

## Electronic Supporting information (ESI)

### An effective approach to enhanced energy-transfer efficiency from up-converting phosphors and increased assay sensitivity

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### Experimental Details

#### Materials and instrumentations

Polyethylenimine (PEI, with an average molecular weight of 25000) and sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (Sulfo-SMCC) were purchased from Sigma-Aldrich. Thrombin (TB), human IgG antibody, and bovine serum albumin (BSA) were from Zhongshan Golden Bridge Biotechnology Co., Ltd. (Beijing, China). The rest of the chemical reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All aqueous solutions were prepared using ultrapure water (Mill-Q, Millipore, 18.2 M $\Omega$  resistivity). The sulfhydryl modified molecular beacons labeled at 3' end with Tetramethylrhodamine (TMR) and the oligonucleotides were purchased from Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China) and purified using high-performance liquid chromatography. Their sequences were as follows:

The ssDNA molecular beacon (named as MB<sub>1</sub>):

5'-TACGAGGTAAAAGGCTCTCTCCCTGTCGTA-3';

The complementary strand: 5'- CAGGGAGAGAGCCTTTTACC -3';

The single-base mismatched (SBM) strand: 5'- CAGGGAGAGTGCCTTTTACC -3';

The non-complementary (NC) strand: 5'-GACGCACTGAATCAGCTCTC-3';

The thrombin aptamer molecular beacon (named as MB<sub>2</sub>):

5'-TACGACTGTTGGTGTGGTTGGTCTCGTA-3'.

The size and morphology of PEI modified UCPs were characterized by a JEM-2010 transmission electron microscope (TEM) with an accelerating voltage of 200 kV. The crystal phase of UCPs was identified by Bruker D8 Discover X-Ray Diffractometer (XRD) with 2 $\theta$  range from 10° to 70° at a scanning rate of 4° per minute, with Cu K $\alpha$  irradiation ( $k = 1.5406 \text{ \AA}$ ). A 980 nm CW laser (Beijing Hi-Tech Optoelectronic Co., Ltd.) was used as the excitation source with the power being set at 500 mW. The up-converting fluorescence spectra were recorded on a DCS200PC Photon Counting (Beijing Zolix Instruments Co., Ltd) with single-photon sensitivity through an Omni- $\lambda$ 300 monochromator (Beijing Zolix Instruments Co., Ltd). The down-converting fluorescence measurements were performed with a RF-5301 PC fluorometry (Shimadzu).

#### Synthesis of Polyethyleneimine (PEI) Modified NaYF<sub>4</sub>: Yb, Er Nanoparticles

The water-soluble NaYF<sub>4</sub>: Yb, Er nano-crystals modified with polyethylenimine (PEI) was synthesized according to our previous work.<sup>1</sup> In a typical synthesis procedure, 0.25 mmol of lanthanide oxides Ln<sub>2</sub>O<sub>3</sub> (Y: Yb: Er = 0.80: 0.18: 0.02, mole-to-mole ratio) were dissolved in hot nitric acid (65 °C) to acquire Ln(NO<sub>3</sub>)<sub>3</sub>, and the solvent was evaporated after 6 h reaction. The as-obtained nitrate salts were added to a solution containing 680 mg of polyethyleneimine. And then another aqueous solution containing 0.126 g of NaF (F<sup>-</sup>/Ln<sup>3+</sup> = 6) was added dropwise to the above solution under vigorous stirring. After stirring at room temperature for 20 min, the mixture (with a total volume of 36 mL, V<sub>ethanol</sub>: V<sub>water</sub> = 1: 1) was transferred into a 50 mL Teflon autoclave and heated to 200 °C. After 6 h hydrothermal treatment, the autoclave was cooled down to room temperature naturally, and then a precipitate was obtained by centrifuging. The precipitate was washed with water and absolute ethanol three times respectively and was dried under vacuum before use.

### Preparation of UCPs-MB-TMR conjugates

UCPs were linked to the TMP labeled MB chains via a sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (Sulfo-SMCC) coupling protocol following the reports.<sup>2,3</sup> 2 mg of PEI modified UCPs was added in 2 mL of N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer (10 mM, pH = 7.4) containing 0.5 mg of Sulfo-SMCC, and the mixture was gently shaken for 1 h at room temperature in darkness. After centrifugation, the precipitate was collected and washed with Tris-HCl buffer (10 mM, 100 mM NaCl, pH = 7.4) for three times to remove the excess Sulfo-SMCC. The activated UCPs particles were incubated overnight at room temperature in 2 mL of Tris-HCl buffer in the presence of 2 μM oligonucleotides (MB<sub>1</sub> and MB<sub>2</sub>, respectively). The final products were washed with Tris-HCl buffer and diluted with 1 mL of the buffer. And the concentrations of UCPs-MB<sub>1</sub>-TMR and UCPs-MB<sub>2</sub>-TMR were both determined as 2 mg/mL. The coverage densities of MB probes were estimated as the amounts of MB-TMR coating onto per milligram of UCPs, which was calculated with the absorbance of TMR at 559 nm after subtracting the contribution of UCPs at this wavelength, using the molar extinction coefficient of 9.5 × 10<sup>4</sup> M<sup>-1</sup> cm<sup>-1</sup>. Using the absorbance and molar extinction coefficient of TMR at 550 nm, the coverage density of the MB probe was calculated to be 1.15 nmol per milligram of UCPs.

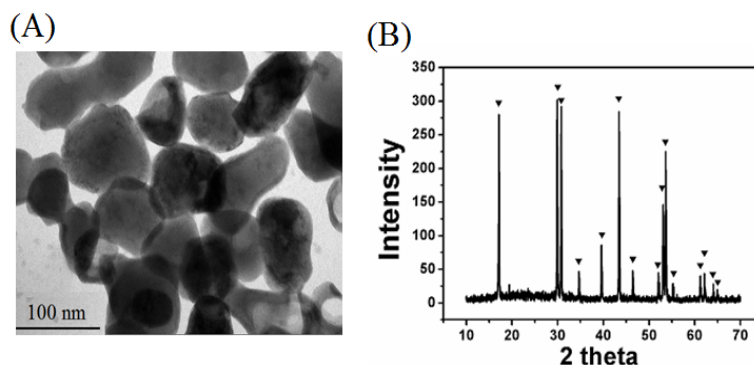
### Recognition of target ssDNA with UCPs-MB<sub>1</sub>-TMR

In a typical procedure, the MB sensor was fixed at 0.080 mg/mL, and different concentrations of complementary ssDNA were individually introduced to the EP tubes. After adjusting the total volume to 600 μL with Tris-HCl buffer (10 mM, containing 100 mM NaCl, pH 7.4), the mixture was incubated at 42 °C for 1 h with gentle shaking. The solution was taken for fluorescence measurement under the excitation of 980 nm with a CW laser. To examine the ability of UCPs-MB<sub>1</sub>-TMR in discriminating complementary, NC, and SBM DNAs, 50 nM NC, SBM DNAs and 5 nM complementary chains were tested with an identical procedure.

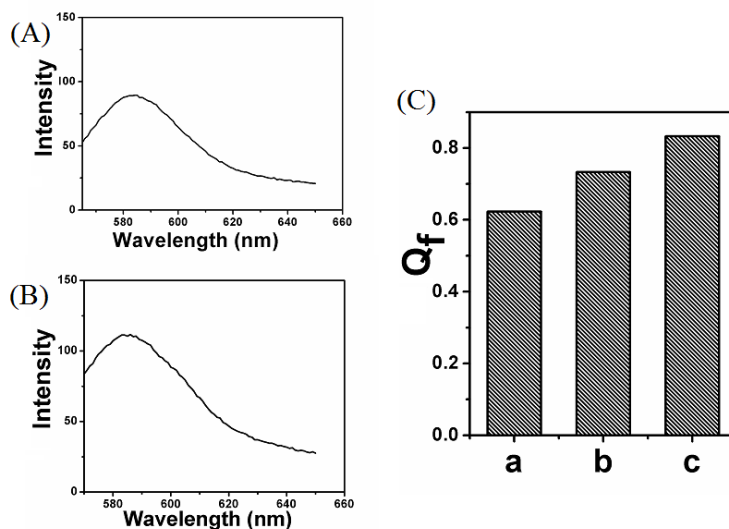
### Thrombin determination in aqueous buffer and human serum

To a solution containing 0.066 mg/mL UCPs-MB<sub>2</sub>-TMR, various concentrations of thrombin were added and incubated at 37 °C with gentle shaking for 1 h. And then the mixture was taken to up-converting fluorescence measurements. To examine the specificity of the UCPs-MB<sub>2</sub>-TMR

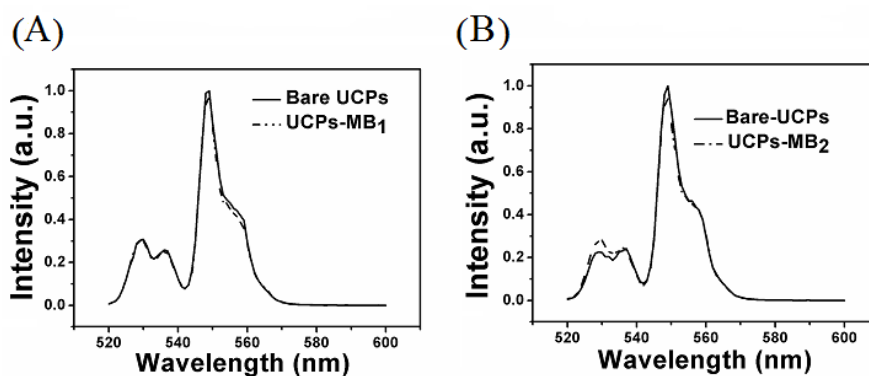
sensor to-wards TB, some other biomolecules and ions (with a concentration of 2000 nM) were added in place of TB with the same experimental conditions. To examine the applicability of the sensor in complicated sample matrix, human serum from healthy volunteer (provided by Zhongnan hospital, Wuhan University) was 40-fold diluted with Tris-HCl buffer, which was then used as the medium for TB assay following an identical procedure as in the aqueous buffer.



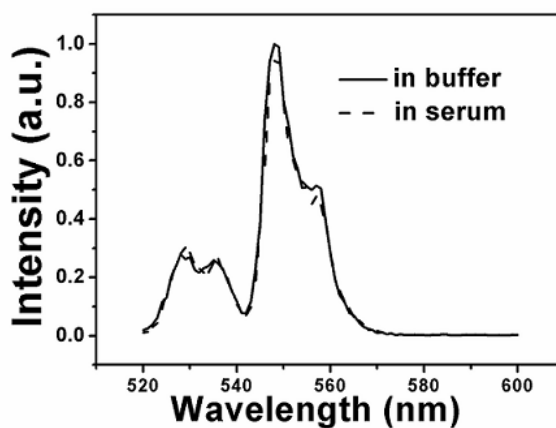
**Fig S1.** (A) The TEM image of the PEI coated NaYF<sub>4</sub>: Yb, Er up-converting phosphors. (B) XRD pattern of NaYF<sub>4</sub>: Yb, Er up-converting phosphors. ▼, hexagonal phase (JCPDs card 16-0334).



**Fig S2.** (A) The down-converting fluorescence of the UCP-MB<sub>1</sub>-TMR complex (0.08 mg/mL) excited with a Xe-lamp at 550 nm. Excitation slit = 10 nm, Emission slit = 10 nm (B) The down-converting fluorescence of and UCP-MB<sub>2</sub>-TMR com-plexes (0.066 mg/mL) excited with a Xe-lamp at 550 nm. Excitation slit = 10 nm, Emission slit = 10 nm (C) Quenching rates of the up-converting fluorescence of UCPs (2 mg/mL) with various dosages of MB<sub>1</sub>-TMR. a: 1 nM, b: 2 nM, and c: 4 nM.



**Fig S3.** (A) Up-converting fluorescence of bare UCPs (0.08 mg/mL) and the UCPs-MB<sub>1</sub> complex (0.08 mg/mL). (B) Up-converting fluorescence of bare UCPs (0.066 mg/mL) and the UCPs-MB<sub>2</sub> complex (0.066 mg/mL).



**Fig. S4.** Up-converting fluorescence spectra of the UCP-MB<sub>2</sub>-TAMRA complex in buffer solution (solid line) and diluted human serum (dashed line).

## References

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- [3] J. Li, C. Lu, Q. Yao, X. Zhang, J. Liu, H. Yang, G. N. Chen, *Biosens. Bioelectron.* 2011, **26**, 3894.