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Electronic Supplementary Information

Functionalized gold nanorods as an immunosensor probe for neuron specific enolase sensing via resonance light scattering

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EXPERIMENTAL SECTION

Regents and Treatments:

Neuron specific enolase (NSE, 0-200 ng mL^{-1}) and rabbit anti-NSE monoclonal antibody (anti-NSE) were purchased from Beijing Biosynthesis Biotechnology Co., LTD (Beijing, China). A 0.1 mg mL^{-1} rabbit anti-NSE working solution was obtained by diluting the stock solution (2.0 mg mL^{-1}) with water and then refrigerated under -20 °C. A NSE enzyme-linked immunosorbent assay (ELISA) Kit was purchased from Zhengzhou Biocell Biotechnology Co., LTD (Zhengzhou, China). PBS buffer solution was prepared with 0.2 mol L^{-1} Na_2HPO_4, 0.2 mol L^{-1} NaH_2PO_4 and 0.2 mol L^{-1} NaCl. Tris-HCl buffer solution was prepared with Tris standard solution and 0.1 mol L^{-1} HCl. Na_2HPO_4-Citric acid buffer solution was prepared with 0.2 mol L^{-1} citric acid and 0.2 mol L^{-1} N_2HPO_4. Silver nitrate ($AgNO_3$), citric acid trisodium salt, $NaBH_4$, and NaCl were purchased from Sinopharm Chemical Reagent Co., LTD. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), cysteamine were purchased from Sigma (St. Louis, Mo, USA). The doubly distilled water was used throughout. All reagents were of analytical grade or the best grade commercially available. And all chemicals used without other illustration were of analytical grade or the best grade commercially available. All above solutions need to be stored at 4 °C.

Apparatus

All RLS spectra were measured on a Perkin-Elmer LS-55 spectrofluoremeter equipped with a 1 cm × 1 cm quartz cuvette. The SEM schemes were obtained on a JEOL-JSM6360LA scanning electron microscope. A Zetasizer Nano ZS90 (Malvern, UK) and a TGL-16G high speed refrigerated centrifuge (Shanghai Anting Scientific Instrument Factory, Shanghai, China) was used. The pH measurements were completed with a SA720 Instrument (Orion Research, USA). A thermostatic water bath (Tianjin Honour Instrument Co., Ltd. Tianjin, China) was used to control the temperature. A model 59KHz

ultrasonic reactor (Kedao Ultrasonic Instrument Limited Company, Shanghai, China) and a model of CC162 magnetic heat agitator (Grandtry Testing Instruments Co., LTD. Guangzhou, China) were used.

Preparation of AuNRs

Before the preparation, all the glassware must be soaked in aqua regia, and then washed by water several times and dried. A seed-mediated method was referenced to prepare AuNRs.[1] Firstly, in the presence of CTAB (7.5×10^{-2} M), 5.0 mL gold seeds solution was prepared by reducing $HAuCl_4\cdot4H_2O$ (2.5×10^{-4} M) with ice-cold $NaBH_4$ (9.0×10^{-4} M). Mixed vigorously for about 30 s, the mixture rapidly changed from yellow into light brown and then aged for 24 h at 25°C before AuNRs synthesis. Then, 25.0 mL AuNRs were made by adding 0.15 mL 0.01 M $AgNO_3$ into the growth solution containing 5.0 mL 2.0×10^{-3} M $HAuCl_4$ $4H_2O$, 7.7 mL H_2O, 11.9 mL 0.20 M CTAB, which was characterized by the color changing from light yellow into orange quickly and then got colorless immediately after the addition of 0.16 mL 0.10 M ascorbic acid. Finally, the color gradually got red when 0.11 mL above Au seed was placed into the mixture, indicating the formation of AuNRs. After undisturbed further growth overnight, the longitudinal PRA band of the mixture was at 776 nm with the aspect ratio about 3.38 and the concentration about 0.80 nM.[2] (diameter 25nm, see Figure S1 in the Supporting Information)

Preparation of rabbit anti-NSE

Rabbit anti-NSE should be dialyzed 24 hours in doubly distilled water to eliminate redundant electrolyte before being modified. Because redundant electrolyte from the rabbit anti-NSE would lower Zeta potential of AuNRs and influence the adsorption of rabbit anti-NSE on AuNRs.

Preparation of functionalized gold nanorods

Functionalized AuNRs that conjugated rabbit anti-NSE were prepared according to the literature method with modification.[3] 1.0 mL AuNRs was mixed with 100 μL 0.4 M deoxygenated cysteamine solution for 16 h at 4°C. Then the cysteamine-modified AuNRs was separated by centrifugation at 6000 rpm for

10 min and washed three times with PBS (pH 7.4) to remove unimmobilized cysteamine. The above solution was redispersed in 2.0 mL PBS (pH 7.4). Then 100 μL rabbit anti-NSE was incubated with 1.0 mL cysteamine-modified AuNRs solution containing 0.25 M EDC for 12 h at 4°C to conjugate rabbit anti-NSE antibody with AuNRs surface. Then antibody-conjugated AuNRs solution was separated by centrifugation at 6000 rpm for 10 min) and washed three times with PBS (pH 7.4) to remove unconjugated rabbit anti-NSE. Finally, the prepared functionalized gold nanorods were redispersed in 1.0 mL PBS buffer (pH 7.4) and saved at 4°C.

RLS detection of NSE

Into a 5 mL colorimetric tube were successively added 1.0 mL PBS (pH 7.5), 1.2 mL functionalized gold nanorods, 0.5 mL 1M NaCl, a certain amount of NSE standard solution or sample solution. The mixture was diluted to 5.0 mL with water, and then incubated in an ultrasonic reactor for 45 min at 37°C. The reacted solution was used to record the RLS spectrum which was obtained by synchronous scanning the excitation and emission monochromators ($\Delta\lambda$= 0.0 nm) from 250.0 to 750.0 nm on a common spectrofluorometer. Both excitation and emission slit widths were kept at 10.0 nm and the RLS intensities were recorded at 452.0 nm. The enhanced RLS intensity was presented as $\Delta I_{RLS} = I_{RLS} - I^0_{RLS}$, where, I_{RLS} and I^0_{RLS} were the RLS intensities with and without NSE, respectively.

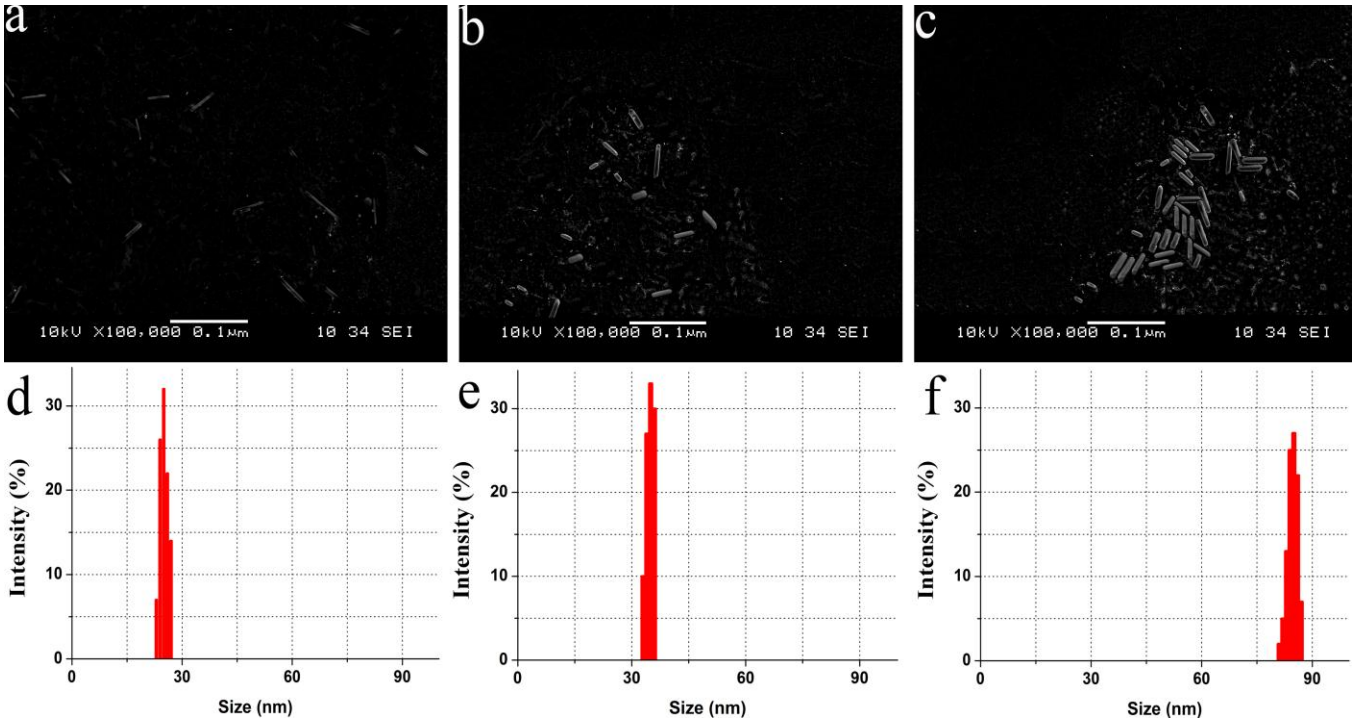

Fig. S1 SEM images and size distributions of (a, d) 0.8 nM AuNRs, (b, e) 0.24x functionalized AuNRs, (c, f) 0.24x functionalized AuNRs with 0.1 ng mL^{-1} NSE. Conditions: pH 7.5 PBS, 0.1 M NaCl.

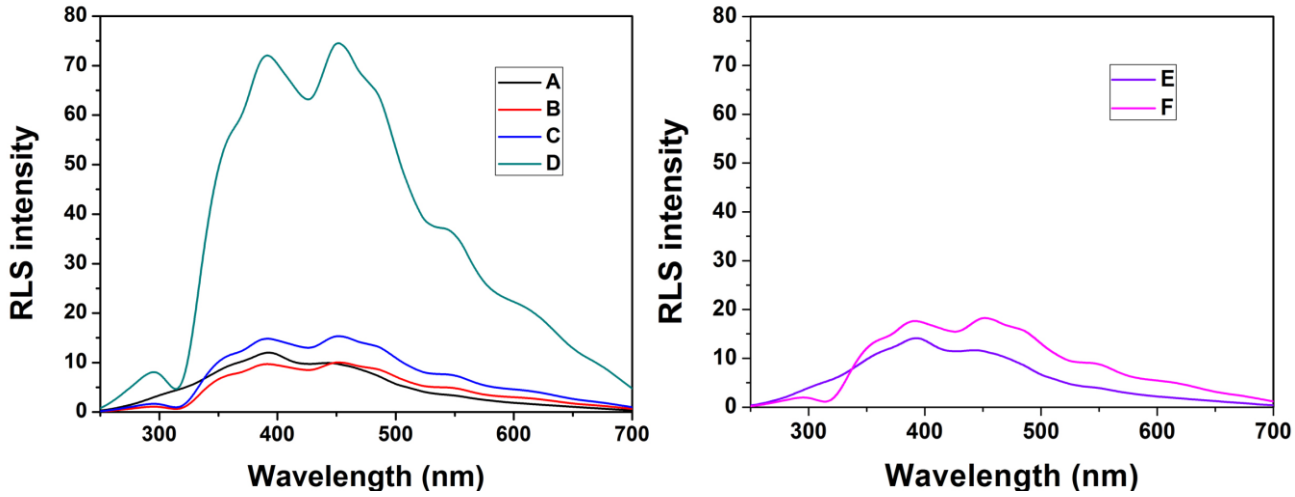

Fig. S2 The RLS spectra of (A) 0.1 mg mL^{-1} rabbit anti-NSE, (B) 0.8 nM AuNRs, (C) 0.24x functionalized AuNRs, (D) 0.24x functionalized AuNRs with 0.1 ng mL^{-1} NSE, (E) 300 mg/mL BSA, (F) 0.8 nM AuNRs with 300 mg/mL BSA. Conditions: pH 7.5 PBS, 0.1 M NaCl.

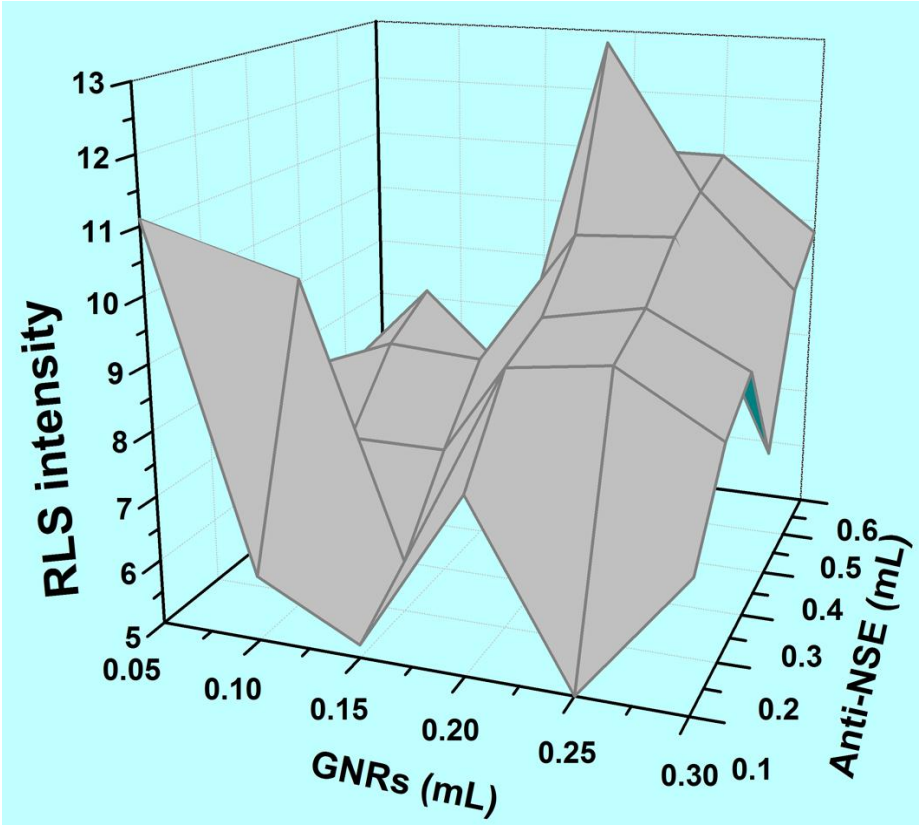

Fig. S3 Effect of the ratio of AuNRs and rabbit anti-NSE in the preparation of functionalized AuNRs.

Conditions: 0.8 nM AuNRs, 0.1 mg mL^{-1} anti-NSE.

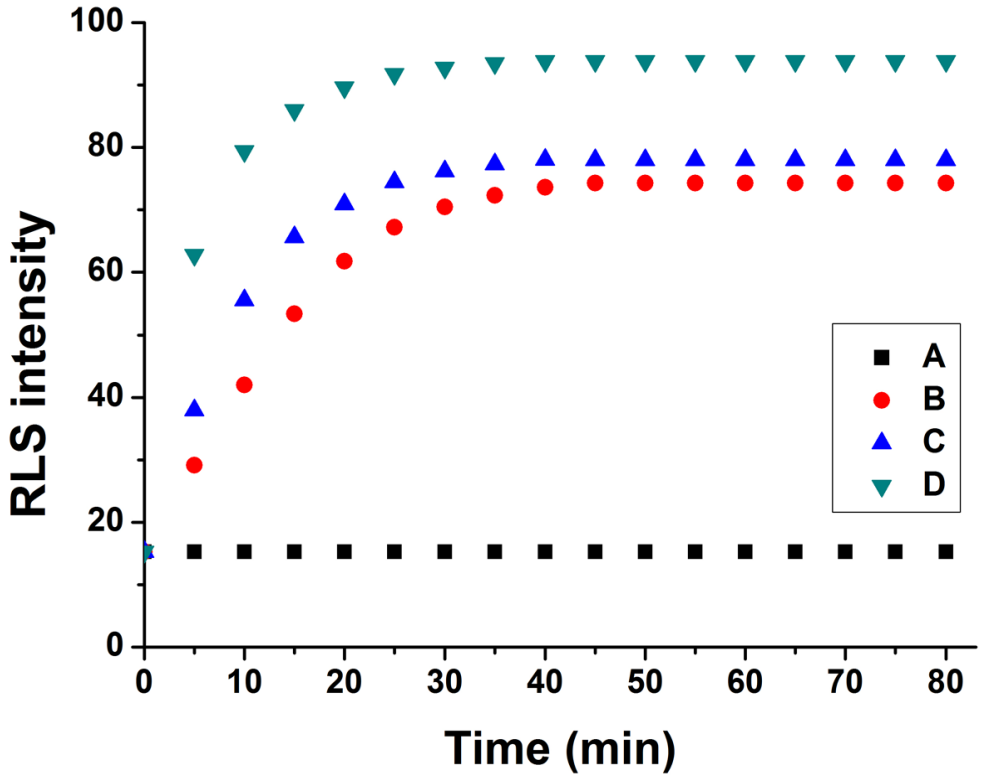

Fig. S4 The kinetics of RLS enhancement of functionalized AuNRs in the presence of different amounts of NSE. Conditions: (A) pH 7.5 PBS, 0.24x functionalized AuNRs; (B) A+0.05 ng mL^{-1} NSE; (C) A+1 ng mL^{-1} NSE; (D) A+15 ng mL^{-1} NSE.

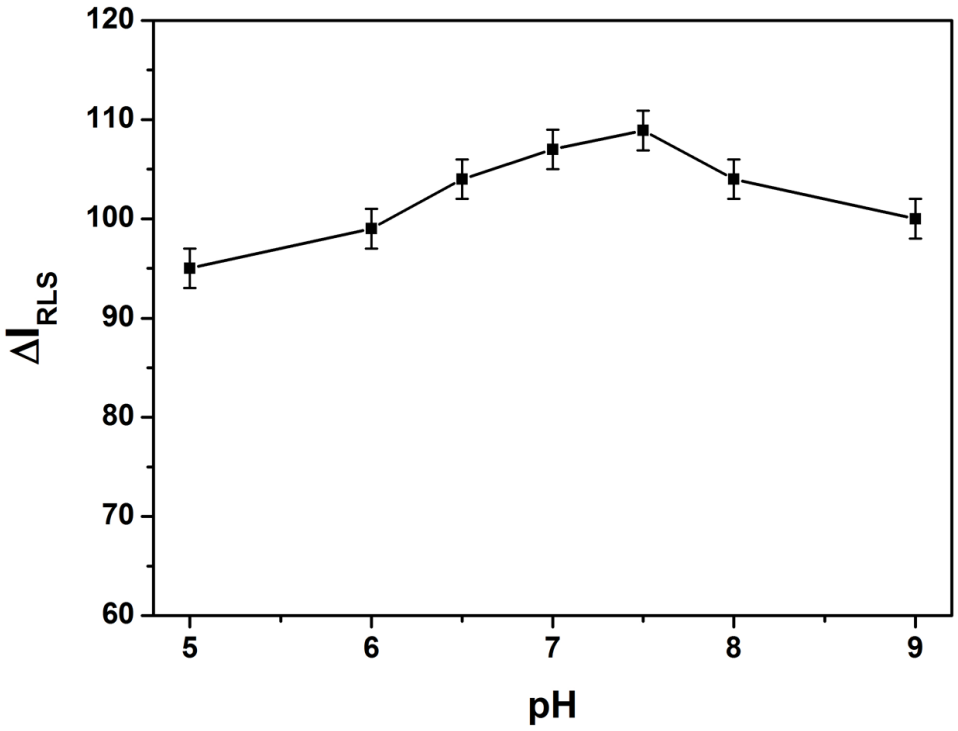

Fig. S5 Effect of pH value on detection of 12.5 ng mL^{-1} NSE. Conditions: 0.24x functionalized AuNRs.

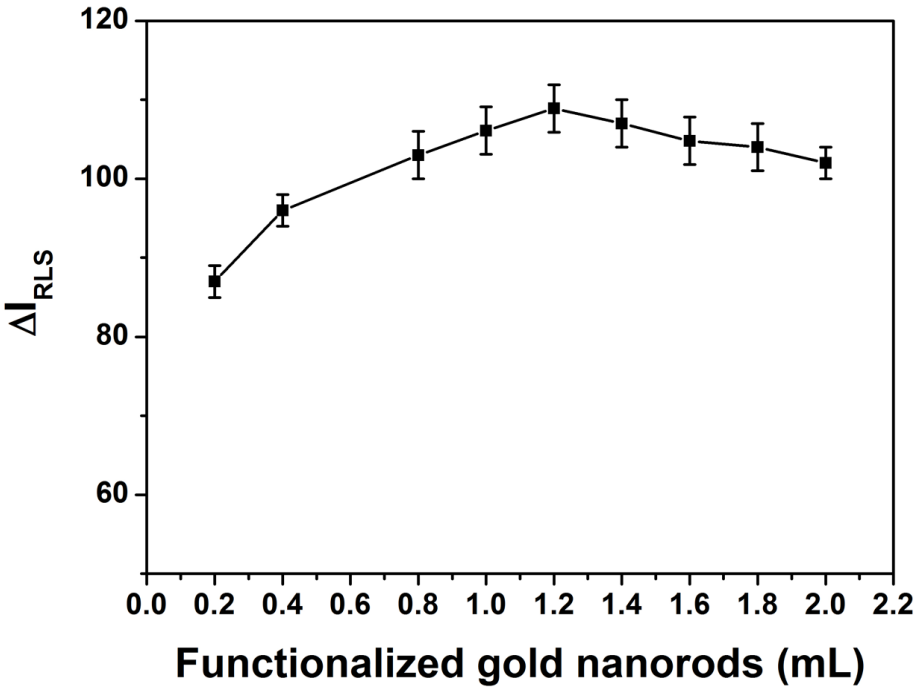

Fig. S6 Effect of functionalized AuNRs concentration on detection of 12.5 ng mL^{-1} NSE. Conditions: pH 7.5 PBS.

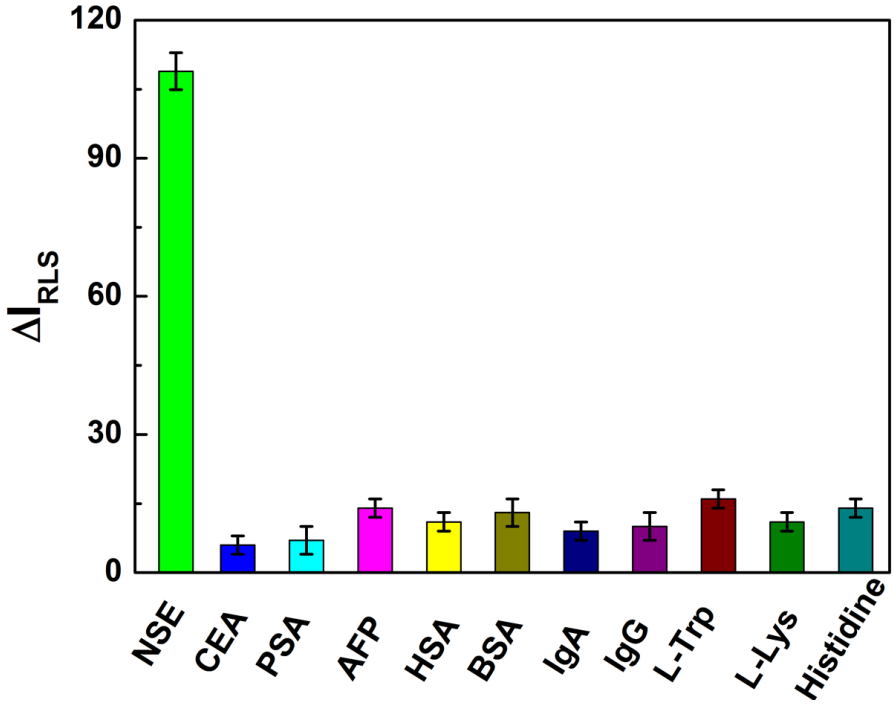

Fig. S7 Specifity of this developed assay. The concentrations of NSE, CEA, PSA, AFP, HSA, BSA, IgA, IgG, L-tryptophan, L-lysine and Histidine were 12.5 ng mL^{-1}, 5, 5, 10, 120, 250, 320, 280, 350, 290, and 300 mg mL^{-1}, respectively. The results were the averages of five experiments.

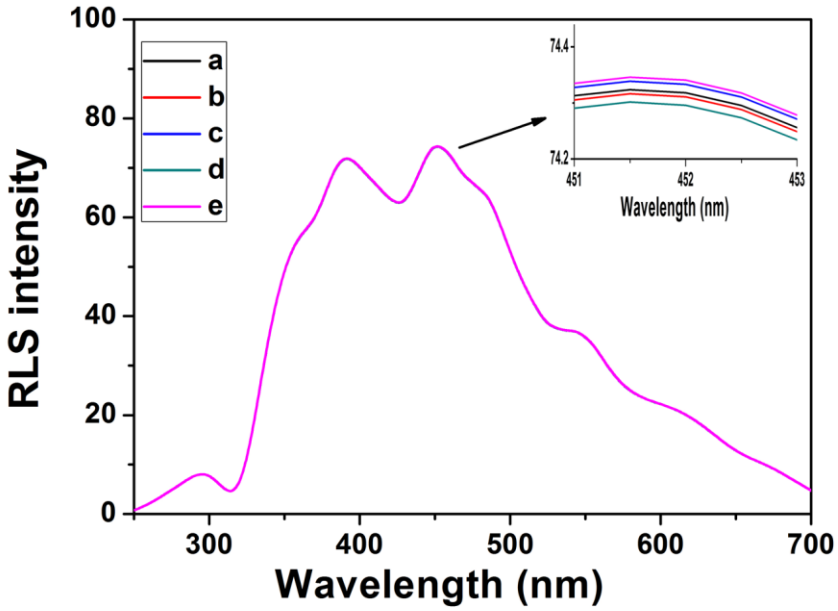

Fig. S8 The RLS spectra of five (a, b, c, d, e) consecutive analysis of 0.048 ng L^{-1} concentration level of NSE. Conditions: pH 7.5 PBS, 0.24x functionalized gold nanorods, 0.1 M NaCl, 0.048 ng L^{-1} NSE.

Table S1. Comparison of the analytical performances of the developed assay with those of other NSE assays.

Assays	Linear range ($ng\ mL^{-1}$)	Detection limit ($ng\ mL^{-1}$)	Ref.
Electrochemical immunosensor using liposomes as enhancer	5.0-100	0.18	[4]
Chemiluminescence enzyme immunoassay using magnetic nanoparticles	0-300	0.2	[5]
Fluoroimmunoassay based on dual-color quantum dots	1.25-80	0.625	[6]
Electrochemical immunosensor using functional carbon nanotubes and gold nanoprobe	0.1-2000	0.033	[7]
Resonance light scattering immunosensor using functionalized gold nanorods as immunosensor	0.048-150	0.015	This paper

Table S2. The recovery of this assay.

Sample No.	Added (ng mL^{-1})	Found (ng mL^{-1})	Recobery (%)	Relative difference (%)
1	1	0.95	95.0	3.1
2	5	5.4	108.0	4.3
3	10	9.6	96.0	5.5
4	25	26.7	106.8	3.7
5	45	48.1	106.9	5.8

Table S3.*

The comparisons of the developed assay** with the enzyme-linked immunosorbent assay (ELISA) method for the detection of NSE in human serum (purchased from the first affiliated hospital of Shantou University Medical College).

Serum samples	This assay (ng mL^{-1})	ELISA (ng mL^{-1})	Relative deviation (%)
1	0.73 ± 0.03	0.69	5.80
2	1.92 ± 0.31	2.03	-5.42
3	1.76 ± 0.28	1.69	4.14
4	5.91 ± 0.37	6.11	-3.27
5	9.82 ± 0.51	10.02	-2.00
6	2.93 ± 0.18	2.79	5.01
7	13.44 ± 0.92	13.84	-2.89
8	6.13 ± 0.32	5.97	2.68
9	15.79 ± 1.03	17.03	-7.28
10	8.25 ± 0.41	8.12	1.60
11	6.29 ± 0.73	6.58	-4.40
12	23.67 ± 1.36	21.84	8.38
13	10.53 ± 0.40	11.12	-5.30
14	28.72 ± 1.76	30.19	-4.87
15	41.27 ± 2.61	38.95	5.96
16	34.29 ± 1.29	37.12	-7.62
17	6.27 ± 0.58	5.92	5.91
18	17.16 ± 0.84	18.69	-8.19
19	29.45 ± 1.03	31.48	-6.45
20	18.36 ± 0.65	17.75	3.44

*The NSE levels are 5-12 ng/mL in normal human beings. Higher than 15 ng/mL NSE is associated with lung cancer and high levels (>100 ng mL^{-1}) of NSE indicate the presence of small cell lung cancer.

** The values shown here are the average values from three measurements.

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