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Review

Gold nanoparticle probes

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Contents

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ABSTRACT

Depending on their size, shape, degree of aggregation and nature of the protecting organic shells on their surface, gold nanoparticles (AuNPs) can appear red, blue and other colors and emit bright resonance light scattering of various wavelengths. Because of this unique optical property, AuNPs have been extensively explored as probes for sensing/imaging a wide range of analytes/targets, such as heavy metallic cations, nucleic acids, proteins, cells, etc. Since their initial discovery, novel synthetic methods have led to precise control over particle size, shape and stability, thus allowing the modification of a wide variety of ligands on the AuNP surfaces to meet different experimental conditions. This review discusses the synthesis and applications of functionalized AuNPs in chemical sensing and imaging.

1. Introduction

The combination of nanotechnology with chemistry, biology, physics, and medicine for the development of ultrasensitive detection and imaging methods in the analytical or biological sciences is becoming increasingly important in modern science [1–14]. Particularly attractive is the use of functional AuNPs in biological and pharmaceutical field, such as the ultrasensitive detection and imaging methods for bioreorganizing events, because AuNPs have unique optical properties (i.e. surface plasma resonance absorption and resonance light scattering), a variety of surface coatings and great biocompatibility [5–7,11–18].

Generally, the optical properties of small metal nanoparticles are dominated by collective oscillation of electrons at surfaces (known as "surface plasmon resonance", SPR or "localized surface plasmon resonance", LSPR) that are in resonance with the incident electromagnetic radiation [4–6,13,19]. For gold, it happens that the resonance frequency of this oscillation, governed by its bulk dielectric constant, lies in the visible region of the electromagnetic spectrum [19]. Because nanoparticles have a high surface area to volume ratio, the plasmon frequency is exquisitely sensitive to the dielectric (refractive index) nature of its interface with the local medium. Any changes to the environment of these particles (surface modification, aggregation, medium refractive index, etc.) leads to colorimetric changes of the dispersions [5,8,20–25]. Due to coupling of the plasmons, assemblies (or aggregations) of AuNPs are often accompanied by distinct color changes. Colorimetric sensors using AuNPs have been widely explored and have important applications [6,8,14]. Not only is light strongly absorbed

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by the plasmons, it is also Rayleigh (elastically) scattered by them, and as the particle gets larger, a larger proportion of the outgoing light is scattered, compared with that absorbed [15,21]. Because the light scattered from AuNPs is in the visible portion of the electromagnetic spectrum in accord with their plasmon bands, it is possible to optically track the position of individual nanoparticles, paving the way for imaging applications [16,23,24]. The tailorable physical properties of AuNPs affect their collective oscillation of electrons offering tunable optical properties. This has further facilitated the application in biodetection via numerous detection methods [1–17]. The versatile surface chemistry of AuNPs could be achieved by linking various biofunctional groups, such as amphiphilic polymers, silanols, sugars, nucleic acids, and proteins, via the strong affinity of gold surface with thiol ligands [1–27]. Some of these studies have recently been reviewed elsewhere with a focus on the materials themselves or as subclassifications in more generalized overviews of biological applications of nanomaterials [1,3,5,6,8,12,14,15,20].

Here, without pretending to being exhaustive, we will focus on the preparation of spherical AuNP probes and AuNP based assays for ions, small molecules, DNA and protein detection and cellular analysis, highlighting some of their technical challenges and the new trends by means of a set of selected recent applications. We also note studies on non-spherical gold nanomaterials, gold nanorods (AuNRs) and gold nanoshells (AuNS, silica nanospheres with a nanoscale overcoat gold) which have tunable absorption in visible and NIR (700–1300 nm); these materials are the subject of other recent reviews [15,28,29].

2. Synthesis, stabilization and functionalization of gold nanoparticle probes

2.1. Synthesis

AuNPs are useful in a broad range of applications, but practical limitations are apparent when monodispersity is required. Numerous preparative methods for AuNPs from about 1 nm to several micrometers diameter are documented in the literature [30–41]. The most widely applied procedures to obtain ca. 10 to 150 nm gold hydrosols are variations of the classic Turkevich–Frens citrate reduction of gold (III) derivatives [30,31]. The AuNP size (between 10 and 147 nm) can be controlled by the ratio between the reducing/stabilizing agents (the trisodium citrate) and gold (III) derivatives (the hydrogen/sodium tetrachloroaurate (III)). This method is very often used even now since the rather loose shell of citrates on the particle-surfaces is easily replaced by other desired ligands (e.g., thiolated DNA) with valuable function [5,8].

Most hydrophobic AuNPs (also sometimes called monolayer-protected clusters (MPCs)) with diameters in 1 to ca. 8 nm ranges are prepared by the Brust–Schiffrin method: the gold (III) derivatives are reduced by sodium borohydride ($NaBH_4$) in an organic solvent in the presence of thiol capping ligands using either a two-phase liquid/liquid system or a suitable single-phase solvent [33,34]. In the Brust–Schiffrin methods, tetrachloroaurate (III) is transferred to toluene using tetraoctylammonium bromide (TOAB) as the phase-transfer reagent and reduced by $NaBH_4$ in the presence of dodecanethiol (DDT). Larger thiol/gold mole ratios give smaller average core sizes, and fast reductant addition and cooled solutions produced smaller, more monodisperse particles. Following the Brust–Schiffrin method, AuNPs with a variety of functional thiol ligands have been synthesized. Recently, a simple protocol for the one-step aqueous preparation of highly monodisperse AuNPs with diameters below 5 nm using thioether- or thiol-functionalized polymer ligands, such as alkyl thioether end-functionalized poly(methacrylic acid), has been developed by Hussain and co-workers [40–42]. In this approach, the particle size and size distribution is controlled by subtle variation of the polymer structure, concentration and "denticity". By varying systematically the polymer to gold ratio, the size of the nanoparticles can be finely tuned and a transition from non-fluorescent to fluorescent nanoparticles is observed for core diameters between 1.7 and 1.1 nm [42].

2.2. Stabilization and functionalization

For further AuNP applications, attaching the molecular recognition motifs (i.e. functional groups) of interest to the nanoparticles has to be readily achieved, and, most importantly, the probes must not bind non-specifically to each other or to anything else present in the system under investigation. In addition, introducing multiple functionalities would be of great value, as it provides more flexibility for multiplexing in bioanalytical applications and new tools to control the bottom-up assembly of nanostructures. Stabilization and functionalization of AuNP probes has been extensively reviewed elsewhere [1,5,6,8,10,14].

Electrostatic interaction, specific recognition (antibody–antigen, biotin–avidin, etc.), and covalent coupling (Au–S covalent, etc.) are three kinds of widely used methods to synthesis AuNP probes to meet the application requirement (as shown in Fig. 1) [1,3,5,6,8,12,14,15,20,43]. Electrostatic interaction or physical adsorption immobilization of ligands for AuNP probes is a simple process with the benefits of time saving and reduced complexity of ligand preparation [6,43,44]. Its relative simplicity gives this approach certain advantages over the more complex covalent immobilization methods. However, the binding is not strong enough to yield stable surfaces capable of standing the necessary washing steps and incubation conditions in biological studies on subsequent reaction. This issue is even more crucial in biological studies under harsh experimental conditions, for example, long time incubation with buffer solution which contains attacking molecules such as dithiothreitol (a small, uncharged molecule with two thiol groups, used to protect proteins from oxidation) as well as high salt concentration (generally used in DNA hybridization experiment). This results in a strong non-specific interaction between the AuNP probes and analytes which leads to decreased detection selectivity.

In comparison with the electrostatic interaction or physical adsorption immobilization techniques discussed above, covalent binding is normally more complex, sometimes requiring intensive synthesis work on the ligands. On the other hand, covalent binding of ligands with AuNPs offers high stability and is demonstrated to be quite robust: they can withstand a very high salt concentration (e.g., 2 M NaCl); they are extremely stable under thermal

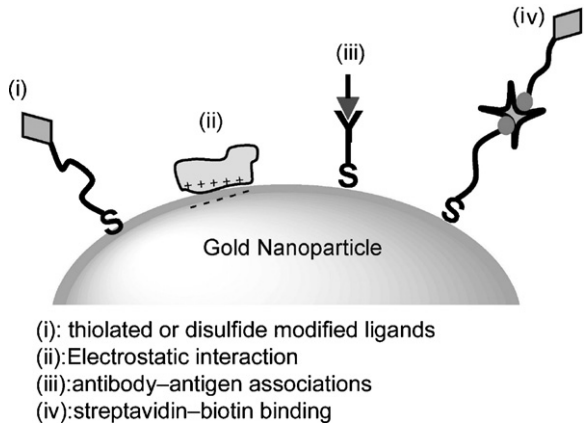

Fig. 1. Schematic representation of formation of gold nanoparticle probes.

conditions (e.g., boiling); and they can also resist, to some extent, attack by molecules such as dithiothreitol or molecules bearing SH, phosphine, and NH_2 groups. As a wide range of applications require high stability assays, in all those cases covalent binding should be considered. Most of the techniques reported for immobilizing ligands to AuNPs surfaces are based on Au–S covalent bond formation between the ligands and the gold atoms on the particle surfaces [1,3,5,6,8,12,14,15,20,45,46]. This approach necessitates the use of a sulfur containing ligand, i.e., thiol, disulfide and thiolester. For example, AuNPs can be stabilized with various molecules including alkanethiolates, glutathione, tiopronin, thiolated poly(ethylene glycol), thiolated or disulfide modified DNA, peptide CALNN and so on, by their facile and robust interaction with thiol or disulfide groups [1,3,5,6,8,12,14,15,20,45,46]. In particular, a tetra(ethylene glycol) spacer was introduced at the nanoparticle–protein interface to overcome the drawback of an alkyl-based monolayer which generally results in protein denaturation [47]. Recently, more and more attention has been given to development of aptamer-AuNP probes for biosensor application since aptamers are single-stranded DNA or RNA molecules created in vitro through systematic evolution of ligands by exponential enrichment (SELEX) for the recognition of target analytes with high affinity and specificity. Aptamer functionalized AuNPs have been applied to the analysis of several analytes, including proteins, metal ions, and small organic compounds [48–53]. For example, Zhou and co-workers combine the amplifying characteristic of Au nanoparticles with the advantage of aptamer technique to design a "pseudo" sandwich reaction for detecting small molecules (adenosine) by SPR spectroscopy [51].

Nanoparticles functionalized with groups that provide affinity sites for the binding of biomolecules have also been used for the specific attachment of proteins and oligonucleotides [54–56]. For example, (1) streptavidin-functionalized AuNPs have been used for the affinity binding of biotinylated proteins (e.g., immunoglobulins and serum albumins) or biotinylated oligonucleotides [54]; (2) protein A conjugate bound to AuNPs was used as a versatile linker to Fc fragments of various immunoglobulins [55] and (3) carbohydrate modified AuNPs was used to recognize their respective binding proteins. This may be advantageous because of a high binding constant between these molecules [56].

3. Applications of gold nanoparticle probes

Potential applications of AuNP probes in analytical and/or biological sciences include chemical sensing and imaging applications. The color changes associated with nanoparticle aggregation were originally exploited by Mirkin et al. who showed that ssDNA stabilized nanoparticles could be used to colorimetrically detect the complementary oligonucleotide [57,58]. The Mirkin's type colorimetrically assay has opened up an avenue to apply gold nanoparticles. In the past decade or so, numerous AuNP-based assays have been developed for the detection of many targets, including: metal ions, small organic compounds, nucleic acids, proteins, cells, etc. [1,3,5,6,8,12,14,15,20]. Here, we provide an overview of recent examples of AuNP probe-based assays involved in (i) determination of heavy metal cations, DNAs, and proteins, (ii) studies of carbohydrate–protein interactions and enzymatic activities and (iii) cellular analysis or imaging. In addition, the application of biocatalytic growth of AuNPs for sensing has also been discussed because of the excellent conductivity and catalytic properties of AuNPs.

3.1. Heavy metal cations determination

Heavy metal cations such as Pb^{2+}, Cr^{2+} and Hg^{2+} are the commonly encountered toxic substances in the environment and pose significant public health hazards when they are present in drinking water even in parts per million concentration [59–61]. In particular, Pb^{2+} is dangerous for children, causing mental retardation [61]. Colorimetric sensors using AuNPs have been widely explored and have important applications in the sensitive detection of metallic ions [62–73]. The AuNP-based colorimetric assay does not utilize organic co-solvents, enzymatic reactions, light-sensitive dye molecules, lengthy protocols, or sophisticated instrumentation thereby overcoming some of the limitations of more conventional methods. Lu's group has developed a fast and simple colorimetric sensor for on-site and real-time heavy metal cation detection based on a DNAzyme modification of AuNP (as shown in Fig. 2) [69–74]. The sensor has a detection limit of 3 nM for Pb^{2+}, which is much lower than the Environmental Protection Agency of United States (EPA) limit for lead ions in drinking water [69,70,72]. In particular, the dynamic range of Lu's sensor can be tuned simply by adjusting

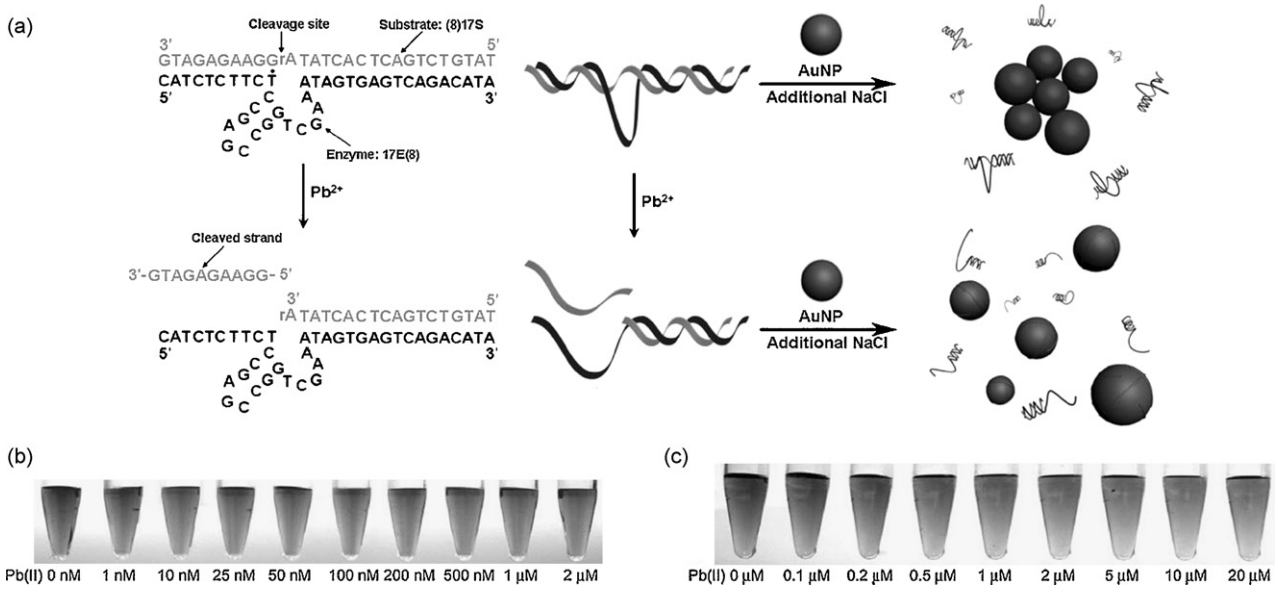

Fig. 2. (a) Left: secondary structure of the DNAzyme complex, which consists of an enzymestrand (17E(8)) and a substrate strand ((8)17S). Right: schematic of the label-free colorimetric sensor. Color change of the AuNP solution with different concentrations of lead in the solution at pH 7.2 (b) and 5.5 (c); the dynamic range of the assay is 3 nM to 1 μM at pH 7.2 and 120 nM to 20 μM at pH 5.5, respectively. Copyright Wiley-VCH Verlag GmbH & Co. KGaA and reproduced with permission [71].

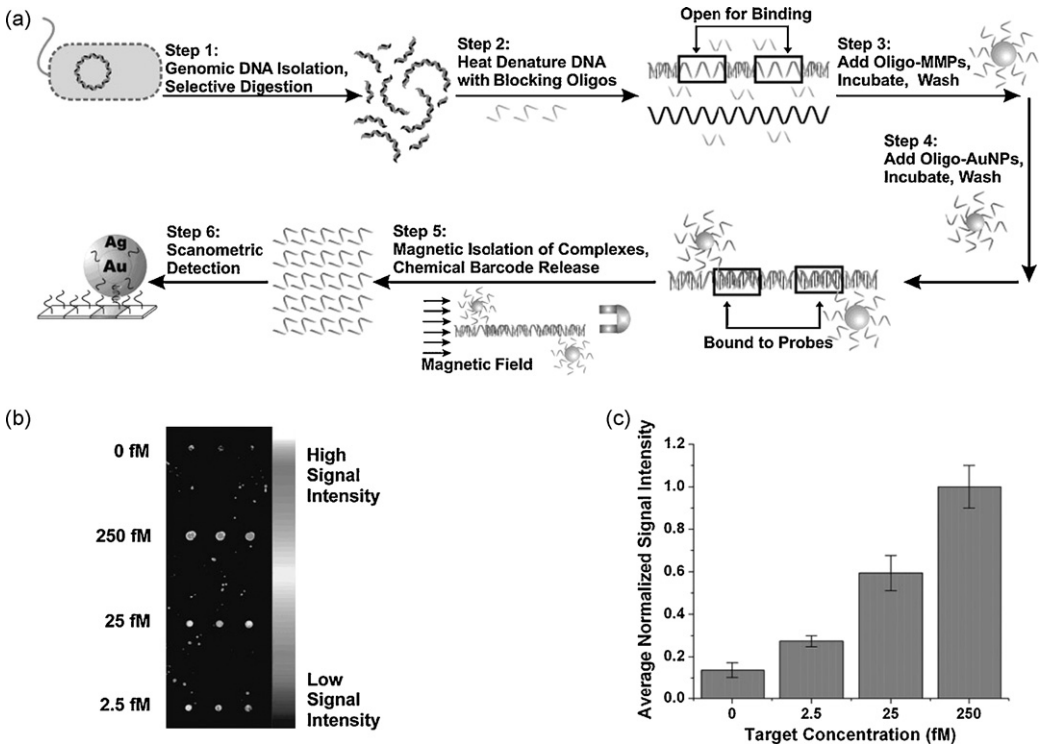

Fig. 3. (a) The schematic representation of Genomic Bio Bar Code Assay. For details of the assay, readers are advised to see Ref. [78]. (b) Representative slide from a single assay showing that 2.5 fM is distinguishable from the 0 fM (no target) sample. (c) The data shown above are the average of 5 independent runs of the genomic DNA bio bar code assay. Copyright ACS and reproduced with permission [78].

the pH [70,71]. Willner and co-workers have developed a method for colorimetric detection of Hg^{2+} ions by a DNA based machine [68]. This method reveals a substantial enhancement in the sensitivity (1 nm, 0.2 ppb) over the reported methods, and comparable sensitivity to the reported DNAzyme method. Based on the Hg^{2+}-mediated formation of T-Hg^{2+}-T base pairs, a highly sensitive and selective Hg^{2+} detection assay has also been developed by Liu's group and Mirkin's group [75,76]. This is based on Hg^{2+} induced thymine–thymine (T–T) mismatches in DNA modified AuNPs to form particle aggregates at room temperature with a concomitant colorimetric response This method is enzyme free and does not require specialized equipment other than a temperature control unit. The concentration of Hg^{2+} can be determined by the change of the solution color at a given temperature or the melting temper-

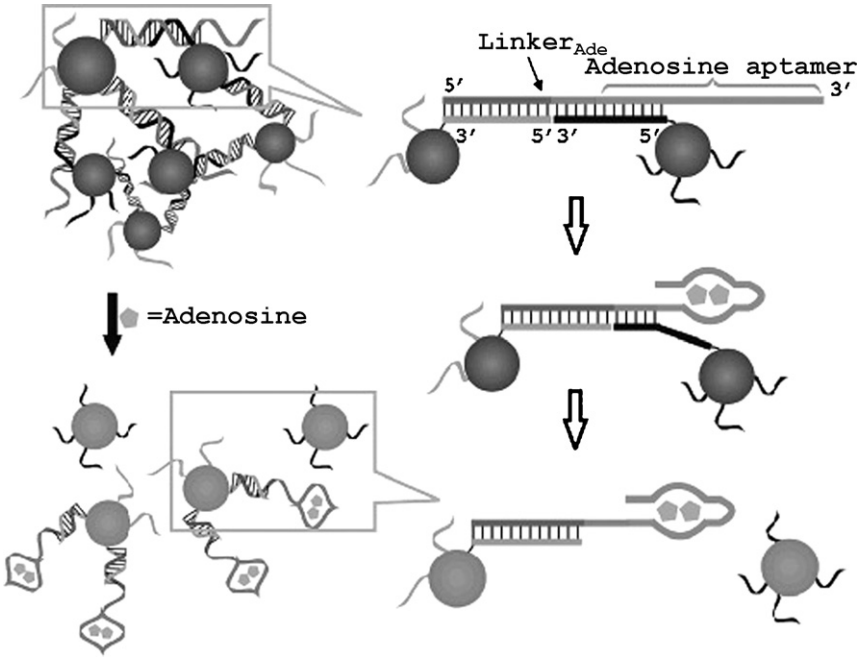

Fig. 4. Schematics of adenosine-induced disassembly of AuNPs linked by a DNA containing an adenosine aptamer. Each aptamer binds two adenosine molecules. Copyright ACS and reproduced with permission [50].

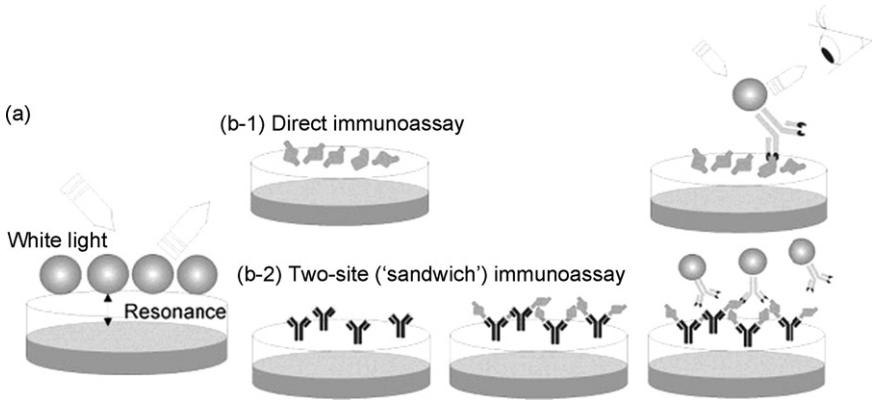

Fig. 5. (a) Schematic illustration of the resonance-enhanced absorption (REA) setup. The nanoclusters are deposited above a highly reflecting mirror surface. Interlayer distance-dependent light that is reflected by the mirror is in phase with the incident field. Thus, the REA setup acts as a nanointerference filter. (b) Schematic representation of the two immunoassay formats. (b-1) Direct immunoassay. Antigen is immobilized onto the surface of the optically transparent distance layer of the immunochips and screened by AuNP-labeled antibodies. (b-2) Two-site (sandwich) assay. The chips are precoated with the first antibody. The antigen is then captured by the antibody and detected with a second AuNP-labeled readout antibody. Copyright ACS and reproduced with permission [100].

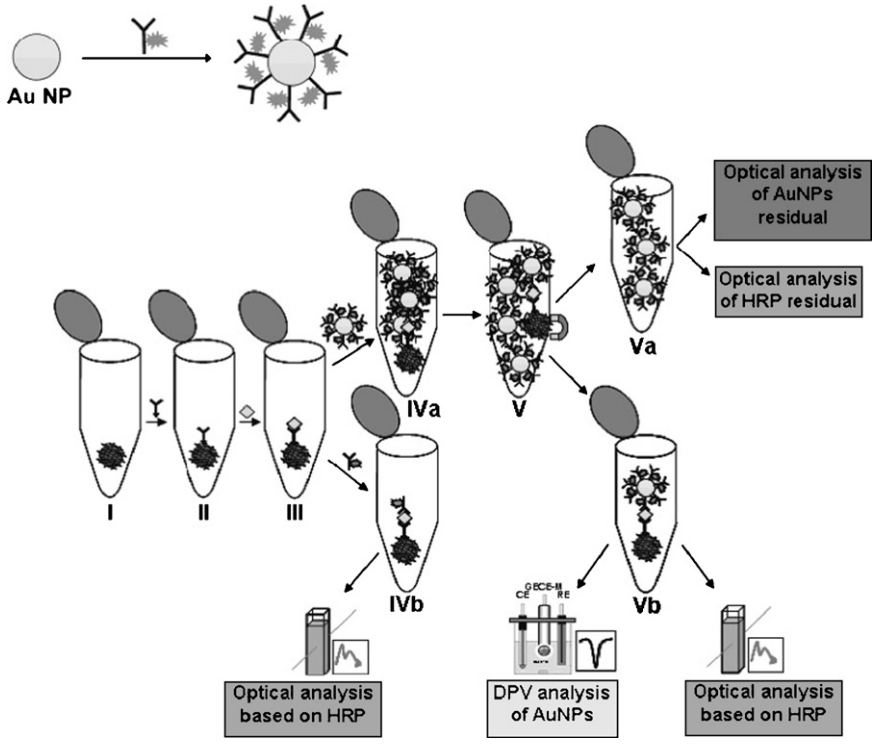

Fig. 6. Schematic (not in scale) of (upper part) preparation of double-codified label using AuNPs (13 nm) and anti-human IgG peroxidase-conjugated antibody (anti-human-HRP) and (lower part) general assay procedure and characterizations. Copyright ACS and reproduced with permission [101].

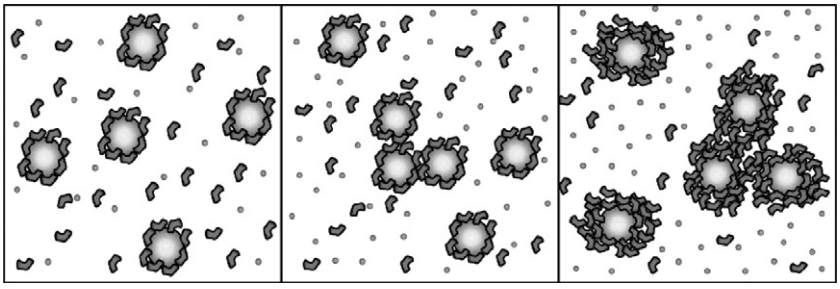

Fig. 7. Schematic diagram of the protein/gold conjugates at solution conditions corresponding to protein interactions that are repulsive or very weakly attractive (left), weakly attractive (center), and strongly attractive (right). Copyright ACS and reproduced with permission [106].

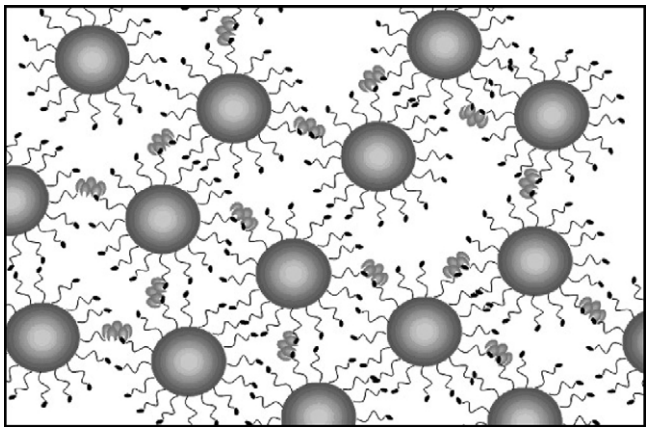

Fig. 8. Schematic representation of the cholera toxin induced aggregation of lactose-stabilized AuNPs showing the relative scale of the toxin with respect to the gold nanoparticles. Copyright ACS and reproduced with permission [27].

ature (T_m) of the DNA–AuNP aggregates. Significantly, this method can, in principle, be used to detect other metal ions by substituting the thymidine in study with synthetic artificial bases that selectively bind other metal ions.

To overcome some drawbacks (e.g., high cost of tags (DNAs or fluorescent dyes) and real life of the sensors) of colorimetric assays, very recently, Darbha and co-workers demonstrate that second-order non-linear optical (NLO) properties of AuNPs can be used for screening mercury from environmental samples without any DNA or fluorescent tag, with excellent sensitivity (5 ppb) and selectivity [77].

3.2. DNA detection

The use of AuNP-based colorimetric methods for detection of DNA has been widely reported and reviewed [1,5,6,8]. Herein, we summarize the new approaches and techniques of AuNP based-DNA assay [78–83]. Combined a bio bar code with microfluidic chip-based format, Mirkin and co-workers have developed a new version of the bio bar code assay that utilizes blocking strands to inhibit target rehybridization and allows one to detect double stranded genomic DNA (named as Genomic Bio Bar Code Assay, as shown in Fig. 3) [78]. This work paves the way for the transition of the bio bar code assay from a laboratory technique to one that can be deployed in the field for the rapid and accurate detection of biological terrorism agents.

Others detection techniques have also been applied in the AuNPs-based DNA assay for improving detection sensitivity and selectivity [79–82]. A one-step homogeneous DNA detection method with high sensitivity was developed using AuNPs coupled with dynamic light scattering (DLS) measurement by Dai et al. [79]. This DLS-based assay is extremely easy to conduct and requires no additional separation and amplification steps. The detection limit is around 1 pM, which is 4 orders of magnitude better than that of light-absorption-based methods. Single base pair mismatched DNAs can be readily discriminated from perfectly matched target

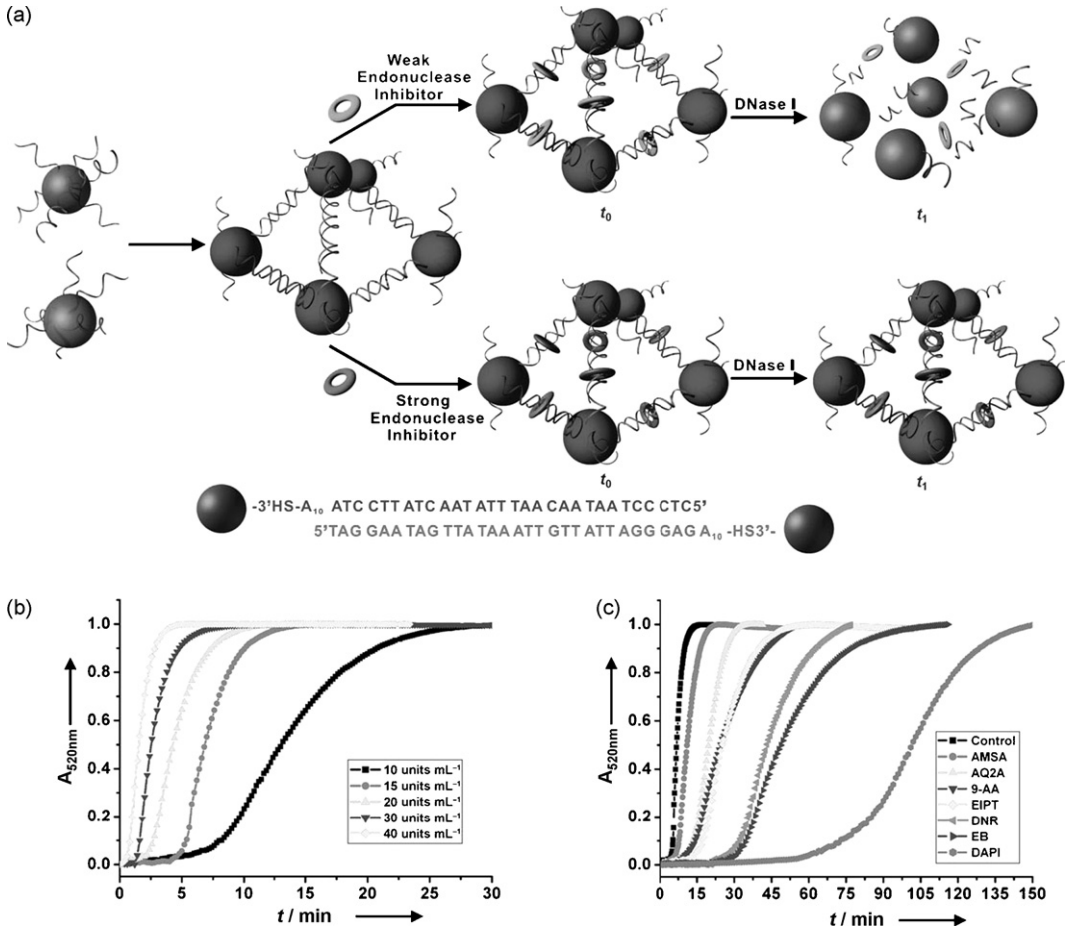

Fig. 9. (a) Illustration of the aggregation and dissociation of the DNA–AuNP probes used in the colorimetric screening of endonuclease inhibitors. (b) and (c) In the absence and presence of endonuclease inhibitors (1 mm) at a DNase I concentration of 15 units/mL. The change in the absorbance at 520 nm in the UV/Vis spectrum was monitored. Copyright Wiley-VCH Verlag GmbH & Co. KGaA and reproduced with permission [134].

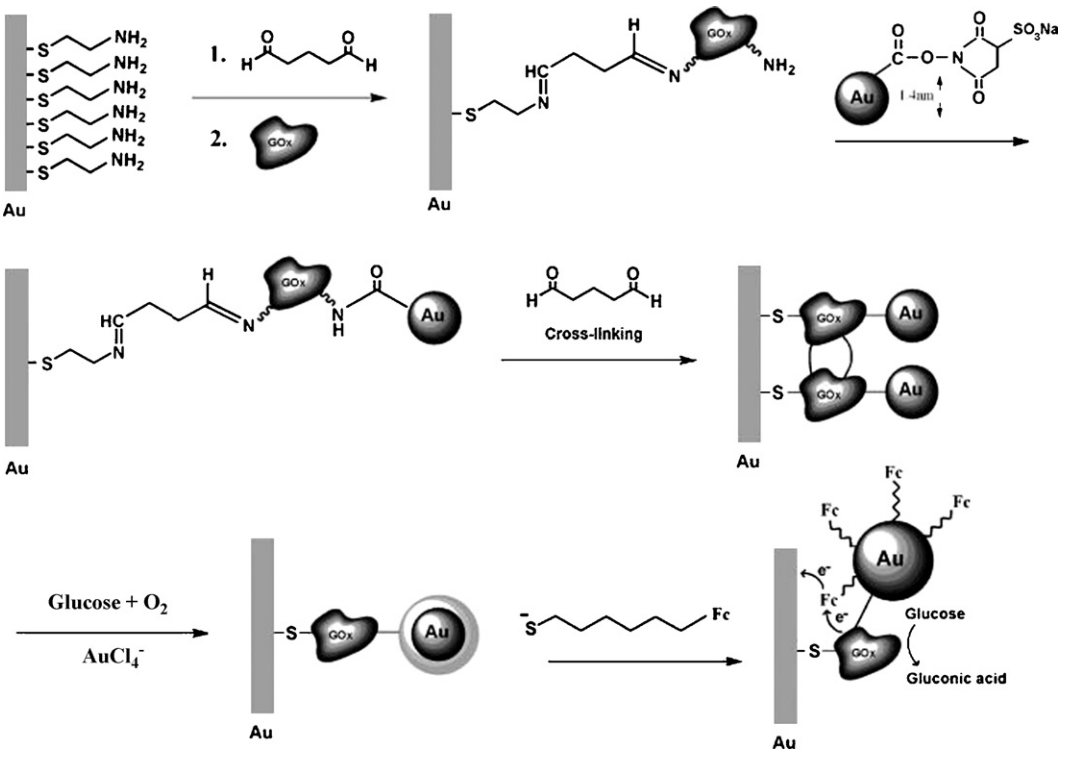

Fig. 10. Preparation of an electrically contacted AuNPs–GOx composite electrode and its enlargement by the GOx-catalyzed reduction of $HAuCl_4$. Copyright Wiley-VCH Verlag GmbH & Co. KGaA and reproduced with permission [145].

DNAs using this assay. Fan's group reported a protocol for the amplified detection of target DNA by using a chronocoulometric DNA sensor (CDS) which is based on a 'sandwich' detection strategy, involving a capture probe DNA immobilized on a gold electrode and a reporter probe DNA loaded on AuNPs [80]. Each probe flanks one of two fragments of the target sequence. A single DNA hybridization event brings AuNPs, along with hundreds of reporter probes, in the proximity of the electrode. They then employ chronocoulometry to interrogate $[Ru(NH_3)_6]^{3+}$ electrostatically bound to the captured DNA strands. Dong and co-workers have carefully demonstrated a significant sensitivity enhancement in electrical detection of DNA hybridization in single-walled carbon nanotube (SWNT) networks (SNFETs) via the introduction of reporter DNA–AuNP conjugates in the hybridization step. With detection limits in the femtomolar range, SNFET-based biosensors and immunosensors may be adapted to the detection of a variety of biomarkers for applications ranging from fM diagnostics to in vitro diagnostics [82].

The oligonucleotide-mediated AuNP aggregation process has been extensively used for the colorimetric screening of DNA binders and triplex DNA binders [83,84]. Furthermore, attractive sensors based on aptamer conjugated AuNPs have been developed for the detection of a wide range of targets with high affinity and specificity [48–53,85–89]. An example is adenosine sensing, using an adenosine specific aptamer (as shown in Fig. 4). The system contained two kinds of DNA-functionalized AuNPs and a linker DNA. The linker DNA contained an adenosine aptamer fragment and an extension. In the presence of adenosine the nanoparticles are deaggregated, with a concomitant purple-to-red color change [50].

3.3. Protein analysis

AuNPs conjugated to antibodies have been widely used in the field of light and electron microscopy, for visualizing proteins in biological samples [90,91]. The use of AuNPs for protein analysis/detection is also a very interesting research field. In the past decade, AuNP/protein conjugates have found increasing application as bioanalytical, diagnostic and/or immunohistochemical probes [3,5,6,92–100]. AuNP-based colorimetric assays for detection proteins or protein–protein interactions have

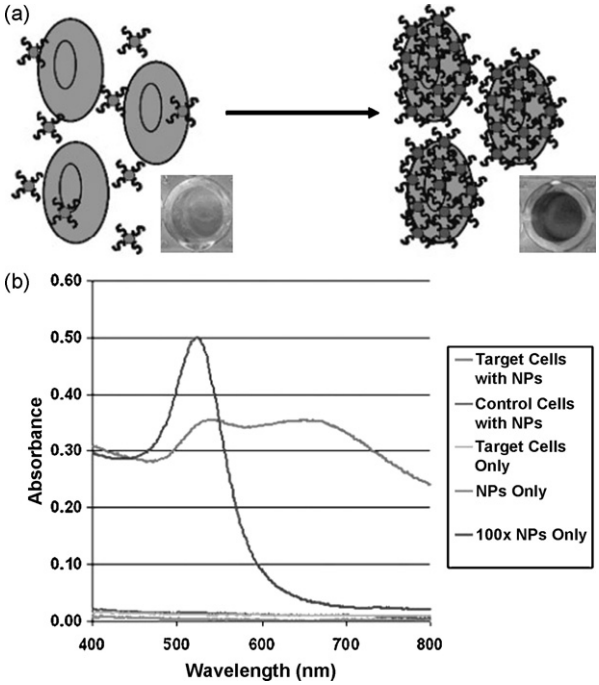

Fig. 11. (a) Schematic representation of the aptamer-conjugated AuNPs based colorimetric assay. (b) Plots depicting the absorption spectra obtained for various samples analyzed using aptamer-conjugated AuNPs. The spectra illustrate the differences in spectroscopic characteristics observed after the aptamer-conjugated AuNPs bind to the target cells. Copyright ACS and reproduced with permission [162].

been documented elsewhere [35,6,94,96,97,99]. Silver-enhanced nanoparticle-labeled immunoassays provide a simple, low-cost, and effective way of detecting antigens in dilute solutions [1,2,8]. Using an adsorption-controlled kinetic model, the approach has been optimized by Gupta et al. [99] who found that the performance of immunoassays by correlating the opacities of silver spots is dependent on the concentrations and incubation times of antigens and AuNPs. The results could allow the development of more rapid and reliable nanoparticle immunoassays.

An optical resonance-enhanced absorption-based near-field immunochip biosensor for allergen detection was developed by Maier et al. (as shown in Fig. 5) [100]. Using this approach, a novel, simple, and rapid colorimetric solid-phase immunoassay on a planar chip substrate was realized in direct and sandwich assay formats, with a detection system that does not require any instrumentation for readout. In particular, semiquantitative immunochemical responses are directly visible to the naked eye of the analyst.

The combination of optical and electrochemical properties of AuNPs with different detection techniques has been demonstrated in protein determination [101–106]. Ambrosi and co-workers synthesized a novel double-codified nanolabel (DC-AuNP) based on an AuNP modified with an anti-human IgG peroxidase (HRP)-conjugated antibody that allows enhanced spectrophotometric and electrochemical detection of antigen human IgG as a model protein (shown in Fig. 6) [101]. The detection limits for this novel double-codified nanoparticle-based assay were much lower than those typically achieved by ELISA tests. Using the same detection principle, Zhu's group, also developed AuNPs/carbon nanotube (CNT) hybrid platforms with horseradish peroxidase (HRP)-functionalized gold nanoparticle labels for the sensitive detection of human IgG (HIgG) as a model protein [102].

Tessier and co-workers developed a self-interaction nanoparticle spectroscopy which involves adsorbing proteins on the surface of AuNPs and adding the protein/gold conjugates to solutions of interest for crystallization (as shown in Fig. 7) [106]. Given the remarkable efficiency of this method, it holds significant potential to aid in the crystallization of proteins that have not been crystallized previously. Moreover, this method may find utility in the analysis of weak homo and heterotypic interactions involved in other biological applications, including preventing protein aggregation and formulating therapeutic proteins.

Since AuNPs provide the key effect for observing enhanced Raman signals for molecules attached to them, a sensitive spectroscopic assay based on surface-enhanced Raman spectroscopy (SERS) using AuNPs as substrates has also been developed for the rapid detection protein–protein interactions [107–109]. Manimaran and co-workers reported the synthesis of 2–5 nm size AuNPs labels for surface-enhanced Raman Spectroscopy (SERS) based immunoassay to detect protein molecules [107]. The AuNPs were conjugated with fluorescein isothiocyanate (FITC) and goat anti-h-IgG (immunoglobin) and the resultant particles were used for the detection of h-IgG in concentration ranging from 1 to 100 ng/μl. A microarray approach based on surface-enhanced Raman spec-

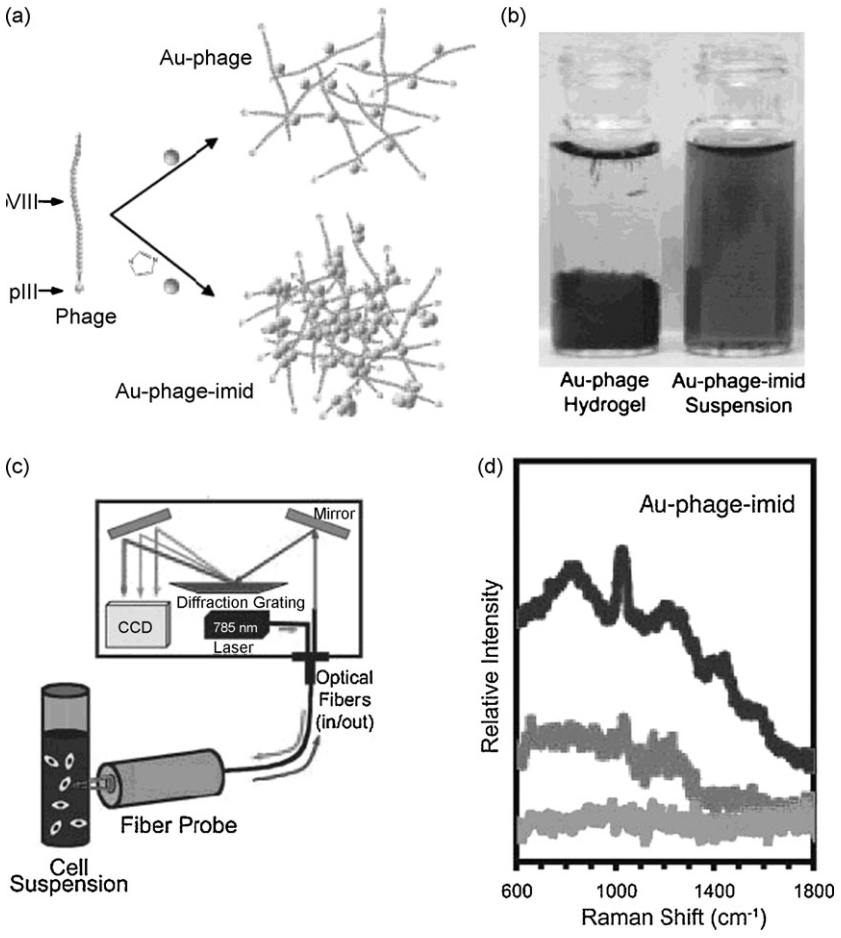

Fig. 12. Targeted cell detection by using SERS. (a) Strategy for Au assembly onto phage nanoparticles. (b) Vials of nanoparticle solutions: Au-phage hydrogel (Left) and suspension of purified Au-phage-imid (Right; suspended from hydrogels precursor). (c) Cell-suspension scheme for SERS detection. (d) SERS and spectra of cells incubated with Au-phage: Au-RGD-4C-imid (black line), Au-fd-tet-imid (gray line), and RGD-4C phage (light gray line; control, no Au) in suspension. See Ref. [165] for details. Copyright (2006) National Academy of Sciences, U.S.A. with permission [165].

troscopic (SERS) was developed for detection of protein–antibody interaction by Li et al. [108]. The procedure involves the attachment of peptide-capped AuNPs followed by silver deposition for signal enhancement.

Understanding the molecular basis for carbohydrate–protein interactions not only provides valuable information on biological processes in living organisms but also aids the development of potent biomedical agents [110,111]. Recently, Russell and co-workers found that simple sugars functionalized with a thiol moiety can be used to stabilize a self-assembled monolayer surrounding AuNPs (as shown in Fig. 8) [26,27,112–115]. Such glyconanoparticles have been used as colorimetric probes for detecting carbohydrate binding proteins (lectins), such as concanavalin A and *Ricinus communis* agglutinin [26,112–114].

Another attractive sensor approach for protein detection uses aptamer conjugated AuNPs that can bind target proteins with high affinity and specificity [49,116–119]. For example, Dong and co-workers have developed a kind of colorimetric sensor for detection of a-thrombin based on AuNPs through the interaction of the aptamer to a-thrombin. The aptasensor has high sensitivity and selectivity and is able to detect targets in complex biological samples such as human plasma [116].

3.4. Enzymatic activity assay

Development of highly sensitive assay for determination of enzymatic activities and kinetic parameters is important in the fabrication of novel pharmaceuticals and medical diagnostic devices. Currently, the protein detection methods have largely been dominated by enzyme-linked immunological assay (known as ELISA) which is limited by the requirement of producing high-quality antibodies and is time consuming. For addressing these limitations, scientists have developed numerous novel detection strategies that incorporate the optical and electronic properties of AuNPs [120–135]. Generally, these procedures circumvent the need for radioactive or fluorescent labeling usually required for assaying enzymatic activities. Some enzyme-responsive (protein kinases and proteases) nanoparticle systems have been reviewed recently by Ghadiali and Stevents [12]. The potential application of AuNPs as colorimetric indicators to evaluate enzymatic activity and to screen enzyme inhibitors has been reported by Mirkin and co-workers (shown in Fig. 9) [134]. In principle, this method can be used to screen libraries of inhibitors of endonucleases in a high throughput fashion by using either the naked eye or a simple colorimetric reader.

The biocatalytic growth of metallic NPs represents a further interesting direction in nanobiotechnology and has been applied to develop optical, electrical (conductivity) or electrochemical biosensors for probing enzyme activities and their substrates [136–145]. Different enzymes catalyze the reduction of gold salts to metallic AuNPs, and enzymes can catalyze the deposition of metals on AuNP seeds. In particular, Willner and co-workers have designed several systems using nanoparticle-enzyme hybrids as electrochemical sensors since the biocatalytic growth or enlargement of AuNPs onto electrodes drastically enhances the conductivity and electron transfer from the redox analytes [138–145]. For example, GOx oxidizes glucose to gluconic acid with the concomitant formation of H_2O_2. The latter product acts as a reducing agent which

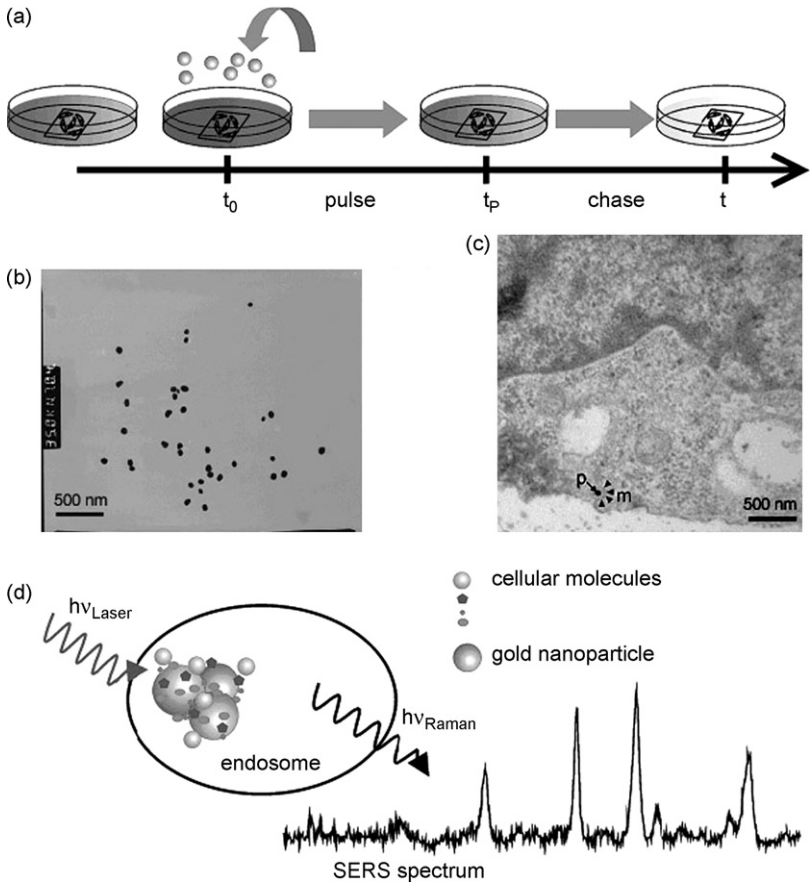

Fig. 13. Schematic of the Kneipp's SERS assay. (a) AuNPs were applied with the cell culture medium at time t_0 as a pulse of duration t_P, followed by incubation in medium without nanoparticles for different times t, at which the Raman experiments were carried out in PBS buffer. (b) TEM graph of AuNPs immersed in cell culture medium. (c) TEM graph showing the endocytotic uptake of an individual gold nanoparticle by an IRPT cell. (d) The SERS measurements inside the endosomal compartment. Copyright ACS and reproduced with permission [170].

reduces $AuCl_4^-$ and deposits metal on the AuNP seeds, which act as catalysts for the metallization process [145] (Fig. 10). As the concentration of H_2O_2 is controlled by the concentration of glucose, the extent of the enlargement of the particles is determined by the concentration of the substrate. The growth of AuNPs and the optical/electrochemical monitoring of the biocatalytic transformations was also extended to other enzymes, such as alcohol dehydrogenase (NAD(P)+-dependent enzyme) [138,141], acetylcholine esterase [140] and tyrosinase [142].

3.5. Cellular analysis

The ease of synthesis and functionalization, unique optical properties, and biocompatibility of gold nanoparticles (AuNPs) are advantages in intracellular diagnostic applications [146–164]. These properties have recently sparked great interest in gold nanoparticles as a scaffold for cell-targeting studies [15,156–164]. Early and accurate detection of cancer often requires time-consuming techniques and expensive instrumentation. To address these limitations, Tan's group developed a colorimetric assay for the direct detection of diseased cells (as shown in Fig. 11) [162]. The assay uses aptamer-conjugated AuNPs to combine the selectivity and affinity of aptamers and the spectroscopic advantages of AuNPs to allow for the sensitive detection of cancer cells. Samples with the target cells present exhibited a distinct color change while non-target samples did not elicit any change in color. The assay also showed excellent sensitivity with both the naked eye and based on absorbance measurements. In addition, the assay was able to differentiate between different types of target and control cells based on the aptamer used in the assay indicating the wide applicability of the assay for diseased cell detection. On the basis of these qualities, aptamer-conjugated AuNPs could become a powerful tool for point of care diagnostics.

Verma and co-workers compare membrane penetration by two nanoparticle 'isomers' with similar composition (same hydrophobic content), one coated with subnanometre striations of alternating anionic and hydrophobic groups, and the other coated with the same moieties but in a random distribution [163]. The results show that the former particles penetrate the plasma membrane without bilayer disruption, whereas the latter are mostly trapped in endosomes. Their results offer a paradigm for analysing the fundamental problem of cell-membrane-penetrating bio- and macro-molecules.

Some studies also employed gold structure as advanced SERS optical sensors for cellular analysis. The advantages of gold nanoparticles over other SERS substrates with respect to mobility, versatility, and biocompatibility will be useful for the development of SERS nanosensors for the probing of a variety of cellular compartments [165–174]. The cellular SERS signature contains information on the molecular composition of the cellular substructure and also other chemical properties, such as the local pH. In particular, SERS nanosensors enable the chemical characterization of the nanometer vicinity of the gold nanoparticles and the measurement of vibrational spectra at a sensitivity and lateral resolution unachieved so far in other experiments. Souza and co-workers generated Au-phage networks that, in addition to targeting cells, can function as signal reporters for fluorescence and dark-field microscopy and near-infrared (NIR) surface-enhanced Raman scattering (SERS) spectroscopy (as shown in Fig. 12) [165]. These networks can effectively integrate the unique signal reporting properties of AuNPs while preserving the biological properties of phage. Kneipp and co-workers have measured SERS spectra from endosomes in living individual epithelial and macrophage cells and investigated the properties of AuNPs in the cells (as shown in Fig. 13) [169–171]. This was achieved by using the SERS spectroscopic information itself and by complementary TEM studies. Differences in the SERS spectra obtained from the molecules in the enhanced local fields of the nanostructures in different cell lines and over time, as well as the direct identification of physiologically relevant molecules, demonstrate that SERS approaches are feasible for the characterization of changing cellular environments and useful for intracellular applications.

4. Conclusion and outlook

AuNPs are excellent platform for a diverse array of developing analytical methods and they have already been used for a wide range of applications both in chemical and biological research. The surface and core properties of these systems can be engineered for individual and multifold applications, including molecular recognition, chemical sensing and imaging. However, there are a number of critical issues that require addressing, including acute reproducible and reliable manufacturing methods/assays and long-term health effects of nanomaterials as well as scalability. We envision, in the near future, that increased collaboration between scientists from different disciplines will yield new fundamental insights into the AuNP-based nanotechnology.

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