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A Review on Functionalized Gold Nanoparticles for Biosensing Applications

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Abstract Nanoparticle technology plays a key role in providing opportunities and possibilities for the development of new generation of sensing tools. The targeted sensing of selective biomolecules using functionalized gold nanoparticles (Au NPs) has become a major research thrust in the last decade. Au NP-based sensors are expected to change the very foundations of sensing and detecting biomolecules. In this review, we will discuss the use of surface functionalized Au NPs for smart sensor fabrication leading to detection of specific biomolecules and heavy metal ions.

Keywords Surface plasmon resonance · Gold nanoparticles · Biosensing · Surface functionalization

Introduction and Background

Nanomaterial with average diameter less than 100 nm is defined as nanoparticles [1]. Among them, gold nanoparticles (Au NPs) are the most extensively investigated one due to their unique tunable optical properties, which can be applied in various applications such as sensing, detecting, and imaging. To date, in the research field of development of new protocols for preparing functionalized gold nanoparticles and using them for biosensing is presently an active research area. The synthesis techniques of gold nanoparticles have been continuously evolving, leading to improvements in the control over their size and shape. In addition to sensing, gold nanoparticles are attractive candidate for photothermal therapeutic, diagnostic, and drug delivery applications [2, 3]. For example, the gold nanoparticles can be used to enhance both Rayleigh and Raman signals for obtaining chemical information on many biological species of interest, which were previously unattainable using simple spectroscopy setting [4–6]. Thus, the manipulation in the size or shape of the gold nanoparticles represents an attractive approach for biosensing applications and will continue to be used as a tool for novel future applications.

Au NPs represent an excellent nanoplatform in developing analytical methods for biosensing and many research groups have demonstrated that they can be used for a wide range of sensing applications, ranging from chemical to biological sample [7–10]. The Au NP surface and core properties can be manipulated for specific applications such as molecular recognition, chemical sensing, and imaging. However, there are challenges that require overcoming before the fabricated colloidal Au NPs formulation can be successfully translated into clinical trial research. This includes the reproducibility, reliability, scalability in man-

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ufacturing Au NP-based biosensing assays and long-term stability. In the near future, we envision that the multidisciplinary research output will yield new fundamental insights into the AuNP-based nanotechnology.

In this article, we review and discuss on current status of using functionalized Au NPs for biosensing applications. More specifically, we discuss the use of Au NPs as a nanoplatform to produce biocompatible sensors in vitro and in vivo. In addition, bioimaging and therapeutic applications of these unique nanomaterials will be described. The Au NPs are particularly interesting from the point of view of their tunable optical properties, which strongly depend on the particle size, shape, composition, and surface coating. The optical properties of Au NPs are first briefly discussed in "Optical Properties of Gold Nanoparticle" section. The general approaches of synthesizing Au NPs are described in "Preparation of Functionalized Gold Nanoparticles" section. "Functionalized Gold Nanoparticles for Biosensing" section focuses on using functionalized Au NPs for biosensing ranging from detecting of DNA to identification of glucose. Finally, the last section briefly describes the summary and future outlook of these functionalized Au NPs for biological applications.

Optical Properties of Gold Nanoparticles

The field of Au NPs research has received tremendous attention due to their unique optical properties. The unique optical properties of Au NPs arise from their size confinement effect [11]. The size confinement effect on the Au NPs provides new electronic and optical properties. A distinct feature of Au NPs is the strong vibrant color of their colloidal solution that is caused by the surface plasmon resonance (SPR) absorption [12, 13]. For the last two decades, many research groups have studied the optical characteristic features of the Au NPs with various sizes and shapes.

It is worth noting that Au NPs were used as a coloring agent for staining church's glass dating back to the seventeenth century. The stained glasses with Au NPs are ruby red in color. Colloidal Au NPs sample is ruby red in color and Mie explained this phenomenon theoretically by solving Maxwell's equation for the absorption and scattering of electromagnetic radiation by spherical particles [14]. Since then, the developed theory is widely used to calculate the particle extinction spectra if the material dielectric function is known and the particle size is smaller than that of the wavelength of the light. The origin of the light absorption by Au NPs is the coherent oscillation of the conduction band electrons induced by the interacting with the electromagnetic field. This effect is generally known as SPR and this effect is absent in the individual atoms and bulk form [3, 8, 15–18].

In general, the SPR absorption peak red shifted to longer wavelength with increasing of particle size as predicted by theory [10]. For detail description of estimating the optical spectra, please refer to reference [12]. To date, the SPR frequency of Au NPs has been shown to depend on particle size, shape, dielectric properties, aggregate morphology, surface modification, and refractive index of the surrounding medium. For example, the SPR peak of 13 nm spherical gold colloids is around 520 nm and that of 5–6 nm silver NPs around 400 nm. The SPR peak of the Au NPs can be systematically tuned by changing the size of the Au NPs (see Figs. 1 and 2).

In addition to their unique SPR effect, Au NPs can also be used as probes for single molecule surface-enhanced Raman scattering (SERS) detection [4–6, 19, 20]. The discovery of SERS has received great attention both in the nano-optics community. Kneipp et al. [20] and Nie et al. [4] are the pioneers in this discovery. They have discovered and confirmed that the large enhancement factors of SERS are on the order of 10^{14}–10^{15}. These nanoprobes are referred as "hot" nanoparticles for SERS optical enhancement. We envision that SERS effect may be able to incorporate into the current Au-NP-based biosensors for enhancing the detection sensitivity of the system. For details of SERS findings, please refer to references [5, 21].

Preparation of Functionalized Gold Nanoparticles

To date, there are various kinds of approaches to synthesize Au NPs [22–30]. In general, most of the Au NPs are prepared using solution phase synthesis method. They can be made in either aqueous or organic phase. For example, Au NPs can be produced by reducing chloroauric acid in aqueous phase. This is the most common way for preparing colloidally stable NPs for the last 10 years. This method was invented by Turkevich et al. [31] in 1951. Later, in 1970, Frens et al. have made an improvement on this synthesis protocol [32–34]. More specifically, it was demonstrated that Au NPs with a diameter of 10–20 nm can be obtained by reacting hot chloraruic acid solution with sodium citrate solution. In this synthesis process, sodium citrate is used as the reducing and capping agent. However, when the NPs size becomes larger than 50 nm, the monodispersity and the shape will be disrupted even in the presence of sodium citrate. In addition to sodium citrate, many research groups are exploring other reducing agent to synthesize monodispersed Au NPs. For example, Perrault et al. recently demonstrated the use of hydroquinone as reducing agent and successfully produced Au NPs with size ranging from 50 to 200 nm [35]. This method represents a good alternative to prepare Au NPs in aqueous phase in addition to the synthetic approaches of Turkevich

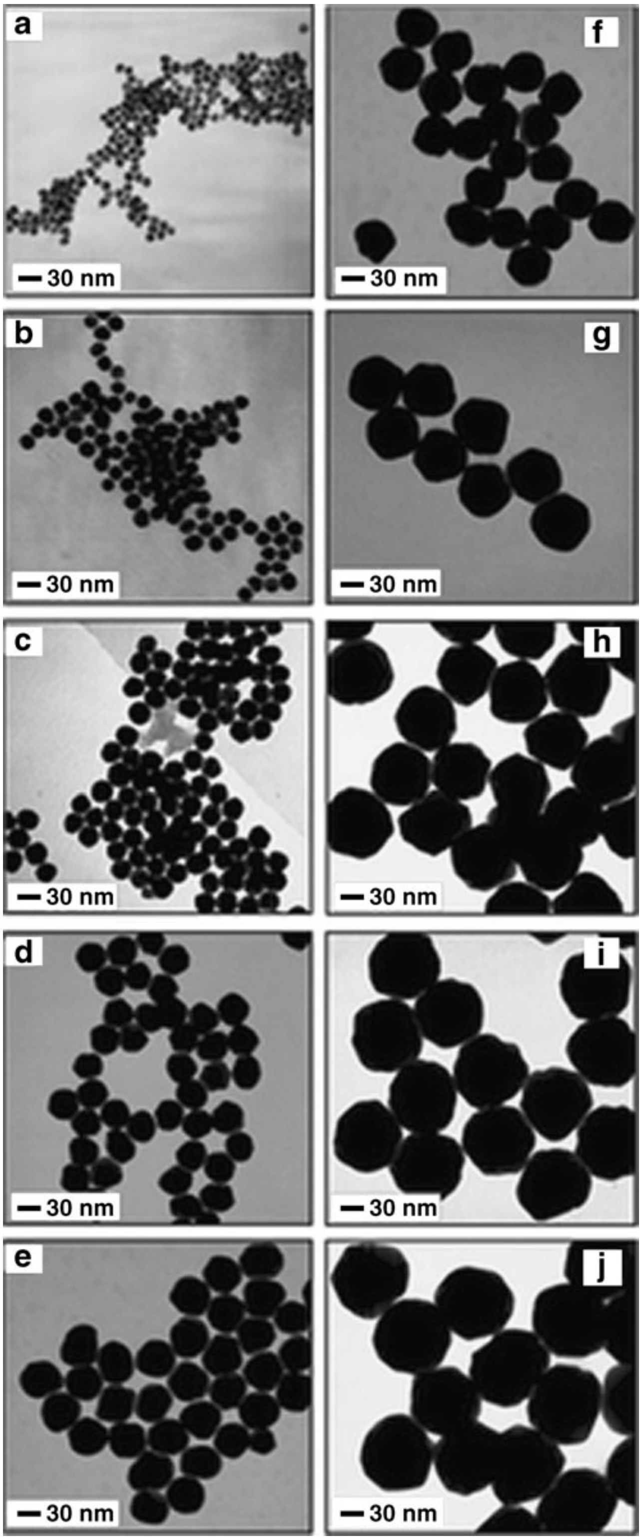

Fig. 1 TEM micrographs of gold nanoparticles of different sizes: 10 nm (**a**), 20 nm (**b**), 30 nm (**c**), 40 nm (**d**), 50 nm (**e**), 60 nm (**f**), 70 nm (**g**), 80 nm (**h**), 90 nm (**i**), and 100 nm (**j**). Figure reprinted with permission from [10]. Copyright 2007 American Chemical Society

et al. and Frens et al. Besides from synthesizing Au NPs in aqueous phase, Au NPs can be produced in organic phase

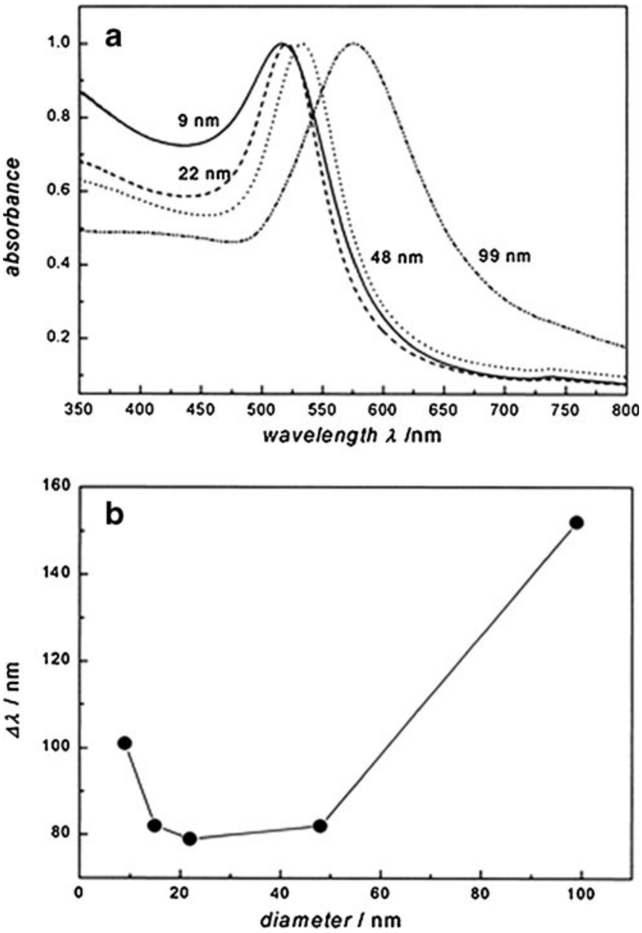

Fig. 2 **a** UV-vis absorption spectra of 9-, 22-, 48-, and 99-nm gold nanoparticles in water. All spectra are normalized at their absorption maxima, which are 517, 521, 533, and 575 nm, respectively. Figure reprinted with permission from [12]. Copyright 1999 American Chemical Society

using Brust et al. method [36]. Basically, Au NPs with size ranging from 2 to 5 nm can be obtained by reducing gold salt with sodium borohydride in the presence of alkenethiol molecules for forming stabilized colloidal Au NPs. The organic solutions generally used for the synthesis of Au NPs are toluene, chloroform, and hexane. Later, the synthesis protocol was further modified where the Au NPs were functionalized with bifunctional thiol ligands for specific applications [37]. The bifunctional thiol molecules were attached to the Au nuclei during the reduction process of the gold salts. In general, these NPs are referred as monolayer-protected clusters. Since then, different kinds of biofunctional groups, such as nucleic acids, proteins, amphiphilic polymers are used to modify the Au NPs surface that laid a promising platform for using functionalized Au NPs for biosensing [38–43]

In recent years, many research groups have made significant effort in developing new synthesis methods for making different size and shape of Au NPs with various combinations of reducing and capping agents. We will highlight a few examples in this section. For example, Jana et al. developed the seed-mediated approach for making Au nanorods (NRs) in a micellar solution [44]. They have demonstrated that alkyl chain length from the CTAB surfactants play an important role in determining the aspect ratio the Au NRs. They have also reported that the NRs formation rate increases in the presence of the gold seeds. In general, they have suggested that Au NRs with aspect ratios ranging from 1 to 10 can be synthesized by carefully controlling the concentration ratio between gold seeds and gold salts in the growth micellar solution. The same group later reported that Au NPs with diameter size of 5–40 nm with narrow size distribution can be produced by using the similar seed-mediated approach reaction process [45]. In addition, using similar approach, they are able to fabricate different shapes and sizes of Au NPs by tailoring the reaction parameters of the growth solution (see Fig. 3).

More recently, Aslam et al. [46] have demonstrated a one-step process for the making of water-dispersible Au NPs using the oleylamine as both reducing and capping agents. The Au NPs prepared were fcc phase and were fairly monodisperse. Kim et al. [47] reported a one-phase synthesis of Au NPs using thiol-functionalized ionic liquids. Thiol-functionalized ionic liquids were served as the stabilizing agents for making colloidally stable Au NPs. The ionic liquids were designed to have thiol groups on either the cation or anion and symmetrical or unsymmetrical positions at the cation. The NPs formed using thiol-functionalized ionic liquids were crystalline with face-centered cubic packing arrangements and have sizes ranging from 3.5 to 2.0 nm. The authors speculated that the NP size and distribution depend on the number and position of thiol groups in the ionic liquids. Malikova et al. [48] reported the preparation of Au NPs by reducing gold salt in the presence salicylic acid. The resulting NPs dispersion was found to have a mixture of triangular/hexagonal and smaller close-to-spherical NPs. Such Au NPs display two differentiated surface plasmon bands at 540 and 860 nm associated with spherical and anisotropic triangular/hexagonal NPs. Otsuka et al. [49] demonstrated that Au NPs with sizes ranging from 1 to 10 nm were prepared upon in situ reducing of HAuCl4 in the presence of heterobifunctional polyethylene glycol derivatives containing both mercapto and acetal groups. The R-acetal-PEG layers formed on gold nanoparticles impart appreciable stability to the nanoparticles in aqueous solutions with elevated ionic strength and also in serum-containing medium. This derivatization of Au NPs with heterobifunctional PEG provides a straightforward method to fabricate various colloidal sensor systems for bioassays and biorecognition applications. Martin et al. [24] reported a fast and highly reproducible chemical synthesis method for preparing colloidal Au NPs which are negatively charged in nonpolar solvents and coated with hydrophobic organic molecules. After evaporation of the solvent, the monolayer film of NPs can be deposited to any substrate without any size limitation. The synthesis does not require a post-synthesis cleaning step, since the two immiscible liquid phases separate the reaction byproducts from Au NPs and a minimal amount of coating molecules is used. Also, it was recently reported that some green synthesis approaches have been developed for making Au NPs. For example, polysaccharides and biopolymers generally are used as both stabilizers and reducing agents to synthesize Au NPs [50, 51]. Sylvestre et al. [51] developed a technique to synthesize Au NPs using laser ablation in the presence of cyclodextrins. The particle size and colloidal stability can be controlled by adjusting the pH value in the solution. For detail synthesis protocol, please refer to the reference [51].

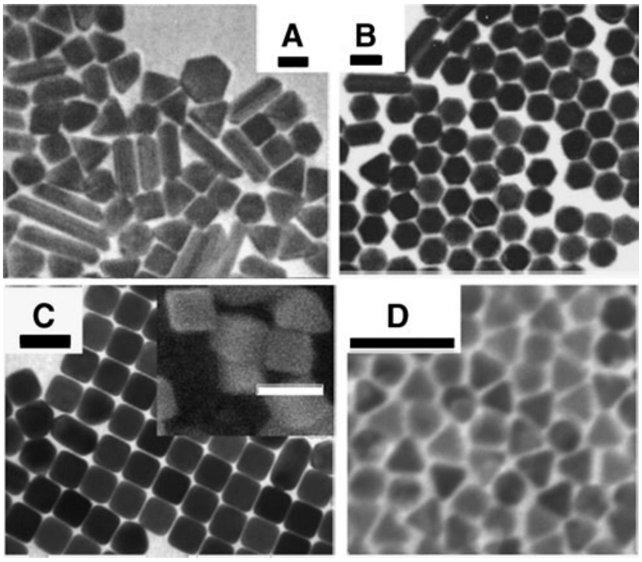

Fig. 3 TEM images of Au NPs with various shapes and sizes using seed-mediated approach synthesis method. Figure reprinted with permission from [28]. Copyright 2004 American Chemical Society

Functionalized Gold Nanoparticles for Biosensing

Because of the sensitivity and selectivity response to the biological environment, the optical properties of Au NPs have been used in sensing biological molecules and cells. Various Au NP formulations have been fabricated for targeting biological targets, such as DNA, RNA, cells, metal ions, small organic compounds, protein, and many more of biological specimens. In the following sections, we have divided seven categories for using functionalized Au NPs for biosensing, namely, sensing of DNA and oligonucleotides, SPR biosensor with functionalized Au NPs, cell detection and labeling with functionalized Au NPs, protein detection, detecting heavy metal ions, sensing of glucose, and sensing of other biological-related molecules with functionalized gold nanoparticles, respectively. In each section, we will focus on reviewing and discussing the latest trend for each topic.

Sensing of DNA and Oligonucleotides

Detection of DNA, aptamers, and oligonucleotide has received great attention in the past few years because it has important applications in medical research and diagnosis and food and drug industry monitoring. Majority of the assays identify specific sequence through hybridization of an immobilized probe to the target analyte after the latter has been modified with a covalently linked optical probe. Currently, many research teams have developed DNA, aptamers, and oligonucleotide detection schemes that involve the use of chemically functionalized Au NPs [52–56]. These approaches are simple and straightforward and use facile NPs surface functionalization chemistry and usually do not require expensive instrumentation. In this section, we will discuss a few examples of using functionalized Au NPs as ultrasensitive tools for sensing DNA, aptamers, and oligonucleotides.

DNA sequencing and sensing are important for pathogen detection and biomedical research. Currently, many DNA sensing systems have been integrated with Au NPs to enhance the detection limit and sensitivity. For example, Li et al. [57] found that single- and double-stranded oligonucleotides have different propensities to adsorb on Au NPs. They have showed that adsorption of single-stranded DNA stabilizes the Au NPs and prevent them from aggregation. Since the color of Au NPs is determined by SPR and the aggregation state of the NPs, one will be able to use the difference in the electrostatic properties of single-stranded DNA and double-stranded DNA to design a simple colorimetric hybridization assay. The assay can be used for sequence-specific detection of untagged oligonucleotides. In addition, it is designed for visual detection at concentration of 100 fmol, and the authors showed that it is easily adapted to detect single-base mismatches between probe and target. Later, the same group [58] have shown the designing of a fluorescent assay for DNA hybridization based on the electrostatic properties of DNA. They have exploited the ability to create conditions specifically that only single-stranded DNA adsorbs onto negatively charged Au NPs, but not in the case for the double-stranded DNA. The dye-tagged probe sequences have their fluorescence quenched upon mixing them with Au NPs. Target sequences in complex mixtures of DNA and single-base mismatches in DNA sequences can be easily detected using this approach. Liu et al. [59, 60] shows that DNAzyme-directed assembly of Au NPs can be used to fabricate effective colorimetric biosensors (see Fig. 4). More specifically, a colorimetric adenosine biosensor based on the aptazyme-directed assembly of Au NPs is reported. The

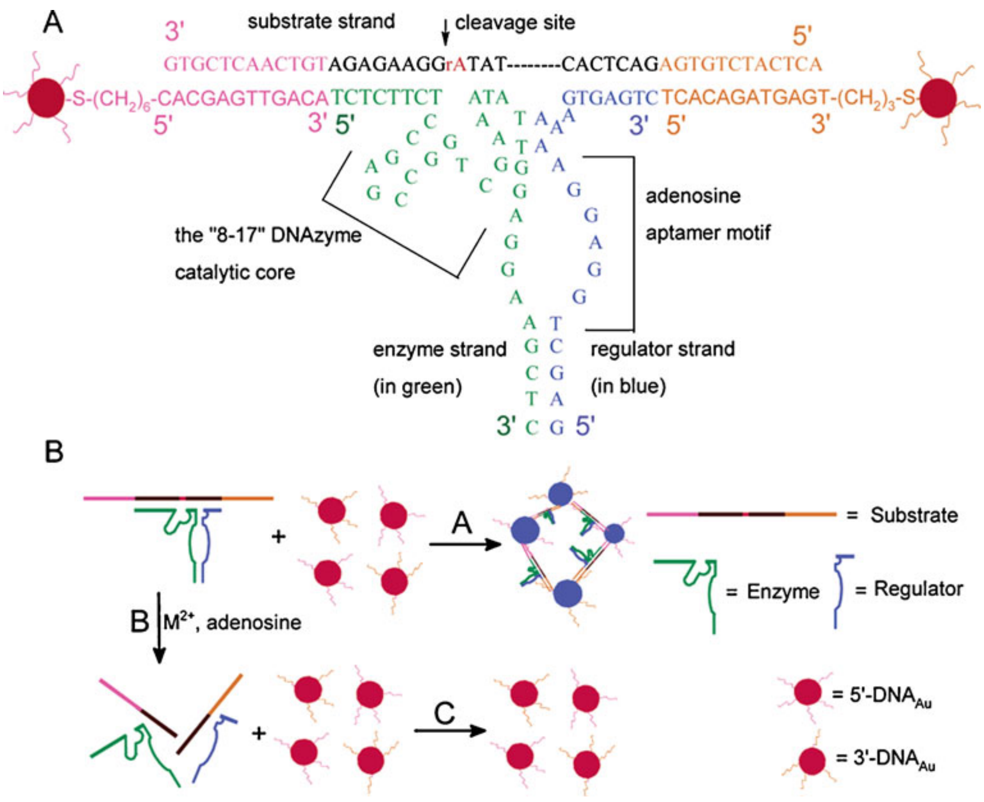

Fig. 4 **a** The primary and the proposed secondary structure of the adenosine aptazyme built on the 8–17 DNAzyme platform. Shown in the figure are four components. The *red balls with pink or orange strings* are 12-mer DNA-functionalized 13-nm-diameter Au NPs. **b** Schematic representation of the colorimetric detection of adenosine. The sequences of the substrate, enzyme, and regulator strands are given in **a**. The substrate strands can act as linkers for the DNA-functionalized Au NPs to form aggregates, which have a *blue* color (reaction A). In the presence of adenosine and metal ions, the substrate can be cleaved (reaction B). The cleaved substrate can no longer act as linker for NPs and the color remains *red* (reaction C). Figure reprinted with permission from [59]. Copyright 2004 American Chemical Society

aptazyme is based on the DNAzyme with an adenosine aptamer motif that can modulate the DNAzyme activity through allosteric interactions with the presence of adenosine. Without adenosine, the aptazyme is inactive and the substrate strands can serve as linkers to assemble DNA-functionalized Au NPs, causing a blue color appearance. On the other hand, the presence of adenosine activates the aptazyme that cleaves the substrate strand and disrupts the formation of NP aggregates. Since aptamers can be used to target many classes of important analytes [54, 61–63], they can be adapted into aptazyme systems for other promising sensing applications. In addition to these biosensors mentioned above, a hybrid type of diffraction grating-based sensor was developed for sensing DNA. Wark et al. [64] engineered a surface bioaffinity sensor based on the adsorption of Au NPs onto Au diffraction gratings (see Fig. 5). An enhanced diffraction was obtained in the SPR geometry due to the optical coupling of the planar surface plasmons in the grating to the localized surface plasmons in the Au NPs. The authors have illustrated that these NP-grating biosensors can be used to detect unmodified DNA at concentration as low as 10 fM.

Elghanian et al. [65] reported the use of Au NPs formulation to develop a colorimetric detection system for detecting polynucleotides molecules. The Au NPs were modified with mercaptoal-kyloligonucleotide molecules. It was demonstrated that the system has a detection limit of fmols for sensing oligonucleotide samples. This technique is based on the change of interparticle distances due to hybridization between mercaptoal-kyloligonucleotide-functionalized Au NPs formulation and the targeted sequence that will lead a red to-pinkish/purple color. Mariotti et al. [66] described the fabrication of a SPR affinity biosensor based on DNA hybridization. This biosensor was applied for genetically modified organism detection. Single-stranded DNA probes were immobilized on the sensor chip of a SPR device and the hybridization between the immobilized probe and the complementary sequence was monitored. The system was optimized for detection employing synthetic oligonucleotides and then applied to real samples analysis. Demers et al. [67] determined the number of thiol-derivatized single-stranded oligonucleotides bound to Au NPs and their extent of hybridization with complementary oligonucleotides in solution using a fluorescence-based method. Oligonucleotide surface coverages of hexanethiol 12-mer oligonucleotides on Au NPs were significantly higher than on planar gold thin films, while the percentage of hybridizable strands on the gold nanoparticles was lower than for gold thin films. The surface coverage of recognition strands can be tailored using coadsorbed diluent oligonucleotides. This provides a means of indirectly controlling the average number of hybridized strands per nanoparticle. The method presented here has important application for optimizing the sensitivity of Au NP-based oligonucleotide detection methods.

The development of a nanostructured electrochemical biosensor based on the integrated assembly of dehydrogenase enzymes and Au NPs was reported by Jena et al. [68]. The Au NPs were self-assembled on a thiol-terminated silicate network. The Au NPs on the silicate network efficiently catalyze the oxidation of NADH with a decrease in overpotential in the absence of any redox mediator. The Au NPs self-assembled network behaves like a nano-electrode ensemble. The nanostructured electrode shows high sensitivity toward NADH with an amperometric detection limit of 5 nM. The electrode displays excellent operational and storage stability. The biosensor is stable and

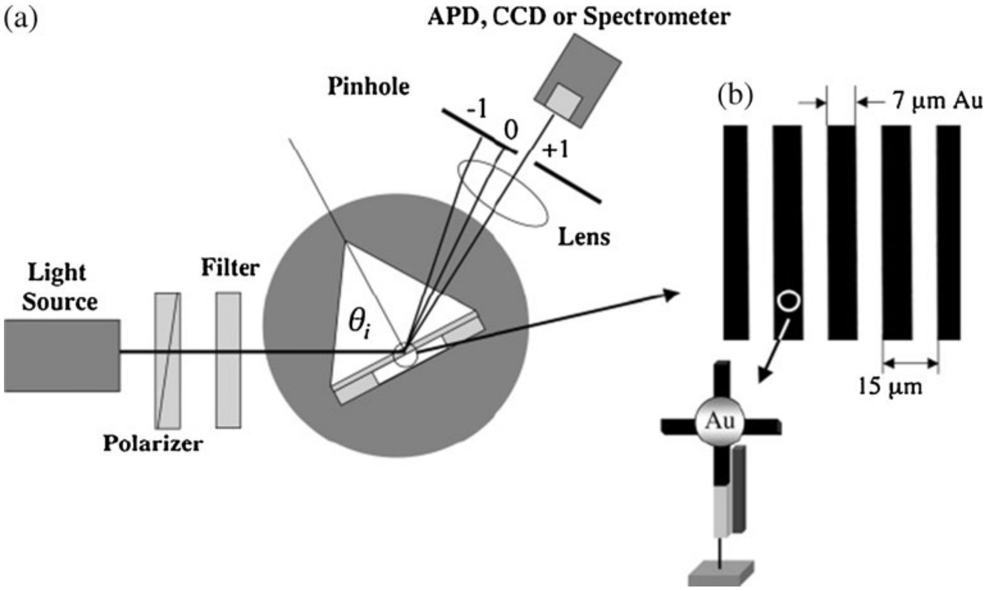

Fig. 5 **a** Schematic of the diffraction grating-based sensor setup. Collimated white light is directed through a polarizer and filter onto a prism/grating chip/flow cell assembly at an incidence angle. The diffraction beam is then collected through a lens and analyzed using one of three detectors: an optical fiber-coupled spectrometer, CCD camera, or avalanche photodiode (APD). **b** A partial image of the gold diffraction grating acquired using a transmission optical microscope. Figure reprinted with permission from [64]. Copyright 2007 American Chemical Society

sensitive, and more importantly it has a fast response time. The fabrication of sensing system for platelet-derived growth factors (PDGFs) and platelet-derived growth factor receptors that employs Au NPs was reported by Huang et al. [69]. They have fabricated aptamer-functionalized Au NPs that is specific to PDGFs and employed them to detect PDGFs upon monitoring the changes in the color and extinction of the bioconjugated NPs that happen as a result of aggregation. The color of the bioconjugated Au NPs changes from red to purple at concentration below 400 nM. The result indicates that there are real-life applications of using bioconjugated NPs for protein analysis and cancer diagnosis. All these examples have illustrated the importance of integrating Au NPs within the sensing system to enhance the selectivity and sensitivity of the sensor for detecting specific DNA, oligonucleotide, and other interesting biomolecules. More importantly, these reports provide important guidelines for the development of the next generation of DNA and oligonucleotide biosensor with a lower cost and able to use them for real-time monitoring in vivo.

SPR Biosensor with Functionalized Au NPs

Au NPs have also been used to enhance SPR biosensor response. Many research groups have used functionalized Au NPs in an immunoassay to demonstrate the amplification in biosensing. Au NPs-enhanced SPR technique has also been employed to develop assays for cholera cells detection. Some other groups have made an Au-amplified SPR sandwich immunoassay and demonstrated picomolar detection of human immunoglobulin. These reports suggest that the detection limit for Au NPs-amplified SPR biosensor system can be further improved by optimizing the setup parameters. This use of functionalized Au NPs for enhancing biosensor response will create new avenues for single molecular interaction detection biosensors and DNA diagnostics. Here, we will briefly discuss the various setups of SPR biosensor and subsequently describe general approaches of using Au NPs to enhance the SPR biosensor response.

The SPR sensor technology has been developed more than a decade ago [9, 70–72]. Currently, commercially available SPR sensors are available in the market and they are used as a powerful tool for biomolecular interaction analysis, drug discovery, and life science research. SPR sensor mechanism involves surface plasmons or electromagnetic waves propagating along a metal/dielectric interface. The wave vector of plasmons is dependent on the dielectric constants of media and it is extremely sensitive towards the properties of the dielectric medium that is in contact with the metal. Surface plasmons are excited in the Kretchmann configuration by directing p-polarized light to a glass prism and reflecting from a gold film. When the tangential x-component of the incident optical wave vector is matched with wave vector of plasmons, the pumping light energy is transferred to surface plasmons.

The plasmon coupling condition is generally accompanied with a dip of reflectivity at a specific combination of the angle of incidence and the wavelength. Here, the sensing effect comes from the fact that this resonance condition depends on the refractive index of a thin 200–300 nm layer, which is in contact with the SPR-supporting gold. Therefore, the information of biological interaction events on the gold film can be obtained by carefully monitoring the SPR coupling characteristics. In most SPR systems, the information of the biomolecular interactions is obtained from measurements of the angular or the spectral characteristics of light reflected under SPR. In this case, the detection limit is usually 10^{-5} in terms of the refractive index change, which corresponds to 1 pg mm^{-2} of the biomaterial accumulating at the biosensor surface [73–76].

Recently, we have developed a homemade SPR biosensor where the SPR phase is measured by using a photoelastic modulator to generate a weak retardation modulation [77]. We found that the variable retarder plays a very important role in the response of the second and the third harmonic signals to the polarization and the phase change caused by SPR. Without compensating the phase introduced by SPR, the second harmonic signal exhibits a shorter dynamic range than the third harmonic signal. We were able to simultaneously monitor the second and the third harmonic signals under condition of full pre-compensation of the initial phase introduced by SPR. It was discovered that these two signals are affected by two different properties of the optical beam, the former predominantly by the polarization amplitude and the latter by the phase. By utilizing these two characteristics, the developed system is able to detect a very small change of refractive index over a wide dynamic range [78].

Later, using the same setup, we have reported the use of functionalized Au NRs as amplification labels for ultrasensitive SPR biosensing [79]. Drastic sensitivity enhancement, owed to the electromagnetic interaction between the Au NRs and the sensing film, was maximized using longitudinal plasmonic resonance of gold nanorods. The detection sensitivity of the nanorod-conjugated antibody is estimated to be ~40 pg/ml, which is 25–100 times more sensitive than the current reported values in the literature. Recently, Matsui et al. [80] have developed an SPR sensor, which is able to detect low molecular weight analytes. Basically, the system was made by preparing molecularly imprinted polymer gel embedded with Au NPs on a gold substrate of a chip for SPR sensor detection. The sensing mode relied on the swelling effect of the polymer gel, which is triggered by the analyte binding within the polymer gel. The swelling effect causes the distance between the Au NPs and substrate to increase and causing a shift in a dip of a SPR curve to a higher SPR angle. The modified sensor chip showed an increasing SPR angle in response to enhanced dopamine concentration. More importantly, the Au NPs were shown to be effective for enhancing the signal intensity upon comparing to a sensor chip without immobilizing with Au NPs. Our finding gives good agreement with their observation indicating that Au NPs are essential for enhancing the sensitivity of the SPR sensor. Obviously, there are rooms for improvement in terms of enhancing the sensitivity of the biosensor upon coupling them with the Au NPs technology. In the next few years, many more research groups will focus on systematic examination of employing various sizes and shapes of Au NPs for SPR biosensing applications.

Cell Detection and Labeling with Functionalized Au NPs

Molecular imaging methods are often used to image and detect tumor cells. Among them, fluorescence microscopy is preferable technique for tissue and cells investigation with high-spatial resolution. However, this technique suffers from low sensitivity and tedious steps are needed to prepare the samples for imaging. For this reason, great efforts have been made to develop efficient biosensors aimed at improving the detection signals for accurate diagnosis of diseases. Functionalized Au NP-based sensor systems have attracted much attention in biomedical diagnostics in view of their usefulness for sensing cells at a small quantity. The design and construction of a successful biosensor with high detection limit is a primary goal toward the development of high detection limit for sensing cancer cells.

Ding et al. [81] reported the preparation of nanocomposite gel by neutralizing a designer nanocomposites solution of chitosan-encapsulated Au NPs. The gel was designed for immobilization and electrochemical study of cells and monitoring adhesion, proliferation, and apoptosis of cells on electrodes. An impedance cell sensor was constructed using K562 leukemia cells as a model. The nanocomposites gel showed improved immobilization capacity for cells and good biocompatibility for preserving the activity of immobilized living cells. The living cells immobilized on glassy carbon electrode exhibited an irreversible voltammetric response and increased the electron transfer resistance (see Fig. 6). This work illustrated that the nanocomposites gel based on biopolymer and NPs have the potential for biosensing and will provide a new avenue for electrochemical investigation of cell adhesion, proliferation, and apoptosis. Kneipp et al. [82] reported the SERS studies on indocyanine green (ICG)-functionalized Au NPs and demonstrated that the probe can be used for sensing of living cells. The ICG Au NPs provides spatially localized chemical information from the cell environment by monitoring the SERS local optical fields of the Au NPs. The functionalized Au NPs offer the potential to enhance the spectral specificity and selectivity of current chemical analysis approaches of living cells based on vibrational information. Souza et al. [83] reported an approach for fabrication of biologically active molecular networks consisting of bacteriophage directly assembled with Au NPs. They have showed that when the phage engineered with peptide is able to preserve the cell surface receptor binding and internalization. The networks can be used as labels for enhanced fluorescence, dark-field microscopy, and surface-enhanced Raman scattering detection of living cells. The physical and biological features of the Au NPs networks offer multimodalities for nanobiomedical imaging applications.

Protein Detection

Proteins have complementary counter parts that are similar to oligonucleotide strands. Protein targets biomolecules can be anchored to Au NPs surface for their specific detection together with other sensing agents and tools. Commonly, the developed detection scheme of using Au NPs can be applied to detect multiple protein targets through a single screening test. For instance, Nath et al. [18, 84] demonstrated that the unique optical properties of Au NPs can be used to develop a label-free biosensor in a chip format. The author demonstrated that the Au NPs significantly affects the sensitivity of the biosensor. Sensor chips were fabricated by chemisorption of Au NPs on amine-functionalized glass. Sensors fabri-

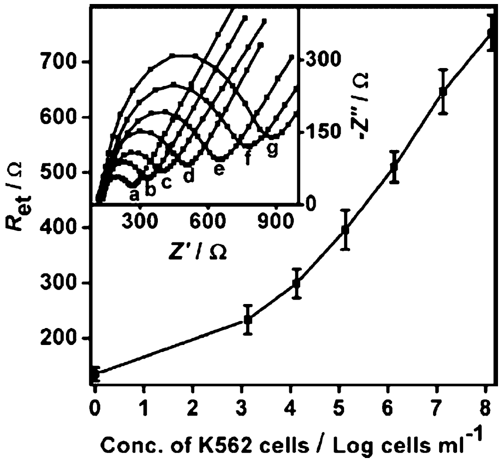

Fig. 6 Linear relationship between electron-transfer resistance and logarithm of K562 cells concentration. *Inset*: Nyquist diagrams of Au NPs-CHIT gel-modified GCE after immersed in various concentration of K562 cells suspension. Figure reprinted with permission from [81]. Copyright 2007 American Chemical Society

cated from 39 nm Au NPs exhibited maximum sensitivity to the change of the bulk refractive index. The detection limit for streptavidin-biotin binding of a sensor fabricated from 39 nm Au NPs was 20-fold better than that of a sensor fabricated from 13 nm Au NPs. Also, Thanh et al. [85] reported the development of a specific immunoassay system for antibodies using Au NPs. The assay is based on the aggregation of Au NPs that are modified with protein antigens in the presence of their corresponding antibodies. The aggregation of the Au NPs results in absorption changes and was monitored using an absorption plate reader. To demonstrate the analytical capabilities of the new technique, monodispersed protein A-coated gold particles were used to determine the level of anti-protein A in serum samples. A dynamic range of two orders of magnitude and a limit of detection of 1 μg/mL of anti-protein A were observed.

In addition to the sensing systems mentioned above, Ahirwal et al. [86] reported the use of Au NPs to covalently attach an antibody (Ab_1) using a spacer arm and subsequently the Ab_1 functionalized Au NPs are linked to Au electrode and used for sandwich electrochemical immunoassay. The detection was performed using cyclic voltammetry and impedance measurements using horse radish peroxidase (HRP) as enzyme label on secondary antibody and tertramethyl benzidine as an electroactive dye. The detection limit of this electrode was 2 ng/mL analyte. The electrochemical impedance spectroscopy studies demonstrate that the formation of antigen–antibody complexes increases the series resistance and thus confirms the assembly on the electrode. This study showed that Au NPs was efficient in preserving the activity of the antibody and can serve as a sensing platform for many clinical immunoassays. Xiang et al. [87] reported the preparation of a composite film based on mixture of Au NPs, ionic liquid, and multi-wall carbon nanotubes modified on glassy carbon (GC) electrode by a layer-by-layer self-assembly technique. Cytochrome c (Cyt c) was immobilized on the nanohybrid film-modified GC electrode by electrostatic adsorption. Direct electrochemistry and electrocatalysis of Cyt c were investigated. The results suggested that Cyt c could be tightly adsorbed on the modified electrode. The nanohybrid film showed a promotion for the direct electron transfer between Cyt c and the underlying electrode. The immobilized Cyt c exhibited an excellent electrocatalytic activity towards the reduction of H_2O_2. These reports have indicates that the functionalization of protein targets on the surface of Au NPs play an important key role in determining the sensitivity and selectivity of the Au NPs-based sensing system. Further studies will be needed in the near future to optimize the functionalization steps for the development of new generation of Au NPs-based sensor in detecting multiple proteins through a less complex preparation.

Detecting Heavy Metal Ions

The development of precise and sensitive metal ion sensors has always been a major research trust in academics and healthcare. The reason is simple; we need a sensor system that we can use to provide on-site and real-time monitoring of toxic metal ions (Pb^{2+}, Cd^{2+}, Hg^{2+}, and Se^{2-}) in applications such as industry waste monitoring, environmental biology, and clinical toxicology. In general, a simple colorimetric sensor is preferable because the cost can be minimized and they can be made for portable real-time detection. Furthermore, a metal ion sensor with a tunable dynamic range is desirable for applications in widely different concentration ranges. So far, many approaches have been reported to widen the dynamic range without having to design new sensors [88–91]. Here, we describe various designs of metal ions sensors based on Au NPs setup and their use for sensitive and selective detection and quantification of metal ions.

The high metal selectivity of DNAzymes and the strong distance-dependent optical properties of metallic nanoparticles are ideal attributes for the development of colorimetric sensors for heavy metal ion detection. Liu et al. [92] recently demonstrated the engineering of a colorimetric lead sensor based on the assembly of Au NPs by a Pb^{2+}-dependent DNAzyme. However, heating and cooling processes were needed to observe the color change in the sensor. Later, the same group reported [90] an improved version of sensor that allows fast detection of lead ions at room temperature (see Fig. 7). This improvement of sensor performance is due to the alignment of the nanoparticles in a "tail-to-tail" manner rather than "head-to-tail" manner, and NP size is the key factor in allowing fast color changes. These results lay an important foundation in understanding the DNAzyme biochemistry and NP technology for dramatically improving colorimetric sensors.

Recently, Wang et al. [93] reported a colorimetric method for lead sensing using combination of both unlabeled DNAzyme and Au NPs. They have demonstrated the use of chelating agent to quench the Pb^{2+}-induced DNAzyme reaction, which is important for reproducible detection. The detection process can be finished within several minutes with a detection limit of 3 nM. The design of label-free DNAzyme colorimetric sensor for lead detection is straightforward. Basically, the setup is based on the 8–17 DNAzymes, and these DNAzymes have been shown to be highly specific for Pb^{2+}. The 8–17 DNAzyme is composed of a substrate strand extended by eight bases at the 50 end and an enzyme strand extended by eight complimentary bases at 30 end. The eight base-pair extension allows hybridization between the substrate and enzyme strands, while allowing the release of single-stranded DNA upon cleavage in the presence of Pb^{2+}.

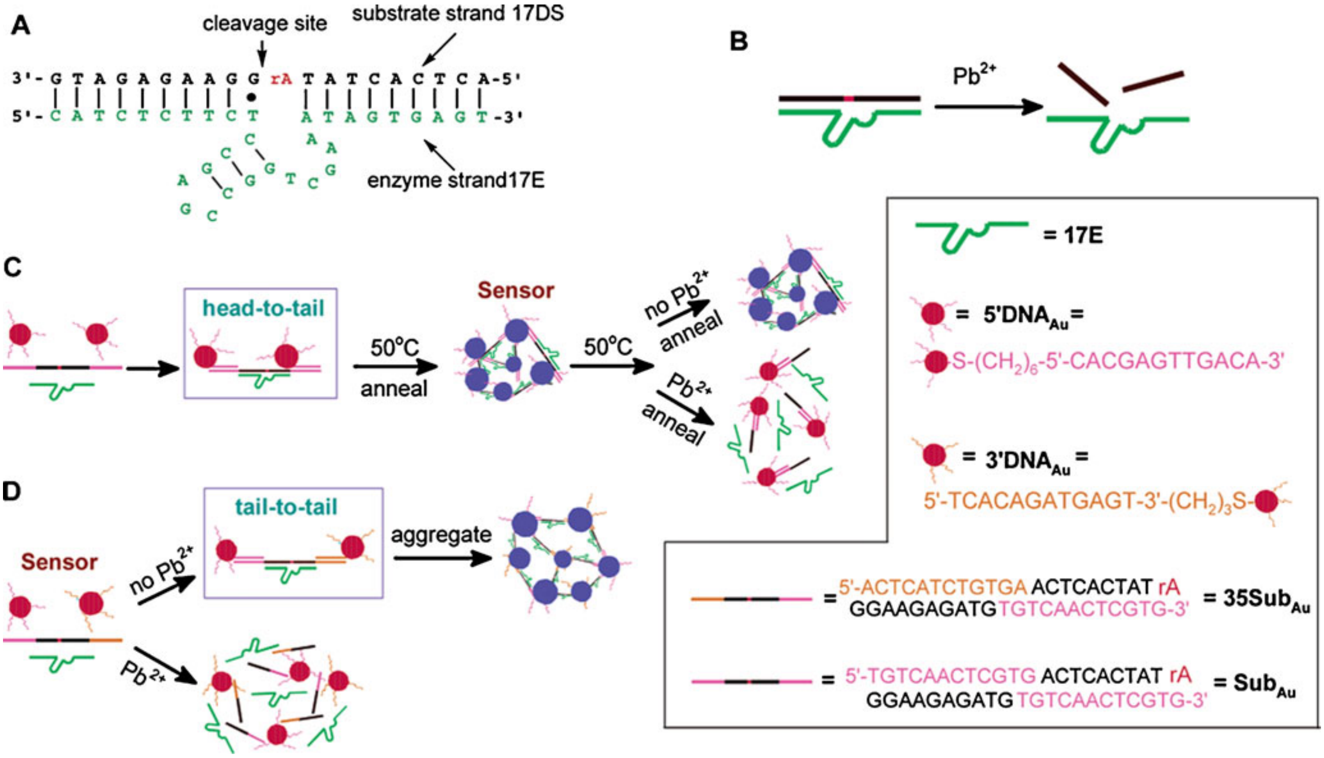

Fig. 7 **a** Secondary structure of the “8−17” DNAzyme. **b** Cleavage of 17DS by 17E in the presence of Pb^{2+}. **c** Schematics of the previous colorimetric Pb^{2+} sensor design. The three components of the sensor (5′ DNA_{Au}, Sub_{Au}, and 17E) can assemble to form blue-colored aggregates. Nanoparticles were aligned in a “head-to-tail” manner. **d** Schematics of the new colorimetric sensor design. The NPs are aligned in the “tail-to-tail” manner. In the absence of Pb^{2+}, nanoparticles can be assembled by the DNAzyme at ambient temperature, resulting in a *blue* color; while in the presence of Pb^{2+}, $35Sub_{Au}$ is cleaved by 17E, inhibiting the assembly, resulting in a red color of separated NPs. Figure reprinted with permission from [90]. Copyright 2004 American Chemical Society

Upon addition of trishydroxymethylaminomethane and NaCl to adjust ionic strength, followed by addition of Au NPs, the released ssDNA can be adsorbed onto AuNP and the individual red AuNPs can be prevented from forming blue aggregates under high-ionic strength conditions. In the absence of Pb^{2+}, however, no cleavage reaction occur, and the enzyme-substrate complex would not be able to stabilize individual red Au NPs and cause the Au NPs to form aggregates and become purple–blue in color. Chai et al. [94] demonstrated a sensitive colorimetric detection method for sensing Pb^{2+} ions by employing glutathione functionalized Au NPs. The functionalized Au NPs formed aggregates in the presence of Pb^{2+}. The Pb^{2+} ions were detected by colorimetric response of Au NPs. This was achieved by monitoring the SPR peak with UV-vis spectrophotometer and naked eyes. The detection limit was estimated to be 100 nM. The GSH-GNPs bound by Pb^{2+} showed excellent selectivity compared to other metal ions. This provides a colorimetric sensor that can be employed for on-site and real-time detection of Pb^{2+}. Huang et al. [89] have developed a colorimetric assay for detecting Pb^{2+} by narrowing the size distribution of gallic acid-functionalized Au NPs and reducing electrostatic repulsion between the bioconjugated Au NPs. The colorimetric sensitivity of the Pb^{2+}-induced aggregation of gallic acid functionalized Au NPs were improved by using narrow-size distribution of Au NPs. Under the optimum conditions, the sensitivity of 9.3 nm functionalized Au NPs for sensing Pb^{2+} concentration is up to 10 nM.

Liu et al. [95] reported the preparation of a colorimetric assay that employs poly-Tn ss-DNA and 13-nm diameter Au NPs for the sensing Hg^{2+} ions. The fundamental concept is based on $Hg2^{+}$/DNA complexes that induce the Au NPs aggregation. More specifically, the DNA molecules adsorb onto Au NP surfaces by electrostatic attraction. When Hg^{2+}/DNA complexes are formed, the conformation of the poly-Tn ss-DNA changes to folded structures. This causes the zeta potential on Au NPs to decrease, resulting in weakening of the electrostatic repulsion between Au NPs and causing the aggregation of the Au NPs, leading to the color change of the solution from red to purple. Similarly, Lee et al. [96] have fabricated a chip-based scanometric method for the detection of mercuric ions detection. This approach utilizes the cooperative binding and catalytic properties of DNA-functionalized Au NPs and the selective binding of a thymine–thymine mismatch for mercuric ions.

The sensitivity of this assay is 10 nM (2 ppb) of mercuric ions and it is able to discriminate mercuric ions from other metal ions. This technique is potential for on-waste samples management due to its high throughput, convenient readout, and portability. These reports have suggested that Au NP-based sensor is able to sense traces of heavy metal ions indicating that these developed sensors have great potential for real-time monitoring of concentration of heavy metal ions in the industries, which use high quantity of heavy metals for catalysis processes.

Sensing of Glucose

The development of fast and reliable sensing devices in monitoring of glucose for the treatment and control of diabetes has always been an important research topic for the last decade [97, 98]. Typically, optical methods are commonly used for the indirect detection of carbohydrates. However, the sensitivity of these methods is poor and the enzyme glucose oxidase/glucose dehydrogenase is required. To date, electrochemical methods are considered to be useful for sensing glucose because higher sensitivity detection can be achieved. Most of the electrochemical methods are based on the use of the enzyme glucose oxidase that selectively catalyzes the oxidation of glucose to glucolactone. The electrochemical biosensing of glucose is based on two mechanisms [97, 99–102]: (1) the redox reaction of mediators that are attached onto the electrode surface along with the enzyme and (2) the electrochemical oxidation of enzymatically generated H_2O_2. In redox reaction case, the internally wired redox mediators electrochemically access the flavin cofactor of glucose oxidase. As for the electrochemical case, it involves the reduction of oxygen to hydrogen peroxide by the enzyme glucose oxidase. Here, we discuss a few examples using such approaches to detect glucose.

Zhang et al. [98] reported the fabrication glucose biosensor by covalent attachment of glucose oxidase to Au NPs-modified Au electrode. Cyclic voltammetry and electrochemical impedance spectroscopy were used to confirm the assembly process of biosensor and suggested that the Au NPs in the biosensing interface effectively enhanced the electron transfer between analyte and electrode surface. Cyclic voltammetry performed in the presence of glucose and artificial redox mediator, ferrocenemethanol, allowed the quantification of the surface concentration of electrically wired enzyme. The sensor provided a linear response to glucose over the concentration range of 2.0×10^{-5} to 5.7×10^{-3} M with a detection limit of 8.2 μM. Jena et al. [103] demonstrated the development of nonenzymatic electrochemical method for sensing glucose upon using Au NPs self-assembled on a three-dimensional silicate network. The Au NPs efficiently catalyzed the oxidation of glucose in phosphate buffer solution in the absence of any enzymes or redox mediators. The Au NP-modified transducer was used for the amperometric sensing of glucose and it showed excellent sensitivity with a detection limit of 50 nM. Recently, Wu et al. [104] illustrated the fabrication of glucose biosensor through a layer-by-layer assembled chitosan/Au NPs/glucose oxidase multilayer films on Pt electrode. The results confirmed that the immobilized glucose oxidase displayed a good catalytic property to glucose, and AuNPs in the biosensing interface efficiently improved the electron transfer between analyte and electrode surface. These examples have proved that by incorporating Au NPs into the sensing system, one can tailor ultrasensitive glucose sensor for achieving high precision detection of glucose. More importantly, we envision that Au NPs can be integrated into sensing devices for real-time monitoring of blood sugar that will enable clinicians to develop personalized medicine for the individual patients.

Sensing of Other Biological-related Molecules with Functionalized Gold Nanoparticles

In addition to sensing DNA, glucose, heavy metal ions, proteins, and cells, Au NPs can be further manipulated for detecting other chemical molecules by modifying their surface with specific ligands [105]. For instance, Astruc et al. [106] recently reviewed the use of dendrimers, alkylthiol-Au NPs, and Au NP-cored dendrimers, containing tethers terminated by a redox group attached to a hydrogen-bonding groups, as selective exo-receptors for the recognition, sensing and titration of oxo-anions, mostly using cyclic voltammetry. Various positive dendritic effects were disclosed, and large Au NP-cored redox dendrimers of this type that contain several hundred equivalent ferrocenyl groups adsorbed on Pt electrodes, providing useful electrochemical sensors. Sanz et al. [107] demonstrated the preparation of a tyrosinase biosensor based on the immobilization of the enzyme onto a glassy carbon electrode modified with electrodeposited Au NPs. The enzyme immobilized by cross-linking with glutaraldehyde retains a high bioactivity on this electrode material. Under the optimized working variables, the biosensor exhibited a rapid response to the changes in the substrate concentration for all the phenolic compounds tested: phenol, catechol, caffeic acid, chlorogenic acid, gallic acid, and protocatechualdehyde. Chen et al. [108] demonstrated that Nile red functionalized Au NPs can be used for sensing thiols. The functionalized Au NPs fluoresce weakly due to the fluorescence resonance energy transfer between Nile red and the Au NPs. The fluorescence of Nile red-functionalized Au NPs increases upon the addition of thiols. This method allows for the selective determination of thiols such as cysteamine and homocysteine.

It is known that conventional methods for lung cancer diagnosis are unsuitable for screening because very often these techniques are not able to detect tumors [109–111]. Gas chromatography/mass spectrometry studies have shown that several volatile organic compounds are elevated to levels between 10 and 100 ppb in lung cancer patients [112–114]. Peng et al. [115] demonstrated that an array of sensors based on bioconjugated Au NPs formulation that can be used to rapidly distinguish the breath of lung cancer patients from the breath of healthy individuals in an atmosphere of high humidity. In combination with solid-phase microextraction, gas chromatography/mass spectrometry was used to identify 42 volatile organic compounds that represent lung cancer biomarkers. Four of these were used to optimize the sensors, demonstrating good agreement between patient and simulated breath samples. The results show that sensors based on biofunctionalized Au NPs could lay a foundation of an inexpensive and non-invasive diagnostic tool for lung cancer.

Luminescent quantum dots are effective fluorescence resonance energy transfer donors with an array of organic dye acceptors. Conversely, Au NPs are fluorescence quencher for conventional dye donors. Pons et al. [116] demonstrated the monitoring of the photoluminescence quenching of quantum dots by Au NP acceptors arrayed around the QD surface by using a rigid variable-length polypeptide as a bifunctional biological linker (see Fig. 8). They measured the Au NP-induced quenching rates for QD bioconjugates using steady-state and time-resolved fluorescence techniques. They have found that nonradiative quenching of the QD emission by Au NPs is due to long-distance dipole-metal interactions.

A horseradish peroxidase biosensor was developed by Jia et al. [117]. They have self-assembled Au NPs to a thiol-containing sol-gel network. Simply, an Au electrode was immersed in a hydrolyzed 3-mercaptopropyl-trimethoxysilane sol-gel solution to assemble three-dimensional silica gel. Next, Au NPs were linked onto the thiol groups of the sol-gel

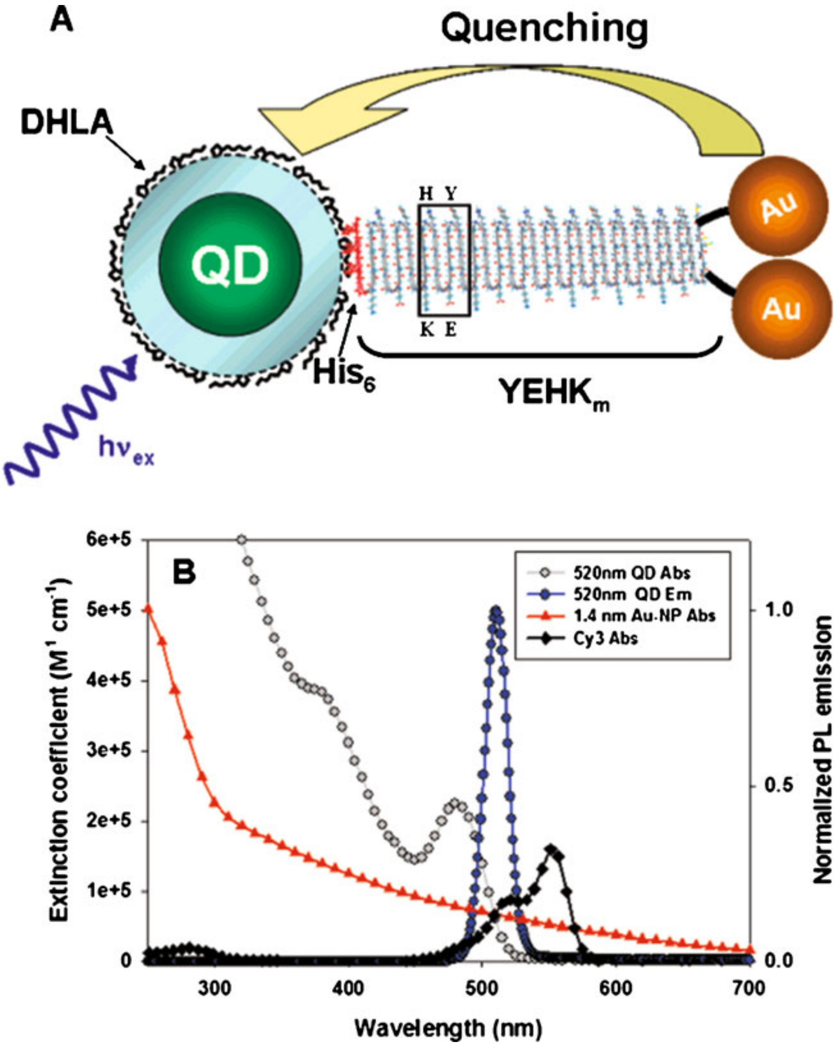

Fig. 8 **a** schematic representation of the QD–peptide–Au NP bioconjugates. The C-terminal His_6 coordinates to the QD surface while the cysteines are used as attachment sites for 1.4 nm monomaleimide-functionalized Au-NPs. Repeat units of 5, 7, 14, or 21 were used; $YEHK_7$ as shown with a single core YEHK boxed. The Au NPs are separated from the cysteine thiol by a maximum of ~8 Å and from each other by a maximum of 18 Å. **b** Extinction coefficient spectra of 520 nm emitting QDs, 1.4 nm Au-NPs and Cy3. Normalized emission of the QDs is also shown. Figure reprinted with permission from [116]. Copyright 2007 American Chemical Society

network. Finally, Au NPs were functionalized with HRP. The immobilized horseradish peroxidase exhibited direct electrochemical behavior toward the reduction of hydrogen peroxide. The resulting biosensor exhibited fast amperometric response to H_2O_2. The detection limit of the functionalized Au NPs-based biosensor was determined to be 2.0 μmol L^{-1}. The examples discussed above have clearly shown that the versatility of the functionalized Au NPs formulation in terms of integrating them to various sensing system in which they can be used as signal enhancer for sensitive detection.

Summary and Future Outlook

Gold nanoparticles have emerged as a promising material for biosensing that provide a useful complement to more traditional sensing techniques. The combination of low toxicity, high surface area, rich surface functionalization chemistry and colloidal stability allow them to be safely integrated into the sensor systems for detection in vitro and in vivo. In the near future, we envision that there are a few research trusts that have the potential, but need further refinements for overcoming the challenges for ultrasensitive biosensing. The first thrust is to develop multifunctional Au NPs for sensing multiple biomolecules at single screening. Also, multifunctional Au NPs will be more suitable to be translated for clinical research. We believe that in the coming few years, there will be a tremendous growth in developing Au NP-based sensor devices for therapeutic and diagnostic applications. In addition, environmental monitoring is another important area where Au NP-based biosensors can play an important role. Due to contamination of biochemicals in the environment that cause harmful effects to human, current available sensing techniques may not be effective for detecting trace of the harmful and life-threatening viruses such as swine flues and anthrax. Therefore, development of Au NP-based sensors integrated with molecular markers that will target viruses would be ideal for novel prophylactic and therapeutic approaches against several human diseases.

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