ACS Macro Letters

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Label-Free DNA Sequence Detection through FRET from a Fluorescent Polymer with Pyrene Excimer to SG

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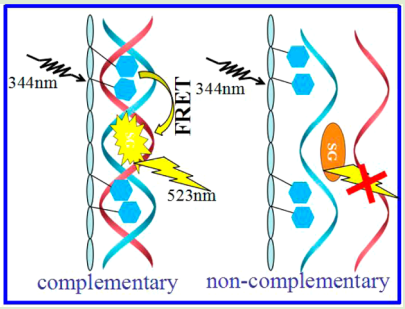
Supporting Information

ABSTRACT: A label-free complex probe composed of a water-soluble fluorescent pyrene-functionalized polymer, ssDNA, and a nucleic acid stain (SG) is presented here, which can detect DNA sequence via FRET from pyrene excimer to SG. Complementary and one-base mismatched strands at nanomolar concentrations can be distinguished by the examination of the FRET fluorescence intensity of SG. This novel strategy for detecting DNA using the fluorescent pyrene-functionalized polymer not only affords a simple label-free method to detect nucleic acid sequence but also endows the detection with high sensitivity and selectivity, which may find wide applications for optical biosensing.



Fluorescent biosensors for DNA detection have received great attention for their potential applications in disease diagnosis and biomedical studies.[1−3] A major challenge in DNA sequence detection is to achieve high sensitivity and excellent selectivity, in particular, the capability of detecting single nucleotide mismatch.[4,5] Water-soluble conjugated polymers (CPs), which are characterized by delocalized π-electronic backbone structures, can function as light-harvesting materials and have been successfully employed as optical probes for DNA detection via fluorescence resonance energy transfer (FRET).[6−8] To improve the sensitivity of DNA sensors, efficient FRET from conjugated polymers through fluorescein attached at a DNA terminus to DNA intercalated dyes[9] and from tetrahedral molecules with specific shape and spatial registry to fluorescence-labeled DNA[10] have been reported, while which lost the advantage of label-free detection and increased the protocol complexity and the cost. Xu et al.[11] used a combination of CPs and DNA intercalator to recognize G-quadruplex, and Tan et al.[12,13] designed multiple-pyrene labeled molecular beacons which could detect DNA sequences with high sensitivity, but their methods were limited to G-rich structure or hairpin like DNA stem-loop structure, which lacked universality. For high selectivity, the detection and recognition of a single mismatch has been the focus for biosensing.[14,15] A variety of mismatch detection methods using oligonucleotide probes have been developed for identifying nucleic acid hybridization and specific sequence variations.[16−20] However, these methods needed complex processes such as multiple steps or layer-by-layer fabrication or chromophore labeling.

Recently, we presented a simple label-free complex probe composed of nonconjugated fluorescent polyelectrolytes and ssDNA,[21−23] which could detect DNA hybridization by a decrease in fluorescence resulted from the intercalation of pyrene into the dsDNA duplex, while the fluorescence turn-off assay for hybridization detection might limit the sensitivity and selectivity. In this report, we demonstrate a novel label-free complex probe composed of a water-soluble fluorescent pyrene-functionalized polymer, ssDNA and nucleic acid stain SYBR Green I (SG), which can detect target DNA sequence via FRET from pyrene excimer to SG. This novel strategy not only affords a simple label-free method to detect nucleic acid sequence, but also endows the detection with high sensitivity and selectivity at nanomolar concentration of target DNA.

The water-soluble fluorescent pyrene-functionalized polymer, poly(pyrenylmethyl acrylate-*co*-(dimethylamino) ethyl methacrylate) (PDP), was synthesized by free-radical copolymerization of 1-pyrenylmethyl acrylate and 2-(dimethylamino) ethyl methacrylate (DMAEMA). The detailed procedure of the synthesis and the ^{1}H NMR, ^{13}C NMR, and FTIR spectra of the polymer PDP are shown in the Supporting Information (Scheme S1 and Figures S1 and S2). The number-average molecular weight (M_n) and molecular weight distribution (M_w/M_n) of the random copolymer were about 1.8×10^4 g/mol and 1.21, respectively, as determined by gel permeation chromatography (GPC). The molar ratio of 1-pyrenylmethyl acrylate to DMAEMA was determined to be 1:7 based on the UV−vis absorption of pyrene. The fluorescence quantum yield (Φ_F) of the polymer PDP was determined to be 0.31, using quinine sulfate as a reference ($\Phi_F = 0.55$). The chemical structure of the polymer PDP and its absorption and fluorescence spectra in PBS buffer (10 mM, pH = 7.4) are shown in Figure 1. The two fluorescence bands in the wavelength ranges of 360−410 nm and 420−600 nm are attributed to the pyrene monomer emission and excimer emission, respectively.[24]

SYBR Green I (SG), which is a DNA intercalating dye, can bind to double-stranded DNA (dsDNA), and thus, the

Received: June 24, 2014
Accepted: August 9, 2014
Published: August 12, 2014

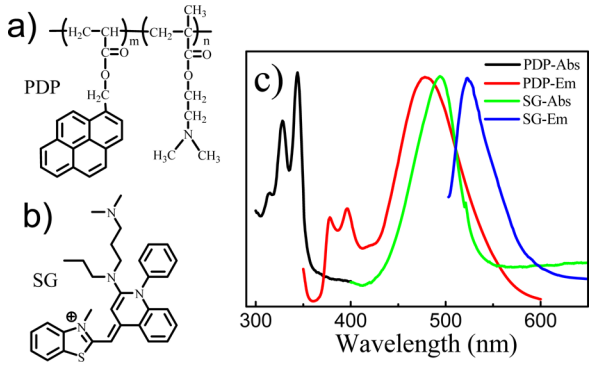
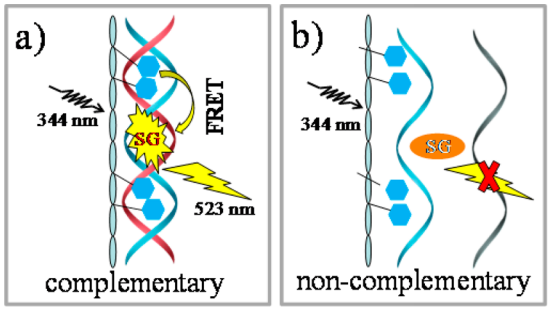


Figure 1. (a, b) Chemical structures of the fluorescent pyrene-functionalized polymer PDP and SG, respectively; (c) UV−vis absorption and fluorescence spectra of PDP and SG in PBS buffer. For fluorescence of PDP, excited at 344 nm; for fluorescence of SG, excited at 490 nm.

fluorescence of SG will be enhanced, since it is known that SG quenching in the free state has a intrinsic intramolecular origin and the SG fluorescence enhancement in complex with dsDNA can be explained by a dampening of its intramolecular motions.[25] However, owing to its nonspecific binding to nontarget nucleic acids, SG has a strong background fluorescence, as will restrict the detection sensitivity and selectivity.[26,27] Here we introduced FRET from pyrene excimer to SG to detect target DNA. Generally, the FRET process requires that the emission band of the donor overlaps the absorption band of the acceptor, and the distance between them can be within the Förster radius. It is noted that the absorption spectrum of SG almost completely overlaps with the emission spectrum of PDP shown in Figure 1c, thus, the efficient energy transfer from PDP to SG can occur. Scheme 1

Scheme 1. Schematic Representation of the Complex Probe (PDP/ssDNA/SG) Detecting Target DNA, in the Presence of the Complementary (a) and Noncomplementary (b) DNA



presents the simple and general protocol for label-free detection of DNA hybridization by the FRET. DNA strands could attach to the polymer PDP through the electrostatic interaction between the protonated amines of PDP and the anionic phosphates of DNA. The complex probe composed of the fluorescent polymer PDP, ssDNA, and SG will exhibit FRET fluorescence in the presence of the complementary DNA, since SG chromophores can be intercalated into the formed duplex and the FRET fluorescence of SG occurs; while in the presence of the noncomplementary DNA, the FRET fluorescence will not occur because of the quenching of SG in its free state and the decrease in distance between the polymer PDP and SG.

The following DNA oligonucleotides were used in this work. $ssDNA_1$ (3′-CGT GTA TGT AAG ATG AAC-5′) was used to form a complex probe. $ssDNA_2$ (5′-GCA CAT ACA TTC TAC TTG-3′) is complementary with $ssDNA_1$, while $ssDNA_a$ (5′-AAA AAA AAA AAA AAA AAA-3′), $ssDNA_c$ (5′-CCC CCC CCC CCC CCC CCC-3′), and $ssDNA_t$ (5′-TTT TTT TTT TTT TTT TTT-3′) are noncomplementary with $ssDNA_1$. $ssDNA_{1nc}$ (5′-GCA CAT A**T**A TTC TAC TTG-3′) and $ssDNA_{3nc}$ (5′-GCA **G**AT AC**C** TTC TA**A** TTG-3′) are one-base mismatched and three-base mismatched DNA strands, respectively.

The fluorescence spectra of the complex probe excited at the absorption wavelength of pyrene (344 nm) with different concentrations of SG in the presence of the complementary ssDNA are shown in the Supporting Information (Figure S4). With the increase of the concentration of SG, the pyrene excimer emission centered at 490 nm decreased and the SG emission centered at 523 nm increased. However, the SG emission did not appear when excited at 344 nm in the absence of dsDNA. The appearance of SG emission and the decrease of pyrene excimer emission when excited at the absorption wavelength of pyrene, where SG has negligible absorption, confirmed the FRET from pyrene excimer to SG in the system. When the concentration of SG increased to 5×10^{-7} M, the FRET fluorescence of SG reached the maximum. Thus, the concentration of SG with 5×10^{-7} M was used to investigate the detection selectivity and sensitivity in this work, where the ratio of SG to pyrene was calculated to be 1−2. Figure 2a shows the emission spectra of the complex probe composed of PDP, $ssDNA_1$, and SG upon adding complementary or non-complementary ssDNA at the concentration of 1.0×10^{-7} M. Upon adding the noncomplementary ssDNA, such as $ssDNA_a$, $ssDNA_c$, and $ssDNA_t$, only a weak fluorescence band of SG

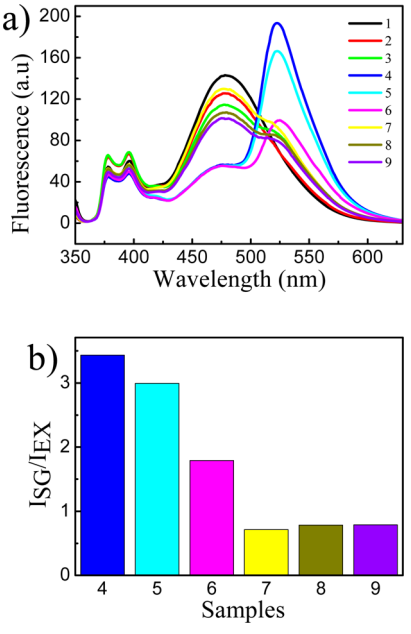


Figure 2. (a) Fluorescence spectra of PDP (1); PDP/SG (2); PDP/$ssDNA_1$/SG (3); PDP/$ssDNA_1$/SG with complementary $ssDNA_2$ (4), one-base mismatched DNA_{1nc} (5), three-base mismatched DNA_{3nc} (6), noncomplementary $ssDNA_a$ (7), $ssDNA_t$ (8) and $ssDNA_c$ (9), respectively. λ_{ex} = 344 nm; [PDP] = 1.0×10^{-7} M; [SG] = 5.0×10^{-7} M; [ssDNA] = 1.0×10^{-7} M; in PBS buffer. (b) Fluorescence intensity ratios (I_{SG}/I_{EX}) for the samples 4−9 obtained from (a).

dx.doi.org/10.1021/mz500378c | ACS Macro Lett. 2014, 3, 845−848

centered at 523 nm could be seen, while upon adding the three-base mismatched $ssDNA_{3nc}$, an obvious FRET fluorescence of SG could be noticed. Upon adding the one-base mismatched $ssDNA_{1nc}$, and the complementary $ssDNA_2$, the FRET fluorescence of SG increased greatly. It should be noted that the difference in FRET fluorescence of SG was quite clear even when the probe encountered the one-base mismatched $ssDNA_{1nc}$ and the complementary $ssDNA_2$. While for the probe using SG only, SG showed strong background fluorescence and the fluorescence intensity when mixed with the one-base mismatched DNA could not be distinguished from that when mixed with the complementary DNA (Figure S5, Supporting Information). Figure 2b shows the SG-to-pyrene excimer fluorescence intensity ratios (I_{SG}/I_{EX}) of the probe when mixed with ssDNAs with different sequences, which showed a general trend of ssDNA2 (3.4) > $ssDNA_{1nc}$ (2.9) > $ssDNA_{3nc}$ (1.8) > $ssDNA_a$ (0.7), $ssDNA_c$ (0.8), and $ssDNA_t$ (0.8) under identical conditions. The distinctive difference in the FRET fluorescence of SG endowed the probe with excellent selectivity.

Besides the selectivity, the sensitivity of the complex probe under different concentrations of target DNA was also investigated. The fluorescence of the complex probe composed of PDP, $ssDNA_1$, and SG in PBS buffer ([PDP] = 1.0×10^{-7} M, [$ssDNA_1$] = 1.0×10^{-7} M, and [SG] = 5.0×10^{-7} M) was explored when mixed with different concentrations of ssDNA for detection ($ssDNA_2$, $ssDNA_{1nc}$, $ssDNA_{3nc}$, $ssDNA_a$, from 100 to 3 nM), excited at the absorption wavelength of pyrene (344 nm), see the Supporting Information (Figure S6). Figure 3

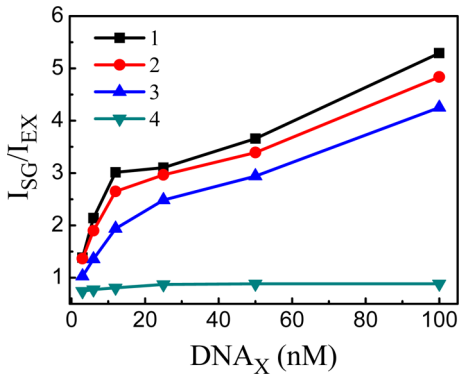


Figure 3. Fluorescence intensity ratios (I_{SG}/I_{EX}) for the complex probe (PDP/$ssDNA_1$/SG) as a function of concentration of $ssDNA_2$ (1), $ssDNA_{1nc}$ (2), $ssDNA_{3nc}$ (3), and $ssDNA_a$ (4). λ_{ex} = 344 nm, [PDP] = 1.0×10^{-7} M, [$ssDNA_1$] = 1.0×10^{-7} M, [SG] = 5.0×10^{-7} M in PBS buffer.

shows the fluorescence intensity ratios (I_{SG}/I_{EX}) as a function of the concentration of the ssDNAs for detection. For the three-base mismatched $ssDNA_{3nc}$, one-base mismatched $ssDNA_{1nc}$ and complementary $ssDNA_2$, the ratio I_{SG}/I_{EX} increased with the increase of their concentrations. While for the noncomplementary $ssDNA_a$, the ratio was very low and increased little with the increase of its concentration. It should be noted that the one-base mismatched and complementary ssDNA could be still distinguished when their concentrations were as low as 6 nM.

The complex probe composed of the fluorescent polymer PDP, ssDNA, and SG could detect DNA sequences not only in PBS buffer with pH 7.4, as shown above, but also in Tris-HCl buffer with pH 9.0 (Figure S8, Supporting Information). In addition, the strategy for detection of DNA sequences based on the FRET fluorescence change can be still practical when the DNA sequence has more bases. Figure S9 (Supporting Information) shows that the fluorescence changes of the complex probe composed of a DNA with 30 bases could be used to distinguish the complementary DNA from the noncomplementary DNA under the concentration of 10 nM.

In summary, here we report a novel label-free complex probe composed of a water-soluble fluorescent pyrene-functionalized polymer, ssDNA and a nucleic acid stain (SG), which can detect DNA sequences via FRET from pyrene excimer to SG. The complementary ssDNA could be distinguished from the single-mismatched strand at nanomolar concentrations by fluorescence changes of the complex probe. This novel strategy with the fluorescent pyrene-functionalized polymer not only affords a simple label-free method to detect nucleic acid sequence, but also endows the detection with high sensitivity and selectivity, which may find wide applications for optical biosensing.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Grant Nos. 21074010 and 51373025), Beijing Municipal Natural Science Foundation (Grant No. 2112029), the Program for New Century Excellent Talents in University (NCET-11-0582), and the Fundamental Research Funds for the Central Universities (FRE-TP-12-004B).

■ REFERENCES

(1) Marras, S. A. E.; Tyagi, S.; Kramer, F. R. *Clin. Chim. Acta* **2006**, *363*, 48−60.
(2) Zhu, J.; Lu, Y.; Deng, C.; Huang, G.; Chen, S.; Xu, S.; Lv, Y.; Mitchelson, K.; Cheng, J. *Anal. Chem.* **2010**, *82*, 5304−5312.
(3) Martin, J.; Meng, L.; Phillips, J. A.; Kim, Y. M.; Tan, W. H. *Leukemia* **2009**, *23*, 235−244.
(4) He, F.; Tang, Y.; Yu, M.; Feng, F.; An, L.; Sun, H.; Wang, S.; Li, Y.; Zhu, D.; Bazan, G. C. *J. Am. Chem. Soc.* **2006**, *128*, 6764−6765.
(5) Ren, X.; Xu, Q. H. *Langmuir* **2009**, *25*, 43−47.
(6) Feng, X.; Liu, L.; Wang, S.; Zhu, D. *Chem. Soc. Rev.* **2010**, *39*, 2411−2419.
(7) Feng, F.; He, F.; An, L.; Wang, S.; Li, Y.; Zhu, D. *Adv. Mater.* **2008**, *20*, 2959−2964.
(8) Feng, X.; Duan, X.; Liu, L.; Feng, F.; Wang, S.; Li, Y.; Zhu, D. *Angew. Chem., Int. Ed.* **2009**, *121*, 5420−5425.
(9) Wang, S.; Gaylord, B. S.; Bazan, G. C. *J. Am. Chem. Soc.* **2004**, *126*, 5446−5451.
(10) Liu, B.; Dan, T. T. T.; Bazan, G. C. *Adv. Funct. Mater.* **2007**, *17*, 2432−2438.
(11) Xu, H.; Gao, S.; Yang, Q.; Pan, D.; Wang, L.; Fan, C. *ACS Appl. Mater. Interfaces* **2010**, *2*, 3211−3216.
(12) Huang, J.; Wu, Y.; Chen, Y.; Zhu, Z.; Yang, X.; Yang, C. J.; Wang, K.; Tan, W. *Angew. Chem., Int. Ed.* **2011**, *50*, 401−404.

(13) Conlon, P.; Yang, C. J.; Wu, Y.; Chen, Y.; Martinez, K.; Kim, Y.; Stevens, N.; Marti, A. A.; Jockusch, S.; Turro, N. J.; Tan, W. *J. Am. Chem. Soc.* **2008**, *130*, 336−342.
(14) Feng, X.; Duan, X.; Liu, L.; An, L.; Feng, F.; Wang, S. *Langmuir* **2008**, *24*, 12138−12141.
(15) Jiang, Z.; Zhang, Y.; Yu, Y.; Wang, Z.; Zhang, X. *Langmuir* **2010**, *26*, 13773−13777.
(16) Feng, X.; Liu, L.; Yang, Q.; Wang, S. *Chem. Commun.* **2011**, *47*, 5783−5785.
(17) Tang, Y.; He, F.; Wang, S.; Li, Y.; Zhu, D.; Bazan, G. C. *Adv. Mater.* **2006**, *18*, 2105−2110.
(18) Kang, J.; Loew, M.; Arbuzova, A.; Andreou, I.; Dähne, L. *Adv. Mater.* **2010**, *22*, 3548−3552.
(19) Karadag, A.; Riminucci, M.; Bianco, P.; Cherman, N.; Kuznetsov, S. A.; Nguyen, N.; Collins, M. T.; Robey, P. G.; Fisher, L. W. *Nucleic Acids Res.* **2004**, *32*, e63.
(20) Rashatasakhon, P.; Vongnam, K.; Siripornnoppakhun, W.; Vilaivan, T.; Sukwattanasinitt, M. *Talanta* **2012**, *88*, 593−598.
(21) Yang, L.; Zhao, M.; Zhang, R.; Dong, J.; Zhang, T.; Zhan, X.; Wang, G. *ChemPhysChem* **2012**, *13*, 1−6.
(22) Zhao, M.; Yang, L.; Zhang, R.; Dong, J.; Dong, H.; Wen, Y.; Zhan, X.; Wang, G.; Lu, Y.; Wang, G. *Polymer* **2013**, *54*, 297−302.
(23) Zhang, R.; Yang, L.; Zhao, M.; Dong, J.; Dong, H.; Wen, Y.; Zhan, X.; Yang, H.; Wang, G. *Polymer* **2013**, *54*, 1289−1294.
(24) Wang, G.; Bobkov, G. V.; Mikhailov, S. N.; Schepers, G.; Van Aerschot, A.; Rozenski, J.; Van der Auweraer, M.; Herdewijn, P.; De Feyter, S. *ChemBioChem* **2009**, *10*, 1175−1185.
(25) Dragan, A. I.; Pavlovic, R.; McGivney, J. B.; Casas-Finet, J. R.; Bishop, E. S.; Strouse, R. J.; Schenerman, M. A.; Geddes, C. D. *J. Fluoresc.* **2012**, *22*, 1189−1199.
(26) Fang, J. M.; Leng, F.; Zhao, X. J.; Hu, X. L.; Li, Y. F. *Analyst* **2014**, *139*, 801−806.
(27) Lee, K.; Maisel, K.; Rouillard, J.; Gulari, E.; Kim, J. *Chem. Mater.* **2008**, *20*, 2848−2850.