Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Iminodiacetate-modified conjugated polyelectrolyte for fluorescent labeling of histidine-tagged proteins

Kai Cai,[†ab] Ying Tan,[†ab] Chunyan Tan,[*ab] Jiatao Wu,[ab] Pan Wu,[ab] Jiamei Liang,[ab] Shuwen Liu,[ab] Bibo Zhang,[ab] and Yuyang Jiang[bc]

[a] Department of Chemistry, Tsinghua University, Beijing 100084, P. R. China.

[b] The State Key Laboratory Breeding Base-Shenzhen Key Laboratory of Chemical Biology, the Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, P. R. China. Email: tancy@sz.tsinghua.edu.cn; Tel: 86-755-26036533.

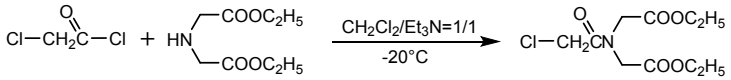
[c] School of Pharmaceutical Sciences, Tsinghua University, Beijing 100084, P. R. China.

Experimental

Materials. Bis(triphenylphosphine)palladium chloride ($Pd(PPh_3)_2Cl_2$), tetrakis(triphenylphosphine)palladium ($Pd(PPh_3)_4$, cuprous iodide (CuI), zinc chloride ($ZnCl_2$) and nickel sulfate hexahydrate ($NiSO_4 \cdot 6H_2O$) were purchased from Aladdin Chemical Co. (Shanghai, China). 1,4-dimethoxy benzene, chloroacetyl chloride, trimethylsilyl acetylene, tetrabutylammonium fluoride (1 M in THF) and iminodiacetic acid diethyl ester were purchased from TCI Chemical Co. (Japan). Phenylmethanesulfonyl fluoride (PMSF), Tris, and glycine were purchased from Beyotime Biotech Co. (Shanghai, China). DL-Dithiothreitol (DTT), sucrose, ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulfate sodium salt (SDS), albumin from bovine serum (BSA), lysozyme and N,N,N',N'-tetramethylethylenediamine (TEMED) were purchased from Sangon Biotech Co. (Shanghai, China). Bromophenol blue was purchased from Sigma-Aldrich Co. LLC. (Shanghai, China). Acrylamide was purchased from GenStar CO. (Beijing, China). His 60 Ni Super Flow Resin was purchased from Takara Bio USA, Inc. (USA). The expression and purification of His-Bcl-xL and His-RFP protein were referred to the method previously reported.[1-3] The plasmid pcDNA3.1-His-B2R was purchased from Biogot Biotechnology CO. (Nanjing, China). The water used in all of the experiments was prepared on a Milli-Q water purification system and displayed a resistivity of ≥18.2 MΩ·cm-1. All the other chemicals were supplied by TCI Chemical Co. and used without further purification unless noted.

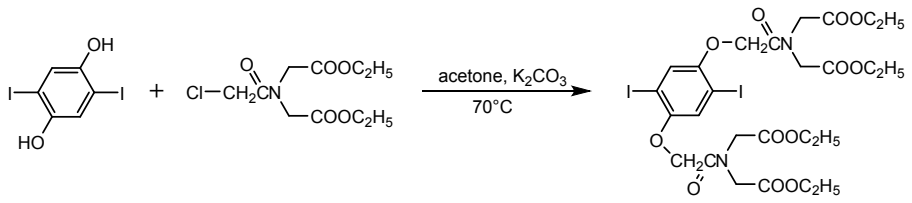
Instrumentation. ^1H spectra were recorded on a Bruker 400 MHz spectrometer. All mass spectra were acquired by a 4700 Proteomics Analyzer MALDI-TOF/TOF-MS (Applied Biosystems, Framingham, MA). Fluorescence experiments were carried out on a SPEX Fluorolog 3-TCSPC

spectrometer with 1 cm path length cuvette, while absorption spectra were recorded on an Agilent Cary 100 UV-Vis spectrophotometer. Fluorescence images were taken by confocal laser scanning microscope (Olympus FV1000-IX81).



Compound1

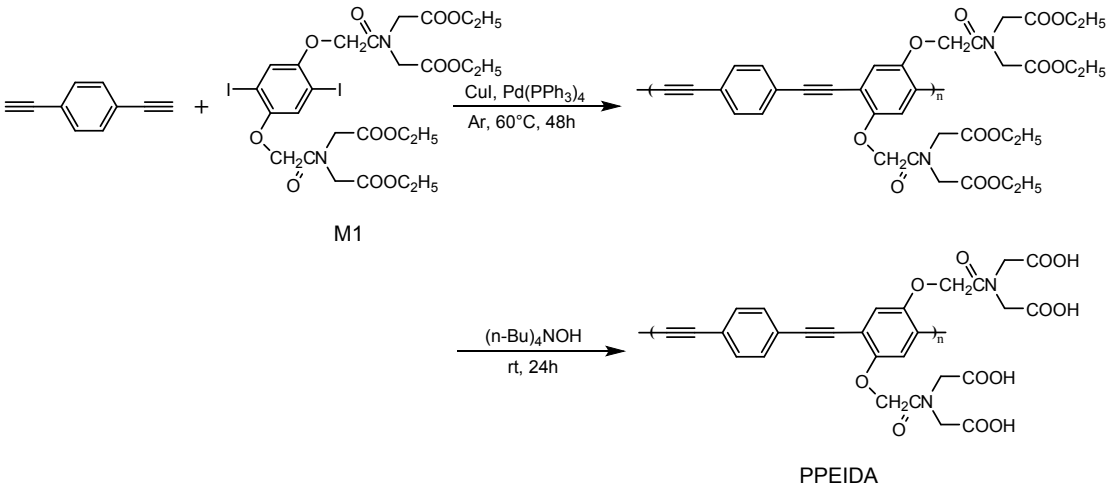
Synthesis of Compound 1. Chloroacetyl chloride (0.27 g, 2.4 mmol) was reacted with iminodiacetic acid diethyl ester (0.38 g, 2.0 mmol) in 1:1 CH_2Cl_2/Et_3N by volume in dichloromethane at -20 °C. After filtration, the crude product was purified by silica column and solid product compound 1 was collected. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 4.2 (m, 8H), 4.1 (d, 2H), 1.2 (m, 6H). MS (ESI): $[M+H]^+$ 266.1, $[M+Na]^+$ 288.1.



M1

Synthesis of M1. A solution of Compound 1 (1.6 g, 6.0 mmol) and potassium carbonate (5.52g, 40.0 mmol) in 150 mL acetone fitted with a condenser were degassed with argon for 5 minutes. Then 1.45g of 2,5-diiodohydroquinone (4.0 mmol) was added slowly under argon. Then the reaction mixture was stirred at 70 ºC for 20 h. After cooling to room temperature, remove potassium carbonate through filtration. The reaction solvent was removed in vacuo. Then the remainders were partitioned between water and CH_2Cl_2. The organic solution was dried by adding anhydrous sodium sulfate, and then poured into 100 mL ethyl acetate, and the precipitation were further purified by two repeated cycles of dissolution in CH_2Cl_2 and precipitation into ethyl acetate and white solid was

collected and dried. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.25 (s, 2H), 4.73 (s, 4H), 4.32 (s, 4H), 4.2 (m, 12H), 1.2 (m, 12H). MS (ESI): $[M+H]^+$ 821.0, $[M+Na]^+$ 843.0.



Poly(p-pheynylene ethynylene)-Iminodiacetate Ester. A solution of the monomer M1 (162.0 mg, 0.2 mmol) and 1,4-diethynylbenzene (25.3 mg, 0.2 mmol) in 20 mL of dry THF/Et_3N/CH_2Cl_2 (v/v/v = 3/1/1) fitted with a condenser were degassed with argon for 15 min. Then 17.4 mg of $Pd(PPh_3)_4$ (15.0 μmol) and 8 mg of CuI (15.0 μmol) were added under argon. The reaction mixture was stirred at 60 °C for 48 h. The obtained reaction solution was poured into 150 mL of cold methanol, and yellow solid was collected and dried (98.3 mg, yield 71%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.5 (d, 4H), 7.1 (d, 4H), 4.8 (t, 4H), 4.1 (m, 16H), 1.2 (m, 12H).

Poly(p-pheynylene ethynylene)-Iminodiacetate. A solution of PPE-iminodiacetate (IDA) ester (142.0 mg, 0.2 mmol) in 30 mL of dioxane/THF (v/v = 5/1) was added to 1.5 mL of 1 M (*n*-Bu)$_4$NOH in methanol and stirred at room temperature for 24 h. During the course of the hydrolysis, 2 mL of water was systematically added to keep the solution clear. Then a solution of 0.2 g of $NaIO_4$ in 3 mL of water was added to the hydrolyzed polymer solution, and the resulting mixture was poured into 400 mL of cold acetone, resulting in the precipitation of PPEIDA as yellow powders

(92.5 mg, yield 76%). The polymer was then dissolved in 50 mL of deionized water (several drops of 1 M NaOH solution were added) and was purified by dialysis against deionized water using a regenerated cellulose membrane (7 kDa molecular weight cutoff). After dialysis, the solution was stored as the stock solution.

Metal ion titration fluorescence spectroscopy. Metal ion titration experiments were carried out by microtitration in a fluorescence cuvette. Metal ions (Ni^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}) were dissolved in deionized water to prepare stock solution (10 mM). PPE-IDA was diluted from a concentrated stock solution (3.25 mM) in H_2O. The concentration of diluted PPE-IDA was calibrated using its molar extinction coefficient at 407 nm as 9.67×10^4 $M^{-1}cm^{-1}$. In titration experiments, 2 mL of 1.125 uM polymer solution was placed in a fluorescence cuvette with light path 1 cm. Then fluorescence spectra were repeatedly acquired after addition of concentrated ion solution with an excitation at 380 nm and an emission at 400-700 nm. Both of the slits for excitation and emission were set to be 5 nm and the integration time was 0.2 sec.

Fluorescence anisotropy. Fluorescence anisotropy (FA) experiments were carried out by microtitration in a micro quartz cuvette. 200 μL PPEIDA solution (1.125 μM PPEIDA) or PPEIDA-Ni^{2+} complex solution (1.125 μM PPEIDA, 200 μM Ni^{2+}) was placed in a cuvette. Anisotropy of sample was measured after 5-min incubation of the polymer solution with added protein at different concentrations. The excitation wavelength was 380 nm and the emission wavelength was 433 nm. Both of the slits for excitation and emission were 5 nm and the integration time was 0.2 sec.

Förster resonance energy transfer analysis. Förster resonance energy transfer (FRET) analysis experiments were carried out by microtitration in a fluorescence cuvette. 2000 μL PPEIDA solution

(1.125 μM PPEIDA) or PPEIDA-Ni^{2+} complex solution (1.125 μM PPEIDA, 200 μM Ni^{2+}) was placed in a 1 cm fluorescence cuvette. Each time RFP or His-tagged RFP was added to the solution, fluorescence spectrum was recorded after 5-min incubation. The excitation wavelength was 380 nm and the emission wavelength range was 400-700 nm. Both of the slits for excitation and emission were 5 nm.

Western blot analysis. His-tagged Bcl-xL were boiled with 5X loading buffer (250 mM Tris, 10% SDS, 0.5% BPB, 50% Glycerol, 5% thioglycol) for 10 min at 100 °C for western blot analysis. A series of different concentration of protein extracts were fractionated by 10% SDS-PAGE and transferred to PVDF membrane (Roche). The transferred membrane was incubated with PPEIDA-Ni^{2+} solution (32.5 μM PPEIDA, 50 μM Ni^{2+}) for 6h at 4 °C. Then, distinct blue fluorescence on the position of His-tagged Bcl-xL was observed under UV irradiation.

Cell Culture. HeLa cells were cultured in MEM medium supplemented with 10% (v/v) fetal bovine serum (FBS) at 37 °C under a humidified atmosphere of 5% CO_2 in air. For all of the experiments, cells were harvested from subconfluent (<80%) cultures using a trypsin-EDTA solution and then resuspended in fresh medium. A subculture was performed every 2 days.

Cytotoxicity Assay. HeLa cells were seeded into a 96-well plate, maintained overnight in MEM medium containing 10% FBS, and then treated with 0-200 μM polymers at 37 °C for 24 h. After 10 μL of freshly prepared 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/mL in 1× phosphate-buffered saline (PBS)) was added to each well, the wells were incubated for 4 h. The supernatant was removed, and the cells were lysed by addition of 100 μL of DMSO per well.

The optical density (OD) at 570 nm was then measured on a microplate reader (Tecan M1000 Pro) to correlate to relative cell viability.

Fluorescence imaging. HeLa cells expressing His-tagged B2R were incubated with PPEIDA-Ni^{2+} PBS solution (3.25 μM PPEIDA, 200 μM Ni^{2+}) and PPEIDA PBS solution (3.25 μM PPEIDA) for 3 min, respectively. After washing by imidazole (5 mM) PBS solution, the cells were analyzed by a confocal laser scanning microscope. Confocal microscope conditions: excitation at 405 nm and 488nm, emission 425-525 nm.

Results

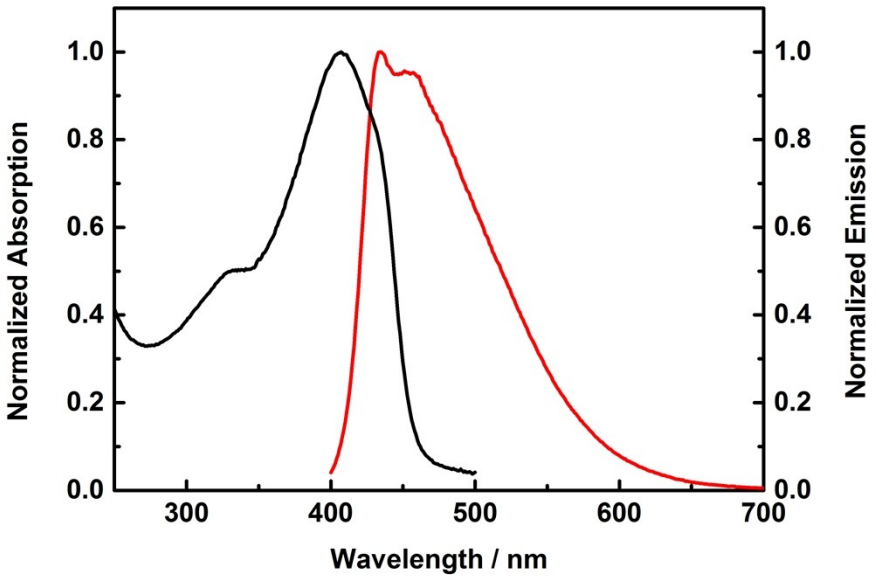


Fig. S1 Normalized UV-visible absorption (black line) and fluorescence emission spectra (red line) of PPEIDA in aqueous solution.

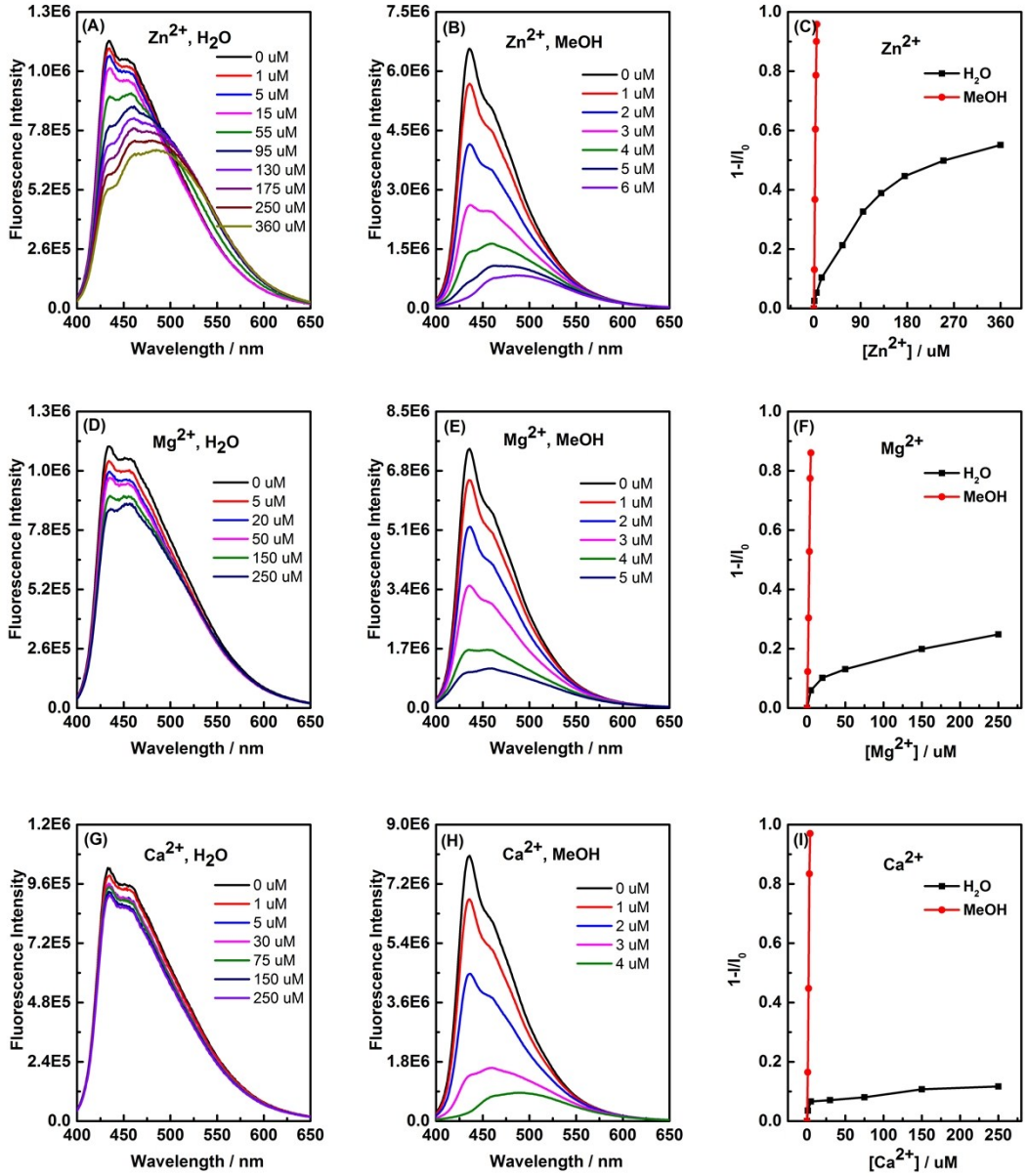


Fig. S2 Emission quenching spectra and quenching efficiency of 1.125 μM PPEIDA with different metal ions in H_2O and MeOH. I_0 and I are the fluorescence intensities of PPEIDA in the absence and presence of metal ions, respectively.

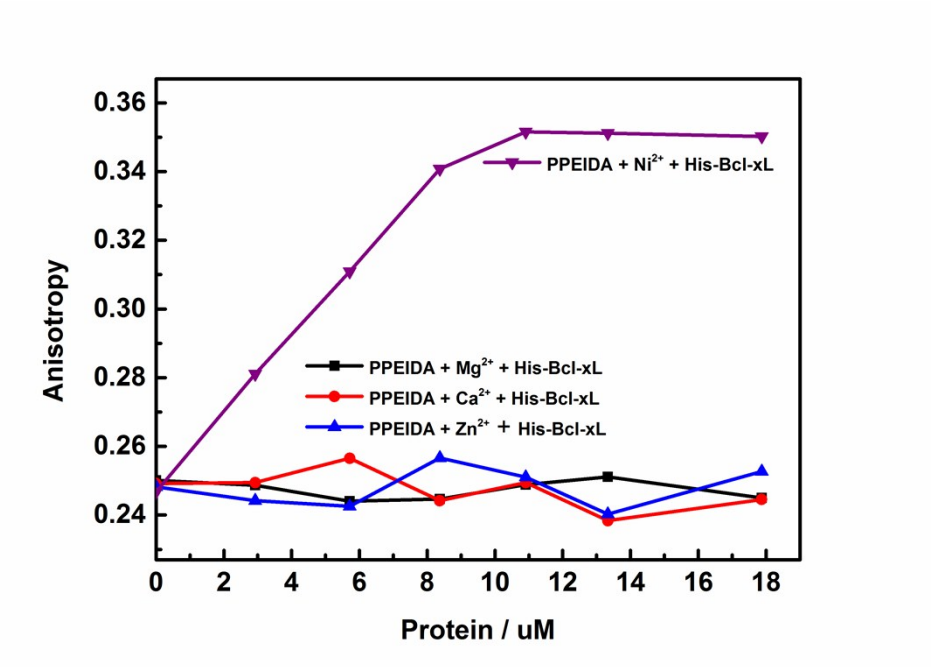


Fig. S3 Fluorescence anisotropy of PPEIDA in response to varying concentrations of His-tagged Bcl-xL in the presence of 200 μM Ni^{2+}, Mg^{2+}, Ca^{2+} and Zn^{2+}. [PPEIDA] = 1.125 μM.

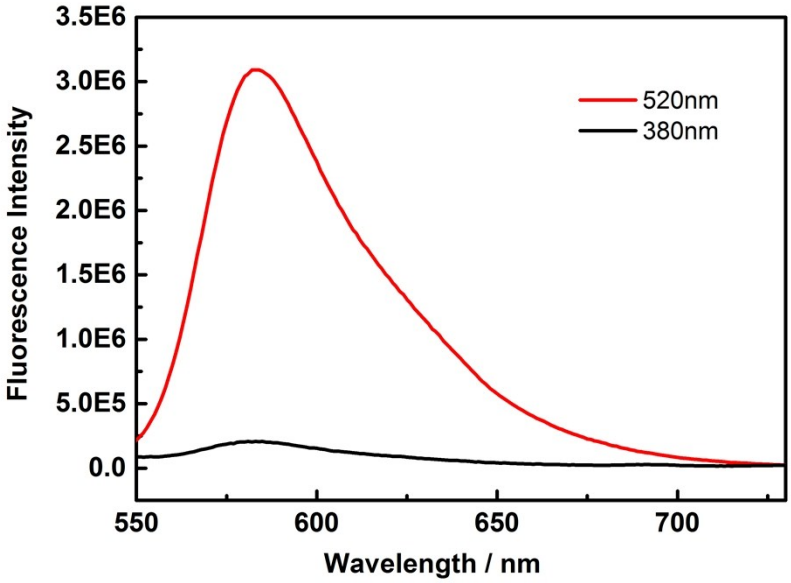


Fig. S4 Fluorescence spectra of His-RFP in aqueous solution when excited at 520 nm (red) and 380 nm (black), respectively. [His-RFP]= 111.0 nM.

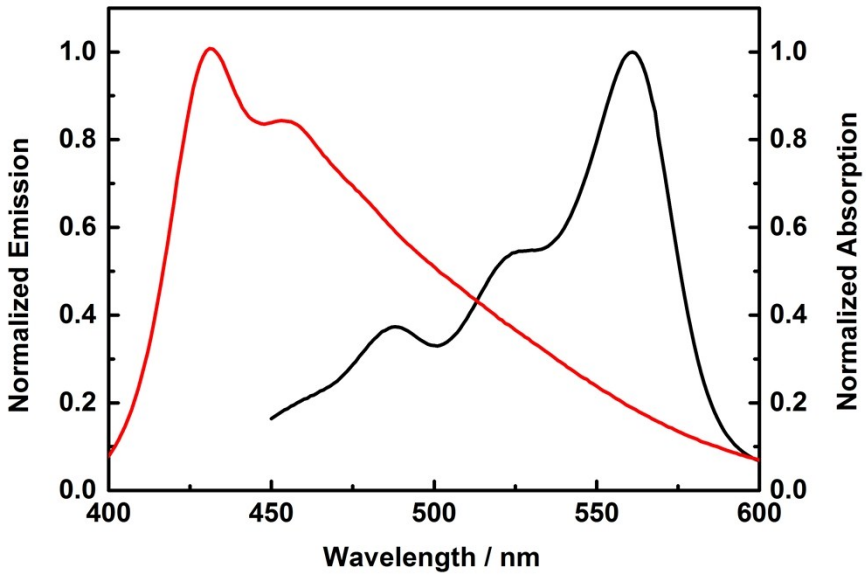


Fig. S5 Normalized UV-visible absorption (black line) of His-RFP and fluorescence emission spectra (red line) of PPEIDA in aqueous solution.

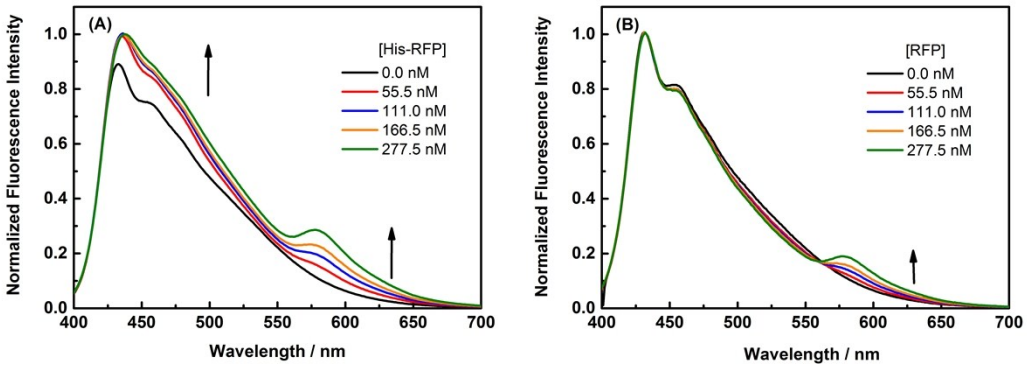


Fig. S6 (A)Normalized fluorescence spectra (λ_{ex} = 380 nm) of PPEIDA upon addition of His-RFP (c = 0-277.5 nM) in aqueous solution. (B)Normalized fluorescence spectra (λ_{ex} = 380 nm) of PPEIDA-Ni^{2+} complex upon addition of RFP (c = 0-277.5 nM) in aqueous solution. Arrows show direction of change of the bands with increasing [His-RFP].

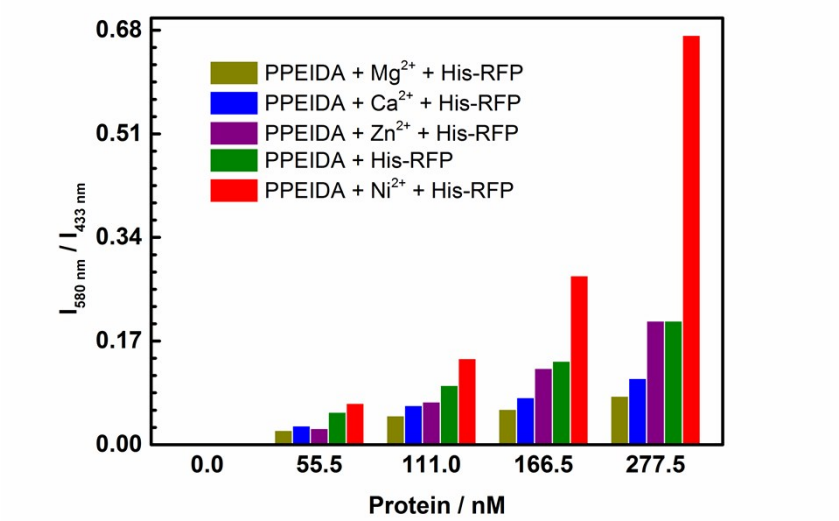


Fig. S7 FRET efficiency of PPEIDA after addition of His-RFP at various concentrations with and without metal ions in aqueous solution. [PPEIDA] = 1.125 μM, [Ni^{2+}] = 200 μM, [Mg^{2+}] = 200 μM, [Ca^{2+}] = 200 μM, [Zn^{2+}] = 200 μM.

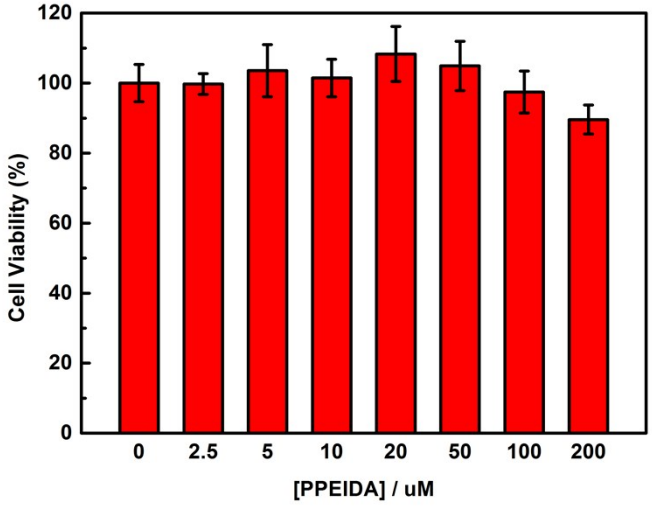


Fig. S8 Viability of Hela cells incubated with PPEIDA measured by the MTT assay. Results are presented as the mean ± SD in triplicate.

Table S1. FRET parameters the PPEIDA-Ni^{2+}-His-tagged RFP system.

Donor	Accepter	Q_D	J (λ)	R_0 (Å)	E	R_1 (Å)	R_2 (Å)
PPEIDA	His-RFP	0.20	3.38×10^{15}	48.31	45.82 %	47.67	103.91

The Förster radius R_0 and efficiency E were calculated by the following equations:

$$R_0 = 0.211 \times [\kappa^2 n^{-4} Q_D J(\lambda)]^{1/6},$$

$$E = 1 - \frac{I_{DA}}{I_D} = \frac{R_0^6}{R^6 + R_0^6}$$

$$R = (\frac{R_0^6}{E} - R_0^6)^{1/6}$$

where κ^2 is the dipole orientation factor, (κ^2=2/3 for randomly oriented donors and acceptors), n is the refractive index of the solvent, Q_D is the fluorescence quantum yield of the donor in the absence of the acceptor, J (λ) is the spectral overlap integral, R_0 is the Förster distance, I_{DA} is the fluorescence intensity of the donor in the presence of acceptor, and I_D is the fluorescence intensity of the donor in the absence of an acceptor, R_1 and R_2 is the average donor-acceptor distance for PPEIDA-Ni^{2+}-His-tagged RFP and PPEIDA-His-tagged RFP.

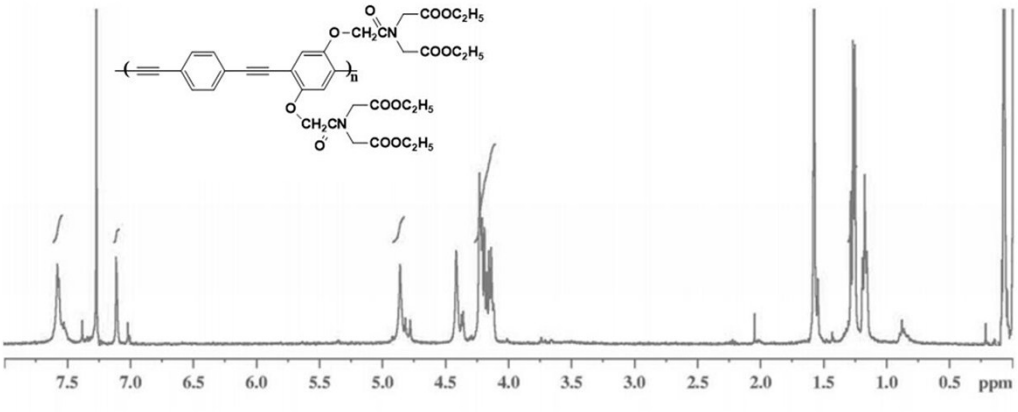


Fig. S9 NMR spectra of poly(p-pheynylene ethynylene)-iminodiacetate ester.

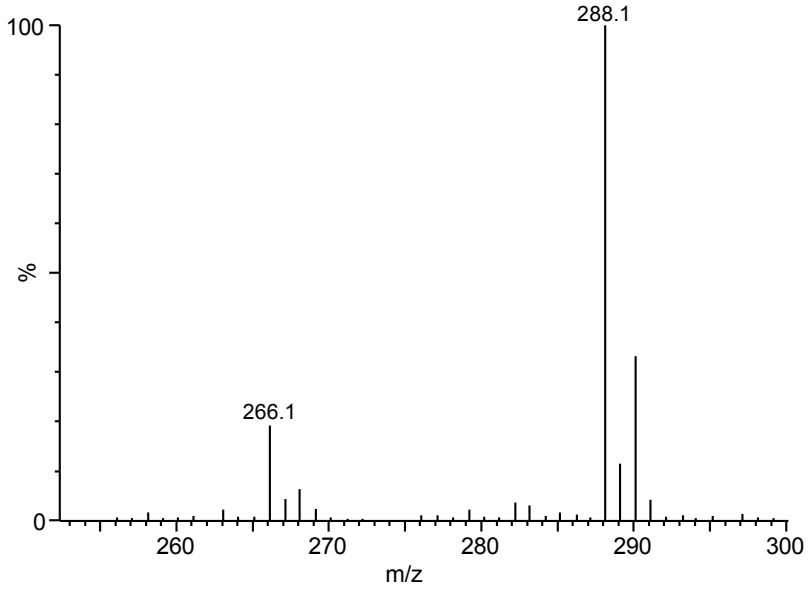


Fig. S10 ESI MS spectra of Compound1.

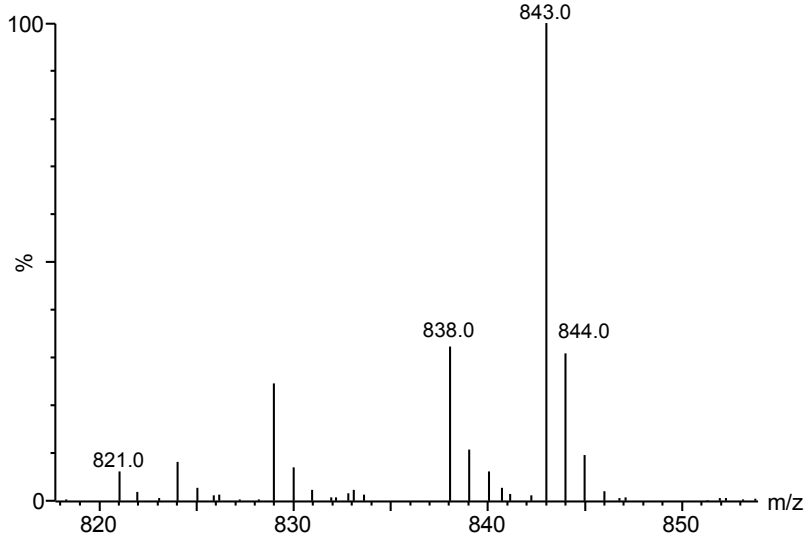


Fig. S11 ESI MS spectra of M1.

References

1. Z. Sun, W. Lu, Y. Tang, J. Zhang, J. Chen, H. Deng, X. Li and J.-N. Liu, *Protein Expr. Purif.*, 2007, **55**, 312-318.
2. Y. Feng, L. Zhang, T. Hu, X. Shen, J. Ding, K. Chen, H. Jiang and D. Liu, *Arch. Biochem. Biophys.*, 2009, **484**, 46-54.
3. X. Zhang, Y. Tan, R. Zhao, B. Chu, C. Tan and Y. Jiang, *Protein Pept. Lett.*, 2012, **19**, 991-996.