Ratiometric Fluorescent Probe based on Peptides Modified $MnFe_2O_4$ Nanoparticles for Matrix Metalloproteinase-7 Activity Detection *in Vitro* and *in Vivo*

Hongda Chen,[a,b] Hua Zhang*[a] and Zhenxin Wang[a,b]

[a]State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, P. R. China.

[b]School of Applied Chemical Engineering, University of Science and Technology of China, Road Baohe District, Hefei, Anhui, 230026, P. R. China

Tel.: +86-431-85262757

Fax: +86-431-85262243

E-mail address: zhanghua@ciac.ac.cn (Hua Zhang)

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1. Additional Experimental Section

Characterization

Transmission electron microscope (TEM) micrographs was obtained on a FEI Tecnai G2 S-Twin TEM (FEI Co., USA) with a field emission gun operating at 200 kV. Fourier transform infrared (FTIR) spectra were captured with a Bruker Vertex 70 spectrometer. All dynamic light scattering (DLS) and Zeta potential distribution measurements were carried out on the Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., UK). The element analysis experiments were performed by an ELAN 9000/DRC ICP-MS system (Perkin Elmer, USA). The fluorescence emission spectra were recorded on a QE65 Pro fiber optic spectrometer (Ocean Optics Ltd., USA). The fluorescence micrographs were performed with a reconstructive Ti-S fluorescent microscope (Nikon Co., Japan).

Cell viability evaluation

The MCF-7, MDA-MB-231 and NCM460 cells (1×10^4 cells per well) were cultured with 100 μL fresh culture medium containing 10% FBS and 100 U mL^{-1} penicillin-streptomycin in 96-well microtiter plate under a humidified 5% CO_2 at 37 °C for 24 h, respectively. After washed with 100 μL PBS (3 times), the cells were treated with various concentrations (0, 6.25, 12.5, 25, 50 and 100 μg mL^{-1}) of $MnFe_2O_4$-pep-dyes and incubated for another 24 h, respectively. Cell viability was determined by conventional MTT assay. The relative viabilities of $MnFe_2O_4$-pep-dyes stained cells and untreated cells (control sample) were calculated via measuring the absorbance at 490 nm by a microplate reader.

2. Additional Figures S1-S19

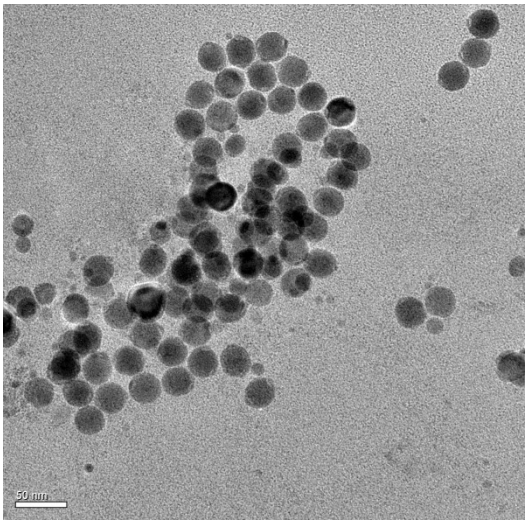

Figure S1 TEM image of $MnFe_2O_4$ NPs.

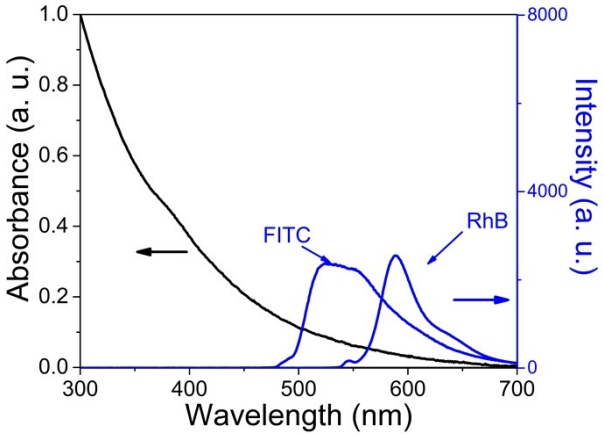

Figure S2 UV-visible spectrum of $MnFe_2O_4$ NPs (black line) and fluorescence spectra of FITC-peptide and RhB-peptide (blue line).

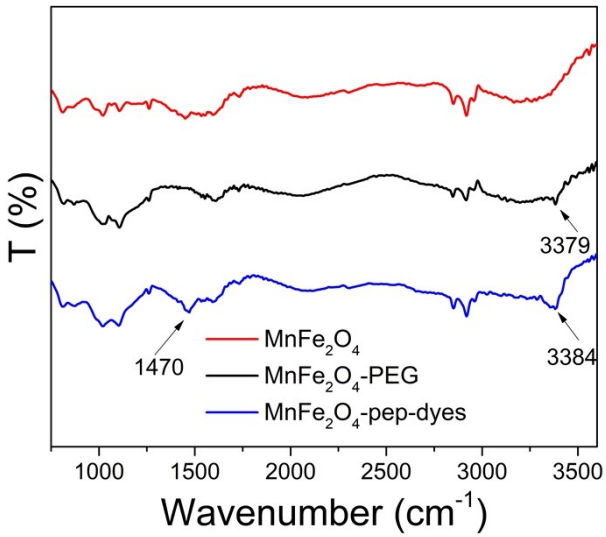

Figure S3 FT-IR spectra of $MnFe_2O_4$ NPs, $MnFe_2O_4$-PEG, $MnFe_2O_4$-pep-dyes.

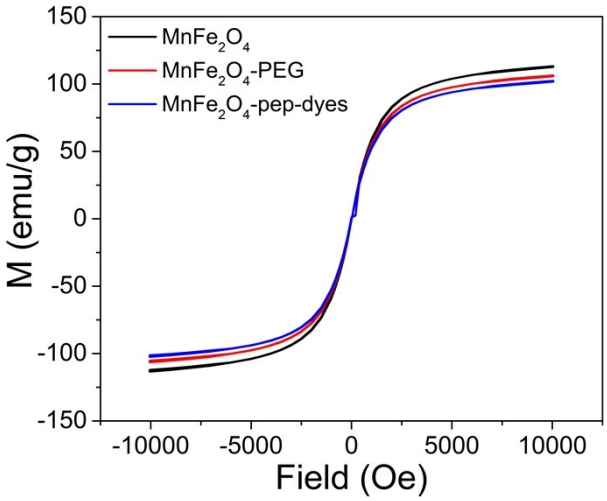

Figure S4 Magnetic Hysteresis loops of $MnFe_2O_4$-oleate, $MnFe_2O_4$-PEG, $MnFe_2O_4$-pep-dyes.

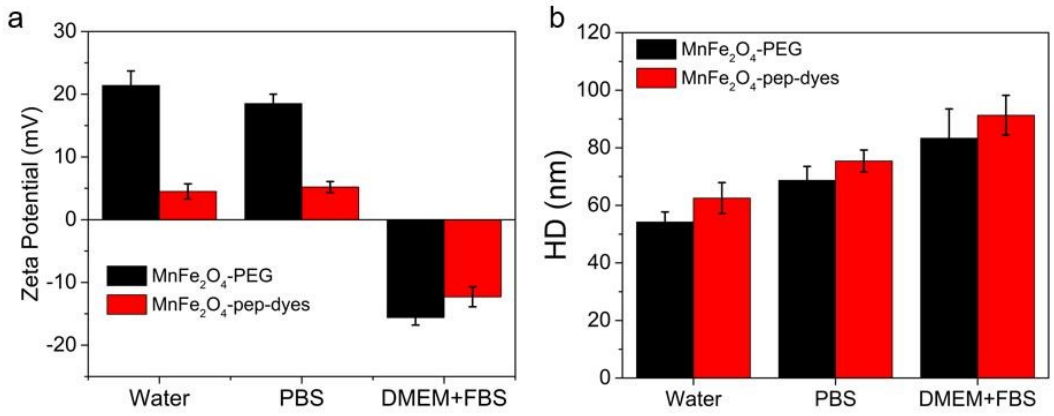

Figure S5 The zeta potentials and hydrodynamic diameters of as-prepared $MnFe_2O_4$-PEG and $MnFe_2O_4$-pep-dyes.

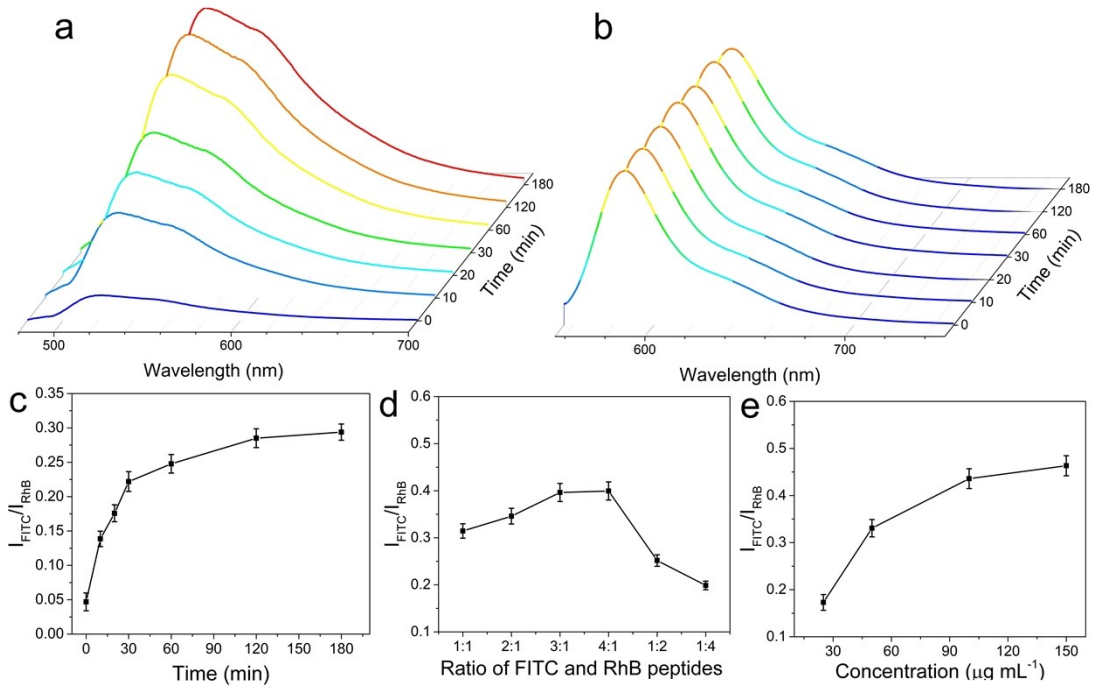

Figure S6 The fluorescence spectra of $MnFe_2O_4$-pep-dyes upon excitation at 488 nm (a) and 530 nm (b) after incubation with activated MMP-7 for different periods of time. (c) I_{FITC}/I_{RhB} of $MnFe_2O_4$-pep-dyes incubated with 5 nM MMP-7 for different periods of time. (d) I_{FITC}/I_{RhB} against different mass ratios of FITC and RhB peptides incubated with 8 nM MMP-7. (e) I_{FITC}/I_{RhB} against different $MnFe_2O_4$-pep-dyes concentrations incubated with 8 nM MMP-7.

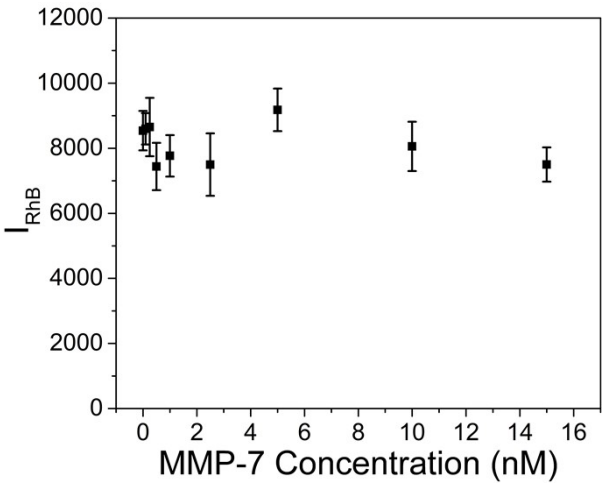

Figure S7 RhB intensity against different concentrations of activated MMP-7.

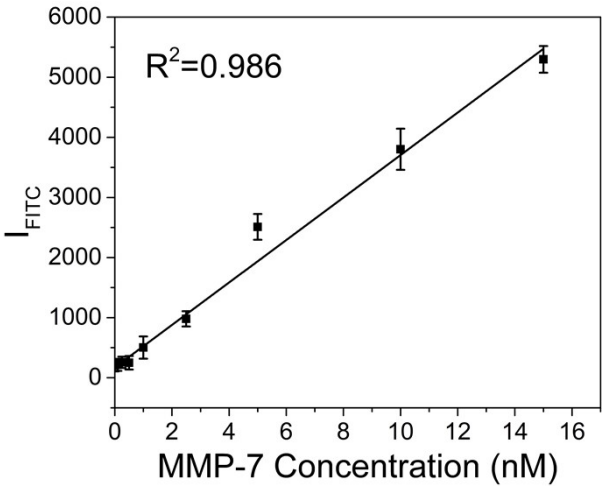

Figure S8 Linear relationship of FITC intensity against different concentrations of activated MMP-7.

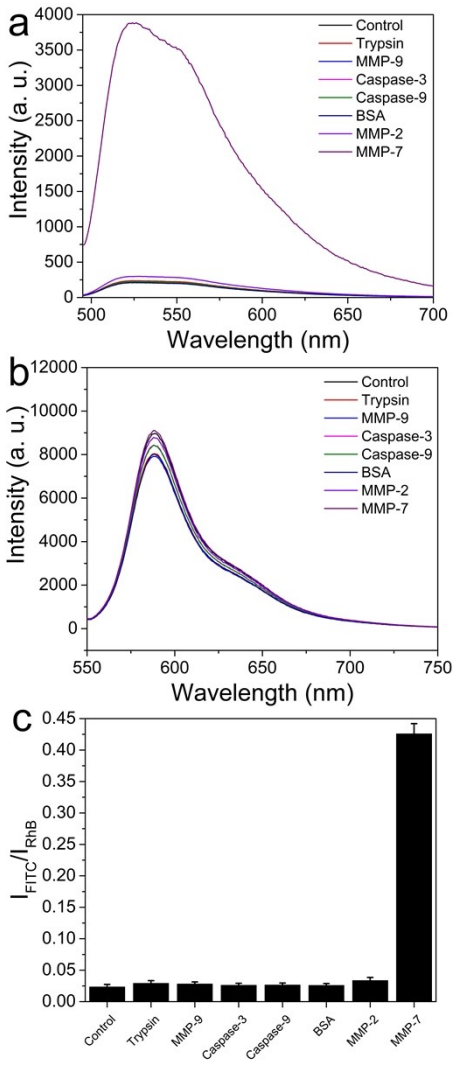

Figure S9 The selectivity of the nanoprobe. The fluorescence spectra of FITC (a) and RhB (b), and the relative I_{FITC}/I_{RhB} (c) after $MnFe_2O_4$-pep-dyes were incubated with different proteases and protein. The error bars mean standard deviations (n=3).

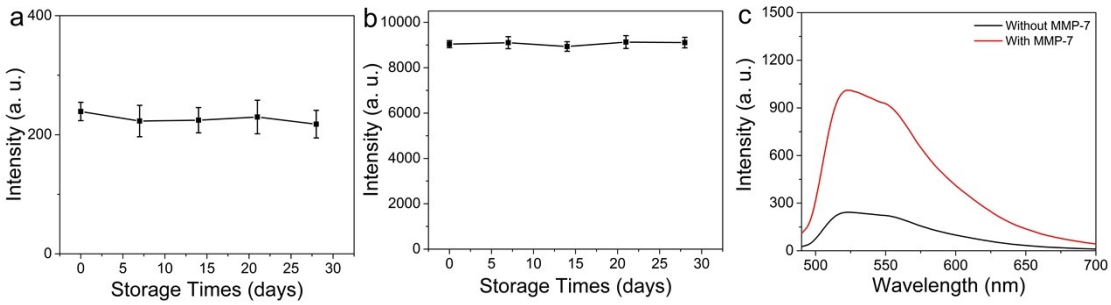

Figure S10 (a) RhB and (b) FITC fluorescence intensity of $MnFe_2O_4$-pep-dyes dispersed in PBS as a function of storage days. (c) Fluorescence spectra of $MnFe_2O_4$-pep-dyes incubated with or without 3 nM MMP-7 after 28 days storage.

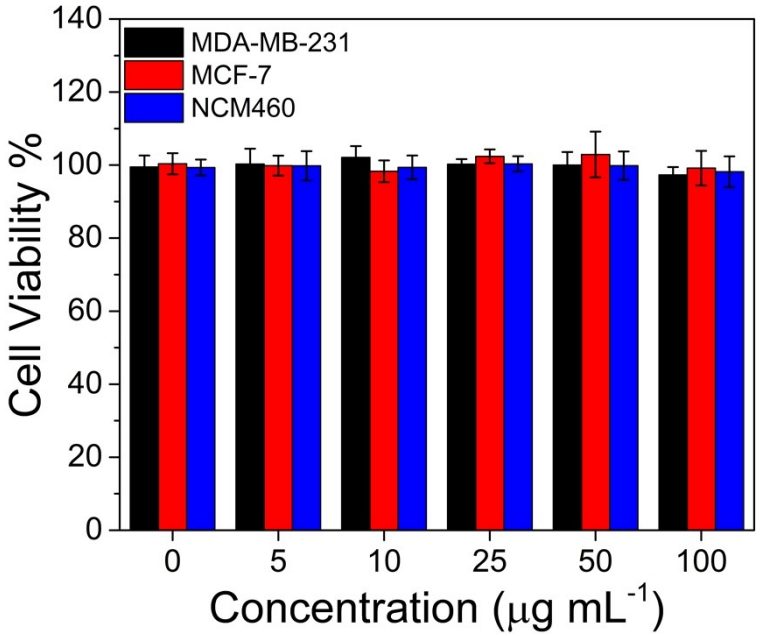

Figure S11 Cell viabilities of MDA-MB-231, MCF-7 and NCM460 cells as a function of $MnFe_2O_4$-pep-dyes concentration.

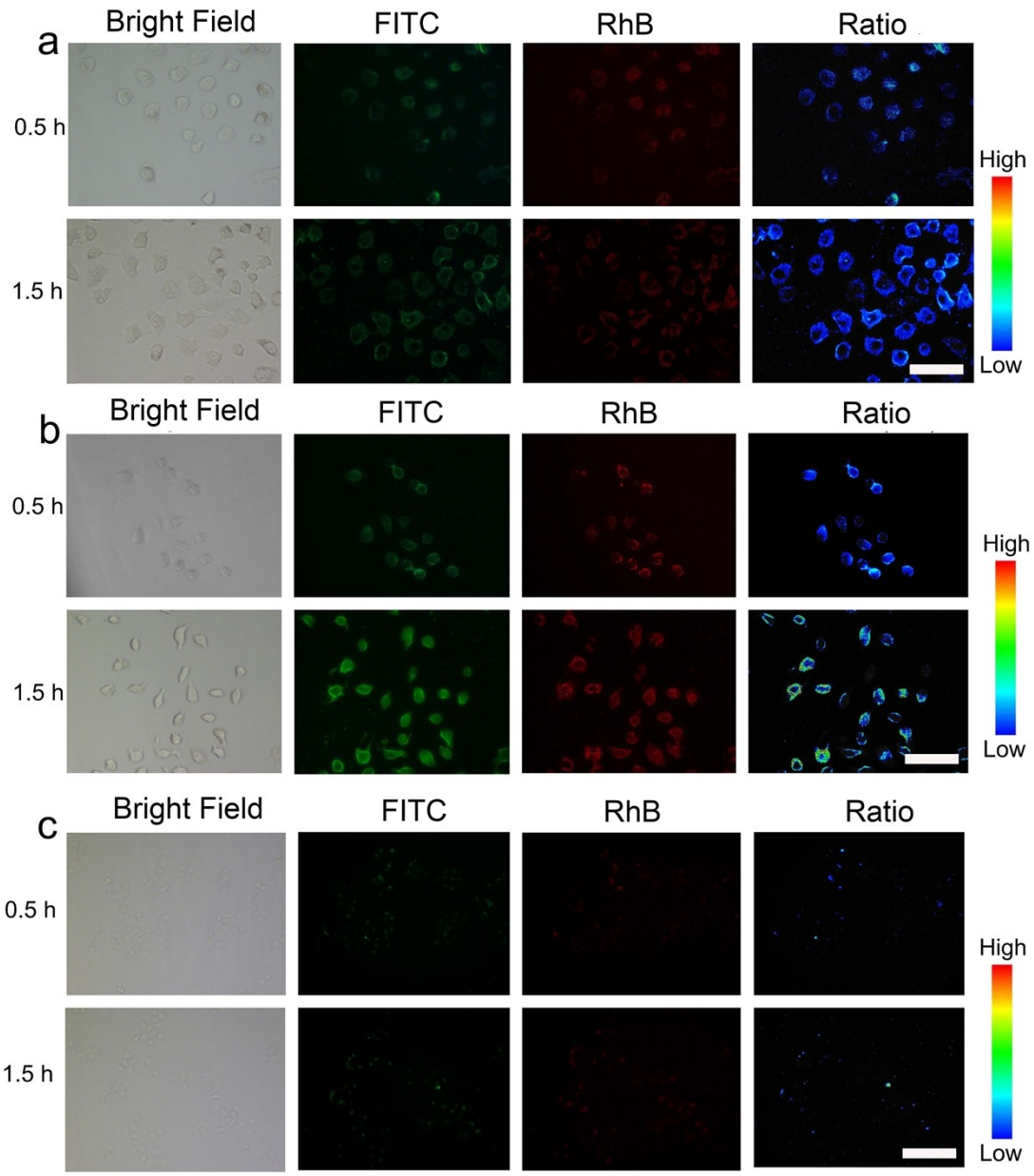

Figure S12 Fluorescence microscopy images of (a) MCF-7, (b) MDA-MB-231 and (c) NCM460 cells after incubation with $MnFe_2O_4$-pep-dyes for 0.5 and 1.5 h. Ratio fluorescence images are obtained by Image-Pro Plus software processing. The scale bars are 50 μm.

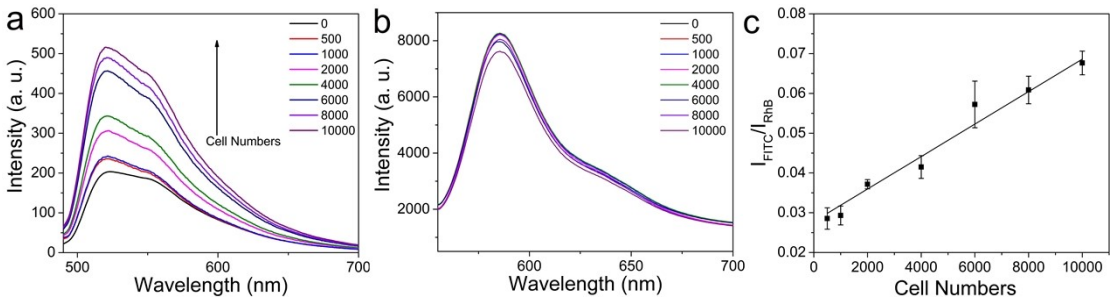

Figure S13 (a) FITC and (b) RhB fluorescence spectra of 100 μg mL^{-1} $MnFe_2O_4$-pep-dyes incubated with the lysates of various amounts of MDA-MB-231 cells. (c) Corresponding I_{FITC}/I_{RhB} versus cell numbers. The error bars mean standard deviations (n = 3).

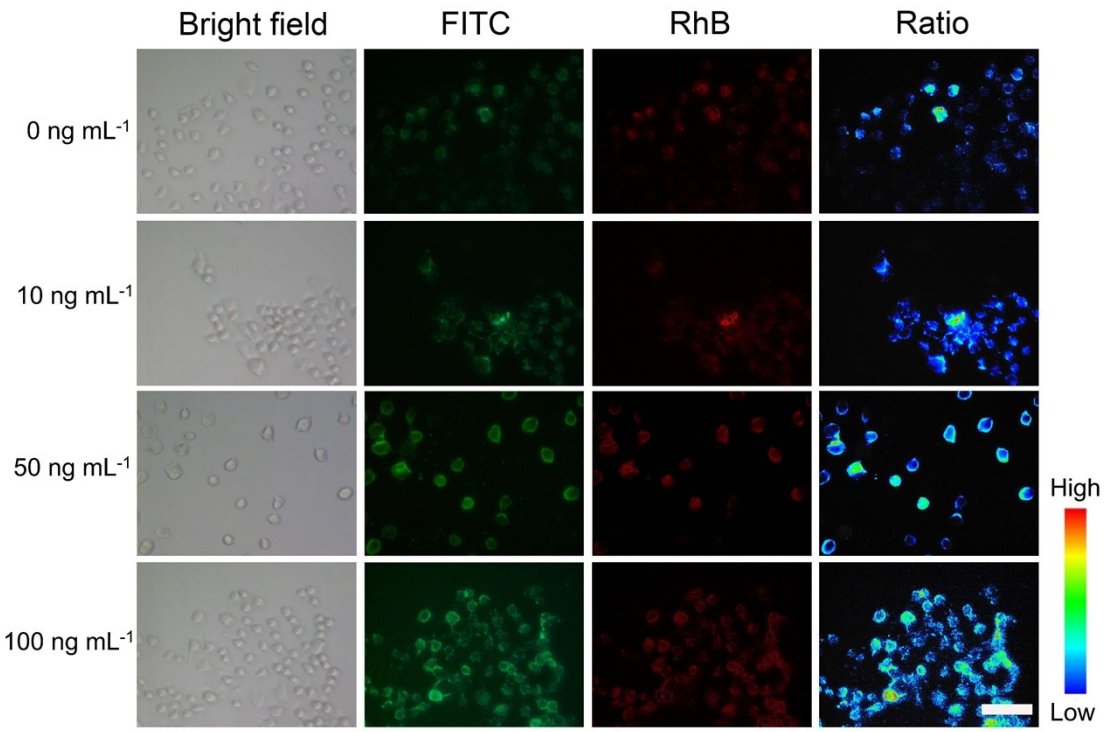

Figure S14 Fluorescence microscopy images of MDA-MB-231 cells co-cultured with $MnFe_2O_4$-pep-dyes for 1 h after treated with PMA for 2 h. Ratio fluorescence images are obtained by Image-Pro Plus software processing. The scale bar is 50 μm.

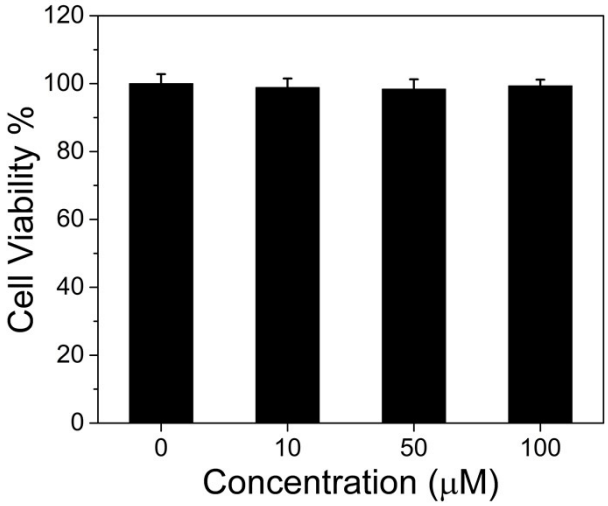

Figure S15 Cell viabilities of MDA-MB-231 cells as a function of oleic acid concentration.

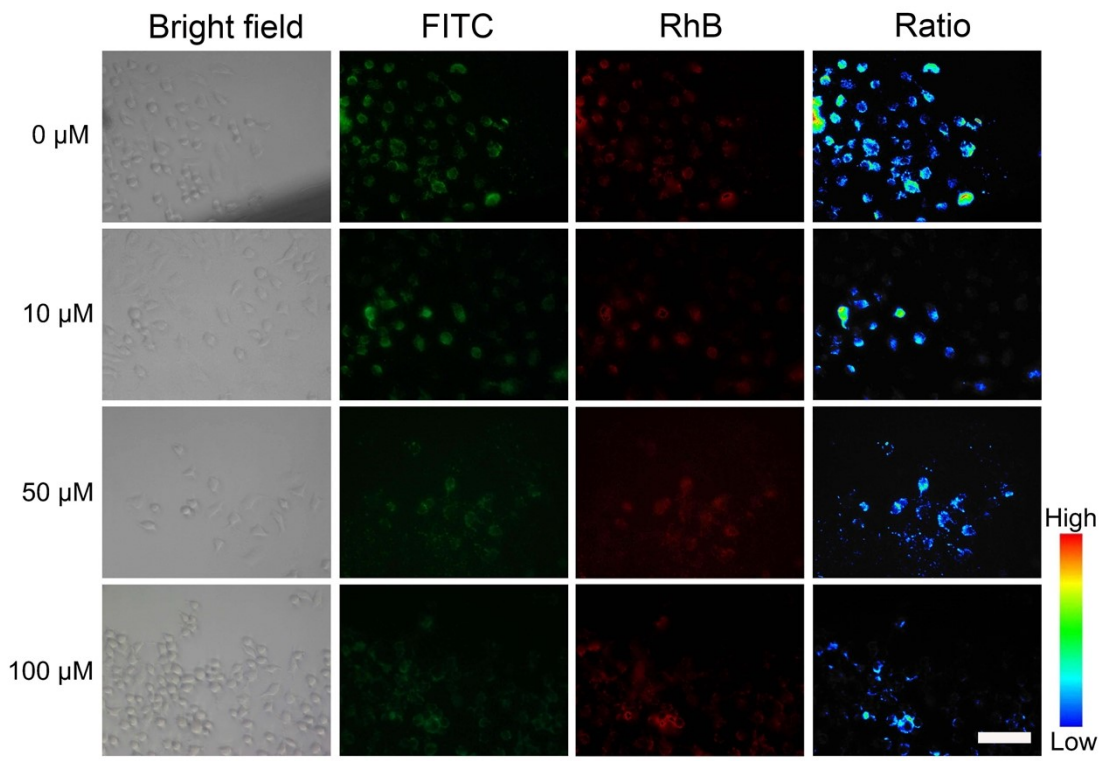

Figure S16 Fluorescence microscopy images of MDA-MB-231 cells co-cultured with $MnFe_2O_4$-pep-dyes in presence of different concentrations of oleic acid. Ratio fluorescence image are obtained by Image-Pro Plus software processing. The scale bar is 50 μm.

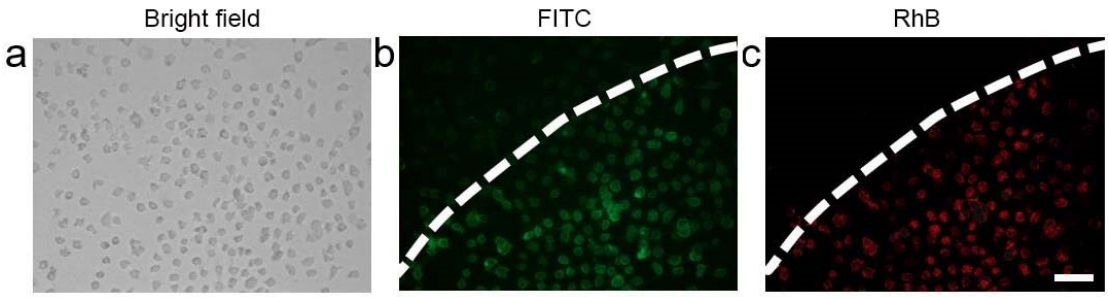

Figure S17 Fluorescence micrographs of MDA-MB-231 cells co-cultured with $MnFe_2O_4$-pep-dyes in the MF for 4 h, (a) bright field mode, (b) FITC mode, and (c) RhB mode. The scale bar is 50 μm.

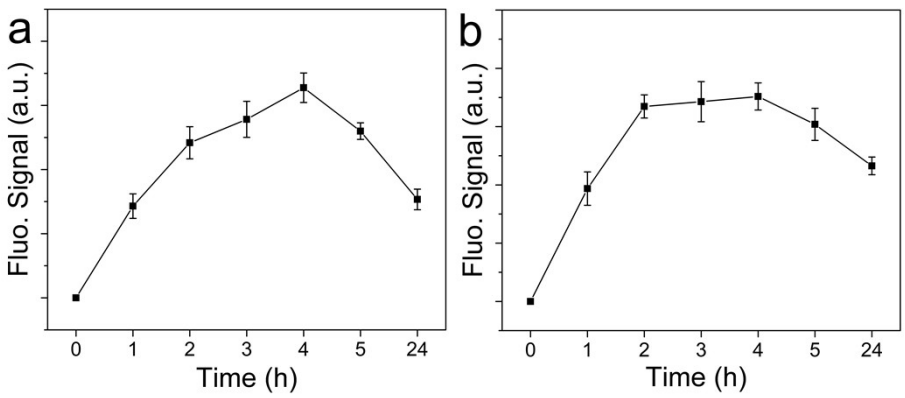

Figure S18 Temporal evolutions of the integrated fluorescence intensity of (a) FITC and (b) RhB at the tumorous region.

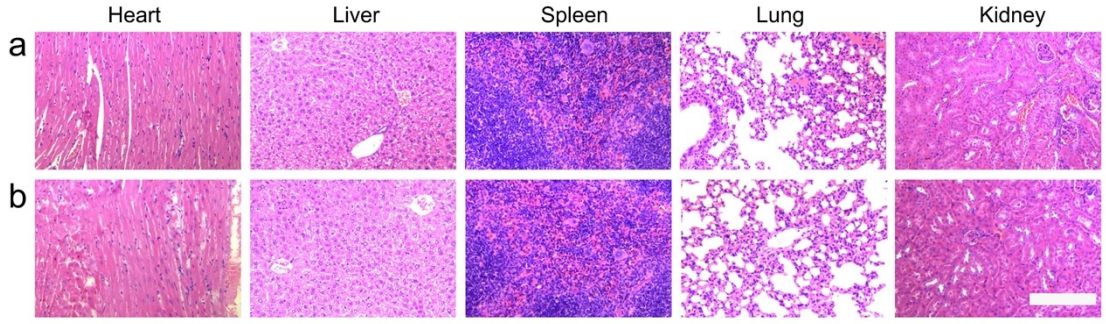

Figure S19 Histological changes of healthy mice at 30-day post-injection with saline (a) and $MnFe_2O_4$-pep-dyes (b), respectively. Scale bar is 100 μm, and all of images were collected under same scale.

3. Additional Tables S1-S2

Table S1 Detection of MMP-7 in serum samples

Sample	Added (nM)	Found (nM)	Recovery (%)	RSD (n=3, %)
1	1	0.943	94.3	3.9
2	5	5.139	102.8	2.5
3	10	9.847	98.5	3.2

Table S2 Results of blood biochemical assays.

Hematological	Units	Control	Treatment
WBC	$\times 10^9$/L	7.34 ± 1.55	6.22 ± 1.24
RBC	$\times 10^{12}$/L	5.38 ±1.41	6.12 ± 1.13
HGB	g/L	170.32 ± 18.54	165.47 ± 32.87
MCV	fL	32.48 ± 5.17	35.16 ± 5.81
MCH	pg	18.65 ± 2.54	17.32 ± 1.51
MCHC	g/L	312.47 ± 32.14	331.84 ± 40.54
PLT	$\times 10^9$/L	750.25 ± 50.36	776.67 ± 101.30
PDW	fL	8.53 ± 0.65	10.11 ± 1.34