Nanostructures in Biodiagnostics

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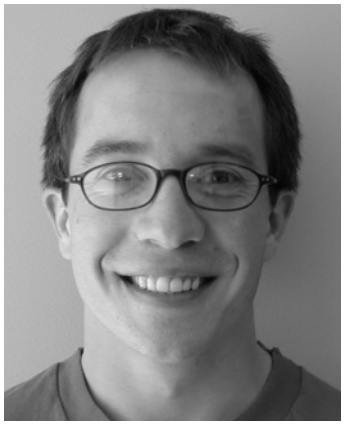

Nathaniel L. Rosi earned his B.A. degree at Grinnell College (1999) and his Ph.D. degree from the University of Michigan (2003), where he studied the design, synthesis, and gas storage applications of metal–organic frameworks under the guidance of Professor Omar M. Yaghi. In 2003 he began postdoctoral studies as a member of Professor Mirkin's group at Northwestern University. His current research focuses on the rational assembly of DNA-modified nanostructures into larger-scale materials.

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1. Introduction

In the last 10 years the field of molecular diagnostics has witnessed an explosion of interest in the use of nanomaterials in assays for gases, metal ions, and DNA and protein markers for many diseases. Intense research has been fueled by the need for practical, robust, and highly sensitive and selective detection agents that can address the deficiencies of conventional technologies. Chemists are playing an important role in designing and fabricating new materials for application in diagnostic assays. In certain cases assays based upon nanomaterials have offered significant advantages over conventional diagnostic systems with regard to assay sensitivity, selectivity, and practicality. Some of these new methods have recently been reviewed elsewhere with a focus on the materials themselves or as subclassifications in more generalized overviews of biological applications of nanomaterials.[1–7] We intend to review some of the major advances and milestones in the field of detection systems based upon nanomaterials and their roles in biodiagnostic screening for nucleic acids,

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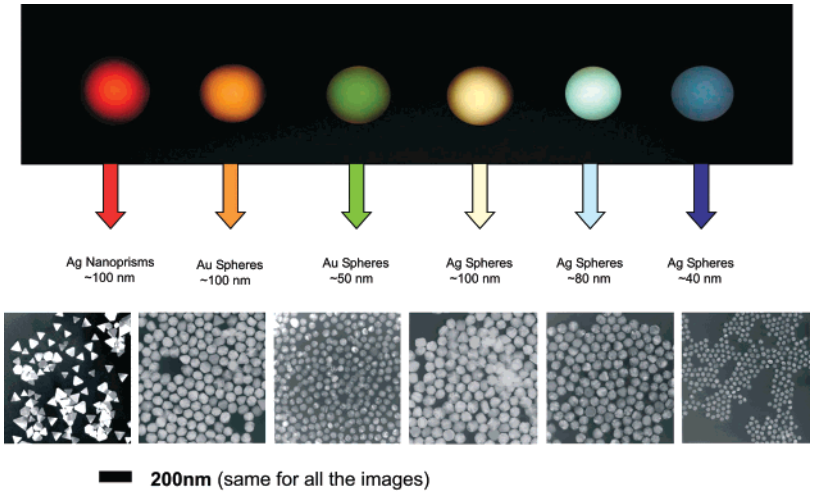

Figure 1. Sizes, shapes, and compositions of metal nanoparticles can be systematically varied to produce materials with distinct light-scattering properties.

proteins, and some biologically relevant small molecules and metal ions. Moreover, we focus on some of the key fundamental properties of certain nanostructures that make them ideal for specific diagnostic applications.

1.1. Background and Perspectives

Nucleic acid sequences unique to every living organism and every bacterium, virus, or pathogen provide practical targets for the identification and diagnosis of various diseases. With the advent of rapid sequencing capabilities, sequence information is now available for many diseases, including those associated with bioterrorism and warfare. To more effectively combat these diseases in the medical arena and accelerate response to bioterrorism threats, early and accurate detection of DNA markers is crucial. In this area, multidisciplinary teams of researchers including chemists, biochemists, and physicists have been evaluating the prospect of using assays based upon nanomaterials to compete effectively with the polymerase chain reaction (PCR) coupled with molecular fluorophore assays.[8−11] PCR, a technology that allows duplication of portions of prospective targets, represents the ultimate in terms of sensitivity[12] but has significant drawbacks including complexity, sensitivity to contamination, cost, and lack of portability and major challenges with respect to multiplexing (detecting multiple targets in a single assay).[13] Many researchers view these limitations as some of the biggest impediments to moving nucleic-acid-based detection to point-of-care settings, including the doctor's office, the battlefield, the third world, and first responder sites in the case of bioterrorism defense. These settings require straightforward, inexpensive, and disposable detection formats that have rapid and accurate readouts and require limited processing and user expertise. For nanomaterials to compete in the area of nucleic-acid detection, they have to make a compelling case, with PCR and molecular fluorophore technology setting the benchmarks for comparison.

Abnormal concentrations of certain proteins often signal the presence of various cancers and diseases. However, current protein detection methods only allow detection after protein levels reach critical threshold concentrations. At these concentrations the cancer or disease is often significantly advanced. More sensitive methods that allow for early detection of protein markers could potentially revolutionize physician treatment of various cancers and diseases and increase patient survival rates. In the area of protein diagnostics, the current gold standard is the enzyme-linked immunosorbent assay (ELISA) (~pM detection limits[14]), which also relies on fluorophore labeling and is extraordinarily general. An equivalent to PCR in the protein detection arena does not exist; therefore, there is greater room with which to compete with respect to sensitivity. It is important to note, however, that molecular fluorophores have many significant drawbacks, including susceptibility to photobleaching, broad absorption and emission bands, and a reliance on relatively expensive equipment to probe their presence in an assay. Again, these properties limit their use in point-of-care settings, so less expensive and more portable detection systems would be beneficial. For nanomaterials to compete in the area of protein detection, they must address one or more of the limitations imposed by the use of molecular fluorophores.

1.2. Why Nanomaterials?

Not all molecular fluorophores make for suitable probes in biodiagnostic assays nor do all nanomaterials offer advantages in biodetection. Certain nanomaterials are attractive probe candidates because of their (1) small size (1−100 nm) and correspondingly large surface-to-volume ratio, (2) chemically tailorable physical properties, which directly relate to size, composition, and shape (Figure 1), (3) unusual target binding properties, and (4) overall structural robustness. The size of a nanomaterial can be an advantage over a bulk structure, simply because a target binding event involving the nanomaterial can have a significant effect on its physical and chemical properties, thereby providing a mode of signal transduction not necessarily available with a bulk structure made of the same material. Tailorable physical properties

are a very important aspect of nanomaterials. Indeed, in this regard, nanomaterials and biology have a long history as nanoparticles have been used in bioconjugation and as cellular labeling agents for the past four decades.[15] However, new synthesis, fabrication, and characterization methods for nanomaterials have evolved to the point that deliberate modulation of their size, shape, and composition is possible, thereby allowing exquisite control of their properties. The ability to carefully tailor the physical properties of nanomaterials is essential for their application in biodetection.[1] Specifically, the sizes, shapes, and compositions of metal nanoparticles and quantum dots can now be systematically varied to produce materials with specific emissive, absorptive, and light-scattering properties (Figure 1), which make these materials ideal for multiplexed analyte detection;[1,16−19] the composition of nanowires and nanotubes also can be controlled, thus allowing for measurement and variation of their conductive properties in the presence of target analytes.[20] Additionally, tools and techniques for surface modification and patterning have advanced to a point that now allows generation of nanoscale arrays of biomacromolecules and small molecules on surfaces.[21−24] Along with synthetic advances for varying the size, shape, and composition of nanostructured materials has come the ability to tailor their binding affinities for various biomolecules through surface modification and engineering.[25−28] Each of these capabilities allows researchers to design materials that can potentially be implemented into new assays having improved modes of signal transduction that can compete favorably with the molecular fluorophore-dominated methods of PCR and ELISA.

2. Nanoparticle-Based Detection Methods

2.1. Optical Detection

2.1.1. Nucleic Acids

An early indication of the potential of nanomaterials as biodetection agents, beyond conventional histochemical staining, was reported in 1996 with the observation that oligonucleotide-modified nanoparticles and sequence-specific particle assembly events, induced by target DNA, could be used to generate materials with unusual optical and melting properties.[25] Specifically, when 13-nm gold particles were used in the assay, the color of the solution changed from red to blue upon the analyte-directed aggregation of gold nanoparticles, a consequence of interacting particle surface plasmons and aggregate scattering properties. This simple phenomenon pointed toward the use of nanoparticles as DNA detection agents in a type of "litmus test" for nucleic acid targets, and indeed, it was found that spotting the solution onto a white support enhanced the colorimetric change and provided a permanent record for each test (Figure 2).[29,30] Further studies indicated that the melting profiles of the nanoparticle-labeled DNA aggregates were extraordinarily sharp, occurring over a temperature range much more narrow than the transition for unlabeled or conventional fluorophore-labeled DNA (Figure 2).[29−32] These two

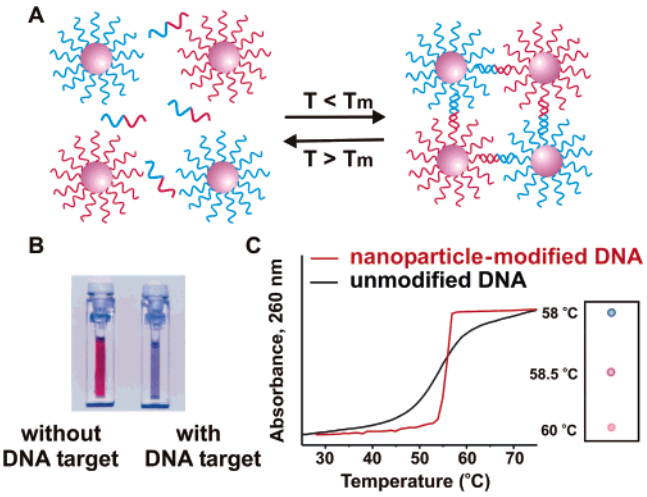

Figure 2. In the presence of complementary target DNA, oligonucleotide-functionalized gold nanoparticles will aggregate (A), resulting in a change of solution color from red to blue (B). The aggregation process can be monitored using UV−vis spectroscopy or simply by spotting the solution on a silica support (C). (Reprinted with permission from *Science* (http://www.aaas.org), ref 29. Copyright 1997 American Association for the Advancement of Science.)

observations, both consequences of the high surface area and unique optical activity of the gold nanoparticles, created worldwide interest in exploring the potential for designer nanomaterials in biodiagnostic applications. The colorimetric change pointed to a simple and inexpensive way of diagnosing disease, and the unanticipated sharp melting profile suggested that assays based upon such nanostructures should have higher selectivities than the conventional molecular fluorophore-labeled structures that exhibit broad melting profiles when hybridized with complementary DNA. The sharp melting transitions associated with these nanoparticle probes derive from the dense loading of oligonucleotides on their surfaces and their ability to bind to complementary DNA in a highly cooperative manner.[32] These properties have not been observed with microparticle probes, partly because the loading efficiency of oligonucleotides does not compare with the gold nanoparticle−thiol system. It is worth noting that colorimetric responses have been utilized in viral detection systems based upon supramolecular polydiacetylene liposomes.[33]

Further exploration of the potential of these materials in DNA detection showed that by virtue of these sharp melting transitions target DNA could be differentiated from DNA with single base-pair mismatches simply by measuring absorbance (or looking at color) as a function of temperature.[29,30] This technique offered several advantages over other techniques such as arrays probed by fluorescence in that (1) it exhibited a high degree of discrimination between perfectly matched target oligonucleotides and targets with single base-pair mismatches, (2) it was "quick and easy", and (3) its optical read-out did not require expensive, sophisticated instrumentation. It should be further noted that this assay had the potential for modest multiplexing simply by synthetically tuning the composition of the nanoparticles to yield particles with different surface plasmon reso-

nances.[34] At least two color pairs are available via this approach (red and blue for gold particles; yellow and black for core−shell, gold-coated silver particles). A limitation of this approach involved its sensitivity, which in the unoptimized format was in the 1−10 nM range. Modest improvements to this assay were made when larger nanoparticles (50 or 100 nm probes) were employed. Specifically, in using 50 nm probes target could be quantitatively detected between 5 nM and 50 pM.[31] However, these values are still not as good as the best results from fluorophore-based assays (typically in picomolar range; best reported ~600 fM),[35] thus limiting its application to assays that require preamplification of target through methods such as PCR.

In addition to DNA hybridization-promoted nanoparticle aggregation, others demonstrated that hybridization reactions involving oligonucleotide-modified gold nanoparticles that do not result in aggregate assembly can result in measurable optical changes that correlate with target concentration.[36] The authors contend that in these systems the aggregation is promoted by a reduction of the repulsive interactions between nanoparticles upon formation of duplex DNA on the surface of the nanoparticles. Specifically, they postulate that the stiffening of the DNA upon formation of duplex raises the binding constant with counterions, which can serve to better shield the negative charges. While an interesting phenomenon, this assay is even less sensitive (detection limit = 60−500 nM range) than the assay involving particles cross-linked through hybridization[29] because it requires more DNA to effect the optical changes. Another DNA detection format involves nanocrystals modified with peptide nucleic acids (PNAs) and hybridized with target DNA.[37] This method relies on the increased salt stability of PNA-functionalized particles in the presence of hybridized target oligonucleotides. By slowly increasing the salt concentration of a suspension of PNA-functionalized particles hybridized with target DNA while also monitoring the colloidal stability, the extent of binding of the target oligonucleotide can be determined. If the target has a mutation, the colloid will aggregate at lower salt concentrations.

Very recently, the interactions between citrate-coated gold nanoparticles and short single-strand DNA were exploited to detect sequences in PCR-amplified genomic DNA.[38,39] Researchers found that short single-strand DNA oligomers stabilize citrate-coated gold nanoparticles and prevent salt-induced aggregation.[39] Thus, exposure of citrate-coated gold nanoparticles to a saline mixture containing amplified, dehybridized genomic DNA and short oligomers that are complementary to regions along the genomic DNA results in particle aggregation (color change from red to blue) because the oligomers hybridize to the target genomic DNA and are therefore not available to stabilize the particles. If the oligomers are not complementary to regions along the genomic DNA, they can then stabilize the gold nanoparticles, resulting in no color change and signaling the absence of target DNA.

Introduction of nanoparticles into some well-studied DNA assays results in improved sensitivity. For example, surface plasmon resonance (SPR) is used to detect and probe real-time DNA hybridization on surfaces with detection limits of ~150 nM target.[40] However, when targets are hybridized in a sandwich format between surface-capture strands and oligonucleotide-functionalized nanoparticle labels, the detection limit improves approximately 1000-fold to less than 10 pM target concentration.[41] Another real-time detection method that utilizes oligonucleotide-labeled gold nanoparticles was recently developed.[42] This method involves the immobilization of capture oligonucleotide strands onto chemoresponsive diffraction gratings followed by capture of target DNA and finally labeling of targets with oligonucleotide-functionalized nanoparticle probes. Monitoring target hybridization in real-time using laser diffraction results in femtomolar concentration detection limits.

Molecular beacons are commonly used for nucleic acid detection.[10,11] A drawback of molecular beacons is the quenching efficiency of the molecular quencher.[43] When the molecular quencher is replaced with a gold nanoparticle, the quenching is much more efficient, resulting in a more sensitive probe.[43] Moreover, these probes have higher single base mismatch selectivity (25:1) compared to conventional molecular beacons (4:1). Nie and co-workers also used gold nanoparticles as quenchers in a molecular fluorophore nucleic acid probe.[44] Their design, however, does not incorporate the DNA hairpin structure used in molecular beacons. Rather, they modify gold nanoparticles with oligonucleotides functionalized on one end with a thiol and the other end with a molecular fluorophore. The thiol end binds to the gold particle surface, and the fluorophore nonspecifically binds to the gold surface, resulting in a "loop" structure in which the gold nanoparticle quenches the emission from the fluorophore. Target binding breaks the "loop" structure, thus distancing the fluorophore from the nanoparticle quencher, resulting in measurable fluorescence.[44]

Tan and co-workers developed fluorescent dye-doped silica nanoparticles functionalized with oligonucleotides as labels for chip-based sandwich DNA assays.[45] The nanoparticles are composed of a silica matrix that encapsulates large numbers of fluorophores. Not only does this increase the fluorescent signal associated with each target recognition event, but the silica matrix also acts as a protective barrier against fluorophore bleaching. This method results in an impressive detection limit of ~1 fM target and provides ~14:1 differentiation between target DNA and DNA with only one base mismatch. Tan's group used similar particles to detect single bacterium cells.[46] Here, they modify the fluorescent nanoparticles with monoclonal antibodies specific for the O-antigen of *E. coli* O157:H7. When mixed with 100 μL samples containing single bacterium cells, the fluorescent particles densely coat the cell walls, allowing detection with typical fluorescent plate readers. These methods improve upon typical molecular fluorophore-based assays, but they remain somewhat limited by the fundamental drawbacks of

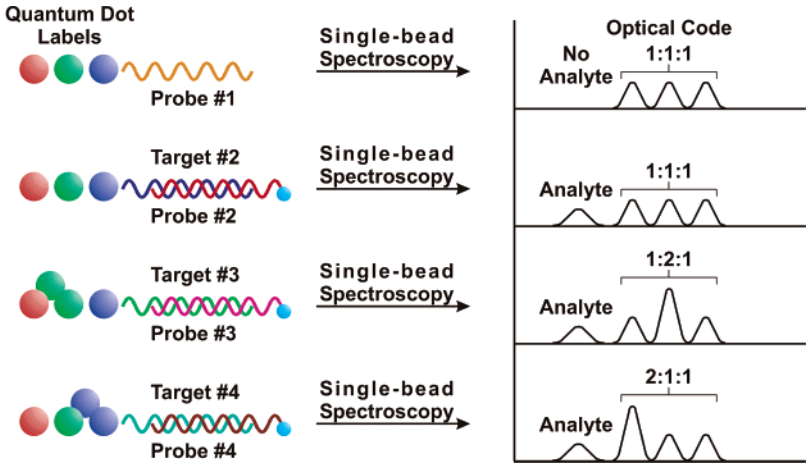

Figure 3. Quantum dots can be employed for detecting multiple targets in a single assay. Specifically, varying the numbers and ratios of different quantum dots per target results in a unique fluorescent signal for each individual target. (Reprinted with permission of Nature Publishing Group. *Nature Biotech.*, Vol. 19, 2001, by Nie, et al.)

molecular fluorophores including broad adsorption and emission profiles, which reduces multiplexing capabilities.

Quantum dots, with their broad excitation spectra, sharp emission spectra, and easily tunable emission properties, are potential candidates for replacing conventional fluorescent markers in biodetection assays. Having already shown considerable promise as intracellular imaging and tracking agents,[47−51] quantum dots made of CdSe and ZnS, with few exceptions,[52−56] have not been widely investigated as materials for biodetection assays. The first example of chemically modifying CdSe quantum dots with DNA involved ligand exchange coupled with particle surface engineering.[53] Recent studies employed quantum dots as labels imbedded in polymeric structures.[54] Using this strategy, Nie and co-workers provided a proof-of-concept study to display the potential of quantum dots as tags for multiplexed DNA detection (Figure 3). In this work they labeled the target DNA with a fluorophore and oligonucleotide-functionalized polymeric microbeads with quantum dots designed to emit at various specified wavelengths other than that of the target DNA. Microbeads with different ratios of quantum dots exhibited different signature fluorescence spectra. After capture of target DNA by the microbead/quantum dot assembly, single-bead spectroscopy studies revealed both the presence and the identity of the target DNA. In essence, this work demonstrates that quantum dot labels can be "mixed and matched" to produce emission signals with variable intensities. This results in a palette of quench-resistant labels for biomolecule detection that compare favorably with molecular fluorophores.

Alivisatos and co-workers recently reported studies using CdSe/ZnS quantum dots in chip-based assays to detect single base-pair mutations in DNA.[56] They detect perfectly complementary target DNA at concentrations as low as ~2 nM in the presence of background oligonucleotides containing various sequence mismatches. Since individual quantum dots have previously been used to detect single molecules under ideal conditions using fluorescence microscopy,[57] it is likely that the sensitivity of these assays can be improved with proper surface modification processes and engineering. However, as for any assay, the ultimate sensitivity of techniques based upon these materials will not be based upon how few quantum dots can be detected but rather the target binding constant for the particles and their selectivity in complex media. They will undoubtedly be used extensively for research applications, but their use in the medical diagnostic arena will be determined by the advances made over the next few years in increasing sensitivity and selectivity and the movement toward materials less toxic than CdSe.[58]

The unique light-scattering properties of nanoparticles (Figure 1) have prompted interest in their potential application as labels for multiplexed analyte detection.[59−65] In 1995 Stimpson and co-workers incorporated light-scattering selenium nanoparticles into a simple proof-of-concept chip-based DNA assay.[59] Since that initial report Yguerabide and Yguerabide demonstrated that light-scattering particles favorably compete with conventional fluorophores as diagnostic labels.[60−62] For example, they showed that a single 80 nm gold particle has a light-scattering power equivalent to the signal generated from ~10^6 fluorescein molecules,[60] and unlike molecular fluorophores, the light-scattering signal from metal nanoparticles is quench resistant. Given these properties, they replaced molecular fluorophores with resonance light-scattering (RLS) particles (essentially 40−120 nm metal nanoparticles) in typical cDNA microarrays[9] to evaluate their potential as labeling agents. In these experiments biotinylated probe DNA binds to specific regions in the cDNA microarrays. Then, anti-biotin-labeled RLS particles signal the presence of these specific regions by binding to the biotinylated probe sequences. In comparing this approach to molecular fluorophore-based approaches, they found that at low probe DNA concentrations (16.7 pg/μL) RLS particles detected ~300 times more genes than Cy3, a commonly used molecular fluorophore.[60] The drawback of light scattering based on nanoparticles is that the signal depends on not only the size and shape of each particle, which is difficult to control on a large scale, but also the orientation of the particles on the surface and their interactions

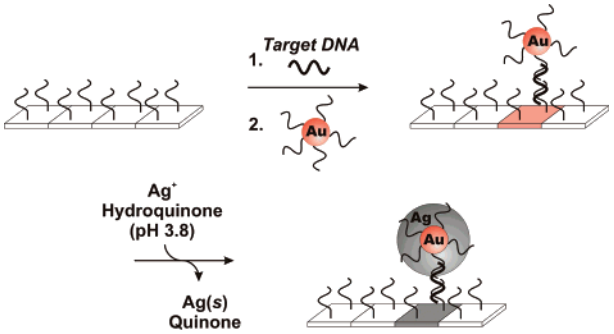

Figure 4. Scanometric DNA assay. In this assay a surface-bound capture oligonucleotide binds one-half of the target of interest, and an oligonucleotide-functionalized gold nanoparticle probe binds to the other half. Catalytic reduction of silver onto the capture/target/probe sandwich results in a signal that can be detected scanometrically. (Reprinted with permission from *Science* (http://www.aaas.org), ref 66. Copyright 2000 American Association for the Advancement of Science.)

with other particles. This makes response calibration very difficult. More recently, researchers used the colorimetric light scattering of nanoparticles to detect synthetic DNA and genomic DNA at concentrations of 333 and 33 fM, respectively.[65] This assay involves pairs of 50-nm diameter gold probes, each modified with oligonucleotides that are complementary to neighboring regions on the target DNA. In the presence of target, the nanoparticle probes scatter orange light as a result of a plasmon band red shift; if the target is absent, the probes scatter green light.

One of the most important advantages offered by the colorimetric nanoparticle approach to DNA detection is the exquisite selectivity that results from the sharp melting transitions of nanoparticle-labeled DNA (Figure 2).[29−32] This advantage has been realized in a chip-based system that relies on a sandwich assay involving an oligonucleotide-modified glass slide, a nanoparticle probe, and target.[66] The assay consists of a capture DNA strand immobilized on a glass chip that recognizes the DNA of interest. A separate sequence on the captured target strand is then labeled with an oligonucleotide-functionalized nanoparticle probe. At this point, a thermal stringency wash removes nonspecifically bound target strands, allowing for over 10:1 selectivity for single base-pair mutations. After catalytic reduction of silver onto the gold nanoparticle surfaces to amplify the target signal (Figure 4), the capture-strand/target/nanoparticle sandwich can be visualized with a flatbed scanner (hence the term "scanometric" is used to describe the approach) at target concentrations as low as 50 fM, a nearly 100-fold increase in sensitivity over traditional fluorescence-based assays. Since the original study the technique has been significantly refined and new research shows that 250 base-pair PCR amplicons of the Factor V Leiden gene can be distinguished from strands containing a single base-pair mismatch at concentrations as low as 100 aM.[35] Moreover, researchers unambiguously detected the MTHFR gene from a 20 μg sample of human genomic DNA (~200 fM in target) without prior PCR amplification.[67] This was a major advance, demonstrating the ability to use nanostructures to detect genomic DNA in samples without PCR at concentrations relevant to real medical diagnostic applications. The use of nanoparticles is the key to these advances. Indeed, the selectivity of this method is a consequence of the sharp melting transitions of DNA-modified gold nanoparticles, and its sensitivity derives from the catalytic properties of the gold nanoparticles and their ability to effect the reduction of silver ions to amplify the detection signal.

Attaching Raman-dye-labeled oligonucleotides to the gold nanoparticle probes generates spectroscopic codes for individual targets of interest, thus permitting multiplexed detection of analytes.[68,69] Specifically, the presence of the target is confirmed by silver staining, and the identity of the target is revealed by detecting the surface-enhanced Raman scattering (SERS) of the Raman dye near the nanoparticle surface (Figure 5). The silver coating enables detection of the signal from the dye-labeled particle (~1 fM).[68] SERS is one of the most sensitive diagnostic approaches available to the analytical chemist.[70] This approach is similar to that which employs multiple fluorophores as labels; however, the spectroscopic lines in Raman spectroscopy are not as broad as the bands in fluorescence spectroscopy, and the spectral window is much broader. This, in principle, will allow a greater degree of multiplexing. Indeed, target DNA sequences specific to multiple different bioterrorism agents have been identified using this approach with spectroscopically distinguishable nanoparticle probes (Figure 5). Furthermore, only single-wavelength laser radiation is needed to scan a highly multiplexed array with numerous target-specific Raman dyes. This is in contrast to array-based detection of molecular fluorophore probes, where different excitation frequencies are needed for each fluorophore.

The catalytic deposition of metals onto gold nanoparticles allowed for signal amplification in the scanometric detection of DNA.[66,67] Indeed, silver enhancement resulted in 100 aM detection limits,[35] nearly a 5 orders of magnitude increase over solution-phase, unamplified colorimetric detection. Even with signal amplification, however, most of the reported assays still require enzymatic-based target amplification steps such as PCR prior to detection steps. A new assay, which couples silver enhancement with an additional indirect target amplification method, pushes nanoparticle-based detection limits to values previously approached only by using PCR. This assay, called bio-bar-code amplification (BCA),[71] employs oligonucleotides that act as bar codes for target DNA (Figure 6). There are two components to the assay: magnetic microparticles functionalized with target capture strands and gold nanoparticles functionalized with both target capture and hundreds of bar-code capture oligonucleotides that are hybridized to bar-code DNA. In the presence of target DNA, the magnetic microparticles and the gold nanoparticles form sandwich structures that are magnetically separated from solution and washed with water to remove the hybridized bar-code DNA. The bar codes (hundreds to thousands per target) are detected using the scanometric approach, resulting in detection limits as low as 500 zM (10 strands in solution).[71]

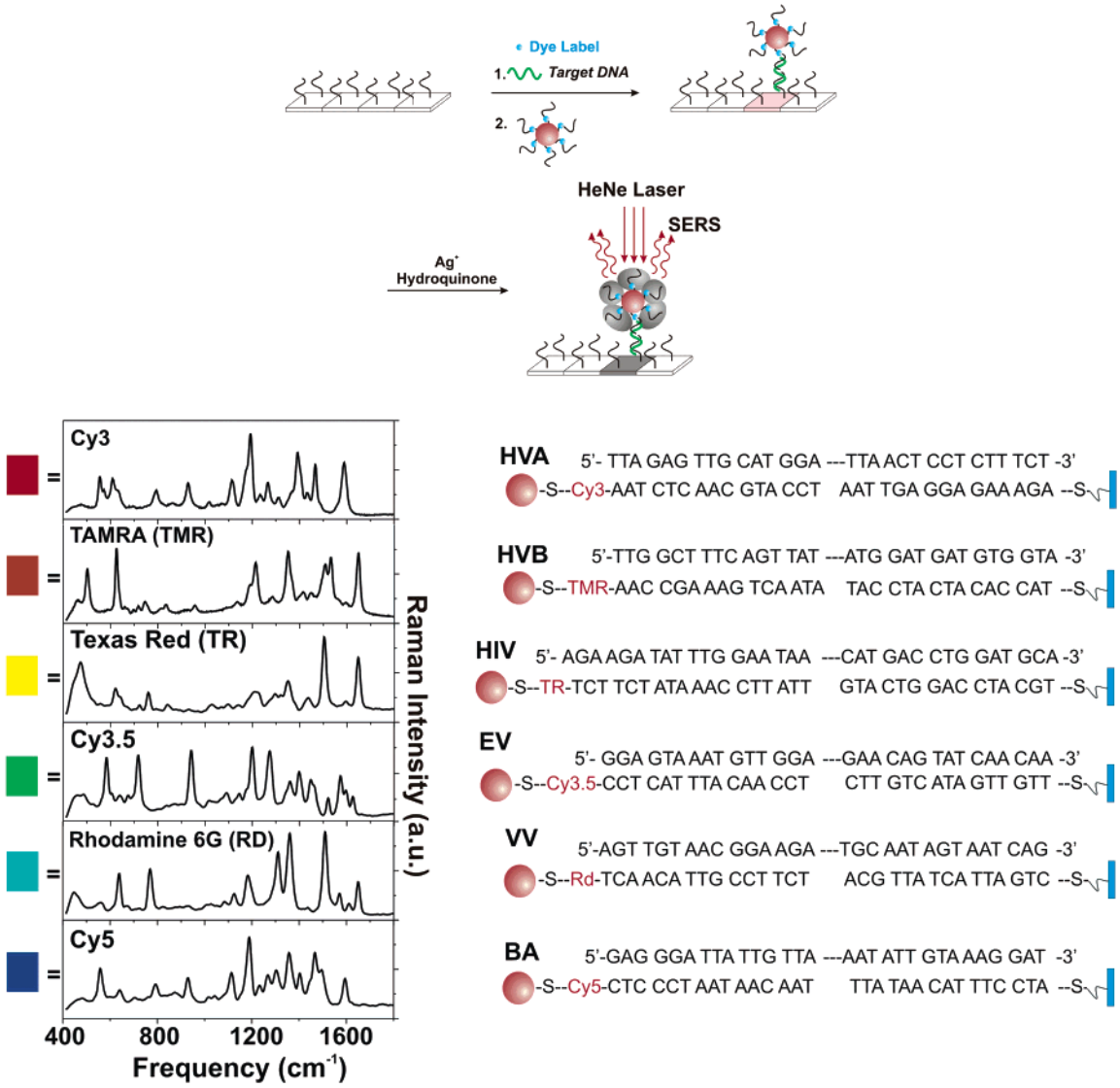

Figure 5. If Raman dyes (blue spheres) are attached to the labeling probe in the scanometric assay, the targets can be encoded and detected via the Raman signal of their labels. (Reprinted with permission from *Science* (http://www.aaas.org), ref 68. Copyright 2002 American Association for the Advancement of Science.)

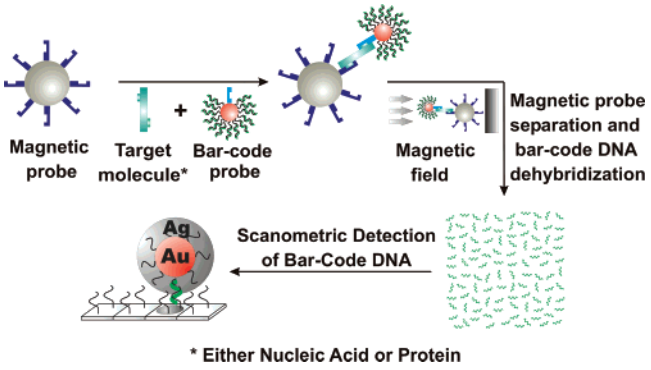

Figure 6. Nanostructure-based bio-bar-code amplification scheme. In this assay magnetic microparticles capture either the target DNA or the protein. Gold nanoparticles loaded with bar-code oligonucleotides and target capture molecules are added to the assay to form a sandwich system. The sandwich complexes are magnetically separated from the assay mixture and then washed with water to remove the bar-code DNA that code for the target DNA or protein of interest. The bar codes are detected using the scanometric approach.

This method obviates the need for PCR in DNA detection and is fast, regardless of target concentration since the kinetics of the target binding process can be controlled by adjusting probe concentrations. Additionally, it is well suited for multiplexing as bar codes can be synthesized for virtually any target of interest.

2.1.2. Proteins and Biologically Relevant Small Molecules

Protein and small molecule detection strategies that incorporate nanoparticles typically rely on the specific interactions between nanoparticle-bound antibodies with the target protein and the resulting effects these interactions have on the optical signature of the nanoparticles. The versatile surface chemistry of nanoparticles is important for these applications in that there are numerous straightforward methods of conjugating antibodies to various types of nanoparticles. One approach, pioneered by Halas, West, and co-workers, uses antibodies conjugated to the surface of gold nanoshells to detect proteins in saline, serum, and whole blood.[72] Upon

interaction with the target protein, the antibody-functionalized nanoshells aggregate, resulting in a corresponding broadening of the nanoshell extinction peak at 720 nm. This assay is simple, fast (10 min), and detects target proteins in the range of 88−0.88 ng/mL, which is within the range of ELISA. An important aspect of this assay that should not be overlooked is its ability to detect proteins in serum and whole blood, which is important for any assay designed to function in nonlaboratory sites where sample preparation and purification is limited. Similar assays involve monitoring the light-scattering properties of gold colloids before and after avidin−biotin-induced particle aggregation.[73] As the concentration of avidin decreases, the light-scattering intensity of the gold colloids also decreases. However, this assay is only sensitive down to 1 nM avidin concentrations.

Another route involves tagging protein recognition molecules with oligonucleotides that are complementary to oligonucleotides coating the surfaces of Au nanoparticles.[74] These molecules are then recognized by specific proteins in solution, resulting in aggregation of the nanoparticle system. This assay has the potential for massive multiplexing as different protein targets can be tagged with specific oligonucleotides. As proof-of-concept, it was shown that both IgG1 (anti-biotin) and IgE (anti-dinitrophenyl) could be detected simultaneously by labeling the small molecules, biotin and dinitrophenyl, with oligonucleotides of different sequence and then hybridizing these moieties to nanoparticles functionalized with the appropriate strands of complementary oligonucleotides. To detect both IgG1 and IgE the nanoparticles were added to a solution containing the two proteins, and then the melting profile of the resulting aggregate was examined. In solutions where both IgG1 and IgE were present there was evidence of melting for both the biotin-labeled oligonucleotide and the dinitrophenyl oligonucleotide. An alternative approach requires isolation and separation of the protein−DNA−nanoparticle aggregate followed by dehybridization of the DNA that tags the individual proteins. This DNA can then be detected using the scanometric approach. Both assays, melting and scanometric, exhibit optimal detection limits in the nanomolar range, 3 orders of magnitude lower in sensitivity than ELISA, thus necessitating optimization and improvement in order to compete. To this end, this methodology has recently evolved into the bio-bar-code amplification (BCA) method used for DNA (vide supra) and protein detection, which is unparalleled in terms of assay sensitivity, especially with respect to protein markers.

Specifically, BCA for proteins involves scanometric detection of DNA bar codes that code for target proteins instead of DNA (Figure 6).[75] As before, there are two components in this assay: magnetic microparticles functionalized with monoclonal antibodies for the target protein and gold nanoparticles coated with both polyclonal antibodies for the target protein and also oligonucleotides hybridized to bar-code strands that code for the target protein. In this method the magnetic microparticles first capture target proteins in solution, and addition of the gold nanoparticles results in the formation of sandwich structures. Following the same procedure in the DNA-BCA assay, protein targets can be detected at attomolar concentrations. While PCR amplification enhances DNA detection limits, protein detection has not benefited from a similar target amplification strategy.[76,77] The BCA assay is impressive in this regard, providing a PCR-less method of amplifying protein concentrations by coding for protein targets with hundreds of bar-code oligonucleotides. With this advance, protein markers that flag the presence of diseases such as prostate and breast cancer, Alzheimer's disease,[78] and AIDS can be detected at levels unachievable with current techniques, thus potentially allowing for earlier detection and perhaps more effective treatment protocols for these ailments.

Heterogeneous chip-based systems also have been explored for protein detection. Niemeyer and colleagues used the scanometric approach as a method for detecting proteins.[79] In this procedure capture antibodies specific for target proteins are immobilized on a surface. After the target proteins bind to the capture antibodies, antibody-labeled gold nanoparticles bind to the proteins to generate a sandwich system. Silver amplification is used to detect protein binding either spectrophotometrically or using a flatbed scanner, resulting in a detection limit of ~200 pM which is comparable with ELISA. A Raman-based approach that relies on many of the principles used in DNA detection (vide supra) provides a method for detecting protein−protein and protein−small molecule interactions.[80] In this approach protein microarrays[81] are screened with gold nanoparticles functionalized with specific antibodies or small molecules in addition to a Raman dye to code for the antibody or small molecule. After the functionalized gold nanoparticles interact with the surface-bound proteins, the arrays are amplified with silver to elucidate particle binding, and then SERS is employed to determine the type of protein−protein or protein−small molecule interactions. In a similar approach Porter and co-workers sandwiched target proteins between an antibody-coated gold substrate and gold nanoparticles coated with both antibody and Raman labels.[82] The resulting Raman signal indicated the presence of target protein, and the intensity of the signal correlated with target concentration. Using this method they detected ~1 pg/mL (~30 fM) prostate-specific antigen in human serum and ~4 pg/mL (~120 fM) in bovine serum albumin.

Many solution-based nanoparticle assays take advantage of analyte-induced aggregation events that result in measurable changes and shifts of nanoparticle surface plasmon absorption bands. Van Duyne and co-workers demonstrated that surface nanostructures can be used to detect proteins by monitoring shifts in their surface plasmon resonance after binding of target proteins.[83,84] In their system triangular silver nanoparticles are generated on surfaces using nanosphere lithography, and then biotin is immobilized on the surfaces of the particles. After adding streptavidin to the system a shift in the surface plasmon resonance of the silver triangles is

observed which results from changes in the refractive index near the nanoparticle surfaces. This method allows detection of streptavidin at concentrations as low as ~0.1−1 pM.[83] Because streptavidin has a total of four biotin binding sites, biotinylated gold nanoparticles can be added to the assay after the detection step to amplify the surface plasmon signal shift. In further studies using the same approach it was shown that anti-biotin, instead of streptavidin, could be detected at ~100 pM concentrations.[84] Most recently, this technique has been used to detect nanomolar amounts of amyloid β-derived diffusible ligands (ADDLs), potential molecular markers for Alzheimer's disease.[85]

2.1.3. Metal Ions

The simplicity of the colorimetric detection format pointed toward its use as a general method to detect wide varieties of analytes. Lu and co-workers provided a particularly elegant example of colorimetric detection by implementing an oligonucleotide-assembled nanoparticle network to detect Pb(II) ions in aqueous media and lead-containing paint samples at concentrations as low as 100 nM.[86] In this assay the nanoparticle network was assembled using a linking strand with 3′ and 5′ ends that were complementary to strands on the Au nanoparticles. The middle region of the linking strand was complementary to a DNAzyme with a high affinity for Pb(II). In the presence of Pb(II), the DNAzyme hydrolyzes the linking strand, causing the nanoparticle aggregate to disassemble, thus resulting in a color change from violet to red.

Spectroscopically silent metal ions such as Hg(I) can induce the aggregation of nanoparticles functionalized with appropriately designed chelating groups such as mercaptocarboxylic acids. Here, metal ions bridge the carboxylate moieties of different gold nanoparticles, resulting in a concomitant colloidal color change from red to blue.[87] Using this technique Pb was detected at concentrations as low as 400 μm. Another approach uses gold nanoparticles functionalized with a phenanthroline ligand designed to bind Li^+ ions in a 2:1 fashion. In the presence of Li^+ ions, the particles aggregate, allowing detection of Li^+ in the 10−100 mM range.[88] Last, gold nanoparticle systems are also useful for selectively detecting K^+ ions versus Na^+ ions in water.[89] When millimolar solutions of K^+ ions are exposed to solutions of 15-crown-5-modified gold nanoparticles that also contain Na^+ ions, the particles aggregate as a result of sandwich complex formation between two 15-crown-5 (from neighboring nanoparticles) and one K^+. In the case of Na^+, there is no sandwich formation and thus no particle aggregation. This technique could provide a useful method of detecting K^+ ions in serum samples that typically have a high background concentration of Na^+.[89] Continued improvement of ligand design that allows for highly specific metal coordination will result in even more selective metal ion detection assays that implement nanostructured probes.

Detection and tracking of metal ions in vivo necessitates the use of robust and highly specific detection agents that can withstand intracellular environments. Nanoparticle-based methods have proven to be viable tools for these tasks in certain cases. In particular, different nanoparticle probes that consist of fluorescent dyes encapsulated in a biocompatible polymer matrix can be designed to detect a wide variety of intracellular cations including calcium,[90] zinc,[91] and magnesium.[92] These probes, called PEBBLEs (probes encapsulated by biologically localized embedding), are advantageous in that the polymer matrix can both reduce the amount of dye photobleaching and nonspecific binding and protect the cell from potential toxic side effects of certain dyes. Variation of the polymer matrixes enables encapsulation of both hydrophilic and hydrophobic dyes. Moreover, multiple different dyes can be encapsulated in one particle, thus allowing signal ratioing for quantification purposes.

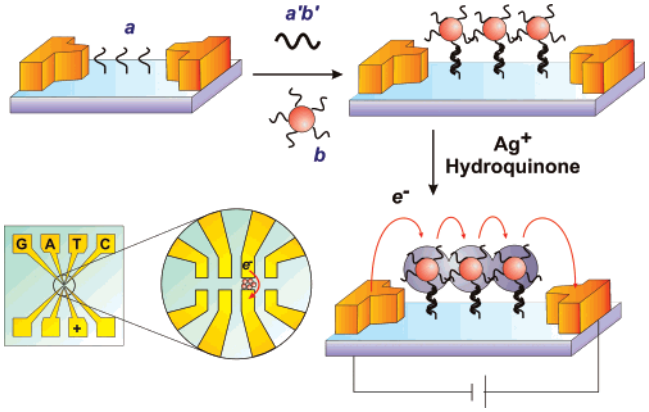

Figure 7. When the capture/target/probe sandwich is positioned in the gap between two electrodes, catalytic reduction of silver onto the sandwich system results in a signal that can be detected electrically. (Reprinted with permission from *Science* (http://www.aaas.org), ref 93. Copyright 2002 American Association for the Advancement of Science.)

2.2. Electrical and Electrochemical Detection

2.2.1. Nucleic Acids

Electrical detection methods offer the possibility of portable assays that could be used in a variety of point-of-care environments. Nanoparticle sandwich assays combined with silver amplification can be used for the electrical detection of DNA in a handheld format (Figure 7).[93] If oligonucleotide capture strands are immobilized in the gap between two electrodes and a sandwich assay analogous to the one used in the scanometric approach is performed, DNA can be detected as a measure of the change in electrical current or resistance between the two electrodes. In the absence of target DNA, there is no current flow across the electrode gap, but in the presence of target DNA, the associated nanoparticle probes, and catalytically deposited silver, current can flow between the electrodes. This method registers an unoptimized detection limit of 500 fM, but more importantly, when coupled with a salt-concentration-based stringency wash, it exhibits a selectivity factor of 10 000:1, which is impressive when compared to the analogous array experiment carried out with a molecular fluorophore

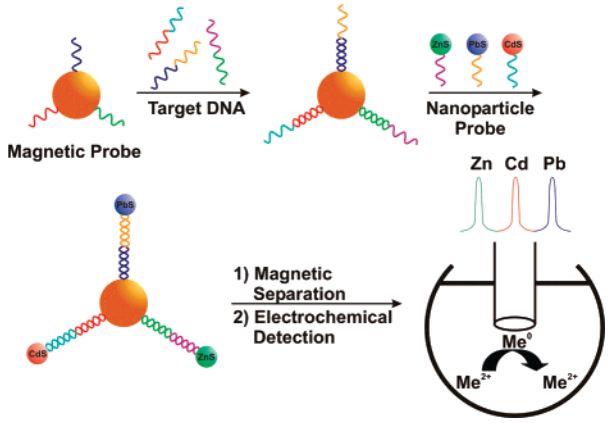

Figure 8. Magnetic microparticles (large brown spheres) labeled with DNA capture strands can bind target DNA, and then oligonucleotide-functionalized nanoparticle labels (small spheres) with different electrochemical signatures can be used to code for the specific target DNA of interest.

probe using identical chip and probe sequences (2.6:1) or even the scanometric approach with a thermal stringency (10:1).[66] This potentially eliminates the need for on-chip temperature control, dramatically reducing the complexity of a hand-held device for DNA detection.

The detection of DNA using a quartz crystal microbalance (QCM) can be amplified using gold nanoparticle probes.[6,94] Here, a DNA capture strand is immobilized on a gold/quartz piezoelectric crystal. After hybridization of the capture strand to one-half of the target, a gold nanoparticle-modified oligonucleotide hybridizes to the second half of the target strand, thus enhancing the signal output of the QCM device. Gold is then electrochemically reduced onto the surface of the gold nanoparticle to provide further signal enhancement. Using this method 1 fM concentrations of target DNA can be detected.[94]

Coupling DNA detection with electrochemical readout has been widely studied.[5] Redox-active nanoparticle probes are attractive because their electrochemical signal can be systematically tuned by changing their compositions and their binding properties to various biomolecules can be controlled. Recently, new assays were developed that involve electrochemical stripping of the nanoparticle portion of DNA−nanoparticle conjugates.[95,96] These systems employ a sandwich assay in which target capture strands are attached to magnetic beads (Figure 8).[96] Once the target oligonucleotide hybridizes to the capture strand, it is then labeled with an oligonucleotide-functionalized inorganic nanoparticle probe that codes for the target strand of interest. The sandwich system can be magnetically separated and transferred to an electrochemical cell where the nanoparticles are dissolved and detected electrochemically. Different nanoparticles yield different voltammetric signals, depending upon their composition. The magnitude of the recorded signal corresponds to the concentration of target DNA, thus making this method amenable to multiplexing and quantification. However, the optimized detection limit of this assay is at 270 pM target concentration, still necessitating target amplification with PCR.

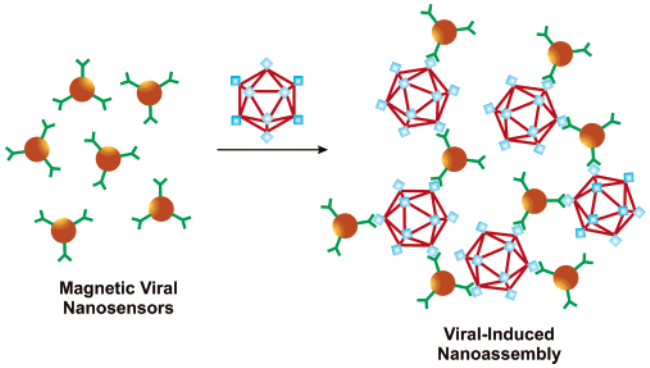

Figure 9. Superparamagnetic iron oxide nanoparticles (brown spheres) labeled with antibodies (green) specific to antigens (blue) presented on viral capsids (red) will form aggregates in the presence of target viruses, which result in detectable perturbations of the T_2 magnetic relaxation times of protons in the surrounding media.

2.3. Magnetic Relaxation Detection

2.3.1. Nucleic Acids

Magnetic nanoparticles also have shown promise in solution-based assays for DNA. Upon aggregation, magnetic nanoparticles can act as magnetic relaxation switches (MRS) by dephasing the spins of the protons in the surrounding water, resulting in an enhancement of the T_2 relaxation times. Weissleder, Perez, and colleagues exploited this phenomenon for use in biodetection.[97] For example, oligonucleotide-functionalized iron oxide particles aggregate in the presence of target oligonucleotides (20 pM limit), resulting in a measurable increase (30 ms) in the T_2 relaxation times of the surrounding water. It was further discovered that base-pair insertions in the target strand resulted in only 2−5 ms increases in the relaxation times, while single base-pair mismatches resulted in 1−21 ms increases in T_2, suggesting that these systems could potentially be used to selectively detect DNA mutations.

2.3.2. Proteins and Viruses

The magnetic relaxation phenomenon exhibited by magnetic nanoparticles also has been exploited for the detection of proteins and viruses (Figure 9).[97,98] To detect viruses in solution and in serum, Perez and Weissleder's team immobilized antibodies specific to surface antigens present on the herpes simplex virus capsid to the surfaces of magnetic nanoparticles and then incubated the particles in the presence of solutions and serum containing the virus. They observed that the virus promotes formation of virus−particle aggregates, and they could therefore measure the concomitant increase in the relaxation time of the surrounding media. As the concentration of virus particles increased, the relaxation time also increased, allowing for quantitative determination of viral concentrations. Given the magnetic basis of this detection method, it might be well suited for in vivo and patient sample diagnostics because the magnetic signal is not affected by the turbidity of the analyte medium.

3. Nanowire- and Nanotube-Based Detection Methods

3.1. Electrical Detection

3.1.1. Nucleic Acids

Nanotubes and nanowires are being explored as new signal transduction motifs in the electrical detection of DNA[99−103] as they have for the detection of gases,[104−107] small molecules,[108] and proteins (vide infra).[20,103,109,110] For example, Lieber and colleagues demonstrated that silicon nanowires functionalized with PNA can be used for real-time, label-free detection of DNA.[99] In their assay the conductance of a PNA-functionalized silicon nanowire bridging two electrodes is measured in the presence of target DNA and mutant DNA with three consecutive base deletions. Introduction of target DNA into the assay caused a rapid and immediate change in conductance, while the effect of mutant DNA was negligible. Furthermore, the conductance changes scale with target concentration, and target DNA can be detected at concentrations as low as 10 fM. In the case of nanotubes, Lieber and colleagues showed that specific sequences of kilobase-size DNA can be detected using single-walled carbon nanotube (SWNT) atomic force microscopy (AFM) probes.[100] Specifically, they marked particular sequences along the DNA strand with streptavidin-labeled complementary DNA probes and then used AFM to identify the streptavidin and thus the location of the target sequences. This technique enabled the detection of specific haplotypes that code for genetic disorders.

Glassy carbon electrodes modified with carbon nanotubes can amplify the electrochemical signal of guanine bases. Wang and co-workers exploited this phenomenon by showing that label-free electrochemical detection of DNA can be performed by carbon nanotube-modified electrodes at nanomolar concentrations.[101] In similar studies carbon nanotube arrays[102] and gold nanoelectrode arrays[111] were used to detect DNA hybridization. Here, the nanotubes or nanoelectrodes in the array are functionalized with a capture oligonucleotide strand. Upon target capture in the nanotube system $Ru(bpy)_3^{2+}$ is introduced to mediate guanine base oxidation, which can then be detected by the carbon nanotube nanoelectrodes.[102] In the case of the gold nanoelectrodes, target capture is monitored by measuring Ru(III)/Fe(III) electrocatalysis at the gold electrodes before and after hybridization.[111]

Recent work utilized carbon nanotubes coated with alkaline phosphatase enzymes as labels in an assay for amplified DNA detection.[103] This assay employs a magnetic microparticle modified with oligonucleotides that are complementary to one-half of the target DNA sequence and carbon nanotubes coated with alkaline phosphatase enzymes and modified with oligonucleotides that are complementary to the other half of the target DNA sequence. Target DNA promotes the formation of a magnetic microparticle−target−carbon nanotube sandwich system that can be magnetically separated from the assay medium. After separation, α-naphthyl phosphate substrate is added to the mixture, resulting in formation of α-naphthol product that is ultimately detected at a carbon nanotube-modified electrode via chronopotentiometric stripping. This method can detect target DNA at concentrations as low as 54 aM.[103]

3.1.2. Proteins, Viruses, and Biologically Relevant Small Molecules

Nanoscale conducting materials such as nanowires and nanotubes also have been used for protein detection. Lieber and co-workers used boron-doped silicon nanowires modified with biotin to detect picomolar concentrations of streptavidin.[20] Specifically, they showed that the conductivity of the silicon nanowire increased in the presence of streptavidin and that the magnitude of the conductivity change depended on the concentration of analyte. Lieber's group also interfaced nanowires functionalized with antibodies specific for influenza A virus particles with a microfluidic sampling system to demonstrate that single virus/nanowire recognition events can be detected by measuring real-time changes in nanowire conductivity.[109]

Similar studies have been performed with carbon nanotubes. Dai's team has shown that poly(ethylene oxide) (PEO) coated carbon nanotubes resist nonspecific adsorption of proteins onto the nanotube surface.[110] At the same time, the PEO coating can be selectively functionalized with antibodies specific to target proteins of interest. In the presence of ∼1 nM concentrations of target protein, the conductance of the nanotube decreases. No change in conductance is observed in the presence of structurally similar proteins. While carbon nanotubes and nanowires currently are not as easily functionalized as quantum dots or spherical nanoparticles, they offer the distinct advantage of rapid, real-time detection. With continued research into methods of surface modification, nanotube/nanowire alignment, and integration with microelectrode devices, nanowire and nanotube systems may become viable options as nanostructured biodiagnostic devices.

4. Nanofabrication

4.1. Nanopatterning

In current chip-based biodiagnostic detection formats (nanomaterial-based or otherwise) the capture molecules on chip surfaces are patterned on the microscale. This format allows for massive parallel screening of various analytes in a small area, a feature that has proven invaluable in genomics and proteomics research. Moreover, microarrays provide a platform for multiplexed DNA and protein detection in small areas.[81] Further miniaturization in the form of nanoarrays would allow for orders of magnitude more massively paralleled multiplexed detection in the same array area as a microarray and potentially improved detection limits resulting from the smaller analyte capture area (Figure 10A).[112] Various methods including dip-pen nanolithography (DPN),[21,113−118] nanografting,[22−24,119] and finely focused ion beam lithography,[120] among others,[121−123] have been devel-

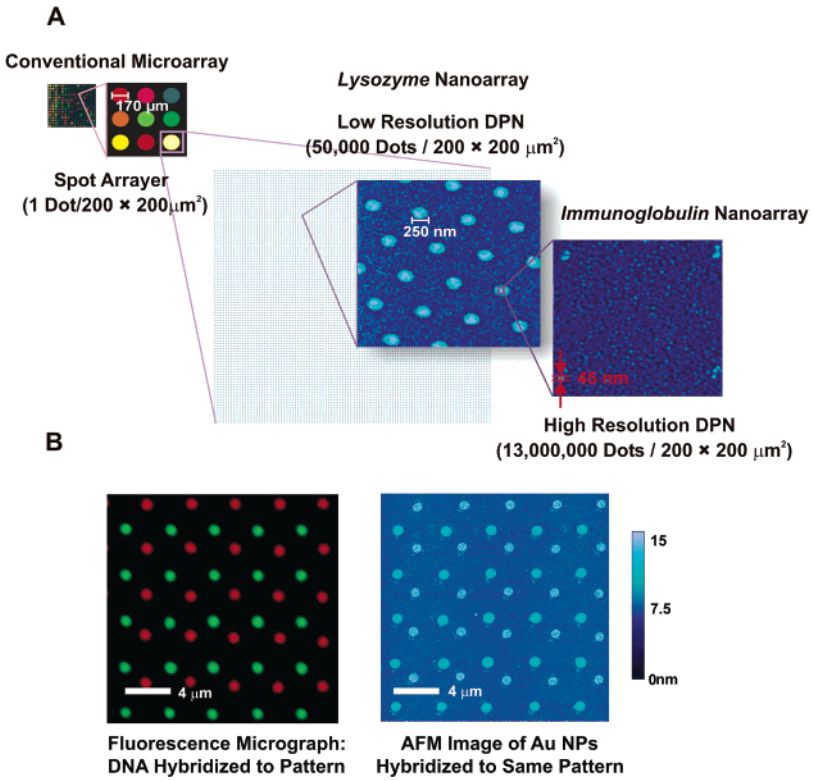

Figure 10. In a conventional microarray spot sizes are typically 200 × 200 μm^2. Using low-resolution dip-pen nanolithography (DPN), 50 000 250-nm protein spots can be spotted in an equivalent area. Patterns can be further miniaturized using high-resolution DPN to generate a total of 13 000 000 spots in a 200 × 200 μm^2 area (A). Similarly, DPN can be used to construct nanopatterns of oligonucleotides on SiO_x surfaces. The reactivity of the patterns can be interrogated using either fluorescence microscopy or atomic force microscopy (AFM) (B). (Reprinted with permission from *Science* (http://www.aaas.org), ref 114. Copyright 2002 American Association for the Advancement of Science.)

oped to fabricate nanoscale patterns of biomolecules such as DNA and proteins on surfaces. DPN can be used to both directly and indirectly pattern reactive protein features and directly pattern reactive DNA features onto various surfaces (Au, silica, Ni) with nanoscale resolution. Nanografting and ion-beam methods rely on indirect deposition processes.

The potential of nanoarrays for detection purposes hinges on their reactivity with targets and the ability to effectively screen for targets using conventional techniques. To this end, it was shown that DNA nanoarrays fabricated using DPN can recognize complementary target DNA labeled with either molecular fluorophores[114] or oligonucleotide-functionalized gold nanoparticles (Figure 10B).[113,114] In the case of molecular fluorophore labeling, the presence of target can be detected with a fluorescence microscope, and for nanoparticle labeling, target presence is assessed using AFM, which measures the change in height profile after nanoparticle probe binding events. For slightly larger spots with nanoparticle probes, light scattering can be used to measure and probe target binding events. The reactivity of protein nanoarrays is determined by rinsing the substrates with antibodies specific to the patterned proteins. The antibodies, tagged with either a molecular fluorophore or a nanoparticle probe, can be detected using either fluorescence microscopy[117,118] or AFM.[115,116] Lithographic techniques also can be used to fabricate nanoscopic wells[124,125] and channels[126] on surfaces. Such features could be used as nanoconfinement vessels for recognition events between probes and target analytes, allowing for significant reduction in sample volume and possibly lower detection limits. While these nanopatterning techniques are still in their infancy, they represent the next step toward further miniaturization of biodetection assays. In principle, they will require smaller sample volumes and thus may result in higher sensitivities than is achieved with microarrays. Recently, DPN was used to fabricate nanoarrays of monoclonal antibodies against HIV-1 p24.[127] These arrays were used to capture HIV p24 proteins from human plasma samples. After capture, the presence of p24 was determined using AFM. To amplify the signal, the nanoarray was rinsed with anti-p24-modified gold nanoparticles, which bind to the spots only when p24 is present and increase the height of the spots. Importantly, only 1 μL of sample is required for this assay, which is critical in cases where sample volumes are small and limited. The detection limit for p24 using this assay is 0.025 pg/mL, which is much better than conventional ELISAs (5 pg/mL).

4.2. Nanoelectromechanical Devices

Advances in photo- and e-beam lithographic techniques continue to enable the fabrication of complex devices on the micrometer and nanometer scale. Microcantilevers with nanoscale thickness allow detection of important biomolecules and microorganisms through measurement of their frequency as a function of target binding. Functionalizing microcan-

Table 1. Detection Limits of Nucleic Acid Assays[a]

	assay	ss DNA	PCR products	genomic DNA
nanostructure-based methods	colorimetric[29] (cross-linked Au nanoparticles)	~10 nM		
	colorimetric[36] (non-cross-linked Au nanoparticles)	60 nM		
	magnetic relaxation[97] (iron oxide nanoparticles)	20 pM		
	electrochemical[96] (nanoparticles)	270 pM		
	scanometric[35,66,67] (Au nanoparticles with Ag amplification)	50 fM	100 aM[b]	200 fM
	Raman spectroscopy[68] (Au nanoparticles with Ag amplification)	~1 fM		
	electrical[93] (Au nanoparticles with Ag amplification)	500 fM		
	electrical[99] (Si nanowire)	10 fM		
	electrical[103] (carbon nanotube)	54 aM		
	resonant light-scattering[61−66] (metal nanoparticles)	170 fM[b]		33 fM
	fluorescence[56] (ZnS and CdSe quantum dots)	2 nM		
	surface plasmon resonance[41] (Au nanoparticles)	10 pM		
	quartz crystal microbalance[94] (Au nanoparticles)	~1 fM		
	laser diffraction[42] (Au nanoparticles)	~50 fM		
	fluorescence[45] (fluorescent nanoparticles)	~1 fM		
	bio-bar-code amplification[71] (Au nanoparticles with Ag amplification)	500 zM		
other non-enzymatic based methods	fluorescence[35] (molecular fluorophores)		~600 fM[b]	
	fluorescence (dendrimer amplification)[134]		2.5 μg	
	electrochemical amplification[136] (electroactive reporter molecules)	100 aM		

[a] Detection limits can vary based on target length and sequence; therefore, it is difficult to compare assays without testing them using identical targets and conditions. [b] Values taken from ref 34.

Table 2. Detection Limits of Protein Assays

	assay	target	protein in saline	protein in serum
nanostructure-based methods	optical[72] (Au nanoshells)	rabbit IgG	0.88 ng/mL (~4.4 pM)[a]	0.88 ng/mL (~4.4 pM)[a]
	optical[74] (Au nanoparticles)	IgE and IgG1	~20 nM	
	magnetic relaxation[98] (iron oxide nanoparticles)	adenovirus (ADV) and herpes simplex virus (HSV)	100 ADV/ 100μL	50 HSV/ 100 μL
	scanometric[79] (Au nanoparticles with Ag amplification)	mouse IgG	200 pM	
	Raman[82] (Au nanoparticles with Raman labels)	prostate-specific antigen		30 fM
	surface plasmon resonance[83,84] (triangular Ag particles on surfaces)	streptavidin(S A) and anti-biotin (AB)	~1 pM SA and ~700 pM AB	
	electrical[110] (single-walled carbon nanotubes)	10E3 antibody to U1A RNA splicing factor	~1 nM	
	electrical[20] (Si nanowires)	streptavidin	10 pM	
	bio-bar-code amplification[75] (Au nanoparticles with Ag amplification)	prostate-specific antigen	30 aM (3 aM)[b]	(30 aM)[b]
molecular fluorophore methods	enzyme-linked immunosorbent assay	various	pM range	pM range
electrochemical methods	electrochemical amplification[137] (oligonucleotide reporter molecules)	IgG	13 fM	
enzyme-based amplification methods	immuno-PCR[76]	bovine serum albumin	2 fM	
	rolling circle amplification[77]	prostate-specific antigen	3 fM	

[a] Reported in ng/mL; authors converted to molar concentration for ease of comparison. [b] These values are the lower limits when PCR is used to amplify the bar-code DNA prior to scanometric detection of bar codes.

tilevers with target capture DNA,[128,129] for example, provides a platform for formation of a sandwich assay between target capture DNA, target DNA, and DNA-modified gold nanoparticle labels. The gold labels provide a site for silver ion reduction, which increases the mass on the cantilever and results in a detectable frequency shift that can be correlated with target detection.[129] The detection of viruses and bacteria is also possible using nanoelectromechanical devices.[130−133] In particular, Craighead and co-workers modified microcantilevers with antibodies for either specific viruses[131] or bacteria.[133] Upon exposure to solutions containing these species, they recorded measurable frequency changes associated with target binding events.

5. Conclusions and Outlook

Throughout this review the promise of nanostructure-based biodiagnostic assays has been assessed with respect to how they compare with the PCR/molecular fluorophore approach for DNA (Table 1) or ELISAs for proteins (Table 2). However, the merit of nanostructure-based assays must also be gauged in light of other assays that have been developed to compete with the conventional approaches. Dendrimers[134,135] (nanostructures in their own right) and molecular electrochemical tags[5] have been successfully incorporated into DNA assays in efforts to improve upon or replace the molecular fluorophore-based assay. Specifically, dendrimers have primarily

been used as a means to increase the number of labels associated with each target binding event. Using a dendrimer probe that contains approximately 250 fluorophores instead of a conventional molecular fluorophore probe, the fluorescent assay sensitivity increases by a factor of ~16.[134] However, the dendrimer introduces an additional level of synthetic complexity to the assay, which might negate the improvement in sensitivity. Electrochemical assays based upon molecular probes are attractive because of their low cost and simplicity.[5] An electrochemical DNA assay in which each target recognition event is indirectly amplified by detecting the electrochemical signal from a microbead imbedded with electroactive molecules exhibits ~100 aM sensitivity.[136] This represents the lowest detection limit reported to date for an electrochemical assay and one that competes favorably with molecular fluorophore-based assays; however, it is still higher than the best reported for a nanostructure-based assay (500 zM).[71] Indirect protein amplification schemes also have received much attention for the sensitive detection of proteins. Immuno-PCR, which involves tagging antibodies specific to target proteins with DNA oligomers followed by PCR amplification after the detection step, offers significantly higher sensitivities than ELISA.[76,77] However, PCR introduces complications,[13] thus making immuno-PCR less favorable than the simpler and more user-friendly ELISAs. The nanoparticle-based bio-bar-code approach for detecting proteins eliminates the need for PCR amplification and is approximately 6 orders of magnitude more sensitive than ELISAs.[75] Very recently, Wang and co-workers adopted a method similar to the bio-bar-code approach for protein detection, but instead of scanometrically detecting bar-code DNA, they fragmented the bar codes and then detected the bases electrochemically, resulting in a detection limit of ~13 fM.[137]

Indeed, some nanostructure-based assays outperform conventional assays in terms of sensitivity, selectivity, and practicality. Continued optimization of these parameters will be necessary to determine the applicability of these assays in point-of-care settings. In particular, many of the assays reviewed herein have only been tested using synthetic single-strand DNA oligomers or commercially available protein samples, but some have proven effective for detecting genomic DNA (Table 1) and proteins from patient serum (Table 2). The ability of an assay to detect analytes in complex environments with high background and competing targets requires exquisite selectivity and sensitivity and will ultimately serve as a yardstick for determining its applicability in laboratory, clinical, and point-of-care settings. In this regard, diagnostic systems based upon many of the aforementioned nanomaterials look promising; however, most have not been studied in real-world settings. The transition to such settings often results in added complexity and affects ultimate assay performance. In most cases, these assays will need to be merged with simple and convenient sample handling systems in a way that does not make them prohibitively complicated or costly. Important advances in microfluidics will certainly complement these systems, but much work needs to be done before their full potential can be realized.[138] Future advances will require continued innovations by chemists in close collaboration with experts in medical and biological fields.

6. Acknowledgments

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