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Review

Nanoparticles-based strategies for DNA, protein and cell sensors

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ABSTRACT

The need for novel biosensing systems has increased enormously in the last few years. In this context nanoparticles with special optical and electrochemical properties are bringing significant advantages in fields such as clinical analysis, environmental monitoring, food and safety/security control. Biosensor technology represents an interesting alternative for the development of efficient, fast, low-cost and user-friendly biosensing devices. Between different biosensing alternatives the nanotechnology and nanomaterial oriented biosensors represent very attractive and cost-efficient tools for real sample applications. The developed devices are based on the use of various platforms which allows their future applications and extension in several fields. Optical detection alternatives based on light absorption and scattering, surface plasmon resonance enhancement, fluorescence (including its quenching strategies) between other methods will be discussed. In addition, a special emphasis on electrical methods (electromechanical, stripping analysis, potentiometric etc.) that use nanoparticles as tracers for biomolecules detection will be given. In most of the examples nanoparticle-based biosensing systems are being offered as excellent screening and advantageous alternatives to existing conventional strategies/assays and the corresponding equipments.

Contents

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1. Introduction

Bottom–up nanotechnology approaches are offering a large series of nanoparticles (NPs) with special interest for biosensing systems. Investigations of these materials are gaining interest due to the size and shape-dependent physical, chemical and electrochemical properties which make them extremely useful in sensing and biosensing applications (Rosi and Mirkin, 2005) The size and composition sometimes make NPs even more attractive than the corresponding bulk structure. A target binding event (i.e. DNA hybridization or immunoreaction) occurring onto NPs surface may have a significant effect on its optical (change of the light absorption/emission) or electrochemical properties (oxidation/reduction current onto a transducing platform) offering novel alternatives for bioanalysis. For example of a special interest are metal nanoparticles of group II–VI compound semiconductors like CdSe, ZnSe, CdTe, etc. called also quantum dots (QDs) (Murphy, 2002) as well as gold nanoparticles (AuNPs). QDs are highly fluorescent and in comparison with organic dyes such as rhodamine are 20 times as bright, 100 times as stable against photobleaching, and one-third as wide in spectral line width (Chan and Nie, 1998). Another intrinsic benefit of NPs is the increased surface area available with special interest for bioapplications between others.

The application of NPs in biosensors is strongly related to their properties that depend in certain mode from the synthesis (quality of nanoparticles) and posterior modifications (chemical and biological). The NPs preparation procedures, either in colloidal solutions or grown on solid substrates, have been extensively reviewed (Parak et al., 2003). Along with synthetic advances for varying the size, shape, and composition of nanostructured materials has come the ability to tailor their binding affinities for various biomolecules through surface modification and engineering.

Many types of NPs of different sizes and compositions are now available, which facilitate their optical (Rosi and Mirkin, 2005; Merkoçi, 2007a,b) and electrochemical (Wang, 2003; Merkoçi et al., 2005a,b) related application in enzyme-based sensors, immunosensors and DNA sensors. Nevertheless biological or molecular coating which will act as a bioactive and selective interface is necessary to be attached to the nanoparticles prior to their application in biosensing systems. The functionalization of inorganic nanoparticles by means of evolutionary optimized biological components concerns an important point. Adsorption, linkage via thiol groups, electrostatic interaction, covalent linkage eyc. are the various reported strategies. Since the nanoparticles and biomolecules typically meet at the same nanometer length scale, this interdisciplinary approach is even contributing to the establishment of the novel field, descriptively termed biomolecular nanotechnology or nanobiotechnology (Niemeyer, 2001).

In addition to the chemical and biological modification (including coatings with antibodies, DNA, cells etc.) the NP characterisation and quantification play a crucial role for the final biosensing application. The optical and electrochemical properties of NPs offer various signal transduction modes, including simultaneous approaches (optical and electrochemical) not available with other materials (Ambrosi et al., 2007; Merkoçi, 2007a,b,).

The range of the used NPs is as large as the range of potential applications in biosensors and depends strongly from the applications, the biomolecules to detect as well as the type of the sample to be analysed. This review will discuss some typical examples of NPs application for DNA, protein and cell analysis using opti-

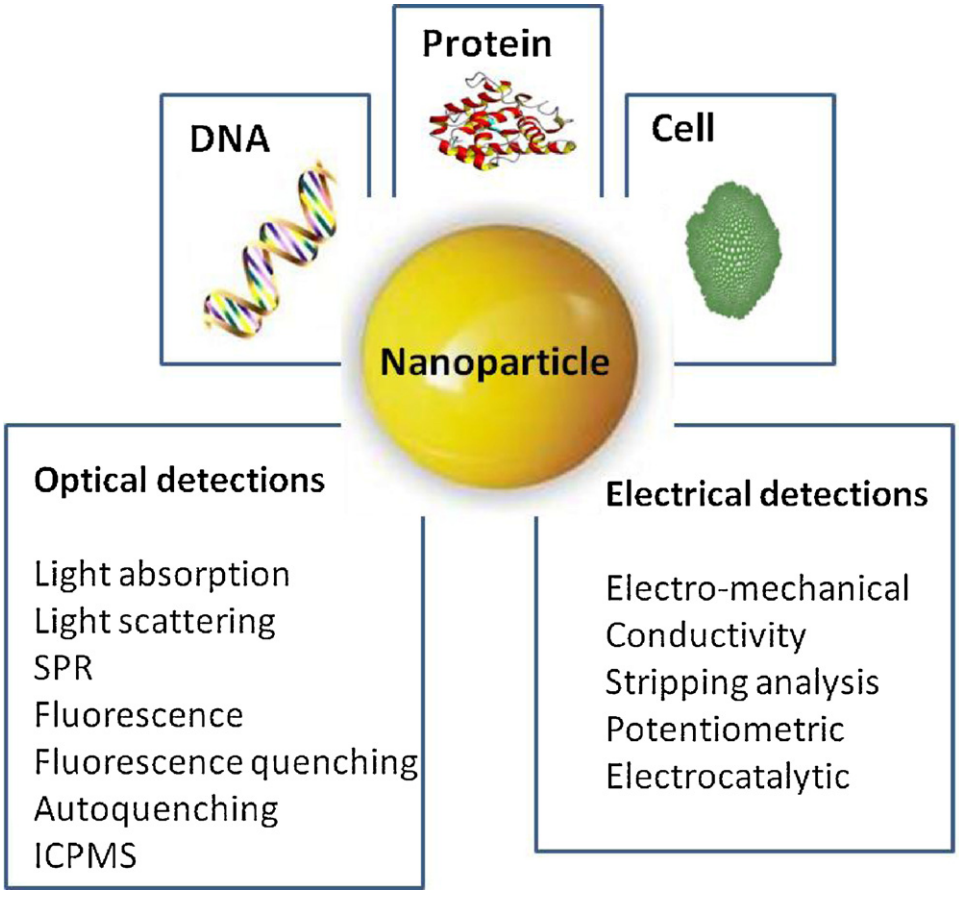

Fig. 1. Schematic (not in scale) of some of the optical and electrical detection alternatives that are being used for DNA, proteins and cells analysis thanks to the use of nanoparticles. SPR: surface plasmon resonance; ICP-MS: inductively coupled plasma mass spectroscopy.

cal and electrical biosensing systems. (see Fig. 1). As some of the detection principles, i.e. optical (Rosi and Mirkin, 2005) or electrical (Merkoçi et al., 2005a,b) detections only along with the corresponding biosensing application, are previously revised the objective now of this review is to give readers in a single source the whole range of the NP detection possibilities offered and discuss novel opportunities for future applications in bioanalysis. Detailed aspects related to the (bio)chemistry of nanoparticles (Niemeyer, 2001) including their modifications and the detection principle are not included as these have been extensively revised in the mentioned earlier reviews.

2. Optical detection alternatives

2.1. Light absorption

Conventional methods that use radioactive labeled nucleic acid probes or the polymerase chain reaction (PCR) coupled with molecular fluorophore assays offer high sensitivity of detection, but they suffer from several drawbacks that include complex handling procedures, easy contamination, high cost, and lack of portability (Xu et al., 2009a,b). In this context the use of NPs along with simple detection alternatives such as those based on light absorption measurement is very attractive.

A system based upon Au nanoparticles chemically modified with 5′- and 3′-(alkanethiol)-capped oligonucleotides that exhibits extraordinary selectivity for DNA detection is reported (see Fig. 2) (Storhoff et al., 1998). It provides a simple means for colorimetric, one-pot detection of a target oligonucleotide in the presence of a mixture of oligonucleotides with sequences differing by one nucleotide, regardless of position, in the target region. The light absorption based mode is extended to many other applications. A new type of rapid, highly selective, and sensitive colorimetric assay for detecting cysteine using gold nanoparticle-oligonucleotide conjugates was developed by the same group. It relies upon the distance-dependent optical properties of gold nanoparticles, the sharp melting transition of oligonucleotide-linked nanoparticle aggregates, and the very selective coordination of Hg^{2+} with cysteine developed by the same group (Lee et al., 2008). High sensitive detection of proteins, i.e. platelet-derived growth factors (PDGFs) and platelet-derived growth factor receptors (PDGFR), that use an aptamer modified AuNP as a probe, are reported (Huang et al., 2005). Using a simple and sensitive aptamer-based colorimetric method based on the use of AuNPs sensing of alpha-thrombin was also reported (Wei et al., 2007).

2.2. Light-scattering

Metal nanoparticles that differ in size and composition can be designed to scatter light of different wavelengths according to their distinct surface plasmon resonances. Considering this property a DNA array imaging technique based on scattered light from oligonucleotide-functionalized nanoparticles was proposed (see Fig. 3A and B) (Taton et al., 2001). It shows to be a sensitive, ultraselective, multicolor labeling method for DNA arrays. This approach was extended by the same group for multiplexing detection of oligonucleotide targets with gold nanoparticle probes labeled with oligonucleotides and Raman-active dyes.

Gold nanoparticles facilitate the formation of a silver coating that acts as a surface-enhanced Raman scattering (SERS) promoter for the dye-labeled particles that have been captured by target molecules and an underlying chip in microarray format (see Fig. 3C and D) (Cao et al., 2002). Multiplexed detection of oligonucleotide targets has been performed with gold nanoparticle probes labeled with oligonucleotides and Raman-active dyes. The

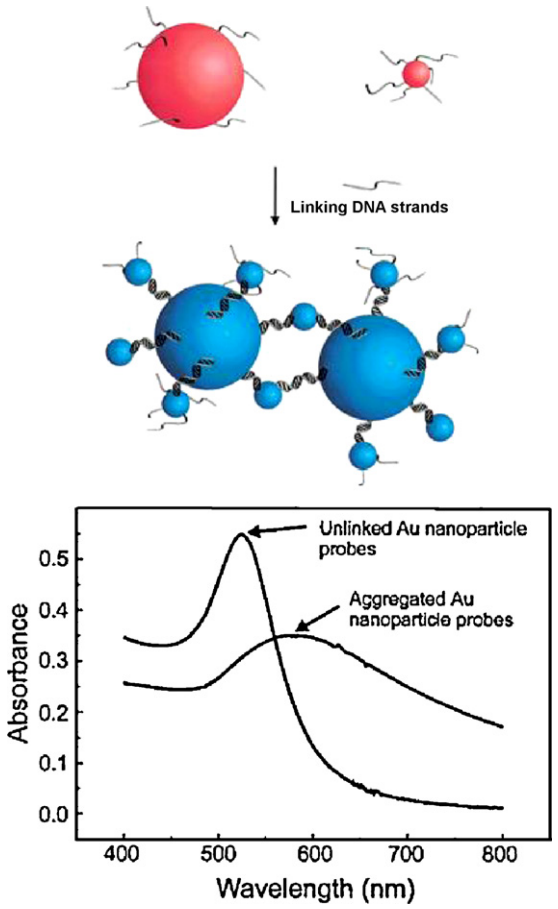

Fig. 2. DNA detection based on light absorption. (A) Schematic shows the gold nanoparticles agregation upon linking with the complementary DNA target. (B) Comparison of UV–vis spectra for of Au nanoparticles without modification, functionalized with 5′-hexanethiol 12-base oligonucleotides and after treatment with a complementary 24-base oligonucleotide. Adapted with permission from reference Storhoff et al. (1998).

strategy provides high-sensitivity and high-selectivity attributes of gray-scale scanometric detection but adds multiplexing and rationing capabilities because a very large number of probes can be designed based on the concept of using a Raman tag as a narrowband spectroscopic fingerprint. Six dissimilar DNA targets with six Raman-labeled nanoparticle probes as well as two RNA targets with single nucleotide polymorphisms were distinguished. The detection limit of this method is 20 fM. The scattering technique is extended also to several other applications. A 'spot-and-read' colorimetric detection method for identifying nucleic acid sequences based on the distance-dependent optical properties of AuNPs is developed by Storhoff et al. (Storhoff et al., 2004). In this assay, nucleic acid targets are recognized by DNA-modified gold probes by using a scatter-based method that enables detection of zeptomole quantities of nucleic acid targets without target or signal amplification. A silver-plated DNA and AuNPs assemblies were used for the detection of sequence-specific protein–DNA interactions (Bonham et al., 2007).

A single AuNP counter in solution based on the photon bursting in a highly focused laser beam due to the plasmon resonance scattering and Brownian motion of gold nanoparticles is used to construct homogeneous sandwich immunoassays for cancer biomarkers, such as carcinoembryonic antigen (CEA) and alpha fetal protein (AFP), and aptamer recognition for thrombin. The reported detection limits were 130 fM for CEA, 714 fM for AFP and 2.72 pM for thrombin (Xie et al., 2009). A microarray for-

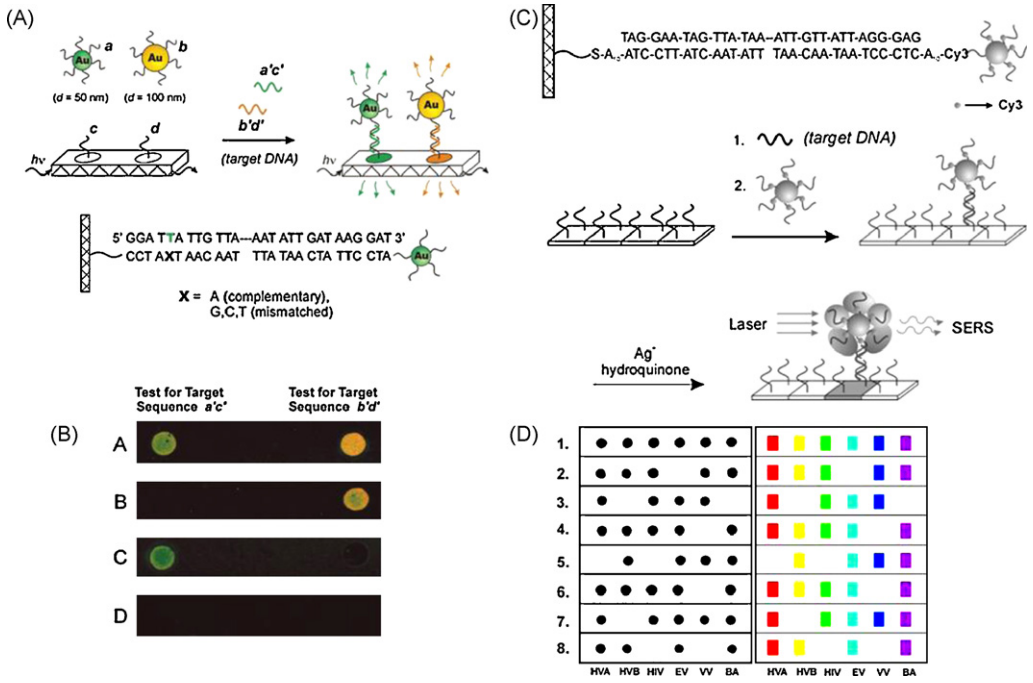

Fig. 3. (A) Schematic of DNA sequences of the array capture strands, the oligonucleotide-functionalized nanoparticle labels and the targets to be detected designed to cohybridize in a three-component sandwich assay. (B) Microscope images of model DNA arrays functionalized with oligonucleotide sequences. (C) Schematic of DNA detection using SERS. A three-component sandwich assay is used in microarray format. Au nanoparticles modified with Cy3-labeled, alkylthiol-capped oligonucleotide strands were used as probes to monitor the presence of specific target DNA strands. After incubation procedure the chip was treated with a Ag enhancement solution and SERS measurements performed. Because the SERS-active substrate in this strategy is generated immediately before the detection event, a large and reproducible SERS response is obtained. (D) Flatbed scanner images of Ag-enhanced microarrays and corresponding Raman spectra. The colored boxes correlate with the color-coded Raman spectra. (A) and (B) adapted from reference Taton et al. (2001); (C) and (D) adapted from Cao et al. (2002).

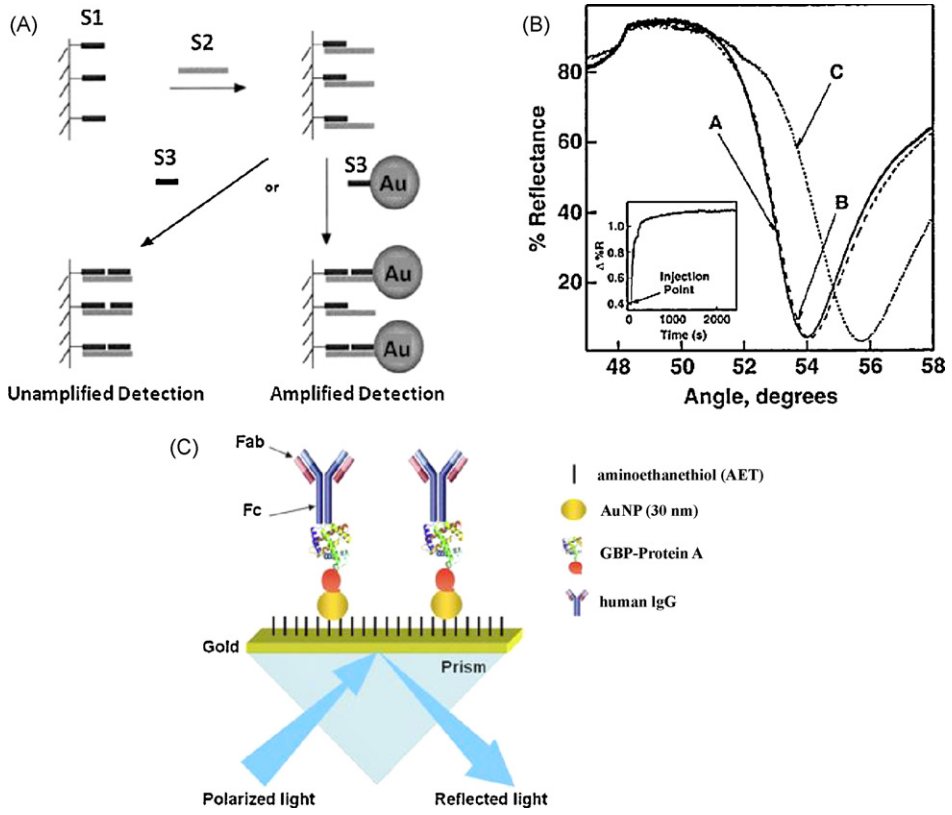

Fig. 4. (A) SPR surface assembly. (B) SPR curves of surfaces prepared in sequential steps as illustrated in Scheme A: a MHA-coated Au film modified with a 12-mer oligonucleotide S1 (curve A), after hybridization with its complementary 24-mer target S2 (curve B), and followed by introduction of S3:Au conjugate (curve C) to the surface. Inset: surface plasmon reflectance changes at 53.2° for the oligonucleotide-coated Au film measured during a 60-min exposure to S3:Au conjugates. Adapted from reference He et al. (2006). (C) Schematic of the oriented immobilization of antibody onto the AuNPs assembled immunosensor chip using gold binding polypeptides-ProA fusion proteins. Adapted from Ko et al., 2009.

mat that uses resonance light scattering as detection principle is also reported. It is applied for the detection of proteins and protein functionality (kinase activity) based on marking either specific antibody-protein binding or peptide phosphorylation events by attachment of AuNPs followed by silver deposition for signal enhancement (Wang et al., 2005). An AuNP-based Raman label for the protein detection by SERS that can be expected to be used for the simultaneous determination of many proteins is also reported (Manimaran and Jana, 2007).

The same technique (SERS spectrum based analysis) in connection to silver nanoparticles (50–80 nm in diameter) modified with 4-mercaptobenzoic acid (Talley et al., 2004), 2-aminothiophenol (Wang et al., 2008) or 4-mercaptopyridine (Nowak-Lovato and Rector, 2009) is used for pH measurements in living cells.

2.3. Surface plasmon resonance

Surface plasmon resonance (SPR) is a surface-sensitive analytical technique based on the ability to detect dielectric constant changes induced by molecular adsorption at a noble metal film. The inability of conventional SPR to measure sometimes extremely small changes in refractive index hinders its application in ultrasensitive detection. To address this drawback the use of AuNPs tags to increase the angle shift in SPR reflectivity is proposed (He et al., 2006). This effect is a combined result of greatly increased surface mass, high dielectric constant of AuNPs and electromagnetic coupling between AuNPs and the Au film used as sensor surface (see Fig. 4). Even without further optimization, the sensitivity of this technique (10 pM limit of quantitation for 24-mer oligonucleotides; surface density $\leq 8 \times 10^8$ molecules/cm^2) begins to approach that of traditional fluorescence-based methods for DNA hybridization. The sensitivity of transmission surface plasmon resonance (T-SPR) spectroscopy was reported to be dramatically increased by the functionalization of the complementary DNA with AuNPs (Hutter and Pileni, 2003).

An ultrasensitive surface bioaffinity sensor based on the adsorption of AuNPs onto gold diffraction gratings is reported. Advantages of an enhanced diffraction due to the optical coupling of the planar surface plasmons in the grating to the localized surface plasmons in the AuNPs are used and applied to detect unmodified DNA at a concentration of 10 fM (Wark et al., 2007). Another interesting application is related to colloidal AuNPs assembled onto the surface of SPR Au chip via 2-aminoethanethiol for the enhancement of sensitivity as a label-free detection system (see Fig. 4C) achieving a 10-fold increased sensitivity in detection of S. typhimurium compared to the bare electrode (Ko et al., 2009).

2.4. Fluorescence

The size-tunable emission and simultaneous excitation render the highly luminescent quantum dots (QDs) ideal fluorophores for wavelength-and-intensity multiplexing. Multicolor optical coding for biological assays has been achieved by embedding different-sized QDs (zinc sulfide–capped cadmium selenide nanocrystals) into polymeric microbeads at precisely controlled ratios (see Fig. 5) (Han et al., 2001). The use of 10 intensity levels and 6 colors could theoretically code one million nucleic acids or protein sequences. Imaging and spectroscopic measurements indicate that the QD-tagged beads are highly uniform and reproducible, yielding bead identification accuracies as high as 99.99% under favorable conditions. DNA hybridization studies demonstrate that the coding and target signals can be simultaneously read at the single-bead level. This spectral coding technology is expected to open new opportunities in gene expression studies, high-throughput screening, and medical diagnostics.

Fluorescent (dye-doped) nanoparticles that amplify the signal intensity corresponding to a single aptamer binding event, resulting in improved sensitivity over methods using individual dye-labeled probes have been used (He et al., 2006). According to this method aptamer-modified magnetic nanoparticles were used for target cell extraction, while aptamer-modified fluorescent nanoparticles were simultaneously added for sensitive cell detection. Combining two types of nanoparticles allows for rapid, selective, and sensitive detection not possible by using either particle alone.

The use of QDs in conjunction to optical spectroscopy has provided an interesting tool to obtain a sensitive, accurate and quantitative immunoprofiling/quantifying of protein expression of

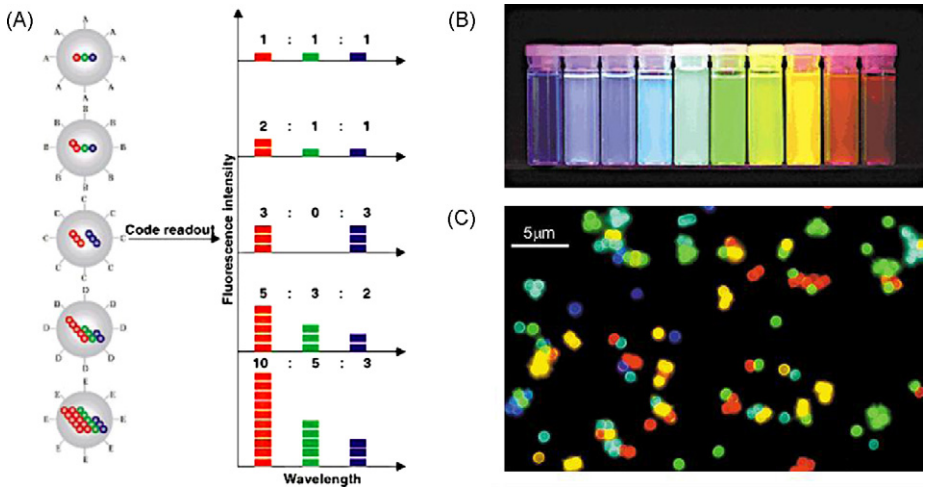

Fig. 5. (A) Schematic illustration of optical coding based on wavelength and intensity multiplexing. Large spheres represent polymer microbeads, in which small colored spheres (multicolor quantum dots) are embedded according to predetermined intensity ratios. Molecular probes (A–E) are attached to the bead surface for biological binding and recognition, such as DNA–DNA hybridization and antibody–antigen/ligand–receptor interactions. The numbers of colored spheres (red, green, and blue) do not represent individual QDs, but are used to illustrate the fluorescence intensity levels. Optical readout is accomplished by measuring the fluorescence spectra of single beads. Both absolute intensities and relative intensity ratios at different wavelengths are used for coding purposes; for example (1:1:1) (2:2:2), and (2:1:1) are distinguishable codes. (B) Ten distinguishable emission colors of ZnS-capped CdSe QDs excited with a near-UV lamp. From left to right (blue to red), the emission maxima are located at 443, 473, 481, 500, 518, 543, 565, 587, 610, and 655 nm. (C) Fluorescence micrograph of a mixture of CdSe/ZnS QD-tagged beads emitting single-color signals at 484, 508, 547, 575, and 611 nm. The beads were spread and immobilized on a polylysine-coated glass slide, which caused a slight clustering effect. Adapted with permission from reference Han et al. (2001).

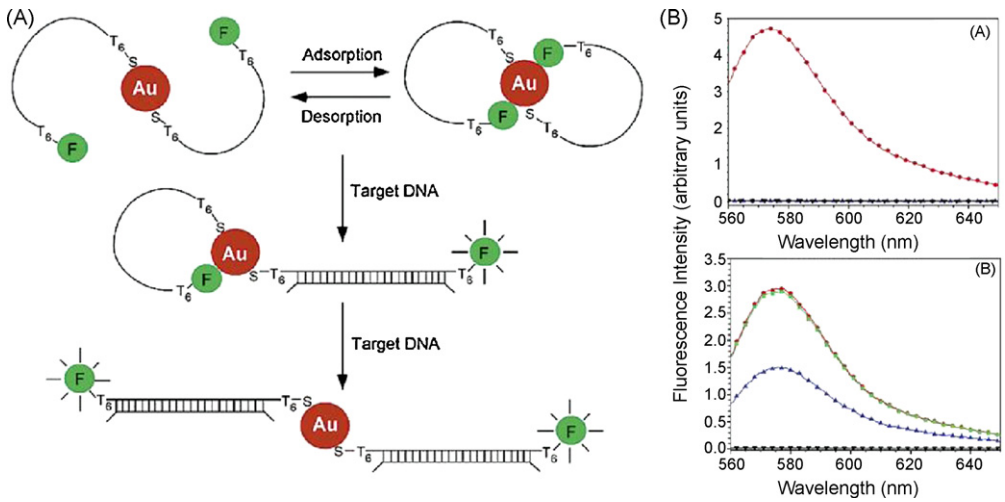

Fig. 6. (A) Nanoparticle-based quenching of fluorescence probes. Two oligonucleotide molecules are shown to self-assemble into a constrained conformation on each gold particle (Au) (2.5 nm diameter). A T6 spacer (six thymines) is inserted at both the 3′- and 5′-ends to reduce steric hindrance. Single-stranded DNA is represented by a single line and double-stranded DNA by a cross-linked double line. In the assembled (closed) state, the fluorophore (F) is quenched by the nanoparticle. Upon target binding, the constrained conformation opens, the fluorophore leaves the surface because of the structural rigidity of the hybridized DNA (double-stranded), and fluorescence is restored. In the open state, the fluorophore is separated from the particle surface by about 10 nm. (B) Spontaneous adsorption of fluorophores on gold particles and complete fluorescence quenching (upper curve): Fluorescence spectra obtained from 0.1 μM TMR (free dye) under the following conditions: without a quencher (red curve), with 2 μM gold nanoparticles (black curve), and after the gold particles were removed by centrifugation (complete adsorption, no dye left in solution) (blue curve) (lower curve). Fluorescence spectra obtained from 0.1 μM TMR-labeled DNA (5′-TMR-T6-TAG GAA ACA CCA AAG ATG ATA TTT-3′, not thiolated) under the following conditions: without a quencher (red curve), with 2 μM Dabcyl (5% quenching, green curve), with 2 μM gold particles (55% dynamic quenching, blue curve), and with 2 μM gold nanoparticles plus 2.0 mM $MgCl_2$ (100% static quenching, black curve). Note that the oligos do not have an anchoring thiol group and are different from the thiolated probes shown in Fig. 3. Adapted from reference Maxwell et al. (2002).

cancer tumours on a continuous scale not attainable by traditional methods (Ghazani et al., 2006). The study of the interactions of endogenous bait proteins, recruited by QDs, with fluorescently tagged prey by using a multiresolution implementation of fluorescence correlation spectroscopy to achieve maximal temporal resolution was recently reported (Zamir et al., 2010).

2.5. Fluorescence quenching

Gold nanocrystals represent a new class of universal fluorescence quenchers that should find applications in molecular engineering and biosensor development. In the case of DNA probes labeled with a thiol at one end and a dye at the other, it was found that the DNA molecules self-organize into a constrained conformation on the nanoparticle surface, and that the fluorophore is completely quenched by the particle (see Fig. 6) (Maxwell et al., 2002). Upon target binding, the constrained conformation opens and the fluorophore is separated from the particle surface. This structural change generates a fluorescence signal that is highly sensitive and specific to the target DNA.

2.6. Auto-quenching of fluorescence

A considerable quenching of the photoluminescence (PL) intensity and lifetime of QDs when conjugated to single-walled carbon nanotube (SWCNT) have been observed (Biju et al., 2006). A controlled conjugation of CdSe-ZnS QDs to sidewall-functionalized SWCNT templates and the effect of conjugation of QDs to SWCNT on the photoluminescence (PL) properties of QDs was performed (see Fig. 7). A Förster resonance energy transfer (FRET) involves nonradiative transfer of energy from a photoexcited QD (energy donor) to a nearby SWCNT (energy acceptor) in the ground state. The quenching of the PL intensity and lifetime is attributed to FRET from QDs to SWCNT. From control experiments involving QD–SWCNT conjugates with different size and density of QDs the authors identified that inter-QD FRET is not important when QDs having larger diameters are close-packed into hierarchical structures. However, based on the current work authors are unable to exclude the contribution of other factors such as electron transfer from QDs to SWCNT and destabilization of excited states in QDs to the quenching of the PL intensity and lifetime in QD–SWCNT conjugates. This phenomenon could be with interest to be used for sensing application although its sensitivity and the interferences in complex matrix must be carefully considered. Luminescent CdSe-ZnS core-shell QDs as energy donors in FRET assays for proteins have been reported. As anticipated by authors a careful preparation of intermediary proteins, polysaccharides, or oligonucleotides in order to ensure sufficient signal change upon acceptor binding is required (Clapp et al., 2004).

2.7. Inductively coupled plasma mass spectrometry

Inductively coupled plasma mass spectroscopy (ICPMS) is showing to be an interesting alternative to detect and quantify

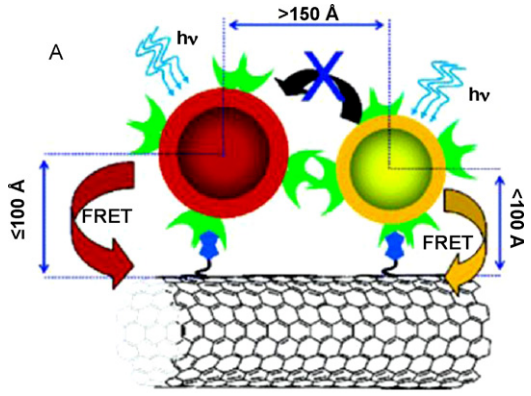

Fig. 7. Schematic presentation of a QD–SWCNT conjugate structure containing QD585 (smaller) and QD605 (bigger) and the possible distances between energy donors and acceptors. The average diameter of QDs is 150 Å. Adapted from reference Biju et al. (2006).

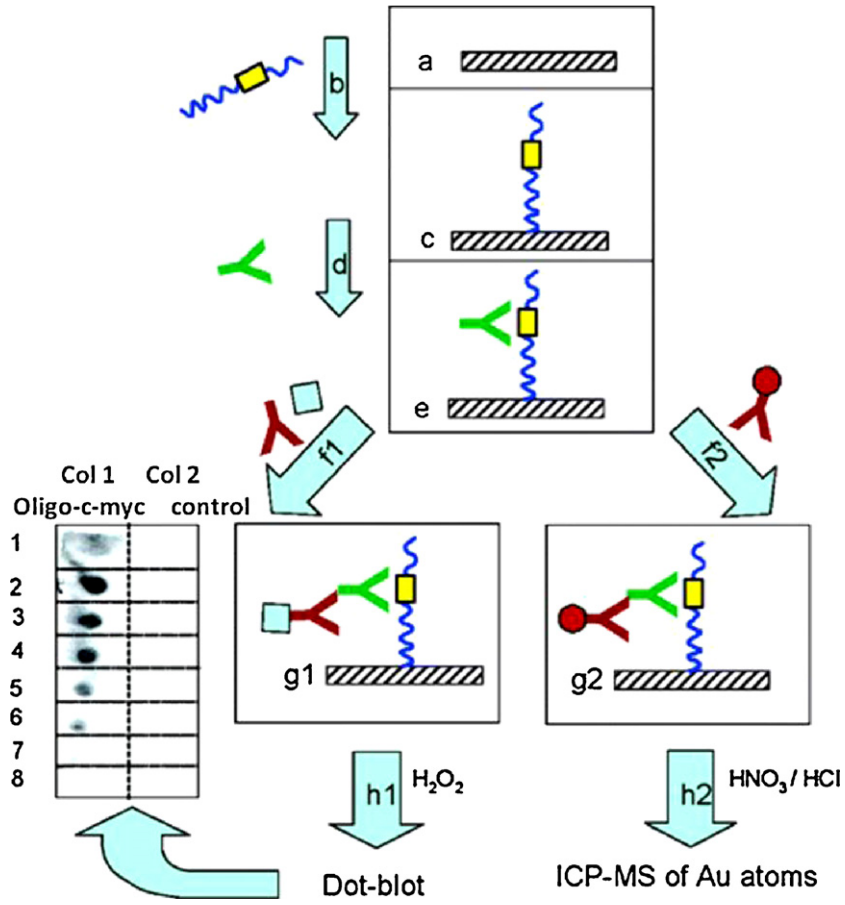

Fig. 8. Schematic presentation of the ICPMS AuNP based assay protocol. The nitrocellulose membrane (a) was introduced into the dot-blot manifold. The oligonucleotide carrying c-myc peptide (b) is immobilized over the membrane (c). It reacts overnight with the anti-cmyc (d). The immobilized oligonucleotide (e) is then treated: 1. According to the dot-blot assay it reacts first with the anti-c-myc and goat antimouse HRP-conjugate antibody (f1) and then developed (h1) following the ECL (Amersham) protocol and exposing to X-ray film. 2. According to the ICPMS-linked assay it reacts for 1 h with goat anti-mouse colloidal Au (f2) and then is dissolved (h2) and detected by ICPMS. Also shown in that figure are the following: autoradiography of the oligonucleotide with peptide c-myc (column 1), and the oligonucleotide without peptide (column 2—blank). Amounts of oligonucleotide–peptide conjugates: 8 (1), 4 (2), 2 (3), 1 (4), 0.5 (5), 0.25 (6), 0.125 (7), and 0 (8) μg of oligonucleotide/dot. From reference Merkoçi et al. (2005a,b).

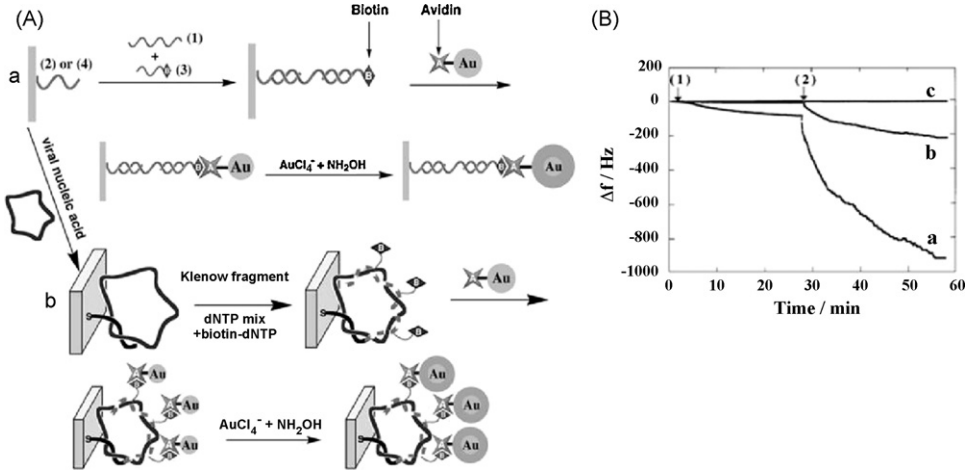

Fig. 9. (A) Amplified detection of DNA by: (a) association of an Au-avidin conjugate and catalytic deposition of Au; (b) polymerase-induced replication of DNA followed by the electroless deposition of gold on an Au-avidin conjugate linked to the double-stranded assembly. (B) Time-dependent frequency changes upon the amplified microgravimetric analysis of DNA by the catalytic deposition of Au on a Au-avidin conjugate according to Scheme A: (a) analysis of 1, 1×10^9 M; (b) analysis of 1, 1×10^{13} M; (c) analysis of 1a, 5×10^7 M. Point (1) indicates the addition of the Au-avidin conjugate to the 2-functionalized Au-quartz crystal pretreated with the respective DNA and the biotinylated nucleic acid 3, 5×10^7 M. The experiment is performed in phosphate buffer, 0.1 M, pH 7.5. Point (2) indicates the interaction of the resulting interface with $AuCl_4$, 0.5 mM, and NH_2OH, 0.5 mM, in a pure aqueous solution. Hybridization of the 2-functionalized Au-quartz crystal with the DNA-biotin nucleic acid complex was performed in phosphate buffer 0.3 M, pH 7.5, for 90 min and room-temperature. With permission from Weizmann et al. (2001).

nanoparticles. Although ICPMS is known as one of the most powerful methods for trace element analysis the possibilities of this technique on the quantification of nanoparticles are not yet deeply explored. AuNPs modified with anti-mouse IgG have been used to trace oligonucleotides carrying a c-myc peptide (see Fig. 8) (Merkoçi et al., 2005a,b). Two strategies, a dot-blot format as well as inductively ICPMS have been used to detect the nanoparticle tracer. ICPMS-linked DNA assay may have significant potential as an important nonradioactive DNA detection method for the simultaneous determination of various sequences by labeling different kinds of inorganic nanoparticles and taking also advantage of the recent development of the ICPMS technique. The possibility of using the large diversity of available antibodies and their many epitope sequences makes oligonucleotide–peptide conjugates good candidates for the directed assembly of complex nanoparticles. The DNA modification via peptide method and consecutive antigen/antibody reaction can be even used for developing multiple genosensor platforms based on the same label. Moreover, this approach can solve the problem of nonspecific adsorption coming from the limited available attachment mechanisms such as biotoin/streptavidin or covalent reactions used to label the DNA strands. The proposed method is a general one with a broad range of application possibilities offered by metallic labels easily to be detected by the ICPMS technique (Allabashi et al., 2009).

3. Electro-mechanical, electrical and electrochemical detection alternatives

In this section several strategies based on electro-mechanical (i.e. quartz crystal microbalance), electrical (measurements of changes in the ohmic response of a circuit) and

electrochemical methods will be discussed. In the last one the current that indicates the presence of a target is faradaic in origin. It arises as a result of the oxidation or reduction of either a redox probe in the detection medium or the redox activity of a conjugated electroactive nanoparticle approaches. This will include both voltammetric- and potenciometric-based detections.

3.1. Electro-mechanical detection

Microgravimetric quartz-crystal-microbalance is also used to transduce the nanoparticle mediated biosensing recations on the piezoelectric crystals. Au-nanoparticle-catalyzed deposition of gold, for the amplification of DNA sensing and the detection of single-base mismatches is an interesting application. An amplification route for DNA detection based on the deposition of gold on a 10 nm Au-colloid/avidin conjugate label acting as a 'seeding' catalyst is used. The amplification of microgravimetric analysis through the catalytic deposition of Au on an Au–avidin conjugate was achieved (see Fig. 9) (Weizmann et al., 2001). Ultrasensitive detection of DNA is accomplished by this catalyzed deposition of gold, with a detection limit of $\sim 1 \times 10^{-15}$ M.

3.2. Conductivity

Measurements of conductivity changes related to nanoparticles involved in DNA hybridization or immunosensing have been also reported. A DNA array detection method in which the binding of oligonucleotides functionalized with gold nanoparticles leads to conductivity changes associated with target-probe binding events is reported. The binding events localize AuNPs in an electrode gap; silver deposition facilitated by these nanoparticles bridges the gap and leads to readily measurable conductivity changes (see Fig. 10A). Using this method, a DNA detection at concentrations as low as 500 fM is achieved (Park et al., 2002). As this system is based on conventional microelectrodes, it is positioned for massive multiplexing through the use of larger arrays of electrode pairs than the four used in the proof-of-concept experiments reported here.

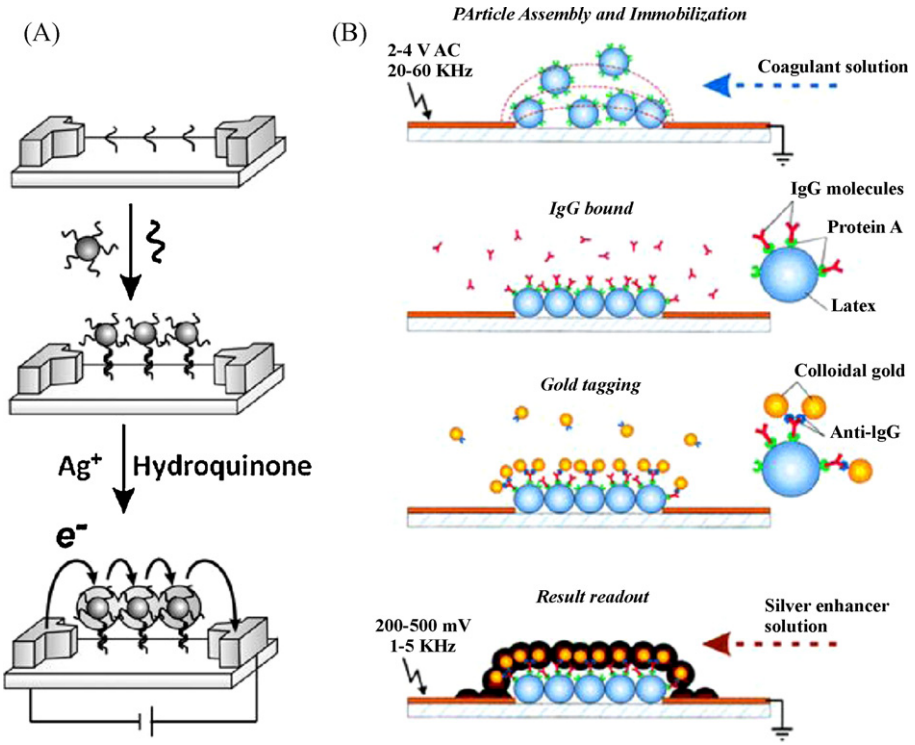

Fig. 10. (A) Scheme showing concept behind electrical detection of DNA. With permission from Park et al. (2002). (B) Schematics of the main stages of sensor assembly and functioning illustrated by an immunoglobulin test. Adapted from Velev and Kaler (1999).

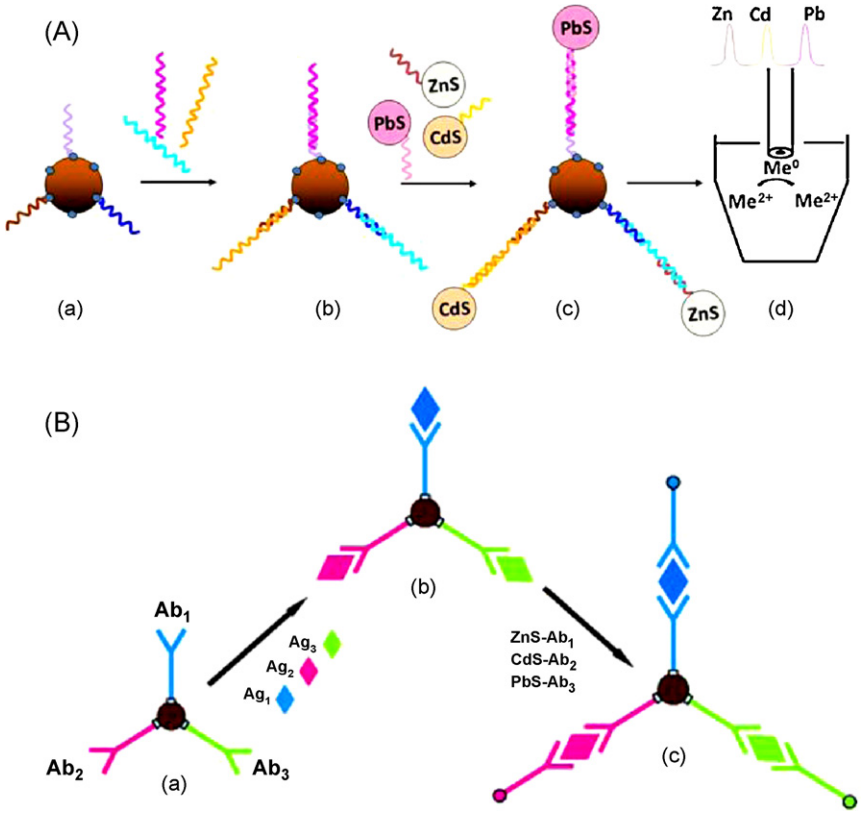

Fig. 11. (A) Multi-target electrical DNA detection protocol based on different inorganic colloid nanocrystal tracers. (a) Introduction of probe-modified magnetic beads. (b) Hybridization with the DNA targets. (c) Second hybridization with the QD-labeled probes. (d) Dissolution of QDs and electrochemical detection. Adpated from Wang et al. (2003). (B) Multiprotein electrical detection protocol based on different inorganic colloid nanocrystal tracers. (a) Introduction of antibody-modified magnetic beads; (b) binding of the antigens to the antibodies on the magnetic beads; (c) capture of the nanocrystal-labeled secondary antibodies. Dissolution of nanocrystals and electrochemical stripping detection as in (A). Adpated from Liu et al. (2004).

A similar method for DNA diagnostics based on direct electrical detection of autometallographically enhanced Au labeled analytes is also reported (Diessel et al., 2004). Unequivocal discrimination of all possible base pairing combinations in the SNP assay has been achieved by this method. The SNP assay in particular indicates the potential of the method for analyte quantification.

Direct electric conductivity readout accomplished after secondary tagging with colloidal gold and its enhancement by silver nucleation is also used for immunosensing. Latex microspheres from suspension are collected via dielectrophoresis in the micrometer-sized gaps between planar electrodes. The assembled particulate patches are fixed by changing the colloidal interactions

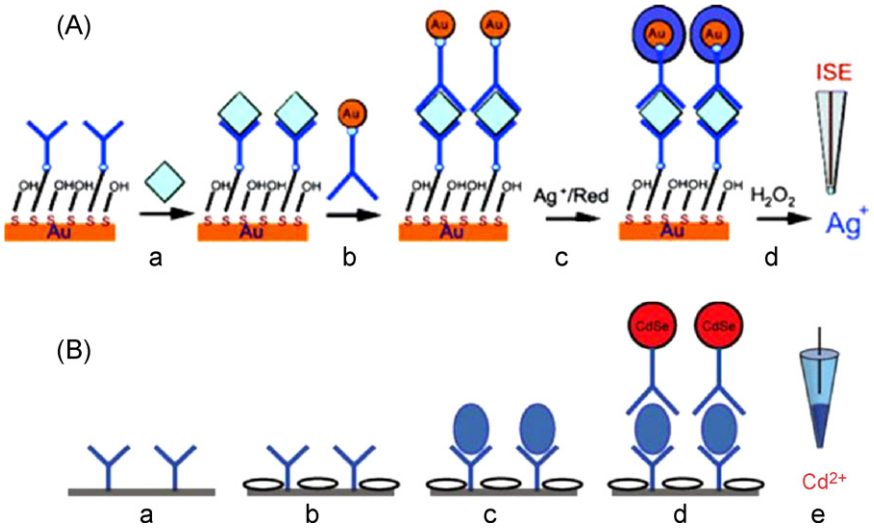

Fig. 12. (A) Potentiometric detection of sandwich immunoassay. (a) Antigen addition, (b) capture of the gold nanoparticles labeled anti-mouse IgG antibody, (c) catalytic deposition of silver ions on the conjugated Au nanoparticles, (d) silver dissolution and potentiometric detection using a Ag^+-selective electrode (ISE). Adpated from Chumbimuni-Torres et al. (2006). (B) Immunoassay protocol based on CdSe quantum dots. (a) Antibody is immobilized on a microwell plate, (b) the plate is blocked with BSA, (c) anti-mouse IgG antigen is incubated, (d) the secondary antibody with CdSe nanoparticle labels is bound, and (e) the label is dissolved with H_2O_2 and the released Cd^{2+} is detected with the Cd^{2+}-ISE. Adapted from Numnuam et al. (2008).

to induce coagulation. Immuno-active sites on the latex surfaces bind the target molecules and detected afterwards accordingly (see Fig. 10B) (Velev and Kaler, 1999).

3.3. Stripping analysis

A multi-target detection capability thanks to the coupling of the amplification feature of stripping voltammetry in the case of quantum dots (QDs) used as labels for DNA hybridization is shown to be a promising alternative for affinity bioassays. Different inorganic-colloid (QDs) nanocrystals (zinc sulfide, cadmium sulfide, and lead sulfide) were used as encoding tags to differentiate the signals of three DNA targets in connection to stripping-voltammetric measurements of the heavy metal dissolution products (see Fig. 11A) (Wang et al., 2003). These products yield well-defined and resolved stripping peaks at −1.12 V (Zn), −0.68 V (Cd), and −0.53 V (Pb) at the mercury-coated glassy-carbon electrode (vs Ag/AgCl reference). The position and size of these peaks reflect the identity and level of the corresponding DNA target. The protocol is illustrated for the simultaneous detection of three DNA sequences related to the BCRA1 breast-cancer gene in a single sample in connection to magnetic beads bearing the corresponding oligonucleotide probes. An effective and inexpensive multitarget electrochemical immunoassay based on the use of similar tags is also reported (see Fig. 11B) (Liu et al., 2004). This multiprotein electrochemical stripping detection capability with fmol detection limits is also combined with the efficient magnetic separation that minimizes nonspecific binding effects.

3.4. Potentiometric analysis

The use of potentiometry for ultrasensitive nanoparticle-based detection of protein interactions has been found to be a very interesting alternative due also to the simplicity of the method. A silver ion-selective microelectrode (ISE) is used to detect silver ions oxidatively released from silver enlarged gold nanoparticle labels (connected with anti-mouse IgG antibody) in a sandwich immunoassay (see Fig. 12A) (Chumbimuni-Torres et al., 2006). It is anticipated that this approach may form the basis for highly sensitive bioaffinity assays. In addition to antibodies quantum dots have also been used to label aptamers. As a result an aptamer-based potentiometric sandwich assay of proteins has been developed (Numnuam et al., 2008). It consists in ISEs that monitor protein–aptamer interactions with semiconductor nanocrystal labels in an ELISA microplate format (see Fig. 12B). A low detection limit of 5 ppb or 28 fmol of thrombin was reached without a preconcentration step typically used in other electrochemical techniques. A reduction of the sample volume and the excellent lower detection limit of the Cd ISE were some of the advantages. Based on this kind of assays various biomolecular interactions can be monitored by using different nanoparticle tracers and corresponding ISEs.

3.5. Electrocatalytic methods

A sensitive and selective electrochemical hybridization detection with the electrocatalytically silver-enhanced gold nanoparticle approach is demonstrated by Hsing group (see Fig. 13A) (Lee et al., 2005). The silver electrodeposition potential is the most important parameter in determining the selectivity and sensitivity of the assay. Streptavidin–5 nm gold is employed as the hybridization indicator, with avidin-modified ITO electrode as the immobilization platform, to enable signal amplification by the silver electrodeposition process at the selected potential. After silver enhancement of the hybrid-bound gold nanoparticle using the electrodeposition method measurement of the deposited silver with linear sweep voltammetry was performed. Following this idea and combined with magnetic collection an electrocatalytical method induced by AuNPs in order to improve the sensitivity of

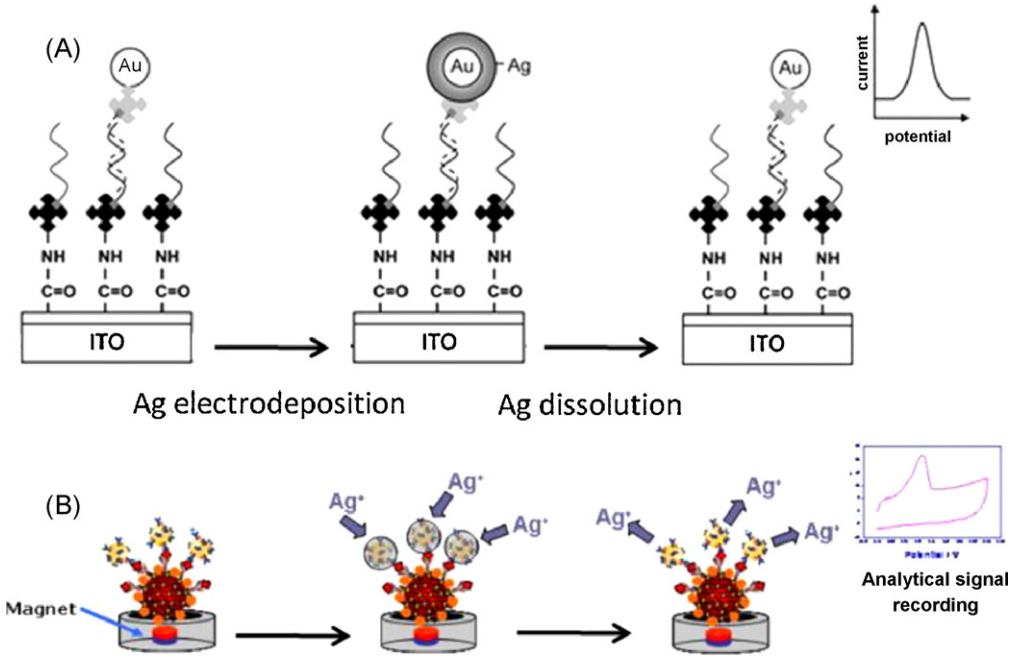

Fig. 13. (A) Partial schematic of DNA hybridization assay protocol with the silver electrodeposition of the 10 nm gold–DNA conjugate tag. The previous steps (not shown) include: electropolymerization of 2-aminobenzoic acid (ABA); covalent attachment of avidin to the carboxyl group of the electropolymerized ABA via EDAC/NHS coupling chemistry; immobilization of biotinylated capture probe via avidin–biotin interaction; hybridization between the capture probe and biotinylated target and specific attachment of the 5 nm gold–streptavidin label. Following the previous steps, shown are: the sandwich with specific attachment of the 5 nm gold–streptavidin label; silver enhancement of the hybrid-bound gold nanoparticle with the electrodeposition method followed by measurement of the deposited silver with linear sweep voltammetry. Adapted from Lee et al. (2005). (B) Magnetoimmunosandwich and steps based on catalytic effect of AuNPs on the silver electrodeposition followed to detect protein. Adpated from De la Escosura-Muñiz et al. (2009a,b).

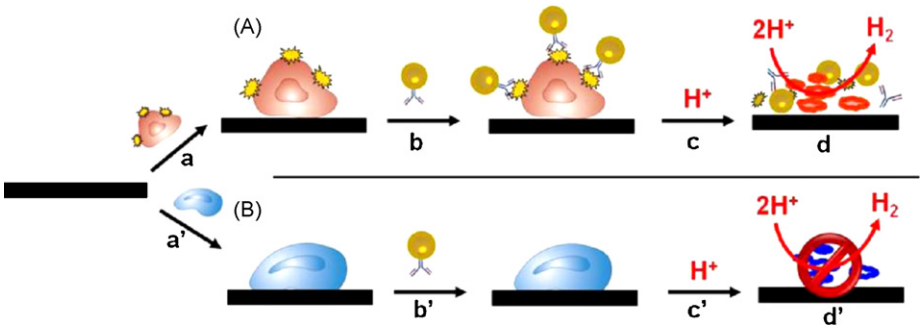

Fig. 14. (A) Tumoral cell line (HMy2) expressing surface HLA-DR molecules compared to a (B) cell line that is negative to this marker (PC-3). Cells were (a,a′) attached to the surface of the electrodes and (b,b′) incubated with AuNP/αDR, (c,c′) an acidic solution was added, and (d,d′) the hydrogen generation was electrochemically measured. Adapted from De la Escosura-Muñiz et al. (2009a,b).

the magnetoimmunosensing technology is reported by our group (see Fig. 13B) (De la Escosura-Muñiz et al., 2009a,b). Microparamagnetic beads as immobilization platforms and AuNPs modified with secondary antibodies as high sensitive electrocatalytical labels are used. A magnet-based carbon electrode allows the collection/immobilization on its surface of the microparamagnetic beads with the immunological sandwich and AuNP used as catalyst tag. The developed magnetoimmunosensing technology allows the antigen detection with an enhanced sensitivity due to the catalytic effect of AuNPs on the electroreduction of silver ions. The low levels of AuNPs detected with this method allow the obtaining of a novel immunosensor with low protein detection limits (up to 23 fg/mL), with special interest for further applications in clinical analysis, food quality and safety as well as other industrial applications.

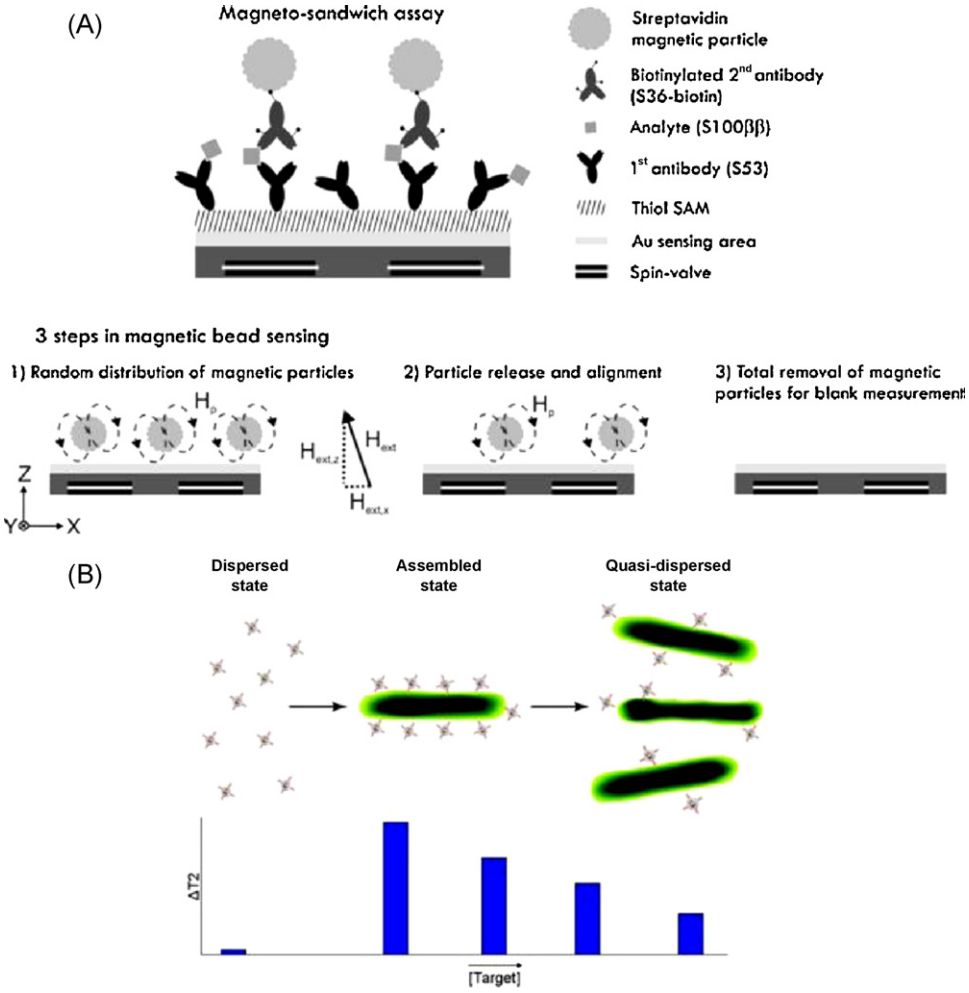

Fig. 15. (A) Schematic diagram illustrating the magnetosandwich assay applied in this work for the detection of S100β and the three steps performed during the MBS measurement. First (1) the MPs are specifically bound (i.e., randomly distributed) to the sensor surface, followed by (2) the regeneration of the biomolecular bonds and alignment of the MPs to the most sensitive part of the spin valves. (3) Finally, all particles are washed away from the sensor surface to perform a blank measurement. Adapted from De Palma et al. (2007). (B) Proposed detection mechanism of bacterial targets using superparamagnetic nanoparticles. Disperse nanoparticles in solution self-assemble on the surface of a bacterial target inducing large changes in *T*2. As the number of bacteria increases, the available number of nanoparticles in solution binds in a more disperse-like state, causing minimal changes in *T*2. Adapted from Kaittanis et al. (2007).

Another interesting property of AuNPs is their electrocatalytic property on hydrogen formation from hydrogen ions at an adequate potential in acidic medium. This property was used lastly by our group to quantify nanoparticles and, in turn, to quantify cancer cells (see Fig. 14) (De la Escosura-Muñiz et al., 2009a,b). Proliferation and adherence of tumor cells are achieved on a carbon-based electrode. In-situ identification and quantification of tumor cells is achieved with a detection limit of 4000 cells per 700 μL of suspension. This novel and selective cell-sensing device is based on the reaction of cell surface proteins with specific antibodies conjugated with AuNPs. The catalytic current generated by the reduction of the hydrogen ions is chronoamperometrically recorded and related to the quantity of the cells of interest.

4. Other techniques

4.1. Magnetic sensors

Surface-functionalized paramagnetic particles are widely employed in various protein and DNA detection systems as immobilization platform with interest for purification/incubation processes. Although they are originally designed to enhance and simplify the protein/DNA isolation and purification process, their field of application has significantly widened.

Sensing based on the combination of giant magnetoresistive (GMR) sensors and magnetic nanoparticles has attracted much attention as a promising alternative, since such a system has the unique merits of portability, low cost, rapid detection, ease for integration into lab-on-a-chip systems, and lack of magnetic background in biological samples.

A magnetic bead sensor (MBS) platform that allows immunosensing over a wide range of target concentrations is developed (De Palma et al., 2007). In this MBS the strong dependence of the MBS signal on the specific location of the magnetic particles relative to the spin valves has been exploited (see Fig. 15A). By repositioning the magnetic particles to the most sensitive location on the spin valves, the MBS signal can be increased to its theoretical maximum.

Superparamagnetic iron oxide nanoparticles to identify Mycobacterium avium spp. paratuberculosis (MAP), through magnetic relaxation, are also reported (see Fig. 15B). The principle underlying the detection mechanism of this nanosensor is based on its ability to switch between a disperse and clustered (or assembled) state upon target interaction, with a concomitant change in the spin–spin relaxation time ($T2$) of the solution's water protons. On the basis of changes in $T2$ ($\Delta T2$) various molecular targets such as nucleic acids (DNA and mRNA), proteins and even viruses have been detected. Even a multiplex detection of DNA sequences was performed by using several sizes of probe-tagged magnetic beads.

4.2. Lateral flow devices

The first dry-reagent strip system that makes use of oligonucleotide-conjugated gold nanoparticles as probes seems to be the one reported by Glynou et al. in 2003 (Glynou et al., 2003). The authors combined the highly specific molecular recognition properties of oligonucleotides with the unique optical properties of AuNPs enabling a simple visual detection of double stranded DNA within minutes in a strip. The assay does not require instrumentation and avoids the multiple incubation and washing steps performed in most current assays. AuNPs reporters with oligo(dT) attached to their surface form an integral part of the strip and are used for the detection of biotinylated PCR products (see Fig. 16). The sensor offers at least eight times higher detectability than ethidium bromide staining of agarose gels and provides confirmation of the amplified fragments.

A disposable nucleic acid biosensor for low-cost and sensitive detection of nucleic acid samples (within 15 min) is also developed by Liu et al. This device also combines the AuNPs properties with the high efficiency of chromatographic separation. Sandwich-type DNA hybridization reactions were realized on the lateral flow strips, which avoid multiple incubation, separation, and washing steps in the conventional nucleic acid biosensors. The same group took advantages of the highly specific molecular recognition properties of aptamers and combined these with the AuNP properties in the

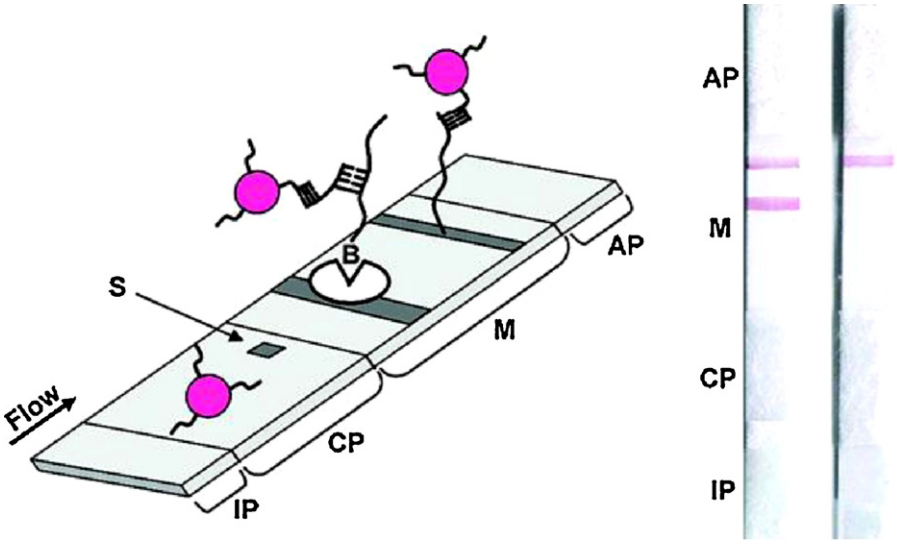

Fig. 16. (A) Parts of the proposed dry-reagent strip and principle of the strip-based hybridization assay for target DNA. Poly(dT) conjugated gold nanoparticles are deposited on the "conjugate pad". Streptavidin (SA) is immobilized on the "test zone". Poly(dA) strands are immobilized on the "control zone". The target DNA (a biotinylated amplified fragment) is hybridized with a poly(dA)-tailed probe and loaded on the "sample application" area. The strip is immersed ("immersion pad") in the running buffer which then migrates and rehydrates the nanoparticle conjugates that hybridize with the sample. The hybrids are captured in the test zone, giving a characteristic red line. Excess nanoparticles are captured in the control zone giving a red line. IP = immersion pad; CP = conjugation pad; S = sample application area; M = membrane; AP = absorbent pad. The two shaded areas in the membrane represent the test zone and the control zone of the strip. (B) A positive and a negative strip-test. In the absence of target DNA, there is no observable signal in the test zone of the strip. From Glynou et al. (2003).

Table 1
Summary of some of the main characteristics of the reported nanoparticles-based biosensing systems.

	Role of nanoparticles	Detection methodology	Main advantages	Main drawbacks	Instruments availability
Optical techniques					
Light absorption	Label	Direct and Indirect	Simplicity, low cost	Low sensitivity	Yes
Light-scattering	Label	Direct	High sensitivity	–	Yes
Surface plasmon resonance	Label, Transducer's modifier	Direct	High sensitivity	–	Yes
Fluorescence	Label	Direct and Indirect	High sensitivity	–	Yes
Fluorescence quenching	Label	Indirect	High sensitivity	–	Yes
Auto-quenching of fluorescence	Label	Direct	High sensitivity	–	Yes
ICPMS	Label	Direct	High sensitivity	Analysis time	Yes
Electrical techniques					
Electro-mechanical detection	Label	Direct	High sensitivity	–	Yes
Conductivity	Label	Direct	Simplicity, high sensitivity	–	Yes
Stripping analysis	Label, Transducer's modifier	Direct	Simplicity, high sensitivity	–	Yes
Potentiometric analysis	L	Indirect	Simplicity	Low sensitivity	Yes
Electrocatalytic methods	Label, Transducer's modifier	Direct	Simplicity, high sensitivity	–	Yes
Other techniques					
Magnetic sensors	Label	Direct	High sensitivity	–	Yes
Lateral flow devices	Label	Direct and Indirect	Simplicity	Low sensitivity	No

As summarized in Table 1 it is interesting to note that in most of the biosensing systems nanoparticles are used as labels although interest for including these as modifiers of trasnducers (both electrical and optical) is also shown. Regarding the NP label detections the most used is the direct one where a physical property of NPs (i.e. light absorption or current produced by their oxidation) is directly related to the NP quantity and consequently to the biomolecule quantity too. Nevertheless indirect detection methods such as catalytic properties are also used.

development of a dry-reagent strip biosensor that enables qualitative (visual)/quantitative detection of protein within minutes.

5. Concluding remarks

Recent advances in nanoscience and nanotechnology have enabled a paradigm shift in biosensing technology. In this review an attempt to give a broad overview on the use of nanoparticles for DNA, protein and cell analysis is made. Nanoparticles are leading to the development of various biosensing devices with interest for applications in several fields. Optical detection alternatives based on light absorption and scattering induced by nanoparticles used as labels are being extended not only to DNA and protein analysis fields but also to other fields (i.e. heavy metal detection (Aragay et al., 2010)). Surface plasmon resonance enhancement by nanoparticles is also a novel alternative that is bringing advantages to this technique in terms of sensitivity. Between the different optical techniques those related to fluorescence measurements including its quenching monitoring while using nanoparticles as tracers of biomolecules are without doubts very interesting not only for the shown sensitivity but the possibility to achieve multidetection through multiplexing alternatives. From the other side the nanoparticle-based SERS are showing to be with special interest for high sensitivity DNA (Sun et al., 2007), viral pathogens (Driskell et al., 2005) and proteins (Song et al., 2009), (Chon et al., 2009) detections with interest for clinical applications.

As summarized in Table 1 it is interesting to note that in most of the biosensing systems nanoparticles are used as labels although interest for including these as modifiers of transducers (both electrical and optical) also is shown. Regarding the detections of NP labels the most used is the direct mode where a measured physical property of NPs (i.e. light absorption or current produced by their oxidation) is directly related to the NP quantity and consequently to the quantity of biomolecule to be analyzed. Nevertheless indirect detection methods such as catalytic properties are also used. In general the NP-based systems use to offer high sensitivity in connection also to the simplicity of the used detection technologies. Almost all the detection technologies are based on conventional/established ones previously used in existing labeling based biomolecule detection techniques. This represents an additional advantage of NP-based system: the NP-based biosensing technology is related to established techniques and instrumentation. NPs are just being used as 'reagent' in the various bioassays. Nevertheless for special applications, i.e. in-field applications, simpler instruments, focused only to NP detection, may be developed and this will depend on market needs.

In terms of simplicity, miniaturization and cost electrical methods (electromechanical, stripping analysis, potentiometric etc.) that use nanoparticles as biomolecules tracers represent an interesting field of research and application.

In most of the examples nanoparticle-based biosensing systems are being offered as excellent screening and advantageous alternatives to existing conventional strategies/assays and the corresponding conventional optical and electrical/electrochemical equipments. Although several nanoparticle-based biosensing strategies have been demonstrated during the last decade most of them have not yet been shown to be effective in a production scale technology. Besides problems related to the homogeneity of the NP related biosensing measurements aspects concerning the toxicology of the used nanomaterials should be carefully addressed. This will probably lead to advances in the theranostic (Ho and Leong, 2010) applications of the developed NP-based systems with interest probably for drug discovery and other applications.

The future development of microfluidic paper-based analytical devices (μPADs) (Martinez et al., 2010), a new class of in-field or point-of-care diagnostic devices that are inexpensive, easy to use in connection to the use of nanoparticles can be another important trend that would bring real application possibilities of future NP-based biosensing systems. The integration of NPs within dry-reagent strip-type (i.e. lateral flow) can open the way to the development of other devices for fast, low-cost, sensitive, and specific detection of DNA, proteins and even cells.

Although the NP-based biosensing systems are relatively new these are strengthening year by year and are showing to be novel alternatives to several other biosensing systems opening in this way new horizons for research and applications with interest for health care, environmental monitoring as well as safety and security of citizens.

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