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Rapid fluorescence detection of immunoglobulin E using an aptamer switch based on a binding-induced pyrene excimer†

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Immunoglobulin E (IgE) is an important immunoglobulin related to allergic reactions and some diseases such as atopic dermatitis, allergic asthma, etc. Sensitive and rapid detection of IgE is desired for disease diagnosis and therapeutics. Taking advantage of a binding-induced pyrene excimer, we developed a sensitive fluorescent aptamer switch for IgE sensing by using a pyrene-labeled aptamer. The aptamer having four base pairs at the stem was labeled with a single pyrene molecule at the 3' end and 5' end and used as an affinity probe. The binding of IgE brought the pyrene moiety at each terminal of the aptamer into proximity, generating an excimer and showing a distinct fluorescence peak. The measurement of the fluorescence peak of the excimer allowed the sensitive detection of IgE. We optimized the experimental conditions such as temperature, incubation time, and metal cations. The detection limit for IgE was about 1.6 nM. This assay displayed good specificity and allowed for the detection of IgE spiked in a diluted human serum sample. This fluorescent aptamer sensor has potential for its application in rapid sensing of IgE.

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Introduction

Human immunoglobulin E (IgE) plays a key role in a variety of allergic reactions, such as atopic dermatitis, allergic asthma, AIDS, etc.^{1–6} Sensitive and selective detection of human IgE is of great importance. Immunoassays are one of the commonly used methods for IgE detection,^{7–9} which use antibodies as affinity ligands. Compared with antibodies, aptamers, the artificial nucleic acid affinity ligands, have some great features, such as easy labelling with functional groups, simple synthesis, good stability, and binding-induced structure switching.^{10–12} They have been applied in biosensing and bioanalysis with great promise in different detection methods, including electrochemistry, optical detection, chromatography, mass spectrometry, etc.^{13–20} A 37-mer DNA aptamer against IgE has been selected (5'-GCA CGT TTA TCC GTC CCT CCT AGT GGC GTG C-3'), and the dissociation constant (K_d) is about 10 nM.²¹ The discovery of aptamers for IgE allows the development of aptamer-based assays for IgE in different formats.^{12,15,17–20,22–28} To date, several aptamer-based optical detection methods (e.g. luminescence and fluorescence) have been developed for IgE detection,^{22–28} such as a label-free aptasensor based on a molecular light

switch,²² fluorescence anisotropy assay relying on IgE-binding induced increase of molecular volume and fluorescence anisotropy,²³ competitive molecular beacon assay,²⁶ etc. The optical assays show advantages in terms of simplicity, sensitivity, and rapidity.

The unique feature of the pyrene excimer allows the fabrication of fluorescent sensors for a variety of targets.^{29–35} The pyrene molecules in proximity generate fluorescence peaks different from those of separated pyrenes. The combination of the aptamer and pyrene excimer provides an aptamer excimer probe for rapid detection of targets based on target-binding induced structure switching of the aptamer.^{36–43} Yang *et al.* reported an excimer aptamer probe to detect platelet-derived growth factor BB (PDGF-BB).³⁶ Pyrene was conjugated on each end of the aptamer against PDGF-BB. This aptamer excimer probe underwent a conformational change upon the binding of PDGF-BB and formed a stem structure. It brought the dual pyrene labels on the ends of the aptamer into proximity, generating an excimer. The measurement of the fluorescence peak of the pyrene excimer at 485 nm allowed the determination of PDGF-BB. With different designs, the aptamer-based excimer probes have been applied to the detection of potassium ions, ATP, thrombin, cocaine, and lysozyme.^{36–43}

Herein, taking advantage of the pyrene excimer and the affinity aptamer in biosensing, for the first time we reported an aptamer switch probe for the determination of IgE based on binding-induced excimer generation by using a pyrene labeled aptamer. After truncating 4 base pairs from the terminals of the 37-mer anti-IgE aptamer, we successfully designed a 29-mer aptamer having pyrene labels at the sequence ends and showing

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Fig. 1 Scheme of the detection of IgE using an aptamer excimer probe, which has single pyrene molecule labelling on each end of the aptamer. The binding of IgE to the aptamer probe causes the formation of a stem structure and brings pyrenes close to generate an excimer, showing distinct fluorescence emission and allowing the detection of IgE.

a fluorescence response to IgE. The binding of IgE to the aptamer probe brought the separated pyrene labels into proximity and generated the excimer of pyrenes (Fig. 1). A fast and sensitive detection of IgE was achieved by monitoring the distinct fluorescence peak of the generated excimer. With this strategy, we achieved the detection of IgE at 1.6 nM with good selectivity. This aptamer probe also enabled the detection of IgE in a diluted serum sample, showing potential for rapid analysis of IgE in different applications.

Experimental

Chemicals and reagents

Human IgE and human immunoglobulin G (IgG) were ordered from Athens Research & Technology, Inc. (Athens, Georgia,

water for preparing solutions was obtained from an Elga Lab water system (Purelab Ultra Genetic, UK).

Fluorescence measurement

A binding buffer (20 mM Tris-HCl, pH 7.5) containing 50 mM NaCl and 1 mM MgCl₂ was used for the detection of IgE. A 50 nM aptamer probe and varying concentrations of IgE were incubated in ice for 10 min before the fluorescence measurement. The fluorescence measurement was performed on a JASCO FP-8300 fluorescence spectrometer (Japan) at 25 °C. The fluorescence spectra in the range from 365 to 600 nm were recorded with an excitation at 349 nm. The slits for excitation and emission were both set to 5 nm.

Determination of K_d

K_d of the aptamer probe IgE-4bp-2Py was determined from the fluorescence response of the aptamer probe upon IgE using the following eqn (1) and (2).⁴⁴ F_a is the fraction of bound IgE. F_f and F_b are the fluorescence intensities (485 nm) of the free aptamer probe and the IgE bound aptamer, respectively. F is the fluorescence intensity (485 nm) of the aptamer measured at a certain concentration of IgE. $[IgE]$ is the concentration of IgE, and $[Aptamer]$ is the total concentration of the aptamer. The stoichiometry between IgE and the aptamer was set to be 1 : 1. The K_d was obtained by fitting the data to eqn (2) through non-linear regression analysis using Graph Pad.

$$F_a = \frac{F - F_f}{F_b - F_f} \quad (1)$$

$$F_a = \frac{[IgE] + [Aptamer] + K_d - \sqrt{([IgE] + [Aptamer] + K_d)^2 - 4[IgE][Aptamer]}}{2[Aptamer]} \quad (2)$$

USA). Oligonucleotides with pyrene conjugated at both the 3' end and 5' end were synthesized and purified by Takara (Dalian, China). Table 1 shows the sequences of tested DNA oligonucleotides. Lysozyme, hemoglobin (Hb), and albumin from bovine serum (BSA) were purchased from Sigma (St. Louis, Missouri, USA). Other chemicals of analytically pure grade were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Human serum was obtained from Zhongke Chenyu Biotechnology (Beijing, China). Distilled deionized

Results and discussion

Excimer aptamer probes for IgE detection

Fig. 1 shows the principle of IgE detection using a dual pyrene labeled aptamer switch. The aptamer probe was labeled with a single pyrene molecule at the 3' end and 5' end. The binding of IgE to the aptamer probe brings the pyrenes close to form an excimer, and the excimer gives a fluorescence emission at about 485 nm with an excitation at 349 nm, allowing the detection of

Table 1 DNA probes used in this study

Name	Sequence
IgE-5bp-2Py	5'-Pyrene- <u>GCACG</u> TTTATCCGTCCTCCTAGTGCGGTG-pyrene-3' ^a
IgE-4bp-2Py	5'-Pyrene- <u>CACG</u> TTTATCCGTCCTCCTAGTGCGGTG-pyrene-3'
IgE-3bp-2Py	5'-Pyrene- <u>ACG</u> TTTATCCGTCCTCCTAGTGCGGTG-pyrene-3'
Control-2Py	5'-Pyrene- <u>CAC</u> CTTTATCCGTCCTCCTAGTGCGGTG-pyrene-3' ^b

^a The letters with an underline represent the nucleotides that can form a stem due to complementary pairing. ^b The bold letter represents the mutant nucleotide.

IgE. Our design strategy benefits from the stem-loop secondary structures of the anti-IgE aptamer induced by IgE binding. The length of the stem of the anti-IgE aptamer probe is critical for the successful detection of IgE in our design, which affects the stability of stem and signal generation. Three aptamer probes (IgE-5bp-2Py, IgE-4bp-2Py, and IgE-3bp-2Py) (Table 1) having 5-, 4-, and 3-base-paired stems were examined, respectively. As shown in Fig. 2, the IgE-5bp-2Py probe displayed a higher background signal at 485 nm (Fig. S1A in the ESI†), suggesting that a large fraction of IgE-5bp-2Py was already in conformation having pyrene molecules close in the absence of IgE owing to the higher stability of the 5-mer stem. Though the aptamer probe IgE-3bp-2Py gave a lower background signal, this probe only showed a weak response to IgE (Fig. 2 and S1B in the ESI†), which may be due to the less stable stem and reduced binding affinity of IgE-3bp-2Py. IgE-4bp-2Py not only showed a low background, but also showed a remarkable fluorescence response to IgE (Fig. 2A). Thus, IgE-4bp-2Py was a favourable aptamer probe for the detection of IgE in the following work.

To test the sequence specificity, we designed a control probe (Control-2py) by mutating the fourth nucleotide G of IgE-4bp-

2Py into C, which is located at the stem part close to the loop region. Upon addition of IgE, no obvious change of fluorescence intensity at 485 nm occurred for Control-2py. This indicates that the affinity binding between the aptamer and IgE is sequence dependent (Fig. S1C in the ESI†), and the stem part is crucial for the anti-IgE aptamer.²¹

Optimization of the experimental conditions

We examined the effect of metal ions (Na^+ , K^+ or Mg^{2+}) on the signal responses of the aptamer probe IgE-4bp-2Py to IgE. In the absence of IgE, when 1 mM MgCl_2 was added to the binding buffer (20 mM Tris-HCl, pH 7.5), the blank signals of IgE-4bp-2Py at 485 nm increased slightly in the range from 0 to 5 mM (Fig. 3), showing that MgCl_2 may enhance the stability of the stem structure. The IgE-binding induced fluorescence increase of the aptamer probe was lower in the presence of MgCl_2 than that in the absence of MgCl_2 . When the solution did not contain MgCl_2 , the obtained $\Delta F/F_{\text{blank}}$ was higher (ΔF was obtained by subtracting the fluorescence signal of the blank sample (F_{blank}) from the fluorescence signal of the sample containing 50 nM IgE). We chose to use a binding buffer containing 1 mM MgCl_2 because the reported selection buffer contained 1 mM MgCl_2

Fig. 2 (A) The emission spectra of the aptamer probe IgE-4bp-2Py (50 nM) in the absence of IgE (black line) and in the presence of 50 nM IgE (red line). (B) The fluorescence responses of the different DNA probes (IgE-5bp-2Py, IgE-4bp-2Py, IgE-3bp-2Py, and Control-2py) to IgE at 485 nm. The binding buffer (20 mM Tris-HCl, pH 7.5) contained 150 mM NaCl, 1 mM MgCl_2 and 5 mM KCl.

Fig. 3 (A) The influence of MgCl_2 on the fluorescence signal of the blank sample (F_{blank}) and IgE-induced signal change (ΔF , obtained by subtracting F_{blank} from the fluorescence signal of the sample containing 50 nM IgE) in the buffer (20 mM Tris-HCl, pH 7.5) containing the aptamer probe IgE-4bp-2Py (50 nM). (B) The relationship between the concentration of MgCl_2 and the ratio of $\Delta F/F_{\text{blank}}$.

and the presence of MgCl_2 was helpful for the anti-IgE aptamer to maintain good binding affinity to IgE.²³ We further tested the effect of NaCl in the binding buffer (20 mM Tris-HCl, pH 7.5) containing 1 mM MgCl_2 (Fig. 4). With the increase of NaCl concentration, F_{blank} decreased and then remained unchanged when the concentration of NaCl was higher than 50 mM. The response of IgE-4bp-2Py to IgE (ΔF) decreased with the increase of NaCl concentration when the concentration of NaCl was higher than 50 mM. The maximum $\Delta F/F_{\text{blank}}$ was obtained at 50 mM NaCl. The use of 50 mM NaCl in the buffer containing 1 mM MgCl_2 is preferred for higher signal responses of IgE-4bp-2Py to IgE.

In the presence of IgE, the addition of KCl in the buffer (20 mM Tris-HCl, pH 7.5) containing 50 mM NaCl and 1 mM MgCl_2 caused a slight decrease of the responses of IgE-4bp-2Py to IgE (Fig. S2 in the ESI†). So, we finally chose to use the binding buffer (20 mM Tris-HCl, pH 7.5) containing 50 mM NaCl and 1 mM MgCl_2 in further work.

A large signal response was immediately observed, when the aptamer probe IgE-4bp-2Py was mixed with IgE, showing that the response of IgE-4bp-2Py to IgE was very fast. Further incubation did not cause a significant signal change (Fig. S3 in the

ESI†). This suggests that the IgE-4bp-2Py can be used for rapid sensing of IgE. The fluorescence signal at 485 nm was measured after the incubation of IgE-4bp-2Py with IgE for 3 min in further experiments. The effect of the temperature on the signal responses of the aptamer probe to IgE was also tested (Fig. S4 in the ESI†). The low temperature caused a high signal response of the aptamer probe IgE-4bp-2Py to IgE at 485 nm, showing that low temperature is favourable for the interaction between IgE and the aptamer probe. With the increase of temperature, the signal response of the IgE-4bp-2Py to IgE decreased as a high temperature may disrupt the secondary structure of the aptamer and reduce the binding affinity of the aptamer. In further study, experiments were conducted at 25 °C.

Detection of IgE

Under the optimized conditions, various concentrations of IgE were detected by using IgE-4bp-2Py (Fig. 5). With the addition of IgE, the peak at 485 nm gradually increased, corresponding to the binding-induced generation of the pyrene excimer. For the

Fig. 4 (A) The effect of NaCl on the fluorescence signal of the blank sample (F_{blank}) and IgE-induced signal change (ΔF , obtained by subtracting F_{blank} from the fluorescence signal of the sample containing 50 nM IgE) in the buffer (20 mM Tris-HCl, pH 7.5) containing 1 mM MgCl_2 and the aptamer probe IgE-4bp-2Py (50 nM). (B) The relationship between NaCl concentration and the ratio of $\Delta F/F_{\text{blank}}$.

Fig. 5 Detection of IgE with the aptamer probe IgE-4bp-2Py. (A) Emission spectra of IgE-4bp-2Py in solution containing increasing concentrations of IgE (curves from the bottom to the top corresponding to 0, 1.6, 3.2, 6.3, 12.5, 25, 50, 100, 200 and 400 nM IgE). (B) The relationship between fluorescence intensity (485 nm) and different concentrations of IgE.

emission of the monomer, it did not show any decrease, which is different from that usually reported,^{31,32,35} and it increased with the addition of IgE. This kind of phenomenon was also observed in some assays using the aptamer excimer probe,^{36,41} but the exact reason remains unknown. The detection limit was determined to be 1.6 nM (S/N = 3), and the dynamic range was from 1.6 nM to 400 nM. We extracted K_d of IgE-4bp-2Py from its fluorescence responses upon varying concentrations of IgE by non-linear fitting according to the protocol described in the Experimental section.⁴⁴ The obtained K_d of the aptamer probe IgE-4bp-2Py was about 25.3 ± 5.5 nM ($R^2 = 0.976$) (Fig. S5†), which is close to the reported value,²¹ showing that the pyrene labeled aptamer probe still maintains good binding affinity to IgE. Table S1† shows the comparison of some other aptamer-based optical assays for IgE with our method,^{22–27} and the detection limit (LOD) of our assay is comparable or higher than that of reported assays. Table S2† provides a summarized comparison of the previous reports of aptamer excimer probe based assays for other target molecules.^{36–39,41–43} For the aptamer having low binding affinity (*e.g.* the aptamers for cocaine, ATP, and potassium ions), the obtained detection limits are high.^{38,39,41} The aptamer with high binding affinity allowed aptamer excimer based assays to have higher sensitivity (*e.g.* the assays for PDGF-BB and thrombin).^{36,42,43} The anti-IgE aptamer has a moderate binding affinity, so the obtained LOD at around the nM level is reasonable. Clearly, the sensitivity of these aptamer excimer assays depends on the binding affinity of the aptamer probe, and the pyrene excimer is not the limiting factor for the aptamer sensor.

Specificity of IgE detection

In order to investigate the specificity of the aptamer excimer probe IgE-4bp-2Py for IgE detection, we tested some other proteins including IgG, Hb, lysozyme, BSA, and thrombin. As shown in Fig. 6, there was no remarkable change of the

Fig. 6 Specificity test for IgE detection using IgE-4bp-2Py as the probe. The concentration of IgG, Hb, lysozyme, BSA, and thrombin was 100 nM. IgE (50 nM) in the presence of 100 nM other proteins was also tested.

Fig. 7 Detection of IgE (50 nM) spiked in 500-fold, 200-fold or 100-fold diluted human serum in comparison with the result of IgE detection in the assay buffer using the aptamer IgE-4bp-2Py as the probe.

fluorescence intensity at 485 nm in the presence of the tested proteins (100 nM), showing that the tested proteins did not cause interference in IgE detection. The simultaneous presence of 100 nM tested proteins did not show any significant effect on IgE detection. These results suggest that the excimer aptamer probe has good selectivity in the detection of IgE.

Detection of IgE in a complex sample matrix

To examine the applicability of our aptamer sensor to complex sample matrices, IgE (50 nM) spiked in a diluted serum sample was tested by the aptamer excimer probe IgE-4bp-2Py. As Fig. 7 shows, IgE in 500-fold, 200-fold and 100-fold diluted human serum showed good recoveries: the recovery rates were 103%, 99%, and 81%, respectively. We further investigated the fluorescence responses of IgE-4bp-2Py to various concentrations of IgE in 500-fold diluted human serum. As shown in Fig. S6 in the ESI,† the fluorescence intensity at 485 nm increased with the addition of IgE. The detection limit was 1.6 nM, which was comparable to that obtained in the assay buffer. The results indicate the potential applicability of our assay for the determination of IgE in complex sample matrices.

Conclusion

In summary, we successfully developed an excimer aptamer switch for IgE detection in a homogeneous solution by using a dual pyrene-labeled 29-mer aptamer that has a 4 base-pair stem. When the aptamer probe was free in solution without containing IgE, both pyrene molecules were spatially separated, showing monomer emission peaks. The binding of the aptamer probe to IgE brought the pyrene molecules at the 3' and 5' ends into close proximity, forming a pyrene excimer and showing a distinct emission peak at 485 nm. The measurement of the excimer emission peak allowed sensitive real-time detection of IgE: as low as 1.6 nM IgE was detected. The excimer aptamer probe showed good selectivity and enabled the detection of IgE spiked in diluted human serum. This aptamer probe has

potential for biosensing applications. This work is helpful for designing aptamer switches to develop sensors for IgE detection in other different formats.

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References

- 1 M. A. Sanjuan, D. Sagar and R. Kolbeck, *J. Allergy Clin. Immunol.*, 2016, **137**, 1651–1661.
- 2 T. A. Platts-Mills, A. J. Schuyler, E. A. Erwin, S. P. Commins and J. A. Woodfolk, *J. Allergy Clin. Immunol.*, 2016, **137**, 1662–1670.
- 3 H. C. Oettgen, *J. Allergy Clin. Immunol.*, 2016, **137**, 1631–1645.
- 4 J. A. Fairley, C. T. Burnett and C. L. Fu, *J. Invest. Dermatol.*, 2007, **127**, 2605–2611.
- 5 H. J. Gould, B. J. Sutton, A. J. Beavil, R. L. Beavil, N. McCloskey, H. J. Coker, D. Fear and L. Smurthwaite, *Annu. Rev. Immunol.*, 2003, **21**, 579–628.
- 6 T. A. Platts-Mills, *Crit. Care Med.*, 2001, **164**, S1–S5.
- 7 S. Y. Chung and E. T. Champagne, *J. Agric. Food Chem.*, 2001, **49**, 3911–3916.
- 8 B. K. Sævik, T. L. Ulstein and H. J. S. Larsen, *Res. Vet. Sci.*, 2003, **74**, 37–45.
- 9 S. Kirsch, S. Fourdrilis, R. Dobson, M. L. Scippo, G. Maquhin-Roqister and E. De Pauw, *Anal. Bioanal. Chem.*, 2009, **395**, 57–67.
- 10 C. Tuerk and L. Gold, *Science*, 1990, **249**, 505–510.
- 11 A. D. Ellington and J. W. Szostak, *Nature*, 1990, **346**, 818–822.
- 12 F. Li, H. Q. Zhang, Z. X. Wang, A. M. Newbigging, M. S. Reid, X. F. Li and X. C. Le, *Anal. Chem.*, 2015, **87**, 274–292.
- 13 B. Deng, Y. W. Lin, C. Wang, F. Li, Z. X. Wang, H. Q. Zhang, X. F. Li and X. C. Le, *Anal. Chim. Acta*, 2014, **837**, 1–15.
- 14 J. Zhao, Q. Zhu, L. Zhao, H. Lian and H. Chen, *Analyst*, 2016, **141**, 4961–4967.
- 15 J. Liu, Z. Cao and Y. Lu, *Chem. Rev.*, 2009, **109**, 1948–1998.
- 16 H. P. Jiang, J. X. Zhu, C. Y. Peng, J. J. Gao, F. Zheng, Y. X. Xiao, Y. Q. Feng and B. F. Yuan, *Analyst*, 2014, **139**, 4940–4946.
- 17 W. H. Zhou, P. J. Huang, J. S. Ding and J. W. Liu, *Analyst*, 2014, **139**, 2627–2640.
- 18 C. Jin, L. P. Qiu, J. Li, T. Fu, X. B. Zhang and W. H. Tan, *Analyst*, 2015, **141**, 461–466.
- 19 X. Yang, Y. Tang, R. R. Alt, X. Xie and F. Li, *Analyst*, 2016, **141**, 3473–3481.
- 20 S. P. Song, L. H. Wang, J. Li, J. L. Zhao and C. H. Fan, *TrAC, Trends Anal. Chem.*, 2008, **27**, 108–117.
- 21 T. W. Wiegand, P. B. Williams, S. C. Dreskin, M. H. Jouvin, J. P. Kinet and D. Tasset, *J. Immunol.*, 1996, **157**, 221–230.
- 22 Y. X. Jiang, X. H. Fang and C. L. Bai, *Anal. Chem.*, 2004, **76**, 5230–5235.
- 23 G. Gokulrangan, J. R. Unruh, D. F. Holub, B. Ingram, C. K. Johnson and G. S. Wilson, *Anal. Chem.*, 2005, **77**, 1963–1970.
- 24 K. Stadtherr, H. Wolf and P. Lindner, *Anal. Chem.*, 2005, **77**, 3437–3443.
- 25 E. Katilius, Z. Katiliene and N. W. Woodbury, *Anal. Chem.*, 2006, **78**, 6484–6489.
- 26 J. L. He, Z. S. Wu, S. B. Zhang, G. L. Shen and R. Q. Yu, *Analyst*, 2009, **134**, 1003–1007.
- 27 K. J. Feng, C. H. Sun, J. H. Jiang and R. Q. Yu, *Anal. Lett.*, 2011, **44**, 1301–1309.
- 28 C. Y. Lee, K. Y. Wu, H. L. Su, H. Y. Hung and Y. Z. Hsieh, *Biosens. Bioelectron.*, 2013, **39**, 133–138.
- 29 K. J. Oh, K. J. Cash and K. W. Plaxco, *J. Am. Chem. Soc.*, 2006, **128**, 14018–14019.
- 30 A. Vallée-Bélisle and K. W. Plaxco, *Curr. Opin. Struct. Biol.*, 2010, **20**, 518–526.
- 31 C. Wu, C. Wang, L. Yan and C. J. Yang, *J. Biomed. Nanotechnol.*, 2009, **5**, 495–504.
- 32 C. M. Wang, C. C. Wu, Y. Chen, Y. L. Song, W. H. Tan and C. J. Yang, *Curr. Org. Chem.*, 2011, **15**, 465–476.
- 33 K. Fujimoto, H. Shimizu and M. Inouye, *J. Org. Chem.*, 2004, **69**, 3271–3275.
- 34 Y. X. Wang, F. H. Geng, Q. L. Cheng, H. Y. Xu and M. T. Xu, *Analyst*, 2011, **136**, 4284–4288.
- 35 L. Gai, H. Chen, B. Zou, H. Lu, G. Lai, Z. Li and Z. Shen, *Chem. Commun.*, 2012, **48**, 10721–10723.
- 36 C. J. Yang, S. Jockusch, M. Vicens, N. J. Turro and W. H. Tan, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 17278–17283.
- 37 S. Nagatoishi, T. Nojima, B. Juskowiak and S. Takenaka, *Angew. Chem., Int. Ed.*, 2005, **44**, 5067–5070.
- 38 C. Wu, L. Yan, C. Wang, H. Lin, C. Wang, X. Chen and C. J. Yang, *Biosens. Bioelectron.*, 2010, **25**, 2232–2237.
- 39 J. Huang, Z. Zhu, S. Bamrungsap, G. Z. Zhu, M. X. You, X. X. He, K. M. Wang and W. H. Tan, *Anal. Chem.*, 2010, **82**, 10158–10163.
- 40 J. Zheng, J. H. Li, X. X. Gao, J. Y. Jin, K. M. Wang, W. H. Tan and R. H. Yang, *Anal. Chem.*, 2010, **82**, 3914–3921.
- 41 C. Shi, H. Gu and C. Ma, *Anal. Biochem.*, 2010, **400**, 99–102.
- 42 J. Zheng, J. S. Li, Y. Jiang, J. Y. Jin, K. M. Wang, R. H. Yang and W. H. Tan, *Anal. Chem.*, 2011, **83**, 6586–6592.
- 43 Q. Zhao and L. Cheng, *Anal. Bioanal. Chem.*, 2013, **405**, 8233–8239.
- 44 M. Jing and M. T. Bowser, *Anal. Chim. Acta*, 2011, **686**, 9–18.