Electronic Supplementary Information

"AIE + ESIPT" activity-based NIR Cu^{2+} sensor with dye participated binding strategy

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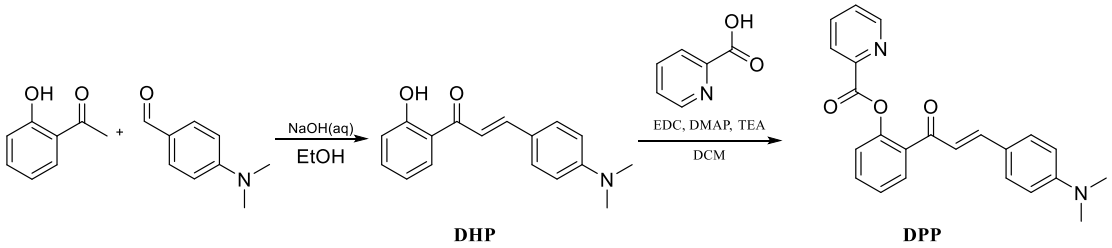
1. General Information

All reagents and solvents were obtained commercially and used without further purification unless otherwise noted. Analytical thin-layer chromatography (TLC) was performed on silica gel plate and analyzed by UV light or by potassium permanganate stains followed by heating. Flash chromatography was carried out utilizing silica gel (200-300 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ at room temperature on a Bruker AM-400 spectrometer (400 MHz ^{1}H, 100 MHz ^{13}C). The chemical shifts are reported in ppm relative to either the residual solvent peak (^{13}C) (δ = 77.00 ppm for $CDCl_3$), (^{1}H) (δ = 7.26 ppm for $CDCl_3$) or TMS (1H) (δ = 0 ppm) as an internal standard. Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, dd = doublet doublet), coupling constant (Hz), integration. Data for ^{13}C NMR are reported as chemical shift. HRMS were performed on a Bruker Apex II mass instrument (ESI).

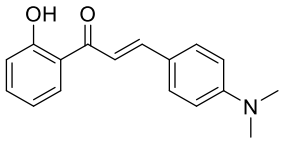
All UV − visible spectra and fluorescence spectra were recorded using a Shimadazu UV-1900 spectrophotometer and Horiba FluoroMax-4 luminescence spectrometer, respectively. Effect of pH was performed on Deutta spectrophotometer. Fluorescent quantum yields were determined to be 17.7 % for probe **DPP**, and 20.5 % for **DHP** as solid, respectively by an absolute method using an integrating sphere on FLS920 of Edinburgh Instrument. Luminescence spectra of solid **DDP** and **DHP** were collected using a Hitachi F7100 luminescence spectrometer.

2. General Procedure for Preparation of Compound DPP



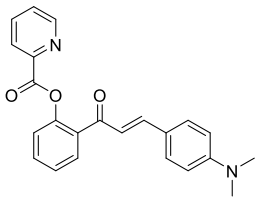
Scheme S1. Synthesis of **DPP**.

(E)-3-(4-(dimethylamino)phenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (DHP)



To the mixture of 1-(2-hydroxyphenyl)ethan-1-one (1.36 g, 10mmol) and 4-(dimethylamino)benzaldehyde (1.49 g, 10 mmol) in 30 ml ethanol, sodium hydroxide solution (1 g NaOH in 1 ml water, 25 mmol) was added. The reaction was kept and stirred at 60 ℃ for 12 hours. After cooling to room temperature, pH was adjusted to 1 with 2 M hydrochloric acid solution. Precipitation was formed and filtered, washed with a small amount of ethanol to obtain dark red solid no need further purification (1.25 g, 47 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.25 (s, 1H), 7.91 (dd, J = 8.4, 6.9 Hz, 2H), 7.56 (d, J = 8.9 Hz, 2H), 7.45 (td, J = 8.4, 4.1 Hz, 2H), 7.01 (dd, J = 8.4, 0.9 Hz, 1H), 6.95 – 6.89 (m, 1H), 6.68 (d, J = 8.9 Hz, 2H), 3.04 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.4, 163.4, 152.3, 146.5, 135.6, 130.8, 129.3, 122.2, 120.3, 118.5, 118.4, 114.1, 111.7, 77.3, 77.0, 76.7, 40.0. HRMS: 266.1164 ([M-H]$^{+}$). Data in agreement with those reported previously.[1]

(E)-2-(3-(4-(dimethylamino)phenyl)acryloyl)phenyl picolinate (DPP)



To a solution of **DHP** (0.534 g, 2 mmol) in anhydrous CH_2Cl_2 (20 mL) was added EDC (0.76 g, 4 mmol), DAMP (0.488 g, 4 mmol), triethylamine (1.2 mL) and picolinic acid (0.295 g, 2.4 mmol), respectively. The mixture was stirred overnight at room temperature and was then poured into water, extracted with CH_2Cl_2 twice. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The crude product was further purified using silica gel chromatography with ethyl acetate/DCM as the eluent to afford **DPP** as an orange solid (0.43 g, 58 % yield). ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.80 (ddd, J = 4.7, 1.7, 0.8 Hz, 1H), 8.19 (dt, J = 7.8, 1.0 Hz, 1H), 8.01 (td, J = 7.7, 1.7 Hz, 1H), 7.87 (dd, J = 7.7, 1.6 Hz, 1H), 7.71 – 7.66 (m, 2H), 7.52 – 7.42 (m, 5H), 7.20 (d, J = 15.6 Hz, 1H), 6.68 (d, J = 9.0 Hz, 2H), 2.99 (s, 6H); ^{13}C NMR

(100 MHz, d_6-DMSO) δ 189.8, 163.7, 152.6, 150.5, 149.0, 147.0, 146.4, 138.1, 133.0, 133.0, 131.1, 130.4, 128.4, 127.0, 126.2, 124.1, 121.9, 119.6, 112.1, 40.1. HRMS: 373.1404 ($[M+H]^+$).

3. General Procedure for Sensing Studies

Stock solution of **DPP** at the concentration of 10 mM was prepared in DMSO. The probe solution at the concentration of 20 μM for spectrometric analysis was freshly prepared by diluting the stock solution with 10 mM PBS buffer (containing 0.2 % DMSO) of pH 7.4. Freshly prepared $Cu(ClO_4)_2$ solution was added into the solution (total volume 3.0 mL), and the spectrum was collected after mixing the solution for 20 min at room temperature. The emission signal was recorded from 490 nm to 820 nm with an excitation of 475 nm and intervals of 1 nm. Solutions of metal ions of Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Al^{3+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Pd^{2+} and Ag^+ were prepared by dissolving their chloride or perclorate salts into double-distilled water.

4. Recognition Mechanism

DPP react with $Cu(ClO_4)_2$: DPP (2.0 mg, 0.53 mmol) was dissolved in 270 μL THF and 217 μL water, further mixed with 53 μL Cu^{2+} solution (0.1 M, 1.0 eq). The mixture was shaken at room temperature. The course of the reaction was monitored by thin layer chromatography (TLC) assay in PE/EA (3 : 2) solution until completion within minutes. In order to explore the nature of the mechanism of Cu^{2+} sensing, TLC assay was developed by comparing five spots including **DPP**, **DPP** mixed with system, system mixed with **DHP** and **DHP** only, respectively. Pictures of the TLC plate were taken under UV lamp (365nm) and shown in Fig. S9. The mixture was then collected for HRMS, and a peak at 268.1374 was found and assigned to **DHP** ($[M+H^+]$).

DPP react with $Cu(CH_3CN)_4PF_6$: DPP (4.0 mg, 1.07 mmol) and $Cu(CH_3CN)_4PF_6$ (4.0 mg, 1.07 mmol) was dissolved in degassed solution of 1080 μL THF and 1080 μL water. The mixture was kept under argon and shaken at room temperature. The course of the reaction was monitored by TLC assay in PE/EA (3:2) solution after 1 h. TLC assay was developed by comparing five spots including **DPP**, **DPP** mixed with system,

system, system mixed with **DHP** and **DHP** only, respectively. Pictures of the TLC plate were taken under UV lamp (365 nm) and shown in Fig. S10.

LC-MS analysis:

The probe reacting with Cu^{2+} was analyzed by liquid chromatography-mass spectrometry (LC–MS) instrument (Bruker Impact II LC–MS/MS, UPLC Dionex U3000) using the following procedure. Sample **DHP** or **DPP** was dissolved at a concentration of 5 mmol in THF/H_2O (1:1). System of picolinic acid (**PA**) -Cu^{2+} (labeled as **PACU**) was prepared with 5 mmol of **PA** and 10 mmol of $Cu(ClO_4)_2$ in THF/H_2O (1:1). System of **DPP**-Cu^{2+} was prepared with 5 mmol of **DPP** and 10 mmol of $Cu(ClO_4)_2$ in THF/H_2O (1:1) and incubated for 15min before the measurement .Then, 10 μL of each sample was injected into a Thermo C18 HPLC column (250 mm × 4.6 mm; particle size, 5 μm) and chromatographed using solution of water/CH_3CN (60/40, containing 0.1% CH_3OOH) for 20 min at a flow rate of 850 μL/min. UV absorption was detected at 264 nm for **PACU** and the **DPP**-Cu^{2+} system, 430 nm for **DHP** and **DPP**. Mass spectra were recorded on a Bruker Impact II mass spectrometer (electron spray ion source, Germany) coupled with a Dionex U3000 UPLC system.

5. Cytotoxicity and Cell Imaging Studies of DPP

Cell Culture and MTT assay: HeLa human cancer cell lines were purchased from American Type Cell Collection. Cells were cultured in DMEM, supplemented with 10 % FBS, 1 % penicillin, 1% streptomycin sulfate in a humidified 5 % CO_2/95 % air incubator at 37 °C. The growth medium was replaced every two days. Cells were routinely detached with trypsin-EDTA solution and then seeded in a 25 mL cell culture bottle. The cells were reached about 80 % confluence prior to experiments. MTT assay of cytotoxicity of **DPP** to HeLa cells was performed by MTT assay method. HeLa cells were seeded at a density of 5×10^4 cells/mL in a 96-well micro-assay culture plate. After growth at 37 °C in a 5 % CO_2 incubator for 24 h, the culture medium was removed and washed with PBS solution thrice and further replaced with the freshly prepared medium containing different concentrations of **DPP**, respectively. The group with the addition of culture medium only was set as the control, and the wells containing culture

media without cells were used as blanks. After incubation at 37 °C in a 5 % CO_2 incubator for 24 h, cell culture medium was removed, and cells were carefully washed three times with PBS. Then, the MTT solution in PBS (100 μL, 0.5 mg/mL) was added into each well for further incubation for 4 h. The excess MTT solution was then carefully removed from each well, and the formed formazan was dissolved in 100 μL of dimethyl sulfoxide (DMSO). The absorbance at 490 nm was measured in a Spark 10M multimode microplate reader. The results from the five individual experiments were averaged. The following formula was used to calculate the viability of cell growth: Vialibity (%) = (mean of absorbance value of treatment group-blank)/(mean absorbance value of control-blank) × 100.

Luminescence Imaging of Cu^{2+} in Live HeLa cells

For luminescence cell imaging, stock solution of **DPP** (10 mM) was prepared in DMSO. HeLa cells were typically seeded at a density of 5×10^4 cells/mL in a cover glassbottomed cell culture dish (ϕ = 20 mm) for the luminescence microscopic cell imaging. After 24 h growth, the culture medium was removed, and the cells were further incubated with freshly prepared medium containing different concentration of Cu^{2+} (10 μM, 20 μM). The cells were incubated at 37 °C in a 5 % CO_2/95 % air incubator for 1 h. The excess Cu^{2+} was discarded, and the cells were washed with PBS for three times and then treated with **DPP** (10 μM, 0.2 % DMSO as the co-solvent) for another 30 min incubation time. The cells were further washed with PBS for three times before being subjected to the luminescence imaging measurements. For comparison, cells pre-treated with 10 μM **DPP** only for 0.5 h were also prepared and used for the imaging measurements.

For endogenous Cu^{2+} imaging, the cells were incubated with freshly prepared medium containing 500 μM of 3,6-dithia-1,8-octanediol as a Cu-chelator for 2h.[2] The cells were washed with PBS for three times and then treated with **DPP** (10 μM, 0.2 % DMSO as the co-solvent) for another 30 min incubation time. The cells were further washed with PBS for three times before being subjected to the luminescence imaging measurements. For comparison, cells pre-treated with 10 μM **DPP** only for 0.5 h were prepared and used for the control of measurements.

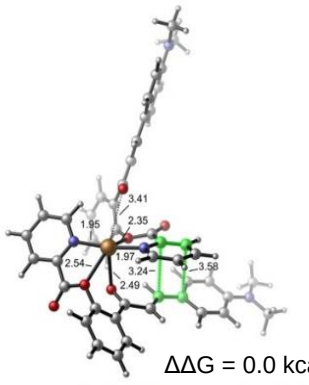
6. Determination of The Detection Limit

The detection limit was calculated based on the fluorescence titration. Fluorescence emission spectrum of probe **1** was measured by thirty times and the standard deviation (σ) of this blank measurement was achieved. The slope (k) was derived from the calibration curve for quantitative analysis of Cu^{2+}. The detection limit was determined with the following equation: detection limit = $3\sigma/k$. (30 nM)

7. Computational details and computed structures

The M06 density functional and a mixed basis set of LANL2DZ for Cu and 6-31G(d) for other atoms were used in the geometry optimizations. All minima have zero imaginary frequency. Single-point energies were calculated by using M06 and a mixed basis set of SDD for Cu and 6-311+G (d, p) for other atoms. Solvation energy corrections were calculated using the SMD model with water as the solvent. All these calculations were carried out with Gaussian 09.[1]

A series of Cu^{2+} complexes with two **DPP** molecules were computed. The coordination number of these complexes vary from 4 to 6. The most stable one is **DPPCU** with 5-coordninated Cu center. The significant π···π stacking is critical for the stability of the complex **DPPCU**. Other less stable complexes were shown in Figure S1.



The most stable complex **DPPCU**

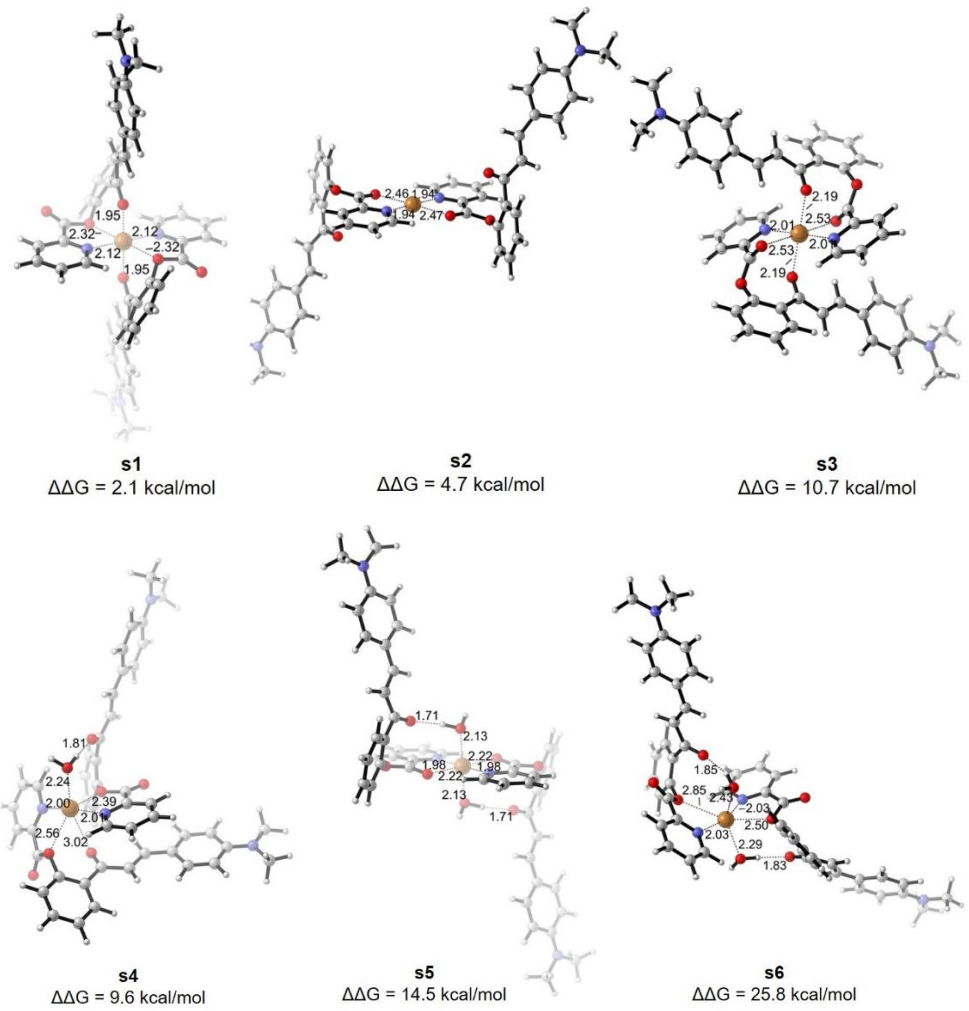


Figure S1. Computed complexes of Cu^{2+} with **DPP** and/or H_2O.

1. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian 09, Revision D.01; Gaussian, Inc.: Wallingford, CT, 2009.

8. Supplementary Figures (S2-S16)

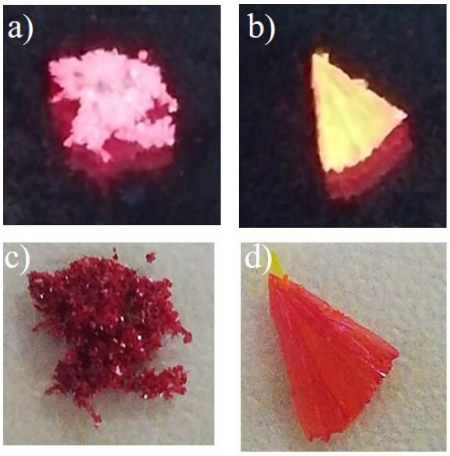


Figure S2. Photographs of solid compounds **DHP** (a, c) and **DPP** (b, d) under 365 nm UV irradiation (a, b) and daylight (c, d).

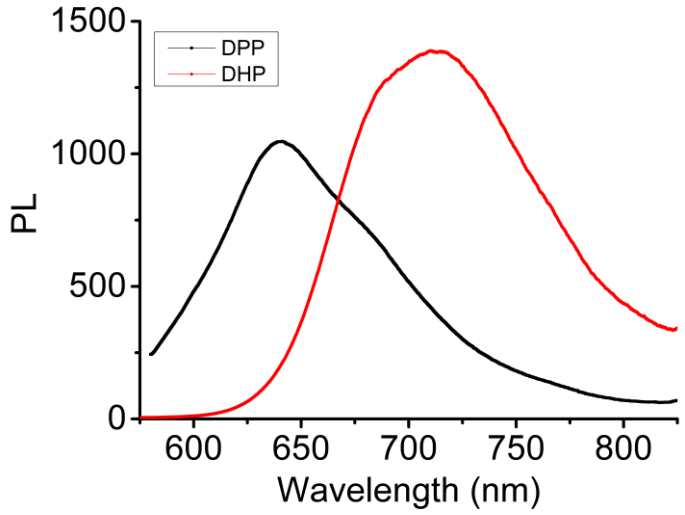


Figure S3. Luminescence spectra of solid **DDP** (black line, Ex = 565 nm, Em = 640 nm) and **DHP** (red line, Ex = 552 nm, Em = 710). Slit: 5 nm/5 nm.

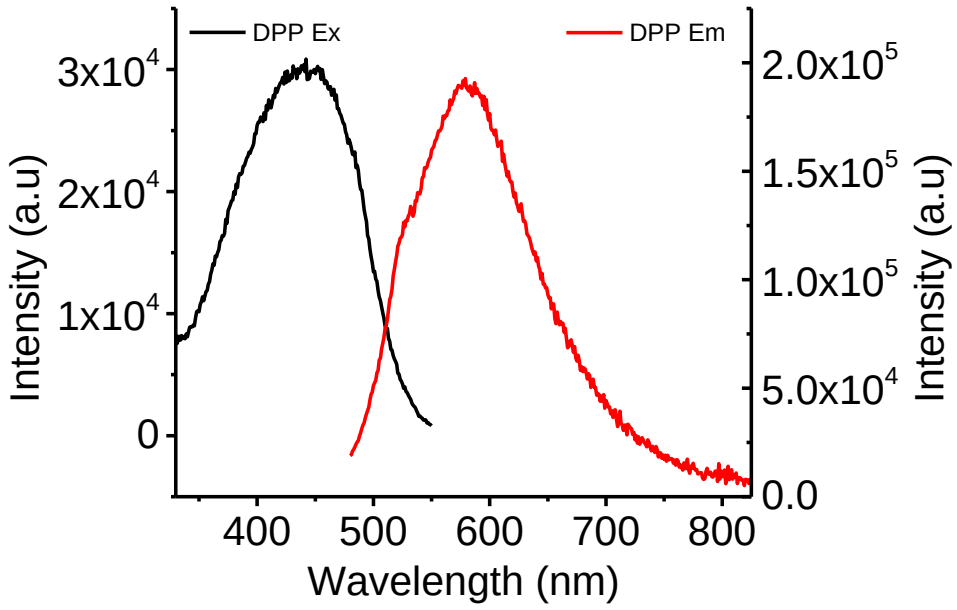


Figure S4. Excitation (black line) and emission (red line) spectra of **DPP** (20 μM) in PBS solutions (0.2% DMSO). Slit: 3 nm/3 nm.

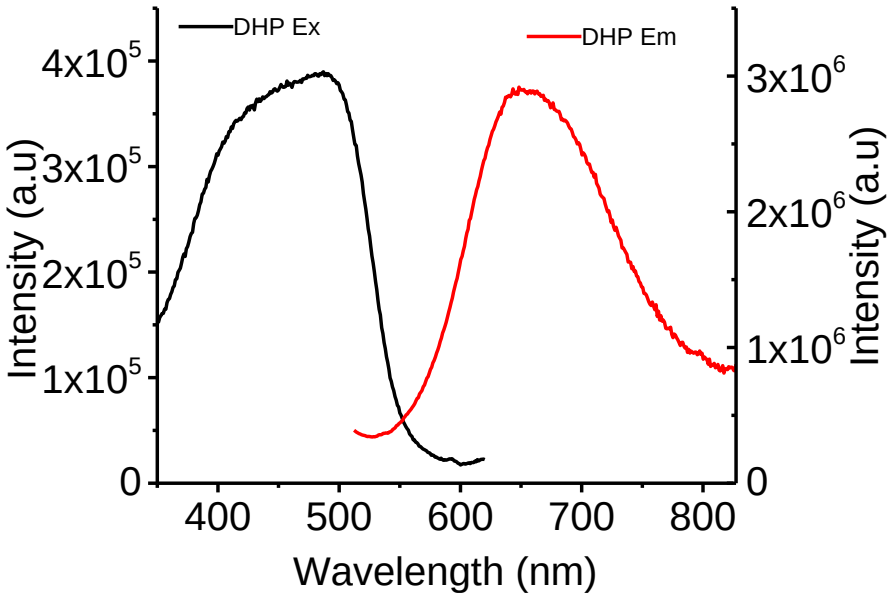


Figure S5. Excitation (black line) and emission (red line) spectra of **DHP** (20 μM) in PBS solutions (0.2% DMSO). Slit: 3 nm/3 nm.

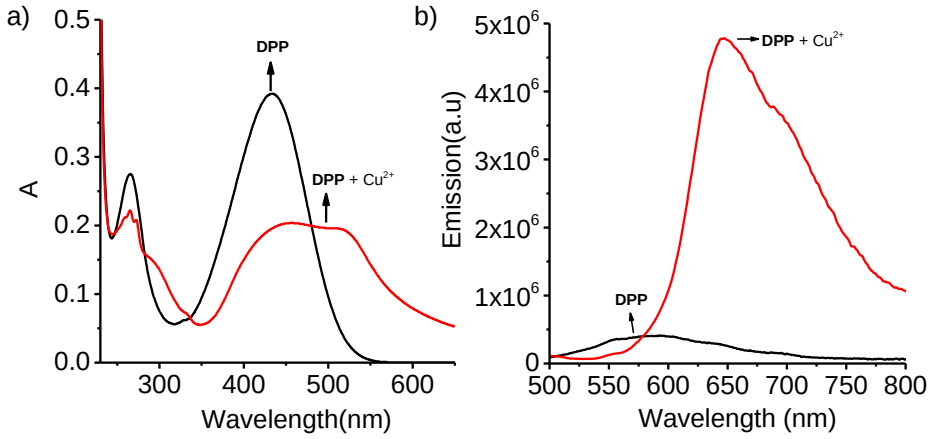


Figure S6. a) UV-vis absorption spectra and b) fluorescence spectra of probe **DPP** in the absence and presence of Cu^{2+} in PBS buffer solutions (pH = 7.4, containing 0.2 % DMSO). [Cu^{2+}] = 20 μM, [**DPP**] = 20 μM.

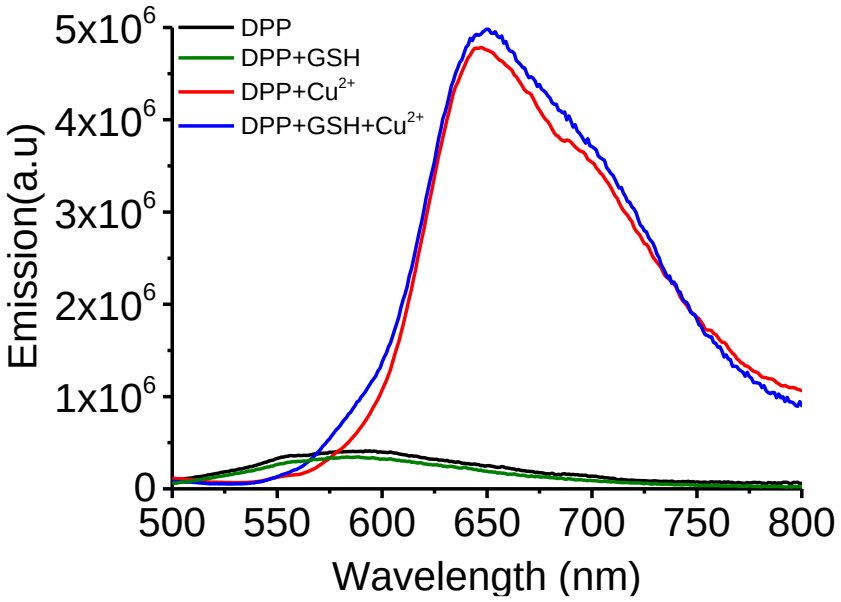


Figure S7. Fluorescence responses of **DPP** (20 μM, ─) towards GSH (20 μM) with (─) or without (─) Cu^{2+} (40 μM) in PBS buffer solutions (pH = 7.4, containing 0.2 % DMSO). Red line represents **DPP** (20 μM, ─) towards Cu^{2+} (20 μM).

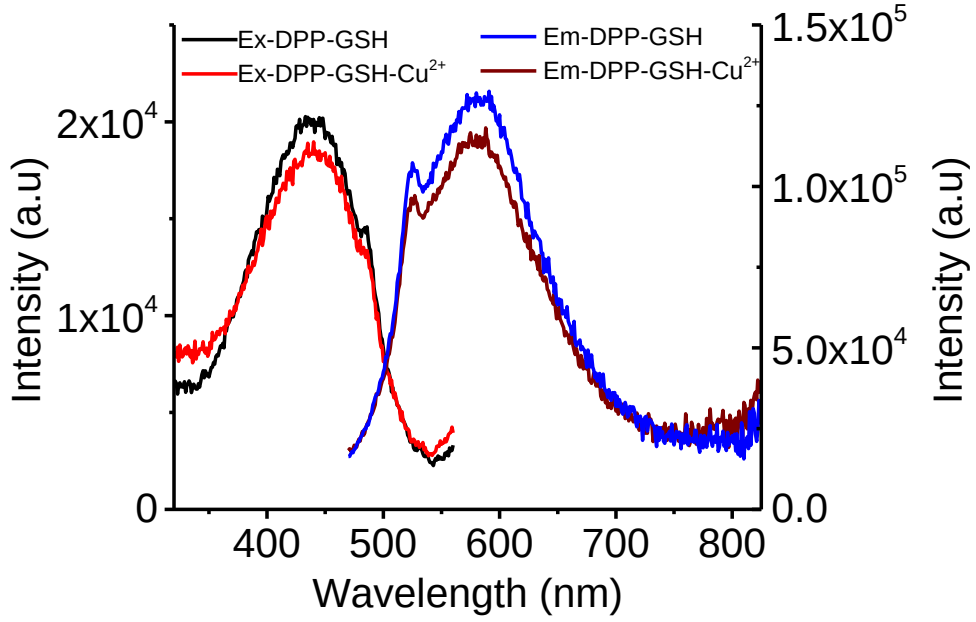


Figure S8. Fluorescence responses of **DPP** (20 μM) towards GSH (2 mM) w/o Cu^{2+} (20 μM) in water (containing 0.2 % DMSO).

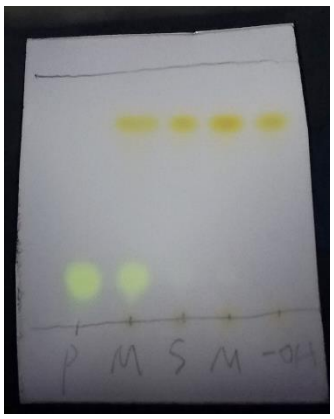


Figure S9. Photo of TLC plate for the reaction between **DPP** and Cu^{2+} under UV lamp (365 nm). From left spot to right one: **DPP** (spot P), **DPP** mixed with **DPP**-Cu^{2+} system (spot M), **DPP**-Cu^{2+} system (spot S)**, DHP** mixed with **DPP**-Cu^{2+} system (spot M), **DHP** (spot -OH). PE : EA = 3:2.

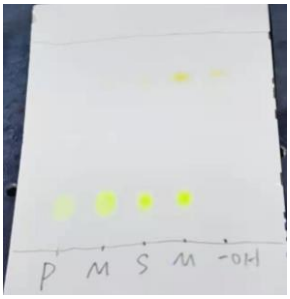


Figure S10. Photo of TLC plate for the reaction between **DPP** and $Cu(CH_3CN)PF_6$ under UV lamp (365 nm). From left spot to right one: **DPP** (spot P), **DPP** mixed with **DPP**-Cu^{+} system (spot M), **DPP**-Cu^{+} system (spot S)**, DHP** mixed with **DPP**-Cu^{+} system (spot M), **DHP** (spot -OH). PE : EA = 3:2.

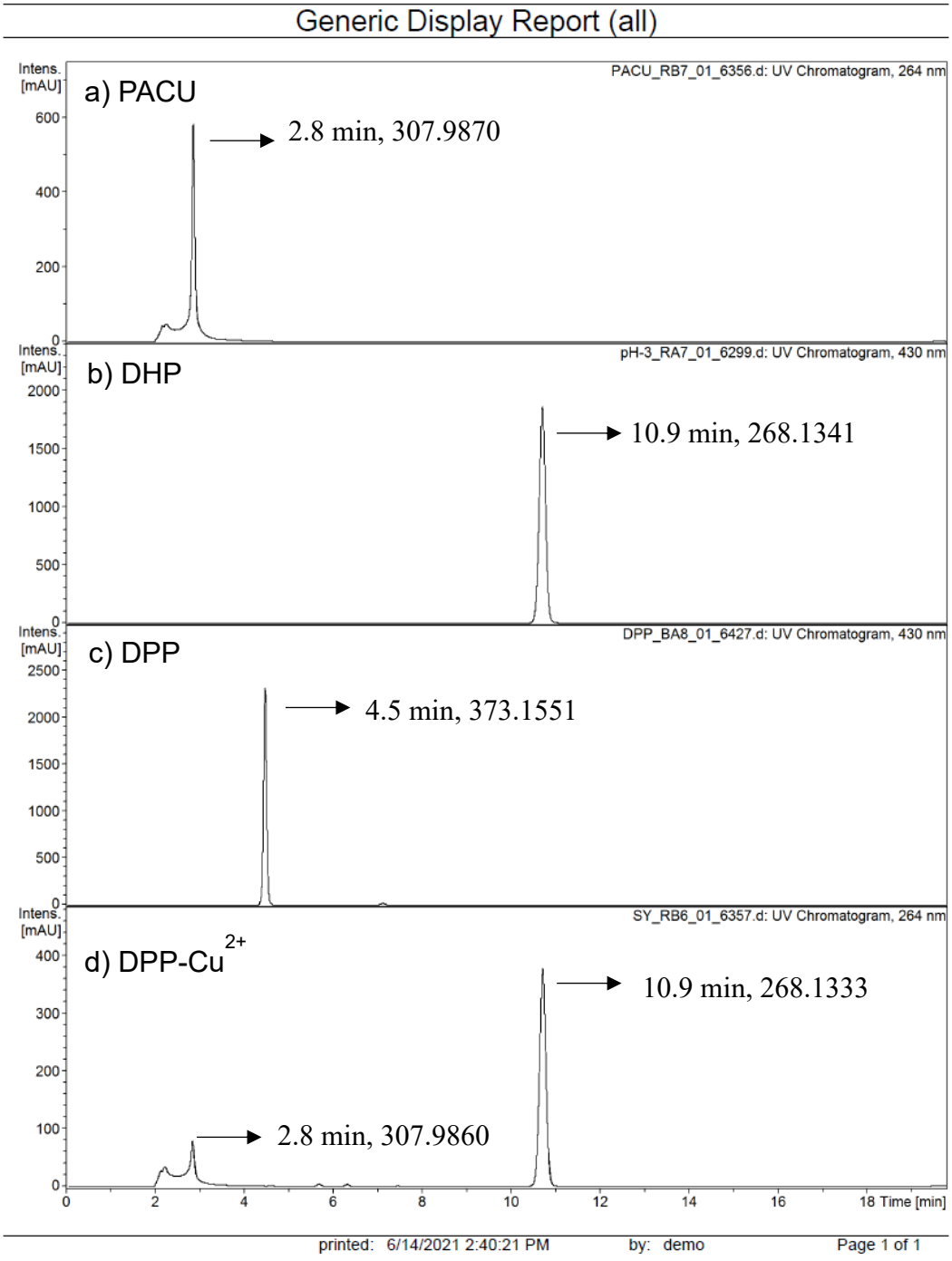


Figure S11. LC-MS analysis with retention time for the reaction between **DPP** and $Cu(ClO_4)_2$ under UV detector. a) **PACU** (picolinic acid- Cu^{2+} 1:2), b) **DHP**, c) **DPP**, d) **DPP** incubated with Cu^{2+} system (1 : 2). All the samples are prepared at a concentration of 5 mmol in THF/H_2O (1:1).

Table S1. LC-MS analysis data with retention time for the reaction between **DPP** and $Cu(ClO_4)_2$.

Name	Time/min	Formula	Found
PACU	2.8	$C_{12}H_8CuN_2O_4$	307.9870 $[M+H]^+$
DHP	10.9	$C_{17}H_{17}NO_2$	268.1341 $[M+H]^+$
DPP	4.5	$C_{23}H_{20}N_2O_3$	373.1551 $[M+H]^+$
DPP-Cu^{2+}	2.8		307.9860
	10.9		268.1333

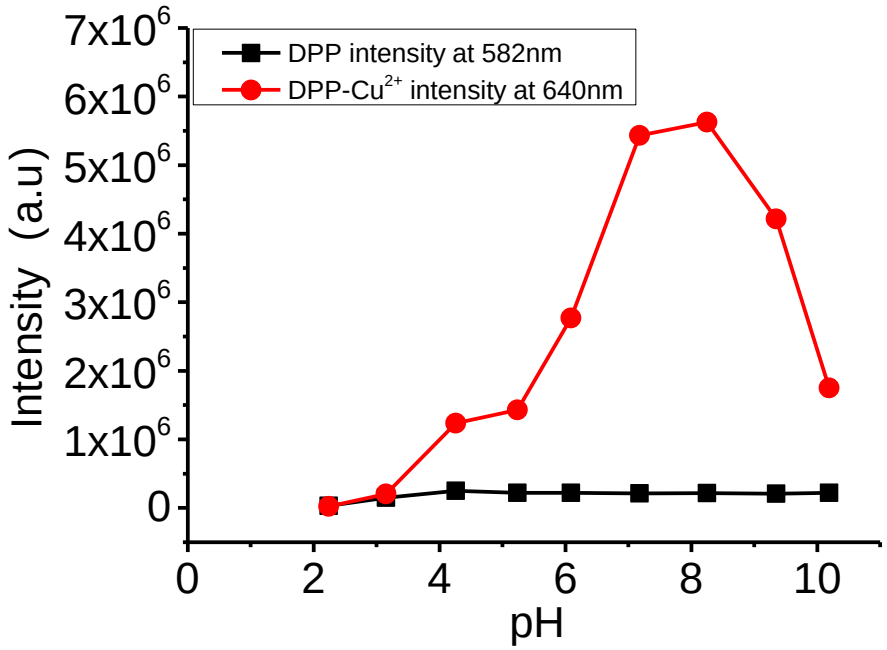


Figure S12. The fluorescence intensity of 20 μM **DPP** (at 582nm) and **DPP**–Cu^{2+} (at 640nm) as a function of pH in aqueous solutions (containing 0.2 % DMSO). Each spectrum was acquired within 20 min until the intensity was stable.

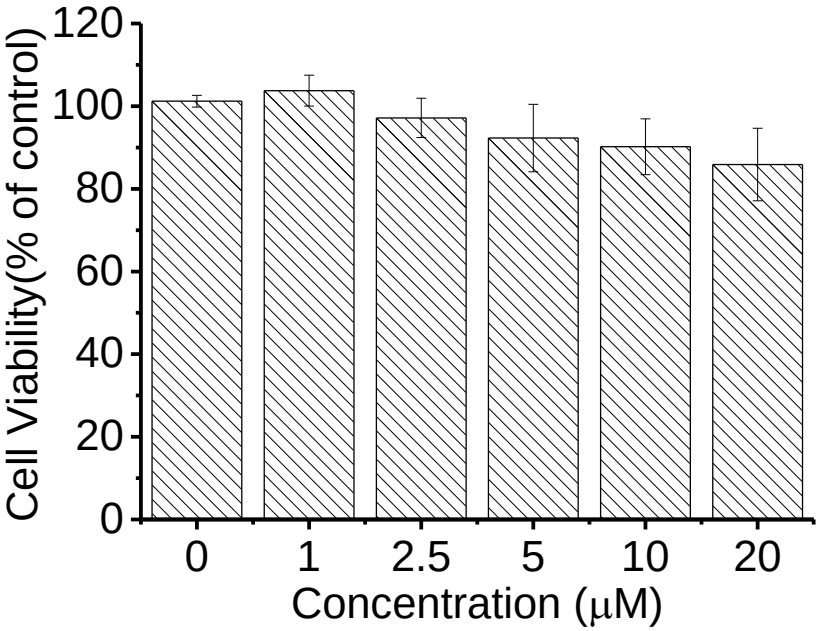


Figure S13. Cell viabilities of **DPP** at various concentrations for HeLa cells after 24 h incubation.

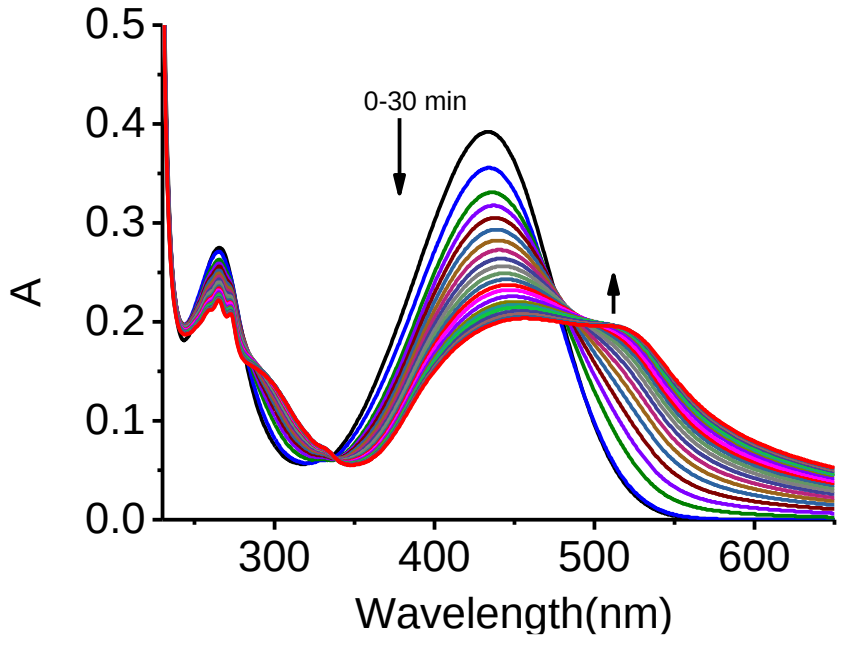


Figure S14. a) Time-dependent of UV-vis absorption spectra of probe **DPP** after treated with Cu^{2+} in PBS buffer solutions (pH = 7.4, 0.2 % DMSO). [Cu^{2+}] = 20 μM, [**DPP**] = 20 μM.

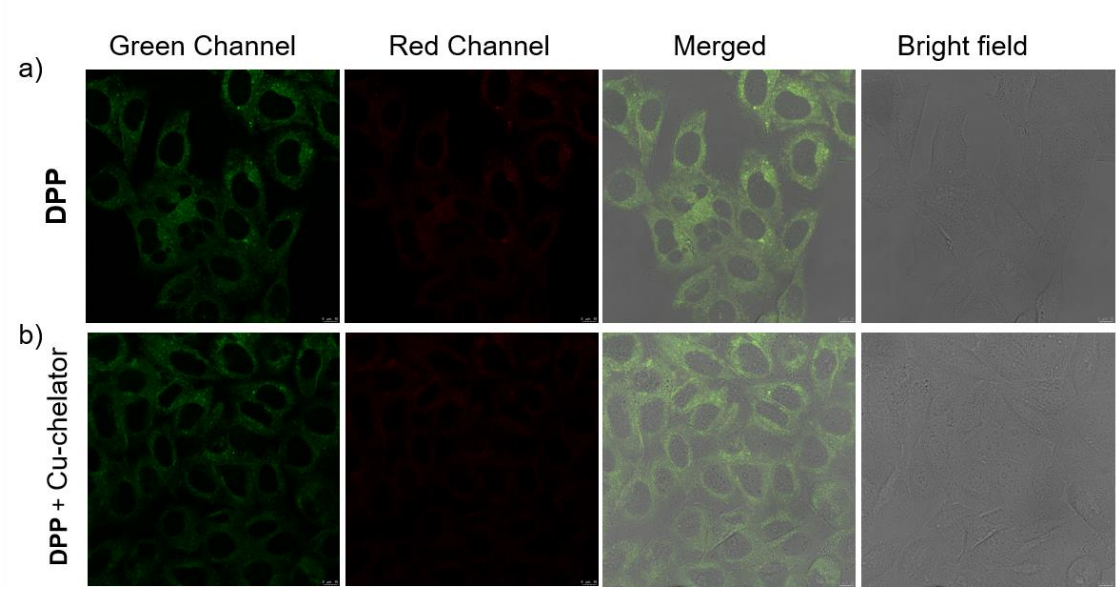


Figure S15. Fluorescence image of HeLa cells treated by **DPP** (10 μM) without **a)** / with **b)** 500 μM 3,6-dithia-1,8-octanediol as a Cu-chelator, respectively. Green channel: λem = 550–610 nm; Red channel: λem = 640–680 nm. Scale bar: 10 μM.

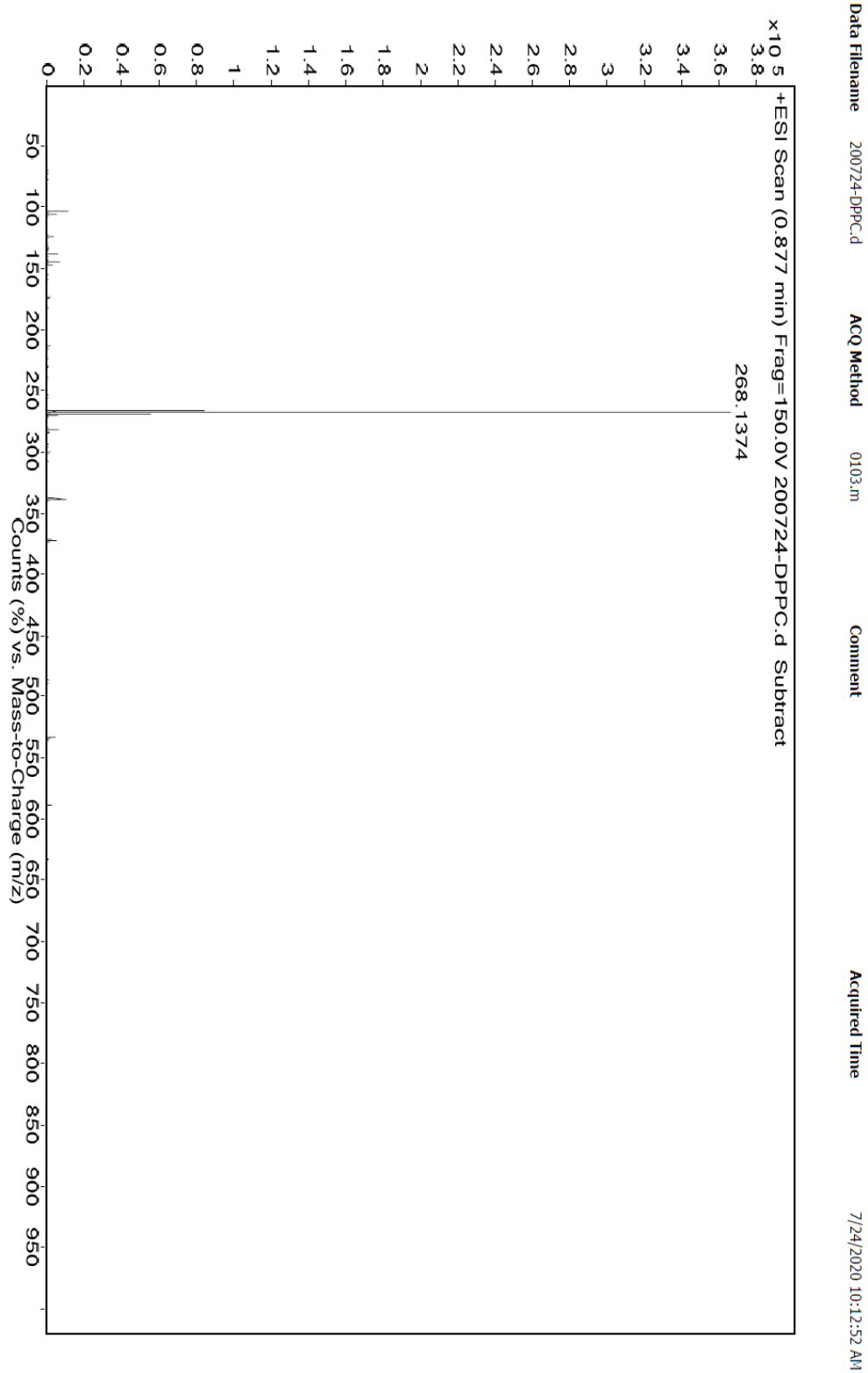
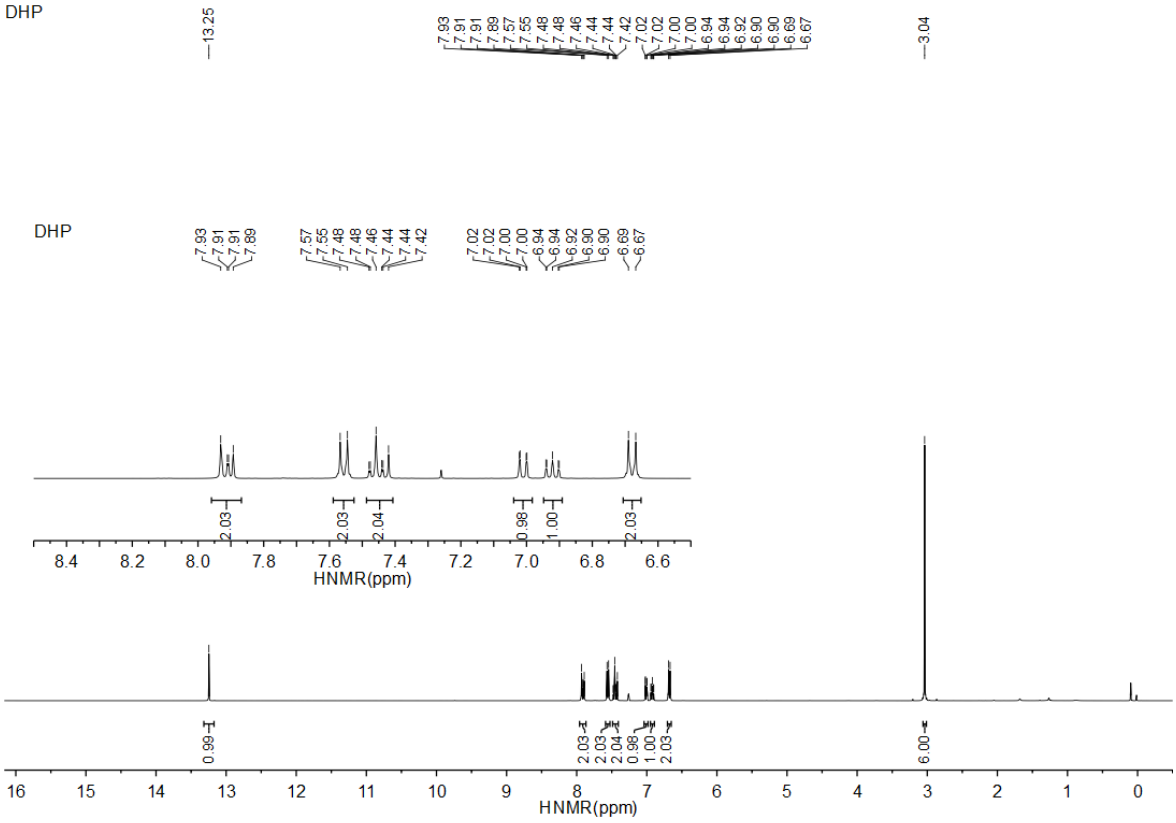
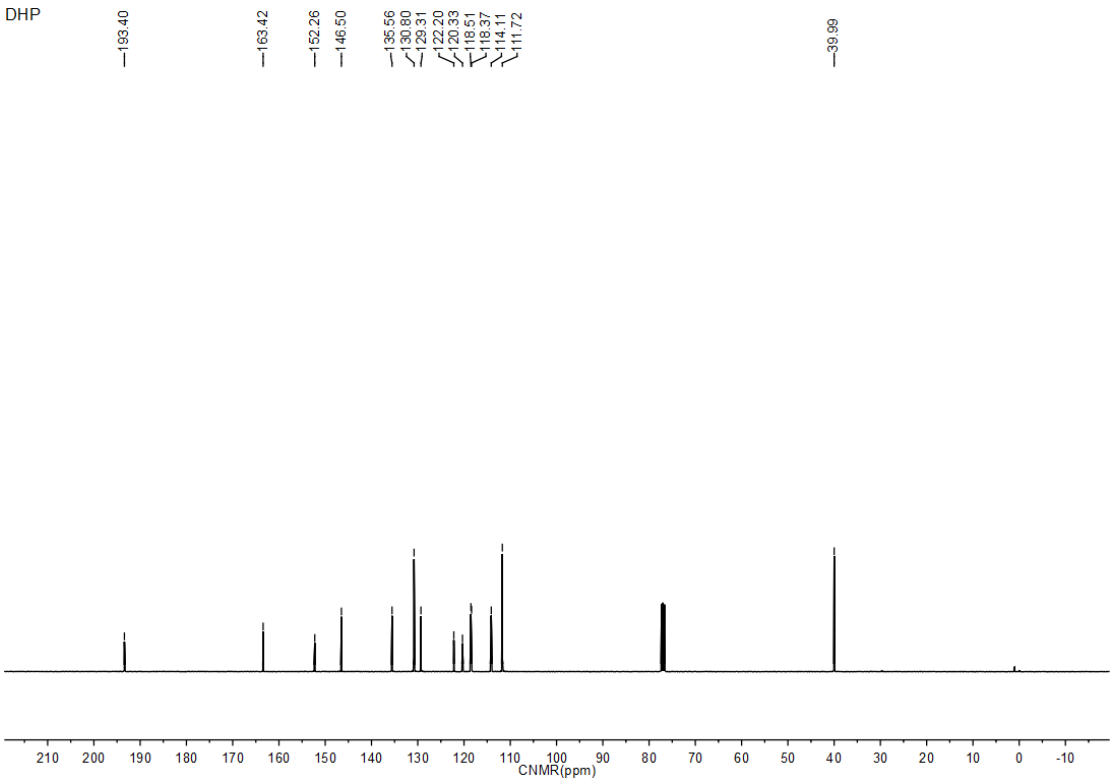


Figure S16. ESI-MS spectra of **DPP** upon addition of Cu^{2+}.

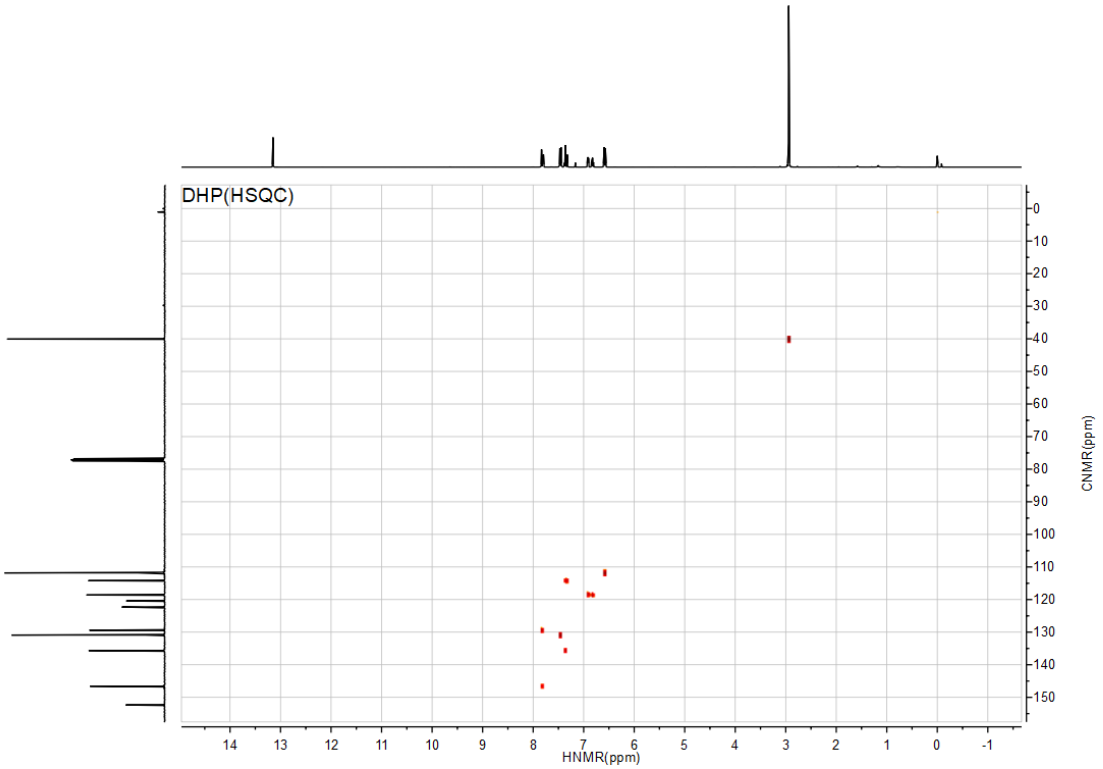
DHP ^{1}H NMR



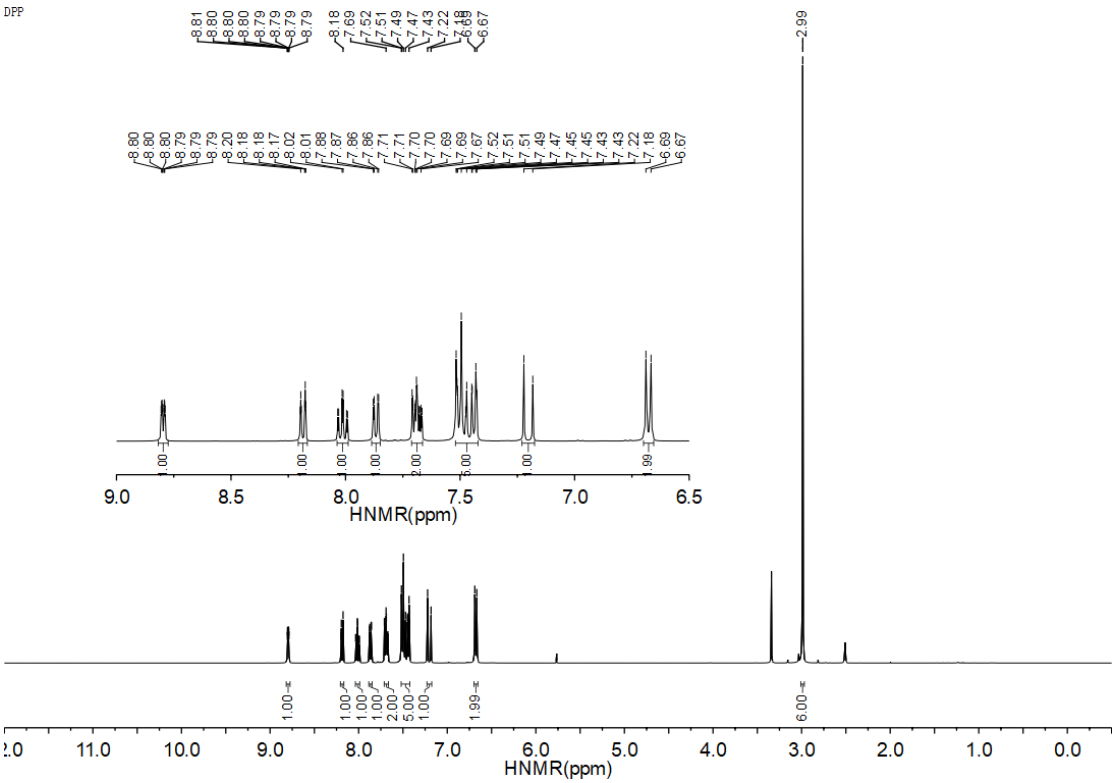
DHP ^{13}C NMR



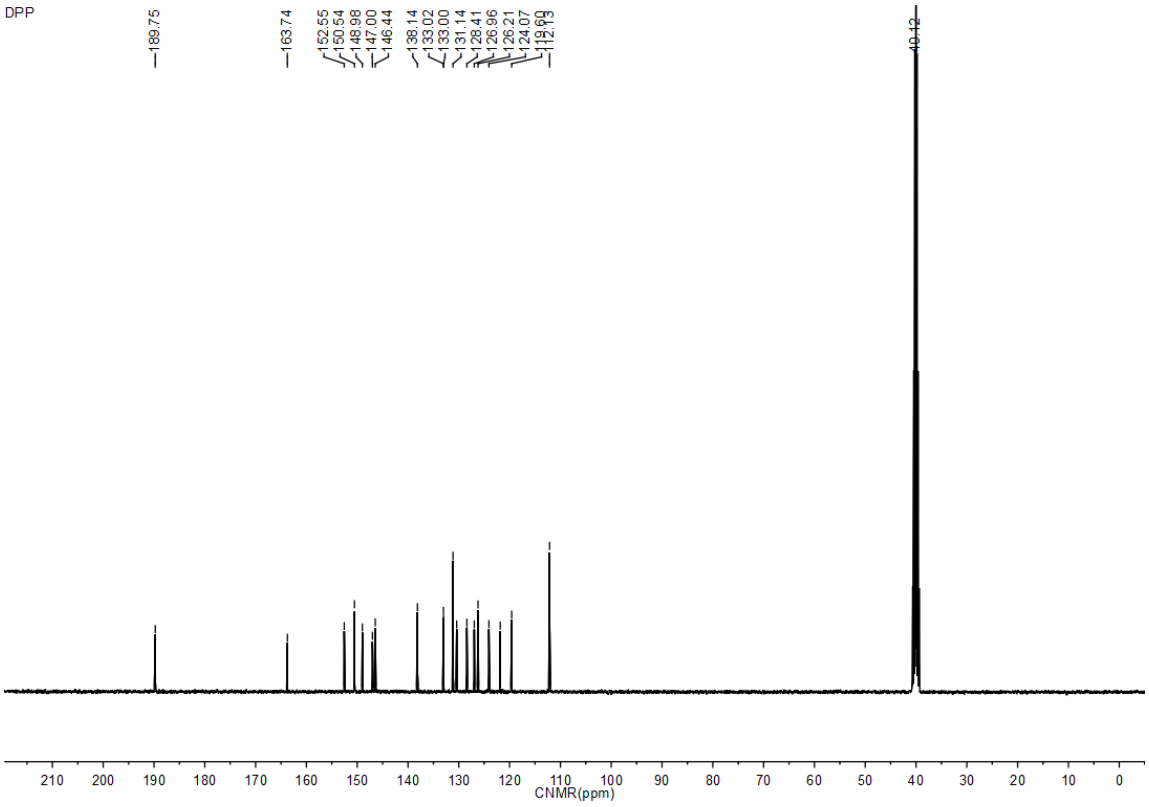
DHP HSQC



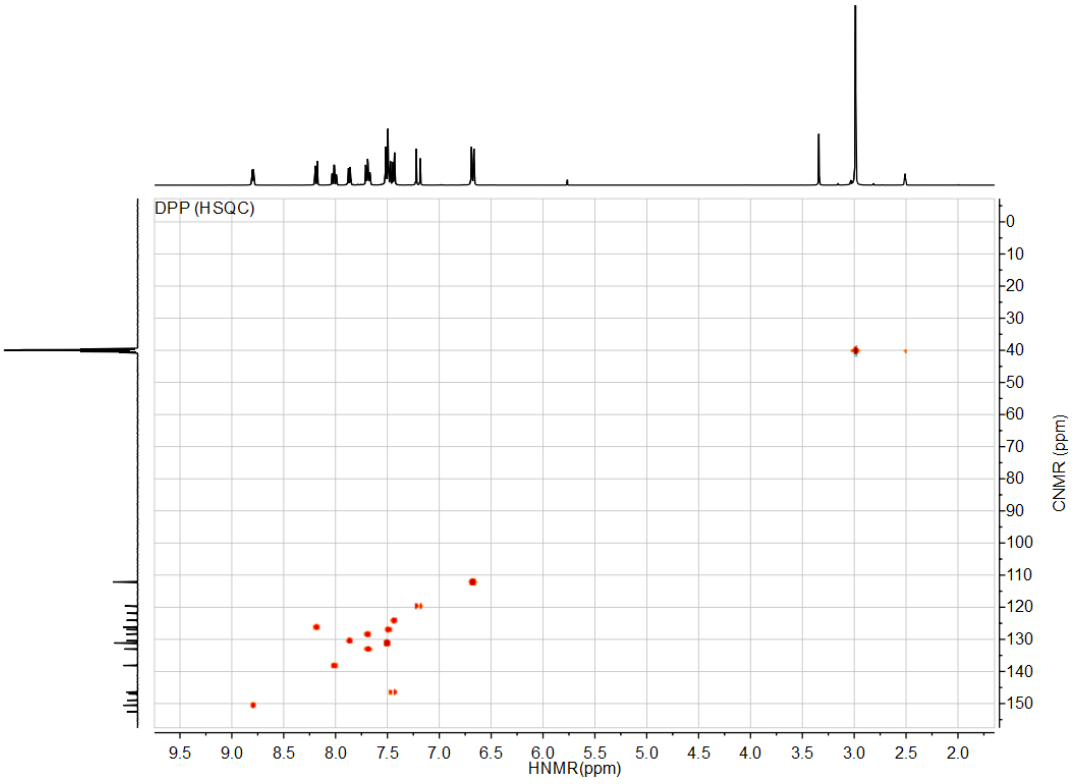
DPP ^{1}H NMR



DPP ^{13}C NMR



DPP HSQC



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