[Frontiers in Bioscience 10, 37-46, January 1, 2005]

ELECTROCHEMICAL BIOSENSORS FOR DNA ANALYSIS

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1. ABSTRACT

Over the past few years, progress in electrochemical biosensors for DNA analysis is outstanding. This article briefly reviewed the principals of such biosensors, followed by a selection of topics that are of particular current interest. Selectivity and sensitivity are the two major challenges in DNA analysis. The former can be accomplished using peptide nucleic acids as probes, or relying on the DNA-mediated electron transfer. Special emphasis has been given to the efforts for high sensitivity, involving combination with polymerase chain reaction techniques, enzyme-labeled methods, direct label-free detection and nano-based techniques.

2. INTRODUCTION

The remarkable achievement of the draft human genomic sequence represents the beginning of an exciting era of life science (1). Analysis of the draft human genomic sequence has already led to the identification of genes for inherited diseases as well as single base variations in the genetic code that play a significant role in the disease process. Such discoveries will necessarily have a profound impact on the diagnosis of diseases requiring rapid and accurate assays of DNA. Moreover, assays of DNA will be required in other applications, such as forensic medicine (2, 3), environmental monitoring (4), and detection of biological warfare agents (5). Developing easy-to-use, rapid, inexpensive, miniaturized analytical devices stays therefore on the center of interest among analytical chemists.

DNA hybridization biosensors, which combine the specificity of biological recognitions with the sensitivity of physicochemical transducers, offer a promising alternative for such demands in DNA analysis. Although optical (6) and microgravimetric (7) sensors are exciting areas of research, much more attention have been paid on electrochemical biosensors due to their high sensitivity in association with portability, disposability, and compatibility with microfabrication. Several reviews (8-13) have put emphasis on electrochemical biosensors for DNA hybridization and on applications of such DNA biosensors. Two major challenges facing DNA sensors are high selectivity (a single base mismatch) and high sensitivity (down to femtomole), to which considerable efforts were dedicated respectively. This article does not provide an exhaustive review of literatures, but instead focus on the recent strategies for electrochemical detection of DNA hybridization and issues needed to understand better for design of ideal DNA biosensors.

3. PRINCIPLE OF ELECTROCHEMICAL DNA BIOSENSING

The DNA techniques, including hybridization, amplification, recombination, are almost based on the double helix structure of the DNA. Watson-Crick base-pairing rules are the underlying principle of DNA biosensors (14). These sensors commonly rely on the immobilization of a ssODN (a DNA probe usually 15-20 bases, i.e. single strand oligodeoxyribonucleic acid) probe on different electrode (or chip) to recognize its complementary target sequence. The hybridization event can be detected via monitoring changes in the current response of a redox indicator, or electronic and interfacial properties resulting from the duplex formation.

Toward a successful operation of DNA biosensors, the probe immobilization should be firstly considered. Conventional probe immobilization procedures include adsorption (15-18), encapsulation/bulk modification (19), avidin-biotin complexation (20-22) and covalent attachment (23-26). The distribution, packing density and orientation of the oligonucleotide probe on surface may influence the performance of DNA biosensors (27-29).

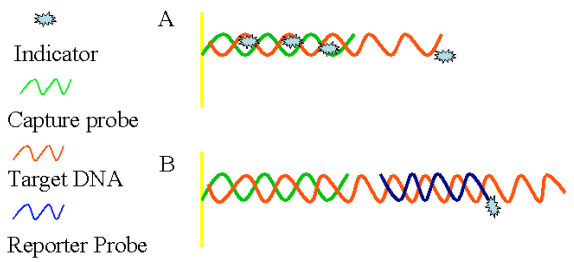

Figure 1. Detection of hybridization using indicators. A) Indicators bind to ssDNA and dsDNA with different ability resulting in a distinguished signal. B) A sandwich-type assay relies on the introduced indicator-labeld oligonucleotide. (Adapted from Ref. 8)

Surface characterization techniques, such as XPS (30), neutron reflectivity (28) and surface-plasmon resonance (29, 31), can shed useful insights into the surface coverage and the structure of surface-bound DNA. Random adsorption of ssODN on electrode surfaces results in multi-site attachment, decreases the specificity of the recognition layer, and therefore is not recommended. Bulk modification of the carbon paste with DNA may result in poorly defined DNA layers and the leakage of water-soluble DNA from the bulk-modified carbon paste during the analysis. Significantly better results can be obtained by immobilizing the probe via one end of the DNA molecule. Generally, a covalently bound probe is better because it provides more structural flexibility and more opportunities for minimizing nonspecific adsorption effects. To achieve high hybridization efficiency, the DNA recognition interface can be formed via coassembly of a thiolated DNA and mercaptohexanol monolayer (28). The purpose of the mercaptohexanol monolayer was to prevent contacts between DNA backbone and the electrode surface, so that the probe was standing up and accessible for hybridization.

Since hybridization of DNA on electrode surfaces is a solid-phase reaction, the rate is only about one tenth to one hundredth of that in solution (32). Proper attention should be given to experimental variables prompting the hybridization event at the transducer-solution interface with consideration to the selectivity of hybridization. These involve the salt concentration, temperature, viscosity, the addition of acceleration agents, hybridization time and length of probe sequence (9). Strict control of hybridization, particularly using elevated temperature, can eliminate the mismatch possibility at the cost of low hybridization efficiency. The use of peptide nucleic acid (PNA) probes, which can significantly enhance the selectivity (33), seems to be a well-suited alternative to solve this problem.

As compared to the newly developed transduction schemes (discussed in Section 5), earlier electrochemical devices have relied typically on the use of electroactive hybridization indicators such as metal chelates (e.g. $Co(phen)_3$ (34)) and organic compounds (e.g. methylene blue (35)). These indicators interact with DNA in three ways: electrostatic binding along the exterior of the DNA backbone, which is generally nonspecific; groove binding, in which the bound molecule interacts directly with the edges of base pairs in the minor or major grooves of DNA; and intercalation of planar aromatic ring systems between base pairs (8). Groove binders and intercalators possess much higher affinity to the resulting dsDNA compared to the ssDNA probe. The difference between the binding ability of the indicator with ssDNA and dsDNA results in a difference in electrochemical responses, which produces a hybridization signal (Figure 1A). Our laboratory used methylene blue (MB) as an electroactive indicator to monitor the recognition of native yeast DNA sequence (36). The yeast single-stranded DNA was covalently bound to a self-assembled 3-mercaptopropionic acid monolayer by using water soluble N-(3-dimethylaminpropyl)-N'-ethylcarbodiimide hydrochloride (EDC) as linker. Upon the hybridization of immobilized ssDNA with its cDNA in the solution, an increase in peak currents of MB was observed. The adsorption constants of MB on ssDNA and dsDNA modified gold electrode surface were found to be $(3.3\pm0.3)\times10^3$ M^{-1} and $(6.6\pm0.4)\times10^3$ M^{-1}, respectively, indicating a higher affinity of MB to dsDNA. An electrochemical biosensor for the hepatitis B virus DNA PCR production was also developed by using $[Os(bpy)_2Cl_2]^+$ (37) or Fc^+ (30) as hybridization indicator. Two unique indicators, threading intercalators (38, 39) and bis-intercalators (40), which both bind to dsDNA more tightly than usual intercalators and have negligible affinities to ssDNA, have been extensively used to offer better distinction between ssDNA and dsDNA. Ferrocenyl naphthalene diimide has three benefits: it binds to dsDNA more strongly than to ssDNA, it does not have a bias against DNA sequence, and it can bind to the DNA-RNA hetero duplex more strongly than to the DNA-DNA duplex. These properties resulted in a detection limit of 10 zmol, and made it suitable for the electrochemical detection of mRNA expression (41). On the other hand, the redox indicator can also be covalently tethered to the end of oligonucleotides as a reporter probe in a sandwich hybridization assay (42, 43). After hybridizing the target DNA with the ssDNA immobilized on electrode surface, the resulting assembly was reacted with the reporter probe complementary to the target in the region adjoining the capture probe binding site. Then the labeled indicator produced a signal (Figure 1B).

Although redox indicators have the apparent advantage of simplicity, the selectivity and sensitivity is relatively poor. The ensuing sections will highlight some of the current progress in the area of DNA biosensors with respect to the selectivity and the sensitivity.

4. SELECTIVITY OF ELECTROCHEMICAL DNA BIOSENSING

For the analysis of genetic diseases, the identification of single nucleotide polymorphs (SNPs) is desired. Generally, two main approaches are used to enhance the selectivity of DNA biosensors. One is based on the selection of the probe (the use of PNA as a probe (44-49)) along with the hybridization conditions (especially the temperature (50)); the other is to differentiate the property of electron transfer between the perfect match and the single-base mismatch (51-53).

PNA originated from efforts during the 1980s to design new nucleic acid sequence-specific reagents for

controlling gene expression associated with the development of antisense and antigen therapeutic drugs (54). So far, several articles (33, 55, 56) have reviewed the PNA chemistry, structure, hybridization and applications. PNAs are DNA analogs in which a 2-aminoethyl-glycine linkage generally replaces the normal phosphodiester backbone. A methyl carbonyl linker connects natural as well as unusual (in some cases) nucleotide bases to this backbone at the amino nitrogens. PNAs are non-ionic, achiral molecules and are not susceptible to hydrolytic (enzymatic) cleavage. Despite all these variations from natural nucleic acids, PNA is still capable of sequence-specific binding to DNA as well as RNA obeying the Watson-Crick hydrogen bonding rules. The neutral backbone results in the independence of the ionic strength and a lack of electrostatic repulsion between the PNA and DNA strands. Hence, its hybrid complexes to its complementary nucleic acid sequence exhibit extraordinary thermal stability and specificity compared to traditional oligonucleotides. However, the presence of mismatches strongly influenced the thermal stability of the PNA/DNA duplex, thus more easily destabilized than a mismatch in a DNA/DNA duplex. This property can remarkably discriminate between perfect matches and mismatches, and makes PNA probes particularly attractive as recognition layers in DNA biosensors (55).

Photoinduced electron transfer through donor/acceptor-labeled duplexes has been observed in a variety of systems (57, 58). Numerous studies of electron transfer through the DNA base stack have demonstrated that long-range charge transfer shows a weak dependence on distance but is exceptionally sensitive to perturbations in the base stack such as mismatches (59). Thus, while single-base mismatches may cause only subtle changes in duplex stability and structure, they appear to induce significant perturbations in the electronic structure of the base-pair stack. Using the efficiency of DNA-mediated charge transport as a signaling device, an alternative approach has been applied to detect the base mismatches. To prepare the biorecognition interface, the thiol-modified prehybridized duplexes have been self-assembled on gold electrodes (51). When the duplexes perfectly matched, redox-active intercalators such as daunomycin yielded well-resolved electrochemical signals. However, the presence of a single mismatch in the DNA duplexes between the daunomycin-binding site and the electrode caused a striking decrease in the electrochemical response (51). In order to increase the inherent sensitivity of the assay, the direct electron transfer was coupled to an electrocatalytic cycle involving the electrocatalytic reduction of ferricyanide by methylene blue (52). Using this protocol, all of the possible single-base mismatches, even the thermodynamically stable GA mismatch, can be readily detected. Recently, Long *et al* (60) observed a difference in redox potential between two ferrocenoyl (Fc)-labeled DNA, which differed only in the Fc attachment point: the first was on the same strand as the thiolate linkage, and the second was on the complementary strand. Although the authors intended to the combination of strand jumping and Fc orientation accounting for this phenomenon, the mechanistic details concerning the electron transfer remained elusive. A close examination would be important in the case of utilizing electron transfer via DNA base stack to detect the mismatches. Such work would also be helpful to provide clues toward explaining the remarkably higher redox potential when using a ferrocene-oligonucleotide conjugate in a sandwich-type assay (61).

5. AMPLIFICATION OF ELECTROCHEMICAL SIGNAL OF DNA BIOSENSORS

5.1. Combination with PCR

PCR technique has been widely used in routine DNA assays, aiming to amplify enormously the specific DNA sequence in a sample. High sensitivity of DNA biosensors can be achieved by coupling of the electrochemical detection with PCR technique. In its early stage, PCR mainly served as an intermediate step independent of the subsequent detection. Microfabrication technologies made it possible to integrate sample amplification with subsequent amplicon detection in a single lab-on-a-chip device, which promised significant advantages in terms of speed, efficiency, contamination and automation (62).

Over the past few years, effective and reliable microchip-based PCR devices have been successfully carried out capable of on-chip amplicon detection (62, 63). Regretfully, the majority of detection modules are constructed on optical systems, which are difficult to miniaturize onto a monolithic microanalytical system. The inherent advantages of electrochemical methods make possible true miniaturization of DNA analysis functions onto a single microfluidic chip. Recently, Lee *et al* (64) integrated microchip-based PCR with electrochemical detection functionality into a microdevice, which comprises of a reaction chamber (volume of 8 nl) formed in a silicon substrate and an electrochemical sensor fabricated onto a glass substrate. Platinum temperature sensors and heaters were patterned on top of the silicon substrate for real-time temperature monitoring and control. Electrode materials were patterned on the glass substrate functioning as electrochemical detection platforms and a seal for the reaction chamber. The ability to detect trace amounts of target DNA (as few as several hundred copies) has been demonstrated.

5.2. Enzyme-amplified electrochemical biosensing

Inspired by the bioelectrocatalytic reaction of enzyme labels in immunosensors, Heller and co-workers (50) realized a scheme for monitoring of a single base mutation in an 18-base oligonucleotide in connection with the enzyme-amplified amperometric detection. In this system, the probe DNA was covalently bound to the electron-conducting redox hydrogel, which electrically wired the enzyme reaction centers upon contact. Upon hybridization with the target DNA labeled with a peroxidase enzyme, the added H_2O_2 was electrocatalytically reduced to water. Hybridization was thereby translated to current of H_2O_2 electrochemical reduction. A problem in point is that each target DNA should be labeled, which is rather inconvenient.

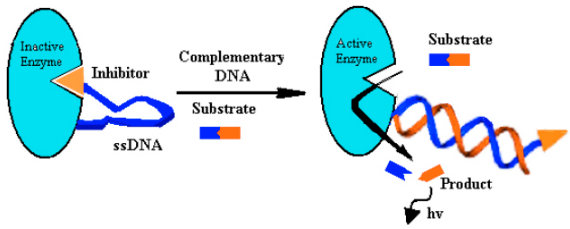

Figure 2. Schematic representation of an intrasterically inactivated inhibitor-DNA-enzyme (IDE) construct (left) and the DNA hybridization triggered enzyme activation (right). IDE can be used to sense low concentrations of complementary DNA because of its built-in capacity for signal amplification via rapid substrate turnover (from Ref. 74).

Later on, an enzyme-amplified sandwich-type amperometric assay for RNA and DNA has been developed (65-69). A film of avidin and redox polymer was coelectrodeposited on a carbon electrode, and then the avidin was conjugated with its biotinylated oligonucleotide, complementary to the assayed sequence. When the electrode was immersed in a test solution mixing the analyte DNA solution, the HRP-labeled oligonucleotide solution and a H_2O_2 solution, the analyte DNA was cohybridized with the probe and the HRP-labeled oligonucleotide at different segments, respectively. Completion of the sandwich brings the HRP label into electrical contact with the redox polymer, converting the nonelectrocatalytic base layer into an electrocatalyst for the electroreduction of H_2O_2 to water (65). In this case, the lower detection limit can be anticipated through the emerging use of nanoparticles and nanoelectrode arrays due to the enhanced wiring of the labeled enzymes (9, 70). DNA was detected at 0.5 fM concentration when the earlier used 3.6-mm-diameter carbon electrode was replaced by a 10-mμm-diameter microelectrode (66). By using ultramicroelectrode array, E. Nebling *et al* (71) proposed direct virus DNA detection from a PCR-amplified clinical probe. The nanoscaled electrodes are used to perform a sensitive detection of enzyme activity by signal enhancing redox recycling of 4-hydroxyaniline resulting in local and position-specific current signals. Another approach relies on the ferrocenyl-tethered dendrimers as a building block offering 2-fold greater capacity to immobilize oligomer probes (68). Ferrocenyl-tethered dendrimers also served as an electrocatalyst. In the electrocatalytic process, *p*-Aminophenol was generated as a result of the enzymic reaction of alkaline phosphatase, and then was electrocatalytically oxidized by electronic mediation of ferrocenyl-tethered dendrimers leading to a great enhancement in signal. Applicability for highly sensitive detection and sequence-selective discrimination was consequently demonstrated. In another amplification means for sensing the DNA, Willner *et al* used a biotinylated oligonucleotide as a label; the linked avidin-HRP conjugate precipitated an insoluble product on the electrode, which could be monitored by Faradaic impedance spectroscopy (72, 73).

Recently, Ghadiri *et al* presented a very interesting and fantastic detection of DNA hybridization based on signal amplification via an engineered allosteric enzyme illustrated in Figure 2 (74). The covalently associated inhibitor-DNA-enzyme (IDE) templates were functioned via DNA hybridization-triggered allosteric enzyme activation, and signal was subsequently amplified through substrate turnover. In this system, a single-stranded DNA (ssDNA) probe was first employed to covalently tether Cereus neutral protease 5 to its small-molecule phosphoramidite inhibitor. The conformational flexibility of the ssDNA tether allowed facile intramolecular binding of the inhibitor to the enzyme active site to furnish the inactive state of the IDE. The presence of its complementary DNA sequence rapidly converted IDE to an active state because formation of a high-affinity DNA duplex structure drastically altered the conformation of the tether, in favor of the release of the inhibitor from the enzyme active site. Because the enzyme turns over many copies of the substrate, in principle each probe hybridization event can be chemically amplified to produce high detection sensitivity. Approximately 10 fmol DNA was detected in connection to the active enzyme acting upon its fluorogenic substrate *in situ*. Such work suggested new avenues for generating the hybridization signal through the conformational changes of single DNA oligomer probe with respect to the host-guest chemistry.

5.3. Direct label-free electrochemical biosensing

Direct label-free electrochemical detection eliminated the external labels or indicators and greatly shortened the assay time, hence attracting increasing interest. In principal, such direct, *in situ* detection originates from the two following effects as a consequence of the DNA hybridization: 1) the introduced intrinsic redox activity of the nucleotide acid target; 2) changes in the electrochemical properties of the interface (e.g. capacitance, impedance, conductance as well as surface charge).

The most redox active among the four nucleic acids bases is the guanine moiety, which is reported in detail by steenken (75). In our laboratory, we proposed a simple and reliable method for simultaneous determination of guanine and adenine in DNA based on adsorptive stripping at an electrochemically pretreated glassy carbon electrode (76). In view of the challenges for directly detecting hybridization, inosine-substituted probes have been exploited and the appearance of a guanine signal upon hybridization indicates the target sequence as well as its concentration (77-84). However, the guanine voltammetric peaks are poorly developed due to the high exponential background current at potentials above +0.90 V, presumably due to the electrode surface fouling. Substantial progress has been achieved with the application of sophisticated baseline correction techniques (82, 85) and the electrocatalytic action of a $Ru(bpy)_3^{2+}$ redox mediator (78, 84), thus providing sufficient sensitivity for DNA analysis. Recently, Ferapont and Dominguez reported on electrochemical oxidation of guanine at +0.73 V through direct DNA adsorption at polycrystalline gold electrodes in connection with square wave voltammetry (86). A label-free electrochemical detection protocol for DNA hybridization by using a gold electrode was later reported

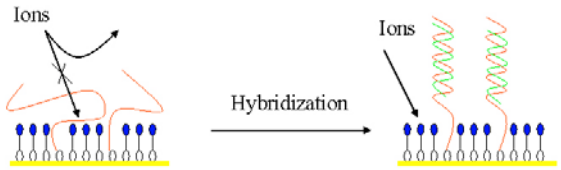

Figure 3. Schematic diagram of the ion gating effect. The ssDNA is flexible and lies across the interface, preventing ions from reaching the gold electrode. After hybridization the rigid dsDNA stands up off the surface, open up the interface to ions, and thus opening an electrochemical signal (Adapted from Ref. 91).

by Kerman and co-workers (87). Label-free detection of hybridization on gold electrodes holds an enormous promise for its applicability to a microarray in comparison with the existing carbon and mercury electrode materials.

Changes in electrical properties of the DNA interface upon hybridization have been also exploited (88-92). For example, Shim *et al* (88) demonstrated a significant change in admittance during duplex formation on the basis of oligonucleotide-functionalized conductive polymer, attributed to the reduction of the resistance after hybridization. A biosensor using impedance measurements was therefore designed. The increase in surface charge upon hybridization can be directly monitored by microfabricated silicon field-effect sensors (89). In this protocol, the probe DNA was electrostatically immobilized on a positively charged poly-L-lysine layer in favor of hybridization at low ionic strength where field-effect sensing is most sensing. Nanomolar concentrations can be detected within minutes, and a single base mismatch within 12-mer oligonucleotides can be distinguished by using a differential detection technique with two sensors in parallel. Janata (90) utilized electrostatic modulation of ion-exchange kinetic of polypyrrole film for a simple and direct detection of a hybridization event. It is based on the exchange of chloride ion between the polypyrrole layer and buffer, which is controlled by the negative charge density at this interface. The probe DNA was linked to the polypyrrole surface via the Mg^{2+} bridging complex between an alkyl phosphonic acid group on the polymer and the phosphate group of the DNA. When the complementary DNA hybridized to the probe DNA, the negative charge density increased, resulting in a distinguished shape of the cyclic voltammogram. Thus, noncomplementay and complementary interactions can be easily detected. In a similar way, Gooding (91) presented a label-free electrochemical method using an ion-gating effect at a gold electrode modified with a mixed SAM of thiolated DNA and alcohol terminated alkanethiol as a diluent layer (Figure 3). The ssDNA is a flexible molecule lying across the interface, hindering ions from reaching the gold surface. Upon hybridization, the formed dsDNA is a rigid rod standing up off the surface, opening up the interface to access of ions. Such ion-gating effect can modulate the oxidative desorption of the oxidized SAM, which allows a direct electrochemical method of detecting DNA hybridization.

5.4. Nano-based electrochemical biosensing

The emerging field of nanobiotechnology, in which nanoparticles are applied to the analysis of biomecules, is opening new opportunities for DNA analysis especially with the current rapid development of nanotechnology and synthesis technology. Nanoparticles display fascinating electronic and optical properties as a result of their dimensions, offering excellent prospects for chemical and biological sensing (93-95). Very recently, Wang (96) reviewed a variety of new nanoparticle/polynucleotide assemblies for advanced electrochemical detection of DNA sequences, and Park (97) described the extended chip-based detection system besides the designed DNA-based assembly of nanoparticles.

Nanoparticles offer innovative and elegant ways for interfacing DNA recognition events with electrochemical signal transduction processes (96). Of considerable interest for numerous bioanalytical applications are colloidal gold tags (98-107) and semiconductor nanocrystals (108, 109). Mirkin *et al* reported a DNA array detection method in which the hybridization of oligonucleotides functionalized with gold nanoparticles leaded to conductivity changes. The hybridization localized gold nanoparticles in an electrode gap; silver deposition facilitated by these nanoparticles bridged the gap and leaded to readily measurable conductivity changes (98). Wang *et al* developed a new nanoparticle-based electrical detection of DNA hybridization, based on electrochemical stripping detection of the colloidal gold tags (99). This protocol involved the hybridization of a target oligonucleotide to magnetic bead-loaded oligonucleotide probe, followed by binding of the streptavidin-coated gold nanoparticles to the captured DNA, acid dissolution of the gold tag, and potentiometric stripping measurements of the dissolved tag at bare carbon strip electrodes. The magnetic processing technique was used to isolate the duplex. Further signal amplification was attained via catalytic silver precipitation on the colloidal gold tag forming the Au/Ag core-shell structure and a magnetic collection of the assembly (100). In another attempt to increase selectively the number of labeled Au-nanoparticles, several layers of nanoparticles were assembled producing significantly higher amounts of metal silver on the multilayer nanoparticles than the monolayer did through silver enhancement (101). The novel Cu@Au core-shell nanoparticles combining the surface modification properties of Au with the good electrochemical activity of Cu core also showed perspective applications in the electrochemical DNA analysis (102). Ozsoz *et al* (103) described the detection of Factor V Leiden mutation from PCR amplicons using the oxidation signal of colloidal gold at disposable pencil graphite electrodes. Wang *et al* (104) developed a sandwich-type voltammetric assay based on the oxidation of ferrocene caps on gold nanoparticles/streptavidin conjugates. Since each Au nanoparticle was covered with a large number of ferrocene and more than one gold nanoparticle might be attached to a single streptavidin molecule, the amount of redox markers per DNA duplex was greatly increased and, as a result, the voltammetric signals were significantly amplified. Inorganic-colloid (quantum dots) nanocrystal tracers with discriminating redox potentials have been used for the simultaneous electrochemical measurements of multiple DNA targets (108). This protocol involved a sandwich assay including

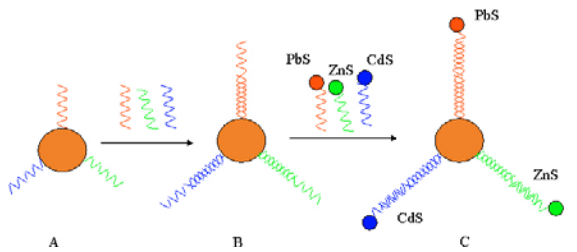

Figure 4. Multi-target electrochemical DNA detection protocol based on different inorganic colloid nanocrystal tracers. (A) Imobilizaition of capture probe on magnetic beads. (B) Hybridization with the DNA targets. (C) Second hybridization with the QD-labeled probes (from Ref. 108).

the introduction of probe-modified magnetic beads, hybridization with the DNA-analyte, second hybridization with QDs-labeled probes, and stripping voltammetry of the corresponding metals (Figure 4). The position and size of the peaks reflect the identity and quantity of the corresponding DNA target. Indium has an excellent electrochemical behavior and is not normally present in biological samples. Taking advantages of its merits, Wang and co-workers (110) also reported on solid-state electrochemical detection of DNA hybridization using cylindrical indium microrod tracers, which were prepared by a template-directed electrochemical synthetic route involving plating of indium into the pores of a host membrane.

Nanoscale sensors based on nanowires (NWs), nanotubes (NTs), and other nanomaterials are attracting enormous interest and becoming increasingly popular (49). Since their discovery in 1991, carbon nanotubes are being developed for a host of biomedical and biotechnological applications attributed to their unique structures and properties (111). Li *et al* (84) fabricated a precisely positioned and well-aligned multiwalled carbon nanotube (MWNT) array embedded in a planarized SiO_2 matrix with a bottom-up scheme. The open ends of MWNTs exposed at the dielectric surface acted as nanoelectrodes, showing a fast electron transfer rate while the sidewalls are inert. Lieber *et al* (49) designed a p-type silicon nanowire nanosensor for high sentivitivity detection in connection with recording the time-dependant conductance, because the increase in negative surface charge density, which is caused by hybridization of negatively charged oligonucleotide targets to the PNA probe at the surface, affects the entire cross-sectional conduction pathway to produce an increase in conductance.

6. SUMMARY AND PERSPECTIVE

Over the past decade, encouraging and amazing advances have been made toward extremely sensitive, selective yet inexpensive and robust detection methods. The clear direction for future work in DNA biosensors is to develop a simple, very fast, direct strategy for simultaneous multi-target detection without the multiple washing step and extraneous addition of electroactive indicators. Selectivity and sensitivity are the primary areas of focus, with the ultimate goal of single-molecule and single-base-mismatch detection in a biological medium (112). High selectivity can be achieved through the formation of specific recognition layer using the PNA and its various derivatives, since it binds to DNA with much greater affinity and stability than corresponding DNA and shows binding with single base specificity. Although enzyme-linked amplified technique exhibits high sensitivity, the labor-intensive procedures seem to make it less practical especially for point-of-care testing. In contrast, the signal amplification based on nanoparticles provides a judicious choice.

New efforts are being undertaken currently to devise DNA chips incorporated into total analysis systems, so-called “lab on a chip”, including sample preparation, DNA extraction and amplification, and signal acquisition. Nanoelectrode arrays based on nanotubes or nanowires open up exciting ways for direct integration with microelectronics and microfluidic system to gain miniaturization devices. Such devices will have definite advantages in terms of cost, speed, sample volume and automation (9, 62). It can be envisioned that such research will undoubtedly hold a leading position in the future rapid determination of DNA sequence.

7. ACKNOWLEDGEMENTS

We gratefully acknowledge the financial support of the Distinguished Young Scholar Fund to HX Ju (20325518), the National Natural Science Foundation of China (20275017, 90206037), the Specialized Research Fund for the Excellent Young Teachers from Ministry of Education of China and the Science Foundation of Jiangsu (BS2001063).

8. REFERENCES

1. International Human Genome Sequencing Consortium: Initial sequencing and analysis of the human genome. *Nature* 409, 860–921 (2001)
2. Erdem A. & M. Ozsoz: Interaction of the anticancer drug epirubicin with DNA. *Anal Chim Acta* 437, 107-14 (2001)
3. Teulade-Fichou M. P, C. Carrasco, L. Guittat, C. Bailly, P. Alberti, J. L. Mergny, A. David, J. M. Lehn & W. D. Wilson: Selective recognition of G-quadruplex telomeric DNA by a bis(quinacridine) macrocycle. *J Am Chem Soc* 125, 4732-4740 (2003)
4. Chiti G, G. Marrazza & M. Mascini: Electrochemical DNA biosensor for environmental monitoring. *Anal Chim Acta* 427, 155-64 (2001)
5. Shah J. & E. Wilkins: Electrochemical biosensors for detection of biological warfare agents. *Electroanal* 15, 157-167 (2003)
6. Piumno P, U. Krull, R. Hudson, M. Damha & H. Cohen: Fiber-optic DNA sensor for fluorometric nucleic acid determination. *Anal Chem* 67, 2635-2643 (1995)
7. Janshoff A, H. J. Galla & C. Steinem: Piezoelectric mass-sensing devices as biosensors--an alternative to optical biosensors. *Angew Chem Int Ed* 39, 4004–4032 (2000)
8. Palecek E. & M. Fojta: DNA hybridization and damage. *Anal Chem* 73, 75A–83A (2001)

9. Wang J: Electrochemical nucleic acid biosensors. *Anal Chim Acta* 469, 63–71 (2002)
10. Bakker E. & M. Telting-Diaz: Electrochemical sensors. *Anal Chem* 74, 2781–2800 (2002)
11. Mascini M, I. Palchetti & G. Marraza: DNA electrochemical biosensors. *Fresen J Anal Chem* 369, 15–22 (2001)
12. Gooding J. J: Electrochemical DNA hybridization biosensors. *Electroanal* 14, 1149–1156 (2002)
13. Drummond T. G, M. G. Hill & J. K. Barton: Electrochemical DNA sensors. *Nat Biotechnol* 21, 1192-1199 (2003)
14. Zhai J. H, H. Cui & R. F. Yang: DNA based biosensors. *Biotechnol Adv* 15, 43–58 (1997)
15. Wang J, X. Cai, G. Rivas, H. Shiraishi, P. A. M. Farias & N. Dontha: DNA electrochemical biosensor for the detection of short DNA sequences related to the human immunodeficiency virus. *Anal Chem* 68, 2629–2634 (1996)
16. Wang J, X. Cai, G. Rivas, H. Shiraishi & N. Dontha: Nucleic–acid immobilization, recognition and detection at chronopotentiometric DNA chips. *Biosens Bioelectron* 12, 587–599 (1997)
17. Wang J, X. Cai, J. R. Fernandes, D. H. Grant & M. Ozsoz: Electrochemical measurements of oligonucleotides in the presence of chromosomal DNA using membrane-covered carbon electrodes. *Anal Chem* 69, 4056–4059 (1997)
18. Marrazza G, L. Chianella & M. Mascini: Disposable DNA electrochemical sensor for hybridization detection. *Biosens Bioelectron* 14, 43–51 (1999)
19. Wang J, J. R. Fernandes & L. T. Kubota: Polishable and renewable DNA hybridization biosensors. *Anal Chem* 70, 3699-3702 (1998)
20. Zhou X. C, L. Q. Huang & S. F. Y. Li: Microgravimetric DNA sensor based on quartz crystal microbalance: comparison of oligonucleotide immobilization methods and the application in genetic diagnosis. *Biosens Bioelectron* 16, 85–95 (2001)
21. Shamansky L. M, C. B. Davis, J. K. Stuart & W. G. Kuhr: Immobilization and detection of DNA on microfluidic chips. *Talanta* 55, 909–918 (2001)
22. Masarik M, R. Kizek, K. J. Kramer, S. Billova, M. Brazdova, J. Vacek, M. Bailey, F. Jelen & J. A. Howard: Application of avidin-biotin technology and adsorptive transfer stripping square-wave voltammetry for detection of DNA hybridization and avidin in transgenic avidin maize. *Anal Chem* 75, 2663–2669 (2003)
23. Millan K. M & S. K. Mikkelsen: Sequence-selective biosensor for DNA based on electroactive hybridization indicators. *Anal Chem* 65, 2317–2323 (1993)
24. Hashimoto K, K. Ito & Y. Ishimori: Sequence-specific gene detection with a gold electrode modified with DNA probes and an electrochemically active dye. *Anal Chem* 66, 3830–3833 (1994)
25. Xu X. H. & A. J. Bard: Immobilization and hybridization of DNA on an aluminum (III) alkanebisphosphonate thin film with electrogenerated chemiluminescent detection. *J Am Chem Soc* 117, 2627 – 2631 (1995)
26. Korri-Youssoufi R, F. Garnier, P. Srivastava, P. Godillot & A. Yassar: Toward bioelectronics: specific DNA recognition based on an oligonucleotide-functionalized polypyrrole. *J Am Chem Soc* 119, 7388–7389 (1997)
27. Watterson J. H, P. A. E. Piunno, C. C. Wust & U. J. Krull: Effects of oligonucleotide immobilization density on selectivity of quantitative transduction of hybridization of immobilized DNA. *Langmuir* 16, 4984-4992 (2000)
28. Levicky R, T. M. Herne, M. J. Tarlov & S. K. Satija: Using self-assembly to control the structure of DNA monolayers on gold: a neutron reflectivity study. *J Am Chem Soc* 120, 9787–9792 (1998)
29. Peterson A. W, R. J. Heaton & R. M. Georgiadis: The effect of surface probe density on DNA hybridization. *Nucleic Acids Res* 29, 5163-5168 (2001)
30. Ye Y. K, J. H. Zhao, F. Yan, Y. L. Zhu & H. X. Ju: Electrochemical behavior and detection of hepatitis B virus DNA PCR production at gold electrode. *Biosens Bioelectron* 18, 1501–1508 (2003)
31. Peterlinz K. A, R. M. Georgiadis, T. M. Herne & M. J. Tarlov: Observation of hybridization and dehybridization of thiol-tethered DNA using two-color surface plasmon resonance spectroscopy. *J Am Chem Soc* 119, 3401–3402 (1997)
32. Bunemann T: Immobilization of denatured DNA to microporous supports: II. Steric and kinetic parameters of heterogeneous hybridization reactions. *Nucleic Acids Res* 10, 7181–7196 (1982)
33. Ray A. & B. Norden: Peptide nucleic acid (PNA): its medical and biotechnical applications and promise for the future. *Faseb J* 14, 1041–1060 (2000)
34. Millan K. M, A. Saraullo & R. Mikkelsen: Voltammetric DNA biosensor for cystic fibrosis based on a modified carbon paste electrode. *Anal Chem* 66, 2943–2948 (1994)
35. Erdem A, K. Kerman, B. Meric, U. S. Akarca & M. Ozsoz: Novel hybridization indicator methylene blue for the electrochemical detection of short DNA sequences related to the hepatitis B virus. *Anal Chim Acta* 422, 139–149 (2000)
36. Gu J. Y, X. J. Lu & H. X. Ju: DNA sensor for recognition of native yeast DNA sequence with methylene blue as an electrochemical hybridization indicator. *Electroanal* 14, 949-954(2002)
37. Ju H. X, Y. K. Ye, J. H. Zhao & Y. L. Zhu: Hybridization biosensor using di(2,2'-bipyridine)osmium (III) as electrochemical indicator for detection of polymerase chain reaction product of hepatitis B virus DNA. *Anal Biochem* 313, 255-261 (2003)
38. Takenaka S, K.Yamashita, M. Takagi, Y. Uto & H. Kondo: DNA sensing on a DNA probe-modified electrode using ferrocenylnaphthalene diimide as the electrochemically active ligand. *Anal Chem* 72, 1334-1341 (2000)
39. Yamashita K, M. Takagi, H. Kondo & S. Takenaka: Electrochemical detection of nucleic base mismatches with ferrocenyl naphthalene diimide. *Anal Biochem* 306, 188-196 (2002)
40. Juskowiak B, E. Galezowska & S. Takenaka: Spectral properties and binding study of DNA complexes with a rigid bisintercalator 1,4-bis((N-methylquinolinium-4-yl)vinyl)benzene. *Spectrochim Acta A* 59, 1083-1094 (2003)
41. Sato S, S. Fujii, K. Yamashita, M. Takagi, H. Kondo & S. Takenaka: Ferrocenyl naphthalene diimide can bind to DNA-RNA hetero duplex: potential use in an

electrochemical detection of mRNA expression. *J Organomet Chem* 637, 476-483 (2001)
42. Ihara T, M. Nakayama, M. Murata, K. Nakano & M. Maeda: Gene sensor using ferrocenyl oligonucleotide. *Chem Commun* 1609-1610 (1997)
43. Umek R. M, S. W. Lin, J. Vielmetter, R. H. Terbrueggen, B. Irvine, C. J. Yu, J. F. Kayyem, H. Yowanto, G. F. Blackburn, D. H. Farkas & Y. P. Chen: Electronic detection of nucleic acids - A versatile platform for molecular diagnostics. *J Mol Diagn* 3, 74-84 (2001)
44. Arlinghaus H. F, M. N. Kwoka & K. B. Jacobson: Analysis of biosensor chips for identification of nucleic acids. *Anal Chem* 69, 3747-3753 (1997).
45. Wang J, P. E. Nielsen, M. Jiang, X. H. Cai, J. R. Fernandes, D. H. Grant, M. Ozsoz, A. Beglieter & M. Mowat: Mismatch sensitive hybridization detection by peptide nucleic acids immobilized on a quartz crystal microbalance. *Anal Chem* 69, 5200-5202 (1997)
46. Sawata S, E. Kai, K. Ikebukuro, T. Iida, T. Honda & I. Karube: Application of peptide nucleic acid to the direction of deoxyribonucleic acid amplified by polymerase chain reaction. *Biosens Bioelectron* 14, 397-404 (1999)
47. Basile A, A. Giuliani, G. Pirri & M. Chiari: Use of peptide nucleic acid probes for detecting DNA single-base mutations by capillary electrophoresis. *Electrophoresis* 23, 926-929 (2002)
48. Gaylord B. S, A. J. Heeger & G. C. Bazan: DNA detection using water-soluble conjugated polymers and peptide nucleic acid probes. *Proc Natl Acad Sci USA* 99, 10954-10957 (2002)
49. Hahm J. & C. M. Lieber: Direct ultrasensitive electrical detection of DNA and DNA sequence variations using nanowire nanosensors. *Nano Lett* 4, 51-54 (2004)
50. Caruana D. J. & A. Heller: Enzyme-amplified amperometric detection of hybridization and of a single base pair mutation in an 18-base oligonucleotide on a 7-µm-diameter microelectrode. *J Am Chem Soc* 121, 769-774 (1999)
51. Kelly S. O, E. M. Boon, J. K. Barton, N. M. Jackson & M. G. Hill: Single-base mismatch detection based on charge transduction through DNA. *Nucleic Acids RES* 27, 4830-4837 (1999)
52. Boon E. M, D. M. Ceres, T. G. Drummond, M. G. Hill & J. K. Barton: Mutation detection by electrocatalysis at DNA-modified electrodes. *Nat Biotechnol* 18, 1096-1100 (2000)
53. Bhattacharya P. K. & J. K. Barton: Influence of intervening mismatches on long-range guanine oxidation in DNA duplexes. *J Am Chem Soc* 123, 8649-8656 (2001)
54. Nielsen P. E, M. Egholm, R. Berg & O. Buchardt: Sequence selective recognition of DNA by strand displacement with a thymine substituted polyamide. *Science* 254, 1497-1500 (1991)
55. Wang J: DNA biosensors based on peptide nucleic acid (PNA) recognition layers. *Biosens. Bioelectron.* 13, 757-762 (1998)
56. Ganesh K. N & P. E. Nielsen: Peptide nucleic acids: analogs and derivatives. *Curr Org Chem* 4, 931-943 (2000)
57. Holmlin R. E, P. J. Dandliker & J. K. Barton: Charge transfer through the DNA base stack. *Angew Chem Int Ed* 36, 2714- 2730 (1997)
58. Kelly S. O. & J. K. Barton: Electron transfer between bases in double helical DNA. *Science* 283, 375-381 (1999)
59. Hall D. B. & J. K. Barton: Sensitivity of DNA-mediated electron transfer to the intervening pi-stack: A probe for the integrity of the DNA base stack**.** *J Am Chem Soc* 119, 5045-5046 (1997)
60. Long Y. T, C. Z. Li, T. C. Sutherland, M. Chahma, J. S. Lee & H. B. Kraatz: A comparison of electron transfer rates of ferrocenoyl-linked DNA. *J Am Chem Soc* 125, 8724-8725 (2003)
61. Nakayama M, T. Zhara, K. Nakano & M. Maeda: DNA sensors using a ferrocene-oligonucleotide conjugates. *Talanta* 56, 857-866 (2002)
62. Liu Y. J, C. B. Rauch, R. L. Stevens, R. Lenigk, J. N. Yang, D. B. Rhine & P. Grodzinski: DNA amplification and hybridization assays in integrated plastic monolithic devices. *Anal Chem* 74, 3063-3070 (2002)
63. Trau D, T. M. H. Lee, A. I. K. Lao, R. Lenigk, I. M. Hsing, N. Y. Ip, M. C. Carles & N. J. Sucher: Genotyping on a complementary metal oxide semiconductor silicon polymerase chain reaction chip with integrated DNA microarray. *Anal Chem* 74, 3168-3173 (2002)
64. Lee T. M. H, M. C. Carles & I. M. Hsing: Microfabricated PCR-electrochemical device for simultaneous DNA amplification and detection. *Lab Chip* 3, 100-105 (2003)
65. Campbell C. N, D. Gal, N. Cristler, C. Banditrat & A. Heller: Enzyme-amplified amperometric sandwich test for RNA and DNA. *Anal Chem* 74, 158-162 (2002)
66. Zhang Y. C, H. H. Kim & A. Heller: Enzyme-amplified amperometric detection of 3000 copies of DNA in a 10-mµL droplet at 0.5 fM concentration. *Anal Chem* 75, 3267-3269 (2003)
67. Aguilar Z. P. & I. Fritsch: Immobilized enzyme-linked DNA-hybridization assay with electrochemical detection for Cryptosporidium parvum hsp70 mRNA. *Anal Chem* 75, 3890-3897 (2003)
68. Kim E, K. Kim, H. Yang, Y. T. Kim & J. Kwak: Enzyme-amplified electrochemical detection of DNA using electrocatalysis of ferrocenyl-tethered dendrimer. *Anal Chem* 75, 5665-5672 (2003)
69. Patolsky F, A. Lichtenstein & I. Willner: Detection of single-base DNA mutations by enzyme-amplified electronic transduction. *Nat Biotechnol* 19, 253-257 (2001)
70. Shipway A. N, E. Katz & I. Willner: Nanoparticle arrays on surfaces for electronic, optical, and sensor applications. *Chemphyschem* 1, 18-52 (2000)
71. Nebling E, T. Grunwald, J. Albers, P. Schafer & R. Hintsche: Electrical detection of viral DNA using ultramicroelectrode arrays. *Anal Chem* 76, 689-696 (2004)
72. Patolsky F, E. Katz, A. Bardea & I. Willner: Enzyme-linked amplified electrochemical sensing of oligonucleotide-DNA interactions by means of the precipitation of an insoluble product and using impedance spectroscopy. *Langmuir* 15, 3703-3706 (1999)
73. Patolsky F, A. Lichtenstein, M. Kotler, I. Willner: Electronic transduction of polymerase or reverse transcriptase induced replication processes on surfaces: Highly sensitive and specific detection of viral genomes. *Angew Chem Int Ed* 40, 2261-2265 (2001)
74. Saghatelian A, K.M. Guckian, D.A. Thayer & M.R. Ghadiri: DNA detection and signal amplification via an engineered allosteric enzyme. *J Am Chem Soc* 125, 344-345 (2003)
75. Steenken S. & S. V. Jovanovic: How easily oxidizable is DNA? One-electron reduction potentials of adenosine

and guanosine radicals in aqueous solution. *J Am Chem Soc* 119, 617-618 (1997)
76. Wang H. S, H. X. Ju & H. Y. Chen: Simultaneous determination of guanine and adenine in DNA using an electrochemically pretreated glassy carbon electrode. *Anal Chim Acta* 461, 243-250 (2002)
77. Wang J, A.N. Kawde & M. Musameh: Carbon-nanotube-modified glassy carbon electrodes for amplified label-free electrochemical detection of DNA hybridization. *Analyst* 128, 912-916 (2003)
78. Yang I. V. & H. H. Thorp: Modification of indium tin oxide electrodes with repeat polynucleotides: Electrochemical detection of trinucleotide repeat expansion. *Anal Chem* 72, 5316-5322 (2001)
79. Wang J. & A. N. Kawde: Pencil-based renewable biosensor for label-free electrochemical detection of DNA hybridization. *Anal Chim Acta* 431, 219-224 (2001)
80. Wang J. & A. B. Kawde: Amplified label-free electrical detection of DNA hybridization. *Analyst* 127, 383-386 (2002)
81. Jelen F, B. Yosypchuk, A. Kourilova, L. Novotny & E. Palecek: Label-free determination of picogram quantities of DNA by stripping voltammetry with solid copper amalgam or mercury electrodes in the presence of copper. *Anal Chem* 74, 4788-4793 (2002)
82. Ozkan D, A. Erdem, P. Kara, K. Kerman, B. Meric, J. Hassmann & M. Ozsoz: Allele-specific genotype detection of factor V leiden mutation from polymerase chain reaction amplicons based on label-free electrochemical genosensor. *Anal Chem* 74, 5931-5936 (2002)
83. Kerman K, D. Ozkan, P. Kara, A. Erdem, B. Meric, P.E. Nielsen & M. Ozsoz: Label-free bioelectronic detection of point mutation by using peptide nucleic acid probes. *Electroanal* 15, 667-670 (2003)
84. Li J., H. T. Ng, A. Cassell, W. Fan, H. Chen, Q. Ye, J. Koehne, J. Han & M. Meyyappan: Carbon nanotube nanoelectrode array for ultrasensitive dna detection. *Nano Lett* 3, 597-602 (2003)
85. Tomschik M, F. Jelen, L. Havran, L. Trnkova, P. E. Nielsen & E. Palecek: Reduction and oxidation of peptide nucleic acid and DNA at mercury and carbon electrodes. *J Electroanal Chem* 476, 71-80 (1999)
86. Elena E. E. & E. Dominguez: Direct electrochemical oxidation of DNA on polycrystalline gold electrodes. *Electroanal* 15, 629-634 (2003)
87. Kerman K, Y. Morita, Y. Takamura & E. Tamiya: Label-free electrochemical detection of DNA hybridization on gold electrode. *Electrochem Commun* 5, 887-891 (2003)
88. Lee T. Y. & Y. B. Shim: Direct DNA hybridization detection based on the oligonucleotide-functionalized conductive polymer. *Anal Chem* 73, 5629-5632 (2001)
89. Fritz J, E. B. Cooper, S. Gaudet, P. K. Sorger & S. R. Manalis: Electronic detection of DNA by its intrinsic molecular charge. *Proc Natl Acad Sc USA* 99, 14142-14146 (2002)
90. Thompson L. A., J. Kowalik, M. Josowicz & J. Janata: Label-Free DNA Hybridization Probe Based on a Conducting Polymer. *J Am Chem Soc* 125**,** 324-325 (2003)
91. Gooding J. J, A. Chou, F. J. Mearns, E. Wong & K. L. Jericho: The ion gating effect: using a change in flexibility to allow label free electrochemical detection of DNA hybridization. *Chem Commun* 15, 1938-1939 (2003)
92. Hahm J. I. & C. M. Lieber: Direct ultrasensitive electrical detection of DNA and DNA sequence variations using nanowire nanosensors. *Nano Lett* 4, 51-54(2004)
93. Caruso F.: Nanoengineering of Particle Surfaces. *Adv Mater* 13, 11-22 (2001)
94. Storhoff J. J., C. A. Mirkin: Programmed materials synthesis with DNA. *Chem Rev* 99, 1849-1862 (1999)
95. Willner I. & B. Willner: Functional nanoparticle architectures for sensoric, optoelectronic, and bioelectronic applications. *Pure Appl Chem* 74, 1773-1783 (2002)
96. Wang J: Nanoparticle-based electrochemical DNA detection. *Anal Chim Acta* 500, 247-257 (2003)
97. Park H. G: Nanoparticle-based detection technology for DNA analysis. *Biotechnol Bioeng* 8, 221-226 (2003)
98. Park S. J., T. A. Taton & C. A. Mirkin: Array-based electrical detection of DNA with nanoparticle probes. *Science* 295, 1503-1506 (2002)
99. Wang J, D. K. Xu, A. N. Kawde & R. Polsky: Metal nanoparticle-based electrochemical stripping potentiometric detection of DNA hybridization. *Anal Chem* 73, 5576-5581 (2001)
100. Wang J, D. K. Xu & R. Polsky: Magnetically-induced solid-state electrochemical detection of DNA hybridization. *J Am Chem Soc* 124, 4208-4209 (2002)
101. Li J, M. Xue, H. Wang, L. Cheng, L. Gao, Z. H. Lu & M. S. Chan: Amplifying the electrical hybridization signals of DNA array by multilayer assembly of Au nanoparticle probes. *Analyst* 128, 917-923 (2003)
102. Cai H, N. N. Zhu, Y. Jiang, P. G. He & Y. Z. Fang: Cu@Au alloy nanoparticle as oligonucleotides labels for electrochemical stripping detection of DNA hybridization. *Biosens Bioelectron* 18, 1311-1319 (2003)
103. Ozsoz M, A. Erdem, K. Kerman, D. Ozkan, B. Tugrul, N. Topcuoglu, H. Ekren & M. Taylan: Electrochemical genosensor based on colloidal gold nanoparticles for the detection of Factor V Leiden mutation using disposable pencil graphite electrodes. *Anal Chem* 75, 2181-2187 (2003)
104. Wang J, J. H. Li, A. J. Baca, J. B. Hu, F. M. Zhou, W. Yan & D. W. Pang: Amplified voltammetric detection of DNA hybridization via oxidation of ferrocene caps on gold nanoparticle/streptavidin conjugates. *Anal Chem* 75, 3941-3945 (2003)
105. Authier L., C. Grossiord, P. Brossier & B. Limoges: Gold nanoparticle-based quantitative electrochemical detection of amplified human cytomegalovirus DNA using disposable microband electrodes. *Anal Chem* 73, 4450-4456 (2001)
106. Lee T. M. H, L. L. Li & I. M. Hsing: Enhanced electrochemical detection of DNA hybridization based on electrode-surface modification. *Langmuir* 19, 4338-4343 (2003)
107. Kawde A. N. & J. Wang: Amplified electrical transduction of DNA hybridization based on polymeric beads loaded with multiple gold nanoparticle tags. *Electroanal* 16, 101-107 (2004)
108. Wang J, G. D. Liu & A. Merkoci: Electrochemical coding technology for simultaneous detection of multiple DNA targets. *J Am Chem Soc* 125, 3214-3215 (2003)
109. Willner I, P. Patolsky & J. Wasserman: Photoelectrochemistry with controlled DNA-cross-linked CdS nanoparticle arrays. *Angew Chem Int Ed* 40, 1861-1864 (2001)

110. Wang J, G. D. Liu & Q. Y. Zhu: Indium microrod tags for electrochemical detection of DNA hybridization. *Anal Chem* 75, 6218-6222 (2003)
111. Martin C. R. & P. Kohli: The emerging field of nanotube biotechnology. *Nat Rev Drug Discov* 2, 29-37 (2003)
112. Orazio P. D: Biosensors in clinical chemistry. *Clin Chim Acta* 334, 41-69 (2003)

Abbreviations: IDE: inhibitor-DNA-enzyme, MB: methylene blue, PCR: polymerase chain reaction, PNA: peptide nucleic acids, SNP: single nucleotide polymorphs, ssODN: single strand oligodeoxyribonucleic acid

Key Words: Biosensors, DNA, PNA, PCR, Enzyme-labeled, Nanoparticles, Electrochemistry, Analysis, Review

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