Electronic Supplementary Material (ESI) for Nanoscale.

Hierarchical manganese dioxide nanoflowers enable accurate ratiometric fluorescence enzyme-linked immunosorbent assay

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EXPERIMENTAL SECTION

Materials and instruments. Potassium permanganate (**(**$KMnO_4$) and hydrochloric acid were obtained from Laiyang Fine Chemical Factory. Citric acid, o-phenylenediamine (OPD), bovine serum albumin (BSA), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Shanghai Macklin biochemical technology Co., Ltd. Carbon dots (CDs) kindly provided by Yang, was synthesized by his previous work.[1] CRP and its antibodies were purchased from Shanghai Linc-Bio Science Co. LTD. Acetic acid (HAC), sodium acetate (NaAC), Na_2HPO_4, KH_2PO_4 and KCl were obtained from Sinopharm Chemical Reagent Beijing Co., Ltd.

Scanning electron microscope (SEM) was operated using a field emission SEM (ZEISS, Germany). Transmission electron microscope (TEM) was recorded by a Philips CM200 UT (Field Emission Instruments, USA). X-ray photoelectron spectroscopy (XPS) was obtained by an XSAM800 multifunctional surface analysis electron spectrometer (Kratos, UK). Fourier transform infrared spectroscopy (FTIR) spectrum was measured by VERTEX 70 spectrometer (Bruker, Germany). The X-ray diffraction (XRD) was conducted from 10° to 70° (Bruker AXS, Germany). All UV-vis spectrum and fluorescent spectrum were measured through a multimode microplate reader (TECAN SPARK 20M, Switzerland).

Preparation of MnO_2 NFs. 80 mg of $KMnO_4$ was dissolved in 40 mL of hydrochloric acid (0.1 mol/L) solution to obtain a homogeneous wine solution. After that, 1 mL of citric acid (100 mM) was added into the solution and stirred 30 minutes at room temperature, emerging a brown suspension from wine solution. The products (MnO_2 NFs) were centrifuged and washed several times and dried under vacuum at 50 ℃.

Ratiometric fluorescence immunoassay of CRP. MnO_2 NFs (0.5 mg/mL, 1 mL) was adjusted by K_2CO_3 to be pH 6.0 under vigorous ultrasonication for 1 hour. Then, MnO_2 NFs was activated by EDC (2 mg/mL) and NHS (4 mg/mL) for gently shaking for 30 minutes. The mixture was centrifuged and washed for 3 times. Ab_2 (100 μg/mL, 1 mL) in PBS was incubated with active MnO_2 NFs for 1 hour at 37 ℃ and centrifuged 3 times to remove unbonded antibodies. The products were passivated with 1% BSA for 30 minutes and dispersed in 1 mL of PBS stored at 4 °C for further use.

100 μL of Ab_1 with a concentration of 1 μg/mL diluted in PBS (pH 7.4) was incubated with 96-

well plates for 12 h at 4 ℃. Next, these plates were washed with PBS (pH 7.4) for 3 times to remove unbonded antibodies. Afterwards, 200 μL of BSA (1%) diluted in PBS (pH 7.4) was added and incubated for 30 minutes at 37 ℃ to block nonspecific active sites and washed for 3 times to remove excess BSA. Next, 100 μL of different concentration of CRP was added into each well and incubated for 30 minutes at 37 ℃. After that, each well was washed with PBS (pH 7.4) for 3 times to remove unbonded antigen. And 100 μL of MnO_2 NFs-Ab_2 (0.5 mg/mL) was subsequently added into each well and incubated for 60 minutes at 37 ℃. Each well was washed 3 times with PBS (pH 7.4) to remove unbonded MnO_2 NFs-Ab_2. Finally, 10 μL of CDs (0.02 mg/mL) was added into each well rapidly and 200 μL of OPD (15 mM) diluted in HAC-NaAC (pH 4.0) was incubated for 7 minutes at 37 ℃. The fluorescence emission spectra were recorded at excitation spectrum of 365 nm.

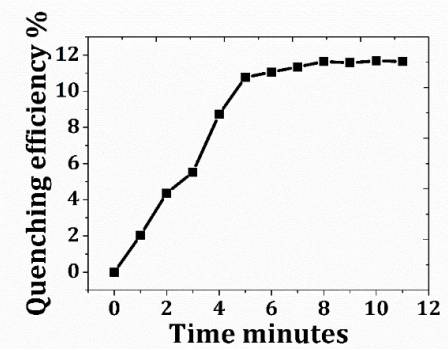

Figure S1 The fluorescent quenching efficiency of MnO_2 NFs towards CDs at different time.

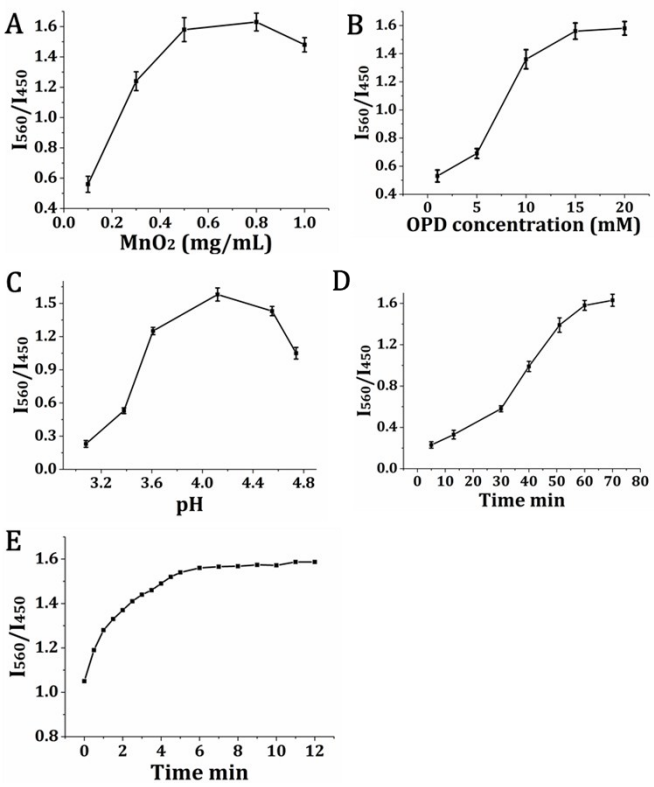

Figure S2 Optimized experimental conditions of ratiometric fluorescence ELISA: (A) MnO_2 NFs concentration; (B) OPD concentration; (C) pH; (D) incubation time of recognition between antigen and MnO_2 NFS-Ab_2; (E) incubation time of immunosensor with OPD and CDs.

Table S1 Comparison of the developed method with other methods

Method	Signal output	Detection ranges	Limit of detection	references
Aptasensor	electrochemical	5 pg/mL-125 ng/mL	0.0017 ng/mL	2
Microfluidic device.	colorimetric	0.005 μg/mL-5 μg/mL	0.0005 μg/mL	3
Microfluidic device	chemiluminescence	50 ng/mL-300 ng/mL	4.27 ng/mL	4
ELISA	Electrochemical	0 pg/mL-200pg/mL	12.5 pg/mL	5
Microfluidic device	Colorimetric	1 ng/mL-81 ng/mL	1.0 ng/mL	6
ELISA	Ratiometric fluorescence	0.001 ng/mL-50 ng/mL	0.67 pg/mL	This work

Table S2 The recovery of the fabricated immunosensor in human serum

Sample	Added (ng/mL)	Found (ng/mL)	Recovery (%)
1	1.00	0.91	91.00
2	1.50	1.46	97.33
3	2.00	2.08	104.00
4	2.50	2.54	101.60
5	3.00	3.09	103.00

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