

Nanoparticles for bioanalysis

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This review covers the emerging field of nanobiotechnology, in which nanoparticles are applied to the analysis of biomolecules. Nanoparticles can be used in a variety of bioanalytical formats, and this review discusses four classes of use. First, nanoparticles as quantitation tags, such as the optical detection of quantum dots and the electrochemical detection of metallic nanoparticles. Second, encoded nanoparticles as substrates for multiplexed bioassays, such as striped metallic nanoparticles. Third, nanoparticles that leverage signal transduction, for example in colloidal gold-based aggregation assays. Fourth, functional nanoparticles that exploit specific physical or chemical properties of nanoparticles to carry out novel functions, such as the catalysis of a biological reaction. In addition, the review discusses the next generation of nanoparticles that will be utilized in the life sciences, such as nanodots and carbon nanotubes.

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Abbreviations

SEM scanning electron microscopy
SERS surface enhanced Raman scattering
SNP single nucleotide polymorphism

Introduction

Nanobiotechnology — a new word — describes the use of nanotechnology in biological systems. Although still in its infancy, nanotechnology is already having an impact in bioanalysis, where nanoparticles of a variety of shapes, sizes and compositions are poised to fundamentally change the bioanalytical measurement landscape. For example, it now appears clear that nanoparticles will overcome many of the significant chemical and spectral limitations of molecular fluorophores. Equally important, methods for preparation and handling of nanoparticle–biomolecule conjugates are proliferating, and such bioconjugation-ready nanoparticles are becoming commercially available. Thus, it seems appropriate to provide

a critical assessment of the state-of-the art, as well as an indication of future directions.

This review focuses on nanoparticles for bioanalysis, covering peer-reviewed publications from 2001–2003. Accordingly, several of the groundbreaking papers from the late 1990s that had a catalytic effect on the field [1–6], fall outside the period under review. Moreover, the earliest work in nanoparticles for bioanalysis, namely the use of 5–50 nm diameter colloidal gold as electron-dense, and immunocytochemical probes for electron microscopy, dates back to the 1970s, and was reviewed extensively in a three-volume series [7].

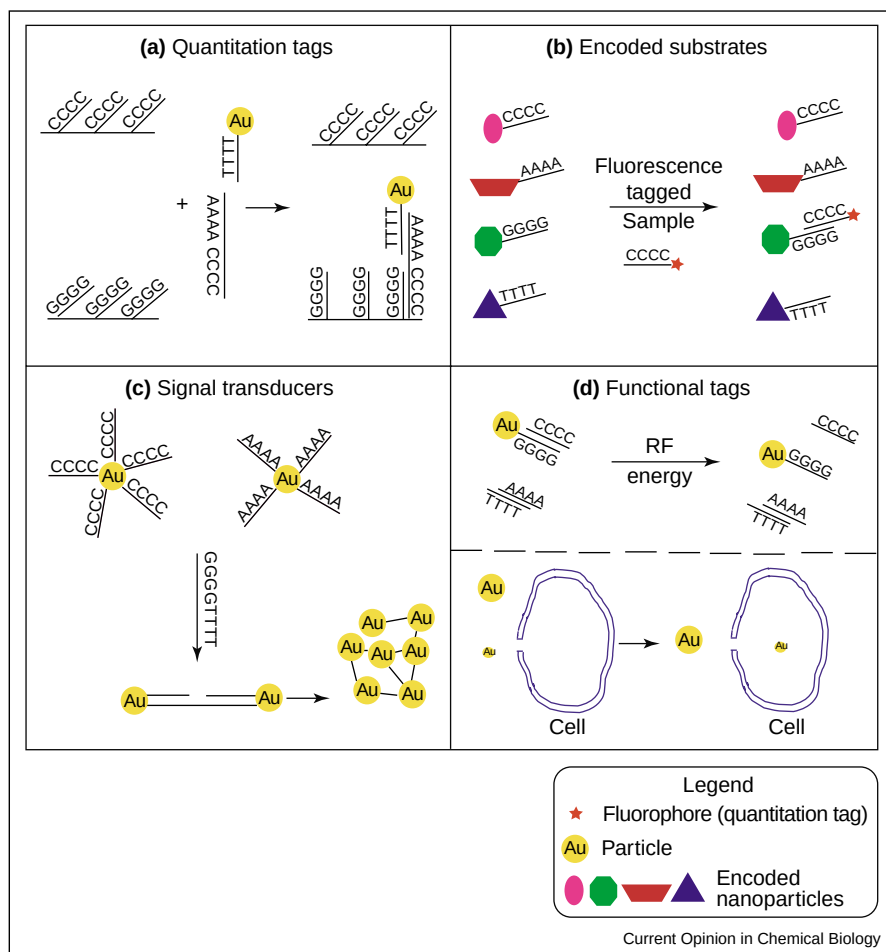
A further narrowing of the scope comes from our decision to focus on nanoparticles that are initially in suspension (and thus, equivalent, in some sense, to traditional biological reagents). Thus, while immobilized nanoparticles have tremendous potential in a variety of bioanalytical detection schemes (see, for example, Van Duyne's recent work on nanoparticle-based sensing of glucose [8]), this field is not discussed herein. In addition, because of space limitations, we are unable to cover the entire landscape of excellent work in the nanoparticles arena, and therefore have instead tried to give the reader a taste of the scope of the work being carried out in the field.

Nanoparticles can be used in a variety of bioanalytical formats, and the review is divided accordingly (Figure 1). When nanoparticles are used as quantitation tags, either the particles themselves or a measurable parameter emanating from the particles (e.g. photons) are quantified. Encoded nanoparticles used as substrates rely on one or more identifiable characteristics to allow them to serve as encoded physical hosts for multiplexed bioassays. This is analogous to the positional encoding of assays on microarrays, but in solution. Nanoparticles that leverage signal transduction involve a change either in the location of nanoparticles relative to one another, or a perturbation in the biological system caused by the nanoparticle — both of which, in turn, lead to a change in a measurable signal. Functional nanoparticles exploit specific physical or chemical properties of nanoparticles to carry out novel functions, such as catalysis of a biological reaction. For certain particles and/or applications, these divisions can be blurred, but they nonetheless serve as a general classification method.

Nanoparticles as quantitation tags in biological assays

The driving force behind the use of nanoparticles as tags in biological assays is to eliminate the need for either

Figure 1



Four formats in which nanoparticles can be divided, in the field of bioanalysis. **(a)** A hybridization assay in which a gold nanoparticle is used as a tag in a DNA sandwich assay. **(b)** A multiplexed assay, in which only one sequence is complementary to the sample and hence fluorescent. **(c)** An assay in which gold nanoparticles undergo aggregation due to cDNA sequences bringing the nanoparticles into close proximity. **(d)** The upper cartoon depicts localized heating of a single DNA sequence tagged with a gold nanoparticle, leading to selective dehybridization. The lower cartoon depicts a cellular assay in which only gold nanoparticles of a particular size can enter a cell.

organic fluorophores or radioactive labeling, both of which have shortcomings. The majority of the work has been focused in two areas, quantum dots and metallic nanoparticles, with some development of novel reporters as well.

Quantum dots take advantage of the quantum confinement effect, giving the nanoparticles unique optical and electronic properties. Quantum dots offer advantages over conventional dye molecules in that they have tunable fluorescence signatures, narrow emission spectra, brighter emission and good photostability. An active area of research is the development of surface coatings that can be derivatized with biomolecules without changing the optical properties of the quantum dots, and several groups are making progress [9–11]. There is similar work on attempts to derivatize colloidal gold with biomolecules [12].

After many years of research on the basic properties of quantum dots, their use as fluorescent probes in real world biological applications has become a reality. For example, Wu *et al.* [13] described a Her2 assay for breast cancer, Parak *et al.* [14] reported on imaging of cell motility, Dubertret *et al.* [15] investigated development of a *Xenopus* embryo, Akerman *et al.* [16] developed a mouse-lung *in vivo* targeting assay, and Xu *et al.* [17] published a multiplexed single nucleotide polymorphism (SNP) assay. This diverse range of assays and applications demonstrates the maturing of quantum dots from interesting curiosity to research tool. In addition to their optical properties, the unique electronic properties of quantum dots have also been exploited. Wang *et al.* [18] described a DNA assay in which the difference in voltammetric signatures of the quantum dot metal components enabled both multiplexability and analyte quantitation.

In the field of metallic nanoparticles as quantitation tags, there are many varieties of detection approaches being developed, including electrochemical, optical, microscopic and mass spectrometric. Electrochemical detection lends itself well to the detection of metallic nanoparticles. Several groups detect the metallic (often colloidal gold) tag directly. Two examples are Authier *et al.* [19] who oxidatively released the gold tag and detected using anodic stripping voltammetry, and Ozsos *et al.* [20•] who used differential pulse voltammetry to detect colloidal gold tagged DNA hybridized to oligonucleotides directly attached onto an electrode. An alternative approach is employed by Wang and colleagues, who indirectly detected the gold colloid tag by precipitation of silver, followed by the use of stripping potentiometry [21–23]. In short, they labeled oligonucleotides with gold nanoparticles, precipitated silver onto the gold particles, and subsequently detected using stripping potentiometry.

Given the historic significance of colloidal gold in electron microscopy, it is not surprising that many groups have investigated microscopy as a method of detection. For example, Csáki *et al.* [24] used scanning force microscopy to determine the surface density of adsorbed DNA quantitatively via hybridization to gold colloid tagged oligonucleotides. Levit-Binnun and co-workers [25] employed scanning electron microscopy (SEM) to detect colloidal gold in a protein array sandwich assay. Although SEM may seem less convenient than today's fluorescence microarray slide scanners, the authors point out the plethora of automated SEM used today in the semiconductor business. Zhang and coworkers [26] used MS for detection of colloidal gold in a sandwich immunoassay with detection by inductively coupled plasma MS. Interestingly, preliminary data indicate that multiplexed detection may be possible by varying the metal.

Optical methods such as light scattering and Raman scattering have been investigated as methods of detection for colloidal gold and silver. Taton *et al.* [27] leveraged the phenomenon that different sized gold particles will scatter light of distinct wavelengths enabling 'two color' detection on microarrays. Bao *et al.* [28•] have demonstrated resonance light scattering particles for use in DNA microarrays. These colloidal metal particles scatter light when illuminated with white light, and the scattered light is of a specific color and intensity depending on the composition of the particles. Raman detection is utilized by Cao *et al.* [29], in which an oligonucleotide is labeled both with a Raman active dye (such as Cyanin5) and gold nanoparticles. After hybridization, the microarray is reacted with a silver and a reductant, such that surface-enhanced Raman scattering (SERS) is achieved. By using different fluorescent dyes, multiplexed detection can be achieved. While they have not yet been applied to biological applications, Mulvaney *et al.* [30] have devel-

oped a glass-coated SERS particle for use as a Raman detection tag that should make the process simpler.

Novel classes of optically active nanoparticles are also being developed as next-generation quantitation tags. Santra *et al.* [31] encapsulated ruthenium complexes in thin silica layers as photostable lumiphore biomarkers for leukemia cell recognition. Van de Reijke *et al.* [32] developed a technology they call 'up-converting phosphor technology' and applied it to DNA microarrays. These particles are 0.4 μm in diameter, and emit visible light when illuminated in the infrared (980 nm) region. Caruso and Schuetz assembled polyelectrolyte-coated lanthanum phosphate nanoparticles, with tunable fluorescence signatures [33].

Nanoparticles as substrates

Encoded nanoparticles can serve as substrates for multiplexed biological assays in solution. Compared with microarray-based methods, these 'suspension arrays' offer, in principle, greater flexibility (via the ability to easily incorporate a new assay via addition of a new bead type), more rapid assay times (radial versus planar diffusion), greater reproducibility (10 s to 100 000 s of replicates for each assay), and can potentially use less sample and reagent. Typically, analyte quantitation involves the use of a quantitation tag (typically an organic fluorophore).

Mixtures of quantum dots have been embedded in micron-sized polymer particles as a multiplexing method in a solution-based array format. Nie and colleagues have demonstrated proof-of-principle for a DNA hybridization experiment using a suspension of polymeric colloids pre-encapsulated with three kinds of quantum dots mixed at various ratios for probe identification [34]. Subsequently, the surfaces of these particles were conjugated to specific oligonucleotide probes for molecular recognition, and an organic dye molecule was used to directly label the target DNA molecules for analyte quantitation. The fluorescence spectrum of each bead illustrates the absence or presence of the target sequence in the original sample based on the organic dye signal, while the identity of the analyte is revealed by the fluorescence fingerprint of the embedded quantum dots. Although no direct quantitation data were reported in the paper, the authors have projected a comparable assay performance to that of conventional microarray systems. Note also that a variation on the same theme can be carried out with organic dyes both for quantitation and identification, embedded in support beads [35].

Nam *et al.* [36] fabricated 'biobarcodes' in which gold nanoparticles are derivatized with distinct sequences of DNA, and then taking advantage of the distinct melting temperature of the different DNA sequences, to determine the 'barcode' identity. A multiplexed immunoassay was then carried out using aggregation as the

end point detection. The sample is centrifuged, and only the aggregated particles were precipitated. A subsequent melting point analysis is carried out to decode the biobarcode.

Striped metal nanoparticles ('Nanobarcodes[®] particles') have been described as substrates for multiplexed assays [37–40]. In contrast to the quantum dots described above, for which fluorescence measurements are needed both to identify the particles and to quantitate an analyte, these particles are intrinsically encoded by virtue of the difference in reflectivity of adjacent metal stripes. The large number of striping patterns available, and the development of automated software for particle identification should facilitate development of very highly multiplexed assays (i.e. hundreds to thousands), although optimization of assay performance is in the early stages.

Fundamental studies on the behavior of nanoparticle–biomolecule interactions are essential to advance their uses. In basic work that is relevant to several substrate-type applications, Nicewarner-Pena *et al.* [41•] carried out a systematic study of hybridization efficiencies and enzymatic extension of DNA attached to gold nanoparticles. They investigated the effect of spacer length, concentration, complement length and oligo length to understand the steric factors that the gold nanoparticle introduced. In addition, Jin *et al.* [42•] have provided a very thorough model of the behavior of DNA hybridization in the presence of gold nanoparticles that explains the very sharp hybridization transition temperature observed (much sharper than a DNA duplex untagged). Also of importance, Zanchet *et al.* [43] describe a gel electrophoretic method to count single-stranded DNA molecules attached to a particle, a useful tool for fundamental studies.

Nanoparticles that leverage signal transduction

Nanoparticles that act as signal transducers show the most promise in diagnostic assays, due to the elimination of the need to tag a biological sample. With the sample preparation steps reduced or eliminated, the diagnostic test will become more robust and less expensive. As previously stated, we define signal transduction as a system in which a change occurs either to the location of nanoparticles relative to one another, or as a perturbation to the biological component of the assay — both of which in turn lead to a change in a measurable signal. An example of a proximity change is a system in which gold nanoparticles aggregate, a phenomenon that several groups have explored. Thanh and Rosenzweig [44] developed an assay in which antibody concentration was determined as a function of aggregation of antigen-coated gold particles. Otsuka *et al.* [45] synthesized a heterobifunctional PEG molecule that enabled reversible aggregation, and demonstrated its use in a lectin assay. The eventual goal

for any diagnostic assay is detection from whole blood. This goal has been attained by Hirsch and co-workers [46•], who developed an aggregation assay to detect immunoglobins in whole blood using gold nanoshells. Nanoshells are spherical nanoparticles made up of a dielectric core (often silica) and surrounded by a thin metal shell. By using gold nanoshells, in which the core is optically active in the near IR, aggregation studies that could otherwise not be 'observed' in whole blood were detected, because of the transparent nature of blood at these wavelengths.

Other signal transduction applications involving metallic nanoparticles include fluorescence quenching by gold nanoparticles, which has been used to great success. Both Maxwell *et al.* [47•] and Dubertret *et al.* [48•] exploited the ability of colloidal gold nanocrystals to quench fluorescence dyes in assays that distinguish oligonucleotides with single base mismatches. Park *et al.* [49] built a microarray in which the capture DNA was spotted between microfabricated electrodes, and hybridized in a sandwich assay format with gold-nanoparticle-labeled oligonucleotides. Silver was deposited that bridged the electrode gap, and thus they were able to detect hybridization events via conductivity measurements.

Several groups have developed environmentally sensitive sol-gels on the nanometer scale. Brasuel *et al.* [50] have developed PEBBLE (Probes Encapsulated By Biologically Localized Embedding) sensors, which are oxygen sensitive and can be used to monitor live cells. Gan and Lyon [51] have developed a temperature sensitive hydrogel that shows promise, although it has not been applied to biological assays. The environmentally sensitive response is tunable by modification of the chemical compositions of the shell layers.

Functional nanoparticles

One of the most exciting areas of nanobiotechnology is the use of nanomaterials to carry out particle-specific functions. In other words, intrinsic physical or chemical properties of the particles effect analytically relevant transformations. Three leading edge studies have been recently published that demonstrate this concept. Hamad-Schifferli *et al.* [52] used a radio-frequency magnetic field to inductively heat a gold nanoparticle attached to a DNA duplex to promote dehybridization. They demonstrated that a temperature rise of between 10 and 20°C is afforded to the duplex, which is enough to denature it. The authors also show that when two DNA duplexes are adjacent, one with a gold particle label and one without, they can selectively denature the former without affecting the latter. More recently, Paunesku *et al.* [53] described a novel TiO₂-DNA nanocomposite that exhibits photo-inducible endonuclease activity. The activity results from (i) the electron- and hole-generating capacity of photo-irradiated n-type semiconducting TiO₂

nanoparticles; (ii) the conductivity of double-stranded DNA; and (iii) the sequence-specific binding properties of the single-stranded oligo attached to the nanoparticle. In a third example, Xiao *et al.* [54] describe work in which they use gold nanoparticles as 'electrical nanoplugs' and thus open the field of what they term nano-bioelectronics. They successfully achieved electron transfer turnover rates from apo-glucose oxidase and gold nanoparticles functionalized with FAD at greater than for the enzyme *in vitro*. Here, the particle is acting both as a carrier for the apo-enzyme and as an electron conduit.

More recently, Tkachenko and co-workers published their initial findings on multi-functional gold nanoparticle-peptide complexes for nuclear targeting [55]. For this function, particles must be small enough to penetrate cells and nuclear membranes, rugged enough to survive harsh endosomal/lysosomal processes, and must be able to carry targeting peptides for nuclear localization. These functions were met by 20 nm diameter gold nanoparticles coated with bovine serum albumin and derivatized with well-defined quantities of combinations of targeting peptides. Presumably, these particles would ultimately carry DNA repair sequences.

In addition to their optical signatures, nanomaterials with magnetic properties have also been exploited in various biological applications. Besides using the magnetism in the preparation of biological samples and contrast enhancement in magnetic resonance imaging applications, Prasad *et al.* have reported an interesting application in which silica-coated paramagnetic nanoparticles were used in magnetocytolysis of selected cancer cells [56]. In their report, silica particles with 20 nm Fe₂O₃ magnetic cores have been prepared and conjugated to the luteinizing hormone releasing hormone receptors, an agent targeted specifically to human breast carcinoma cell surfaces. The results show that breast cancer tissue samples have uptaken these silica particles with very high specificity in less than 30 min, followed with a selective cytolysis under a 7-tesla DC magnetic field.

Another interesting application worth mentioning is reported by Weissleder *et al.* in which superparamagnetic crystalline Fe₂O₃/Fe₃O₄, caged in epichlorohydrin cross-linked dextran, has been used as nanosensors in DNA hybridization experiments [57]. The presence of a particular DNA sequence brings the particles coated with the complementary probes in close proximity, leading to a significantly enhanced spin-spin relaxation of the resulting clusters. As a result, networks of these particles were formed and were stabilized long enough to report the presence of specific DNA targets. Additionally, they demonstrated that these aggregates could be separated using a hand-held magnet to further elucidate the target structures to provide additional information regarding the identities of captured species.

The next generation of nanoparticles

There are several nanoparticles currently being developed that show promise for bioassays, but have not yet been exploited to their full potential. A key advance has been the ability to control the shape of metallic nanoparticles, such as the Xia group's [58] 'nanocubes'. The same group has also synthesized hollow nanoparticles, which may be important in simultaneous bioanalysis and drug delivery regimes [59], particularly in light of their interesting optical properties [60]. Carbon nanotubes will also play a role in this arena, and several groups have successfully derivatized carbon nanotubes with a variety of biomolecules, including DNA [61] and proteins [62]. We can expect major advances in this field in the next few years. If nanoparticles are to become useful in field diagnostic tests, very robust materials will also be required. Cao *et al.* [63] described a nanoparticle in which the core is silver with a single monolayer shell of gold. The spectroscopic properties of these particles are identical to silver while their binding properties are identical to gold, leading to better properties for biomolecular interactions.

A potentially revolutionary tag has been developed by the Dickson group [64–66]. They have successfully synthesized water-soluble 'nanodots' of gold and silver. A poly(amidoamine) dendrimer cage stabilizes and solubilizes single nanodots ranging in size from two to eight silver atoms. These are the smallest tags yet developed at 0.8 nm. They have large quantum yields, are more robust than organic dyes, are water soluble, and therefore are expected to be important in single-molecule detection applications.

Summary

While there can be no denying that instrumentation has had a major role in accelerating the pace of biological knowledge generation, the fundamental reality is that life science research is reagent driven. Indeed, even the most important instrumentation (e.g. DNA sequencing) incorporates reagent-intensive processes. The focus of this review has been to highlight advances in nanoparticles for bioanalysis, and in particular, nanoparticles that are used, or could eventually be used, as reagents. Ultimately, nanoparticles will become valuable for bioanalysis when protocols involving them become widely adopted. This will happen when nanoparticles offer a measurable improvement in performance (sensitivity, cost, ease of use, etc.) compared to existing technologies. Importantly, this appears to be the case for many of the systems described above. Of course, widespread adoption also requires a scale of manufacture, reliability and distribution capability that comes only with commercial implementation. It is thus encouraging that some of the particles and particle-based assay systems described above are well along a commercialization path, and further gratifying that new sensing modalities involving nanoparticles continue to occupy a high profile in the

research literature. As this basic research is moved to the development stage, and as commercially available materials achieve broad user acceptance, there will come a time, say 3–5 years from now, when nanoparticles will be routinely used across the entire spectrum of bioanalysis. At such a point in time, nanobiotechnology will lose its moniker of a ‘made-up word’.

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