Functional nanoprobes for ultrasensitive detection of biomolecules

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There has been great interest in developing new nucleic acid and protein detection methods for both clinical and numerous non-clinical applications. In a long-lasting effort to improve the detection ability of bioassays, functional nanomaterials have been actively explored to greatly enhance the sensitivity during the last two decades. This *tutorial review* focuses on recent progress in biosensor development by exploiting several unique optical, electronic and catalytic properties of a range of nanomaterials, such as gold nanoparticles, quantum dots, silicon nanowires, carbon nanotubes and graphene. In addition, a perspective on new opportunities offered by emerging technologies (*e.g.* DNA nanotechnology) is provided.

1. Introduction

Detection of biomolecules (*e.g.* DNA and proteins) has become increasingly important in a variety of areas including medical diagnostics, food safety and anti-bioterrorism.[1] While a large number of bioassay kits have been on the market, and these state-of-the-art methods have been able to satisfy many practical requirements, there are ever-growing efforts to develop new biodetection methods with ultrasensitivity, and ultimately, to improve the limit of detection to very few molecules. Enhanced detection ability is expected to significantly strengthen the capability of medical diagnostics, *e.g.*, to detect low-abundance cancer biomarkers in sera.

Parallel to research efforts devoted to such high-end requirements, much recent interest has been directed towards the development of low-cost and portable biosensors, which possess comparable high sensitivity to conventional instruments, but with greatly reduced cost/mass/power requirements. In both directions, it is critically important to introduce new signal amplification strategies to realize high-sensitivity biodetection. With the rapid emergence of nanotechnology, hybrid bio/nano-structures have been popularly employed to amplify bioassay signals *via* various electronic, optical, or microgravimetric transduction approaches.

Nanomaterials provide almost unlimited combinations of various compositions, sizes, dimensions and shapes of materials, which can be tailored to couple different biomolecules in order to develop nanoprobes with desired properties. A prominent example is the gold nanoparticle (AuNP), which is among the most explored nanomaterials in biology.[2] This is because of the AuNP's excellent biocompatibility as well as readily available conjugation chemistry at gold surfaces. AuNPs also possess highly attractive plasmonic coupling, fluorescent quenching as well as high conductivity. In addition, some recent advances have demonstrated that multiple biomolecules can be assembled onto one AuNP because of its high surface-to-volume ratio. However, while biologically functionalized

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nanoprobes are expected to lead to exciting progress in this area, it remains challenging to precisely control biomolecular assembly on complicated nanostructures. In this review, we aim to summarize recent progress of nanoprobe design and different strategies for nanoprobe-based ultrasensitive bio-detection. Due to the space limit, we provide only several selected examples for each strategy described herein. Readers who have interest in learning more may refer to several elegant and more comprehensive reviews.[2–6]

2. One nanomaterial with multiple biomolecules: An effect of high surface-to-volume ratio

The size dimensions of biomolecules (DNA or proteins) are typically in the nanometre range. For example, DNA molecules are perfect nanowires with a uniform diameter of 2 nm, and most proteins are nanoparticle-like with diameters ranging from several to up to 100 nanometres. Given that biomolecules and nanomaterials are of comparable sizes, it is possible to couple biomolecules to nanomaterials with high efficiency, and more importantly, one nanomaterial unit may accommodate many biomolecules (high surface density) due to the high surface-to-volume ratio property of nanomaterials. In 1996, Mirkin and coworkers reported their pioneering work on the design of novel bionanostructures, DNA-modified gold nanoparticles (DNA-AuNPs).[7] Since then, DNA-AuNPs have been popularly employed as nanoprobes in a variety of ultrasensitive DNA-based assays.[4] The highly dense DNA layer at the surface of AuNPs not only leads to high selectivity due to collective behavior of the densely-loaded DNA

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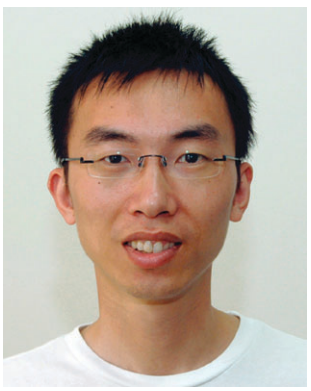

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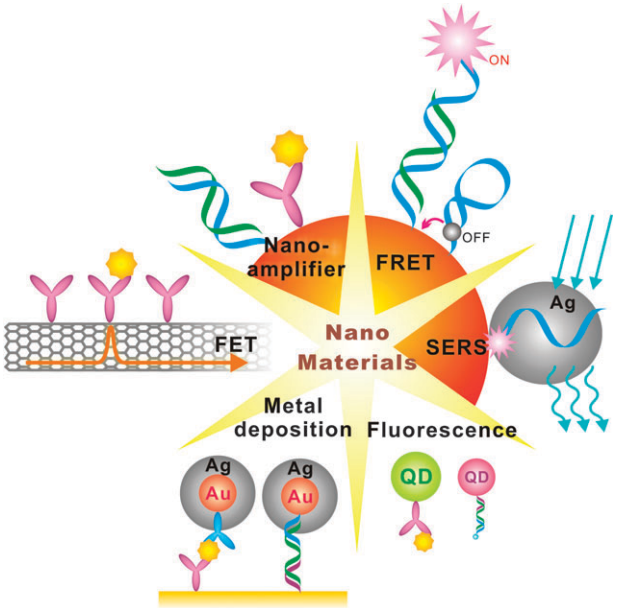

Fig. 1 Schematic illustration for various ultrasensitive biodetection strategies by using biofunctionalized nanomaterials.

but also offers new opportunities to amply hybridization signals. In a recently developed electrochemical DNA sensor, surface-confined DNA molecules were employed as reporter probes. Since one AuNP of 20 nm could carry hundreds of DNA molecules, one DNA hybridization event occurring at the Au electrode surface brought hundreds of reporter probe DNAs to the surface, resulting in a large signal amplification factor of 1000, and a low detection limit of 10 fM (Fig. 1).[8–10]

Multi-component and multi-functional nanoprobes can also be conveniently designed by attaching different types of biomolecules on the surface of AuNPs. Nam *et al*. developed a bio-barcode assay format for detecting proteins with PCR-like sensitivity.[11] In their design, AuNPs were coated with both a polyclonal antibody that was specific for the target protein and hundreds of barcode DNA strands. With this bio-barcode nanoprobe, the antibody-target protein recognition was translated into a DNA detection process, which could be realized with extremely high sensitivity *via* PCR amplification. An optimized bio-barcode assay can detect down to attomolar (aM) proteins, an unprecedented sensitivity that allows direct detection of protein biomarkers that would be otherwise impossible. This novel assay method has been employed to detect protein markers for early-stage diagnostics of Alzheimer's disease[12] and monitoring of biochemical recurrence after radical prostatectomy.[13] Enzymes can also be anchored on the surface of inorganic nanomaterials and remain functional. Li *et al*. developed an enzyme-based multi-component colorimetric gold nanoprobe that integrated DNA recognition, signal amplification and non-specific blocking.[14] The nanoprobes were prepared by co-assembling thiolated DNA detection probe, HRP and bovine serum albumin (BSA) at the surface of AuNPs. This nanoprobe was employed in a magnetic particle-based "sandwich-type" assay, providing colorimetric signal readout arising from enzymatic catalysis (Fig. 2).

Alternatively, carbon nanotubes (CNTs) provide an extremely large surface area for biomolecular conjugation and subsequent signal amplification. Wang and coworkers immobilized both enzymes and DNA on multi-walled carbon nanotubes (MWNTs),[15] which coupled DNA hybridization with catalytic amplification of the alkaline phosphatase (ALP) enzyme. Due to the presence of numerous enzymes on CNTs and the enzyme-based high turn-over catalysis, this electrochemical DNA sensor reached a remarkably low detection limit of 54 aM. Similarly, MWNTs with ALP and antibodies could be employed to detect up to 500 fg mL^{-1} protein. Rusling and coworkers developed a CNT-based electrochemical platform for immunoassays.[16] In their approach, detection antibodies and HRP were covalently conjugated to MWNTs with a high HRP/Antibody ratio. In addition to this MWNT-based amplification, they further employed single-walled carbon nanotube (SWNT) forest electrodes to anchor capture antibodies. Their immunosensors revealed highly sensitive detection of cancer markers in real biological samples (sera and lysates), with a detection limit of 100 fM for prostate-specific antigen (PSA) in undiluted sera.

In order to further maximize the sensitivity, Wang and coworkers reported a method to increase the enzyme loading on MWNTs by using electrostatic layer-by-layer self-assembly of ALP and a reverse-charged polyelectrolyte to MWNTs.[17] The enzyme-incorporated multilayer structure greatly increased the ratio of ALP tags per binding event, offering greatly enhanced signal amplification that allowed ultrasensitive detection of DNA down to 80 copies (5.4 aM) or proteins down to 2000 molecules (67 aM).

3. Fluorescent nanomaterials with high brightness: An effect of high fluorescent quantum yield

Quantum dots (QDs) are a class of semiconductor nanoparticles whose charge carriers are quantum confined, emitting stable fluorescence with a high quantum yield. Compared to the conventional organic fluorophores, QDs usually have a tunable and more narrow emission spectrum, brighter fluorescence, and are resistant to photobleaching over a long period of time.[3] In addition, QDs can be readily conjugated with proteins and DNA, which lead to high detection sensitivity due to their highly bright fluorescence. More recent studies revealed that QDs also possesses interesting electrochemiluminescent properties.[18,19] Because of these attractive properties, great efforts have been taken for biological applications of QDs.[20,21] In this section, we focus on reviewing recent advances for protein and DNA detection with QDs (Fig. 3).

Bakalova *et al*. demonstrated that the use of QD labels brought about significantly improved sensitivity in western blots for protein detection,[22] which could elucidate information from low-abundant proteins in proteomic studies. Yan *et al*. developed a nano- and micro-integrated protein chip (NMIPC) that combined the advantages of microfluidic network with QDs. This chip allowed facile patterning of proteins and subsequent biomolecular detection, leading to a

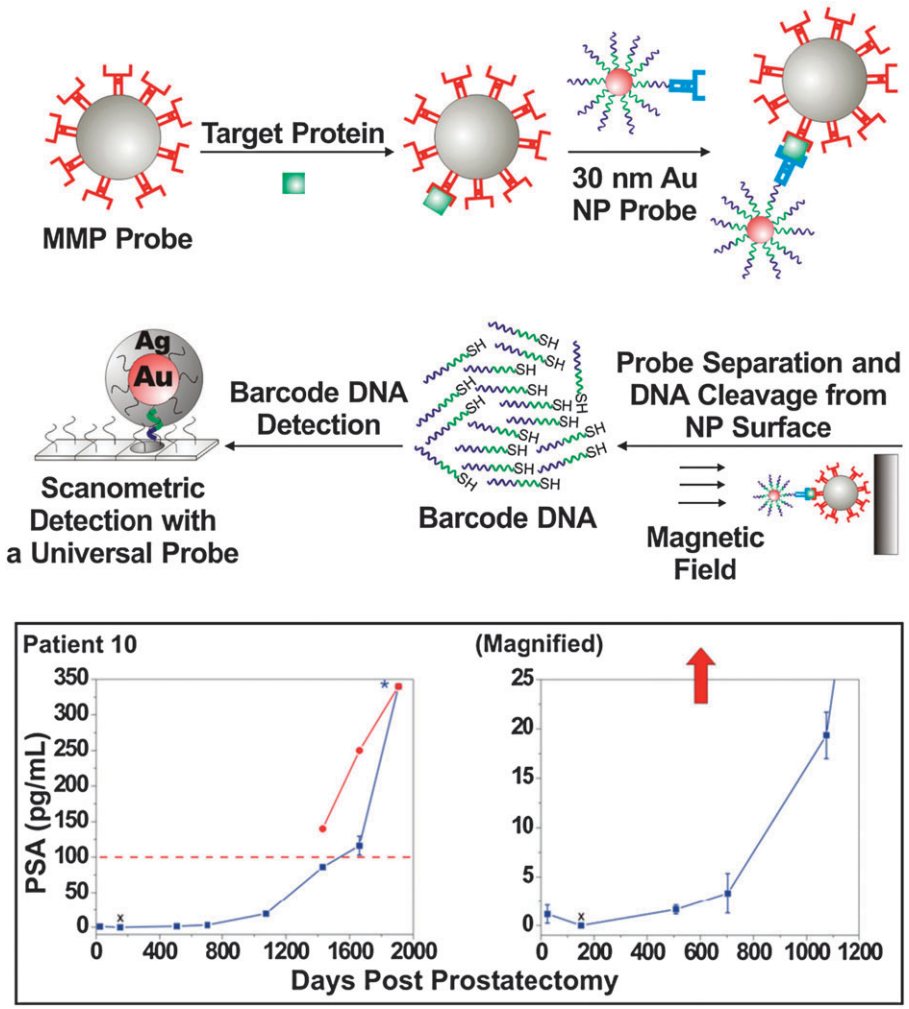

Fig. 2 AuNPs-based bio-barcode assays for the detection of prostate specific antigen. Scheme for the bio-barcode detection strategy and immunoassay data for PSA values of postoperative samples from prostate cancer patients [reprinted with permission from ref. 12].

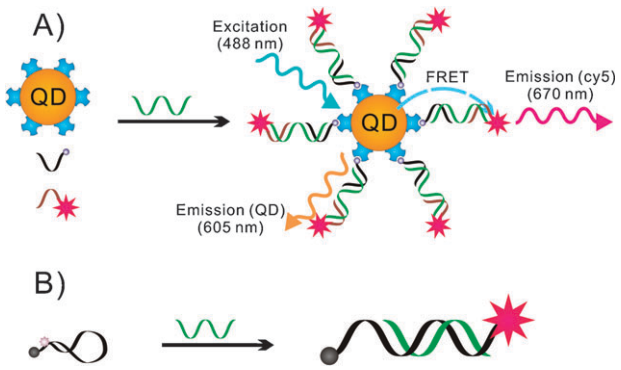

Fig. 3 Scheme for the design of a single-QD-based DNA nanosensor system. (A) In the presence of targets, a sandwich complex is formed that allows efficient FRET between the Cy5 acceptor and the QD donor, leading to fluorescence emission of the nanosensor [reprinted with permission from ref. 25]. (B) A molecular beacon that shows target hybridization-induced conformational change (See ref. 25 for more details on performance comparison between the nanosensor and the conventional molecular beacon).

detection limit of 500 fM for carcinoembryonic antigen (CEA) with streptavidin-adsorbed QDs.[23] Further studies showed that covalent conjugation of QDs with antibodies could slightly improve the performance, with a two-fold increase in sensitivity.[24]

Wang and coworkers reported an ultrasensitive DNA sensor by using QDs. In their detection scheme, the target DNA was flanked by a biotinylated DNA probe and a dye-labeled DNA probe. Specific hybridization of these three pieces of DNA led to the binding of streptavidin-coated QDs to biotinylated DNA and the occurrence of fluorescence resonance energy transfer (FRET) between the QD and the dye due to their close proximity. This sensor was highly specific as almost no background fluorescence was observed. By using the single-molecule fluorescence technique, even 50 copies of target DNA could be readily detected, representing a very high sensitivity.[25] More recently, the same group employed this QD-based sensor to detect DNA methylation, which relied on a methylation-specific PCR and the QD-based FRET detection of PCR products. Since DNA methylation has been well known to be associated with the occurrence of tumors, this quantitative, high-throughput and ultrasensitive sensor has shown great promise as a tool for early-stage cancer diagnosis.[26]

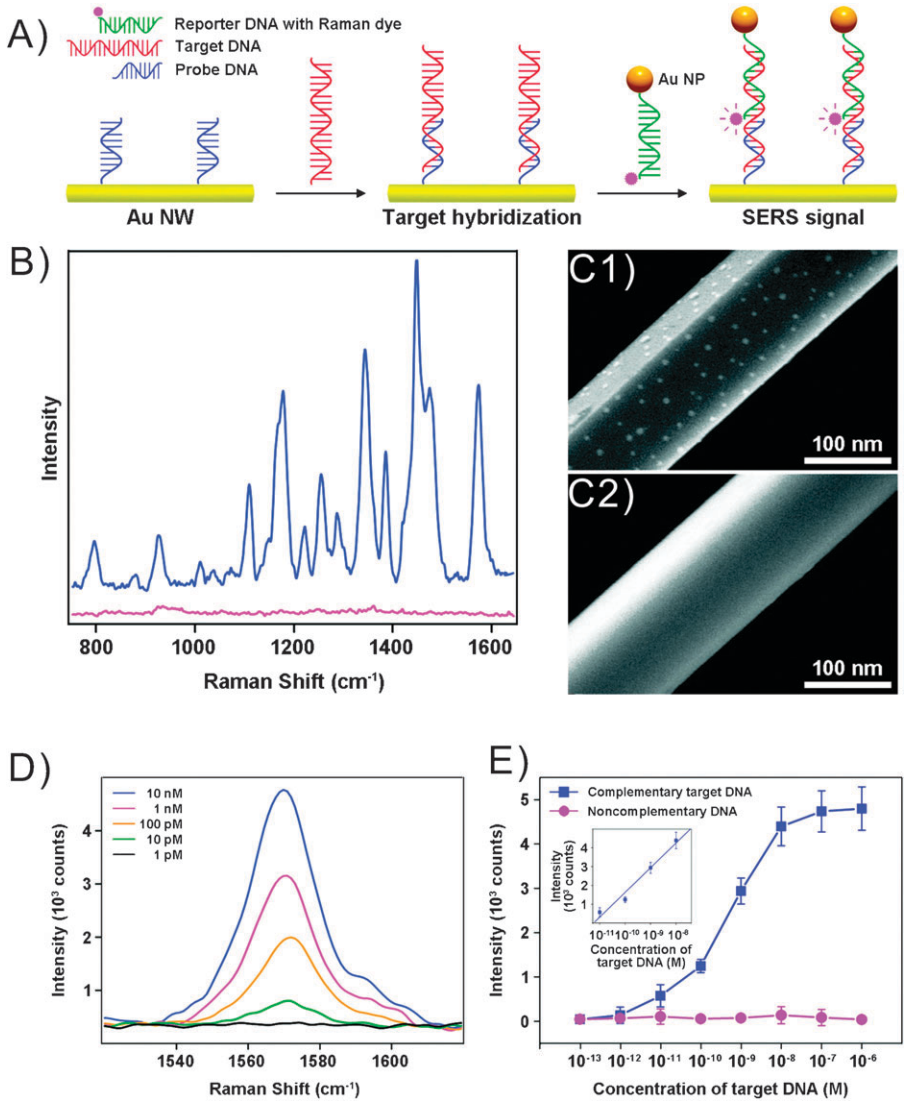

Fig. 4 (A) Scheme for a gold particle-on-wire biodetection system for DNA analysis. (B) SERS spectra of Au particle-on wire system prepared by probe-complementary target-reporter DNA hybridization (blue) and that of a single Au NW prepared by noncomplementary target DNA (magenta). (C) SEM image of a typical Au particle on-wire structure (C1, complementary DNA) and a clean NW by (C2, noncomplementary DNA). (D) SERS intensities changed with the concentration of target DNA. (E) Dose-response curve of SERS-based DNA detection [reprinted with permission from ref. 34].

4. Nanomaterial-based Raman detection: An effect of nanoscale surface-enhanced Raman resonance scattering

Surface-enhanced Raman scattering (SERS) has intrigued intense interest during the last several decades. Compared with conventional Raman, SERS can dramatically enhance signals by factors up to $\sim 10^{14}$, which is attributed to the strong light-induced electric field at locations in metallic nanostructured spaces (so-called "hot spot"). In addition to strong signal intensity, SERS possesses other attractive merits, such as narrow Raman band, and high resistance to interferences of environmental factors (*e.g.*, humidity, oxygen, and foreign species).[27] Consequently, SERS is recognized as one of the most promising tools for ultrasensitive chemical and biological analysis with the target of attaining the single-molecule level.

Various kinds of nanomaterial-based SERS biosensors have been developed over the past few years. In 2002, Mirkin and coworkers demonstrated that six different oligonucleotide targets were simultaneously detected with AuNPs labeled with different Raman probes. Significantly, the detection limit was down to 20 fM due to the strong SERS signal intensity.[9] Thereafter, metal nanoparticles (*e.g.* AgNPs or AuNPs)-based SERS biosensors have been extensively optimized for sensitive and specific detection of biomolecules. Braun *et al.* reported AgNPs-based probes for the detection of single-stranded DNA using SERS. Their study proved that only a few AgNPs were sufficient to produce intense and reliable SERS signals, suggesting that highly reproducible signals could be obtained at the near-single nanoparticle level.[28] Graham and coworkers utilized dye-coded, DNA functionalized AgNPs as SERS probes to enhance Raman scattering, and reliably transduce biological recognition events. Importantly, the electromagnetic

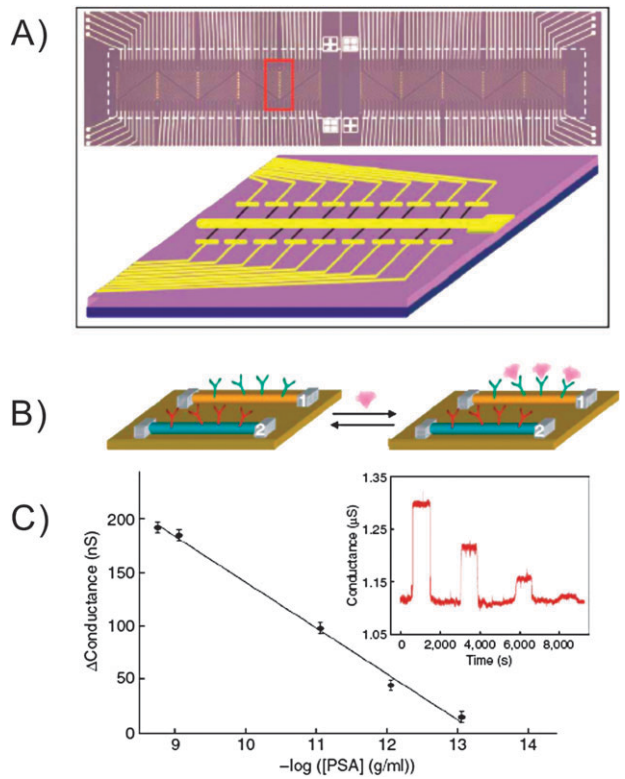

Fig. 5 Scheme for a silicon nanowire-based biosensor array for label-free and multiplexed electrical detection of tumor markers. (A) Optical image of a nanowire device array. (B) The nanowire device modified with antibodies that can bind specifically to target proteins. (C) Dose-response curve for PSA detection based on a silicon nanowire device [reprinted with permission from ref. 39].

enhancement mechanism was extensively explored in their study, which opens new opportunities to elaborate upon the design of SERS-based sensors.[29] Huh *et al.* reported a novel SERS-ligase detection reaction system for the detection of single nucleotide polymorphisms (SNPs) associated with mutation oncogenes of low concentration (~20 pM) by using AgNPs probes, which provided insight for understanding the basis for phenotypes and diseases.[30] Also noteworthy, Nie and coworkers recently presented the first example of AuNPs-based probes for *in vivo* tumor targeting and SERS detection. Significantly, these nanoprobes produced strong signals, which were 4200 times brighter than near-infrared-emitting quantum dots. This SERS technology allowed spectroscopic detection of small tumors (0.03 cm^3) at a penetration depth of 1–2 cm.[31]

On the other hand, alternative nanostructures (*e.g.*, nanoflowers, nanowires, and nanospheres) have been proposed as efficient SERS hot spots with higher enhancement factors (EF) up to 10^7–10^9,[32] which is several factors enhanced in comparison to the relatively low EF (10^4–10^5) of silver or gold nanoparticles. Lee and coworkers recently reported a novel and highly effective silicon nanowires (SiNWs)-based SERS-active substrate (*e.g.*, SiNWs coated with AgNPs) with a high EF up to 10^9, providing a platform for ultrasensitive detection of various biological molecules.[33] More recently, Kim and coworkers developed a new SERS-active substrate, *i.e.* Au-nanowire coated Au-nanoparticles, as a high-sensitivity DNA biosensor (10 pM) (Fig. 4).[34]

While various nanostructured probes have been developed for sensitive biological detection, the in-depth mechanisms for SERS have not been well understood. In addition, there still remain a number of open questions on how to design sophisticated nanomaterial-based SERS-active probes, as well as how to achieve robust and sensitive signals in SERS detection, demanding further systematic investigations and understanding for sensitive, specific and reproducible Raman biodetection.

5. Nanomaterial-based electrical detection: An effect of nanoscale surfaces

Electrical transducers are usually simple in structure, small in size, rapid in response and possess low-cost, which make them well suited to the development of inexpensive and portable biomedical devices. Nanomaterials have been widely employed to design electrical biodetection strategies because of their unique nanoscale structures and electronic properties. Nanomaterial-based electrodes can be either integrated in field-effect transistors (FETs),[35,36] or employed as electrochemical nanoelectrodes.[37,38] In this section, we focus on FET-type biosensors, which exploit changes in resistivity induced by target-binding to the sensing surface.

SiNWs are popular choices to develop FET-type biosensors. Since all electrical currents flow through the nanoscale cross-section of nanowires, SiNWs-based FET-type biosensors are extremely sensitive to minute variations in the surrounding environment. SiNWs-based FET sensors have shown excellent performance due to the nanoscale dimensions of SiNWs and their suitability for biofunctionalization. In addition, they can be easily integrated to provide a high-throughput platform for multiplexed biosensing. Lieber and coworkers first reported the development of a single-SiNW-based nanobiosensor for highly sensitive and selective detection of biomolecules.[35] They demonstrated that biotin-modified silicon nanowires exhibited a significant increase in conductance upon the addition of streptavidin with the concentration as low as 10 pM. This proof-of-concept SiNWs-based FET sensor architecture has become the paradigm for the development of a range of biosensors toward DNA, proteins, and even viruses (Fig. 5).

A SiNWs-based DNA sensor with higher sensitivity was later developed by using peptide nucleic acid (PNA) probes.[36] PNA is an uncharged DNA analogue, and a PNA probe was designed to recognize a piece of sequence in a transmembrane receptor gene. The specific PNA-DNA binding was found to produce a pronounced increase in conductance, an effect arising from the change in surface charges. This nanoscale DNA sensor could identify and distinguish a perfectly matched DNA from a single-base mismatched one, with a remarkable detection limit of 10 fM. With a similar design, SiNWs-based FET sensors were employed for highly sensitive, label-free and multiplexed electrical detection of tumor markers with femtomolar sensitivity.[39] As an example, a SiNWs-based nanosensor array was employed to simultaneously detect prostate specific antigen (PSA), PSA-a1-antichymotrypsin, CEA and mucin-1 in undiluted serum samples. In an effort

to demonstrate the ultimate sensitivity of nanowire-based sensors, Patolsky *et al*. interrogated the detection of viruses.[40] Anti-virus antibodies were immobilized on a single SiNW, which captured virus particles, leading to conductance changes in response to the virus binding. Remarkably, this nanosensor revealed that even a single positively charged influenza virus could be detected.

Carbon nanomaterials such as SWNTs and graphene have demonstrated utility in FET-type biosensors. Because of the high conductivity of carbon nanomaterials, even a very small amount of target binding to the surface may alter electrical properties of nanomaterials, leading to extremely sensitive nanobiosensors.[5] Dai and coworkers developed a two-step functionalization approach for SWNTs,[41] which effectively prevent nonspecific binding that often hampers detection with FET sensors. Their FET sensor with inter-connected SWNTs could detect nanomolar antibodies. Star *et al*. reported a FET biosensor with SWNT networks for DNA detection with picomolar sensitivity.[42] In another study, Sudibya *et al*. demonstrated that a cell-modified nanotube biosensor could detect the stimulated release of biomolecules with high sensitivity and in real time.[43] We note that all these sensors employed multiple SWNTs rather than a single nanotube, which might be the reason that SWNTs-based biosensors have lower sensitivity than single-SiNW-based biosensors. It is expected that single SWNT-based technology has the potential to greatly enhance the sensitivity of SWNT-based FET biosensors.

Graphene, a single layer of carbon atoms, is a recently discovered two-dimensional carbon nanomaterial with numerous unique optical and electronic properties. Particularly, this extremely thin nanomaterial possesses remarkably high conductivity. Mohanty *et al*. reported the first electrical biosensor using few-layer graphene for the detection of DNA and a bacterium.[44] Electrical measurements showed that DNA hybridization caused an increase in conductivity, and this increase was reversible during repetitive denaturing and rehybridization cycles. Also, the adsorption of a single bacterium on the surface of graphene caused a clear increase in conductivity, suggesting that this sensor has single-cell sensitivity. Wu *et al*. reported an electrical nanosensor for glucose by using glucose oxidase and functionalized graphene sheets.[45] The detection limit of this glucose sensor was 0.6 μM, and it had good reproducibility, long-term stability and negligible interfering signals. Very recently, Li and coworkers developed a FET-type DNA sensor by using CVD-grown graphene sheets, which showed single-base specificity with 10 pM sensitivity.

6. Fluorescent background suppression: An effect of fluorescence "superquenching"

Fluorescent detection methods are still the predominantly employed detection technologies for biological assays, which is due to the commercial availability of a wide spectrum of fluorophores, the ease of fluorescent labeling, and the inherent capability for real-time detection. While fluorescent detection is usually highly sensitive, the detection sensitivity is often limited by the presence of background emission. For example, high quenching efficiency is critically important for molecular beacons (MBs) since the sensitivity of MBs relies on internally quenched fluorescence.[46] In a typical MB, a fluorophore and a quencher are attached to both ends of a stem-loop structured oligonucleotide. The formation of the stem-loop holds the quencher in close proximity to the fluorophore, preventing the emission of the fluorophore; while target hybridization opens the stem-loop, which competitively forces apart the dye and the quencher and turns on the signaling fluorescence.

While organic quenchers have proven their utility in designing MBs, they suffer from relatively low quenching efficiency, and, more importantly, quenching efficiencies often vary significantly from one dye to another. Interestingly, metallic gold (macroscopic gold or AuNPs) is known to possess ultrahigh fluorescence quenching ability both from theoretical prediction and experimental observations. Dubertret *et al*. designed an AuNPs-based nanobeacon that replaced organic quenchers with 1.4-nm AuNPs, and found that AuNPs served as a universal quencher that could quench a range of fluorophores with 100-fold higher quenching efficiency than organic quenchers.[47] This nanobeacon not only led to improved sensitivity but also remarkably high selectivity for single-base mismatch discrimination. Nie and coworkers later reported a stem-less probe on 2.5-nm AuNPs that still demonstrated target-induced conformational change and fluorescence variation in response to the binding of target DNA.[48] It is worth noting that these small-sized AuNPs can accommodate only one to several oligonucleotides at each particle.

Recently, Song *et al*. employed larger-sized AuNPs of 15 nm in diameter to construct multicolor nanobeacons.[49] Surface plasmon resonance (SPR) absorption of 15-nm AuNPs exceeds smaller-sized ones (1–2 nm) by orders of magnitude,[50] which may enhance the quenching of fluorophores. More importantly, it is possible to anchor many DNA probes at one single particle due to the increased surface area. As a proof-of-concept experiment, three DNA probes designed for three tumor-suppressor genes (P16, P21 and P53) were immobilized at the AuNP surface, each carrying a unique fluorophore. This nanobeacon exhibited rapid hybridization kinetics (minutes), and could respond specifically to the presence of different gene targets. This nanobeacon design could be easily extended to detect non-nucleic acid targets *via* the use of aptamers. A recent example showed that three targets, adenosine, potassium ion and cocaine could be simultaneously detected in homogeneous solution with AuNPs coated with three specific fluorophore-tagged aptamers.[51] Importantly, Mirkin and coworkers have demonstrated that DNA-loaded AuNPs can be efficiently taken up by cells, which makes it possible to perform intracellular detection with AuNPs-based nanoprobes.[52]

In an alternative approach, several nanomaterials including AuNPs, SWNTs and graphene have been observed to interact differentially with ss- and ds-DNA, which provides a tool to detect biomolecular recognition with unmodified nanomaterials. Li and Rothberg developed a colorimetric DNA assay method with unmodified AuNPs, which could selectively differentiate unhybridized and hybridized DNA molecules.[53,54] This method was shown to be applicable for aptamer-based detection of a range of molecular targets. Similarly, SWNTs

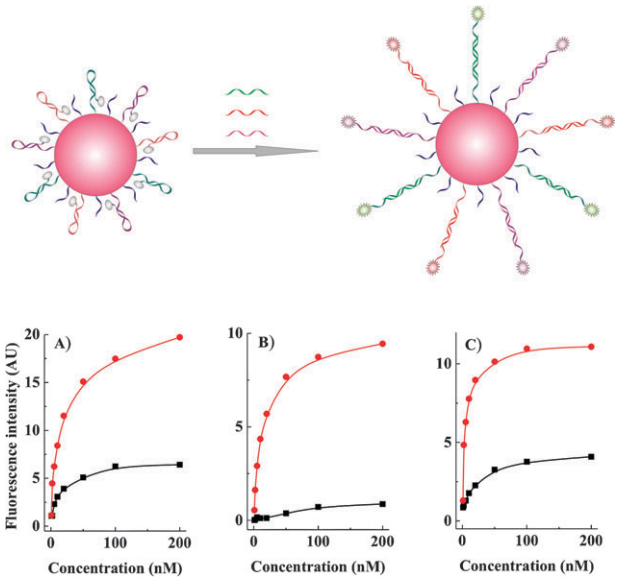

Fig. 6 Multicolor nanobeacons for multiplexing detection of three different cancer DNA markers. The multicolor nanobeacons are hybridized with three perfectly matched targets and three single-mismatched targets, showing high sequence specificity that arises from the conformational constraint [reprinted with permission from ref. 49].

were found to induce fluorescence quenching with extremely high efficiency, based on which Yang *et al*. developed a DNA sensor.[55] They demonstrated that fluorophore-labeled DNA probes were efficiently quenched in the presence of SWNTs while they were released from SWNTs upon hybridization, leading to fluorescence recovery (Fig. 6).[55]

More recently, graphene was found to be a super-quencher due to its long-range nanoscale resonance energy transfer.[56,57] Molecular dynamics (MD) studies revealed that ssDNA adsorbed strongly to graphene oxide (GO) sheets while dsDNA did not.[57] Similar to the SWNT-based sensor, Lu *et al*. employed GO to adsorb and quench dye-labeled DNA probes, which could be liberated upon hybridization, leading to fluorescence increment.[56] He *et al*. designed a new approach that relied on different binding kinetic of ssDNA and dsDNA to GO, which led to sequence-specific detection with high sensitivity and rapid response time (~5 min).[57] More significantly, due to the availability of large planar surface of GO, multicolor DNA detection was realized by using differentially labeled DNA probes.[57]

7. Nanoparticle-induced metal deposition: An effect of nanoparticle-seeded growth

Metal nanoparticles (*e.g*. AuNPs) are often employed as labels for biomolecular detection. While metal nanoparticles themselves can amplify signals as described above, they may further greatly increase the signal magnitude *via* nanoparticle-induced metal deposition. AuNPs are particularly useful in this regard. AuNPs-induced silver deposition (or silver enhancement) has been widely used for decades, *e.g*. in immunohistochemistry.[2] AuNPs can selectively reduce silver salts to silver in the presence of a reducing agent, resulting in significantly increased size and optical absorption. Similarly, AuNPs can induce the deposition of gold itself, which potentially has inherently low background.[58]

Taton *et al*. developed an ultrasensitive DNA detection method by using DNA-AuNPs conjugates and silver enhancement.[59] The sensitivity of their scanometric array exceeded that of conventional fluorescent detection by two orders of magnitude. A similar strategy was employed to perform electric DNA detection in a microfabricated electrode gap.[60] DNA hybridization entrapped the DNA-modified AuNPs in the gap, which was further enhanced with silver, leading to greatly enhanced conductivity that served as the signal readout. This method led to a low detection limit of 500 fM. Silver enhancement has also been used for ultrasensitive biodetection with Surface Enhanced Raman Scattering (SERS, see section 4) (Fig. 7).[9]

Silver enhancement can also enhance the sensitivity for protein detection. Sia *et al*. demonstrated that AuNPs-induced silver deposition was compatible with the microfluidic technique.[61] Under continuous flow in microchannels, both the time required for the assay and reagents consumption were significantly reduced. This microfluidic-based immunoassay method was used for quantification of HIV-1 antibodies in sera of HIV-infected patients in a point-of-care diagnostics format.

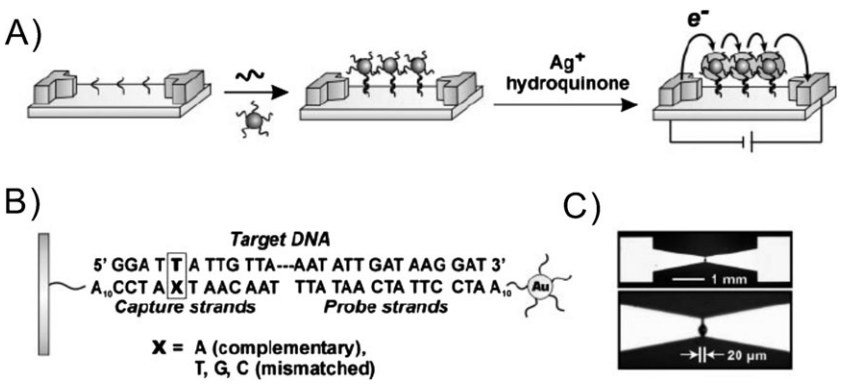

Fig. 7 Electrical detection of DNA in an electrode gap. (A) The DNA hybridization leads to electrical signal when coupled with AuNP probes and silver enhancement. (B) Sequences of capture, target, and probe DNA strands. (C) Optical microscope images of the electrodes employed in a typical detection experiment [reprinted with permission from ref. 60].

8. Conclusions and perspectives

In this review, we summarized six strategies for nanomaterial-based ultrasensitive biodetection, either *via* biomolecular nanoprobes that offer signal amplification, or by exploiting the inherent nanoscale effects of biomolecule-functionalized nanomaterials. All these strategies have greatly enhanced sensitivity for both DNA and protein detection. Some elaborately designed nanoprobes have led to extremely low detection limits down to the attomolar level, which represents sensitivity improvement up to six orders of magnitude compared to conventional fluorescent detection (1–10 pM). Despite tremendous progress, there remain several challenges for nanomaterial-based bioassay technologies before they can be widely accepted in clinical or food-related applications. First, the use of inorganic nanomaterials introduces heterogeneous interfaces in bioassays that are usually in homogenous solution. Consequently, while many nanomaterials provide great signal amplification, they also bring about problems such as low recognition efficiency and slow binding kinetics that are closely related to the presence of heterogeneous interfaces. Second, some ultrasensitive assays require multiple steps that significantly increase the operation complexity. Third, elimination of non-specific binding is critically important to avoid interferences, particularly for detection in complex biological matrixes (*e.g.* serum). Forth, the ultrahigh amplification offered by nanomaterials often lacks generality, therefore bioassay reactions should be individually optimized and amplification factors usually vary from case to case. Notably, recently developed DNA origami-based chips, while rather immature in their current form, provide a potentially promising solution to overcome the interface problem since DNA origami structures are essentially in aqueous solution.[62,63] Other DNA nanostructures may also serve as nanoprobes for generic signal amplification. We thus expect that the emerging DNA nanotechnology could offer new opportunities to meet the above-mentioned challenges, and, if combined with high-speed and high throughput readouts as well as automated devices,[64] will form the basis of high-performance detection platforms for a range of biomolecules.

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