Biosensing with Fluorescent Carbon Nanotubes

Julia Ackermann\(^+\), Justus T. Metternich\(^+\), Svenja Herbertz, and Sebastian Kruss*
Biosensors are powerful tools for modern basic research and biomedical diagnostics. Their development requires substantial input from the chemical sciences. Sensors or probes with an optical readout, such as fluorescence, offer rapid, minimally invasive sensing of analytes with high spatial and temporal resolution. The near-infrared (NIR) region is beneficial because of the reduced background and scattering of biological samples (tissue transparency window) in this range. In this context, single-walled carbon nanotubes (SWCNTs) have emerged as versatile NIR fluorescent building blocks for biosensors. Here, we provide an overview of advances in SWCNT-based NIR fluorescent molecular sensors. We focus on chemical design strategies for diverse analytes and summarize insights into the photophysics and molecular recognition. Furthermore, different application areas are discussed—from chemical imaging of cellular systems and diagnostics to in vivo applications and perspectives for the future.

1. Introduction

Future challenges in medicine such as early disease detection, point-of-care diagnostics, and tailored therapies require novel methods of biosensing. Additionally, biosensors can provide insights into the complex dynamics of biological and chemical systems. Consequently, they are essential tools for both fundamental research and biomedicine. In particular, optical sensing approaches possess a great potential for contactless real-time readouts that are required in biomedical research, as well as industrial healthcare and agriculture applications.[1–3] During the last decade, the field of biosensors based on nanomaterials has seen vast improvements.[4,5] These materials include carbon-based nanomaterials such as graphene, graphene quantum dots, and carbon nanotubes (CNTs).[4,6–8] Here, single-walled carbon nanotubes (SWCNTs) are of particular interest. Their optoelectronic properties are sensitive to the surrounding environment, which makes them suitable for highly selective biosensing.[8–16] When dispersed in aqueous solutions, SWCNTs fluoresce without bleaching in the near-infrared region (NIR, around $\lambda = 870-2400$ nm).[17,18] This region of the electromagnetic spectrum is beneficial for detection and imaging as it offers an ultralow background and high penetration depths in biological tissues (tissue transparency window).[1,2,9–21] Fluorescence methods using common visible fluorophores often suffer from high scattering, absorption, and autofluorescence, which limits the penetration depth and signal to noise ratios.[1] Additionally, phototoxicity is increased by excitation of common fluorophores with visible (Vis) or ultraviolet (UV) light. Consequently, SWCNTs offer an advantage as they combine the biocompatibility and photostability required for optical sensing and imaging with emission in the NIR region.[14,22,23] Furthermore, the structural diversity of SWCNTs promises tunable emission wavelengths.[10,12] SWCNTs are highly sensitive to environmental changes, which is the basis for molecular recognition and was pioneered by optical sensors for glucose detection and DNA polymorphism.[24,25] Both covalent and noncovalent functionalization approaches play an essential role in tailoring molecular interactions close to the SWCNT surface.[10,14,17,23,26] By using such concepts, SWCNT-based biosensors for many highly important biomolecules have been developed.

More recently, this allowed chemical signaling to be mapped in a completely new manner, for example, release patterns of neurotransmitters from cells with high spatial and temporal resolution, which provides unique insights into fundamental biological questions.[27,28] Moreover, recent advances have been made in remote in vivo biosensing applications by the multimodal optical detection of several analytes. By combining multiple nanosensor elements and integrating them into functional arrays, analytes can be identified and distinguished on the basis of their characteristic image signatures.[29] Such a combination of optical nanosensors could pave the way for the next generation of fast and reliable in situ diagnostics. In addition, these approaches provide completely new opportunities for standoff process controlling, for example, fabrication of antibodies or monitoring in food and agriculture industries (smart plant sensors).[3,19,30–35]

In this Review we focus on optical biosensing with SWCNTs to give an update on this fast-evolving field. We evaluate in detail the specificity, sensitivity, spatial resolution, and biocompatibility of different SWCNT-based biosensors. This Review follows on from previous reviews,[2,10,14,23,34,35]
and discusses new chemical strategies developed in the last few years. SWCNTs can also serve as NIR labels. However, this is not discussed here and we refer to other excellent reviews.\cite{1,2}

In Section 2, the basic structural properties and photophysics of SWCNTs as well as functionalization strategies are described. To conclude this section, we touch on the most important aspects of SWCNT biocompatibility. Section 3 contains an overview of general chemical recognition strategies. We provide a detailed and up-to-date summary of all currently accessible biomolecular target groups, including reactive oxygen species (ROS), neurotransmitters, proteins, antibodies, lipids, and sugars. This overview is complemented with mechanistic insights into how these sensors work. Finally, we provide a perspective on the field (Sections 3 and 4) and discuss possible future directions. This includes novel biological topics such as plants, advanced chemical tools (defects), methods for improved (hyperspectral) imaging, novel screening approaches, and multiplexing.

2. Functionalization Concepts

Since the report of their structure, CNTs have attracted wide interest within the scientific community and beyond. Their remarkable mechanical, electrical, and photophysical properties have paved the way for applications in the fields of advanced materials, microelectronics, biosensing, imaging, drug delivery, and many more.\cite{8,36} Here, we will briefly describe the structure and photophysics of SWCNTs, followed by approaches to tailor their surface chemistry and biocompatibility.

2.1. SWCNT Structure and Photophysics

CNTs can be conceptualized as rolled-up cylinders of graphene.\cite{37} Their properties are determined by the exact sp²-hybridized carbon lattice as well as by the number of cylinders that are stacked into each other.\cite{37} CNTs derived from a single graphene cylinder are called single-walled carbon nanotubes (SWCNTs),\cite{38} whereas tubes consisting of multiple layers are called multiwalled carbon nanotubes (MWCNTs).\cite{7} SWCNTs are commonly labeled using the chiral index \((n,m)\), where \(n\) and \(m\) are integers that describe the carbon lattice structure (Figure 1a).\cite{37,39} In this notation, the SWCNT is conceptually rolled up along the vector \(c = n \mathbf{a}_1 + m \mathbf{a}_2\) (\(\mathbf{a}_1\) and \(\mathbf{a}_2\) are the graphene lattice vectors). Consequently, the roll-up vector also determines the diameter. For SWCNTs, the reported diameters range from 0.4 nm to 10 nm.\cite{8,40}

The roll-up vector affects the density and energy of the electronic states of SWCNTs and consequently the optoelectronic properties are directly related to chirality. As a result, for \(n−m = 0\) (armchair configuration), SWCNTs are metallic, for \(n−m = 3j\) (\(j \in \mathbb{N}\), \(\{0\}\)), semimetallic, and semiconducting for all other \((n,m)\) chiralities.\cite{10} When SWCNTs are excited with light, an electron–hole pair (exciton) can be created and diffuses along the SWCNT axis.\cite{41} For semiconducting SWCNTs,\cite{18} the absorption of photons with energies corre-
and colloidally stabilize single SWCNTs. The functionalization also serves the purpose to a) interact (specifically) with other molecules and b) translate this interaction into a fluorescence change.

In the past years, different covalent and noncovalent modification strategies (Figure 2) have been developed. For a complete overview, we refer the reader to several excellent reviews and discuss only concepts relevant for sensing here.\[26,51–53\]

On a more abstract level, two strategies to assemble selective SWCNT-based sensors have been used, namely screening and rational design (Figures 3 and 5). The first one relies on permutations of the organic corona around the SWCNT (e.g. deoxyribonucleic acid (DNA) sequence) whereas the second one uses known recognition motifs (e.g. antibodies).

### 2.2.1. Noncovalent Functionalization

Noncovalent functionalization in aqueous solution is achieved by sonication with surfactants that form micellar structures around the SWCNT or through strong π-π interactions with the SWCNT surface (Figure 2). Prominent examples of surfactants are sodium dodecylsulfonate (SDS), sodium dodecylbenzenesulfonate (SDBS), sodium cholate (SC), sodium deoxycholate (DOC), lithium dodecyl sulfate, Triton X-100, and pluronic F127.\[17,26\] Additionally, functional surfactants—for example, with a perylene core together with a hydrophilic dendron—adsorb through strong π-π stacking and enable energy transfer.\[54\] In general, a surfactant concentration above the critical micelle concentration is required to stabilize dispersed SWCNTs in solution.\[17\] Thus, these approaches are limited with regards to experiments in complex (biological) systems.

In contrast, functionalization with larger biopolymers enables the formation of stable conjugates. Here, DNA and ribonucleic acid (RNA) form strong π-stacking interactions between the nucleobases and the SWCNT surface, thereby exposing their negatively charged phosphate backbones and solvating the SWCNT–nucleic acid complex (Figure 2),\[22,53\]

As the conformation of the SWCNT–nucleic acid complex is affected by changes in the local ion concentration,\[25,56,57\] locked nucleic acids have been used as more rigid synthetic derivatives at higher salt concentrations.\[57\]

As alternative to nucleic acids,\[59\] certain polycyclic aromatic compounds carrying hydrophilic moieties have effectively solubilized SWCNTs. Similar to the π-stacking of those compounds, the functionalization of SWCNTs with peptides,\[60,61\] proteins,\[60,61\] and other polymers has been widely demonstrated (Figure 2). SWCNT-based biosensors have been rationally designed by the attachment of antibodies (or analogues; Figure 4a,b,d)\[64,65\] and peptides (Figure 4c)\[66,67\] to polymers or by the adsorption of boronic acids (Figure 4c)\[68\] and aptamers (Figure 4f) on SWCNTs.\[27\] In cases when sonication would destroy the structural integrity of the (bio-)polymers, primary suspension of the SWCNTs in a surfactant, followed by subsequent exchange to the polymer by dialysis has been employed.\[24,40,49\] An alternative to this rational design is the screening/search for

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**Figure 1.** Structure and properties of single-walled carbon nanotubes (SWCNTs). a) The structure of SWCNTs can be rationalized by rolling a sheet of graphene along its roll-up vector, for example, ε = 6α1 + 5α2. b) The Band gap structure gives rise to fluorescence emission in the near-infrared (NIR) region. c) The E2 transition of SWCNTs\[49\] overlaps with the tissue transparency window, thus offering the advantage of reduced light absorption,\[50\] scattering (e.g. Rayleigh), and background fluorescence. Here, the emission spectrum of SWCNTs of (6,5)-chirality is shown, but the emission wavelength for other chiralities span the whole NIR region.

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2.2. Surface Functionalization

The extended π-system makes SWCNTs hydrophobic and consequently they easily aggregate in solvents like water. Therefore, an important step in the preparation of SWCNT-based sensors is their functionalization to isolate, solubilize...
novel organic phases. This concept was named corona-phase molecular recognition (CoPhMoRe).

Here, a heteropolymer adsorbs onto the carbon nanotube surface and forms a new structure (corona) that serves as a molecular recognition site for interaction with an analyte. The biomolecules used are typically amphiphilic with hydrophobic domains that enable SWCNT adsorption and hydrophilic domains to be responsible for the entropic stabilization of the SWCNT in suspension and formation of a binding site for the analyte.

It is important to note that the biomolecules/polymers alone do not necessarily need to interact selectively with the analyte of interest. As such, the formation of these recognition sites cannot be predicted and are typically found by screening or high-throughput approaches. Prominent examples of CoPhMoRe screenings are the identification of SWCNT-based neurotransmitter sensors as well as the adaptation of the CoPhMoRe concept to proteins (Figure 5).

Figure 2. Covalent and noncovalent (bio)functionalization of SWCNTs. Note that only covalent approaches that preserve the NIR fluorescence are included and that double bonds in the SWCNT carbon lattice are not shown for clarity.

Figure 3. Chemical concepts for the design of SWCNT-based NIR fluorescent sensors. a) Screening of different organic phases identifies biopolymer/SWCNT conjugates with the desired analyte response. b) Rational concepts use known recognition motifs and assemble them on the SWCNT surface. Note that for both concepts colloidal stability in aqueous solution determines the usable reactions.
2.2.2. Covalent Functionalization

The covalent functionalization of SWCNTs introduces new o-bonds into the sp²-hybridized SWCNT structure. In contrast to noncovalent functionalization methods, the conjugates promise higher stability.[10] However, the uncontrolled introduction of covalent sp³ bonds (defects) destroys the electronic and optical properties and diminishes the intrinsic NIR fluorescence.[10] One strategy to overcome this problem preserves the sp²-hybridized structure of SWCNTs during their covalent functionalization.[70] In contrast, a certain number of sp³ defects give rise to novel properties, such as red-shifted emission features that are capable of single photon emission.[53,73–75] Therefore, these defects are also called quantum defects or quantum color centers.[53]

These sp³ defects have, at low densities, been shown to increase the fluorescence of SWCNTs.[53,74,75] Incorporation of these defects at low concentrations leads to the trapping of excitons and an alternative decay pathway (E₁₁*) that results in a new red-shifted fluorescence feature (Figure 6a).[74,75] A wide range of sp³ defects has been incorporated into SWCNTs to increase the fluorescent properties by using diazo ether, aryl halide, (bis-)diazonium, as well as Billup-Birch and alkyl halide reductions.[53] Additionally, O-doping approaches using...
ozone and light,
sodium hypochlorite,
as well as hydroperoxides of polyunsaturated fatty acids have been reported to increase the red-shifted emission of SWCNTs at low defect concentrations (Figure 2).

Covalent functionalization approaches also offer opportunities beyond changes in the photophysics. Defects that can be further functionalized enable the conjugation of important biomolecules. Recently, maleimide defects were used to link proteins such as nanobodies and phenylalanine defects to grow peptides directly on the SWCNT surface, similar to solid-phase peptide synthesis (Figure 6b). The covalent conjugation approach with dichlorotriazine allows subsequent nucleophilic aromatic substitution of the chlorides using amine-containing linkers (Figure 2). Another example are defects that are already able to interact with other biomolecules, such as phenyl boronic acids that interact with saccharides, and change the $E_{11}^*$ ($S_{11}^*$) and $E_{11}$ ($S_{11}$) emissions.

It is interesting to note that the resulting bathochromic shifts ($E_{11}^*$) caused by sp$^3$ defects can be tuned using the electronic properties of the incorporated moieties. That said, defects provide a rich chemical playground and interested readers are referred to several excellent reviews. With regards to SWCNTs with aryl defects, electron-withdrawing substituents generally introduce red-shifts to the $E_{11}^*$ emission that can be correlated to the Hammet constants ($\sigma$) of the substituents. Furthermore, the $E_{11}^*$ red-shift shows a $1/d^2$ dependence on the diameter ($d$) of the SWCNT. The protonation of diethylamino-substituted aryl defects ($\sigma_{HR2N} = +0.82$ vs. $\sigma_{R2N} = -0.66$) is a good example of the effect caused by the inductive effects of substituents. Furthermore, this type of defects allows a precise sensing of
Apart from the introduction of defects for sensing, the covalent functionalization of SWCNTs can be used for site assembly using different (bio)polymers and linkers. Defects also change the exciton decay pathways and, therefore, affect the photophysics and sensing mechanism of SWCNT-based sensors. This approach was recently used to perturb the sensing and elucidate the rate constants that are involved. In this study it was also found that a small number of defects can reverse the sensing response from a strong increase to a strong decrease in fluorescence.

2.3. SWCNT Biocompatibility

Biocompatibility is highly important for materials in direct contact to biological matter. Even though many biocompatibility studies exist, the conclusions are difficult to compare. The main reason is that different materials, surface reactions, and biological systems are compared, which leads to a noncoherent view. Moreover, the SWCNT field has evolved dramatically over the years and well-defined chirality pure SWCNTs-based sensors with ultrahigh purity are available today, whereas older studies used less well-defined materials. In addition, the application itself determines the stability functionalization is (Figure 6d).

As a consequence, it is fundamentally important to compare. Moreover, the SWCNT field has evolved dramatically over the years and well-defined chirality pure SWCNTs-based sensors with ultrahigh purity are available today, whereas older studies used less well-defined materials. In addition, the application itself determines the stability functionalization is (Figure 6d).

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For example, endocytosis experiments have shown that the DNA sequence length plays an important role in endocytosis and retention time scales of DNA-functionalized SWCNTs within mammalian cells. Additionally, experiments using a combination of NIR fluorescence spectroscopy and resonance Raman scattering have been used to analyze the fate of DNA-functionalized SWCNTs through the endosomal process. Based on the experimental findings, the authors propose that DNA-SWCNTs enter the cell, where they are transported into early endosomes. Maturation of the endosome begins with a decrease in the luminal pH value, which is followed by a series of physicochemical processes that transform the endosome into a lysosome, where the SWCNTs finally aggregate.

As correct functionalization has been shown to alleviate the pathogenicity of SWCNTs, stable functionalization is one possible way to safeguard the future design of SWCNT-based sensors in environments where long-term stability is of the highest importance. Adequately functionalized SWCNTs have been shown to possess excellent biocompatible properties. A good example is the recently published long-term biodistribution and compatibility assessment of DNA-encapsulated SWCNTs after intravenous administration in mice. After an initial increase in the SWCNT fluorescence in the liver, the SWCNT fluorescence decreased rapidly over the course of 14 days. The same trend is also seen in the long-term SWCNT biodistribution in different organs. By using hyperspectral microscopy, low levels of SWCNTs were detected in murine hearts, lungs, livers, kidney, and spleen tissues one month after injection. Assessment of these tissues after three and five months showed no SWCNT fluorescence in lung tissue, or in heart and lung tissues. Moreover, no abnormalities were found in chronically exposed tissues after hematoxylin and eosin (H&E) staining at all observed time points and the assessed biomarkers showed negligible changes up to four months, and minor changes after five months.

The aforementioned studies suggest remarkable opportunities for SWCNTs in biomedical applications. As a consequence of the interplay between different materials, surface reactions, and biological systems it becomes evident that biofunctionalized SWCNTs represent a class of different materials. As for all new materials, the biocompatibility should be evaluated for every type of chirality, purity, functionalization, and route of administration. The scientific community is well-aware of this problem and it has been pointed out that an assessment of these parameters in the context of biocompatibility depends on the context of the experiment, timescale, and the application of the nanomaterial. As a consequence, it is fundamentally important to place experimental data in the right context.

An important requirement for a biocompatible design of SWCNT-based sensors is an in-depth understanding of the composition of the protein corona in biological media. In this regard, a recent study characterized the enrichment of certain proteins in the SWCNT corona.

In the future, long-term studies comparing the biocompatibility of different SWCNT subclasses (purity, chirality, surface chemistry) would be desirable to safeguard the development of biocompatible sensors. A foundation for the standardization of protocols could be the MIRIBEL (Minimum Information Reporting in Bio-Nano Experimental Literature) reporting standard. As the functionalization of the SWCNT plays a critical role in the biocompatibility of SWCNT-based sensors, the design of stable SWCNT functionalizations needs to be carefully ensured for long-term applications. In particular, the recent advances in covalent functionalization strategies might, therefore, offer interesting starting points for future development.

3. SWCNT-Based Sensors

3.1. Development of Chemical Design Strategies

The discovery of band gap fluorescence from SWCNTs and their structure-dependent NIR emission wavelength marks the starting point for SWCNT-based sensors. Given the high surface to volume ratio of SWCNTs, it was quickly anticipated that SWCNT fluorescence would be sensitive to the chemical environment. The first generation
of sensors targeted mainly smaller molecules including protons and reactive oxygen/nitrogen species (ROS/RNS). In these cases, the fluorescence changes were most likely caused by direct quenching. At the same time, it was discovered that different surface reactions with biopolymers lead to molecular interactions that are surprisingly specific even without using a standard approach with antibodies. This idea was conceptualized as corona-phase molecular recognition (CoPhMoRe).[70] During the last few years, great progress has been made in the chemical design of sensors for both biomedical and environmental applications. In the next sections we will give an overview on different sensing strategies, organized according to the molecular target. Here, sensing of the target molecule has partly environmental as well as biomedical applications. We focus on the advances in the last few years but also report previous studies (see Tables 1–9 in the Appendix).

3.2. Biosensing of Target Analytes

The ongoing advances in the development of recognition strategies have led to powerful biosensors based on SWCNTs. Various targets can be detected with high selectivity and sensitivity by combining a recognition unit with the SWCNTs. Recognition strategies (Figure 3) can mainly be categorized into a screening (Figure 5) and a rational approach (Figure 4). The first approach is in principle achieved with a library of synthetic organic phases (coronas) consisting of different amphiphilic polymers wrapped around SWCNTs and screened against a panel of various analytes to find a selective interaction. [13,17,71,100,101] The latter approach is mostly applied for the detection of larger molecules such as protein or sugars by conjugating a known binding partner of the target analyte to the SWCNT surface. Several approaches are based on the use of SWCNTs wrapped by single-stranded DNA (ssDNA), whereby different lengths of the (GT) sequence is probably the most used sequence up to now.

It has shown its versatility in the detection of divalent ions,[25,109] genotoxins,[110] NOX,[10] H$_2$O$_2$,[20,32,110–112] riboflavin,[49,113,114] doxorubicin,[115] β-carotene,[116] endolysosomal lipids,[117] arsenite,[13] and neurotransmitters, especially dopamine.[15,49,63,80,87,118–121] This section highlights the major advances from the last few years for different categories of biomolecules. A detailed overview of most of the known fluorescent SWCNT-based sensors, subdivided into the target categories, can be found in Tables 1–9 in the Appendix.

3.2.1. ROS/RNS

ROS/RNS are important signaling molecules in many organisms,[122] but their detection is challenging because they diffuse fast and have short lifetimes due to their high reactivity with O$_2$ and other molecules.[10,123] Since the finding of the first NO sensor based on SWCNTs,[124] the performance of SWCNT-based ROS sensors has grown from the first selective detection of NO and H$_2$O$_2$ at the single molecule level,[13,125,126] and first in vivo applications[127] to a new approach to study NO generation and spatiotemporal imaging of intracellular NO signaling.[128] Recently, a mathematical model that calculated the NO concentration based on the change in the SWCNT fluorescence was derived.[128] This was previously not possible due to a nonlinear fluorescence quenching rate in response to NO.[13]

ROS play a mediating role in the cell-to-cell communication of plants to activate defence mechanisms[122] whereby it has become clear that H$_2$O$_2$ is the primary mediator that responds to different stresses in plants.[129] This has led to novel SWCNT sensor approaches to study ROS within plants.[19,20,31,32] Wu et al. demonstrated remote H$_2$O$_2$ monitoring of plant health with sensitivity in the plant physiological range by using fluorescent SWCNT-based sensors.[31] Their rational approach was based on a DNA aptamer that specifically binds to the porphyrin hemin (HeAptDNA-SWCNT). Hemin binds ferric ions, which undergo a Fenton-like reaction with H$_2$O$_2$ to produce hydroxyl radicals (Figure 7a) that directly quench the SWCNT fluorescence. For spatiotemporal in vivo monitoring, SWCNTs were embedded in leaves of plants and the plants exposed to different stresses such as UV-B, high light intensities, and a pathogen-associated peptide (flg22; Figure 7b). The decrease in fluorescence reported remotely different aspects of the stress. These differences in fluorescence intensity quenching offer the possibility to interpret stress patterns in plants.

Similar to this approach, Lew et al. developed a platform for H$_2$O$_2$ detection in leaves of different plant species[32] This sensor platform used a ratiometric approach, with (GT)$_2$$_2$ SWCNTs (G-SWNT) that respond to H$_2$O$_2$ by quenching, possibly as a result of a charge-transfer phenomenon, and (AT)$_2$$_2$ (6,5)-SWCNTs (A-SWNT) as an invariant reference (Figure 7c). They infiltrated both G-SWNT and A-SWNT into spinach leaves and monitored the H$_2$O$_2$ signal with a standoff detection platform in real time (Figure 7d). In the presence of different stresses, for example, tissue wounding, distinct waveform characteristics were observed (Figure 7e), whereby the wave speeds in different plant species postwounding differed in the range of 0.44 to 3.10 cm min$^{-1}$.

In the same manner, Lew et al. developed a SWCNT-based sensor system for the specific detection of arsenite in plants to monitor the uptake of the toxic heavy-metal pollutant arsenic by using a self-powered microfluidic system in real time.[13] For this purpose, they infiltrated the sensors and the invariant reference into leaves of spinach, rice plants, and hyperaccumulating fern, which is able to preconcentrate and extract arsenic from soil (Figure 8a). The intensity of the sensors increased steadily over several days, with the sensor response of the hyperaccumulating plant being significantly higher than those of the rice and spinach plants (Figure 8b). Based on a kinetic model, the arsenite concentration in the leaf and the limit of detection (LOD) were calculated to be 4.7 nm and 1.6 nm as a function of the root fresh weight and uptake solution volume after 7 and 14 days (Figure 8c). These examples show that SWCNT-based H$_2$O$_2$ sensors are able to report plant stress on a microscopic and macroscopic level with potential applications in smart agriculture.

Another macroscopic situation in which H$_2$O$_2$ plays an important role is wound healing. Safaee et al. developed
a wearable optical microfibrous material with encapsulated SWCNT-based sensors (Figure 8d) to monitor the \( \text{H}_2\text{O}_2 \) concentration in wounds\[^{123}\]. Their approach was based on the ratiometric signal of (8,7)\/(9,4)-SWCNT chiralities, which differed in their response to \( \text{H}_2\text{O}_2 \) (Figure 8e). The fluorescence signal was invariant to the excitation source distance, and exposure time, which enabled detection within commercial wound bandages with a wireless readout (Figure 8f). These microfibers encapsulated the SWCNTs for at least 21 days without structural changes.

Furthermore, Zheng et al. reported the selective interactions of SWCNTs coated with ten different ssDNA sequences in response to dissolved oxygen.\[^{131}\] The SWCNT emission intensity was quenched between 9 to 40\% depending on both the ssDNA sequence and SWCNT chirality in response to 1 atm \( \text{O}_2 \) compared to samples purged with 1 atm argon, thus indicating that stronger coating interactions lead to reduced \( \text{O}_2 \) access to the SWCNT surface. Since the quenching reversed completely after the removal of dissolved oxygen, it is probably based on physisorption on the SWCNT. Thus, the screening for fluorescence quenching by dissolved oxygen provides a simple approach to explore the structure-selective interactions of ssDNA with SWCNTs.

ROS can also be generated by enzymes and SWCNTs. Yaari et al. demonstrated the first SWCNT-based sensor that reports the degree of enzymatic suicide inactivation.\[^{201}\] The approach was based on enzyme-bound SWCNTs, which report fluorescence modulations by quenching and red-shifting selectively in response to substrate-mediated suicide inactivation of tyrosinase. Mechanistic insights revealed that the red-shifted response is most likely a result of the generation of singlet oxygen during the enzymatic reaction, which leads to the binding of ssDNA on the SWCNT surface.\[^{132}\]

### 3.2.2. Neurotransmitters

Neurotransmitters are an important class of signaling molecules. To understand neuronal networks and linked neurological diseases, imaging with high selectivity and spatiotemporal resolution is necessary, which existing methods are currently not able to provide.\[^{133}\] In the last few years...
several SWCNT-based sensors based on functionalization with DNA have been explored and it has been shown that sensitivity and selectivity depend on the exact DNA sequence.\(^{100,118,119}\)

The first SWCNT-based sensors for the detection of neurotransmitters were reported by Kruss et al.\(^{15}\) By using a screening approach, it was found that certain ssDNA-SWCNTs change their fluorescence in the presence of catecholamine neurotransmitters such as dopamine. These sensors were reversible and showed sensitivities in the nanomolar range. Similar sensors were used for the high-resolution imaging of cellular dopamine efflux from stimulated neuroprogenitor cells.\(^{100}\) Here, the sensors were immobilized on a collagen-coated glass substrate to increase cell adhesion, and dopamine-releasing neuroprogenitor (PC12) cells were cultured on top. In response to a stimulation event, the fluorescence intensity of the sensor layer image consisting of up to 20000 pixels increased (Figure 9a,b). This allowed both the spatial and temporal dynamics of dopamine release events to be studied with extraordinary high resolution and to identify hotspots (Figure 9c,d).

This approach of imaging many nanosensors under cells is also applicable to other neurotransmitters. Dinarvand et al. imaged the release of serotonin from human blood platelets in real time,\(^{27}\) as most of the serotonin is stored in blood platelets and not in the brain of humans. This serotonin sensor (NIRSer) consisted of a serotonin-binding aptamer on a SWCNT, which exhibited an increased fluorescence emission of up to 80% in response to serotonin (Figure 10a). High-resolution images of serotonin release patterns from single cells were obtained by placing the sensors below and around serotonin-releasing cells (Figure 10b.c). This approach allows serotonin release to be studied with unprecedented resolution and the time delay between stimulation and release to be resolved.

In a similar fashion, artificially added serotonin was detected in acute brain slices by Jeong et al.\(^{114}\) In this case, a DNA sequence was found by an expanded screening approach from a library of around $6.9 \times 10^{10}$ different ssDNA-SWCNTs. These ssDNA-SWCNTs exhibited a selective response to serotonin over serotonin analogues, metabolites, and receptor-targeting drugs.

The high spatiotemporal resolution of SWCNT-based neurotransmitter sensors is especially useful when it comes to resolving parallel processes on the subcellular to cell-network length scale. However, placing the sensors below cells can be a drawback, especially with cells that need to differentiate on this layer for several weeks. Elizarova et al. developed a new sensor paint approach (AndromeDA) to use sensors and study the dopaminergic signaling in primary neurons.\(^{135}\) In this case, the sensors were adsorbed (‘painted’) onto the complex cell networks, which included different cell types. This approach allowed the heterogeneity of dopamine release events to be quantified from up to 100 release sites (varicosities), which is highly important to understand the information processing and plasticity of neurons.

An effect that has to be accounted for in these studies is that SWCNT fluorescence is affected by changes in the local cation concentration, which is also a hallmark of neuronal activity.\(^{146,25}\) To circumvent this problem, Gillen et al. used...
Figure 9. High-resolution imaging of dopamine using SWCNT-based sensor arrays. a) Specific ssDNA-functionalized fluorescent SWCNTs respond selectively to dopamine. Sensors are immobilized on a glass substrate and dopamine-releasing neuroprogenitor cells are cultivated on top. The SWCNT fluorescence changes in response to stimulation of the cells. b) Fluorescence intensity change of a single sensor in response to dopamine. c) Three-dimensional release profiles for dopamine along the border of neuroprogenitor (PC12) cells at different time points relative to the stimulation at $t_0$. The height and color indicate the relative fluorescence change normalized to the maximum fluorescence change. d) Bright-field image of the cell stimulated on top of the nanosensor array and corresponding hotspots (blue circles) along the cell border. Arrows indicate positions belonging to the hotspots in the response profile. Reprinted from Ref. [100] with permission.

Figure 10. High-resolution imaging of serotonin (5HT) release from cells. a) NIRSer sensor: SWCNTs functionalized with a serotonin aptamer respond selectively to serotonin. b) NIRSer increases its fluorescence in response to serotonin. c) Sensors are immobilized on a surface and serotonin-releasing cells are cultured on top. Here, blood platelets are used, which contain most of the body’s serotonin. d) Color-coded image of serotonin release from a single platelet at three time points (before, during, and after serotonin release). e) Fluorescence response from a region of interest (ROI, green circle in (d)). The activation and delay time of the onset of serotonin release are marked with arrows. Reprinted from Ref. [27] with permission. Copyright 2020 American Chemical Society.
locked nucleic acids to develop sensors with improved stability to cation-induced fluctuations of the fluorescence intensity. By systematically introducing locked bases along the (GT)$_{15}$-DNA sequence they found that the fluorescence stability in the presence of Ca$^{2+}$ ions depends on the type of the locked bases. Certain SWCNT chiralities exhibited improved stability against Ca$^{2+}$ ions and retained their ability to detect dopamine in the presence of Ca$^{2+}$ ions, thus highlighting the importance of the exact conformation of the nucleic acid sequence. Moreover, the detection of both Ca$^{2+}$ and dopamine was possible by monitoring multiple chiralities simultaneously.

Interestingly, the fluorescence responses of SWCNTs suspended in sodium cholate to dopamine and serotonin can be altered by modulating the exposed area by the surfactant concentration. However, such surfactants would not be compatible with cellular systems.

A central challenge in biomedicine is the controlled delivery (uptake, transport, and release) of (nano)materials such as sensors and pharmaceuticals. Even whole cells can serve as vehicles to take up such materials. Certain immune cells (neutrophils) have been shown to be suitable for cargo delivery by hijacking a process known as neutrophil extracellular trap formation (NETosis). During NETosis, cells lyse after rupture of the cellular membrane by chromatin swelling.[136] Meyer et al. showed that human immune cells take up ssDNA-SWCNT-based sensors and can be triggered to release the cargo after a certain time by using NETosis. Moreover, the sensors maintained their functionality to detect dopamine and H$_2$O$_2$, which offers opportunities for in vivo delivery.

All these discussed neurotransmitter sensors responded by a fluorescence increase. Interestingly, the introduction of a small number of aryl defects into ssDNA-functionalized SWCNTs completely reversed the sensing response (Figure 6d).[87] The E$_{11}$ emission slightly decreased and strongly decreased the red-shifted E$_{11}^*$ emission. Apart from new insights into the sensing, this approach enables ratiometric detection schemes. For a more detailed overview on the biological relevance of catecholamine neurotransmitters and alternative detection methods (e.g. electrochemical) readers are referred to the literature.[28,114]

### 3.2.3. Other Small Molecules

Beyond neurotransmitters, recognition strategies for other small molecules have been developed, for example, adenosine 5'-triphosphate,[140] nitroaromatics,[30,141] riboflavin,[149,70,142] l-thyroxine,[70] oestradiol,[70] doxorubicin,[115,143] and steroids.[144]

Finding a specific molecular recognition element is difficult for many of these biomolecules, such as hormones, due to their chemical similarity. Therefore, Lee et al. used a polymer self-templating synthetic approach, which is based on the attachment of a chemical appendage similar in molecular weight and structure to the target analyte to create a binding pocket within the corona. This approach reduced the library size for screening and led to implantable SWCNT-based sensors for the selective detection of the human steroid hormones cortisol and progesterone.[144]

Recently, the first reversible fluorescent SWCNT-based sensor for volatile organic compounds was also reported, which has potential for the detection of wine spoilage.[145] For this, Shumeiko et al. used peptide-encapsulated SWCNTs, which were adsorbed onto a polystyrene cuvette to detect low concentrations of acetic acid (down to 0.05 % (v/v)) in air. Using (6,5)-SWCNTs, which fluoresce below 1000 nm they demonstrated the detection with a low-cost Si-based camera (Figure 11a). The sensor was exposed to different concentrations of acetic acid, which quenched the fluorescence but was reversible when switching to clean air (Figure 11b). The ability to identify wine spoilage was investigated by using two wine types with and without the addition of acetic acid to simulate an undesirably high acetic acid concentration (Figure 11c).

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**Figure 11.** Detection of volatile compounds in the gas phase. a) Air with analyte (acetic acid) flows to the SWCNT-based sensors, which produces an optical readout. b) Dynamic response of the sensor to rising acetic acid concentrations. c) Dynamic response of the sensor to wine spiked with acetic acid. Reprinted from Ref. [145] with permission. Copyright 2021, Elsevier.
In an extension of this study, this system was expanded to an array of five different peptide-encapsulated SWCNTs on a nitrocellulose paper. The optical patterns enabled the distinction of volatile molecules such as ethanol, methanol, and 2-propanol as well as the aromas of red wine, beer, and vodka by linear discriminant analysis and machine learning.[49]

One way to detect or study small molecules is to mimic parts of larger biomolecules, such as enzymes. Dong et al. screened a library of 24 amphiphilic polymers to find a corona phase which demonstrates a binding specificity very similar to the enzyme phosphodiesterase type 5 (PDE5), which catalyzes the hydrolysis of secondary messengers.[117] The SWCNT-based sensor consisted of a poly(methacrylic acid-co-styrene) motif. This synthetic corona mimics the H loop of the native enzyme and is, thus, able to bind to Vardenafil, a PDE5 inhibitor, and its molecular variant as a result of the unique corona phase configuration. It is selective over other off-target inhibitors, but not completely over the chemically similar inhibitor Sildenafil.

One of the challenges in SWCNT-based sensing is the heterogeneous material that is used for most sensors. Even though purification has made tremendous progress, getting access to chirality-pure SWCNTs with a tailored surface chemistry has been a challenge. Nißler et al. showed sensing of small molecules such as riboflavin and ascorbic acid as well as pH value with chirality-pure SWCNTs by using aqueous two-phase extraction and a subsequent surface functionalization exchange process.[49] The chirality-pure sensors were up to ten-times brighter than mixtures of SWCNT chiralities, and enabled insights into the impact of chirality and handedness of SWNCTs and the sensing mechanism. Additionally, long-time stability over 14 days was demonstrated as well as ratiometric and multiplexed sensing based on the non-overlapping fluorescence spectra (Figure 22.b,c).

On a macroscopic level, monochiral SWCNTs were used by Nißler et al. to detect polyphenols in and around plants.[146] Polyphenols are secondary metabolites and messenger molecules that are released from leaves and roots as a chemical defence against pathogens and herbivores (Figure 12.a). Certain polyethylene glycol phospholipids (PEG-PL) were identified for the selective detection of different polyphenols over interfering molecules such as sugars or H$_2$O$_2$. The SWCNT-based sensors responded through quenching and red-shifting of up to 20 nm, for example, to tannic acid (TaA, Figure 12b). To image the plant polyphenol release over time, the sensors were embedded in agar, soybean seedlings were plated on top, and polyphenol secretion was triggered with a pathogen-derived elicitor, which resulted in a decrease in the NIR fluorescence over time (Figure 12c). These sensors help to understand this plant defence mechanism and could improve the breeding of stress-resistant plants for precision agriculture.

3.2.4. Lipids

The investigation of lipid-linked diseases is challenging because methods for accurate in vivo monitoring of lipid accumulation have been missing. The Heller group was the first to address this issue by developing a SWCNT-based optical sensor, which non-invasively detects the lipid flux within the lumen of endolysosomal vesicles in vitro and in vivo.[117,149] In a first approach, they used (GT)$_n$-functionalized (8,6)-SWCNTs, which fluoresce at 1200 nm and responded through a wavelength shift to biological lipids and water-soluble lipid analogues.[117]

By incubating the sensors with fibroblasts from a lysosomal storage disorder Niemann-Pick-type C patient in vitro, the sensors localized in the lumen of endolysosomal organelles without affecting their properties and resolved the lipid accumulation down to the subcellular level in real time. The authors proved the reversibility of this sensor by administering a drug which reverses the disease phenotype.

The second approach was based on the screening of several ssDNA-SWCNT chirality combinations to identify CTTC$_2^{34}$_TTC$_{10}$(9,4)-SWCNTs with the greatest wavelength shift of up to 8 nm in response to lipid accumulations.[149] The emission wavelength at 1125 nm is spectrally separated from the lipid absorption band at 1210 nm, thus facilitating promising in vivo applications. Molecular dynamics calculations led to the assumption that the lipid molecules sphingomyelin and cholesterol bind to the SWCNT surface by hydrophobic interactions, thereby decreasing the water density in the SWCNT environment and leading to the

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**Figure 12.** Detection and imaging of plant polyphenols. a) Polyphenols from leaves and roots are released in the immediate vicinity in response to pathogens or herbivores as a defence mechanism. b) SWCNTs functionalized with polyethylene glycol phospholipids (PEG-PL) respond selectively to polyphenols such as tannic acid (TaA) through a red-shift and decrease in the fluorescence emission. c) Visible and NIR images of a soybean root plated on top of the sensors (embedded in agar). The plant is challenged with a pathogen elicitor and the NIR fluorescence intensity decreases over time in response to the release of polyphenols in the immediate vicinity of the root. Reprinted from Ref. [148] with permission.
observed blue shift (Figure 13a). To validate the sensor functionality in live cells, endolysosomal lipid accumulation was induced in macrophage cells with chemical inhibitors to mimic different lipid phenotypes. After sensor incubation, a blue-shift was observed for all sensors within the drug-treated cells compared to the control (Figure 13b). For in vivo applications, two mouse models were used with lipid accumulation within the organelles of many cell types, for example, in Kupffer cells. By intravenously injecting the SWCNT-based sensors, a rapid decrease of the SWCNT fluorescence and consequently removal of SWCNTs from all parts of the mice except for the liver were observed. The sensors were also able to report uptake and endolysosomal lipid accumulation of oxidized low-density lipoprotein (oxLDL; Figure 13c). Therefore, these types of sensors provide novel insights into the complexity of lipid metabolism and related health states.

### 3.2.5. Proteins

Proteins are one of the major biomacromolecules. Consequently, the study of protein–protein interactions helps to understand the function of proteins or to find novel drugs. As discussed above, the detection of proteins with SWCNTs is either based on attaching a known natural recognition element onto all kind of materials changes the actual corona structure and might affect sensing. To improve the performance of sensors in those protein-rich environments, a fundamental understanding of the interaction between sensors and their biological environment is necessary. Pinals et al. addressed this issue by studying the protein corona formation on (GT)_{16}-SWCNTs in cerebrospinal fluid and blood plasma by mass spectroscopy.

Their results showed strong binding to fibrinogen and other proteins involved in blood clotting, lipid transport, and complement activation. The identification of interactions responsible for the formation of protein corona revealed that...
the outer corona formation can be reduced by optimizing the electrostatic interactions through the sensor design and dynamic flow conditions (e.g., with lateral flow assays or microfluidic systems), while entropic calculations must be considered for the inner corona. This study highlights the urgent need to investigate sensors not only in simple buffers but biologically complex environments. Most recently, Ehrlich et al. developed a complementary approach using an insulin aptamer (found within the natural insulin gene promoter) functionalized to the SWCNT surface through an (AT)$_{15}$ ssDNA anchor sequence. In contrast to the synthetic PEGylated-lipid, which has no prior affinity to insulin, this aptamer possesses a known affinity to insulin. However, the observed sensitivity was lower than the previous approach.

Most of the SWCNT-based sensors that have been discussed so far were measured in solution. The immobilization of SWCNTs on different porous paper matrices, for example, nitrocellulose, has several advantages for a robust assay. Paper-based immobilization enabled analyte detection within non-aqueous solvents such as edible oil, which was previously not possible. To further extend this system the authors used wax to pattern hydrophobic regions onto the paper to create a multiplexed one-dimensional sensor barcode consisting of different ssDNA-wrapped SWCNTs.

Another important class in biomedical diagnostics are antibodies. The detection of immunoglobulin G (IgG) with SWCNTs was realized by using chitosan-wrapped SWCNT noncovalently modified with immunoglobulin-binding proteins. Recently, Kozawa et al. designed a flexible fiber optic interface coupled to nanosensors that was capable of detecting the aggregation status of human IgG by reporting the relative fraction of monomers and dimer aggregates with sizes of 5.6 and 9.6 nm. For this purpose, the SWCNT-based sensors were incorporated into a hydrogel (HG) and attached to the end of a fiber waveguide. Proteins are also part of pathogens and consequently disease markers. Pinals et al. developed a SWCNT-based sensor which is functionalized with the angiotensin-converting enzyme 2 (ACE2), a host protein which shows a high binding affinity for the SARS-CoV-2 spike protein (Figure 14e). A twofold NIR fluorescence increase was detected 90 min after the addition of the purified spike protein (Figure 14f). Passivation with a hydrophilic polymer was used to enable detection of the spike protein in saliva and viral transport medium.

However, antibodies are not always available for all targets. In addition, the development of new recognition units can be expensive and tedious, which is why new approaches are directed to the development of multiplexed sensor arrays to overcome the limited selectivity of existing single sensors. Recently, Yiaari et al. developed a SWCNT solution-based sensor platform to detect multiple gynecologic cancer biomarkers in uterine lavage samples. The array consisted of eleven different ssDNA-SWCNT sensors, and the
optical change in the intensity and wavelength was extracted for twelve chiralities present in the sample, which resulted in 132 individual ssDNA-SWCNT complexes. With machine learning algorithms a classification accuracy (F1 score) of 0.95 was achieved. With retraining, this sensor platform may not be limited to the detection of cancer biomarkers. The large variety of possible SWCNT chiralities in combination with unlimited SWCNT wrappings opens possibilities to meet the rising demand of new recognition strategies.

3.2.6. Sugars

Sugars are important building blocks and metabolites. Glucose, in particular, is a major target, for which continuous monitoring of the glucose level in blood is desired. SWCNT-based sensing ranges from the use of glucose-specific enzymes,[24,161,162] or proteins[108] to the first affinity sensor based on the competitive binding between glucose and its polymer dextran.[11] Although improvements were made, the first approaches suffered from limited reversibility and/or physiological detection range. One sensor that meets the requirements is based on the functionalization of SWCNTs with glucose oxidase (GOX), a glucose-specific enzyme.[163] The addition of glucose causes an increase in fluorescence emission. The proposed mechanism is based on SWCNT fluorescence being quenched by defect sites on the SWCNT surface, which are hole-doped through oxygen adsorption. The addition of glucose causes an oxidation of the GOX wrapping, which behaves as an electron donor and passesivate the oxygenated sites of the SWCNT, thereby resulting in a fluorescence increase. This effect is reversible by removing the glucose. The sensor showed responses to five other tested saccharides, but with the highest response to glucose.

Another recognition element for saccharides is phenylboronic acids, which have been used to functionalize SWCNTs noncovalently for the detection of sugars.[164] Recently, covalent aryl-boronic acid defects were also incorporated in SWCNTs.[81] Upon interaction with fructose and glucose, these sensors decreased in fluorescence intensity and the E_{11}^* signal shifted, which can be used for spectrally encoded sensing.

3.2.7. DNA/RNA

One of the most abundant and important types of biomacromolecules are nucleic acids that store and process genetic information. Using a construct of a complementary capture sequence connected to a (GT)$_{15}$-sequence serving as an anchor, thus providing colloidal stability, SWCNTs were recently used to detect hybridization events of microRNA and other oligonucleotides directly in serum, urine, and in mice in vivo.[165] Upon the addition of complementary nucleic acids, a specific blue-shift for different chiralities upon hybridization was observed. Additionally, the sensor response was reversible through toehold-mediated strand displacement and the sensors possessed a LOD in the picomolar range.

Further development of this sensor led to the first SWCNT-based sensor for the detection of HIV in serum.[166] Harvey et al. discovered that SDS-denaturized serum proteins lead to an enhanced optical response of the SWCNTs in response to DNA hybridizations. They hypothesized that the addition of SDS ensured both the liberation of the viral RNA genome and the denaturation of the proteins which competitively bind to the freed surface of the sensor. The interaction leads to a blue-shift in the SWCNT emission (Figure 15a). This was first shown for hybridization with complementary target miR-19 DNA compared to control R23 DNA (Figure 15b). A dose-dependent enhancement of the blue-shift occurred in the region of the critical micelle concentration of SDS; thus, the denaturation of proteins by SDS is considered to involve an unfolding process of the tertiary structure of the protein to complete denaturation. As the SDS concentration was increased, the amount of denatured protein absorbed onto the SWCNT surface after hybridization of the DNA increased and saturated at 2% SDS. For HIV detection, the recognition strategy was based on a sensor consisting of (GT)$_{15}$-T$_{15}$-SWCNTs, which hybridize with the polyadenylation elements of HIV RNA (Figure 15c). In a similar way, a control sensor with the noncomplementary capture sequence, namely (GT)$_{15}$-A$_{15}$-DNA, was constructed. The wavelength shifts of both the sensor and negative control in the presence of HIV particles treated with 1% SDS were recorded over time and and the sensor displaying the complementary capture sequence showed a blue-shift of around 3 nm after 180 min.

Figure 15. Detection of viral (HIV) RNA. a) Detection of viral RNA using a HIV lentivirus model. The virus is denatured by SDS, which liberates the RNA genome. It hybridizes to the complementary ssDNA on the SWCNT-based sensor and increases the free surface area, which is then occupied by serum proteins and causes a blue-shift of the spectrum. b) Wavelength shift in response to the target miR-19 DNA or control R23 DNA in bovine serum albumin with various SDS concentrations. c) Kinetics of the response of sensors with complementary (GT)$_{15}$-T$_{15}$ and negative control ((GT)$_{15}$-A$_{15}$) ssDNA in the presence of HIV particles, FBS, and 2% SDS. Reprinted from Ref. [164] with permission. Copyright 2019 American Chemical Society.
A completely different application of DNA chemistry on SWCNTs was established by Cha et al. Based on the consumption of chemical energy delivered by the RNA molecules, they developed a synthetic motor that transports nanoparticles through the mechanical motion of DNA conformation changes along the SWCNTs.\textsuperscript{[165]} Movements of the motor of over 3 µm with a speed of 1 nm min\(^{-1}\) were observed.

3.2.8. Enzymes

So far, the detection of enzymes with SWCNTs has been shown for the characterization of proteases and DNases as well as cellulases and pectinases. The hydrolytic enzyme activity was measured by Kallmyer et al., who used hydrolytic enzyme wrapped SWCNTs that respond to the target enzyme with a fluorescent intensity quenching because of the degradation of the enzyme-wrapping.\textsuperscript{[166]} Different polymer wrappings consisting of polysaccharides and polypeptides were used to study cellulase, pectinase, and bacterial protease. Most recently, they used this approach to evaluate the enzyme activity in soil using a low-cost multiplexed and portable fluorimeter able to perform the measurement outside the laboratory only minutes after extraction from the field.\textsuperscript{[167]} As a consequence of the fresh nature of the soil sample, field tests indicated activities an order of magnitude larger than those obtained in benchtop experiments.

Enzymes are also released by microorganisms, which can be used to fingerprint them. Nißler et al. chemically tailored SWCNTs to detect enzymes such as DNases and proteases.\textsuperscript{[29]} For targeting extracellular proteases, SWCNTs were modified with bovine serum albumin (BSA), which serves as an enzymatic substrate, while SWCNTs were functionalized with calf thymus (CT) DNA for reporting DNase I and S. aureus nuclease activity. The sensors showed a fluorescence decrease, most likely as a result of decomposition of the BSA surface coating. These SWCNT-based sensors were further used for the discrimination of bacteria, which are known to alter their chemical environment through the release of signaling molecules, enzymes, and metabolites (see section below).

In contrast, Shumeiko et al. used peptide-encapsulated SWCNTs, which also responded through a fluorescence decrease upon enzymatic digestion of the SWCNT wrapping.\textsuperscript{[168]} They utilized a low-cost paper-based dipstick system, with which they evaluated the trypsin activity in urine samples as a mimic for acute pancreatitis, where abnormal trypsin concentrations are common.

To study the enzyme myeloperoxidase, which is involved in the regulation of inflammation processes, He et al. used a ratiometric system based on graphene oxide (GO) wrapped SC-SWCNT sensors and GO-wrapped carboxymethylcellulose (CMC)-SWCNTs as a reference.\textsuperscript{[169]} GO and SC-SWCNTs showed an opposed fluorescence signal in response to enzymatic degradation. Whereas the blue fluorescence intensity of GO was increased due to oxidation and degradation of GO leading to the formation of graphene quantum dots, the NIR emission of SWCNTs decreased due to the generation of defects on the SWCNT surface. In contrast, the CMC-SWCNT reference was almost stable as a result of the better surface protection of the CMC-wrapping.

3.2.9. Epitopes and Metabolites from Pathogens

Microbial infections are one of the major causes of mortality worldwide. Currently, the limited number of diagnostic methods in combination with increasing antibiotic resistances demonstrate the rising need for the rapid, contactless, and specific detection of pathogens such as bacteria. The optical properties of SWCNTs promise advantages for pathogen detection. Bardhan et al. developed M13 bacteriophage functionalized SWCNTs (M13-SWNTs, Figure 16a), which are able to distinguish F\(^{-}\)-positive and F\(^{-}\)-negative bacterial strains by modulation of the fluorescence intensity.\textsuperscript{[170]} The M13 bacteriophage has a known binding affinity to F\(^{-}\)-positive E. coli strains. Therefore, they intramuscularly infected the right flank of living mice with E. coli strains, either F\(^{-}\)-negative DH5-α strains (Figure 16b, left) or targeted F\(^{-}\)-positive JM109 strains (Figure 16b, right) and observed a 1.6-fold intensity increase over the nonspecific DH5-α strains. Injection of PBS into the right flank served as a control. To extend this SWCNT-based label to a wider range of bacterial strains lacking F\(^{-}\)-pili, they additionally attached an antibacterial antibody on those M13-SWCNTs (anti-S. aureus-M13-SWNT) through a streptavidin–biotin reaction.

![Figure 16](image_url)
This complex specifically detected \textit{S. aureus} intramuscular infections in a mouse model in vivo (3.1-fold intensity increase over the nontargeted M13-SWCNTs, Figure 16c).

This approach is a NIR labeling rather than sensing. In contrast, Nißler et al. created a concept to remotely distinguish six important pathogens using an array of SWCNT-based sensors:[29] four for specific bacterial target detection based on a rational approach and four generic lower sensitivity sensors together with an invariant reference consisting of NIR fluorescent Egyptian Blue nanosheets (Figure 17a).[171]

To overcome unspecific effects arising from the complex composition of bacterial media, the sensors were incorporated into a hydrogel (HG) array, in which the pore size was varied in accordance to the size of the analyte. For the detection of small molecules (such as siderophores), HGs with a low porosity were used, whereas HGs with a high porosity allowed large enzymes to diffuse to the sensors. The sensors were exposed to clinical isolates of bacteria and the unique change in the fluorescence intensity for each sensor was monitored remotely. Within 24–72 h, a unique fingerprint in response to bacterial metabolites and virulence factors, which are released by bacteria growing on top of this HG, was visible (Figure 17b), which further allowed differentiation of the pathogens by principal component analysis (PCA; Figure 17c). Within the dimensions of the exciton, the fluorescence of SWCNTs is very sensitive to the surrounding environment. To

3.3. Mechanism of Fluorescence Modulation

In SWCNT-based sensors or probes, the SWCNTs serve as transducer elements that translate chemical changes caused by an analyte in the vicinity of the SWCNT into a fluorescent signal. Thus, SWCNT-based sensing involves molecular recognition and signal transduction. The precise mechanisms are most likely different for different analytes and different surfaces. However, from the literature one can distinguish several possible generic mechanisms (Figure 18).

3.3.1. Direct Quenching

Direct quenching, that is, the decrease of fluorescence, is caused by adsorption of the analyte onto the SWCNT surface. Changes in the pH value caused by the addition of an acid can cause protonation of the SWCNT sidewall and result in direct, reversible quenching of the SWCNT fluorescence (Figure 19a). Mechanistically, this can be explained by the injection of an electron hole into the $\pi$-system near the protonation site.[17] Excitons encountering such an electron hole will be quenched through a nonradiative Auger process.[172] Furthermore, electron transfer between the valence band of 3,4-diaminophenyldextran-functionalized SWCNTs and the lowest unoccupied molecular orbital (LUMO) of nitrogen oxide results in rapid, reversible quenching of the SWCNT fluorescence.[134] Both of these interactions take part in the vicinity of the SWCNT and, consequently, solvent effects should play a large role.

3.3.2. Impact of Conformational Changes and Solvation

Within the dimensions of the exciton, the fluorescence of SWCNTs is very sensitive to the surrounding environment. To
study the contributions of the solvent to the fluorescence of SWCNTs, fluorescence spectra were analyzed in different dielectric environments.\textsuperscript{[173,174]} By using the solvatochromic shifts, a semiempirical scaling model was developed that linked optical with structural parameters and suggested an inverse dependence of exciton polarizability on the diameter and the square of the transition energy.\textsuperscript{[173]} In nonpolar solvents, the solvatochromic modulation of the fluorescence intensity becomes more pronounced for larger diameters.\textsuperscript{[174]} Changes in the solvatochromic shift, on the other hand, were more pronounced for SWCNTs with smaller diameters.\textsuperscript{[174]}

In general, the displacement of H\textsubscript{2}O or DNA from the surface of SWCNTs by surfactants leads to a strong blue-shift and increase in the fluorescence intensity. Interestingly, the change in the fluorescence characteristics of pristine SWCNTs and DNA-coated SWCNTs immobilized in gel are highly similar. This suggests that considerable portions of the nanotube surface are exposed to H\textsubscript{2}O.\textsuperscript{[175]} However, to date, a local model of solvatochromism that accounts for the nonhomogeneous structure around SWCNTs is missing.

One of the best understood systems is the recognition of small molecules by DNA-functionalized SWCNTs. Here, the mechanism of sensors for catecholamine neurotransmitters such as dopamine have been studied in greater detail (Figure 19d,e).\textsuperscript{[100]} As these molecules are redox-active, they could reduce or oxidize either the SWCNT or the surrounding organic phase, thereby affecting the fluorescent properties. To study this potential mechanism, the redox potential and the fluorescent response of certain analytes were correlated. This study showed that molecules with a negative redox potential are more likely to increase the SWCNT fluorescence (Figure 19b).\textsuperscript{[100]} However, as molecules of the same redox potential can induce drastically different fluorescence responses, the redox potential alone cannot account for the fluorescence changes observed.\textsuperscript{[63]} Likewise, fluorescent responses of ssDNA-functionalized SWCNTs to dopamine and riboflavin cannot be correlated to the amount of adsorbed nucleotides/DNA molecules on the SWCNT surface (Figure 19c), which suggests that more complex conformational changes are responsible for the change in the SWCNT fluorescence.\textsuperscript{[176]} Molecular dynamics simulations showed that a stacking of dopamine with DNA-functionalized SWCNTs leads to interactions between the phosphate backbone of the DNA as well as the hydroxy and amine groups of dopamine (Figure 19d,e).\textsuperscript{[100]} As a result of this interaction, the phosphate backbone moves toward the SWCNT surface and the electrostatic potential at the SWCNT surface changes (Figure 19d,e).\textsuperscript{[100]} It is also known that the diffusion coefficient of the excitons in surfactant-containing systems changes with the surfactant identity and, furthermore, correlates with the fluorescence intensity.\textsuperscript{[48]}

As pristine SWCNTs do not show any fluorescence response to dopamine\textsuperscript{[100]} and differently functionalized SWCNTs display different affinities to neurotransmitters,\textsuperscript{[118]} it follows that the organic phase (DNA) governs both the sensitivity as well as selectivity for this neurotransmitter.\textsuperscript{[118]} The used biopolymers are typically charged and, consequently, electrostatic interactions play an integral part for biosensing.\textsuperscript{[177]} It has been shown that the presence of certain salts alters the conformation of the DNA wrapping on the SWCNT\textsuperscript{[56,57]} and decreases the electrostatic repulsions between equally charged molecules.\textsuperscript{[277]} To reduce ion-induced fluorescence effects, the flexibility of the DNA can be altered by using xeno nucleic acids.\textsuperscript{[57]} A good example of the influence of electrostatic repulsion and screening effects is the increased SWCNT surface accessibility at higher salt concentrations (Figure 19b).\textsuperscript{[177]} In a surfactant-containing

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure18.png}
\caption{Mechanisms of fluorescence modulation in SWCNT-based biosensors. Note that the size of the objects and their arrangement is simplified and not to scale.}
\end{figure}
The exposed surface is covered by the surfactant, which causes a blue-shift of the NIR fluorescence, again demonstrating that the dynamics of the organic phase around the SWCNT is crucial for the sensing mechanism. By modulating the exposed surface area, it is furthermore possible to tune surfactant-suspended SWCNTs to respond to different bio-analytes.[137] Together, these findings indicate that fluorescence is modulated by the precise 3D arrangement of the molecules, ions, and water molecules in the vicinity of the nanotube.

3.3.3. Exciton Decay and Defects

As described above, the introduction of certain sp^3 defects into the carbon lattice of SWCNTs can increase the NIR fluorescence through the trapping of excitons.[53,54] The defined introduction of quantum defects, thus, provides a way to perturb the exciton decay and elucidate the involved processes that ultimately affect the fluorescent response. As the NIR fluorescent response of SWCNT-based sensors to dopamine in H_2O and D_2O did not show major differences, electronic-to-vibrational energy transfer (EVET)[176] seems not to be a main factor in (dopamine) sensing.[87] In contrast, the correlation between the length of the SWCNTs and their fluorescent response seems to indicate that quenching at the ends plays a role. However, the fluorescence response was independent of the variation of defect density.[87] Together with the finding that a small number of quantum defects reverse the fluorescent response of DNA-functionalized SWCNTs to dopamine (Figure 6d), it follows that multiple rate constants are affected by the analyte.[87] Computationally, the experiments were best explained by a three rate constant model (3RC) that includes a decrease in the nonradiative decay from the E_{11} state (k_{nr}), an increase in the exciton diffusion constant (D_e), and an increase in the nonradiative decay constant from the E_{11}^* (k_{nr}^*) caused by dopamine.[87] Together, these insights highlight the complex interplay between photophysics and molecular recognition as well as new avenues to tailor sensing using defects.
3.4. Considerations on Kinetics and Imaging

The processes related to sensing happen on a certain time scale. How fast an analyte binds or dissociates from a sensor is determined by its kinetics. Additionally, the optical signal is detected in setups that determine aspects such as spatial resolution or imaging speed. In the following sections we discuss how these hallmarks of fluorescence sensors affect their performance.

3.4.1. Kinetics of Sensors and Impact on Spatiotemporal Resolution

Chemical imaging with many SWCNT-based sensors at one time is a highly effective strategy to gain chemical information from a sample with outstanding spatial and temporal resolution. To understand how the collective image of such an array of sensors reflects the concentration of an analyte, Meyer et al. used stochastic Monte Carlo simulations to study the kinetic requirements for spatiotemporal chemical imaging with nanoscale sensors such as SWCNTs.[180]

The subject of the simulation was a nanosensor array being exposed to a changing concentration gradient of an analyte. To predict the image one gains from many sensors, single sensor responses were first simulated. In a typical scenario, the time-dependent concentration/diffusion profile of a dynamic process, such as release of signaling molecules (e.g. neurotransmitters) from cells and the stochastic binding site state of the sensors for certain rate constants, was simulated to calculate the expected fluorescence change of single sensors (Figure 20a,b). The overall image was then calculated considering the individual fluorescence emission point spread functions and technical considerations, such as the frame rate of the camera. This simulation allows the prediction of how the rate constants of a sensor (\(k_{on}, k_{off}\)) and other factors affect the spatiotemporal resolution, for example, to resolve fast concentration changes such as neurotransmitter release from cells. It can serve as guiding principle for the chemical design but also for the interpretation of signals from a given biological problem. Phase diagrams (Figure 20c) indicated that the sensors need a surprisingly low affinity (\(K_d = 100 \, \mu M\)) to resolve fast processes.

3.4.2. Ratiometric Detection

So far, optical biosensors based on SWCNTs have mainly been fabricated from mixtures of multiple chiralities. This leads to a large spectral overlap in the fluorescence emission, which complicates multiplexing and reduces sensitivity.

Advances in the separation and functionalization of SWCNTs have recently enabled the sole use of SWCNTs emitting below 1000 nm together with low-cost silicon-based detectors[145,181] for the development of ratiometric sensors, in which two or more distinct NIR signals are detected simultaneously. Here, one SWCNT chirality is typically not responsive to the analyte and acts as a reference. Besides the fact that single chirality SWCNTs lead to six- to tenfold higher fluorescence intensities compared to multichirality SWCNT mixtures at the same concentration,[49,182] ratiometric approaches are more stable to external noise. Despite the clear advantages, the implementation was not possible for a long time because of difficulties in gaining chirality-pure SWCNTs.

Although progress was made in the synthesis of chirality-enriched SWCNT samples,[183] only a few chirality-enriched samples are commercially available. Different separation and purification methods have been developed. They range from density gradient centrifugation,[184] gel chromatography,[185] ion-exchange chromatography,[55] and aqueous two-phase extraction[186] to wrappings of special macromolecules that preferably solubilize certain chiralities.[22,187] What they all have in common is that the resulting pure chiralities are solubilized in certain polymers or surfactants. However, for a biosensing application it is necessary to tailor the surface chemistry. Therefore, straightforward processes are required that yield chirality-pure SWCNTs with tunable functionalization.[121]

The first ratiometric SWCNT-based sensor was demonstrated by Giraldo et al., who integrated into the leaves of plants two sensors: one for the detection of \(H_2O_2\) and one for...
NO$_2$.[20] The sensor system was based on the ratio of the distinct emission bands of two chirality species (Figure 21a). Whereas the (GT)$_{15}$-(7,6)-SWCNT-based sensor was quenched by 20% within 10 min in the presence of 100 μM H$_2$O$_2$, the reference sensor remained mostly invariant to the analyte. The overall sensor response was similar to in vitro tests.

Most recently, Nißler et al. combined the isolation of specific SWCNTs and their subsequent functionalization for the detection of neurotransmitters and other small molecules.[49] They used aqueous two-phase extraction to obtain chirality-pure (6,5)-, (7,5)-, (9,4)-, and (7,6)-SWCNTs and applied a surface exchange through dialysis to remove the DOC wrapping of the SWCNTs and replace it with specific ssDNAs sequences or aptamers. This approach enabled the fabrication of ratiometric sensors, for example, for the detection of dopamine and H$_2$O$_2$ (Figure 22b,c). For these sensors, SWCNTs were functionalized either with a (GT)$_{40}$ sequence or, in the case of H$_2$O$_2$ detection, with an aptamer (hemin binding aptamer, HeApta) that binds the protoporphyrin hemin and catalyzes the decomposition of H$_2$O$_2$. PEG-PL-functionalized SWCNTs of another chirality served as an invariant reference. These examples show the potential of ratiometric or multiplexed sensing and given the large wavelength range of SWCNT fluorescence, there are plenty of opportunities for advanced sensing schemes.

3.4.3. Remote Imaging and Alternative Excitation Pathways

Remote imaging—the spatial separation of molecular sensors and detectors—is particularly beneficial to observe biochemical processes non-invasively, for example, in biomonitoring or in vivo. As only a small portion of the emitted light is captured by a camera within a certain distance to the sensors, remote detection requires either bright fluorophores or cameras with a high quantum yield in the spectral window of the fluorophore emission.

An example for remote imaging are NIR fluorescent nanosheets, which were remotely detected in a customized portable setup.[171,188] Due to the high brightness and emission closer to the visible range (910 nm), a low-cost CMOS camera (Complementary Metal Oxide Semiconductor, Si based),
which typically has a low quantum efficiency of around 5% in the NIR region, could be used here.

Additionally, this setup was modified for SWCNT detection by using an NIR-sensitive InGaAs camera, a white-light LED, and corresponding filters for wavelength-specific excitation and detection of emission (Figure 22a). Up to now, remote imaging of fluorescent SWCNT-based sensors has been used for the identification of bacteria and monitoring of plant health, for which resolutions in the (sub-)millimeter range were achieved with standoff distances of up to 1 m.

Apart from exploiting the decreasing sensitivity of Si-detectors in the NIR, efforts have been made to promote NIR-fluorescent transducers using straightforward designs for inexpensive NIR fluorimeters. Instead of using high-cost InGaAs photodiode arrays, single InGaAs diodes were combined with a motorized stage controlled with an open source programming language. The robustness of these devices outside of the laboratory was demonstrated in a high-throughput format with field-side measurements of soil samples. Future developments might also show remote detection using pure (6,4)-SWCNTs with Si-based cameras and also versatile use as smart surfaces, for example, for monitoring contamination with pathogens, such as bacteria, on medical surfaces or even implants.

Fluorophore brightness is not the only performance-related factor when measurements in live biological tissues are carried out. Fluorophore stability as well as wavelength-dependent tissue scattering, absorption, and the background originating from autofluorescence must also be considered. From this point of view, SWCNTs have excellent photophysical properties.

However, imaging at the single molecule level is always accompanied by considerations to achieve a high signal to noise ratio. Long-term single SWCNT imaging has, in particular, become established for (6,5)-SWCNTs in living cells and brain tissue.

Although the second-order excitonic transition, $E_{22} (s_{22})$, is typically used for excitation, alternative excitation pathways such as the K-momentum exciton-phonon sideband (KSB) excitation and up-conversion excitation have been successfully demonstrated, but excitation efficiency, photostability, as well as absorption and scattering of molecules in tissue differ depending on the excitation wavelength. Danné et al. evaluated the different excitation options (Figure 22b) for optimal single (6,5)-SWCNT imaging.

Here, $E_{22}$ excitation of PEG-PL SWCNTs was found to be four times more efficient than KSB excitation and an order of magnitude more efficient than upconversion excitation, while the signal to noise ratio was more than five times higher for KSB and up-conversion excitation. However, the excitation at the $E_{22}$ transition is not ideal due to limited tissue penetration depth and autofluorescence. In addition, simulations to quantify the impact of tissue absorption showed that a higher temperature rise of the tissue induced by upconversion excitation might be an issue, thus suggesting that KSB excitation is the best choice when considering all the factors. Nevertheless, it still requires relatively high excitation doses in the kW cm$^{-2}$ regime.

Recently, single SWCNT imaging was performed in brain tissue in vivo by using ultralow excitation doses of 0.1 kW cm$^{-2}$ (Figure 22c). To achieve this, sp$^3$ defects were introduced into (6,5)-SWCNTs, which lead to a fluorescence emission at $E_{11}^*$ (1160 nm) when exciting at the first-order excitonic transition $E_{11}$ (985 nm). This approach is beneficial as a result of excitation in the NIR window, but also because of the increased brightness by channeling free excitons to defect sites and subsequent $E_{11}^*$ emission.

Another way to both excite and detect in the NIR region is the use of multiphoton microscopy. This technique relies on the nonlinear excitation of at least two photons and is optimal for in vivo tissue applications when using NIR radiation. Del Bonis-O’Donnell et al. demonstrated two-photon 1560 nm excitation of dopamine-sensitive SWCNT-based sensors, which showed only 4% scattering (one-photon excitation: 42% scattering). However, the frame rate is significantly reduced for single sensor imaging compared to wide-field one-photon excitation.

3.4.4. Hyperspectral and Spinning Disc Microscopy

Hyperspectral microscopy (i.e. simultaneous imaging at different wavelengths) provides another approach to exploit the spectral variety for multiplexed SWCNT imaging. Roxbury et al. resolved up to 17 distinct chiralities with single nanotube spatial resolution. In contrast to organic fluorophores, more chiralities can be imaged simultaneously in a certain emission window due to the narrow emission bands of SWCNTs. The hyperspectral imaging is based on the use of a volume Bragg grating (VBG) placed between the emission port of an inverted fluorescence microscope and the NIR camera. The VBG filters one specific emission wavelength depending on optical properties such as the incident angle and the grating period $\Lambda$ (Figure 23a).

The spatial imaging of different chiralities is then achieved by measuring a continuous stack of 152 images within a time frame of 20 s and 10 min depending on the signal intensity and, thus, the integration time. By using this approach 12 different chiralities from DOC-SWCNTs in live human cervical cancer cells could be detected (Figure 23b). Additionally, individual SWCNTs adsorbed on a surface in live mammalian cells, murine tissues ex vivo, and zebrafish endothelium in vivo were imaged.

Another challenge of typical wide-field microscopy setups is the poor $z$-resolution. To improve this limitation, a NIR spinning-disc confocal laser microscope with an increased resolution and imaging contrast was demonstrated by Zabkovs et al. (Figure 23c). The custom-built microscope was based on a spinning-disc module integrated between a cooled InGaAs camera and the microscope body, which rejects out of focus light.

To achieve a maximized photon intensity, the lenses in the spinning disc unit were optimized for the NIR region. The authors showed in different biological applications the advantages of an improved lateral/axial resolution of $0.5 \pm 0.1 \mu m / 0.6 \pm 0.1 \mu m$ (enhancement of 17% / 45%) compared to the wide-field configuration, reaching from single-particle tracking over the spatial distribution of nanoparticles within...
an organelle to the optical in situ monitoring of glucose by GOx-SWCNT-based sensors embedded within an agarose gel. Overall, this approach showcases the opportunities for in vitro and in vivo imaging and sensing with improved spatiotemporal resolution.

4. Outlook and Perspectives

SWCNTs have shown their large potential as versatile building block for biosensors. The last few years have provided fundamental mechanistic insights, novel reactions, and novel applications such as sensing in plants or primary cells. The advent of covalent quantum defect chemistry as well as the broader availability of monochiral samples will further advance typical figures of merit such as selectivity and robustness in demanding biochemical environments. One of the key challenges remains a basic understanding of molecular recognition and also signal transduction in the organic phase around these materials. Advances in this space can directly translate into superior selectivities. Additionally, high-throughput or screening approaches will increase the speed of chemical discoveries. This is particularly interesting for applications in complex biological environments, where interactions become too complex to be predicted. Several milestones remain to be achieved before transfer into commercially available products. In vivo applications require long-term evaluation of each sensor’s toxicology and stability profile. Here, new covalent quantum defect functionalization strategies as well as the standardization of protocols and the purity of SWCNTs offer promising opportunities. Additionally, higher degrees of multiplexing and the adaptation of conventional cameras and readout systems will increase the application potential of SWCNT-based sensors. In summary, SWCNT-based biosensors offer a rich playground for chemistry and related disciplines and promise further advances and breakthroughs in the near future.

Appendix

Figure 23. Advances in spatial and spectral resolution. a) A Volume Bragg grating filters one specific emission wavelength depending on the incident angle $\theta$, refractive index $n$, and grating period $A$, which enables hyperspectral imaging. b) Detection of 12 different SWCNT chiralities without deconvolution in live human cervical cancer cells. Reprinted from Ref. [197] with permission. c) Improved NIR image contrast of NIR fluorescent beads with a spinning disc NIR fluorescence microscope in comparison to wide-field microscopy. Reprinted from Ref. [198] with permission.
| Target     | Biological system                          | Recognition strategy                                      | Sensitivity | Selectivity | SWCNT chirality/ wavelength | Spatial resolution | Temporal resolution | Reversibility |
|------------|--------------------------------------------|----------------------------------------------------------|-------------|-------------|-----------------------------|--------------------|--------------------|---------------|
| Arsenite   | In buffer (NaCl, MES, TES), embedded in plant tissue by syringe infiltration, uptake by plants through the roots | Screening approach: (GT)_n-SWCNTs FI increase TO-PRO-1-(GT)_n-SWCNTs (dye) FI decrease | In buffer (NaCl) LOD 122 nm | Over other heavy-metal ions present in soil, e.g. Mn^{2+}, Cd^{2+}, Pb^{2+}, Ni^{2+}, Hg^{2+} | HiPCO (9.4) at 1128 nm Dye for additional fluorescence in the visible range: 540 nm | Sub-mm for a standoff distance of 1 mm | Real time | No |
| Group 2 and 12 metal ions[^20,109] | In solution (NH_4OH, NaOH) | SDBS-SWCNTs FI decrease | 0.5—5 mM tested | Varying quenching efficiency for different ions and SWCNT chiralities | HiPCO Analysis of each individual chirality at 925—1425 nm | Measurement after 1 h of metal ion addition | ND | Yes |
| Divalent ions[^20,109] | In Tris buffer, blood, black ink, tissue, within living mammalian cells | ssDNA-SWCNTs ssDNA tested: (GT)_n, (GG)_n, 5’-TAG CTA TGG AAT TCC TCG TAG GCA-3’ Red-shift | Concentration and chirality dependent red-shift for different cations | Responds to different divalent ions | Chirality mix (6.5) peak mostly analyzed | ND | Within minutes | Yes, with dialysis to remove ions |
| H_2O_2, H_1, Fe(CN)_6^{3–}[^20] | Embedded in collagen film | Collagen-SWCNTs FI changes in discrete quenching steps | Different quenching equilibrium constants: 1.59/1.37 for H_2O_2/Fe(CN)_6^{3–} at 20 μM, similar constant for H^+ at 0.1 M | HiPCO | Single molecule 4 pixels, 1600 nm × 900 nm | Real time | 1 s** | H_2O_2 and H^+ induced quenching reversible with MnO_2 catalyst for H_2O_2/NaOH decomposition |
| H_2O_2[^21] | H_2O_2 emitted from A431 human epidermal carcinoma cells | Collagen-SWCNTs FI changes in discrete quenching steps | Single molecule | HiPCO | Sub-μm | Real time | Typical observation time 3000 s | Yes |
| H_2O_2[^22] | H_2O_2 generated from live human umbilical vein endothelial cells stimulated by vascular endothelial growth factor (VEGF) and artificial proangiogenic factor Eu(OH)_3 nanorods | Collagen-SWCNTs FI changes in discrete quenching steps | Single molecule 12.5—400 nm | HiPCO | Single molecule 300 nm pixel size** | Real time | 1 s** | Yes |

[^1]: Authors. Angew. Chem. Int. Ed. 2022, 61, 202111272 (Of 50)
Table 1 (Continued)

| H₂O₂[11] (GT)₂-SWCNTs FI quenching Alteration of Raman G-band intensity | m mol L⁻¹ range tested | ND | HiPCO Raman G-band intensity at 1590 cm⁻¹ | µm | Real time Measurement after 0-48 h of gemcitabine/irinotecan-treatment | Yes |
| H₂O₂[11] Embedded in leaves of Arabidopsis plants, in vivo monitoring of stresses (UV-B light, high intensity light, pathogen-related peptide) | Real-time | Yes |
| H₂O₂[11] Embedded in leaves of Arabidopsis plants, in vivo monitoring of wound-induced H₂O₂ waves and other stresses (high intensity light, heat stress, pathogen-related peptide) | 10—100 µm | Over Ca²⁺, sugar (sucrose, glucose), plant hormone levels (methyl salicylate, abscisic acid (ABA), jasmonic acid (JA)), mechanical wounding | HiPCO >900 nm-1250 nm | Sub-mm with standoff detection | Real time 60/120 min for saturation after pathogen/environmental-related stress (UV-B/high intensity light) | Yes |
| H₂O₂[11] Embedded in wearable microfibrous textiles, in presence of peroxide-producing macrophages, incorporated in wound bandages | 5 µm—5 mm | Over other plant analytes (JA, auxin, ABA, salicylic acid, glutamates, Ca²⁺ ions), NO, NO₂⁻, antioxidant O₃, OH caused FI decrease, but not reversible | HiPCO 950 nm—1250 nm | Sub-mm for a standoff distance of 1 m Wave speeds due to different stress treatments from 0.44 to 3.10 cm min⁻¹ | Real time Response within 4 min after wound infliction, recovery after 10-20 min | Yes, due to unbinding or consumption by antioxidants and peroxides |
| NO/H₂O₂[11] Leaves mounted on microfluidic chamber, in vivo in leaves of Arabidopsis plants | See Refs. [20, 32, 110] | HiPCO (8.7)/(9.4) | Sub-µm | Real time within 5—30 min | Yes, within 50 min |
| NO/H₂O₂[11] In PBS, macrophage cells, mouse model | See Ref. [110] | Monochiral (7.6) | Sub-µm | Real time in vitro/in vivo: >600 s/50 s for saturation | Yes |
| NO[12] Immobilized in gelatin | LOD in solution/cells 70 nm/200 nm | Over other ROS/RNS (e.g. NO−, NO₂⁻, ONOO−, HNO, OCl−, OH·, H₂O₂) Chirality mix 950—1350 nm | Sub-µm in cells | Real time Bleaching rate k=0.856 s⁻¹ for (10,5)-SWCNTs | With reducing agent |
| NO[12] Screening approach: (AT)₂-SWCNTs FI changes in discrete quenching steps | Single molecule LOD 300 nm | Over other ROS/RNS, dopamine, NADH, L-ascorbic acid, riboflavin | HiPCO 900—1400 nm | Single molecule 4 pixels, 580 nm x 580 nm² | Real time Response time for NO: 1.1 s | Yes |
Table 1 (Continued)

| NO[127] | Intraavenous injection into mice, localization within the liver Implantation of alginate-encapsulated SWCNTs within specific tissue | PEG-(AAAT)$_2$-SWCNTs FI decrease | LOD 1 μM | Less quenching with other ROS/RNS | CoMoCAT-SWCNTs (6,5) at 990 nm | Sub-mm | For injection/implantation within s min$^{-1}$ (93% quenching after 30 min) | Yes |
|---|---|---|---|---|---|---|---|---|
| NO[128] | In cultures of A375 melanoma cells through micropinocytosis, NO production using NO-releasing anticancer drug JS-K VEGF-mediated NO production in endothelial cells | (AT)$_2$-SWCNTs FI decrease | JS-K concentrations of 16–28 μM tested VEGF concentrations between 10 ng mL$^{-1}$ (LOD) and 100 ng mL$^{-1}$ | See Ref. [13] | HiPCO | Sub-μm | Real time | Yes |
| NO[129] | In extracted chloroplasts and leaves of Arabidopsis plants, in vivo by infiltration through the leaf lamina | (AT)$_3$-SWCNTs FI decrease | 40%—60% FI decrease | See Ref. [13] | HiPCO | (8,6), (12,1), (11,3), (8,7), (10,5) 1150—1450 nm | Sub-μm | Real time | Yes |
| O$_2$[131] | In buffer (NaH$_2$PO$_4$/Na$_2$HPO$_4$) | Screening approach: 10 ssDNA sequences tested, e.g. (GT)$_{10}$, (GT)$_{20}$, (ATT)$_{10}$-SWCNTs FI decrease | Depending on ssDNA and SWCNT chirality, 9—40% quenching with 1 atm O$_2$ compared to samples purged with 1 atm Ar 0.01—1 m M of tyrosinase substrates Response curves for different tyrosinase inhibitors measurable, IC$_{50}$ values of suicide inactivation, e.g. 0.5 mM for kojic acid Single molecule | Relatively insensitive to pH | HiPCO | ND | Within seconds | Yes |
| 'O$_2$[130] | SWCNTs challenged with different tyrosinase substrates for enzyme-catalyzed reactions Tyrosinase-conjugated-PEG$_2$-(GT)$_{15}$-amine-SWCNTs Red-shift and FI decrease | For tyrosinase inhibitors which induce suicide inactivation of the enzyme | | | HiPCO | 950—1350 nm | Single-sensor | Within minutes 0.0281 nm min$^{-1}$ with l-tyrosine, 0.75 nm min$^{-1}$ with pyrogallol | ND |
| Single protons/pH[45] | Embedded in agarose gel SDBS-SWCNTs FI changes in discrete steps | Response to chemical reactions with acid, base, diazonium reagents | HiPCO individual chiralities, e.g. (8,6) at 1175 nm, (8,3), (11,1) | Single molecule 4 pixels, 670 n m x 670 n m | Single-sensor | 54 ms | For acid reactions | **|

[a] FI: fluorescence intensity, ND: not determined, **: limited by the Abbe limit, diffusion, and detection speed.
| Target                  | Biological system                  | Recognition strategy                                                                 | Sensitivity                                                                 | Selectivity                                                                 | SWCNT chirality/ wavelength | Spatial resolution | Temporal resolution | Reversibility |
|-------------------------|------------------------------------|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------|----------------------------------------------------------------------------|----------------------------|-------------------|--------------------|---------------|
| Dopamine (DA)\(^{[15]}\) | In PBS, immobilized                | Screening approach: (GT)\(_5\)-SWCNTs and other candidates FI increase                  | Depending on chirality LOD in solution 11 nm                               | Response to other catecholamines with different magnitude                  | HiPCO (6,5) at 991 nm, (7,5) at 1044 nm, (10,2) at 1077 nm, (9,4) at 1132 nm, (8,6) at 1203 nm | Single sensor 4 pixels, 585 nm × 585 nm \(^{[2]}\) | Real time within seconds | Yes           |
| Reducing and oxidizing molecules, e.g. ascorbic acid/oxidized ascorbic acid, cysteine/cystine, DA, epinephrine (EPI), glutathione/oxidized glutathione, riboflavin, trolox\(^{[20]}\) | In PBS                             | FI increase with negatively charged polymer wrapping (e.g. ssDNA, poly(acrylic acid)) FI decrease with positively charged wrapping (polyallylamine-SWCNTs) No FI change with PLPEG-SWCNTs | 100 µm tested Polymer-wrapped SWCNTs that respond to reducing molecules (e.g. +141%, ascorbic acid) also respond to oxidizing molecules (e.g. 81%, riboflavin) Up to 250% FI increase with reducing molecules such as ascorbic acid, EPI, trolox with negatively charged wrapping, but redox potential alone cannot explain FI changes Different responses for different small molecules | Chirality-dependent FI change, e.g. smaller diameter SWCNTs with (TAT)_4 wrappings are less responsive than larger diameter SWCNTs | CoMoCAT (6,4), (8,3), (9,1), (6,5), (7,5) 800—1000 nm | ND                | ND                | ND            |
| Catecholamine (DA, EPI, norepinephrine (NE), amino acids, saccharides, riboflavin\(^{[20]}\) | In PBS                             | ssDNA-SWCNTs (24 sequences tested, e.g. (ATT)_4, (TAT)_4, (ATTTT)_3) FI increase for catecholamines and ascorbic acid | Different responses for different small molecules | Chirality-dependent FI change, e.g. smaller diameter SWCNTs with (TAT)_4 wrappings are less responsive than larger diameter SWCNTs | HiPCO (8,3), (6,5), (7,5), (10,2), (9,4), (7,6), (12,1), (11,3) | ND                | ND                | ND            |
| DA\(^{[100]}\)            | Collagen-coated SWCNTs for increased cell adhesion, immobilized on glass, PC12 neuroprogenitor cells cultivated on top | Screening approach: (GA)_5-SWCNTs FI increase Interaction of hydroxy groups of DA with phosphate groups of DNA backbone pulls phosphate groups closer to the SWCNT surface, which increases the FI. | Single molecule LOD 100 pm | Not over catecholamine homologues (e.g. EPI, NE, L-ascorbic acid), but PC12 cells mainly release dopamine | (6,5)-enriched chiralities (6,5) at 980 nm >20,000 sensors per cell 850 nm pixel size \(^{[2]}\) | <100 ms           | No                 | Yes           |

\(^{[1]}\) Table 2: Detailed overview of fluorescent SWCNT-based sensors for neurotransmitters.\(^{[4]}\)
Table 2 (Continued)

| Catecholamine (DA, EPI, NE) [118] | In PBS, DA in presence of NE | Screening approach: different ssDNA-SWCNTs, e.g. A30, (GT)10, (GC)15, (AT)15, (GT)10 for different selectivity and sensitivity | LOD mostly in single-digit nm regime | Dissociation constants vary between 2.3 nm ((GC)15-SWCNTs + NE) and 9.4 nm ((AT)15-SWCNTs + DA) | (6,5)-enriched at 987 nm | Single nano-sensor | Real time | Within seconds | Yes |
|---|---|---|---|---|---|---|---|---|---|---|
| Catecholamine release, e.g. DA [120] | Brain slices from dorsal striatum of mouse incubated in artificial cerebrospinal fluid (ACSF) | (GT)10-SWCNTs FI increase | 10 nm—100 μm | Over γ-aminobutyric acid, glutamate, acetylcholine in the presence of pharmacological DA receptor ligands | HiPCO (9.4) at 1128 nm | μm | Sub-second | Yes |
| DA [121] | In PBS | (GT)10-SWCNTs FI increase | >20% intensity increase (100 nm) | Not for DA over NE | ND | Monochiral | ND | ND | ND |
| DA, riboflavin, H2O2, pH [122] | In PBS | ssDNA-SWCNTs, e.g. (GT)10, (GT)10, A30, T30, C30 | In most cases FI change does not correlate with the number of adsorbed ssDNA molecules | Different responses for different small molecules | HiPCO | Sub-μm | Real time | 111 ms | Yes |
| DA [123] | Acute mouse brain slices in ACSF, electrically evoked neurotransmitter release | PEG:PL-(GT)10-SWCNTs FI increase | Improved sensor response due to PL-induced quenching of SWCNT baseline FI | Reduced protein adsorption of 28% compared to nonpassivated sensors | HiPCO | Sub-μm | Real time | 111 ms | Yes |
| DA [124] | Self-assembled on neutravidin-coated microscopy slides | Screening approach: Combination of covalent and noncovalent SWCNT functionalization (GT)10-amine-PEG2k-biotin-SWCNTs (GT)10 is able to detect DA, while biotin is able to detect neutravidin and streptavidin | Δf/f0 = 0.8658 ± 0.0218 for 25 μM DA | ND | HiPCO (10,2) at 1053 nm | ND | Real time | Sub-seconds | Yes |
| Compound | SWCNT uptake, transport, and programmed release by human immune cells (neutrophilic granulocytes, neutrophils) |
|----------|----------------------------------------------------------------------------------------------------------------|
| DA, H₂O₂ | DA: (GT)₁₅-SWCNTs, FI increase H₂O₂: Heminaptamer-SWCNTs, FI decrease 100 nM DA and 100 μM H₂O₂ tested Sensors functional after cargo transport and release |
| DA¹¹⁹ | Immobilized on surface in complex medium (e.g. Schneider’s medium) In presence of CaCl₂, Drosophila neurons |
| DA, NE¹¹⁶ | In PBS, NaCl, protein-rich media (DMEM + 10% fetal bovine serum (FBS)) and ACSF |
| DA, riboflavin, pH, H₂O₂ | Immobilized Release from primary neurons (varicosities) using a sensor paint (AndromeDA) |
| DA¹³⁹ | Release from primary neurons (varicosities) using a sensor paint (AndromeDA) |
| Serotonin²⁷ | Release from human blood platelets |
| Serotonin²⁷ | Screening approach: Identification of polynucleotides by exponential enrichment for selective ssDNA-SWCNTs |

[a] FI: fluorescence intensity, ND: not determined, —: limited by the Abbe limit, diffusion and detection speed.
| Target                      | Biological system                                                                 | Recognition strategy                                                                 | Sensitivity          | Selectivity                                               | SWCNT chirality/ wave-length | Spatial resolution | Temporal resolution | Reversibility               |
|-----------------------------|-----------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|----------------------|-----------------------------------------------------------|------------------------------|---------------------|---------------------|--------------------------|
| Acetic acid                 | In DI water, wine; Analytes injected into glass bottle with inlet and outlet tubes | Screening approach: Peptide-encapsulated SWCNTs (YK-SWCNT) detect volatile molecules  | In DI water: concentrations of 0.05%–3.2% (v/v) tested     | Slightly increased sensor response at higher humidity results in stronger quenching due to additional binding of water molecules to the sensor | CoMoCAT (6,5) at 970–1050 nm | ND                  | Real time           | Yes, but slow; illumination with UV light enhances recovery speed |
| Adenosine-5'-triphosphate (ATP) | In Tris-HCl buffer, cellular ATP detection in living HeLa cells                    | Screening approach: PL-PEG-functionalized SWCNT/luciferase enzyme conjugate  | LOD 240 nM          | Over Adenosine 5'-monophosphate, adenosine 5'-diphosphate, cytidine 5'-triphosphate, guanosine 5'-triphosphate | Chirality mix μm             | Real time           | Measurement after 15 min of analyte addition | No                       |
| DA, riboflavin, pH, H₂O₂   | Immobilized                                                                       | DA: (GT)₉₀-SWCNTs Riboflavin: (GT)₉₀-SWCNTs pH: PEG-PL-SWCNTs H₂O₂: hemin-binding aptamer-SWCNTs | LOD 8 μm            | Affected by surfactant residues, differences in sensing magnitudes for different sequences/analytes          | Chirality mix (6,5) at 990 nm, (7,6) at 1130 nm | ND                  | ND                  | ND                       |
| Doxorubicin                 | In water or water/DMSO mixture, human blood plasma, mouse tissue                  | Screening approach: CCCCCCCCCAGAATTACTTCCCCCCCC-SWCNTs FI change and red-shift (shift for readout) | LOD 50 μm           | Over dacarbazine (chemotherapeutic, commonly co-injected with doxorubicin)                                      | Chirality mix (7,6), (6,5) | ND                  | Steady state within minutes | Yes                      |
| Doxorubicin                 | In saline-sodium citrate buffer, FBS, intracellularly (incubated in Raw 264.7 murine macrophage cells), incorporated into membrane and implanted into peritoneal cavity of living mice | (GT)₁₀-SWCNTs FI decrease and red-shift                                             | LOD 50 μm           | Over other anthracycline chemotherapeutic drugs (epirubicin, daunorubicin) Not over other DNA-intercalating agents (SYBR green, Hoechst 33258, ethidium bromide, 1-pyrenebutyric acid) | HiPCO (9.4) | Sub-μm             | Real time           | Within minutes            | No                       |
| Table 3 (Continued) |
|----------------------|
| **Human steroid hormones (cortisol, progesterone)**<sup>[144]</sup> | In DMSO, PBS, 10% mouse serum, subcutaneously implanted in mice (progesterone detection, SWCNTs in HG) | Semirational approach: Cortisol sensor: p(AA<sub>197</sub>-ran-AC<sub>5</sub>)-SWCNTS Progesterone sensor: p(AA<sub>53</sub>-ran-S<sub>22</sub>-ran-AC<sub>4</sub>)-SWCNT F1 increase | In solution: cortisol: 10–100 μM, progesterone: 5–100 μM | Over other steroids by a factor of 2 Progesterone additionally over other small molecules and large proteins | HiPCO (6.5) for cortisol sensor, (7.6) for progesterone sensor | Sub-mm | 3 h needed for stabilization of fluorescence signal in HG | Yes |

| **Nitroaromatics**<sup>[141]</sup> | In solution, immobilized on glass in Tris buffer | Screening approach: Bombolitin II-SWNTs Wavelength shift (AT)<sub>15</sub>-SWCNTs for TNT detection F1 changes in discrete quenching steps | Distinction with PCA possible: over several containing nitroaromatic and non-aromatic compounds containing nitro groups, e.g. picric acid, cycloheximethylenetrimine, 2,4-dinitrophenol, over other nitroaromatics (e.g. RDX, TFM) but 2,4-dinitrotoluene and 2-nitrophenol show quenching to a lesser degree | HiPCO Analysis of 8 chiralities, e.g. (7,5), (11,3) | Single molecular | Real time 500 ms–1 s | Yes |

| **Nitroaromatics/picric acid**<sup>[30]</sup> | Within spinach plant leaf mesophyll by root uptake or through direct uptake through leaf surface | Bombolitin II-SWNTs F1 quenching | 85%/78% quenching with 400 μM picric acid by root/leaf uptake | HiPCO (6.5) enriched chiralities | 0.5 mm pixel size for a standoff distance of 0.85 m | Real time 5–15 min transport time of picric acid uptake from the roots to the leaves, 40–50 min for saturation Sensor response after 10 s by leaf uptake | No |

<sup>[144]</sup> Human steroid hormones including cortisol and progesterone were detected using semirational approaches. Cortisol was detected using a sensor containing polypeptide (AA<sub>197</sub>-ran-AC<sub>5</sub>)-SWCNTS, while progesterone detection utilized a sensor composed of polypeptide (AA<sub>53</sub>-ran-S<sub>22</sub>-ran-AC<sub>4</sub>)-SWCNT. In solution, cortisol was detected at concentrations ranging from 10 to 100 μM, and progesterone was detected at concentrations ranging from 5 to 100 μM. Over other steroids, cortisol and progesterone showed a factor of 2 increase in detection sensitivity compared to other small molecules and large proteins. Progesterone detection was particularly sensitive, showing an additional increase over other small molecules and large proteins.

<sup>[141]</sup> Nitroaromatics were detected using a screening approach involving Bombolitin II-SWNTs. Wavelength shifts observed in the presence of nitroaromatic compounds were indicative of distinct quenching steps. Distinction from non-aromatic compounds, such as picric acid, cycloheximethylenetrimine, 2,4-dinitrophenol, was achieved through PCA analysis. The detection was performed using HiPCO analysis of 8 chiralities, e.g. (7,5), (11,3).

<sup>[30]</sup> Nitroaromatics and picric acid were detected within spinach plant leaves via root uptake or direct uptake through the leaf surface. Quenching effects were observed at concentrations of 85% and 78% with 400 μM picric acid. Detection was performed using HiPCO enriched chiralities. The real-time transport time of picric acid from the roots to the leaves was measured to be 5–15 min, while the saturation sensor response was observed after 10 s by leaf uptake.

<sup>[144]</sup> and <sup>[141]</sup> references are cited for further detailed information on the methodologies and results.
| Odors, volatile molecules \(^{[14]}\) | Odors injected into glass bottle with inlet and outlet tubes | Array of five different peptide-encapsulated SWCNTs adsorbed on a nitrocellulose paper, recognition by LDA and machine learning | LOD around 216 ppm of ethanol | Slightly increased sensor response at higher humidity | Discrimination of a mixture of ethanol and methanol | CoMoCAT (6.5) at 1000 nm | ND | Real time | Yes, within 160 s |
|-----------------------------------|-------------------------------------------------------------|-----------------------------------------------------------------|-----------------------------|-----------------------------|-----------------------------------------------------|------------------------|--------|------------|-----------------|
| Alcoholic vapors (ethanol, methanol, propanol, 2-propanol) | | | | | | | | | Time within seconds |
| Distinction between aromas of red wine, beer, vodka | | | | | | | | | Steady state after 24 s, some continue to rise after 45 s |
| Distinction between limonene, undecanal, and geraniol vapors | | | | | | | | | |
| Phosphodiesterase type 5 (PDE5) inhibitor Vardenafil \(^{[14]}\) | In PBS | Screening approach: Poly(methacrylic acid-co-styrene)-SWCNTs with methacrylic acid and styrene at 90:10 ratio Synthetic corona mimics H loop of native enzyme PDE5 Sensor has smaller binding affinity than the enzyme, interaction is disrupted in the presence of PDE5a | LOD 0.02–0.2 μm by varying the polymer length | Over 22 tested small molecules, not over inhibitor Silde-nafil (chemical similar), but smaller emission modulation | HiPCO (8.3), (6.5) | ND | 5 min incubation time | ND |
| Polyphenols (tannins, flavonoids, ...) \(^{[14]}\) | In plant extracts, tissue culture media Sensors embedded in agar, pathogen-induced release from soybean roots (stimulated with pathogen-derived elicitor or mechanical wounding) | PEG-PL-SWCNTs FI decrease, red-shift | | | | | | | |
| Porphyrins/heme \(^{[14]}\) | Detection of plasma samples with solvent extraction method | Heme-binding-aptamer-SWCNTs FI decrease | LOD 20 nm | Over other porphyrins, BSA, lysozyme, phthalocyanine | CoMoCAT (7.5) | ND | Real time | ND |

\([14]\) Angew. Chem. Int. Ed. 2022, 61, e202112372 (34 of 50)
Table 3 (Continued)

| Riboflavin, l-thyroxine, oestradiol\(^{[b]}\) | In buffer | Screening approach: Oestradiol: Rhodamine isothiocyanate-difunctionalized poly(ethylene glycol)-SWCNTs FI decrease l-thyroxine: Fmoc-L-phenylalanine PEG-SWCNTs FI decrease Aliphatic chain and Fmoc group adsorb onto SWCNTs, strong interaction between Fmoc and l-thyroxine leads to molecular recognition Riboflavin: 53 mol/mol boronic-acid-substituted phenoxydextran-wrapped-SWCNTs (BA-PhO-Dex-SWCNTs) Red-shift through polymer dielectric change 0.6 FI change to 100 µm oestradiol/l-thyroxine and 11 nm shift to riboflavin | Over 35 biological molecules Chirality mix Deconvoluted into 8 chiralities (7,6) at 1147 nm for tracking of riboflavin in macrophage cells Sub-µm 15 mm in macrophage cells within seconds Yes, for riboflavin through riboflavin-binding protein |
| Riboflavin\(^{[113]}\) | SWCNTs embedded in HG with water and barium chloride, subcutaneously implanted into mice (GT)\(_{15}\)-SWCNTs FI decrease 10 nm riboflavin results in 48% quenching for HG SWCNTs with a concentration of 10 mg L\(^{-1}\) LOD in 5.4 mm depth tissue Pore size of HG can be engineered to exclude large molecular weight interfering molecules, average pore size: 3.2 nm (6,5)-enriched SWCNTs Sub-mm CoMoCAT \(^{[142]}\) mm In vitro: immediate response, fluorescence decreases continuously while riboflavin diffuses to CoMoCAT sensors | 0.6 FI change to 100 µm oestradiol/l-thyroxine and 11 nm shift to riboflavin | Chirality mix Deconvoluted into 8 chiralities (7,6) at 1147 nm for tracking of riboflavin in macrophage cells Sub-µm 15 mm in macrophage cells within seconds Yes, for riboflavin through riboflavin-binding protein |

Riboflavin\(^{[142]}\) | In HG for in vitro and ex vivo tissue measurement of marine organisms (Sparus aurata, Stenotomus chrysops, Galeus melastomus) (AC)\(_{15}\)-SWCNTs FI decrease In vitro: 1–100 µm in vivo: LOD in 7 mm skin and muscle tissue Pore size of HG can be engineered to exclude large molecular weight interfering molecules, average pore size: 15 nm | Two characteristic quenching times: 14.1 min (riboflavin-SWCNT reaction), 5.8 h (riboflavin diffusion) for HGs with 10 mg L\(^{-1}\) SWCNT concentration ND | CoMoCAT ND |

[a] FI: fluorescence intensity, ND: not determined, HG: hydrogel.

\(^{[b]}\) HG: hydrogel.  
\(^{[113]}\) GT: graphene nanotube.  
\(^{[142]}\) AC: alginate.

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Table 4: Detailed overview of fluorescent SWCNT based sensors for lipids.\(^a\)

| Target                  | Biological system                                                                 | Recognition strategy                                                                 | Sensitivity   | Selectivity                                      | SWCNT chirality/wavelength | Spatial resolution | Temporal resolution | Reversibility                                                                 |
|-------------------------|------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|---------------|-------------------------------------------------|---------------------------|--------------------|---------------------|-------------------------------------------------------------------------------|
| Endolysosomal lipids\(^{117}\) | In PBS, in fibroblasts from an NPC patient (Niemann-Pick type C, lysosomal storage disease) | Screening approach: (GT)\(_{51}\)-SWCNTs adsorbed onto nitrocellulose paper          | Dissociation constant 2.2 \(\mu\)m | Less FI increase to fat-soluble vitamins (menadione, retinyl acetate and \(\alpha\)-tocopherol) | HiPCO (8.6) at 1200 nm | Subcellular         | < 2 min for incubation of fibroblasts from NPC patient with SWCNTs         | Yes, upon administration of a drug (hydroxypropyl-\(\beta\)-cyclodextrin) that reverses disease phenotype |
| Endolysosomal lipids\(^{149}\) | In cell culture medium with 10% FBS, intravenously injected into mice with NPA/B and NPC disease to detect lipid accumulation in the Kupffer cell endolysosomal organelles | Screening approach: Chitosan-SWCNTs modified with Cu\(^{2+}\)-NTA/His-tagged protein A interacting with human IgG leading to ion based proximity quenching | LOD below 660 nm | ND                                              | CoMoCAT (6.5) | mm                  | Real time            | ND                                                                           |

[a] FI: fluorescence intensity, ND: not determined.

Table 5: Detailed overview of fluorescent SWCNT based sensors for proteins.\(^b\)

| Target                  | Biological system                                                                 | Recognition strategy                                                                 | Sensitivity   | Selectivity                                      | SWCNT chirality/wavelength | Spatial resolution | Temporal resolution | Reversibility                                                                 |
|-------------------------|------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|---------------|-------------------------------------------------|---------------------------|--------------------|---------------------|-------------------------------------------------------------------------------|
| \(\beta\)-Carotene\(^{114}\) | In canola oil                                                                      | Screening approach: (GT)\(_{51}\)-SWCNTs adsorbed onto nitrocellulose paper          | LOD 3 mg L\(^{-1}\) | Potential interfering proteins such as transferrin, \(\gamma\)-globulins, degraded albumin showed either negligible change or red-shift | HiPCO (9.4) | ND                  | Saturation after 20 min | ND                                                                           |
| Aggregation status of human IgG\(^{139}\) | In HG                                                                             | Chitosan-SWCNTs modified with Cu\(^{2+}\)-NTA/His-tagged protein A interacting with human IgG leading to ion based proximity quenching | LOD below 660 nm | ND                                              | CoMoCAT (6.5) | mm                  | Real time            | ND                                                                           |
| Albumin\(^{138}\)        | In clinical urine samples of patients with microalbuminuria                        | Screening approach: Constructed polymer mimics fatty binding to albumin Carboxylate-functionalized poly-carboxidiimide polymer-SWCNTs incorporated into acrylic-based paint Blue-shift, FI increase | LOD 1–5 nm    | Response to BSA, but with three orders less sensitivity | HiPCO 900—1500 nm | ND                  | ND                  | Yes                                                                          |
| Avidin                  | In solution                                                                        | SWCNTs noncovalently bound to dye-ligand conjugates (biotinylated anthracene), which are covalently bound to a biological receptor ligand (biotin), which binds to the analyte | LOD 1–5 nm    | FI increase                                     | HiPCO 900—1500 nm | ND                  | ND                  | Yes                                                                          |
| Cardiac biomarker troponin T<sup>64</sup> | In HG | Rational approach: Chitosan-wrapped SWCNTs non-covalently modified with troponin antibody using a Ni<sup>2+</sup>-NTA/hexa-histidine-tagged protein mechanism for divalent ion-based proximity quenching | LOD 2.5 nm | Over BSA, IgG, cancer biomarker Pisum sativum agglutinin (PSA), 10000 times diluted human plasma (7 μg·mL<sup>-1</sup> proteins) | Affected by viscosity change (e.g. 100 times diluted plasma), unable to quantitatively detect in full plasma | ND | Yes | Within 5 min saturation |
| Cell surface receptors CD20, HER2/ neu<sup>205</sup> | Incubation of cells in SWCNT solution | Rational approach: PL-PEG-NH<sub>2</sub>-SWCNTs conjugated to antibodies Rituxan antibody to recognize CD20 cell surface receptor on B-cell lymphoma Herceptin to recognize HER2/neu positive breast cancer cells | ND | ND | ND | 1 h incubation time before measurement |
| Electrostatic charge accumulation mediated by cell membrane proteins<sup>206</sup> | SWCNTs in contact with cell monolayer (HeLa cells, murine fibroblast cell line (NIH/3T3), human lymphocyte cell line (Jurkat)) | Emission energy correlated with the degree to which a cell adheres to a substrate and the zeta potential of the cell Jurkat cells: Δλ = 0.62 ± 0.28 nm NIH/3T3: Δλ = 4.97 ± 0.23 nm HeLa cell: Δλ = 2.72 ± 0.12 nm | Cell type dependent trend | HiPCO 900–1600 nm | 20 μm pixel size<sup>**</sup> | ND | ND |
| Fibrinogen<sup>71</sup> | In serum (10% FBS in PBS) | Screening approach: Dipalmitoyl-phosphatidylethanolamine (DPPE)-PEG (5 kDa)-SWCNT | Negligible response of < 5% intensity to 13 proteins (some are highly abundant in blood and others are relatively rare but of clinical significance) | HiPCO (9,4), (7,6) | Single sensor | Real time | ND |
| Glutathione-S-transferase (GST) fusion proteins<sup>154</sup> | In solution | Glutathione-(TAT)<sub>6</sub>-SWCNTs | Responds to multiple classes of GST-tagged proteins (e.g. cell-cycle proteins, RNA-binding proteins, ovarian cancer protein biomarkers) | HiPCO (8,6), (9,1) | ND | Real time | ND |

<sup>A</sup> Angew. Chem. Int. Ed. 2022, 61, 202112372 (37 of 50)
<sup>**</sup> 10 mm mineral particle size (measured at 40% relative humidity and 22°C temperature)
Table 5 (Continued)

| Green fluorescent protein (GFP), GFP-fusion proteins<sup>[79]</sup> | Incubation with GFP | GFP-binding nanobody-SWCNTs, covalently bound aryl maleimide. Quantum defects introduced by light-driven diazonium chemistry serve as anchor points for peptides and proteins, conjugation of cysteine-containing proteins to maleimide | ND | Over negative control, where no diazonium salt was added | E<sub>1</sub><sup>1</sup> at 1135 nm μm | ND | ND |

| Gynecologic cancer biomarker human epididymis protein 4, cancer antigen 125, chitinase-3-like protein, mesothelin<sup>[100]</sup> | In 10% FBS, uterine lavage samples | Machine-perception-based sensor array consisting of 132 distinct DNA-SWCNT complexes: 11 ssDNA-SWCNT-based sensors with 12 chiralities, e.g. (AT)<sub>12</sub>, (GT)<sub>12</sub>, (ATT)<sub>4</sub>, (TCT)<sub>5</sub>-SWCNTs FI increase/decrease and wavelength shift | LOD in pm range | Simultaneous distinction/detection of biomarkers with classification accuracy (F1-score) of ca. 0.95 | HiPCO | ND | ND | ND |

| Human α-thrombin<sup>[207]</sup> | In PBS | Rational approach: Dye (FAM)-labelled human α-thrombin binding aptamer-SWCNTs | LOD 1.8 nm Dynamic range 4–150 nm | In presence of 100 nm of other proteins (BSA, HAS, IgG) | HiPCO | ND | Incubation of target 2–3 h | ND |

| Immunoglobulin IgG<sup>[100]</sup> | In HG | Rational approach: Chitosan-wrapped SWCNT noncovalently modified with immunoglobulin-binding proteins A using a Cu<sup>2+</sup>-NTA/His-tagged protein mechanism for divalent ion based proximity quenching | LOD 10 ng mL<sup>–1</sup> | ND | HiPCO | ND | Real time | ND |

| Immunoglobulin (human IgG, mouse IgM, rat IgG2a, human IgD)<sup>[111]</sup> | Ink printed | Rational approach: Chitosan-SWCNTs noncovalently modified with immunoglobulin-binding proteins. Cu-NTA chelating chemistry with histidine tag for divalent ion based proximity quenching Binding proteins: protein A, protein G, protein L | LOD 25 μg mL<sup>–1</sup> of human IgG, 2.5 μg mL<sup>–1</sup> at 20x magnification | Different association constants for different antibody/binding proteins | CoMoCAT (6, 5) at 980 nm | Sub-μm | Real time | Yes |

| Insulin<sup>[159]</sup> | In buffer | Insulin-binding aptamer-SWCNTs FI quenching | Lowest concentration tested: 9 nm | Over BSA, proteinase K | HiPCO (6, 5) | Sub-μm | ND | ND |
### Table 5 (Continued)

| Insulin$^{[106]}$ | In collagen extracellular matrix | Insulin-binding aptamer-SWCNTs | LOD 10 nm | Over BSA, proteinase K, cell culture media RPMI 1640, glucose | CoMoCAT (7,5) at 1044 nm | Sub-μm | Real time | Yes, through enzymatic proteolysis within 1 h |
|----------------|----------------------------------|---------------------------------|-----------|------------------------------------------------------------|--------------------------|---------|-----------|-------------------------------------------|
|                | Insulin secreted by pancreatic β-cells due to glucose addition | FI quenching upon analyte binding by photoinduced charge-transfer mechanism (electron transfer from conduction band of SWCNTs to LUMO of the bound insulin) |           | Within seconds | F1 quenching rate | 5.85x10^{14} M^{-1} s^{-1} diffusion reaction rate | 0.129 s^{-1} | Measurement after 30 min of insulin addition |
| Insulin$^{[107]}$ | In PBS, blood serum (10% FBS in PBS) | Screening approach of PEG-conjugated lipids C_{16}PEG(2000 Da)-ceramide-SWCNTs | F1 decrease | Over library of proteins (<5% intensity change) | HiPCO (10,2) | ND | 5 min incubation time | ND |
|                | Insulin secreted by pancreatic β-cells due to glucose addition | C_{16}PEG(2000 Da)-ceramide-SWCNTs (AT)$_{15}$-Insulin-binding aptamer-SWCNT | 8.1 nm-74 nm tested | Not over apolipoprotein A-I and longer fragments of isolated α- and β-peptide chains of insulin | HiPCO (10,2) | (6,5) | Detection in real time | ND |
| Integrins$^{[66]}$ | Immobilized on epithelial cells | Arg-Ly-Asp (RGD) peptide conjugated to different ssDNA sequences in linear ssDNA-RGD or bridged ssDNA-RGD-ssDNA geometries | Integrin affinity of RGD motif depends on its conformational freedom due to ssDNA-RGD geometry | DNA sequence affects overall RGD affinity | CoMoCat | ND | ND | ND |
| Human platelet integrin α_{IIb}β_{3} | | | | | | | | |
| Green fluorescent protein (GFP), GFP-fusion proteins$^{[70]}$ | Tracking of motor protein (Kinesin-S-GFP) in embryos of Drosophila melanogaster | Rational approach: GFP binding nanobodies conjugated to DNA-wrapped SWCNT GBP-(GT)$_{15}$-SWCNTs | ND | CoMoCAT (6,5) | Single sensor | Real time | Resolving the velocity of a molecular motor in vivo at 1340 nm s$^{-1}$ | ND |
| Ovarian cancer biomarker HE4 (human epididymis protein 4)$^{[66]}$ | In serum (10% FBS), living mice, sensors loaded into semipermeable PVDF membrane capillary | Rational approach: HE4 Antibody (Ab) is goat polyclonal anti-HE4 IgG antibody Ab (TAT)$_{15}$-amine-SWCNT passivated with BSA | In serum: 10 nm–500 nm | Mostly over nontarget proteins like uPA, CA-125 (another ovarian biomarker), BSA or 93% FBS | HiPCO 900–1400 nm | Single sensor | Change after 1 min, stabilized signal after 1 h | ND |
| Table 5 (Continued) |
|----------------------|
| **Prostate cancer biomarker uPA**[106] |
| In PBS, blood, FBS, plasma |
| Rational approach: Mouse monoclonal IgG anti-uPA-(TAT)_3-SWCNTs passivated with BSA to increase sensitivity Red-shift |
| LOD In buffer 100 pm In serum 25 nm In plasma 100 nm |
| Increased selectivity in complex protein environment due to BSA passivation of SWCNTs |
| HiPCO (9,4), (8,6), (8,7) |
| ND Saturation after 30/90 min in human serum/plasma |

| **Prostate tumors**[21] |
| In tissues/ tissue-like phantoms, serum (FBS), incubated on human prostate cancer cell lines, in vivo in mice (intravenous injection) |
| Rational approach: Prostate specific membrane antigen (PSMA) antibody conjugated to M13-phage SWCNTs |
| LOD At 2 μg ml⁻¹ SWCNTs detection depth up to 2.5 cm in tissue-like phantoms |
| 4-fold improved uptake in PSMA positive prostate tumors compared to control, stable in serum |
| HiPCO mm in vivo |
| After 2-4 h post injection ND |

| **RAP1**[105] |
| Release from E. coli cell lysates |
| Rational and screening approach: RAP1-aptamer-(AT)_11-SWCNTs With spacers between (AT)_11 enhanced FI increase (4x) due to less adhesion of aptamer to SWCNT surface |
| LOD Zeptomolar protein detection |
| Over eight tested other proteins (Chirality mix) |
| Single cell ms⁻¹ |
| Yes |

| **SARS-CoV-2 spike protein**[157] |
| In saliva, viral transport medium |
| Rational approach: ACE2-(GT)_6-SWCNTs, passivation with PE-PEG FI increase |
| LOD 12.6 nM/10⁵–10⁶ viral copies per μL in solution/on surface |
| Less increase with other viral spike-like proteins (SARS-CoV-1 S RBD, MERS S RBD, FLU hemagglutinin subunit, serum albumin), in biological fluids (e.g. saliva, viral transport medium) |
| HiPCO 1130 nm |
| ND Within 90 min/5 s in solution/immobilized |

| **Wheat germ agglutinin (WGA)** (sugar-binding lectin protein)[153] Sugar/N-acetylglutamic acid (GlcNAc) and N-acetylneuraminic acid (Neu5Ac) |
| In buffer, complex biological media, immobilized |
| Screening approach: Peptoid-SWCNT FI decrease in response to WGA |
| LOD 3.4 μM |
| Minor responses to protein A, BSA, NeutrAvidin, lysozyme FI increase to peanut agglutinin, ConA |
| Attenuated ability to detect WGA in complex biological media (DMEF, FBS) Over other sugars commonly bound by lectins (fructose, galactose, glucose, mannitol mannose, sucrose), but not fucose |
| HiPCO (7,6) |
| Single sensor Equilibrium reached within 1 h |
| ND |

[a] FI: fluorescence intensity. ND: not determined. HG: hydrogel.
Table 6: Detailed overview of fluorescent SWCNT based sensors for sugar[^1]

| Target | Biological system | Recognition strategy | Sensitivity | Selectivity | SWCNT chirality/ wavelength | Spatial resolution | Temporal resolution | Reversibility |
|--------|-------------------|----------------------|-------------|-------------|-----------------------------|--------------------|--------------------|--------------|
| d-glucose[^2][^3] | SWCNT solution loaded into dialysis capillary imaged through human epidermal tissue sample | Rational approach: GOX-SWCNTs with Fe(CN)$_6^{3-}$ addition as electroactive species results in FI decrease or Fermi level shift into the VB due to irreversible adsorption on the SWCNT surface. Addition of analyte results in increasing restored FI. GOX catalyzes the reaction of d-glucose to d-glucono-1,5-lactone with a H$_2$O$_2$ co-product. | LOD 34.7 µM | ND | HiPCO | μm | Within 80 s | ND |
| d-glucose[^4] | In PBS, immobilized | Rational approach: Glucose-binding protein (GBP) covalently conjugated to PVA-SWCNTs. Quenching in discrete steps in response to glucose due to conformation change of GBP. | 2.5–50 nm linear until 10 nm | Over fructose, mannose | CoMoCAT (8,3), (6,5), (7,5) | Single-sensor 500 nm pixel size^* | Immediately (s) | Yes |
| Glucose[^5] | In BSA + PBS to prevent concanavalin A (ConA, lectin with four glucose-binding sites) adsorption to the walls of the cuvette | Rational approach: Phenoxydextran-SWCNTs with ConA Competitive binding between target analyte and dextran, ConA introduces protein-controlled aggregation and FI decrease, introduction of glucose causes dissolution of aggregate and FI recovery. | LOD 3.8 nm | Response range based on ConA concentration | Chirality mix | ND | 3–28 min to reach steady state | Yes |
| Glucose[^6] | In solution, 36°C | Rational approach: GOX-DNA-SWCNTs with 2 mM/5 mM potassium ferricyanide (PFC) as electron transfer mediator. FI increase. | LOD 1 nm | With increasing amount of glucose portions (1–2 µM) less sensitive (FI restoration decreases) | ND | HiPCO (9,2) at 1136 nm | ND | With in minutes | No |
| Glucose[^7] | In solution | Screening approach: Boronic acid derivatives complexed with SC-SWCNTs. Identified derivatives: 4-cyanophenylboronic acid, 4-chlorophenylboronic acid. Blue-shift and FI increase. | 5–30 nm | ND | CoMoCAT (6,5) | ND | Within seconds | Yes |
| Glucose, fructose[^8] | In solution | SWCNTs locally functionalized with a phenylboronic acid group. Blue-shift and FI increase. Rational approach: GOX-SWCNTs | 13.5 nm shift with 8.8 mM fructose/19 mM glucose | ND | CoMoCAT E$_{1/2}$ of (6,5) at 1138 nm | ND | ND | ND |
| Glucose[^9] | In PBS, human serum, incorporated into membrane device | Rational approach: GOX-SWCNTs | In PBS: 3 mm—30 mm tested | Different FI increase to mannose, galactose, xylose, Over maltose, fructose | ND | CoMoCAT (6,5) at 995 nm | ND | Without/ with membrane setup saturation after 1/12–15 min | Yes |
| Glycan\[107\] | Free biotinylated glycans, glycans tethered to streptavidin | Rational approach: Chitosan-SWCNTs noncovalently modified with lectins using a Ni\textsuperscript{2+}-NTA/His-tagged protein mechanism for divalent ion based proximity quenching FI increase | LOD 2 µg/100 ng of glycosylated protein/free glycan to 20 µg of lectin | Greater response of known high affinity pairs than to cross reactions | CoMoCAT (6.5) | Single sensor | Within seconds | Yes |
|---|---|---|---|---|---|---|---|---|
| Glycoforms with high mannose content\[209\] | In PBS, metabolically induced hypermannosylation of human IgG from CHO cells, SWCNTs in HG | Rational approach: Chitosan-SWCNTs noncovalently modified with PSA-lectin using a Ni\textsuperscript{2+}-NTA/His-tagged protein conjugation for divalent ion based proximity quenching FI increase | Dissociation constant 1.3–55 µM | Over human/mouse IgG with absent mannose content | HiPCO | ND | Within 5 min | ND |
| Saccharide\[210\] | In PBS | Semirational approach: Phenylboronic acid (PBA) grafted, polyethylene glycol 8-membered branched polymers (PPEG8) wrapped SWCNTs Three PBAs to measure saccharide binding: 4-carboxyphenylboronic acid, N-(4-phenylboronic)-succinamic acid, 3-carboxy-5-nitrophenylboronic acid FI quenching | FI changes varied for different saccharide, e.g., 4-carboxyphenylboronic acid-PPEG8-SWCNTs respond with –20% FI change upon 10 mM ribose and 15% upon arabinose | Not selective over DA | Most responsive to pentoses, such as arabinose, ribose, and xylose | Chirality mix (9,4) | ND | 1 h incubation of analytes | ND |
| Saccharide\[211\] | In DMSO/PBS | Screening approach: Polymer or surfactant SWCNTs modified with noncovalent adsorption of PBAs Saccharide responses occur only within corona phases that have allowed PBAs to adsorb and quench SWCNT emission (e.g., most PBAs fail to penetrate SDS coronas) Recognition upon saccharide adsorption into SWCNT corona, followed by surface reaction of PBA mm range tested | Corona-phase environment has a profound effect on selectivity of saccharide binding | Chirality mix | ND | Within minutes | ND |

[a] FI: fluorescence intensity, ND: not determined, HG: hydrogel, **: limited by the Abbe limit, diffusion and detection speed.
| Target      | Biological system | Recognition strategy                                                                 | Sensitivity | Spatial resolution | Sensitivity | Temporal resolution | Reversibility | S/WCNT chirality/wavelength | S/WCNT nanodimensionality | Selectivity |
|-------------|-------------------|---------------------------------------------------------------------------------------|-------------|--------------------|-------------|----------------------|--------------|-----------------------------|--------------------------|-------------|
| DNA hybridization | ssDNA-SWCNTs | Hybridization of 24-mer oligonucleotide sequence with its complementary DNA sequence(s) causes blue-shift. | LOD 6 nm    | ND                 | ND          | ND                   | 13 h to reach steady state (for 62.5 nm DNA) | Yes, but time-limited due to shelf life of DNA or SWCNTs. | HiPCO (6,5) | Single sensor | Within 10 min/buffer, 120 min/in vivo | LOD 100 pm |
| MicroRNA/DNA hybridization | MicroRNA-SWCNTs | Rational approach: SWCNTs functionalized with 23-mer microRNA capture sequence (complementary to target oligonucleotide) and (GT)₁₅ (for SWCNT colloidal stability). Low concentrations of SDBS (5.7 mM) cause an increased blue-shift (and FI enhancement) by an order of magnitude. | LOD 10 pM/mM - 1 mM | Single sensor | Within 10 min/buffer, 120 min/in vivo | 1.8 x faster for DNA vs. RNA | Reaction kinetics are 1.8 times faster for DNA vs. RNA | HiPCO (6,5) | Single sensor | Within 10 min/buffer, 120 min/in vivo | LOD 100 pm |
| Single nucleotide polymorphism (SNP) | ssRNA genome of intact HIV | Rational approach: (GT)₁₅-SWCNTs | 0.8 meV blue-shift | Distinction from response caused by complementary DNA due to higher energy shift of 1.2 meV | ND | ND | 8 h to reach steady state | HiPCO (6,5) | Single sensor | Within 10 min/buffer, 120 min/in vivo | LOD 100 pm |
| DNA hybridization | ssDNA-SWCNTs | Hybridization of 24-mer oligonucleotide sequence with its complementary DNA sequence(s) causes blue-shift. | LOD 6 nm    | ND                 | ND          | ND                   | 13 h to reach steady state (for 62.5 nm DNA) | Yes, but time-limited due to shelf life of DNA or SWCNTs. | HiPCO (6,5) | Single sensor | Within 10 min/buffer, 120 min/in vivo | LOD 100 pm |
| MicroRNA/DNA hybridization | MicroRNA-SWCNTs | Rational approach: SWCNTs functionalized with 23-mer microRNA capture sequence (complementary to target oligonucleotide) and (GT)₁₅ (for SWCNT colloidal stability). Low concentrations of SDBS (5.7 mM) cause an increased blue-shift (and FI enhancement) by an order of magnitude. | LOD 10 pM/mM - 1 mM | Single sensor | Within 10 min/buffer, 120 min/in vivo | 1.8 x faster for DNA vs. RNA | Reaction kinetics are 1.8 times faster for DNA vs. RNA | HiPCO (6,5) | Single sensor | Within 10 min/buffer, 120 min/in vivo | LOD 100 pm |
| Single nucleotide polymorphism (SNP) | ssRNA genome of intact HIV | Rational approach: (GT)₁₅-SWCNTs | 0.8 meV blue-shift | Distinction from response caused by complementary DNA due to higher energy shift of 1.2 meV | ND | ND | 8 h to reach steady state | HiPCO (6,5) | Single sensor | Within 10 min/buffer, 120 min/in vivo | LOD 100 pm |
| DNA hybridization | ssDNA-SWCNTs | Hybridization of 24-mer oligonucleotide sequence with its complementary DNA sequence(s) causes blue-shift. | LOD 6 nm    | ND                 | ND          | ND                   | 13 h to reach steady state (for 62.5 nm DNA) | Yes, but time-limited due to shelf life of DNA or SWCNTs. | HiPCO (6,5) | Single sensor | Within 10 min/buffer, 120 min/in vivo | LOD 100 pm |
| MicroRNA/DNA hybridization | MicroRNA-SWCNTs | Rational approach: SWCNTs functionalized with 23-mer microRNA capture sequence (complementary to target oligonucleotide) and (GT)₁₅ (for SWCNT colloidal stability). Low concentrations of SDBS (5.7 mM) cause an increased blue-shift (and FI enhancement) by an order of magnitude. | LOD 10 pM/mM - 1 mM | Single sensor | Within 10 min/buffer, 120 min/in vivo | 1.8 x faster for DNA vs. RNA | Reaction kinetics are 1.8 times faster for DNA vs. RNA | HiPCO (6,5) | Single sensor | Within 10 min/buffer, 120 min/in vivo | LOD 100 pm |
| Single nucleotide polymorphism (SNP) | ssRNA genome of intact HIV | Rational approach: (GT)₁₅-SWCNTs | 0.8 meV blue-shift | Distinction from response caused by complementary DNA due to higher energy shift of 1.2 meV | ND | ND | 8 h to reach steady state | HiPCO (6,5) | Single sensor | Within 10 min/buffer, 120 min/in vivo | LOD 100 pm |

[a] FI: Fluorescence intensity, ND: not determined.
| Target               | Biological system               | Recognition strategy                                                                 | Sensitivity | Selectivity | SWCNT chirality/ wavelength | Spatial resolution | Temporal resolution                         | Reversibility |
|---------------------|---------------------------------|--------------------------------------------------------------------------------------|-------------|-------------|-----------------------------|-------------------|---------------------------------------------|---------------|
| Enzyme activity<sup>[a]</sup> | In solution                     | BSA-SWCNTs for bacterial protease, citrus pectin-SWCNTs for pectinase, carboxymethylcellulose (CMC)-SWCNTs for cellulase. Target enzyme degrades the polymer-wrapping, resulting in F I quenching. Albumin-SWCNTs for proteolytic activity. Lignosulfonic acid (LSA)-SWCNTs for lignin-modifying activity. CMC-SWCNTs for cellulolytic activity. Target enzyme degrades the polymer-wrapping resulting in F I quenching or target enzyme further protects the SWCNT resulting in F I increase. | LOD 5 f m  
Sensitivity and repeatability of sensors varies due to affinity of polymers to the SWCNT. | ND          | ND          | CoMoCAT, (6.5) at 975 nm     | ND                | Real time  
Signal stabilized 1–3 h after incubation (24–29 h for 5 f m) | No            |
| Enzyme activity<sup>[a]</sup> | In soil, diluted with DI water  | Albumin-SWCNTs for bacterial protease, citrus pectin-SWCNTs for pectinase, carboxymethylcellulose (CMC)-SWCNTs for cellulase. Target enzyme degrades the polymer-wrapping, resulting in F I quenching. | LOD 5 f m  
Sensitivity and repeatability of sensors varies due to affinity of polymers to the SWCNT. | ND          | ND          | ND                           | ND                | No                                          |               |
| Myeloperoxidase (MPO)<sup>[a]</sup> | MPO/H<sub>2</sub>O<sub>2</sub>/Cl<sup>-</sup> system | GO-SC-SWCNTs  
Increasing F I at 420/430 nm due to oxidation and degradation of GO.  
Decreasing NIR SWCNT F I due to generation of defects on the SWCNT surface | Linear response to MPO-catalyzed degradation | ND          | ND          | CoMoCAT, 998 nm GO: 420 nm | ND                | Real time, within 5 days                      | No            |
| Trypsin<sup>[a]</sup> | SWCNTs drop-casted and dried on glass fiber paper, fixed on plastic strips, Urine samples diluted in PBS | Screening approach: Peptide-SWCNTs (HexCo<sub>i</sub>-Ala)  
Target enzyme degrades the peptide-wrapping resulting in F I quenching.  
Urine samples: reduced sensitivity | 1–20 μg mL<sup>-1</sup>  
Urine samples: reduced sensitivity | ND          | ND          | CoMoCAT, (6.5) at 975 nm     | ND                | Significant F I decrease after 2 h/1 h of incubation at concentrations of 1/5–20 μg mL<sup>-1</sup> | No            |

<sup>[a] </sup>F I: fluorescence intensity, ND: not determined.
Table 9: Detailed overview of fluorescent SWCNT based sensors for epitopes and metabolites from pathogens.[4]

| Target | Biological system | Recognition strategy | Sensitivity | Selectivity | SWCNT chirality/wavelength | Spatial resolution | Temporal resolution | Reversibility |
|--------|-------------------|----------------------|-------------|-------------|-----------------------------|-------------------|-------------------|--------------|
| Distinction between F'-positive and F'-negative bacterial strains, specific F'-negative bacteria<sup>74</sup> | In tissue phantom, mice, SWCNTs injected intravenously | Rational approach: For distinction: M13 bacteriophage-SWCNTs F'-negative bacteria: Anti-bacterial antibody-SWCNTs via streptavidin-biotin interaction Spatially encoding, distinction with PCA: Sensors for specific bacterial targets (rational approach): 1) Lipopolysaccharides (LPS) sensor: LPS-binding peptide linked to NH<sub>2</sub>-<sup>(GT)</sup><sub>10</sub>-SWCNTs, FI increase 2) Siderophore sensor: hemin-binding aptamer-<sup>(HeApta)</sup>SWCNTs, HeApta binds to hemin, which brings Fe<sup>3+</sup> into proximity of the SWCNT and quenches it. Siderophore can reverse this effect by removing iron, which increases FI again 3) Nuclease activity sensor: calf-thymus-(CT)-DNA-SWCNTs, report to DNase I and S. aureus nuclease activity due to degradation of CT-DNA via FI modulation 4) Protease sensor: BSA-SWCNTs, FI decrease in response to protease Generic lower-selectivity sensors to increase the discrimination power of the sensor array: (GT)<sub>10</sub>, C<sub>30</sub>, (GC)<sub>15</sub>, PEG-PL-SWCNTs react to changes in pH, oxygen or protein concentration For spectrally encoding: LPS-(6,5)-SWCNTs, PEG-PL-(9,4), -(8,6),(9,5) SWCNTs and EB-NS reference | Over control (injection of PBS) | Distinction of P. aeruginosa, S. aureus, S. epidermidis, E. coli and of 43 different clinical isolates of S. aureus and S. epidermidis with 80% likelihood E. faecalis and S. pyogenes not distinguishable Over human synovial fluid LPS sensor: responds to different LPS structures with different sensitivity, siderophore sensor: not responsive to weaker chelators such as ethylenediaminetetraacetic acid citrate | HiPCO | mm for a stand-off distance of ≥25 cm | Limited by ND diffusion of analytes through HG | Within 24–72 h distinction possible |

Distinction between F'-positive bacterial strains, specific F'-negative bacteria<sup>74</sup> | SWCNTs in HG array with low porosity for small molecules (e.g. siderophores) and high porosity for large enzymes, bacteria plated on top, in human synovial fluid, tissue phantom | Spatial encoding: CoMoCAT, >900 nm Spectral encoding: EB-NS: 920 nm, (6,5): 920 nm, (9,4), (8,6), (9,5) > 1110 nm | Spatial encoding: ND Imaging of mice 8–48 h after injection |

[a] FI: fluorescence intensity, ND: not determined, HG: hydrogel.
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Conflict of Interest

The authors declare no conflict of interest.

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