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Gene sensors: Detection of specific targeted sequences on DNA

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Electrochemical interrogation of conformational changes as a reagentless method for the sequence-specific detection of DNA

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Abstract

We report a strategy for the reagentless transduction of DNA hybridization into a readily detectable electrochemical signal via a conformational change analogous to the molecular beacon optical approach. The strategy involves an electroactive, ferrocene-tagged DNA stem-loop structure that self-assembles onto a gold electrode via facile gold-thiol chemistry. Hybridization induces a large conformational change in this surface-confined DNA structure, which in turn significantly alters the electron-transfer tunneling distance between the electrode and the redoxable label. The resulting change in electron transfer efficiency is readily measured via cyclic voltammetry at target DNA concentrations as low as 10 pM. In contrast to existing optical approaches, an electrochemical DNA (E-DNA) sensor built upon this strategy can detect fmol of target DNA without employing cumbersome and expensive optics, light sources, or photo-detectors. In contrast to previously reported electrochemical approaches the E-DNA sensor achieves this impressive sensitivity without the use of exogenous reagents or sacrificing selectivity or reusability. The E-DNA sensor thus offers the promise of light-weight, reusable, pM detection of DNA.

Introduction

The detection of DNA hybridization is of significant scientific and technological importance, as manifested in, for example, the growing interest in chip-based characterization of gene expression patterns and the detection of pathogens in both clinical and civil defense settings (1). Consequently, a variety of optical (2-4), acoustic (5, 6) and electrochemical (7, 8) "gene detection" approaches have been reported. Among these, a solution-phase optical approached termed "molecular beacons" has attracted significant interest (9) because of its applicability to uses ranging from *in vitro* genotyping (10) to *in vivo* studies within single cells (11). Molecular beacons are comprised of a hairpin-like DNA stem-loop structure, with a fluorescent moiety and a fluorescence quencher attached to either terminus. In the absence of target the molecular beacon is in the folded configuration in which its termini are held in close proximity and fluorescence emission is thus suppressed. Upon hybridization with a complementary target sequence, the stem-loop is converted into a rigid, linear double helix, removing the fluorophore from proximity to the quencher and greatly enhancing emission. Previous studies demonstrate that molecular beacons can discriminate even single-base mismatches (12). More recently, a reagentless solid-state version of the optical molecular beacon has been described that may prove suitable for chip-based optical detection (13, 14).

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While optical detection methods have historically dominated state-of-the-art real-time or near real-time genosensors (1, 15, 16), the application of electrochemical methods to the sensing of biologically related species may provide very significant advantages (17-19). Specifically, the advantages of bioelectronic approaches include: 1) the speed,

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sensitivity and low cost/mass/power requirements of electrochemical detection (20); 2) the relatively high stability and environmental insensitivity of electroactive labels; and 3) the availability of electroactive labels with non-overlapping redox potentials for "multi-color" labeling and the simultaneous detection of multiple analytes (21).

DNA bases are electrochemically silent at moderate applied voltages. Thorp et al reported a novel approach to electro-catalytically oxidize guanine via inorganic metal complexes (22). However typical electrochemical detection of hybridization typically requires the use of exogenous reporter groups (23). The first sequence-selective electrochemical method for DNA detection was based on the electrochemical interrogation of exogenous, redox-active intercalators that bind preferably to double-stranded DNA (24-26). Barton and coworkers have improved the sensitivity of this approach by employing exogenous electrocatalytic species for amplification (27). They also report that the current flow through the double helix is sensitive even to single-base mismatches, paving the way for the direct electrochemical identification of point mutations (8). In an attempt to reduce high backgrounds arising from the inappropriate binding of hybridization indicators to unhybridized DNA, "sandwich" type detectors have also been developed and are commercialized (28, 29). Sandwich approaches employ a surface-confined, electrochemically silent probe sequence to bring target DNA strands to the electrode surface. A signal is generated when this bound target strand is hybridized with an exogenous, redox-labeled signaling sequence. Lastly, Mirkin and co-workers have developed an electronic DNA detection approach with high sensitivity and selectivity (7). In this resistance-based method, the probe-captured target undergoes a second hybridization with exogenous, Au nanoparticle-labeled DNA strands. Subsequent

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catalytic deposition of exogenously added silver onto the Au nanoparticles produces electrical contact between a closely spaced electrode pair.

Despite these advances, there has been relatively little progress toward the important goal of creating electrochemical DNA detection methods that are simultaneously sensitive, selective and *reagentless* (30); all of the above described electrochemical hybridization detectors require post-hybridization treatment with either hybridization indicators or other exogenous signaling molecules. Here we report the development of a sensitive, reagentless, reusable electrochemical DNA sensor that combines the significant advantages of electrochemical detection with the versatility of reagentless, reusable, surface-attached molecular beacons. This "E-DNA" sensor employs an electrode-attached, molecular beacon-like DNA stem-loop labeled with an electroactive reporter as the hybridization sensing element (Scheme 1). Upon hybridization, the distance between the label and the electrode is significantly altered, leading to a large, readily measurable signal change. The E-DNA sensor thus provides a ready means for the reagentless, reusable detection of hybridization.

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Materials and Methods

Materials

Oligonucleotides were obtained from Synthegen (Houston, TX). The sensor oligonucleotide, 5'-NH_2-$(CH_2)_6$-GCGAGGTAAAACGACGGCCAGTCCTCGC-$(CH_2)_6$-SH-3' (oligo 1), contains a 5' hexamethylene amine and a 3' hexamethylene thiol. A ferrocene tag was conjugated to oligo 1 through coupling the succinimide ester of ferrocene carboxylic acid (Fc-NHS) with the 5' amine of oligo 1 (31). The final product

(oligo 1-Fc) was purified by HPLC on a C18 column and confirmed by electrospray mass spectroscopy. The sequences of the target and control DNA oligos were 5'-TTTTTACTGGCCGTCGTTTTACTCTTT-3' (oligo 2) and 5'-CGTATCATTGGACTGGCCATTTAT-3' (oligo 3) respectively. The control DNA oligo is a random sequence which is irrelevant to the probe.

Cyclic Voltammetry (CV) was performed at room temperature using a CHI 603 workstation (CH Instruments, Austin, TX). Polycrystalline Au disks (1.6 mm diameter) (BAS Inc., West Lafayette, IN) were used as working electrodes. While potentials are reported versus the normal hydrogen electrode (NHE), in actuality we employed a platinum electrode as a quasi-reference electrode at an assigned potential of 0.495 V relative to NHE (calibrated with Ag/AgCl, 3 M NaCl reference electrode from BAS Inc. in 1 M $NaClO_4$ with 1 mM ferricyanide). The electrolyte is 1 M $NaClO_4$ in all experiments. In certain cases, recorded CV curves were background-subtracted in Origin 7.0 (Microcal Software, Inc.) through extrapolation to the baseline in regions far from the peak (32).

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Construction of E-DNA sensor

The E-DNA sensor was constructed by assembling the ferrocene-labeled DNA stem-loop at a bioelectronic interface. This surface assembly was achieved by self-assembly process given the fact that DNA-thiol has strong tendency to diffuse from diluted aqueous solutions to clean gold surfaces (33). The stem-loop unit (oligo 1) has been such designed that it has five complementary bases at its 5'-end and 3'-end (four of them are G-C pairs), in the hope that the DNA strand will be closed by the thermostable G-C pairs and thus

forms a stem-loop with either end close to the gold surface. We expect this folding localizes the ferrocene unit at the 5'-end to proximity of the gold surface.

In order to construct the sensor as demonstrated in Scheme 1, a 1 μM solution of the stem-loop oligo 1-Fc was self-assembled on a extensively cleaned gold surface (34). Self-assembly was performed in 10 mM phosphate buffer with 0.1 M NaCl, pH 7.4 in order to produce a loosely packed surface that minimizes steric or electrostatic effects (35). The prepared surface was subsequently passivated with excess 2-mercaptoethanol (2-ME) at 1 mM. This process has been reported to "cure" relatively disordered self-assembled monolayers (SAMs) by gradually displacing nonspecifically adsorbed oligonucleotides (33). This oligonucleotide-containing, passivated surface does not interact significantly with non-cognate DNA sequences, as reported previously (36) and independently confirmed in our lab by monitoring via quartz crystal microbalance measurements (data not shown). The modified electrode was thoroughly rinsed, dried and then incubated in 1 M $NaClO_4$ prior to use.

Results

Characterization of the E-DNA modified electrode

In the absence of target DNA, a ferrocene redox peak pair is observed with the E-DNA modified gold electrodes (Figure 1). Bare gold electrodes and gold modified with either 2-ME or 2-ME/ferrocene-free oligo 1, in contrast, do not produce CV peaks in the relevant potential range (data not shown). The apparent formal potential ($E^{0\prime}$) of the electroactive label is 0.492 V, as estimated from $E_{1/2} = (E_{red}+E_{ox})/2$. This value falls within the typical redox potential range of ferrocene (21, 28). We thus ascribe this peak

pair to the redox conversion of ferrocene labels in close proximity to the gold electrode. Because of electrostatic repulsion between negatively charged DNA strands high ionic strength is required for the formation of stem-loop structure (36). The observation that freshly modified electrodes do not produce significant redox peaks without prior incubation in 1 M $NaClO_4$ (data not shown) thus provides strong evidence that the formation of the stem-loop structure is required for efficient electron transfer. This result also suggests that the use of uncharged peptide nucleic acids (PNA) in place of the sensor DNA might allow hybridization to occur at low ionic strength.

Modulating the scan rate of the CVs provides further evidence that ferrocene is confined at the electrode surface by the formation of the stem-loop structure. Peak currents of the ferrocene redox reaction (I_p) are directly proportional to scan rates (Figure 1, inset), consistent with a surface-confined electrochemical reaction (in contrast to I_p being proportional to the square-root of the scan rate characteristic of diffusion-controlled electrochemical reactions) (20).

Target Detection

Hybridization of the stem-loop structure with a target sequence complementary to the 17-base loop region competes with the less stable stem structure, moving the ferrocene away from the electrode surface (Scheme 1). Thus, incubating a stem-loop modified electrode with 5 μM cDNA (oligo 2) in 1 M $NaClO_4$ completely eliminates the ferrocene reduction and oxidation peaks within ~30 min (Figure 2). At lower target concentrations, only partial loss of signal is observed after 30 minutes (Figure 2). For example, after 30-min incubation at 500 pM target the electrochemical signal attenuates to approximately 70% of its initial value (Figure 3).

Employing a fixed 30-minute incubation time, we have tested the sensitivity and selectivity of the E-DNA sensor. Under these conditions we observe easily measurable decreases in peak current at target DNA concentrations as low as 10 pM (Figure 2). We currently employ a sample volume of 500 μl, equating to an absolute detection limit of 5 fmol. The exceptional signal-to-noise of the approach (Figure 2) suggests, however, that large improvements in electrode size –and thus sample volume and absolute sensitivity- will be straightforward. In contrast, no significant signal change is observed for electrodes incubated in DNA-free hybridization buffer or in the presence of the highest non-cognate DNA concentrations we have investigated (10 μM oligo 3). Thus the selectivity of the sensor is in excess of 10^6. The E-DNA sensor also exhibits exceptional dynamic range: peak currents are logarithmically related to target concentration across the almost six decade range we have investigated (Figure 2; inset). While similar logrithimic signal-versus-concentration relationships have been reported for other solid-state DNA sensors (30, 37), the mechanism underlying the relationship has not been determined.

Sensor regeneration

The electrochemical DNA sensor is readily reusable. We have successfully recovered up to ~80 % of the original signal by washing the electrode with 1 M $NaClO_4$ at 95°C and re-challenging with the target sequence. We believe the minor signal loss during recovery arises due to the relative instability of ferrocene in aqueous solution at high temperature.

Discussion

The E-DNA sensor is thus a reusable, solid-state, electrochemical genosensor. Unlike all previous sequence-specific electrochemical genosensors, however, the E-DNA sensor is reagentless and thus ideally suited for the continuous, rather than batch, monitoring of a flow of analyte. Moreover, the E-DNA sensor achieves the significant advantages of reagentless, reusable operation without compromising sensitivity or selectivity: the ~10 pM sensitivity and greater than million-fold selectivity of the E-DNA sensor is competitive with the very best current electrochemical DNA sensors. For example, E-DNA's 10 pM detection limit (~5 fmol in 0.5 mL) matches both sandwich approaches [J. F. Kayyem, pers. com.] and a recently proposed enzyme-amplified electrochemical detection method (30, 37), and is only surpassed by the 0.5 pM detection limit achieved by the reagent-intensive, non-reusable catalytic amplification approach of Mirkin and co-workers (7).

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The E-DNA sensor also offers significant advantages over optically-detected molecular beacons. For example, while the most highly optmized optical molecular beacon approaches can detect fM target in the laboratory, the pM sensitivity of the E-DNA sensor is comparable to or significantly better than the fluorescence-based techniques employed in the "real world" (*i.e.* with lower-power light sources and off-the-shelf detectors) (4, 38). For example, the sensitivity of the E-DNA exceeds that of typical, CCD-based fluorescent detectors by at least an order of magnitude (4). The E-DNA sensor also vastly surpasses solid state optical molecular beacons, for which a ~1 nM detection limit is reported (13). However, we note that the current E-DNA sensor, like all other state-of-the-art electrochemical or optical DNA sensors, does not meet the high-end

requirements of many real-world gene-targeting applications; while pM sensitivity is orders of magnitude more sensitive than necessary for the detection of *amplified* targets (and offers the very real possibility of replacing cumbersome gel- or optical-based detection schemes in this role), pM sensitivity is not sufficient for the *unamplified* detection of most pathogens. We are currently working on several methods of improving the sensitivity of the E-DNA sensor that may enable direct pathogen detection.

The preparation of the E-DNA sensor is quite straightforward. The key sensing element, the electroactive stem-loop, is compatible with normal solid-state synthesis of oligonucleotides, and the surface assembly chemistry is facile. Since the entire set-up can be conveniently prepared and generalized to be consistent with chip-based electrode arrays, the novel, reagentless detection described here appears to provide a promising alternative to traditional, fluorescence-based gene arrays.

While the signal generation mechanism of the E-DNA sensor has not been determined in detail, our experimental results provide strong support for the claim that the signal change arises from the "on-and-off" states provided by the DNA stem-loop (Scheme 1). When in the "on" state the ferrocene label is localized to the electrode surface via hybridization of the 5 base-pair stem region of the sensor DNA. Presumably, this spatial proximity allows facile electron transfer between the electroactive label and the gold. In the presence of a complementary target, the stem-loop is disrupted in favor of the thermodynamically more stable, rigid "rod-like" (39) target-sensor duplex, thereby separating the ferrocene from the electrode and blocking the exponentially distance-dependent electron transfer process. The separation of the electroactive label from the electrode surface may be facilitated by repulsion between the negatively charged DNA

and the negative dipole of the 2-ME hydroxyl group (33, 40). Previous neutron reflectivity studies demonstrate that, both single and double stranded DNA project away from surfaces covered with similar SAMs (33). This agrees with our observation that stem-loop formation is required in order to generate electrochemical signals in the E-DNA sensor.

A critical aspect of the described E-DNA sensor is the electrochemical detection of a target-induced conformational change of a biopolymer. This suggests that the E-DNA approach may be generalizable to other sensor designs in which significant protein (41, 42) or aptamer (31, 43, 44) conformational changes occur upon target binding.

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Figure legends

Scheme 1. A stem-loop oligonucleotide possessing terminal thiol and a ferrocene groups is immobilized at a gold electrode through self-assembly. In the absence of target, the stem-loop structure holds the ferrocene tag into close proximity with the electrode surface, thus ensuring rapid electron transfer and efficient redox of the ferrocene label. Upon hybridization with the target, electron transfer between the ferrocene and the electrode is blocked, presumably because the ferrocene label is separated from the electrode surface.

Figure 1. A cyclic voltammogram for a gold electrode modified with the ferrocene tagged, stem-loop oligonucleotide in the absence of target DNA (scan rate of 0.1 V/s). The electrolyte is 1 M $NaClO_4$. Inset: the linear relationship between peak currents and scan rates confirms that the redox species is confined to the electrode surface (20).

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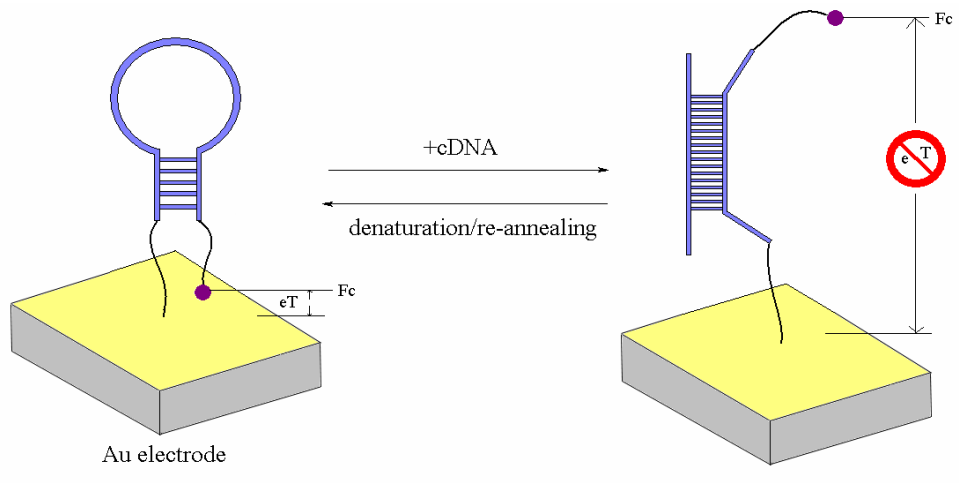
Figure 2. Background-subtracted (32) voltammograms (anodic scan) for the E-DNA sensor in the presence of complementary DNA at 0 M, 30 pM, 500 pM, 30 nM, 800 nM and 5 μM (from top to bottom). The hybridization time was fixed at 30 minutes. Inset: A calibration curve demonstrating peak height versus target concentration; E-DNA sensor response is logarithmically related to target concentration over at least six orders of magnitude.

Figure 3. The E-DNA signal develops in minutes (peak currents have been background subtracted). At a target concentration of 500 pM, the signal change observed after one

hour of hybridization implies that 35% of the probe DNA has hybridized with target molecules. At 5 μM target, in contrast, the peak current is entirely abolished within 30 minutes.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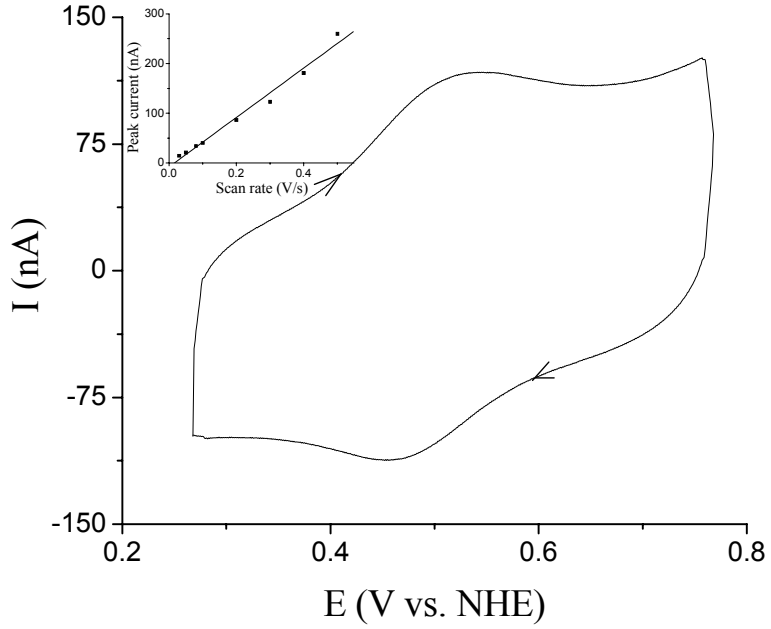
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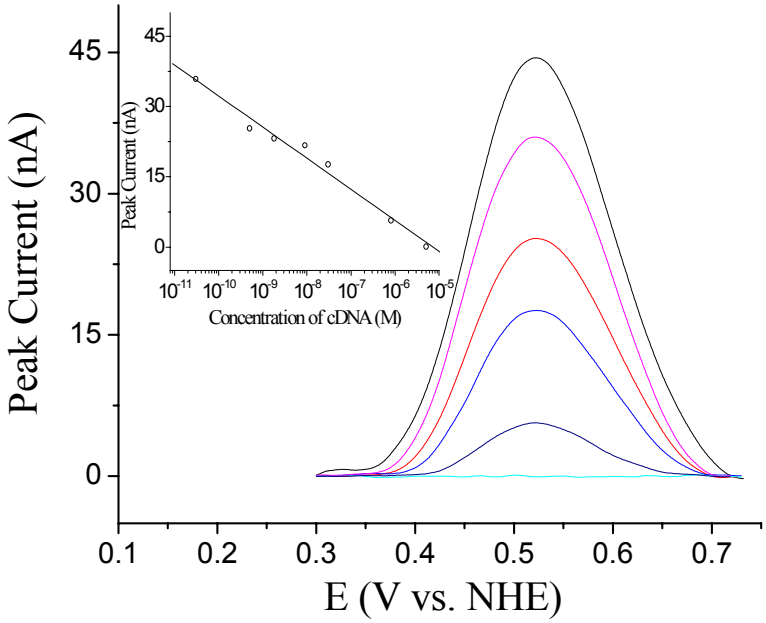
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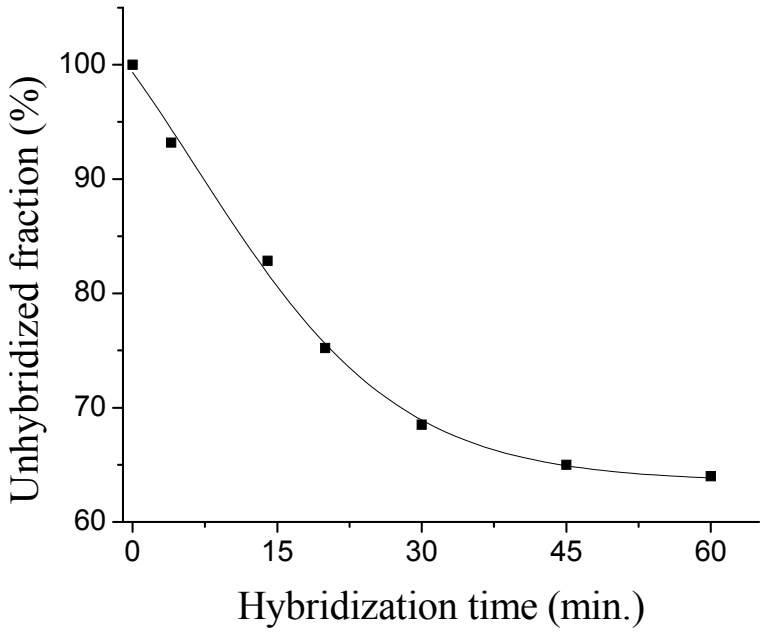
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DNA Detection Using Water Soluble Conjugated Polymers and Peptide Nucleic Acid Probes

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Classification: Major--> Physical Sciences Minor--> Applied Physical Sciences
Manuscript Information: 10 pages of text, 1 scheme (double column), 2 structures/schemes (single column) and 3 figures (single column).
Word and Character Count: 115 words in the abstract and 28530 characters in the paper.

Abbreviations Footnote: PNA-Peptide Nucleic Acid
ssDNA-Single Stranded DNA
dsDNA-Double Stranded DNA
CPs-Conjugated Polymers
CCP-Cationic Conjugated Polymer
C*-Chromophore Dye
FRET-Fluorescence Resonance Energy Transfer

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Abstract:

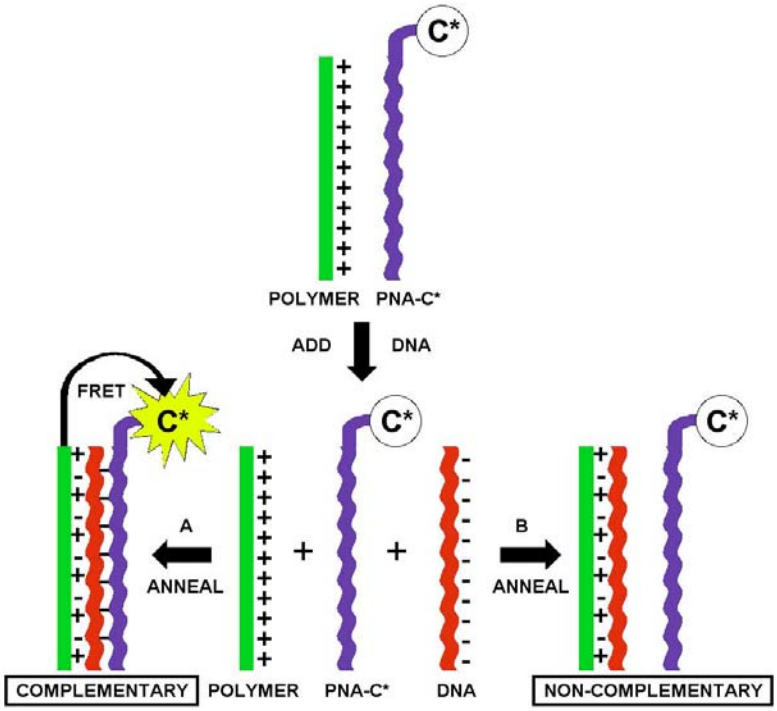
The light harvesting properties of cationic conjugated polymers are used to sensitize the emission of a dye on a specific PNA sequence for the purpose of homogeneous, "real time" DNA detection. Signal transduction is controlled by hybridization of the neutral PNA probe and the negative DNA target. Electrostatic interactions bring the hybrid complex and cationic polymer within distances required for Förster energy transfer. Conjugated polymer excitation provides fluorescein emission >25 times higher than that obtained by exciting the dye, allowing detection of target DNA at concentrations of 10 pM using a standard fluorometer. A simple and highly sensitive assay with optical amplification and which utilizes the improved hybridization behavior of PNA/DNA complexes is thus demonstrated.

Methods for DNA sequence identification in real time and with high sensitivity are of great scientific and economic interest. (1,2,3) Their applications include medical diagnostics, identification of genetic mutations, gene delivery monitoring and specific genomic techniques. (4) Cationic organic dyes, such as ethidium bromide and thiazole orange, emit when intercalated into the grooves of double strand DNA (dsDNA), and serve as direct DNA hybridization probes, but lack sequence specificity. (5,6) Energy/electron transfer chromophore pairs for strand specific assays exist, but require chemical labeling of two nucleic acids, or dual modification of the same altered strand (i.e. molecular beacons). (7,8) Difficulties in labeling two DNA sites results in low yields, high costs and singly labeled impurities, which lower detection sensitivity. (9) Much of the motivation behind improving DNA sensing is to develop simple and economic methods for evaluating strand specific hybridization that utilize the ease of homogeneous fluorescence assays with minimal DNA modification and enhanced signal amplification.

Conjugated polymers (CPs) are characterized by a delocalized electronic structure and can be used as highly responsive optical reporters for chemical and biological targets. (10,11) Because the effective conjugation length is substantially shorter than the number of repeat units, the backbone serves to hold a series of conjugated segments in close proximity. Thus, conjugated polymers are efficient for light harvesting and enable optical amplification via Förster transfer. (12) Water-soluble CPs show exceptional fluorescence quenching efficiencies in the presence of oppositely charged acceptors and are of particular interest for transduction of biological recognition events. (13)

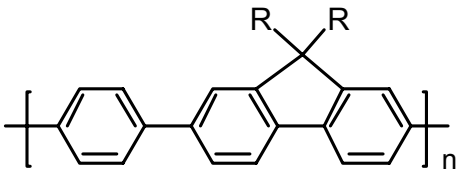
Spontaneous interpolymer complexation between cationic polyelectrolytes and DNA is known and is largely the result of cooperative electrostatic forces. (14,15,16) Hydrophobic interactions between aromatic polymer units and DNA bases were also recently recognized. (17) The free energy of polyelectrolyte/DNA interactions is controlled by the structure of the participating species, in conjunction with solution variables such as pH, ionic strength, and temperature. (18) The strength and specificity of these interactions has recently been coordinated to recognize the tertiary structure of plasmid DNA. (19)

The recent introduction of peptide nucleic acids (PNAs) opened the door for new research and diagnostic applications. (20,21) In PNAs, the negatively charged phosphate linkages in DNA are replaced with peptomimetic neutral amide linkages. PNA/DNA complexes form more quickly and are tighter and more specific than analogous DNA/DNA complexes. (22) These properties are largely due to the absence of the Coulombic repulsion found between negatively charged DNA strands. PNA complexes are thus more thermally stable and, by virtue of their backbone, less susceptible to biological degradation by nucleases, proteases and peptidases. (23,24) Additionally, their general insensitivity to ionic strength and pH during hybridization provides a wider platform for DNA detection.



Scheme 1. Schematic representation for the use of a water soluble conjugated polymer with a specific PNA-C* optical reporter probe to detect a complementary ssDNA sequence.

A novel scheme for detecting PNA/DNA interactions based on the considerations given above is shown in Scheme 1. Consider a solution which contains a cationic conjugated polymer (CCP, shown in green) and a PNA strand (shown in blue) labeled with a chromophore dye (C*). The optical properties of the CCP and C* are chosen to favor Förster energy transfer (FRET) from CCP (donor) to C* (acceptor). (25) In the initial solution no electrostatic interactions are present, resulting in an average CCP---C* distance too large for effective FRET. Single strand DNA (ssDNA) is then added and an appropriate annealing protocol is followed. Situation **A** corresponds to addition of a complementary ssDNA (shown in red), which hybridizes with the target PNA. Hybridization endows the C* bearing macromolecule with multiple negative charges. Electrostatic interactions should cause the formation of a complex and a decrease in the average CCP---C* distance, allowing for FRET. When a ssDNA that does not match the PNA sequence is added (shown in red), situation **B**, hybridization does not take place. Electrostatic complexation occurs only between the CCP and DNA while the CCP-PNA-C* distance remains too large for FRET. PNA/ssDNA hybridization is therefore measured by FRET efficiency, or the enhanced C* emission. The overall scheme serves as a probe for the presence of specific ssDNA sequences in solution.



1 -R = -$(CH_2)_6NMe_3^{\oplus}I^{\ominus}$

PNA-C*: 5'-FI-CAGTCCAGTGATACG-3'
2: 5'-CGTATCACTGGACTG-3'
3: 5'-ACTGACGATAGACTG-3'

Scheme 2.

Scheme 1 was tested using the cationic water soluble conjugated polymer poly(9,9-bis(6'-N,N,N-trimethylammonium)-hexyl)-fluorene phenylene) containing iodide counteranions (**1**, in Scheme 2). (26) A PNA probe corresponding to a CAGTCCAGTGATACG base sequence (in Scheme 2) with fluorescein at the 5' position of the strand was used as PNA-C* (blue fragment in Scheme 1). The absorption and emission spectra of **1** and PNA-C* in Figure 1 show an optical window for the excitation of **1**, between the DNA, PNA and C* absorptions. There is excellent overlap between the emission of **1** and the absorption of C* to ensure FRET.

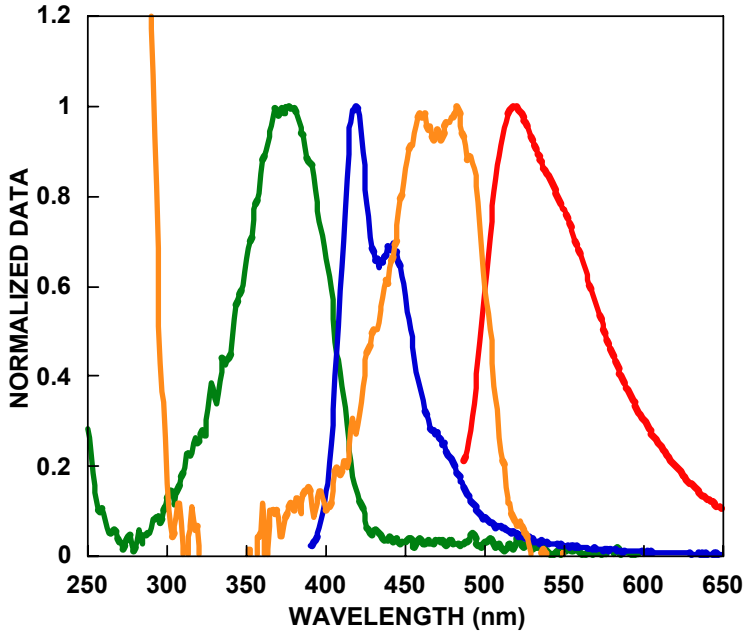


Figure 1. Absorption (green and orange) and emission (blue and red) spectra of polymer **1** and ssPNA (DNA) probe **2**, respectively. Fluorescence was measured by exciting at 380 and 480 nm, for **1** and **2** respectively.

The PNA-C* probe ([PNA-C*] = 2.5×10^{-8} M) was annealed at 2°C below its T_m (72°C at 10^{-8}M, pH = 5.5) in the presence of an equimolar amount of its complementary 15 base pair ssDNA, **2**, and in an identical fashion with a non-complementary 15 base ssDNA, **3**. (27,28) Annealing was accomplished in the absence of buffer, *i.e.* at low ionic strength, and the subsequent melting was monitored by UV/Vis spectroscopy. (29) Addition of **1** in water ([**1**] = 2.3×10^{-7} M) and comparison of the resulting fluorescence (Figure 2) reveals a FRET ratio > 11 times higher for the PNA/DNA hybrid, relative to the non-complementary pair. (30) These FRET differences demonstrate the validity of Scheme 1. Furthermore, the fluorescein emission is greater than 8 times more intense than that obtained from direct C* excitation in the absence of **1**. (31) The increased C* emission in the energy transfer complex indicates the optical amplification provided by the conjugated polymer. The sensitized acceptor emission is turned on by the addition of complementary DNA.

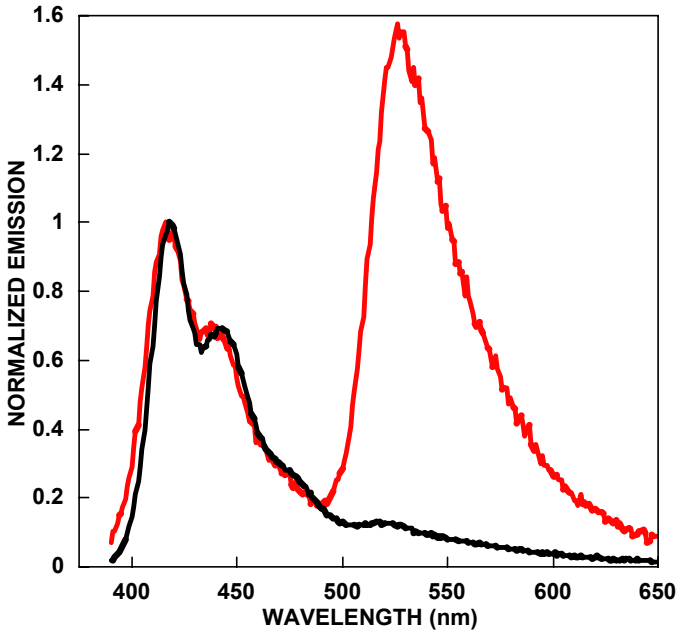
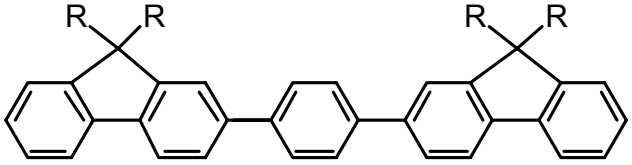


Figure 2. Emission spectra of PNA-C* in the presence of complementary (red) and non-complementary (black) DNA by excitation of polymer **1**. Conditions are in water at pH=5.5. The spectra are normalized with respect to the emission of polymer **1**.

Energy transfer was optimized by varying the ratio of **1** to PNA-C*. At a concentration of [PNA-C*] = 2.5×10^{-8} M, initial additions of **1** cause an immediate rise in the FRET ratio. When [**1**] far exceeds [PNA-C*], a decrease is observed. The maximum in the FRET ratio corresponds to a near 1:1 ratio of polymer chains to PNA strands, according to previously published molecular weight information. Such a relationship should not be surprising, since when [**1**]/[PNA-C*] < 1, not all ssDNA/PNA-C* hybrid strands can be complexed efficiently to independent polymer chains. Conversely, in the [**1**]/[PNA-C*] > 1 regime, not all the photons harnessed by **1** (the donor) can be transferred to the DNA/PNA-C* hybrid (the acceptor). Note that the C* emission at the saturation point is

>25 times more intense than that obtained by direct C* excitation (480 nm), giving further evidence of signal amplification. *This amplification allows detection of C* emission when [PNA-C*/DNA complex] = 10 pM using a standard fluorometer*. (32)

Examination of Figure 2 shows a small fluorescein signal from the non-hybridized PNA probe (situation B, Scheme 1), which result from hydrophobic interactions between **1** and PNA-C*. (33,34) Solutions containing 10% ethanol, under the identical conditions as the experiments shown in Figure 2, show a decrease in C* emission. The presence of the organic solvent decreases hydrophobic interactions and reduces C* emission by a factor of 3, at which point the signal is almost undetectable using a standard fluorometer.



4 -R = $-(CH_2)_6\overset{\oplus}{N}Me_3\ \overset{\ominus}{I}$

Scheme 3.

A water soluble conjugated oligomer, (**4**, in Scheme 3), of similar chemical structure to that of **1** was also examined within the context of Scheme 1. Although the smaller molecule will not display the same signal amplification, it is useful to deconvolute structure property relationships, which are difficult to determine with the inherent polydispersity and batch-to-batch variations found in polymers. Further, in aqueous media, **4** is considerably more soluble than **1**, and hydrophobic interactions with neutral PNA should not be as severe. Figure 3 ([**4**]=6.7×10^{-8} M and [PNA-C*]=2.5×10^{-8} M) shows C* emission only when the complementary ssDNA was present. Comparison of Figures 2 and 3 implies that use of conjugated polymers with higher molecular weights will lead to higher FRET ratios. We thus anticipate that significantly higher sensitivity can be achieved.

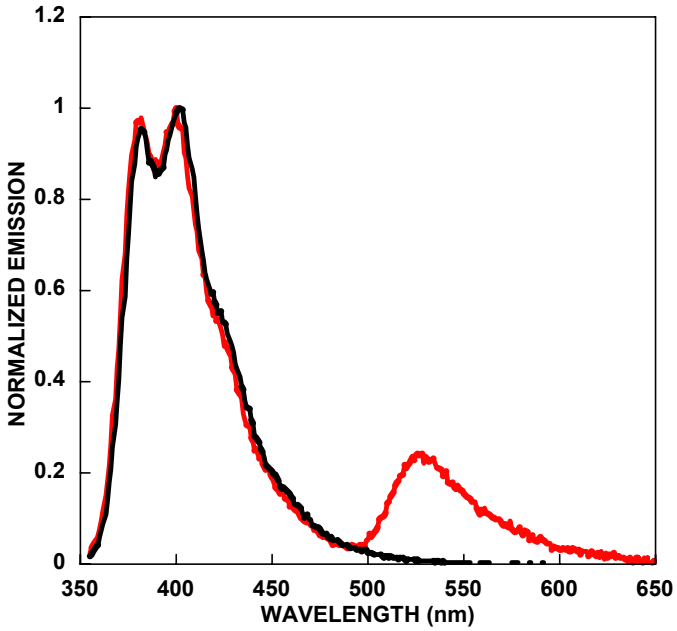


Figure 3. Emission spectra of PNA-C* in the presence of complementary (red) and non-complementary (black) DNA by excitation of **4**. Conditions are in water and pH=5.5. The spectra are normalized with respect to the emission of **4**.

In summary, it is possible to take advantage of the optical amplification of CPs to detect DNA hybridization to a singly labeled PNA strand. This method provides a homogeneous assay that utilizes the ease of fluorescence detection methods and capitalizes on the enhanced hybridization behavior found in PNA-DNA interactions. As shown in Figure 2 and 3, the reporter emission "turns on" only when the target ssDNA is present in solution. The overall strategy also eliminates the need for multiple probes and complex DNA structures. In a practical assay, donor emission could easily be filtered off and light intensity measured with a simple photodiode. The concept could also be used in post PCR analysis or, because of the large signal amplification, as a stand alone assay for the detection of a specific DNA sequence. Additionally, PNA's also have the ability to form triplex structures by binding to dsDNA and to invade a dsDNA sequence and displace the DNA strand of the same sequence. (35,36,37) We foresee that such interactions could lead to the use of PNA-C*/CP sensor platforms to be used in direct dsDNA detection. Further optimization of CP structure/optical properties with a better understanding of the forces that control the association between conjugated polyelectrolytes, DNA and PNA will likely yield viable detection platforms.

Acknowledgments

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31. Fluorescein at pH=5.5 is not in its high quantum yield dianionic form, thus we would expect higher C* emission at higher pH, but at the expense of charge neutrality on the PNA-C* complex.
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