



Paper-based upconversion fluorescence aptasensor for the quantitative detection of immunoglobulin E in human serum

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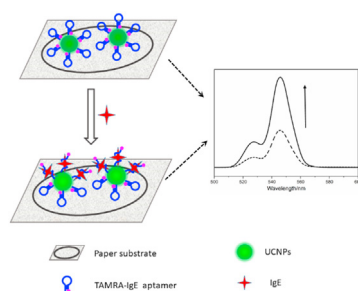
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HIGHLIGHTS

- Novel paper-based UC-LRET aptasensor was developed for sensitive, selective and accurate detection of IgE in human serum.

GRAPHICAL ABSTRACT



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ABSTRACT

Immunoglobulin E (IgE), a biomarker of allergic diseases, plays a critical role in allergic mechanism. Because of its low abundance in serum, the demand of developing sensitive, selective and simple methods for IgE detection is still very urgent. Paper-based analytical devices using upconversion nanoparticles (UCNPs) as the label can be promising point-of-care test (POCT) methods in rapid diagnosis, owing to their NIR-excitation and visible light emission nature, which can avoid the interference of autofluorescence and scattering light from biological samples and paper substrates. In this work, we proposed a paper-based analytical device for the sensitive, selective and accurate detection of total immunoglobulin E (IgE) in human serum. The assay was based on resonance energy transfer between UCNPs and organic dye tetramethylrhodamine (TAMRA), and IgE aptamer with stem-loop structure was used as the recognizing probe. The existence of IgE change the conformation of IgE aptamer, enlarge the distance between donor and acceptor, and block the energy transfer process. Thus, the luminescence of UCNPs recovered with an IgE concentration independent manner. A linear calibration was obtained in the range of 0.5–50 IU/mL, with a detection limit of 0.13 IU/mL. The results of our method were well correlated with that of commercial ELISA kit (20 human serum samples). This work suggests promising prospect of the paper-based UC-LRET analytical devices in real samples and may promote the application of paper-based analytical devices in clinical diagnosis.

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1. Introduction

As the least abundant isotype of immunoglobulin in human serum, Immunoglobulin E (IgE) plays a critical role in allergic diseases [1]. IgE can initiate the release of chemicals such as histamine, leukotrienes, and certain interleukins through binding to basophils and mast cells. These chemicals can further cause symptoms associated with allergies such as dermatitis, asthma, and even shock which can bind to basophils and mast cells to trigger the release of chemicals like histamine [2]. The concentration of total IgE in a healthy adult is about 80 IU/mL and will be increased 4–30 fold in the event of allergic disease, in which 1.0 IU/mL is equivalent to 2.4 ng IgE/mL [3]. So, IgE is chosen as allergy biomarker and used to support the diagnosis of allergy and predict allergen immunotherapy effectiveness. There are mainly four kinds of clinical methods used for measuring serum IgE, i.e., reflective immunosorbent assay (IRST), indirect hemagglutination test, enzyme-linked immunosorbent assay (ELISA), and chemiluminescence assay. These methods are all based on immunoassay techniques, with the limitation of high detecting cost, long time-consuming and the complexity for IgE detection [4]. In order to achieve sensitive yet simple and rapid methods for the quantitative analysis of proteins, the use of aptamer with high affinity and specificity towards the target, can be a good alternative. D17.4 IgE aptamer, the most commonly used IgE aptamer was first selected by Wiegand and co-workers in 1996 [5]. Since then, a series of aptamer-based probes for IgE detection have been reported, including optical [6], electrochemical [7,8], and mechanical [9] sensing schemes. But because of its very low content in serum, the requirement of researching and developing new methods with excellent sensitivity and selectivity for the detection of IgE is still very pressing.

Paper-based analytical devices (PADs), using paper as analytical substrate materials, integrate the advantages of paper and microfluidic analytical devices and show great prospect in clinical diagnosis, especially in point-of-care diagnosis [10–12]. Luminescence nanomaterials possess unique physical and chemical properties such as quantum size effect, plasmonics and resonance energy transfer. So, more and more of researchers have sought effective ways to integrate luminescence nanomaterials with paper-based analytical devices in recent years. Gold nanoparticles (AuNPs) were one of the first luminescence nanomaterials used in PADs, owing to the color change caused by particle aggregation [13,14]. Other nanoparticles, such as semiconductor quantum dots (QDs) [15,16] and upconversion nanoparticles (UCNPs) [17–19] were introduced into PADs subsequently. Compared to those traditional down-conversion luminophores, UCNPs, exciting with near-infrared (NIR) light, could effectively avoid the interference of autofluorescence and scattered light from biological samples and paper substrate, which considerably improved the robustness and sensitivity of fluorescence biosensors [20–22]. Therefore, paper-based analytical devices using UCNPs as labels are likely to be promising tools with a combination of simplicity, accuracy, and sensitivity, promoting the application of PADs in point-of-care diagnosis.

Luminescence resonance energy transfer (LRET) is a homogeneous analysis technology with high sensitivity, in which the energy transfer nonradiatively from excited energy donor to energy acceptor by means of dipole-dipole coupling. UCNPs have been used as the energy donors of LRET technology to develop a series of excellent analytical methods in homogeneous solution [23–27]. In 2013, Liu's group introduced LRET technology using UCNPs as energy donors to paper-based analytical devices for the first time, realizing the quantitative detection of protein in human serum and whole blood with high sensitivity [28]. Thereafter, contributions from other groups have demonstrated that the combination of

PADs with UCNPs and LRET can be useful tools for the analysis of oligonucleotide targets, enzyme, protein and explosive. Krull and co-workers reported a paper-based analytical method for DNA detection, in which UCNPs were fixed on the surface of paper as energy donors, and fluorescent dyes were drawn to the surface of UCNPs through nucleic acid hybridization [29]. Compared to paper-based LRET assay using QDs as energy donors, this UCNPs-based assay possessed lower limit of detection and wider dynamic range. They also verified the possibility to concurrently use the green emission and red emission of UCNPs to quantitative duplexed analysis of oligonucleotide targets [30]. By making full use of the multicolor emission characteristics of UCNPs, Yuan's group developed a paper-based radiometric luminescence device for the successful visualization of TNT explosives [31].

Herein, we proposed a paper-based UC-LRET aptasensor for quantitative IgE detection by utilizing the specific recognition between IgE aptamer and IgE, in which UCNPs and organic dyes were used as energy donor-acceptor pairs. The secondary structure of IgE aptamer is a stem-loop structure and the recognition site is in the part of the loop, and the conformation of the aptamer readily changes upon binding with IgE. Through the reasonable design of aptamer sequence, a novel molecular beacon sensor was developed on paper substrate. In the case of molecular beacon mode, the distance between donor and acceptor could be reduced to molecular size, increasing the quenching efficiency of UCNPs and improving detection sensitivity. Meanwhile, the “turn-off” TAMTA-aptamer-UCNPs complex could be loaded on paper substrate and realized the one-step detection of IgE without any washing and separation steps, providing a simple, rapid, sensitive and reliable detection method for IgE detection [32].

2. Materials and methods

2.1. Materials

Polyethylenimine (PEI, with an average molecular weight of 25000) and sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (Sulfo-SMCC) were obtained from Sigma-Aldrich. Immunoglobulin E (IgE) was purchased from Shanghai Linc-Bio Science Co., Ltd. (Shanghai, China). The rest of the chemical reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Whatman no.1 filter paper was purchased from Whatman (UK). The sulfhydryl modified IgE aptamers labeled at 3' end with Tetramethylrhodamine (TAMRA) were purchased from SBS Genetech Co., Ltd. (Shanghai, China). Their sequences were as follows: (1) DNA 1: 5'-SH-AAAAACGTTTATCCGTCCTCC-TAGTGGCG-TAMRA-3'; (2) DNA 2: 5'-SH-AAAAAGCACGTT-TATCCGTCCTCCTAGTGGCGTGC-TAMRA-3'; (3) DNA 3: 5'-SH-AAAAAGGGGCACGTTTATCCGTCCTCCTAGTGGCGTGCCCC-TAMRA-3'.

2.2. Apparatus

The size and morphology of UCNPs were characterized by a JEM-2010 transmission electron microscope (TEM) with an acceleration voltage of 200 kV (Hitachi H-7000FA, Japan). The crystal phase of UCNPs was identified by Bruker D8 Discover X-ray Diffractometer (XRD) (Bruker, Germany) with 2θ range from 10° to 70° at a scanning rate of 4 deg/min, with Cu K α irradiation ($k = 1.5406 \text{ \AA}$). FT-IR spectra of PEI-UCNPs were measured on a NICOLET 5700 FT-IR Spectrometer (Thermo Scientific, USA) with the KBr pellet technique. UV–vis absorption spectra were measured on an UV-2550 spectrophotometer (Shimadzu, Tokyo, Japan). Upconversion luminescence spectra were collected with a RF-5301PC fluorescence spectrophotometer (Shimadzu, Tokyo, Japan). An external 980 nm

CW laser (Beijing Hi-Tech Optoelectronic Co., Ltd.) were used as excitation source. The laser power was set as 0.2 W. The optical density of 96-well plates were measured by Thermo Scientific Multiskan Mk3 microplate reader (Thermo Scientific, USA). The luminescence lifetime was measured with FLS1000 photoluminescence spectrometer (Edinburgh Instruments, UK).

2.3. Synthesis of PEI-UCNPs

Oleic acid stabilized NaYF_4 : Yb, Er nanoparticles were first synthesized according to previously reported method and dispersed in chloroform solution [33]. Then, the PEI-modified UCNPs were prepared through ligand exchange strategy. Firstly, a certain amount of PEI (600 mg) and DEG (30 mL) were mixed in a 100 mL three-necked flask. The mixture were heated to 110 °C and reacted in vacuum until the solution became clear. 100 mg of OA-UCNPs were then added to the flask dropwise. The solution was reacted at this temperature for 1 h under argon protection and then heated to 240 °C. After reacting for another 6 h, the solution was cooled down to room temperature. Precipitates were obtained with the addition of ethanol, which were washed several times with ethanol and ultrapure water. Then the as-obtained PEI-UCNPs were dispersed in ultrapure water for further use.

2.4. Attachment of the TAMRA modified IgE aptamer to UCNPs

Sulfhydryl modified IgE aptamer labeled at 3' end with TAMRA was covalently conjugated to UCNPs with the assistance of cross-linking agent Sulfo-SMCC. Briefly, PEI-UCNPs (1 mg) was dispersed in HEPES buffer (pH 7.2, 10 mM). Then Sulfo-SMCC (0.5 mg) was added and reacted at room temperature without light for 1 h with gentle shaking. To remove excess Sulfo-SMCC, the mixture was centrifuged and washed with HEPES buffer for three times. Thereafter, the maleimide-activated UCNPs were redispersed in HEPES buffer (pH 7.4, 10 mM) and incubated with TAMRA-aptamer-SH (1 nmol) at room temperature for 12 h under gentle shaking. After washing with HEPES buffer for three times, the obtained TAMRA-aptamer-UCNPs conjugate was finally dispersed in 2 mL of HEPES buffer (pH 7.4, 10 mM) solution.

2.5. Paper-based IgE determination in aqueous solution and serum samples

Paper-based analytical devices were prepared through our previously reported one-step plotting method [28], using Whatman no.1 filter paper as substrate. The test zones were circular with a diameter of 2 mm. A set of 2 μL solutions of the TAMRA-aptamer-UCNPs conjugate in HEPES (0.02 mg/mL) were spotted on test zones. The paper-based analytical devices were kept in 37 °C for 10 min. Thereafter, 2 μL of IgE solutions at various concentrations (0, 0.5, 5, 10, 25, and 50 IU/mL) were added and incubated for 30 min at given temperature. With 980 nm laser as excitation source, the upconversion fluorescence tests were performed and the emission intensity at 545 nm was taken for quantification. In order to obtain better detection result, we have optimized the reaction conditions, such as DNA chain length, the reaction temperature, and the binding buffer. Three different length of DNA chains with different number of hybridized base pairs were attached to UCNPs and reacted with IgE at different temperatures (30 °C, 37 °C and 42 °C). Three different binding buffers were used to dilute IgE: (1) binding buffer 1: PB buffer (10 mM, pH 7.4); (2) binding buffer 2: PBS buffer (10 mM, 138 mM NaCl, 2.7 mM KCl, pH 7.4); (3) binding buffer 3: PBS buffer (10 mM, 138 mM NaCl, 2.7 mM KCl, 1 mM MgCl_2 , pH 7.4). To examine the specificity of this aptasensor, a series of distractors including Na^+ , K^+ , Mg^{2+} , glucose, glycine, BSA,

lysozyme and IgG were detected at the same experimental procedures for IgE. To detect the content IgE in human serum, freshly collected serum samples from 20 volunteers, which were obtained from ZhongNan Hospital, Wuhan University, were 10-fold diluted with HEPES buffer and detected with the same experimental procedure as in the buffer solution.

2.6. Determination of IgE in human serum by ELISA kit

To further verify the practicability and reliability of the paper-based analytical devices, the total level of IgE in human serum samples were determined via ELISA kit (EUROIMMUN Medizinische Labordiagnostika AG, Germany). Briefly, 100 μL of standard with different concentrations, the total IgE positive/negative control, and the serum samples were added to wells coated with anti-human IgE polyclonal antibody respectively. The plate was incubated at room temperature for 30 min. 100 μL /well anti-IgE antibodies conjugated to horseradish peroxidase were added to wells. Between each step, the plate was washed 3 times with 10-fold diluted washing buffer. 100 μL /well TMB substrate was then added and the plate was incubated at room temperature without light for 15 min. 100 μL /well TMB peroxidase stop solution was added when the color developed. The optical density at 450 nm was measured for quantitative analysis.

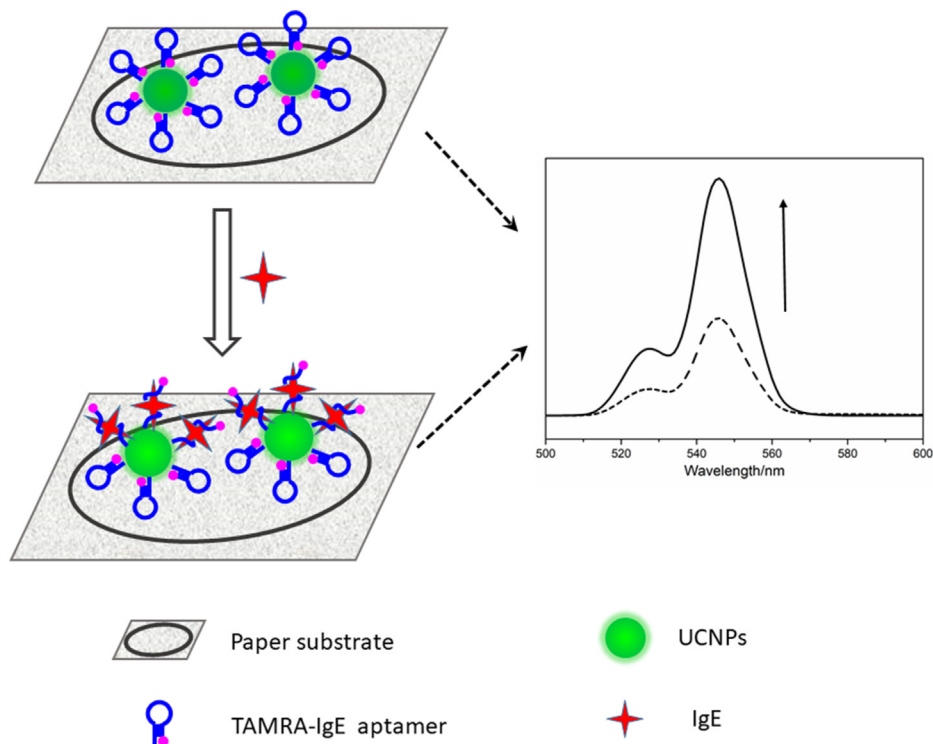
3. Result and discussion

3.1. Principle of the paper-based UC-LRET biosensor for IgE detection

The paper-based UC-LRET biosensor was constructed on the basis of the conformation change of IgE aptamer (Scheme 1). This aptamer has a stem-loop structure and the recognition site is located in the loop region, which makes it act as a molecular beacon (MB) probe when an energy donor is tagged at the end of the stem. In this case, the distance between energy donor and energy acceptor is reduced effectively and the energy transfer efficiency is dramatically increased [34]. TAMRA was chosen as the energy acceptor and tagged to the 3' end of IgE aptamer since its absorption band (around 560 nm) matches well with the emission of NaYF_4 : Yb, Er nanoparticles (around 550 nm). TAMRA could quench the fluorescence of UCNPs when the TAMRA modified IgE aptamer coupled to the surface of UCNPs as a result of the energy transfer from UCNPs to the TAMRA dye. when IgE was introduced into test zone, IgE aptamer could recognize IgE specifically and form an IgE-aptamer complex, destroying the stem-loop structure and enlarging the distance between UCNPs and TAMRA. Thus, the energy transfer was blocked and the fluorescence emission of UCNPs should be restored.

3.2. Characterization of NaYF_4 : Yb, Er nanoparticles

PEI-UCNPs were prepared through a developed ligand exchange method. TEM images revealed that the as-prepared OA-UCNPs had an average diameter of about 35 nm (Fig. 1a). After modifying with PEI ligand, the diameter of PEI-UCNPs particles was slightly increased to about 37 nm with good water dispersibility (Fig. 1b). The XRD pattern showed that the position of the diffraction peak of as-obtained PEI-UCNPs were well coincided with cubic phase NaYF_4 (JCPDs 06–0342) (Fig. 1c). To confirm the successful modification of PEI molecules on the surface of UCNPs, the FT-IR spectra of PEI-UCNPs were measured (Fig. 1d). The peak at 3436 and 1635 cm^{-1} are attributed to stretching vibration and in-plane bending vibration of N–H bond. The peaks between 997 and 805 cm^{-1} were due to the out-of-plane bending vibration of N–H



Scheme 1. Schematic illustration of the paper-based UC-LRET aptasensor for IgE detection.

bond. The peaks at 2962, 2923, 2850, 2838 cm^{-1} were assigned to asymmetric and symmetric stretching of the C–H bond and those at 1463 cm^{-1} is attributed to the in-plane bending vibration of C–H bond. The peaks at 1375 and 1166 cm^{-1} are associated with the stretching vibration of C–N and C–C bond, respectively. All of these results indicated the presence of PEI molecule on the surface of UCNP. The luminescence intensity of UCNP kept stable in 15 days when stored at 4 °C (Fig. S1), which ensured the reliability of this nanomaterials in further use.

3.3. Characterization of TAMRA-aptamer-UCNPs complexes

The successful conjugation of UCNP with aptamer was verified by UV–vis absorption spectrum and fluorescence spectrum. The absorption peak at 260 nm and 552 nm on the curves of the TAMRA-aptamer-UCNP complexes was belong to the absorption of ssDNA and TAMRA respectively (Fig. S2a), which confirms the successful conjugation of aptamer-TAMRA and UCNP. After conjugating with TAMRA modified IgE aptamer, the luminescence intensity of UCNP decreased significantly (Fig. S2b), which further verified this conclusion. As a control, free TAMRA were added to UCNP directly and the luminescence intensity showed quite faint reduction (Fig. S2c), indicating the quenching were caused by resonance energy transfer process. To further confirm this conclusion, we performed luminescence lifetime measurement. The lifetime of the 545 nm emission of UCNP was reduced from 72.3 to 45.5 μs upon the introduction of energy acceptor TAMRA (Fig. S2d), thus indicating the occurrence of resonance energy transfer. To obtain better sensitivity, three different IgE aptamer chains with different numbers of hybridizing base pairs, named DNA 1–3 as illustrated in the above experimental section, were attached to UCNP respectively, which could quench the luminescence of UCNP in varying degrees. It was seen that the degree of luminescence quenching increased along with the increase of hybridizing

base pairs (Fig. S2b). When different concentration of IgE were added to three different TAMRA-aptamer-UCNP complexes, aptamer could combined with IgE and form an IgE-aptamer complex, enlarging the distance between UCNP and TAMRA and causing the recovery of luminescence. The UCNP-DNA 2 sensor provided the highest extent of fluorescence recovery after reacting with IgE (Fig. S3), probably due to the reason that the too less hybridizing base pairs in DNA 1 cannot form a stable MB structure, while overmuch hybridizing base pairs in DNA 3 may hinder the structure change of the aptamer when reacting with IgE. So, UCNP-DNA 2 were chosen to continue the construction of UC-PADs for IgE detection.

3.4. Construction of paper-based analytical devices modified with TAMRA-aptamer-UCNP complex

The paper-based analytical devices were constructed through one-step plotting method, in which the permanent markers were used to draw patterns on the surface of paper with the help of plastic template. After volatilizing of the solvent in the ink, the hydrophobic substances such as resin remaining in the paper could form hydrophobic barrier. Through the comparison between SEM images of pure cellulose filter paper (Fig. 2a) and the cellulose filter paper patterned with permanent markers (Fig. 2b), the resin barriers could be observed clearly. Considering the coverage of test zone and the consumption of reagent, 2 μL of solution was used to fill the zone with uniformity. TAMRA-aptamer-UCNP complexes were dropped on the surface of test zones and dried at room temperature. The SEM image revealed that the TAMRA-aptamer-UCNP complex, which were immobilized on the surface of test zone, show relative well dispersion, although some degree of aggregation was observed (Fig. 2c). Under this circumstances, most UCNP in the test zone could be excited, which is conducive to signal stability.

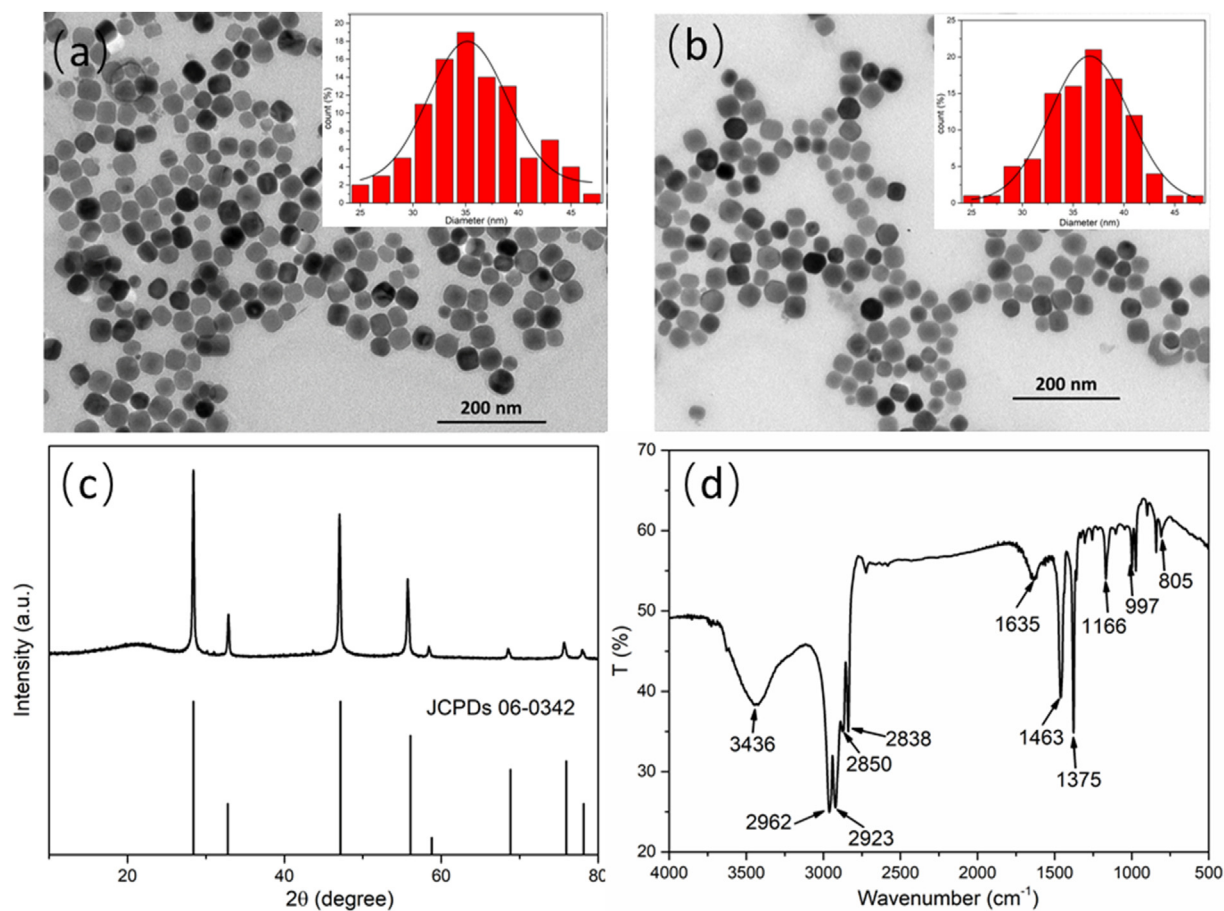


Fig. 1. (a) TEM image of OA-UCNPs. (b) TEM image of PEI-UCNPs. (c) XRD pattern of the PEI-UCNPs and the standard pattern of cubic phase NaYF_4 (JCPDs 16–0334). (d) FT-IR spectra of PEI-UCNPs.

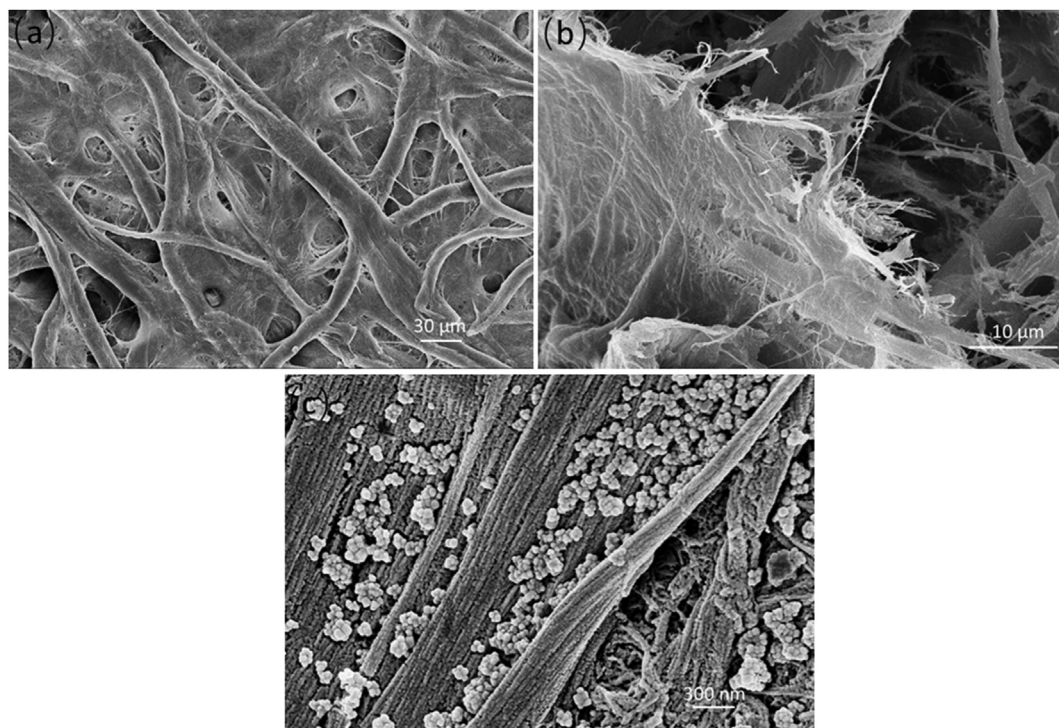


Fig. 2. (a) SEM image of pure cellulose filter paper. (b) SEM image of the cellulose filter paper patterned with permanent markers. (c) SEM image of the test zone loaded with UCNPs-DNA-TAMRA complex.

3.5. Optimal assay conditions for the paper-based UC-LRET aptasensor

We optimized the performance of the biosensor before moving on to the assay of samples. The effect of reaction temperature on the extent of fluorescence recovery was tested (Fig. S4a). The extent of fluorescence recovery increased initially with the increase of reaction temperature and reached a maximum at 42 °C. Further elevation of the temperature impaired the signal, probably because of the helicase of double-stranded DNA. We also found that the addition of Mg^{2+} in the assay buffer was beneficial to the extent of fluorescence recovery (Fig. S4b). We attributed this phenomenon to the capability of Mg^{2+} ions to stabilize the stereo-structure of aptamer [35,36].

3.6. The detection of IgE in aqueous solution

Under the optimal conditions, we tested the analytical performance of the sensor for IgE with a series of concentrations in aqueous solution. When the target IgE was added to test zones modified with TAMRA-aptamer-UCNPs complex, IgE aptamer would bind to IgE specifically, enlarging the distance between the UCNPs and TAMRA and blocking the energy transfer process. Moreover, the change of the reaction signals could be directly measured and no separation steps were required, which will remarkably simplify the procedure and shorten the detecting time. With the increasing of IgE concentration, the luminescence of UCNPs recovered gradually (Fig. 3a). The relative fluorescence intensity, $(F-F_0)/F_0$, where F and F_0 were the fluorescence intensities in the presence and in the absence of IgE respectively, were linearly correlated to the IgE concentration within the range of 0.5–50 IU/mL (Fig. 3b). The correlation coefficient could reach 0.9951 and the detection limit was as low as 0.13 IU/mL. To further estimate the performance of this paper based upconversion aptasensor, the limit of detection, detection time and detection instrument of our strategy were compared with previously reported IgE detection methods on paper substrate (Table S1). Compared to paper-based UC-FRET aptasensor using UCNPs and OCNPs as donor-acceptor pairs, our strategy could significantly shorten the detection time and simplify operation steps. Meanwhile, the detection sensitivity showed significant advantages over most of the existing paper-based lateral flow assay. On balance, this paper-based molecular beacon strategy showed relatively good analytical performance.

3.7. The specificity of the paper-based UC-LRET aptasensor

To verify the specificity of this paper-based UC-LRET aptasensor, a series of other distractors including Na^+ , K^+ , Mg^{2+} , glucose, glycine, BSA, lysozyme and IgG were added into the test zones modified with TAMRA-aptamer-UCNPs complexes and the same procedure as IgE was followed. No obvious restoring fluorescence of the UCNPs was observed even the concentration of interfering species (120 ng/mL) was 5 times higher than that of IgE (10 IU/mL, about 24 ng/mL) (Fig. 4), indicating the good specificity of this paper-based UC-LRET aptasensor for IgE.

3.8. Comparison with ELISA kit assay

To validate the accuracy of this paper-based UC-LRET aptasensor, human serum samples from 20 volunteers were analysed both by the total IgE ELISA kit and our method (10-fold dilution with binding buffer 3). The concentrations from ELISA assay and the concentrations detected by our method were compared by linear regression analysis. The concentrations from ELISA kit assay were regarded as actual value and the concentrations detected by our method were distributed around the straight line (Fig. 5). The linear least-squares method, with an equation of $y = 1.09x - 16.85$, was used to calculate the correlation. The correlation coefficient between UC-PAD and ELISA kit was 0.9838, suggesting good consistency and clinical comparability between these two methods. The relative standard deviations of our method between three independent measurements varied from 2.7% to 19.7% (Table S2). This result indicated that our UC-PAD biosensor can be used for quantitative detect total IgE level in human serum.

4. Conclusions

Paper-based analytical devices with UC-LRET technique are likely to be promising tools for point-of-care diagnosis. We constructed a paper-based UC-LRET aptasensor for IgE with high sensitivity, selectivity and accuracy. Moreover, this aptasensor could be applied to quantitative detection of IgE in both aqueous solution and human serum samples. The detection result of our method showed good consistent with that of commercial ELISA kit (confirmed by 20 human serum samples). This work suggests promising prospect of the paper-based UC-LRET sensor in real samples and may promote the practical application of paper-based analytical devices in clinical diagnosis. We hope our method could

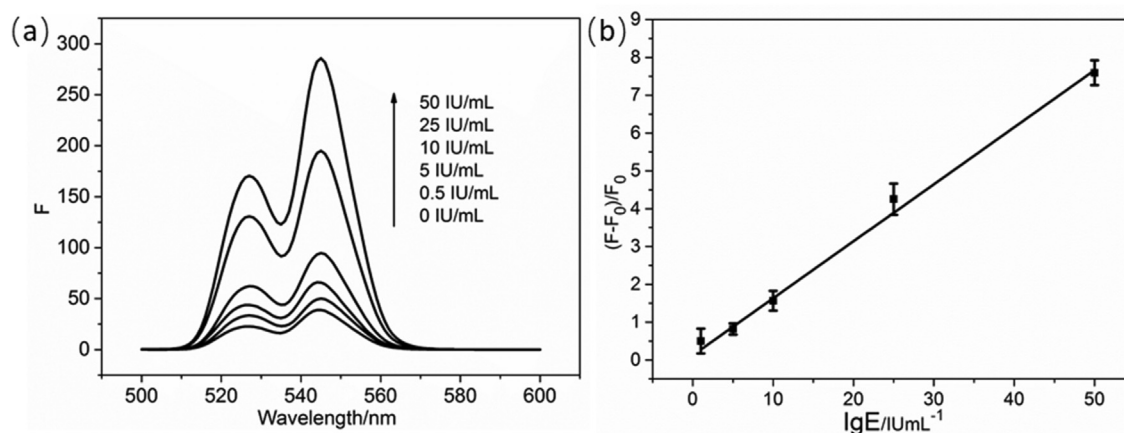


Fig. 3. (a) Upconversion luminescence spectra of the test zones with the introduction of various amounts of IgE (0, 0.5, 5, 10, 25, and 50 IU/mL). (b) The linear relationship between the relative fluorescence intensity $(F-F_0)/F_0$ and the concentration of IgE within the range of 0.5–50 IU/mL.

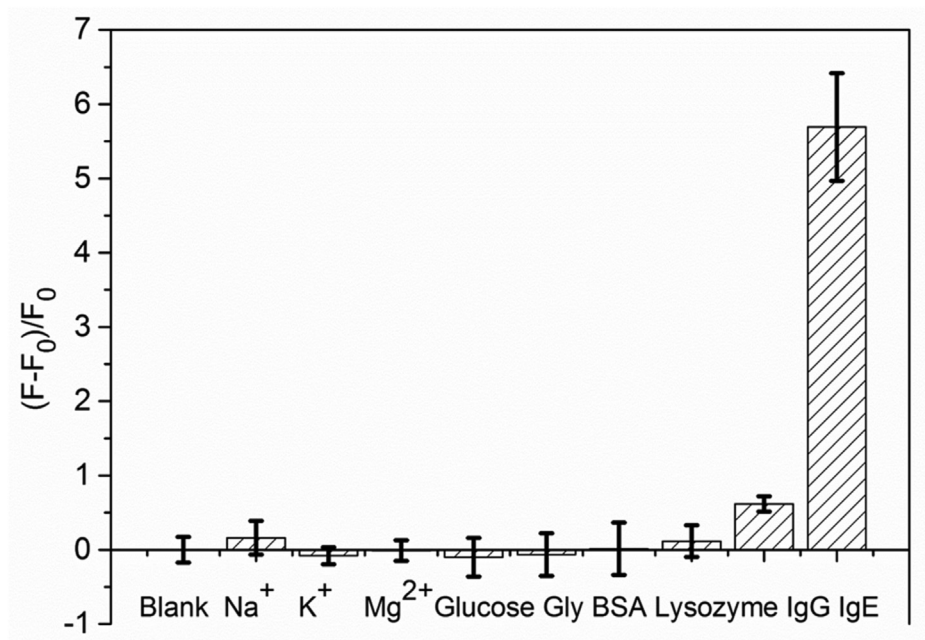


Fig. 4. Relative fluorescence intensity $((F-F_0)/F_0)$ of the paper-based UC-LRET aptasensor in the presence of different distractors. The concentration of IgE was 24 ng/mL, and that of the other distractors was 120 ng/mL.

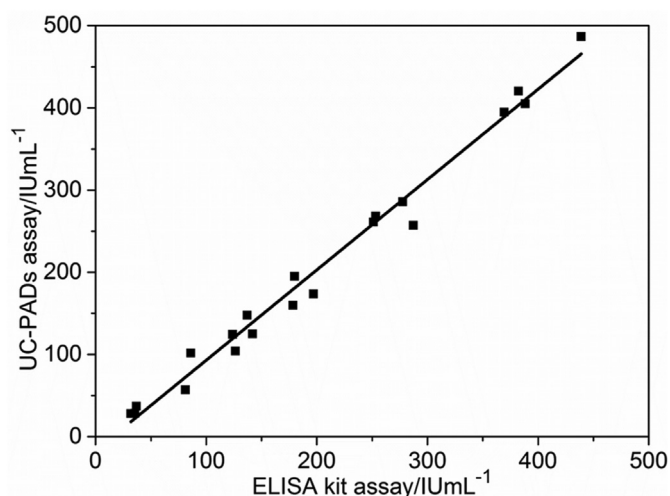


Fig. 5. Relationship between our developed paper-based UC-LRET aptasensor and the ELISA kit assay for IgE in 20 human serum samples.

promote the use of paper-based UC-LRET analytical devices in point of care testing (POCT) for individual diagnostic and real-time detection in the future.

CRediT authorship contribution statement

Mengyuan He: Conceptualization, Funding acquisition, Methodology, Project administration, Investigation, Writing - original draft. **Ning Shang:** Validation, Investigation, Data curation, Software. **Qianru Zhu:** Investigation, Data curation. **Jing Xu:** Formal analysis, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing

financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aca.2020.11.036>.

References

- [1] S. Anvari, J. Miller, C.Y. Yeh, C.M. Davis, IgE-Mediated food allergy, *Clin. Rev. Allergy Immunol.* 57 (2019) 244–260.
- [2] A.N. Berlina, N.A. Taranova, A.V. Zherdev, M.N. Sankov, I.V. Andreev, A.I. Martynov, B.B. Dzantiev, Quantum-dot-based immunochromatographic assay for total IgE in human serum, *PLoS One* 8 (2013), e77485.
- [3] S. Morais, L.A. Tortajada-Genaro, A. Maquieira, M.-A. Gonzalez Martinez, Biosensors for food allergy detection according to specific IgE levels in serum, *Trac. Trends Anal. Chem.* 127 (2020) 115904.
- [4] C.Y. Yao, Y.Z. Qi, Y.H. Zhao, Y. Xiang, Q.H. Chen, W.L. Fu, Aptamer-based piezoelectric quartz crystal microbalance biosensor array for the quantification of IgE, *Biosens. Bioelectron.* 24 (2009) 2499–2503.
- [5] T.W. Wiegand, P.B. Williams, S.C. Dreskin, M.H. Jouvin, J.P. Kinet, D. Tasset, High-affinity oligonucleotide ligands to human IgE inhibit binding to Fcε receptor 1, *J. Immunol.* 157 (1996) 221–230.
- [6] C.C. Chang, C.Y. Chen, X. Zhao, T.H. Wu, S.C. Wei, C.W. Lin, Label-free colorimetric aptasensor for IgE using DNA pseudoknot probe, *Analyst* 139 (2014) 3347–3351.
- [7] B.Y. Jiang, F.Z. Li, C.Y. Yang, J.Q. Xie, Y. Xiang, R. Yuan, Aptamer pseudoknot-functionalized electronic sensor for reagentless and single-step detection of immunoglobulin E in human serum, *Anal. Chem.* 87 (2015) 3094–3098.
- [8] S. Khezrian, A. Salimi, H. Teymourian, R. Hallaj, Label-free electrochemical IgE aptasensor based on covalent attachment of aptamer onto multiwalled carbon nanotubes/ionic liquid/chitosan nanocomposite modified electrode, *Biosens. Bioelectron.* 43 (2013) 218–225.

- [9] K. Maehashi, T. Katsura, K. Kerman, Y. Takamura, K. Matsumoto, E. Tamiya, Label-free protein biosensor based on aptamer-modified carbon nanotube field-effect transistors, *Anal. Chem.* 79 (2007) 782–787.
- [10] D.M. Cate, J.A. Adkins, J. Mettakoonpitak, C.S. Henry, Recent developments in paper-based microfluidic devices, *Anal. Chem.* 87 (2015) 19–41.
- [11] M.R. Wu, Q.Y. Lai, Q. Ju, L. Li, H.D. Yu, W. Huang, Paper-based fluorogenic devices for in vitro diagnostics, *Biosens. Bioelectron.* 102 (2018) 256–266.
- [12] P.P. Wang, L. Ge, S.G. Ge, J.H. Yu, M. Yan, J.D. Huang, A paper-based photo-electrochemical immunoassay for low-cost and multiplexed point-of-care testing, *Chem. Commun.* 49 (2013) 3294–3296.
- [13] C.Y. Qin, W. Wen, X.H. Zhang, H.S. Gu, S.F. Wang, A double-enhanced strip biosensor for the rapid and ultrasensitive detection of protein biomarkers, *Chem. Commun.* 51 (2015) 8273–8275.
- [14] J.Y. Huang, H.T. Lin, T.H. Chen, C.A. Chen, H.T. Chang, C.F. Chen, Signal amplified gold nanoparticles for cancer diagnosis on paper-based analytical devices, *ACS Sens.* 3 (2018) 174–182.
- [15] M.O. Noor, U.J. Krull, Camera-based ratiometric fluorescence transduction of nucleic acid hybridization with reagentless signal amplification on a paper-based platform using immobilized quantum dots as donors, *Anal. Chem.* 86 (2014) 10331–10339.
- [16] Y. Zhou, X. Huang, C. Liu, R. Zhang, X. Gu, G. Guan, C. Jiang, L. Zhang, S. Du, B. Liu, M.Y. Han, Z. Zhang, Color-multiplexing-based fluorescent test paper: dosage-sensitive visualization of arsenic(III) with discernable scale as low as 5 ppb, *Anal. Chem.* 88 (2016) 6105–6109.
- [17] Q. Ju, U. Uddayasankar, U.J. Krull, Paper-based DNA detection using lanthanide-doped LiYF_4 upconversion nanocrystals as bioprobe, *Small* 10 (2014) 3912–3917.
- [18] F.M. Wang, W. Li, J.S. Wang, J.S. Ren, X.G. Qu, Detection of telomerase on upconversion nanoparticle modified cellulose paper, *Chem. Commun.* 51 (2015) 11630–11633.
- [19] Z. Huang, Y.H. Liu, Y. Chen, Q.R. Xiong, Y.H. Wang, H.W. Duan, W.H. Lai, Improving the performance of upconversion nanoprobe-based lateral flow immunoassays by supramolecular self-assembly core/shell strategies, *Sensor. Actuator. B Chem.* 318 (2020) 128233.
- [20] H.Y. Chen, Q.J. Lu, K.L. He, M.L. Liu, Y.Y. Zhang, S.Z. Yao, A cyclic signal amplification strategy to fluorescence and colorimetric dual-readout assay for the detection of H_2O_2 -related analytes and application to colorimetric logic gate, *Sensor. Actuator. B Chem.* 260 (2018) 908–917.
- [21] Z. Luo, Q. Qi, L. Zhang, R. Zeng, L. Su, D. Tang, Branched polyethylenimine-modified upconversion nanohybrid-mediated photoelectrochemical immunoassay with synergistic effect of dual-purpose copper ions, *Anal. Chem.* 91 (2019) 4149–4156.
- [22] Z.L. Qiu, J. Shu, J.F. Liu, D.P. Tang, Dual-channel photoelectrochemical ratiometric aptasensor with up-converting nanocrystals using spatial-resolved technique on homemade 3D printed device, *Anal. Chem.* 91 (2019) 1260–1268.
- [23] R.R. Deng, X.J. Xie, M. Vendrell, Y.T. Chang, X.G. Liu, Intracellular glutathione detection using MnO_2 -nanosheet-modified upconversion nanoparticles, *J. Am. Chem. Soc.* 133 (2011) 20168–20171.
- [24] Z. Li, T. Liang, S.W. Lv, Q.G. Zhuang, Z.H. Liu, A rationally designed upconversion nanoprobe for in vivo detection of hydroxyl radical, *J. Am. Chem. Soc.* 137 (2015) 11179–11185.
- [25] Q.Q. Wu, H.Y. Chen, A.J. Fang, X.Y. Wu, M.L. Liu, H.T. Li, Y.Y. Zhang, S.Z. Yao, A universal multifunctional nanoplatform based on the target-induced in situ promoting Au seeds growth to quench fluorescence of upconversion nanoparticles, *ACS Sens.* 2 (2017) 1805–1813.
- [26] F. Chen, Q.J. Lu, Y.Y. Zhang, S.Z. Yao, Strand displacement dual amplification miRNAs strategy with FRET between $\text{NaYF}_4\text{:Yb,Tm/Er}$ upconversion nanoparticles and Ti_3C_2 nanosheets, *Sensor. Actuator. B Chem.* 297 (2019) 126751.
- [27] Y.R. Guo, R.B. Zou, F.F. Si, W.L. Liang, T.Y. Zhang, Y.Y. Chang, X.S. Qiao, J.H. Zhao, A sensitive immunoassay based on fluorescence resonance energy transfer from up-converting nanoparticles and graphene oxide for one-step detection of imidacloprid, *Food Chem.* 335 (2021) 127609.
- [28] M.Y. He, Z.H. Liu, Paper-based microfluidic device with upconversion fluorescence assay, *Anal. Chem.* 85 (2013) 11691–11694.
- [29] F. Zhou, M.O. Noor, U.J. Krull, Luminescence resonance energy transfer-based nucleic acid hybridization assay on cellulose paper with upconverting phosphor as donors, *Anal. Chem.* 86 (2014) 2719–2726.
- [30] F. Zhou, U.J. Krull, Spectrally matched duplexed nucleic acid bioassay using two-colors from a single form of upconversion nanoparticle, *Anal. Chem.* 86 (2014) 10932–10939.
- [31] X.X. Hu, T. Wei, J. Wang, Z.E. Liu, X.Y. Li, B.H. Zhang, Z.H. Li, L.L. Li, Q. Yuan, Near-infrared-light mediated ratiometric luminescent sensor for multimode visualized assays of explosives, *Anal. Chem.* 86 (2014) 10484–10491.
- [32] P. Jiang, M.Y. He, L. Shen, A.N. Shi, Z.H. Liu, A paper-supported aptasensor for total IgE based on luminescence resonance energy transfer from upconversion nanoparticles to carbon nanoparticles, *Sensor. Actuator. B Chem.* 239 (2017) 319–324.
- [33] M.Y. He, N. Shang, L. Shen, Z.H. Liu, A paper-supported sandwich immuno-sensor based on upconversion luminescence resonance energy transfer for the visual and quantitative determination of a cancer biomarker in human serum, *Analyst* 145 (2020) 4181–4187.
- [34] Y.X. Yuan, Z.H. Liu, An effective approach to enhanced energy-transfer efficiency from up-converting phosphors and increased assay sensitivity, *Chem. Commun.* 48 (2012) 7510–7512.
- [35] E.J. Cho, J.R. Collett, A.E. Szafranska, A.D. Ellington, Optimization of aptamer microarray technology for multiple protein targets, *Anal. Chim. Acta* 564 (2006) 82–90.
- [36] X.L. Yan, Z.J. Cao, M. Kai, J.Z. Lu, Label-free aptamer-based chemiluminescence detection of adenosine, *Talanta* 79 (2009) 383–387.

Supporting Information

Paper-based upconversion fluorescence aptasensor for the quantitative
detection of Immunoglobulin E in human serum

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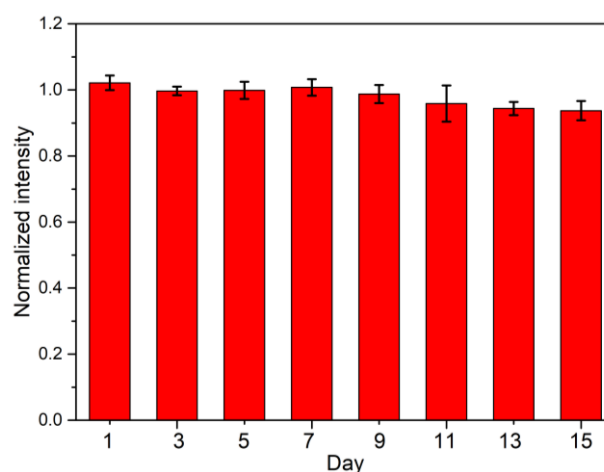


Figure S1 The luminescence intensity of UCNPs after being stored at 4 °C for different times. The signals were normalized to the intensity of UCNPs in the first day.

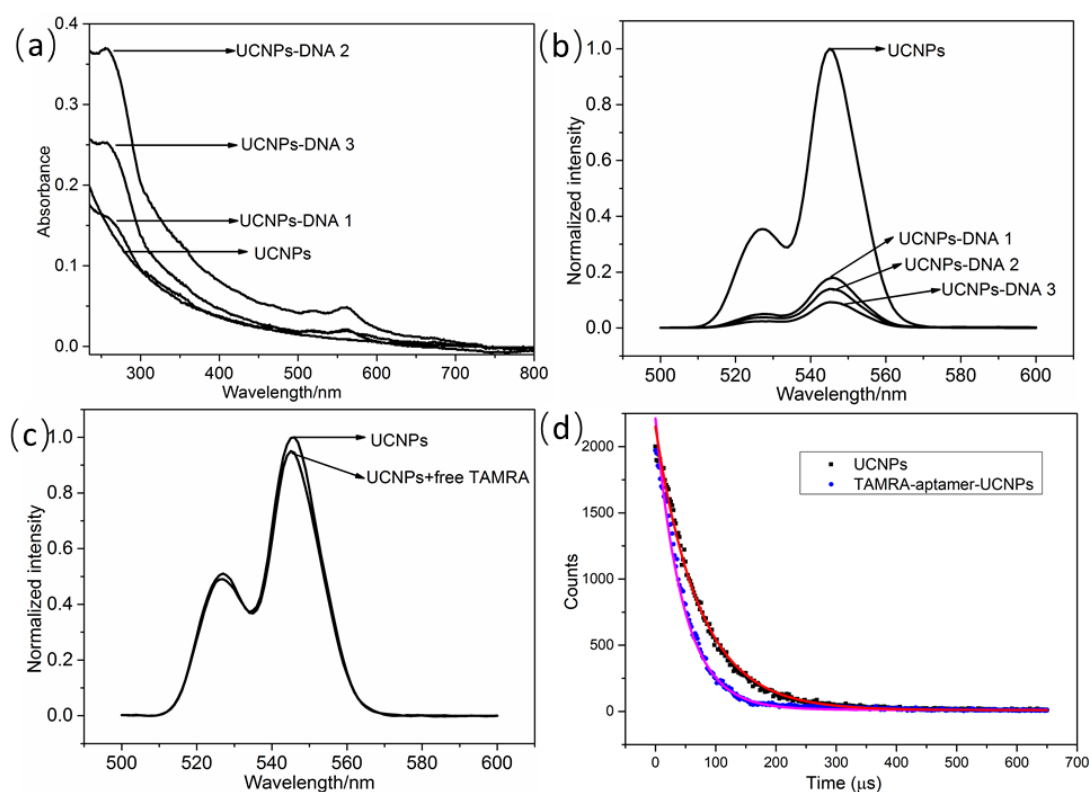


Figure S2 (a) The UV-vis absorption spectra of UCNPs and the TAMRA-aptamer-UCNPs complexes. (b) The upconversion fluorescence spectra of UCNPs and the conjugates of UCNPs and three different lengths of IgE aptamers. (c) The upconversion fluorescence spectra of UCNPs and UCNPs plus free TAMRA. (d) The decay curves of the donor emission at 545 nm in the absence and existence of energy acceptor TAMRA.

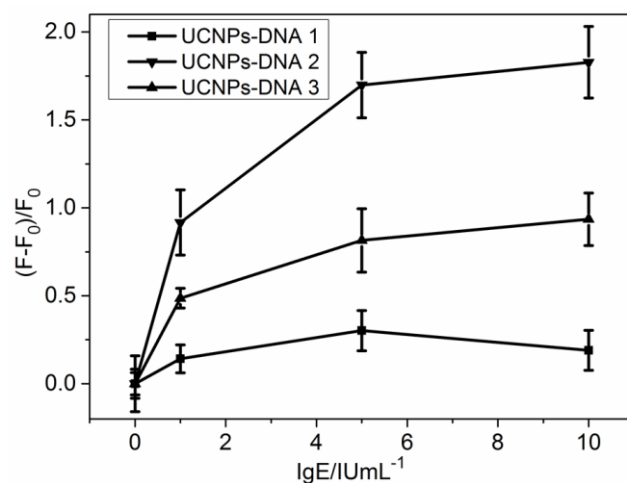


Figure S3 The dependence of the relative fluorescence intensity on IgE concentration (0, 1, 5, 10 IU/mL) when UCNPs were conjugated with the three different aptamers in binding buffer 2 at 37 °C.

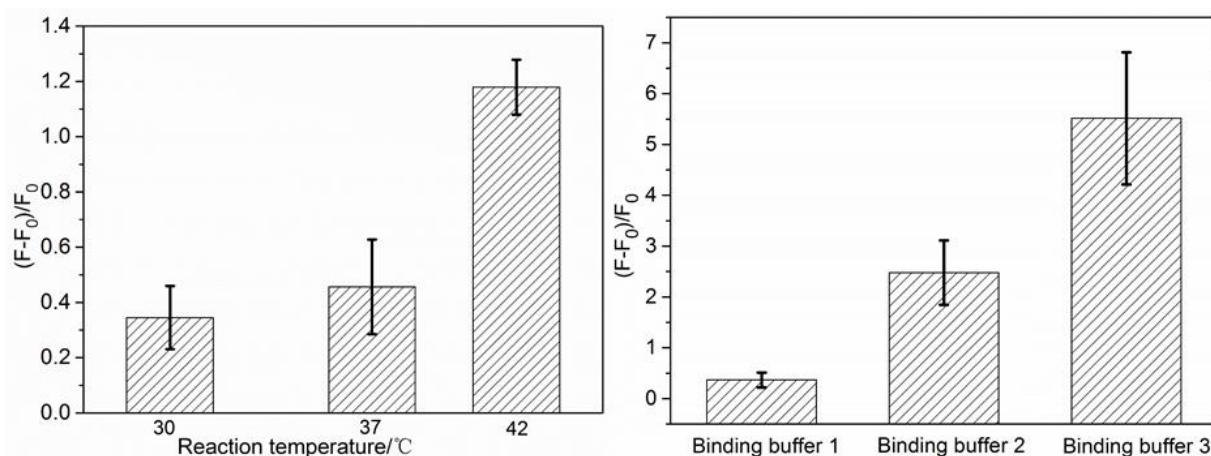


Figure S4 (a) The relative fluorescence intensity of the test zone when UCNPs-DNA 2 complex reacted with IgE (10 IU/mL) in binding buffer 2 at different temperatures (30 °C, 37 °C, 42 °C). (b) The relative fluorescence intensity of the test zone when UCNPs-DNA 2 conjugate reacted with IgE in different binding buffers at 42°C (binding buffer 1: PB (10 mM, pH 7.4); binding buffer 2: PBS (10 mM, 138 mM NaCl, 2.7 mM KCl, pH 7.4); binding buffer 3: PBS (10 mM, 138 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, pH 7.4). F represents the fluorescence intensity of the system in the presence of IgE, F₀ represents the fluorescence intensity of the system in the absence of IgE.

Table S1 Comparison on analytical performance for IgE detection between previously reported methods and this method.

Detection method	Strategy	LOD (ng/mL)	Detection time (min)	Detection instrument	ref
paper-based lateral flow microarrays	dual labelled gold nanoparticle	2.4	10	flatbed scanner	[1]
paper-based vertical flow microarray assay	reverse phase detection with conjugated gold nanoparticles	1900	8	flatbed scanner	[2]
allergen-coated strips	line blot enzyme immunoassay	0.84	240	fully automated analyzer	[3]
paper-based lateral flow assay	quantum dot-based fluorescence immunoassay	0.21	15	clinical BioIC microfluidic system	[4]
immunochromatographic assay	quantum-dot-based immunochromatographic test	12	10	portable detector REFLEKOM-UV	[5]
paper-based aptasensor	UCNPs/OCNPs LRET	0.5	50	fluorescence spectrophotometer	[6]
paper-based molecular beacon aptamer	UCNPs/TAMRA LRET	0.31	30	fluorescence spectrophotometer	This work

Table S2 Comparison of total IgE detection results with paper-based UC-LRET aptasensor and ELISA kit assay (n = 3).

Sample	UC-PAD assay/IUmL ⁻¹		ELISA kit assay/IUmL ⁻¹
	Content	RSD, %	Content
1	195.14	14.7	178.035
2	28.25	3.6	31.98
3	394.78	8.1	367.33
4	420.38	6.2	381.54
5	268.3	2.8	251.37
6	104.43	18.5	125.23
7	159.81	17	178.39
8	147.92	2.8	136.98
9	285.74	6.9	277.6
10	173.81	9.3	196.89
11	124.55	18.5	123.82
12	125.08	19.7	141.9
13	28.4	8.5	35.4
14	375.64	7	428.2
15	261.16	9.1	251.19
16	37.25	2.7	36.9
17	257.28	4.9	287.25
18	405.027	4.5	388.3
19	57.11	12	81.28
20	101.82	6.8	85.93

References:

- [1] T. Chinnasamy, L. I. Segerink, M. Nystrand, J. Gantelius, H. A. Svahn, A lateral flow paper microarray for rapid allergy point of care diagnostics, *Analyst*, 139 (2014) 2348-2354.
- [2] P. Reuterswärd, J. Gantelius, H. A. Svahn, An 8 minute colorimetric paper-based reverse phase vertical flow serum microarray for screening of hyper IgE syndrome, *Analyst*, 140 (2015) 7327-7334.
- [3] J. H. Oh, M. K. Park, S. W. Kim, J. K. Park, A fully automated analyzer for multiple detection of allergen-specific immunoglobulin E, *Analytical Methods*, 7.20 (2015) 8889-8895.

- [4] Z. Y. Liang, Y. Q. Deng, Z. Z. Tao, A quantum dot-based lateral flow immunoassay for the rapid, quantitative, and sensitive detection of specific IgE for mite allergens in sera from patients with allergic rhinitis, *Anal. Bioanal. Chem.*, 412 (2020) 1785-1794.
- [5] A. N. Berlina, N. A. Taranova, A. V. Zherdev, M. N. Sankov, I. V. Andreev, A. I. Martynov, B. B. Dzantiev, Quantum-dot-based immunochromatographic assay for total IgE in human serum, *PLoS One*, 8 (2013) e77485.
- [6] P. Jiang, M. Y. He, L. Shen, A. N. Shi, Z. H. Liu, A paper-supported aptasensor for total IgE based on luminescence resonance energy transfer from upconversion nanoparticles to carbon nanoparticles, *Sensor. Actuat. B-Chem.*, 239(2017), 319-324.