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Nanoparticle-based Detection Technology for DNA Analysis

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Abstract With the current rapid development of nanotechnology and synthesis technology for designed oligonucleotides or oligonucleotide-modified nanoparticle conjugates, the combined strategies have become one of the most valuable methods in detection technology for DNA analysis. Using the uniquely recognizable interactions of pre-designed DNA molecules in assembling nanoparticles, various novel approaches have been recently developed towards detecting specific DNA sequences. Here we describe the key fundamentals and issues of this promising strategies ranging from the initial findings of rationally designed DNA-based assembly of nanoparticles to the extended chip-based detection system. Some limitations of these new strategies and possible approaches will be also discussed for the practical application in the area of DNA microarray detection.

Keywords: nanobiotechnology, DNA analysis, DNA chip, nanoparticle, gold (Au)

INTRODUCTION

Nanostructures are highly respected materials, as their exquisite physical and chemical properties [1-3] impose remarkable specificity and sensitivity, and recent advances in nanotechnology require us to utilize techniques and concepts from almost every area of science and engineering. Probably one of the most impressive results of nanotechnology thus far is the coupling of nanostructure-based programmed assembling techniques to the detection strategies of DNA analysis [4-9].

Currently, the main challenge is to develop nanoparticle-based strategies for DNA sequence detection. The basic rationale is that a nanoparticle-DNA conjugate is designed to assemble only in the presence of a complementary DNA sequence and a change in the material properties induced by nanoparticle assembly can be used as an indicator of whether a particular sequence is present in a sample or not. Such events cause changes in the optical, mechanical, and electrical properties of the nanoparticles. For example, Au nanoparticles change from red to purple in a DNA-mediated assembly and this change can be correlated to the presence of a DNA target [5-7].

The purpose of the current review is to summarize the advances that have been made in involving new strategies that rely on the use of oligonucleotide-modified nanoparticles to assemble nanoscale building blocks into extended meso- and macroscopic structures. In this area, Professor Mirkin at Northwestern University is the undoubted pioneer and his research group has published a number of outstanding research results [5-14]. Therefore, selected publications from this research group will be the main focus of this review.

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Rational Assembly of Nanoparticles Based on Watson-Crick Base-pairing Interactions

Advances in oligonucleotide synthesis and subsequent modification have paved the way for the use of DNA as a tool in material applications. In particular, the unique molecular recognition properties of DNA combined with the preparatory methods for modifying nanoparticles, provide many opportunities for the development of the novel detection technology for DNA analysis. Such opportunities include efforts that focus on using DNA molecules to direct the formation of periodic structures from inorganic building blocks functionalized with oligonucleotides [5-8].

Assembly of DNA-gold conjugates linked by specific DNA hybridization interactions was first reported in 1996 [4,5]. These reports present a synthetic strategy that merges the chemistries of oligonucleotides and inorganic nanoparticles to yield designed materials consisting of nanoparticles functionalized with oligonucleotides linked by complementary DNA (Fig. 1). These methods involved the use of Au particles functionalized with oligonucleotides terminated with thiol groups at one ends. A beneficial consequence of capping Au colloids with oligonucleotides is that they are much more stable than bare Au colloids at higher temperatures and salt concentrations, which is necessary for DNA hybridization events, presumably because the DNA-modified surface of the nanoparticles prohibits them from getting close enough

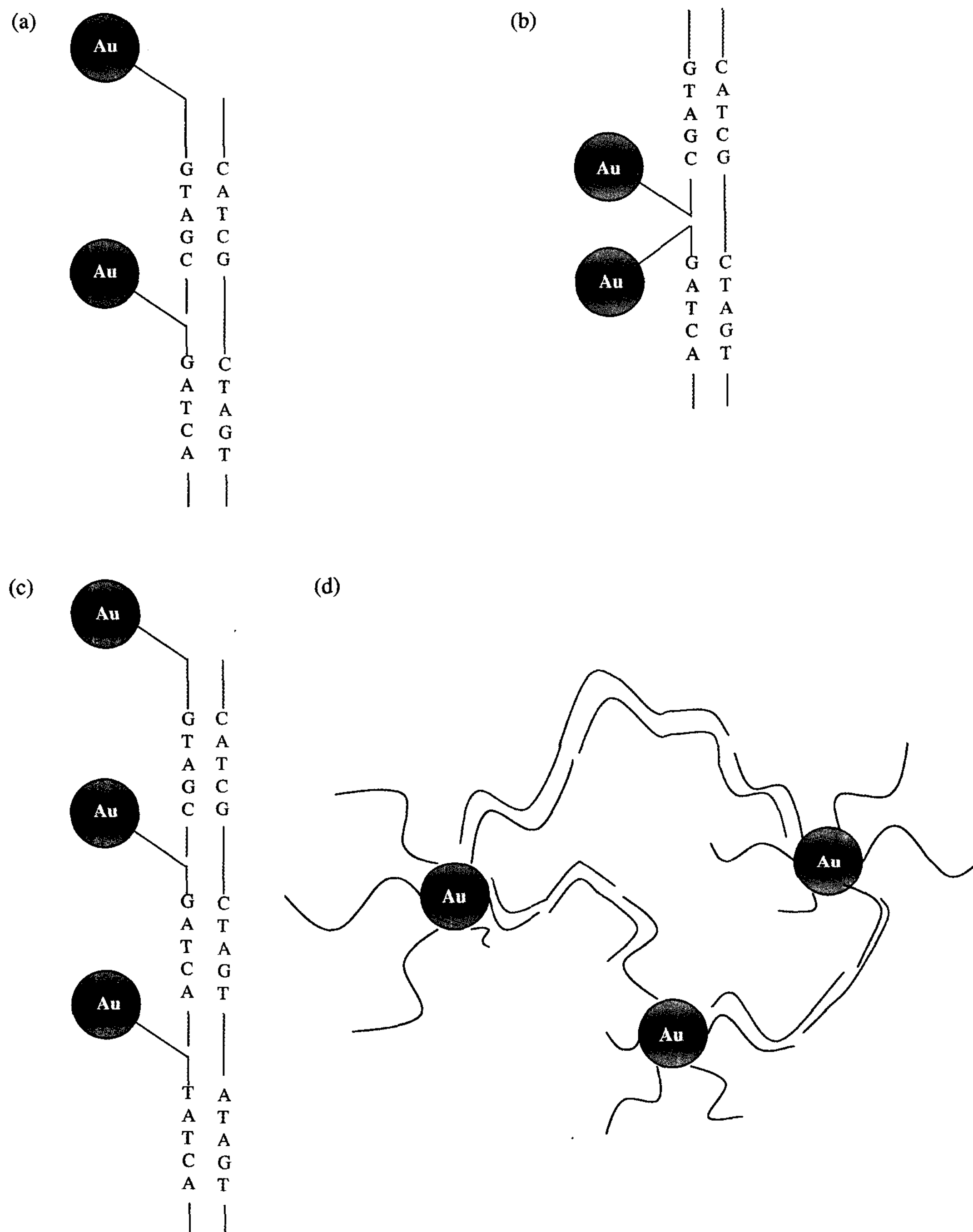

Fig. 1. Various nanoparticle assemblies based on the uniquely recognizable interactions of DNA molecules. (a) Head-to-tail dimer with single stranded linking DNA. (b) Head-to-head dimer with single stranded linking DNA. (c) Parallel trimer with single stranded linking DNA. (d) Duplex DNA with sticky ends as a linking molecule.

to undergo particle growth.

Alivisatos *et al.* [4] used 1.4 nm Au particles conjugated by single-stranded DNA with a defined sequence. These particles assembled into dimers or trimer on the addition of complementary single stranded DNA templates. Depending on the template sequence used, head-to-tail and head-to-head dimers or parallel trimers were prepared (Figs. 1(a), (b) & (c)). In contrast to the approach of the Alivisatos group in which single stranded DNA was used as a linking molecule, Mirkin *et al.* [5] achieved particle linking by adding duplex DNA with sticky ends that were complementary to two grafted sequences of Au particles, and the nanoparticles self-assembled into aggregates (Fig. 1(d)). The initial 13 nm

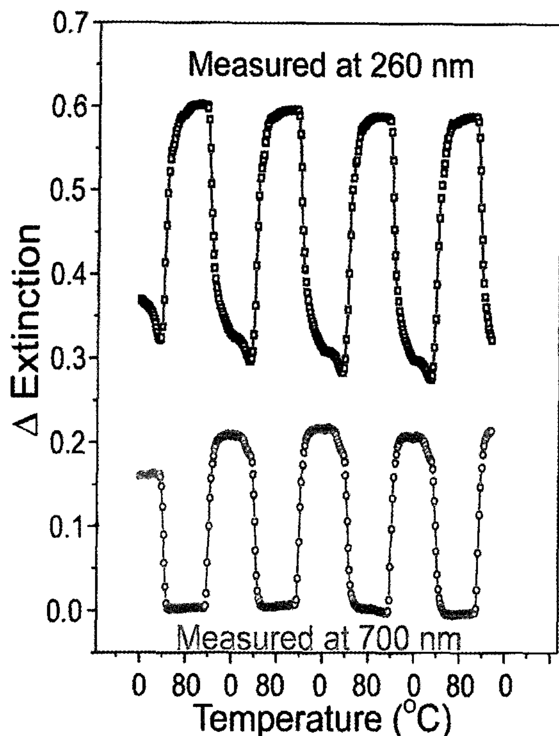

Fig. 2. Absorbance versus temperature/time profile for DNA/colloid hybridized materials (adapted from Mirkin *et al.* [5]).

Au nanoparticle solution exhibited a dark red color (typical for colloidal Au of this size), which immediately changed to purple with the addition of the connecting DNA. Over the course of several hours, the solution became clear with a pinkish-gray precipitate, indicating the formation of DNA-colloid precipitates.

This oligonucleotide-based method offers several advantages over non-biological-based linker molecules. For example the molecular recognition properties of an oligonucleotide can be controlled based on the oligonucleotide composition and length, and this assembly process can be simply reversed by cycling the temperature of the reaction vessel above and below the melting temperature of the DNA, as evidenced by the temperature traces in Fig. 2. Another property of this system is the optical change that accompanies the particle assembly. The plasmon band that is characteristic of Au nanoparticles, experiences a red shift from 520 to 600 nm as the particles are assembled into extended structures, resulting in a striking color change from red to blue. These initial findings may be useful when studying the factors that control the optical properties of nanoparticle aggregates and in the colorimetric detection of DNA.

Colorimetric Detection of Polynucleotides Using Au Nanoparticle Probes

The Mirkin group extended their initial findings to the highly selective colorimetric detection of polynucleotides based on the optical change of Au nanoparticles [6,7].

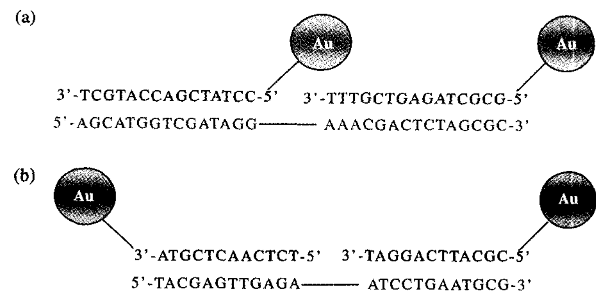

Fig. 3. Detection of polynucleotide sequences using Au nanoparticles modified with oligonucleotide. (a) Head to Tail alignment of Au nanoparticle probes. (b) Tail to Tail alignment of Au nanoparticle probes.

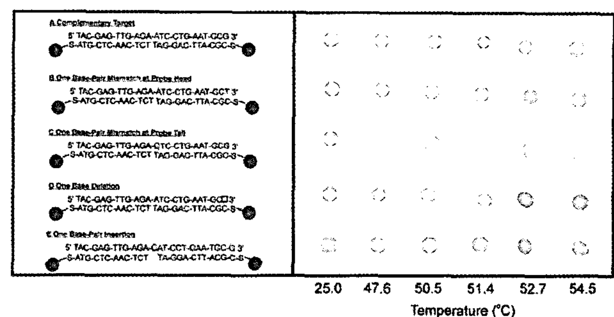

Fig. 4. Spot method on a C_{18} reverse phase plate for polynucleotide detection, demonstrating selectivity toward single base imperfections (adapted from Storhoff and Mirkin [19]).

Their study utilized a three-component system, wherein two probes were used for one target sequence. The probe sequences were linked to the Au nanoparticles and selected so that in the case of hybridization, the recognition segments of the probes would align contiguously on the target sequence (Fig. 3). The target molecules serve as linking strands and, as long as the sequence of a target to be detected is known, two appropriate nanoparticle probes can be designed. When encountering target strands, the particle probes are polymerized and form network structures composed of thousands of particles. In addition, the polymerization process is accompanied by a red-to-blue color change, providing a means of detection. The color changes associated with hybridization are significantly enhanced and easily visualized if the hybridized sample is developed on a solid support. This heterogeneous assay involves pipetting a droplet of a solution of the nanoparticle probes and target onto a silica gel plate as a function of temperature. If the two sequences of the nanoparticle probes are complementary to the target sequence, the nanoparticles are linked by the target and form a blue spot when transported to a reverse phase silica gel plate, as shown in Fig. 4. Conversely, if the nanoparticle probes are dispersed in the solution, they form a red spot. The probe/target aggregate structures, which have a purple color in the solution, develop a blue

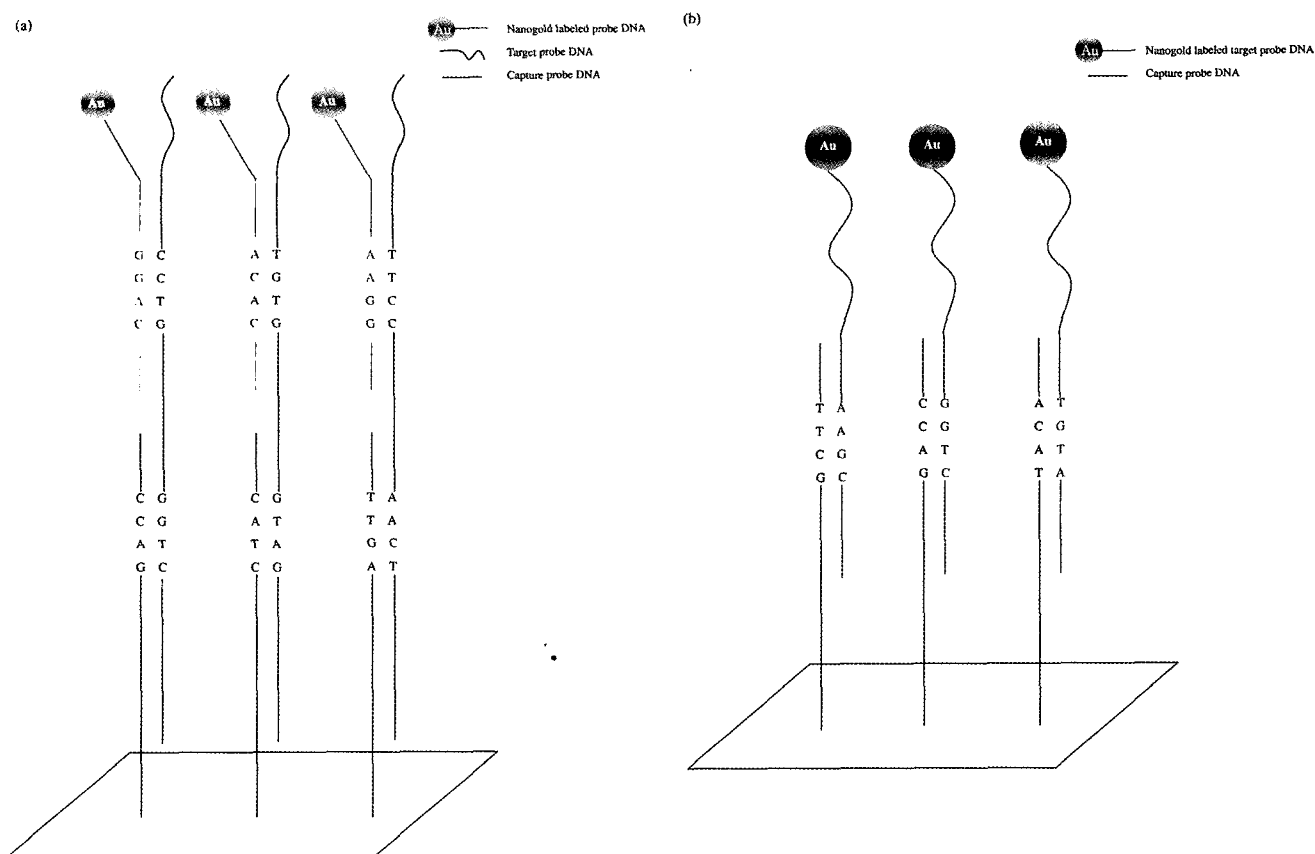

Fig. 5. Au nanoparticle-based detection of hybridization between capture probes and target sequences on chip. (a) Indirect method. (b) Direct method.

color upon drying on the reverse-phase plate.

In their first study, the Mirkin group used 5'-(alkanethiol)-capped oligonucleotides as two probe sequences to prepare Au nanoparticle probes. This type of two probes aligned with the target strand in a 'head-to-tail' fashion, as shown in (Fig. 3(a)). Subsequently, another study was conducted with a very similar system based on Au nanoparticles chemically modified with both 5'- and 3'-(alkanethiol)-capped oligonucleotides. Upon hybridization with target sequence, the system formed a tail-to-tail alignment of the Au nanoparticle probes (Fig. 3(b)). In tests, this latter system exhibited extraordinary selectivity and could identify almost any mismatch, deletion, or insertion in the polynucleotide sequence as distinct from the fully complementary target sequence, as demonstrated in Fig. 4. With the initial 'head-to-tail' alignment, polynucleotides containing one additional base insertion were not distinguished from the fully complementary target.

No matter which alignment method is adopted, the strategies described above are limited in that only a single sequence of a target sample can be analyzed per test solution, thereby preventing the high parallelization needed for high-throughput DNA analyses.

Optical Detection of DNA Array with Nanoparticle Probes

Solid substrate-based analysis has many typical advantages over solution experiments, including ease of incubation/washing and the simultaneous detection of numerous target sequences. Therefore, solution-based strategies with DNA-modified nanoparticles and complementary DNA have been extended to solid substrate-based systems such as a DNA microarray.

Taton *et al.* [8] used single-nanoparticle probes for recognizing DNA segments immobilized on a chip, which provides substantially sharper and higher temperature melting profiles than those obtained with analogous, conventional fluorophore-based systems. In this system, Au nanoparticles modified with a oligonucleotide are used to indicate the presence of a particular DNA sequence hybridized to the capture probes on a substrate in a three-component assay format. The conjugated sequence of nanoparticle probes is designed to be complementary to the unhybridized part of the target sequence by the capture probes (Fig. 5(a)). As such, the nanoparticle probes and target strands of interest are cohybridized to the capture probes on the solid substrate. If the target strands

are complementary to the capture probes and hybridized with them, the nanoparticle probes are subsequently hybridized to the target strands on the substrate and generate a pink color on the surface.

One of the outstanding features of this system is an extremely high sensitivity caused by a signal amplification method based on nanoparticle-promoted reduction of silver(I). In the amplification process, silver ions are reduced by hydroquinone to silver metal on the surfaces of the Au nanoparticles. The sensitivity was found to be 100 times greater than that of conventional Cy3-labeled assays [8]. The signal intensity can be enhanced by a factor as large as 10^5, which enables very low surface coverage of nanoparticles to be visualized using a simple flatbed scanner. Furthermore, this nanoparticle probe-based detection system could also discriminate between perfect matched and single base mismatched target samples in contrast to conventional fluorescence-based assays.

However, this kind of indirect detection method also has certain drawbacks, as it requires an extra nanoparticle probe for each target sequence and an additional hybridization step between the nanoparticle probe sequence and the target sequence. Meanwhile, standard fluorescence procedures include a direct detection method, in which the fluorescent compound, such as Cy3, is directly incorporated into the target strand and hybridization of the labeled target sequence is sufficient to generate the signal without any extra probe sequence or additional step.

Another direct detection method based on nanoparticle labeling was also reported by Reichert *et al.* [15], as shown in Fig. 5(b). In this strategy, capture DNA probes are arrayed on a glass chip via standard photolithographic techniques and the target DNA probes are directly labeled with 30 nm Au nanoparticles. After incubation of the chip with the target solution, the hybridization events are monitored by optical means, using reflected and transmitted light to detect surface-bound nanoparticles. When compared with fluorescent techniques, this optical detection is significantly simplified by relying on optical transmission or reflection. With the enhanced stability of the target samples, high light intensities can be applied for the readout. This is in stark contrast to the sensitive fluorescence dyes and their bleaching problems, which could result in a significantly shorter detection time. Furthermore, the influence of the chemical and physical environment on the signal intensity is significantly reduced, resulting in robust measurements.

Using very similar systems, Niemeyer *et al.* [16] demonstrated the site-selectivity of surface adsorption by immobilizing Au colloids on a DNA microarray on glass cover slides, while Csáki *et al.* [17] achieved a discrimination ratio of approximately 100:1 between complementary and noncomplementary sequences with a higher concentration of a DNA-gold complex.

In the direct methods described above, 3' or 5'-alkylthiolated oligonucleotides were chosen as a target DNA, conjugated with Au nanoparticles, and used in the experiments. However, in a real system, the target DNA probes to be analyzed are usually prepared via a polymerase chain reaction (PCR) process and the nanoparticle-labeling process for the PCR product is not yet available, whereas the fluorescence-labeling PCR process is already fully established and widely used. Therefore, if concurrent advances can be made in PCR technology related to nanoparticle-modified dNTP, this would certainly accelerate the application of direct detection methods based on nanoparticle-labeling beyond the academic level.

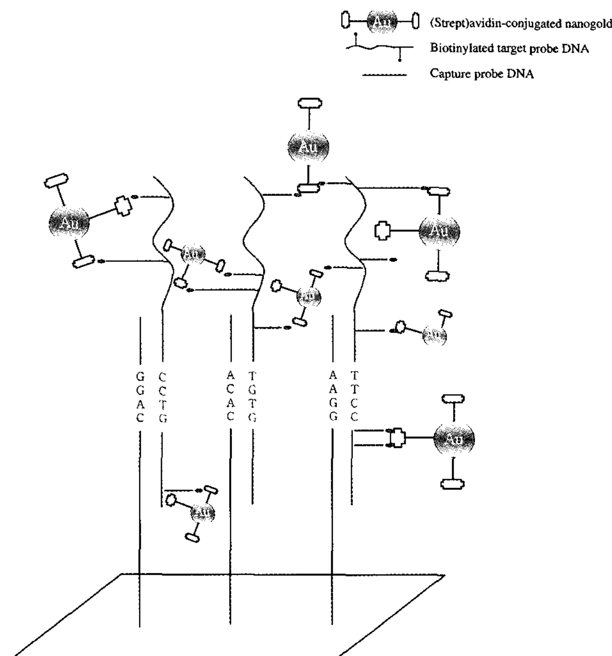

Fig. 6. Universal detection strategy of the biotin[]-(strept) avidin[] coupling for binding of biotinylated DNA to (strept)avidin-conjugated Au colloids.

Another approach is the development of universal nanoparticle-based labeling probes. For example, the current authors are developing a universal process based on the use of biotin-(strept)avidin coupling for binding biotinylated target DNA to (strept)avidin-conjugated Au colloids (Fig. 6). With this strategy, only one type of nanoparticle conjugated with (strept)avidin is sufficient for the simultaneous detection of numerous target sequences, as long as the target samples are labeled with biotin using conventional PCR techniques.

CONCLUSION

The current question is how best to combine the advanced methods of nanotechnology and biotechnology into the new promising field of nano-biotechnology. Biomolecules, like nucleic acids and proteins, seem to be particularly suitable candidates for such combined technology, as they can be geometrically defined and function in a very specific manner determined by every single unit sequence. Accordingly, the special attractiveness of nano-

biotechnology in the development of detection strategies for DNA analysis is becoming more apparent and Au nanoparticle labeling strategies based on the uniquely recognizable interactions of DNA molecules are certainly promising candidates for a more robust and reliable detection method in DNA analysis.

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