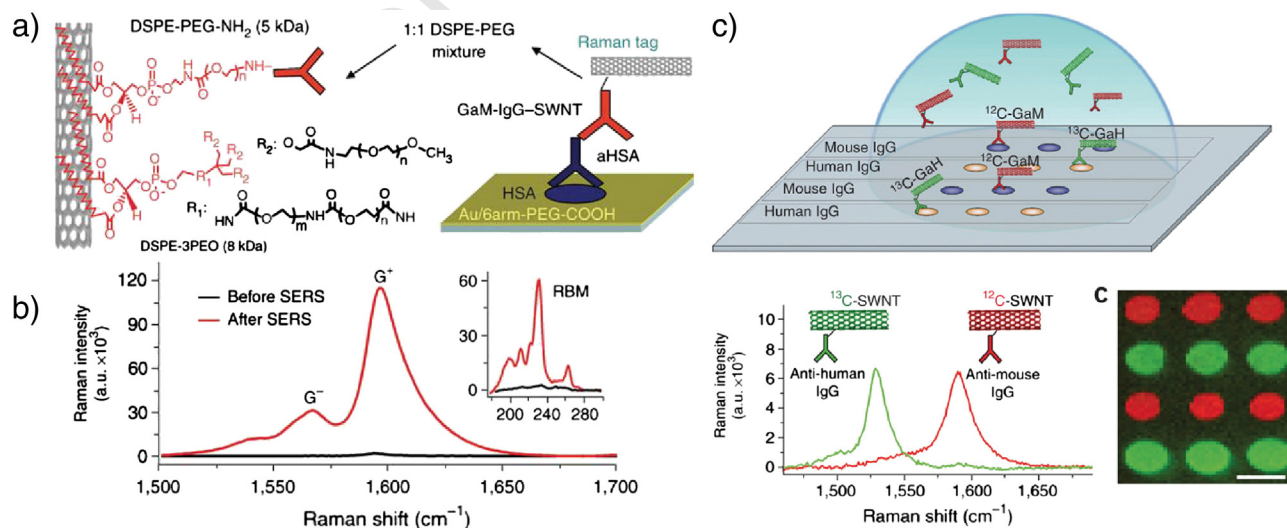


Fig. 6. SWCNTs as Raman tags for protein detection: a) Schematic of the surface chemistry of SWCNT Raman tags and the sandwich assay used to detect antibodies/proteins. b) Example of the Raman spectra with/without the SWCNT Raman label. c) Multiplexing with different carbon isotopes. The representative Raman spectra of functionalized ¹²C- and ¹³C-SWCNTs with distinct G-mode features and a false-color protein array are shown. Reproduced with permission from [69].

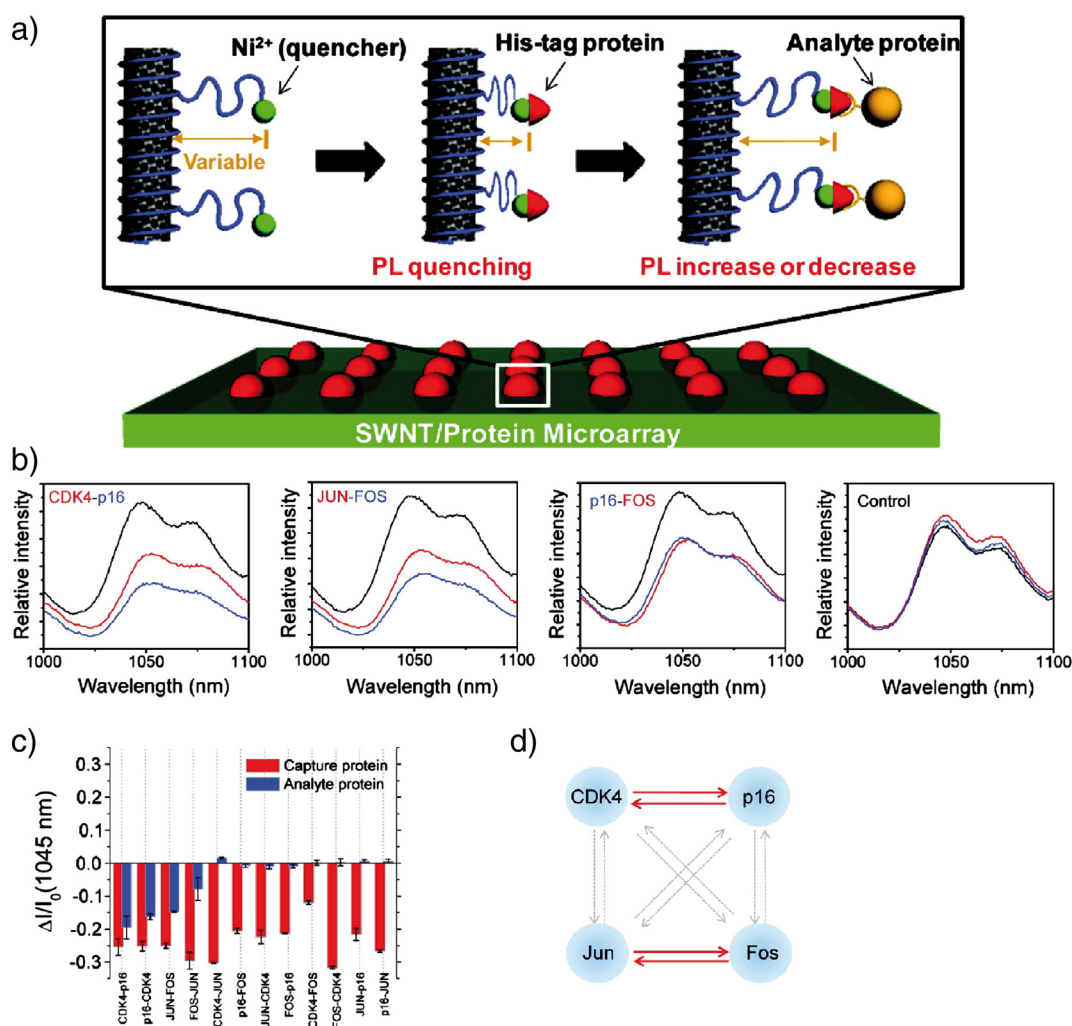


Fig. 7. SWCNT-based microarray for protein detection: a) Schematic of the SWCNT array and the signal transduction. NTA-bound Ni^{2+} ions act as proximity quenchers of the nanotube fluorescence. Upon binding of a his-tagged capture protein, the distance of the ion, from the SWCNT surface, decreases, resulting in a fluorescence quenching response. Upon binding of an analyte protein to the his-tagged capture protein, a further fluorescence modulation occurs, allowing for direct detection of the binding event. b) NIR fluorescence spectra of SWCNT/chitosan gels functionalized and probed with different proteins of interest. c) Fluorescence response map of tested protein–protein interactions. d) Schematic of the interaction network, generated by this approach. Red arrows indicate positive interactions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Reproduced with permission from [71].

4.3. H_2O_2 sensing

As the first demonstration of using SWCNT for detecting reactive oxygen species, Hong, et al. developed a sensing film containing type I collagen wrapped SWCNTs [121,122]. Each SWCNT in the film is an individually addressable optical sensor, and therefore the entire array is able to report spatial H_2O_2 production in real-time. As H_2O_2 adsorbs onto the SWCNT surface, a stepwise fluorescence quenching is observed. Using hidden Markov modeling, the authors identified an increase in forward (adsorption) rate constant as H_2O_2 concentration increases, while the reverse (desorption) rate constant remains independent of concentration. Applying the sensing array to quantify H_2O_2 production of cultured human carcinoma epidermal A432 cells, it was found that EGF produces 2 nmol H_2O_2 over a period of 50 min in A431 cells, but not in liver 3T3 cells. Using the array, a new mechanism was proposed, in which oxygen is first converted to superoxide by NAD (P)H oxidase, and then further converted to singlet oxygen through superoxide dismutase. The latter can form H_2O_2 upon binding of EGF to EGFR. This platform offers a quantitative way of monitoring cellular production at single-cell level in real-time for the first time.

4.4. NO sensing

Nitric oxide (NO) is involved in many biological functions in the body, including signaling, host defense, and vasodilation [125,126]. The concentration of NO and the location of production are crucial to its biological function [127,128]. The Strano group has developed the first SWCNT-based NO sensor [129]; 3,4-diaminophenyl-functionalized dextran (DAP-dex)-wrapped SWCNT was synthesized via five synthetic steps and the DAP-dex wrapped SWCNT sensor was prepared using subsequent dialysis in the presence of sodium cholate-suspended SWCNT. The DAP-dex wrapping imparts SWCNT with high selectivity for NO with a detection limit down to 70 nM. The sensor was able to detect endogenously produced NO inside macrophage cells with spatio-temporal resolution, demonstrating the first SWCNT-based NO measurements in a cell.

In a more recent study [123], it was found that SWCNTs wrapped in a particular DNA oligonucleotide, d(AT)₁₅, exhibit a selective optical response to NO, while SWCNTs suspended with eight other variant DNA sequences change fluorescence intensity upon exposure to analytes such as dopamine, NADH, L-ascorbic acid, and riboflavin.

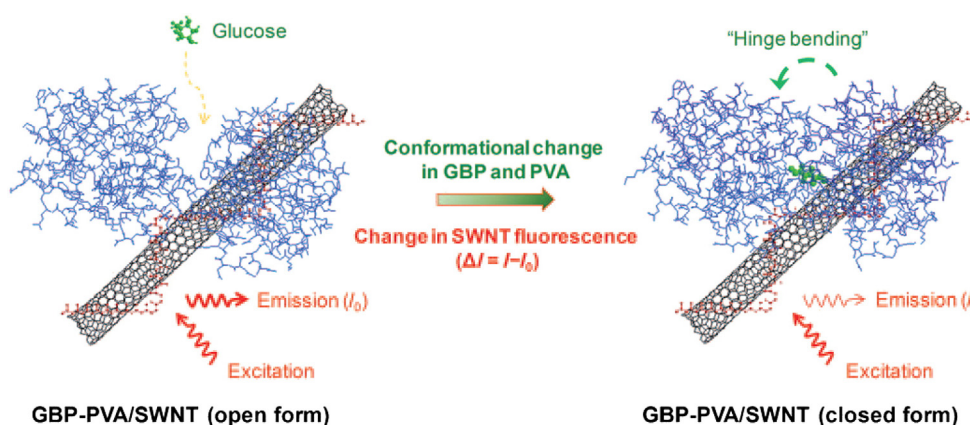


Fig. 8. General concept of an optical near-infrared glucose sensor based on SWCNTs functionalized with glucose binding proteins (GBP). Reproduced with permission from [161].

Stepwise fluorescence change was observed when the (AT)₁₅-SWCNT was exposed to NO, reporting single-molecule NO adsorption and desorption events. A birth-and-death Markov model was applied to describe the data and extract the kinetic information [130]. As expected, the adsorption rate was found to be proportional to the NO concentration, allowing for the quantification of local NO concentrations. The ability to detect NO at single-molecule level may find future applications in new cellular assays on disease diagnostics, molecular signaling, and detection of inflammatory signals.

5. Protein and biomarker detection

Protein biomarkers are specific proteins, either circulating in body fluid, or displayed on cell surfaces, which are indicative of diseased states. The research area of biomarker detection focuses on rapid, sensitive detection of known biomarkers in order to diagnose diseases in a timely and accurate fashion. Methods for biomarker detection can be divided into two separate categories: those, which are label-based, and those, which are label-free. Label-based methods require the labeling of query molecules with observable tags, such as fluorophores, radiolabels, or enzymes, which aid in the detection process [131]. Despite recent advances and widespread-usage, even in portable point of care devices [132,133], labeling assays have several limitations, the foremost being that they often require multiple application and washing steps, making them tedious, labor-intensive, and time consuming. Further, the intermediate washing steps, which aid in the reduction of non-specific background signal, intrinsically limit the usage of this technique to strong binding pairs. In addition to requiring multiple steps, the chemical labeling of the query protein adversely influences its native structure, thereby reducing its binding affinity, and if the labeling reaction occurs randomly on the protein surface, heterogeneous protein populations will result. Both of these can adversely affect test results [134]. Further, the development of antibody pairs for sandwich based assays can be difficult and expensive, limiting the widespread applicability of this approach [135].

As opposed to label-based methods, label-free methods allow for direct detection of a target analyte without the need of secondary antibodies or intermediate washing steps. This enables simplified detection of the target analyte while minimizing interference due to tagging molecules [136]. Label-free technologies include (1) field-effect transistors [137,138], (2) other electrical methods, including cyclic voltammetry [139] and impedimetric sensors [140,141], and (3) spectroscopy or fluorescence-based methods [142–144]. Many of these measurement techniques have employed a variety of nanomaterials in their detection scheme, including nanowires [137], nanoparticles [142], nanoclusters [145], microspheres [146], gold nanorods [147] and carbon nanotubes [141,148,149].

Optical biosensors can utilize the intrinsic Raman scattering properties of carbon nanotubes. Raman scattering of typical biomolecules is very weak. In contrast, SWCNTs are ideally suited as Raman labels because they provide characteristic spectral features and extremely high Raman scattering cross-sections. Chen et al. used carbon nanotubes as Raman labels for highly sensitive protein detection [69]. The SWCNTs were functionalized with IgG-antibodies and used as a label similar to organic fluorophores (Fig. 6a). Instead of using fluorescence microscopy Raman spectra were collected and the presence of the characteristic SWCNT Raman features indicated the binding event and the presence of the analyte (65b). In this particular case, a sandwich assay with a protein, a first antibody and the second antibody bound to the SWCNT-tag was used. The authors detected autoantibodies, a disease marker of Wegener's granulomatosis, with a detection limit in the femtomolar range. This approach provided up to 3 orders of magnitude higher sensitivity than with fluorophore-based methods, which can be attributed to the very low Raman scattering background. In contrast, the background of organic fluorophores is much higher due to the autofluorescence of many biomolecules. By using different carbon isotopes SWCNT-labels with shifted Raman features were generated and used for multiplexing (Fig. 6c).

Satishkumar et al. demonstrated avidin detection with nanomolar sensitivity by using fluorescent SWCNT sensors [150]. Their approach was based on redox-active dyes that were conjugated with recognition elements (biotin). The biotinylated dyes (anthracene and phenylazoaniline) adsorbed onto the SWCNT surface and quenched the fluorescence by a charge-transfer mechanism. When avidin bound to the biotin the quenching was disrupted and the fluorescence was recovered. This approach is not limited to avidin/biotin. Using a reversible charge-transfer process, which is changed by the analyte is a general principle and could be useful for many important analytes.

Ahn et al. employed carbon nanotubes as optical sensors for the label-free detection of protein–protein interactions [71]. In this platform, each nanotube is encased within a chitosan polymer wrapping, which has been modified with nitrilotriacetic acid (NTA) moieties (Fig. 7). Upon chelation of Ni²⁺, these NTA–Ni²⁺ complexes serve as proximity quenchers, modulating the intrinsic SWCNT fluorescence intensity as a function of distance from the SWCNT surface. The Ni²⁺–NTA group binds to any capture protein, which possesses a hexahistidine tag. Upon the subsequent binding of a target protein to the capture protein, the Ni²⁺–SWCNT distance changes, allowing the detection of protein binding events through changes in fluorescence intensity [71]. Thus far, the platform has been engineered to measure protein–protein interactions down to the single-protein level, as well as protein concentrations and protein glycoprofiles [151].

More recently, this platform has been extended to detect the cardiac biomarker troponin T (cTnT), with a detection limit of 100 ng/ml [152].

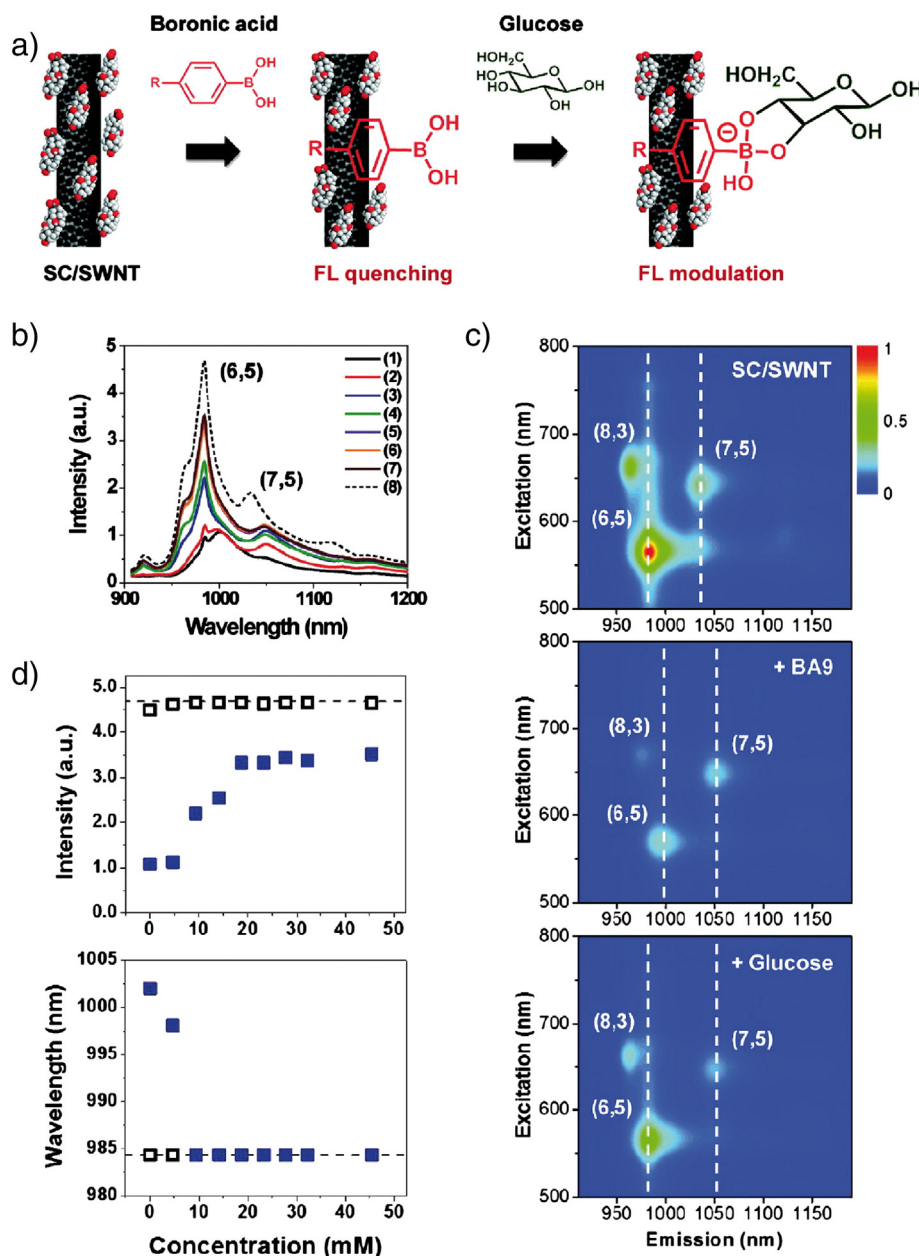


Fig. 9. Schematic of optical glucose detection with boronic acid functionalized carbon nanotubes: a) Phenyl boronic acid derivatives adsorb to SWCNT via its aromatic rings. Molecules with two adjacent OH-groups like glucose bind to the boronic acid moiety. This binding event induces a change of the SWCNT fluorescence and can be used to quantify glucose concentrations. b) Fluorescence spectra of a 4-cyanophenylboronic acid-SWCNT complex in the presence of glucose. The glucose concentration was increased from 0 mM (1) to 56.6 mM (7). c) Respective calibration curve of the peak intensities and wavelengths. Black open squares show the response of SWCNTs without boronic acids. d) Excitation maps of sodium cholate wrapped SWCNTs. Raw (top), with 50 mM 4-cyanophenylboronic acid (BA9) (middle) and BA9 + 50 mM glucose (bottom). Reproduced with permission from [173].

The SWCNT-based cTnT detection platform presents a higher detection limit in comparison with traditional ELISA-based methods (0.01–0.2 ng/ml) [153]. However, the SWCNT platform does not require a secondary antibody or washing processes during detection. Through further engineering to improve the detection limit of the fluorescent SWCNT sensor, this platform could potentially offer an alternative to current point-of-care diagnostics for acute myocardial infarction.

Insulin is a very important hormone regulating e.g. carbohydrate metabolism in our body and measuring insulin levels promises great potential in monitoring diabetes. Cha et al. wrapped SWCNTs in an insulin-binding aptamer [72]. They found that SWCNT-fluorescence was quenched upon insulin binding via a charge transfer mechanism. The detection limit was 10 nM, which would not be sufficient to measure insulin in the human blood but was sufficient to detect insulin in

a cellular system. When pancreatic INS-1 cells were stimulated with glucose quenching events were detected and correlated with insulin release. These proof of principle experiments showed insulin detection with SWCNT fluorescence sensors. For applications in diabetes care the sensitivity and possible unspecific responses in the blood/tissue have to be taken into account.

6. Glucose detection

Diabetes mellitus is a severe disease and continuous monitoring of glucose levels is critical for its treatment [154,155]. For this reason glucose detection has become a major task for biosensor development.

Although nanomaterials have aided in the sensitivity, efficiency and size of glucose monitors, electrochemical sensors still suffer from the

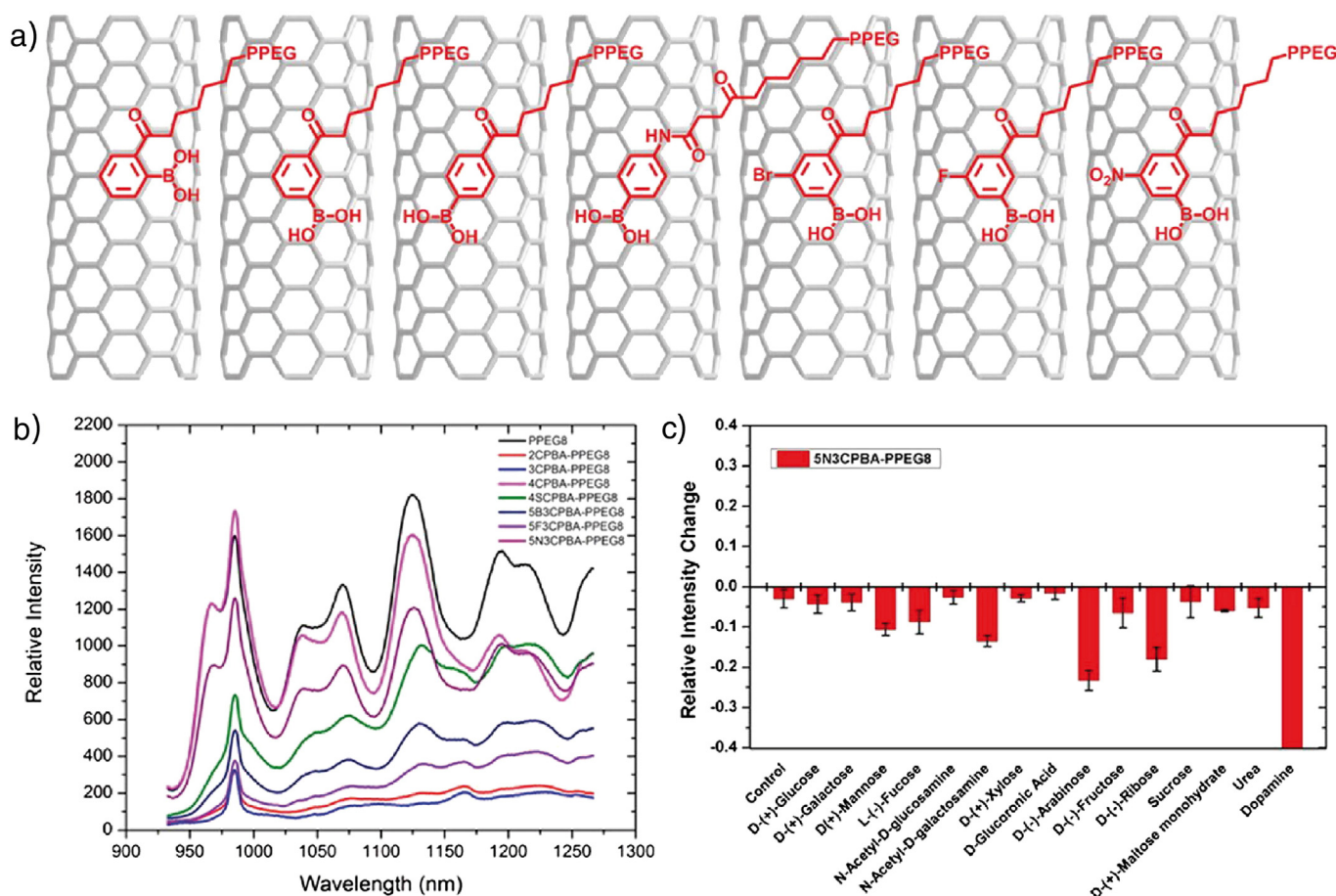


Fig. 10. Screening approach to identify the most promising carbon nanotube surface functionalization for selective glucose/saccharide detection: a) Structure of several branched poly(ethylene glycol) (PPEG) boronic acid derivatives that wrap carbon nanotubes. b) Optical modulation of polymer-SWCNT complexes in the NIR. c) Fluorescence modulation of 5N3CPBA-PPEG8 wrapped SWCNTs (last structure in (a)) by different saccharides and control molecules. Reproduced with permission from [174].

need for relatively invasive sampling means such as transcutaneous electrode placement or direct blood withdrawal. As such, a great deal of attention has been placed on developing a continuous and minimally invasive means for glucose detection. Fluorescent-based sensors have emerged as strong candidates to accomplish this minimally invasive detection. In particular, SWCNT have several advantages as fluorophores for biomedical applications such as high sensitivity [156–158], tissue transparency [159] and no photobleaching [160]. Resulting from these benefits, significant focus and progress have been made to derive a continuous implantable blood glucose module based on SWCNT fluorescence. By using this approach SWCNT wrapped in carboxylated polyvinyl chloride (cPVA) functionalized with glucose binding protein (GBP) could be used to monitor glucose concentrations in real-time [161]. GBP is a periplasmic binding protein characterized by two domains linked by a “hinge” region. It is known that, upon binding to glucose, the protein undergoes a conformational change in which the two domains close around the glucose molecule. It is believed that this actuation resulted in changes of the polymer-SWCNT interaction, ultimately causing a highly selective and reversible SWCNT fluorescent quenching proportional to the glucose concentration (Fig. 8). This fluorescent modulation was observed over long time periods (>60 h) with no observed degradation in signal intensity or sensor response. Furthermore, this was accomplished over the same physiologically relevant glucose concentrations as were observed for the electrochemical sensors outlined previously. In addition to the sensitive detection of glucose, this sensor formation was demonstrated as directly applicable

to an implantable microcapillary. It is likely that such a construct could be planted subcutaneously in order to achieve glucose detection [160].

Enzyme based electrochemical detection of glucose is limited by enzyme degradation [162]. Another approach is based on highly stable small such as boronic acids that are well known to bind saccharides through a diol-coupling reaction [163–167]. This interaction has been heavily investigated in the context of creating a glucose sensor [168–171]. Interestingly, fluorescent sensors for glucose based on boronic acid have been developed in the form of non-invasive contact lenses which monitor tear glucose concentrations [172]. However, potential problems such as photobleaching and low concentrations of tear sampling fluid still remain with such constructs. Recently, the application of phenylboronic acid derivatives as the glucose-binding moiety in a SWCNT based fluorescent glucose sensor has been investigated [173]. In this work, a library of boronic acids was developed. It was observed that all phenyl boronic acid derivatives investigated adhered strongly to the SWCNT surface via π - π stacking, resulting in a quenching of SWCNT fluorescence (Fig. 9a). The quenched complex was then subjected to glucose and the SWCNT fluorescent intensity and peak position were monitored (Fig. 9b–d). This screening experiment allowed for a few boronic acid candidates to be identified for potential inclusion in a boronic acid mediated SWCNT fluorescent glucose sensor. Interestingly, this included examples where glucose binding resulted in a strong increase in SWCNT fluorescence. It is believed that this observation results from a glucose mediated “lift-off” of the phenyl boronic acid

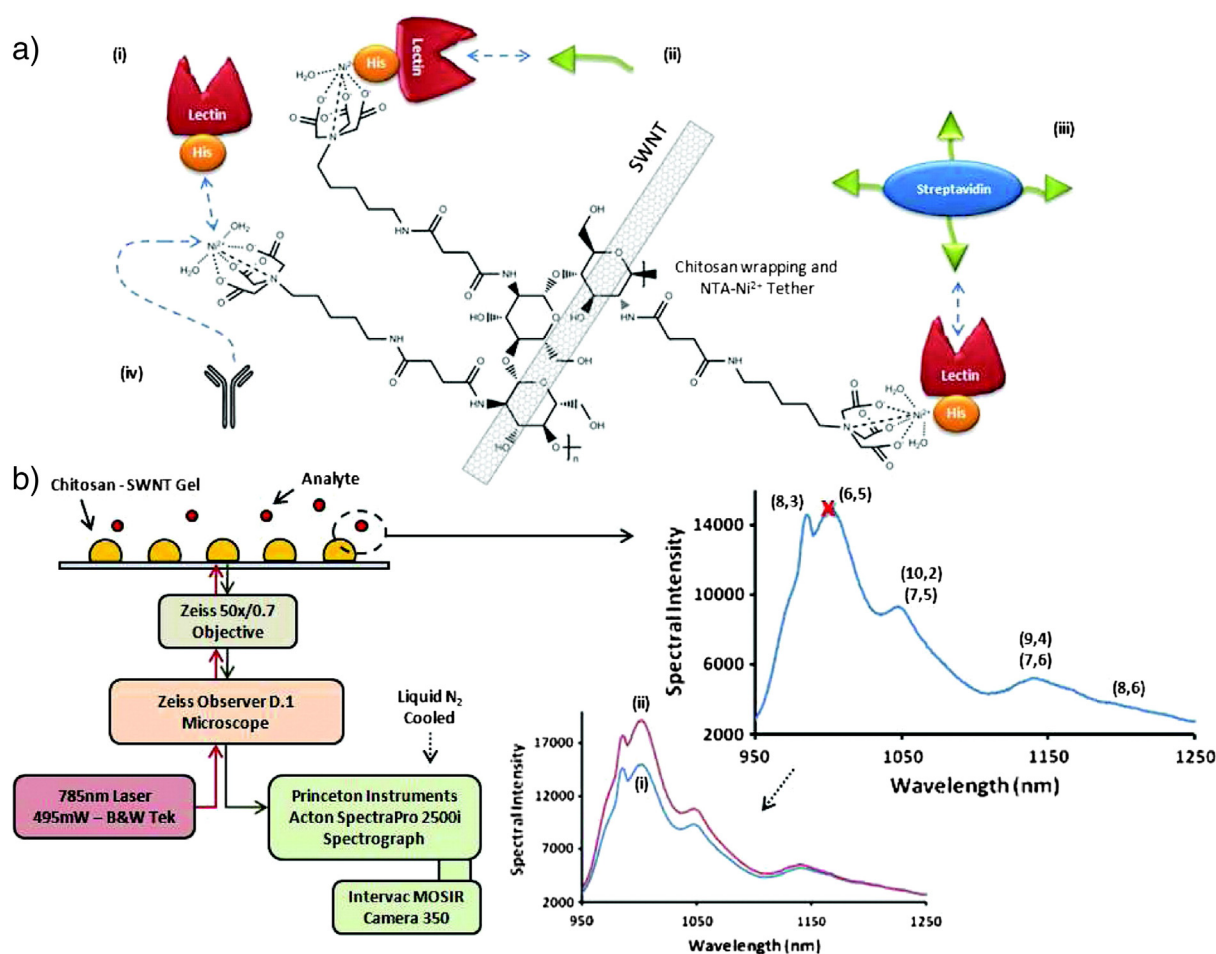


Fig. 11. Optical sensor for glycans based on carbon nanotubes: a) SWCNTs are wrapped in chitosan and functionalized with a chelated nickel ion, which acts as both the docking site for a His-tagged lectin and modulator of the SWCNT intensity. b) Nanotube gel is excited with a laser, and the SWCNT emission is collected via an inverted microscope. When a free glycan or glycoprotein interacts with the sensor, the signal is increased. Reproduced with permission from [157].

from the SWCNT surface, and thus a restoration of the SWCNT fluorescence. In addition, phenylboronic acid polymers have also been explored as dispersion agents of SWCNT for saccharide detection (Fig. 10) [174].

Both functions were demonstrated by using a homologous series of seven phenyl boronic acids conjugated to a polyethylene glycol, eight-member, branched polymer (PPEG8) that allows aqueous dispersion of single walled carbon nanotubes (SWCNTs) and quenching of the near infrared fluorescence in response to saccharide binding. A polymer library consisting of 2-carboxyphenylboronic acid (2CPBA), 3-carboxy (3CPBA), 4-carboxy (4CPBA), N-(4-phenylboronic)succinamic acid (4SCPBA), 5-bromo-3-carboxy (5B3CPBA), 3-carboxy-5-fluoro (5F3CPBA), and 3-carboxy-5-nitro (5N3CPBA) was investigated and a clear link between SWCNT photoluminescence quantum yield and boronic acid structure was observed. Moreover, saccharide recognition was demonstrated, including selectivity towards pentoses such as arabinose, ribose, and xylose to the exclusion of the expected fructose, which has a high selectivity on PBA due to the formation of a tridentate complex between fructose and PBA. It is envisioned that such sensors are integrated into “smart tattoos” and used for continuous in vivo glucose detection.

7. Glycoprofiling

Glycans, or surface carbohydrates, coat every cell and many proteins in the human body. They act as identification tags for differentiation, signaling, and transport. Thus glycans play a pivotal role in human health and disease. Glycans, unlike proteins and nucleic acids, are more

structurally diverse due to monosaccharide building blocks that have multiple, anomeric linkage sites. This gives rise to branched oligosaccharides that are difficult to characterize. The established procedure to profile glycoproteins is to enzymatically cleave and digest the glycans from the protein, label the released glycan pieces (optional), separate with chromatographic methods, analyze the separated glycan peaks with mass spectrometry, and reconstruct the probable glycan from the digested pieces [175]. The current state of the art is provided by Agilent Technologies that reduces the release, digestion and separation to an automated, microfluidic chip which then injects the separated glycan peaks into a MALDI-TOF spectrometer [176]. Although this simplifies and speeds up glycan analysis, such a capital intensive tool is prohibitive to many medicinal glycan analysis applications – such as point of care biomarker detection or on-line glycosylation monitoring during protein therapeutic production. A new sensing paradigm based on lectin detection [177–179], further augmented by quantitative nanosensors may provide the solution for these emerging applications. These nanoengineered platforms for glycoprofiling have been recently reviewed according to their method of signal transduction (mass, electrical, and optical changes) [180]. Maintaining the theme of this review, only the optical carbon based platform will be highlighted.

The Strano group has been developing an optical, carbon-nanotube based platform for glycoprofiling [70]. They recently demonstrated the concept with two glycan–lectin binding pairs (fucose (Fuc) to PA-III and N-acetylglucosamine (GlcNAc) to GaFD). The platform (Fig. 11a,b) consists of functionalized carbon nanotube sensors that were immobilized in a chitosan hydrogel. The fluorescent, semiconducting

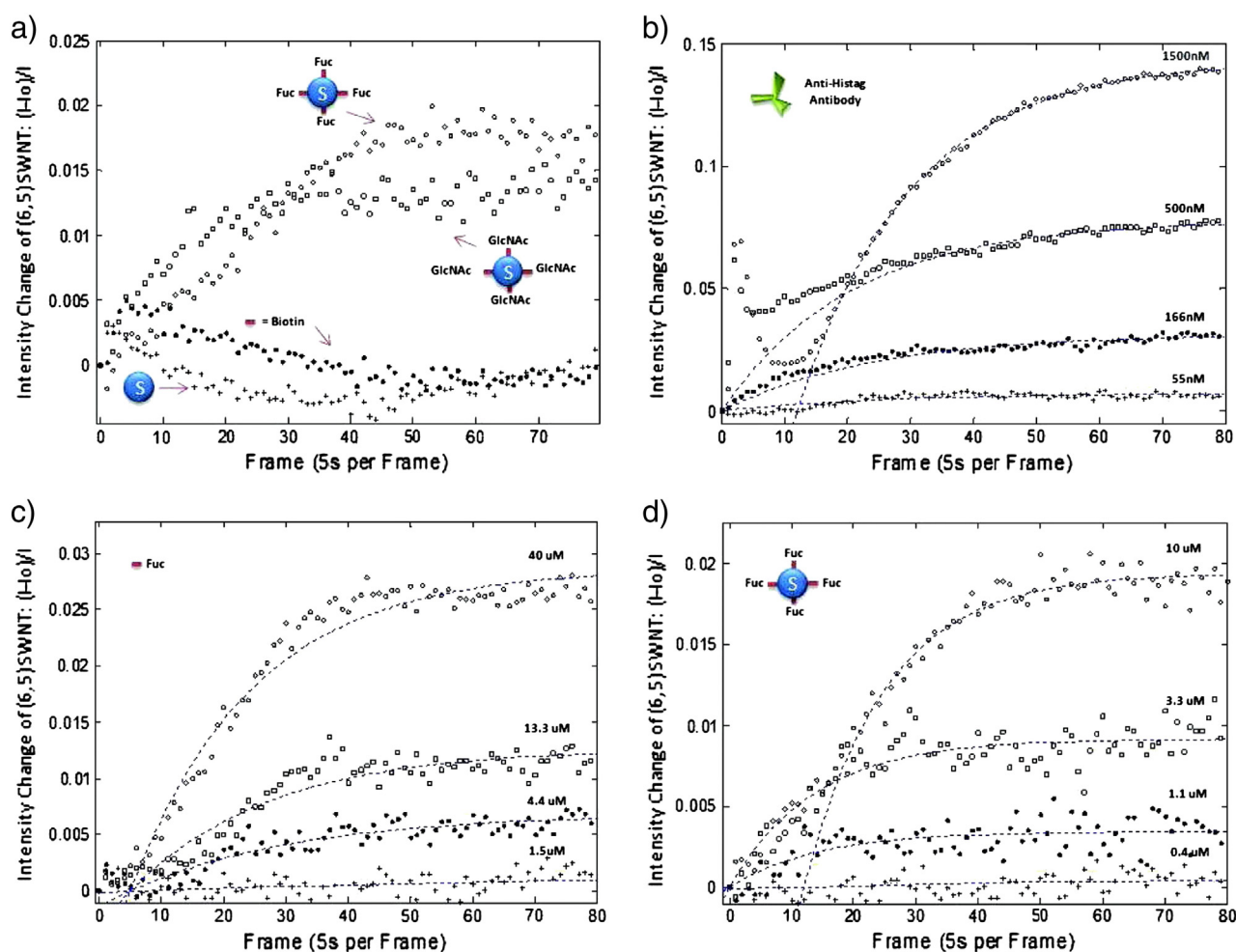


Fig. 12. Concentration dependent loading curves of various analytes to SWCNT–chitosan sensors: a) Control curves showing positive response of GlcNAc–strept to GafD and Fuc–strept to PA-III and null responses to biotin and blank streptavidin. Concentration dependent loading curves for (b) anti His-tag antibody to GafD lectin, (c) biotinylated Fuc to PA-III, and (d) Fuc–streptavidin to PA-III. The dotted lines denote the kinetic model fit and resulting parameters are reported in Table 1 of the publication. Reproduced with permission from [157].

nanotubes are wrapped in chitosan and the amine groups are used to tether a chelated nickel group. This divalent cation serves as both a docking site for the His-tagged capture lectin and as the signal modulator. The nickel initially quenches part of the nanotube fluorescence; when a glycan or glycoprotein binds to the lectin it displaces the nickel and the nanotube fluorescence returns. The ensemble response (integrated signal from many nanotubes in a single hydrogel spot (Fig. 12)) resembles the loading curve observed in surface plasmon resonance experiments (Biacore). These loading curves likewise can be fit by a kinetic model and forward and reverse kinetic rates are obtained as well as the dissociation constant. These sensors were also assayed on the single nanotube level and it was found that only a small sub-population of the sensors was responsive.

The optical, carbon nanotube glycoprofiling platform has many advantages and a few limitations. One advantage is the ease of fabrication. No electronic interfacing is necessary (the major limitation of FET sensors) and the functionalization can be completed on many chips simultaneously. The nanoscale sensor requires significantly less glycoprotein sample than macroscale assays. The nanotubes are independent reporters and as such can be patterned into a nanoscale, multiplexed array (multiple lectins can be probed with a single glycoprotein sample). One limitation of the current platform was the accessibility of the sensor to the analyte, as evidenced by the single sensor results. To make the sensors equally responsive, one would need to engineer an optimized hydrogel platform to allow equal access to all nanotubes

within the focal plane. Another limitation to the current setup is the quality of the nanotube sensors. This platform used a heterogeneous mix of nanotube chiralities that decreases the overall brightness and detectable sensitivity. This can be further improved with current advances in nanotube separations, tuning to a single chirality.

An optical carbon nanotube based platform could have many future applications in biomedicine. Multiplexed arrays of lectins have been utilized in the past decade to study disease biomarkers, such as aberrant glycosylation due to cancer, however these platforms were limited in their sensitivity and have found little practical application. Nanotube sensors could provide robust, quantitative results rather than relative brightness maps provided by the current tools. Kinetic measurements to multiple lectins could be used as a fingerprint for each glycan, and basic profiling could be accomplished. Although this platform will not have the exquisite detail of LC/MS techniques (i.e. reading core glycosylation pieces), it would be able to rapidly distinguish terminal glycan groups which are the dominant players in cell–cell and cell–protein carbohydrate interactions.

8. Conclusions and outlook

Carbon nanotubes are becoming a promising building block for biosensor technology. Although the first biosensors with integrated carbon nanotubes were electrical or FET sensors, in the past few years several examples of optical carbon nanotube based sensors were demonstrated. An optical signal transduction has intrinsic advantages

compared to electrical transduction such as contactless signal transduction or simple up scaling and multiplexing of surface assays. In this review article we reported on carbon nanotube optical sensors and the most recent examples of sensors based on NIR-fluorescence, Raman-scattering and SWCNT-mediated fluorophore quenching were discussed. A large body of work has been dedicated to detect biomolecules that are important for cell biology such as NO, H₂O₂, ATP or DNA. Apart from that, clinically relevant applications like glucose/glycane or disease marker detection have been demonstrated. From a methodological point of view single-molecule detection or multiplexing could be achieved by using carbon nanotubes as optical transducers.

In the next step, these concepts have to show their potential beyond scientific basic research [181]. A current trend in the biosensor field is point-of care applications, which are fueled by the broad availability of cell phones. Recently, a disposable device for surface plasmon resonance measurements based on cell phone optics was demonstrated [182]. In the next years carbon nanotube based sensors might be used for new sensor applications such as fingerprinting of complex samples, artificial noses, glycan-profiling or in vivo sensing.

Another field that could benefit from some of the advantages of carbon nanotube based optical sensors is integrated devices. Simultaneous capturing and profiling of circulating cancer cells (CTCs) from patient blood samples could be one such example. Moreover, the increasing evidence for heterogeneity of cell populations makes it necessary to develop sensors that can collect data of single cells instead of acquiring ensemble data.

In summary, optical carbon nanotube based sensors have shown their potential in several challenging biosensing applications. Some of the intrinsic carbon nanotube properties such as near-infrared fluorescence make them unique building blocks for biosensor devices and have advantages compared to other materials. Although several milestones have been achieved, ongoing efforts are necessary to engineer new biosensing devices for complex media like blood and to promote commercialization.

Acknowledgment

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