

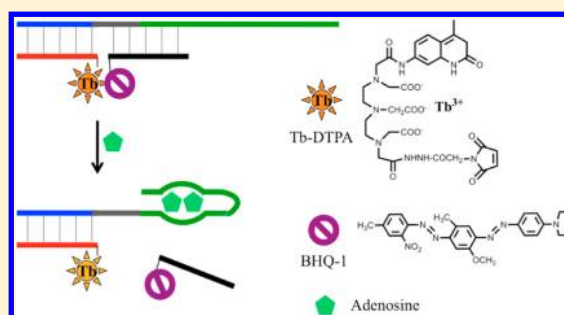
Direct Detection of Adenosine in Undiluted Serum Using a Luminescent Aptamer Sensor Attached to a Terbium Complex

Le-Le Li,^{†,§} Pinghua Ge,[‡] Paul R. Selvin,[‡] and Yi Lu^{*,†,§}

[†]Department of Chemistry, [‡]Department of Physics, Center for the Physics of Living Cells, and [§]Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana–Champaign, Urbana, Illinois 61801, United States

Supporting Information

ABSTRACT: Aptamers, single-stranded nucleic acids that can selectively bind to various target molecules, have been widely used for constructing biosensors. A major challenge in this field, however, is direct sensing of analytes in complex biological media such as undiluted serum. While progress has been made in developing an inhomogeneous assay by using a preseparation step to wash away the interferences within serum, a facile strategy for direct detection of targets in homogeneous unprocessed serum is highly desired. We herein report a turn-on luminescent aptamer biosensor for the direct detection of adenosine in undiluted and unprocessed serum, by taking advantage of a terbium chelate complex with long luminescence lifetime to achieve time-resolved detection. The sensor exhibits a detection limit of 60 μM adenosine while maintaining excellent selectivity that is comparable to those in buffer. The approach demonstrated here can be applied for direct detection and quantification of a broad range of analytes in biological media by using other aptamers.



Detection and quantification of various analytes related to health and environment has attracted intense attention recently due to their applications in areas such as biomedical diagnostics and environmental monitoring. While significant progress has been made in developing sensors for the detection of these analytes,^{1–4} it is still very difficult to apply the sensors in detecting their targets in biological fluids effectively.⁵ Aptamers, obtained through a combinatorial selection process called systematic evolution of ligands by exponential enrichment (SELEX), are single-stranded nucleic acids that bind a specific target molecule.^{6–15} Mimicking natural evolution, SELEX has resulted in many aptamers that can bind a broad range of biological targets with high affinity and with specificity comparable to that of antibodies, such as small organic molecules, proteins, viruses, and cells.^{6–15}

Aptamers offer distinct advantages over antibodies, such as small size, high stability, low cost, ease of modification, and simple production without the need of live animals or cells.¹¹ Because of these properties, aptamers have been used widely for constructing biosensors.^{11–23} The design strategy is to transform the specific binding event into detectable signals, such as fluorescence,^{24–35} colorimetry,^{36–45} magnetic field,^{46–48} electrochemistry,^{49–58} and glucose,⁵⁹ so that portable devices can be used for on-site and real-time detection and quantification. Although the performance of these sensors such as limit of detection is excellent, the use of aqueous buffer in most of these studies has limited their applications in biomedical research and disease diagnostics. To overcome this limitation, a few studies have been reported detection in real biological samples such as in serum. However, the serum used was often diluted significantly (e.g., 10% serum),^{25,46,60}

due to the strong interference from species in the biological fluids, which can result in a very high background that masks the detection signal. To date, only a few aptamer-based biosensors have been developed for the detection in undiluted and unprocessed serum.^{43,61,62} For example, aptamer-based sensors for detection of cocaine in 100% serum were reported based on lateral flow separation⁴³ or microfluidic electrochemical approach.⁶² In a more recent example, Huang and Liu reported the aptamer-based sensor for detection of adenosine in 30% or 90% serum (with detection limits of 167 μM and 200 μM , respectively) using flow cytometry.⁶¹ Since such a method is inhomogeneous, requiring a preseparation step based on the immobilization of DNA on magnetic beads to wash away the interferences within serum, a facile strategy for direct detection of targets in homogeneous unprocessed serum is highly desired. To meet such a challenge, we report herein a simple “turn-on” luminescence resonance energy transfer (LRET) aptamer biosensor for the direct detection of adenosine in 100% serum without any sample dilution or separation procedure, which shows a detection limit comparable to the inhomogeneous fluorescent assay for adenosine detection in serum.⁶¹

Lanthanide chelate complexes are well established probes for luminescence-based biological assays, which have two distinct properties that enable sensitive biosensing application.^{63,64} First, multiple sharp emission bands (<10 nm at half-maximum) and large Stoke’s shifts (>150 nm) enable efficient

Received: June 5, 2012

Accepted: August 15, 2012

spectral separation of emission signals. Second, the long-lived luminescence lifetime of lanthanide complexes allows time-resolved luminescence (TRL) detection methods to completely eliminate the interference of background fluorescence originating from autofluorescence and excitation light scattering by setting appropriate delay time.⁶³ For these reasons, recent efforts have sought to conjugate lanthanide probes to antibodies, oligonucleotides, and proteins for various applications.^{63–68} Most recently, TRL microscopy was developed, making lanthanide probes based TRL imaging a promising technology for next-generation bioimaging.⁶⁸ Even though biological fluids contain numerous species that could interfere with detection, it has been demonstrated that aptamers can still selectively recognize their targets in such media.^{43,61,62,69} In contrast to the common steady-state fluorescence measurements of most fluorescent sensors, TRL are rarely affected by autofluorescence in the biological samples. In our biosensor design, terbium luminescence was introduced to combine with aptamer for the construction of a time-resolved LRET-based assay for the direct detection of adenosine in serum.

The aptamer luminescence sensor was designed as shown in Figure 1. The sensor contains three nucleic acid strands: a

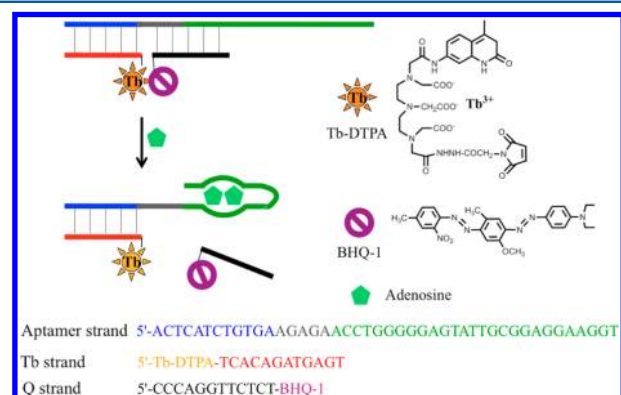


Figure 1. Scheme of the design of the adenosine sensor based on a terbium complex conjugated to a DNA aptamer.

DNA strand labeled with a terbium chelate complex at the 5'-end (Tb strand), a DNA strand modified with a quencher at the 3'-end (Q strand), and a linker DNA strand (aptamer strand). The linker DNA can be divided into three segments: the first segment (in blue) hybridizing with the Tb strand; the second segment (in gray) hybridizing with the last five nucleotides of the Q strand; and the third segment (in green) containing 27 nucleotides that code for the adenosine aptamer, seven of which are complementary to those on the Q strand. In the absence of the adenosine, the three DNA molecules assemble into the tripartite duplex structure, which lead to the close proximity of the terbium chelate complex and the quencher and thus the efficient luminescence quenching due to the luminescence resonance energy transfer (LRET). In the presence of target, the aptamer changes its structure to bind adenosine, leaving only five base pairs to hybridize with the Q strand, which is unstable at room temperature due to lower melting temperature. As a result, the Q strand dissociates from the aptamer strand, resulting in an increase of luminescence intensity of the terbium complex.

LRET, a modified spectroscopic technique of the widely used fluorescence resonance energy transfer (FRET), has shown great potentials in bioanalysis, such as detection of DNA,

determination of protein–protein interactions, and analysis of ion channels in living cells.^{63,67,68,70,71} The lanthanide complex used here as an energy transfer donor contains a chelate (diethylenetriaminepentacetic acid) to protect the lanthanide from solvent quenching effects, a covalently attached organic chromophore (carbostyryl 124) to act as an antenna to absorb the excitation light and transfer the energy to lanthanide ions, and a reactive site (maleimide) to attach the complex to biomolecules (see structure in Figure 1).^{72,73} The 3'-BHQ-1-modified oligonucleotides is used as a quencher. The structure of the linkage of the terbium complex and BHQ-1 with DNA was shown in Figure S1 in the Supporting Information. As shown in Figure 2, the Tb strand exhibits characteristic terbium

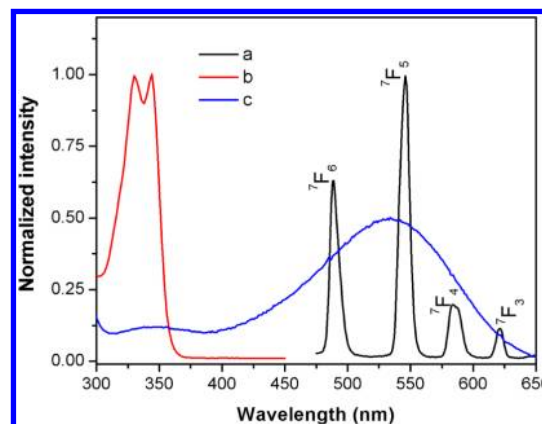


Figure 2. Normalized emission (a) and excitation (b) spectrum of the Tb-DNA donors and the absorption spectrum of Q-DNA acceptors (c). The radiative transitions of Tb³⁺ from the ⁵D₄ energy level to different ground levels (⁷F_j) are assigned to the corresponding emission bands.

luminescence arising from transitions from a ⁵D₄ excited state to different ground levels (⁵D₄–⁷F_j) upon excitation with near-UV light ($\lambda_{\text{ex}} = 344$ nm). The absorption spectrum of the Q strand has a maximal absorption in the 450–600 nm range, which overlaps extensively with the terbium transitions, thus providing for efficient LRET between the donor and acceptor.

The spectra of the terbium emission of the assembled duplex in HEPES buffer (20 mM, 200 mM NaCl, and pH 7.4) before and after addition of different concentrations of adenosine are presented in Figure 3. An increase in the characteristic terbium luminescence bands can be observed after addition of increasing concentrations of adenosine, indicating that the binding of adenosine by the adenosine aptamer caused the release of the Q strand and thus the increase of terbium luminescence. The real-time signaling test shows that the binding and subsequent switching was fast, and the response reached equilibrium within 1 min after adenosine addition (Figure S2 in the Supporting Information). The selectivity of this method toward adenosine over two other nucleotides was also tested. Addition of 5 mM uridine or cytidine did not induce any luminescence enhancement.

To evaluate the luminescence sensor performance in complex biological media, the steady-state fluorescence emission spectra of the aptamer sensor in 100% serum before and after addition of 5 mM of adenosine were collected (Figure 4A). There was almost no luminescence enhancement in this case, most likely due to the strong autofluorescence and light scattering effect of the serum, which is known to contain a

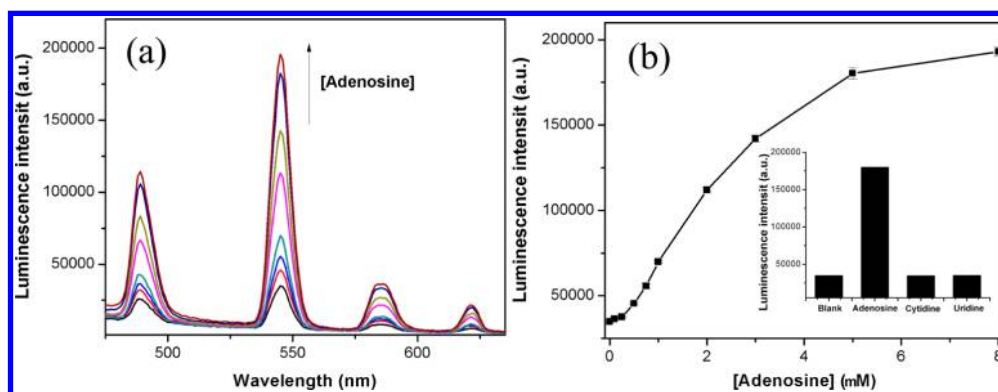


Figure 3. (a) Steady-state emission spectra of the aptamer sensor upon the addition of increased concentrations of adenosine in the HEPES buffer solution ($\lambda_{\text{ex}} = 344$ nm). (b) Emission intensity of the sensor at 545 nm as a function of adenosine concentrations. The inset shows the selectivity of the sensor toward adenosine over other nucleosides at 5 mM concentration (Guanosine was not tested due to its low solubility in aqueous solution at such a concentration).

number of endogenous components that produce a high autofluorescence background in the visible emission range. These results suggest that the autofluorescence and scattering interferences led to a serious limitation of the fluorescent aptamer biosensor assay on analytical accuracy and test sensitivity in a complex environment.

Since most of the background fluorescence of organic fluorophores and biological molecules has a lifetime of <10 ns, the much longer luminescence lifetime of the Tb^{3+} ions (on the order of milliseconds) enables the use of a time-resolved technique to eliminate short-lifetime background fluorescent signals. First, we test the stability of the terbium complex in serum, which shows that it is very stable in serum even after incubation for 1 day (Figure S3 in the Supporting Information). To confirm that our aptamer sensor is feasible for time-resolved luminescence detection in a complex environment, the LRET of the same samples with varying concentrations of adenosine was measured in 100% serum using a delay time of 50 μs . As shown in Figure 4B, the luminescence signal of the Tb^{3+} emission band at 545 nm increased with increasing adenosine concentration, reaching a ~ 4 -fold increase at ~ 5 mM adenosine, in contrast to a mere 1.03-fold increase when a steady-state luminescence measurement was performed. The inset of Figure 4B shows that the time-resolved response curve at the low concentration region can be fit to a linear response with a detection limit of 60 μM adenosine. This detection limit is comparable to other homogeneous adenosine aptamer-based sensors in buffer solution^{25–27,34,44–47} and lower than that of the reported adenosine aptamer assays in undiluted serum.⁶¹ The selectivity of the sensor in the serum was also tested with 5 mM of various nucleotides. As shown in Figure 4C, 5 mM uridine or cytidine induced little luminescence change compared with the untreated sensor, suggesting that the high selectivity of the aptamer was maintained. On the other hand, for most aptamer-based fluorescent sensor, at least 100–500 μL of sample is needed to fill a fluorescence cuvette. For our method, we demonstrate that as little as 20 μL of serum is sufficient for each assay, which benefits their practical applications.

In summary, by taking advantage of the long lifetime of the Tb^{3+} luminescence that favors a time-resolved LRET assay method to eliminate background fluorescent signals and light scattering in complex biological media, we have demonstrated the use of aptamer for direct detection of target in undiluted serum with a terbium chelate complex conjugated to the

aptamer. This system has sensitivity and selectivity in undiluted serum comparable to other homogeneous adenosine sensor systems in buffers. Since aptamers specific for many other analytes can be obtained through SELEX, the described method is independent of exact sequences used and the conjugation of lanthanide complexes to DNA can be easily realized based on various reactive lanthanide chelate complexes, this approach can be further extended to other aptamer-based sensing systems for detecting and quantifying many analytes of interest. Furthermore, considering strong autofluorescence in vitro and in vivo, the lanthanide probes conjugated to the aptamer will greatly improve bioimaging and biosensing based on the most recently developed time-resolved luminescence microscopy.

EXPERIMENTAL SECTION

Materials. All DNA samples (including the linker DNA, the quencher-labeled DNA, and the thiol-modified DNA) were synthesized in Integrated DNA Technologies, Inc. (Coralville, IA). The terbium complex conjugated DNA was synthesized according to our reported method based on the reaction of thiol-modified DNA with maleimide-functionalized terbium complex.^{72,73} Human serum (type AB), adenosine, cytidine, and uridine were purchased from Aldrich. Buffers were prepared in Millipore water.

Sensor Preparation and Luminescence Measurements. Aptamer stock solution was prepared by incubation of 400 nM terbium complex labeled DNA, 800 nM aptamer linker, and 1.2 μM quencher-labeled DNA in HEPES buffer (20 mM, 200 mM NaCl, pH 7.4) or in the human blood serum for 1 h to ensure that nucleic acids are hybridized to each other completely. For the steady-state luminescence measurement, the aptamer stock solution was then diluted 4-fold with HEPES buffer or pure serum. The luminescence response of the sample for adenosine was monitored on a FluoroMax-P fluorimeter (HORIBA Jobin Yvon Inc., Edison, NJ). A volume of 200 μL of the above sensor solution was mixed with a small volume of concentrated adenosine solution. After 10 min, the emission spectra of the sample were recorded with the excitation at 344 nm. For TRL measurement in serum, the aptamer serum stock solution was first diluted 2-fold with pure serum, and a 10 μL volume of this solution was placed in separate wells of a black 96-well Molecular Devices HE high-efficiency microplate (Molecular Devices, Inc., Sunnyvale, CA). Then a 10 μL of serum solution containing different concentrations of adenosine

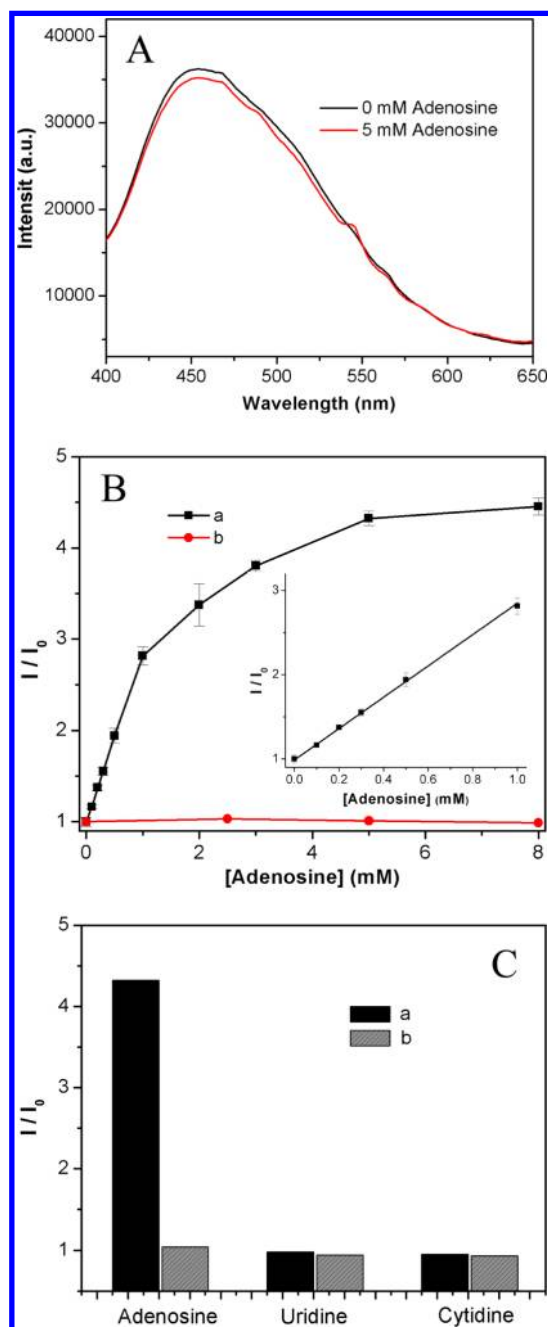


Figure 4. (A) Steady-state emission spectra of the aptamer sensor upon the addition of adenosine in the 100% serum. (B) Time-resolved (a) and steady-state (b) emission intensity enhancement of the aptamer sensor at 545 nm as a function of adenosine concentration in 100% serum. Delay time = 50 μ s. The inset shows the luminescence response at low concentrations of adenosine, and the black line shows a linear fitting of the data. (C) The selectivity of the sensor in the 100% serum toward different 5 mM nucleosides based on time-resolved (a) and steady-state (b) emission measurement.

was added to the stock aptamer solution in the 96-well plate. The final 20 μ L of the each sensor solution was allowed to incubate for 10 min at room temperature. The emission intensity at 545 nm of each sensor solution (20 μ L) in the 96-well plate was directly read out at a Wallac Victor2 V, 1420 multilabel HTS counter (Perkin-Elmer, Waltham, MA) using an excitation filter at 340/10 nm and emission filters at 545/20 (delay time 50 μ s) (Figure S4 in the Supporting Information).

All samples were prepared in triplicate to measure the luminescence.

■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Fax: (+1) 217-244-3186. E-mail: yi-lu@illinois.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by and the U.S. National Institute of Health (Grant ES016865 to Y.L. and Grant AR044420 to P.R.S.) and the National Science Foundation (Grant CMMI 0749028 to Y.L. and Grants 0968976 and 0822613 to P.R.S.). We also thank Dr. Teresa Anne Martin and Dr. John A. Katzenellenbogen for the help on the time-resolved emission measurement.

■ REFERENCES

- (1) Nolan, E. M.; Lippard, S. J. *Chem. Rev.* **2008**, *108*, 3443–3480.
- (2) Chen, P. R.; He, C. *Curr. Opin. Chem. Biol.* **2008**, *12*, 214–221.
- (3) Xue, X.; Wang, F.; Liu, X. *J. Am. Chem. Soc.* **2008**, *130*, 3244–3245.
- (4) Ohmichi, T.; Kawamoto, Y.; Wu, P.; Miyoshi, D.; Karimata, H.; Sugimoto, N. *Biochemistry* **2005**, *44*, 7125–7130.
- (5) Fernie, A. R.; Trethewey, R. N.; Krotzky, A. J.; Willmitzer, L. *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 763–769.
- (6) Ellington, A. D.; Szostak, J. W. *Nature* **1990**, *346*, 818–822.
- (7) Tuerk, C.; Gold, L. *Science* **1990**, *249*, 505–510.
- (8) Shamah, S. M.; Healy, J. M.; Cload, S. T. *Acc. Chem. Res.* **2008**, *41*, 130–138.
- (9) Famulok, M.; Hartig, J. S.; Mayer, G. *Chem. Rev.* **2007**, *107*, 3715–3743.
- (10) Joyce, G. F. *Annu. Rev. Biochem.* **2004**, *73*, 791–836.
- (11) Liu, J.; Cao, Z.; Lu, Y. *Chem. Rev.* **2009**, *109*, 1948–1998.
- (12) Lee, J. H.; Yigit, M. V.; Mazumdar, D.; Lu, Y. *Adv. Drug Delivery Rev.* **2010**, *62*, 592–605.
- (13) Lu, Y.; Liu, J. *Acc. Chem. Res.* **2007**, *40*, 315–323.
- (14) Xing, H.; Wong, N.-Y.; Xiang, Y.; Lu, Y. *Curr. Opin. Chem. Biol.* **2012**, *16*, 429–435.
- (15) Wang, Z.; Lu, Y. *J. Mater. Chem.* **2009**, *19*, 1788–1798.
- (16) Fowler, C. C.; Li, Y. *Chem. Biol.* **2007**, *14*, 736–738.
- (17) Lu, Y.; Liu, J. *Curr. Opin. Biotechnol.* **2006**, *17*, 580–588.
- (18) Li, D.; Song, S.; Fan, C. *Acc. Chem. Res.* **2010**, *43*, 631–641.
- (19) Navani, N. K.; Li, Y. *Curr. Opin. Chem. Biol.* **2006**, *10*, 272–281.
- (20) Potyrailo, R. A.; Conrad, R. C.; Ellington, A. D.; Hieftje, G. M. *Anal. Chem.* **1998**, *70*, 3419–3425.
- (21) Mok, W.; Li, Y. *Sensors* **2008**, *8*, 7050–7084.
- (22) Famulok, M.; Mayer, G. *Acc. Chem. Res.* **2011**, *44*, 1349–1358.
- (23) Jhaveri, S.; Rajendran, M.; Ellington, A. D. *Nat. Biotechnol.* **2000**, *18*, 1293–1297.
- (24) Pei, R.; Rothman, J.; Xie, Y.; Stojanovic, M. N. *Nucleic Acids Res.* **2009**, *37*, e59/51–e59/56.
- (25) Xu, W.; Lu, Y. *Anal. Chem.* **2010**, *82*, 574–578.
- (26) Liu, J.; Lee, J. H.; Lu, Y. *Anal. Chem.* **2007**, *79*, 4120–4125.
- (27) Song, P.; Xiang, Y.; Xing, H.; Zhou, Z.; Tong, A.; Lu, Y. *Anal. Chem.* **2012**, *84*, 2916–2922.
- (28) Xiang, Y.; Tong, A.; Lu, Y. *J. Am. Chem. Soc.* **2009**, *131*, 15352–15357.
- (29) Huang, C.-C.; Chang, H.-T. *Chem. Commun.* **2008**, 1461–1463.
- (30) Zhou, Z.; Du, Y.; Dong, S. *Anal. Chem.* **2011**, *83*, 5122–5127.

- (31) Zhang, H.; Li, X.-F.; Le, X. C. *J. Am. Chem. Soc.* **2008**, *130*, 34–35.
- (32) Nutiu, R.; Li, Y. *Angew. Chem., Int. Ed.* **2005**, *44*, 5464–5467.
- (33) Nutiu, R.; Li, Y. *J. Am. Chem. Soc.* **2003**, *125*, 4771–4778.
- (34) Dave, N.; Liu, J. *Chem. Commun.* **2012**, *48*, 3718–3720.
- (35) Li, J.; Zhong, X.; Zhang, H.; Le, X. C.; Zhu, J.-J. *Anal. Chem.* **2012**, *84*, 5170–5174.
- (36) Zhang, J.; Wang, L.; Pan, D.; Song, S.; Boey, F. Y. C.; Zhang, H.; Fan, C. *Small* **2008**, *4*, 1196–1200.
- (37) Zhao, W.; Chiuman, W.; Lam, J. C. F.; McManus, S. A.; Chen, W.; Cui, Y.; Pelton, R.; Brook, M. A.; Li, Y. *J. Am. Chem. Soc.* **2008**, *130*, 3610–3618.
- (38) Zhao, W.; Brook, M. A.; Li, Y. *ChemBioChem* **2008**, *9*, 2363–2371.
- (39) Wei, H.; Li, B.; Li, J.; Wang, E.; Dong, S. *Chem. Commun.* **2007**, 3735–3737.
- (40) Elbaz, J.; Moshe, M.; Shlyahovsky, B.; Willner, I. *Chem.–Eur. J.* **2009**, *15*, 3411–3418.
- (41) Zhu, Z.; Wu, C.; Liu, H.; Zou, Y.; Zhang, X.; Kang, H.; Yang, C. J.; Tan, W. *Angew. Chem., Int. Ed.* **2010**, *49*, 1052–1056.
- (42) Wang, J.; Wang, L.; Liu, X.; Liang, Z.; Song, S.; Li, W.; Li, G.; Fan, C. *Adv. Mater.* **2007**, *19*, 3943–3946.
- (43) Liu, J.; Lu, Y. *Angew. Chem., Int. Ed.* **2006**, *45*, 7955–7959.
- (44) Liu, J.; Lu, Y. *Nat. Protoc.* **2006**, *1*, 246–252.
- (45) Liu, J.; Lu, Y. *Anal. Chem.* **2004**, *76*, 1627–1632.
- (46) Yigit, M. V.; Mazumdar, D.; Kim, H. K.; Lee, J. H.; Odintsov, B.; Lu, Y. *ChemBioChem* **2007**, *8*, 1675–1678.
- (47) Xu, W.; Lu, Y. *Chem. Commun.* **2011**, *47*, 4998–5000.
- (48) Bamrungsap, S.; Shukoor, M. I.; Chen, T.; Sefah, K.; Tan, W. *Anal. Chem.* **2011**, *83*, 7795–7799.
- (49) Wen, Y.; Pei, H.; Wan, Y.; Su, Y.; Huang, Q.; Song, S.; Fan, C. *Anal. Chem.* **2011**, *83*, 7418–7423.
- (50) Wu, Z.-S.; Guo, M.-M.; Zhang, S.-B.; Chen, C.-R.; Jiang, J.-H.; Shen, G.-L.; Yu, R.-Q. *Anal. Chem.* **2007**, *79*, 2933–2939.
- (51) Guo, S.; Du, Y.; Yang, X.; Dong, S.; Wang, E. *Anal. Chem.* **2011**, *83*, 8035–8040.
- (52) Liu, G.; Wan, Y.; Gau, V.; Zhang, J.; Wang, L.; Song, S.; Fan, C. *J. Am. Chem. Soc.* **2008**, *130*, 6820–6825.
- (53) Li, B.; Du, Y.; Wei, H.; Dong, S. *Chem. Commun.* **2007**, 3780–3782.
- (54) Willner, I.; Zayats, M. *Angew. Chem., Int. Ed.* **2007**, *46*, 6408–6418.
- (55) Xiao, Y.; Lai, R. Y.; Plaxco, K. W. *Nat. Protoc.* **2007**, *2*, 2875–2880.
- (56) Zhang, S.; Xia, J.; Li, X. *Anal. Chem.* **2008**, *80*, 8382–8388.
- (57) Baker, B. R.; Lai, R. Y.; Wood, M. S.; Doctor, E. H.; Heeger, A. J.; Plaxco, K. W. *J. Am. Chem. Soc.* **2006**, *128*, 3138–3139.
- (58) Zayats, M.; Huang, Y.; Gill, R.; Ma, C.-a.; Willner, I. *J. Am. Chem. Soc.* **2006**, *128*, 13666–13667.
- (59) Xiang, Y.; Lu, Y. *Nat. Chem.* **2011**, *3*, 697–703.
- (60) Huang, D.-W.; Niu, C.-G.; Zeng, G.-M.; Ruan, M. *Biosens. Bioelectron.* **2011**, *29*, 178–183.
- (61) Huang, P. J.; Liu, J. *Anal. Chem.* **2010**, *82*, 4020–4026.
- (62) 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.
- (63) Selvin, P. R. *Annu. Rev. Biophys. Biomol. Struct.* **2002**, *31*, 275–302.
- (64) Mathis, G. *Clin. Chem.* **1993**, *39*, 1953–1959.
- (65) Xiao, M.; Li, H.; Snyder, G. E.; Cooke, R. G.; Yount, R.; Selvin, P. R. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15309–15314.
- (66) Saha, A. K.; Kross, K.; Kloszewski, E. D.; Upson, D. A.; Toner, J. L.; Snow, R. A.; Black, C. D. V.; Desai, V. C. *J. Am. Chem. Soc.* **1993**, *115*, 11032–11033.
- (67) Posson, D. J.; Ge, P.; Miller, C.; Bezanilla, F.; Selvin, P. R. *Nature* **2005**, *436*, 848–851.
- (68) Rajapakse, H. E.; Gahlaut, N.; Mohandessi, S.; Yu, D.; Turner, J. R.; Miller, L. W. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 13582–13587.
- (69) Zuo, X.; Xiao, Y.; Plaxco, K. W. *J. Am. Chem. Soc.* **2009**, *131*, 6944–6945.
- (70) Bünzli, J. -C. G. *Chem. Rev.* **2010**, *110*, 2729–2755.
- (71) Li, J.; Zhou, W.; Ouyang, X.; Yu, H.; Yang, R.; Tan, W.; Yuan, J. *Anal. Chem.* **2011**, *83*, 1356–1362.
- (72) Chen, J.; Selvin, P. R. *Bioconjugate Chem.* **1999**, *10*, 311–315.
- (73) Ge, P.; Selvin, P. R. *Bioconjugate Chem.* **2003**, *14*, 870–876.