Electronic Supplementary Information (ESI) for Chemical Communications

Core-shell nanoparticle-peptide@metal−organic framework as dual-recognition switch for pH and enzyme stepwise-responsive imaging in living cells

Hong Shen, Jintong Liu, Jianping Lei* and Huangxian Ju

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China

Experimental

Materials and reagents. Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), 2-methyl imidazole (2-MIm), gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), and trisodium citrate dehydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), cathepsin B, cathepsin D (CaD), cathepsin L (CaL) and CA-074-Me (CaB inhibitor) were purchased from Sigma-Aldrich (USA). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo (Japan). Lysotracker Green DND-26 was purchased from Cell Signaling Technology, Inc. mPEG-thiol (mPEG-SH, M.W. 5000) was obtained from Nanocs (USA). Cy3-GRRGKC (Cy3-peptide, Mn=1115.41) was synthesized as CaB-specific substrate by ChinaPeptides Co., Ltd. (Suzhou, China). Tris-HCl solution (1.0 M, pH 7.4, Sterile) and 20X phosphate buffer saline (PBS) buffer (DEPC Treated) were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, PRC). All aqueous solutions were prepared using ultrapure water (≥ 18 MΩ cm, Milli-Q, Millipore).

Characterizations. The transmission electron microscopic (TEM) images were acquired on a JEM-2800 transmission electron microscope (JEOL Ltd., Japan). The scanning electron

microscopic (SEM) images were obtained by Hitachi S-4800 scanning electron microscope (Japan). Powder X-ray diffraction data was measured using a X'TRA diffractometer (ARL, Switzerland). Dynamic light scattering (DLS) was recorded on a 90 Plus/BI-MAS equipment (Brook Haven, USA). Absorption spectra were observed on an UV-3600 UV–vis-NIR spectrophotometer (Shimadzu, Japan). Infrared (IR) spectra were obtained on a Nicolet NEXUS870 Fourier transform infrared spectrometer (Madison, WI). Fluorescence spectra were recorded on an F-7000 spectrometer (Hitachi, Japan). Zeta potential analysis was carried out on a Zetasizer instrument (Nano-Z, Malvern, UK). Confocal fluorescence imaging of cells was performed on a TCS SP5 confocal laser scanning microscope (Leica, Germany).

Preparation of Au-Cy3P. Firstly, AuNPs (13 nm in diameter) were prepared using the sodium citrate reduction method according to previously reported.[S1] Then, the resulting AuNPs were concentrated by centrifugation at 10000 rpm for 10 min and resuspended in Tris-HCl 7.4 buffer. 10 μL of mPEG-SH (1.0 mg mL^{-1}) and 10 μL of Cy3-peptide (1.0 mg mL^{-1}) were sequentially added to 1.0 mL concentrated AuNPs solution. After shaken gently at room temperature for 8 h, the mixture was concentrated by centrifugation and washed with water twice. The resulting Cy3-peptide functionalized AuNPs (named as Au-Cy3P) were resuspended in water for further use.

Synthesis of Au-Cy3P@ZIF-8. Au-Cy3P@ZIF-8 was prepared by the encapsulation of Au-Cy3P into ZIF-8 through a previous procedure with some modifications.[S2] Firstly, 30 mg of $Zn(NO_3)_2 \cdot 6H_2O$ and 66 mg 2-MIm were individually dissolved in 4.0 mL methanol. Subsequently, 0.2 mL of Au-Cy3P in water was added dropwise into the zinc nitrate solution under stirring vigorously. After agitation for 15 min, 2-MIm solution was injected into the jar and stirred continuously for another 15 min. The color of reaction solution changed from transparent faint red to milky gradually. Then the as-synthesized nanoparticles were centrifuged

at 7500 rpm for 10 min and washed with 8.0 mL of methanol three times to obtain the precipitation of Au-Cy3P@ZIF-8 nanoprobe.

Detection of CaB activity. For fluorescence analysis, 10 μL of Au-Cy3P@ZIF-8 (1.0 mg mL^{-1}) was firstly immersed in 200 μL 10 mM PBS buffer for 6 h at 37 °C, and then was incubated with human recombinant CaB with different concentrations from 0, 2.5, 5, 10, 15 to 25 U mL^{-1} at 37 °C for 1 h. The fluorescence spectra were recorded with excitation at 514 nm and emission wavelength range from 530 to 700 nm.

Selectivity towards CaB. To investigate the selectivity toward CaB, Au-Cy3P@ZIF-8 nanoprobe (50 μg mL^{-1}) was incubated in CaB assay buffer with 15 U mL^{-1} human recombinant CaB, CaD, CaL, or CaB pretreated with its inhibitor (CA-074-Me, 10 ng mL^{-1}). The reaction solutions were kept for 1 h at 37 °C, and the fluorescence intensities at 560 nm were immediately monitored under the excitation at 514 nm.

Cell culture. HeLa cells were cultured in high-glucose DMEM (Gibco) medium in a flask containing 10% fetal bovine serum and 1% penicillin/streptomycin at 5% CO_2 humidified atmosphere at 37 °C. Cell numbers were determined with a Petroff-Hausser cell counter (USA).

Cell cytotoxicity assay. A total of 200 μL DMEM medium containing HeLa cells was pipetted into the wells of a 96-well plate to provide a cell density of ~5×10^5 cells per well. The plate was then incubated for 24 h at 37°C in 5% CO_2 atmosphere. After the removal of medium, HeLa cells were incubated with 200 μL of serum-free DMEM medium containing series of concentrations (5, 10, 25, 50, 75 μg mL^{-1}) of Au-Cy3P@ZIF-8 for different time (2, 4, 8, 12, 24 h). Then the plate was incubated with100 μL of PBS buffer containing 10 μL of CCK-8 reagent

at 37 °C for 3 h. The cell viability was calculated as a ratio of absorbance at 450 nm of treated and untreated cells.

Colocalization assay. For colocalization studies, HeLa cells were incubated with 50 μg mL^{-1} nanoprobe for 8 h. The cells were washed with PBS (1×) three times, and further incubated with 1.0 μM Lysotracker Green DND-26 at 37 °C for 10 min. After that, the medium was removed, and the cells were washed with cold PBS for three times. Fluorescence imaging was performed under a Leica TCS SP5 confocal laser scanning microscope. Emission from Cy3 was collected at red channel from 540−700 nm wavelength with the excitation at 514 nm, and emission from Lysotracker Green DND-26 was collected at green channel from 500 to 530 nm with excitation at 488 nm.

Confocal fluorescence imaging. HeLa cells (~5×10^4) were planted onto glass-bottom dish (In Vitro Scientific, D35-20-1-N) for 24 h. The cells were then incubated with Au-Cy3P@ZIF-8 nanoprobe (50 μg mL^{-1}) in serum-free DMEM at 37 °C for 8 h. After that, the medium was removed, and the cells were rinsed with PBS (1×) buffer 3 times. The fluorescence images of the cells were then captured on the microscope from 530 to 700 nm with the excitation wavelength of 514 nm. All images were digitized and analyzed with Leica Application Suite Advanced Fluorescence (LAS-AF) software package.

Supporting Figures

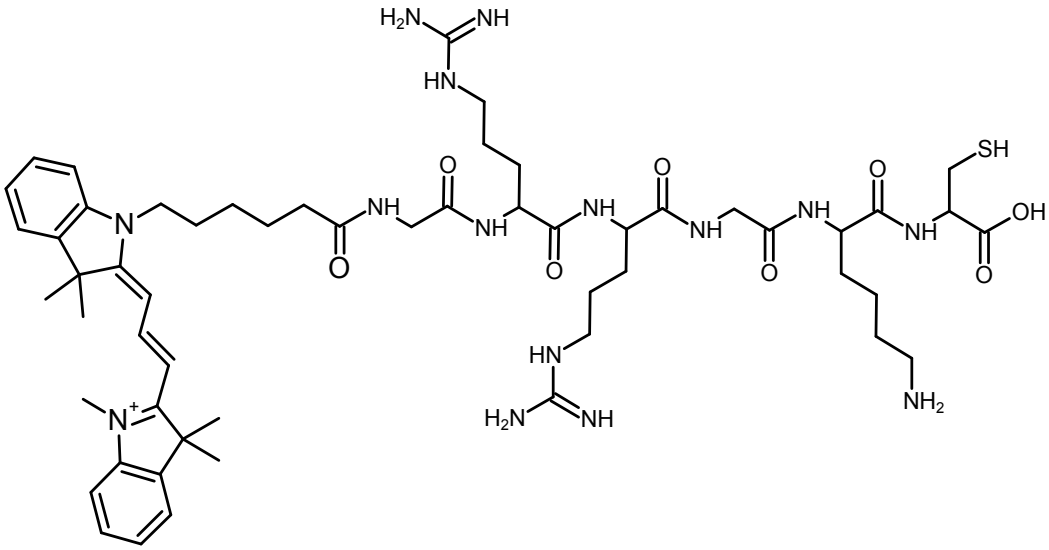

Fig. S1. Chemical structure of Cy3-GRRGKC.

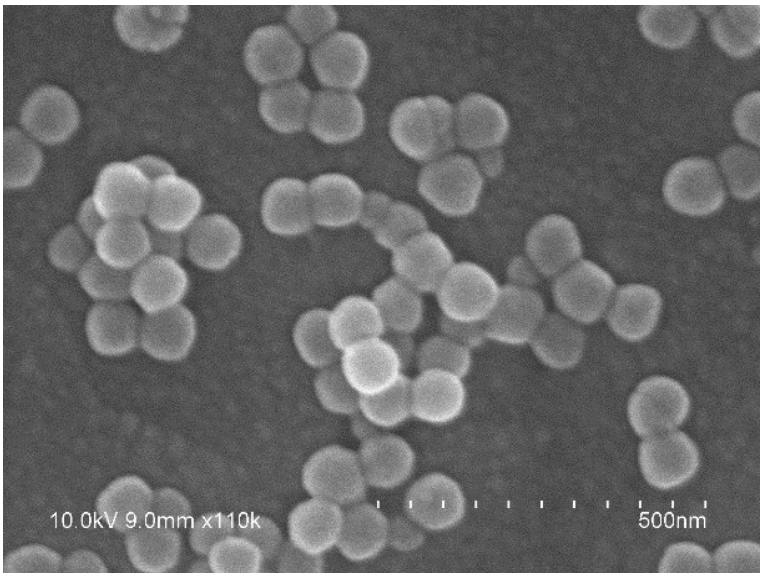

Fig. S2. SEM image of pure ZIF-8.

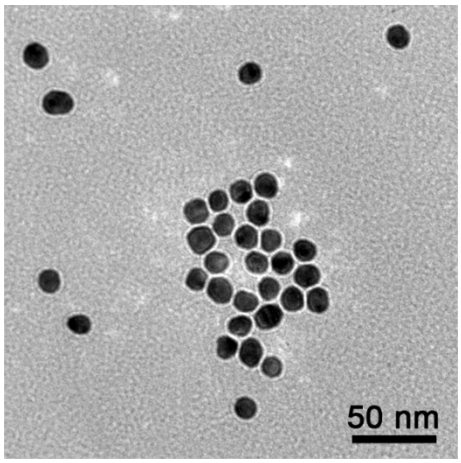

Fig. S3. TEM image of Au-Cy3P.

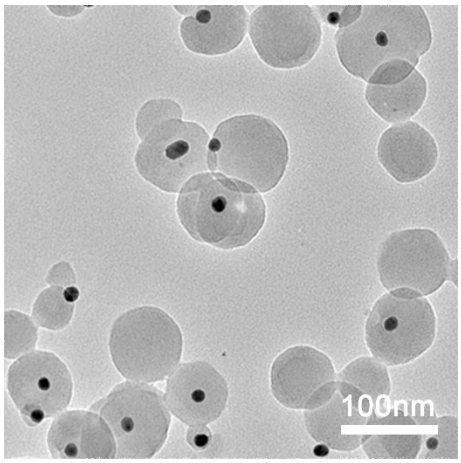

Fig. S4. TEM image of Au-Cy3P@ZIF-8.

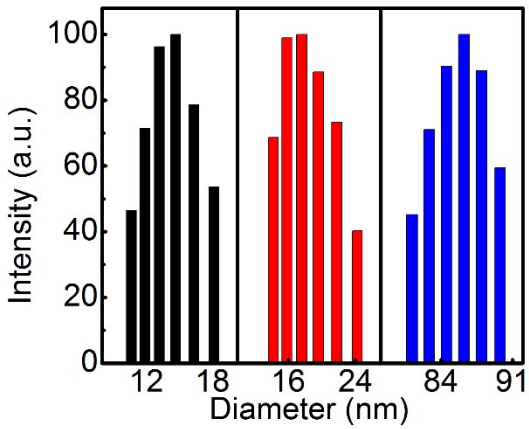

Fig. S5. Dynamic light scattering assay of AuNP (black), Au-Cy3P (red), and Au-Cy3P@ZIF-8 (blue).

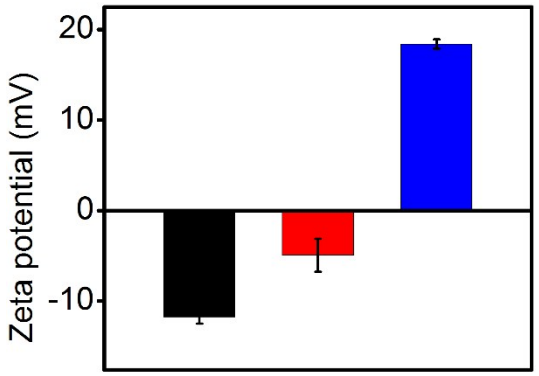

Fig. S6. Zeta potentials of AuNP (black), Au-Cy3P (red), and Au-Cy3P@ZIF-8 (blue).

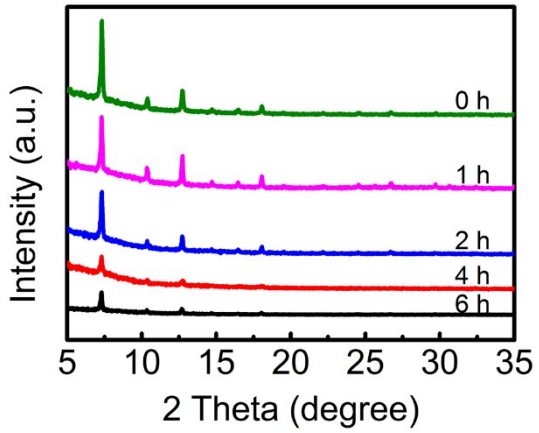

Fig. S7. Effect of powder X-ray diffraction patterns of Au-Cy3P@ZIF-8 on the incubation time in pH 4.5 buffer.

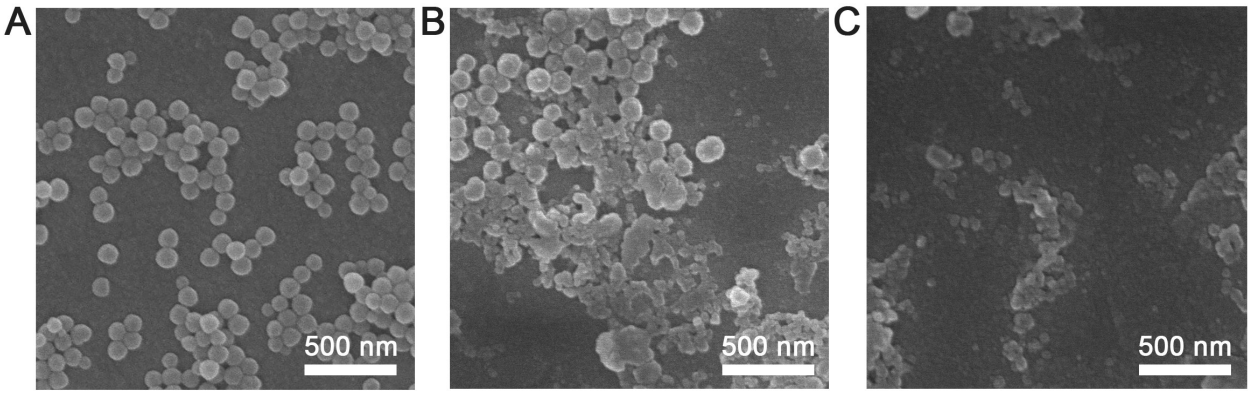

Fig. S8. SEM images of Au-Cy3P@ZIF-8 incubated in pH 4.5 buffer for (A) 0, (B) 3 and (C) 6 h.

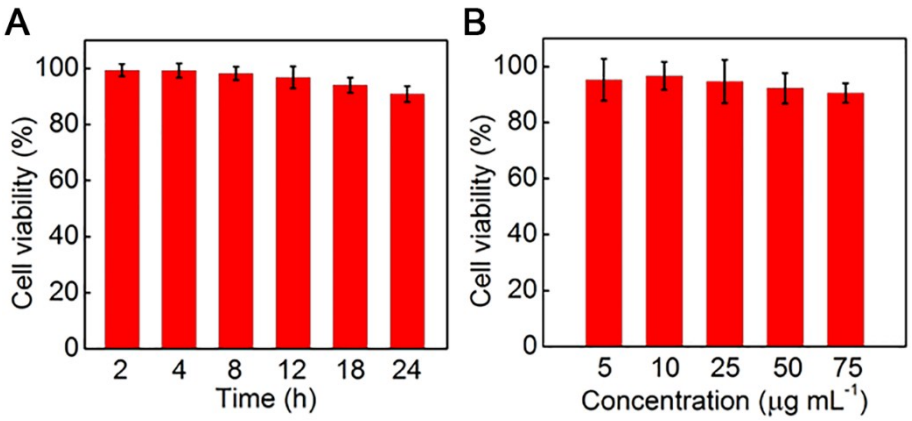

Fig. S9. Cell Counting Kit-8 cell viability assay of HeLa cells in response to (A) 50 μg mL^{-1} Au-Cy3P@ZIF-8 nanoprobe for different time, and (B) different concentrations of Au-Cy3P@ZIF-8 nanoprobe for 8 h. Error bars are standard deviation (n = 3).

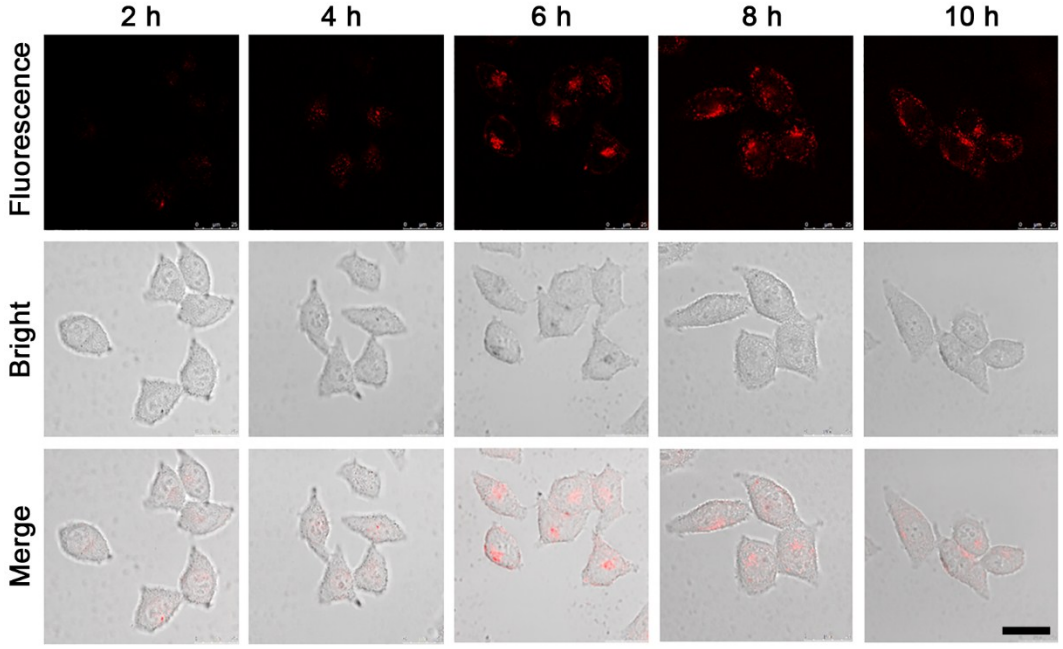

Fig. S10. Confocal fluorescence images of HeLa cells incubated with 25 μg mL^{-1} Au-Cy3P@ZIF-8 nanoprobe for different time. Scale bar: 25 μm.

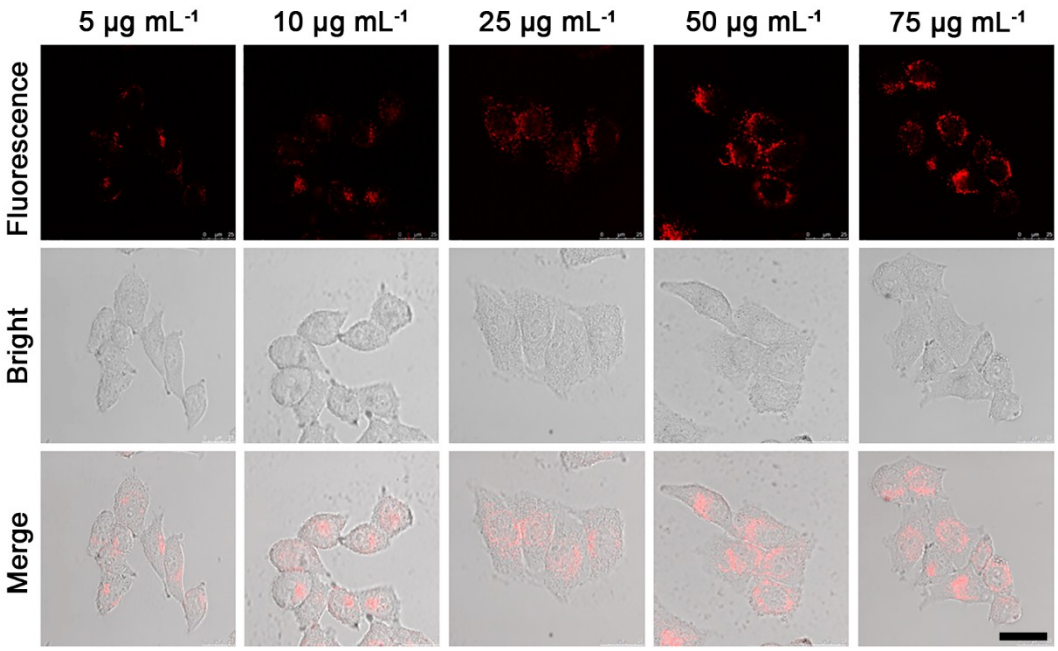

Fig. S11. Confocal fluorescence images of HeLa cells incubated with different concentrations of Au-Cy3P@ZIF-8 nanoprobe for 8 h. Scale bar: 25 μm.

Supporting references

S1 K. C. Grabar, R. G. Freeman, M. B. Hommer and M. J. Natan, *Anal. Chem.*, 1995, **67**, 735.

S2 G. Lu, S. Z. Li, Z. Guo, O. K. Farha, B. G. Hauser, X. Y. Qi, Y. Wang, X. Wang, S. Y. Han, X. G. Liu, J. S. DuChene, H. Zhang, Q. C. Zhang, X. D. Chen, J. Ma, S. C. Joachim Loo, W. D. Wei, Y. H. Yang, J. T. Hupp and F. W. Huo, *Nat. Chem.*, 2012, **4**, 310.