Mn2+-triggered an in situ ratiometric fluorescence immunosensor via rapidly aggregation-induced emission transformation of levodopa fluorescent copolymer nanoparticles

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1. Experimental section

1.1 Materials and reagents.

Alkaline Phosphatase (ALP, specific activity = 5000 U/mg Protein), Trypsin, Pyrophosphatase (PPase), Tyrosinase (TYR), Bovine serum albumin (BSA), Glucose oxidase (GOD), Pepsin, COD, Levodopa, Ethylenediamine (EDA), $MnCl_2$, Na_5P_3O10, Ethylenediaminetetraacetic acid (EDTA) and other reagents were purchased from Aladdin reagent Co., Ltd. (Shanghai, China). Human blood samples were purchased from Youqing Biological Reagent Co., Ltd. (Nanjing, China). All chemicals were of analytical reagent grade and used without further purification.

1.2 Apparatus.

A UV-Vis spectrophotometer (UV-1750, SHIMADZU, Japan) was applied to record the UV-Vis absorption spectra. An F-7000 fluorescence spectrophotometer (HITACHI, Japan) was adopted to record the fluorescence spectra with a standard 10×10 mm quartz cell and a xenon lamp as the excitation source. Ultrapure water (18.2 MΩ) was acquired utilizing a Milli-Q Advantage A10 purification system (Millipore S.A, Bedford, USA). Transmission electron microscopy (TEM) images were obtained using a JEM-2010 UHR transmission electron microscopy (JEOL, Japan). Photos were taken by a Canon PowerShot SX50 digital camera (Japan).

1.3 Synthesis of levodopa fluorescent copolymer (LFC).

LFC was prepared according to room temperature-aqueous synthesis. The synthesis process was as follows: 4 μL levodopa (10 mM) and 2 μL EDA (10 g/mL) were added into 400 μL Tris-HCl buffer (10 mM, pH = 7.4). After mixing thoroughly,

the fluorescence intensity of LFC was checked after mixing for 15 min at room temperature.

1.4 Sensing Mn^{2+}.

80 μL levodopa (10 mM) and 40 μL EDA (10 g/mL) were added into 8 mL Tris-HCl buffer (10 mM, pH = 7.4) to obtain LFC. After mixing thoroughly, Mn^{2+} aqueous solution with different concentrations was added to 400 μL LFC solution, respectively. The fluorescence intensity of LFC was checked after mixing for 15 min at room temperature. To evaluate the selectivity of this method for Mn^{2+}, The common metal ions (Ag^{+}, Zn^{2+}, Cr^{3+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Al^{3+}, Ni^{2+}, Mg^{2+}, Ba^{2+}, K^{+}, Fe^{2+}, Fe^{3+}, Pd^{2+}, Hg^{2+}, Cu^{2+}) and various anions in water (CO_3^{2-}, SO_4^{2-}, HCO_3^{-}, Cl^{-}, OH^{-}) were investigated under the same conditions. The concentration of all ions was 2 μM.

1.5 Sensing ALP.

Different concentrations of the ALP were joined to 40 μL $P_3O_{10}^{5-}$ (500 μM) solution in Tris-HCl buffer (pH = 9.0) at 37 °C for 30 min. Then the volume of 40 μL ALP/ $P_3O_{10}^{5-}$ mix solution was added to the LFC/Mn^{2+} system. After incubation for 15 min, the fluorescence intensity of LFC was recorded. To assess the selectivity of the assay to ALP, a series of competitive enzymes or proteins, including Trypsin, PPase, TYR, BSA, GOD, Pepsin, and COD were investigated under the same condition. The concentration of all substances was 200 mU/mL.

1.6 Sensing of cTnI.

The immunoassay was performed as follows. Firstly, 100 μL of diluted mouse monoclonal antibody (1 μg/mL) in coating solution was injected into the wells of a 96-

well polystyrene plate and incubated at 4 °C overnight. After removing the solutions, the plate was washed four times with 200 μL of wash buffer (TBST), and blocked with 2% BSA at 37 °C for 1 h to reduce nonspecific binding. After washing again, 100 μL of cTnI standard solutions with different concentrations ranging from 0 to 125 ng/mL were injected into each well and incubated at 37 °C for 1 h, followed by washing procedures. Second, 100 μL of goat-cTnI antibody (2 μg/mL, 100 μL) was injected and incubated at 37 °C for 1 h. Third, the wells were washed four times and 100 μL of donkey goat secondary antibody labeled with ALP (1.5 μg/mL) was added and subsequently incubated at 37 °C for 1 h. After washing the plates, 40 μL $P_3O_{10}^{5-}$ (500 μM) and 160 μL Tris-HCl (10 mM, pH 9.0) buffers were injected into each well, incubated at 37℃ for 30 min, then the alp coupled second antibody was removed and washed four times. Afterward, 400 μL of Tris-HCl (10 mM, pH 7.4), 4 μL levodopa (10 mM), 2 μL EDA (10 g/mL) and Mn^{2+} (200 nM) were added into each well of the plates, and the real-time fluorescence curves of the resultant solutions were directly recorded.

2. Data Chart Supplement

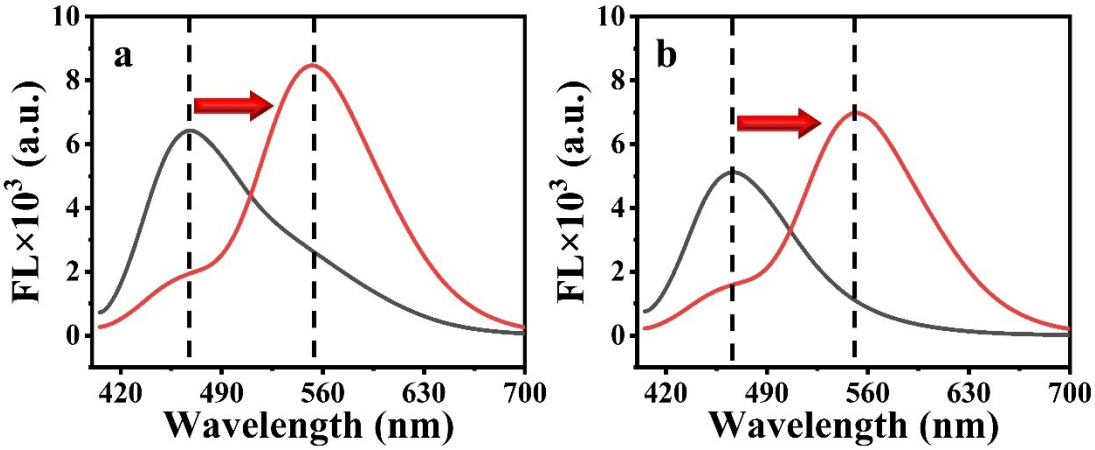

Fig. S1. Luminescence spectra of LFC nanoparticles and LFC nanoparticles -Mn^{2+} before (a) and after (b) dialysis.

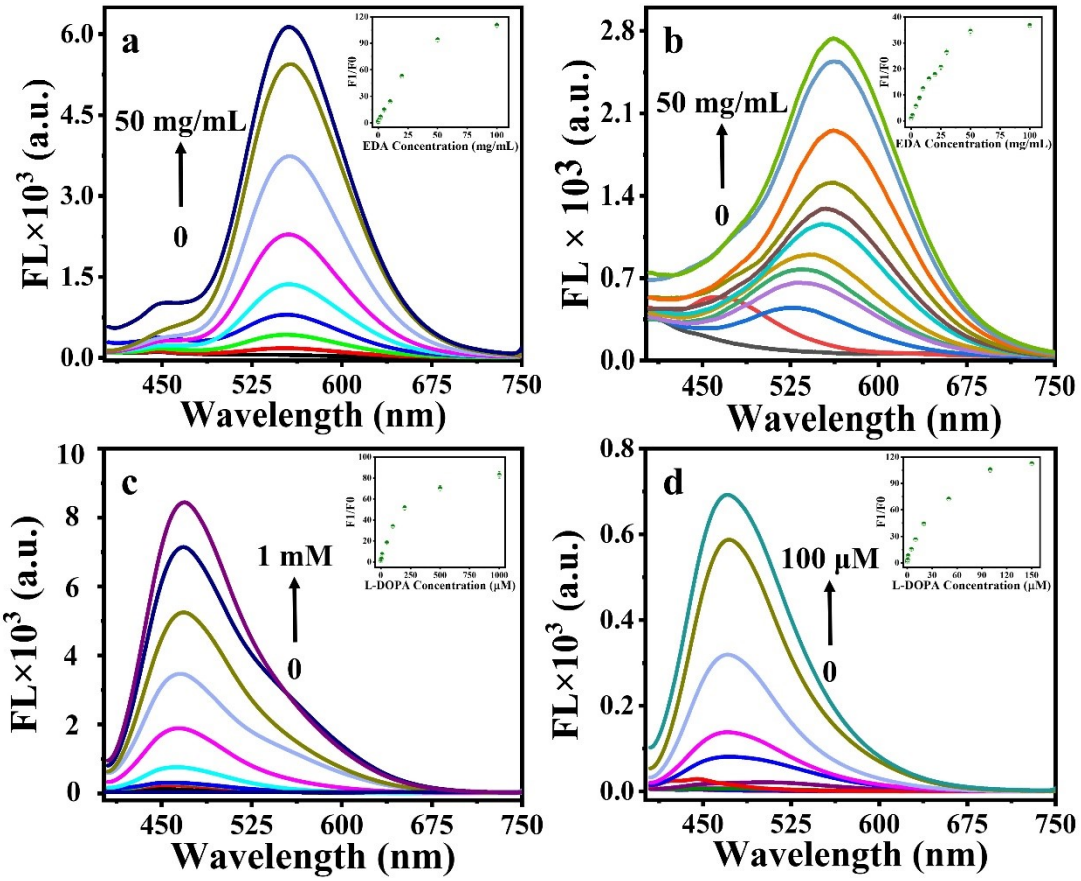

Fig. S2. Variations in luminescence intensity of LFC nanoparticles and LFC nanoparticles -Mn^{2+} with the change of concentration of EDA (a, b) and L-DOPA (c, d) at 385 nm excitation. Inset: Relationship between F/F_0 and the EDA/L-DOPA concentration.

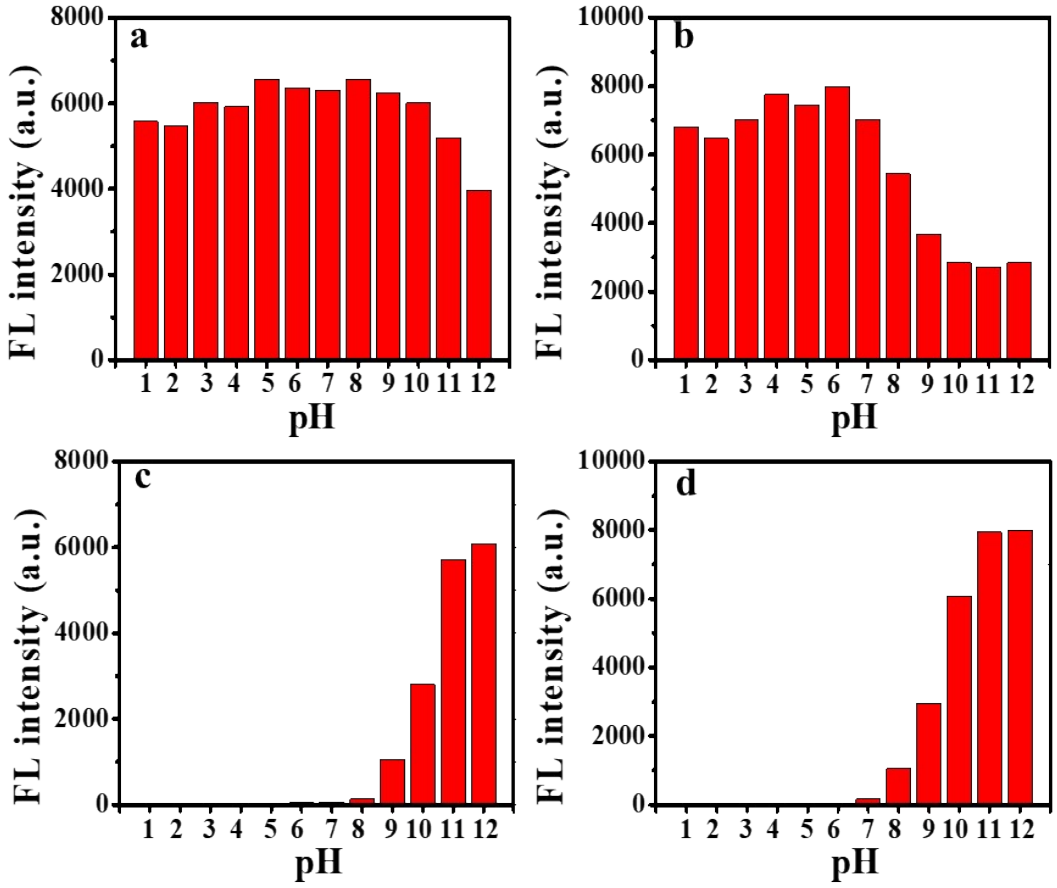

Fig. S3. The influence on the luminescence intensity of LFC nanoparticles with the pH change of Tris-HCl buffer (a) and EDA (c); The influence on the luminescence intensity of LFC nanoparticles -Mn^{2+} with the pH change of Tris-HCl buffer (b) and EDA (d).

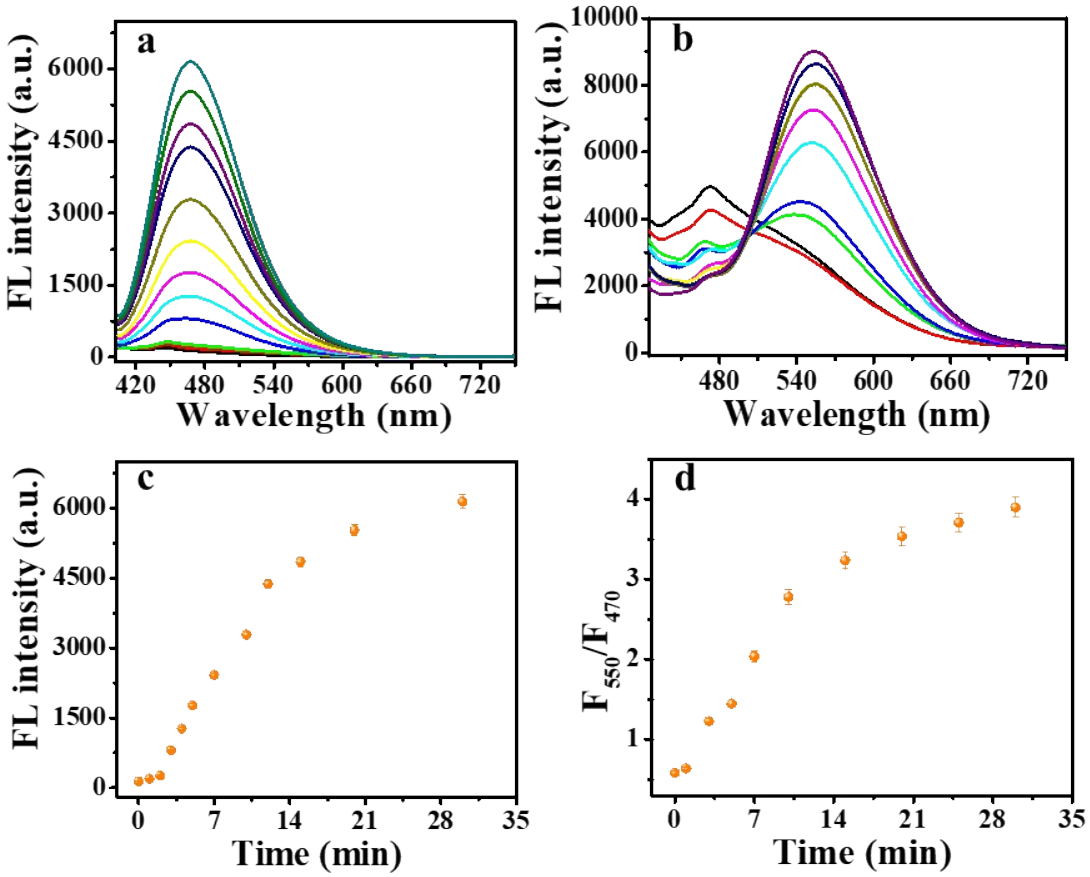

Fig. S4. Luminescence spectra of the LFC nanoparticles (a) and LFC nanoparticles - Mn^{2+} (b) at different times; (c) Variations in luminescence intensity with the change of reaction time without Mn^{2+} (d) Variations in luminescence intensity with the change of reaction time with Mn^{2+}; [L-DOPA] = 100 μM, [Mn^{2+}] = [2 μM].

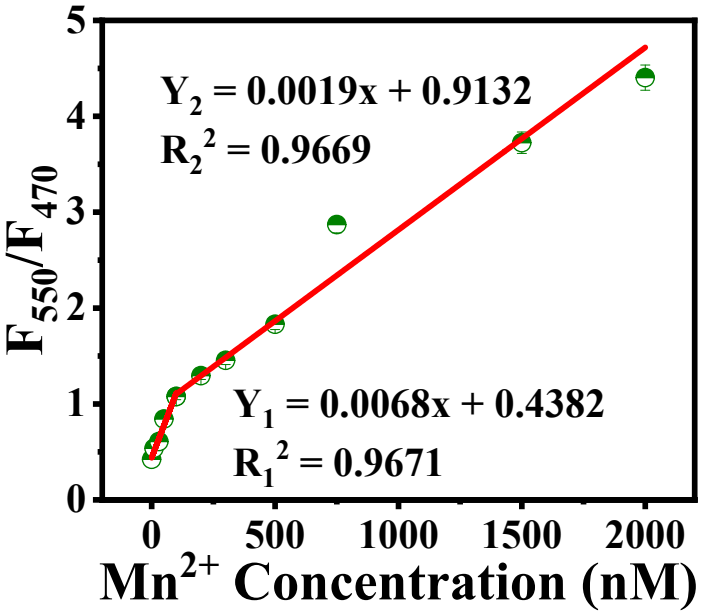

Fig. S5. The relationship between the fluorescent ratio (F_{550}/F_{470}) and the amounts of Mn^{2+}.

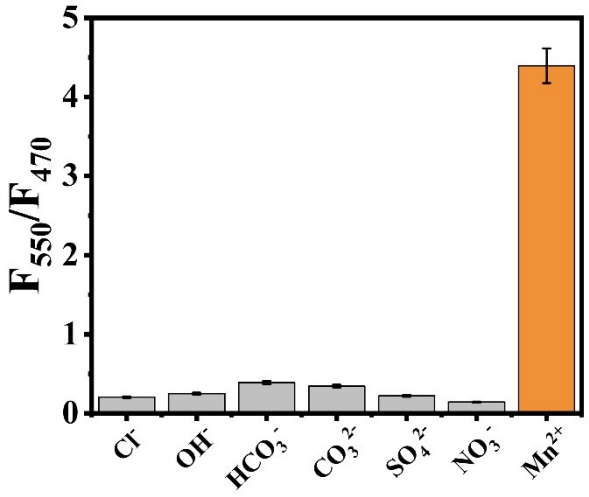

Fig. S6. Relative responses of the luminescence ratio of LFC at 550 nm and 470 nm to different anions ([L-DOPA]=100 μM, [Anion]=[2 μM]).

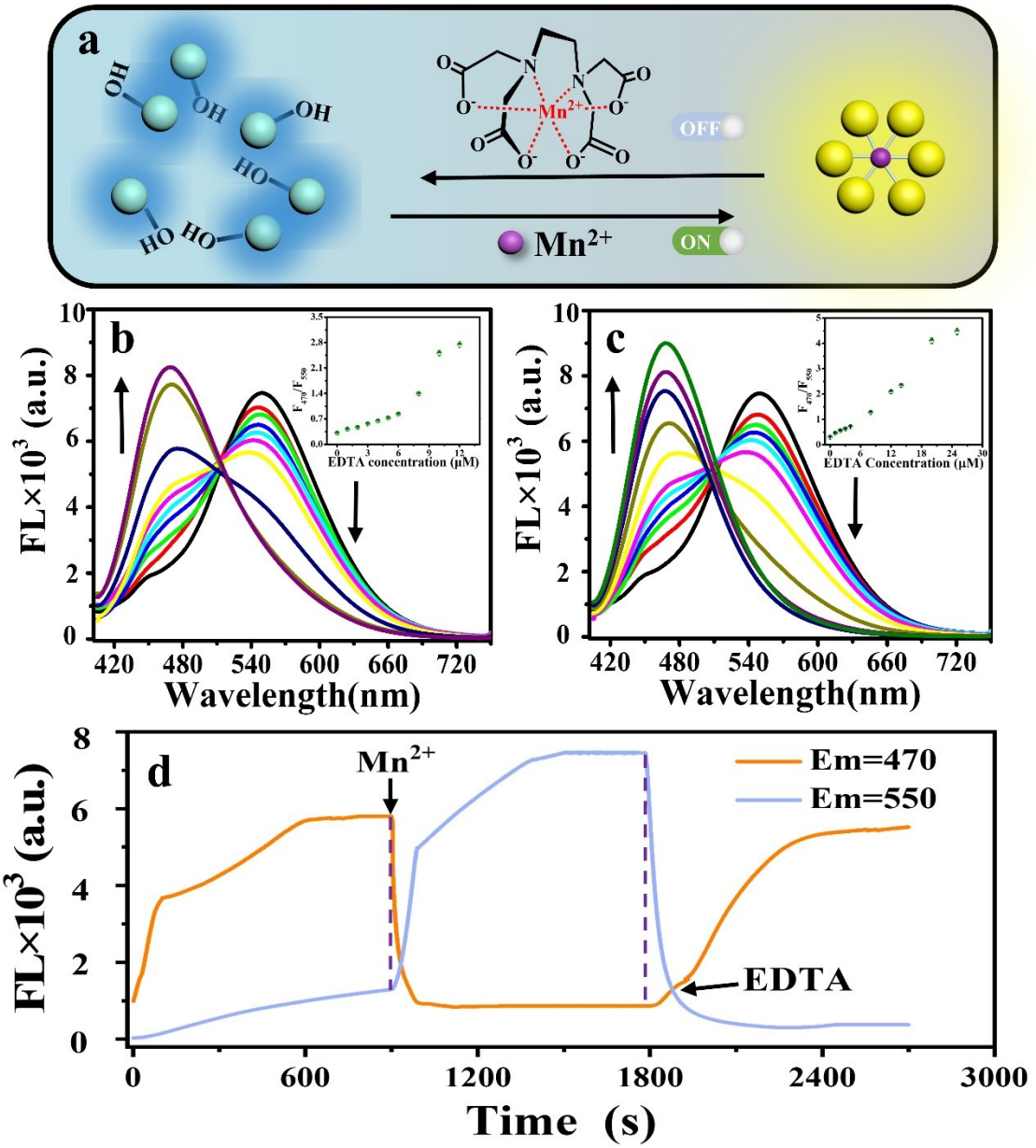

Fig. S7. (a) Scheme of the formation of Mn^{2+}-induced LFC nanoparticles aggregates by introducing Mn^{2+} into LFC nanoparticles, and the property of luminescence "On/Off" upon Mn^{2+}/EDTA; (b) Luminescence spectra of the Mn^{2+}-induced LFC nanoparticles aggregates at different concentrations of EDTA based on the pre-mixing strategy (c) Luminescence spectra of the Mn^{2+}-induced LFC nanoparticles aggregates at different concentrations of EDTA based on the post-mixing strategy; (d) Luminescence spectra in different time.

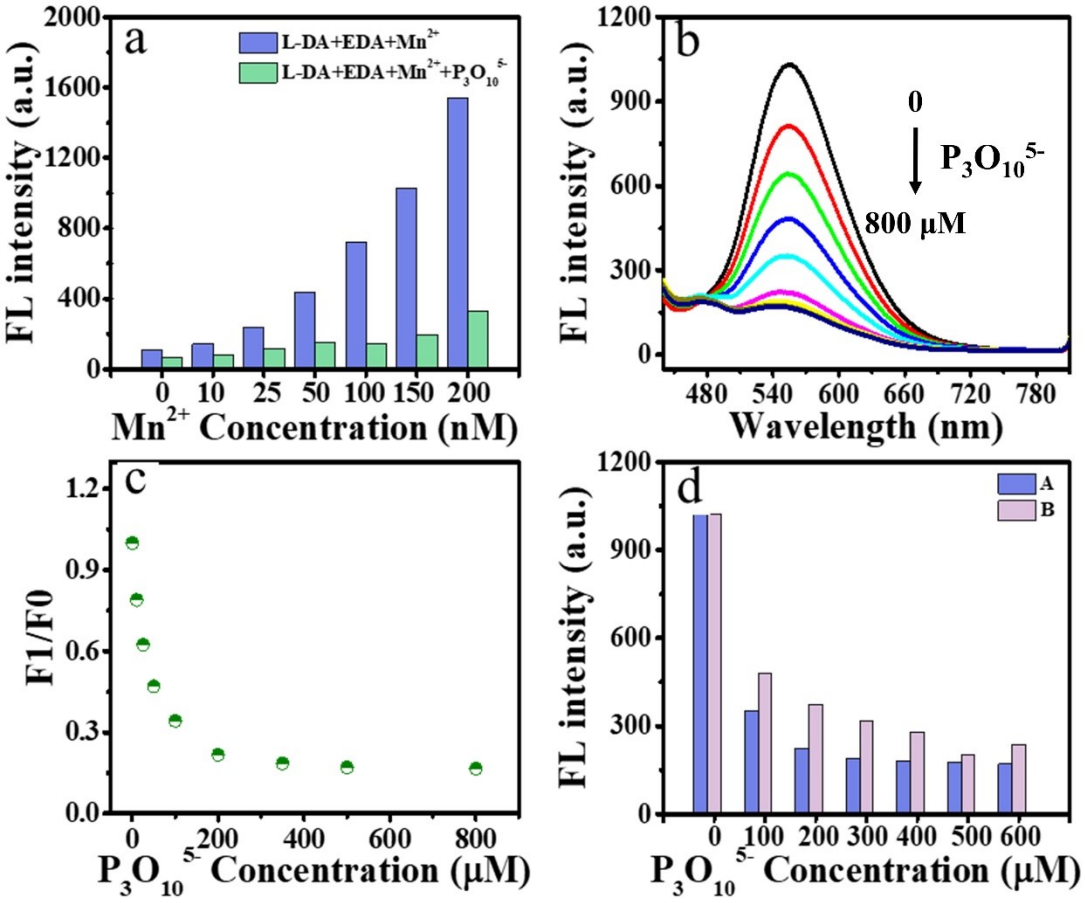

Fig. S8. (a) Luminescence histograms of the LFC nanoparticles at different concentrations of Mn^{2+} (blue) at 500 μM $P_3O_{10}^{5-}$(green); luminescence spectra (b) and F1/F0 (c) of the Mn^{2+}-induced LFC nanoparticles aggregates at different concentrations of $P_3O_{10}^{5-}$; (d) Luminescence histograms of the Mn^{2+}-induced LFC nanoparticles aggregates and different concentration $P_3O_{10}^{5-}$ incubated at 37 ℃ for 30 min at pH 7.4 (A) and pH 9 (B).

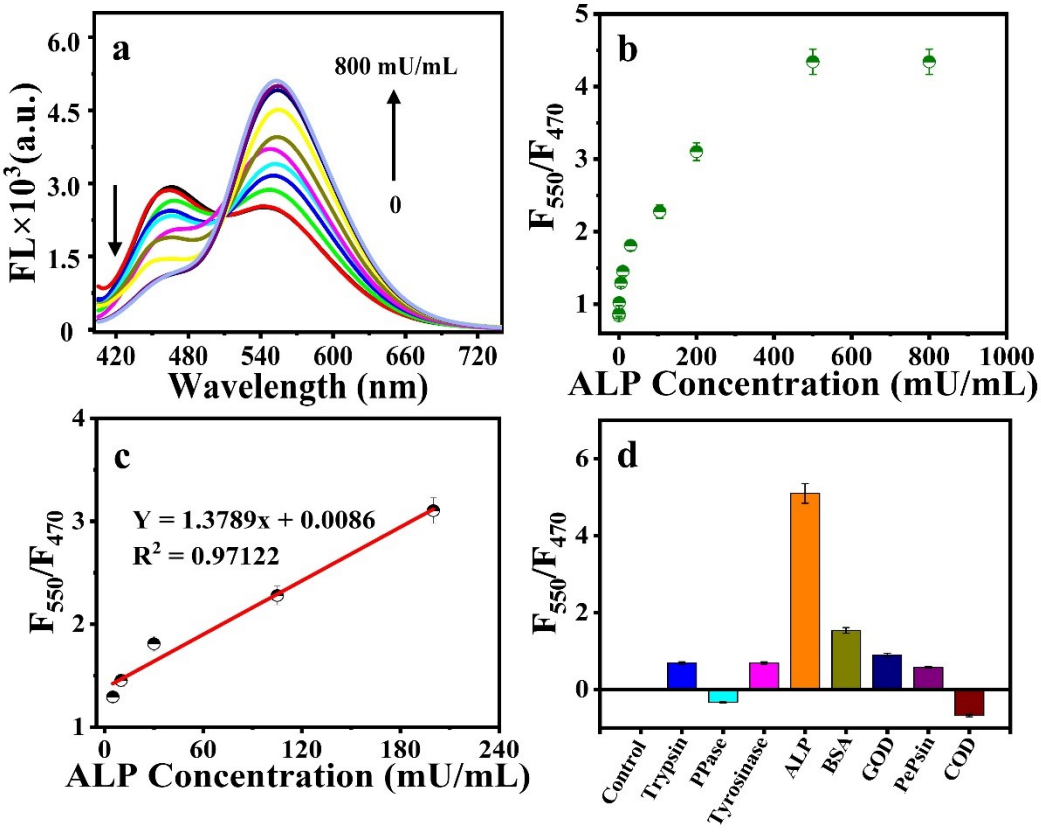

Fig. S9. (a) Luminescence spectra of the Mn^{2+}-LFC nanoparticles obtained after the addition of 0-800 mU/mL ALP; (b) A plot of the $1/(F_{470}/F_{550})$ value versus the ALP concentration (c) Linear curve (ALP concentrations 5-200 mU/mL); (d) Luminescence response of the proposed nanoimmunosensor to different kinds of proteins. The concentrations of the proteins were all 200 mU/mL.

3. Supplementary tables

Table S1 Comparison of ratiometric luminescence Mn^{2+} sensor with other Mn^{2+} sensors

Method	Linear range	Limit of detection	Ref
fluorescence	1 - 22 mM	0.713 mM	[1]
silver nanoparticles	50 - 200 μM	16 μM	[2]
colorimetric	5 - 70 μM	20 nM	[3]
thermosensitive fluorescent	0 - 55 μM	247 nM	[4]
fluorescence	0.5 - 700 μM	0.45 μM	[5]
ratiometric luminescence	0 - 200 nM	3.33 nM	this work

Table S2 Recoveries for the Detection of Mn^{2+} in the Water Samples (n = 3)

Samples	Spiked (nM)	Found (nM) mean[a]±SD[b]	Recovery (%)
Drinking pure water	0	not detected	not available
	40	41.40±2.84	103.50
	80	83.35±3.72	104.19
	120	115.44±5.04	96.20
Tap water	0	not detected	not available
	40	38.21±1.99	95.53
	80	77.27±4.03	96.59
	120	115.16±6.67	95.97
River water	0	not detected	not available
	40	41.71±1.81	104.28
	80	78.49±5.75	98.11
	120	123.65±4.83	103.04

a Mean of three independent experiments. b Standard deviation.

Table S3 Recoveries for the Detection of ALP in the human serum at different concentrations (n = 3)

Serum concerntration (%)	ALP Spiked (mU/mL)	Found (mU/mL) mean[a]±SD[b]	Recovery (%)
1		50.56±1.78	101.12
2		50.37±1.36	100.70
3	50	49.13±1.14	98.27
5		53.68±3.59	107.40
10		61.41±4.51	122.80

a Mean of three independent experiments. b Standard deviation.

Table S4 Recoveries for the Detection of ALP in the human serum (n = 3)

Samples No	Spiked (mU/mL)	Found (mU/mL) mean[a]±SD[b]	Recovery (%)
1	0	1.67±1.21	-
2	5	4.93±0.36	98.56
3	10	10.11±1.54	101.10
4	15	14.93±1.59	99.53
5	20	19.14±0.97	95.70
6	50	50.56±2.78	101.12
7	100	103.12±3.56	103.12
8	200	197.38±4.51	98.69

a Mean of three independent experiments. b Standard deviation.

Table S5 Comparison of ratiometric luminescence ALP assay with other ALP analytical techniques

Method	**Sensing system**	**Linear range (mU/mL)**	**Limit of detection (mU/mL)**	**Time (min)**	**Ref**
photothermal	AAP-MnO_2 nanosheet	0.5 - 200	0.1	40	[6]
photoelectrochemical	$CsPbBr_3$/PbS heterojunctions	0.5 - 40	42.1	30	[7]
fluorescence	DNA/AgNCs	30 - 240	5	30	[8]
fluorescence probe	TPEPy-pY	1 - 1000	6.6	60	[9]
test strips platform	HRP-DNA paper	1 - 20	0.7	23	[10]
ratiometric luminescence	CDs	1×10^{-5}-0.1	1×10^{-5}	40	[11]
ratiometric luminescence	MnO_2-based RF	0.25 -14	0.25	30	[12]
ratiometric luminescence	LFC-Mn^{2+}	0 - 800	1.67	15	this work

Table S6 Comparison of ratiometric luminescence cTnI with other methods

Method	Linear range	Limit of detection	Ref
electrochemistry	0 - 24 ng/mL	1 ng/mL	[13]
colorimetric	0.1 - 500 ng/mL	0.2 ng/mL	[14]
lateral flow immunoassays	1 - 1000 ng/mL	4 ng/mL	[15]
fluorescence immunoassay	1 - 250 ng/mL	1 ng/mL	[16]
paper-based fluorescence	1 - 50 ng/mL	1 ng/mL	[17]
ratiometric luminescence	0.1-125 ng/mL	0.033 ng/mL	this work

Table S7 Analytical results for cTnI in the human serum samples (mean; n = 3). The results of the normal adults (samples 1 and 2) and patients with AMI (samples 3, 4, 5 and 6) are obtained by the dilution for 0 and 20 times.

Samples No	This assay		TMB-based standard ELISA method	
	Detected (ng/mL)	RSD (%, n=3)	Detected (ng/mL)	RSD (%, n=3)
1	0.27	2.91	0.32	1.94
2	0.39	4.79	0.41	2.97
3	6.52	2.18	8.91	3.17
4	21.0	1.25	22.4	2.76
5	59.2	7.34	61.6	6.29
6	94.3	3.63	97.0	3.91

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