

Aptamer-based detection and quantitative analysis of human immunoglobulin E in capillary electrophoresis with chemiluminescence detection

Yan-Ming Liu^{1,*}, Jun-Tao Cao¹, Ying-Ying Liu¹, Jing-Jing Zhang¹, Min Zhou¹, Ke-Jing Huang¹, Yong-Hong Chen², Shu-Wei Ren²

¹ College of Chemistry and Chemical Engineering, Xinyang Normal University, Xinyang 464000, P. R. China

² Xinyang Central Hospital, Xinyang 464000, P. R. China

Correspondence: Prof. Dr. Yan-ming Liu. College of Chemistry and Chemical Engineering, Xinyang Normal University, 237 Chang'an Road, Xinyang 464000, P. R. China

E-mail: liuym9518@sina.com;

Tel & Fax: +86-376-6392889

Abbreviations: AuNPs, gold nanoparticles; CBS, carbonate buffer solution; CL, chemiluminescence; ECL, electrochemiluminescence; HAuCl₄·3H₂O, hydrogen tetrachloroaurate(III) trihydrate; IA, immunoassay; IgE, immunoglobulin E; OED, orthogonal experimental design; PMT, photomultiplier tube; RSDs, relative standard deviations; SELEX, systematic evolution of ligands by exponential enrichments; SH-aptamer, thiol-modified aptamer; TGK, Tris, glycine and KH₂PO₄.

Keywords: Capillary electrophoresis, Chemiluminescence, Aptamer, Gold nanoparticles, Human immunoglobulin E

Received: 24-Mar-2015; Revised: 27-Apr-2015; Accepted: 18-May-2015.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/elps.201500158.

This article is protected by copyright. All rights reserved.

Abstract

A novel aptamer-based capillary electrophoresis (CE) with chemiluminescence (CL) assay was developed for highly sensitive detection of human immunoglobulin E (IgE). The IgE aptamer was conjugated with gold nanoparticles (AuNPs) to form AuNPs-aptamer which could specifically recognize the IgE to produce an AuNPs-aptamer-IgE complex. The mixture of the AuNPs-aptamer-IgE complex and the unbounded AuNPs-aptamer could be effectively separated by CE and sensitively detected with luminol-H₂O₂ CL system. By taking the advantage of the excellent catalytic behavior of AuNPs on luminol-H₂O₂ CL system, the ultrasensitive detection of IgE was achieved. The detection limit of IgE is 7.6 fM ($S/N = 3$) with a linear range from 0.025 to 250 pM. Successful detection of IgE in human serum samples was demonstrated and the recoveries of 94.9-103.2% were obtained. The excellent assay features of the developed approach are its specificity, sensitivity, adaptability, and very small sample consumption. Our design provides a methodology model for determination of rare proteins in biological samples.

1. Introduction

Human immunoglobulin E (IgE) plays an important role in the allergic reaction discovered by Ishizaka et al. [1] and Johansson et al. [2], where its concentration can be used as a marker for several allergic diseases [3,4]. The level of IgE significantly increases in patients afflicted with allergic asthma, atopic dermatitis and other immune deficiency-related diseases, including AIDS [5]. Therefore, highly sensitive and selective determination of human IgE in biological samples is essential. The traditional methods established for IgE detection are mainly radioallergosorbent test [6] and enzyme-linked immunosorbent assay (ELISA) [7-9]. Although antibody-based method possesses distinct advantages, the drawbacks of antibody such as the instability in the production, irreversible denaturation and a relative short shelf life, limit the application of ELISA [10]. Additionally, ELISA requires highly trained personnel to perform the assay. The multistage washing and sample addition process in ELISA are also time-consuming. Accordingly, new bio-recognition elements are highly desirable to explore novel assays for proteins detection including IgE.

Aptamers are short single-stranded oligonucleotides *in vitro* selected by SELEX (systematic evolution of ligands by exponential enrichments) technology [11,12]. With advantages of simple synthesis and modification, high selectivity and affinity toward targets [13-16], and long-term stability [17], aptamers have been extensively investigated as an alternative to traditional antibody. To date, several aptamer-based assays have been developed for IgE detection. For example, Lee et al. [18] developed an ultrasensitive electrochemical biosensor using aptamer as a capture probe for IgE detection with a limit of detection (LOD) of 0.16 pM. Cheow et al. [19] described an electrokinetic concentration

enhanced aptamer affinity probe electrophoresis assay for detection of IgE in a microfluidic device with a LOD of 4.4 pM. Using polystyrene beads as the amplification platform, Peng et al. [20] proposed a highly sensitive aptameric assay based on chemiluminescence (CL) detection for detection of IgE with a LOD of 4.6 pM. Yu et al. [21] designed an electronic channel switching-based aptasensor for ultrasensitive IgE detection with a low LOD of 22.7 fM.

Capillary electrophoresis (CE) offers many advantages including high separation efficiency, short run time, simple instrumentation, minimum operation cost, and compatibility with small sample volumes [22]. CL detection has been proven to be one of the most sensitive detection technique, and the cost of the instrumental setup is also relatively low [23]. CL detection has become an attractive detection scheme for sensitive detection in CE [24,25]. Up to now, nanomaterials have received great attention and are widely used in analysis of aminothiols [26], metalloproteinases [27], and peanut allergen [28] because of their unique physical and chemical properties [29]. Chen's group [30] developed an ultrasensitive IgE assay based on silver nanoparticle hybrid probes and metal-enhanced fluorescence with LOD of 211 fM. Zhao et al. [31] constructed a gold nanoparticles (AuNPs) sensing platform for detection of thrombin by chemiluminescence resonance energy transfer method with a LOD of 15 pM. A colorimetric method for detection of thrombin and adenosine triphosphate (ATP) was also described by Li et al. based on cyclic enzymatic signal amplification and hairpin aptamer probe [32]. The LODs of thrombin and ATP are 50 pM and 100 nM, respectively. In the previous work, we proposed an antibody-based CE-CL method for detection of tumor marker CEA in human serum with a LOD of 0.21 pM

[33]. Recently, our group has further developed an AuNPs enhanced antibody-based CE-CL method for detection of human IgG in human serum with a LOD of 7.1 pM [34].

In this work, a novel aptamer-based CE-CL assay was developed for detection of IgE. AuNPs-aptamer was prepared to specifically capture target protein IgE to produce an AuNPs-aptamer-IgE complex. AuNPs-aptamer-IgE complex and unbound AuNPs-aptamer could be separated by CE and detected by luminol-H₂O₂ CL system. The conditions for CE separation and CL detection were investigated. Ultrasensitive detection of IgE was performed. The proposed method has been applied to determination of IgE in human serum.

2 Materials and methods

2.1 Chemicals

IgE was purchased from Athens Research and Technology (Athens, GA, USA). The SH-aptamer for IgE [35] (5'-SH-(CH₂)₆-TTTTTTTTTTGGGGCACGTTTATCCGTCCCTCCTAGTGGCGTGCCCC-3'), blocker DNA (5'-SH-(CH₂)₆-TTTTTTTTTT-3'), and 10 mM phosphate buffer saline (PBS, pH 7.4) were obtained from Sangon Biological Engineering Technology and Services (Shanghai, China). Both aptamer and protein solutions were prepared with 10 mM pH 7.4 PBS containing 137 mM NaCl, 2.7 mM KCl and 1 mM MgCl₂ and stored at 4 °C.

TGK buffer contains 25 mM Tris, 182 mM glycine and 5 mM KH₂PO₄ (pH = 8.5). Hydrogen tetrachloroaurate (III) trihydrate (HAuCl₄·3H₂O, Au 49.5% min) was obtained from Alfa Aesar (A Johnson Matthey Company, Ward Hill, MA). Tween 80 and bovine serum albumin (BSA) were from Seebio Biotechnology Inc. (Shanghai, China). Luminol was from Sigma-Aldrich (St. Louis, MO, USA). All reagents used were of analytical grade. The

pure water ($18.2 \text{ M}\Omega\cdot\text{cm}$) obtained from an ultrapure water purification system (Kangning Water Treatment Solution Provider, China) was used in all runs. All solutions were stored at 4°C and filtered through $0.22 \text{ }\mu\text{m}$ cellulose acetate membrane filters (Shanghai Xingya Purification Material Factory, China) before use.

2.2 Apparatus

The CE-CL apparatus was built in the laboratory as previously described [33]. The separation voltage was provided by a 0-30 kV power supply (Shanghai Institute of Applied Physics, Chinese Academy of Sciences, China). The CL signal was collected with a BPCL ultraweak luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China).

A $50 \text{ cm} \times 75 \text{ }\mu\text{m}$ id capillary (Hebei Yongnian Ruifeng Chromatographic Apparatus, China) was used for separation. On one end of the separation capillary, 5 cm of the polyimide coating was burned off. After etching with 40% HF for 2.5 h, the capillary was preconditioned sequentially by flushing with 2.0 M $\text{CH}_3\text{OH-NaOH}$, 1.0 M NaOH, 1.0 M HCl and water for 20 min, respectively, and then equilibrated with the running buffer for 20 min. After that, the treated end of the capillary was inserted into a $20 \text{ cm} \times 530 \text{ }\mu\text{m}$ id reaction capillary, on which a 1 cm detection window was made and located just in front of the photomultiplier tube (PMT, CR-135, Binsong Photonics, Beijing, China). The pair of $40 \text{ cm} \times 320 \text{ }\mu\text{m}$ id capillaries was used to introduce post column CL reagents to a tee reservoir from two vials located 20 cm above it. The CL reagents were delivered by gravity through the reagent-introducing capillaries and mixed, flowing coaxially along the reaction capillary. The CL reaction occurred at the outlet of separation capillary when analyte eluted out from

the separation capillary. The ground electrode was also put into one joint of the tee.

The UV-vis spectroscopic experiment was performed with an UVmini-1240UV-vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The CL spectrum was recorded by a Cary Eclipse fluorescence spectrophotometer (Varian Inc., Walnut Creek).

2.3 Conjugation of SH-aptamer and AuNPs

AuNPs were prepared according to the literature reported previously [36]. The concentration of AuNPs was calculated to be about 3.2×10^{-10} M. The AuNPs-aptamer conjugates were prepared by following the reported procedure [37]. 4 μ L 10% (v/v) Tween 80 was firstly added into 1 mL AuNPs, the mixture was then incubated at 25 °C for 30 min under gentle stirring. Subsequently, 50 μ L 10 μ M SH-aptamer was added to the mixture and then incubated at 25 °C for 30 min. Afterwards, 50 μ L 30 μ M blocker DNA were added into the above mixture. Prior to aging at 50 °C for 2.5 h, the mixture was brought to 1 mL 0.1 M PBS. The resulted solution was centrifuged at 10 000 rpm for 15 min to remove the unconjugated DNA. The precipitate was washed three times with pH 7.4 0.1 M PBS containing 0.05% (v/v) Tween 80, with repetitive centrifugation and dispersion. Final conjugates were redispersed in the same PBS solution and stored at 4 °C for further use.

2.4 Preparation of AuNPs-aptamer-IgE complex

10 μ L IgE standard solution or serum sample was mixed with 10 μ L AuNPs-aptamer in a 200 μ L microcentrifuge tube. The mixture was diluted to 40 μ L with 10 mM pH 7.4 PBS containing 137 mM NaCl, 2.7 mM KCl and 1 mM $MgCl_2$. The mixture solution was then incubated at room temperature for 0.5 h and subsequently analyzed by CE-CL system.

2.5 CE-CL system

Prior to analysis, the separation capillary was rinsed with 0.1 M NaOH, water, and running buffer for about 3 min successively. Between three consecutive injections, the separation capillary was flushed with 0.1 M NaOH, water, and running buffer for 2 min, respectively. The running buffer is 25 mM TGK, 0.30 mM luminol and 3.5 μ M BSA at pH 8.5. The postcolumn CL reagent contains 25 mM sodium carbonate buffer solution (CBS) and 50 mM H₂O₂. The voltage of PMT was set at -800 V in the detection process. The sample was introduced by electrokinetic injection at 12 kV for 6 s, and the electrophoresis was performed at 20 kV. The peak area of AuNPs-aptamer-IgE was used for quantification in this work.

2.6 Preparation of human serum sample

Human blood samples were provided by Xinyang Central Hospital (Xinyang, China). The fresh blood samples were centrifuged immediately at 1000 rpm for 15 min to obtain serum. The treated serum samples were stored at -20°C .

3 Results and discussion

3.1 Principle of the proposed protocol for human IgE detection

It is well known that the formation of thiol-gold bonding is easy and it has been commonly used to immobilize SH-aptamer on AuNPs. In this work, AuNPs were used as a platform for introduction of SH-aptamer and an effective catalyst of luminol-H₂O₂ CL system for signal enhancement. SH-aptamer was immobilized onto the surface of AuNPs as a capture reagent for target protein. In order to avoid the nonspecific adsorption, the blocker DNA was used to cover any remaining unoccupied surfaces of AuNPs [38]. When IgE was added, the AuNPs-aptamer-IgE complex was preferred to form instead of noncovalent binding to

AuNPs because of the high specificity and affinity of the aptamer to the target molecules. Subsequently, the mixture of AuNPs-aptamer-IgE complex and unbound AuNPs-aptamer was separated by CE and detected by CL. This new protocol is an alternative to the antibody-based CE-CL protein assay.

3.2 Characterization of AuNPs-aptamer and AuNPs-aptamer-IgE

The UV-vis absorption and CL spectra were used to verify the formation of AuNPs-aptamer and AuNPs-aptamer-IgE. The size of the AuNPs could be estimated to be 35 nm from its absorption peak at about 526 nm (Fig. 1A, curve a). The size was also substantiated from the TEM image of the AuNPs (Supporting Information Fig. S1). Compared with the AuNPs, the UV-vis spectra of AuNPs-aptamer (Fig. 1A, curve b) and AuNPs-aptamer-IgE (Fig. 1A, curve c) show a red shift of the absorption peaks, and another strong absorption at approximately 260 nm due to the adsorption of aptamer and IgE. The results demonstrate that the aptamer and the IgE were immobilized on the AuNPs. Compared with the bare AuNPs, the CL intensities of AuNPs-aptamer and AuNPs-aptamer-IgE were decreased successively (see Fig. 1B), which further proves the formation of AuNPs-aptamer and AuNPs-aptamer-IgE.

3.3 Optimization of CE separation conditions

In order to gain desired CE separation results, the parameters including concentration and pH of TGK, and concentration of BSA were optimized with orthogonal experimental design (OED) method (factors and levels for $L_9 (3^4)$ OED experiments have been listed in Supporting Information Table S1). To decrease the adsorption of protein on the inner wall of capillary, BSA was selected as an additive into the electrophoretic buffer. The optimal

running buffer is 25 mM TGK and 3.5 μ M BSA at pH 8.5. The separation voltage has also a significant effect on resolution and CL intensity (Supporting Information Fig. S2). The optimized voltage is 20 kV.

3.4 Optimization of CL detection conditions

To increase the sensitivity of CL detection following CE separation, the CL detection conditions including the concentration of luminol, H_2O_2 , and CBS as well as the pH of CBS were examined and the results were shown in Supporting Information Fig. S3. The maximum CL signal was obtained with luminol of 0.3 mM and H_2O_2 of 50 mM. The optimal values for concentration and pH of CBS were 25 mM and pH 11.5, respectively.

3.5 Detection of IgE by the proposed protocol

Under the optimum conditions, the AuNPs-aptamer-IgE complex and the unbound AuNPs-aptamer were well separated and detected within 5 min. A typical electropherogram of AuNPs-aptamer was presented in Fig. 2A. After the addition of the different concentration of IgE, the peaks of the complex appeared as shown in Fig. 2B-D. It can be seen that the peak area of the complex increased with the IgE concentration increasing. The calibration plot reveals that the CL intensity increases linearly with the logarithm of IgE concentration in the range of 0.025-250 pM. The linear regression equation is $Y = 278615 + 16010 X$ with a correlation coefficient of $R = 0.996$. The LOD of IgE is 7.6 fM ($S/N = 3$). The relative standard deviation values (RSDs) of the migration time and the peak area ($n = 5$) for 5.0 pM IgE are 1.5%, 2.3% (intra-run), and 2.2%, 5.4% (inter-run, for a 4-day period), respectively.

3.6 Determination of IgE in human serum samples

To evaluate applicability of the proposed method, seven human serum samples including 4

non-atopic patients (No. 1-4) and 3 atopic patients (No. 5-7) from Xinyang Central Hospital were tested by the proposed method (X) and an electrochemiluminescence (ECL) method by Cobas E601-3 instrument (Y) employed in Xinyang Central Hospital as control. When the level of IgE in serum sample was over the calibration range, serum sample was appropriately diluted with water prior to assay. Compared with the values obtained by ECL method, the analytical results of the proposed method show an acceptable agreement, with relative errors less than 9.9% (Table 1). The two methods coincide well with a regression equation of $y = 1.03816x - 0.05333$ with a correlation coefficient R of 0.998. In addition, the recovery studies were carried out by standard addition method for human serum samples. Three different IgE concentration (0.25, 5.0, 25.0 pM) were added to the human serum samples respectively. The electropherograms of serum samples and spiked with IgE were shown in Fig. 3A and Fig. 3B, respectively. The recoveries from 94.9 to 103.2% and the RSDs of peak area from 2.3 to 3.7% ($n = 5$) were obtained, indicative of the satisfactory accuracy and reliability of the proposed method.

4 Concluding remarks

This work describes a novel aptamer-based CE-CL method for IgE detection. Aptamer, a new recognition element, was introduced in CE analysis. AuNPs were used as the platform for immobilization of SH-aptamer and signal enhancer for luminol- H_2O_2 CL system. With the proposed protocol, ultrasensitive detection of fM level human IgE was achieved. The applicability of this strategy has been demonstrated by measuring IgE in human serum samples. The present method has many outstanding merits including high specificity and affinity of aptamer, good separation efficiency of CE, excellent catalytic behavior of AuNPs,

and high sensitivity of CL detection. This approach might open a new door for proteins detection.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant 21375114, 21405129), the Project of Science and Technology development of Henan Province (142300410197), the Foundation of Henan Educational Committee (14A150013), and Program for Innovative Research Team (in Science and Technology) in University of Henan Province (15IRTSTHN001).

The authors have declared no conflict of interest.

5 References

- [1] Ishizaka, K., Ishizaka, T., I, *J. Immunol.* 1967, 99, 1187-1198.
- [2] Johansson, S. G., Bennich, H., *Immunology* 1967, 13, 381-394.
- [3] Gould, H. J., Sutton, B. J., Beavil, A. J., Beavil, R. L., McCloskey, N., Coker, H. J., Fear, D., Smurthwaite, L., *Ann. Rev. Immunol.* 2003, 21, 579-628.
- [4] Platts-Mills, T. A., *Am. J. Respir. Crit. Care Med.* 2001, 164, S1-S5.
- [5] Maehashi, K., Katsura, T., Kerman, K., Takamura, Y., Matsumoto, K., Tamiya, E., *Anal. Chem.* 2007, 79, 782-787.
- [6] Wide, L., Bennich, H., Johansson, S.G., *Lancet* 1967, 2 1105-1107.
- [7] Kirsch, S., Fourdrilis, S., Dobson, R., Scippo, M. L., Maquhin-Roqister, G., De Pauw, E.,

- Anal. Bioanal. Chem.* 2009, 395, 57-67.
- [8] Sævik, B. K., Ulstein, T. L., Larsen, H. J. S., *Res. Vet. Sci.* 2003, 74, 37-45.
- [9] Chung, S.Y., Champagne, E. T., *J. Agric. Food Chem.* 2001, 49, 3911-3916.
- [10] Wang, Y. X., Ye, Z. Z., Si, C.Y., Ying, Y. B., *Chin. J. Anal. Chem.* 2012, 40, 634-642.
- [11] Ellington, A. D., Szostak, J. W., *Nature* 1990, 346, 818-822.
- [12] Tuerk, C., Gold, L., *Science* 1990, 249, 505-510.
- [13] Deng, N., Liang, Z., Liang, Y., Sui, Z. G., Zhang, L.Y., Wu, Q., Yang, K. G., Zhang, L. H., Zhang, Y. K., *Anal. Chem.* 2012, 84, 10186-10190.
- [14] Billinge, E. R., Broom, M., Platt, M., *Anal. Chem.* 2014, 86, 1030-1037.
- [15] Khezrian, S., Salimi, A., Teymourian, H., Hallaj, R., *Biosens. Bioelectron.* 2013, 43, 218-225.
- [16] Haes, A. J., Giordano, B. C., Collins, G. E., *Anal. Chem.* 2006, 78, 3758-3764.
- [17] Radi, A. E., Sanches, L. A., Baldrich, E., O'Sullivan, C. K., *J. Am. Chem. Soc.* 2006, 128, 117-124.
- [18] Lee, C.Y., Wu, K. Y., Su, H. L., Hung, H. Y., Hsieh, Y. Z., *Biosens. Bioelectron.* 2013, 39, 133-138.
- [19] Cheow, L. F., Han, J. Y., *Anal. Chem.* 2011, 83, 7086-7093.
- [20] Peng, Q. W., Cao, Z. J., Lau, C. W., Kai, M., Lu, J. Z., *Analyst* 2011, 136, 140-147.
- [21] Li, H. B., Wang, C., Wu, Z. S., Lu, L. M., Qiu, L. P., Zhou, H., Shen, G. L., Yu, R. Q., *Anal. Chim. Acta* 2013, 758, 130-137.
- [22] Wang, J. H., Huang, W. H., Liu, Y. M., Cheng, J. K., Yang, J., *Anal. Chem.* 2004, 76, 5393-5398.

- [23] Qi, Y. Y., Li, B. X., *Chem. Eur. J.* 2011, *17*, 1642-1648.
- [24] Jiang, J., Zhao, S. L., Huang, Y., Qin, G. X., Ye, F. G., *J. Chromatogr. A* 2013, *1282*, 161-166.
- [25] Wang, J. H., Li, L. M., Huang, W. H., Cheng, J. K., *Anal. Chem.* 2010, *82*, 5380-5383.
- [26] Li, Q. Q., Liu, F., Lu, C., Lin, J. M., *J. Phys. Chem. C* 2011, *115*, 10964-10970.
- [27] Cheng, W., Chen, Y., Yan, F., Ding, L., Ding, S., Ju, H., Yin, Y., *Chem. Commun.* 2011, *47*, 2877-2879.
- [28] Pollet, J., Delport, F., Janssen, K. P., Tran, D. T., Wouters, J., Verbiest, T., Lammertyn, J., *Talanta*, 2011, *83*, 1436-1441.
- [29] Zamborini, F. P., Bao, L. L., Dasari, R., *Anal. Chem.* 2012, *84*, 541-576.
- [30] Li, H., Qiang, W. B., Vuki, M., Xu, D. K., Chen, H.Y., *Anal. Chem.* 2011, *83*, 8945-8952.
- [31] Qin, G. X., Zhao, S. L., Huang, Y., Jiang, J., Liu, Y. M., *Biosens. Bioelectron.* 2013, *46*, 119-123.
- [32] Li, J., Fu, H. E., Wu, L. J., Zheng, A. X., Chen, G. N., Yang, H. H., *Anal. Chem.* 2012, *84*, 5309-5315.
- [33] Liu, Y. M., Yue, H. Y., Tian, W., Chen, Y. H., Li, F. R., *Anal. Lett.* 2009, *42*, 45-57.
- [34] Liu, Y. M., Mei, L., Liu, L. J., Peng, L. F., Chen, Y. H., Ren, S.W., *Anal. Chem.* 2011, *83*, 1137-1143.
- [35] Wiegand, T. W., Williams, P. B., Dreskin, S. C., Jouvin, M., Kinet, J., Tasset, D., *J. Immunol.* 1996, *157*, 221-230.
- [36] Frens, G., *Nat. Phys. Sci.* 1973, *241*, 20-22.

[37] Xu, S. M., Yuan, H., Xu, A., Wang, J., Wu, L. J., *Langmuir*, 2011, 27, 13629-13634.

[38] Huang, Y., Chen, J., Zhao, S. L., Shi, M., Chen, Z. F., Liang, H., *Anal. Chem.* 2013, 85, 4423-4430.

Accepted Article

Fig. 1. UV-vis absorption spectrum (A) and CL spectrum (B). Curves: a. bare AuNPs; b. AuNPs-aptamer; c. AuNPs-aptamer-IgE; d. luminol + H_2O_2 + bare AuNPs; e. luminol + H_2O_2 + AuNPs-aptamer; f. luminol + H_2O_2 + AuNPs-aptamer-IgE; g. luminol + H_2O_2 .

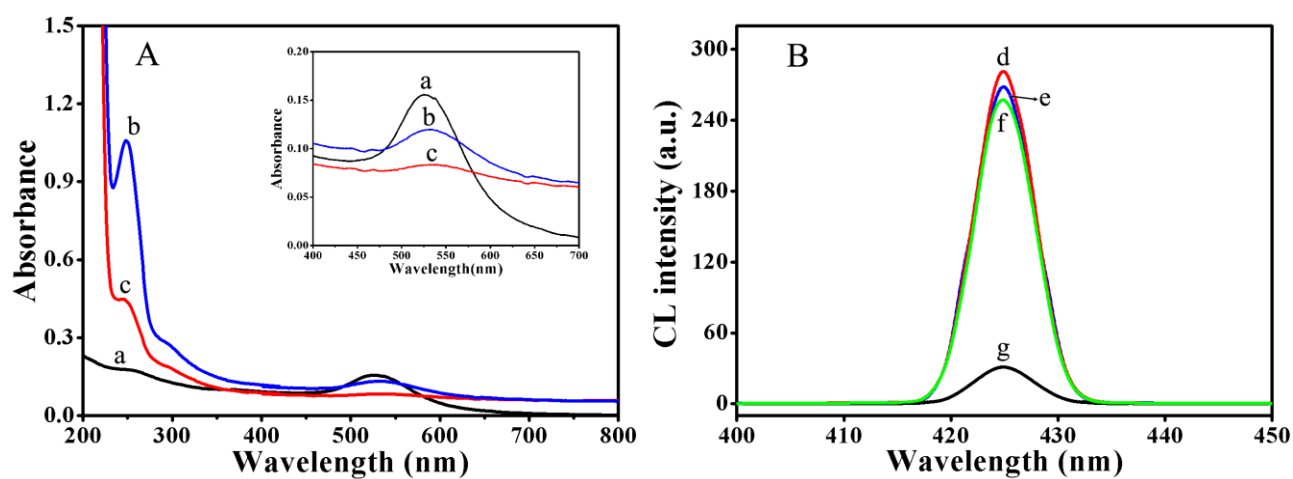


Fig. 1

Fig. 2. Electropherograms of AuNPs-aptamer (A) and the separation of AuNPs-aptamer-IgE complex and unbound AuNPs-aptamer (B-D). Parts A, B, C, and D correspond to 0, 5, 25, and 50 pM human IgE, respectively; Peak a, AuNPs-aptamer-IgE complex; peak b, unbound AuNPs-aptamer. Conditions: running buffer, 25 mM TGK with 0.30 mM luminol and 3.5 μ M BSA at pH 8.5; the postcolumn CL reagents, 50 mM H_2O_2 and 25 mM pH 11.5 CBS; electrokinetic injection with 12 kV for 6 s; separation voltage, 20 kV.

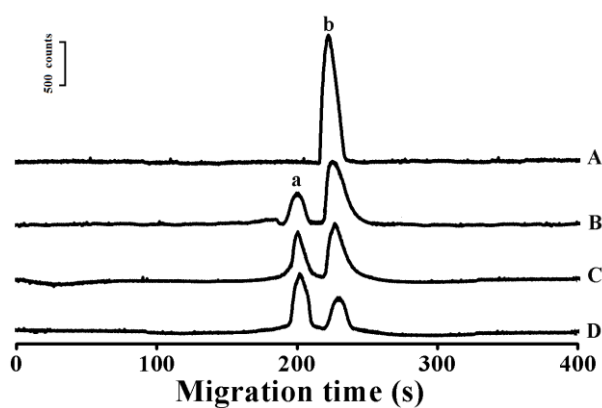


Fig. 2

Fig. 3. Electropherograms of the serum sample (A) and spiked with IgE (B). Peak a, AuNPs-aptamer-IgE complex; peak b, unbound AuNPs-aptamer.

Table 1. Analytical results of seven human serum samples

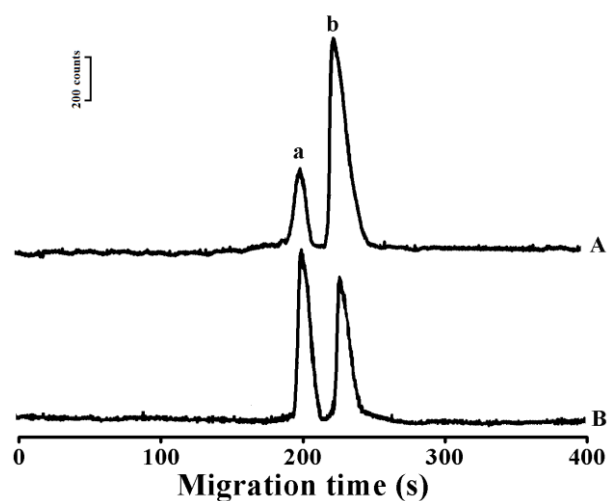


Fig. 3

Table 1. Analytical results of seven human serum samples ($n = 3$)

No. of samples	Human IgE contents (nM)		
	ECL method	This method (RSDs, %)	Relative error (%)
1	0.206	0.187 (6.6)	-9.22
2	0.414	0.455 (4.3)	9.90
3	0.316	0.304 (2.1)	3.80
4	0.436	0.450 (2.0)	3.21
5	1.87	1.90 (8.7)	1.60
6	1.03	1.12 (7.6)	8.74
7	3.73	3.60 (6.9)	-3.48

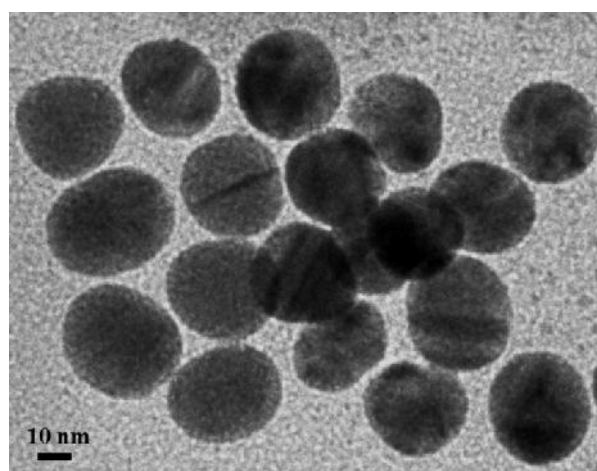


Fig S1. TEM images of the AuNPs.

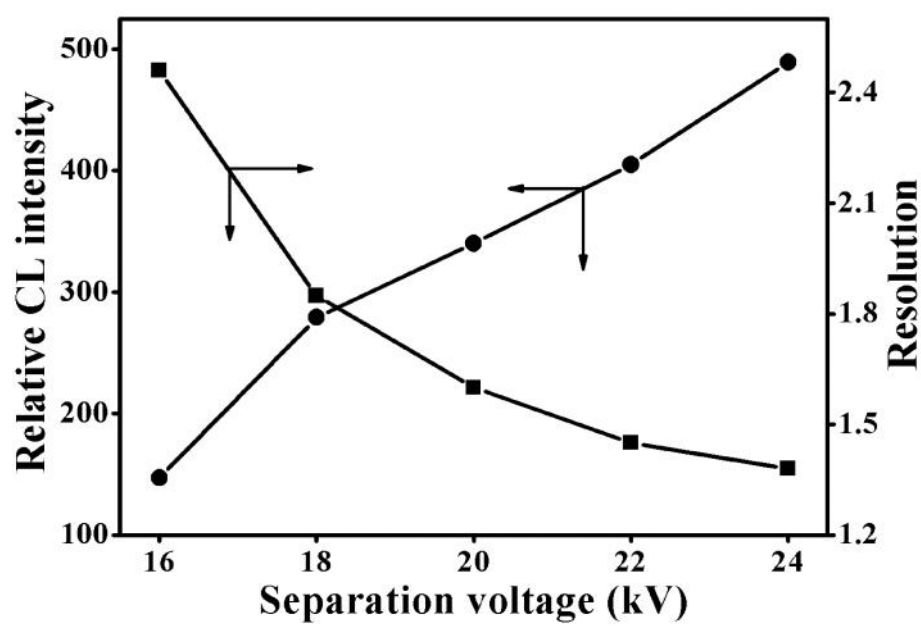


Fig. S2. Effects of separation voltage on CL intensity and resolution.

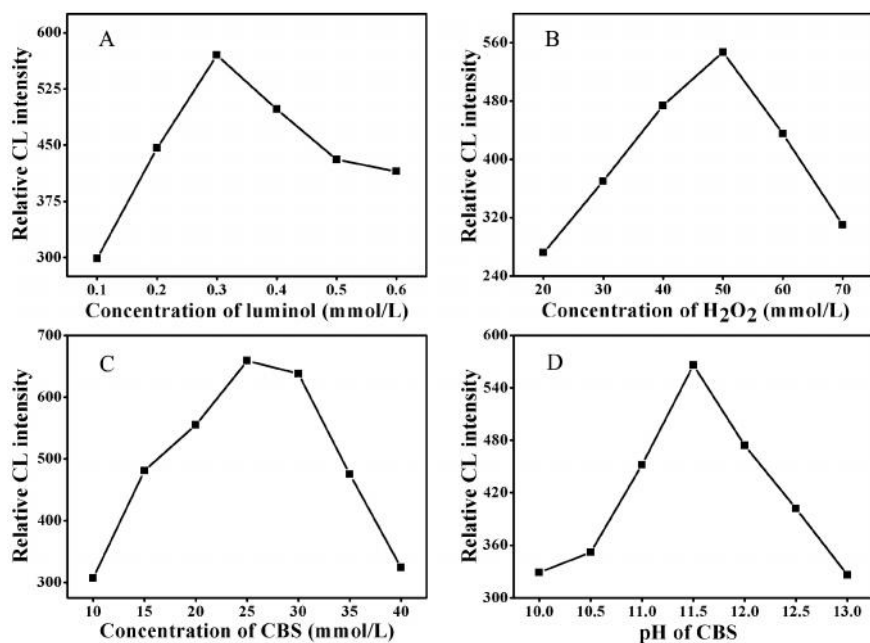


Fig. S3. Effects of concentrations of luminol (A), H₂O₂ (B), and CBS (C), and pH of CBS (D). Conditions: running buffer, 25 mM TGK with 0.30 mM luminol and 3.5 μ M BSA at pH 8.5, electrokinetic injection with 12 kV for 6 s; separation voltage, 20 kV; separation capillary, 50 cm $\times$ 75 μ m i.d.

Table S1 Factors and levels for L₉ (3⁴) OED experiments

No.	Concentration of buffer (mM)	pH of buffer	Concentration of BSA (μ M)
1	20	8.0	2.5
2	25	8.0	3.0
3	30	8.0	3.5
4	20	8.5	3.0
5	25	8.5	3.5
6	30	8.5	2.5
7	20	9.0	3.5
8	25	9.0	2.5
9	30	9.0	3.0