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Recent Advances in Molecular Recognition Based on Nanoengineered Platforms

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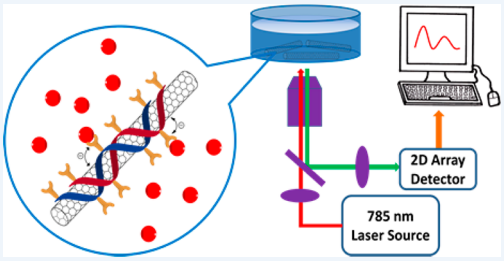
ABSTRACT: Nanoparticles and nanoengineered platforms have great potential for technologies involving biomoleuclar detection or cell-related biosensing, and have provided effective chemical interfaces for molecular recognition. Typically, chemists work on the modification of synthetic polymers or macromolecules, which they link to the nanoparticles by covalent or noncovalent approaches. The motivation for chemical modification is to enhance the selectivity and sensitivity, and to improve the biocompatibility for the in vivo applications.



In this Account, we present recent advances in the development and application of chemical interfaces for molecular recognition for nanoparticles and nanoengineered platforms, in particular single-walled carbon nanotubes (SWNTs). We discuss emerging approaches for recognizing small molecules, glycosylated proteins, and serum biomarkers. For example, we compare and discuss detection methods for ATP, NO, H_2O_2, and monosaccharides for recent nanomaterials. Fluorometric detection appears to have great potential for quantifying concentration gradients and determining their location in living cells.

For macromolecular detection, new methods for glycoprofiling using such interfaces appear promising, and benefit specifically from the potential elimination of cumbersome labeling and liberation steps during conventional analysis of glycans, augmenting the currently used mass spectrometry (MS), capillary electrophoresis (CE), and liquid chromatography (LC) methods. In particular, we demonstrated the great potential of fluorescent SWNTs for glycan−lectin interactions sensing. In this case, SWNTs are noncovalently functionalized to introduce a chelated nickel group. This group provides a docking site for the His-tagged lectin and acts as the signal modulator. As the nickel proximity to the SWNT surface changes, the fluorescent signal is increased or attenuated. When a free glycan or glycosylated probe interacts with the lectin, the signal increases and they are able to obtain loading curves similar to surface plasmon resonance measurements. They demonstrate the sensitivity and specificity of this platform with two higher-affined glycan-lectin pairs: fucose (Fuc) to PA-IIL and *N*-acetylglucosamine (GlcNAc) to GafD. Lastly, we discuss how developments in protein biomarker detection in general are benefiting specifically from label-free molecular recognition. Electrical field effect transistors, chemi-resistive and fluorometric nanosensors based on various nanomaterials have demonstrated substantial progress in recent years in addressing this challenging problem. In this Account, we compare the balance between sensitivity, selectivity, and nonspecific adsorption for various applications. In particular, our group has utilized SWNTs as fluorescence sensors for label-free protein−protein interaction measurements. In this assay, we have encapsulated each nanotube in a biocompatible polymer, chitosan, which has been further modified to conjugate nitrilotriacetic acid (NTA) groups. After Ni^{2+} chelation, NTA Ni^{2+} complexes bind to his-tagged proteins, resulting in a local environment change of the SWNT array, leading to optical fluorescence modulation with detection limit down to 100 nM. We have further engineered the platform to monitor single protein binding events, with an even lower detection limit down to 10 pM.

1. INTRODUCTION

A wide variety of nanomaterials have been explored in biomolecular detection or cell-related biosensing. Most of these approaches are used to generate either electrical/electrochemical or optical readouts. One dimensional nanomaterials such as nanowires have been used to record action potentials of cardiomyocytes and neuronal activity, or to guide light to subcellular locations where it can be used for sensing.[1−3] Nanoparticles have been widely used for in vitro as well as in vivo sensing and imaging. Examples include metal and metal oxide nanoparticles, core−shell nanoparticles, polymeric nanoparticles, and quantum dots.[4−6] Another emerging field is carbon-nanomaterials such as single-walled carbon nanotubes (SWNTs), carbon nanodots, and graphene quantum dots.[7,8] The recent discovery that the corona phase of a nanoparticle can structure a polymer such that a unique molecular recognition site is created opens new opportunities in this field.[9] Corona phase molecular recognition (CoPhMoRe) generates unique recognition sites necessarily integrated with the nanoparticle or nanotube transducer surface, and hence stands to impact the field of nanosensor development substantially. In this Account, we summarize recent progress in the field of molecular recognition based on nanoengineered platforms including CoPhMoRe phases and advances in nanosensor design.

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2. SMALL MOLECULE DETECTION

Bioactive small molecules have been playing important roles in elucidating basic biology,[10] such as serving as cell signaling molecules, as tools in molecular biology, as drugs in medicine, as pesticides in farming, and in many other roles. For example, adenosine 5′-triphosphate (ATP) is a universal energy storage molecule, and its depletion is related to pathogenesis such as hypoglycemia, ischemia, and Parkinson's disease.[11] ATP concentration detection has been used to determine bacterial contamination,[12] and to help the study of energetic processes in cell physiology from ion-channel regulation to intercellular signaling cascades.[13] In addition, nitric oxide (NO) is an important cellular signaling molecule, critical for maintain vascular physiology and regulating immune defense.[14] Accurate detection of NO concentration and its location of production are essential to understanding the diverse biological roles of NO.[15] Another significant small molecule in cellular signaling pathways is hydrogen peroxide (H_2O_2).[16] H_2O_2 and several other reactive oxygen species (ROS) are byproducts of aerobic metabolism when cells are stimulated with various growth factors, cytokines, and other signaling molecules, and are known to activate specific downstream targets.[16] Last, accurately monitoring blood glucose levels is important for the overall treatment of patients inflicted with diabetes. Thus, the knowledge of small molecules' concentration and distribution in biological systems is central to the study of cell physiology, chemical biology, pharmacokinetics, and chemical genetics.[10,17]

2.1. Adenosine 5′-Triphosphate (ATP)

The attachment of biological recognition elements to nanomaterials has opened new avenues for the exploitation of modern biosensors. Up to date, several approaches for ATP detection have been described, either based on electrochemical or spectroscopic methods including bioluminescence, fluorescence, color, and Raman spectroscopy. Bioluminescence based on ATP-dependent luciferase–luciferin reaction has been the most commonly used method since 1947 when McElroy[18] was the first to apply this reaction to determine ATP level. In optical methods, organic fluorophores have always been used as the signal source, which would undoubtedly suffer from the same problem of photobleaching among all organic dyes. To overcome this issue, our group recently developed a nIR fluorescent ATP sensor based on a SWNT-luciferase complex ($SWNT^{Luc}$),[19] in which the luciferase was conjugated with phospholipids that wrapped SWNTs so that ATP can bind to the luciferase in the presence of D-luciferin by the bioluminescent reaction to modulate the fluorescence of SWNTs (Figure 1). The nIR fluorescence is ultimately quenched by a two-step reaction that involves detection of a target and generation of a redox quenching intermediate. This $SWNT^{Luc}$ sensor is very selective to ATP, but not to AMP, ADP, CTP, and GTP, and is also able to detect ATP temporally and spatially in living HeLa cells, which is the first one among this kind of sensors.

2.2. Nitric Oxide (NO)

Currently, electrochemical and spectroscopic methods including absorbance, fluorescence, chemiluminescence, and electron paramagnetic resonance (EPR) are widely explored to detect NO. However, each method proposed to date has significant limitations. Basically, the electrochemical methods could be excluded for any in vivo test due to the spatial resolution. Widely used diaminofluoresceins[20] in fluorescence detection

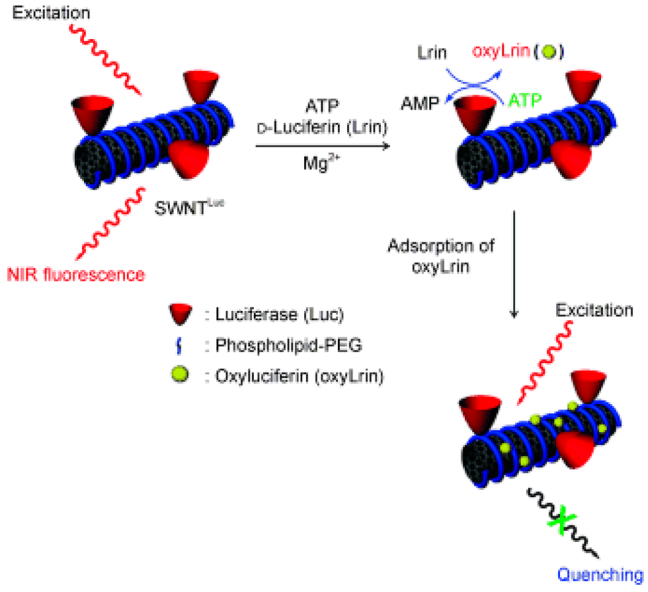

Figure 1. Illustration of the $SWNT^{Luc}$ sensor for ATP detection. SWNTs were suspended with distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[carboxy(polyethylene glycol)-2000] (ammonium salt) (PLPEG-COOH) in aqueous solution. Reproduced with permission from ref 19. Copyright 2010 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

methods only indirectly detect NO through its oxidation products. Other metal–fluorophore complexes[21] lack significant optical penetration through biological tissues and have the issue of photobleaching.

Near infrared fluorescence-based probes combined with microscopy are particular useful for resolving spatial and temporal aspects of NO levels. Our laboratory recently developed an optical NO sensor based on fluorescent SWNTs functionalized with a 3,4-diaminophenyl-functionalized dextran polymer (DAP-dex), which exhibits excellent selectivity and rapid detection response in vitro experiments.[22] Furthermore, intracellular testing demonstrated the successful quantitative tracking of NO production in Raw 264.7 macrophage cells by fluorescence images, and in vivo testing in a CO_2-asphyxiate mouse illustrates the great potential for its practical application. Encouraged by this success, we continued to develop a fluorescence-based SWNT sensing array comprising single-stranded $d(AT)_{15}$ DNA oligonucleotide-wrapped SWNTs (AT_{15}-SWNT) (Figure 2).[23] We found that the AT_{15}-SWNT complex is unique in its high selectivity toward NO, which exhibits a single molecule NO detection sensitivity.

2.3. Hydrogen Peroxide (H_2O_2)

H_2O_2 and other ROS are produced in all kinds of biological and physiological processes. Proper ROS concentrations play a significant role to maintain the homeostasis, and the balance between ROS production and antioxidant defenses determines the degree of oxidative stress. A lower level below the homeostatic set point may interrupt the physiological role of oxidants in cellular proliferation and host defense, while an intracellular concentration rise would cause two potentially important effects: damage to various cell components and triggering of the activation of specific signaling pathways, both of which link to aging and the development of age-related diseases. However, it is still unclear how ROS affects cellular

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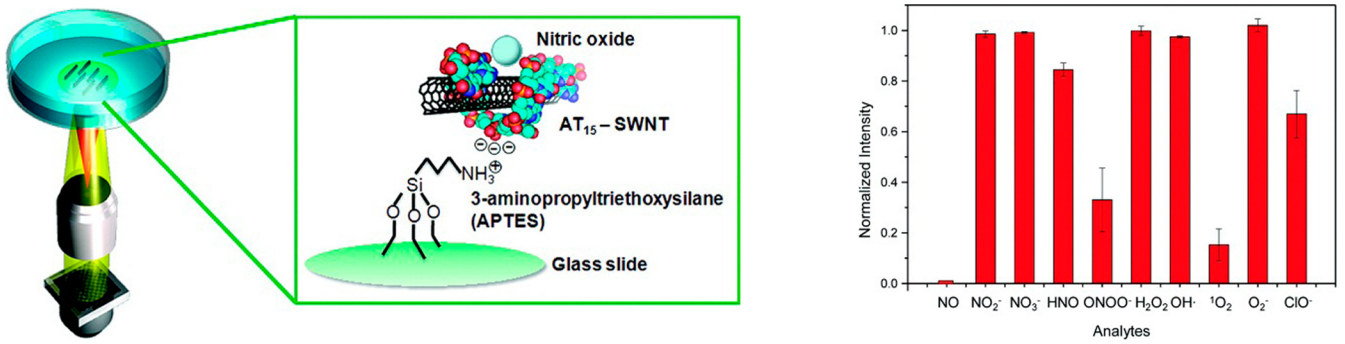

Figure 2. Left: Schematic of the microscope setup. A 658 nm laser beam (red) excites at the SWNT array deposited on the glass-bottomed Petri dish. The emission light (yellow) is collected by a near-infrared array detector through a 100× TIRF objective mounted on an inverted microscope. Right: Fluorescence intensity (I/I_0, intensity/initial intensity) of (7, 5) species of AT_{15}-SWNT measured 10 min after addition of 60 μM of each analyte. Reprinted with permission from ref 23. Copyright 2011 American Chemical Society.

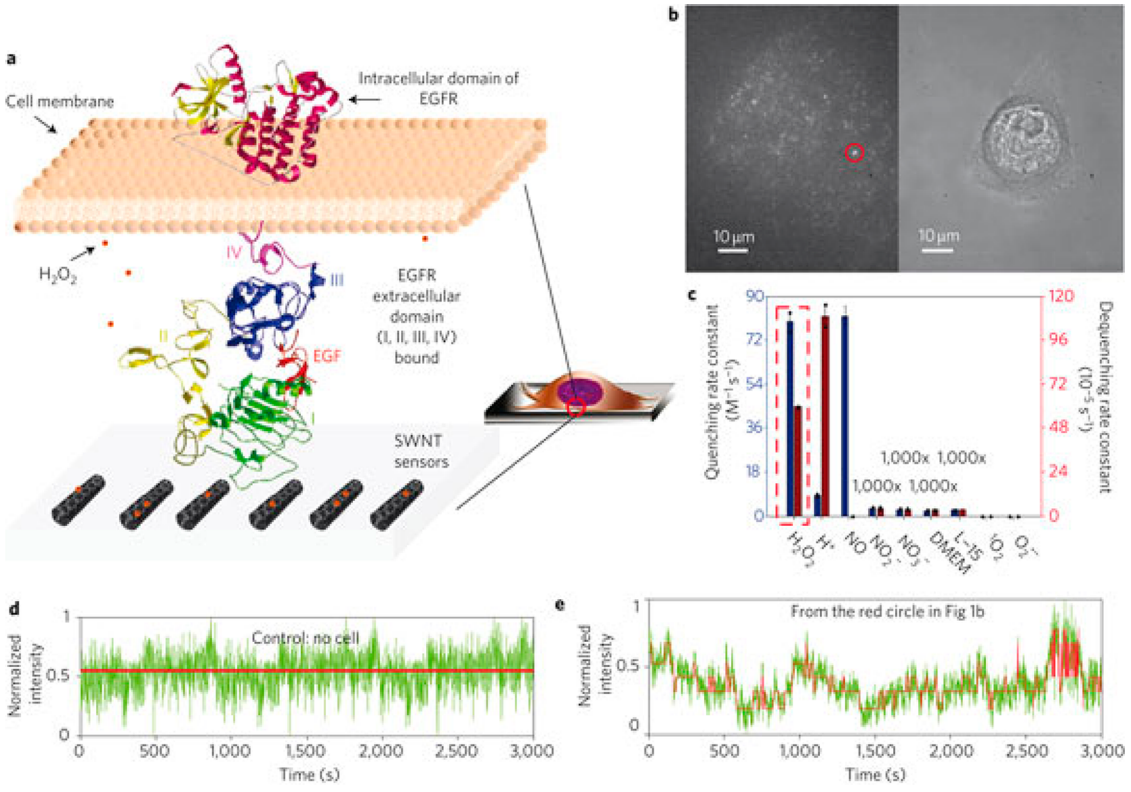

Figure 3. (a) A431 cell cultured on a collagen−SWNT film. The enlarged representation of the red circle shows EGFR domains spanning the cell membrane. Domains I and III bind to EGF (red) and generate H_2O_2. (b) Near-infrared (NIR) image of SWNTs under the A431 (left), and phase contrast image of an A431 cell (right) cultured on SWNT sensors. (c) Forward and reverse binding rates of the SWNT sensor for various analytes show selectivity for H_2O_2. (d) Fluorescence trace for control (no cells) shows no steps. (e) Trace for the SWNTs in the red circle in (b) show reversible, stepwise quenching (green trace), modeled by a hidden Markov algorithm (red). Reprinted by permission from Macmillan Publishers Ltd: Nature Nanotechnology (ref 24), copyright (2010).

mechanisms where redox changes have been demonstrated, what is the fate of ROS generated by cellular NADPH oxidases, and how to identify and track intracellular ROS. Thus, there is an urgent need for developing novel techniques for ROS detection in living systems, which remains highly challenging due to their low concentration and short lifetime. Our laboratory has developed an array of SWNTs that can selectively record, in real time, the discrete, stochastic quenching events that occur as H_2O_2 molecules are emitted from individual human epidermal carcinoma cells stimulated by epidermal growth factor (Figure 3).[24]

2.4. Monosaccharides

Although nanomaterials have aided in the sensitivity, efficiency, and size of glucose monitors, electrochemical sensors still suffer from the need for relatively invasive sampling means such as transcutaneous electrode placement or direct blood withdraw. As such, a great deal of attention has been placed on developing a continuous and minimally invasive means for glucose detection. Fluorescent based sensors have emerged as strong candidates to accomplish this minimally invasive detection. Early work by our group discovered that SWNT wrapped in carboxylated polyvinyl chloride (cPVA) functionalized with glucose binding protein (GBP) could be used to monitor

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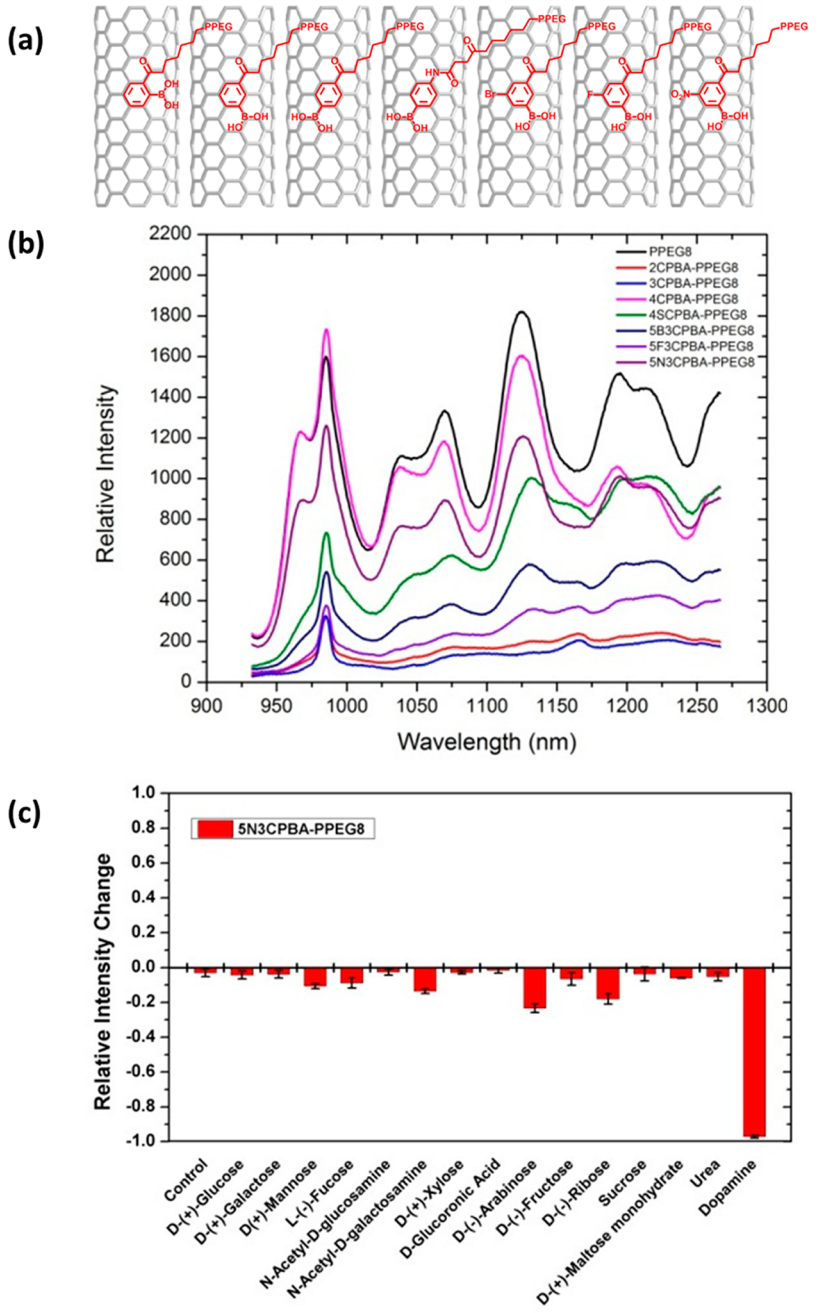

Figure 4. (a, b) Series of phenylboronic acid (PBA) polymers demonstrated a systematically optical modulation of polymer−SWNT complexes. (c) 5N3CPBA-PPEG8 polymer functionalized SWNTs demonstrated a selective saccharide detection system (50 mM sugar solution was used in the test). Reprinted with permission from ref 28. Copyright 2012 American Chemical Society.

glucose concentrations in real time.[25] However, protein stability is an important concern in such protein−nanomaterial constructs. As such, using highly stable small molecules as receptors may help in addressing these concerns.

Boronic acids are well-known to bind saccharides through a diol-coupling interaction. This interaction has been heavily investigated in the context of creating a glucose sensor. Interestingly, fluorescent sensors for glucose based on boronic acid have even been developed in the form of noninvasive contact lenses which monitor tear glucose concentrations.[26] However, potential problems such as photobleaching and low concentrations of tear sampling fluid still remain with such constructs. Recently, the application of phenylboronic acid derivatives as the glucose binding moiety in a SWNT based fluorescent glucose sensor has been investigated.[27] In this work, a library of 30 boronic acids was investigated. It was observed that all phenyl boronic acid derivatives investigated adsorbed to the SWNT surface via $\pi-\pi$ stacking, resulting in a quenching of SWNT fluorescence. The quenched complex was then subjected to glucose and the SWNT fluorescent intensity and peak position were monitored. In addition, SWNTs engineered by phenylboronic acid polymers have also been explored for saccharide detection (Figure 4).[28]

Ultimately, the idea of a "smart tattoo" based on glucose sensitive fluorescent ink is one that is being investigated through several means. However, challenges still remain in such

constructs. Sensor migration, optimized tissue depths, and biocompatible encapsulation persist as issues which will need to be investigated more completely before such constructs can be applied to patient use. However, this minimally invasive, continuous glucose detection form factor remains one of the more promising tools currently under development.

3. GLYCOPROFILING

Surface carbohydrates, or glycans, are dominant players in cell−cell interactions and protein recognition.[29] Glycans coat the surface of every cell (creating the glycocalyx, literally a "sugar coat") and provide means of defense and recognition. These surface carbohydrates dictate self-identity and signal rejection of foreign materials in blood transfusion, tissue grafts, and organ transplants. Because of glycans' dominant roles in signaling, immunology, and transport, they are of immense interest to basic researchers and applied therapeutics. Due to the omnipresence of glycans in vivo, nature has created very few strong binding antibodies to these antigens as they would nonspecifically bind instantly and irreversible to many surfaces. Instead, glycan recognition is mediated by a delicate dance of multiple, low-affinity binding events, otherwise known as avidity. Thus, standard assaying techniques that rely upon strong binding to withstand labeling and washing steps are not as efficient and accurate with glycan recognition. Furthermore, within a single organism and a single cell type there exists a "microheterogenity" that changes due to extrinsic environmental factors, so once a glycoform has been determined, it does not extend to all other cells or protein(s) in solution or over time. Thus, timely detection or profiling of glycans on a protein or cell still remains a difficult task. Nanoengineered platforms, especially those enabled by carbon materials, may provide more convenient assay methods for measuring glycan interactions and determining glycan profiles.

3.1. Current Platforms for Glycan Profiling

Because the protein's presence will confound the separation technique or influence the spectrometric analysis in most situations, glycans have to be released from the core protein before the analysis, which is referred to as the liberation step. Liberation is done by either chemical (hydrazinolysis) or enzymatic release (general peptide N-glycanases for N-glycans and more specialized enzymes for O-glycans).[30] This liberation step must be done carefully to ensure that the glycans are neither destroyed nor altered so the subsequent analysis is an accurate representation of the protein's glycan profile. The released glycans are often labeled with fluorescent tags or fully methylated for the following profiling steps. Current glycan profiling efforts require multiple analytical techniques and many specialists. Several comprehensive reviews[31,32] well summarized both advantages and limitations of the established methods, including mass spectrometry (MS), capillary electrophoresis (CE), liquid chromatography (LC), and more recent microarray methods, as shown in Figure 5. Another text, the *Essentials of Glycobiology*,[29] provided a greater list of specialized tools. These tools for the analysis of glycans have their own strong/weak points, and appropriate methods should be selected for each glycan sample. Although these techniques are useful for both quantitative determination and structural analysis, they still remain expensive and time-consuming, and at the expense of destroying the protein and glycan, which is not suitable in cases such as screening the expressed production of a protein therapeutic. Thus, there has been an interest in techniques that

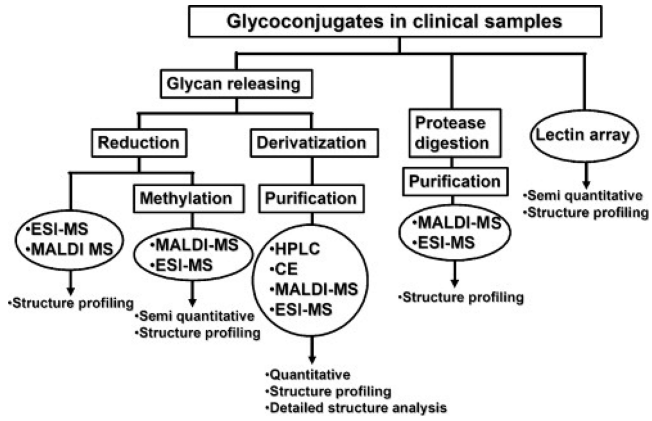

Figure 5. Analysis of glycans from glycoproteins. Reprinted from ref 32, Copyright 2011, with permission from Elsevier.

are more rapid and can do profiling without labor intensive glycan liberation.

3.2. Nanoengineered Platforms for Glycan Profiling

Nanoengineered platforms strive to maintain the quantitative detail of the established "acronym" systems while including the simplicity and convenience of lectin microarrays. Although no readily accepted tool has emerged to date from this field, many are currently being researched. These platforms can be broadly categorized by transduction methods: electrical, optical, and mass changes and have been extensively reviewed recently.[33] Many nanoscale materials have been proposed as the sensing elements for glycan−lectin interaction (gold, polymers, ferrousmagnetic, quantum dot, and multilayer nanoparticles); however, to maintain the theme of this current Account, herein we only cover current methods that utilize nanoscale carbon in their signal transduction.

The Star group has pioneered the electrical measurements of carbon materials to elucidate glycan−lectin interactions. In their first scheme[34] (Figure 6), the glycans are porphyrin-based glycoconjugates which noncovalently functionalize single-walled carbon nanotube field-effect transistors. When lectins specific to the conjugated glycans are added to solution (ConA and PA-IIL in this study), conductance measurements indicate specific binding and dissociation constants can be obtained. Lectin concentrations down to 2 nM could be assayed with this device, and selectivity was confirmed with conventional, fluorescent colocalization assays.

The Ju group has developed electrochemical assays based on glassy carbon electrodes to detect cell surface glycosylation. In their founding work for this field,[35] they bind ferrocene to ConA and allow the ConA conjugates to interact with the mannose receptors of added K562 cells (Figure 7). This reduces the available, free ConA conjugates to interact with the electrode, and a change in current is detected. They report a simple, yet robust sensor that can determine the mannose content of specific cell types and distinguish between cancerous and not cancerous cells.

The final emerging area of carbon materials for glycan−lectin interactions are the optical sensors developed by the Strano group for glycan−lectin interactions.[36] In this case, fluorescent SWNTs are noncovalently functionalized to introduce a chelated nickel group. This group provides a docking site for the His-tagged lectin and acts as the signal modulator. As the nickel proximity to the SWNT surface changes, the fluorescent signal is increased or attenuated. When a free glycan or

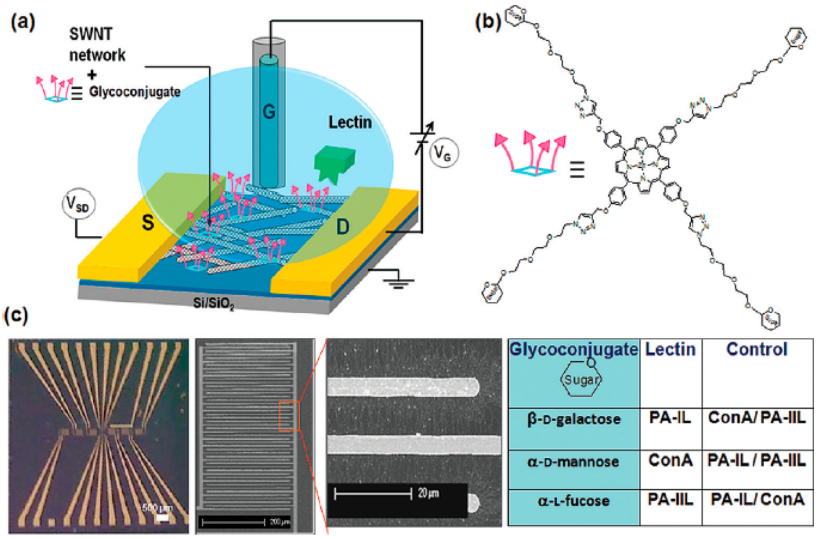

Figure 6. First generation carbon nanotube FET based sensor for glycan–lectin interactions. Reprinted with permission from ref 34. Copyright 2011 American Chemical Society.

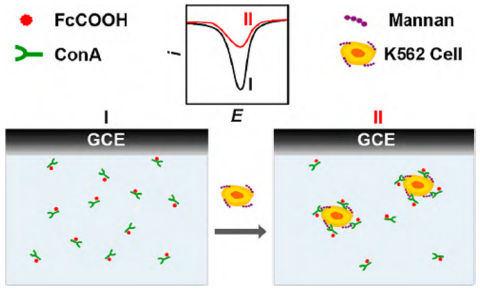

Figure 7. Electrochemical assay for glycan–lectin interaction based on electric measurements from a glassy carbon electrode (GCE). Reprinted from ref 35, Copyright 2010, with permission from Elsevier.

glycosylated probe interacts with the lectin, the signal increases and they are able to obtain loading curves similar to surface plasmon resonance measurements. They demonstrate the sensitivity and specificity of this platform with two higher-affined glycan-lectin pairs: fucose (Fuc) to PA-IIL and *N*-acetylglucosamine (GlcNAc) to GafD.

4. LABEL-FREE DETECTION OF PROTEIN BIOMARKERS: ENGINEERING TOWARD POINT-OF-CARE DIAGNOSTICS

Biomarkers are proteins, microRNA, DNA, or DNA fragments, either circulating in body fluid (blood, serum, urine) or presented on diseased cell surfaces, which are indicative of certain disease states. Biomarker detection has been focusing on rapid, sensitive detection of known biomarkers with minimal sample preprocessing, to diagnose certain diseases in a timely and accurate fashion. One of the potential applications is for point-of-care (POC) diagnostics. The detection of certain biomarkers, especially at low concentration, is the key to diagnosing the onset of diseases, such as cancer, enabling the timely treatment of patients and reducing medical costs.[37,38]

Techniques for biomarker detection can be divided into two separate categories: labeling methods and label-free methods. Traditional POC protein biomarker detection techniques mostly rely on benchtop, commercialized, multistep antibody-based immunoassays, such as ELISA (enzyme-linked immunosorbent assay), which is a labeling method. Label-free technologies include field-effect transistors,[39] cyclic voltammetry, and impedimetric sensors,[40] and spectroscopy or fluorescence-based methods.[41] Within the field of nanosensor biomarker detection, various nanomaterials have been used, including nanowires,[39] nanoparticles,[41] gold nanorods, and carbon nanotubes.[40]

The Ren group reported a one-step immunoassay for cancer biomarker detection using resonance light scattering correlation spectroscopy (RLSCS).[41] Technically, this approach does require labeling; however, it avoids washing steps that are needed in many traditional labeling immunoassays. The diffusion time of gold nanoparticles (GNPs) increases upon binding of the antigen, and the change in diffusion time can be captured and analyzed by RLSCS. The detection method is very sensitive, with a linear range from 1 pM to 1 nM in serum. However, the instrumentation is fairly complicated. In addition, the linear range only applies at low concentration, and therefore, the authors need to dilute healthy samples by 400 times and diseased patient samples by 4000 times to work within the linear range.

The Lee group devised a detection scheme for vascular endothelial growth factor-165 ($VEGF_{165}$), a predominant biomarker of cancer angiogenesis, by taking advantage of surface plasmon enhancement.[42] In the absence of VEGF, unfolded VEGF aptamer is electrostatically bound to a positively charged PLL-coated GNP surface; because the aptamer is labeled with Cy3B, adhering it close to the surface creates surface-enhanced fluorescence. Addition of VEGF pulls the specific aptamer off the gold surface, and the previously enhanced fluorescence disappears. The sensor has a high selectivity toward VEGF with linear detection from 25 pg/mL to 25 μg/mL (= from 1.25 pM to 1.25 μM) in buffer. However, the sensor shows weak interaction toward human serum albumin (HSA). Specifically, at physiologically relevant concentrations, VEGF (2.5 ng/mL) and HSA (40 mg/mL) show similar signals.

Our group has utilized SWNTs as fluorescence sensors for label-free protein–protein interaction measurements. In this assay, each nanotube is encapsulated in a biocompatible polymer, chitosan, which has been further modified to conjugate nitrilotriacetic acid (NTA) groups. After Ni^{2+}

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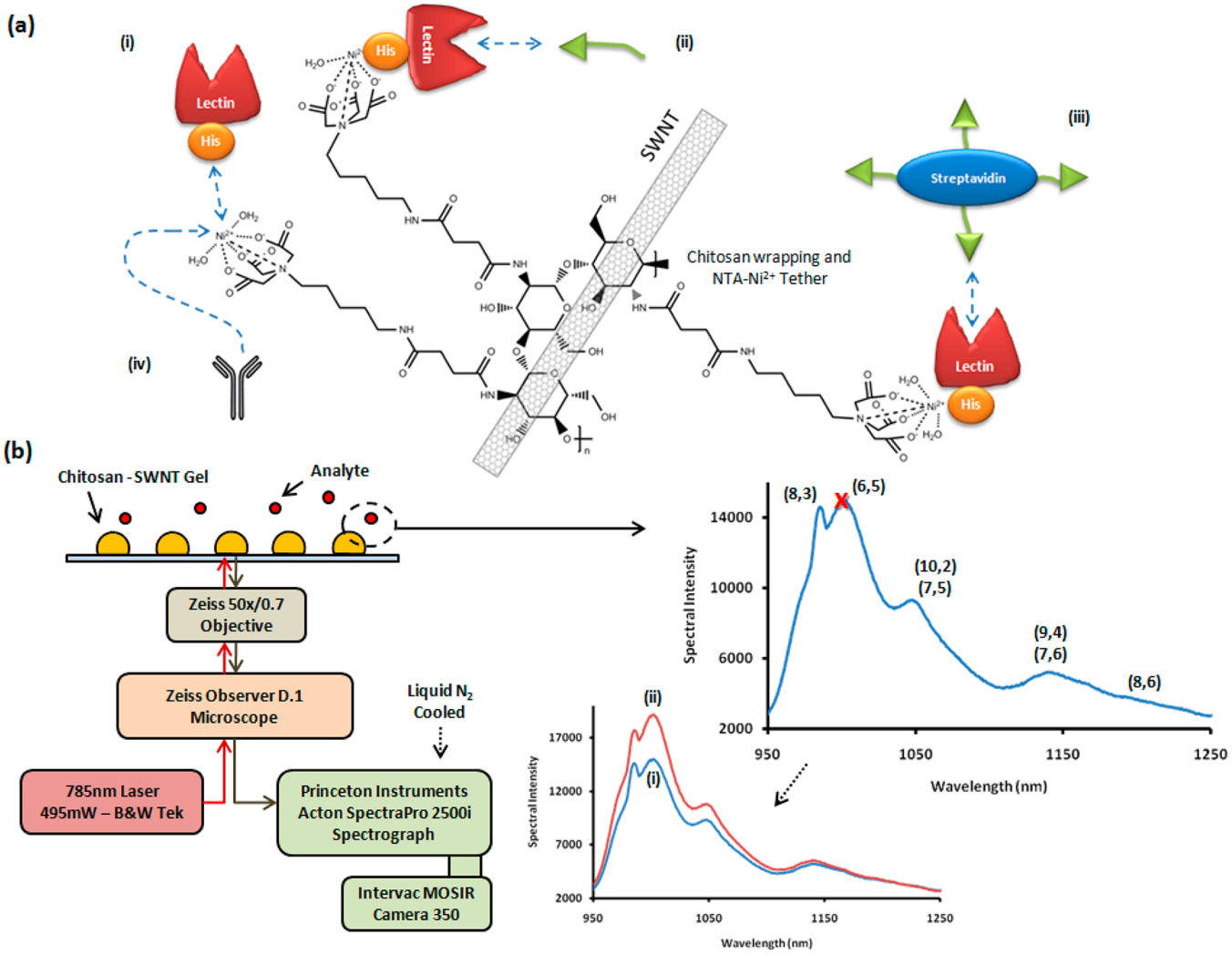

Figure 8. Optical nanotube-based sensor for glycan–lectin interactions. Reprinted with permission from ref 36. Copyright 2011 American Chemical Society.

chelation, NTA Ni^{2+} complexes bind to his-tagged proteins, resulting in a local environment change of the SWNT array, leading to optical fluorescence modulation[43] with a detection limit down to 100 nM. The platform has been further engineered to monitor single-protein binding events, with an even lower detection limit down to 10 pM.

Finally, the advantages, disadvantages, sensitivities, and applications of different techniques are summarized in Table 1.

5. THE DEVELOPMENT OF SYNTHETIC RECOGNITION SITES USING CoPhMoRe

In general, most biosensors rely on known biological recognition units such as antibodies. This limits many applications if there is no recognition unit available with sufficient affinity, size, or sufficient signal transduction. Recently, we introduced the concept of corona phase molecular recognition (CoPhMoRe).[9] If a polymer is confined to the surface of a nanoparticle, it forms a unique organic phase around this nanoparticle. Even if there is no known affinity of the free polymer for a certain analyte, organic phases exist that selectively interact with the analyte. We showed the feasibility of this concept by synthesizing different organic corona phases on carbon nanotubes and detecting analytes such as riboflavin, L-thyroxine, and estradiol. In an additional work, we applied this concept also for neurotransmitters such as dopamine.[53] Several examples in this Account are based on CoPhMoRe such as NO, H_2O_2, and glucose detection. In all cases, the free organic phase/polymer or the carbon nanotube itself did provide selectivity for these compounds before bringing them together. This concept holds very much promise for the future, because it indicates that by screening a large polymer library a selective corona phase for a specific analyte can be found.

6. CONCLUSIONS

From small molecule sensing to the detection of protein–protein interaction, nanoengineered platforms are emerging as a new research direction, holding great promise for a host of applications. In particular, a SWNT-based optical sensing system presents an opportunity for significant progress in many fields. Excellent photostability and the unique tissue transparency property of nIR fluorescence emitted from SWNTs are major advantages compared with other nanoparticles. Thus, sensor developments based on nIR fluorescence of SWNTs have already extended beyond mere molecular recognition and into living cell imaging and in vivo study. Definitely, the future directions of this platform will focus on the selectivity,

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Table 1. Summary of Advantage, Disadvantage, Sensitivities, and Applications of Different Techniques

detection method	sample preprocessing	advantages	disadvantages	detected biomarker	detection limit	physiological concentration in diseased patients
In_2O_3 nanowires, electrical measurements[39]	yes, filter off blood cells	very high sensitivity	(1) works in 1.5 mM ionic strength; (2) serum needs to be diluted or desalted into buffer; (3) and each device has a different calibration curve	CA-125, an epithelial ovarian cancer biomarker	0.5 pM in 100× diluted serum	100−275 U/mL or 0.5 −1.375 nM
				IGF-II, an epithelial ovarian cancer biomarker	8 ng/mL (1.5 mM buffer)	800 ng/mL
Si-FET, reference buffer method[44]	no	can work with serum directly		cancer marker, carcinoembryonic antigen (CEA)	0.2−114 ng/mL (in serum)	0−2.5 ng/mL healthy people; cancer patients significantly vary[45]
aligned carbon nanotube on quartz FET[46]	unclear, only demonstrated in buffer		currently only demonstrated in buffer	PSA	100 pM (buffer)	further screening for cancer may be required for elevated PSA levels in serum in the order of > ∼4 ng/mL (> ∼150 pM)
impedimetric apatasensor[40]	unclear, only demonstrated in buffer	cheap	detection limit is in ug/mL range; response is not linear	lysozyme	12.09 μg/mL (equal to 862 nM) in buffer	
nanochannel/nanoparticle-based filtering and sensing[47]	no, size-exclusion effect removes blood cells		still needs AuNP label to enhance sensitivity; label-free method has lower sensitivity.	CA-153	52 U/mL (in blood)	<30 U/mL in normal person, but levels as high as 100 U/mL can sometimes be seen in women who do not have cancer
				IgG	50 ng/mL (in buffer)	<30 U/mL in normal person
break-junction[48]	only tested in buffer		(1) seem to be complicated to make; (2) control samples also show some binding	EGFR	50 ug/mL (in buffer)	45−78 ng/mL, or 200 fmol/mL to 27 ng/mL[49]
impedimetric, diamond-based immmunosensor[50]	no	seem to be sensitive; linear response	data are difficult to analyze	C-reactive protein, cardiovascular diseases biomarker	10 nM	>25 nM is indicative of cardiovascular disease
resonance light scattering correlation spectroscopy (RLSCS)[51]	no	high sensitivity	require diluting the sample quite a bit to obtain meaningful results; and the setup is complicated	liver cancer biomarker alpha-fetoprotein (AFP)	linear 1 pM to 1 nM (in buffer); tested patients in 400× to 4000× diluted serum)	
zeta potential based colorimetric immunoassay[52]	only tested in buffer		has only been tested in buffer.	glycosylated hemoglobin (HbA1c)	1.5 ug/mL (in buffer)	5.5% of the total hemoglobin content
surface-enhanced fluorescence with Cy3-labeled VEGF-aptamer[42]	tested in buffer, has high response to physiological concentration of human serum albumin	very simple, cheap, one-step	has relatively high response toward human serum albumin	vascular endothelial growth factor-165 ($VEGF_{165}$), a predominant biomarker of cancer angiogenesis	25 pg/mL to 25 μg/mL (=from 1.25 pM to 1.25 μM) in buffer	500 pg/mL to 8 ng/mL
SWCNT-based optical sensing[43]	tested in in-house produced cell extract	simple, high sensitivity	have not tested in whole blood	34 proteins	100 nM ensemble, 10 pM single molecule measurements	

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sensitivity toward the single-molecule level, and the high-throughput microarray chips.

AUTHOR INFORMATION

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