nature biotechnology

Electrochemical DNA sensors

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Electrochemistry-based sensors offer sensitivity, selectivity and low cost for the detection of selected DNA sequences or mutated genes associated with human disease. DNA-based electrochemical sensors exploit a range of different chemistries, but all take advantage of nanoscale interactions between the target in solution, the recognition layer and a solid electrode surface. Numerous approaches to electrochemical detection have been developed, including direct electrochemistry of DNA, electrochemistry at polymer-modified electrodes, electrochemistry of DNA-specific redox reporters, electrochemical amplifications with nanoparticles, and electrochemical devices based on DNA-mediated charge transport chemistry.

Over the past 15 years, many important technological advances have been made that provide us with the tools needed to develop new techniques to monitor biorecognition and interaction events on solid devices and in solution. Coupled with the ability to fabricate features on solid substrates with nanoscale precision, biosensing offers unprecedented opportunities for genetic screening and detection. 'Gene chips' featuring dense arrays of oligonucleotides have been successfully applied to problems in transcriptional profiling and single-nucleotide polymorphism (SNP) discovery, where massively parallel analysis is required[1]. However, because the fluorescence-based readout of these chips involves not only highly precise and expensive instrumentation but also sophisticated numerical algorithms to interpret the data, these methods have been generally limited to use in research laboratories.

Recently, an impressive number of inventive designs for DNA-based electrochemical sensing have appeared. These types of sensors combine nucleic acid layers with electrochemical transducers to produce a biosensor and promise to provide a simple, accurate and inexpensive platform for patient diagnosis. In this review, we include illustrative examples and compare and contrast these designs (see **Table 1**). We also discuss how these technologies might be implemented to produce sensitive multiplexed assays for clinical diagnostics of genetic and infectious disease. Several recent and comprehensive reviews have also been published[2–4]. Given the pace of advances in this field, the development of clinical and even point-of-care DNA diagnostics based on these technologies seems a realistic goal.

Principles of biosensor function

All molecular-based biosensors rely on highly specific recognition events to detect their target analytes. The essential role of the sensor is to provide a suitable platform that facilitates formation of the probe-target complex in such a way that the binding event triggers a usable signal for electronic readout. The minimal elements of any biosensor include a molecular recognition layer and a signal transducer that can be coupled to an appropriate readout device. DNA is especially well suited for biosensing applications, because the base-pairing interactions between complementary sequences are both specific and robust.

In a typical configuration, a single-stranded probe sequence is immobilized within the recognition layer, where base-pairing interactions recruit the target DNA to the surface (**Fig. 1**). The repetitive, essentially uniform structure of DNA makes its assembly on the recognition surface well defined. It is at this interface that the critical dynamics of target capture take place to generate the recognition signal; therefore, immobilizing nucleic acid probe sequences in a predictable manner while maintaining their inherent affinity for target DNA is crucial to overall device performance. How this recognition event is reported depends ultimately on the method of signal transduction, whether it be optical, mechanical or electrochemical.

Optical readout. The use of photolabile protection schemes, coupled with photochemical screening techniques and combinatorial phosphoramidite chemistry, allows the fabrication of 'gene chips' for massively parallel detection of target DNA sequences[5]. Optical biosensors based on fluorescence are extraordinarily sensitive, with detection limits approaching ~10^7 molecules/cm^2, and arrays containing thousands of unique probe sequences have been constructed[6]. Because the instrumentation required is sophisticated and expensive, gene-chip technology is best suited for laboratory applications. Cases in which large numbers of genes or sequences need to be simultaneously sampled, as in transcriptional profiling or SNP discovery, are well suited to gene-chip analysis[7–10]. Clinical diagnostics do not generally require this massive data accumulation. What is required for the molecular diagnostic, however, is reliability and generality irrespective of sequence. Besides the cost and sophistication of the instrumentation, the inconsistent yields of target synthesis and labeling, as well as nonuniform rates of fluorophore photobleaching can result in readout accuracies lower than what is required for patient diagnosis[11].

Surface plasmon resonance (SPR), another optical technique, reports changes in the refractive index of a thin metal film substrate that occur upon adsorption of the analyte and is suitable for target detection in an array-based format[12]. To achieve detection limits sufficient for a diagnostically useful signal, it is usually necessary to amplify the hybridization signal by increasing the amount of material deposited at the metal film surface, either before or after target

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Published online 30 September 2003; doi:10.1038/nbt873

Table 1 Comparison of platforms for DNA electrochemical sensing

Type of sensor	Advantages	Disadvantages	References
Direct DNA electrochemistry	Highly sensitive (femtomoles of target); requires no labeling step; amenable to a range of electrodes	High background signals; cannot be multiplexed; destroys the sample	19–25
Indirect DNA electrochemistry	Highly sensitive (attomoles of target); usually requires no labeling step; multiple-target detection at same electrode	Probe substrate can be difficult to prepare; destroys the sample	26–28
DNA-specific redox indicator detection	Moderate to high sensitivity (femtomoles of target); well suited to multiple-target detection; samples remain unaltered	Chemical labeling step required unless 'sandwich' method used; sequence variations can be problematic	29–40
Nanoparticle-based electrochemistry amplification	Extremely sensitive (femtomole to zeptomole range, 10^{-15} to 10^{-21} moles); well suited to multiple-target detection with different nanoparticles	Many development steps in assay; reliability and robustness of surface structures problematic; sample usually destroyed	53–57
DNA-mediated charge transport	Highly sensitive (femtomole range) and simple assay; requires no labeling; uniquely well suited for mismatch detection; sequence independent; amenable to multiplexing; applicable to DNA-protein sensing step	Biochemical preparation of target sample required	43–47

capture. Moreover, as with fluorescence-based techniques, SPR systems can be complex and costly (although the cost is decreasing), making them generally more suitable for research applications.

Perhaps the most straightforward optical readout technology involves single-stranded DNA labeled with gold nanoparticles that simply change color upon hybridization of the target sequences[13]. Using photographic silver developing solutions, a 'scanometric' small-array platform for DNA analysis at a flatbed scanner has been demonstrated using this technology with 100-pM sensitivity[14]. This technology, offering simplicity and sensitivity, could provide a useful approach for clinical diagnostic development with optical readout.

Mass readout. An alternative readout strategy is to monitor mass changes in the immobilized recognition layer that occur upon target binding, most frequently using a quartz crystal microbalance (QCM)[15]. These devices are sensitive and can provide real-time monitoring of hybridization events. Although reliable operation of the QCM in aqueous solution has been a technical challenge, new amplification strategies may overcome this limitation[16].

Changes in mass can also be measured using microfabricated cantilevers[17]. Here, the increase in mass that accompanies hybridization is detected by the deflection of a laser beam reflected from the cantilever surface. This technique is well suited for linear array development and provides continuous internal correction for nonspecific interactions and thermal drift. The primary limitations of microcantilever techniques again are the associated expensive instrumentation and the technical difficulties involved with fabricating the cantilever features.

Electrochemical readout. Electrochemical methods are well suited for DNA diagnostics. Because electrochemical reactions give an electronic signal directly, there is no need for expensive signal transduction equipment. Moreover, because immobilized probe sequences can be readily confined to a variety of electrode substrates, detection can be accomplished with an inexpensive electrochemical analyzer. Indeed, portable systems for clinical testing and on-site environmental monitoring are now being developed[18]. Sensitive electrochemical signaling strategies based on the direct or catalyzed oxidation of DNA bases, as well as the redox reactions of reporter molecules or enzymes recruited to the electrode surface by specific DNA probe-

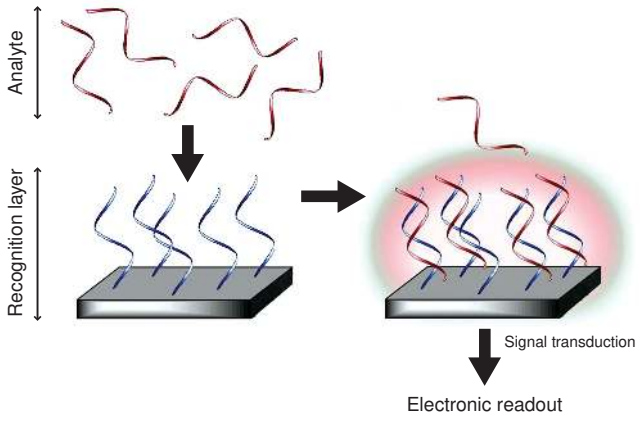

Figure 1 General DNA biosensor design. Target DNA is captured at the recognition layer, and the resulting hybridization signal is transduced into a usable electronic signal for display and analysis. In the case of electronic and electrochemical biosensors, signal transduction is greatly simplified, because the incoming signal is already electronic in origin.

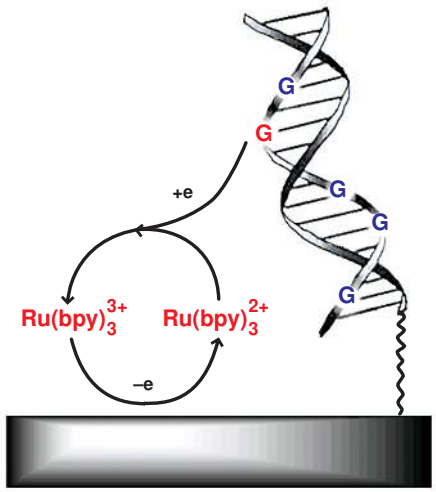

Figure 2 Schematic representation of guanine oxidation mediated by a ruthenium complex in solution. The electrode is held at a potential that oxidizes the reduced metal complexes, which then come into contact with DNA. Guanine residues in DNA can reduce the metal complex, regenerating the reduced mediator. The enhanced signal thus reflects the amount of guanine available for oxidation.

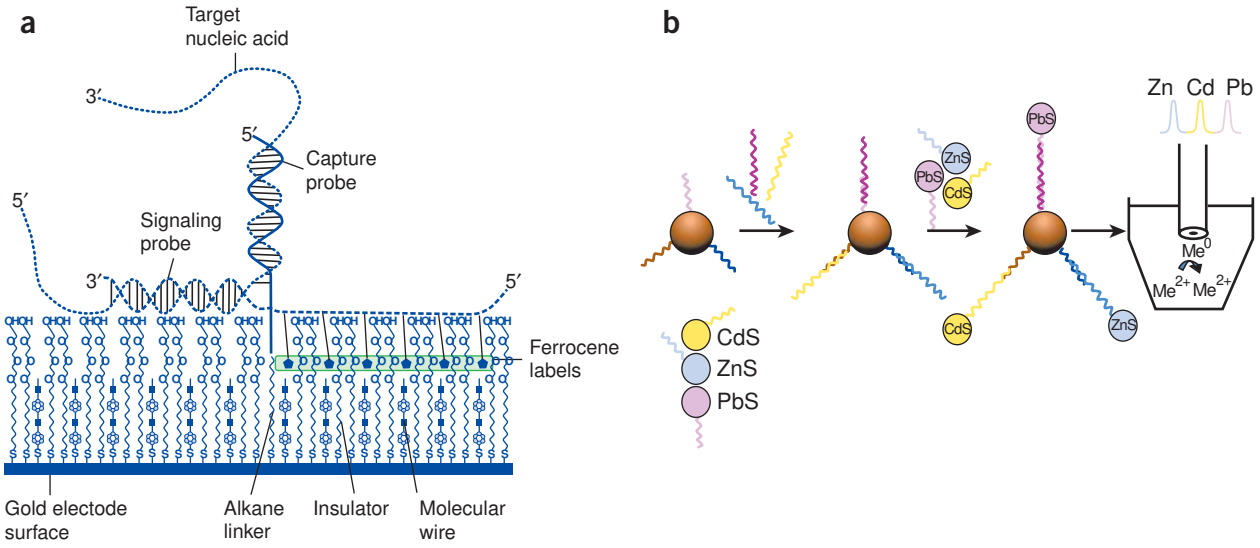

Figure 3 Electrochemical 'sandwich' assays. (**a**) The gold electrode is functionalized with the DNA probe sequence, the oligophenylethynyl molecular wires, and the polyethylene glycol insulator molecules. The target sequence, once captured on the electrode, hybridizes also to a second reporter sequence, labeled with ferrocene. Thus, the target is not labeled but is instead 'sandwiched' by the capture probe and signal probe. The molecular wires provide a pathway for electron transfer between the ferrocene label and the electrode, and the insulators block the access of redox species in solution to the electrode. Interfacial electron transfer from ferrocene to the gold electrode is detected as a Faradaic current. Adapted from ref. 31. (**b**) Multitarget sandwich hybridization scheme based on different inorganic nanoparticle tags for DNA detection. Probe-modified magnetic beads are hybridized with target DNA strands. A second hybridization with the nanoparticle-labeled indicator strands confers the ability to identify multiple targets upon dissolution of the beads and electrochemical analysis. Adapted from ref. 34.

target interactions and by charge transport reactions mediated by the π-stacked base pairs have all been demonstrated.

Direct electrochemistry of DNA as a detection platform

The earliest electrochemical DNA sensing strategy was based on reduction and oxidation of DNA at a mercury electrode; simply, the amount of DNA reduced or oxidized would reflect the amount of DNA captured. More than 40 years ago, Palecek[19] and co-workers developed methods to discriminate single- versus double-stranded DNA through direct DNA reduction. More recently, DNA oxidation has been carried out through adsorption stripping voltammetry (ASV)[20]. This technique achieves its high sensitivity by inducing an electrostatic buildup of analyte at the electrode surface before the detection step. The purine bases of DNA can be oxidized electrochemically, and this process can be carried out using carbon, gold, indium tin oxide (ITO) and polymer-coated electrodes[21].

Although this methodology is inherently quite sensitive, its application is complicated by significant background currents at the relatively high potentials required for direct DNA oxidation. Numerical methods to improve the signal-to-noise ratio have been developed, but more recent designs employ physical separation techniques to remove the sources of background interference. For example, Palecek and colleagues[22] and Wang and Kawde[23] have separately reported a two-step strategy for capturing target sequences using probe DNA immobilized onto magnetic beads. After target hybridization, the beads are magnetically separated from the pool of analytes. The collected DNA is depurinated in acidic solution, and the free guanine and adenine nucleosides are collected and analyzed using ASV. As few as 40 femtomoles (~2×10^{10} molecules) of substrate have been detected by this assay.

A similar technique using the direct guanine oxidation signal at carbon paste electrodes has recently been reported, in which specific genotypes of the Factor V Leiden mutation in PCR amplicons were identified[24]. The use of peptide nucleic acid probes[25] affords more stringent control over hybridization, and recent studies have shown that point mutations in target DNA can be more readily discerned using this methodology.

Indirect electrochemistry of DNA as a detection platform

Methods to oxidize target DNA indirectly through the use of electrochemical mediators have also been explored (**Fig. 2**). An especially attractive approach uses polypyridyl complexes of Ru(II) and Os(II) to mediate the electrochemical oxidation of guanine. For example, Yang and Thorp[26] have used this method to detect trinucleotide-repeat expansions, in which catalytic currents due to the oxidation of guanine residues immobilized within the target sequence show a linear dependence on the repeat number. This same technique has been coupled to a reverse transcription–PCR assay to monitor the overexpression of genes in tumor samples[27]. Experiments with model PCR products have shown that the sensitivity of this system extends down to 550 attomoles of target DNA (~3×10^8 molecules).

A noteworthy coding method has also been reported using this approach[28]. In this strategy, chemically modified bases are incorporated into PCR products, and the resulting DNA is detected at an ITO electrode by catalytic oxidation of the modified base. Though remarkably sensitive, it is not clear whether this technique is well suited to clinical diagnostics. The ITO electrode material may or may not be amenable to microfabrication in a multiplexed configuration. Nonetheless, the methodology does provide high sensitivity without complex instrumentation through redox-mediated DNA oxidation.

DNA-specific redox indicator detection platforms

By analogy to fluorescence-based methods, several strategies have been pursued in which target DNA sequences are labeled with redox-active

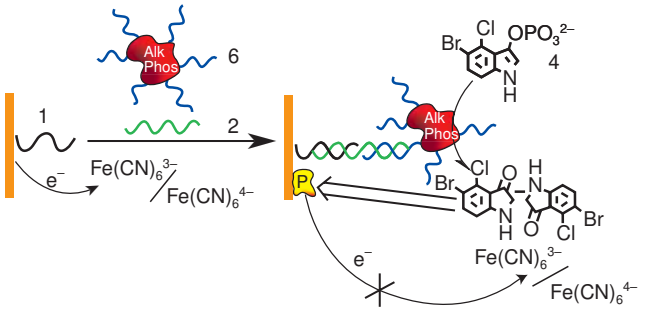

Figure 4 Scheme for an enzymatic amplification of DNA hybridization signal. DNA probes are immobilized onto the electrode surface. Target DNA is pretreated with enzyme-modified probe, which binds the 5′ end of the target. The target-enzyme complex is hybridized to the probe, and the biocatalyzed precipitation of product dimer provides amplification of the hybridization signal, which is detected as an increase in electron transfer resistance between the electrode and ferricyanide in solution. Adapted from ref. 45.

reporter molecules. Appearance of the characteristic electrochemical response of the redox reporter therefore signals the hybridization event[29,30]. Using physical separation methods to isolate the labeled sequences, detection limits on the order of ~10^{10} molecules have been reported.

A variation on this approach involves a three-component 'sandwich' assay, in which the redox label has been attached to a synthetic sequence specifically designed to bind an overhang portion of the probe-target complex[31]. This dual-hybridization approach eliminates the need to modify the target strand, whose function is to bring together the probe and labeled sequences. In one example, ferrocene-labeled reporter strands signal the presence of target DNA hybridized to thiolated probe sequences immobilized onto gold electrodes (**Fig. 3a**). Incorporation of a second redox reporter (dimethylcarbamyl ferrocene), with an oxidation potential 170 mV more positive than the ferrocene analog, allows the detection of two targets simultaneously and without spatial separation, much like a multicolor fluorescence labeling assay[32]. Using AC voltammetry, the detection of 50 nM target concentrations and the identification of a GT single-base mismatch was accomplished.

Colloidal gold nanoparticles have also been used to signal hybridization in a sandwich-based assay. In one study, the labeled target is captured by probe strands immobilized on a pencil graphite electrode, and hybridization is detected electrochemically with the appearance of a characteristic gold-oxidation signal[33]. The signal is greatly enhanced because of the large electrode surface area and the availability of many oxidizable gold atoms in each nanoparticle label. Indeed, the detection limit for PCR amplicons was found to be as low as 0.8 femtomoles of DNA (~5×10^8 molecules).

Employing nanoparticle labels with different redox potentials, Wang *et al.*[34] have developed a technique in which these nanoparticles encode DNA sequences (**Fig. 3b**). Probe-modified magnetic beads are hybridized with target DNA, separated magnetically from the pool of analytes and hybridized again with the nanoparticle-labeled reporter strands. The products are isolated, and the nanoparticles are dissolved and analyzed by ASV. Electrochemical detection of three targets simultaneously was demonstrated with CdS, ZnS and PbS nanoparticle tags.

In yet another innovative application of this approach, Willner and Willner[35] have developed nanoparticle architectures of CdS particles and DNA to provide photoelectrochemical detection of DNA hybridization. Immobilized probe DNA is hybridized to target DNA, which is subsequently hybridized with a CdS-labeled reporter strand. This CdS-DNA aggregate can be further elaborated by repetitive treatment of oligonucleotide-modified nanoparticles and complementary bridging DNA strands to produce larger assemblies of the nanoparticle labels. Exposure of this aggregate to visible blue light triggers a photoelectrochemical current between the CdS nanoparticle aggregate and the gold electrode.

Many applications of DNA sensing (for example, real-time pathogen detection) involve extremely small numbers of target analytes, with correspondingly few hybridization events. Analyte amplification has also been accomplished in an innovative way indirectly using electrochemical detection of nanoparticles (**Box 1**).

In an effort to improve transduction of the hybridization signal, an electrode surface may also be modified with a polymer layer that confers desirable properties, such as electrical conductivity, amenability to probe immobilization or protection of the electrode from nonspecific analyte adsorption. Heller and colleagues[36] have reported an enzyme-amplified DNA sensing technique involving the electropolymerization of a polycationic redox polymer, upon which amine-terminated oligonucleotide single strands are electrodeposited by ligand exchange with bipyridyl osmium complexes impregnated within the polymer gel. Target DNA is captured at the electrode surface and subsequently hybridized to a reporter strand modified with HRP. This technique has been extended to screen-printed carbon electrodes on polyester sheets, which are inexpensive and potentially amenable to mass manufacture[37].

Biocatalyzed production of insoluble products has been used by Willner and colleagues[38] to sense DNA hybridization electrochemically at probe-modified electrodes. Target DNA is captured at a probe-modified gold electrode, where a redox-active DNA intercalator electrocatalytically generates peroxide, which, in turn, is oxidized by

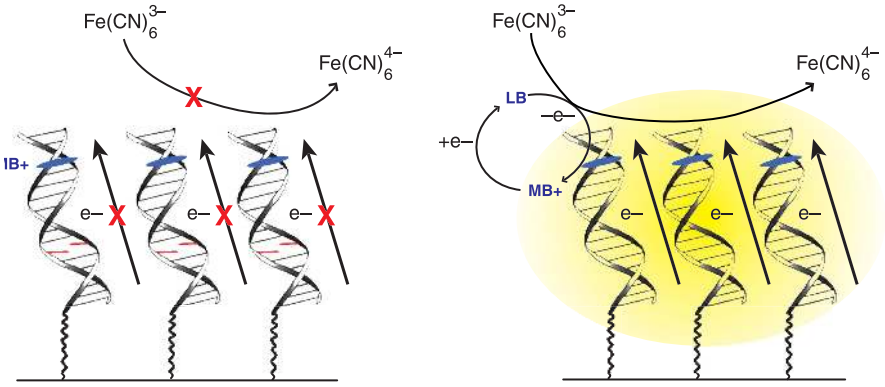

Figure 5 Electrochemical assay for mismatches through DNA-mediated charge transport. On the right is shown an electrode modified with well-matched duplex DNA. Current flows through the well-stacked DNA to reduce methylene blue (MB^+) intercalated near the top of the film, to leucomethylene blue (LB). LB goes on to reduce ferricyanide in solution, thereby regenerating MB^+ catalytically, leading to an amplification of the hybridization signal. In the case of a DNA film containing mismatched duplexes (left), current flow through the DNA duplex is attenuated, MB^+ is not reduced, and the catalytic signal is lost.

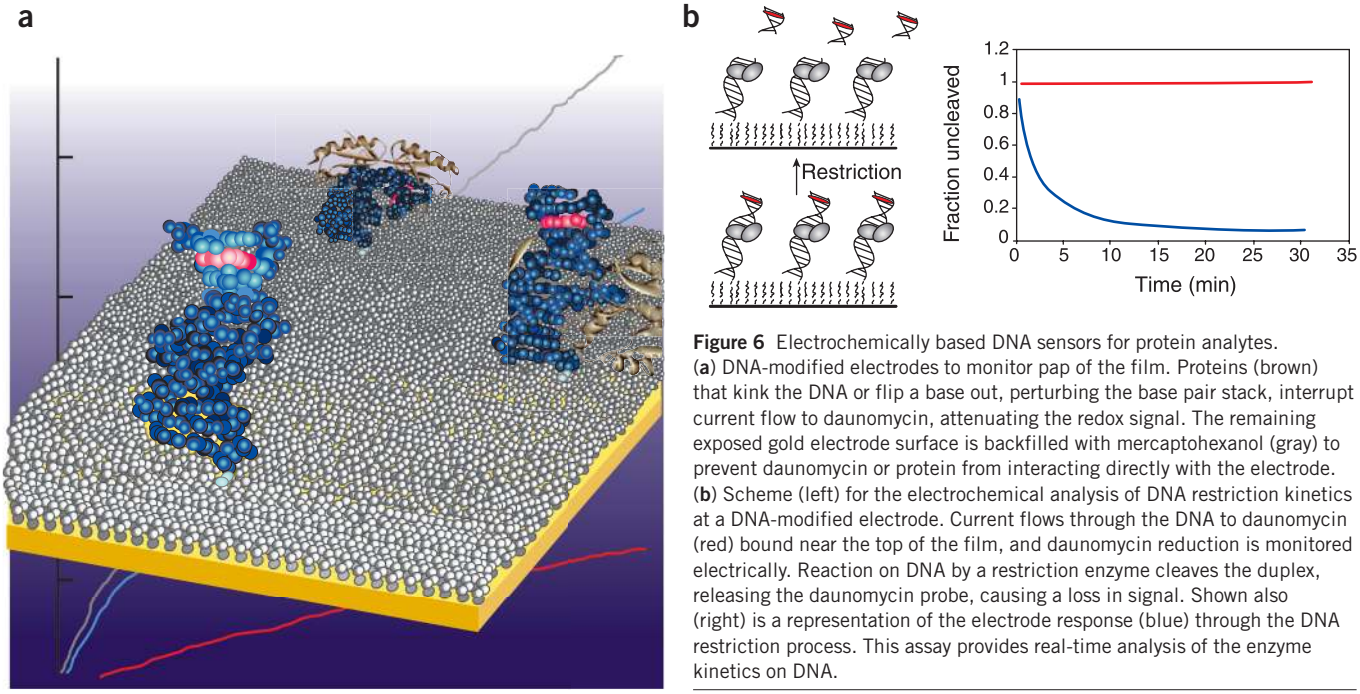

Figure 6 Electrochemically based DNA sensors for protein analytes. (**a**) DNA-modified electrodes to monitor pap of the film. Proteins (brown) that kink the DNA or flip a base out, perturbing the base pair stack, interrupt current flow to daunomycin, attenuating the redox signal. The remaining exposed gold electrode surface is backfilled with mercaptohexanol (gray) to prevent daunomycin or protein from interacting directly with the electrode. (**b**) Scheme (left) for the electrochemical analysis of DNA restriction kinetics at a DNA-modified electrode. Current flows through the DNA to daunomycin (red) bound near the top of the film, and daunomycin reduction is monitored electrically. Reaction on DNA by a restriction enzyme cleaves the duplex, releasing the daunomycin probe, causing a loss in signal. Shown also (right) is a representation of the electrode response (blue) through the DNA restriction process. This assay provides real-time analysis of the enzyme kinetics on DNA.

horseradish peroxidase (HRP). The product precipitates onto the electrode, blocking the reaction of ferricyanide and providing the basis for DNA detection. In a variation on this approach, 5-bromo-4-chloro-3-indoyl phosphate is enzymatically converted to an insoluble indigo product, blocking ferricyanide; using this method, the detection limit for target DNA was extended down to 5×10^{-14} M (**Fig. 4**)[39]. An enzyme-based hybridization assay for detection of two DNA targets has also been described in which alkaline phosphatase and β-galactosidase are used to differentiate between two DNA targets by measuring the chronopotentiometry of their electroactive products[40]. These products yield well-resolved oxidation signals at carbon electrodes to allow amplified dual-target electrochemical detection.

DNA-mediated charge transport electrochemistry

In an alternative approach to chemical labeling schemes, redox-active reporter molecules that intrinsically associate with the double helix noncovalently have been successfully used for electrochemically based DNA analysis. In these analyses, rather than serving as a reactant, the DNA is the *mediator*. These assays can provide high sensitivity and simplicity.

Electrostatic probe molecules. The notion that electrochemical reactions could be used to signal DNA hybridization was first explored by Milan and Mikkelsen[41]. Their assay featured a single-stranded probe sequence adsorbed onto glassy carbon, wherein hybridization of target DNA caused an increase in the surface concentration of electrostatically bound $Co(phen)_3^{3+}$ as a result of the higher negative charge density at the hybridized surface. Characteristic redox reactions of the cobalt probe provided the electrochemical readout. More recently, Steel *et al.*[42] reported the effective use of $Ru(NH_3)_6^{3+}$ probe molecules to signal DNA hybridization at gold films modified with thiol-bearing DNA probe sequences. As the DNA is captured to form double-stranded product, proportionately more ruthenium hexammine binds, yielding a higher signal. It is noteworthy how simple and quantitative this redox reporter is, providing a reliable and robust assay for the amount of DNA present on the electrode surface.

Intercalative probe molecules. Our work in this field has taken advantage of the electronic structure of double-helical DNA, using intercalated redox probe molecules to report on perturbations in base stacking[43]. The intercalator is thus not used in this assay to report the amount of DNA or whether it is double stranded versus single stranded. Instead, the DNA base pair stack mediates charge transport to the intercalator bound at the top of the film. If the base pair stack is intact, current can flow. Therefore, we take advantage of a different inherent characteristic of duplex DNA, its ability to mediate charge transport. We have found this chemistry to be exquisitely sensitive to DNA structure and perturbations in structure. Assays of DNA-mediated electrochemistry are therefore uniquely suited to sense changes in DNA: damage, mistakes, mismatches and even protein binding.

In a typical assay, thiolated duplexes are self-assembled into densely packed films on gold surfaces, then treated with micromolar concentrations of a redox-active intercalator[44]. Upon intercalation, the reporter molecule is electrochemically reduced by DNA-mediated charge transport. Importantly, the reaction occurs only if the individual duplexes in the film contain well-stacked base pairs; the presence of just a single intervening mismatch is sufficient to shut off the reaction completely[45]. Because of its remarkable specificity to the electronic structure of the π-stack, this assay is especially well suited for mutational analysis[46]. The detection of mismatches does not depend on the thermodynamic destabilization at a mismatch site; rather, it depends on the change in base stacking that alters current flow. The need for stringent hybridization control to distinguish between matched and mismatched sequences near their melting temperature is therefore completely eliminated. The assay is carried out using DNA films wherein the DNA, matched or mismatched, is in the duplex form, irrespective of melting temperature or sequence.

To increase the inherent sensitivity of the assay, we have developed a coulometric readout strategy based on the electrocatalytic reduction of ferricyanide by methylene blue[47]. In the electrocatalytic process, electrons flow from the electrode surface to intercalated methylene blue in a DNA-mediated reaction. The reduced form of methylene

Box 1 Amplification strategies with nanoparticles

The deposition of silver metal onto gold nanoparticles exemplifies the ability of electrochemical methods to amplify the electrical signal. In a typical approach, a sandwich assay is carried out to recruit gold nanoparticles to a magnetic bead by means of the target DNA. Once mechanically separated, the chimeric duplexes are treated with a silver developing solution (silver ions and hydroquinone) to deposit silver metal onto the gold labels[53,54]. An acid treatment to redissolve the silver and subsequent ASV of the adsorbed metal ions extends the detection limits down to 1.5 femtomole, or ~9×10^8 molecules, in a 50-μl hybridization solution[55].

Mirkin and colleagues[56] have exploited the silver deposition technique to construct a sensor based on conductivity measurements (**Fig. 7**). In their approach, a small array of microelectrodes with gaps (20 μm) between the electrode leads is constructed, and probe sequences are immobilized on the substrate between the gaps. Using a three-component sandwich approach, hybridized target DNA is used to recruit gold nanoparticle–tagged reporter strands between the electrode leads. The nanoparticle labels are then developed in the silver enhancer solution, leading to the precipitation of silver metal onto the gold nanoparticles. The deposition of silver closes the electrical connection between the two flanking microelectrodes, and target capture is signaled by a sharp drop in the resistance of the circuit. This technique demonstrated a sensitivity of ~5×10^{-13} M in target DNA. An unusual dependence of hybridization stringency based on salt concentration, probably involving the cooperative interactions among targets on the nanoparticle, allows excellent discrimination of single-base mutations within the target sequences.

Another improvement in sensitivity has been reported for a sandwich assay in which the gold nanoparticles are replaced with polystyrene microsphere tags impregnated with ferrocenecarboxaldehyde (FCA)[57]. Because each microsphere contains on the order of 10^{11} FCA molecules, capture and subsequent dissolution of the polystyrene shell in acetonitrile to liberate the FCA for electrochemical quantification results in an enormous amplification of the hybridization event. This technique has a demonstrated detection limit of 5×10^{-21} moles (or ~30,000 molecules), the record thus far.

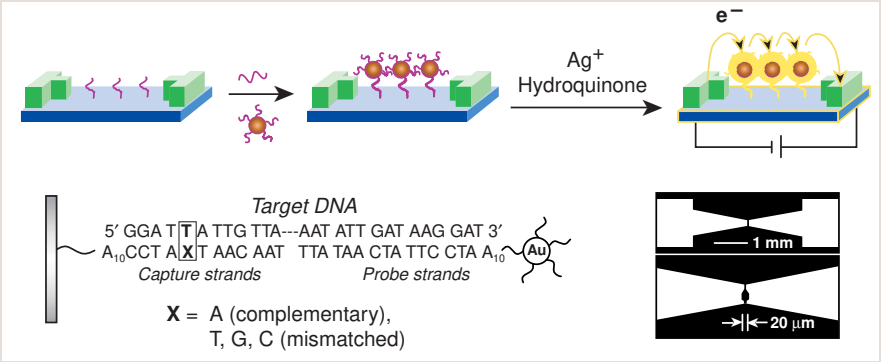

Figure 7 High-sensitivity conductivity assay. Probe DNA immobilized in a small gap between two electrodes is hybridized to a portion of the unmodified target DNA. Gold nanoparticle–labeled probes are then hybridized to the unbound portion of the target, leading to the accumulation of gold in the gap. Silver metal is precipitated onto the gold nanoparticles, improving the sensitivity of the assay by lowering the resistance across the electrode gap. If the target DNA is not present, no gold nanoparticles are captured, silver is not deposited across the gap, and the circuit resistance remains high. This strategy has been extended to produce an array of electrode pairs with a different oligonucleotide capture strand in each electrode gap. Adapted from ref. 42.

blue then reduces ferricyanide in solution, so that additional electrons can flow to methylene blue and the DNA base stack is repeatedly interrogated. If the DNA contains a mismatch, the bound methylene blue is not catalytically active and the electrochemical signal is greatly diminished. Using this assay (**Fig. 5**), all of the possible single-base mismatches (including the thermodynamically stable GA mismatch) have been readily detected[46]. The reaction has also been carried out on an addressable 18-electrode array featuring microelectrode gold pads. At a 30-μm electrode, as few as ~10^8 duplexes have been detected. Thus DNA-mediated charge transport provides specificity in mutation detection, sensitivity through electrocatalysis, facile access to an array format, and a wholly different approach to DNA sensing electrically.

Detecting proteins and small molecules bound to DNA

Given all these innovative strategies for sensing DNA electrochemically, can a DNA-based sensor be used to assay molecules that bind to DNA or react with it? DNA, because of its general structural uniformity, is reliably assembled on an electrode surface, essentially independently of sequence or length. The same cannot be said about other analytes of interest, with proteins the most obvious example. Thus, different laboratories are beginning also to take advantage of the ease of DNA sensor design in developing assays for proteins and small molecules that bind to DNA.

One illustration, recently described, is an electrochemical DNA-based sensor for the detection of arsenic trioxide[48]. In this example, the voltammetric signal, reflecting direct guanine oxidation, decreases with exposure time and concentration of As_2O_3, presumably as a result of a reaction between guanine and arsenic trioxide that damages purine bases. Although other contaminants can also interfere through their preferential reaction with DNA, it is the detection of molecules that bind irreversibly to DNA in the environment that is of interest. Thus, this strategy may provide the basis for a range of sensitive environmental sensors. Currently, using PCR for biochemical amplification, coupled with a redox-label probe assay, a portable sensor to analyze for anthrax is being developed[49].

We have taken advantage of DNA-mediated charge transport chemistry to assay for protein binding and reaction electrically[50]. In this study, duplex DNA containing a protein binding site is covalently modified with daunomycin, an electroactive intercalator, and assembled in a loosely packed monolayer onto a gold surface. Mercaptohexanol to backfill the monolayer prevents direct interaction between the daunomycin probe and the electrode surface. Upon exposure of the DNA-modified electrode to a solution of M.*Hha*I, a base-flipping enzyme, the

daunomycin redox signal is greatly attenuated, indicating that protein binding disrupts the integrity of the base stack because of base flipping. When a K237W mutant *Hha*I enzyme is used instead, a tryptophan residue is inserted into the vacant site after base flipping, restoring both the π-stack and the daunomycin signal. When the electrochemical assay is used to detect TATA-binding protein (TBP), a transcriptional activator that kinks DNA, TBP binding is associated with substantial diminution in current flow through the DNA electrode. Thus, the DNA surface provides a sensitive way to assay for a variety of proteins (**Fig. 6a**). An extension of this assay could be developed to screen for small-molecule inhibitors of transcription factor binding.

In the same study, we also presented a real-time electrochemical analysis of the kinetics of an enzyme, R.*Pvu*II, restricting DNA; the signal from the chronocoulometry of daunomycin is diminished as the enzyme cleaves away the portion of DNA containing the redox label (**Fig. 6b**). Comparison of the electrochemical data to an analogous gel electrophoresis experiment shows good agreement between the measured kinetics of DNA restriction. The assay, unlike gel electrophoresis, however, provides a basis for real-time monitoring of protein reactions on DNA as well as a foundation for developing sensitive electrical screening assays in an array format for small molecules that interfere with protein binding or reaction.

Conclusions and perspectives

Despite the enormous opportunities clearly offered by electrochemical DNA sensing, some important hurdles remain. The first depends upon the electrode probes themselves and their fabrication into useful arrays. Array sizes on the order of 10 have thus far been demonstrated, but more typically arrays of 50–100 sequences will be needed for clinical application. For example, genetic screening for cystic fibrosis carriers requires testing for 25 different mutations plus positive and negative controls[51]. Although it is not difficult to fashion electrode pads with reproducible dimensions of a micron or less, the electrochemical readout requires mechanical connections to each individual electrode. The construction of very large, multiplexed arrays (on the order of 10^3) therefore presents a major engineering challenge. Electronic switches in the form of an on-chip electronic multiplexer may provide a possible solution for this problem.

Another challenge that requires attention involves the biological complexity of a genomic DNA sample. Thus far, even with the high level of sensitivity one can achieve through electrochemical amplification, assays routinely start with a round of PCR or other biochemical amplification. Without this biochemical preamplification, low copy numbers present particular challenges for interfacial hybridization and lead to slow associated kinetics. Moreover, there is inherent complexity associated with the biological sample itself, before DNA isolation and purification. The real goal, for pathogen detection specifically, is an assay involving rapid DNA isolation with the detection of few copies in half an hour or less. With electrochemical sensors this goal is realizable, but we are not yet there.

The application of sensors to assaying of the proteome is a problem of even greater complexity. There is a tremendous need for high-throughput protein screening to catalog the human proteome in its biologically active form. Can the techniques and methodologies developed for DNA-based electrodes be extended to proteins? The irregular shapes, sizes, variety of post-translational modifications and general functional characteristics of proteins add many more dimensions of complexity. The wiring up of proteins to electrode surfaces for diagnostic and even screening applications is in its infancy[52], but the lessons being learned through DNA electrodes, if not reactions mediated by DNA itself, should provide useful starting points.

Converting genomic information to clinical advantage can be successfully accomplished with DNA-based sensors. Their low cost, small size and inherent sensitivity will certainly provide important new tools for the diagnosis of disease.

ACKNOWLEDGMENTS

We are grateful to the National Institutes of Health for their financial support of our research.

Published online at http://www.nature.com/naturebiotechnology/

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