Electronic Supporting information (ESI)

MnO_2 Nanosheet as Label-Free Nanoplatform for Homogenous Biosensing

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Experimental details

Materials. Polyacrylic acid (PAA, with an average molecular weight of 1800), Polyethylenimine (PEI, with an average molecular weight of 25000) 1-ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride (EDC•HCl) and sodium dodecyl benzene sulfonate (Sulfo-NHS) were purchased from Sigma-Aldrich. 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was purchased from Alfa Aesar (Ward Hill, MA). 2-(N-morpholino) ethanesulfonic acid (MES) were obtained from Biosharp (USA). Ochratoxin a (OTA) and a flatoxins B1, B2 was supplied by Fermentek Ltd (Australia). The human cathepsin D (Cat D) was obtained from Sino Biological Inc. (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 MΩ resistivity). All chemicals were of analytical grade or better and were used as received without further purification. The oligonucleotides and the polypeptide were purchased from Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China) and purified using high-performance liquid chromatography. The sequences were as follows:

OTA aptamer: 5'-NH_2- GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGG-3';

The single-strand DNA: 5'-NH_2-AATGTGCTTACTATGCGGTAACTC -3';

The double-strand DNA: 5'-NH_2-AATGTGCTTACTATGCGGTAACTC-3'; 5'-GAGTTACCGCATAGTAAGCACATT-3'

The polypeptide: N-C: GHHHYYYGGKPISFFRLGC; the sequence KPISFFRL is the Cat D-specific substrate.

Instrumentations: The size and morphology of MnO_2 nanosheet and PAA modified UCPs were characterized by a JEM-2010 transmission electron microscope (TEM) with an accelerating voltage of 200 kV. Zeta potential measurements were performed on a Zetasizer Nano-ZS90 (Malvern Instruments Ltd., U. K.). The crystal phase of UCPs were identified by Brucker D8 Discover X-Ray Diffractometer (XRD) with 2θ range from 10° to 70° at a scanning rate of 4° per minute, with Cu Ka irradiation (k = 1.5406 Å). The EDX spectra were recorded with scanning electron microscopy (SEM) (FEI Quanta 200). FT-IR spectra were measured on infrared spectrometric analyzer (Nicolet-Magna-IR 550, USA) with the KBr pellet technique. The UV-Vis spectra of MnO_2 nanosheets were recorded with a UV-2550 UV-Vis spectrophotometer (Shimdzu). The upconversion fluorescence spectra were measured on a RF-5301 PC fluorometry (Shimdzu) with an external 980 nm CW laser (Beijing Hi-Tech Optoelectronic Co., China).

Preparation of MnO_2 nanosheet: In a typical preparation procedure, 0.3 M $MnCl_2$•$4H_2O$ was firstly prepared in 10 mL of ultrapure water, and then 20 mL of aqueous solution containing 0.6 M tetramethylammonium hydroxide (TMA•OH) and 3.0wt% H_2O_2 was added to the above solution within 15 s. The consequent dark brown suspension was stirred vigorously overnight in the open air at room temperature, which was accompanied by the generation of oxygen. Thereafter, the as-obtained dark brown solution was centrifuged at 10000 rpm for 5 min to collect the precipitate, which was washed with water and methanol several times and was finally dissolved in water with a concentration of 5 mg/mL.

Synthesis of PAA-modified $NaYF_4$: Yb, Tm nanoparticles: The water-soluble $NaYF_4$: Yb, Tm nanocrystals modified with PAA was synthesized according to our previous work. Briefly, 0.25 mmol of lanthanide oxides Ln_2O_3 (Y: Yb: Tm =0.7965: 0.18: 0.035, mole-to-mole ratio) were dissolved in hot nitric acid (65 ℃) to acquire $Ln(NO_3)_3$, and the solvent was evaporated after 6 h reaction. The as-obtained nitrate salts were added to a solution containing 900 mg of PAA. And then another aqueous solution containing 0.21 g of NaF (F^-/Ln^{3+} = 10) was added dropwise to the above solution under vigorous stirring. After reacting at room temperature for 20 min, the mixture (with a total volume of 36 ml, $V_{ethanol}$: V_{water} = 1: 1) was transferred into a 50 ml Teflon autoclave and heated to 200 ℃. After 10 h of 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.

Synthesis of PEI-modified $NaYF_4$: Yb, Tm nanoparticles: In brief, 0.25 mmol of $LnCl_3$ (Y: Yb: Tm =0.7965: 0.18: 0.035, mole-to-mole ratio) dissolved in ethylene glycol (EG) were prepared as the similar procedure with $Ln(NO_3)_3$. The as-obtained hydrochloride salts were added to a EG solution containing an appropriate amount of PEI and NH_4F under vigorous stirring. After reacting at room temperature for 20 min, the mixture was transferred into a 50 ml Teflon autoclave and heated to 200 ℃. After 6 h of hydrothermal treatment, a precipitate was obtained by centrifuging. The precipitate was washed with water and absolute ethanol three times respectively and was dried before use.

Preparation of UCPs-aptamer conjugates: Briefly, 2 mg UCPs was dissolved in 2 mL of MES buffer (10 mM, pH 5.5) containing 1.8 mg EDC and 3.6 mg Sulfo-NHS

to active the carboxyl groups on the surface of UCPs. After 1 h of reaction, a precipitate was harvested by centrifugation and washed with water three times. Then the activated UCPs were diluted in 2 mL of HEPES buffer (10 mM, pH 7.2) and incubated with 2 nmol amine modified OTA aptamer under gentle shaking overnight at room temperature. Afterwards the UCPs-aptamer conjugates were obtained by centrifugation and washing with HEPES and finally dissolved in HEPES buffer (10 mM, 100 mM NaCl, pH 7.4) with a concentration of 1 mg/mL.

Preparation of UCPs-peptide conjugates: 2 mg of PEI-modified UCPs was added to 2 mL of HEPES buffer solution (pH 7.4, 10 mM) containing 2 mg of Sulfo-SMCC, and the mixture was gently shaken for 1 h at room temperature. Then the solution was centrifuged to remove excess Sulfo-SMCC and the precipitate was harvested and washed with HEPES three times. Thereafter, 3 mg of polypeptide was incubated with the activated UCPs solution with gentle shaking overnight at room temperature. The excess peptide was removed by centrifuging and washing, and the obtained UCPs-peptide was finally dissolved in HAC-NaAC buffer (pH 4.0, 20 mM).

Sensing of OTA in aqueous buffer, red wine and human serum samples: For the fluorescence quenching experiments, the UCPs-aptamer complex was fixed at 0.02 mg/mL, and varying concentrations of MnO_2 nanosheet were individually introduced into the EP tubes. The mixtures were incubated for 10 min at room temperature followed by upconversion fluorescence measurements. For the assay of OTA in aqueous buffer, to a solution containing 0.02 mg/mL UCPs-aptamer complex, various concentrations of OTA were individually added and incubated at 37 ℃ with gentle shaking for 2 h. And then MnO_2 nanosheet was added with 0.5 mg/mL final concentration. After 10 min of incubation at room temperature, upconversion fluorescence detection was performed. Fluorescence emission of the donor was measured under excitation of 980 nm with a CW laser.

Analysis of Cat D in aqueous solution: In a typical experiment, UCPs-peptide (0.025 mg/mL) was prepared in HAC-NaAC buffer (pH 4.0, 20 mM) with the addition of Cat D. The final Cat D concentration in solution ranged from 1 to 100 ng/mL. After allowing this mixture to bind for about 1h min at room temperature, 0.9 mg/mL of MnO_2 nanosheet were introduced and incubated for another 30 min, and then the fluorescence of the mixture was detected. Fluorescence emission of the donor was measured under excitation of 980 nm with a CW laser.

Supplementary Figures:

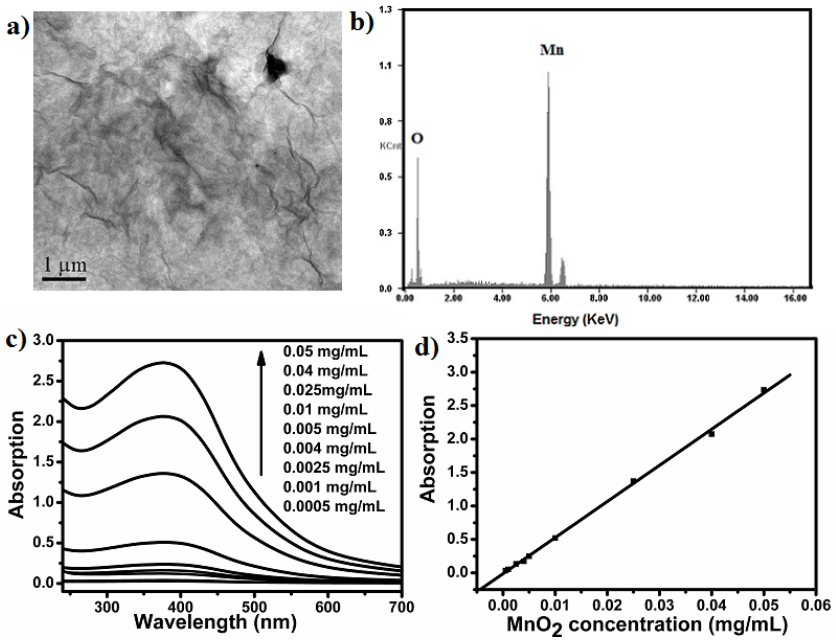

Fig. S1 a) The TEM image of MnO_2 nanosheet. b) EDS spectrum of MnO_2 nanosheet. c) UV-Vis spectra of MnO_2 nanosheet at various concentrations. d) The plot of absorbance at 378 nm against the concentration of MnO_2 nanosheet.

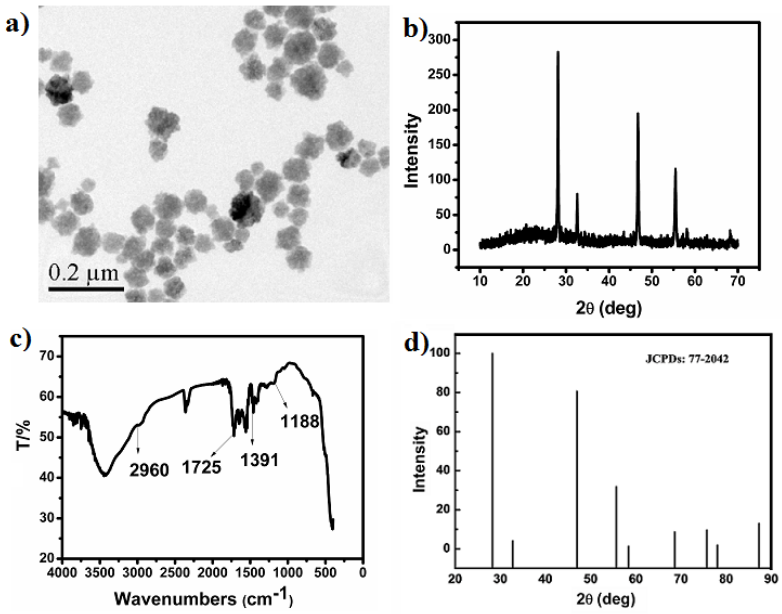

Fig. S2 The TEM image (a), XRD pattern (b) and FT-IR spectrum (c) of PAA-$NaYF_4$: Yb, Tm upconversion phosphors. The standard pattern of cubic phase (JCPDs card 77-2042) $NaYF_4$ is also offered (d).

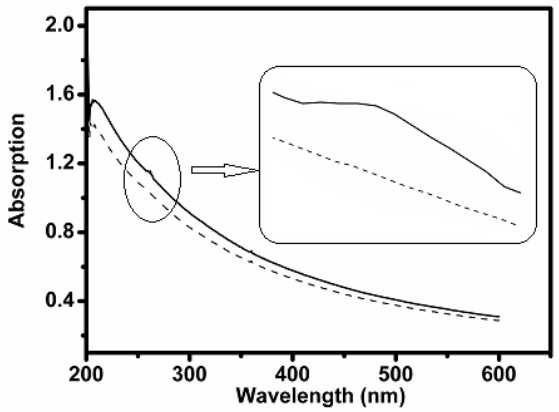

Fig. S3 The UV-Vis absorption spectra of UCPs-aptamers (solid line) and UCPs (dash line).

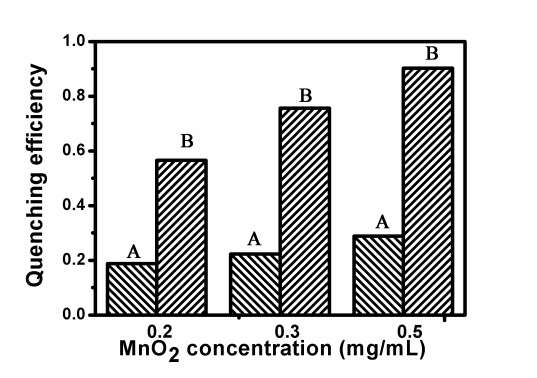

Fig. S4 Fluorescence quenching degrees of bare UCPs (A) and UCPs-aptamer complex (B) at different concentrations of MnO_2 nanosheet (0.2, 0.3, 0.5 mg/mL).

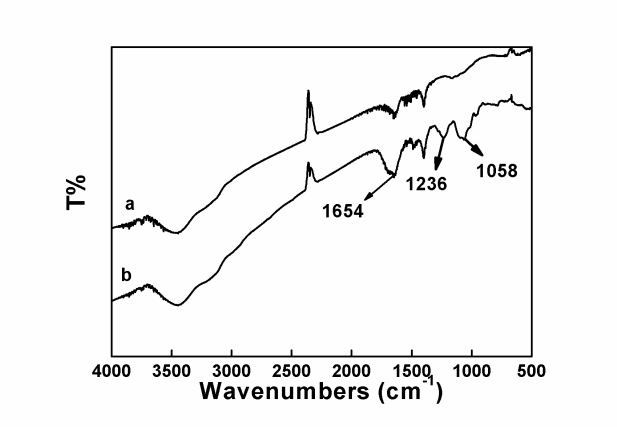

Fig. S5 FT-IR spectra of MnO_2 nanosheet (a) and MnO_2-ssDNA (b).

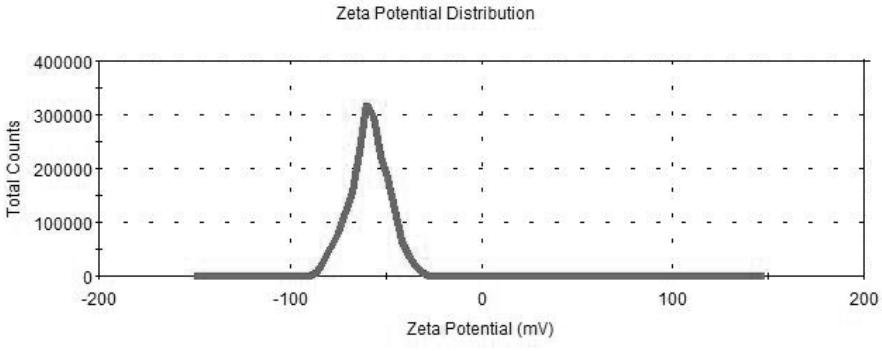

Fig. S6 The zeta potential distribution of MnO_2 nanosheet.

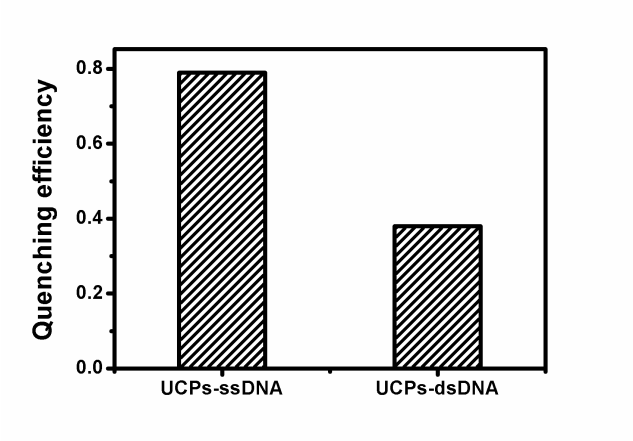

Fig. S7 Fluorescence quenching efficiency of UCPs-ssDNA (0.02 mg/mL) and UCPs-dsDNA (0.02 mg/mL) by the same amount of MnO_2 nanosheet (0.4 mg/mL).

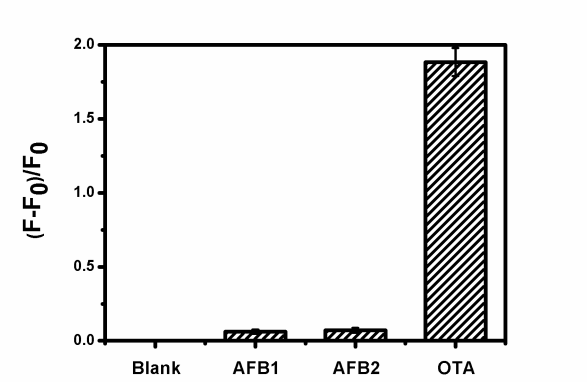

Fig. S8 The relative fluorescence intensity of UCPs-aptamer/MnO_2 nanosheet complex (denoted as blank) in the presence of OTA (2.0 ng/mL) and its analogues AFB1 (200 ng/mL), AFB2 (200 ng/mL) in aqueous solution. Excitation wavelength: 980 nm.

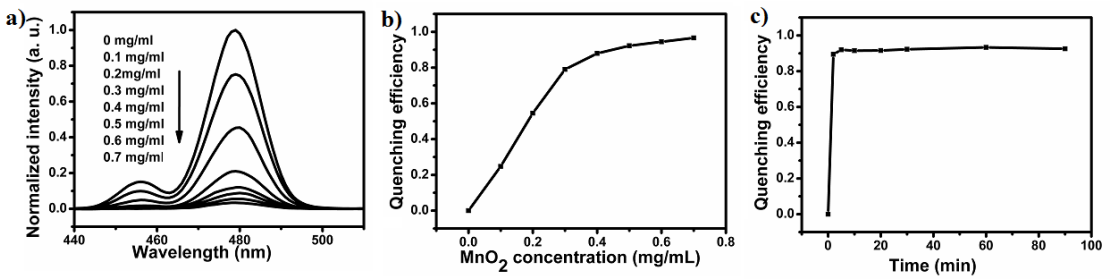

Fig. S9 a) Fluorescence quenching of UCPs-aptamer (0.02 mg/mL) with varying amounts of MnO_2 nanosheet in red wine sample. b) Fluorescence quenching efficiency versus MnO_2 nanosheet concentration (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 mg/mL) in red winesample. c) Time dependence of the fluorescence quenching of 0.02 mg/mL UCPs-aptamer by 0.5 mg/mL MnO_2 nanosheet in red wine sample. Excitation wavelength: 980 nm.

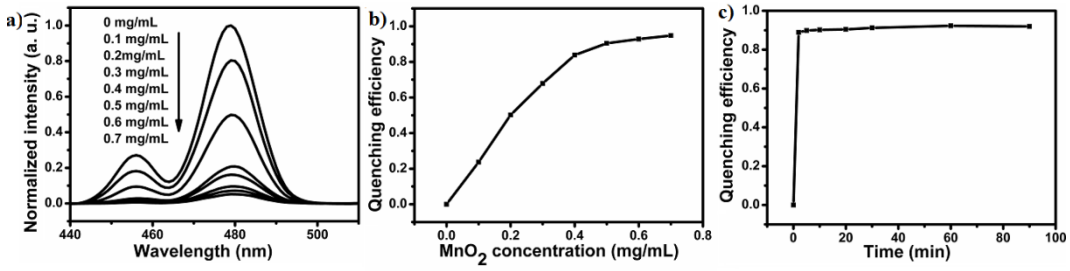

Fig. S10 a) Fluorescence quenching of UCPs-aptamer (0.02 mg/mL) with varying amounts of MnO_2 nanosheet in human serum sample. b) Fluorescence quenching efficiency versus MnO_2 nanosheet concentration (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 mg/mL) in human serum sample. c) Time dependence of the fluorescence quenching of 0.02 mg/mL UCPs-aptamer by 0.5 mg/mL MnO_2 nanosheet in human serum sample. Excitation wavelength: 980 nm.

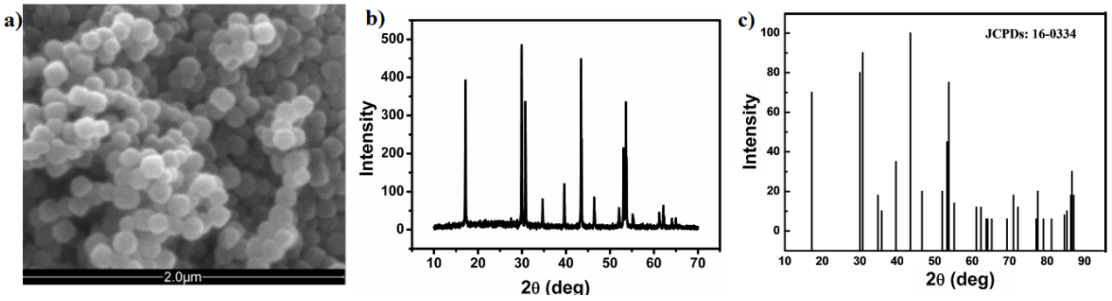

Fig. S11 The TEM image (a) and XRD pattern (b) of PEI-$NaYF_4$: Yb, Tm upconversion phosphors. The standard pattern of hexagonal phase (JCPDs card 16-0334) $NaYF_4$ is also offered (c).

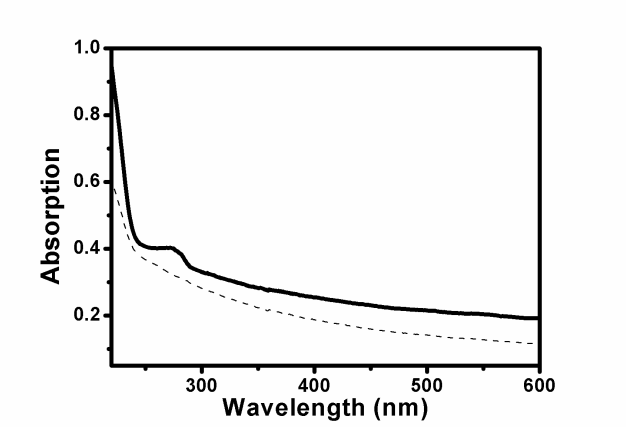

Fig. S12 The UV-Vis absorption spectra of UCPs-peptide (solid line) and UCPs (dash line).

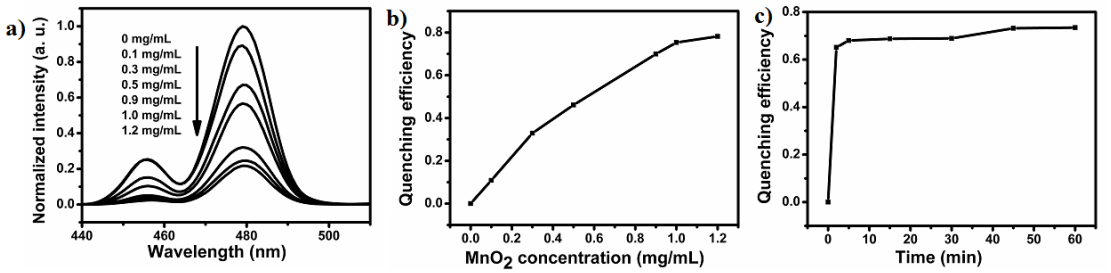

Fig. S13 a) Fluorescence quenching of UCPs-peptide (0.025 mg/mL) with varying amounts of MnO_2 nanosheet. b) Fluorescence quenching efficiency versus MnO_2 nanosheet concentration (0, 0.1, 0.3, 05, 0.9, 1.0, 1.2 mg/mL). c) Time dependence of the fluorescence quenching with 0.025 mg/mL UCPs-peptide and 0.9 mg/mL MnO_2 nanosheet. Excitation wavelength: 980 nm.

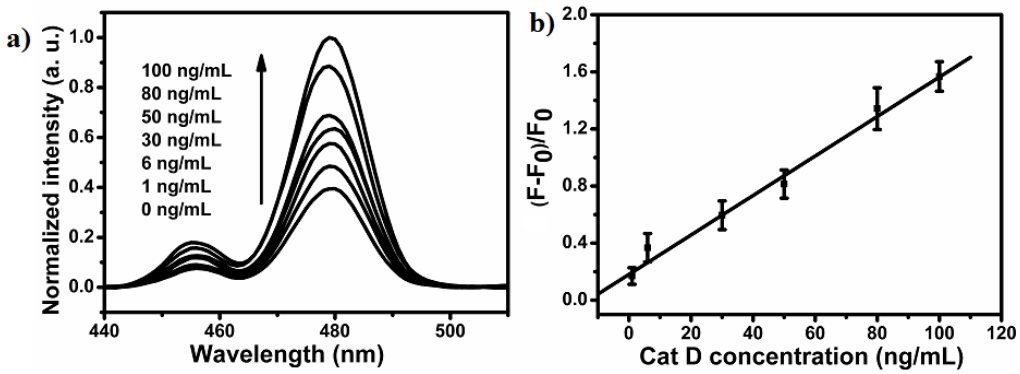

Fig. S14 a) Fluorescence of UCPs-peptide/MnO_2 nanosheet in the presence of different concentrations of Cat D (0, 1, 6, 10, 30, 50, 80, 100 ng/mL) in HAc-NaAc buffer (pH 4.0, 20 mM). b) The linear relationship between the relative fluorescence intensity and the concentration of Cat D within the range of 1-100 ng/mL. Data were presented as average ± sd from three independent measurements. Excitation wavelength: 980 nm.