Nucleic Acids Research, 2005, Vol. 33, No. 9 ***e83***
doi:10.1093/nar/gni084

Magnetically assisted DNA assays: high selectivity using conjugated polymers for amplified fluorescent transduction

Hui Xu[1,2], Haiping Wu[1], Fei Huang[3], Shiping Song[1], Wenxin Li[1], Yong Cao[3] and Chunhai Fan[1,*]

[1]Division of Nanobiology and Nanomedicine, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China, [2]Graduate School of the Chinese Academy of Sciences, Beijing 10039, China and [3]Institute of Polymer Optoelectronic Materials and Devices, South China University of Technology, Guangzhou 510640, China

Received April 6, 2005; Revised and Accepted May 3, 2005

ABSTRACT

We report a strategy for conjugated polymer (CP)-based optical DNA detection with improved selectivity. The high sensitivity of CP-based biosensors arises from light harvesting by the CP and the related amplified fluorescent signal transduction. We demonstrate that the use of magnetic microparticles significantly improves the selectivity of this class of DNA sensors. Compared with previously reported DNA sensors with CP amplification, this novel sensing strategy displays excellent discrimination against non-cognate DNA in the presence of a protein mixture or even human serum. We also demonstrate that the magnetically assisted DNA sensor can conveniently identify even a single-nucleotide mismatch in the target sequence.

INTRODUCTION

The detection of DNA hybridization is a topic of major scientific and technological interest. Application areas include clinical diagnosis, single-nucleotide polymorphism (SNP) genotyping, environmental studies, antiterrorism and forensic analysis (1). This utility has motivated the development of novel DNA sensors with optical (2–5), acoustic (6,7) or electronic (8–12) 'read-out', among which optical (fluorescent) detection methods have historically dominated state-of-the-art genosensors (1,13).

Aromatic, intercalating dyes, e.g. ethydium bromide (EB), served as the first-generation fluorescent DNA hybridization indicator; the fluorescence quantum yield of EB increases significantly upon intercalating into double-stranded (ds) DNA. However, because of the non-specific, hydrophobicity-driven binding of EB to single-stranded (ss) DNA, EB indicators show only limited selectivity against non-cognate DNA (14). In order to overcome this problem, fluorophore-tagged DNA probes were used for hybridization-based assays, which exhibited much improved detection selectivity over indicator-based detection (1). 'Molecular beacon' sensing strategy coupled fluorescence detection with stem–loop structured DNA probes, which further enhanced specificity for discrimination of single-nucleotide mismatches (15–17). More recently, novel materials ranging from inorganic nanocrystals (18,19) to rare earth elements (20) have been incorporated to further improve the performance of fluorescent DNA sensors. Conjugated polymer (CP)-based genosensors represent a new opportunity along these lines.

Conjugated polymers possess a unique combination of optoelectronic properties that have found use in a variety of areas (21). Relatively recently, the use of CPs as components in biosensors has stimulated significant research interest (3,22–25). In the biosensor application, CPs serve as a light-harvesting 'antenna'. The light-harvesting feature originates from their very high absorption coefficients (as high as 10^6 M^{-1} cm^{-1}) (23). Because CPs exhibit efficient energy migration along their delocalized backbones, the collected energy can be efficiently transferred to acceptors via either excited-state electron transfer or Förster resonance energy transfer (FRET) (23,25–27). The light-harvesting properties of CPs create an opportunity to amplify biosensor signals and thereby to develop highly sensitive optical biosensors (23). Based on the so-called superquenching phenomenon arising as a result of rapid excited-state electron transfer from the polymer to quenchers, Chen *et al*. (22) have developed a novel CP-based biosensing strategy that can sensitively detect a variety of bio-recognition events (28–32).

*To whom correspondence should be addressed. Tel: +86 21 5955 6902; Fax: +86 21 5955 6902; Email: fchh@sinap.ac.cn

Gaylord *et al*. (3) demonstrated a different, FRET-based approach that employed CPs as the sensing element to detect specific DNA sequences. In their method, a fluorescein-tagged peptide nucleic acid (PNA) sequence served as the probe sequence. Hybridization of the neutral PNA probe to the negatively charged DNA target significantly creates an electrostatic attraction between the PNA/DNA and a cationic luminescent CP, thus bringing the tagged dye and the polymer sufficiently close to allow efficient FRET. This light-harvesting polymer-based FRET signal amplification provides a means to transduce DNA hybridization to optical sensing with improved sensitivity of more than an order of magnitude (3). A note of caution is that the use of the expensive PNA is key to this sensor (3). Replacement of PNA probes with fluorophore-tagged DNA probes leads to much worse selectivity as a result of the strong Coulomb attraction between the cationic luminescent polymer and the ssDNA probe (33).

In order to be able to quantitatively detect the specific sequences on target DNA, false positive signals that arise from non-specific interactions (i.e. other than the fundamental base pairing within dsDNA) must be eliminated. Thus, it is desirable to develop a DNA sensor that is essentially 'signal-off' in the unhybridized state. We note that the use of magnetic particles has attracted much recent attention in controlling bio-related systems owing to its operational convenience and separation efficiency (34,35). In the study reported here, by introducing magnetic particles to CP-based sensors, we developed a magnetically assisted DNA detection platform with polymer amplification that quantitatively identifies target DNA with perfect discrimination against non-cognate DNA; even a single-nucleotide mismatch in the target DNA sequence can be conveniently recognized. As a further step, we demonstrated the ability to detect specific sequences on DNA in the presence of either a mixture of proteins or human serum. This initial success suggests that this DNA sensor can be used for the detection of DNA hybridization in real samples.

MATERIALS AND METHODS

Materials

Oligonucleotides were obtained from TaKaRa Corporation and subsequently purified with either high-performance liquid chromatography or PAGE. Their sequences are listed in Table 1. Capturing probe **1** is terminated with a biotin that links it to streptavidin-coated magnetic microparticles (MMPs). Capturing probe **1** contains a 15-base probe sequence and a spacer (10 'T's) that reduces surface effects from the MMPs (2). Signaling probe **2** is a 15-base sequence tagged with fluorescein (6-FAM) at the 5′-terminus. Target DNA **3** is a 40-base sequence which contains complementary sequences to **1** on one end and to **2** on the other end. DNA **4** contains a complementary sequence to probe **1** but includes a single-nucleotide mismatch to probe **2**. DNA **5** is a random sequence that is non-complementary to either **1** or **2**. Oligonucleotides **6–9** are related to the BRCA1 breast cancer gene and represent capture probe, signaling probe, target DNA and single-mismatched DNA, respectively.

Lysozyme (Lys), hemoglobin (Hb) and BSA were purchased from Sigma. The water-soluble polyfluorene (PF), Poly[(9,9-bis(3′-((*N*,*N*-dimethyl)-*N*-ethylammonium)-propyl)-2,7-fluorene)-alt-1,4-phenylene] Dibromide, was synthesized as previously reported (36). MagnetSphere® and streptavidin-coated MagnetSphere® paramagnetic particles (~1.0 μm diameter, 1 mg/ml) were obtained from Promega Corporation. All other reagents were of analytical grade. The buffer solutions were hybridization buffer (750 mM NaCl, 150 mM sodium citrate, pH 7.4), washing buffer (10 mM Tris–HCl, pH 7.4, 50 mM NaCl) and TTA buffer (250 mM Tris–HCl, pH 8.0, 0.1% Tween-20 and 5% BSA). All solutions were prepared using Milli-Q water.

Table 1. Oligonucleotide sequences

Capturing probe **1**	5′-ATGGCTGTAACTGAATTTTTTTTTT-(Biotin)-3′
Signaling probe **2**	5′-Fluorescein-TAGTCAGTGATACGT-3′
Target DNA **3**	5′-TTCAGTTACAGCCATTTTTTTTTT TACGTATCACTGACTA-3′
Single-mismatched DNA **4**	5′-TTCAGTTACAGCCATTTTTTTTTT TACGTATCTCTGACTA-3′
Non-cognate DNA **5**	5′-ACACGCTTGGTAGACTTTTTTTTT TAGCATCGATAACGTT-3′
Capturing probe (BRCA1) **6**	5′-GAAACCCTATGTATGCTCTTTTTT TTTT-(Biotin)-3′
Signaling probe (BRCA1) **7**	5′-Fluorescein-GTATGAATTATAATCA AA-3′
Target DNA (BRCA1) **8**	5′-GAGCATACATAGGGTTTCTCTTGG TTTCTTTGATTATAATTCATAC-3′
Single-mismatched DNA (BRCA1) **9**	5′-GAGCATACATAGGGTTTCTCTTGG TTTCTTTGATTATCATTCATAC-3′

Spectroscopic measurements

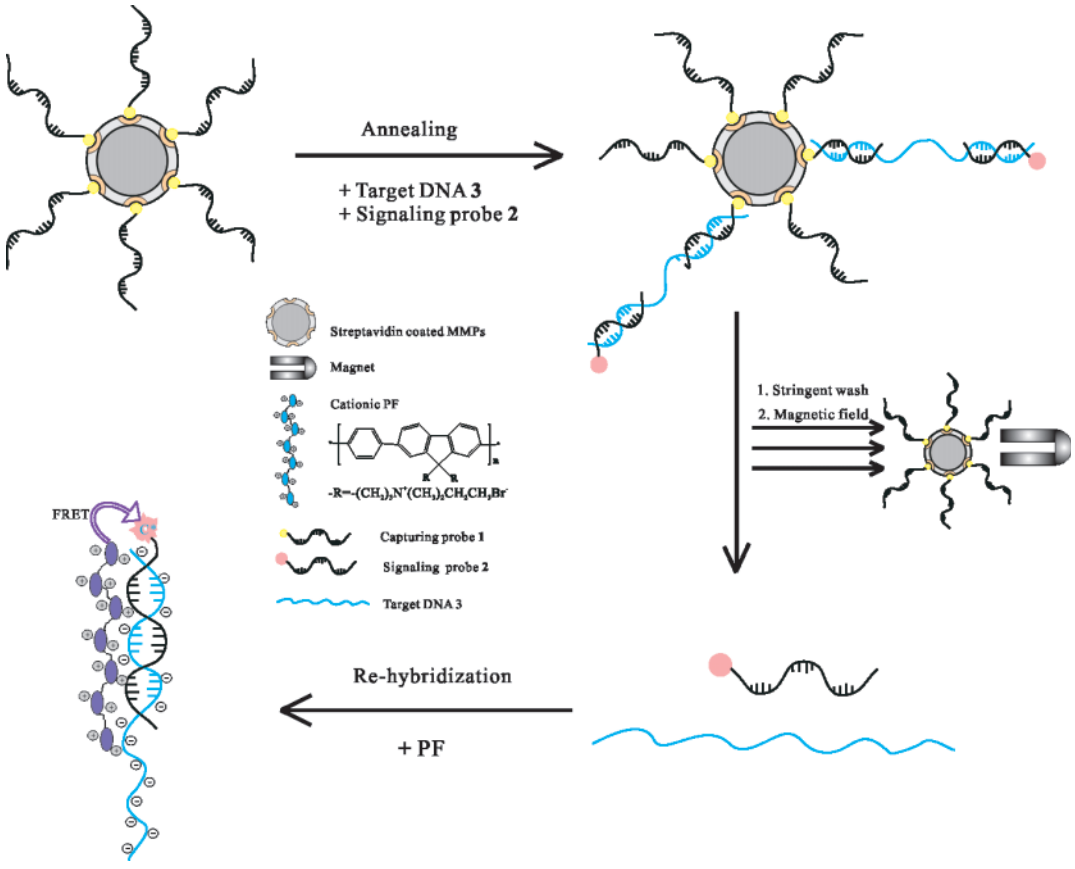
Absorption spectra were collected with a Zeiss UV-visible recording spectrophotomer, and fluorescence spectra were collected with a F-4500 fluorometer equipped with a xenon lamp excitation source (Hitachi). The excitation wavelength is 380 nm, and the spectra were recorded between 390 and 700 nm.

The magnetically assisted DNA sensing strategy

In our design, the DNA sensor is composed of three components: a cationic CP (PF, the light-harvesting polymer that serves as the signal amplification factor), a fluorescein-tagged DNA probe (signaling probe) and a streptavidin-coated MMP labeled with biotinylated DNA probes (capturing probe). Both the signaling probe and the capturing probe are designed to be complementary to different parts of the DNA target sequence, which forms the basis of 'sandwich-type' detection. The detection strategy is described in Scheme 1. In the presence of target DNA, the capturing probe brings the target DNA, along with the signaling probe, proximal to the MMP. In contrast, in the absence of target DNA, the 'sandwich' complex cannot be formed, and thus the signaling probe is not attached to the MMP during the magnetic separation step. As a result, only in the presence of target DNA can the cationic luminescent polymer meet the signaling probe to form a FRET pair and emit amplified fluorescence from the fluorescein that reflects the quantity of target DNA.

Preparation of capturing probe 1-labeled MMPs

The streptavidin protein at MMP surfaces serves as a bridge to link biotinylated DNA to the MMP. MMPs from the stock



Scheme 1. Strategy for the magnetically assisted DNA sensor with CP amplification. In the presence of target DNA, the capturing probe and the signaling probe form the 'sandwich' complex with the target and anchor at the MMP surfaces. After the stringent washing step and magnetic separation, the cationic luminescent polymer meets the signaling probe to form a FRET pair and emit amplified fluorescence from the fluorescein, which reflects the quantity of target DNA.

solution were first washed with buffers as recommended by Promega (http://www.promega.com/tbs/tb246/tb246.pdf). Subsequently, a solution of biotinylated capturing probe **1** was added to the collected MMPs (at a ratio of 1.25 nmol probe **1** to 1 mg MMPs) and the mixture was incubated for ~10 min with gentle mixing. Note that the binding process of biotin–streptavidin at surfaces is fairly rapid (usually within 1 min) according to previous surface plasma resonance studies (37). The surface density of probe **1** was estimated to be $4–6 \times 10^{11}$ strands/cm^2 (http://www.promega.com/tbs/tb246/tb246.pdf). The MMP–**1** complexes were then washed twice with TTA buffer and suspended in the hybridization buffer. After preparation, the MMP–**1** complex was stored in a refrigerator for further use.

Detection of DNA hybridization

In a typical assay, both MMPs with capturing probe **1** (50 μg) and excess signaling probe **2** were added to solutions (1 ml) containing DNA sequences (**3**, **4**, **5**) and incubated for hybridization at 37°C (~20 min). The resulting MMPs were magnetically collected (~30 s), then washed twice with hybridization buffers. In the case of SNP detection, we washed MMPs with washing buffers. In order to avoid light scattering associated with these relatively large MMPs, we separated signaling probe **2** from the MMPs by rinsing the MMPs with 50 mM NaOH solution. Previous studies have demonstrated well that the use of this alkaline solution effectively denatures DNA duplexes within 1 min whereas biotin–streptavidin binding is essentially undisturbed under this condition (37). The rinsed solution containing both **2** and **3** was neutralized by adding an acid solution (50 mM HCl and 100 mM Tris–HCl, pH 7.4) of the same volume as the NaOH solution. Note that **2** and **3** re-form a duplex in this solution containing salts (see Supplementary Figure 1S). PF was added to form the FRET pair with the fluorescein tag at probe **2**. Fluorescence measurements were performed by exciting PF at 380 nm. The spectra were normalized to the polymer emission, and background-subtracted fluorescence intensity was used as the quantitative index.

Detection of target DNA from the mixture

The artificial matrix was a 1.0 ml solution containing 1 nM target DNA **3**, large amounts of random DNA **5** (100× more concentrated) and proteins that are often encountered in real applications (Lys, Hb and BSA, 1000× more concentrated). As a further step, we used human serum samples (1:10 dilution, a gift from Shanghai Orion Diagnostics Co. Ltd) containing 1 nM target DNA **3**. All separation, collection and detection procedures were the same as described above, except that the MMPs were washed twice with TTA buffer after hybridization.

RESULTS

PF-amplified fluorescent transduction

Previous spectroscopic studies have proven that the emission of PF has sufficient spectral overlap with the fluorescein absorption that they form an excellent FRET pair (38). Consistent with this, we observed well-defined FRET signals upon addition of PF to the test solution containing probe **2** (Figures 1 and 2). The light-harvesting PF sensitized the emission of the fluorescein (the energy-transfer acceptor), leading to fluorescent signal amplification. In fact, we could still observe well-defined PF-sensitized fluorescein emission at probe **2** concentrations that were sufficiently dilute (100 pM) that fluorescence emission was negligible by direct excitation of fluorescein (Figure 1). These results verify that light-harvesting polymers do provide optical signal amplification and improve the detection sensitivity.

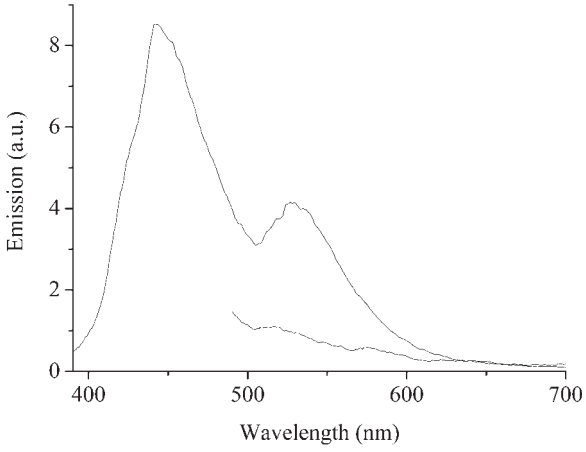


Figure 1. Signal amplification is offered by PF amplification, which is demonstrated by the comparison between the FRET signal sensitized by PF excitation and the signal generated from direct excitation of the fluorescein-tagged ssDNA signaling probe. Upper curve: the excitation wavelength was 380 nm, which excited PF (2.7×10^{-8} M). In the presence of fluorescein-tagged ssDNA probe (1×10^{-10} M), the energy was efficiently transferred from PF to the proximal fluorescein via FRET. Lower curve: the excitation wavelength was 480 nm, which directly excited the fluorescein of the ssDNA probe (1×10^{-10} M). Note that the emission of fluorescein at 520 nm was much more intense in the case of PF amplification than in the case of direct excitation.

Detection of DNA hybridization

In our initial study, we employed a 40-base oligo **3** as the model target DNA sequence. Incubation of MMP–**1** with both **2** and **3** in the hybridization buffer allows annealing of these DNA strands, resulting in the formation of the 'sandwich' complex at MMP surfaces. Upon applying the magnetic force, the MMPs were rapidly separated from the bulk solution (~30 s). After the addition of PF to the rinsed solution containing the duplex (formed by signaling probe **2** and target **3**), we observed a strong FRET signal that increases in strength with the probe concentration. We then performed a quantitative assay for detection of target DNA. We observed that the intensity of PF-sensitized fluorescein emission was linearly proportional to the concentration of target DNA **3** in the range 0–10 nM (Figure 2). Note that the FRET signal can still be clearly identified from the background even for 100 pM target; thus we estimate that the limit of DNA detection is as low as 100 pM (100 fmol). This DNA sensor also shows excellent discrimination against non-cognate DNA. FRET signals were not observed when a non-cognate DNA **4** (5 μM) replaced the target **3**. These indicate that we could selectively identify the target DNA sequence without interferences from excess non-cognate DNA.

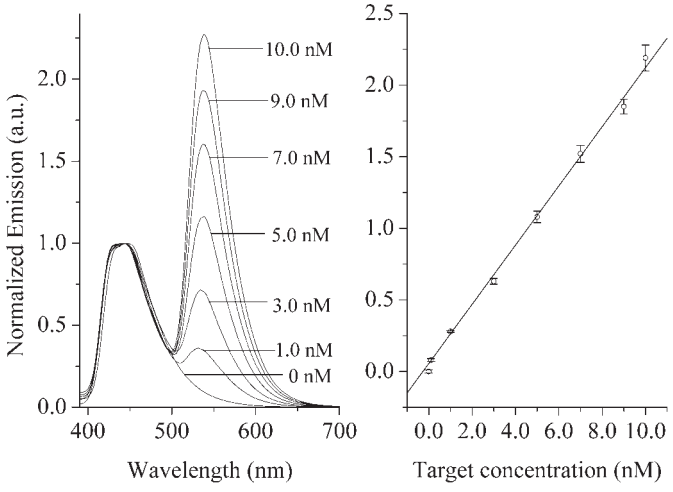


Figure 2. Left panel: normalized fluorescence spectra for DNA sensing based on PF-amplified FRET signals ([PF] = 5.4×10^{-7} M). The concentrations of target DNA are 0, 1.0, 3.0, 5.0, 7.0, 9.0 and 10.0 nM, respectively, from bottom to top. Right panel: dependence of FRET signals on target DNA concentrations. Error bars were estimated from at least three independent measurements.

Detection of a single-nucleotide mismatch

It is essential to identify a single-nucleotide mismatch in order to meet the strict requirement of certain applications, e.g. SNP genotyping. DNA duplex stability is known to be a function sensitive to the ionic strength of solutions. Because the DNA strands are negatively charged, cations are required to screen the Coulomb repulsion that would otherwise prevent the formation of the double helix (39). Mirkin and coworkers have demonstrated that mismatched DNA is much more susceptible to solutions of low ionic strength than perfectly matched DNA (40). Although Mirkin's work was based only on DNA probes at the surface of gold nanoparticles, we observed that this also offered the opportunity to distinguish single-nucleotide mismatches by salt-stringency in our magnetically assisted DNA assays.

We performed a stringent washing step that was expected to destabilize dsDNA containing single-nucleotide mismatches. In this case, MMPs collected from the test solution were rinsed with the washing buffer of low ionic strength. We reason that DNA sequences containing a single-nucleotide mismatch can be largely removed during this stringent washing, while target DNA remains bound to the MMP surfaces. Indeed, we observed that the DNA sensor showed marked differences in FRET signals corresponding to perfectly matched DNA and single-nucleotide mismatched DNA (Figure 3), indicating that this DNA sensor exhibits excellent discrimination against even a single-nucleotide mismatch.

In order to demonstrate that this proposed strategy is applicable to real applications, we further employed the above-mentioned protocol to detect the BRCA1 breast cancer

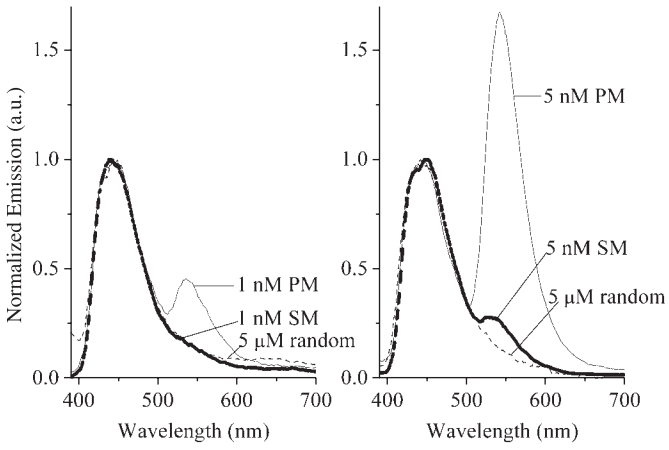


Figure 3. Normalized fluorescence spectra for DNA hybridization with 1 nM perfectly matched (PM) target DNA, 1 nM single-nucleotide mismatched (SM) DNA and 5 µM non-cognate DNA of random sequence ([PF] = 8.1×10^{-8} M); 5 nM PM target DNA and 5 nM SM DNA ([PF] = 4.3×10^{-7} M).

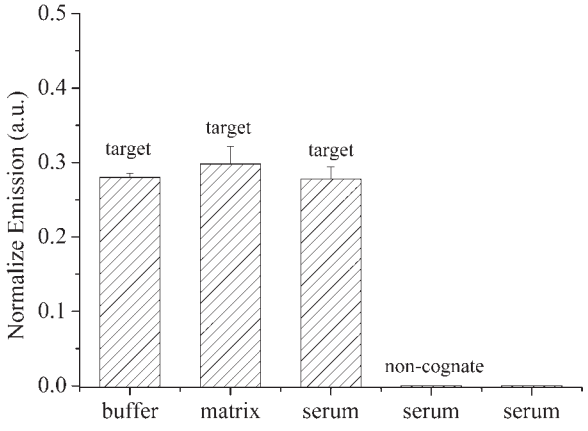


Figure 4. Histograms for fluorescence intensity at different conditions. From left to right: 1 nM target DNA in the pure buffer; 1 nM target DNA in the artificial matrix containing proteins; 1 nM target DNA in the human serum sample; 5 µM non-cognate DNA in the human serum sample and a human serum sample ([PF] = 5.4×10^{-7} M). Error bars were estimated from at least three independent measurements.

gene, a tumor suppressor gene. It is well known that women who inherit mutations of BRCA1 are highly susceptible to the development of breast cancer (41); thus it is important to detect mutations of the BRCA1 gene. By using the magnetically assisted DNA assay with CP amplifications, we could selectively detect 1 nM BRCA1-related oligonucleotide sequence **8**. In contrast, we could not observed any FRET signal for 1 nM single-mismatched oligonucleotide **9** (Supplementary Figure 4S).

Detection of target DNA in complex samples

In an attempt to test the applicability of this DNA sensor to detection in real samples, we prepared an artificial matrix containing 1 nM target DNA **3** in a solution containing a 1000-fold higher concentration of proteins (Hb, Lys and BSA) and random DNA sequences. As a further step, we employed diluted human serum samples (1:10) containing either target DNA or non-complementary DNA. With the assistance of magnetic separation, we were able to observe FRET signals corresponding to the presence of target DNA. More importantly, we have shown that the FRET signal intensity is comparable to that obtained in pure DNA solutions (Figure 4), implying that the DNA detection is insensitive to non-specific species, and that it is possible to perform DNA detection even in blood samples.

DISCUSSION

The use of CPs as highly responsive optical reporters formed the basis of the highly sensitive CP-based DNA sensor proposed by Gaylord *et al*. (3). However, since the signal transduction of this sensor relies mainly on discrimination of electrostatic interactions between neutral PNA and the negatively charged PNA/DNA complex, the selectivity is imperfect. According to recent time-resolved spectroscopic studies (42), electrostatic interactions dominate in dilute PNA solutions, thus leading to fairly good selectivity between unhybridized PNA and hybridized PNA/DNA. In relatively concentrated PNA solutions, however, hydrophobic interactions between the PF backbone and PNA contribute to bringing the PF and fluorescein-tagged PNA within the FRET distance. As a result, non-specific FRET signals are observed even for hybridization with non-cognate DNA (42). Note that, even in dilute solutions, hydrophobic interactions exist, leading to relatively small, non-specific FRET signals (3,42). Moreover, replacement of PNA probes with DNA probes achieves only limited discrimination ability because of the relatively small difference in the Coulomb binding strength of ssDNA and dsDNA to PF (33).

The magnetically assisted sensor system represents an opportunity to develop high-performance CP-based DNA sensors even without the use of expensive PNA. The use of magnetic forces instead of simply relying on electrostatic forces is essential for the significant improvement of the sensor selectivity. As we have demonstrated with this magnetically assisted DNA sensor, target DNA leads to strong FRET emission whereas essentially no signal is observed for non-cognate DNA. In the present experimental setup, we could detect as little as 100 pM target DNA. Note that we could detect only 1 nM of fluorescein-tagged DNA without CP amplification. This clearly demonstrates that the use of CPs offer a signal amplification of an order of magnitude. This detection limit could be further lowered by using state-of-the-art fluorometers, or by finely tuning the backbone or side chains of CPs, which might lead to much improved amplification (43,44).

More importantly, single-nucleotide mismatches can be conveniently identified by a stringent wash with low ionic strength buffer with this strategy. Note that SNP detection is conventionally achieved by a thermal-stringency washing step, relying on the fact that the melting point of mismatched DNA is lower than that of the corresponding perfectly matched DNA. The salt-stringency washing first proposed by Mirkin and coworkers (40) is much more suitable for magnetically assisted assays. First, MMPs have only limited stability over high temperature (<65°C); second, salt-stringency washing steps can be done within a minute, whereas it is not convenient to perform thermal-stringency washing in the presence of the magnet (magnets are also susceptible to high temperature).

Other stringent conditions include the use of denaturing reagents of appropriate concentrations (e.g. urea) (45) or organic solvents (e.g. formamide) (46). Nevertheless, none of these approaches is as convenient as the salt-stringency approach employed here. Also of note is the fact that the current detection strategy might be generalized to multiplex detection, that is, distinguishing multiple targets in only one tube. A recent report by Liu *et al*. (47) has proved that it is possible to design CPs emitting several different colors, which is highly promising progress toward the goal of multiplexing.

Detection in real samples is more problematic for the previously established CP-based biosensor (3). Polymer fluorescence has proved susceptible to environmental changes, such as solvent environments (42) and non-specific interactions with proteins (48). This has largely prevented the use of CP-based biosensors in real applications. Wang *et al*. (48) reported that compensation for charges of CP significantly reduced non-specific signals arising from electrostatic interactions. However, in reality it is often difficult to identify all factors that lead to non-specific signals. We have demonstrated that the use of MMPs offers unprecedented advantages in this respect. MMPs are robust enough to allow repetitive washing under moderately stringent conditions, thus exhibiting the ability to efficiently remove non-specific species.

Very recently, Gaylord *et al*. (49) also reported SNP detection by combining their CP/PNA detection with an S1 nuclease enzyme. Excellent selectivity was offered by the S1 nuclease enzyme, which specifically digested all ssDNA and mismatched DNA in the absence of protection by the enzyme-resistant PNA. However, their assay strategy relies on enzyme digestion that requires incubation at 37°C for 1 h. In contrast, it typically takes <30 min to perform SNP detection with our magnetically assisted DNA assay. This takes advantage of rapid magnetic separation (~30 s), which is in sharp contrast to conventional, tedious bioseparation processes (usually hours), e.g. chromatography and centrifuging.

Based on fluorescence superquenching, Kushon *et al*. (31) reported the use of CP-coated polystyrene microspheres to detect DNA hybridization. Their system is also suitable for SNP detection by utilizing PNA probes as well as optimizing assay temperature (32). We note that both polystyrene microspheres and MMPs provide a nearly homogeneous environment that facilitates DNA hybridization processes, and at the same time possess the advantages of solid-state sensors (e.g. washability and reusability). Moreover, separation of polystyrene microspheres requires exhaustive diafiltration, whereas MMPs can be separated from other species simply by applying the magnetic force. It is also possible to convert the present magnetically assisted DNA assay strategy to an automated chip-based assay format given the availability of microfluidic cells with MMP trapping abilities (50). We thus expect that such magnetically assisted assays should be highly generalizable to other biosensor systems with CP amplification and lead to a broad class of highly sensitive and highly selective CP-based biosensors.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at NAR Online.

ACKNOWLEDGEMENTS

We thank Professor Alan Heeger for discussions and for help in the preparation of the manuscript. We also appreciate helpful discussions with professors Zhizhou Zhang, Lin He and Jun Hu. This work was supported by the National Natural Science Foundation (20404016), the Shanghai Municipal Commission for Science and Technology (0452nm068, 03DZ14025), the Bio-X DNA computer consortium, the Ministry of Personnel and the Chinese Academy of Sciences. Funding to pay the Open Access publication charges for this article was provided by the Chinese Academy of Sciences.

Conflict of interest statement. None declared.

REFERENCES

1. Heller,M.J. (2002) DNA microarray technology: devices, systems, and applications. *Annu. Rev. Biomed. Eng*., **4**, 129–153.
2. Cao,Y.W.C., Jin,R.C. and Mirkin,C.A. (2002) Nanoparticles with Raman spectroscopic fingerprints for DNA and RNA detection. *Science*, **297**, 1536–1540.
3. Gaylord,B.S., Heeger,A.J. and Bazan,G.C. (2002) DNA detection using water-soluble conjugated polymers and peptide nucleic acid probes. *Proc. Natl Acad. Sci. USA*, **99**, 10954–10957.
4. Miao,W. and Bard,A.J. (2003) Electrogenerated chemiluminescence. 72. Determination of immobilized DNA and C-reactive protein on Au(111) electrodes using tris(2,2′-bipyridyl)ruthenium(II) Labels. *Anal. Chem*., **75**, 5825–5834.
5. Peterson,A.W., Wolf,L.K. and Georgiadis,R.M. (2002) Hybridization of mismatched or partially matched DNA at surfaces. *J. Am. Chem. Soc*., **124**, 14601–14607.
6. Cooper,M.A., Dultsev,F.N., Minson,T., Ostanin,V.P., Abell,C. and Klenerman,D. (2001) Direct and sensitive detection of a human virus by rupture event scanning. *Nat. Biotechnol*., **19**, 833–837.
7. Hook,F., Ray,A., Norden,B. and Kasemo,B. (2001) Characterization of PNA and DNA immobilization and subsequent hybridization with DNA using acoustic-shear-wave attenuation measurements. *Langmuir*, **17**, 8305–8312.
8. Fan,C., Plaxco,K.W. and Heeger,A.J. (2003) Electrochemical interrogation of conformational changes as a reagentless method for the sequence-specific detection of DNA. *Proc. Natl Acad. Sci. USA*, **100**, 9134–9137.
9. Boon,E.M., Ceres,D.M., Drummond,T.G., Hill,M.G. and Barton,J.K. (2000) Mutation detection by electrocatalysis at DNA-modified electrodes. *Nat. Biotechnol*., **18**, 1096–1100.
10. Fritz,J., Cooper,E.B., Gaudet,S., Sorger,P.K. and Manalis,S.R. (2002) Electronic detection of DNA by its intrinsic molecular charge. *Proc. Natl Acad. Sci. USA*, **99**, 14142–14146.
11. Patolsky,F., Lichtenstein,A. and Willner,I. (2001) Detection of single-base DNA mutations by enzyme-amplified electronic transduction. *Nat. Biotechnol*., **19**, 253–257.
12. Yu,C.J., Wan,Y.J., Yowanto,H., Li,J., Tao,C.L., James,M.D., Tan,C.L., Blackburn,G.F. and Meade,T.J. (2001) Electronic detection of single-base mismatches in DNA with ferrocene-modified probes. *J. Am. Chem. Soc*., **123**, 11155–11161.
13. Winzeler,E.A., Schena,M. and Davis,R.W. (1999) Fluorescence-based expression monitoring using microarrays. *Methods Enzymol*., **306**, 3–18.
14. Cox,M.M. and Nelson,D.L. (2000) *Lehninger Principles of Biochemistry, 3rd edn*. Worth Publishers, NY.
15. Tyagi,S. and Kramer,F.R. (1996) Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol*., **14**, 303–308.
16. Tyagi,S. and Kramer,F.R. (1999) Thermodynamic basis of the enhanced specificity of structured DNA probes. *Proc. Natl Acad. Sci. USA*, **96**, 6171–6176.
17. Dubertret,B., Calame,M. and Libchaber,A.J. (2001) Single-mismatch detection using gold-quenched fluorescent oligonucleotides. *Nat. Biotechnol*., **19**, 365–370.
18. Han,M., Gao,X., Su,J.Z. and Nie,S. (2001) Quantum-dot-tagged microbeads for multiplexed optical coding of biomolecules. *Nat. Biotechnol*., **19**, 631–635.

19. Alivisatos,P. (2004) The use of nanocrystals in biological detection. *Nat. Biotechnol.*, **22**, 47–52.
20. Dejneka,M.J., Streltsov,A., Pal,S., Frutos,A.G., Powell,C.L., Yost,K., Yuen,P.K., Müller,U. and Lahiri,J. (2003) Rare earth-doped glass microbarcodes. *Proc. Natl Acad. Sci. USA*, **100**, 389–393.
21. Heeger,A.J. (2001) Nobel Lecture: Semiconducting and metallic polymers: The fourth generation of polymeric materials. *Rev. Mod. Phys.*, **73**, 681–700.
22. Chen,L., McBranch,D.W., Wang,H., Helgeson,R., Wudl,F. and Whitten,D.G. (1999) Highly sensitive biological and chemical sensors based on reversible fluorescence quenching in a conjugated polymer. *Proc. Natl Acad. Sci. USA*, **96**, 12287–12292.
23. Heeger,P.S. and Heeger,A.J. (1999) Making sense of polymer-based biosensors. *Proc. Natl Acad. Sci. USA*, **96**, 12219–12221.
24. Fan,C., Plaxco,K.W. and Heeger,A.J. (2002) High-efficiency fluorescence quenching of conjugated polymers by proteins. *J. Am. Chem. Soc.*, **124**, 5642–5643.
25. Fan,C., Wang,S., Hong,J.W., Bazan,G.C., Plaxco,K.W. and Heeger,A.J. (2003) Beyond superquenching: hyper-efficient energy transfer from conjugated polymers to gold nanoparticles. *Proc. Natl Acad. Sci. USA*, **100**, 6297–6301.
26. Swager,T.M. (1998) The molecular wire approach to sensory signal amplification. *Acc. Chem. Res.*, **31**, 201–207.
27. Swager,T.M. and Wosnick,J.H. (2002) Self-amplifying semiconducting polymers for chemical sensors. *MRS Bull.*, **27**, 446–450.
28. Kumaraswamy,S., Bergstedt,T., Shi,X., Rininsland,F., Kushon,S., Xia,W., Ley,K., Achyuthan,K.E., McBranch,D. and Whitten,D. (2004) Fluorescent-conjugated polymer superquenching facilitates highly sensitive detection of proteases. *Proc. Natl Acad. Sci. USA*, **101**, 7511–7515.
29. Xia,W., Rininsland,F., Wittenburg,S.K., Shi,X., Achyuthan,K.E., McBranch,D.W. and Whitten,D.G. (2004) Applications of fluorescent polymer superquenching to high throughput screening assays for protein kinases. *Assay Drug Dev. Technol.*, **3**, 183–192.
30. Pinto,M.R. and Schanze,K.S. (2004) Amplified fluorescence sensing of protease activity with conjugated polyelectrolytes. *Proc. Natl Acad. Sci. USA*, **101**, 7505–7510.
31. Kushon,S.A., Ley,K.D., Bradford,K., Jones,R.M., McBranch,D. and Whitten,D. (2002) Detection of DNA hybridization via fluorescent polymer superquenching. *Langmuir*, **18**, 7245–7249.
32. Kushon,S.A., Bradford,K., Marin,V., Suhrada,C., Armitage,B.A., McBranch,D. and Whitten,D. (2003) Detection of single nucleotide mismatches via fluorescent polymer superquenching. *Langmuir*, **19**, 6456–6464.
33. Gaylord,B.S., Bazan,G.C. and Heeger,A.J. (2003) DNA hybridization detection with water-soluble conjugated polymers and chromophore-labeled single-stranded DNA. *J. Am. Chem. Soc.*, **125**, 896–900.
34. Willner,I. and Katz,E. (2003) Magnetic control of electrocatalytic and bioelectrocatalytic processes. *Angew. Chem. Int. Ed. Engl.*, **42**, 4576–4588.
35. Nam,J.-M., Thaxton,C.S. and Mirkin,C.A. (2003) Nanoparticle-based bio-bar codes for the ultrasensitive detection of proteins. *Science*, **301**, 1884–1886.
36. Huang,F., Wu,H., Wang,D., Yang,W. and Cao,Y. (2004) Novel electroluminescent conjugated polyelectrolytes based on polyfluorene. *Chem. Mater.*, **16**, 708–716.
37. Nilsson,P., Persson,B., Uhlen,M. and Nygren,P.-A. (1995) Real-time monitoring of DNA manipulations using biosensor technology. *Anal. Biochem.*, **224**, 400–408.
38. Lakowicz,J.R. (1999) *Principles of Fluorescence Spectroscopy, 2nd edn*. Kluwer Academic Publishers, NY.
39. Nielsen,P.E. and Egholm,M. (1999) *Peptide Nucleic Acids: Protocols and Applications*. Horizon Scientific Press, Portland, OR.
40. Park,S.J., Taton,T.A. and Mirkin,C.A. (2002) Array-based electrical detection of DNA with nanoparticle probes. *Science*, **295**, 1503–1506.
41. Easton,D.F., Ford,D. and Bishop,D.T. (1995) Breast and ovarian cancer incidence in BRCA1-mutation carriers. *Am. J. Hum. Genet.*, **56**, 1284–1297.
42. Xu,Q., Gaylord,B.S., Wang,S., Bazan,G.C., Moses,D. and Heeger,A.J. (2004) Time-resolved energy transfer in DNA sequence detection using water-soluble conjugated polymers: the role of electrostatic and hydrophobic interactions. *Proc. Natl Acad. Sci. USA*, **101**, 11634–11639.
43. Liu,B., Wang,S., Bazan,G.C. and Mikhailovsky,A. (2003) Shape-adaptable water-soluble conjugated polymers. *J. Am. Chem. Soc.*, **125**, 13306–13307.
44. Liu,B., Gaylord,B.S., Wang,S. and Bazan,G.C. (2003) Effect of chromophore-charge distance on the energy transfer properties of water-soluble conjugated oligomers. *J. Am. Chem. Soc.*, **125**, 6705–6714.
45. Nelson,B.P., Grimsrud,T.E., Liles,M.R., Goodman,R.M. and Corn,R.M. (2001) Surface plasma resonance imaging measurements of DNA and RNA hybridization adsorption onto DNA microarrays. *Anal. Chem.*, **73**, 1–7.
46. Hames,B.D. and Higgins,S.J. (1985) *Nucleic acid hybridization-A Practical Approach*. Oxford Univeristy Press, Oxford.
47. Liu,B. and Bazan,G.C. (2004) Interpolyelectrolyte complexes of conjugated copolymers and DNA: platforms for multicolor biosensors. *J. Am. Chem. Soc.*, **126**, 1942–1943.
48. Wang,D., Gong,X., Heeger,P.S., Rininsland,F., Bazan,G.C. and Heeger,A.J. (2002) Biosensors from conjugated polyelectrolyte complexes. *Proc. Natl Acad. Sci. USA*, **99**, 49–53.
49. Gaylord,B.S., Massie,M.R., Feinstein,S.C. and Bazan,G.C. (2005) SNP detection using peptide nucleic acid probes and conjugated polymers: applications in neurodegenerative disease identification. *Proc. Natl Acad. Sci. USA*, **102**, 34–39.
50. Mirowski,E., Moreland,J., Russek,S.E. and Donahue,M.J. (2004) Integrated microfluidic isolation platform for magnetic particle manipulation in biological systems. *Appl. Phys. Lett.*, **84**, 1786–1788.