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Label-free colorimetric aptasensor for IgE using DNA pseudoknot probe

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Abstract

The development of simple and low-cost approaches for the detection of immunoglobulin E (IgE) would provide a means for the early diagnosis and prevention of atopic diseases. However, the current methods are generally tedious and multiple-step processes, and are limited by the high cost of the labeled proteins. Herein, we describe a label-free structure switching colorimetric method for the simple measurement of IgE using DNA pseudoknot probes and gold nanoparticles. In the absence of a target, the IgE aptamer probe adopts a pseudoknot conformation that dissociates a capture probe from the unmodified gold nanoparticle. However, when IgE binds to the aptamer probe, the pseudoknot is resolved, leading to a favorable hybridization between the 3′ terminal loop of the aptamer probes and capture probes that support the behavior of gold nanoparticles from the dispersion to the aggregation. As a result, the colorimetric IgE sensor employing this structure switching mechanism is sensitive, specific, and convenient, and the assay works even when challenged with complicated biological matrixes such as vaginal fluid. The proposed method is expected to be of great clinical value for IgE detection and could be used, after an appropriate design, for sensing applications of other structured aptamers.

Introduction

Immunoglobulin E (IgE), which has been linked to acute allergic reactions, is a class of immunoglobulin found only in mammals. Normally, serum IgE is present at very small levels, whereas abnormal IgE amounts is a symptom of parasitic infection or some other environmental issues [1]. Accordingly, IgE is a major concern of a specific allergy diagnosis. The most popular and well-established specific IgE detection methods are adapted from traditional immunoenzymatic techniques (enzyme-linked immunosorbent assay, radio allergo sorbent test, and microarray) [2, 3]. These approaches, although very reliable, are often expensive and need tedious labeling procedures and long incubation time, making them unsuitable for on-site detection. Moreover, the labeling steps that might occupy the binding site influence the affinity of the antibody.

As an alternative to the antibody-based strategies, a suitable approach can be through the selection of a specific aptamer binding to the IgE proteins. Aptamers are specific oligonucleotide sequences capable of recognizing and binding to their respective targets through spontaneous formation of a secondary structure. Compared with monoclonal antibodies, aptamers have many advantages, including excellent stability, high affinity, ease of labeling, and simplicity of synthesis [4]. Because of their distinctive properties, aptamers have been extensively used as molecular recognition

elements for bioassay applications [5-7]. As it is well-known, various immunoassays based on aptamer probes for IgE detection have been recently developed. Commonly used methods include surface plasmon resonance [8], fluorescence [9], electrochemical analysis [10], field-effect transistor [11], and quartz crystal microbalance [12]. These approaches exhibit promising results for high sensitivity and feasibility of quantification of IgE molecules. However, these methods still have a few disadvantages such as being limited to laboratory scientific research, including the utilization of radioactive substances, requirement of a large sample volume, washing steps before analysis, and specialized and cumbersome equipment [13]. Moreover, the detection of IgE is slow because of the laboratory-bound processes and multistep detection reaction. Therefore, sensitive, simple, and rapid methods for IgE quantitative analysis are highly desirable.

Recently, nanomaterials have become extremely popular in developing novel sensing systems with advanced functions because of their unique physical and chemical properties [14]. Nanoparticles have been successfully employed in sensing, nanoelectronics, and catalysis applications. In particular, gold nanoparticles (AuNPs), which are detected through their plasmon resonance absorbance, have gained considerable interest in bioanalytical applications because of their unique optical characteristics [15]. Moreover, single-stranded DNA (ssDNA) protects AuNPs because

of electrostatic forces, while double-stranded DNA (dsDNA) cannot electrostatically stabilize AuNPs because of its double-helix geometry. Thus, a combination of complementary oligonucleotides (or aptamers) and AuNPs has been employed to develop simple and direct colorimetric detection of DNA [16], proteins [17], and other small molecules [18]. Although numerous aptamers have been reported in constructing AuNP-based naked-eye analysis, only one colorimetric sensor for IgE involving crosslinking aggregation of surface-functionalized AuNPs has been described [19]. Because IgE aptamer contains secondary structures even in the absence of a target, it cannot be directly sensed using an unmodified strategy. Therefore, exploring label-free and simple colorimetric sensors, which utilize unmodified AuNPs as the IgE sensing indicator, is still challenging and appealing. Here, we developed an alternative strategy based on label-free colorimetric detection by combining a pseudoknot DNA switch probe and AuNP.

Experimental

Materials and Apparatus

Human IgE was purchased from Abbiotec (San Diego, CA, USA). Anti-MUC1 antibody (IgG), interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ) proteins were purchased from Abcam (Cambridge, MA, USA). Bovine serum albumin (BSA) was obtained from Sigma (St. Louis, MO, USA).

Psuedoknot DNA oligonucleotide was synthesized by Purigo Biotech (Taipei, Taiwan), and other oligonucleotides were prepared at the Center for Biotechnology, National Taiwan University (Taipei, Taiwan). The sequences are as follows:

Aptamer probe (pseudoknot):

GGGGCACGTTTATCCGTCCCTCCTAGTGGCGTGCCCCTTTTTTTTTTTTTTTT

TAGGAGGGTA

Capture probe (DNA_C): TTTACCCTCCTATT

Random probe (DNA_R): ATTACGGTCTCAAA

The stock solutions of 10 μM oligonucleotides were prepared in Tris-borate-EDTA (TBE) buffer (90 mM Tris, 90 mM boric acid, 4 mM Na^+, 1 mM Mg^{2+}, pH 7.4) and diluted to desired concentration with the same TBE buffer. The 13-nm citrate-capped AuNPs were prepared by the reduction of $HAuCl_4$, as described previously [20]. The concentration of AuNPs was 8.5 nM, which was estimated using the Beer–Lambert law; extinction coefficient = 2.7×10^8 M^{-1} cm^{-1}. Ultrapure water was provided by a Milli-Q system (Millipore, Bedford, MA, USA) and employed in all experiments. UV–vis spectra were recorded on a Varian Cary 50 spectrophotometer (Varian Medical Systems,Inc., Palo Alto, CA) with the wavelength range of 400–800 nm. Quartz crystal microbalance (QCM) measurements were performed at room temperature by using the Affinity Detection System (ADS) (Taipei,

Taiwan) from Asia New Technology. The photographs were taken with Sony Xperia S.

Vaginal specimens were obtained as previously described [21]. Briefly, semen-free samples were collected by sterile cotton swabs, which were then immersed in 3 mL of PBS and vigorously vortexed to evenly suspend the vaginal specimens throughout the solution. The study was approved by the Institutional Review Board of the Mackay Memorial Hospital.

IgE detection

Solutions of 100 nM psuedoknot DNA probes were incubated with different concentrations of aqueous IgE solution in 1 mM potassium phosphate buffer containing 0.3 mM KCl, 14 mM $NaCl_2$, and 0.005% Tween 20 (solution A). Simultaneously, 8 μL of 1 μM capture DNA probe was added to 200 μL of AuNP solution (4.3 nM) to form the DNA/AuNP complex (solution B). After incubation at room temperature for 60 min, solution A was mixed with solution B, and the volume of this solution (solution C) was adjusted to 386 μL by adding 1 mM PBS. Next, solution C was reacted for 15 min, and then 14 μL of NaCl (1 M) was added in the reaction mixture for 20 min. UV–Vis spectrum of the solution was measured.

Results and discussion

Design strategy

It has been demonstrated that aptamer structures can be rationally adapted to conformation switching for the construction of biosensors [22-24]. Namely, their ligand binding abilities are still preserved without significantly sacrificing the performance arising from the modification of nonconserved elements or the partial hybridization of the conserved ones. Particularly, Willner's and Plaxco's groups developed structure switching aptamer-based sensors for small-molecule targets [25, 26]. Inspired by their findings, we designed the pseudoknot aptamers illustrated in Fig. 1A, and challenged them with AuNP-based colorimetric methods. The architecture of a probe consists of the aptamer sequence (yellow) and an additional tethered sequence (blue) complementary to the loop region of the IgE aptamer. In the absence of a target, the pseudoknot structure maintains its thermodynamically stable conformation, and the additional tethered sequence does not associate with the capture strands presented on the AuNP surfaces. In the presence of a protein, the IgE−aptamer complex is stabilized, resulting in the dehybridization of the 3′ terminal loop. Following the conformational change from psuedoknot to hairpin aptamer, the 3′ terminal loop of the aptamer probe can associate with the capture probe and induce the aggregation of AuNPs.

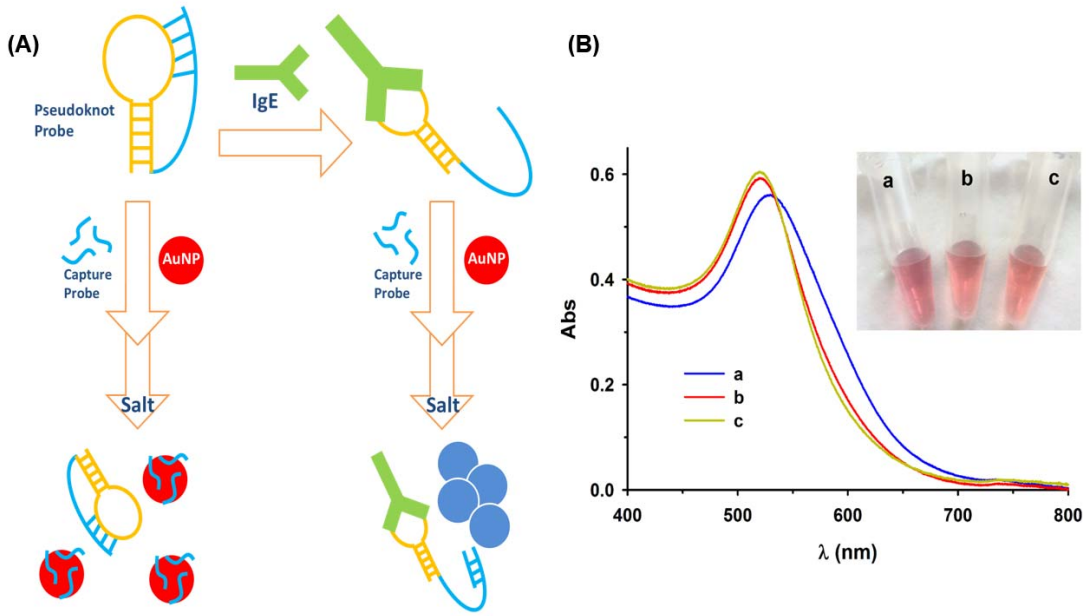

Fig. 1. (A) Schematic of pseudoknot probe-based colorimetric sensing of IgE. (B) UV–vis adsorption spectra of colorimetric aptasensors in different conditions. (a) IgE/pseudoknot probe + capture probe, (b) pseudoknot probe + capture probe, and (c) IgE/pseudoknot probe + random DNA. The final concentrations of the IgE protein, the pseudoknot probe, the capture probe, and the random DNA were 5, 200, 200, and 200 nM, respectively. The inset shows the corresponding samples.

Characterization of the colorimetric assay

To investigate the feasibility of the colorimetric assay, the UV–vis spectrum of the AuNPs solution was measured (Fig. 1B). We treated the AuNP solution with the capture probe (DNA_C) for 30 min in the presence of pseudoknot probe/IgE complex and pseudoknot probe alone. Upon the addition of salt, the former solution exhibited a color change to purple, while the latter remained red (the inset in Figure 1B). It seems that the stabilization effect of the capture probes to AuNPs was insignificant in the presence of IgE, which is the initiator for the formation of pseudoknot probe/IgE/capture probe complex. To rule out the possibility that the IgE molecule may affect the stability of AuNPs, we performed a control experiment. We found that

the control DNA (DNA_R) did not undergo visible color change in the presence of the pseudoknot probe/IgE complex, suggesting that the formation of particle aggregates was mainly caused by IgE-induced hybridization events. The sensing mechanism was further confirmed by quartz crystal microbalance (QCM) measurements, which showed a notable decrease in the frequency upon the addition of the pseudoknot probe/IgE complex onto the DNA_C-modified sensing surface (Fig. S1). Taken together, these results showed that the assembly of AuNPs certainly depends on the hybridization between the capture and pseudoknot probes in the presence of IgE. The spectral absorption of IgE aptamer/AuNP solution at 520 nm (A_{520}) is related to the amount of dispersed AuNPs. In addition, the absorbance with the addition of IgE at 600 nm (A_{600}) increased more than that at other wavelengths compared to the blank absorbance (without IgE). Accordingly, the ratio A_{600}/A_{520} is employed as the indicator to express the aggregation behavior of AuNPs for the following experiments.

Sensitivity of IgE

The experimental conditions, including the concentration of the pseudoknot probe and sodium ion, and the time of incubation and response were optimized to achieve short analysis time and obtain the best detection sensitivity (Fig. S2). Under optimal experimental conditions, aggregation of AuNPs was observed upon addition of different target concentrations. When the concentration of IgE increased, the

aggregation of capture probe-stabilized nanoparticles, resulting from binding of the capture probes to the 3′ terminal sequences of the pseudoknot probes, caused a visible color change from red to violet (the inset in Fig. 2A) because the aggregation led to spectral broadening and red shift of the absorbance of the unmodified AuNPs. Figure 2B shows a direct relationship between the aggregation of the AuNPs and the concentration of IgE. The change of the difference $A-A_0$, where A and A_0 represent the spectra ratio (A_{600}/A_{520}) in the presence and absence of IgE, respectively, increases with increasing amounts of IgE because of the formation of larger AuNPs aggregates. A linear correlation was obtained in the concentration range from 1 nM to 25 nM with a linear coefficient $R^2 = 0.991$ (inset in Figure 2B) and a detection limit (LOD) of IgE as low as 0.2 nM (39.2 ng/mL, on the basis of the molecular weight of 196 kDa) at a signal-to-noise ratio of 3σ (n = 3). Therefore, when the IgE concentration was over 25 nM, it could not be accurately determined, and an appropriate dilution within the linear range of the sensor was necessary in the preincubation step. Considering the sample volume (400 μL) obtained per measurement, this proposed aptasensor could efficiently detect down to 80 fmol of IgE. The normal physiological levels for IgE are believed to be approximately 0.85 nM, whereas the concentrations in patients are found to be significantly increased to at least 10-fold (approximately 8.5 nM) [27]; therefore, our method has the potential for routine clinical applications. In comparison

with previously reported methods, our approach avoids constructing complicated aptasensors and shows better or comparable sensitivity toward IgE molecules [28-31]. Of note, compared with the sticky-end pairing-based colorimetric aptasensor reported by Wu et al.[19], our approach is less sensitive. This existing assay, however, requires slow, multistep preparation processes of nanoparticle-aptamer conjugates that take 2 d, and thus our approach is much more convenient, faster, and cheaper.

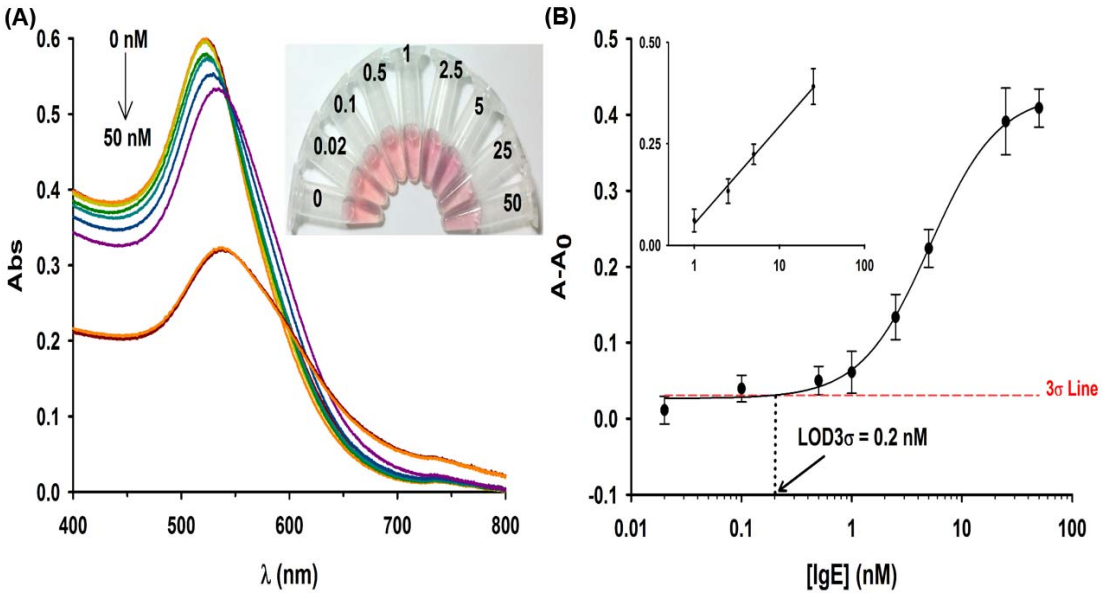

Fig. 2. Colorimetric sensing of IgE. (A) Spectra of the AuNP solutions at various concentrations of IgE (0, 0.02, 0.1, 0.5, 1.0, 2.5, 5, 25, and 50 nM). The inset shows the corresponding samples. (B) Spectral response ($A-A_0$) of the assay against increasing IgE concentrations. A and A_0 represent the spectra ratio (A_{600}/A_{520}) in the presence and absence of IgE, respectively. Error bars represent the standard deviations of three replicates. The inset shows the linear relationship between the spectral response and the concentration of IgE (from 1 nM to 25 nM).

Selectivity of Colorimetric Detection

The selectivity of this AuNP sensor for IgE was examined by visual assay to other physiologically related molecules. As shown in the inset of Fig. 3, only IgE (5

nM) caused a red-to-purple color change, while the solutions of other proteins that were tested at a concentration of 10 nM remained red. Namely, IgE led to a notable spectral ratio change, whereas others caused slight ratio changes that overlap with the background signal, indicating that only IgE effectively triggered the aggregation of AuNPs. Therefore, this proposed aptasensor shows the expected selectivity. In addition, further experiments were performed in diluted clinical samples, and the results exhibited great potential for practical application (Fig. S3).

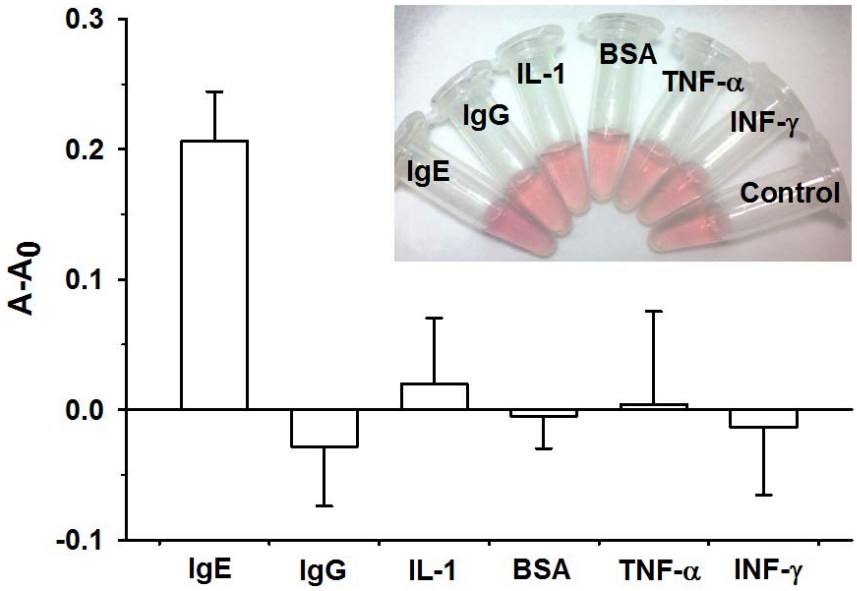

Fig. 3. Selectivity of the pseudoknot probe toward IgE. The inset shows the samples containing the probe with different proteins. The concentration of IgE was 5 nM, and the concentration of each of the other proteins was 10 nM.

Conclusions

We have developed a label-free pseudoknot switch probe-based colorimetric biosensor strategy for simple but effective sensing of IgE with high sensitivity and selectivity. This assay provided a detection limit of 0.2 nM for instrumental detection,

and a linear range up to 25 nM. Moreover, this method can identify IgE spiked into vaginal fluid, providing a good option for the area of clinical analysis. Compared with existing IgE detection techniques, this sensing approach had advantages of speediness, simplicity, and ease of operation. In addition, this method could be further improved by modifying the probe geometry or length. More inspiringly, our aptasensor is facile and cost-effective because of the avoidance of modifying, immobilizing, or labeling processes that result in relatively complicated operation and high cost. Given these features, this proposed colorimetric assay could further facilitate the analytical applications of other hairpin-based structure aptamers with proper probe designs.

Acknowledgments

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References

1. T. A. E. Platts-Mills, *Am. J. Respir. Crit. Care Med.*, 2001, **164**, S1-S5.
2. T. Bacarese-Hamilton, L. Mezzasoma, C. Ingham, A. Ardizzoni, R. Rossi, F. Bistoni and A. Crisanti, *Clin. Chem.*, 2002, **48**, 1367-1370.
3. M. Ollert, S. Weissenbacher, J. Rakoski and J. Ring, *Clin. Chem.*, 2005, **51**, 1241-1249.
4. F. Radom, P. M. Jurek, M. P. Mazurek, J. Otlewski and F. Jeleń, *Biotechnol. Adv.*, 2013, **31**, 1260-1274.
5. Z. Yi, X.Y. Li, Q. Gao, L.J. Tang and X. Chu, *Analyst*, 2013, **138**, 2032-2037.
6. C.C. Chang, S. Lin, S.C. Wei, Y. Chu-Su and C.W. Lin, *Anal. Bioanal. Chem.*, 2012, **402**, 1-9.
7. M. Citartan, S. C. B. Gopinath, J. Tominaga, S.C. Tan and T.H. Tang, *Biosens. Bioelectron.*, 2012, **34**, 1-11.
8. Z. Wang, T. Wilkop, D. Xu, Y. Dong, G. Ma and Q. Cheng, *Anal. Bioanal. Chem.*, 2007, **389**, 819-825.
9. X. Wei, H. Li, Z. Li, M. Vuki, Y. Fan, W. Zhong and D. Xu, *Anal. Bioanal. Chem.*, 2012, **402**, 1057-1063.
10. E. J. Nam, E. J. Kim, A. W. Wark, S. Rho, H. Kim and H. J. Lee, *Analyst*, 2012, **137**, 2011-2016.
11. Y. Ohno, K. Maehashi and K. Matsumoto, *J. Am. Chem. Soc.*, 2010, **132**, 18012-18013.
12. C. Yao, T. Zhu, Y. Qi, Y. Zhao, H. Xia and W. Fu, *Sensors*, 2010, **10**, 5859-5871.
13. M. Citartan, S. C. B. Gopinath, J. Tominaga and T.H. Tang, *Analyst*, 2013, **138**, 3576-3592.
14. G. Aragay, F. Pino and A. Merkoçi, *Chem. Rev.*, 2012, **112**, 5317-5338.
15. K. Saha, S. S. Agasti, C. Kim, X. Li and V. M. Rotello, *Chem. Rev.*, 2012, **112**, 2739-2779.
16. P. Liu, X. Yang, S. Sun, Q. Wang, K. Wang, J. Huang, J. Liu and L. He, *Anal. Chem.*, 2013, **85**, 7689-7695.
17. C.C. Chang, S.C. Wei, T.H. Wu, C.H. Lee and C.W. Lin, *Biosens. Bioelectron.*, 2013, **42**, 119-123.
18. K.M. Song, E. Jeong, W. Jeon, M. Cho and C. Ban, *Anal. Bioanal. Chem.*, 2012, **402**, 2153-2161.
19. Z.S. Wu, H. Lu, X. Liu, R. Hu, H. Zhou, G. Shen and R.Q. Yu, *Anal. Chem.*, 2010, **82**, 3890-3898.
20. P. Zhao, N. Li and D. Astruc, *Coord. Chem. Rev.*, 2013, **257**, 638-665.

21. C.Y. Chen, C.C. Chang, C. Yu and C.W. Lin, *Sensors*, 2012, **12**, 3879-3890.
22. L. Pan, Y. Huang, C. Wen and S. Zhao, *Analyst*, 2013, **138**, 6811-6816.
23. C.C. Chang, S. Lin, C.H. Lee, T.L. Chuang, P.R. Hsueh, H.C. Lai and C.W. Lin, *Biosens. Bioelectron.*, 2012, **37**, 68-74.
24. H. Zhang, B. Jiang, Y. Xiang, Y. Chai and R. Yuan, *Analyst*, 2012, **137**, 1020-1023.
25. R. Freeman, J. Girsh, A. F.j Jou, J.a. A. Ho, T. Hug, J. Dernedde and I. Willner, *Anal. Chem.*, 2012, **84**, 6192-6198.
26. A. Vallée-Bélisle and K. W. Plaxco, *Curr. Opin. Struct. Biol.*, 2010, **20**, 518-526.
27. C. L. King, R. W. Poindexter, J. Ragunathan, T. A. Fleisher, E. A. Ottesen and T. B. Nutman, *J. Immunol.*, 1991, **146**, 1478-1483.
28. I. Y. Huang, M. C. Lee, C. H. Hsu and C. C. Wang, *Sens. Actuator B-Chem.*, 2012, **162**, 184-193.
29. S. Schachermeyer, J. Ashby and W. Zhong, *J. Chromatogr. A*, 2013, **1295**, 107-113.
30. Y. Wang, Z. Ye and Y. Ying, *Chin. Sci. Bull.*, 2013, **58**, 2938-2943.
31. D. T. Tran, V. Vermeeren, L. Grieten, S. Wenmackers, P. Wagner, J. Pollet, K. P. F. Janssen, L. Michiels and J. Lammertyn, *Biosens. Bioelectron.*, 2011, **26**, 2987-2993.

Graphic abstract

A simple design of label-free colorimetric assay for IgE was demonstrated based on a structure-switching aptamer with a low detection limit of 0.2 nM and high selectivity.