Nanoscale labels: nanoparticles and liposomes in the development of high-performance biosensors

Technology for the detection of biological species has generated considerable interest in a variety of fields including healthcare, defense, food and environmental monitoring. In a biosensor, labeled specific binding partners are used to emit a detectable signal. Owing to their unique properties, nanomaterials have been proposed as a novel label category and have led to the development of new assays and new transduction mechanisms. In this article, the role of three major types of nanoscale labels (metallic, semiconductor and liposome nanoparticles) in the development of a new generation of optical, electrochemical or gravimetric biosensors will be presented. The underlying transduction principles will be briefly explained and assay strategies relying on the use of these 'nanolabels' will be described. The contribution to increased assay performance and sensitivity will be highlighted. Approaches towards simple, cost efficient and sensitive assays are essential to meet the demands of a growing number of applications.

KEYWORDS: biosensing ▪ liposome ▪ metallic nanoparticle ▪ quantum dot ▪ semiconductor nanoparticle

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Over the last decade, sensors for the detection and identification of specific biological compounds, commonly proteins or DNA strands, have seen their application field grow considerably. These so-called biosensors include portable field-oriented devices for applications in, for example, food technology and environmental monitoring as well as high-performance instruments for use in medical diagnostics, or biological and pharmaceutical research.

Biosensors commonly take advantage of the highly specific interactions between two biological binding partners (e.g., antigen–antibody or complementary oligonucleotides) to detect the presence of the biomolecule of interest (target molecule) with accuracy. The most common assay format is the sandwich assay, where a capture element immobilized on the sensor surface captures the target from solution, which is subsequently detected upon binding of the signal-generating labeled reporter molecule. Other assay formats include direct assays, where the (labeled or unlabeled) target is detected directly from the solution after binding (i.e., without any subsequent biomolecular binding step) or reverse phase assays, where a complex biological sample is directly immobilized on the surface (i.e., without any capture element) and the target subsequently detected using a reporter molecule [1–3]. One can also distinguish between competitive and noncompetitive assays. In a noncompetitive assay, the labeled reporter molecule binds to the surface in the presence of the analyte, generating a signal directly proportional to the target concentration. Alternatively, in a competitive assay, a labeled analyte competes with the unlabeled analyte of interest for a limited number of binding sites, resulting in an indirectly proportional signal.

Although label-free biosensors are also available for certain applications [4,5], most current biosensors still rely on biomolecule labeling for signal generation. Initially, radiolabeling had been used in various biological assays; however, nowadays, optical detection is the most widespread. Optical transduction relies on the generation of a colorimetric, fluorescent or chemiluminescent signal with fluorescent dyes or enzyme labels commonly being used for signal generation. More recently, electrochemical transduction methods have also started to appear with the advantage of exhibiting miniaturization capability (for the production of portable devices), and of being low cost and less sophisticated compared with, for example, conventional fluorescence readers [6,7].

Recently, much effort has been put into the development of biosensors with increased performance and sensitivity. This includes research on novel assay formats, amplification schemes and transduction mechanisms as well as the development of new high-performance devices. The combination of nanotechnology and biotechnology has gained considerable momentum in various scientific communities bringing physicists, chemists, biologists and material scientists

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closer together. Owing to their unique properties, nanometer-sized materials, including nanoparticles and vesicles self-assembled from amphiphilic molecules, have been applied to biosensors in various situations, including surface modification, labeling or as 'nanosensors' in solution-based assays. Their applications in biotechnology have been reviewed recently in several publications [7–25]. These reviews give either a general overview on the use of nanomaterials in biosensing or restrict themselves to one specific area, focusing either on a specific particle type or on a specific transduction mechanism.

In this review, we systematically address the function of nanoparticles as signal-generating labels, focusing on situations where the biorecognition event is detected after formation of a complex between the target and a tagged reporter molecule on the sensor surface. An overview of three major nanoscale labels, namely metallic and semiconductor nanoparticles as well as phospholipid vesicles, will be provided. We will demonstrate how these 'nanolabels' can provide the means for signal amplification in optical, electrical or microgravimetric biosensors while overcoming limitations of traditional labels. Their main physical or chemical properties will be described and then their contribution to the development of more efficient biosensing devices highlighted. Relevant examples of biological assays will be discussed for each case.

Metallic nanoparticle labels

Metallic nanoparticles are not new in biosciences. Gold colloids have long been used for histological staining and visualization with transmission-electron or light microscopy [26]. Metallic nanoparticles are also emerging in biosensing applications owing to their unique optical, electronic and catalytic properties. For general reviews on the properties and the biological or biosensing applications of metallic nanoparticles refer to the following references [6,7,11,15,21,27,28]. Moreover, assay protocols can take advantage of a silver enhancement step, involving the wet chemical deposition of metal on the nanoparticle in the presence of a reducing agent, in order to increase the particle size after biorecognition, and therefore increase the sensitivity (Figure 1A) [29].

An additional advantage of gold particles for DNA sensing is the fact that oligonucleotides labeled with gold nanoparticles exhibit melting profiles sharper than fluorophore-labeled strands, making a sensitive discrimination of hybridized strands with a few mismatches possible [30]. This is especially important for the detection of single nucleotide polymorphisms (SNPs).

In this section, we will focus on the use of metallic labels in conjunction with optical and electrochemical detection setups. Other transduction mechansims not addressed here rely, for example, on scanning-electron microscopy for microarray readout [31], photothermal imaging [32], scanning electrochemical microscopy [33] or microgravimetric signal amplification [34–37].

Metal labels for optical biosensors

Visual & scanometric detection

The increase in particle size resulting from silver staining makes it possible to detect low particle concentrations by the naked eye [38,39], using optical microscopy [40] or a charge-coupled device (CCD) camera [41], or with a conventional flatbed scanners (Figure 1A).

Metallic staining and naked-eye detection was reported for immunoblots [39] and in membrane strip sensors [38,42]. For the immunoblot, naked-eye DNA detection down to 10 pg/ml was achieved with an optimized gold-enhancement protocol. In the lateral flow assay presented recently by Mao *et al.*, the sensitivity reached 50 pM if the signal was further enhanced by the generation of a colored enzymatic product generated by particle-bound horseradish peroxidase [42].

Scanometric detection of nanomolar DNA target concentrations on a microarray with a conventional flatbed scanner and silver amplified gold colloids was first described in 2000 by Taton and coworkers [30]. With a commercially available optical setup using side illumination and evanescent wave induced light scattering (Nanosphere Inc., Verigene, USA [301]), the detection limit was reduced to less than 0.0025 probes/μm^2, or an 1000-fold increase in sensitivity when compared with conventional fluorescence scanners [43]. This technology was applied, for example, to the detection of RNA expression levels [44] and SNPs [45] from unamplified targets in a DNA sandwich assay.

Scanometric detection of prostate-specific antigen [46,47], Alzheimer's disease marker (amyloid-β-derived diffusible ligands) in cerebrospinal fluid [48], double-stranded genomic DNA [49] and protein was achieved using a biobarcode assay developed at Northwestern University (IL, USA) and illustrated in Figure 1B. This approach uses magnetic beads for target detection and separation as well as nanoparticle labels tagged with a DNA barcode. Indirect detection of the released

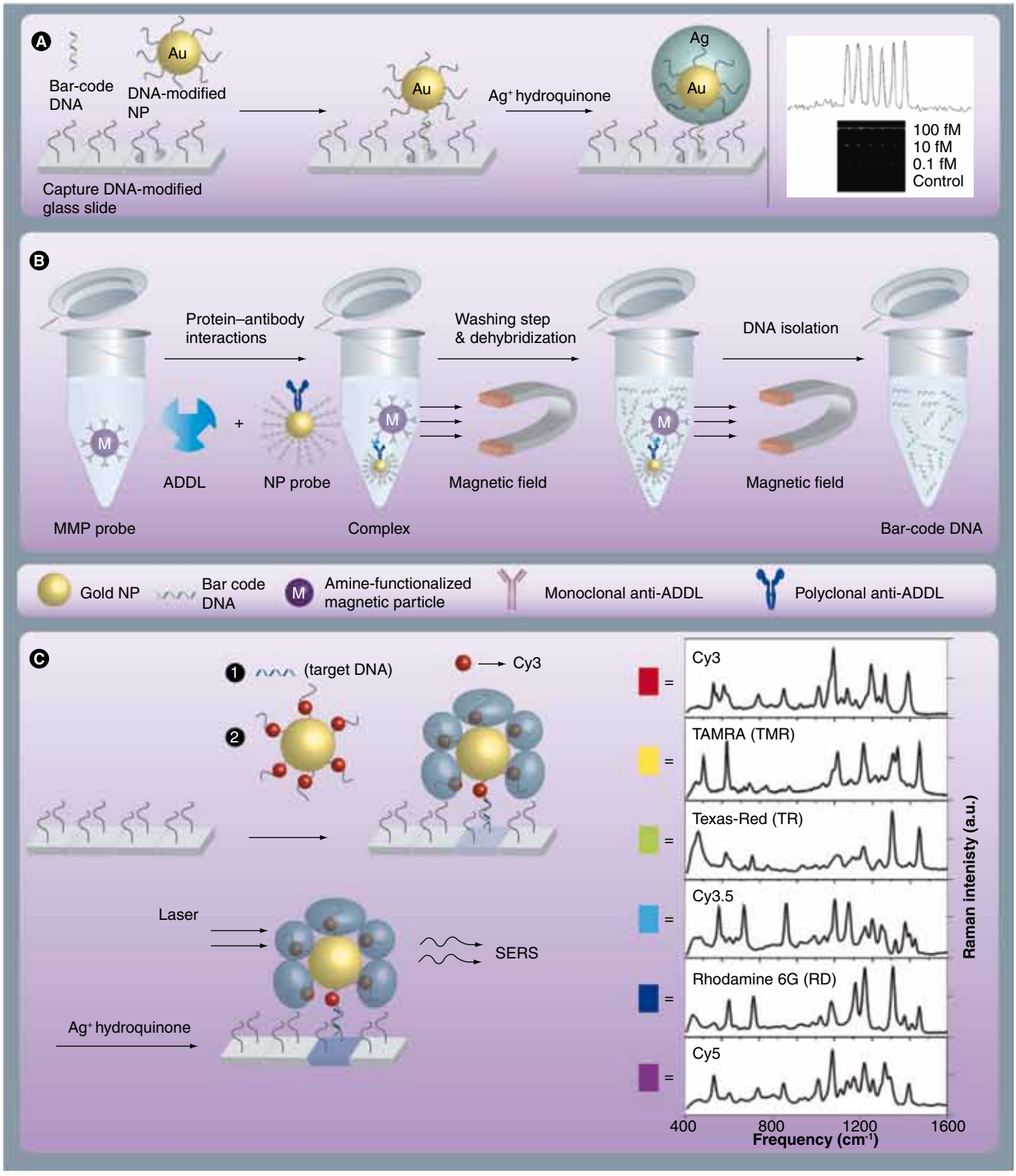

Figure 1. Detection of gold colloid labels after silver enhancement. (A) Optical DNA detection using a sandwich assay format and gold-labeled reporter DNA. **(B)** In the biobarcode assay for the detection of Alzheimer disease marker, a sandwich assay is first performed on the surface of magnetic bead using nanoparticle labels carrying dsDNA. After collection of the magnetic beads and dehybridization of the DNA barcode, the DNA is collected and detected according to the principle shown in **(A)**. **(C)** Multiplexing capability can be added to the assay by using Raman-active dyes and their specific spectra as barcodes.
ADDL: Amyloid-β-derived diffusible ligand; Ag: Silver; Au: Gold; a.u.: Arbitrary units; NP: Nanoparticle; SERS: Surface-enhanced Raman scattering.
Adapted with permission from [48,58].

DNA barcode strands results in signal amplification owing to the high number of strands associated with one particle. In addition, DNA as a barcode is well suited for assay multiplexing [50,51].

Assay multiplexing with surface-enhanced Raman scattering

Recently, it was demonstrated that multiplexing can be added to visual detection using the nanoparticle labels together with Raman-active dyes, since gold and silver colloids have been shown to significantly amplify Raman scattering. This effect is commonly referred to as surface-enhanced Raman scattering (SERS). In SERS experiments, the sample is illuminated with a laser and the spectral properties of the scattered light are analyzed. The pattern of the Raman spectrum is dependent on the molecule and results from the excitation of low-frequency vibrational and rotational states of chemical bonds. Immunoassays taking advantage of the SERS effect have been recently reviewed by Xu *et al.* [52] and include the use of colloidal labels for the generation of a strong SERS signal. Although a metal–protein–metal configuration for the direct detection of the protein cytochrome C was reported [53], often nanoparticle labels were used together with Raman-active dyes to generate a spectral barcode for the identification of the reporter molecule after binding and thus assay multiplexing (**Figure 1C**). The advantage over conventional fluorescence labeling is that a greater variety of molecules (fluorescent and nonfluorescent) are available and sharper peaks are observed, so spectral overlap can be easily avoided. Furthermore, excitation of several Raman dyes with the same wavelength is possible, thus simplifying the assay setup. Several biosensors based on the transduction of the SERS signal of Raman dyes in the vicinity of silver [54], gold [55] or silver-enhanced gold nanoparticles have been described [56,57]. Multiplexing capability using up to six different Raman-labeled nanoparticles was demonstrated by several groups [55,58,59]. Femtomolar detection limits were reached [58]. Finally, in a very recent publication, semiconductor nanoparticles with Raman signal were used instead of dye molecules to generate a Raman signal. A sensitivity of 1 fM for a model DNA assay was achieved with zinc oxide/gold nanocomposites [60].

Detection based on resonance light scattering

Metallic nanoparticles have also attracted attention because of their unique plasmon scattering effect (commonly referred to as resonance light scattering [RLS]). It was shown that upon excitation with white light, metallic colloids scatter light at a specific wavelength with an intensity equivalent to 500,000 fluorophores without suffering from quenching or bleaching effects [22,61]. The scattered wavelength depends on colloid composition, size and shape, so that RLS nanoparticles are well suited to multiplexing (**Figure 2**) [61,62]. RLS-based detection using a waveguide sensor and selenium particles was first published in 1995 [63]. Such evanescent wave setups based on white light side illumination and CCD camera imaging were applied to a variety of DNA array applications [64,65]. More than a 50-fold increase in signal was observed when compared with state-of-the-art fluorescence scanners [65]. Other interesting RLS-based assays include an assay for the detection of kinase activity [66] and as assay for the detection of a polymorphic site in the breast cancer gene *BRCA1*, using plasmonic silver particles and dark-field illumination [67].

Surface plasmon resonance biosensors

Gold colloidal labels have also been shown to significantly increase the response of surface plasmon resonance (SPR) biosensors. In SPR, light is coupled into a thin gold film via a prism. At a specific angle of incidence, surface plasmons (i.e., collective oscillations of the conduction electrons in the metal surface) are excited, resulting in a decrease in reflectivity. Changes in the refractive index in the vicinity of the sensor surface caused, for example, by the adsorption of biomolecules affect this angle, so that binding events can be monitored *in situ* [4,68]. When the reporter molecule is tagged with a colloidal label, larger shifts in plasmon angles, together with an increased and broadened minimum resonance, are measured [69–71]. This phenomenon was associated with the high dielectric constant of gold nanoparticles, as well as with electromagnetic coupling effects between the nanoparticle and the gold film [68,71]. Both detection of DNA [71] and proteins [69,72,73] were reported. In the approach proposed by Hsu *et al.* [73], additional amplification was achieved by attaching multiple gold colloids to an antibody carrying an oligonucleotide by hybridization of tagged short complemetary strands. Picomolar amounts were detected in a sandwich immunoassay [69]. Gold tags were also used together with imaging SPR, an imaging technique based on SPR [74]; in a model DNA array, a 10 pM limit of quantification (i.e., a value close to the ones achieved with conventional fluorescence scanners) was achieved [71].

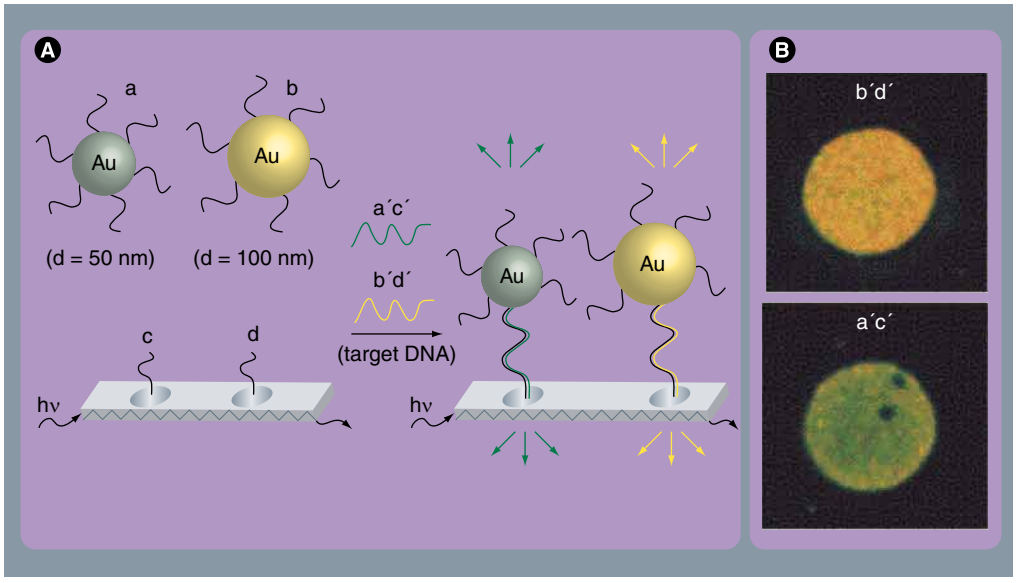

Figure 2. Resonance light scattering-based detection of gold colloids. (A) Resonance light scattering is induced by side illumination of the nanoparticles with white light. The scattered wavelength depends on colloid composition and size so that multiplexing is possible. **(B)** Microscope image of a DNA spot presenting gold colloids of two sizes.
Au: Gold.
Adapted with permission from [62].

Metal labels for electrochemical & electrical biosensors

Electrochemical biosensors

Metallic particles have also attracted attention for their electrochemical and catalytic properties and have therefore been used in conjunction with a variety of electrochemical biosensors [75]. Here, the label is typically detected either directly after immobilization of the reporter molecule or indirectly after a label dissolution step. Metallic nanoparticles have also been used as catalysts for electrochemical reactions.

Direct electrochemical detection

Metallic particle oxidation was detected directly with voltammetry [76–80] or constant-current chronopotentiometry [81]. Picomolar sensitivities were obtained using silver-enhanced gold nanoparticles [82] or gold colloids for the detection of *Factor V Leiden* mutation [77] and DNA from *Escherichia coli* [79]. Wang *et al.* proposed an assay scheme where DNA recognition occurs on a magnetic bead followed by silver deposition, magnet-directed immobilization of the particles on the electrode and silver quantification with

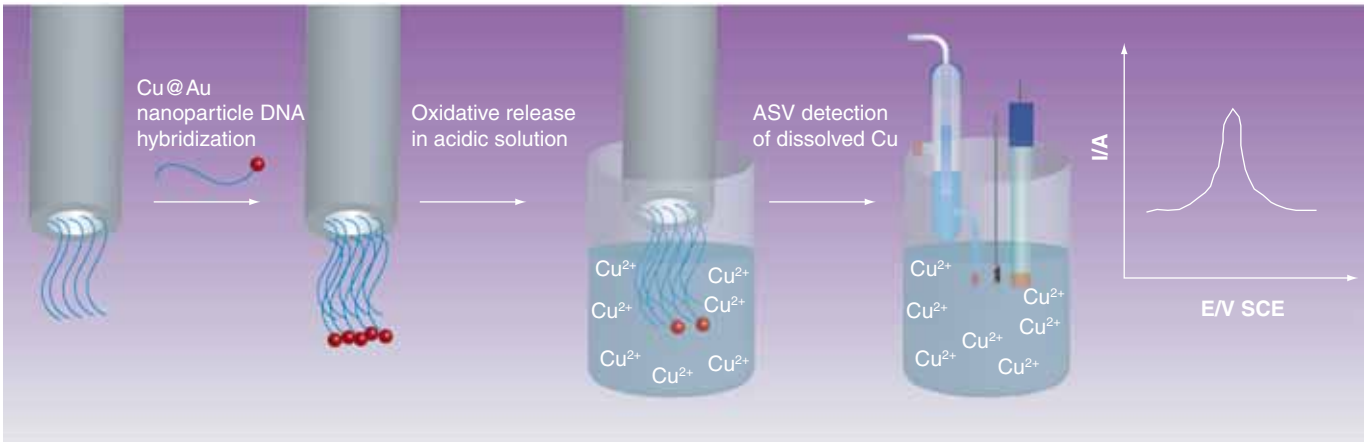

Figure 3. Indirect detection of metallic labels by anodic stripping voltammetry. After hybridization of an oligonucleotide tagged with a metallic label (here Cu/Au nanoshells) and subsequent dissolution of the nanoshells, the Cu is detected by electrochemistry, using ASV.
ASV: Anodic stripping voltammetry; Au: Gold; Cu: Copper.
Adapted with permission from [88].

constant current chronopotentiometry [81]. Gold nanoparticles were also used as carriers of redox markers (e.g., ferrocene) for signal amplification and voltammetric detection of DNA with a detection limit reduced to 2 pM [83,84].

Electrochemical detection based on label dissolution

Indirect approaches involving the dissolution of the metallic label after biomolecular recognition have been proposed as a valuable alternative to direct electrochemical detection. Here, the metal ions in the solution are first concentrated by electrodeposition on the working electrode and then stripped from the electrode by reoxidation. Ion, and thus label, quantification is achieved via anodic stripping voltammetry (ASV) [85–88] or potentiometry [89–92] (FIGURE 3). Picomolar and sub-nanomolar target concentrations are usually detected with this approach [85]. ASV-based detection was performed with variety of particle labels including gold [86,93] and silver nanoparticles [87], copper/gold nanoshells [88], silver-enhanced gold particles [94] and indium microrods [90]. Additional signal amplification can be achieved by associating multiple metallic tags to the biorecognition event using, for example, polyelectrolyte shells containing silver nanoparticles [95] or polystyrene spheres carrying gold nanoparticles [91]. The quantitative detection of cytomegalovirus DNA using ASV was reported with a detection limit of 5 pM [86]. Cathodic stripping voltammetry measurements of iron tracers from gold-coated iron core-shell nanoparticles was also reported [96].

Nanoparticle labels as catalysts for electrochemical reactions

In a different approach, nanoparticle labels act as catalysts for a detectable electrochemical reaction [97–102]. For example, Willner *et al.* used platinum labels to catalyze the reduction of H_2O_2 followed by amperometric [98] or chemiluminescent signal detection in the presence of luminol [99]. By electrochemical means, the detection of thrombin and DNA was reported with sensitivities of 10 nM and 10 pM, respectively (FIGURE 4). In the assay by Kerman *et al.* for the detection of protein phosphorylation, a gold tag is associated to the phosphorylation site and detected by monitoring Cl^- oxidation at the particle surface [100,101].

Enzymes such as horseradish peroxidase have also been associated to nanosilver [78] or nanogold labels [103] for the voltammetric detection of the current resulting from an enzymatic reaction. The high number of enzymes associated to one binding event leads to an additional signal amplification.

Electrical biosensors

Electrical biosensors based on changes in conductivity [104–111] or in capacitance [112,113] between two electrodes have also been described. Such sensors rely on the formation of conductive domains in a micro-/nano-gap. In the approach by Velev and coworkers [104] or Mirkin and coworkers [105], conductive domains are formed between two electrodes upon biorecognition using reporter molecules tagged with gold colloids followed by silver deposition (FIGURE 5). Detection limits of 200 [104] and 500 fM [105] were reported. An assay for the detection of nucleic acids with approximately 1 fM sensitivity was recently proposed by Kong *et al.*, who deposited silver between the electrodes on hematin molecules introduced into the target DNA strand [106].

Furthermore, several conductivity-based electrical biosensors, for the detection of biomolecular interactions in nanometer-sized gaps, which do not rely on a metal-amplification step have been described [107–111]. Here, the conductive domains are formed, for example, by nanoparticle aggregation [108–109] or by the formation of nanoparticle multilayer networks [110].

Semiconductor nanoparticles

Owing to their small size (2–10 nm) and to the associated quantum confinement, semiconductor nanocrystals exhibit unique optical and electronic properties. In fact, in a semiconductor particle of nanoscale dimensions, the gap between the conduction band and the valence band depends on the nanocrystal size, so the wavelength of the emitted photon upon electron relaxation to its ground state can be tuned [10,19,20]. Since original difficulties associated with water solubility and functionalization have been overcome with new protocols for surface modification, quantum dots have been applied to a variety of biological labeling applications [16,20,114,115]. Their properties, synthesis and functionalization strategies, as well as applications of this new class of nanomaterials, have been reviewed in a variety of publications [7,9,10,12,14,16,19,20,75,116]. Several research groups have taken advantage of their luminescent characteristics, as well as their electric and photoelectrochemical properties, for a variety of biosensing applications, which will be reviewed in the following section.

Semiconductor particles for optical biosensors

Semiconductor nanocrystals (quantum dots) are promising label candidates for biological applications, owing to their luminescent properties and their stability against photobleaching. These include tissue staining and cell imaging, as well as a variety of biosensing and microarray applications [8,10,14,19,116]. Quantum dots are usually composed of elements of the II–VI or III–V columns of the periodic table [16,19]. Cadmium selenide (CdSe) nanocrystals (often in the form of a core-shell CdSe/zinc sulfide (ZnS) nanocrystal) with a diameter of 2–8 nm are the most widely found in biological labeling applications, since they emit electromagentic radiation in the visible range [16].

The unique spectral properties of quantum dots make them particularly well suited for fluorescent assay multiplexing and thus for applications in microarray technology: their absorption spectrum is very broad, meaning that one single light source can be used to excite different labels, while their emission spectrum is narrow and well-defined, thus limiting the risk of crosstalk between dyes. Their limitation is the fact that they 'blink' (emit light intermittently), making quantification difficult, especially on the single molecular level. Several groups have started applying quantum dots to multiplexed fluoroimmunoassays [117,118] or together with fluorescent microarray readout for a variety of applications including miRNA expression analysis [119] and the detection of SNPs and DNA from pathogens [120], cancer markers [121], small molecules [122] or toxins [123]. Application of quantum dots in reverse-phase cell lysate microarrays was also demonstrated [124]. Multiplexing using a single light source was demonstrated by several groups with up to six label types detected simultaneously [125,126]. Finally, quantum dots were shown to be well suited for detection by surface plasmon enhanced fluorescence spectroscopy. [127]. This transduction mechanism takes advantage of the evanescent field generated by surface plasmons to excite the quantum dots leading to a considerable increase in sensitivity [128].

Semiconductor particles for electrochemical biosensors

As for metallic particles, stripping voltammetry was also performed with semiconductor crystals such as cadmium sulfide (CdS) [129–133], lead sulfide [134,135] or CdSe/ZnS nanoparticles [136,137]. Potentiometric detection of Cd^{2+} ions released from CdS [138] or CdSe [139] labels using an ion selective electrode, as well as the direct detection of CdS tags [140], was also reported. Advantages over gold colloids include milder dissolution conditions and better electrochemical activity of, for example, Cd^{2+} ions [132]. Furthermore, semiconductor nanoparticles exhibit sharp and well-defined stripping peaks, so the position of the peak is a barcode for the simultaneous detection of multiple targets (FIGURE 6A). Wang *et al.* showed that five different ions can be clearly distinguished [130] and four labels were successfully applied to the stripping detection of SNPs in a solution-based assay using magnetic microparticles [141]. Three semiconductor labels were also used simultaneously in a heterogeneous competition assay. Here, addition of the target strand results in specific release of the nanolabel from the surface and DNA strands could be detected with femtomolar sensitivity (FIGURE 6A) [142].

Limits of detection as low as 0.2–0.3 pM were reported for DNA detection in a model assay [132,135]. In a biologically relevant assay, a detection limit of approximately 3–4 pM was achieved for a DNA biosensor detecting indicators for genetically modified organisms, such as the nopaline synthase terminator gene sequence [131] and a sequence from the cauliflower mosaic virus (*CaMV35 S* sequence) [134]. As already demonstrated for metallic particles, signal amplification can be achieved by associating multiple nanoparticle tags to the biorecognition event. Approximately 500-fold signal amplification was achieved by loading carbon nanotubes with CdS tags (FIGURE 6B) [130].

Photoelectrochemical detection of copper sulfide particles in a DNA assay was also reported [143]. In this approach, illumination of a substrate

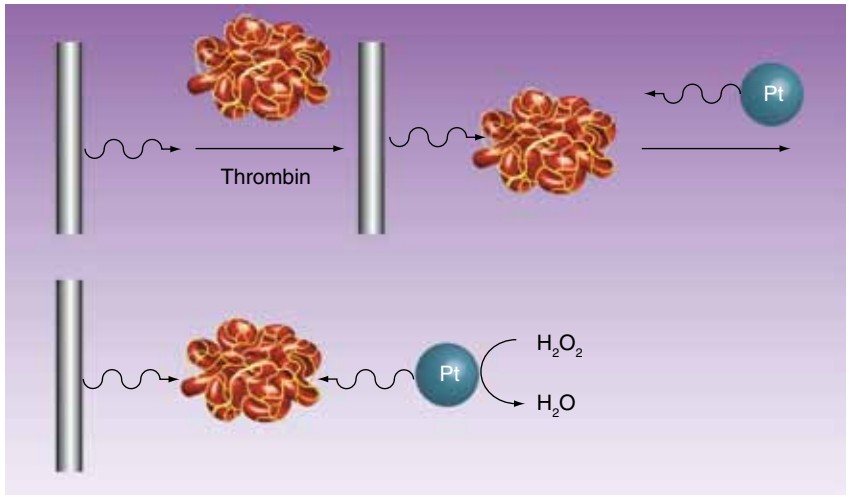

Figure 4. Metallic nanoparticles as catalytic labels. Thrombin is detected using platinum nanoparticle-labeled aptamers. The metallic labels catalyze the reduction of H_2O_2, which is detected by amperometry.
Pt: Platinum.
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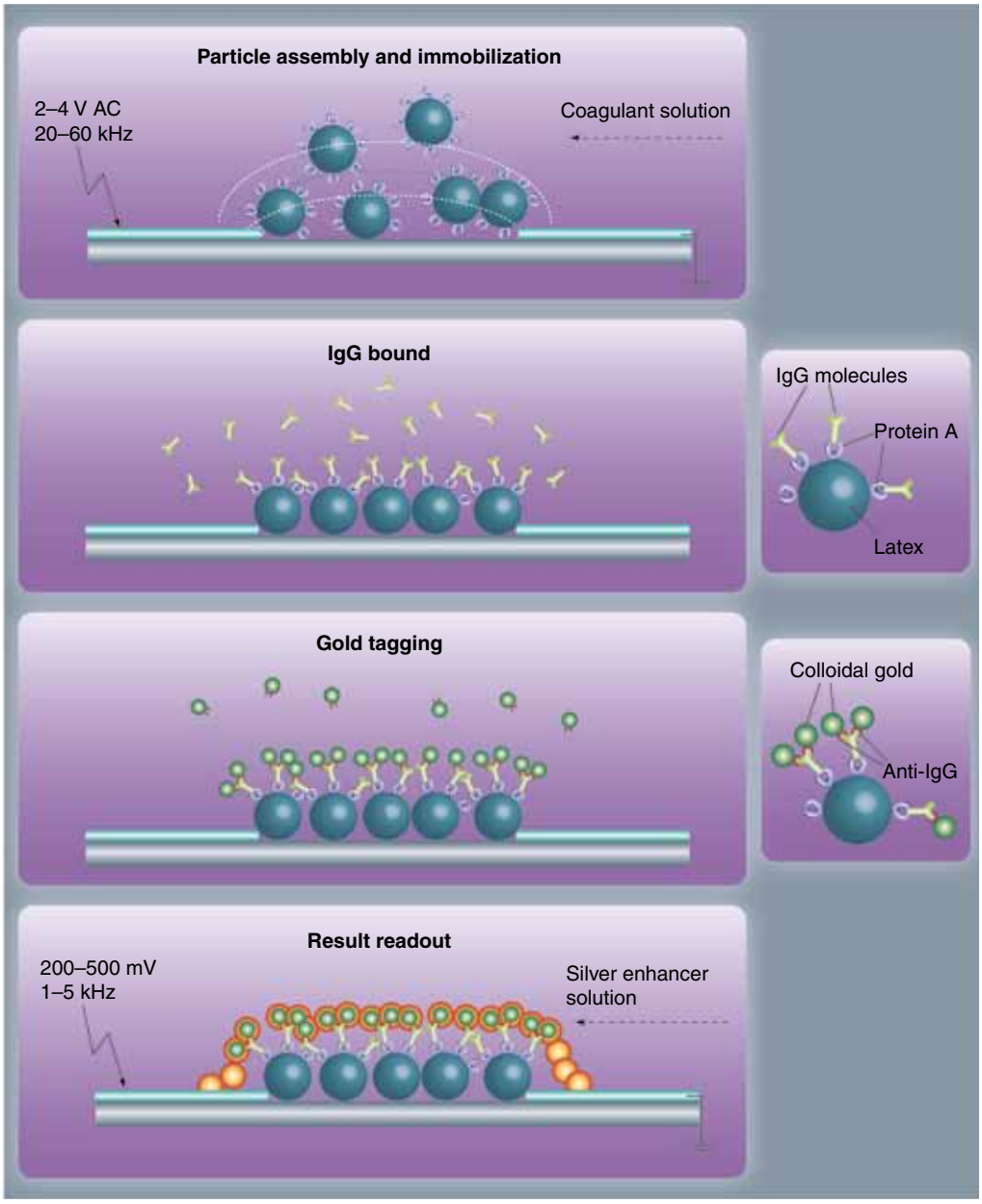

Figure 5. Biosensors based on changes in conductivity. The capture molecules are immobilized between two microelectrodes. Binding of gold nanoparticle-tagged reporter molecules followed by silver amplification leads to an increase in conductivity.
Adapted with permission from [104].

carrying a sandwich complex with copper sulfide tags results in the generation of photocurrent and transport of the conduction band electrons to the electrode, using DNA bound $[Ru(NH_3)_6]^{+3}$ to enhance the photocurrent. Signal amplification can be achieved with a 3D layer-by-layer assembly of the CdS nanoparticles (Figure 7).

Liposome labels

Liposomes are self-assembled colloidal structures formed from amphiphilic molecules, most commonly phospholipids. Because of their amphiphatic properties, phospholipids organize in a bilayer structure with hydrophobic chains forming the core and the hydrophilic heads oriented towards the exterior. In a vesicle one (unilamellar vesicles) or more (multilamellar vesicles) phospholipid bilayers are arranged concentrically as a hollow shell that entraps part of the solvent (Figure 8) [144].

Because marker molecules, such as dyes or enzymes, can be easily encapsulated in the inner cavity or immobilized via covalent or hydrophobic interactions on the membrane surface, phospholipid vesicles have attracted attention as potential labels for a variety of biosensing applications.

Signal enhancement relies on the fact that a high amount of markers is attached to one reporter molecule (e.g., antibody and DNA), therefore generating a much stronger signal than for a standard assay where the biorecognition element is tagged with individual labels. Furthermore, a variety of protocols for the immobilization of biorecognition molecules are also available [13,18].

Different assay formats involving liposomes have been described. They include homogeneous assays as well as heterogeneous assays. Assays based on complement-mediated or melittin-mediated liposome lysis upon biorecognition [13], as well as assays where the binding event induces a colorimetric change (e.g. using polydiacetylene vesicles [17]), fall into the first category, but are beyond the scope of this review. We will focus on heterogeneous assays that involve the immobilization of the vesicle label at the biosensor surface followed by signal readout.

Vesicle assays in optical biosensors

Phospholipid vesicles have been proposed as alternative labels for conventional optical assays based

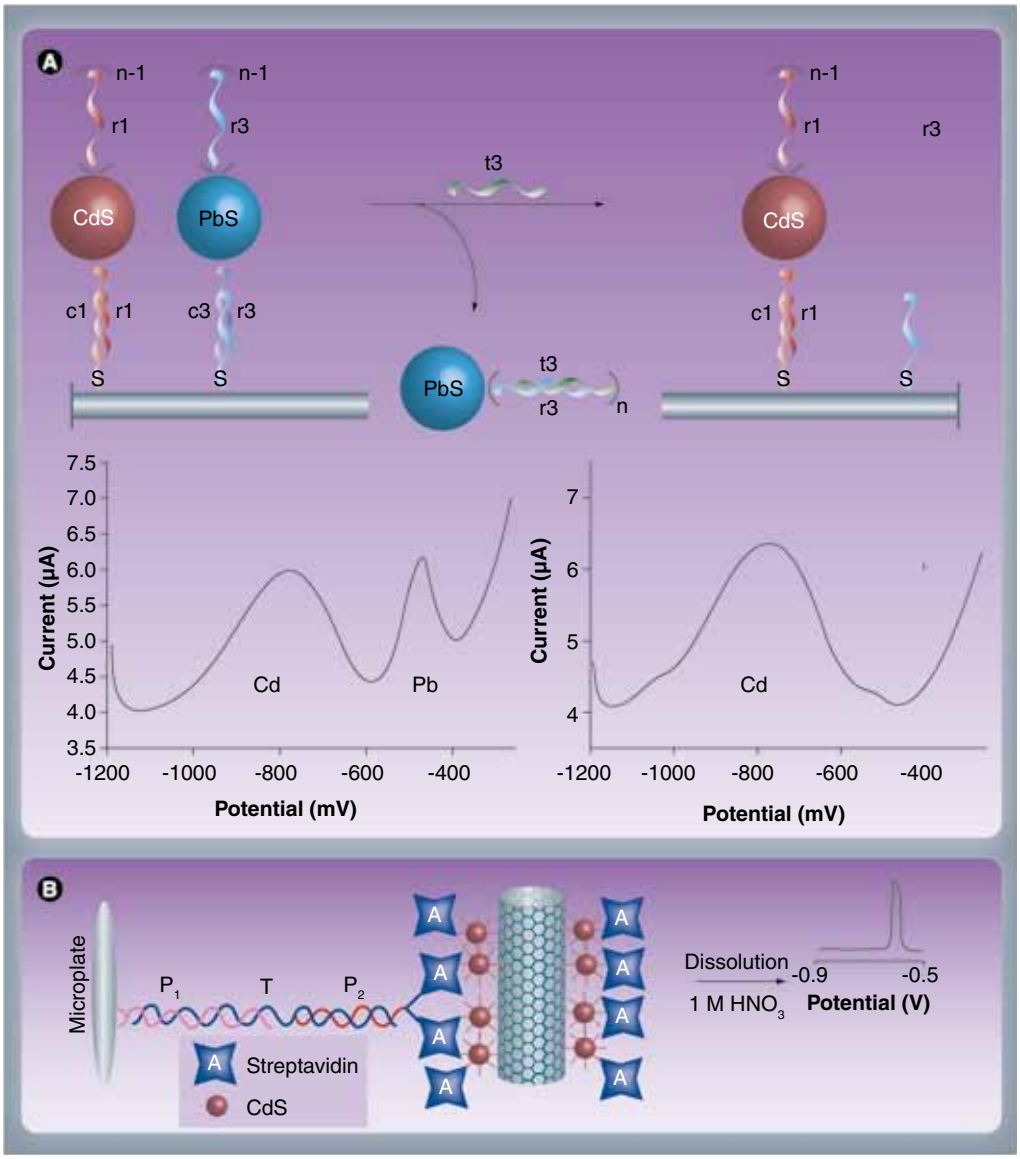

Figure 6. Detection of semiconductor nanoparticles using stripping voltammetry.
(A) Nanocrystals exhibiting different stripping profiles can be used for multiple analyte detection in one experiment. In the competitive assay proposed by Hansen *et al.,* binding of the complementary DNA results in release of the nanoparticles. **(B)** Signal amplification can be achieved by associating a large number of nanoparticle tags to one binding event. Here, this was achieved by loading carbon nanotubes with CdS nanoparticles.
CdS: Cadmium sulfide; PbS: Lead sulfide.
Adapted with permission from [130,142].

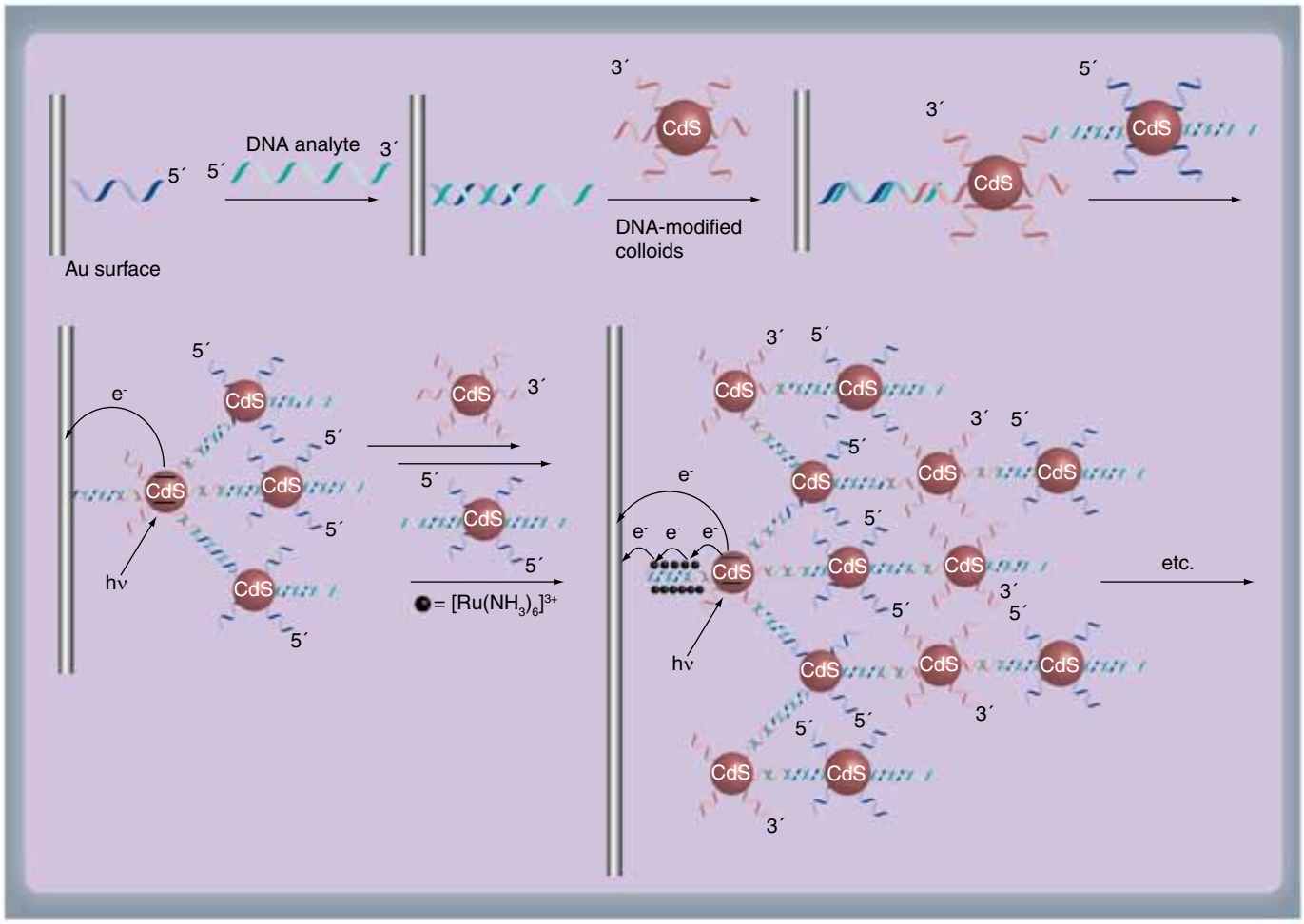

Figure 7. Photoelectrochemical detection of semiconductor nanoparticles. Illumination of the sensor leads to the generation of photocurrent which is transported to the electrode. DNA-bound $[Ru(NH_3)_6]^{+3}$ improves electron transport and particle multilayers contribute to signal amplification.
Au: Gold; CdS: Cadmium sulfide.
Adapted with permission from [143].

on fluorescent or colorimetric reporter molecules mainly because they offer efficient and relatively simple means to generate a strong optical signal. A great amount of effort was put into the development of novel biosensing assay formats compatible with conventional optical technology for readout. This section will present a selection of optical biosensors that make use of phospholipid vesicles.

Flow-through assays

A variety of portable biosensors for the rapid, inexpensive and easy detection of food and water contaminants, environmental pollutants or pathogens have been reported. These sensors include dipstick-like sensors (lateral flow assays), flow injection analysis systems or microfluidic channels.

Lateral flow assays

Membrane strip (dipstick) sensors use nitrocellulose [145–149] or polyethersulfone [150–152] membranes, where the reagents, including the liposomes, flow by capillary migration. In such an assay, dye-loaded vesicles carrying a biorecognition molecule are incubated within the sample and migrate to the detector zone, where they are captured and form a sandwich complex (**Figure 9A**). Detection can be performed either visually by densitometric measurement with a scanner [145,147–149,153] or by measuring the absorbance with a hand-held reflectometer, as proposed more recently [150–152,154–156]. The electrochemiluminescent signal, emitted by ruthenium-containing liposomes, was also detected by placing the detection zone of the nitrocellulose membrane directly in contact with electrodes [157].

Both competitive [145,146,149] and direct assays [147,148,151–156] were reported. Immunosensors for the detection of toxins [145,147], allergens [149] and pathogens [148] were described, while DNA/RNA sensors were used to detect viruses [155], waterborne or foodborne pathogens [152,153],

pathogenic spores [151,154,156] or environmental contaminants [146]. Nanomolar detection limits were achieved with a hand-held reflectometer for readout [150,151].

Flow injection analysis

Another flow-through assay is based on flow injection analysis. Here, the reagents (target molecules and vesicle labels) were passed, in a continuous buffer flow, through a bead-filled column [158–160] or a microcapillary [158,161–163] functionalized with capture molecules. Readout was performed after liposome lysis and transport of the dye to a fluorescence detector. Flow injection analysis systems were applied to the detection of waterborne pathogens [164], herbicides [158] and toxins [145,162], and insulin monitoring [161]. Phospholipid vesicles are particularly well suited to the immobilization of small amphiphatic glycolipid receptors, such as gangliosides, so several assays for the detection of biological toxins with lipidic assemblies were proposed. In a lateral flow assay, the detection limit for cholera toxin was 5.5 pM [162].

Microfluidic channels

Alternatively, microfluidic channels were used, which have the advantage of being smaller, less reagent consuming and allowing multiple analysis to be performed in parallel. Dye-loaded liposomes were captured on the channel walls and detected either directly with a fluorescence microscope [165] or after liposomes lysis (FIGURE 9C) [166,167]. The lysis step reduced dye quenching and thus increased the sensitivity of the assay by a factor of two resulting in a detection limit of approximately 50 pM [168].

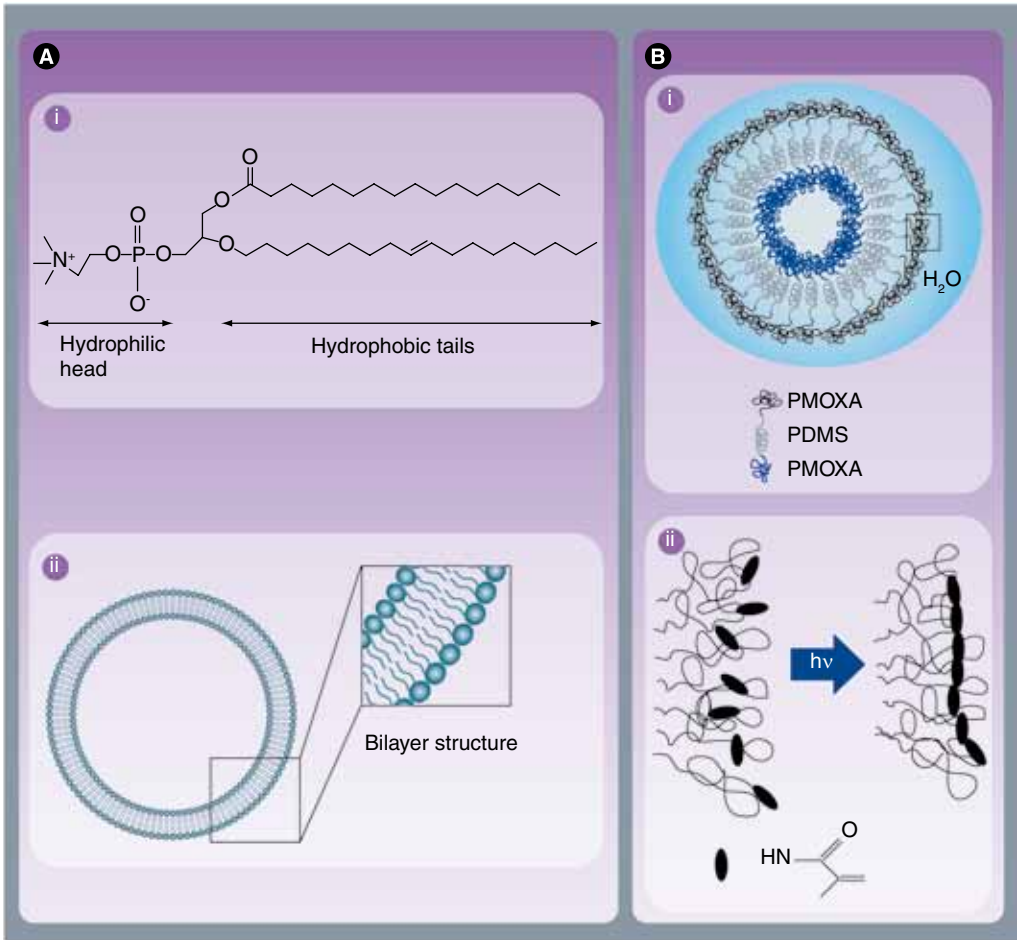

Figure 8. Liposome and polymerosomes. (A) Liposomes are self-assembled structures from amphiphilic phospholipids such as **(i)** 1-palmitoyl-2-oleyl-*sn*-glycero-3-phophocholine. In contact with water, the phospholipids self-organize in a bilayer structure that is arranged concentrically forming a hollow shell **(ii)**. **(B)** Polymerosomes represent an alternative to conventional liposomes. **(i)** For example, polymerosomes can be built from the ABA block-copolymers poly(2-methyloxazoline)-block-poly(dimethylsiloxane)-*block*-poly(2-methyloxazoline). **(ii)** The self-assembled structure can be stabilized by crosslinking.
PDMS: Poly(dimethylsiloxane); PMOXA: Poly(2-methyloxazoline).
Adapted with permission from [208].

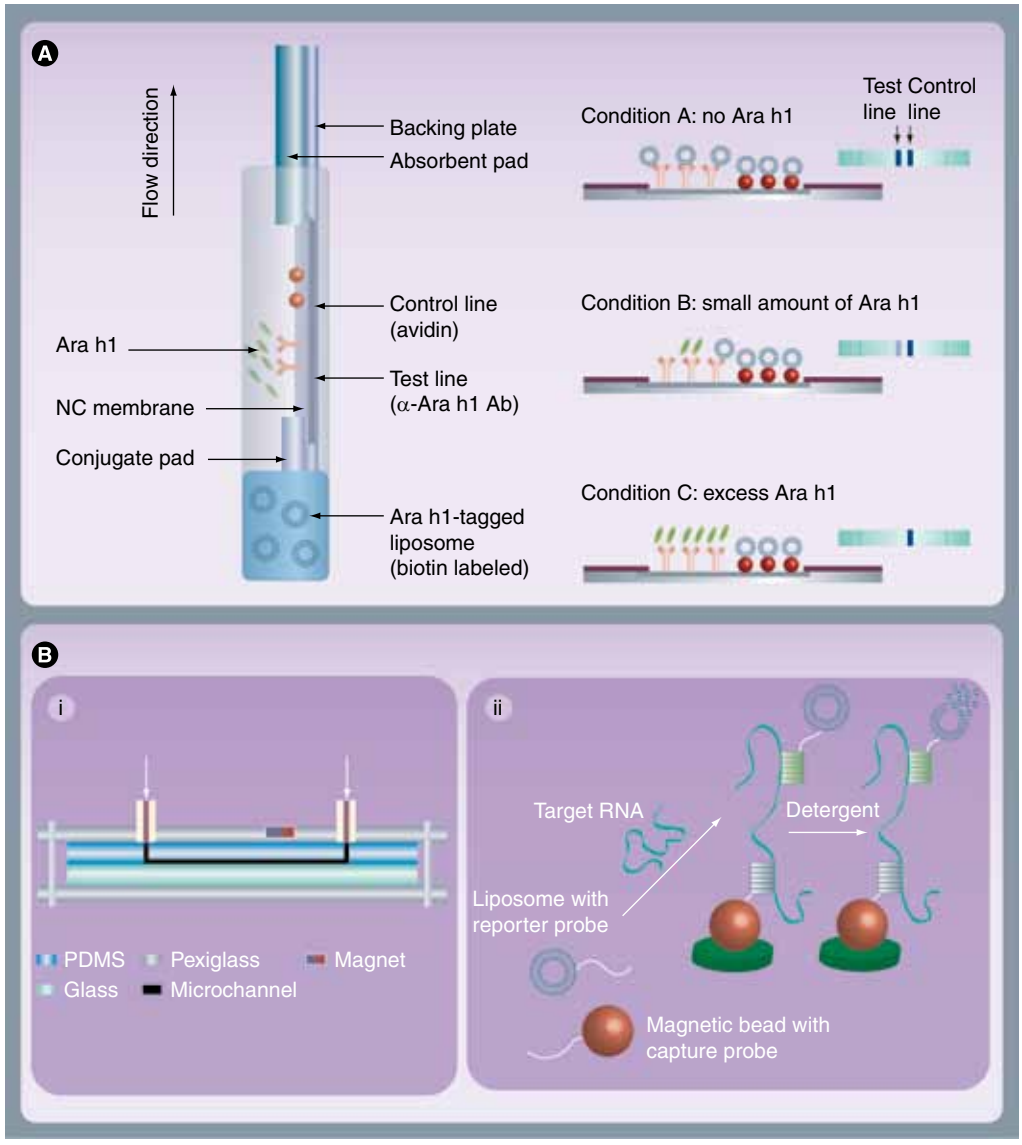

Figure 9. Lateral flow assay using phospholipid vesicles. (A) Assay setup of a competitive lateral flow assay for the detection of a peanut allergen. The reagents migrate along the nitrocellulose strip by capillary action. Here, allergen (Ara h1)-tagged vesicles loaded with sulforhodamine B are added to the strip after the sample. The vesicles are immobilized at the test line (consisting of anti Ara h1) and the signal is detected visually, or by densitometry. To verify the proper functioning of the test strip, the biotinylated vesicles are also immobilized on the control line. In a competitive assay, the signal in indirectly proportional to the target concentration. **(B) (i)** Microfluidic biosensor using a magnet for target immobilization. **(ii)** A sandwich complex is formed on the surface of a magnetic bead. Vesicles tagged with marker molecules (fluorophore, electrochemically active substance) are used for signal generation and are detected after vesicle rupture.
NC: Nitrocellulose; PDMS: Poly(dimethylsiloxane).
Adapted with permission from [149,168].

Multiplexed assays

Liposomes labels have also been proposed as alternatives to conventional enzyme-linked immunosorbent assays for applications with multiplexing capability, such as assays in microtiter plates [169–173] or microarrays [174,175]. Sandwich assays performed in a microtiter plate and making use of vesicles loaded with fluorescent marker [169,170] or carrying horseradish peroxidase on their surface [171–173,176] were already proposed in the 1990s. Fluorescence detection of vesicles in immunowells can be performed both directly and after release of the marker. Although lysis requires an additional step, a 500-fold increase in sensitivity and a limit of detection of approximately

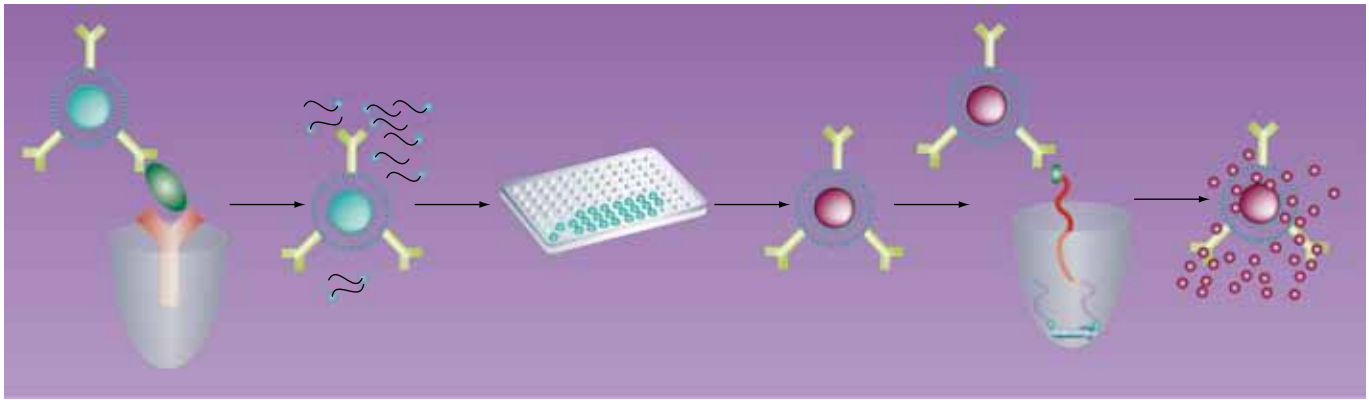

Figure 10. Microwell assay using DNA encapsulating liposomes. A sandwich immunoassay is performed in a microwell using DNA-encapsulating liposomes tagged with an antibody. After release of the encapsulated DNA tagged with fluorescein, the DNA is transferred to a second microtiter plate and detected with vesicles containing sulforhodamine B and tagged with antifluorescein.
Adapted with permission from [177].

1 pM can be achieved [177]. Toxins [178,179] as well as several bacterial pathogens [176] were detected with this approach. A universal assay for the detection of mRNA in a microwell format, using liposomes tagged with streptavidin, was tested recently for the detection of several pathogens [180]. Furthermore, an approach for protein detection, based on the detection of barcode DNA encapsulated in liposome labels, was presented (FIGURE 10). Besides adding multiplexing capability to the assay, a tenfold signal enhancement was reported compared with the direct detection of the antigen with lysed fluorescent liposomes (limit of detection for a protective antigen protein from *Bacillus anthracis*: 4.1 ng/ml). Additionally, the increase in sensitivity was 400-fold when the oligonucleotides were detected using rhodamine-labeled liposomes instead of fluorescein-labeled complementary oligonucleotide strands [181].

Liposomes in electrochemical biosensors

Assays based on liposomes containing electroactive markers

Assay formats based on the electrochemical detection of encapsulated redox labels upon release from a liposome were already presented over 20 years ago (e.g., [182]). More recently, an assay for the detection of cholera toxin with ganglioside

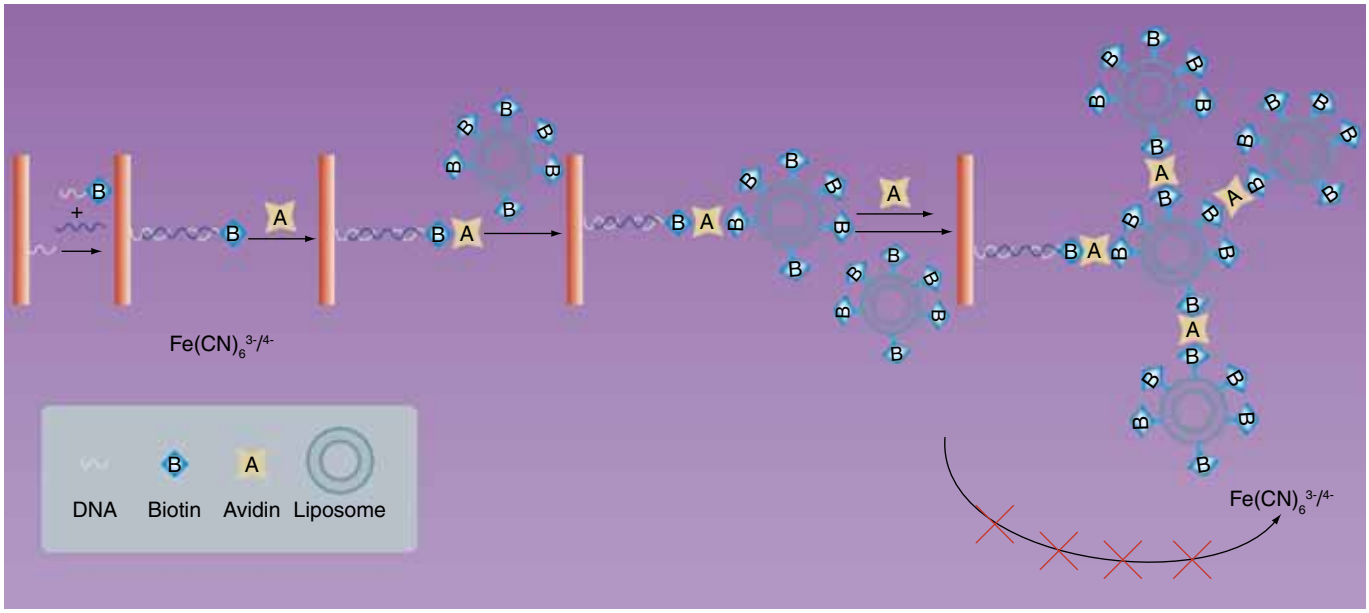

Figure 11. Biosensors based on changes in electron transfer resistance. The negatively charged redox species are repelled by the surface-bound negatively charged species (DNA and vesicles), resulting in a change in electron-transfer properties. Additional amplification can be achieved with vesicle networks.
Adapted with permission from [188].

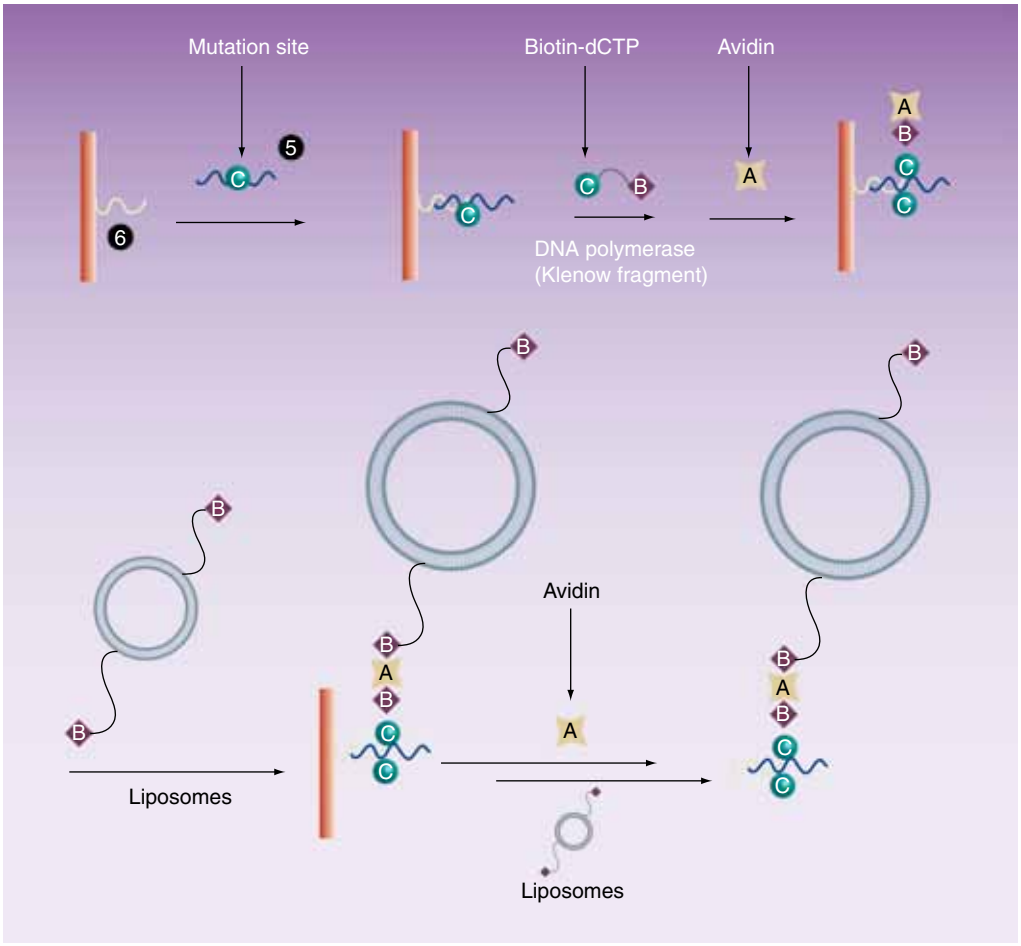

Figure 12. Gravimetric detection of single nucleotide polymorphisms. After hybridization of the target, an oligonucleotide complementary to the target up to the base situated before the mutation site is hybridized. This is followed by the addition of a biotinylated complementary base to the target by DNA polymerase. The base is detected using vesicles as mass tags.
Adapted with permission from [188].

liposomes containing potassium ferrocyanide was described [183]. Here, the electroactive marker released upon liposome lysis is detected by stripping voltammetry. Electrochemical amperometric detection was also suggested as an alternative to optical detection for flow-through assays. In the membrane strip sensor by Baumner *et al.* [184], ascorbic acid is released from the immobilized vesicles and quantified in the detection zone. Electrochemical detection based on vesicle labels containing the electroactive marker ferro/ferricyanide was also applied to the microfluidic channel sensor shown in FIGURE 9C. For the latter setup the sensitivity was in the nanomolar range (10 nM) [185,186].

Detection by impedance spectroscopy

Alternative electrochemical approaches rely on changes in the interfacial electron-transfer resistance measured by Faradaic impedance spectroscopy [187]. In a first assay format, negatively charged liposomes act as labels for the detection of a DNA target. Upon hybridization of the tagged reporter DNA, the interface between the sensor and the solution is highly negatively charged and electrostatically repels redox probes present in the electrolyte, therefore increasing the electron-transfer resistance. Further amplification can be achieved if vesicle networks are constructed using, for example, biotin–streptavidin as a linker (FIGURE 11A) [34,188]. A second assay format for protein or DNA detection utilizes horseradish peroxidase-tagged vesicles. The enzyme catalyzes the oxidation of 4-chloro-1-naphtol in the presence of H_2O_2, which results in the precipitation of an insoluble product on the electrode, enhancing its resistance [189,190]. A detection limit of 0.1 pM was achieved with both assay formats [188,190]. Biosensors based on this transduction principle were proposed for the detection of SNPs [34] or cholera toxins [190].

Signal amplification in microgravimetric transduction

Because of their size and high water content, liposomes were also proposed as mass tags for microgravimetric signal amplification using a quartz crystal microbalance sensor. A quartz crystal microbalance detects *in situ* adsorption events by monitoring changes in the resonance frequency of an oscillating quartz crystal associated with the increased mass at the sensor surface [191]. Willner and coworkers showed that liposomes (and also gold colloids) can be applied to the sensitive detection of single base mutations using microgravimetric transduction [192]. In their assay, the mutation is detected after coupling of a liposome to the mismatch site (FIGURE 12). Note that the same assay strategy can also be applied to other transduction mechanisms (e.g., electrochemical [188]). Furthermore, a dendritic amplification method based on 3D vesicle networks such as the one shown in FIGURE 11 can also be applied to microgravimetric detection [193].

Microgravimetric immunoassays were performed for the detection of haptens with lipid-tagged antibodies produced by bacteria, and incorporated into the liposome membrane (sensitivity: 10 nM) [194] or cholera toxins using ganglioside liposomes (sensitivity: 750 pM) [195]. A sensitivity of 50 pM was achieved in a model assay for the detection of mouse IgG published recently [196].

Conclusion

This article describes the role of three major nanoscale labels for a variety of biosensing applications. In most cases, the benefit of such nanoscale labels is related to the increased signal due to their size. Therefore, many systems exist where the unique chemical and physical properties of metallic and semiconductor nanoparticles, as well as phospholipid vesicles, have contributed to the development of novel transduction mechanisms and novel bioanalytical assays.

However, several limitations, mainly associated with the increased label size, should be pointed out. The label size limits the dynamic range for high target concentrations due to steric hindrance effects and the possibility for multiple bonds (one particle binds several target molecules). As such, the nanolabels are more interesting for applications that require high sensitivity but a relatively narrow dynamic range. In addition, multivalency increases the particle's stickiness so that nonspecific-binding effects might play a more critical role in nanoparticle-based assays, ultimately limiting the assay sensitivity. Lower diffusion rates related to the larger size of particles and the necessity for additional assay steps might increase the total assay time.

Nevertheless, as highlighted in this article, metallic and semiconductor nanoparticles, as well as phospholipid vesicle labels, provide valuable means for signal amplification and sensitivity increase in a variety of analytical assays. Optical signal amplification using metallic particles and phospholipid vesicle opens up the possibility of sensitive target detection with standard optical readout systems or even detection by the naked eye. Owing to their unique electrical and electrochemical properties, nanoparticles have contributed to the development of miniaturized electrochemical biosensors which can now, often with a simple instrumentation, reach sensitivity levels comparable to those achieved using optical devices.

Future perspective

The application of nanoscale labels for biosensing has become a reality and they are now valuable components of commercially available bioanalytical systems. Yet, nanoscale labels of a new generation have already appeared and are likely to contribute to the development of even more efficient biosensors. These include noble metal nanoclusters (nanodots) [197], core-shell fluorescent silica nanoparticles (Cornell dots) [198] and apoferritin nanocontainers [199]. Immunoassays based on lanthanide-doped nanoparticle labels have also been reported [12] and biosensors based on magnetic transduction systems and magnetic nanoparticle labels are starting to appear [200,201].

Polymeric vesicles (FIGURE 8B) with chemical and physical properties tuned towards reduced nonspecific binding and increased stability in complex biological samples will provide a valuable alternative to conventional liposome labels. Furthermore, polymeric systems offer the possibility for more sophisticated vesicle designs incorporating specific functionalities, which are likely to play a key role in the development of bioanalytical devices. For example, oxidation-responsive vesicles with potential applications in biosensing and drug delivery have been described [202]. Moreover, by providing a platform for the immobilization of membrane proteins such as ion channels, the role amphiphilic nanocontainers will play in membrane protein sensing is undeniable. Complex proteoliposomes containing, for example, enzymes or indicator dyes for signal generation and biological process monitoring could soon be a reality [203,204].

Furthermore, the role of nanoparticles and vesicles in biosensing applications is not limited to signal generation in heterogeneous assays. Their potential in the development of nanoscale sensors in particular (i.e., systems where the particle itself is the sensor) has attracted interest recently and is likely to see a dramatic development. Such systems, while being simple (they are often based on the detection of colorimetric changes) offer unique opportunities to combine miniaturization with sensitivity reaching single-molecule levels [205].

Beyond biosensor devices, nanomaterials can be applied to *in vivo* labeling, while their potential in therapeutics, for example for cancer therapy via radiation or heat adsorption, or for targeted drug delivery is currently being reported [206,207]. Thus, smart nanoscale labels offer unique opportunities to combine diagnostics and therapeutics (i.e., establishing theranostics) in our goal for personalized medicine, which is likely to be the major healthcare challenge of the coming decades.

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Executive summary

Metallic nanoparticles

- Sensitive visual or scanometric detection is achieved after a metal-deposition step. Multiplexing capability can be added using Raman-active dyes.
- Metallic nanoparticles show resonance light scattering and generate a strong signal upon illumination with white light. The wavelength of the scattered light is material and size dependent so that multiplexing can be easily achieved.
- Detection schemes based on metal dissolution and stripping voltammetry/chronopotentiometry were proposed. With additional amplification schemes pM detection sensitivities are reached.
- High sensitivities (lower picomolar range), together with arraying and miniaturization capability, can also be achieved with conductivity measurements. In this case, detection is based on conductivity changes induced by particle immobilization between two electrodes.

Semiconductor nanoparticles

- Now that a variety of functionalization protocols are available, quantum dots provide an alternative to conventional fluorophores. Quantum dots are photostable and their spectrum is ideal for assay multiplexing with limited crosstalk.
- Semiconductor labels can also be detected by anodic stripping voltammetry. Their well-defined electrochemical fingerprints make electrochemical assay multiplexing possible.

Phospholipid vesicles

- Signal amplification is achieved by associating a high number of marker molecules to one binding event. The molecules (dyes, enzymes and redox active substances) can be encapsulated in the vesicle inner cavity or bound to its surface.
- A variety of biological assays relying on vesicle-based signal amplification have been described. Biosensor formats include membrane strip sensors and flow injection analysis systems. Thus, phospholipid vesicles are particularly well suited for field-oriented applications involving visual detection.
- Phospholipid vesicles also provide an alternative to conventional labeling for assays performed in microwells with up to a 500-fold increase in sensitivity.
- Liposomes were used as labels in a biosensor based on changes in electron transfer resistance and impedance spectroscopy. The detection limit was 0.1 pM.
- Because of their large size and water content, phospholipid vesicles are well suited for gravimetric signal amplification.

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