Supporting Information

Ultrasensitive detection of specific IgE based on nanomagnetic capture and separation with AuNP-anti-IgE nanobioprobe for signal amplification

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Materials and methods

Materials and instruments

Magnetic NPs, N6-Cbz-L-Lysine, histamine, N-hydroxysuccinimide (NHS) and sodium hydroxid were purchased from Shanghai Aladdin Bio-Chem Technology Co. Ltd. TMB (3,3',5,5'-Tetramethylbenzidine) color liquid and nickel column were purchased from Costar (USA), Nanjing KeyGen Biotech. Co. Ltd. Human serum albumin (HSA) was purchased from Sigma-Aldrich. IL-3 was purchased from PeproTech Inc. Tryptase was purchased from CUSABIO Co. Ltd. The 96-well plate was purchased from Suzhou Zhong analytical Instrument Co., LTD. 1-ethyl-3-[3-(dimethylamino) propyl]carbodiimide hydrochloride (EDC) were purchased from Sinopharm Chemical Reagent Co. Ltd. HRP-labeled signal goat anti-human IgE was obtained from Kirkegaard and Perry Lab., Inc. Isopropyl-b-D-thiogalactopyranoside (IPTG) was purchased from TakaRa Biotechnology (Dalian) Co., Ltd. Bromoacetic acid and nickel chloride were obtained from San Chemical Technology (Shanghai) Co., Ltd. Whatman chromatography paper #1 (WCP#1) (200 mm×200 mm) was purchased from Shanghai Si Rui Technology Co., Ltd. Hydrochloric acid was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. All chemicals and reagents were of analytical grade used without further purification. His-tagged rCanf1 was cloned and expressed as described in our previous work.[1] The human sera used in these studies were provided by the First Affiliated Hospital of Nanjing Medical University. The negative sera were all clinically tested true negative sample. These sera were further confirmed by SPT and ImmunoCAP (ThermoFisher, Fremont CA). The Canf1 sIgE levels in patients' sera were analyzed by the ImmunoCAP system, using Canf1 covalently bound to the CAP solid support (kindly supplied by Prof. Jinlyu Sun from the Department of Allergy, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences).

The chemical modified process was confirmed through the FT-IR spectra by using a Nicolet IS50 (Thermo Fisher, U.S.A). The OD value of AuNPs was measured by Shimazu's UV spectrophotometer UV-1780. Transmission electron microscope (TEM) images were performed with TECHAI-12 (Philips, Holland). Zeta potentials and particle sizes were measured by zeta/nano particle analyzer (NanoPlus, USA). Thermogravimetric analysis (TGA) were conducted by using TG209F1 thermal analyzer. The samples were heated from 25 °C to 1000 °C with a heating rate of 10 °C per min under nitrogen. The paper-based detection platform was created by ColorQube 8580 N wax printer (Xerox, Japan). The chemiluminescence (CL) intensity was measured and recorded with a model Omega Lum W CL Analyzer (Aplegen, U.S.A).

Preparation of Fe_3O_4@SiO_2-NTA

Fe_3O_4@SiO_2-NTA was prepared according to the procedures reported in our previous

work.[2] Briefly, Ni-NTA was synthesized and modified on the magnetic NPs by amidation reaction to capture proteins through the interaction between Ni-NTA and His-tag of recombinant proteins. Firstly, 12 mg of **Fe_3O_4@SiO_2** was suspended into 6 mL of 0.01 M MES buffer assisting with ultrasound. 10 mg of EDC (2.5 mg/mL) and 10 mg of NHS (5 mg/mL) were added to the solution in sequence. After shaking incubation at 37℃for 1 h, the extra NHS, EDC and byproducts were removed by magnetic separation. Then phosphate buffer solution (PBS, 0.01 M, pH = 7.4) was used to wash and resuspend the activated magnetic NPs. Secondly, 8 mg of NTA was dispersed into the activated magnetic NPs solution. The mixture was treated by ultrasound for 30 s and stirred to react for 12 h at 37℃. Last, the NTA-pendant ligand-immobilized NPs were reacted with 0.1M $NiCl_2$ for 1 h. The **Fe_3O_4@SiO_2-NTA** nano-sensor was then redispersed in deionized water and stored at 4°C for subsequent detection.

Synthesis of AuNPs and AuNP-anti-IgE

Three mL of 0.16% aqueous solution of chloroauric acid was added into 80.5 mL of water. The solution was heated to 200℃ with vigorous magnetic stirring (1000 rpm). And then 3.64 mL of newly prepared 1% sodium citrate was added quickly. The solution was boiled for another 25 min with stirring. The color of the solution changed from pale yellow to wine red, indicating the formation of AuNPs. The solution was then cooled to room temperature with stirring, and stored at the 4℃ before use.

For preparing AuNP-anti-IgE, 25 μL of HRP-labeled anti-IgE was added into 25 μL of AuNPs. After shaking incubation at 4℃ for 20 min, the solution was centrifuged. The resulted precipitate was suspended in deionized water by ultrasonic dispersion for further characterization and detection.

Immunoassay procedure

The principle of the proposed immunoassay was shown in Scheme 1. First, 20 μL of **Fe_3O_4@SiO_2-NTA** (5 mg/mL) were mixed with 5 μL of rCanf1 (7.5 μg/mL) in each zone of 96-well-plate and incubated for 5 min followed by washing with PBS for once. Then 5 μL of diluted human serum was added into each well and incubated for 5 min. After washing each hole with PBS for twice, 13 μL of AuNP-anti-IgE (OD=0.47) were added and incubated for 20 min. The excess AuNP-anti-IgE was removed by rinsing three times with PBS. In the process of detection, some AuNPs would be retained in 96-well plate because of nonspecific binding to plate rather than binding with sIgE, which increased the background signal and decreased the sensitivity. To lower the background signal, 10 μL of PBS was added to suspense the NPs in each hole and then dripped onto the prepared paper. The paper used here was treated by the plasma cleaner, wax printer, oven sequentially in advance to form the hydrophilic-hydrophobic detection region. 5 μL of BeyoECL Plus was dropped onto the

paper to produce a signal change of CL intensity. Then the signal changes were captured by Omega Lum W CL Analyzer. The results were analyzed by Image J to obtain different intensity values, so that the concentrations of sIgE were able to be quantified by the brightness of the CL signal. CL intensity was measured in a uniform circle at each microzone, and the mean of 3 replicate wells was used to calculate the sIgE value of each sample.

Optimize experimental parameters.

To achieve the best analytical performance, various parameters were optimized. We changed the experimental parameters to evaluate the influence of concentrations of the rCanf1 and anti-IgE on distinguishing positive serum samples. At the same time, the incubation time of the rCanf1 and anti-IgE were also optimized. Firstly, the concentration of rCanf1 was optimized from 2.5 to 10.0 μg/mL to observe the change of CL intensity when the anti-IgE concentration was kept at the dilution of 1/1000. Similarly, AuNPs were mixed with different dilutions of HRP-labeled anti-IgE to study its effect on CL intensity. In the same way, the incubation time of the rCanf1 and AuNP-anti-IgE were optimized. By optimizing these conditions, the best combination of parameters was obtained for subsequent sIgE detection.

Supplementary figures

The method for synthesizing compound NTA

Preparation of N-(5-carbobenzyloxyamino-1-carboxypentyl) iminodiacetic acid . Bromoacetic acid (1.05 g, 7.6 mmol) was dissolved in sodium hydroxide solution (4 mL, 2 M), and n-carbon benzyloxy lysine (0.5 g, 1.8 mmol) was also dissolved in 10 mL sodium hydroxide solution. The dissolved N-carbon benzyloxy lysine was added to bromoacetic acid at 0°C and stirred at room temperature for 2 h. And then we go to 50 degrees for 19 hours. After the reaction stopped, 2.5 mL of concentrated hydrochloric acid was slowly added to the flask at 0°C to form a white precipitate. Compound 2 was obtained by filtering the solids under reduced pressure and drying them in a vacuum drying oven.

Preparation of N-(5-amino-1-carboxypentyl) iminodiacetic acid (NTA). The above product 2 (0.5 g, 1.3 mmol) was dissolved in 25 mL methanol. 10% palladium carbon 50 mg was added and stirred at room temperature in an atmosphere of hydrogen. After stirring for 12 h, the palladium and carbon were filtered and removed under reduced pressure, and then the excess solvent was removed by rotary evaporation, namely compound 3.

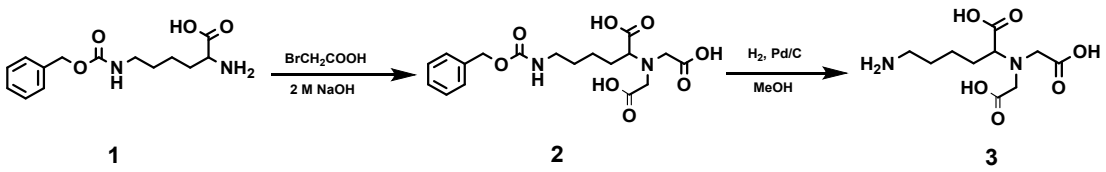

Fig. S1. Synthesis of compound NTA: (i) $BrCH_2COOH$, 2 M aqueous NaOH; (ii) H_2, Pd/C, MeOH.

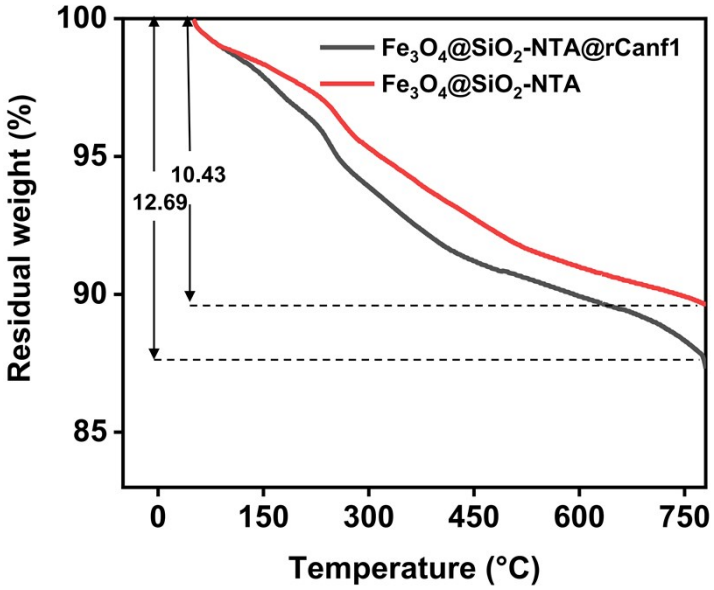

Fig. S2. TGA data of the **Fe_3O_4@SiO_2-NTA** and **Fe_3O_4@SiO_2-NTA@rCanf1**. The amount of rCanf1 coupled onto the **Fe_3O_4@SiO_2-NTA** was calculated according to TGA measurements (1.07 μmol/g).

It could be seen from Fig. S2 that the weight loss of **Fe_3O_4@SiO_2-NTA** and **Fe_3O_4@ SiO_2-NTA@rCanf1** were 10.43% and 12.69% respectively, which was numerically equivalent to the loss of **Fe_3O_4@SiO_2-NTA** with or without modification of rCanf1.

The calculation formula of mass fraction of rCanf1 is as follows:

$$\text{rCanf1}(\%) = \frac{m_{b\,(core)} \times \left(\frac{m_{b\,(shell)}}{m_{b\,(core)}} - \frac{m_{a\,(shell)}}{m_{a\,(core)}}\right)}{m_{b\,(core)} + m_{b\,(shell)}} \times 100\% = \frac{87.31 \times \left(\frac{12.69}{87.31} - \frac{10.43}{89.57}\right)}{100} \times 100\% = 2.52\%$$

Thus, the amount of rCanf1 immobilized on the surface of **Fe_3O_4@SiO_2** was calculated to be 1.07 μmol/g.

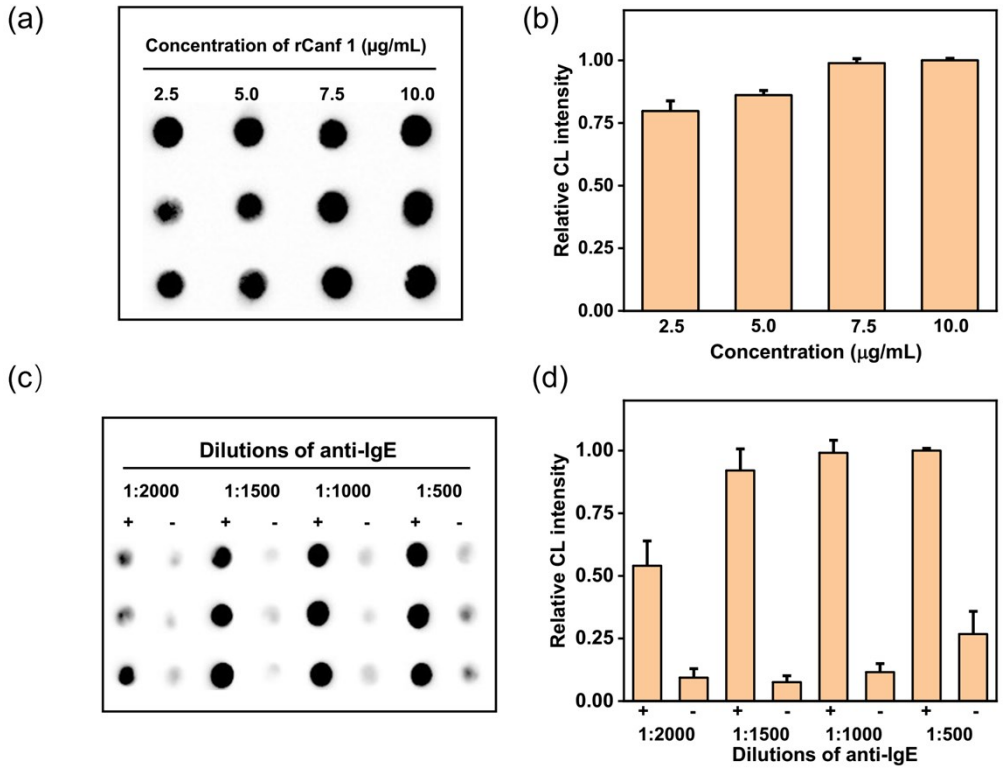

Fig. S3. The concentration optimization of rCanf1 and anti-IgE. The CL image (a) of different concentration of allergen and their corresponding CL intensity (b). The CL image (c) of different dilutions of anti-IgE and their corresponding CL intensity (d).

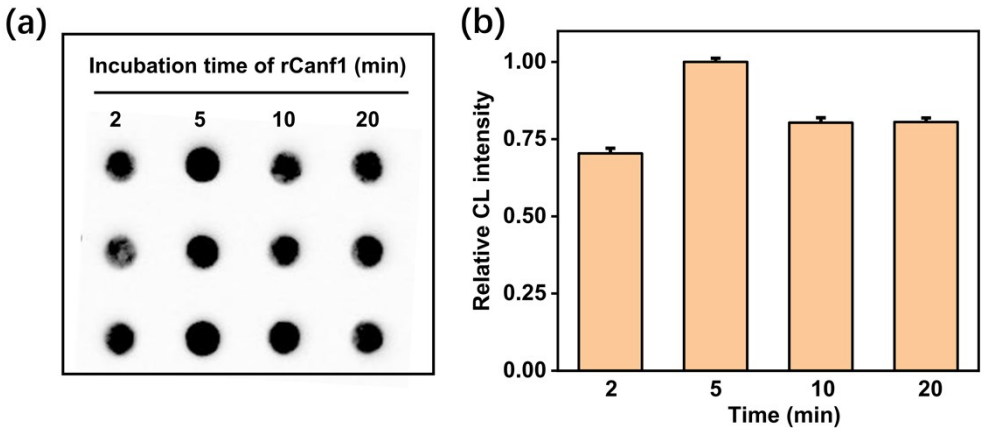

Fig. S4. The incubation time optimization of rCanf1. The CL image (a) of different incubation time of allergen and their corresponding CL intensity (b).

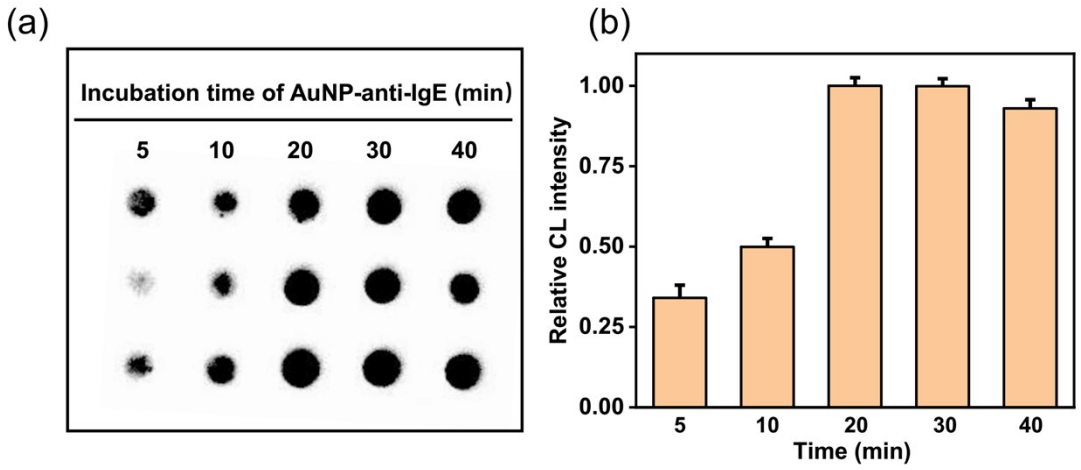

Fig. S5. The incubation time optimization of AuNP-anti-IgE. The CL image (a) of different incubation time of anti-IgE and their corresponding CL intensity (b).

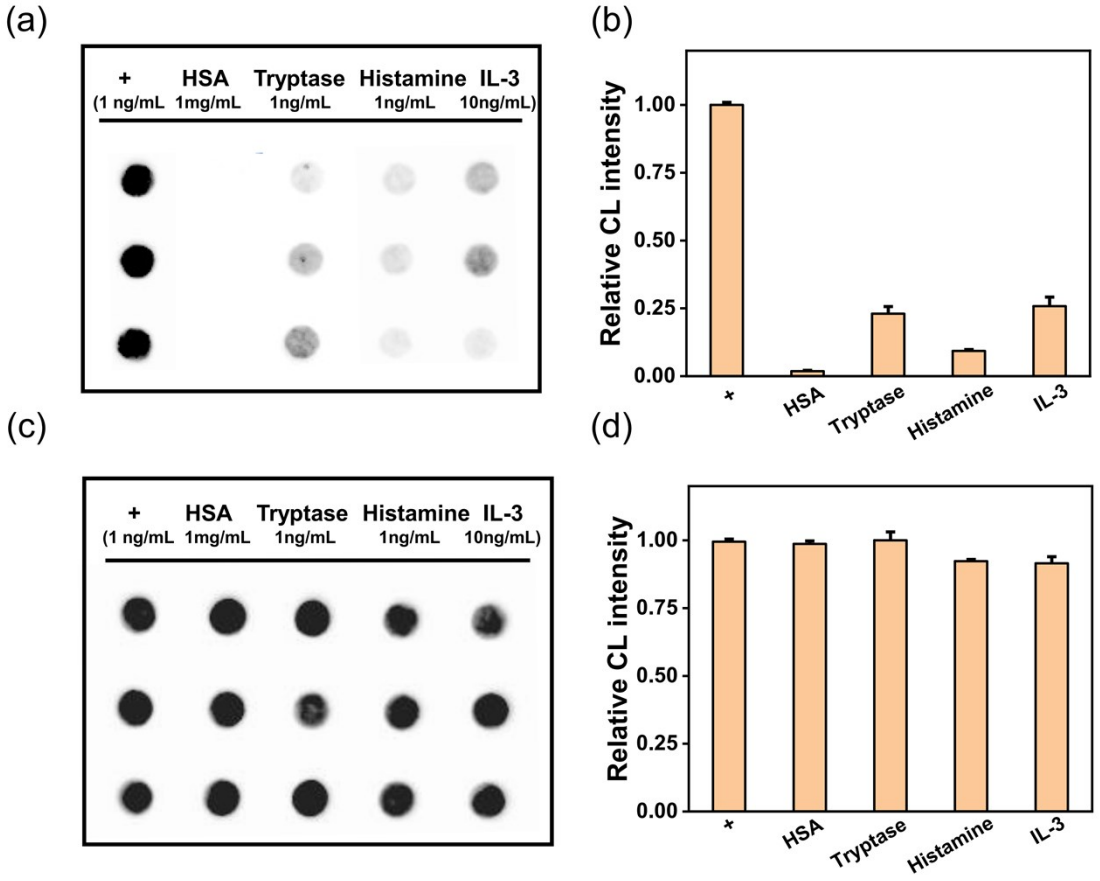

Fig. S6. Specific experiment on interfering substances. The CL image (a) of different potential interfering substances and their corresponding CL intensity (b). The CL image (c) of IgE in the presence of different potential interfering substances and their corresponding CL intensity (d).

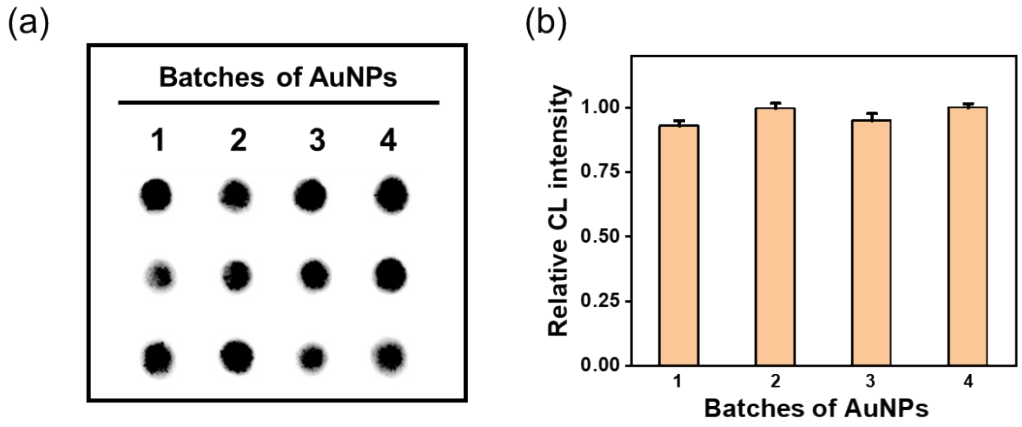

Fig. S7. The test results of different batches of AuNPs. The CL image (a) of different batches of AuNPs and their corresponding CL intensity (b).

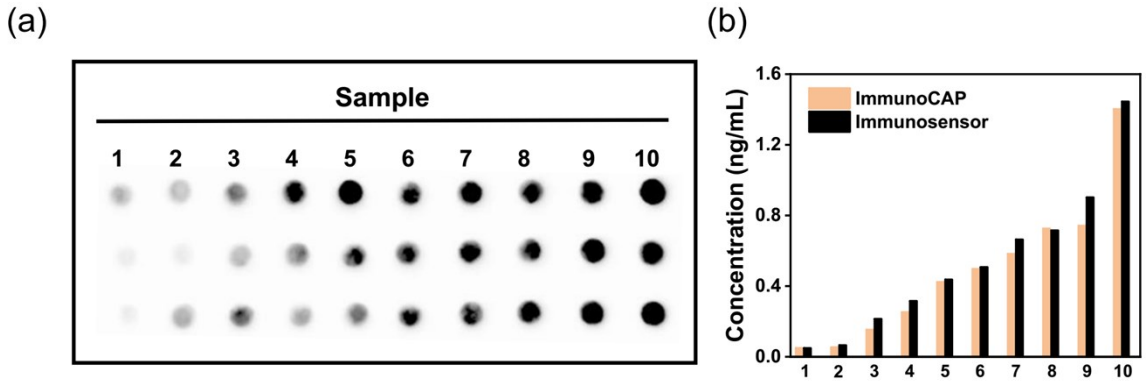

Fig. S8. (a) The CL image of 10 serum samples from patients allergic to rCanf1 determined by our immunosensor. (b) The rCanf1 sIgE level of 10 serum samples determined by our immunosensor and ImmunoCAP.

The method for determining the limit of detection (LOD)

Table S1. Multi-recorded CL intensity of ultrasensitive immunosensor and the data for standard deviation (σ) of blank measurement.

CL intensity of blank measurement					X	SD
14.773	13.159	13.325	13.151	14.017	13.221	0.756
13.111	13.413	13.023	13.189	11.454		
13.189	12.107	13.029	13.101	13.402		
13.462	13.158	12.448	14.829	13.081		

The LOD is the lowest analyte concentration that can reliably distinguish between the limit of

blank and at which detection is feasible. It mainly determines the standard deviation by repeatedly measuring blank samples, and then calculates according to the formula. A traditional and typical approach to estimate LOD consists of measuring replicates (usually n=20) to obtain 3 or more standard deviation of the analytical signals divided by the slope of the calibration curve to provide a more conservative LOD.

The LOD of sIgEs for **Fe_3O_4@SiO_2-NTA** was performed based on the calibration curve in Fig. 2. The LOD was calculated according to the equation: LOD = 3SD / S, where S was the slope of the calibration curve, and SD represented standard deviation of the blank responses[3,4]. From Fig. 3b, the slope of the plot was 90.32 (R^2 = 0.97). The value of σ was 0.756 from Table S1. The detection limit of sIgE was calculated to be 0.02 ng/mL in term of the formula.

In addition, the limit of quantitation (LOQ) of sIgEs for **Fe_3O_4@SiO_2-NTA** could be calculated for this assay. Usually, LOQ is calculated directly from 10 times of standard deviation of the analytical signals of the blank divided by the slope of the calibration curve[5]. The LOQ of the nano-sensor under optimum conditions was calculated to be 0.08 ng/mL.

The relative standard deviation (RSD) was calculated by the equation: RSD = SD / X*100%, where SD was the standard deviation of the blank responses, and X represented the average value of the CL intensity of blank measurements[6]. Thus, RSD was calculated to be 5.72%.

Table S2. Multi-recorded CL intensity of nano-sensor using free anti-IgE method and the data for standard deviation (σ) of blank measurement.

CL intensity of blank measurement					X	SD
28.711	32.983	28.685	32.273	33.272	31.633	2.242
28.643	28.643	33.942	29.685	32.059		
33.608	28.299	33.679	32.55	33.978		
28.643	33.755	32.302	33.408	33.536		

The LOD of sIgEs for **Fe_3O_4@SiO_2@rCanf1** with pure anti-IgE was performed based on the calibration curve in Fig. 2. The slope of the plot was 33.2 (R^2 = 0.92). The LOQ was calculated to be 0.68 ng/mL. The value of σ was 2.242 from Table S2. Meanwhile, the detection limit of sIgE was calculated to be 0.20 ng/mL in term of the above formula. In the same way, RSD was calculated to be 7.09% according to Table S2.

Table S3. Detailed information of serum samples that are allergic to other allergen extracts.

	Dog hair	Cat hair	Cockroach	Juniperus chinensis	Dermatophagoides farinae	Dermatophagoides pteronyssinus
Q1	-	-	+	+	+	+
Q2	-	-	-	+	+	-
Q3	-	-	-	-	-	+

Table S4. The clinical information of dog-allergic patients involved in the study

No.	Gender	Age	Diagnosis	Can f 1 sIgE (ng/mL)
1	Female	39	Asthma	4.87
2	Female	54	Cough	5.30
3	Male	60	Eczema, Cough	15.25
4	Female	60	Rhinitis, Asthma	25.20
5	Male	50	Rhinitis	42.24
6	Female	53	Rhinitis	49.68
7	Male	56	Rhinitis	58.20
8	Female	55	Rhinitis, Eczema	72.52
9	Male	25	Rhinitis	74.16
10	Male	10	Rhinitis, Asthma	140.16

Table S5. Comparison of the LOD of various IgE detection systems with the present ultrasensitive sensor.

	Detection methods	LOD (ng/mL)	Ref.
Commercial devices	Fluorescence (ImmunoCAP assay)	0.24	7
	Chemiluminescence (Immulite®2000 system)	0.24	8
Published IgE sensors	SPR	1	9
	Chemiluminescence	0.51	10
	Colorimetric immunoassay	0.408	11
	SPR	0.6	12
	Electrochemical	150	13
	Electrochemical	11.28	14
	Fluorescence	18.8	15
	Chemiluminescence	0.02	This study

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