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Current trends in nanobiosensor technology

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Abstract

The development of tools and processes used to fabricate, measure, and image nanoscale objects has lead to a wide range of work devoted to producing sensors that interact with extremely small numbers (or an extremely small concentration) of analyte molecules. These advances are particularly exciting in the context of biosensing, where the demands for low concentration detection and high specificity are great. Nanoscale biosensors, or nanobiosensors, provide researchers with an unprecedented level of sensitivity, often to the single molecule level. The use of biomolecule-functionalized surfaces can dramatically boost the specificity of the detection system, but can also yield reproducibility problems and increased complexity. Several nanobiosensor architectures based on mechanical devices, optical resonators, functionalized nanoparticles, nanowires, nanotubes, and nanofibers have been demonstrated in the lab. As nanobiosensor technology becomes more refined and reliable, it is likely it will eventually make its way from the lab to the clinic, where future lab-on-a-chip devices incorporating an array of nanobiosensors could be used for rapid screening of a wide variety of analytes at low cost using small samples of patient material.

Introduction

Advances in diagnostic technology have been essential to the progress of medicine. The ability to identify diseases and pathogens by detecting associated proteins, nucleic acid sequences, or other markers can provide biomedical researchers, disease specialists, and healthcare professionals with highly detailed knowledge of patients' conditions, disease pathways, or the presence of contamination. However, many of the tests currently commercially available (and thus used ubiquitously) are slow, require large amounts of sample materials, and can lead to false positive or negative results. The results of these problems range from mere annoyance on behalf of the patient to misdiagnosis, inability to

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detect potentially lethal pathogens, and difficulty in understanding the development and propagation of diseases. Thus there is a strong push towards developing improved diagnostic technologies that would allow for rapid, trustworthy, low-cost, multiplexed screening to detect a wide range of biomaterials. The current state-of-the art diagnostic biosensors are based on several technologies, often including either the enzyme-linked immunosorbent assay (ELISA) or (for nucleic acids) amplification of a sample by polymerase chain reaction (PCR) using appropriate primers and detection methods (gel electrophoresis, radioactive or fluorescent labels, etc.). In a typical configuration, ELISA indicates the binding of analyte molecules to an immobilized probe (i.e. a "sandwich assay" configuration) by exploiting the activity of an enzyme conjugated to a detection antibody that binds to the captured analyte. With the sandwich complete, any bound enzyme is indicative of the presence of analyte, and thus enzymatic activity upon introduction of an appropriate substrate can be used to quantify the amount of analyte.[1–3] On the other hand, PCR "amplifies" the signal due to the presence of analyte by selectively replicating the desired nucleotide sequences.[4,5] Both of these techniques require significant sample preparation and intensive sample handling, thereby increasing the potential for error in the diagnosis, as well as the cost and time required. Moreover, in many cases, the sensitivity of the assays may not be sufficient to detect the desired levels of analyte.

To overcome the issues associated with current diagnostic techniques, a wide range of new biosensors are being developed. Several of these biosensors rely on nanotechnological platforms. While what in particular constitutes "nanotechnology" is hardly well-defined, there is some general consensus that the term refers to devices or processes that occur and can be controlled at scales below the wavelength of visible light (sometimes the threshold is instead given as 100nm). At this scale, physical processes are often manifested differently than at the macroscale due to quantum effects (e.g. quantum dots, tunnel junctions, etc.). Moreover, surface-to-volume ratios become extremely large, a fact that is beneficial for sensors that often interact with analyte only on a surface, but exploit a change in the sensor volume. It is at this scale that the analyte molecules of interest exist and interact, and thus it is appealing to attempt to probe them with technologies that are controllable at this scale. The chief motivation for driving these technologies to nanoscale architectures is increased overall sensitivity, though the mechanisms for this improvement are somewhat specific to the particular nanobiosensor type. The field of nanobiosensors is vast, and this review aims to cover the more common configurations of nanobiosensors in brief. At the end of each section covering a particular nanobiosensor architecture, the reader is pointed to more focused reviews that cover each type of nanobiosensor in more detail.

Sensitivity and Specificity

Sensitivity

One of the many potential advantages of nanobiosensors over more conventional sensing systems is dramatically improved sensitivity. To better compare these sensors, it is important to discuss what is meant by "sensitivity" in this context. In fact, there are several important parameters that relate to how well a sensor can detect an analyte.[6,7] The internal sensitivity of a sensor is defined as the ratio of the sensor output signal to a change in a

property of the sensor (presumably due to an amount of analyte material bound to the sensor). This parameter can be thought of as a slope defining the ability of the sensor to transduce an input signal (bulk sensor property change) to an output signal. A sensor with a large internal sensitivity value is able to pick out a minute change in a bulk sensor property (due to bound analyte) more easily than one with a small internal sensitivity value.

There is some variation in the literature as to what kind of value the denominator of the sensitivity parameter should be. To take into account the fact that a sensor (often) interacts with an analyte on the sensor surface, the sensitivity may be defined as output signal per (mass/unit sensor area). This method of normalizing away the sensor surface area can often better define the inherent ability of a sensor. While a sensor is often inherently sensitive to an amount of bound analyte, the quantity of interest is typically not a total bound mass (or number of molecules), but a concentration of analyte. This leads one to define a quantity that is expressed as output signal per analyte concentration. The subtlety of this parameter is that it takes into account not only the inherent transducer sensitivity, but also the total exposed area of the sensor, the kinetics of the binding interaction between the sensor and analyte, and other such effects. These factors are particularly important for nanoscale biosensors, as the total exposed sensor surface area may be particularly small, the kinetics of the interaction between the sensor and the analyte may be slow, the affinity of the analyte to the sensor may be poor, etc. Thus, while a sensitivity defined with respect to analyte concentration is in many cases a more useful parameter than one defined with respect to total analyte mass, the effects included in this definition are more complex.

Regardless of the definition of sensitivity used, it can be related to a minimum detectable quantity, called the limit of determination (LOD) or limit of detection or minimum detectable concentration (or mass). This refers to the smallest quantity that can be resolved above a background signal. To define this quantity, the resolution of the sensor must be characterized. If all the various noise processes in the sensor system contribute to a standard deviation in a measurement σ, then the resolution of the sensor is restricted by this standard deviation (often the acceptable spacing between measurements is taken to be 3σ, and thus this is the minimum measurable value above background noise). This leads to a LOD defined by LOD= 3σ/sensitivity. This can be considered the minimum detectable mass or concentration of analyte.[6]

The term “sensitivity” can also refer to test (or assay or diagnostic) sensitivity, which describes how well a diagnostic test is able to correctly identify a certain population of samples as containing (or not containing) an analyte of interest.[8] This definition encompasses several issues, including reproducibility and accuracy of the diagnostic.

Dynamic Range—One parameter rarely discussed in the context of nanobiosensors is the sensor dynamic range. This quantity describes the range over which the sensor is able to accurately produce an output signal indicative of the analyte quantity. The dynamic range is limited on the lower end by the LOD, and on the upper end by saturation of the sensor, breakage of the sensor, or unpredictable changes in the sensor sensitivity. The response curve of the sensor describes the entire range of analyte quantity and corresponding sensor output, and should either be linear or have a corresponding calibration dataset or known

scaling law to allow the user to correctly interpret the sensor output. The upper limit of this range may be extended by performing a kinetic (i.e. non-equilibrium) measurement instead of a steady-state measurement; however, this is uncommon because the increased complexity (increased requirement for controlling flow rates, solution properties, temperature, measurement time, etc.) can reduce measurement reproducibility. Depending on the sensor application, dynamic range may or may not be of interest.

Specificity

Besides being able to produce an output signal indicative of the presence of analyte, a sensor must also be able to distinguish between analyte and any "other" material. This quality, called specificity, is what renders a sensor useful in a non-controlled environment containing quantities of unknown material. Unlike sensitivity, specificity can be difficult to measure and confirm, as the number of possible materials that should *not* produce an output signal is effectively infinite. While several researchers demonstrate specificity by comparing sensor response to the analyte of interest to sensor response to a similar material, the true test of a sensor's specificity is in the field. The specificity of a sensor becomes particularly important when trying to detect an analyte at low concentration in an environment containing a high concentration of other materials, many of which may bind non-specifically to the sensor and thus produce an anomalous signal.

Biosensors often exploit the complex, specific binding interactions provided by nature, such as antibody-antigen, nucleic acid hybridization, biotin-streptavidin, and enzymatic activity. Several groups have developed artificially synthesized (or discovered) ligands, such as aptamers (artificial nucleic acid ligands that can be selected to specifically bind to a wide range of molecules through an iterative process called systematic evolution of ligands by exponential enrichment (SELEX))[9–21] and molecularly imprinted polymers (copolymers made from functional and crosslinking monomers that are polymerized around a template, or "print", molecule, which is subsequently removed by extraction, leaving a polymer with binding sites in the specific shape and size of the template)[22–28], that may have better performance than natural ligands, or may be applicable to a wide range of target molecules. Another method that can be used to obtain some level of specificity is to use an array of non-specific sensors that each react differently to exposed materials; the resulting signals from the sensor array can be used to identify analytes with a known, "signature" response (from a previous calibration experiment). This technique is often used in "electronic nose" or "electronic tongue" sensors, but is currently less commonly used in biosensors.[29–32]

The techniques used to attach specific capturing molecules to sensor surfaces depend on the sensor surface chemistry. Perhaps the most simplistic method is physical adsorption, where the capture molecules are exposed the sensor surface and allowed to physically adsorb onto the surface (i.e. bind to the surface non-covalently). For gold substrates, it is common to see self-assembled monolayers of molecules with a thiol anchoring group connected (often via an organic chain) to a functional group used for molecular recognition. A description of a multitude of surface functionalization techniques for gold substrates can be found in a review by Shankaran and Miura.[33] When silicon surfaces (which, after exposure to atmospheric conditions, contain hydroxyl end groups -Si-OH) are silanized, covalent -Si-O-

Si- bonds are formed between the silicon surface and organofunctional alkoxysilanes. The organofunctional alkoxysilanes are used to bind probe molecules using various functional groups, including amines (such as in APTES[34]), epoxides and thiols. Hydrosilylation of unoxidized silicon (often freshly etched material unexposed to atmosphere, having exposed -Si-H groups on the surface) inserts a carbon-carbon unsaturated bond in the silicon-hydride bond and can create alkyl or alkenyl monolayers which can be further modified to bind probes.[35] Magnetic nanoparticles (consisting of iron-based metal oxides) are usually coated with natural or synthetic polymers such as dextran[36] and polyethylene glycol (PEG)[37] containing desired functional groups. Thus these polymers can interact with functional groups on the probe molecule, such as amines, aldehydes and carboxylic acids, to form covalent bonds and immobilize the probe. A description of many functionalization techniques and polymer coatings for magnetic nanoparticles can be found in a review by McBain et al.[38] There are a wide range of processes for functionalizing carbon nanotubes (CNTs) either non-covalently or covalently.[39,40] It has been found that proteins and DNA will non-specifically adsorb onto the CNT surface, and aromatic molecules will interact strongly with CNT sidewalls via π-stacking.[39] Covalent linking of capture probes to CNTs can be achieved using linking molecules that interact with defects on the CNT (for example, carboxylic acid groups formed by oxidation). The above examples are only a small sample of the available chemistries used to immobilize capture molecules onto sensor surfaces.[41]

Nanobiosensor Systems

Mechanical Resonators and Static Deflection devices

Sensors exploiting mechanical motion (often called micro/nano-electromechanical systems, or MEMS/NEMS, sensors) to detect analyte can generally be divided into two categories: static deflection devices and resonators (Figure 1). Static devices, also known as deflection based sensors, are typically beams supported at one end by a larger substrate (cantilevers). The top of each cantilever is functionalized with a specific receptor, such as oligonucleotides used for hybridization, aptamers or antibodies. The cantilever deflects up or down because of changes in surface stress when the desired analyte binds to the receptor. The position of the cantilever can then be detected and interpreted.[42–45] Detection of cantilever deflection can be accomplished using several different optical and electrical methods, including using a piezoresistive element (an element that will exhibit a change in resistance due to mechanical stress) or reflecting a laser off the cantilever surface and detecting the angle of reflection (typically using a two- or four-quadrant photodiode in a geometry identical to that used in the majority of atomic force microscopes). Static deflection devices employing functionalized cantilevers have been used to detect oligonucleotides (some studies demonstrated the ability to detect a single base-pair mismatch, some studies demonstrated LOD on the ten picomolar range)[46–49] and proteins (LOD in the nanomolar range).[46,50]

Dynamic, or resonant, devices are oscillators that have a resonant frequency dependent on the resonator mass. When analyte binds to receptors attached to the device, the frequency will shift; this frequency shift can then be interpreted as a mass change (Figure 2). There is significant interest in producing nanoscale mechanical resonator devices because, as the

device size decreases, the device mass (*m)* decreases. Thus the addition of bound analyte molecules (Δ*m*) causes an increased relative change in effective resonator mass *(m+*Δ*m)/m*, leading to a larger frequency shift. Smaller devices also benefit from an increased surface-to-volume ratio. Moreover, the high quality factors (Q, a parameter that determines the width of the resonance peak; a higher Q corresponds to a more narrow resonance peak and thus allows the discrimination of a smaller frequency shift) of micro- and nano- mechanical devices allow for extremely sensitive mass measurements. These devices have a variety of different geometries such as cantilevers, domes, doubly clamped beams, torsional resonators, trampolines, etc. Methods for driving a dynamic device include thermal excitation, mechanical coupling,[51] electrostatic,[52] magnetic,[53] and piezoelectric.[54] Thermal excitation can cause resonance through thermal expansion, causing stress. Response can sometimes be improved using a bilayer of materials with different thermal expansion coefficients. Heat can be introduced to the system by fabricated resistors near the device (to heat via Joule heating), or by a focused, pulsed laser. The simplest actuation methods, piezoelectric and optical, can be external to the resonator and do not require additional fabrication on the resonator. A piezoelectric device (a “buzzer”) can be attached to a chip with a resonator device in order to induce resonance mechanically. To optically excite the resonator, a pulsed focused laser beam can be used to thermally excite oscillation.[55] To optically detect resonance, a focused laser beam can be either reflected of the resonator surface at an angle (similar to optical detection of a static deflection device) or used as the illumination source for a Fabry-Pérot interferometer formed by the resonator (as one reflecting surface), the media in which the nanobiosensor operates, and the plane of the chip over which the resonator is suspended (the other reflecting surface).[55,56] In this second configuration, the laser is able to probe minute changes in the gap between the reflecting surfaces, even if the resonator width is significantly smaller than the size of the focused laser spot. When the device is optically driven, the detecting laser should be of a different wavelength. Each excitation method and detection method has advantages and disadvantages that depend on the intended use of the device. For example, while excitation and detection methods that employ on-chip circuitry (such as wire-loops, piezoresistors, or capacitive plates) require less complex macroscale experimental apparatus to interrogate the resonator (compared to optically driven and detected systems), they are more complicated to fabricate.

The Craighead group has used mechanical resonator nanobiosensors to detect a wide variety of biomaterials, including single molecules of DNA (1587 base pairs),[57] viruses,[58] bacteria,[59] prostate-specific antigen (LOD of 1.5 fM using secondary mass labels),[60] prion proteins (LOD of 2ng/mL using secondary mass labels),[61] other proteins,[62] and cells (single-cell detection)[63]. Some of these results are from multiplexed devices incorporated in fluidic channel systems that are cycled through liquid and vacuum (for detection) cycles.[62] Other groups have used mechanical resonators to detect anthrax spores (LOD of 2 spores when detection performed in air, and 50 spores when performed in water),[64] viruses (single-virus detection, non-specific),[65] and prostate-specific antigen (LOD of 10 pg/mL).[66]

Both static and dynamic devices are often fabricated in silicon or silicon nitride, though a wide range of other materials, including polymers,[67,47,68] have been used. The techniques

used in semiconductor manufacturing to make microelectronics, such as photolithography, are also used to fabricate the majority of mechanical biosensor devices. These techniques allow additional circuitry to be added for actuation and detection, as well as the addition of other components needed for integration (fluidics, on-board readout circuitry, etc).

Static devices have an advantage over dynamic devices in that they operate well in both gas and liquid environments, as well as vacuum. However, stress-based detection is limited to the near monolayer regime; single molecule binding typically cannot cause detectable deflection. Dynamic devices have a significantly lower limit of detection compared to static devices. Masses on the attogram and zeptogram scale have been detected using resonant techniques; however these measurements are performed at ultrahigh vacuum and low temperature.[69,70] Due to viscous losses and damping, dynamic devices perform poorly in gaseous and aqueous conditions (with quality factors on the order of 10s or 100s), making it difficult to use mechanical resonators for biosensing without the additional steps of drying and evacuating the resonator after its exposure to analyte solutions. These additional steps have, until recently, dramatically inhibited the use of mechanical resonators for real-time sensing in aqueous environments.

Recent work in the area of resonant mechanical nanobiosensors has shown promise in overcoming this hurdle. Manalis and colleagues showed in 2007 that very small masses of cells, proteins and nanoparticles could be resolved using a variation of resonant cantilevers that contained microfluidic channels (Figure 3).[71] The novel use of channels inside the resonator allowed the resonator to be operated in a low pressure environment (and thus achieve a high quality factor of 15,000) while still providing a mechanism for liquid solutions to be introduced to a part of the resonator surface (in this case, via an interior volume). Resonator devices containing embedded microfluidic channels have been used to measure the mass of single cells during their growth cycle,[72,73] detect cancer biomarkers (LOD of 10ng/mL),[74] detect IgG protein (LOD sub-nM),[71] and weigh individual bacteria.[71] Barton et al. have recently evaluated the performance of doubly clamped beam resonators that contain nanofluidic channels with fluids.[75] They expect a minimum detectable mass with this configuration on the femtogram scale. Similar work performed by the Manalis group using resonators with embedded nanofluidic channels demonstrated detection of masses (Au nanoparticles) in fluid at the level of tens of attograms (single nanoparticle sensitivity).[76] These “inside-out” mechanical resonators overcome the major limitation of mechanical resonator nanobiosensors: reduced quality factor in liquid and gas environments. As this technology becomes more refined, detection of lower mass entities in solution seems likely. In general, there is still a strong push towards reducing the sizes of mechanical resonator sensors, with carbon nanotubes[77,78] and single atomic layers of carbon, called graphene,[79] being the ultimate limit. A thorough review on mechanical nanobiosensors can be found in an article by Waggoner and Craighead.[80]

Optical Resonators

Like mechanical resonators, optical resonator sensors rely on a change in resonant frequency to indicate binding of an analyte. While mechanical resonators are based on physical motion, optical resonators are based upon light oscillating within a resonant cavity. Whispering

gallery mode (WGM) resonators are a commonly used optical resonator and consist of a circular cavity (such as a microsphere or microdisk) into which light is introduced, often by evanescent coupling of a tapered optical fiber placed next to the cavity.[81] The coupled light is confined within the cavity by total internal reflection, and if the wavelength of the light is such that it is able to propagate around the cavity and interfere constructively with itself (in other words, if the cavity circumference is equal to an integer number of wavelengths), then the light will resonate within the cavity. This resonance is measured as a drop in the transmission of light through the nearby optical fiber at the resonant wavelength. Because the resonating light passes multiple times around the cavity, it is able to interact with bound molecules via the evanescent field produced on the cavity surface multiple times before dissipating. The dissipation is inversely related to the quality factor Q of the resonator. The resonant frequencies of a given cavity are ultrasensitive to changes in the effective path length travelled by the resonating light caused by bound analyte at the surface of the cavity. This is illustrated in Figure 4.

Soon after theoretical work describing a WGM resonator biosensor configuration was published (note that this work did not treat the more commonly exploited resonance frequency shifts due to bound molecules as described above, but transmission changes due to nearby absorbing molecules),[82] several groups dedicated effort to realizing the exciting potential sensitivity of the WGM resonator architecture. As a first experimental demonstration of a WGM nanobiosensor, Vollmer et al used a 300μm diameter silica glass microparticle as the cavity for a WGM sensor with a Q of 2×10^6 to detect both non-specific binding of BSA and specific binding of streptavidin to bound BSA-biotin conjugates.[83] They concluded that their sensor saturated at BSA concentrations as low as 20nM, defining an upper limit to their dynamic range; this work did not examine the lower concentration limit of detection. Subsequent theoretical work provided some suggestions to improve WGM biosensor system sensitivity.[84] Work with a similar experimental system demonstrated multiplexed measurement of DNA hybridization to probes bound on the cavity surface.[85] In this system, two microspheres (each with its own “signature” resonance spectrum) were brought near to a tapered optical fiber, one after the other, and thus the resonance dips in the multiplexed transmission spectrum could be uniquely assigned to each cavity. When each microsphere was modified with a different probe oligonucleotide, a resonance dip shift assigned to one of the cavities correctly indicated DNA hybridization upon the introduction of appropriate complementary oligonucleotide into the system. Subsequent introduction of oligonucleotide complementary to the probes on the second microsphere caused a shift in the dip assigned to that cavity, but didn’t cause a detectable shift in the dips assigned to the cavity functionalized with non-complementary probes. The authors estimate the detection limit of this system to be ~6 pg/mm^2 nucleic acid. This nanobiosensor system was able to identify a single nucleotide mismatch with high specificity (the cavity functionalized with a matched probe showed a wavelength shift 10× larger than the one functionalized with a mismatched probe, at a final concentration of 1 μM) and a signal-to-noise ratio of 54. The next major step in WGM nanobiosensor technology was achieved by Armani et al[86] using a micro-toroid cavity with a Q greater than 10^8. In this work, the authors demonstrated single-molecule detection sensitivity for interleukin-2 (IL-2) binding to a surface layer of IL-2 antibody on top of Protein G. Not only

was this nanobiosensor able to perform real-time single-molecule detection, but it also exhibited a dynamic range of 12 orders of magnitude (working range of 5 aM to 1 μM) with a sigmoidal response curve. The physical mechanism for the improved sensitivity of this measurement system, however, is still under debate in the current literature.[87]

Optical resonators have been integrated with fluidics in a geometry called a capillary ring resonator (CRR) (sometimes called a liquid core optical ring resonator (LCORR) or opto-fluidic ring resonator (OFRR)). In this sensor configuration, the walls of a glass capillary are used both as the resonator cavity and as a fluid transport system.[88] A tapered optical fiber brought near the capillary will couple light into the cavity, and changes in the refractive index near the interior surface of the capillary wall are detected as shifts in the transmission dips corresponding to resonant modes. In this system, liquids flowing through the inside of the capillary may be used to deliver analyte to the interior wall, which may be functionalized to provide sensor specificity (Figure 5). These nanobiosensors have been used to detect BSA (without specificity, LOD below 10pM),[89,90] DNA (demonstrated LOD of 10pM),[91] and viruses (demonstrated LOD of 2.3×10^3 pfu/mL of M13 filamentous bacteriophage, dynamic range of 7 orders of magnitude).[92]

While WGM nanobiosensors promise single-molecule sensitivity and are easily functionalized to attain high specificity, their inherent complexity has so far prevented their widespread use. They require tunable lasers to inject light into the cavity and high quality optical detectors with sensitivity as the laser wavelength (both of which are costly), careful alignment of the tapered fiber and the cavity, and potential microfabrication processes to form the cavity itself. It is also important to maintain thermal stability in these systems. However, these issues may all be overcome by systems entirely integrated “on-chip”, and thus there is the potential to reduce cost and complexity of these devices by incorporating most or all of the components on a single chip using a scaled-up manufacturing technique. Capillary ring resonators may help overcome scalability problems by integrating the resonant cavity and fluidic transport device into a single system. An in-depth overview of the current state of WGM-based nanobiosensors and the wide range of potential applications may be found in a review by Vollmer and Arnold.[81]

There are a wide variety of other optical nanobiosensors, including zero mode waveguides,[93–95] single metallic nanoparticles,[96] nanobiosensors based on surface enhanced Raman scattering (SERS)[97,98,9,99–104], Förster (or fluorescence) resonance energy transfer (FRET) between chromophores,[105–107] and systems based on the aggregation of nanoparticles upon binding (see below). A review by Erickson et al provides a discussion of a wide range of nanobiosensor configurations, with particular focus on optical nanobiosensors.[108]

Aggregating Nanoparticles

Nanoparticle-based biosensors are particularly attractive because they can easily be synthesized in bulk using standard chemistry techniques and do not require advanced fabrication approaches (i.e. production is bottom-up instead of top-down). They also offer particularly high surface area due to their extremely small size and the fact that they are typically used suspended in solution (during the time at which they interact with the

analyte). Due to their small size, nanoparticles may be taken up by cells,[109–111] and thus are promising for *in vivo* sensing applications. Moreover, nanoparticles can have unique optical or magnetic properties that may exploited. Several sensing platforms have been developed that exploit a change in output signal due to aggregation of suspended nanoparticles caused by the presence of analyte. Typically, nanoparticles are functionalized with a ligand for the desired analyte, and thus upon exposure to analyte in solution the nanoparticles will form a network.

Early work in this field performed in the Mirkin group demonstrated colorimetric detection of oligonucleotides using Au nanoparticles functionalized with probe oligonucleotides.[112] Gold nanoparticles suspended in solution exhibit a strong optical absorbance at particular visible optical wavelengths due to plasmon absorption ; this property is dependent on the nanoparticle size.[113] Upon hybridization with the target oligonucleotide, the probe molecules attached to the nanoparticles induce the formation of a nanoparticle network, causing a change in solution color from red to blue (Figure 6). This color transition is extremely sharp, and the specificity of this sensing system is good enough to easily detect single base mismatches. The resulting signal upon binding is significant enough to be detected by the naked eye without any artificial equipment to provide amplification or additional transduction. These colorimetric nanoparticle assays work on the principle that aggregation of small metal nanoparticles causes a change in the nanoparticle surface plasmon resonance, and thus the resulting macroscale optical properties of the nanoparticle suspension.[114] Similar nanoparticle assays (some of which monitor changes in solution absorbance at a given wavelength due to aggregation) have been used to detect not only nucleic acids,[112,115] but metal ions (typical LOD ranging from 1–100μM),[116–118] proteins,[10,119] adenosine (LOD of 100μM)[120] and cocaine (LOD of ~50μM).[11] Assays based on nanoparticle aggregation employing amplification by “bio-barcodes”,[121] or indicating oligonucleotide strands, have been used to detect both nucleic acids (with sub-attomolar sensitivity)[122] and proteins (with attomolar sensitivity),[123] and have been combined with colorimetric nanoparticle aggregation detection.[124] More information on nanoparticle-based biosensors may be found in a review by Rosi and Mirkin.[125]

A similar architecture, developed by Weissleder and co-workers, is based on the aggregation of superparamagnetic nanoparticles and may be used in conjunction with MRI for biosensing purposes. The use of nanoscale particles is necessary because the superparamagentic property depends on the particles each having only a single magnetic domain. Instead of a change in an optical signal, these assays rely on an aggregation-induced change in the spin-spin relaxation time (T2) of water molecules near the nanoparticles, which can be detected using magnetic resonance techniques.[126] Thus, this technology is very promising for *in vivo* sensing applications, as it could be used in conjunction with already established NMR/MRI systems. Similar to the architecture described above, this technology relies on nanoparticles functionalized with ligands that bind the analyte desired, and thus aggregate upon exposure to analyte. This system has been used to detect herpes simplex virus and adenovirus in biological samples (best demonstrated LOD of 5 viral particles in 10μL of liquid),[127] influenza,[128] nucleic acids,[126,129] adenosine,[12] enzyme activity,[130–133] avadin,[134] and other proteins (demonstated LOD often at the nanomolar

range).[129,135,136] This technology has also been utilized with a Boolean logic analyte binding system, whereby sensor response occurred either in the presence of matrix-metalloproteinase-2 (MMP2) AND matrix-metalloproteinase-7 (MMP7), or in the presence of MMP2 OR MMP7 (where AND and OR refer to the logic functions).[137] This provides an attractive opportunity to produce custom, “smart” sensing systems that are carefully tuned to produce an output signal only upon the presence of a particular combination of analytes, and the absence of others. Magnetic nanoparticle-based sensing systems have been integrated into lab-on-a-chip devices, providing a handheld detection system that has been demonstrated for use with bacteria, mammalian cells, and various biomarkers.[138] Several reviews on magnetic nanoparticle aggregation biosensors provide a more in-depth discussion of this technology.[139,36]

Other nanoparticle-based nanobiosensors are based on single metallic nanoparticles,[96] mechanical resonators biosensors employing nanoparticle secondary mass labels,[60] and nanobiosensors exploiting the SERS effect.[99,101–104,140]

Nanowires, Nanotubes, and Nanofibers

Several one-dimensional nanostructures have been used as nanobiosensors, typically in an electrical field-effect transistor (FET) configuration with analyte molecules acting as a gate to control the sensing structure’s electrical resistance by causing depletion or accumulation of charge carriers. One or several of the one-dimensional nanostructures are placed between metallic electrodes to allow interfacing to external electronics equipment (Figure 7). These devices offer improved sensitivities due to large surface-to-volume ratios, which enable bound analyte molecules to more significantly affect the bulk electrical properties of the structure. In some cases (e.g. carbon nanotubes), the inherent electrical properties of the device are particularly extraordinary and lend themselves to improved sensor sensitivity.

Carbon nanotubes (CNTs) have received a great deal of attention due to their extremely small size and extraordinary mechanical, optical, and electrical properties. While easily synthesized, they are difficult to work with for several reasons. One major hurdle of working with CNTs is controlling their placement; typical synthesis techniques result in unaligned, “hairy” tangles of CNTs. Recently, techniques have been developed to control the alignment and placement of single and multiple CNTs.[141–144] Other improvements have allowed the growth of nanotubes to extremely long lengths, thereby producing structures with extremely high aspect ratios.[145,146] Another issue encountered when attempting to produce electrical CNT sensors is that the electrical properties of each CNT depend on its chiral vector, a parameter that describes how the graphene sheet that forms the nanotube is rolled up. CNTs can be metallic or semiconducting (with a wide range of bandgap values), depending on this parameter.[147] Typical synthesis processes (laser ablation, chemical vapor deposition, arc-discharge)[148] result in a heterogenous distribution of CNTs chiralities and diameters (the two parameters the define the electrical behavior of the CNT). Thus, to achieve production of electrical CNT-based devices with a high level of consistency and reproducibility, techniques have been developed to isolate CNTs with more controlled electrical properties.[149,150] CNTs can also be categorized as either single-walled nanotubes (SWNTs) or multi-walled nanotubes (MWNTs), depending on how many concentric cylinders of

wrapped graphene form the tube. Several reviews on novel techniques to control alignment, placement, and electrical properties offer more details on CNT synthesis and fabrication advancements.[148,147,151]

Due to their extreme electrical sensitivity to nearby molecules, CNTs have been incorporated into electrical nanobiosensors by several researchers. A common configuration utilizes semiconducting CNTs as FETs. Dai and coworkers demonstrated CNT-based nanobiosensors based on biofunctionalized SWNT networks used to specifically sense several biomolecules (including several monoclonal antibodies, streptavidin (Figure 8), and other proteins) with nanomolar sensitivity.[152,153] They have also discussed in depth the issue of non-specific binding of peptides to CNTs, and techniques to reduce this effect by coating the CNTs with polyethylene oxide (PEO).[152,154] Other groups have demonstated CNT-based nanobiosensors able to detect glucose (via functionalization with glucose oxidase),[155,156] streptavidin,[157,158] thrombin,[21] other proteins,[157,159] and nucleic acids (with sensitivity in the picomolar range).[160,161] CNT networks have also been used as electrodes in electrochemical biosensors, providing improved sensing ability due to both their high surface area and their excellent electrical characteristics.[162,163] More discussion of CNT-based biosensors can be found in several review articles written on the subject.[125,164]

Nanowires produced from other semiconductors have also been used as nanobiosensors. While their sizes and electrical behavior are typically not as extreme as CNTs, they can typically be produced with more reproducible properties and thus may be more amenable to industrial manufacturing. While they can be patterned lithographically,[165–168] nanowires are more often synthesized using a bottom-up chemical growth approach.[17,159,169–171] The Lieber group has exploited FETs made using bottom-up synthesis of doped silicon nanowires to specifically sense a wide range of biomaterials, including streptavidin (LOD of roughly 10pM),[169] antibodies,[169] nucleic acids (LOD of approximately 10fM),[170] viruses (at the single virus level),[172] and other proteins.[173] Some of these studies have incorporated arrays of differently functionalized sensing elements, allowing multiplexed sensing of several analytes on the same chip. Semiconducting polymeric nanowires,[174,175] sometimes called nanofibers, can be used in the same configurations as other semiconducting one-dimensional nanostructures.[176–178] They are attractive because polymeric materials are in general less expensive than other semiconductors, and offer the possibility of more complex functionalization strategies by choosing appropriate monomers (including biomolecules) to integrate during synthesis. However, they in general exhibit poorer electrical behavior than other semiconducting materials. Further information on nanowire and nanofiber biosensors can be found in several reviews.[179,180]

Integration and Scalability

The ability to integrate nanobiosensors with the macroscale world is extremely important; without this capability the sensor is (in the majority of cases) effectively useless. Nanobiosensor technology is often paired with micro/nanofluidic devices to deliver analyte to the sensor surface. This allows the usage of small volumes of sample solution. Theoretical treatments of nanobiosensor configurations suggest that sensitivity may in many cases be limited not by the inherent ability of the sensor, but by transport of analyte to the sensor

surface within an acceptable timeframe.[181–183] Novel fluidic designs based on wicking of solutions in patterned paper,[184–188] or on evaporation-driven flow[189–191] may help reduce the cost and complexity associated with many fluid delivery systems and have shown tremendous promise in applications in third-world countries. Several nanobiosensor architectures require a high level of integration with external equipment (detectors, pumps, electrical components, lasers, and optics), thereby increasing the cost and complexity (and reducing the portability) of the resulting device.

Nanobiosensors produced using top-down fabrication approaches are in many cases not easily (and inexpensively) scaled up to commercial manufacturing levels (with necessary reproducibility and yield). While one-dimensional nanostructure biosensors are attractive due to their extreme sensitivity, there are several issues regarding scalability and reproducible fabrication (i.e. producing sensing components with reproducible electrical behavior, wiring to external electronics, etc.) that may hinder commercial usage of this architecture. Improved fabrication techniques that allow for multiplexing[173] and reproducible device production may aid in the transfer of this technology to commercial diagnostic tools. Diagnostic tools containing several multiplexed highly sensitive nanobiosensors would be invaluable for identifying diseases, and thus the ability to include a large number of sensors specific to different analytes on a single platform is highly attractive. However, only a small fraction of the literature on nanobiosensor technology has demonstrated any multiplexing ability. Nanobiosensors produced using bottom-up synthesis techniques have issues with scalability due to potential difficulty in controllably synthesizing the nanoparticle/nanowire/nanotube with well defined properties (shape, size, or electrical properties). The stability of suspensions of functionalized nanoparticles must also be examined to determine an appropriate “shelf-life”, and they must not be exposed to materials that could cause them to aggregate prematurely.

Conclusion

A wide range of nanobiosensors have been developed in the past two decades, and yet the futuristic goal of low-cost, high throughput, multiplexed clinical diagnostic lab-on-a-chip devices has yet to be truly realized. It is still unclear which nanobiosensor architectures are best matched to which diagnostic tasks. Moreover, nanobiosensors that are functional in the lab may not be of use in the field or clinic for several reasons. Sensitivity and dynamic range should be matched to the analyte and what truly needs to be sensed, and thus it is unclear whether single-molecule sensitivity nanobiosensors will be of clinical use unless they exhibit a dynamic range that extends through a useful concentration range. Preconcentration or pre-dilution of sample could be used to match the analyte concentration to the appropriate concentration range of the nanobiosensor, but this additional step introduces complications and renders the assay inefficient and potentially less accurate. Another consideration when transitioning sensors from the lab to the clinic is their specificity and ability to be fouled by non-specific binding of materials in the sample being interrogated. Finally, the robustness of the sensor architecture, as well as the ease of use, become important parameters when novel diagnostic technology transitions from the development phase to being usable.

Nanobiosensors have generated a great deal of excitement due to their ability to detect a wide range of materials at incredibly small concentrations. As the field (and the multitude of different technologies that it encompasses) matures, it seems highly likely that the diagnostic techniques of today will soon become antiquated, and a new class of low cost, robust, reliable, easy-to-use, and ultra-sensitive diagnostics will become available. This may even spur a dramatic increase in the number of point-of-care diagnostics, as well as diagnostic tools that can be used by patients on their own. Whether a single nanobiosensor architecture will become dominant, or several will transition to commercial devices has yet to be seen. However, it is almost certain that these sensors will allow the detection of pathogens and diseases like never before.

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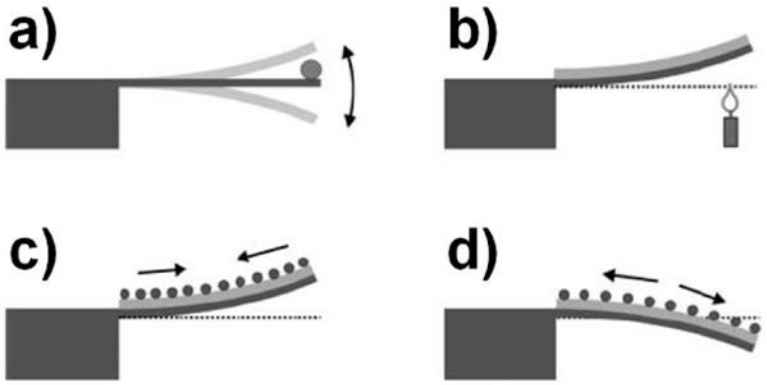

Figure 1.

Illustration of several mechanical device configurations. a) A resonating mechanical device (cantilever) that indicates a change in resonator mass (due to bound analyte) by a shift in resonant frequency. b) A static deflection device formed from two materials with different thermal expansion coefficients that bends due to a change in temperature. c,d) Static deflection devices that bend due to analyte binding causing a surface stress.[192] Fritz J. Cantilever biosensors. *Analyst* 2008, 133:855. http://dx.doi.org/10.1039/b718174d Reproduced by permission of The Royal Society of Chemistry

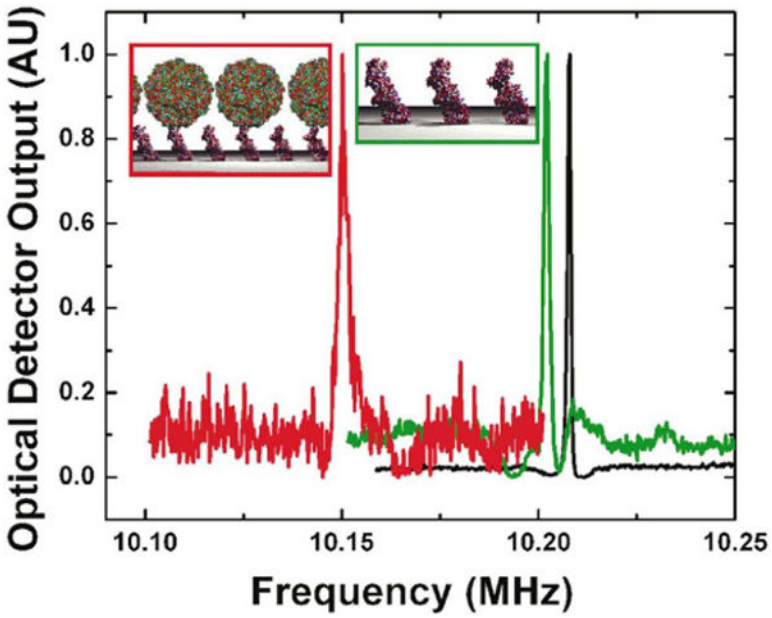

Figure 2.

Frequency shifts of a NEMS resonator due to binding of AcV1 antibody (green) and baculovirus particles (red).[58] Reprinted with permission Ilic B, Yang Y, Craighead HG. Virus detection using nanoelectromechanical devices. *Applied Physics Letters* 2004, 85:2604. Copyright 2004, American Institute of Physics.

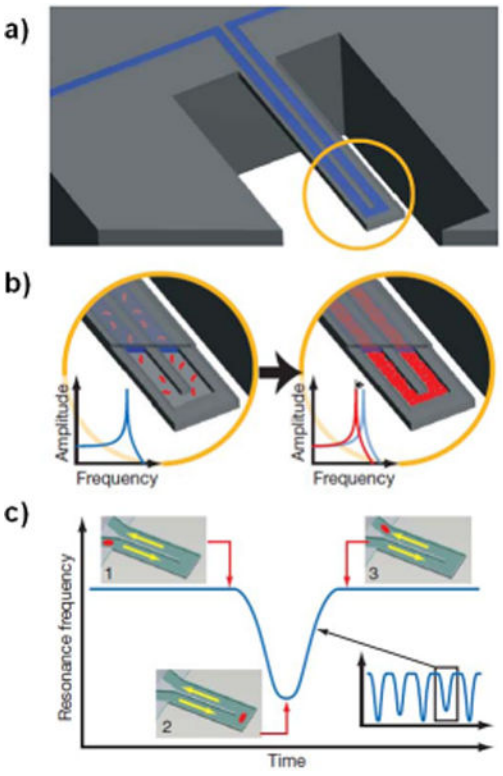

Figure 3.

a) Illustration of a mechanical cantilever resonator containing an embedded microfluidic channel. b) Illustration showing a decrease in resonant frequency as the density inside the embedded channel. If molecules that selectively bind to the channel walls are present, they will accumulate in the channel, causing an increased density in the channel that can be detected as a frequency shift. c) If a single particle is cycled through the channel, it will cause a frequency shift that varies with the particle's position along the cantilever. This allows the measurement of the mass of single, isolated, unbound particles in solution.[71] Reprinted by permission from Macmillan Publishers Ltd: Nature. Burg TP, Godin M, Knudsen SM, Shen W, Carlson G, Foster JS, Babcock K, Manalis SR. Weighing of biomolecules, single cells and single nanoparticles in fluid. *Nature* 2007, 446:1066–1069. Copyright 2007.

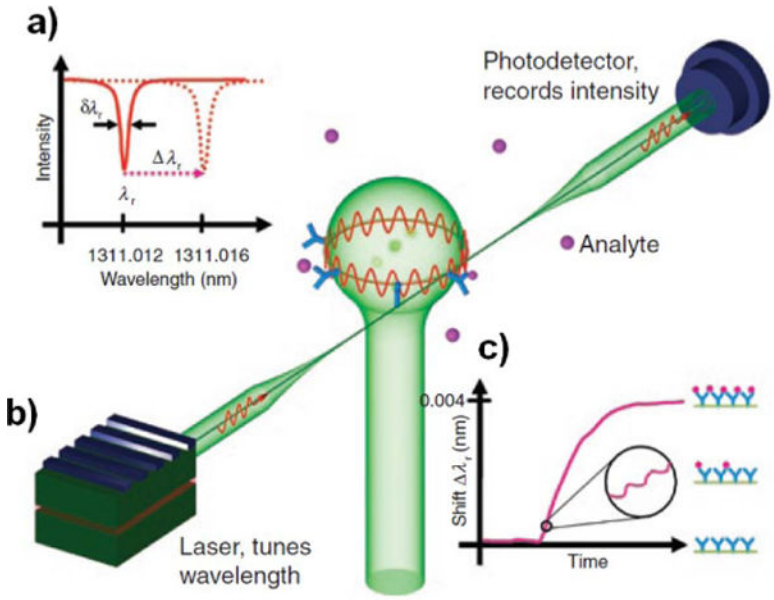

Figure 4.

Illustration of a whispering gallery mode biosensor. Light is coupled into a microsphere evanescently via a tapered optical fiber. Certain wavelengths of light will resonate in the cavity, causing dips in transmission through the optical fiber at those wavelengths. Binding of molecules on the cavity surface will cause a shift in the resonant frequency spectrum of the cavity, and thus the resonant frequency dips monitored at the detector will shift. As more analyte binds to the surface, this resonant frequency shift will increase.[81] Reprinted by permission from Macmillan Publishers Ltd: Nature Methods. Vollmer F, Arnold S. Whispering-gallery-mode biosensing: label-free detection down to single molecules. *Nat Meth* 2008, 5:591–596. Copyright 2008.

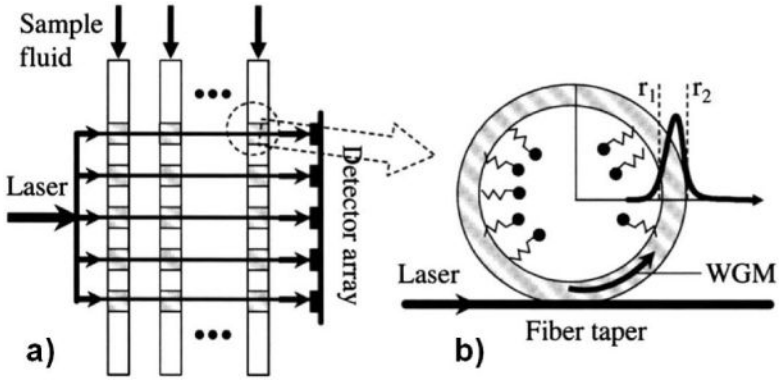

Figure 5.

Illustration of a liquid core optical ring resonator sensing system. a) Illustration of a potential method to multiplex multiple liquid core optical ring resonators using a single laser (to excite multiple cavities) and multiple detectors. b) Illustration of a laser coupled to an optical resonator cavity formed by the wall of a glass capillary.[88] Reprinted by permission from White IM, Oveys H, Fan X. Liquid-core optical ring-resonator sensors. *Optics letters* 2006, 31:1319–1321. Copyright 2006.

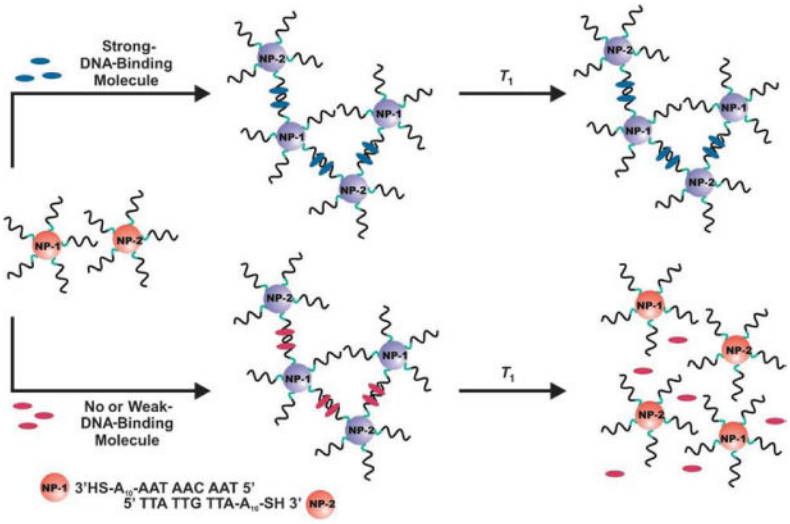

Figure 6.

Illustration showing DNA-functionalized Au nanoparticles aggregating upon hybridization with complementary strands[115] Han MS, Lytton-Jean AKR, Oh B, Heo J, Mirkin CA. Colorimetric Screening of DNA-Binding Molecules with Gold Nanoparticle Probes. *Angew. Chem. Int. Ed.* 2006, 45:1807–1810. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

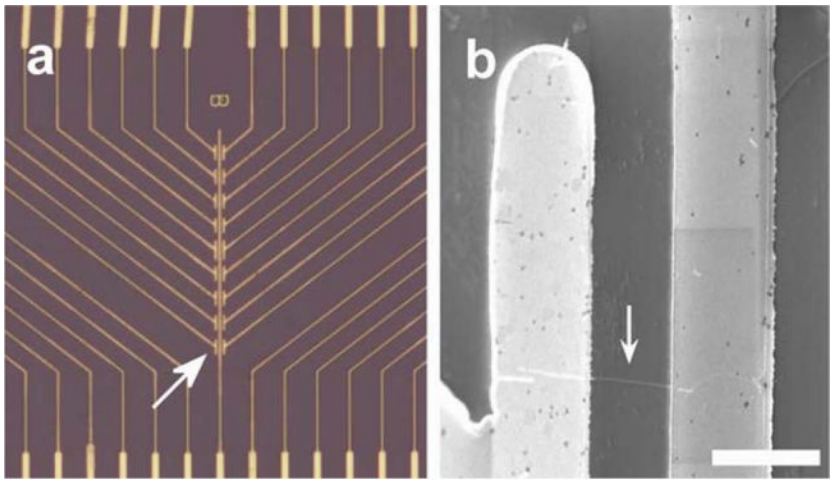

Figure 7.

a) Optical microscopy image of a metal electrode array used to interface to nanowire biosensors (field of view is 350×400 μm). b) An individual silicon nanowire bridging two electrodes (scale bar is 2 μm).[173] Reprinted by permission from Macmillan Publishers Ltd: Nature Biotechnology. Zheng G, Patolsky F, Cui Y, Wang WU, Lieber CM. Multiplexed electrical detection of cancer markers with nanowire sensor arrays. *Nat Biotech* 2005, 23:1294–1301. Copyright 2005.

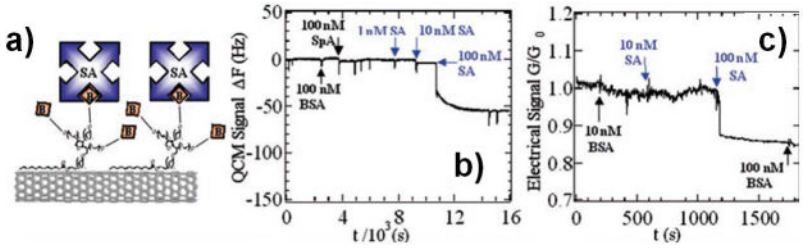

Figure 8.

a) Diagram illustrating binding of streptavidin to a biotin-functionalized CNT sensor. b) Frequency shift of a quartz crystal microbalance mass sensor as streptavidin solution is introduced to the functionalized nanobiosensor. Note that the response is specific to the streptavidin solution; negligible response is seen with solutions of other biomolecules. c) Electrical response of the CNT-based nanobiosensor as streptavidin solution is introduced at increasing concentrations. Again, the response is specific to streptavidin.[152] Reproduced with permission from Chen RJ, Bangsaruntip S, Drouvalakis KA, Wong Shi Kam N, Shim M, Li Y, Kim W, Utz PJ, Dai H. Noncovalent functionalization of carbon nanotubes for highly specific electronic biosensors. *Proceedings of the National Academy of Sciences of the United States of America* 2003, 100:4984. Copyright 2003 National Academy of Sciences, U.S.A.