Supporting Information

Bioimaging and Biosensing of Ferrous Ion in Neurons and HepG2 Cells Upon Oxidative Stress

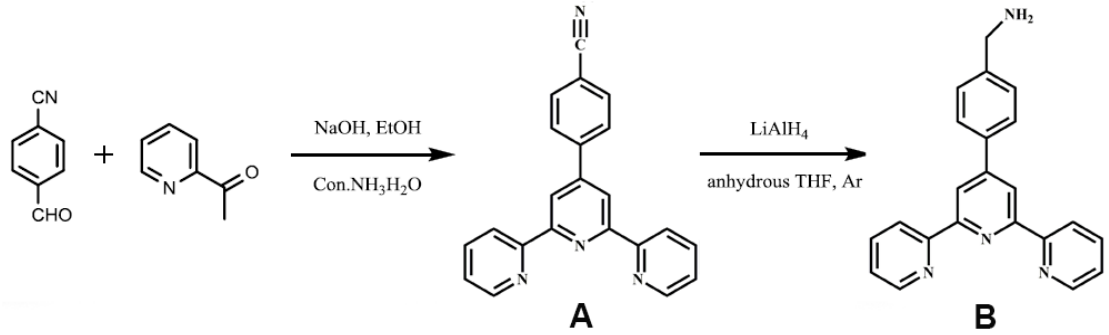
Zhichao Liu, [+] Shengnan Wang, [+] Wanying Li, and Yang Tian*

Shanghai Key Laboratory of Green Chemistry and Chemical Processes, Department of Chemistry, School of Chemistry and Molecular Engineering, East China Normal University, Dongchuan Road 500, Shanghai 200241, China

*E-mail: ytian@chem.ecnu.edu.cn

Contents

1. Synthetic route of FeL (Scheme S1).



Scheme S1. Synthetic route for Fe^{2+} ligand (FeL).

2. Characterization of FeL (Figure S1-S4).

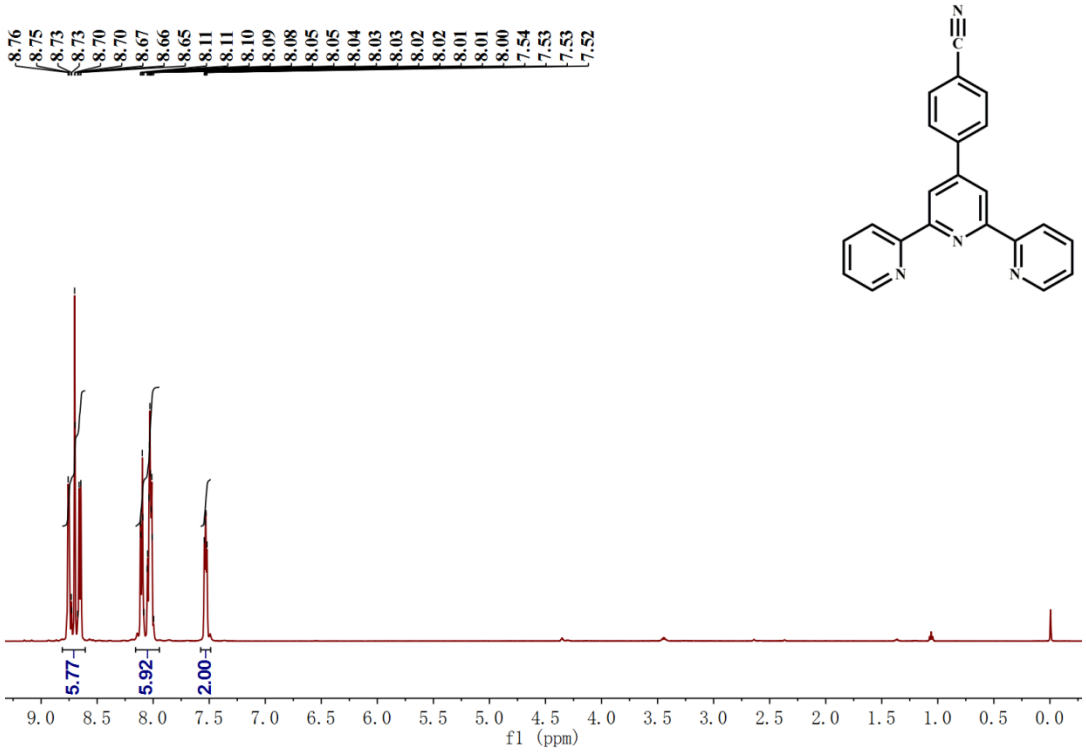


Figure S1. ^{1}H NMR spectrum (500 MHz) of compound A in DMSO.

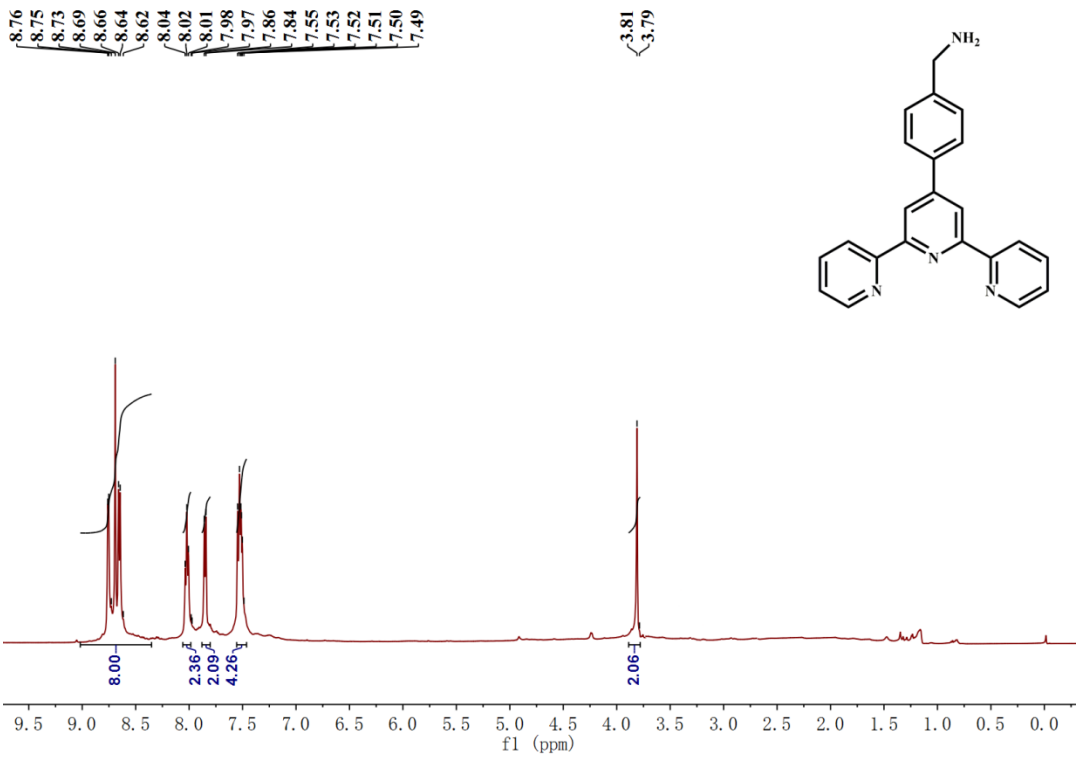


Figure S2. ^{1}H NMR spectrum (500 MHz) of FeL in DMSO.

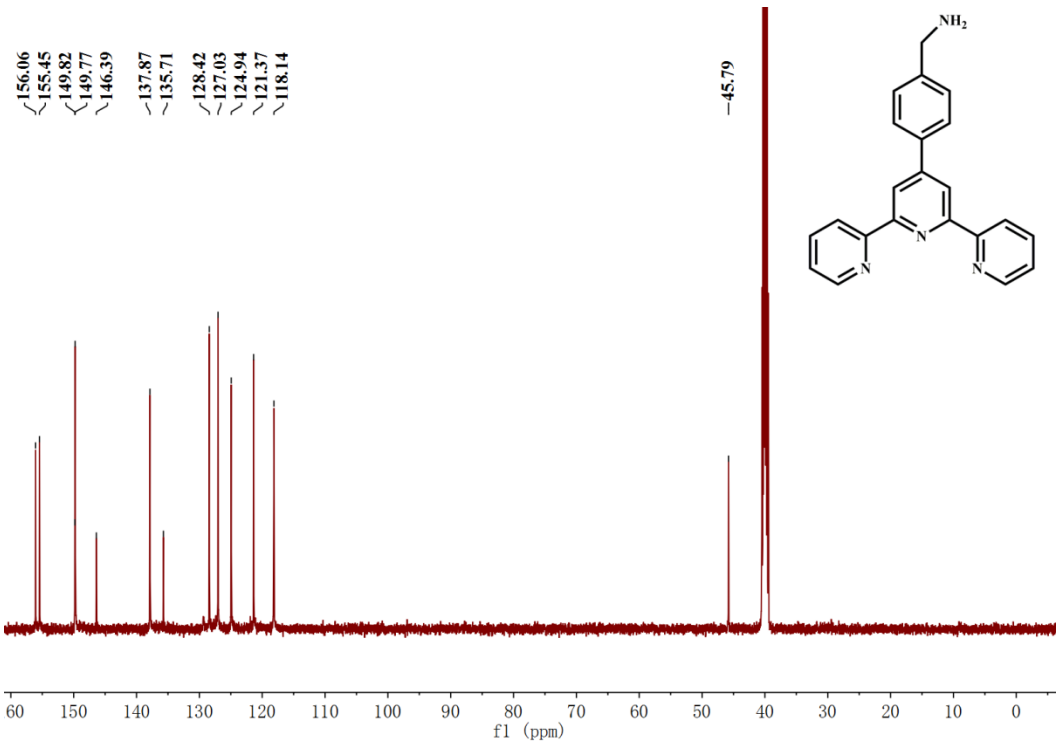


Figure S3. ^{13}C NMR spectrum (500 MHz) of FeL in DMSO.

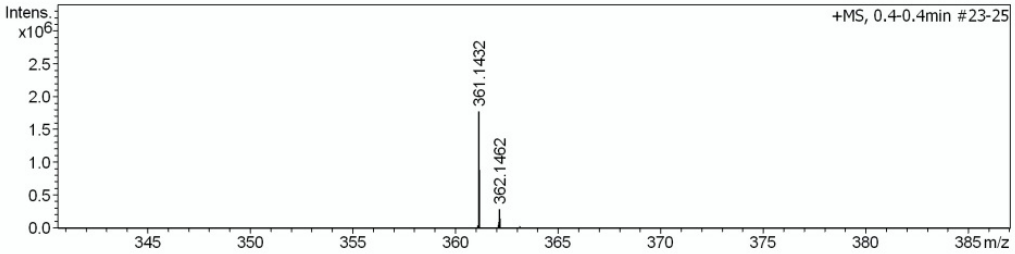
Mass Spectrum List Report

Analysis Info

Analysis Name	D:\Data\chem. dep\tianyang\LZC-0725-1_P1-D-1_01_5781.d	Acquisition Date	7/25/2017 12:13:14 PM
Method	Tune_pos_low_LC with calibration_2min.m	Operator	ECNU-Chem
Sample Name	LZC-0725-1	Instrument	maXis impact 282001.00122
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.5 Bar
Focus	Active	Set Capillary	3700 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min



#	m/z	Res.	S/N	I	I %	FWHM
1	361.1432	45767	29640.0	1771220	100.0	0.0079
2	362.1462	32695	4901.7	292777	16.5	0.0111
3	363.1494	21670	291.1	17379	1.0	0.0168

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma		Score	rdb	e⁻ Conf	N-Rule
361.1432	1	C22H18N4Na	361.1424	-2.2	53.0	2	47.03	15.5	even	ok

Figure S4. Elemental composition of MS for FeL.

3. UV-vis absorption spectrum of AuNCs and sulfo-cyanine 7 NHS ester (Figure S5 and S6).

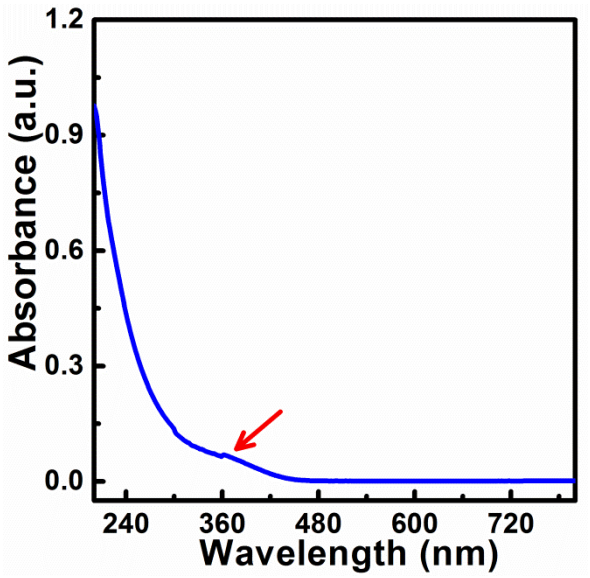


Figure S5. UV-vis absorption spectrum of AuNCs.

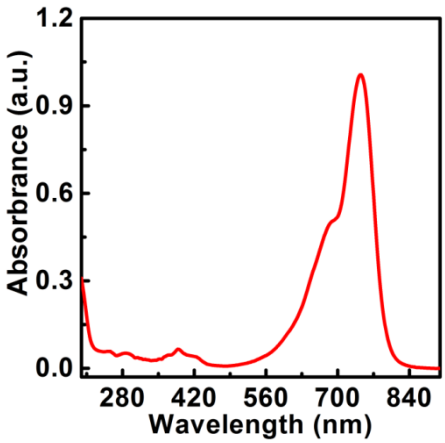


Figure S6. UV-vis absorption spectrum of sulfo-cyanine 7 NHS ester.

4. FT-IR characterization of AuNC@FeL@Cy7 probe (Figure S7).

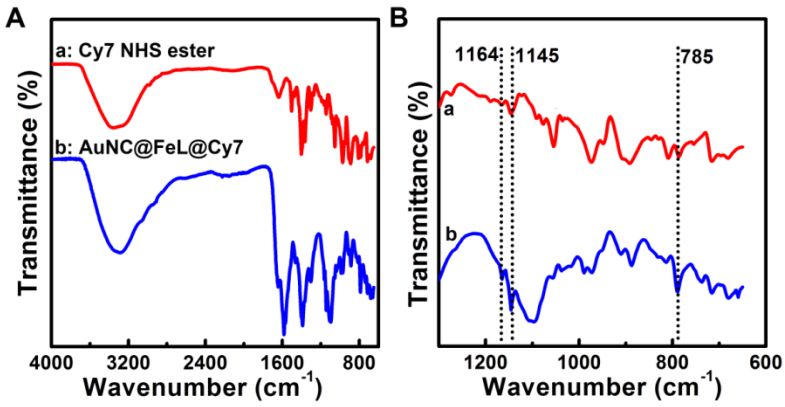


Figure S7. (A) FT-IR spectra of (a) Cy7 NHS ester and (b) AuNC@FeL@Cy7 between 4000-600 cm^{-1}. (B) FT-IR spectra of (a) Cy7 NHS ester and (b) AuNC@FeL@Cy7 between 1300-600 cm^{-1}.

5. Absorption spectra of FeL in the presence of different metal ions and titration of Fe^{2+} (Figure S8).

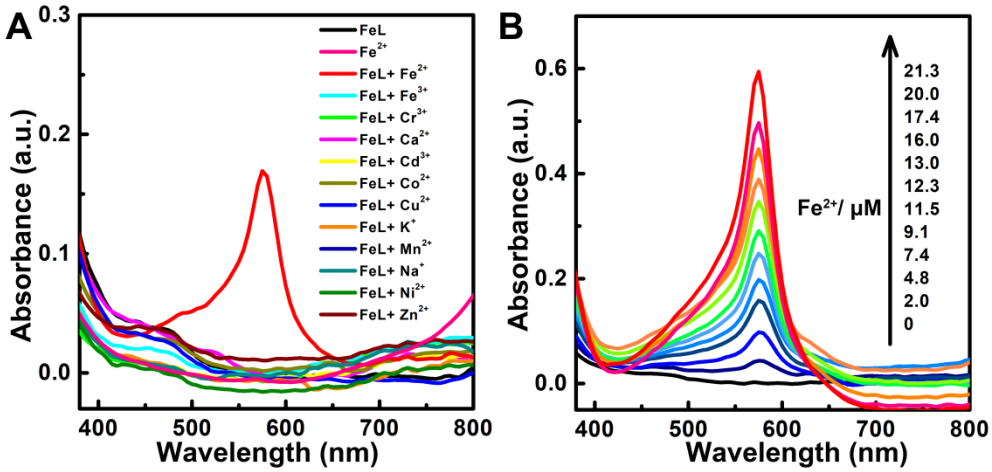


Figure S8. (A) Absorption spectra of FeL in the presence of various metal ions. The concentration of metal ions (except K^+, Na^+ and Ca^{2+}) is 9 μM. The concentrations of K^+, Na^+ and Ca^{2+} are 100 mM, 50 mM and 10 mM, respectively. (B) Absorption spectra of FeL in the presence of different concentrations of Fe^{2+} from 0 to 21.3 μM.

6. Selectivity of AuNC@FeL@Cy7 probe (Figure S9).

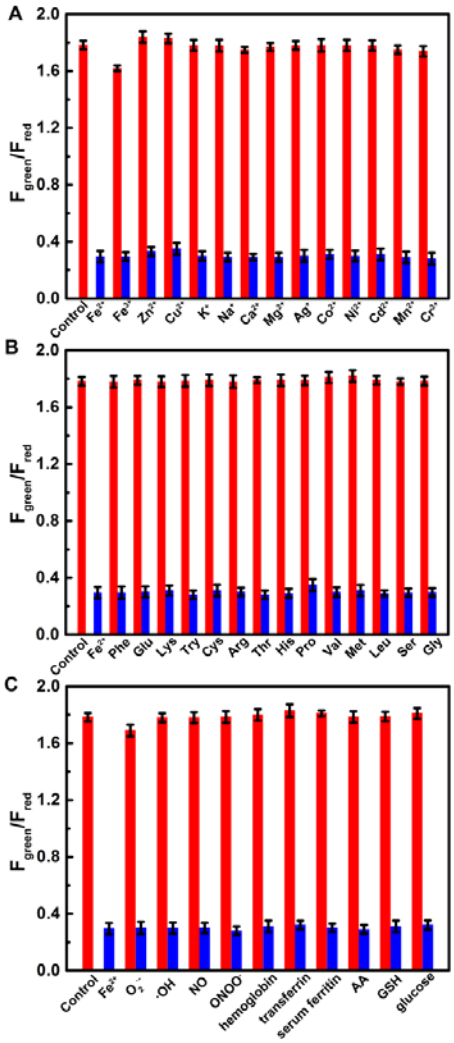


Figure S9. Selectivity and competition tests of AuNC@FeL@Cy7 probe toward various (A) metal ions, (B) amino acids, and (C) common ROS, Fe-containing proteins, ascorbic acid (AA), glutathione (GSH), glucose. The red bars represent the fluorescence signal ratio (F_{green}/F_{red}) of the fluorescent probe after addition of potential interferences, while the blue columns represent the fluorescence signal ratio (F_{green}/F_{red}) of the developed probe in the presence of potential interferences followed by addition of Fe^{2+}. The concentration of amino acids, GSH, ROS and metal ions (except K^+, Na^+ and Ca^{2+}) is 45 μM. The concentrations of K^+, Na^+, Ca^{2+}, AA and glucose are 100 mM, 50 mM, 10 mM, 1 mM and 100 mM, respectively. F_{green} and F_{red} represent the average fluorescence intensity between 580-760 nmand 765-900 nm, respectively. The excitation wavelength was 552 nm.

7. Stability investigation of AuNC@FeL@Cy7 probe (Figure S10-S12).

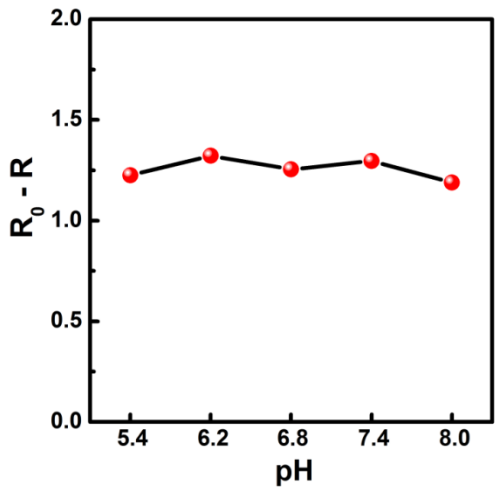


Figure S10. The difference value of fluorescence ratio (F_{green}/F_{red}) of AuNC@FeL@Cy7 probe at various pH from 5.4 to 8.0. R_0 and R represent the F_{green}/F_{red} of AuNC@FeL@Cy7 probe in the present of 0 and 50 μM Fe^{2+}, respectively. F_{green} and F_{red} represent the average fluorescence intensity between 580-760 nm and 765-900 nm, respectively. The excitation wavelength was 552 nm.

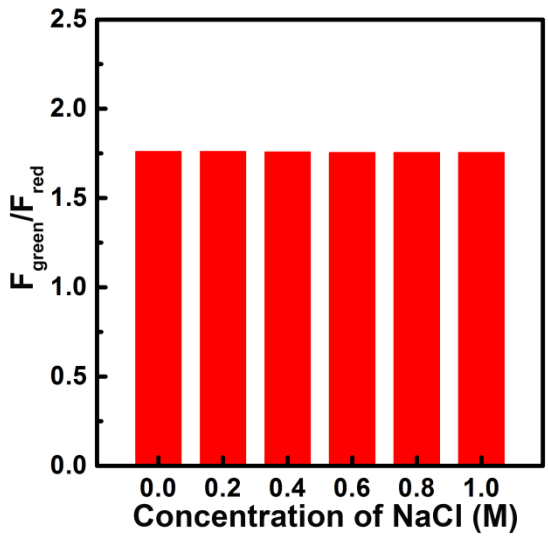


Figure S11. Fluorescent signal ratios (F_{green}/F_{red}) of AuNC@FeL@Cy7 probe in the present of different concentrations of NaCl. F_{green} and F_{red} represent the average fluorescence intensity between 580-760 nm and 765-900 nm, respectively. The excitation wavelength was 552 nm.

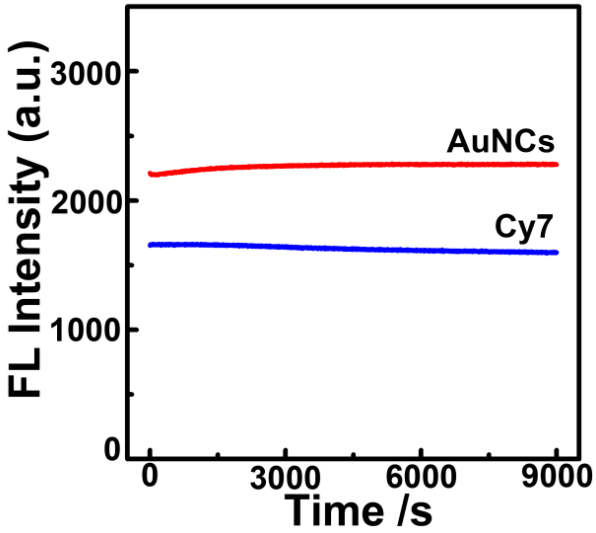


Figure S12. Fluorescence intensity of AuNCs and Cy7 obtained from AuNC@FeL@Cy7 probe as a function of time under a 552 nm excitation.

8. Cytotoxicity and biocompatibility study of AuNC@FeL@Cy7 probe(Figure S13-S15).

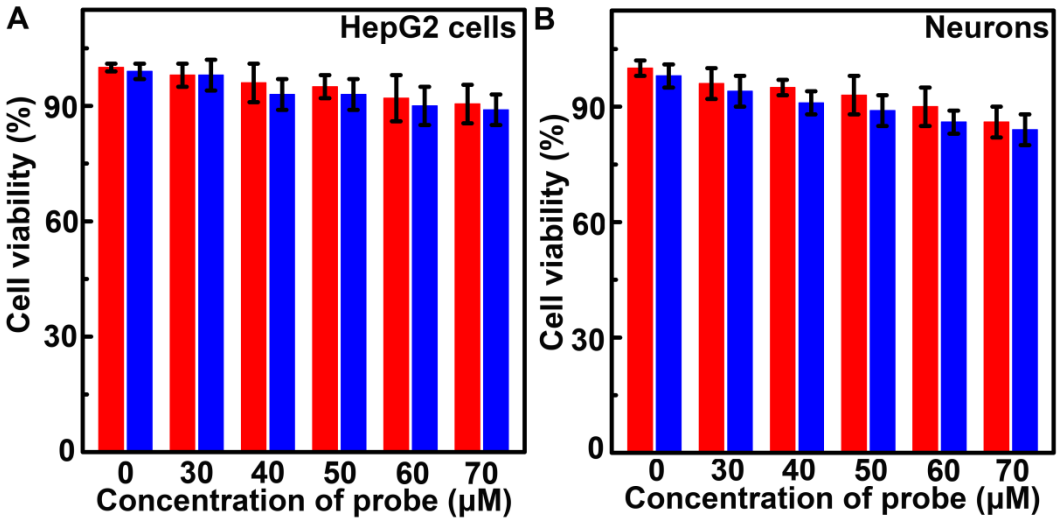


Figure S13. Cell viability of (A) HepG2 cells and (B) neurons incubated with different concentrations of AuNC@FeL@Cy7 probe for 24 h (red bars) or 48 h (blue bars).

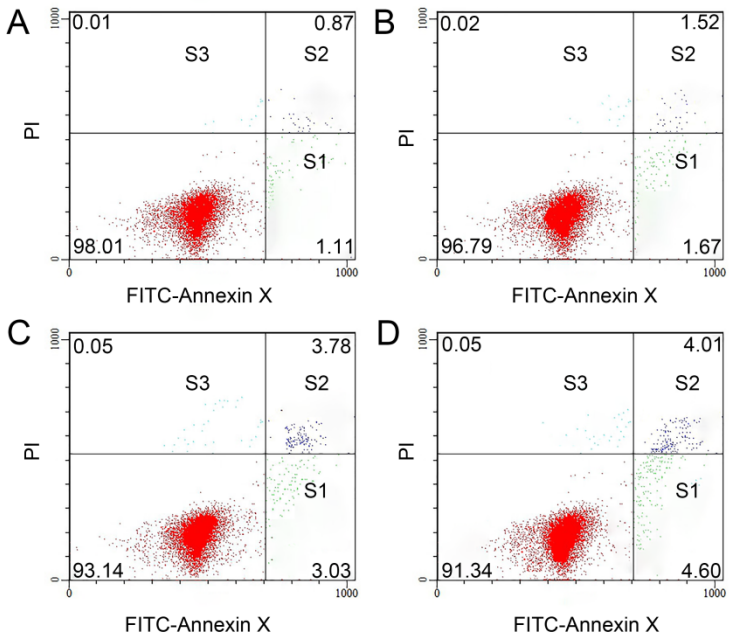


Figure S14. HepG2 cells incubated with AuNC@FeL@Cy7 probe at concentrations of (A) 0 μM, (B) 30 μM, (C) 40 μM, and (D) 60 μM for 24 h. S1, S2, and S3 represent the regions of early apoptotic, late apoptotic and dead cells, respectively.

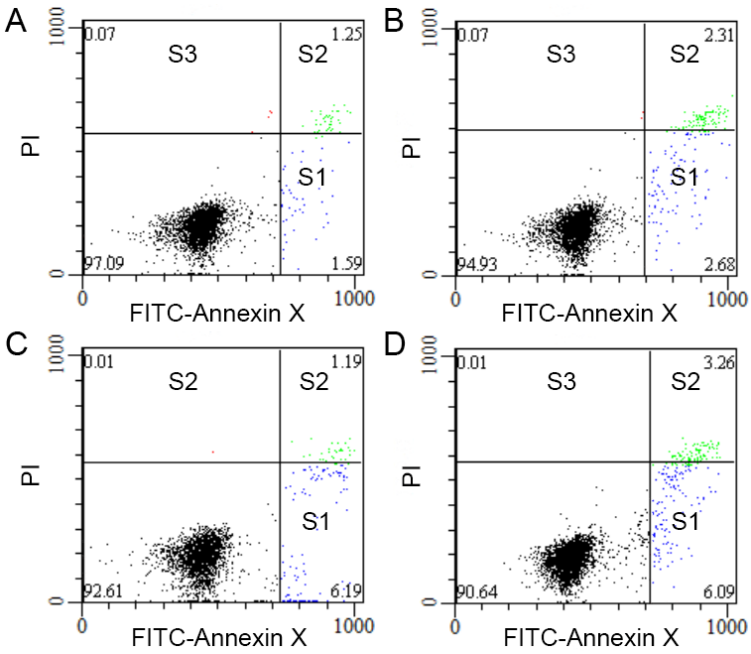


Figure S15. Neurons incubated with AuNC@FeL@Cy7 probe at concentrations of (A) 0 μM, (B) 30 μM, (C) 40 μM, and (D) 60 μM for 24 h. S1, S2, and S3 represent the regions of early apoptotic, late apoptotic and dead cells, respectively.

9. Confocal fluorescence imaging of HepG2 cells with different concentrations of Fe^{2+} (Figure S16).

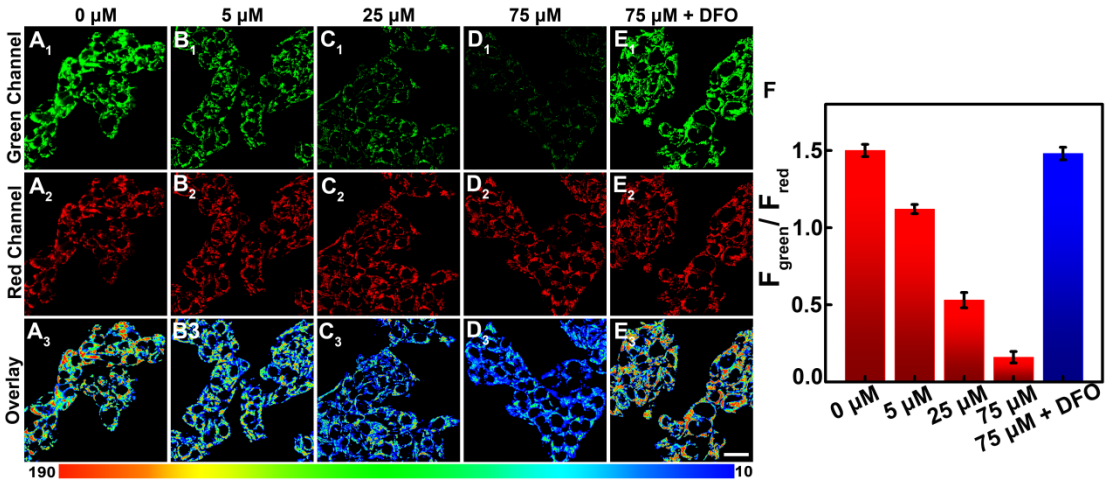


Figure S16. Confocal fluorescence microscopic images of HepG2 cells collected from different channels treated with AuNC@FeL@Cy7 probe (30 μM) in the presence of different concentrations of Fe^{2+}: (A_1-A_3) 0, (B_1-B_3) 5, (C_1-C_3) 25, and (D_1-D_3) 75 μM. (E_1-E_3) Confocal fluorescence microscopic images of HepG2 cells collected from different channels treated with AuNC@FeL@Cy7 probe (30 μM) in the presence of 75 μM Fe^{2+} followed by adding 100 μM DFO for 1 h. (F) Summarized data of average fluorescence intensity ratio (F_{green}/F_{red}) obtained from A-E. The data were obtained based on the statistical analysis of more than 30 neurons. F_{green} and F_{red} represent the average fluorescence intensity collected from green channel (580-760 nm) and red channel (765-900 nm), respectively. The excitation wavelength was 552 nm. Scale = 25 μm.

10. Co-localization imaging of HepG2 cells with AuNC@FeL@Cy7 probe (Figure S17).

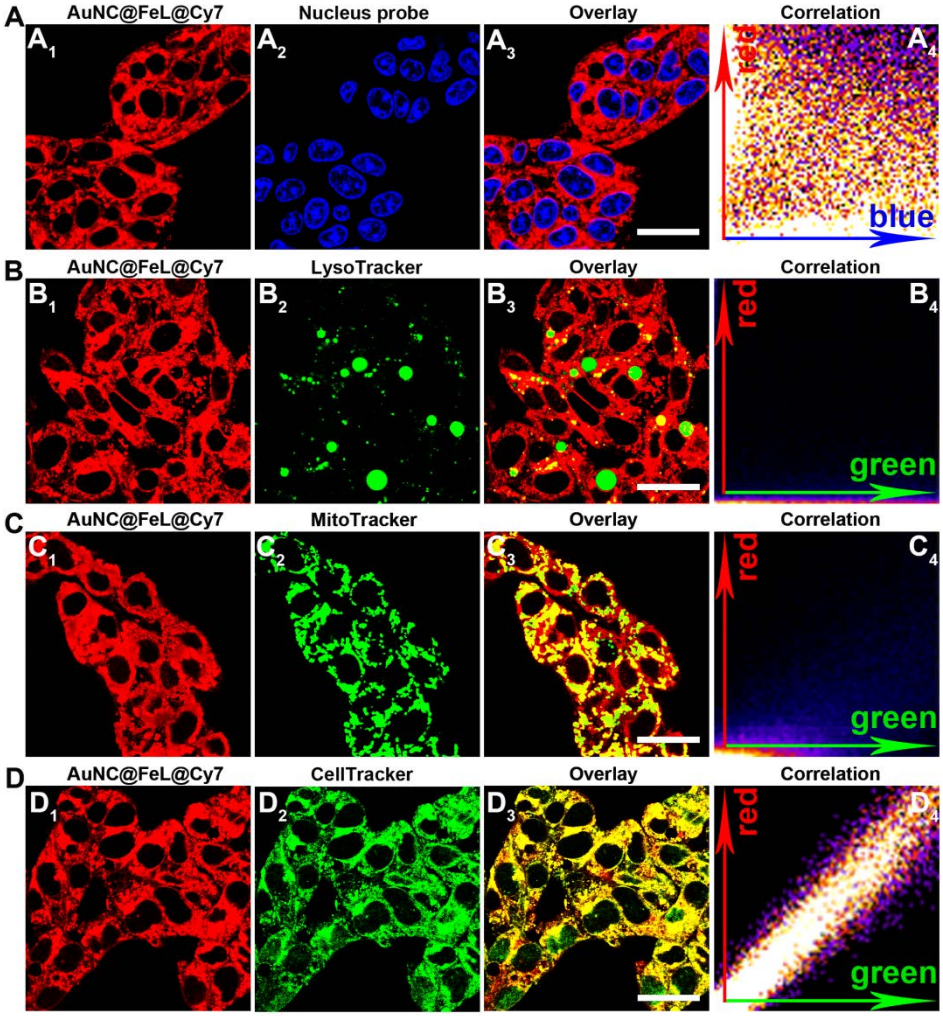


Figure S17. Co-localization imaging of AuNC@FeL@Cy7 probe with (A) nucleus, (B) lysosomes, (C) mitochondria, and (D) cytoplasm probes. (A_1, B_1, C_1, and D_1) represent the fluorescence images of AuNC@FeL@Cy7 probe collected from 765-900 nm excited at 552 nm. (A_2, B_2, C_2, and D_2) represent the fluorescence images of nucleus probe (Hoechst 33342) collected from 410-450 nm at excitation of 405 nm, the images of lysosomes probe (LysoTracker Green) collected from 500-550 nm at excitation of 488 nm, those of mitochondrial probe (MitoTracker Green) collected from 500-550 nm at excitation of 488 nm, and those of cytoplasm probe (CellTracker Green CMFDA) collected from 500-550 nm at excitation of 488 nm, respectively. (A_3, B_3, C_3, and D_3) are the overlay images of A_1 and A_2, B_1 and B_2, C_1 and C_2, D_1 and D_2, respectively. (A_4, B_4, C_4, and D_4) are the fluorescence correlation plots of A_1 and A_2, B_1 and B_2, C_1 and C_2, D_1 and D_2, respectively. Scale = 25 μm.

11. XPS characterization of AuNC@FeL@Cy7@AMP probe and AuNC@FeL@Cy7@TPP probe (Figure S18).

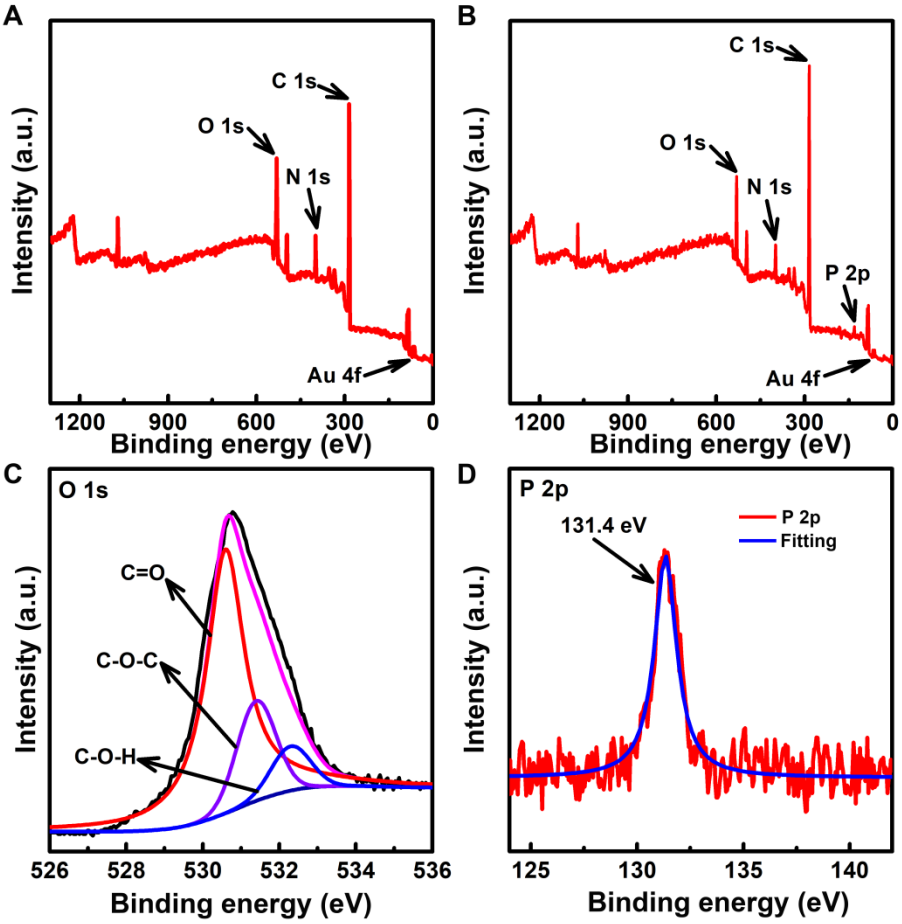


Figure S18. (A and B) XPS survey spectra of (A) AuNC@FeL@Cy7@AMP and (B) AuNC@FeL@Cy7@TPP probes. (C) XPS spectrum of O 1s region of AuNC@FeL@Cy7@AMP probe. (D) XPS spectrum of P 2p region of AuNC@FeL@Cy7@TPP probe.

12. Co-localization imaging of HepG2 cells with AuNC@FeL@Cy7@AMP probe and AuNC@FeL@Cy7@TPP probe (Figure S19).

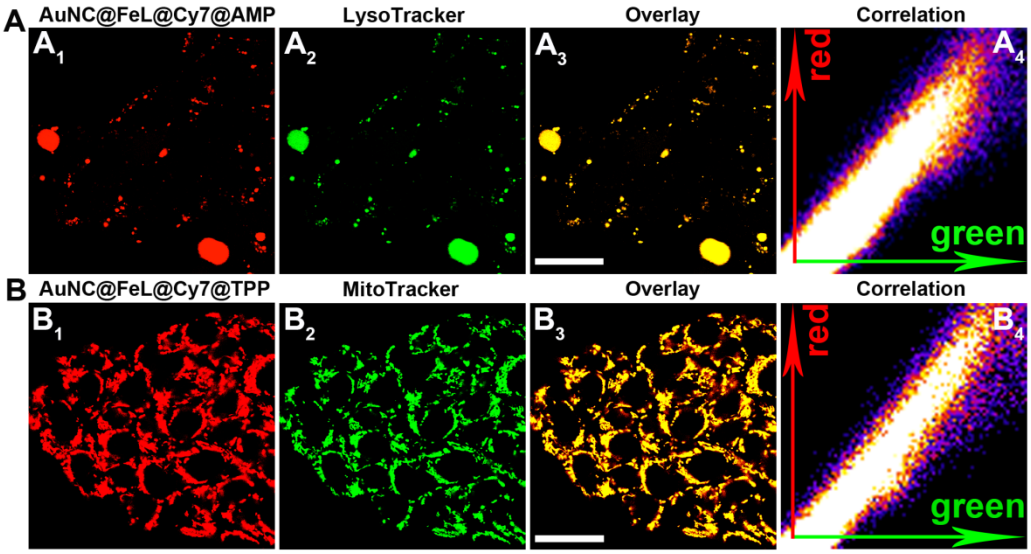


Figure S19. (A) Confocal fluorescence microscopic images of HepG2 cells treated with AuNC@FeL@Cy7@AMP probe and LysoTracker (LysoTracker Green). (A_1 and A_2) represent the fluorescence images collected from AuNC@FeL@Cy7@AMP probe and LysoTracker, respectively. (A_3) is the overlay images of A_1 and A_2. A_4 shows the fluorescence correlation plot of A_1 and A_2, respectively. (B) Confocal fluorescence microscopic images of HepG2 cells treated with AuNC@FeL@Cy7@TPP probe and MitoTracker (MitoTracker Green). (B_1 and B_2) represent the fluorescence images collected from AuNC@FeL@Cy7@TPP probe and MitoTracker, respectively. (B_3) is the overlay image of B_1 and B_2. (B_4) shows the fluorescence correlation plot of B_1 and B_2, respectively. Scale = 25 μm.

13. Confocal fluorescence imaging of HepG2 cells with organelle-targeted probes (Figure S20).

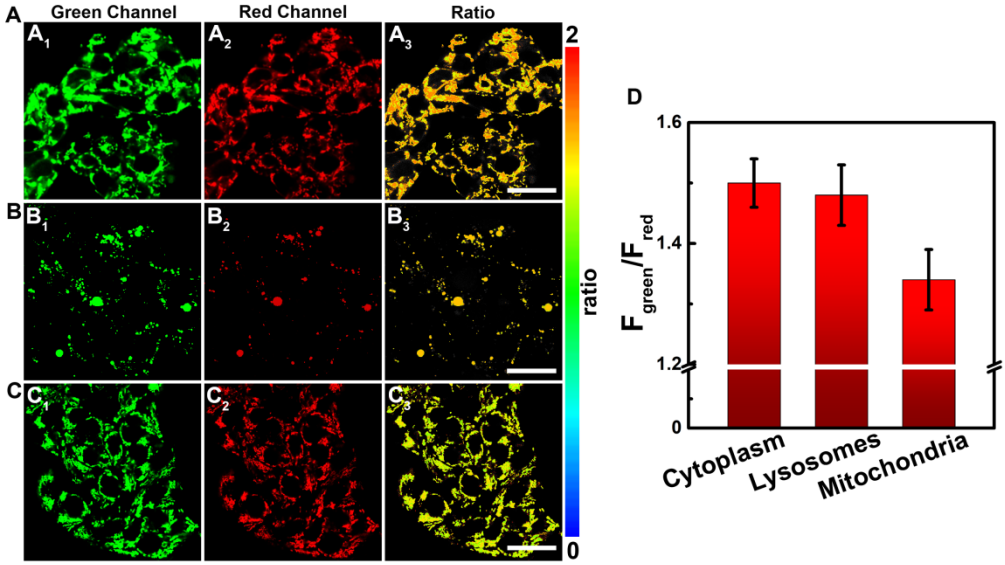


Figure S20. Confocal fluorescence microscopic images of HepG2 cells collected from different channels treated with (A) AuNC@FeL@Cy7 probe, (B) AuNC@FeL@Cy7@AMP probe, and (C) AuNC@FeL@Cy7@TPP probe, respectively. (A_1, A_2, and A_3) represent the fluorescence images of AuNC@FeL@Cy7 probe collected from green channel (580-760 nm), red channel (765-900 nm) and ratio of green channel to red channel, respectively. (B_1, B_2, and B_3) represent the fluorescence images of AuNC@FeL@Cy7@AMP probe collected from green channel (580-760 nm), red channel (765-900 nm) and ratio of green channel to red channel, respectively. (C_1, C2, and C_3) represent the fluorescence images of AuNC@FeL@Cy7@TPP probe collected from green channel (580-760 nm), red channel (765-900 nm) and ratio of green channel to red channel, respectively (D) Summarized data of average fluorescence intensity ratio (F_{green}/F_{red}) obtained from A-C. F_{green} and F_{red} represent the average fluorescence intensity collected from green channel (580-760 nm) and red channel (765-900 nm), respectively. The excitation wavelength was 552 nm. Scale = 25 μm.