Electronic Supplementary Information (ESI)

Target-modulated sensitization of upconversion luminescence by NIR-emissive quantum dots: a new strategy to construct upconversion biosensor

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Experimental Section

Materials. Poly-(acrylic acid) (PAA, MW 1800), EDC•HCl and thrombin were purchased from Sigma-Aldrich. Sulfo-NHS was obtained from Shanghai Medpep Co., Ltd. (Shanghai, China). 1-Octadecene was supplied by Aladdin Reagent, Ltd. (Shanghai, China). GSH was purchased from Amresco. Amino modified thrombin aptamers (TBA1: 5'-NH_2-TTTTTAGTCCGTGGTAGGGCAGGTTGGGGTGACT-3' and TBA2: 5'-NH_2-TTTTTGGTTGGTGTGGTTGG-3'), amino modified CEA aptamers (CEA1: 5'-NH_2-AAAAAAGGGGGTGAAGGGATACCC-3'and CEA2: 5'-NH_2-AAAAAATACCAGCTTATTCAATT-3') and c-TBA2-TAMRA (5'-TAMRA-CCAACCACACCAACCAAAAA-3') were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). Fresh human serum samples were supplied by Zhongnan Hospital of Wuhan University. Other chemical reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All reagents were of analytical grade or higher grade and were used without further purification. All aqueous solutions were prepared by ultrapure water (Mill -Q, Millipore, 18.2 MΩ resistivity).

Instrumentation. Ag_2Se nanocrystals and their conjugated products were purified by Amicon Centrifugal Filter Unit (MWCO 10 kDa). Transmission electron microscopy (TEM) images of UCNPs were taken on Hitachi H-7000FA at 75 kV. TEM image and EDX spectra of Ag_2Se QDs were taken on JEOL JEM-2100 at 100 kV. TEM images of the conjugate of UCNPs, Ag_2Se QDs and thrombin was taken on JEOL JEM-2100 at 200 kV. XRD characterization was conducted on XPert Pro. FT-IR spectra were recorded with Nicolet 5700 FTIR Spectrometer (Thermo Fisher Scientific, USA). UV-vis spectra were acquired by a UV-2550 spectrophotometer (Shimadzu, Japan) and the NIR-UV-vis spectra were acquired by a UV-3600 spectrophotometer (Shimadzu, Japan). NIR fluorescence spectra were obtained by Fluorolog-3 spectrophotometer (Horiba JobinYvonInc). The upconversion luminescence

spectra were obtained by a RF-5301 fluorophotometer (Shimadzu, Japan) equipped with an external 980 nm CW laser as excitation source (Beijing Hi-Tech Optoelectronic Co., Ltd.).

Synthesis of PAA Modified UCNPs. The OA-UCNPs were synthesized through a co-precipitation route.[1] 6.4 mL of oleic acid, 10.4 mL of 1-octadecene and 0.8 mmol Ln(oleate)$_3$ (Y^{3+}:Yb^{3+}:Er^{3+} = 78:20:2) were added into a three-necked flask. After adjusting the temperature to 50 ℃, 10 ml of methanol solution containing 3.2 mmol NH_4F and 2 mmol NaOH were added to the mixture, and the reaction was carried out for 30 min. Next, the temperature was increased to 100 °C under the protection of argon atmosphere, and the residual methanol liquid in the three-necked flask was withdrawn by a vacuum pump. After that the temperature was raised to 290 °C and the reaction kept for another 90 min. Naturally cooling to room temperature, it was centrifuged by adding ethanol to obtain a precipitate, wash twice with a mixed solution of cyclohexane/ethanol (v/v=1:2). The product, oleate acid capped $NaYF_4$:Yb,Er naonaparticles were dispersed in cyclohexane. Before the modification with poly-(acrylic acid) (PAA), oleate acid was removed as we reported previously.[2] After the removal of oleic acid, the bared UCNPs, 20 mL of water, 200 mg PAA and 233 mg $NaHCO_3$ were added to a flask, stirring vigorously overnight. Finally, after washing three times with ultrapure water, the PAA-UCNPs were dispersed in ultrapure water for further use.

Synthesis of GSH Stabilized Ag_2Se QDs. 0.173 g Na_2SeO_3 was added to a mixture consisting of 2 mL water and 2 mL ethylene glycol, stirring at room temperature until completely dissolved. The concentration of obtained Se precursor was 0.25 M. The whole reaction was carried out in argon atmosphere. After 10 mL of ethylene glycol was added to a three-necked flask, the temperature was raised to 130 °C, 0.0153 g glutathione and 0.0086 g $AgNO_3$ were sequentially added under stirring. 2 min later, 100 μL of Se precursor was injected and maintained at this temperature. Stop the heating after reacting for a period of time, the reaction solution was cooled to room temperature. At last, the QDs were collected through centrifugation by adding appropriate water to the reaction solution. The precipitate was

washed again with ethanol and stored in an aqueous solution of NaOH with the pH at 8.0. For the concentration of QDs, we measured the absorption of a certain amount of diluted QDs and calculated the concentration based on their size and the molar extinction coefficients of Ag_2Se QDs with reference to the literature.[3]

Attachment of TBA1 to UCNPs. The aptamers of thrombin, TBA1 and TBA2, were both modified with amino groups, so that TBA1 was covalently conjugated to PAA-UCNPs using the cross-linking agents, EDC•HCl and Sulfo-NHS. Specifically, 1 mg PAA-UCNPs was added into 1 mL of MES buffer solution (10 mM, pH 5.5). After sonication for 5 min, 0.5 mg EDC•HCl and 1 mg Sulfo-NHS were added to the mixture followed by mildly shaking for 40 min at room temperature. The activated PAA-UCNPs were washed with ultrapure water for three times by centrifugation, then the acquired precipitate was dispersed in 1 mL of HEPES buffer solution (10 mM, pH 7.2) containing 1 nmol TBA1. Subsequently, the reaction was allowed to incubating overnight at room temperature with mild shaking. The coupled product was collected by centrifugation and washed with ultrapure water for three times. Finally, the product UCNPs-TBA1 was dispersed in 1 mL of Tris buffer solution (10 mM, pH 7.4) and stored at 4 °C.

Quantification of TBA1 on UCNPs. The luminescence of varying concentration of PAA-UCNPs (0, 0.1, 0.2, 0.4, 0.8, 1.6 mg/mL) in aqueous solution at 546 nm and UV-vis absorption of varying concentration of TBA1 (0, 0.05, 0.1, 0.2, 0.35, 0.5, 0.8, 1 μM) in aqueous solution at 260 nm were measured for the calculation of coefficients. Then the luminescence intensity of UCNPs-TBA1 complex at 546 nm and its absorption at 260 nm were detected. The copies of TBA1 on each UCNP was calculated based on the coefficients and detected optical values.

Attachment of TBA2 to Ag_2Se QDs. Similar to the conjugation of TBA1 to PAA-UCNPs, TBA2 was also combined with GSH-Ag_2Se QDs via EDC•HCl and Sulfo-NHS. 5 nmol QDs

was added into 1 mL of PBS buffer solution (10 mM, pH 6.8), after sonication for 5 min, 10 mg EDC•HCl and 5 mg Sulfo-NHS were introduced into the mixture and shaken mildly for 30 min at room temperature. The activated QDs were harvested by centrifugation and washed with PBS for three times, then dispersed in 1 mL of PBS buffer solution (10 mM, pH 7.2) containing 2 nmol TBA2. Afterwards, the cross-coupling reaction was incubated for 4 h at room temperature with mild shaking. Finally, the coupled product was washed with PBS for three times and dispersed in 1 mL of Tris buffer solution (10 mM, pH 7.4) and stored at 4 °C.

Quantification of TBA2 on QDs. The luminescence of varying concentration of QDs (0, 5.83, 11.66, 23.32, 29.15 μM) in aqueous solution at 980 nm and UV-vis absorption of varying concentration of c-TBA2-TAMRA (0, 0.2, 0.25, 0.5, 1, 1.5, 2, 2.5 μM) in aqueous solution at 560 nm were measured for the calculation of coefficients. Then 0.25 nmol QDs-TBA2 and 2 nmol c-TBA2-TAMRA were added into 0.2 mL of Tris buffer solution (10 mM, pH 7.2, 500 mM NaCl, 1 mM $MgCl_2$) and shaken mildly for 1 h at 37℃. The product was washed with Tris buffer solution for three times by centrifugation, then the luminescence intensity at 980 nm and the absorption at 560 nm of the complex were detected. The copies of TBA2 on each QD was calculated based on the coefficients and detected optical values.

Thrombin Detection in Aqueous Solution and Serum. For the experiment in aqueous solution, 0.01 mg UCNPs-TBA1, 0.064 nmol QDs-TBA2 and varying amount of thrombin (0, 0.1, 0.5, 1, 2, 5, 10, 15, 40, 75, 100, 125 nM) were respectively added into HEPES buffer (10 mM, pH 7.2), then the mixture was incubated for 4 h at 37℃. Subsequently, the luminescence was measured at 546 nm with the excitation of a 980-nm continuous-wave laser. For the experiment in human serum, the serum samples were firstly 100-fold diluted with HEPES buffer (10 mM, pH 7.2). 0.01 mg UCNPs-TBA1, 0.064 nmol QDs-TBA2 and different concentration of thrombin (0, 0.1, 1, 5, 10, 15, 40, 75, 100, 125 nM) were respectively added into the diluted serum, then the mixture was incubated for 4 h at 37℃. The luminescence

measurement was the same as in aqueous solution.

Thrombin Detection in Plasma. The plasma samples provided by Zhongnan Hospital of Wuhan University were pretreated following previously reported method.[16,26] 0.03 M $CaCl_2$ was then added to the samples to convert prothrombin, the precursor form in plasma, to thrombin. After reaction for 2 h, the plasma containing thrombin was 100-fold diluted with HEPES buffer (10 mM, pH 7.2). The subsequent steps of thrombin assay were the same as in serum.

Specificity Assessment of the Sensor toward Thrombin. 0.01 mg UCNPs-TBA1 and 0.064 nmol QDs-TBA2 were mixed with 100 mM thrombin or interfering substances with different concentrations (the concentration of Ca^{2+}, Zn^{2+}, K^{+}, Na^{+}, Mg^{2+}, L-Cys, glucose, BSA and albumen was 1 μM while the concentration of AFP, CEA, PSA and thrombin was 100 nM) in HEPES buffer (10 mM, pH 7.2). Incubating the mixture at 37 °C for 4 h and measuring the luminescence. To examine the influence of thrombin itself on the UCL, 0.05 mg UCNPs-TBA1 was mixed with thrombin of different concentration (0, 1, 10, 20, 40, 75, 100, 125 nM) in HEPES buffer (10 mM, pH 7.2). Then the mixtures were also incubated at 37 °C for 4 h and the luminescence was measured.

CEA Detection in Aqueous Solution. For the CEA assay in aqueous solution, 0.01 mg UCNPs-CEA1, 0.064 nmol QDs-CEA2 and varying amount of CEA (0, 0.1, 0.5, 1.5, 4, 6, 10, 12, 17, 20 ng/mL) were respectively added into HEPES buffer (10 mM, pH 7.2), then the mixture was incubated for 4 h at 37℃. Subsequently, the luminescence was measured at 546 nm with the excitation of a 980-nm continuous-wave laser.

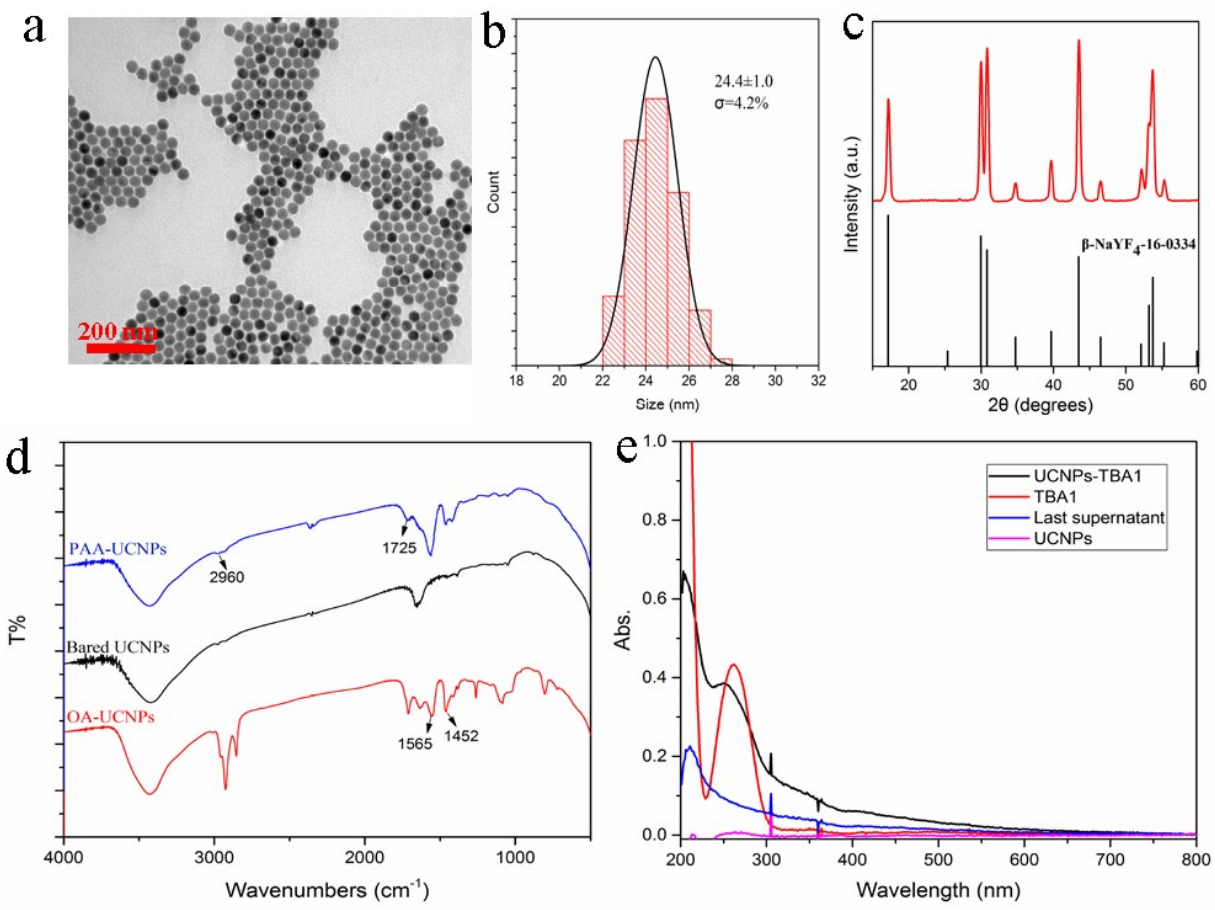

Fig.S1(a) Transmission electron microcopy (TEM) image of oleic acid coated $NaYF_4$:Yb,Er. (b) Corresponding size distribution histogram of the $NaYF_4$:Yb,Ernanocrystals. (c) XRD (X-ray powder diffraction) pattern of the $NaYF_4$:Yb,Er. (d) FT-IR spectra of PAA-UCNPs, bared UCNPs and OA-UCNPs. After removing the oleate ligands, the absorption bands of methylene asymmetric and symmetric C–H stretching vibration (2927 and 2852 cm^{-1}), and asymmetric and symmetric C=O stretching vibration (1452 and 1565cm^{-1}) in oleic acid disappeared. Compared to the bared UCNPs, PAA-UCNPs displayed symmetric stretching vibration band of $–CH_2–$ at 2960 cm^{-1} and C=O stretching vibration band at 1725 cm^{-1}, confirming the successful functionalization of UCNPs. (e) UV-vis absorption spectra of UCNP-TBA1, TBA1, the last supernatant and UCNPs.

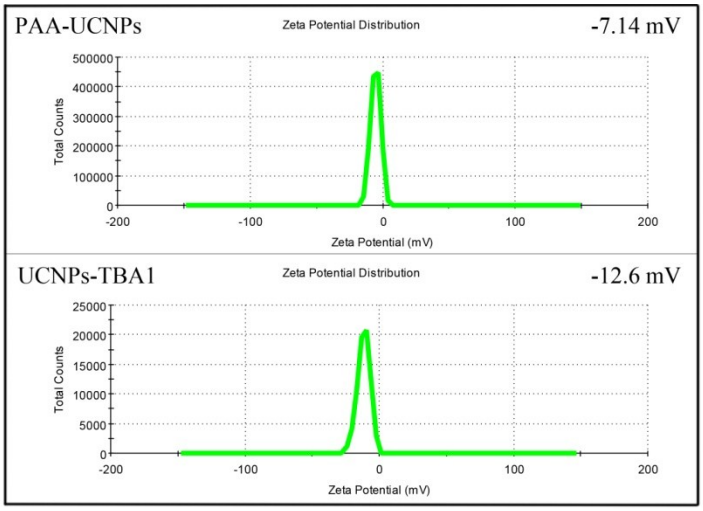

Fig. S2 Zeta-potential analysis of PAA-UCNPs and UCNPs-TBA1.

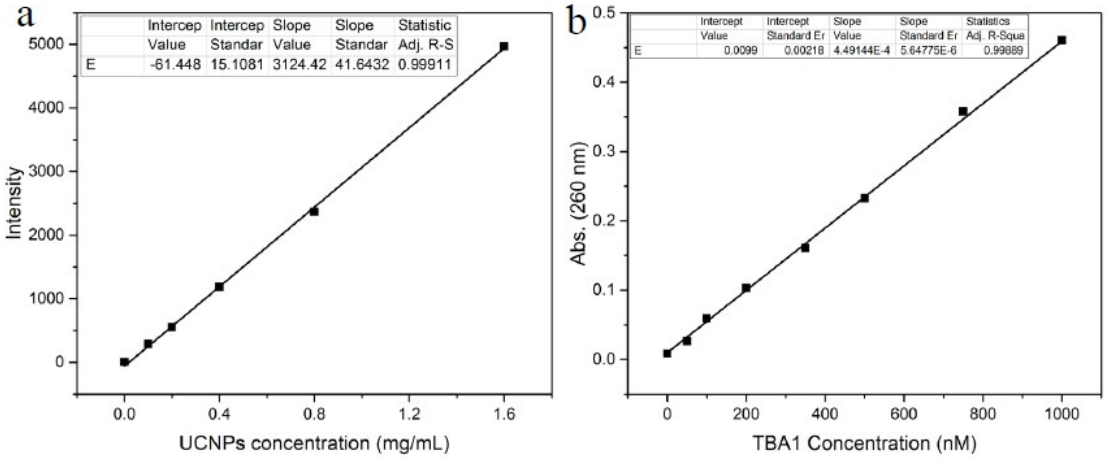

Fig. S3(a) Linear relationship between UCNPs concentration and luminescence intensity at 546 nm. (b) Linear relationship between TBA1 concentration and absorption at 260 nm.

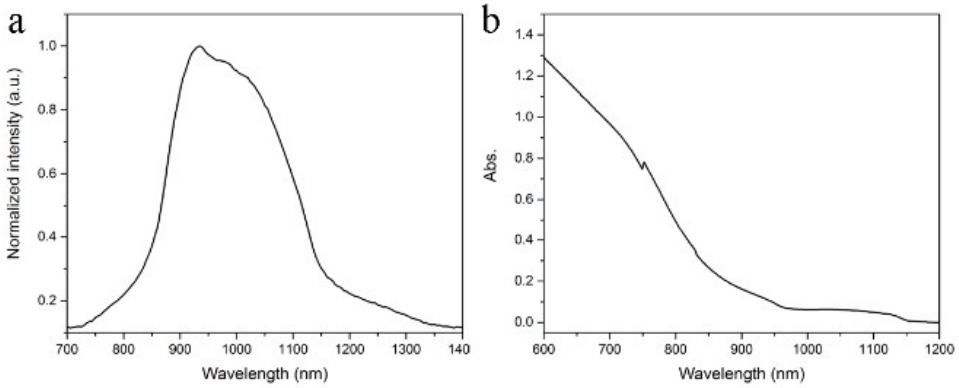

Fig. S4 Luminescence spectra (a) and corresponding absorption (b) of Ag_2Se nanocrystals.

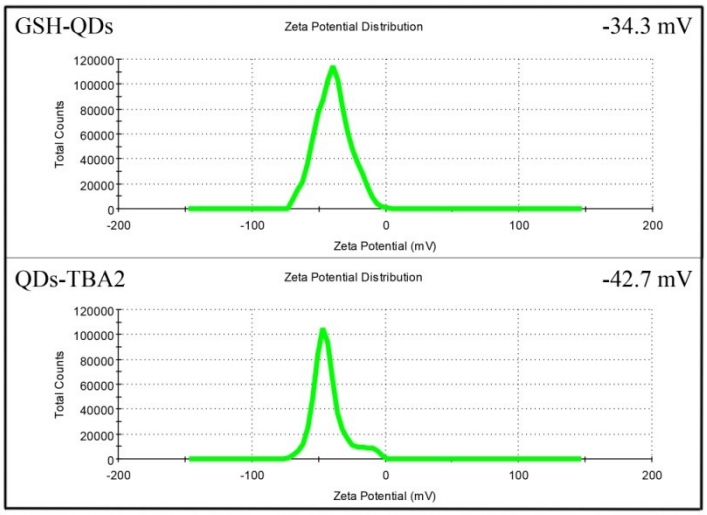

Fig. S5 Zeta-potential analysis of GSH-QDs and QDs-TBA1.

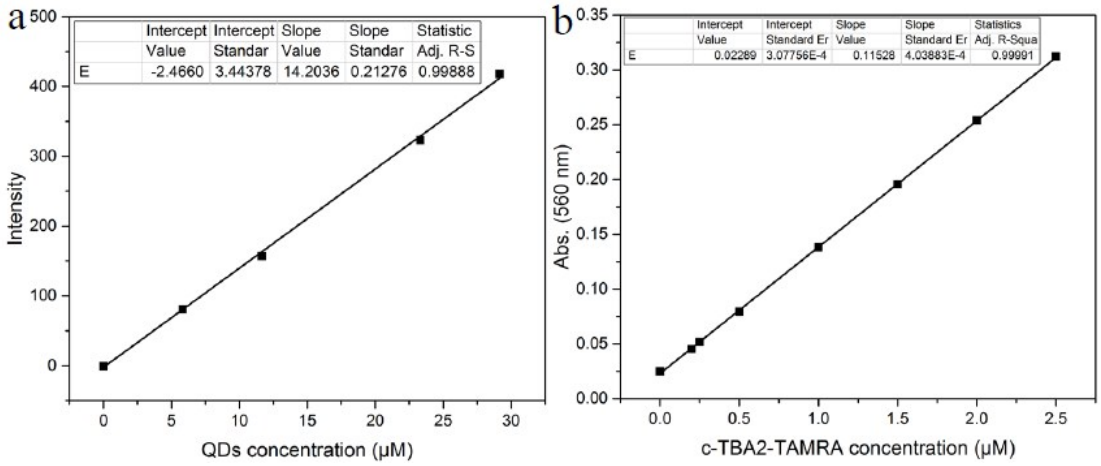

Fig. S6(a) Linear relationship between QDs concentration and luminescence intensity at 980 nm. (b) Linear relationship between c-TBA2-TAMRA concentration and absorption at 560 nm.

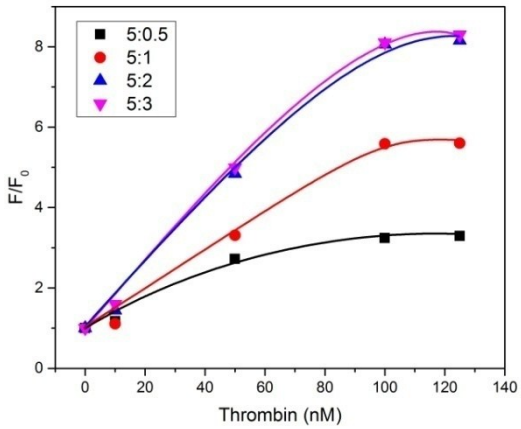

Fig.S7 Relative fluorescence (F/F_0) of biosensor responding to different concentration of thrombin with various ratio of QDs to TBA2.

Table S1. Comparison of the reported UC-LRET biosensors for thrombin.

detection method	LOD	linear range	ref.
LRET beacon sensors based on UCNPs and TAMRA	50 pM	50-2000 pM	3
LRET aptamersor based on UCNPs and CNPs	0.18 nM	0.5-20 nM	4
LRET aptamersor based on UCNPs and AuNRs	0.118 nM	0.375-11.25 nM	5
LRET aptamersor based on UCNPs and AuNRs	1.5 nM	2.5-90 nM	6
Target-modulated direct UCL enhancement by QDs	34 pM	0.1-100 nM	this method

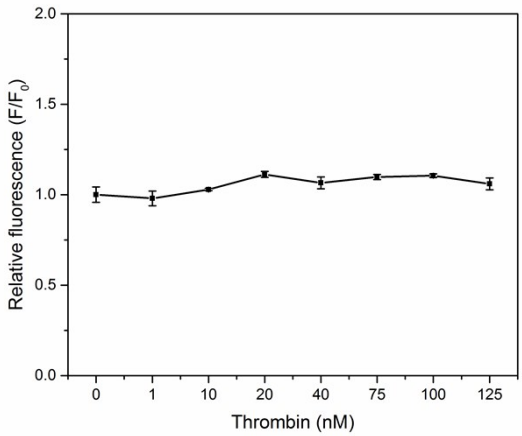

Fig.S8 Stability of UCNPs-TBA1 to different concentration of thrombin.

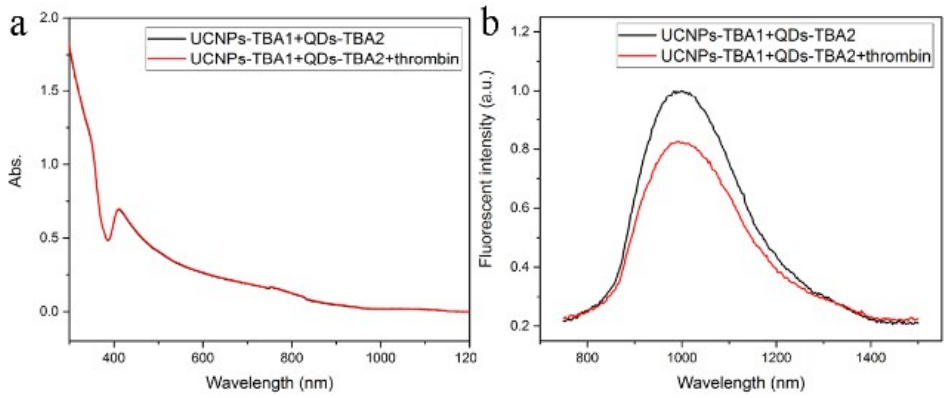

Fig.S9 Absorption (a) and luminescence spectra (b) of the mixture of UCNPs-TBA1 and QDs-TBA2, and the mixture with target thrombin. Excitation wavelength was at 400 nm.

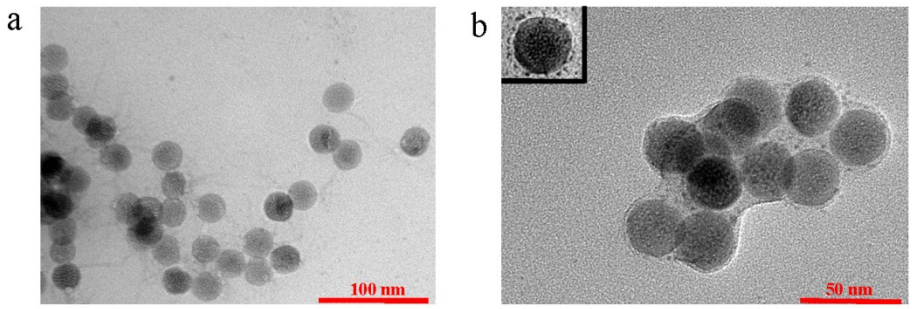

Fig.S10TEM images of the connection of UCNPs-TBA1 and QDs-TBA2 (a) in the absence of thrombin and (b) in the presence of thrombin.

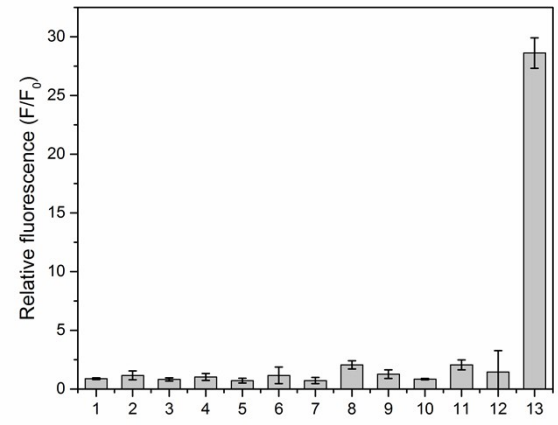

Fig.S11 Specificity of the biosensor in presence of different species (1: Ca^{2+}; 2: Zn^{2+}; 3: K^{+}; 4: Na^{+}; 5: Mg^{2+}; 6: L-Cys; 7: glucose; 8: BSA; 9: albumen; 10: AFP; 11: CEA; 12: PSA; 13: thrombin). The concentration of Ca^{2+}, Zn^{2+}, K^{+}, Na^{+}, Mg^{2+}, L-Cys, glucose,

BSA and albumen were 1 μM, the concentration of AFP, CEA, PSA and thrombin were 100 nM.

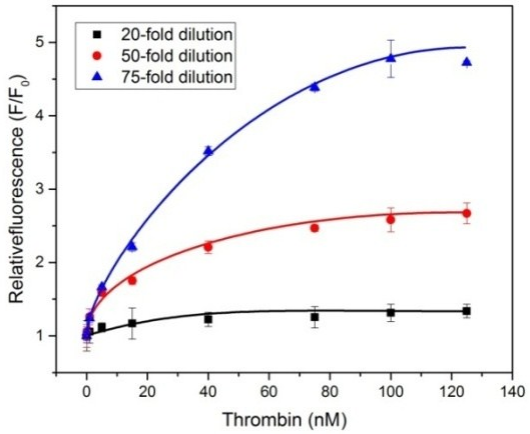

Fig.S12 The biosensor responded to thrombin in different diluted human serum.

Table S2.Detection Results and Recoveries of Thrombin in Diluted Serum Samples

samples	added (nM)	found (nM)	recovery (%)	RSD (%, n=3)
1	1.5	1.48	98.92	6.97
2	5	5.14	102.80	0.75
3	10	9.15	91.46	5.66
4	30	27.39	91.31	1.11

Table S3.The Thrombin Levels in Plasma Obtained by Our Method and Commercial ELISA Kit

samples	found concentration [nM, n=3]		t_{exp}
	our method	thrombin ELISA kit	
1	85.45±2.67	85.35±2.61	0.49
2	114.58±0.72	117.11±1.42	0.09
3	107.31±2.44	106.67±1.71	0.22
4	123.04±3.47	122.89±0.73	0.48

5	117.98±3.96	118.5±2.98	0.87
6	134.53±4.48	126.95±1.86	0.06
7	129.64±3.82	129.37±3.81	0.93

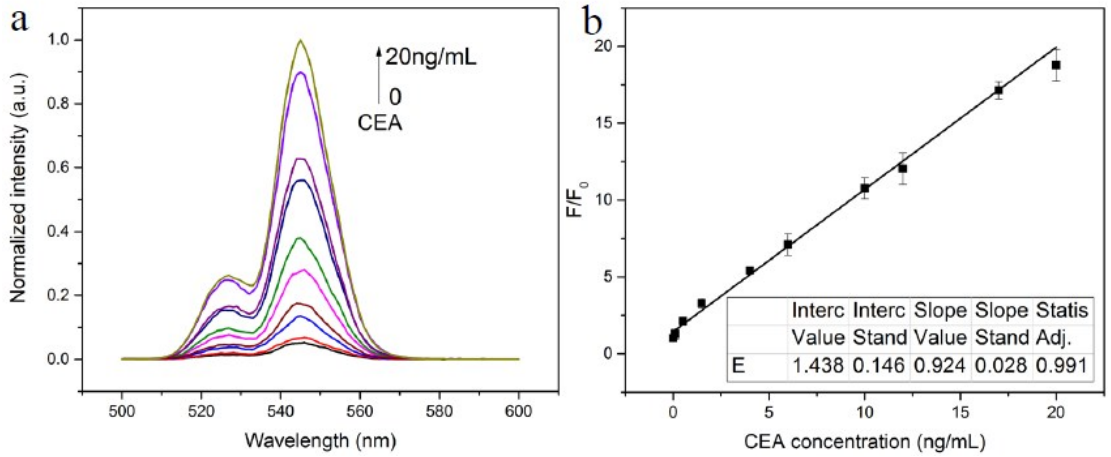

Fig. S13 (a) Upconversion luminescence spectra of the CEA biosensor in aqueous buffer, and (b) the linear relationship between F/F_0 and the concentration of CEA within the range from 0.1 ng/mL to 20 ng/mL. F_0 and F represent upconversion luminescence intensity in the absence and presence of CEA, respectively.

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