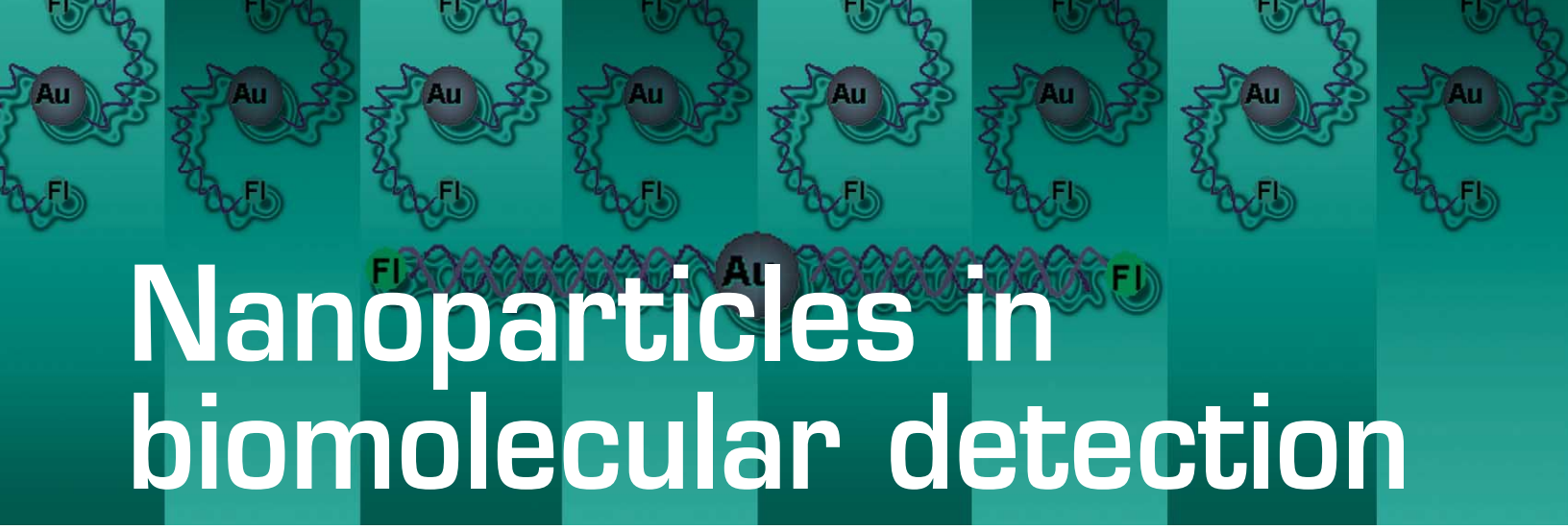

Nanoparticles in biomolecular detection

The use of nanoparticles as labels in biomolecular detection in place of conventional molecular fluorophores has led to improvements in sensitivity, selectivity, and multiplexing capacity. Nevertheless, further simplification is needed to take these technologies from the laboratory to point of care. Here, we summarize the latest developments in the use of nanoparticles as labels, especially in bioaffinity sensors for the detection of nucleic acids and proteins.

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Over the past decade, many important technological advances have been made in the use of nanotechnology for biomolecular detection[1,2]. Coupled with the development of optical, electrochemical, and various other techniques for monitoring biorecognition events on solid devices and in solution, a lot of effort has been put into realizing accurate, sensitive, selective, and practical biosensing devices for both laboratory and point-of-care applications.

Currently, DNA assays rely on a combination of amplification by polymerase chain reaction (PCR) and detection using molecular fluorophores as labels. The typical detection limit is in the picomolar range. Arrays containing thousands of unique probe sequences have also been constructed. However, several major drawbacks still remain. Broad absorption and emission bands and nonuniform rates of fluorophore photobleaching may reduce accuracy. Sophisticated algorithms and expensive instrumentation are needed for fluorescence readout, restricting application to laboratories. Furthermore, the selective and nonlinear target amplification in PCR may distort gene expression.

Proteins are generally detected using immunoassays. Enzyme-linked immunosorbent assay (ELISA) remains the gold standard in protein detection, with detection limits in the picomolar range. Again, the drawbacks of using molecular fluorophores apply. Because of the low abundance of protein and the limited number of procedures for protein amplification, sensitivity needs to be improved.

Nanoparticles, in particular, have been used extensively in bioaffinity sensors for nucleic acids and proteins[3,4]. These particles are unique because their nanometer size gives rise to a high reactivity and beneficial physical properties (electrical, electrochemical, optical, and magnetic) that are chemically tailorable. Their usage can potentially translate into new assays that improve on current methods of DNA and protein detection, as will be discussed in this article. Nanofabrication and other nanostructures such as nanowires and nanotubes are beyond the scope of this review.

Au nanoparticles

Optical detection

In a milestone discovery in 1996, Mirkin *et al.*[5] reported unique optical and melting properties for an aggregate of Au nanoparticles and oligonucleotides. Specific interactions between oligonucleotides brought about the assembly of the 13 nm Au nanoparticles to which they were attached. A color change was observed, caused by the aggregate

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scattering properties and the interaction between particle surface plasmons as the distance between Au nanoparticles varied. This distance-dependent optical property has seen the use of Au nanoparticles in a plethora of biomolecular detection methods, started off by colorimetric systems[6,7].

In DNA detection, Au nanoparticles are modified with oligonucleotide detection probes and introduced into a solution of the single-stranded target oligonucleotide. A polymeric network of nanoparticles is formed and a color change can be detected visually. Spotting the test solution on a white support enhances the color contrast and provides a permanent record of the test, with a detection limit of 10 nM. Subsequent reports have confirmed that the nanoparticle-oligonucleotide aggregates also exhibit an unusually sharp melting transition[6,8,9]. Complementary target sequences can thus be better distinguished from one-base-mismatched sequences through a thermal stringency wash. The level of selectivity allows one-pot colorimetric detection of a target sequence in a complex mixture[7].

The sensitivity of colorimetric detection is improved by catalytic deposition of Ag on Au nanoparticle labels. Scanometric array detection, illustrated in Fig. 1, has been achieved using a flatbed scanner to detect the Ag-enhanced labels with a detection limit of 50 fM[10], 100 times more sensitive than the fluorophore-based system.

An extension of scanometric detection – bio-barcode amplification – was reported recently[11]. This involves magnetic microparticles with capture probes and Au nanoparticles with both reporter probes and numerous barcode oligonucleotides. The presence of target molecules that match both capture- and reporter-probes results in the formation of a magnetic microparticle-Au nanoparticle sandwich that is subsequently isolated from the system. The barcode oligonucleotides are then dissolved and detected using scanometric detection with Ag amplification, from which a detection limit as low as 500 zM has been obtained. This method is applicable to both DNA and proteins.

Au and Ag nanoparticles also have very high light-scattering power, as first reported by Yguerabide *et al.*[12]. These particles, also called plasmon-resonant particles, are quench resistant and generate very high signal intensities (a 60 nm Au particle is equivalent to 3.3 x 10^5 fluorescein molecules). Attaching biomolecules, such as antibodies and DNA, to these nanoparticles does not affect their optical properties. The nanoparticles have thus been used successfully as labels in nucleic acid[13] and protein detection[14]. More recently, colorimetric detection based on evanescent-wave-induced light scattering has been developed[15,16]. The 50 nm Au nanoparticle labels on the detection probes scatter green light, but switch to red upon hybridization with target nucleic acids. Combining this technique with waveguide detection, 333 fM of oligonucleotides and 33 fM of genomic DNA have been detected[15].

The combination of Au nanoparticles and Raman spectroscopy is attractive because of: (1) the greatly amplified Raman scattering upon adsorption of Raman dyes on metallic nanoparticles (surface-enhanced Raman scattering, or SERS); and (2) the narrow spectral characteristics of Raman dyes, which allow multiplexed detection. Raman dyes and detection probes are first attached to Au nanoparticles and hybridized with the captured targets. An Ag coating is then catalytically deposited on the Au nanoparticles to promote SERS of the Raman dyes, and the amplified signal is captured by spectroscopy. To prevent particle agglomeration and protect the Raman dyes, Mulvaney *et al.*[17] deposited a glass shell on the nanoparticles. This method has been used in the multiplexed detection of nucleic acids[18,19] and proteins[17], with an unoptimized detection limit of 20 fM for nucleic acids[18]. Compared with colorimetric and scanometric detection, this method offers more multiplexing capabilities afforded by the narrow-band spectroscopic fingerprint of the Raman dyes. The absence of photobleaching is an advantage compared with fluorophore labeling.

In 1997, Kubitschko *et al.*[20] proposed the use of 85 nm latex nanoparticles as mass labels to enhance the refractometric signal in an immunoassay. Building on a similar principle, surface plasmon resonance (SPR) measurement was recently adopted alongside Au nanoparticle labeling. The nanoparticles bring about an amplified shift in angle because

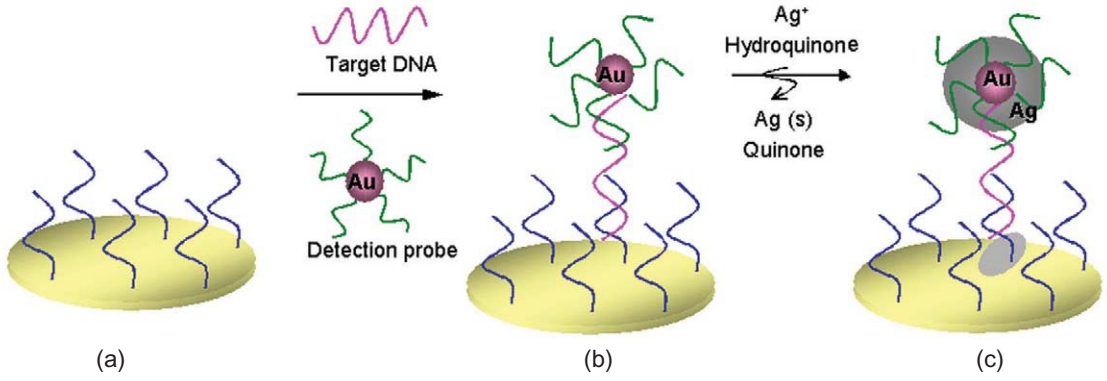

Fig. 1 Scanometric DNA assay[10]. (a) Immobilization of capture probes on electrode. (b) Hybridization with target DNA and labeled detection probe. (c) Amplification by reductive deposition of Ag followed by scanometric detection. (Reprinted with permission from[10]. © 2000 AAAS.)

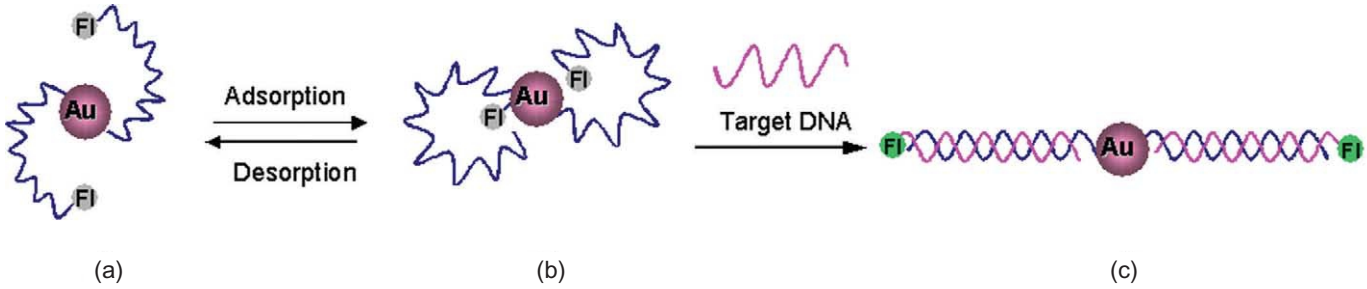

Fig. 2 Optical detection with Au nanoparticles as fluorescent quenchers[27]. (a, b) Owing to the conformation, the fluorescence labels are in close proximity to the Au nanoparticles and their signals are quenched. (c) Upon hybridization, the rigid double-helical DNA molecules 'open up' their conformation and fluorescence is restored. (Reprinted with permission from[27]. © 2004 American Chemical Society.)

they: (1) have a high dielectric constant; (2) cause electromagnetic coupling with the SPR Au film; and (3) increase the surface mass. This results in more sensitive detection. SPR biosensing using Au nanoparticle enhancement has been reported for the detection of nucleic acids[21] as well as proteins[22-25]. Detection limits for 24 mer oligonucleotides[21] and human immunoglobulin G[22] were in the picomolar range. Earlier this year, Aslan *et al.*[26] reported a real-time bioaffinity monitoring system based on an angular-ratiometric approach to plasmon-resonance light scattering. As nanoparticles grow through bioaffinity reactions, they deviate from Rayleigh theory and scatter more light in a forward direction relative to the incident geometry. By comparing the scattered light intensity at 90° and 140° relative to the incident light, the authors managed to monitor the formation of aggregates of biotinylated 20 nm Au nanoparticles as they are crosslinked by the addition of streptavidin.

Au nanoparticles can also function both as a scaffold and fluorescence quencher for the homogenous detection of nucleic acids[27]. Fluorophore-tagged oligonucleotides are attached to the nanoparticles, forming arch-like conformations that cause quenching of fluorophores on the particle surface. The binding of target molecules results in a conformational change that restores the fluorescence signal, as depicted in Fig. 2.

Electrical detection

Direct electrical detection is one of the simplest methods for bioaffinity sensing[28]. In this scheme (Fig. 3), capture probes are immobilized in micron-sized gaps between electrodes in a DNA array. Hybridization with target DNA and Au nanoparticle-labeled detection probes localizes the nanoparticles in the gap, while subsequent Ag deposition creates a 'bridge' across the gap. The detection of a conductivity change results in a detection limit of 500 fM. A mutation selectivity ratio of ~100 000:1 is also achieved by exploiting the unique salt-concentration-dependent hybridization of the labeled

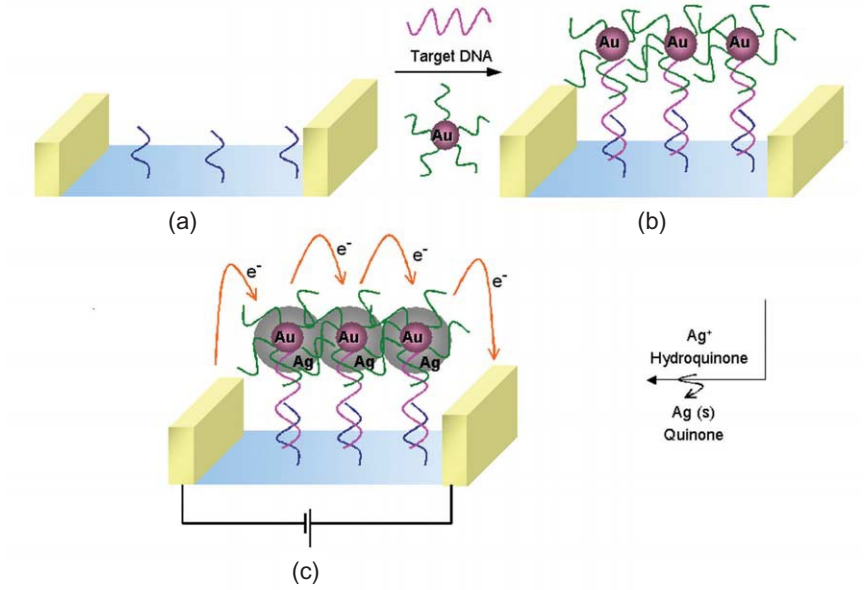

Fig. 3 Electrical detection of DNA hybridization using Au nanoparticle labels[28]. (a) Immobilization of capture probes in the gap between two electrodes. (b) Hybridization with target DNA and Au nanoparticle-labeled detection probe. (c) Reductive deposition of Ag, creating a bridge that decreases resistance. (Reprinted with permission from[28]. © 2002 AAAS.)

probes. This is much higher than the selectivity level in the fluorescence-based approach (2.6:1) and the previously described scanometric approach (11:1). The feasibility of detecting single nucleotide polymorphisms (SNPs) using this method has been confirmed by Burmeister *et al.*[29]. The same group has devised a method for continuously monitoring the autometallographic enhancement process, eliminating the need for multistep enhancement and all the washing, drying, and measurement cycles in between[30].

A further improvement in the electrical detection method has been reported for immunoassays. By reducing the size of the electrode gap to below 100 nm, a conductivity change can be detected even without Ag enhancement[31].

Electrochemical detection

The redox properties of Au nanoparticles have led to their widespread use as electrochemical labels in nucleic acid detection, with numerous configurations being explored. Ozsos *et al.*[32] immobilized target DNA on an electrode, followed by hybridization with complementary probes labeled with Au nanoparticles. The hybrid displayed a Au oxide wave at around 1.20 V (Fig. 4).

Initially, this method was not sensitive enough, such that PCR amplification was still needed. The detection limit for the PCR amplicons of Factor V Leiden mutation was 0.78 fM. Signal amplification can again be achieved by Ag enhancement[33]. Au-catalyzed deposition of Ag on the nanoparticle labels brings a large number of Ag atoms closer to the electrode. A detection limit of 50 pM and selectivity against one-base mismatches were achieved.

In another configuration, Au nanoparticle labels are attached to a single-stranded-DNA binding protein[34]. Hybridization of capture probes with matching targets hinders the binding of labeled proteins, as indicated by the decrease in the Au redox signal. A detection limit of 2.17 pM was achieved.

Kerman *et al.*[35] reported a strategy to not only detect the presence of an SNP but also *identify* the bases involved using monobase nucleotides labeled with Au nanoparticles. If the SNP contains a certain base that matches the labeled monobase, Au nanoparticles accumulate on the electrode surface in the presence of DNA polymerase I. This is indicated by a significant increase in the Au oxidation wave. A model study was performed successfully on a synthetic 21 base oligonucleotide target (Fig. 5).

Another signal amplification strategy is to attach electroactive 6-ferrocenylhexanethiol molecules onto the Au nanoparticle labels[36,37]. Hybridization brings these compounds into close proximity with the underlying electrode, causing a reversible electron-transfer reaction. With detection limits in the picomolar range, this method is simpler than the Ag-enhancement scheme, but just as sensitive[33]. As a side note, remarkable sensitivity was achieved when a larger number of ferrocene units were packed into a micron-sized polystyrene bead[38].

Electrochemical stripping is a sensitive electrochemical measurement protocol. It was first used alongside Au nanoparticle labels in 2001[39,40]. Hybridization of target oligonucleotides to capture probes is followed by binding of the Au nanoparticle labels, dissolution of the labels, and the potentiometric stripping measurement of the dissolved metal ions on an electrode. Initially, a PCR step was needed, and the detection limit for an amplified 406 base pair human cytomegalovirus DNA fragment was 5 pM of PCR product[41]. Precipitation of Au or Ag onto the Au nanoparticle labels leads to PCR-free detection with amplified signals and lower detection limits[41,42]. In protein detection, *Schistosoma japonicum* antibody[43], human immunoglobulin G[44], and rabbit immunoglobulin G[45] have been detected. An optimized detection limit of 0.25 pg/ml (1.5 pM) and a dynamic range of 1-500 pg/ml have been reported[45]. This is 100 times more sensitive than conventional immunoassays.

A common problem in Ag enhancement is the high background signal as a result of nonspecific precipitation of Ag onto the substrate electrode. This can be avoided by coating the electrode with multilayer films of polyelectrolyte or by using materials with low Ag-enhancing properties, such as indium tin oxide[46]. Various electrode surface

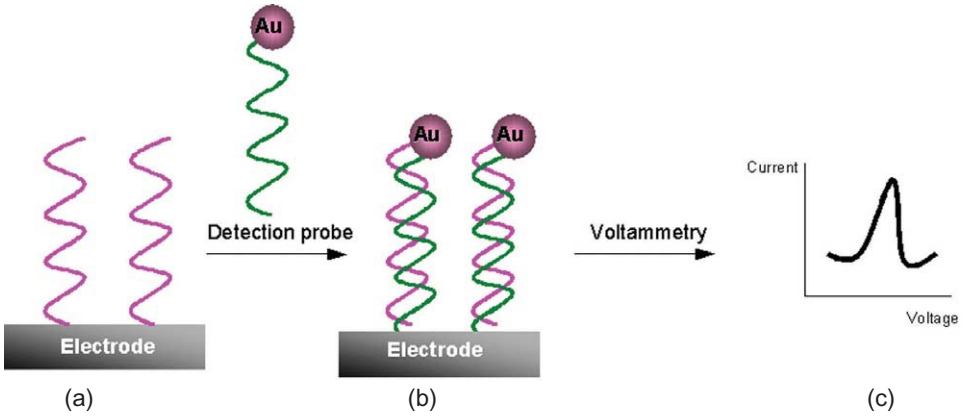

Fig. 4 Electrochemical detection of DNA hybridization using Au nanoparticle labels[32]. (a) Immobilization of target DNA. (b) Hybridization with Au nanoparticle-labeled detection probe. (c) Voltammetric detection of Au redox signal.

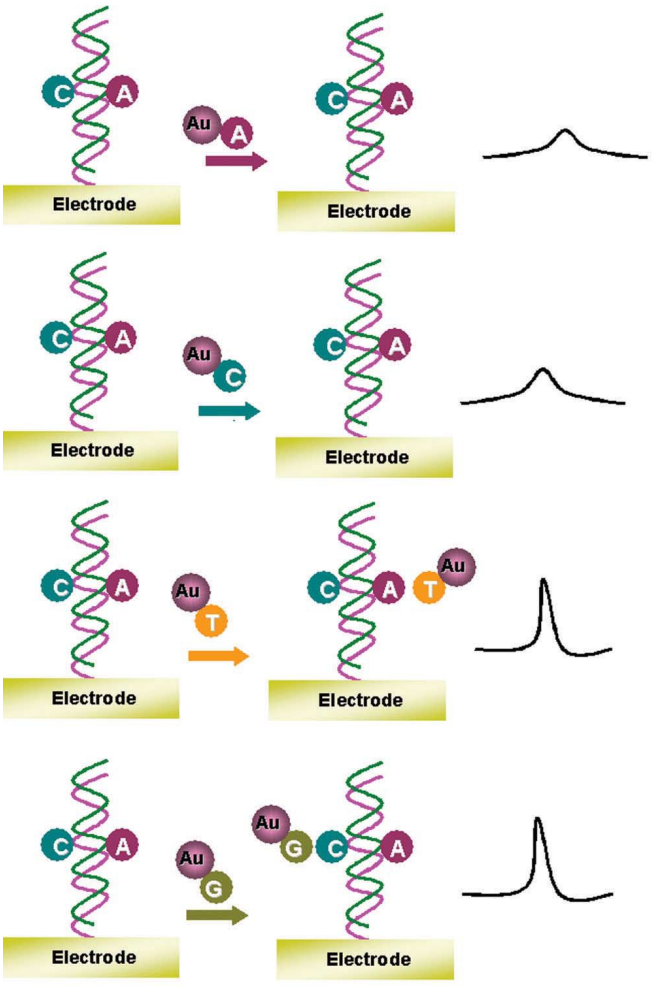

Fig. 5 Electrochemical identification of a single-nucleotide polymorphism[35]. Four Au nanoparticle-labeled monobases are added in separate assays. The comparison of the Au redox signal generated from each assay indicates the identity of the mismatch. (Reprinted with permission from[35]. © 2004 American Chemical Society.)

treatments[47], and electrochemically[48] and enzymatically controlled[49] deposition methods of Ag have been reported, all of which aim to reduce the Ag-related background signal and thus to increase the sensitivity.

Alternatively, signal amplification has been achieved by loading numerous Au nanoparticles on a polymeric bead[50]. The nanoparticles can later be catalytically enlarged, dissolved, and measured by electrochemical stripping. The combined amplification effects allow the detection of DNA targets down to 300 amol. A further reduction in background signal, and hence improvement in sensitivity, has been achieved using magnetic beads for the immobilization of DNA capture probes[39] or capture antibodies[51] (Fig. 6). Upon hybridization, the magnetic beads can be magnetically collected for easy discrimination against unhybridized DNA or nonbinding antibodies. It is worth noting that Willner and coworkers[52] have extensively studied the use of magnetic beads for the control of bioelectrocatalytic processes in DNA and protein detection. The beads in these cases do not function as labels, and thus are outside the scope of this review.

Aside from Au, Ag[53,54] and Cu/Au alloy[55] nanoparticles have also been used as oligonucleotide labeling tags in conjunction with stripping electrochemical detection.

Other detection methods

In addition to optical and electrochemical detection, Au nanoparticle labels have been used in combination with other detection platforms. Scanning electrochemical microscopy[56], based on the increase in conductivity in the assay spot, is fairly sensitive. However, its application may be limited by low sample throughput (a scan of an area of 0.24 cm x 0.24 cm lasts 38 minutes). When used together with a quartz crystal microbalance (QCM), Au nanoparticle labels result in a detection limit of 1 fM[57]. This is more sensitive than any other QCM-based method available to date[58]. In an immunoassay, Au nanoparticle labels were detected through a chemiluminescent reaction[59]. Compared with stripping voltammetry, this method may be more sensitive because of the higher sensitivity of chemiluminescent detection.

Quantum dots

Optical detection

Quantum dots (QDs) were initially used as fluorescent biological labels[60,61]. These semiconductor nanoparticles have narrow, size-tunable, symmetric emission spectra and are photochemically stable. These features, in addition to their binding compatibility with DNA and proteins[60,61], render QDs prime candidates to replace fluorophores as biological labeling agents[62]. In fluorescence-based applications, core-shell structured QDs are more favorable[63-65]. Many core-shell QDs have been prepared by capping an emissive semiconductor core (CdSe, CdTe, etc.) with a thin shell of a higher band gap material (ZnS, CdS, ZnSe, etc.). This increases the photostability of the core and prevents surface quenching of the excitons, hence increasing the quantum yield[66,67].

In nucleic acid assays, QDs take the role of the fluorophore labels. SNP detection of TP53 DNA and multiallele detection for hepatitis B and hepatitis C viruses have been achieved in a microarray format[68]. Detection was performed at room temperature within minutes, with true-to-false signal factors greater than ten. Unique *in situ* detection of chromosome abnormalities or mutations has also been realized using QDs as optical labels[69]. In immunoassays, cancer-cell-marker protein her2[70], diphtheria toxin, and tetanus toxin[71] have been detected successfully using QD-labeled antibodies.

Hybrid inorganic-bioreceptor conjugates made of QD-protein assemblies can also function as chemical sensors. In these assemblies, multiple copies of dye-labeled proteins self-assemble to each QD, enabling fluorescence resonance energy transfer (FRET) between the

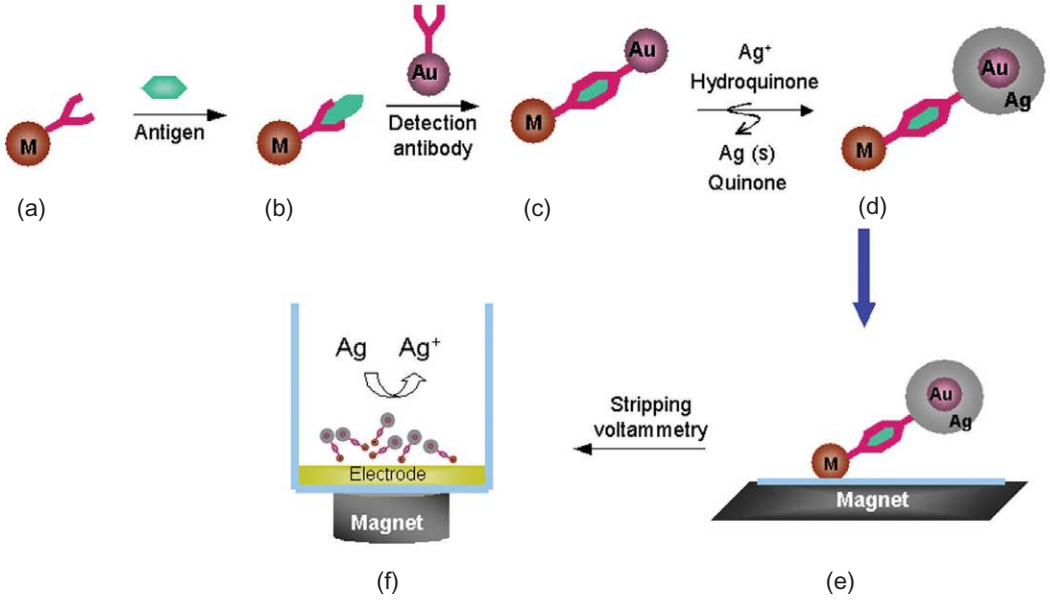

Fig. 6 Electrochemical stripping detection of antigen using magnetic beads and Au nanoparticle labels[51]. (a) Immobilization of capture antibody on a magnetic bead. (b) Binding with target antigen. (c) Recognition by detection antibody. (d) Nanoparticle-catalyzed enlargement by Ag deposition. (e) Collection of magnetic beads and the antibody-Ag complex using an external magnet. (f) Stripping voltammetric detection of the collected Au/Ag nanoparticle label.

QD and the dye molecules. In a scheme proposed by Willard *et al.*[72], biotinylated bovine serum albumin is attached to CdSe-ZnS nanoparticles, followed by specific binding of tetramethylrhodamine-labeled streptavidin. This binding event leads to an enhanced tetramethylrhodamine fluorescence signal caused by FRET. In another scheme, where a dark quencher dye is used at the protein binding site, the addition of analyte displaces the dye and restores the QD emission signal[73]. With two cyanine dyes, a two-step FRET takes place and the presence of analyte is signaled by the wavelength of emission[73]. These schemes are possible because of the size-tunable emission of QDs, such that they resonate with the absorption of the acceptor dyes. For this application, a combination of spectroscopic and crystallographic analyses has been developed to study the orientation of proteins on QD surfaces[74].

DNA hybridization detection using QD labels has recently been achieved using a novel technique combining surface plasmon field enhancement and fluorescence spectroscopy[75]. In this technique, capture probes are immobilized on a metal substrate. Upon binding, the surface plasmon resonance of the metal surface excites and enhances the fluorescence of the QD label on the target DNA, resulting in enhanced sensitivity. By applying this microscopic method in an array format, multiplexed hybridization detection has been achieved. This is facilitated by the broad absorption and sharp emission spectra of QDs, allowing the excitation of multicolored QDs with a single light source. Gerion *et al.*[76] have demonstrated the feasibility of sorting four different QDs into different locations on the basis of complementary DNA (cDNA) interaction. The same group later reported the application of DNA-QD conjugates in a cDNA microarray for SNP and multiallele detection using a commercial scanner and two sets of nanocrystals with orthogonal emission[77].

In another set of experiments, Han *et al.*[78] and Rosenthal[79] have used QDs as identification tags to recognize various DNA sequences in a wavelength-and-intensity multiplexed detection scheme. Differently sized (and thus differently colored) ZnS-capped CdSe nanoparticles were embedded into polymeric microbeads at precisely controlled ratios. Each of these unique microbeads codes for one particular capture probe sequence, while the same fluorophore was used to label the entire target oligonucleotide. Simultaneous reading of coding and target signal reveals the identity of targets that are present in the mixture, as illustrated in Fig. 7. The combination of QDs with ten intensity levels and six colors could theoretically code one million unique nucleic acid sequences or proteins, enabling massively parallel and high-throughput analysis. However, the implementation depends on the uniformity and reproducibility of QD synthesis. It is also important to note that in this protocol, in contrast to the previous protocols, QDs are used for identification rather than signal generation.

Electrochemical detection

The use of QDs for electrochemical monitoring of DNA hybridization was first reported by Wang *et al.*[80] using CdS nanoparticles. Based on an electrochemical stripping protocol analogous to those used with Au nanoparticle labels, this method exploits the attractive stripping behavior of Cd or Pb ions. The nanoparticle-promoted Cd precipitation enlarges the nanoparticle labels and amplifies the stripping signal, resulting in a detection limit of 100 fM for a 32 base target DNA. This

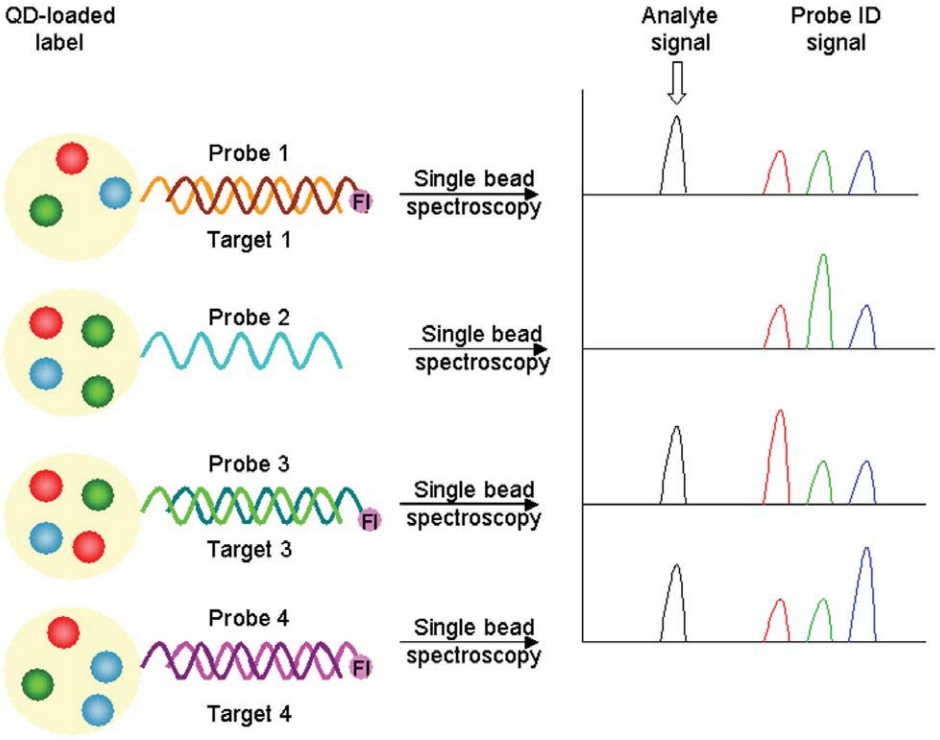

Fig. 7 Wavelength-and-intensity multiplexed detection of antibodies using QD-loaded beads[78]. The unique probe identification (ID) signal is used to identify individual sequences, while the analyte signal is generated by an attached fluorophore. (Reprinted with permission from[78]. © 2001 Nature Publishing Group.)

is slightly more sensitive than the corresponding Au nanoparticle-based system proposed by the same group[39,42].

QD labels made of PbS[81] and ZnS[82] have also proven useful in electrochemical stripping detection of DNA, with detection limits in the subpicomolar range. An amplification strategy involving the use of CdS nanoparticle-loaded carbon nanotubes as labels was reported subsequently[83].

Further exploration of QDs with dissimilar oxidation potentials has led to their use as 'electrochemical codes' in the simultaneous detection of multiple DNA targets[84]. By attaching PbS, CdS, and ZnS to various detection probe sequences and subsequently stripping the labels at various potentials, the different target sequences can be detected and quantified (Fig. 8). The same strategy has also been applied in a multiplexed immunoassay of proteins[85], with measurements of four antigens achieved in a single run. A greater degree of multiplexing may be achieved in connection with the encapsulation of various proportions of different nanoparticles into polymeric carrier beads – an electrochemical equivalent of the optical coding proposed by Han *et al.*[78].

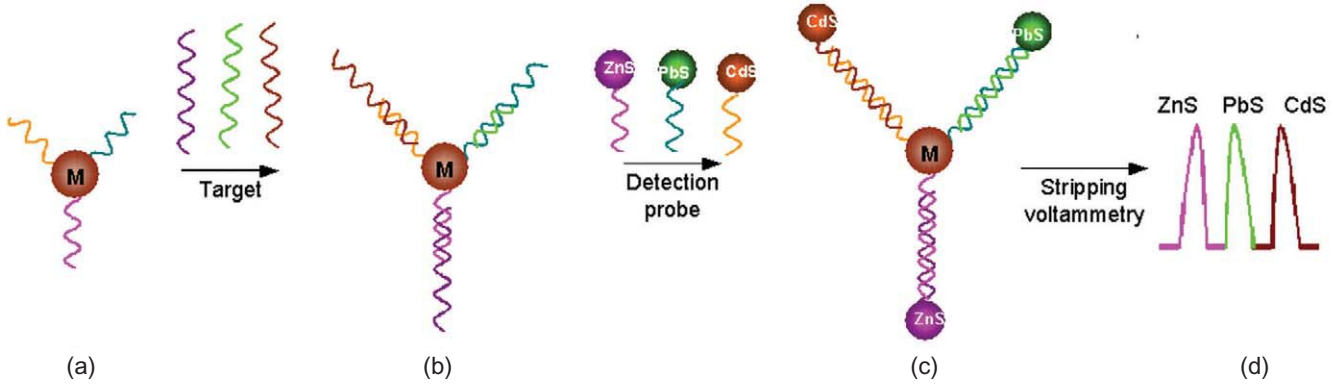

Fig. 8 Multitarget electrochemical detection of DNA using QD labels[84]. (a) Introduction of probe-modified magnetic beads. (b) Hybridization with DNA targets. (c) Second hybridization with QD-labeled probes. (d) Dissolution of QDs and electrochemical detection. (Reprinted with permission from[84]. © 2003 American Chemical Society.)

An exciting development has been reported recently involving the use of QD labels for SNP identification[86]. Four monobases were uniquely labeled with four different QDs and then captured in different combinations for specific SNPs, yielding a distinct electronic fingerprint. As a result, in contrast with other electrochemical methods[35,87,88], SNP identification has been achieved in a single voltammetric run. This new protocol may eventually facilitate simple, fast, and cost-effective screening of important SNPs in high-throughput automated operations.

QD labels have also been used in electrochemical impedance spectroscopic (EIS) detection[89]. Detection is based on the change in electron transfer resistance between the electrode and a redox marker in the solution. As expected, resistance increases upon hybridization. This increase is amplified up to 100 times when the target DNA is labeled with CdS nanoparticles, owing to the labels' space resistance and semiconducting properties. The detection of resistance change by EIS is thus made possible.

Magnetic nanoparticles

Magnetic detection

Because of their extensive use for separation and preconcentration in electrochemical and optical biosensors, magnetic beads are readily available. In recent years, magnetic nanoparticles have been finding use not only as carriers but also as labels that indicate binding events[90]. Superparamagnetic nanoparticles make ideal labels because they are readily magnetized to large magnetic moments, which facilitates detection, and yet the mutual magnetic attraction can easily be switched off to prevent irreversible aggregation.

Chemla *et al.*[91] have reported an immunoassay using magnetic nanoparticle labels. Target antigens are immobilized on a Mylar-film-coated well, to which a suspension of labeled antibody is added. Upon applying a magnetic field in one-second pulses, the nanoparticles develop a net magnetization that relaxes when the field is turned off. Detection is achieved by differentiating the relaxation process: unbound nanoparticles relax rapidly by Brownian rotation, while the bound nanoparticles undergo a slow Néel relaxation, giving out a magnetic flux that can be detected by a superconducting quantum interference device. The ability to distinguish between bound and unbound labels obviates the need for separation and allows homogeneous detection. The same method has also been used to detect bacteria in a suspension on the basis of antibody interaction[92].

Magnetoelectronics has recently emerged as a promising platform technology for biosensors[93,94]. This technique is based on the detection of the magnetic fringe field given off by a magnetically labeled target interacting with a complementary biomolecule bound to a magnetic field sensor. Efforts have focused primarily on the fabrication of various sensor designs rather than on the nanoparticle labels. In earlier attempts to detect DNA using sensors based on giant magnetoresistance (GMR)[95,96], micron-sized Fe_2O_3 beads were used as labels. These sensors focus on determining the areal density of the beads rather than counting individual beads[97,98]. In more recent research, nanosized labels have been used. For example, 200 nm magnetic labels were used recently in single-bead detection on a submicron GMR sensor[99]. Single-bead detection of nanosized magnetic labels has also been performed on spin-valve sensors[100-103], with the successful detection of 11 nm Co[103] and 16 nm Fe_3O_4[104] nanoparticles being reported by Grancharov *et al.*[105]. Another development in magnetoresistive sensors was made in 2005 through the use of an alternating current field to accelerate DNA hybridization[106]. Surface-bound DNA probes rapidly hybridize with magnetically labeled complementary target DNA when oscillating external magnetic fields are applied through current-carrying lines. In 2005, 12 nm manganese ferrite nanoparticles were synthesized and biofunctionalized for use in a magnetic tunnel junction based sensor[105].

Silica nanoparticles

Optical detection

More attention has been focused on dye-doped silica nanoparticles since their first use by Tan and coworkers[107] as labels in DNA detection. The nanoparticles, synthesized by reverse microemulsion, comprise numerous organic dye molecules in a silica matrix. The result is better resistance against photobleaching and an amplified signal for each bound label. Unlike organic fluorophores, these nanoparticles do not suffer from blinking. An impressive detection limit of 0.8 fM and a selectivity ratio of 14:1 against one-base mismatches have been achieved.

Unique core-shell-structured silica nanoparticles have been reported by Zhou *et al.*[108], in which the fluorophore molecules in the core are protected and separated from the conjugated biomolecules. These nanoparticles are stable in both aqueous electrolytes and organic solvents, hence they do not aggregate. When used as labels in DNA detection, a detection limit of 1 pM and a dynamic range of about four orders of magnitude have been achieved. While this system is better than molecular fluorophore-based systems, it is not as sensitive as optical detection using Au nanoparticles. Successful immunofluorometric assays[109-111] and cell quantitation on the basis of antibody recognition[112-114] have also been attempted using silica particles doped with fluorescein isothiocyanate[110,111,114] and tris(2,2'-bipyridyl)-ruthenium(II)[109,112,113]. The detection limits were typically less than 1 ng/ml[110,111].

Electrochemical detection

Doping the nanosized silica matrix with a large quantity of redox active compounds such as tris(2,2-bipyridyl)cobalt(III)[115] results in sensitive electrochemical labels. A detection limit of 200 pM has been achieved. This is a reasonable value considering the simplicity of this assay compared with the more sensitive but multistep method using Au-loaded polymeric beads[50].

Other nanoparticles

Aside from the widely used metal and semiconductor nanoparticles, several other materials have also been explored as labels in biomolecular detection.

Eu_2O_3 is a simple inorganic phosphor with a spectrally narrow red emission and a long fluorescence lifetime. Harma *et al.*[116] trapped chelated Eu in polystyrene nanoparticles, conjugated biomolecules on their surfaces, and used them as labels for time-resolved bioassays. Subsequently, the detection of various biomolecules such as prostate-specific antigen[117], viruses[118], thyroid-stimulating hormone[119], and DNA[120] have all been demonstrated using the Eu-doped nanoparticles. The detection limits are ~1 pg/ml for proteins[117] and 6.1 x 10^4 copies for DNA[120]. Simpler synthesis methods have also been proposed[121,122].

A novel lanthanide-based nanoparticle has been proposed[123]. These nanoparticles consist of a Tb^{3+}-doped Gd_2O_3 core (providing high fluorescence intensity) in a functionalized polysiloxane shell (enabling dispersion in aqueous solution and conjugation with biomolecules).

Organic fluorescent nanoparticles, formed by the precipitation of 1-pyrenebutyric acid, have also been used in fluorescence-based assays[124]. These are photochemically stable and water soluble, and exhibit ideal excitation and emission spectra, high room-temperature fluorescence quantum yields, and long fluorescence lifetimes. Since they do not require coating, synthesis is greatly simplified. Under optimized conditions, human serum albumin, bovine serum albumin, and γ-immunoglobulin G (γ-IgG) can be detected with detection limits in the nanogram per milliliter range by using such labels.

Concluding remarks and future outlook

Many recent articles have reported the potential of nanoparticles as labels in biomolecular detection. Compared with current state-of-the-art DNA detection using fluorophore labels and protein detection using ELISA, significant advances have been made in terms of sensitivity, selectivity, and multiplexing capacity.

Of the various materials, Au nanoparticles seem to be the most versatile and extensively studied. QDs can be detected using optical and electrochemical detection platforms similar to those used for Au nanoparticles, and make excellent substitutes for organic fluorophores. Silica nanoparticles, despite showing great promise in biomolecular detection, need to be explored further in order to realize their full potential as labels. The eventual use of these materials will depend on the ease with which they can be synthesized and manipulated.

In comparing the detection techniques, colorimetric and direct electrical approaches are among the simplest systems available. However, there is still room for improvement in the sensitivity of these methods. Optical detection methods suffer from the drawback of bulky and expensive instrumentation. Electrochemical methods, on the other hand, have become more attractive owing to their simplicity, low cost, and excellent portability. Unfortunately, the current amplification strategy for electrochemical signal generation often involves multiple steps of deposition and stripping. Hence, there is a tradeoff between simplicity and sensitivity. Within the scope of this review, it is not possible to explore in detail the mechanisms and implications of each detection system. For further reading, please refer to other articles[125,126].

Tremendous opportunities exist in the application of nanoparticles for biomolecular detection. Novel nanoparticles have been fabricated that might be useful for molecular detection. For example, magnetic-QD hybrid nanoparticles[127] may offer a unique combination of sample manipulation and sensitive detection with negligible interference. OsO_2 nanoparticles, which have electrocatalytic properties, have been exploited for the chemically amplified detection of microRNA (miRNA)[128]. The association of these nanoparticles with hybridized miRNA molecules leads to the formation of an electrocatalytic system that generates a measurable current upon the addition of substrate to the solution. Novel types of nanoparticle label could be developed by entrapping or immobilizing multiple bioluminescent enzymes, such as luciferase, on nanoparticle carriers. The addition of a substrate will result in a luminescent signal with little interference from the background.

It is also interesting to compare the performance of nanoparticle labels to other labels in biomolecular detection, such as electrochemical tags[129] and dendrimers[130]. Nonetheless, the eventual acceptance of nanoparticle-based detection schemes for diagnostic applications will depend on how these novel schemes compare with the current gold standards, i.e. PCR and ELISA, in terms of simplicity, sensitivity, specificity, and reliability. nt

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