Immunosensing using nanoparticles

Immunosensing technology is taking advantage of the lastest developments in materials science and inparticular from the nanomaterials field. Because of their unprecedented optical tunability as well as electrical and electrochemical qualities, we are seeing significant developments in the design of novel immunoassays; various conventional optical and electrical platforms which allow for future applications in several fields are being used. Properties of nanoparticles such as light absorption and dispersion are bringing interesting immunosensing alternatives. Nanoparticles are improving the sensitivity of existing techniques used for protein detection in immunoassays based on Surface Plasmon Resonance, Quartz Crystal Microbalance, Fluorescence spectroscopy etc. Electrochemical techniques are also taking advantage of electrical properties of nanoparticles. Redox properties of metal based nanoparticles, surface impedance change and conductance changes once nanoparticles are present as labelling tags or modifiers of transducer surfaces are also improving the technology. In most of the examples nanoparticle based biosensing systems are being offered as excellent screening and superior alternatives to existing conventional strategies/assays with interest for fields in clinical analysis, food quality, safety and security.

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Recent developments in nanotechnology are having marked effects on different industries ranging from electronics to biomedical engineering, by producing a new group of nanomaterials such as nanoparticles (NPs), including quantum dots (QDs), with unique semiconducting and light-emitting properties amongst other characteristics. Nanomaterials are defined as having a size regime of less than 100 nanometers and, more specifically, QDs range from 2-10 nanometers. From the material point of view, NPs are so small that they exhibit characteristics that are often not observed in the bulk materials. For example, gold nanoparticles (AuNPs) have unique properties such as a strong absorption in the UV-Vis up to the NIR region. QDs have unique properties because at this size they behave differently to their bulk equivalents and exhibit unprecedented tunability, enabling completely new applications in science and technology. This has required the continuous search for novel routes of synthesis of fabricated NPs from different materials such as Au, Pt, Fe, Co, CdS, Pd, Cu etc.

Between the different applications the use of NPs for biosensing is showing an increased interest for several areas such as clinical analysis[1,2], environmental monitoring[3] as well as safety and security.

NPs involvement in DNA, protein and even cell sensing systems have recently been the hottest topics in nanobiotechnology. Given the special importance of protein analysis we intend in this review to overview some of the major advances and milestones in the field of immunodetection systems based on NPs underlying the different approaches reported so far. Particular emphasis will be on the different optical[4] and electrochemical[5,6] detection methodologies where NPs are showing significant impact. In certain cases assays based upon nanomaterials have offered significant advantages over conventional diagnostic systems with regard to assay sensitivity, selectivity, and practicality[7].

Antibodies, modifications with nanoparticles and immunosensing

The structure of IgG, the most used antibody in immunosensig assays, has been determined by X-ray crystallography (Fig. 1a, upper part) which shows a Y-shape form consisting of three equal-sized portions, loosely connected by a flexible tether[8]. The antibodies are constituted by two heavy and two light polypeptide chains linked between them as shown in Fig. 1a (lower part). The C regions determine the isotype
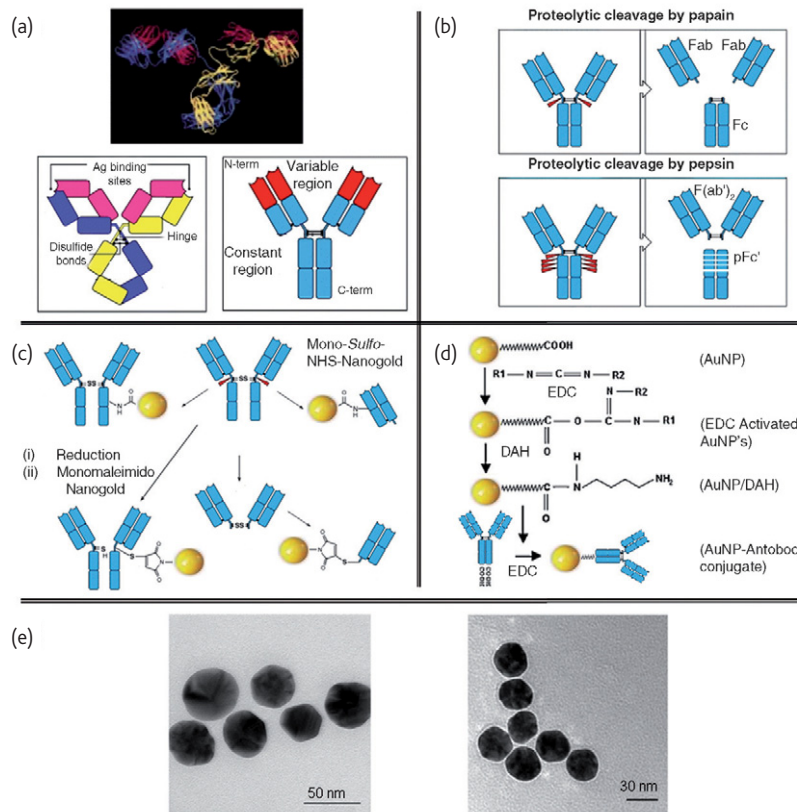

Fig. 1 (a) X-ray crystallographic structure of an IgG antibody (upper part); A schematic representation of IgG and a simplified scheme of the antibody's structure (lower part). (b) The papain (upper part) and the the pepsin (lower part) cleavages. (c) Functionalization of a whole IgG or a Fab by a Mono-Sulfo-NHS-AuNP via free amino groups (upper part); monomaleimido AuNP binds the S atom after the reduction of the disulfide bond starting from the whole IgG (lower part, left) or from $F(ab')_2$ (lower part, right). (d) An activated by EDC AuNP coated with gluthatione, as spacer arm, reacts with an IgG, also activated by EDC. (e) TEM images of AuNPs (left part) and AuNPs conjugated with IgG (right part). Image shows a thin white layer, called "halo" effect, surrounding the surface of the AuNPs indicating coating with protein. Adapted from[8-10] and www.nanoprobes.com with permission.

of the antibody whereas the variable regions of one heavy and one light chain constitute an antigen binding site (ABS). The digestion of antibody with papain (Fig. 1b upper part) and pepsin (Fig. 1b lower part) proteases produces several smaller fragments that may be used instead of the original antibody for immunosensing applications.

Immunoassays are based on the interaction between the antibody and the antigen, in particular between the ABS and the epitope. In fact this interaction gives high specificity and sensitivity to the immunoassay. In order to functionalize the Ig, the connection of labels through three main groups: -NH$_2$, -COOH and –SH have been performed. Fig. 1c (upper part) shows two examples of conjugation of mono-sulfo-NHS-AuNPs with an antibody through the amino group. A conjugation of monomaleimido AuNPs (Fig. 1c, lower part) with an antibody, through the –SH group, is also shown. The use of the thiol group for the functionalization is a good way to control the direction of the bond between the label and the antibody, and prevent the involvement of the ABS. Fig. 1d is an example of conjugation through

the carboxyl group reported by Ahirwal et al.[9]. They connected the carboxi-term of the antibody with a AuNP by glutathione used as a spacer. The C-term region is a good point to attach the label, since it is far from the ABS and should allow the molecule interaction with the antigen. The AuNP modification with protein can even be observed by TEM (Fig. 1e)[10].

In the following part the different immunosensing technologies ranging from optical (i.e. colorimetric) to electrical (i.e. voltammetry) techniques that involve the use of NPs will be discussed.

Colorimetric detection
AuNPs have an extraordinarily high extinction coefficient, emanating from the inherent plasmonic properties[11]. Their optical properties are strongly dependent on the interparticle separation distance, and aggregation that cause a massive shift in the extinction spectrum manifested as a color change of suspensions from red to purple[12]. The clearly distinguishable color shifts facilitate a very simple sensor
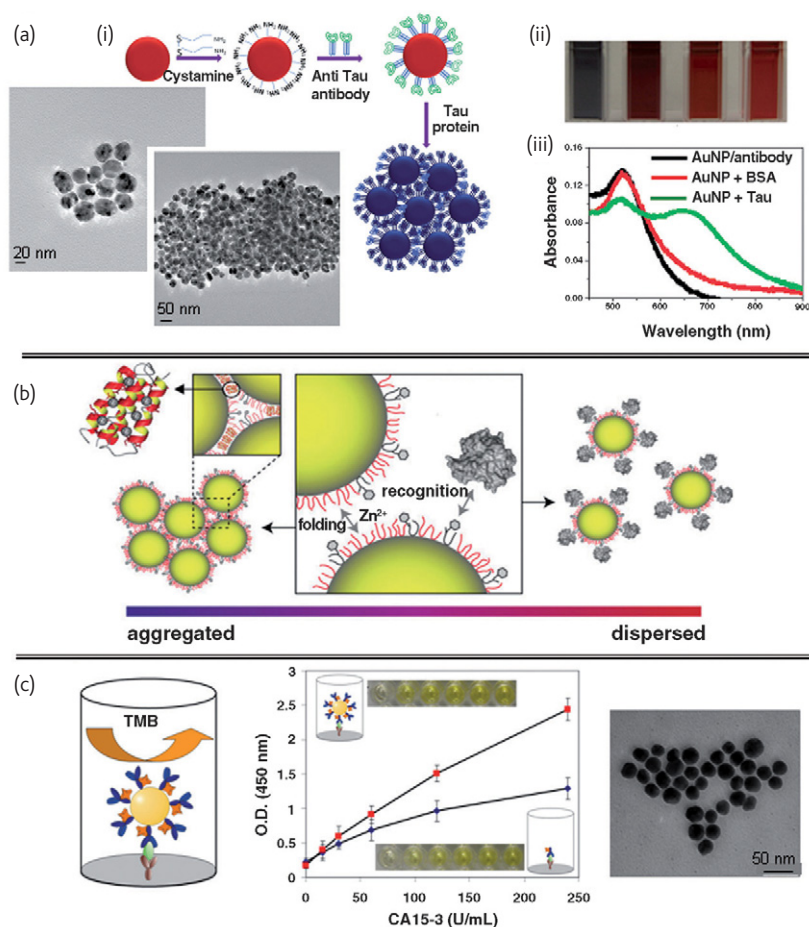

Fig. 2 (a) Colorimetric assay based on aggregation of AuNPs. (i) Schematic representation of the assay and TEM images of the antibody conjugated AuNPs before and after the aggregation; (ii) Photograph showing colorimetric change upon addition of increasing concentrations of specific antigen; (iii) Absorption profile variation of AuNP/antibody due to the addition of specific protein. (b) Colorimetric assay based on dissagregation of AuNPs. AuNPs modified with a polypeptide designed to allow folding induced particle aggregation triggered by Zn^{2+} and a polypeptide-based synthetic receptor for binding of protein analytes (c) Enhancement of enzymatic colorimetric assay by NPs carriers. AuNPs modified with antibodies labelled with an enzymatic label -HRP- exert an amplification effect on the enzymatic colorimetric signal. Adapted from[16,20,22] with permission.

readout that often can be performed by the naked eye. The use of controllable aggregation of AuNPs for biosensor applications was pioneered by Mirkin et al.[13,14] using DNA hybridization to induce an assembly of particles modified with single-stranded DNA. Most nanoparticle-based colorimetric sensors are designed in such a way that binding of an analyte causes particle aggregation, and consequently a colorimetric response[15].

This sensing principle is recently used by Neely et al.[16] for the detection of Alzhemer's tau protein in the 1 pg/mL level using anti-tau antibody-coated AuNP. When anti-tau antibody-coated AuNPs are mixed with tau protein, a displacement in the wavelength of maximum absorbance of AuNPs is observed, allowing its sensitive detection (Fig. 2a). Interesting similar approaches have been reported for the detection of thrombin[17] and rabbit IgG[18] at 0.04 pM and 1.7 nM levels respectively. Glyconanoparticles have also been used as platforms of colorimetric assays for the detection of for example Cholera toxin[19] at 54 nM levels based on its interaction with the lactose contained in the glyconanoparticles.

Some recent works also approach the disaggregation of AuNPs due to the immunological reaction. This is the basis of the work reported by Aili et al.[20] based on polypeptide-functionalized AuNPs. The polypeptide is designed to specifically bind to human carbonic anhydrase II (model protein) and the AuNPs aggregation is induced by the Zn^{2+} triggered dimerization. Folding of a second polypeptide also present on the surface of the AuNP, gives a readily detectable colorimetric shift that is dependent on the concentration of the target protein, allowing its detection at 10 nM levels (Fig. 2b).

In addition to AuNPs, other NPs such as AgNPs[21] have been used in a minor extent, as sensing elements of colorimetric detection methods.

Finally, it must be mentioned the use of AuNPs as carriers of enzymatic labels in colorimetric assays[22], i.e. ELISAs, exerting an amplification effect of the enzymatic signal and improving the detection limits of the proteins (Fig. 2c).

Fluorescence detection

QDs, semiconductor nanoparticles with spectral characteristics, have been intensively studied as unique fluorescent markers[23,24]. QDs with different emission bands that depend on their diameters can be used as labels and all can be excited by a single wavelength. This detection mode is reported by Soman et al.[25]. They used QDs with different emission bands labelled with different antibodies. The simultaneous detection of angiopoietin-2 and mouse IgG model proteins is achieved in a simple way, with high sensitivity and selectivity (Fig. 3a). CdTe[26], ZnO[27] and CdSe@ZnS[28,29] QDs are other examples where NPs have been used as direct fluorescent labels for the detection of proteins at very sensitive levels.

Another strategy for immunodetection approached over the last few years consists of the use of core-shell NPs formed by inert core nanoparticles (made of silica or polystyrene) and a shell of a fluorescent dye, mainly lanthanide chelates containing Europium (III). These modified NPs take advantage of the flexible surface modification of inert NPs and the narrow absorption and long tailing of the mentioned dyes, resulting in very sensitive immunoassays for prostate specific antigen[30], human hepatitis B surface antigen[31] and interleukin-6[32] amongst others at clinical relevant concentrations.

Finally, it can be highlighted the use of NPs associated with charge complementary fluorescent polymers to produce quenched complexes, as performed by You et al.[33] (Fig. 3b).
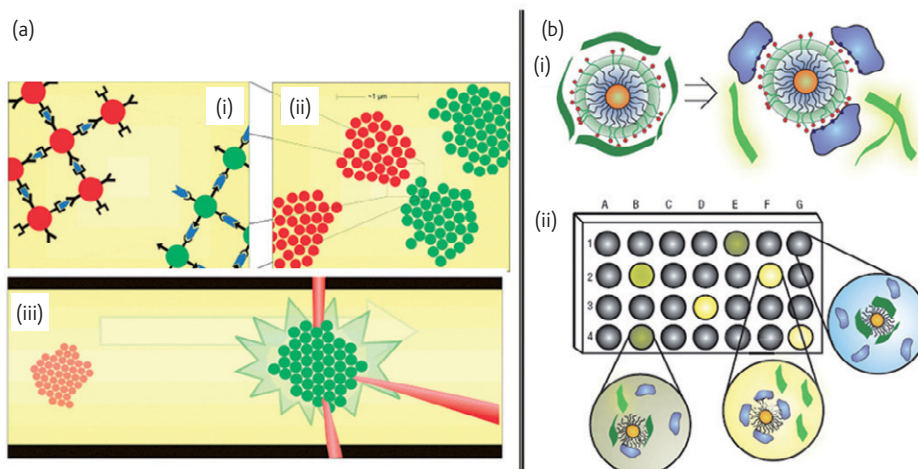

Fig. 3 (a) Fluorimetric detection of QDs. (i) Different QDs are functionalized with polyclonal antibodies for different antigens. Antigens bridge polyclonal antibodies forming two body QDs agglomerates; (ii) Continued agglomeration yields microparticles capable of characterization by i.e. flow cytometry. (iii) The fluorescence of the agglomerates flowing through a microfluidic channel is measured at multiple wavelengths. (b) Fluorophore displacement protein sensor array. (i) Displacement of quenched fluorescent polymer (dark green strips, fluorescence off; light green strips, fluorescence on) by protein analyte (in blue) with concomitant restoration of fluorescence; (ii) Fluorescence pattern generation through differential release of fluorescent polymers from AuNPs. The wells on the microplate contain different nanoparticle–polymer conjugates, and the addition of protein analytes produces a fingerprint for a given protein. Adapted from[25,33] with permission

Surface plasmon resonance based detection

Surface plasmon resonance (SPR) offers a flow-through biosensing technology that measures very small changes in refractive index on a noble metal surface when mass binds to that surface. To conduct a typical protein-protein binding study, one of the protein partners is immobilized on a thin gold film. When the target protein from solution binds to the immobilized protein partner, the binding will cause a refractive index change at the surface layer, which is detected by SPR through resonance angle change of the reflected light[34,35]. One of the most popular commercial SPR systems available is the BIAcore™. The sensitivity of these label-free SPR-based immunosensors can be greatly enhanced by using for example AuNPs tags through the electronic coupling interaction between the localized surface Plasmon (LSP) of the NPs and the surface plasmon wave associated with the SPR gold film for example. In addition, the active area of the LSP-based sensor is smaller, decreasing the minimum detectable number of molecules involved in the binding event. In this way, human IgE at 1ng/mL levels in an aptamer based immunosensor[36] (Fig. 4a) and testosterone[37] at physiologically relevant range have been detected between others. The sensitivity of these assays can be improved even more by secondary enzymatic precipitation produced by enzymes that can be loaded on the surface of the AuNPs[38] and by the fluorescence coming from fluorescence-labeled antibodies loaded on the AuNPs, giving rise to the technique called localised surface Plasmon Resonance Coupled Fluorescence (LSPCF), with fiber optic detection[39,40].

In addition to their surface plasmon resonance absorption, light scattering (LS) is another optical property of AuNPs that is of great interest for biomolecular detection[41,42]. The light-scattering cross-section of an AuNP with a diameter of 60 nm is 200-300 times stronger than that of a polystyrene bead of the same size, and 4-5 orders of magnitude stronger than that of a strong fluorescence dye, e.g., fluorescein[41]. For these reasons, several works have been recently reported developing immunoassays based on either the static, linear, or nonlinear scattering properties of AuNPs[43,44].

Finally, it can also be highlighted the use of the surface-enhanced Raman scattering (SERS) microscopy as very sensitive technique for the detection of NPs tags in immunoassays. SERS is observed for molecules on or nearby the surface of metallic nanostructures that can support localized surface Plasmon resonances. The signal levels observed in SERS are several orders of magnitude higher than in conventional Raman scattering, providing the sensitivity required for bioanalytical and biomedical applications[45-47].

In addition to their extensive use as labels, AuNPs have been directly assembled on the sensing surface, in order to enhance the SPR[48] and SERS[49] signal as a label-free detection system. Furthermore, the AuNPs allow a better oriented immobilization of antibodies, improving the sensitivity and selectivity of the immunoassay (Fig. 4b).

Direct electrical detection

The excellent electroactivity of AuNPs and heavy metal based QDs (i.e. CdS, ZnS, PbS QDs) for example allows the use of both electrical or electrochemical techniques for their detection, reaching low limits of detection (LOD) and consequently detects low concentrations of proteins[50]. NPs can be directly detected due to their own redox properties (of the gold or heavy metals atoms constituents for example) or indirectly due to their electrocatalytic properties toward other species like silver reduction etc.

Several NPs present excellent electroactivity, making direct electrical detection possible, being not necessary any preliminary dissolution step to liberate the metal ions in solution. This direct electrical detection of NPs comprises solid state analysis, where the metals forming the NPs are electrically detected. However, this type of detection needs direct contact between the electrode surface and the metal, and excludes from detection a large portion of non-touching particles. This phenomenon could result in a loss of sensitivity, in contrast with techniques exploiting the total NP dissolution where all metal ions are
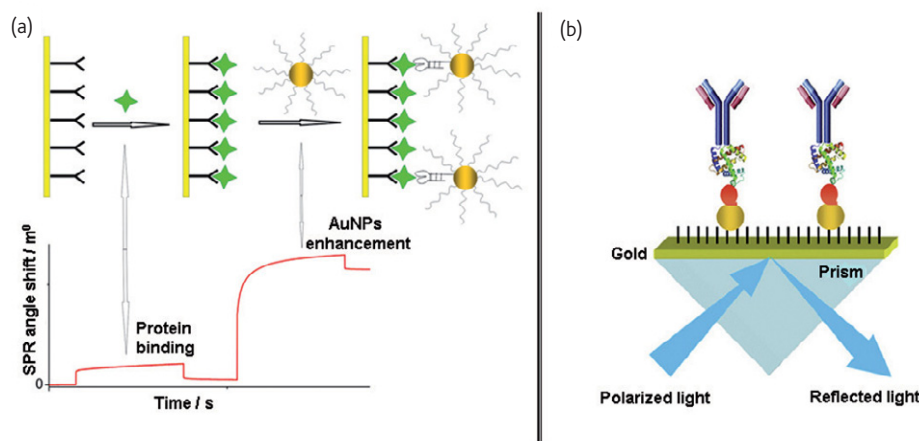

Fig. 4 (a) SPR enhancement by NPs labels. The use of AuNPs conjugated with aptamers in a sandwich assay allows the amplification of the SPR signal of the label-free assay. (b) SPR enhancement by NPs modifiers of the sensing surface The use of AuNPs platforms allows the oriented immobilization of antibody onto the AuNPs assembled SPR immunosensor chips for the further immunoreaction. Adapted from[36,49] with permission.

detected. However, more rapid responses with acceptable LODs with a shorter analysis time and a more compact / integrated immunosensing assays are achieved with this direct detection.

The first work describing direct electrochemical detection of NPs was reported by Costa-García et al.[51]. Important contributions have since been made to direct electrochemical characterization or detection of metal and semiconductor NPs by our group and others, and some of the work has been reported regarding the voltammetric analysis of AuNPs and CdS QDs. In the case of the AuNPs[52], the procedure involves absorption of AuNPs onto the electrode, followed by their electrochemical oxidation in a hydrochloric medium. The resulting tetrachloroaurate ions generated near the electrode surface are detected by differential pulse voltammetry. For CdS QDs[53,54], the procedure is quite similar, but in this case the Cd (II) ions contained as defects in the CdS QDs crystalline structure are electrochemically reduced to Cd (0) and then immediately electro-oxidised to Cd (II), registering the oxidation peak. Based on these principles, solid-state detection of AuNPs[55] (Fig. 5a) and Cds QDs[56] have been applied in immunoassays for the detection of human IgG and carcinoembryonic antigen *respectively* at pM levels.

Indirect electrical detections

The most reported way to electrochemically detect NPs involved in bioassays consists in their preliminary oxidative dissolution in acidic mediums, followed by the detection of the metal ions by a sensitive powerful electroanalytical technique such as anodic stripping voltammetry. Very low LODs, in the order of pM, are achieved due

to the release of a large number of metal ions from each NP and its effective ''built-in'' preconcentration step ensured by electrochemical stripping analysis. Dequaire et al.[57] pioneered the application of this NP detection method for the quantification of IgG as model analyte at pg/mL levels. Since then, AuNPs as well as other NPs, such as CdS@ZnS QDs[58,59] and CdSe@ZnS QDs[60] have been used for the sensitive detection (always in the pM level) of proteins as prostate specific antigen or interleukin 1-α respectively. Furthermore, one of the most important advantages that offers the use of QDs as electroactive labels is their ability to perform multidetection by using QDs made of different inorganic crystals, with different electrochemical responses. This electrochemical coding technology has been approached by Liu et al.[61] for the simultaneous measurements of proteins (β_2-microglobulin, IgG, bovine serum albumin and C-reactive protein) using ZnS, CdS, PbS and CuS QDs as tracers. (Fig. 5b).

It must also be mentioned that the high surface coupled with the easy bioconjugation, make NPs excellent carriers of other electroactive labels in immunoassays. The loading of NPs with other NPs (i.e. AuNPs loaded by CdS QDs[62]) (Fig. 5c) and with enzymes (i.e. AuNPs loaded by HRP[63] and SiNPs loaded by HRP[64] or AP[65]) (Fig. 5d) so as to obtain labels with an enhanced signal have been reported as an interesting alternative for electrochemical immunosensing systems.

Another alternative to detect NPs used as labels in protein detection assays consists in using their catalytic properties toward reactions of other species. The well-known catalytic properties of the AuNPs on the silver chemical reduction have been extensively approached in the last years.
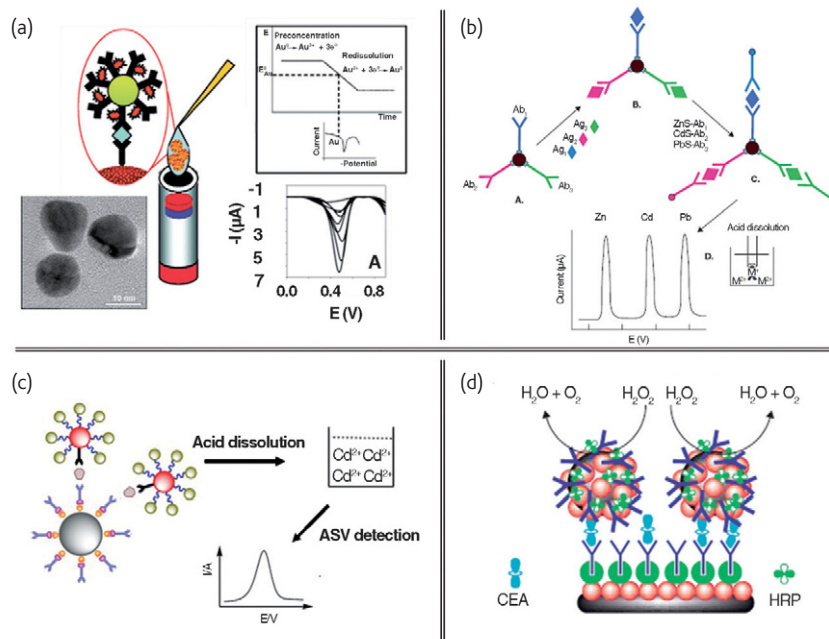

Fig. 5 (a) Electrical detection of NPs. Direct electrical detection of AuNPs tags without acidic dissolving, based on a pre-oxidation and later reduction of Au(III) ions generated. (b) Electrochemical detection of NPs. The electrochemical stripping detection of different QDs labels after their acidic dissolution, allows the multiple detection of proteins. (c) NPs as carriers of other NPs. AuNPs can be used as carriers of a high number of CdS QDs, giving rise to an amplified signal in the further stripping detection after acidic dissolution. (d) NPs as carriers of enzymes. AuNPs can also be used as carriers of a high number of HRP enzymes, exerting an amplification effect in the electrochemical enzymatic signal. Adapted from[56,62-64] with permission.

Recently, our group has developed a very sensitive methodology based on the selective electroreduction of silver ions on the surface of AuNPs in magnetosandwich assays, achieving LODs of human IgG in the fM level[66], and has also exploited the catalytic properties of AuNPs on the hydrogen ions electroreduction in an acidic medium for the antigen-antibody interaction monitoring, applied for cancer cells detection[67] (Fig. 6).

Silver nanoparticles (AgNPs) also exhibit catalytic activity on the chemical reduction of silver ions added as 'substrate'. After that, the silver can be dissolved in for example an acidic medium and measured by anodic stripping voltammetry. This property has been exploited in sandwich immunoassays, for the sensitive detection of human IgG[68] at pM levels.

Finally, in addition to the catalytic activity on metal ions reduction the catalysis of other electrochemical reactions is also reported. For example, the catalytic activity of PtNPs on the reduction of H_2O_2 to H_2O[69], and of the core-shell Au@Pd NPs on the O_2 reduction to H_2O[70] have been exploited by Polsky et al., for the detection of thrombin (nM levels) and cytokine TNF-α (fM levels) respectively.

Electromechanical detection: quartz crystal microbalances and microcantilevers

Microcantilevers and quartz crystal microbalances have emerged in the last few years as versatile biosensors demonstrating remarkable achievements such as high sensitivity and label free detection[71-73] (Fig. 7a, left part). In the case of the quartz crystal microbalance, the bioreaction generates a change in the mass, reordering the charges in the surface of the piezoelectric material and giving rise to a change in the resonant frequency of the microbalance. The use of NP labels in sandwich assays can increase both the surface stress and the mass of the immunocomplex, allowing the increase in sensitivity of these electromechanical assays.

This was the case in work reported by Lee et al.[74] for the detection of prostate specific antigen at pg/mL levels using SiNPs as labels in a dynamic mode microcantilever based biosensor (Fig. 7a, right part and Fig. 7b). In a similar way, quartz crystal microbalance biosensors have been developed using AuNPs, as amplification agents for the detection of human IgG[75] and aflatoxin B1[76] at clinical relevant levels.

NPs as modifiers of electrotransducing surfaces

The presence of NPs on the electrotransducer surface promotes the electron transfer, improving the electrochemical response coming from potentiometric and conductimetric responses. Furthermore, some NPs provide congenial microenvironments similar to that of redox proteins in a native system for retaining their bioactivity giving the protein molecules more freedom in orientation and
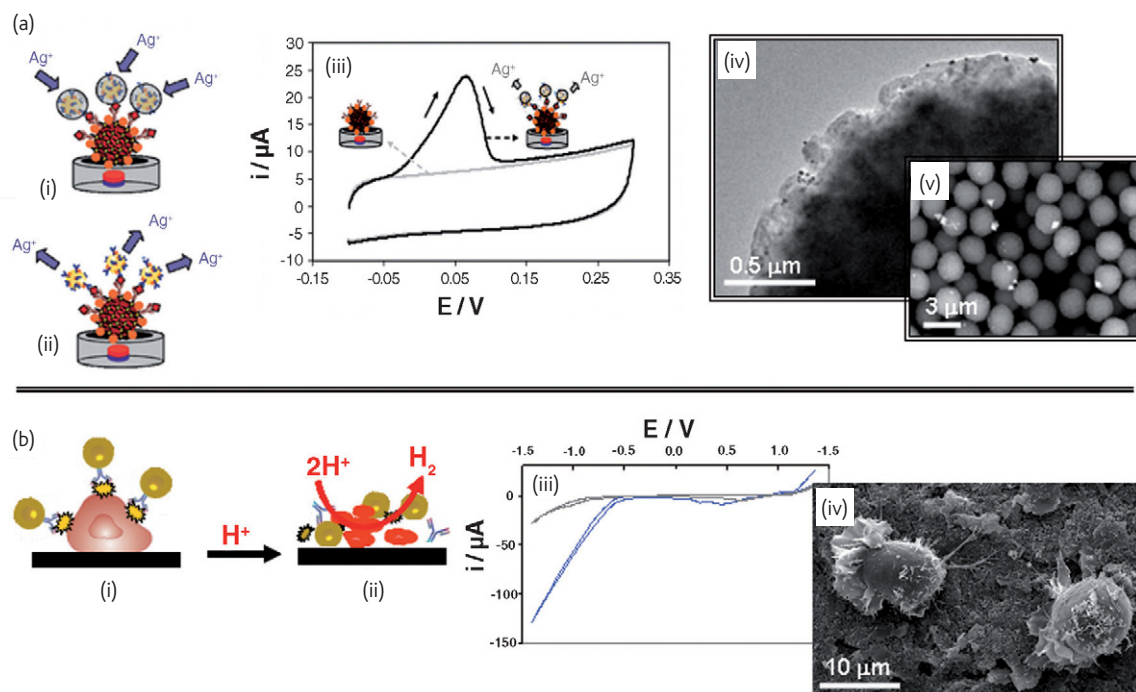

Fig. 6 Electrocatalytic detection of AuNPs. (a) Detection based on the catalytic properties of AuNPs on the silver electroreduction in a magnetosandwich immunoassay (i); the further silver reoxidation (ii) gives rise to a peak current that can be related with the protein concentration (iii); the AuNPs (small black points) can be observed by TEM analysis around the magnetic beads used as immobilization platforms (big black sphere) (iv); the silver crystals formed after the silver electrodeposition (white points) can be observed by SEM analysis around the magnetic beads (v). (b) Detection of cells (i) based on the catalytic properties of AuNPs on the hydrogen evolution in an acidic medium (ii); the presence of AuNPs gives rise to a shift in the reduction potential of the hydrogen ions to hydrogen (lower voltammetric curve) (iii); the cells on the electrotransducer surface can be observed by SEM (iv). From[67,68] with permission.

avoiding their denaturation. The introduction of NPs into the tranducing platform is commonly achieved by their adsorption onto conventional electrode surfaces in various forms including that of a composite.

PtNPs directly electrogenerated[77], AuNPs adsorbed[78], AuNPs integrated in composites[79,80] or AuNPs immobilised on the electrotransducer surface through electrostatic forces on cationic polymeric layers[81] are representative examples of these nanostructurated surfaces built for the detection of proteins such as human IgG, α-fetoprotein, prostate specific antigen and hepatitis B surface antigen at clinical relevant levels.

In addition to the final conductometric, potentiometric and voltammetric detection it is remarkable to see the use of nanostructured surfaces containing AuNPs[82,83], ZrO_2 NPs[84] and $CoFe_2O_4@SiO_2$ NPs[85] for improving the performance of quartz crystal microbalance based immunosensors.

New trends: lateral flow and lab-on-a-chip

A lateral flow assay (LFA) is a prefabricated strip of a carrier material containing dry reagents that are activated by applying the fluid sample[86]. The fluid movement is driven by capillary forces and controlled by the wettability and feature size of the porous or microstructured substrate[87]. When the recognition elements are

antibodies, the test is called lateral flow immunoassay (LFIA). The first LFIA was the pregnancy test, which was based in the detection of human chorionic gonadotropin (HCG), and was introduced into the market in the middle of the '80s[88]. Nowadays several LFIAs to detect drug abuse, cardiac markers, and allergens are commercially available. The LFIA strip is generally divided in 4 zones: sample pad, conjugation pad, detection pad and absorbent pad used to wick the fluid through the membrane. In this way the amount of sample can be increased resulting in an increased sensitivity[86] (see details at Fig. 8a, upper part).

Two formats can be used in a LFIA: competitive and sandwich format. In the competitive format the sample analyte competes with a labelled analyte for the antibodies on the test line[89]. In another type of competitive format an analyte-protein conjugate is sprayed at the test line and the labelled antibody is applied onto the conjugation pad. In this way if the sample contains the analyte then the labelled antibody cannot bind to the test line[90] (Fig. 8b). The sandwich format can be used for analytes with more than one epitope. In this format the sample line is made of an antibody specific for one epitope of the analyte whereas the antibody conjugate with the labelled particles is specific for another epitope. In this way the analyte first binds the antibody of the conjugation pad and then the antibody of the test line. This format gives a signal proportional to the analyte concentration[91] (Fig. 8a, lower part).
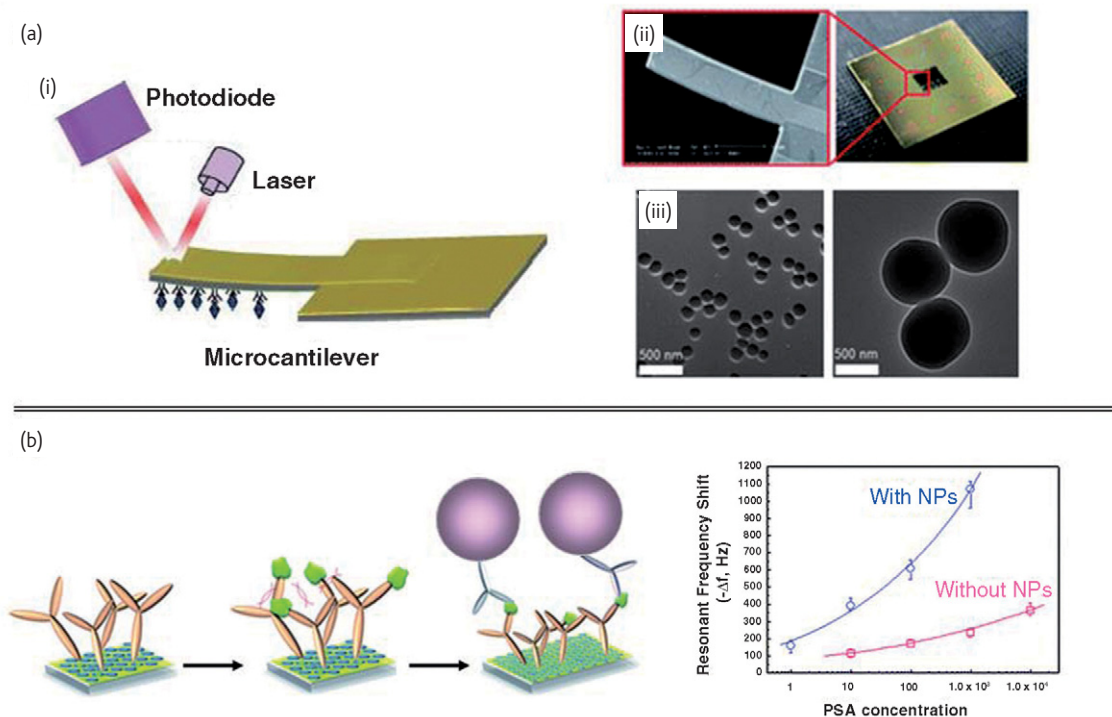

Fig. 7 (a) Electromechanical immunosensing using microcantilevers. (i) The immunoreaction produces a change in the laser reflectance angle on the microcantilever; (ii) SEM image of single microcantilever with dimension of 50 mm/150 mm/1.75 mm (widt/ length/thickness) (left) and photo image (right) of a unit device with twelve microcantilevers (right); (iii) TEM images of SiNPs used to amplify the label-free immunoassay. (b) Electromechanical detection enhancement by NPs. The sandwich assay using polyclonal antibody conjugated SiNPs allows the enhancement of the resonant frequency shift of the microcantilever described in (a-ii). Adapted from[74,75] with permission.

The detection methods are mainly colorimetric and give results mostly qualitative or semi quantitative, even if, in the last year, fluorescent and electrochemical detection produce some quantitative results using a readout device[92,93]. The responses usually come within 2 and 15 minutes. The highest optical detection limit obtained with a sandwich format LFIA is about 1 pg/mL[94], whereas the electrochemical detection limit is about 10 pg/mL. The LFIA strips already have an important market presence due to their ease of use, low cost, at the point of care and fast. These qualities make them suitable for the home care and *in vitro* diagnostics. However, their applications in more complex samples are facing problems related to reproducibility and long-term operation.

Currently, the trend in the development of biosensing systems is towards the miniaturization of the whole analytical chain, from sampling to the detection of the analytes. The advantages of miniaturized systems in general and particularly of microfluidic based biosensors are for reducing the amount of sample and reagents, detection facility, minimal handling of hazardous materials and multiple and parallel sample detection capability. Techniques such as fluorescence, absorbance and electrochemistry have been used for the final detection of the immunoassay. The combination of these microfluidics chips with the use of NPs, gives rise to systems with improvemed performance for immunosensing.

With regard to the electrochemical detection, PDMS–glass hybrid electro-immunosensing chips have recently been reported by Lee's group[95,96] for the multiplex detection of cancer biomarkers (α-phetoprotein, carcinoembryonic antigen and prostate specific antigen) at pM levels, using microbeads as antibody immobilization platforms and measuring the electrical resistance between microelectrodes and taking advantage of AuNPs labels and silver enhancement (Fig. 9a). In addition to the lower volumes of sample needed, the overall assay time were demonstrated to be reduced from 3–8 hours to about 55 minutes when compared to conventional immunoassays.

Alternatively, the multiplex detection of cancer biomarkers at clinical relevant levels has also been performed approaching the fluorescence properties of CdSe@ZnS in an anisotropically etched silicon chip[97] (Fig. 9b).
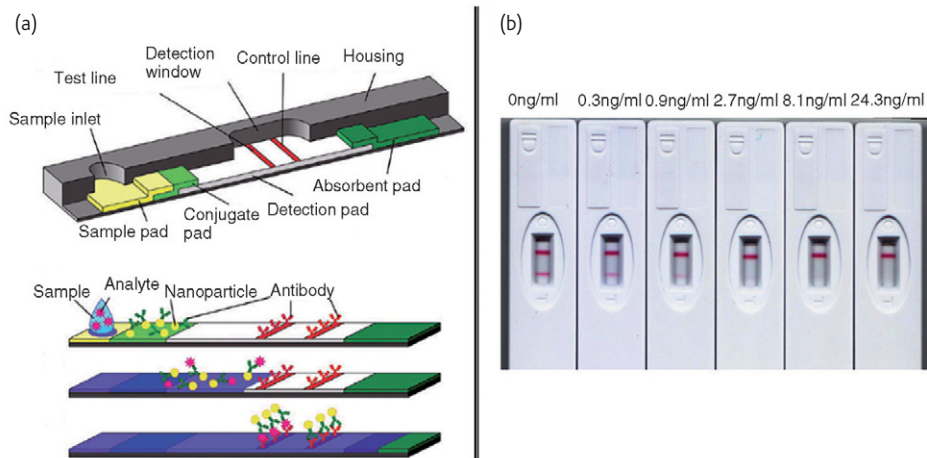
Fig. 8 Lateral flow immunoassay using NPs. (a) Schematic of a lateral flow strip (upper part) and lateral flow immunoassay (LFIA) based on a sandwich format (lower part). (b) Example of results obtained for a competitive LFIA. When increasing the concentration of the analyte, the intensity of test line decreases as expected. Adapted from[88,91] with permission.
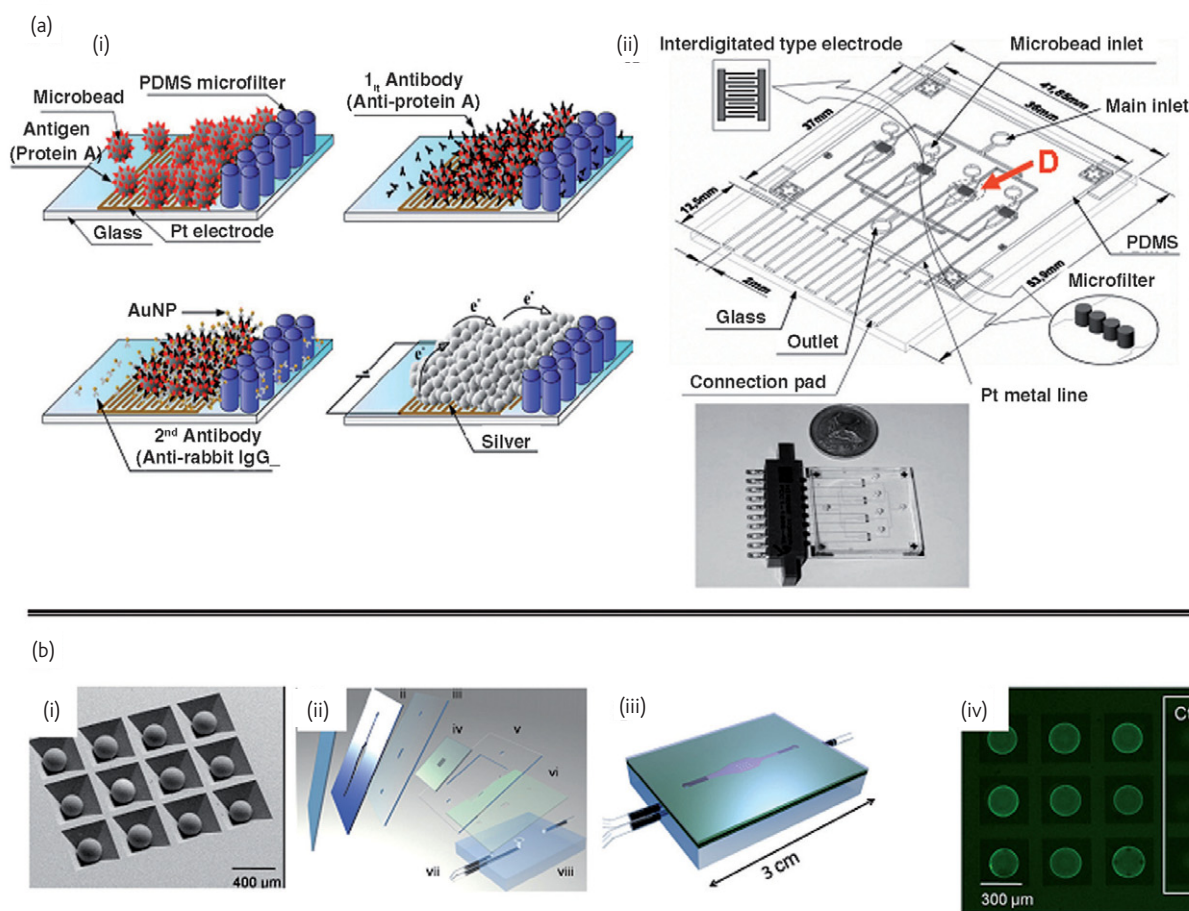
Fig. 9 (a) Electrochemical detection in a microfluidic chips using AuNPs labels. Illustration of the electrical immunoassay. (i) A sandwich immunoassay is carried out in a PDMS-glass chip, using magnetic beads as platforms and AuNPs labels and the final detection is performed by measuring the electrical resistance between microelectrodes, taking advantage of the silver enhancement catalyzed by AuNPs. (ii) 3-D drawing of the design of the PDMS-glass electro-immunosensing chip (the detection zone in the chip is indicated as "D") and photograph of the fabricated chip. (b) Fluorescent detection in a microfluidic chip using QDs labels. (i) SEM photomicrograph of beads in anisotropically etched silicon chip. (ii) PMMA chip layers fabrication; (iii) Sealed LOC assembly; (iv) Fluorescent image of beads after immunoassay including negative controls as imaged with one second of CCD camera integration (exposure) time. Adapted from[96-98] with permission.

Conclusions and outlook

This is a brief overview of a rapidly increasing research field in immunosensing using nanoparticles. The research and applications in this field are at the crossroads of materials research, nanosciences, and molecular biotechnology. Since the nanoparticles and biomolecules typically meet at the same nanometer length scale, this interdisciplinary approach will contribute to the establishment of a novel field, descriptively termed immunosensing nanotechnology or nanoimmunosensing.

Future development of immunosensing technology will continually profit from the rapid current advances in material science and particularly that of nanomaterials science thus producing novel nanoparticles with improved physical and chemical properties. In addition, the current proteome research will also benefit since it provides data that allows the production of even more relevant bioreceptors crucial for this technology. These developments will open the door to more effective nanoimmunosensors with a focus on biomedical applications.

In addition to the long term perspectives, today's advanced nanomaterials as well as the modern optical and electrochemical techniques originally developed for materials research, physics and biology are already being used in immunosensing applications or as model systems with an interest in basic research as well as to solve biological and clinical analysis problems. NP based immunosensing systems are proving to assist in the development of new and versatile protein detection methods. In most of the reported applications NPs are seen as immunoassay labels achieving higher sensitivities as well and multiplexing analysis. They are also used as modifiers of the electrochemical or optical tranducing platforms.

Clearly, we are still far from the real nanosensors that can operate in an automatic mode in living organisms. However the progress in nano and microsystem technology including nanomotors may open the way to real nanosize sensors and even to a new class of immunosensors that in

an intelligent way could combine diagnostic and therapy in the so called theranostic[98] devices. This can probably significantly shorten the time from protein sensing (diagnostic) to therapy (i.e. cancer cell fighting) that would improve an individuals life and ensure a better safety and security. [mt]

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