ACCOUNTS
of chemical research

Target-Responsive Structural Switching for Nucleic Acid-Based Sensors

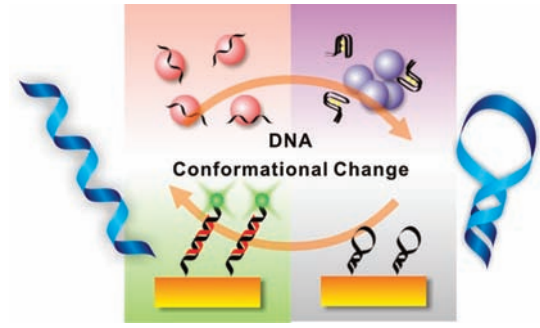
DI LI, SHIPING SONG, AND CHUNHAI FAN*

Laboratory of Physical Biology, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China

RECEIVED ON SEPTEMBER 22, 2009

CONSPECTUS

Interest in the development of sensitive, selective, rapid, and cost-effective biosensors for biomedical analysis, environmental monitoring, and the detection of bioterrorism agents is rapidly increasing. A classic biosensor directly transduces ligand−target binding events into a measurable physical readout. More recently, researchers have proposed novel biosensing strategies that couple ligand-induced structural switching of biomolecules with advanced optical and electronic transducers. This approach has proven to be a highly general platform for the development of new biosensors. In this Account, we describe a series of electrochemical and optical nucleic acid sensors that use target-responsive DNA structures.



By employing surface-confined DNA structures with appropriate redox labels, we can monitor target-induced structural switching of DNA or aptamer-specific small molecule probes by measuring electrochemical currents that are directly associated with the distance between the redox label and the electrode surface. We have also demonstrated significant improvements in sensing performance through optimization of the DNA self-assembly process at electrode surfaces or the introduction of nanomaterial-based signal amplification. Alternatively, gold nanoparticles interact differently with folded and unfolded DNA structures, which provides a visual method for detecting target-induced structural switching based on the plasmonic change of gold nanoparticles. This novel method using gold nanoparticles has proven particularly suitable for the detection of a range of small-molecule targets (e.g., cocaine) and environmentally toxic metal ions (e.g., Hg^{2+}). Rational sequence design of DNA aptamers improves the sensitivity and increases the reaction kinetics. Recently, we have also designed microfluidic devices that allow rapid and portable mercury detection with the naked eye.

This Account focuses on the use of bulk and nanoscale gold and DNA/aptamer molecules. We expect that researchers will further expand the analyte spectrum and improve the sensitivity and selectivity of nucleic acid sensors using functional biomolecules, such as DNAzymes, peptide aptamers and engineered proteins, and nanomaterials of different sizes, dimensions and compositions, such as carbon nanotubes, graphene, silicon nanowires, and metal nanoparticles or nanorods.

1. Introduction

There has been tremendous interest in the development of sensitive, selective, miniaturized, and cost-effective biosensing devices for widespread applications ranging from in-hospital analysis to battlefield detection of warfare agents. DNA sensors are of particular interest since DNA analysis plays a crucial role in a wide range of areas including diagnostics of genetic diseases, monitoring of infectious bacteria, analysis of forensic samples, and screening of bioterrorism agents.[1] While a classic DNA sensor detects nucleic acid targets (DNA or RNA), as well as biorelated variations of nucleic acids such as single-nucleotide polymorphisms (SNPs) and DNA methylation, the introduction of nucleic acid aptamers provides unprecedented opportunities to detect non-nucleic acid targets,

Published on the Web 03/11/2010 www.pubs.acs.org/acr
10.1021/ar900245u

which dramatically widens the application areas of nucleic acid sensors.[2−4] Aptamers are in vitro selected functional oligonucleotides that can bind specifically to target molecules. In principle, aptamers with high affinity can be selected for virtually any given target via a process termed SELEX (systematic evolution of ligands by exponential enrichment).[2,5] As an increasingly important biosensing element, aptamers have well exhibited their competitive advantages over other natural or artificial receptors (e.g., antibodies or molecularly imprinted polymers), such as synthesis convenience, chemical stability, and flexibility in biosensor design.[4]

A biosensor is typically composed of three components, that is, probe-target recognition, signal transduction, and physical readout (e.g., optical, electronic, and acoustic). For nucleic acid sensors, there have been numerous existing strategies for signal transduction. For example, a typical DNA hybridization sensor employs probe DNA strands immobilized on a solid surface; upon capture of target DNA molecules that are complementary to the probe, the hybridization process is transduced to either fluorescent or electrochemical signals.[6] A classic and straightforward transduction model relies on the hybridization-induced physical enrichment of opto- or electroactive tags that are either covalently or noncovalently (via intercalation) attached to nucleic acids on the solid surface. Relatively recently, target-responsive variations of probe structures have been exploited as a new transduction mechanism with many attractive features. It is well-known that biomolecular recognition often induces conformational changes or even folding or unfolding of biomolecules.[7] Coupling of such variations with advanced optical and electronic transducers has proven of great utility in designing simple and potentially generalizable biosensor architectures that are free of target labeling (reagentless), highly sensitive, and exceptionally selective.[8]

In this Account, we focus on nucleic acid sensors with target-responsive structures, largely based on the recent progress of our laboratory. As will be discussed in the following sections, bulk gold electrodes are employed to electrochemically transduce DNA hybridization and aptamer−target binding, and nanoscale gold particles (AuNPs) serve as optical nanoprobes for these recognition processes. Noble metal gold is preferred in our studies because it not only is biocompatible and easily functionalizable with biomolecules but also possesses many interesting optical and electronic properties such as high conductivity (macroscopic gold) and unique plasmonic properties (particularly nanoscale gold).

2. Target-Responsive DNA-Based Electrochemical Sensors

Electrochemical transducers have become popularly used for the detection of DNA hybridization and aptamer−target binding due to their combined advantages of sensitivity, low power, mass, and volume requirements, and low cost, as well as the possibility of mass production via the microelectronic industry.[6] While Palacek pioneered the area of DNA electrochemistry as early as 1960,[9] the first electrochemical DNA sensor was developed by Millan and Mikkelsen in 1993, which electrochemically distinguished hybridized double-stranded (ds)-DNA from single-stranded (ss)-probe DNA by using carbon electrodes and exogenous, redox-active hybridization indicators (double-helix intercalators).[10] Hashimoto et al. replaced carbon electrodes with gold electrodes and employed Au−S chemistry to assemble thiolated DNA probes onto a gold surface.[11] Later Tarlov and co-workers developed a two-step protocol that employed mercaptohexanol to help DNA strands to stand up at the surface, a molecular orientation facilitating DNA hybridization.[12,13] The original intercalator-based method suffers from inherent nonspecific binding that often results in high background. In order to overcome this problem, a sandwich-type strategy was introduced, which involves a pair of DNA probes (capture and redox-labeled reporter probes) that flanked the target DNA sequence.[14,15]

In 2003, Fan, Plaxco, and Heeger developed a first-generation E-DNA sensor (electrochemical DNA sensor).[8] This design adopts a single surface-confined stem−loop (hairpin) DNA structure that integrates the capture probe and the reporter probe (redox labels) in a single hairpin-like architecture (Figure 1A). Hairpin probes dually labeled with a fluorophore−quencher pair (termed "molecular beacons") have been extensively used in homogeneous fluorescent DNA analysis.[16,17] The fluorescence of molecular beacons is internally quenched when the stem−loop holds the fluorophore−quencher pair in close proximity and turned on in the presence of the target, that is, target hybridization in the loop region competitively breaks the stem and separates the fluorophore−quencher pair. Analogous to fluorescent molecular beacons that rely on conformation-associated energy transfer, the E-DNA sensor exploits the hybridization-induced conformational change occurring at the gold surface, which can be electrochemically interrogated based on the distance-dependent electron transfer (ET) property of the coupled redox molecule.[8] A typical E-DNA sensor employs a stem−loop structure dually labeled with thiol and ferrocene at either end, which is self-assembled at the Au electrode surface. In the initial state, the E-DNA sensor leads to an intense electrochemical current corre-

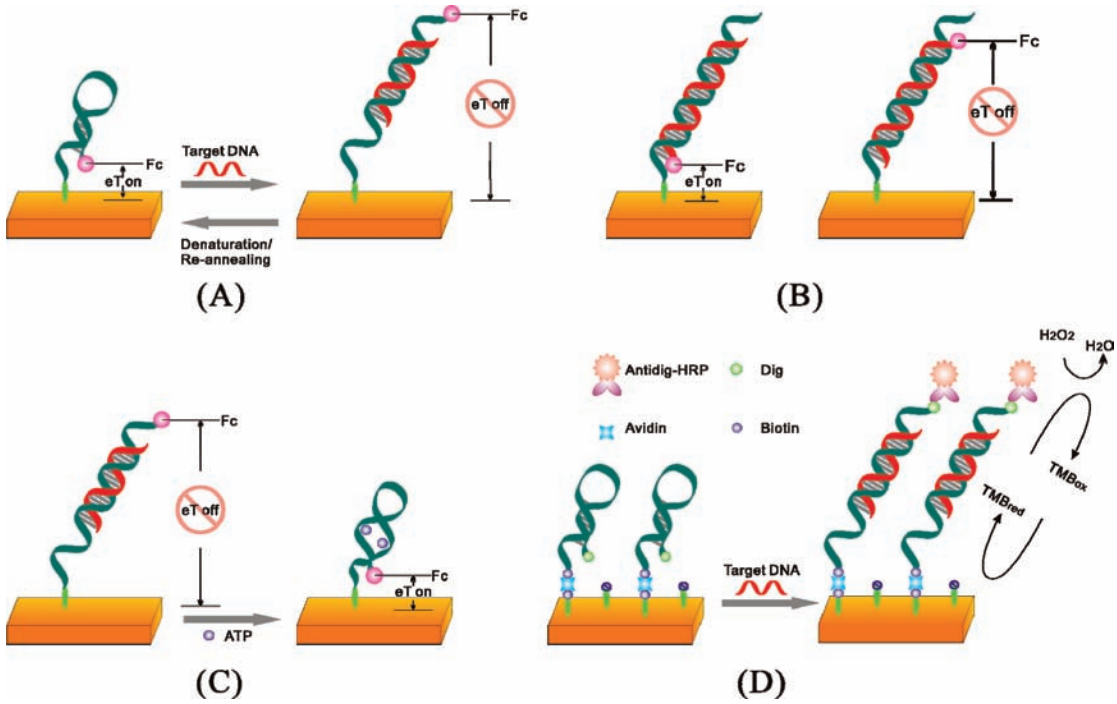


FIGURE 1. Schemes for (A) an E-DNA sensor based on structural switching of a ferrocene-tagged stem−loop DNA probe, (B) electron transfer of ferrocence at different locations of a DNA duplex, (C) an electrochemical aptamer sensor ATP based on target-induced structural switching of a ferrocence-tagged anti-ATP aptamer, and (D) an enzyme-based E-DNA sensor.

sponding to ferrocene redox when the stem−loop holds the ferrocene in proximity to the Au surface, while the gradual turn-off of the current signals when target DNA binds to the probe and liberates the ferrocene from the electrode surface.[8]

We ascribe the large measurable change in electrochemical signals to the hybridization-induced transition from the stem−loop to a linear duplex structure.[8] Given that ET rates fall off exponentially with donor−acceptor distances,[18] even relatively small target-induced conformational changes of a surface-confined, redox-labeled DNA molecule may lead to significant variations in ET rates. In order to test this signal generation mechanism in our DNA−ferrocene system, we designed an experiment with a simple architecture as shown in Figure 1B.[19] A rigid DNA duplex was attached to the Au surface via the end sulfur of one strand, while the other strand was tagged with an electroactive ferrocene moiety that was located either distally or proximally to the Au surface. We expected that the distance from the distal ferrocene to the surface was sufficiently large to suppress efficient ET (the distance between the distal and proximal ferrocene was ∼4−5 nm, assuming a tilting angle of ∼60° for immobilized DNA). Indeed, cyclic voltammetric interrogation showed that the former led to nearly featureless capacitive currents while the latter produced a pair of well-defined reduction and oxidation peaks that correspond to the redox reaction of ferrocene.[19]

The E-DNA sensor possesses several unprecedented advantages. For example, it is a reusable, solid-state sensor that can be integrated within a small sized microfluidic device and easily detected with portable and inexpensive electrochemical detectors, which represents an important improvement over solution-phase fluorescent molecular beacons. E-DNA is also highly selective and robust enough to perform assays in adulterated samples and biological fluids (e.g., serum).[20,21] More significantly, the integration of capture and reporter probe within a single stem−loop provides a way to detect target DNA without the need for exogenous reagents (i.e., reagentless). This interesting property, combined with its high reusability, makes the E-DNA sensor particularly suited for the continuous monitoring of DNA hybridization within microfluidic channels.[22] More recently, the E-DNA sensor has found an interesting application for the detection of authentication DNA markers.[23] DNA is known to possess inherent high sequence complexity, which makes it a highly promising molecular marker for physically labeling merchandise. Compared with existing decoding technologies such as PCR and electrophoresis, the E-DNA sensor provides a means to reliably identify DNA markers with combined advantages of high speed, high sensitivity, and low cost.[23]

The mechanism underlying the E-DNA sensor provides a generic platform for designing electrochemical biosensors by exploiting ET-associated target-responsive structural transi-

tions. A straightforward extension is to develop aptamer-based electrochemical sensors that can detect a range of analytes. A series of E-DNA based sensors for proteins or small molecules were developed in parallel by several groups including ours.[3,19,20,24−26] We recently developed a target-responsive electrochemical aptamer switch (TREAS) strategy for the detection of adenosine triphosphate (ATP) with an anti-ATP aptamer.[19] This TREAS strategy is in fact a nearly reversed process of E-DNA. That is, the anti-ATP aptamer (27-base) dually labeled with 3′-SH and 5′-ferrocene was first hybridized with its complementary sequence and then the duplex was self-assembled on gold (Figure 1C). Ferrocene labeled at the distal end could not efficiently exchange electrons with the underlying electrode (OFF state) due to its large distance separation to the electrode surface (9−10 nm). In the presence of ATP, the target stabilized the tertiary aptamer structure, which responsively separated the duplex and liberated the cDNA. This structural transition from the duplex to the tertiary aptamer structure (duplex-to-aptamer) held the ferrocene moiety in proximity to the electrode surface, producing measurable electrochemical signals (ON state). For example, 1 mM ATP led to a signal gain (ON/OFF ratio) up to 10-fold. This ATP sensor is highly sensitive with a low detection limit of 10 nM. More importantly, this sensor only responds to ATP molecules and not to a range of analogous molecules (e.g., cytidine triphosphate (CTP), guanosine triphosphate (GTP), and uridine triphosphate (UTP)), exhibiting excellent selectivity. We also demonstrated that this sensor is suitable for direct ATP detection in cell lysates.[19]

It is worthwhile to note that the original E-DNA design leads to a signal-off sensor with a detection limit of ∼10 pM.[8] Signal-off sensors are relatively susceptible to false positives, and the picomolar sensitivity does not satisfy the high-end requirements of many real-world applications, for example, PCR-free pathogen detection. In order to develop a signal-on E-DNA sensor with improved sensitivity, we recently designed an enzyme-based E-DNA sensor with femtomolar sensitivity (Figure 1D).[27] The gold surface was first coated with a mixed self-assembled monolayer (SAM) with terminal carboxylic acid groups, which was activated and conjugated with a layer of streptavidin. A stem−loop probe was dual labeled with digoxigenin (DIG) and biotin at the 5′- and 3′-ends, respectively. The probe readily bound to the streptavidin layer via the strong biotin−streptavidin bridge, and the DIG label was expected to bind specifically to a horseradish peroxidase-linked-anti-DIG antibody (anti-DIG−HRP). Of note, the hybridization-induced structural transition induced change in the steric effect in this design rather than distance-dependent ET variations in the original E-DNA sensor.[27] In the initial state, the stem−loop structure of the immobilized probe sterically interfered with the binding of the bulky anti-DIG−HRP conjugate to the DIG label. However, probe−target hybridization broke the stem, leading to the formation of the linear duplex structure that exposed the DIG to anti-DIG−HRP. Of note, this is a signal-on sensor, that is, one hybridization event brings about one HRP enzyme that turns on the catalyzed electrochemical current signals. Besides its signal-on feature, this new E-DNA sensor possesses large signal amplification since the hybridization-associated bound HRP efficiently catalyzes thousands of reduction reactions of hydrogen peroxide (H_2O_2) in this new sensor.[27] This is in contrast to the original E-DNA sensor that only tranduces one hybridization event to one-electron communication of ferrocene with the electrode. As a result, this enzyme-based E-DNA sensor has a detection limit of 10 fM, which excels the original E-DNA sensor by 3 orders of magnitude.

This enzyme-based E-DNA sensor represents new progress in the family of E-DNA sensors, with the signal-on feature and significantly improved sensitivity. More significantly, it possesses high sequence specificity that can identify target DNA in the presence of million-fold excess of noncognate DNA and even distinguish a single-base mismatch from its fully complementary counterpart.[27] Solution-phase molecular beacons are able to effectively differentiate single-nucleotide polymorphisms (SNPs) due to the internal conformational constraints of the stem−loop structure.[16] However, our original E-DNA sensor exhibits limited ability for the discrimination of single-base mismatches, which possibly arise from a surface-induced destabilization effect on the stem. We attribute the increased sequence specificity to the new sensor architecture.[27] Here, the stem−loop probe stays far away from the electrode surface (>10 nm), with an underlayer much thicker than previously employed monolayers (∼1 nm). Therefore, the stem−loop structure is minimally perturbed by the surface and retains high sequence specificity in a solution-phase-like environment.

While the femtomolar sensitivity is impressive and we have indeed demonstrated its real applicability via detecting PCR amplicons from the *uidA* gene of *Escherichia coli*, it remains a challenge to detect low-copy pathogens without prior PCR amplification. The sensitivity of a sensor depends on its signal/background ratio; therefore there are two possible approaches to improve the sensitivity, that is, background suppression via surface design and signal increase via amplification. While these two approaches have not been rigorously tested in E-DNA sensors, their successful applications in several previ-

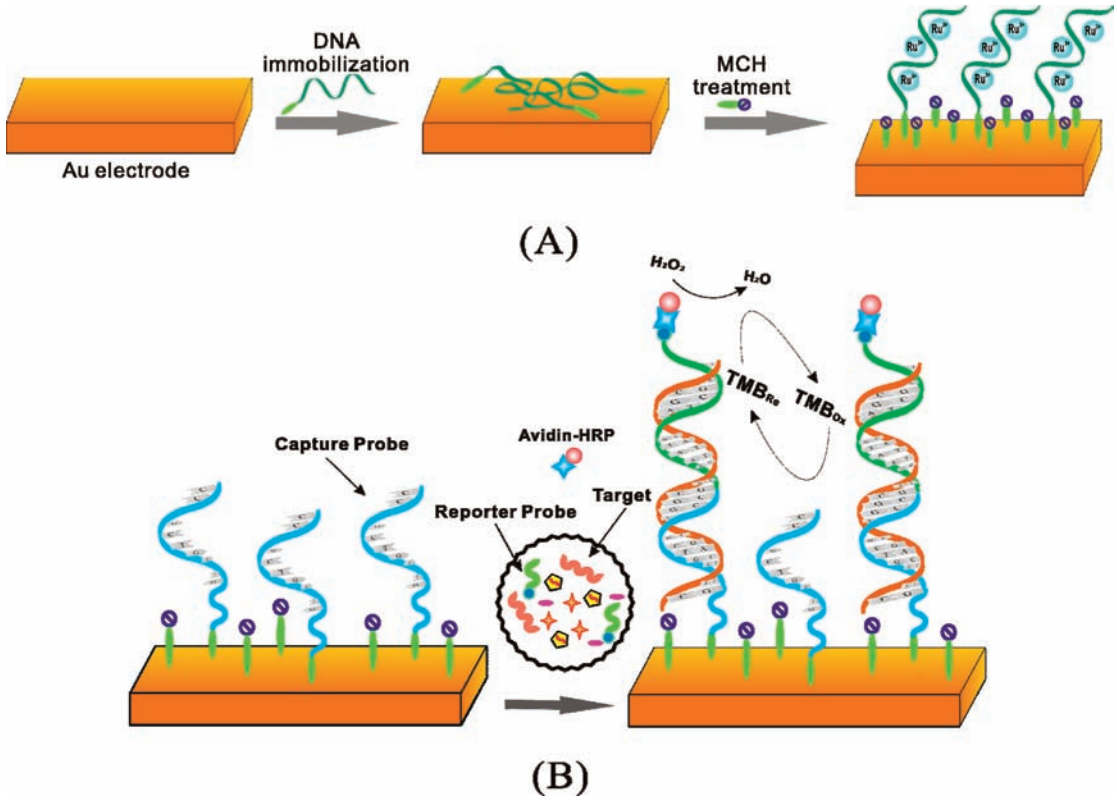


FIGURE 2. Schemes for (A) two-step self-assembly based DNA immobilization at gold electrode surfaces (with MCH treatment); (B) OEG-incorporated nonfouling surfaces that support electrochemical DNA detection with high protein resistance.

ously reported DNA sensors with linear probes strongly imply their promising applications in E-DNA sensors as well. DNA assembly processes at gold electrodes have been previously investigated via both electrochemical and quartz crystal microbalance (QCM) means.[13,28] Thiolated DNA probes are expected to stick to gold surfaces via the specific Au−S interaction; however nonspecific adsorption of DNA does occur on gold due to the relatively weak interaction between Au and nitrogen atoms of DNA bases. The formation of mixed SAMs via MCH adsorption significantly suppresses nonspecific DNA adsorption and makes DNA probes stand up in a highly hybridizable orientation (Figure 2A).[12] This two-step assembly protocol has proven of high utility to develop various electrochemical DNA sensors.[12,29−31] Nevertheless, the MCH passivation layer does not possess high resistance to strong nonspecific adsorption of proteins. In order to improve the applicability of electrochemical DNA sensors in real samples, we developed a nonfouling surface by incorporating oligo(ethylene glycol) (OEG) in the mixed SAM (Figure 2B).[32] Previous reports have well documented that OEG-incorporated SAMs are known to be highly protein-resistant and effectively repel nonspecific adsorption.[33] We found that a mixed SAM structure with both thiolated oligonucleotides and OEG thiols (SH-DNA/OEG) not only showed high resistance to protein adsorption but still supported facile electron transfer across the monolayer and was fully compatible with electrochemical detection.[32] Based on this finding, we developed an enzyme-based DNA sensor that was resistant to nonspecific enzyme adsorption and did not sacrifice performance even in serum detection. This nonfouling surface was also recently employed to develop an enzyme-based DNA sensor with electrochemiluminescent detectors.[34]

The sensitivity of DNA sensors is also critically dependent on the signal amplitude corresponding to a single DNA hybridization event. Clearly, incorporation of nanoparticle amplifiers may transduce one hybridization event into many electrochemical turnovers, leading to large signal amplification and high sensitivity.[35,36] Gold and platinum nanoparticles and quantum dots were previously employed to amplify electrochemical signals based on metal-based stripping or catalysis.[37] We recently proposed a new strategy by using AuNPs heavily loaded with reporter DNA probes (Figure 3).[35,38,39] We employed a small redox molecule, $[Ru(NH_3)_6^{3+}]$, that could bind stoichiometrically to negatively charged DNA strands, which could be quantitatively measurement with choronocoulometry.[35] Since one AuNP carried several hundred reporter DNA strands, the hybridization signal was amplified by over 3 orders of magnitude, leading to a detection limit of 10 fM.[35]

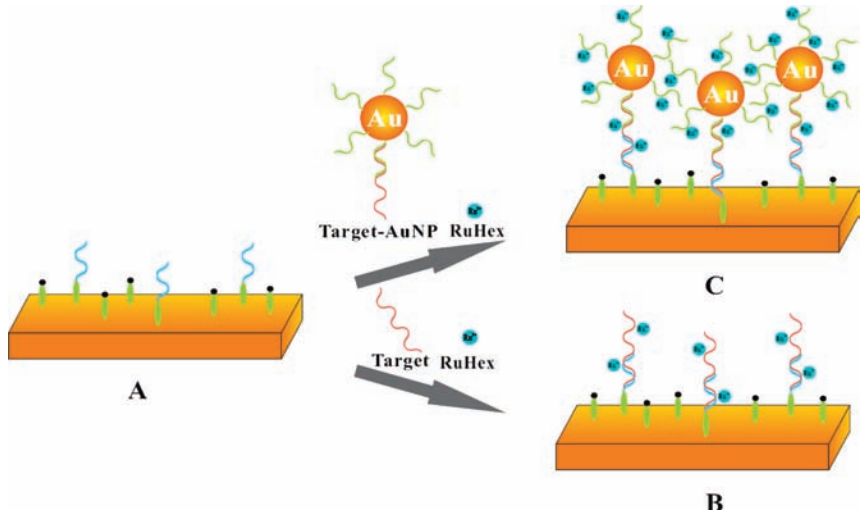


FIGURE 3. Scheme for an AuNP-amplified chronocoulometric DNA sensor.

3. Target-Responsive DNA-Based Optical Sensors

AuNPs are a class of highly attractive nanomaterials with widespread biological applications. The sizes of these nanoparticles are comparable to biomolecules, and they are biocompatible and easily functionalizable with biomolecules. Different from bulk gold with a golden color, AuNPs are small enough to scatter visible light and produce distinct, size-dependent colors. They also possess interesting surface plasmon resonance (SPR) properties and ultrahigh fluorescence quenching abilities. More recently, the crystal structure of a 102-atom AuNP has been solved.[40] AuNPs conjugated with DNA have shown unparalleled abilities in bottom-up construction of nano-to-micro structures.[41,42] These milestone works have strongly implied the pivotal role of AuNPs in nanobiotechnology.

The Mirkin group pioneered the use of AuNP−DNA conjugates in bioassays.[43] The color of AuNPs is dependent on not only their sizes but their interparticle distances. DNA hybridization is able to interconnect AuNPs modified with two different DNA sequences, leading to aggregation of AuNPs, which changes their color from red to blue (or purple). This SPR-related color change has proven highly useful for the detection of DNA targets with extraordinarily high sequence specificity. More recently, several groups including ours have demonstrated various aptamer-based colorimetric sensors by exploiting target-induced assembly and disassembly of AuNP−aptamer conjugates.[4,44] Since there have been several excellent reviews in this area,[4,45,46] we will not describe these strategies in detail in this Account.

Metal nanoparticles are known to strongly quench the fluorescence of dyes with ultrahigh efficiency. This property is particularly useful for the construction of nanoscale molecular beacons (nanobeacons). Dubertret et al. conjugated dye-tagged stem−loop probes at the functionalized surface of small-sized AuNPs (1.4 nm).[47] Similar to MBs, this nanobeacon shows fluorescence ON/OFF in response to a target DNA-induced structural switch, while SNP discrimination ability is significantly improved.[47] In addition to organic dyes, AuNPs can effectively quench the fluorescence of quantum dots, which forms the basis for a recently developed aptamer-based sensor for small molecules.[48] We recently employed large-sized AuNPs (15 nm) to develop multicolor nanobeacons (Figure 4).[49] Relatively large-sized AuNPs not only possess higher quenching ability than small ones but allow the anchoring of many oligonucleotides at a single particle. This multicolor nanobeacon can rapidly respond to target DNA hybridization with high sequence specificity, and one nanobeacon can simultaneously recognize three tumor DNA markers with individual characteristic fluorescent colors.[50] It is interesting to note that AuNP−DNA conjugates can be efficiently taken up by cells;[51] therefore it might be possible to develop real-time intracellular nanoprobes that signal cellular events.

In addition to the use of DNA-modified AuNP conjugates, interactions between DNA and as-prepared AuNPs from citrate reduction provide new opportunities for DNA-based detection. Previous studies have found that AuNPs possess higher affinity for ssDNA than dsDNA.[52] This difference comes from at least three effects.[52,53] First, DNA bases attach to AuNPs with high affinity via Au−N coordination. Importantly, DNA bases are encapsulated within the nega-

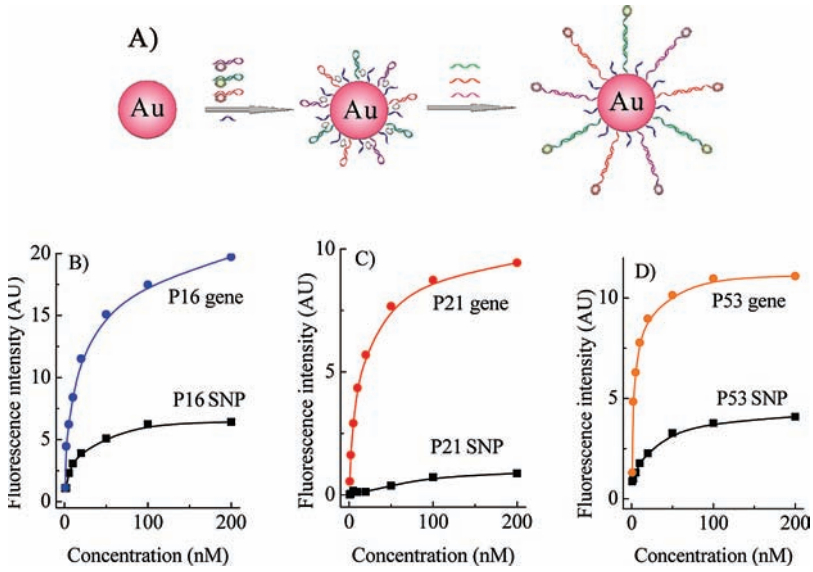


FIGURE 4. (A) Scheme for an AuNP-based multicolor fluorescent nanobeacons. Multiplexing detection with the nanobeacon for (B) the P16 gene (carboxyfluorescein (FAM), emission at 520 nm with blue color), (C) the P21 gene (Cy5, emission at 667 nm with red color), and (D) the P53 gene (5-carboxy-X-rhodamine (Rox), emission at 607 nm with orange color).

tively charged phosphate backbone of dsDNA, while they are exposed in ssDNA. Second, the surface of AuNPs is negatively charged because they are stabilized with citrate ions. Since the charge density of dsDNA is higher than that of ssDNA, the electrostatic repulsion between AuNPs and DNA is larger for dsDNA. Third, dsDNA is much more rigid than ssDNA; thus ssDNA possesses higher freedom to wrap on AuNPs than dsDNA.

Inspired by this finding, we developed a series of aptamer sensors by exploiting interactions between aptamers and AuNP. In the first example, we employed a potassium ion-specific G-quartet structure (Figure 5A).[54] Similar to the ssDNA−dsDNA change, AuNPs could differentiate the structural switching of the G-quartet in response to K^+ binding. The adsorption of K^+-free ssDNA to AuNPs stabilized them from being aggregated by salt addition. As a result, the solution of AuNPs retained its red color in the absence of K^+, while the K^+-induced G-quartet formation led to a characteristic red-to-purple color change that reflects the SPR shift arising from the AuNP aggregation.[54] This strategy was also applicable for the detection of mercury ions with a T-rich DNA probe[55] and solution pH values with a C-rich DNA probe (termed i-motif[56]).[57] Both probes underwent random coil-to-rigid structure changes that were translated into color change of the AuNPs.[57]

In an alternative approach, we first hybridized an anti-ATP aptamer with its complementary sequence and employed AuNPs to differentiate the structural switching from dsDNA to ssDNA in response to the target-induced

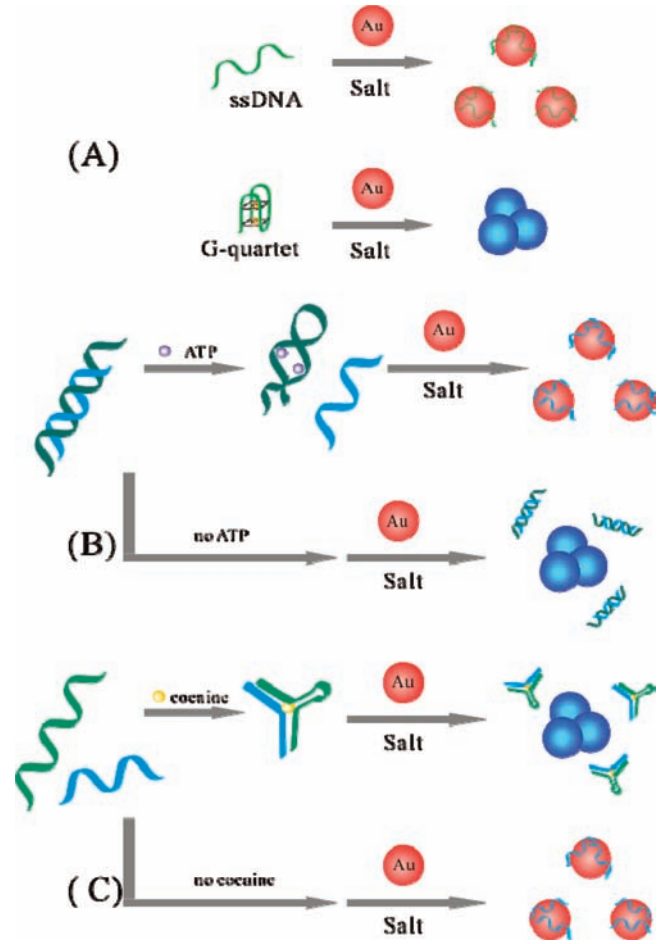


FIGURE 5. Schemes for AuNP-based assays for (A) K^+-induced structural variation of a K^+-specific G-quartet, (B) ATP-induced aptamer strand displacement, and (C) cocaine-induced assembly of an engineered cocaine aptamer.

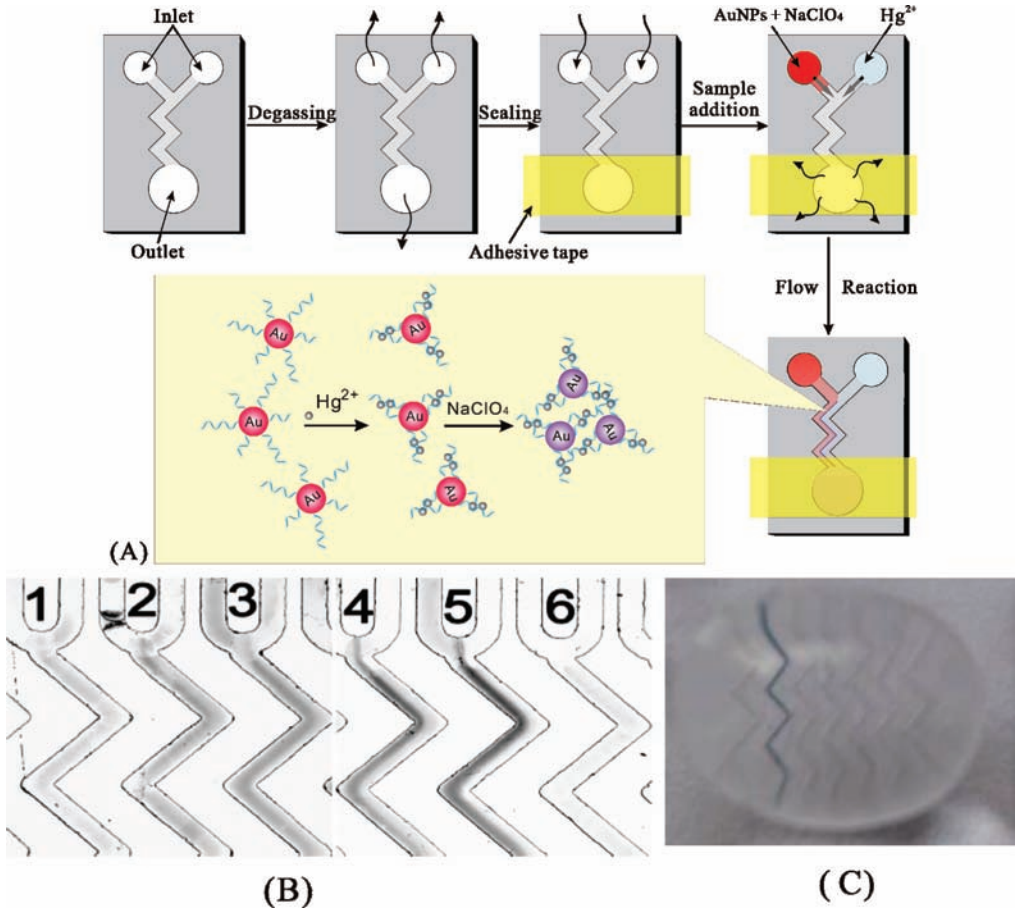


FIGURE 6. (A) Scheme for Hg^{2+} detection with a power-free PDMS microfluidic chip. The black arrows in each step indicate the directions of air transfer. Also shown is the scheme for optical detection of Hg^{2+} using poly-T-modified AuNPs (bottom left). (B) Microscopic images for the PDMS channels corresponding to gold nanoprobes in the presence of a series of Hg^{2+} concentrations (5, 10, 25, 50, and 100 mM and control, from left to right). (C) With the help of a droplet of water cast on the chip, Hg^{2+} detection can be visualized with the naked eye due to the "magnifying glass" effect.

aptamer binding and competitive liberation of the complementary strand (Figure 5B).[58] This displacement-based strategy does not rely on the specific aptamer structure, thus circumventing possible problems associated with internal secondary structures of target-free aptamers. This new strategy provides a generic platform for aptamer-based detection. Indeed, we demonstrated the applicability of this strategy in sensing potassium ions and cocaine.[58]

Despite the generality of this displacement-based strategy, the presence of competition between aptamer binding and duplex makes this process kinetically slow (~30 min). In order to improve the kinetics and reduce the assay time, we further proposed a new strategy by using rationally engineered aptamers (Figure 5C).[59] An anti-cocaine aptamer was divided into two pieces of random coil-like ssDNA. We found that the presence of cocaine reassembled the two pieces into an intact aptamer tertiary structure. AuNPs could effectively differentiate between these two states via their characteristic SPR color change. Importantly, this strategy is similarly generic while inherently fast in kinetics since it does not involve molecular competition. The assay time is usually within only several minutes (vs ~30 min for the displacement-based strategy).[59]

The use of as-prepared AuNPs to perform aptamer-based detection has several advantages. The assay is simple and straightforward, based on visual detection at room temperature. Also it is usually fast and does not require time-consuming preparation of AuNP−DNA conjugates (typically 1−2 days). In order to develop a sensor for real world applications, we further attended to coupling the AuNP-based assays with microfluidic chips, which provide unparalleled advantages since they are inexpensive single-use chips that can be automated. Very recently, we have proven that the AuNP-based visual detection is compatible with a power-free PDMS microfluidic chip (Figure 6).[60] Through an elaborate design, the difference in air pressure in the PDMS channel leads to power-free unidirectional flow and automatic mixing. The Hg^{2+}-induced AuNP aggregation resulted in clearly visible deposition lines in the channel, suggesting that this simple and cost-ef-

fective strategy may become a point of care or field detection tool in future.[60]

4. Conclusions and Perspective

In this Account, we have summarized recent design and applications of nucleic acids by exploiting target-induced structural switching of DNA molecules. We have demonstrated that electrochemical means are particularly suitable for the interrogation of structural switching of surface-confined DNA structures tagged with redox labels. Both DNA hybridization and aptamer−target binding can induce large structural switching of the probe DNA, which alters the distance between the redox label and the bulk electrode surface, leading to measurable electrochemical currents. More recent advances have shown promise for even better sensing performance when the size of electrodes shrinks to the nanoscale range.[61] AuNPs provide an alternative approach to optical detection of target-induced structural switching. This approach is based on either the superquenching ability or the unique size-dependent plasmonic properties of AuNPs. This AuNP-based strategy is highly attractive as a simple and rapid screening method for small-molecule targets (e.g., cocaine) or environmentally toxic metal ions (e.g., Hg^{2+}).

While this Account is not a comprehensive review and only summarizes a small fraction of research activities that exploit target-responsive DNA structures, we have demonstrated that this strategy is a powerful and generic platform for the design of novel DNA biosensors with high performance. Besides the electrochemical and the optical transducers as described above, it is also possible to employ mechanical (e.g., cantilever[56]), magnetic,[62] and acoustic (e.g., QCM[63]) transducers to translate DNA conformational changes into measurable signals. We also note that the rise of DNA nanotechnology provides new opportunities to integrate DNA structural switches to artificial DNA nanostructures that are assembled via the base-pairing property of DNA,[64,65] which has shown great promise for nanoscale DNA sensing with unprecedented advantages. Self-assembled DNA nanostructures could be employed as scaffolds to regulate positions of macromolecules or nanopaticles in three dimensions,[65] based on which nanoscale, solution-phase and spatially addressable DNA arrays with ultrahigh sensitivity have been developed.[66,67] It can be foreseen that the merging of programmable DNA self-assembly nanostructures with structural switching and addressable multiple physical readouts possess enormous potential for DNA sensing or even sequencing.

We also expect to greatly expand the spectrum of analytes with the introduction of functional biomolecules such as DNAzymes, peptide aptamers, and engineered proteins. On the other hand, given the availability of a large variety of nanomaterials of different sizes, dimensions, and compositions (e.g., carbon nanotubes, graphene,[68] silicon nanowires, and metal nanoparticles or nanorods), it is possible to further improve the sensitivity and selectivity of nucleic acid sensors by optimizing interactions between these nanomaterials and DNA structures. Particularly, nanocomplexes that combine two or more different nanomaterials may provide unprecedented abilities to spatially and kinetically control surface assembly of different biomolecules, which offers opportunities to develop "machine"-like biosensing devices that can simultaneously detect multiple analytes with high precision and sensitivity.[69,70]

This work was supported by National Natural Science Foundation (Grants 20725516, 90913014, 20873175, and 20805055), Ministry of Science and Technology (Grants 2006CB933000 and 2007CB936000), Ministry of Health (Grant 2009ZX10004-301), Shanghai Municipal Commission for Science and Technology (Grant 0952nm04600) and Chinese Academy of Sciences.

BIOGRAPHICAL INFORMATION

Di Li was born in Changchun, China, in 1977. He received his B.S. degree (2000) in environmental engineering from Dalian University of Technology and Ph.D. degree (2005) in chemistry from Changchun Institute of Applied Chemistry, Chinese Academy of Sciences. After postdoctoral research with Professor Itamar Willner at the Hebrew University of Jerusalem, he became an associate professor in Shanghai Institute of Applied Physics (SINAP), Chinese Academy of Sciences, in 2008. His research interests are DNA molecular machines and electrochemical DNA sensors.

Shiping Song was born in Shandong, China, in 1969. He received his B.S. degree (1995) in biochemistry from South-Central University for Nationalities, M.S. degree (1998) in chemistry from Shanghai Institute of Nuclear Research, and Ph.D. degree (2004) in chemistry in Shanghai Institute of Applied Physics (SINAP), Chinese Academy of Sciences. He became an associate professor in SINAP in 2005 and was promoted to a professor in 2009. His research interests are nanomaterial-based biosensors and biochips.

Chunhai Fan was born in Zhangjiagang, China, in 1974. He received his B.S. (1996) and Ph.D. (2000) degrees in biochemistry and molecular biology from Nanjing University. After postdoctoral research at the University of California, Santa Barbara, he became a professor in Shanghai Institute of Applied Physics (SINAP), Chinese Academy of Sciences, in 2004. He is now the director of the Division of Physical Biology of SINAP. He was the

recipient of the Chinese Chemical Society Prize for Young Scientists (2006) and NSFC Outstanding Young Investigators (2007). His research interests focus on electrochemical and optical biosensors and the design of nanoprobes for biological systems.

FOOTNOTES

*To whom correspondence should be addressed. E-mail: fchh@sinap.ac.cn.

REFERENCES

1 Debouck, C.; Goodfellow, P. N. DNA microarrays in drug discovery and development. *Nat. Genet.* **1999**, *21*, 48–50.

2 Joyce, G. F. Directed evolution of nucleic acid enzymes. *Annu. Rev. Biochem.* **2004**, *73*, 791–836.

3 Song, S.; Wang, L.; Li, J.; Zhao, J.; Fan, C. Aptamer-based biosensors. *TrAC, Trends Anal. Chem.* **2008**, *27*, 108–117.

4 Liu, J. W.; Cao, Z. H.; Lu, Y. Functional nucleic acid sensors. *Chem. Rev.* **2009**, *109*, 1948–1998.

5 Osborne, S. E.; Ellington, A. D. Nucleic acid selection and the challenge of combinatorial chemistry. *Chem. Rev.* **1997**, *97*, 349–370.

6 Drummond, T. G.; Hill, M. G.; Barton, J. K. Electrochemical DNA sensors. *Nat. Biotechnol.* **2003**, *21*, 1192–1199.

7 Hermann, T.; Patel, D. J. Biochemistry - Adaptive recognition by nucleic acid aptamers. *Science* **2000**, *287*, 820–825.

8 Fan, C.; Plaxco, K. W.; Heeger, A. J. Electrochemical interrogation of conformational changes as a reagentless method for the sequence-specific detection of DNA. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 9134–9137.

9 Palacek, E. Oscillographic polarography of highly polymerized deoxyribonucleic acid. *Nature* **1960**, *188*, 656–657.

10 Millan, K. M.; Mikkelsen, S. R. Sequence-selective biosensor for DNA-based on electroactive hybridization indicators. *Anal. Chem.* **1993**, *65*, 2317–2323.

11 Hashimoto, K.; Ito, K.; Ishimori, Y. Sequence-specific gene detection with a gold electrode modified with DNA probes and an electrochemically acive dye. *Anal. Chem.* **1994**, *66*, 3830–3833.

12 Herne, T. M.; Tarlov, M. J. Characterization of DNA probes immobilized on gold surfaces. *J. Am. Chem. Soc.* **1997**, *119*, 8916–8920.

13 Steel, A. B.; Herne, T. M.; Tarlov, M. J. Electrochemical quantitation of DNA immobilized on gold. *Anal. Chem.* **1998**, *70*, 4670–4677.

14 Yu, C. J.; Wan, Y. J.; Yowanto, H.; Li, J.; Tao, C. L.; James, M. D.; Tan, C. L.; Blackburn, G. F.; Meade, T. J. Electronic detection of single-base mismatches in DNA with ferrocene-modified probes. *J. Am. Chem. Soc.* **2001**, *123*, 11155–11161.

15 Patolsky, F.; Lichtenstein, A.; Willner, I. Detection of single-base DNA mutations by enzyme-amplified electronic transduction. *Nat. Biotechnol.* **2001**, *19*, 253–257.

16 Tyagi, S.; Kramer, F. R. Molecular beacons: Probes that fluoresce upon hybridization. *Nat. Biotechnol.* **1996**, *14*, 303–308.

17 Tan, W. H.; Wang, K. M.; Drake, T. J. Molecular beacons. *Curr. Opin. Chem. Biol.* **2004**, *8*, 547–553.

18 Marcus, R. A. Chemical-electrochemical eletron-transfer theory. *Annu. Rev. Phys. Chem.* **1964**, *15*, 155–196.

19 Zuo, X.; Song, S.; Zhang, J.; Pan, D.; Wang, L.; Fan, C. A target-responsive electrochemical aptamer switch (TREAS) for reagentless detection of nanomolar ATP. *J. Am. Chem. Soc.* **2007**, *129*, 1042–1043.

20 Xiao, Y.; Lubin, A. A.; Heeger, A. J.; Plaxco, K. W. Label-free electronic detection of thrombin in blood serum by using an aptamer-based sensor. *Angew. Chem., Int. Ed.* **2005**, *44*, 5456–5459.

21 Baker, B. R.; Lai, R. Y.; Wood, M. S.; Doctor, E. H.; Heeger, A. J.; Plaxco, K. W. An electronic, aptamer-based small-molecule sensor for the rapid, label-free detection of cocaine in adulterated samples and biological fluids. *J. Am. Chem. Soc.* **2006**, *128*, 3138–3139.

22 Ferguson, B. S.; Buchsbaum, S. F.; Swensen, J. S.; Hsieh, K.; Lou, X. H.; Soh, H. T. Integrated microfluidic electrochemical DNA sensor. *Anal. Chem.* **2009**, *81*, 6503–6508.

23 Lubin, A. A.; Fan, C.; Schafer, M.; Clelland, C. T.; Bancroft, C.; Heeger, A. J.; Plaxco, K. W. Rapid electronic detection of DNA and nonnatural DNA analogs for molecular marking applications. *Forensic Sci. Commun.* **2008**, *10*, 1.

24 Zhang, J.; Wan, Y.; Wang, L.; Song, S.; Li, D.; Fan, C. Switchable charge transport path via a potassium ions promoted conformational change of G-quadruplex probe monolayer. *Electrochem. Commun.* **2008**, *10*, 1258–1260.

25 Zayats, M.; Huang, Y.; Gill, R.; Ma, C. A.; Willner, I. Label-free and reagentless aptamer-based sensors for small molecules. *J. Am. Chem. Soc.* **2006**, *128*, 13666–13667.

26 Xiao, Y.; Piorek, B. D.; Plaxco, K. W.; Heeger, A. J. A reagentless signal-on architecture for electronic, aptamer-based sensors via target-induced strand displacement. *J. Am. Chem. Soc.* **2005**, *127*, 17990–17991.

27 Liu, G.; Wan, Y.; Gau, V.; Zhang, J.; Wang, L.; Song, S.; Fan, C. An enzyme-based E-DNA sensor for sequence-specific detection of femtomolar DNA targets. *J. Am. Chem. Soc.* **2008**, *130*, 6820–6825.

28 Lao, R.; Song, S.; Wu, H.; Wang, L.; Zhang, Z.; He, L.; Fan, C. Electrochemical interrogation of DNA monolayers on gold surfaces. *Anal. Chem.* **2005**, *77*, 6475–6480.

29 Zhang, J.; Wang, L. H.; Pan, D.; Song, S. P.; Fan, C. H. DNA hybridization "turns on" electrocatalysis at gold electrodes. *Chem. Commun.* **2007**, *xxx*, 1154–1156.

30 Wan, Y.; Zhang, J.; Liu, G.; Pan, D.; Wang, L.; Song, S.; Fan, C. Ligase-based multiple DNA analysis by using an electrochemical sensor array. *Biosens. Bioelectron.* **2009**, *24*, 1209–1212.

31 Zhang, L.; Sun, H.; Li, D.; Song, S.; Fan, C.; Wang, S. A conjugated polymer-based electrochemical DNA sensor: Design and application of a multi-functional and water-soluble conjugated polymer. *Macromol. Rapid Commun.* **2008**, *29*, 1489–1494.

32 Zhang, J.; Lao, R.; Song, S.; Yan, Z.; Fan, C. Design of an oligonucleotide-incorporated nonfouling surface and its application in electrochemical DNA sensors for highly sensitive and sequence-specific detection of target DNA. *Anal. Chem.* **2008**, *80*, 9029–9033.

33 Mrksich, M.; Whitesides, G. M. Using self-assembled monolayers that present oligo(ethylene glycol) groups to control the interactions of proteins with surfaces. *ACS Symp. Ser.* **1997**, *680*, 361–373.

34 Zhang, L.; Li, D.; Meng, W.; Huang, Q.; Su, Y.; Wang, L.; Song, S.; Fan, C. Sequence-specific DNA detection by using biocatalyzed electrochemiluminescence and non-fouling surfaces. *Biosens. Bioelectron.* **2009**, *25*, 368–372.

35 Zhang, J.; Song, S.; Zhang, L.; Wang, L.; Wu, H.; Pan, D.; Fan, C. Sequence-specific detection of femtomolar DNA via a chronocoulometric DNA sensor (CDS): Effects of nanoparticle-mediated amplification and nanoscale control of DNA assembly at electrodes. *J. Am. Chem. Soc.* **2006**, *128*, 8575–8580.

36 Li, J.; Song, S.; Liu, X.; Wang, L.; Pan, D.; Huang, Q.; Zhao, Y.; Fan, C. Enzyme-based multi-component optical nanoprobes for sequence-specific detection of DNA hybridization. *Adv. Mater.* **2008**, *20*, 497–500.

37 Wang, J. In *Electrochemical Sensor Analysis*; Alegret, S., Merckoçi, M. A., Eds.; Elsevier: Amsterdam, 2007; Vol. 9.

38 Zhang, J.; Song, S.; Wang, L.; Pan, D.; Fan, C. A gold nanoparticle-based chronocoulometric DNA sensor for amplified detection of DNA. *Nat. Protoc.* **2007**, *2*, 2888–2895.

39 Zhu, Z.; Su, Y.; Li, J.; Li, D.; Zhang, J.; Song, S.; Zhao, Y.; Li, G.; Fan, C. Highly sensitive electrochemical sensor for mercury(II) ions by using a mercury-specific oligonucleotide probe and gold nanoparticle-based amplification. *Anal. Chem.* **2009**, *81*, 7660–7666.

40 Jadzinsky, P. D.; Calero, G.; Ackerson, C. J.; Bushnell, D. A.; Kornberg, R. D. Structure of a thiol monolayer-protected gold nanoparticle at 1.1 angstrom resolution. *Science* **2007**, *318*, 430–433.

41 Nykypanchuk, D.; Maye, M. M.; van der Lelie, D.; Gang, O. DNA-guided crystallization of colloidal nanoparticles. *Nature* **2008**, *451*, 549–552.

42 Park, S. Y.; Lytton-Jean, A. K. R.; Lee, B.; Weigand, S.; Schatz, G. C.; Mirkin, C. A. DNA-programmable nanoparticle crystallization. *Nature* **2008**, *451*, 553–556.

43 Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles. *Science* **1997**, *277*, 1078–1081.

44 Li, F.; Zhang, J.; Cao, X.; Wang, L.; Li, D.; Song, S.; Ye, B.; Fan, C. Adenosine detection by using gold nanoparticles and designed aptamer sequences. *Analyst* **2009**, *134*, 1355–1360.

45 Rosi, N. L.; Mirkin, C. A. Nanostructures in biodiagnostics. *Chem. Rev.* **2005**, *105*, 1547–1562.

46 Zhao, W.; Brook, M. A.; Li, Y. F. Design of gold nanoparticle-based colorimetric biosensing assays. *ChemBioChem* **2008**, *9*, 2363–2371.

47 Dubertret, B.; Calame, M.; Libchaber, A. J. Single-mismatch detection using gold-quenched fluorescent oligonucleotides. *Nat. Biotechnol.* **2001**, *19*, 365–370.

48 Liu, J. W.; Lee, J. H.; Lu, Y. Quantum dot encoding of aptamer-linked nanostructures for one-pot simultaneous detection of multiple analytes. *Anal. Chem.* **2007**, *79*, 4120–4125.

49 Song, S.; Liang, Z.; Zhang, J.; Wang, L.; Li, G.; Fan, C. Gold-nanoparticle-based multicolor nanobeacons for sequence-specific DNA analysis. *Angew. Chem., Int. Ed.* **2009**, *48*, 8670–8674.

50 Zhang, J.; Wang, L.; Zhang, H.; Boey, F.; Song, S.; Fan, C. Aptamer-based multicolor fluorescent gold nanoprobes for multiplex detection in homogeneous solution. *Small* **2010**, *6*, 201–204.

51 Rosi, N. L.; Giljohann, D. A.; Thaxton, C. S.; Lytton-Jean, A. K. R.; Han, M. S.; Mirkin, C. A. Oligonucleotide-modified gold nanoparticles for intracellular gene regulation. *Science* **2006**, *312*, 1027–1030.

52 Li, H. X.; Rothberg, L. Colorimetric detection of DNA sequences based on electrostatic interactions with unmodified gold nanoparticles. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 14036–14039.

53 Li, H.; Huang, J.; Lv, J.; An, H.; Zhang, X.; Zhang, Z.; Fan, C.; Hu, J. Nanoparticle PCR: Nanogold-assisted PCR with enhanced specificity. *Angew. Chem., Int. Ed.* **2005**, *44*, 5100–5103.

54 Wang, L.; Liu, X.; Hu, X.; Song, S.; Fan, C. Unmodified gold nanoparticles as a colorimetric probe for potassium DNA aptamers. *Chem. Commun.* **2006**, *xxx*, 3780–3782.

55 Liu, X.; Tang, Y.; Wang, L.; Zhang, J.; Song, S.; Fan, C.; Wang, S. Optical detection of mercury(II) in aqueous solutions by using conjugated polymers and label-free oligonucleotides. *Adv. Mater.* **2007**, *19*, 1471–1474.

56 Shu, W. M.; Liu, D. S.; Watari, M.; Riener, C. K.; Strunz, T.; Welland, M. E.; Balasubramanian, S.; McKendry, R. A. DNA molecular motor driven micromechanical cantilever arrays. *J. Am. Chem. Soc.* **2005**, *127*, 17054–17060.

57 Wang, L.; Zhang, J.; Wang, X.; Huang, Q.; Pan, D.; Song, S.; Fan, C. Gold nanoparticle-based optical probes for target-responsive DNA structures. *Gold Bull.* **2008**, *41*, 37–41.

58 Wang, J.; Wang, L.; Liu, X.; Liang, Z.; Song, S.; Li, W.; Li, G.; Fan, C. A gold nanoparticle-based aptamer target binding readout for ATP assay. *Adv. Mater.* **2007**, *19*, 3943–3946.

59 Zhang, J.; Wang, L.; Pan, D.; Song, S.; Boey, F. Y. C.; Zhang, H.; Fan, C. Visual cocaine detection with gold nanoparticles and rationally engineered aptamer structures. *Small* **2008**, *4*, 1196–1200.

60 He, S.; Li, D.; Zhu, C.; Song, S.; Wang, L.; Long, Y.; Fan, C. Design of a gold nanoprobe for rapid and portable mercury detection with the naked eye. *Chem. Commun.* **2008**, 4885–4887.

61 Liu, G.; Sun, C.; Li, D.; Song, S.; Mao, B.; Fan, C.; Tian, Z. Gating of redox currents at gold nanoelectrodes by DNA hybridization. *Adv. Mater.* **2010**, DOI: 10.1002/adma.200903590.

62 Yigit, M. V.; Mazumdar, D.; Kim, H. K.; Lee, J. H.; Dintsov, B.; Lu, Y. Smart "Turn-on" magnetic resonance contrast agents based on aptamer-functionalized superparamagnetic iron oxide nanoparticles. *ChemBioChem* **2007**, *8*, 1675–1678.

63 Ma, H.; He, J.; Zhu, Z.; Lv, B.; Li, D.; Fan, C.; Fang, J. A quartz crystal microbalance-based molecular ruler for biopolymers. *Chem. Commun.* **2010**, 949–951.

64 Rothemund, P. W. K.; Ekani-Nkodo, A.; Papadakis, N.; Kumar, A.; Fygenson, D. K.; Winfree, E. Design and characterization of programmable DNA nanotubes. *J. Am. Chem. Soc.* **2004**, *126*, 16344–16352.

65 Lin, C.; Liu, Y.; Yan, H. Designer DNA nanoarchitectures. *Biochemistry* **2009**, *48*, 1663–1674.

66 Ke, Y. G.; Lindsay, S.; Chang, Y.; Liu, Y.; Yan, H. Self-assembled water-soluble nucleic acid probe tiles for label-free RNA hybridization assays. *Science* **2008**, *319*, 180–183.

67 Zhang, Z.; Wang, Y.; Fan, C.; Li, C.; Li, Y.; Qian, L.; Fu, Y.; Shi, Y.; Hu, J.; He, L. Asymmetric DNA origami for spatially addressable and index-free solution-phase DNA chips. *Adv. Mater.* **2010**, DOI: 10.1002/adma.201000151.

68 He, S.; Song, B.; Li, D.; Zhu, C.; Qi, W.; Wen, Y.; Wang, L.; Song, S.; Fang, H.; Fan, C. A graphene nanoprobe for rapid, sensitive and multicolor fluorescent DNA analysis. *Adv. Func. Mater.* **2010**, *20*, 453–459.

69 Benenson, Y.; Paz-Elizur, T.; Adar, R.; Keinan, E.; Livneh, Z.; Shapiro, E. Programmable and autonomous computing machine made of biomolecules. *Nature* **2001**, *414*, 430–434.

70 Zhu, C.; Wen, Y.; Li, D.; Wang, L.; Song, S.; Fan, C.; Willner, I. Inhibition of the in vitro replication of DNA by an aptamer-protein complex in an autonomous DNA machine. *Chem.—Eur. J.* **2009**, *15*, 11898–11903.