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Rational Designed Bipolar, Conjugated Polymer-DNA Composite Beacon for the Sensitive Detection of Proteins and Ions

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ABSTRACT: Nature owns remarkable capabilities in sensing target molecule. While, the artificial biosensor lags far behind nature. Inspired by nature, we devise a new sensing platform that can specifically bind the molecules and synchronized initiate a specific signal response. We rationally designed a type of bipolar probe that is comprised of hydrophilic DNA part and hydrophobic conjugated polymer (CP) unit. In aqueous solution, they can form micelles with a hydrophobic CP core and a hydrophilic DNA shell. The aggregation-caused quenching suppresses the fluorescence of CP. Adding telomerase, the hydropathical profile of the bipolar probes is drastically regulated that results the collapse of micelles and liberates fluorescence simultaneously. The probe has been used in both mimic systems and real urine samples (38 samples). We achieve sensitive and specific detection of telomerase and obtain clearly classification for normal people and cancer patients. It can also be used in signal off sensor that used to detect mercury ions.

INTRODUCTION

The development of biosensing technology largely relies upon the invention of powerful bioprobes, which determine the overall performance of biosensors including sensitivity and specificity[1-4]. Molecular beacons, by reporting the presence of specific nucleic acids targets in homogenous solution upon target hybridization, have revolutionized the nucleic acids detection both in vivo and in vitro[5-8]. However, the targets of molecular beacons are limited to nucleic acids. As an expansion of molecular beacon, aptamers are widely employed as aptamer-beacon with the detection capability of various targets including proteins[9-11], small molecules[12,13], and ions[14,15]. Both the molecular beacons and aptamer beacons undergo conformational switch upon target binding, which can be translated into fluorescent[16-18], electrochemical[19-26] or colorimetric signal[14,27,28]. In order to transduce the binding events into physical/chemical signals, fluorescent or redox labels are usually required to be conjugated on molecular beacons or aptamers[29-38]. Although the signal producing capability of the labels have been employed and investigated very well, the utility of some other chemical and physical properties (e.g. hydrophobic properties) are usually ignored.

Here, we report on a new class of bipolar, conjugated polymer-DNA composite beacon, which can be employed as a beacon for the sensitive detection of proteins and ions. Based on our new bipolar probe, we can readily design both signal-on sensor mode and signal-off sensor mode (Scheme 1). In our design, we firstly synthesize conjugated polymer-DNA (CP-c-DNA) bipolar beacon. The hydrophobic nature of conjugated polymer drives the formation of a micelle with a hydrophobic CP core and a hydrophilic DNA shell. Then, the target binding induces the collapse of micelles (signal-on sensor) or the further aggregation of micelles (signal-off sensor). As test beds, we select telomerase as a protein biomarker and mercury ion as metal ion marker. The binding of telomerase and the initiated elongation reaction by telomerase would significantly change the hydropathical property of the bipolar beacon that stimulates the collapse of micelles and liberates the fluorescence simultaneously. On the other hand, the binding of mercury ions drives the further aggregation of micelles based on the thymine-Hg-thymine interaction and quenches the fluorescence.

EXPERIMENTAL SECTION

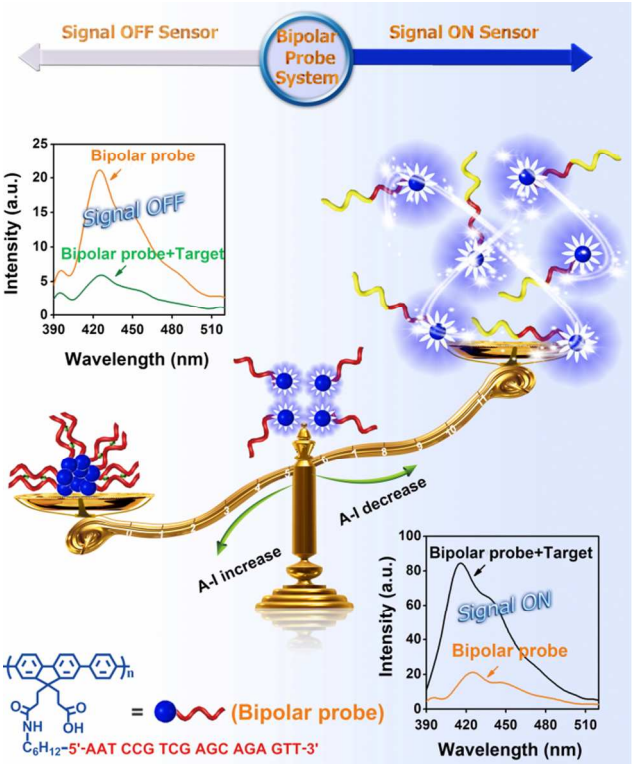
Materials and Measurements. Chemical solvents and reagents used in this work are purchased from TCI and Sigma-Aldrich and Alfa Aesar. The oligonucleotides and deoxynucleotide triphosphates (dNTPs) are purchased by TaKaRaba Biotech (Dalian, China). Carboxylated Polyfluorene is synthesized according to the literature[39]. The fluorescence spectra are recorded on a Cary Eclipse F-4500 spectrofluorometer. ^{1}HNMR spectra are obtained with a Bruker AVANCE 400 (400 MHz) Fourier transform NMR spectrometer. Native gel

electrophoretic analysis is performed by ELITE 300 electrophoresis apparatus (Taiwan Wealtec corp). The reagents are removed by rotary evaporation (RE-5IAA, Shanghai). All aqueous solutions are prepared with ultrapure water purified using a Millipore filtration system.

Increasing the Relative Content of Hydrophilic Parts. Mixtures of equal amounts of single-stranded oligonucleotides (CP-c-Ts & LDNA) are diluted with buffer to the desired concentration (total sample volume: 50 μL), and T4 DNA ligase incubated at 16 °C for 1d. Then the reaction mixture solution is heated at 95 °C for 20 min. Finally, 150 μL of water is added, and the emission spectrum is immediately measured. Fluorescence is measured on a Peltier temperature controlled Varian Cary Eclipse Fluorimeter with the following settings: λex = 380 nm, 5 nm slit, PMT detector voltage = 600 V.

Increasing the Relative Content of Hydrophobic Parts. 50 μL of aqueous solution of CP-c-Ts and CF-c-DNA are prepared for fluorescence measurements respectively. Fluorescence is measured on a Peltier temperature controlled Varian Cary Eclipse Fluorimeter with the following settings: λex = 380 nm, 5 nm slit, PMT detector voltage = 600 V.

RESULTS AND DISCUSSION



Scheme 1. Sensor fabrication. The bipolar probe is an amphiphilic molecule. The amphiphilic nature of the bipolar probes drives the formation of micelles with a hydrophobic CP core and a hydrophilic DNA shell. The target binding induces the further aggregation of micelles, which is beneficial to the ACQ effect, quenches the fluorescence. Thus it can be used in signal-off sensor. While the target binding induces the collapse of micelles which is opposite to the ACQ effect liberates the fluorescence. So it can be used in signal-on sensor.

Sensor fabrication. The principle of the sensor is described in Scheme 1. The bipolar probe (CP-c-DNA) has two components, one is poly [9, 9-bis (3'-propanoate) fluorene-co-phenylene fluorene] (CP) that is a typical fluorogen material with aggregation-caused fluorescence quenching (ACQ) characteristics[40-42] and another one is DNA (Figure S1-5). Its amphiphilic nature arises from the hydrophilicity of DNA backbone containing charged phosphodiester bonds[43-46] and chemical attachment of hydrophobic conjugated polymer containing benzene ring structures. The amphiphilic nature of the bipolar probes drives the formation of micelles with a hydrophobic CP core and a hydrophilic DNA shell. The target binding induces the further aggregation of micelles, which is beneficial to the ACQ effect, quenches the fluorescence. Thus it can be used in signal-off sensor. While the target binding induces the collapse of micelles which is opposite to the ACQ effect liberates the fluorescence. So it can be used in signal-on sensor. We also can tune its aggregation states by simply changing the relative content of DNA in the bipolar probe. At low content of DNA, the hydrophobic property of the bipolar probe is high. The aggregate state of hydrophobic CP core is favored and we observe low fluorescence signal according to ACQ effect. In contrast, at high content of DNA, hydrophilic property of the bipolar probe is high. The aggregate state of hydrophobic CP core is destabilized and we observe high fluorescence signal due to ACQ effect (Figure S6-13).

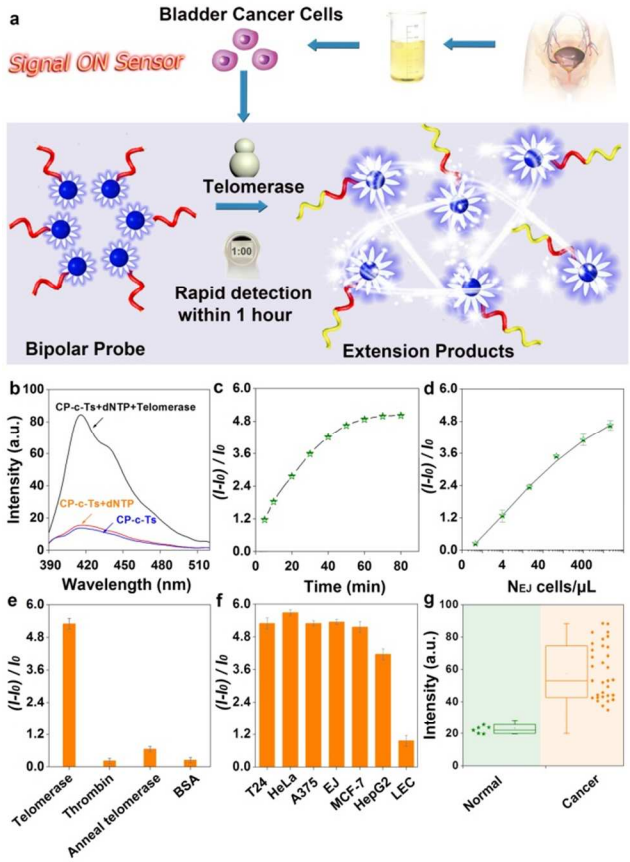


Figure 1. (**a**) Schematic representation of telomerase activity detection method using the hydrophilic/hydrophobic properties of the bipolar probes by changing the relative content of DNA. (**b**) Effect of telomerase on CP-c-Ts fluorescence signal in the presence of 250 nM CP-c-Ts and 2800 cancer cell extracts, respectively. (**c**) Time dependent fluorescence responses of our assay. (**d**) Fluorescence emission spectra are obtained in the presence of various concentrations of EJ cells. (**e**) Histogram for fluorescence intensities to investigate the specificity of our assay. (**f**) Fluorescence responses of this sensing system to the telomerase extracts

from 2800 cells of different kinds of cancer and non-cancer cell lines. (**g**) Fluorescence responses of this sensing system to the telomerase extracts from different bladder cancer patients and normal samples. Concentration of CP-c-Ts is 250 nM. Excitation wavelength is 380 nm, respectively, with 5-5 nm bandwidths. Error bars are calculated from three independent experiments.

Detection of protein. By achieving the successful regulation the hydropathical profile of our bipolar probes, we are inspired to employ this capability to detect stealthy biomarkers. Here, we choose telomerase as a model biomarker. Telomerase is a ribonucleic protein complex that functions as telomere terminal transferees by adding multiple TTAGGG hexamer repeats using its integral RNA as the template[47-49]. Its activity is associated with tumor aggressiveness because it can be detectable in most immortal cell lines and primary human tumors[50-54]. In the presence of telomerase, the signal-on sensor, CP-c-Ts is elongated, resulting in the increasing of the relative content of DNA which leads to higher hydrophilic property of extension product relatively to the original one. Thus, the aggregate state of hydrophobic CP core is destabilized and we observe a high fluorescence signal according to ACQ effect (Figure 1a). As shown in Figure 1b, when CP-c-Ts is mixed with dNTPs and telomerase in an aqueous buffer solution, after incubation at 37 ^{o}C for 60 min, drastic increase in fluorescence signal are observed. Figure 1c shows the fluorescence signal changes of CP-c-Ts in real time in the presence of telomerase and dNTPs. We observe a gradual increase of fluorescence signal and finally reach a plateau at approximately 60 minutes (Figure S14-15). Figure 1d depicts the fluorescence emission spectra of the sensing system in response to cell extracts from different number of bladder cancer (EJ) cells. The fluorescence intensities enhance quickly when the number is raised from 0 to 20000 (Figure S16-17). The limit of detection (LOD)[55], based on F+ 3σ_b, where F is the average signal value of the blank samples and σ_b is standard deviation of the blank samples, is estimated to be 4 EJ cell/μL. To the best of our knowledge, the sensitivity is comparable or even superior to most previously reported methods[56-59] (Table S2). As shown in Figure 1e, an obvious decrease in fluorescence signal nearly down to background level for the heat-inactivated EJ cells extract, thrombin and BSA, implying that the signal response is only dependent on telomerase activity. As shown in Figure 1f, the fluorescence signal enhancements for MCF-7 (breast cancer cell), T24 (bladder cancer cell), EJ (bladder cancer cell), HepG2 (liver cancer cell), A375 (melanoma cancer cell) and HeLa (cervical cancer cell) cells extracts are much higher than LEC (human lens epithelial cell), which illustrates the generality and reliability of our design.

Our telomerase probe is also tested in real clinic samples, bladder cancer patients' urine samples. As shown in Figure 1g, 38 clinical samples have been detected. The orange part is the fluorescence signal change of bladder cancer patients. The green part is the fluorescence signals change of normal people. We achieve sensitive and specific detection of the stealthy biomarker-telomerase and obtain clearly classification for cancer patients according to our results (Figure S18-20). Therefore, our assay based on tuning the aggregate state of the dipolar probe by simply changing the relative content of DNA offers a cost effectiveness, simplicity and non-invasive method for bladder cancer diagnosis, reducing the hurt comparing other diagnosis methods.

Detection of metal ion. Our bipolar probe can also be used for detecting ions based on signal-off mechanism by changing the aggregate state of hydrophobic parts which is beneficial to ACQ effect. We select mercury (Hg^{2+}) as a model ion in this case. Mercury (Hg^{2+}) is a widespread and severe environmental pollutant and has serious adverse effects on human health and the environment[60-64]. Therefore, it is highly desirable to develop a sensitive and selective mercury ion detection method that can provide simple, practical, and high-throughput routine determination of levels of Hg^{2+} ions for both environmental and food samples.

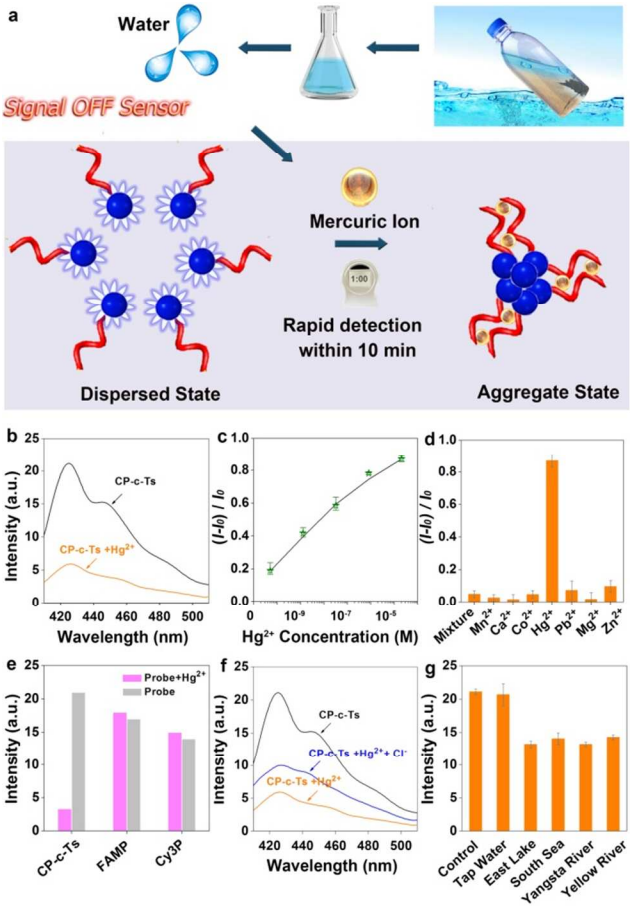


Figure 2. (**a**) Schematic illustration of the strategy of Hg^{2+} ion detection by tuning the aggregate state of the bipolar probe. (**b**) The fluorescence emission intensities without and with the addition of Hg^{2+}. (**c**) Fluorescence emission spectra of CP-c-DNA in the presence of various concentrations of Hg^{2+}. (**d**) Histogram for fluorescence signal to the presence of other metal ions. (**e**) Histogram for fluorescence signal to the presence of different composite probe. (**f**) Fluorescence spectra of probe in aqueous solution upon additions of Hg^{2+} and Cl^{-} ions. (**g**) Histogram for fluorescence signal to the presence of different aqueous solution of the bipolar probe. The concentration of CP-c-Ts is 250 nM. Excitation wavelength is 380 nm, respectively, with 5-5 nm bandwidths. Error bars are calculated from three independent experiments.

The configuration of the assay for Hg^{2+} detection is shown in Figure 2a. In the presence of Hg^{2+} ions, the hydrophobic parts CP are aggregated together because of the formation of T-Hg^{2+}-T complexes (Figure S21). It is beneficial to ACQ effect and we observe low fluorescence signal (Figure 2b). To evaluate the sensitivity of our assay, the assay is tested on

different concentrations of Hg^{2+} ions (0~2.1×10^{-4} M). The detection limit (LOD)[55] is determined to be 2.1×10^{-10} M, which shows that Hg^{2+} could be detected with high sensitivity in our assay (Figure 2c). The selectivity of the method has also been investigated by testing the response of the assay to other metal ions, including Mg^{2+}, Mn^{2+}, Pb^{2+}, Co^{2+}, Ca^{2+} and Zn^{2+} at a concentration of 2.1×10^{-3} M - 1000 times greater than that of the Hg^{2+} ions (Figure 2d). The results indicate that the bipolar probe CP-c-Ts exhibit excellent selectively responsive to Hg^{2+} against other possible competing ions. While, the presence of Hg^{2+} has no influence on the FAM or Cy3 modified bipolar probes' fluorescence signal (Figure 2e). What is more, this Hg^{2+} ion sensor based on our assay is readily reusable. The fluorescence of bipolar probe CP-c-Ts can be recovered upon addition of chlorine anions to the T-Hg^{2+}-T complexes. As shown in Figure 2f, the fluorescence of CP-c-Ts is quenched upon adding Hg^{2+} ion. While, the fluorescence signal of CP-c-Ts is recovered upon the addition of 2 equiv. of chlorine anions to the solution. Because of the lower solubility of $HgCl_2$ in aqueous solution in comparison to that of Hg^{2+} ions, the Cl^- ions can form a precipitate with Hg^{2+} ions. It is reasonable to design a reversible sensor for Hg^{2+} ions based on the fluorescence recovery of the composite probe CP-c-Ts.

To test the practical application of our method, several environmental water samples are tested by using our method. The environmental water samples that are used in the study are tap water, river water, lake water and sea water samples. The water samples are filtered by qualitative filter paper and then centrifuged for 20 min at 12000 rpm at 4 ^oC. As shown in Figure 2g, the concentrations of total mercury in tap water, river water, lake water and sea water samples are measured to be less than 2.1×10^{-4} nM by our method. The results revealed that the present sensor can also work in environmental samples.

CONCLUSIONS

In summary, we have developed an amphiphilic bipolar probe CP-c-DNA which is conjugated by conjugated polymers and DNA. Compared with other probes, it has the following advantage: First, it can be used in signal on sensor based on tuning its hydrophilic/hydrophobic property by simply changing the relative content of DNA. On the basis of this observation, a PCR free, sensitive, selective strategy for the stealthy biomarker-telomerase activity detection has been developed. In comparison with the golden method, the TRAP based method; our assay is accuracy and simplicity by avoiding numerous artifacts and sophisticated optimization. Our assay has been used in both mimic systems and real urine samples (38 clinical samples). We envision that this amphiphilic bipolar probe CP-c-Ts could be employed for the development of novel sensing techniques for the detection of various biomolecules and could be used for clinical bladder cancer therapy. Second, it can be used in signal off sensor based on tuning the aggregate state of hydrophobic parts which is beneficial to ACQ effect. On the basis of this observation, a highly sensitive and selective Hg^{2+} ion determination method at room temperature using T-Hg^{2+}-T coordination chemistry and fluorescence signal changes has been developed. Although we demonstrate here the detection of Hg^{2+} ions only, this sensing strategy can in principle be used to detect various analysts, such as other metal ions or proteins, by substituting the T-Hg^{2+}-T complexes with other specificity structures the selectively bind the other analysts. It is believed that this sensing strategy may be an alternative method for the analysis of Hg^{2+} in environment, water, and food samples.

ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

‡These authors contributed equally.

Notes

The authors declare no competing financial interests.

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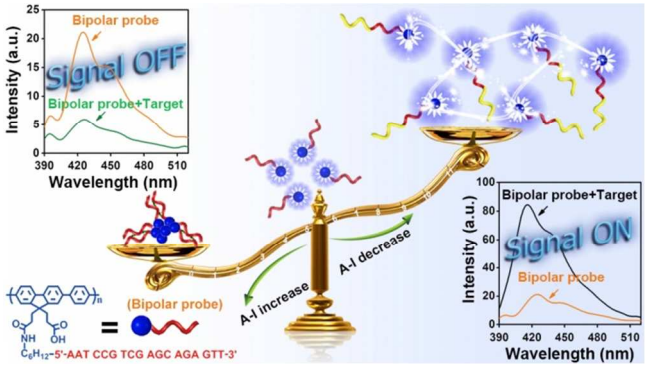
REFERENCES

(1) Vo-Dinh, T.; Alarie, J. P.; Cullum, B. M.; Griffin, G. D. *Nat. Biotechnol.* **2000**, *18*, 764-767.
(2) Yeh, P. H.; Li, Z.; Wang, Z. L. *Adv. Mater.* **2009**, *21*, 4975-4978.
(3) Tu, D. T.; Liu, L. Q.; Ju, Q.; Liu, Y. S.; Zhu, H. M.; Li, R. F.; Chen, X. Y. *Angew. Chem. Int. Ed.* **2011**, *50*, 6306-6310.
(4) Yu, Y.; Feng, C.; Hong, Y.; Liu, J.; Chen, S.; Ng, K. M.; Luo, K. Q.; Tang, B. Z. *Adv. Mater.* **2011**, *23*, 3298-3302.
(5) Tyagi, S.; Kramer, F. R. *Nat. Biotechnol.* **1996**, *14*, 303-308.
(6) Tyagi, S.; Bratu, D. P.; Kramer, F. R. *Nat. Biotechnol.* **1998**, *16*, 49-53.
(7) Zhang, X. B.; Wang, Z.; Xing, H.; Xiang, Y.; Lu, Y. *Anal. Chem.* **2010**, *82*, 5005-5011.
(8) Ricci, F.; Vallée-Bélisle, A.; Porchetta, A.; Plaxco, K. W. *J. Am. Chem. Soc.* **2012**, *134*, 15177-15180.
(9) Rotem, D.; Jayasinghe, L.; Salichou, M.; Bayley, H. *J. Am. Chem. Soc.* **2012**, *134*, 2781-2787.
(10) Zhou, W. J.; Halpern, A. R.; Seefeld, T. H.; Corn, R. M. *Anal. Chem.* **2011**, *84*, 440-445.
(11) Liu, J. B.; Fu, S. H.; Yuan, B.; Li, Y. L.; Deng, Z. X. *J. Am. Chem. Soc.* **2010**, *132*, 7279-7281.
(12) Yang, K. A.; Barbu, M.; Halim, M.; Pallavi, P.; Kim, B.; Kolpashchikov, D. M.; Pecic, S.; Taylor, S.; Worgall, T. S.; Stojanovic, M. N. *Nat. Chem.* **2014**, *6*, 1003-1008.
(13) Das, J.; Cederquist, K. B.; Zaragoza, A. A.; Lee, P. E.; Sargent, E. H.; Kelley, S. O. *Nat. Chem.* **2012**, *4*, 642-648.
(14) Freeman, R.; Liu, X.; Willner, I. *J. Am. Chem. Soc.* **2011**, *133*, 11597-11604.
(15) Kim, B.; Jung, I. H.; Kang, M.; Shim, H. K.; Woo, H. Y. *J. Am. Chem. Soc.* **2012**, *134*, 3133-3138.
(16) Liu, Z. B.; Chen, S. S.; Liu, B. W.; Wu, J. P.; Zhou, Y. B.; He, L. Y.; Ding, J. S.; Liu, J. W. *J. Am. Chem. Soc.* **2014**, *86*, 12229-12235.
(17) Yan, L. a.; Shi, H.; He, X. X.; Wang, K. M.; Tang, J. L.; Chen, M.; Ye, X. S.; Xu, F. Z.; Lei, Y. L. *Anal. Chem.* **2014**, *86*, 9271-9277.
(18) Zhang, W.; Liu, W.; Li, P.; Xiao, H. B.; Wang, H.; Tang, B. *Angew. Chem. Int. Ed.* **2014**, *126*, 12697-12701.

(19) Schoukroun-Barnes, L. R.; Wagan, S.; White, R. J. *Anal. Chem.* **2013**, *86*, 1131-1137.
(20) Das, J.; Cederquist, K. B.; Zaragoza, A. A.; Lee, P. E.; Sargent, E. H.; Kelley, S. O., *Nat. Chem.* **2012**, *4*, 642-648.
(21) Swensen, J. S.; Xiao, Y.; Ferguson, B. S.; Lubin, A. A.; Lai, R. Y.; Heeger, A. J.; Plaxco, K. W.; Soh, H. T. *J. Am. Chem. Soc.* **2009**, *131*, 4262-4266.
(22) Chai, X. L.; Zhou, X. G.; Zhu, A. W.; Zhang, L. M.; Qin, Y.; Shi, G. Y.; Tian, Y. *Angew. Chem. Int. Ed.* **2013**, *52*, 8129-8133.
(23) Lu, X. L.; Cheng, H. J.; Huang, P. C.; Yang, L. F.; Yu, P.; Mao, L. Q. *Anal. Chem.*, **2013**, *85*, 4007-4013.
(24) Zheng, T. T.; Tan, T. T.; Zhang, Q. F.; Fu, J. J.; Wu, J. J.; Zhang, K.; Zhu, J. J.; Wang, H., *Nanoscale*, **2013**, *5*, 10360-10368.
(25) Zhang, W. Y.; Zhang, W. H.; Liu, Z. Y.; Li, C.; Zhu, Z.; Yang, C. J. *Anal. Chem.* **2011**, *84*, 350-355.
(26) Wang, J.; Wei, T.; Li, X. Y.; Zhang, B. H.; Wang, J. X.; Huang, C.; Yuan, Q. *Angew. Chem. Int. Ed.* **2014**, *126*, 1642-1646.
(27) Zhang, H. Q.; Li, F.; Dever, B.; Li, X. F.; Le, X. C. *Chem. Rev.* **2012**, *113*, 2812-2841.
(28) Yin, B. C.; Ye, B. C.; Tan, W. H.; Wang, H.; Xie, C. C. *J. Am. Chem. Soc.* **2009**, *131*, 14624-14625.
(29) Jeong, J. E.; Kim, B.; Woo, S.; Hwang, S.; Bazan, G. C.; Woo, H. Y. *Chem. Sci.* **2015**, DOI: 10.1039/c 4 sc03258f
(30) Xu, J. G.; Dong, H. Y.; Shen, W. Y.; He, S. D.; Li, H. L.; Lu, Y. S.; Wu, Z. S.; Jia, L. *Biosens. Bioelectron.* **2015**, *66*, 504-511.
(31) Lu, L.; Si, J. C.; Gao, Z. F.; Zhang, Y.; Lei, J. L.; Luo, H. Q.; Li, N. B. *Biosens. Bioelectron.* **2015**, *63*, 14-20.
(32) Lin, C. S.; Chen, Y. Y.; Cai, Z. X.; Zhu, Z.; Jiang, Y. Q.; Yang, C. J.; Chen, X. *Biosens. Bioelectron.* **2015**, *63*, 562-565.
(33) Zang, Y.; Lei, J. P.; Hao, Q.; Ju, H. X. *ACS Appl. Mater. Inter.* **2014**, *6*, 15991-15997.
(34) Zheng, J.; Hu, Y. P.; Bai, J. H.; Ma, C.; Li, J. S.; Li, Y. H.; Shi, M. L.; Tan, W. H.; Yang, R. H. *Anal. Chem.* **2014**, *86*, 2205-2212.
(35) Wang, Y. H.; He, X. X.; Wang, K. M.; Ni, X. Q. *Biosens. Bioelectron.* **2010**, *25*, 2101-2106.
(36) Yamamoto, R.; Kumar, P. K. *Genes Cells*, **2000**, 5, 389-396.
(37) Fang, X. H.; Li, J. J.; Perlette, J.; Tan, W. H.; Wang, K. M. *Anal. Chem.* **2000**, *72*, 747 A-753A.
(38) Fang, X. H.; Li, J. J.; Tan, W. H. *Anal. Chem.* **2000**, *72*, 3280-3285.
(39) Zhang, Y.; Liu, B.; Cao, Y. *Chem. Asian. J.* **2008**, *3*, 739-745.
(40) Wang, S.; Bazan, G. C. *Chem. Commun.* **2004**, 2508-2509.
(41) Yuan, Y.; Kwok, R. T. K.; Tang, B. Z.; Liu, B. *J. Am. Chem. Soc.* **2014**, *136*, 2546-2554.
(42) Kwok, R. T.; Leung, C. W.; Lam, J. W.; Tang, B. Z. *Chem. Soc. Rev.* **2015**, DOI: 10.1039/C4CS00325J.
(43) Gregory, M. T.; Park, G. Y.; Johnstone, T. C.; Lee, Y. S.; Yang, W.; Lippard, S. J. *Proc. Natl. Acad. Sci. USA*, **2014**, *111*, 9133-9138.
(44) Miyazono, K. I.; Furuta, Y.; Watanabe-Matsui, M.; Miyakawa, T.; Ito, T.; Kobayashi, I.; Tanokura, M. *Nat. Commun.* **2014**, *5*, DOI:10.1038/ncomms4178.
(45) Jin, J.; Xing, Y. Z.; Xi, Y. L.; Liu, X. L.; Zhou, T.; Ma, X. X.; Yang, Z. Q.; Wang, S. T.; Liu, D. S. *Adv. Mater.* **2013**, *25*, 4714-4717.
(46) Lin, L.; Liu, Y.; Yan, J.; Wang, X. S.; Li, J. H. *Anal. Chem.* **2012**, *85*, 334-340.
(47) Wu, L.; Wang, J. S.; Feng, L. Y.; Ren, J. S.; Wei, W. L.; Qu, X. G., *Adv. Mater.*, **2012**, *24*, 2447-2452.
(48) Chen, Y.; Qu, K. G.; Zhao, C. Q.; Wu, L.; Ren, J. S.; Wang, J. S.; Qu, X. G., *Nat. Commun.* **2012**, *3*, DOI: 10.1038/ncomms2091.
(49) Zong, S. F.; Wang, Z. Y.; Chen, H.; Cui, Y. P. *Small*, **2013**, *9*, 4215-4220.
(50) Xiao, Y.; Dane, K. Y.; Uzawa, T.; Csordas, A.; Qian, J.; Soh, H. T.; Daugherty, P. S.; Lagally, E. T.; Heeger, A. J.; Plaxco, K. W. *J. Am. Chem. Soc.* **2010**, *132*, 15299-15307.
(51) Toghi Eshghi, S.; Li, X. D.; Zhang, H. *Anal. Chem.* **2012**, *84*, 7626-7632.
(52) Zhao, Y. X.; Qi, L.; Chen, F.; Zhao, Y.; Fan, C. H. *Biosens. Bioelectron.* **2013**, *41*, 764-770.
(53) Zhang, Z.; Sharon, E.; Freeman, R.; Liu, X.; Willner, I. *Anal. Chem.* **2012**, *84*, 4789-4797.
(54) Tian, L.; Weizmann, Y. *J. Am. Chem. Soc*. **2012**, *135*, 1661-1664.
(55) Zuo, X.; Xiao, Y.; Plaxco, K. W. *J. Am. Chem. Soc.* **2009**, *131*, 6944-6945.
(56) Kim, N.; Piatyszek, M.; Prowse, K.; Harley, C.; West, M.; Ho, P.; Coviello, G.; Wright, W.; Weinrich, S.; Shay, J. *Science* **1994**, *266*, 2011-2015.
(57) Xiao, Y.; Dane, K. Y.; Uzawa, T.; Csordas, A.; Qian, J.; Soh, H. T.; Daugherty, P. S.; Lagally, E. T.; Heeger, A. J.; Plaxco, K. W. *J. Am. Chem. Soc.* **2010**, *132*, 15299-15307.
(58) Xiao, Y.; Pavlov, V.; Niazov, T.; Dishon, A.; Kotler, M.; Willner, I. *J. Am. Chem. Soc.* **2004**, *126*, 7430-7431.
(59) Zuo, X. L.; Xia, F.; Patterson, A.; Soh, H. T.; Xiao, Y.; Plaxco, K. W. *Chem. Biochem.* **2011**, *12*, 2745-2747.
(60) Liu, X. F.; Tang, Y. L.; Wang, L. H.; Zhang, J.; Song, S. P.; Fan, C. H.; Wang, S. *Adv. Mater.* **2007**, *19*, 1471-1474.
(61) Tang, Y. L.; He, F.; Yu, M. H.; Feng, F. D.; An, L. L.; Sun, H.; Wang, S.; Li, Y. L.; Zhu, D. B. *Macromol. Rapid Comm.* **2006**, *27*, 389-392.
(62) Pu, K. Y.; Liu, B. *Adv. Funct. Mater.* **2011**, 21, 3408-3423.
(63) Lee, J. S.; Han, M. S.; Mirkin, C. A. *Angew. Chem. Int. Ed.* **2007**, *119*, 4171-4174.
(64) Li, D.; Wieckowska, A.; Willner, I. *Angew. Chem. Int. Ed.* **2008**, *120*, 3991-3995.

For TOC only


Bipolar probe
Signal OFF
Bipolar probe+Target
Intensity (a.u.)
Wavelength (nm)
A-I increase
A-I decrease
(Bipolar probe)
5'-AAT CCG TCG AGC AGA GTT-3'
Bipolar probe+Target
Signal ON
Bipolar probe
Intensity (a.u.)
Wavelength (nm)