Electronic Supplementary Information

Monitoring of Au^{3+} in plant with a ratiometric fluorescent probe

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Contents

1. Reagents and Apparatus

All reagents and solvents were commercially purchased. Thin-layer chromatography (TLC) was performed on efficient silica gel plates. Chromatographic purification of chemical products by using 300-400 mesh chromatography silica gel. ^{1}H NMR and ^{13}C NMR spectra were recorded in the particular solvent stated by using Bruker DRX-400. Mass spectra were carried out by Mass Spectrometry Facility at Nanjing University. The infrared spectra were received by fourier transform infrared spectrometer and the type was NEXUS870. All fluorescence measurements were recorded on Hitachi Fluorescence Spectrophotometer F-7000. The imaging experiments were performed by using two-photon confocal fluorescent microscope (Leica TCS SP8 MP, Nanjing University). All pH measurements were carried out on pH meter of PHS-25.

The metal chloride salts were dissolved in distilled water for obtaining the stock solutions of metal ions: Ca^{2+}, Co^{2+}, Au^{+}, Cu^{2+}, Fe^{2+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Hg^{2+}, Pt^{2+}, Zn^{2+}, Pd^{2+}, Al^{3+} and Au^{3+} (1 mM). Other concentrations of metal ions can be obtained by gradual dilution. All the tested anions were prepared from NaCl, NaF, $NaHCO_3$, $NaHSO_4$, NaSCN, Na_2S, and $NaHSO_3$ with ddH_2O. Probe was dissolved in DMSO to get a 1.0 mM stock solution. 10×PBS buffer, pH 7.4, without calcium & magnesium was purchased from Wisent Bio Product (components: KH_2PO_4, NaCl, Na_2HPO_4); CTAC (Hexadecyltrimethylammonium chloride) was purchased from Aladdin, which was dissolved in distilled water to get a stock solution (100 mM).

The testing system for the effect of Au^{3+} ions on absorption and emission spectroscopic properties towards probe AuP was set: 2 μL of probe stock solution, 2 μL of CTAC stock solution, 10 μL metal ions stock solution, 100 μL PBS buffer (pH 7.4), and then added distilled water to finally get a 200 μL solution system. For all measurements, the excitation wavelength was set at 457 nm; both excitation and emission slit widths were set at 10 nm; the photomultiplier voltage was set at 550 V. After incubating with various analytes for 2 min, the emission spectrum was measured and scanned from 500 nm to 800 nm at 1200 nm/min by fluorescence spectrophotometer.

2. Arabidopsis Thaliana culture and imaging

The wild type of *Arabidopsis Thaliana* was used, the seeds were dipped in 1 mL of 75% alcohol for 10 min. Medium configuration contained 50% basal medium with vitamins (MS), 1% sucrose and 1% agar. Set the culture medium with potassium hydroxide as pH 5.8 at the same time. The seeds were

cultivated in the prepared culture medium. It was encased with tin foil, and then placed in the refrigerator at 4 ℃ for two days. Remove the tin foil, transferred in the incubator to shine the light for 5 days. The 5 days-old *Arabidopsis* root tissues were selected to perform the imaging experiments.

3. Determination of fluorescence quantum yield

The fluorescence quantum yield Φ_u was estimated through participation ratio method, where using the ethanol solution of rhodamine B (10 μM, Φ = 0.69, λ_{ex} = 365 nm) for the sample and reference. Through testing the absorption and fluorescence spectra of **AuP** (10 μM), the fluorescence quantum yield was calculated using equation as follows:

$\Phi_u = [(A_s F_u n^2)/ (A_u F_s n_0^2)]\Phi_s$.

Φ_s is the quantum yields of reference substance, A_s and A_u represents the absorbance of the reference and testing sample at the excitation wavelength, F_s and F_u refer to the integrated emission band areas under the same conditions, n and n_0 are the solvent refractive indexes of determined and reference, respectively. In the process of detection should control the absorbance to be lower than 0.05.

Quantum yield: Φ = 0.47.

4. The limit of detection (LOD) of AuP

The emission spectrum of free **AuP** in PBS buffer (10 mM, 1% DMSO, 1 mM CTAC, pH 7.4) was collected for 25 times to confirm the background noise σ. The linear regression curve was then fitted according to the data in the range of Au^{3+} from 0 to 10 μM and obtained the slope (Figure S5). The detection limit (3σ/slope) was then determined to be 1.54 μM.

5. Detection of Au^{3+} ions with AuP in real water samples

Au^{3+} (50 μM) was previously spiked into the real-water samples: Tap water, Surface water from the Yangtze River, Rice sample buffer (200 mg/5 mL), and then incubated with **AuP** (10 μM, 1% DMSO, 1 mM CTAC) for 2 min at 25 ℃, the fluorescence intensity was measured and their ratios (I_{574}/I_{636})

were calculated. The results reveal that this probe is suitable for detecting Au^{3+} in real-environmental samples.

6. Determination of Au^{3+} with a testing paper

The circular testing paper was firstly soaked into **AuP** (1 mM) solution and then evaporated to remove the solvent in the filter paper by dry naturally. Subsequently, various metal ions solutions (1 mM) were added dropwisely to the testing paper. The visual colors and visual fluorescence colors of testing paper were imaged under visible light and irradiation by a 365 nm UV lamp, respectively.

7. Synthesis of compounds

*Synthesis of 2-(3, 5, 5-trimethylcyclohex-2-en-1-ylidene) malononitrile (**2**)*

Malononitrile (1.82 g, 27.6 mmol) was dissolved in anhydrous ethanol (100 mL), then piperidine (0.23 g, 2.76 mmol) and isophorone (**1**) (3.8 g, 27.6 mmol) were added into the above solution. The result mixture was refluxed at 65 ℃ for 8 h. After cooling down to room temperature, the mixture was poured into ice-water, and the collected precipitate was further recrystallized by anhydrous ethanol (15 mL) to afford brown crystal. Yield: 1.2 g (24%). M.p. 72-74 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.60-6.62 (m, 1H), 2.50(s, 2H), 2.19(brs, 2H) 2.04 (brs, 3H), 1.02(s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 170.5, 160.1, 120.5, 113.2, 112.4, 78.0, 45.6, 42.6, 32.3, 27.8, 25.3.

*Synthesis of 2-(3-(4-aminostyryl)-5, 5-dimethylcyclohex-2-en-1-ylidene) malononitrile (**3**)*

Compound **2** (759 mg, 4.08 mmol) and *p*-aminobenzaldehyde (492 mg, 4.08 mmol) were dissolved in a solution of anhydrous ethanol (50 mL), then piperidine (34.8 mg, 0.408 mmol) was added, the resulted mixture was further refluxed at 85 ℃ for 8 h. The solution was extracted, the organic phase was dried with anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (PE: EA = 10:1, V: V) to afford compound **3** as dark red solid. Yield: 423 mg (46.1%). M.p. 130-132 ℃.^{1}H NMR (400 MHz, $CDCl_3$): 7.37 (d, *J* = 8.6 Hz, 2H), 7.01 (d, *J* = 15.96 Hz, 2H), 6.80 (d, *J* = 16 Hz, 2H), 6.76 (s, 1H), 6.68 (d, *J* = 8.64 Hz, 2H), 4.01 (brs, 2H), 2.57 (s, 2H), 2.45 (s, 2H), 1.07 (s, 6H). ^{13}C NMR (CDCl3, 100 MHz) δ (ppm): 169.4,

155.1, 148.6, 137.8, 130.6, 129.5, 125.9, 125.2, 121.9, 115.1, 114.6, 114.1, 113.3, 76.5, 43.0, 39.2, 32.0, 28.0.

Synthesis of 2-(5, 5-dimethyl-3-(4-(prop-2-yn-1-ylamino) styryl) cyclohex-2-en-1-ylidene) malononitrile (AuP)

Compound **3** (400 mg, 1.75 mmol) and KI (348 mg, 2.1 mmol) was dissolved into anhydrous ethanol (5 mL), and then 3-bromoprop-1-yne (247 mg, 2.1 mmol) was added. The reaction mixture was further stirred at 85 ℃ for 12 h. After cooling down to room temperature, the solvent was concentrated under reduced pressure to afford crude product, extracted, and further purified by column chromatography on silica gel (PE: EA = 8:1 V: V) to afford 154 mg of corresponding compound **4** (**AuP**) as a dark red solid (33%) M.p.162-164 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): 7.4 (d, *J* = 8.6 Hz, 2H), 7.02 (d, *J* = 15.9 Hz, 2H), 6.83 (d, *J* = 16.0 Hz, 2H), 6.77 (s, 1H), 6.74-6.67 (m, 2H), 4.32 (s, 1H), 4.01 (d, *J* = 2.5 Hz, 2H), 2.59 (s, 2H), 2.46 (s, 2H), 2.28 (t, *J* = 2.4 Hz, 1H), 1.08 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 169.4, 155.1, 148.5, 137.8, 129.4, 126.0, 125.3, 121.9, 114.1, 113.5, 113.3, 80.2, 76.5, 71.8, 43.0, 39.2, 33.2, 32.0, 28.1.

8. Supplementary Figures

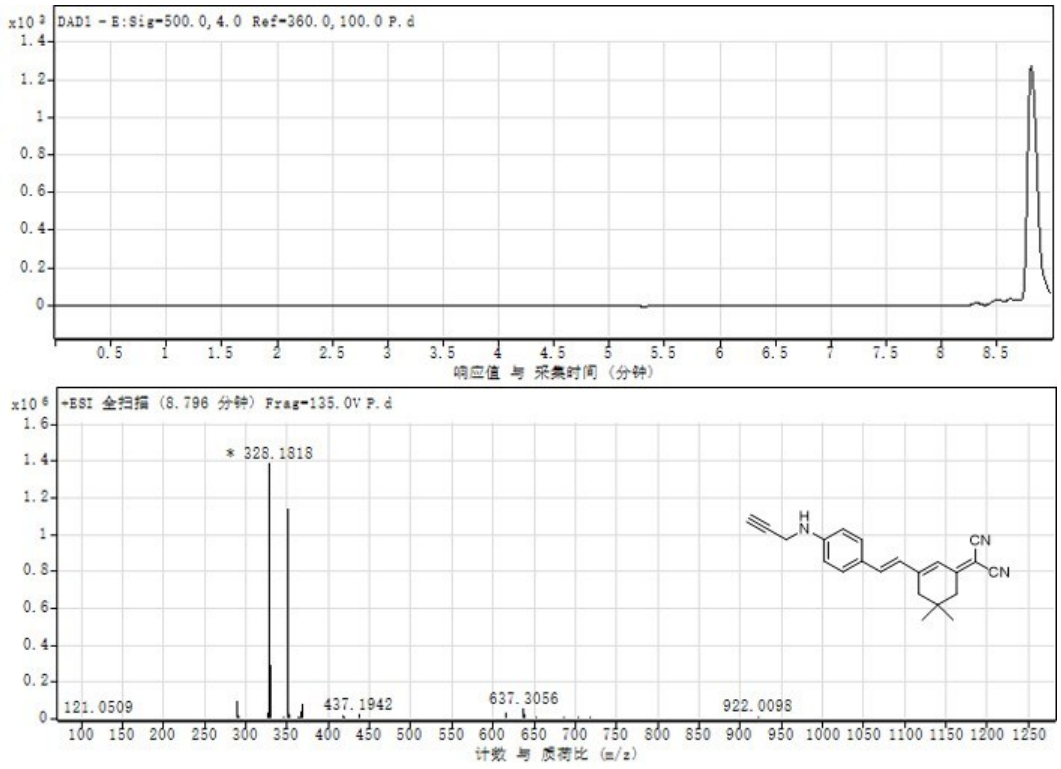
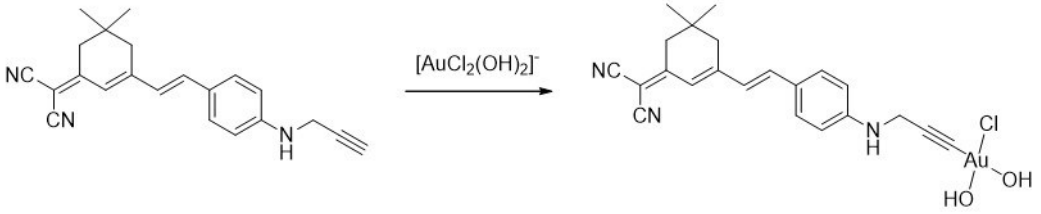


Fig.S1a. MS spectrum of **AuP** (calculated for $C_{22}H_{21}N_3$ $[M+H]^+$ 327.1735, found 328.1818. $C_{22}H_{21}N_3Na^+$ $[M+Na]^+$ 350.1633, found 350.1626). LC-HRMS was conducted using Agilent 6530 Accurate-Mass Q-TOF LC/MS with Agilent EC-C18 column, 2.7 μm, 4.6*50 mm. HPLC runs used a linear gradient from 70% H_2O/30% CH_3OH to 0% H_2O/100% CH_3OH over 6 min and then an extra 3 min with H_2O/100% CH_3OH. The current velocity is set at 0.4 mL/min.

(1) $HAuCl_4 \longrightarrow [AuCl_2(OH)_2]^-$ (ref: *Colloids and Surfaces A: Physicochem. Eng. Aspects* 146 (1999) 139-152; *Geochimica et Cosmochimica Acta* 73 (2009) 3359-3380)

(2)



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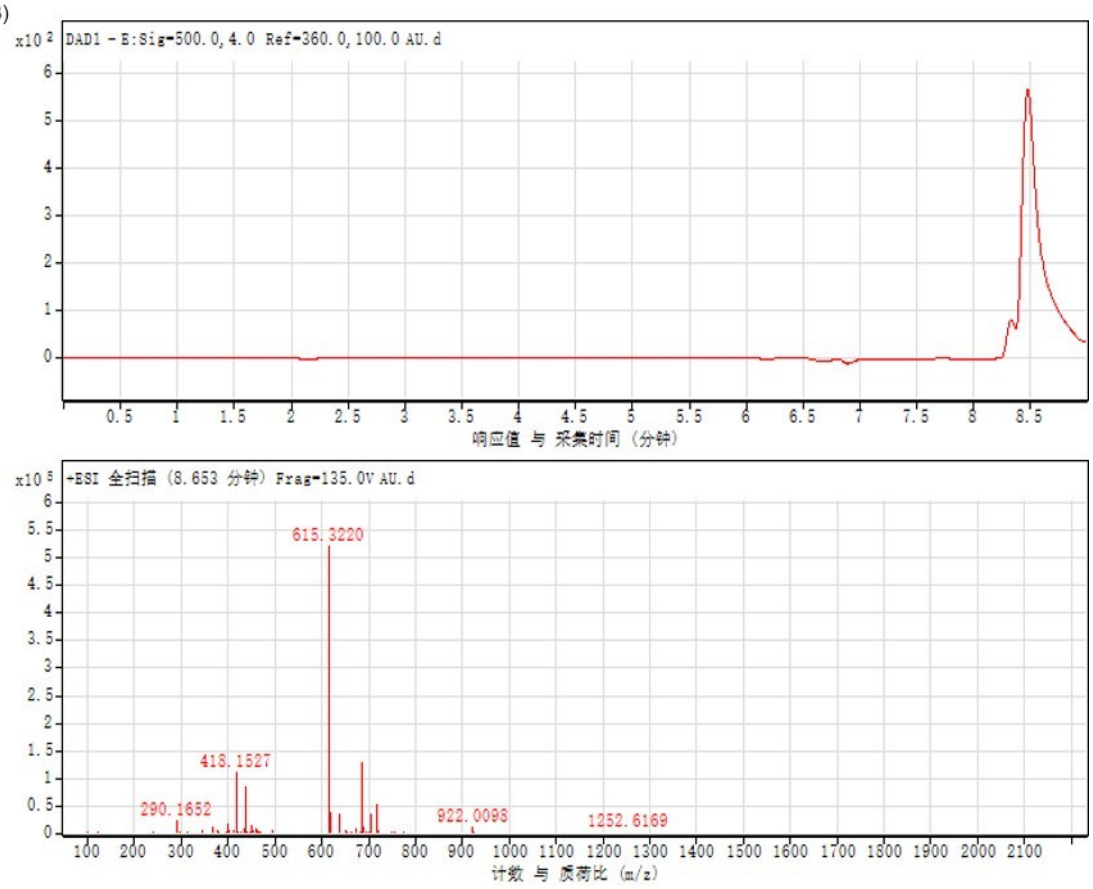

Fig.S1b. MS spectrum of **AuP** solution after incubation with Au^{3+}. (1). In aqueous solution (pH = 7) at room temperature, $[AuCl_2(OH)_2]^-$ would arise from the replacement of Cl^- ligands for OH^- ligands, which has been proposed by previous studies. (2). The proposed reaction mechanism of AuP with Au(III) chloride-hydroxide complex at 25 ^oC. (3). A new peak (m/z = 615.3220) identified as the AuP gold complexes (calculated for $C_{22}H_{20}N_3[AuCl(OH)_2]$ $[M+Na]^+$ 615.1408, found: 615.3220). LC-HRMS was conducted using Agilent 6530 Accurate-Mass Q-TOF LC/MS with Agilent EC-C18 column, 2.7 μm, 4.6*50 mm. HPLC runs used a linear gradient from 70% H_2O/30% CH_3OH to 0% H_2O/100% CH_3OH over 6 min and then an extra 3 min with H_2O/100% CH_3OH. The current velocity is set at 0.4 mL/min.

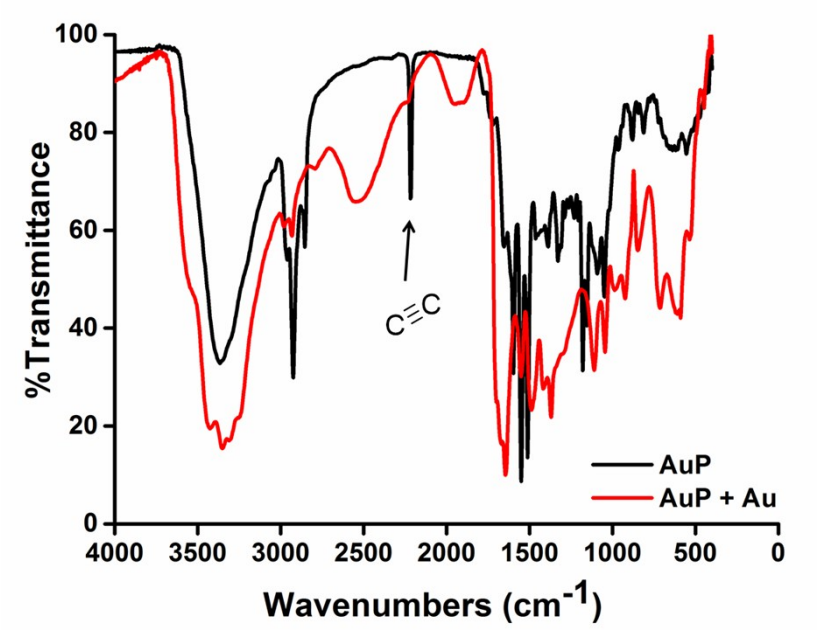

Fig.S2. **IR** spectra of **AuP** before (black) and after (red) treatment with Au^{3+}. The characteristic signal of terminal alkyne group (2217 cm^{-1}) was disappeared, accompanying with a new peak at 2546 cm^{-1} was formed.

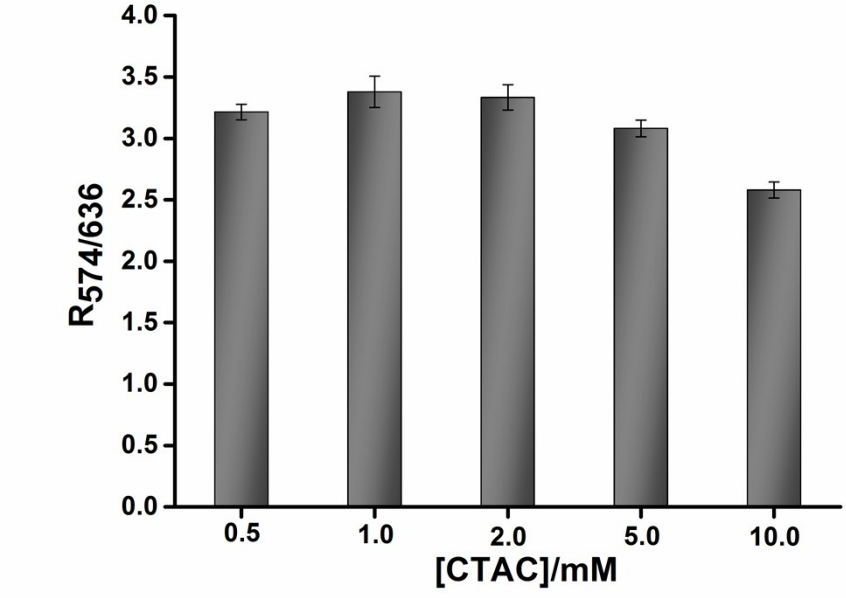

Fig.S3. The fluorescence ratios (I_{574}/I_{636}) of **AuP** (10 μM) towards Au^{3+} (50 μM) were measured with various concentrations of CTAC (0.5-10 mM) in PBS buffer (10 mM, 1% DMSO, pH 7.4) at 25 °C for 2 min. Excitation wavelength = 457 nm, excitation and emission slit widths = 10 nm. The data represent the average of three independent experiments.

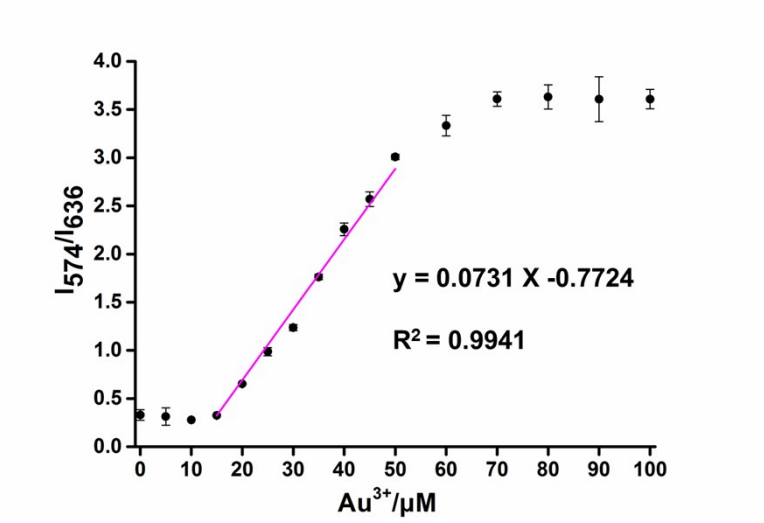

Fig.S4. The fluorescence ratio (I_{574}/I_{636}) of **AuP** (10 μM) was measured with various concentrations of Au^{3+} ions (0-100 μM) in PBS buffer (10 mM, 1% DMSO, 1 mM CTAC, pH 7.4) at 25 °C for 2 min. A good linear relationship between the fluorescence ratio (I_{574}/I_{636}) and Au^{3+} ions (15-50 μM) was observed. Excitation wavelength = 457 nm, excitation and emission slit widths = 10 nm. The data represent the average of three independent experiments.

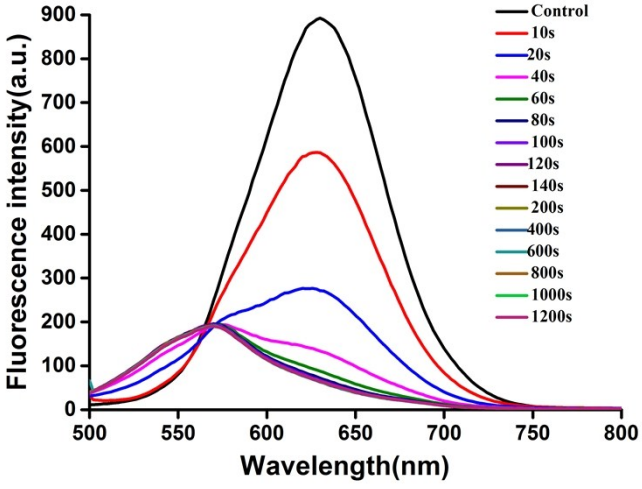

Fig.S5. Time-dependent fluorescence spectra changes of **AuP** (10 μM) towards Au^{3+} (50 μM) in PBS buffer (10 mM, 1% DMSO, 1 mM CTAC, pH 7.4) at 25 °C for the different incubation time. Time points are set at 0, 10, 20, 40, 60, 80, 100, 120, 140, 200, 400, 600, 800, 1000, 1200 s. Excitation wavelength = 457 nm, excitation and emission slit widths = 10 nm. The data represent the average of three independent experiments.

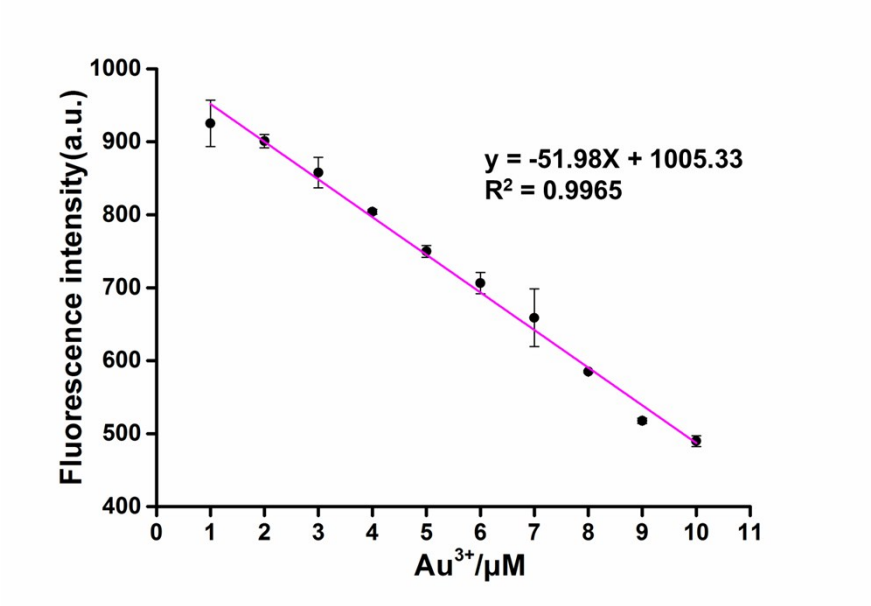

Fig.S6. The fluorescence intensity of **AuP** (10 μM) at 636 nm was recorded at the different concentration of Au^{3+} (0-10 μM) in PBS buffer (10 mM, 1% DMSO, 1 mM CTAC, pH 7.4) at 25 °C for 2 min. Excitation wavelength = 457 nm, excitation and emission slit widths = 10 nm. The data represent the average of three independent experiments.

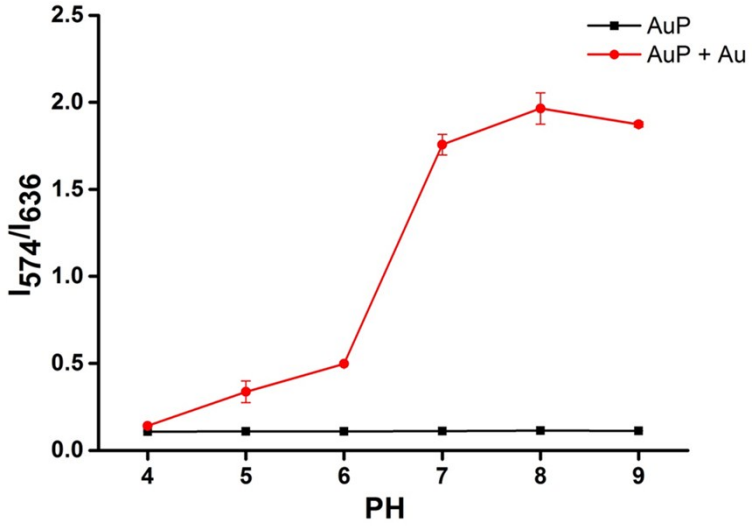

Fig.S7. The changes of fluorescence intensity ratio (I_{574}/I_{636}) of AuP (10 μM) *versus* the reaction incubated without (Black) or with (Red) Au^{3+} ions (50 μM) in PBS buffer with different pH values at 25 °C for 2 min. Excitation wavelength = 457 nm, excitation and emission slit widths = 10 nm. The data represent the average of three independent experiments.

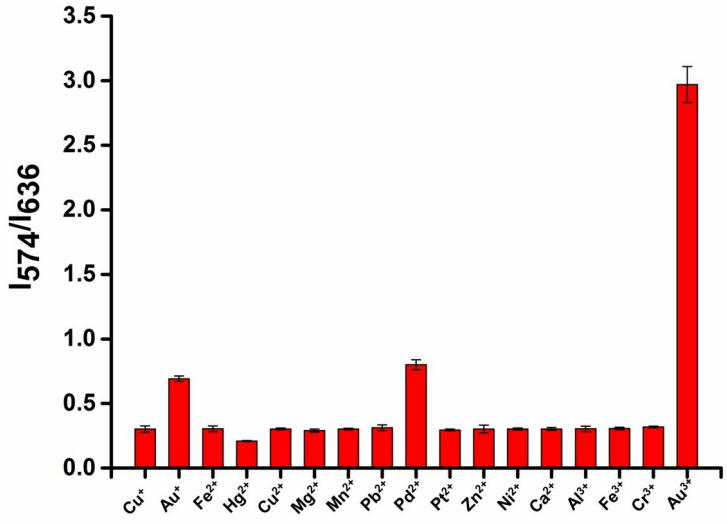

Fig.S8. Fluorescence intensity ratio (I_{574}/I_{636}) of **AuP** (10 μM) towards various metal ions (5 equiv.) in PBS buffer (10 mM, 1% DMSO, 1 mM CTAC, pH 7.4) at 25 °C for 2 min. Excitation wavelength = 457 nm, excitation and emission slit widths = 10 nm. The data represent the average of three independent experiments.

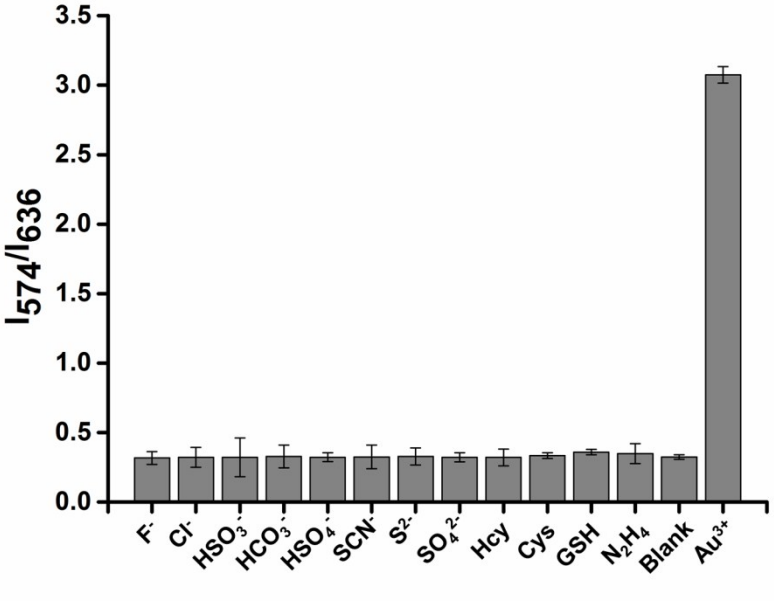

Fig.S9. Fluorescence ratio (I_{574}/I_{636}) of **AuP** (10 μM) towards various anions (5 equiv.) in PBS buffer (10 mM, 1% DMSO, 1 mM CTAC, pH 7.4) at 25 °C for 2 min. Excitation wavelength = 457 nm, excitation and emission slit widths = 10 nm. The data represent the average of three independent experiments.

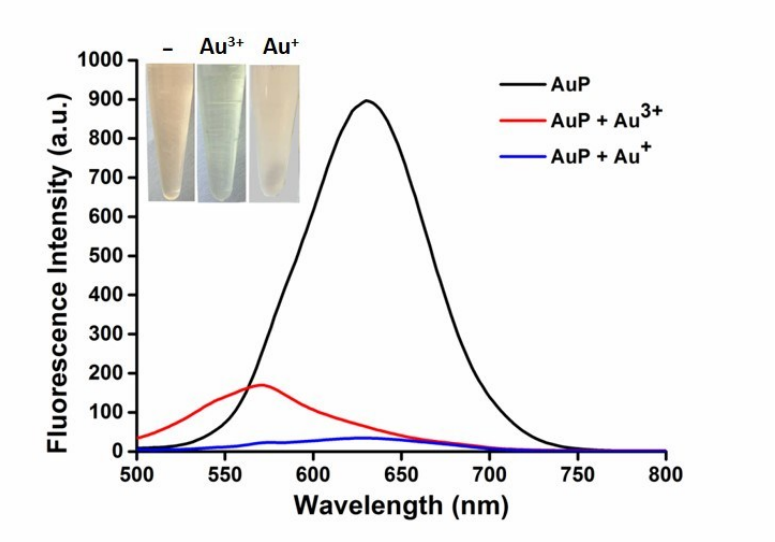

Fig.S10. Fluorescence spectra changes of **AuP** (10 μM) towards Au^{3+} ions and Au^{+} ions (5 equiv.) in PBS buffer (10 mM, 1% DMSO, 1 mM CTAC, pH 7.4) at 25 °C for 2 min. Inset: The photographs were shown under visible light. Excitation wavelength = 457 nm, excitation and emission slit widths = 10 nm. The data represent the average of three independent experiments.

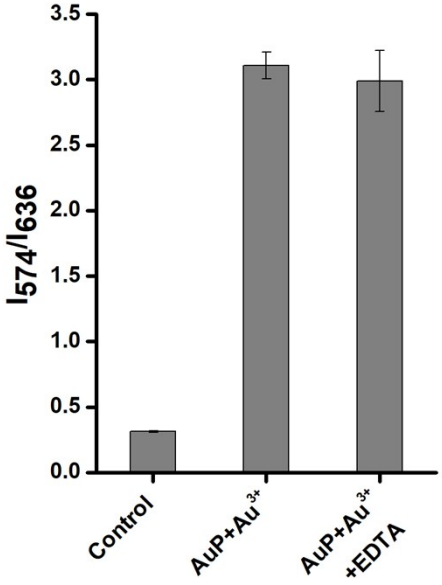

Fig. S11. Fluorescence ratio (I_{574}/I_{636}) responses of **AuP** (10 μM) in the absence and presence of Au^{3+} (50 μM) in PBS buffer (10 mM, 1% DMSO, 1 mM CTAC, pH 7.4) at 25 °C for 2 min. After incubating **AuP** with gold ion, additional EDTA (1 mM) was added into the reaction and further incubated for another 20 min, the results indicated that the formed AuP-gold complex was relatively stable and AuP could not reversibly respond to Au^{3+}. Excitation wavelength = 457 nm, excitation and emission slit widths = 10 nm. The data represent the average of three independent experiments.

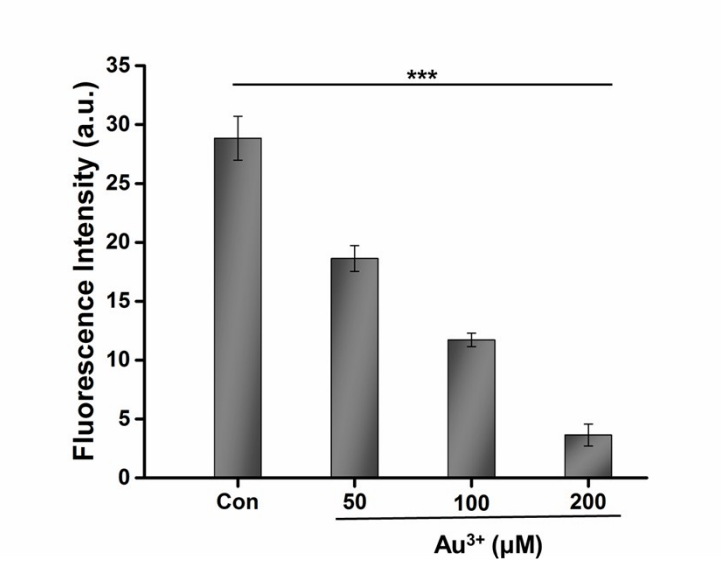

Fig.S12. Quantification of imaging data correspond to samples with different concentration of Au^{3+} ions treatment. Statistical analyses performed with a two-tailed Student's t-test with unequal variance, ***p value < 0.001, n = 3, error bars were ± SD.

Table S1. Determination of spiked Au^{3+} in real-water samples (Tap water; Rice sample buffer (200 mg/5 mL); Surface water from the Yangtze River) (n = 10). Recovery % = 100% × (concentration found / concentration added).

Samples	Spiked (μM)	Found (μM)	Recovery /%	RSD
Tap water	20	18.34	91.7	0.0587
	30	29.73	99.1	0.0334
	40	37.96	94.9	0.0158
Rice sample	20	19.90	95.5	0.0269
	30	29.85	99.5	0.0405
	40	37.84	94.6	0.0681
Yangtze River	20	18.68	93.4	0.0098
	30	27.75	92.5	0.0115
	40	36.52	91.3	0.0352

9. ^{1}H and ^{13}C NMR spectra

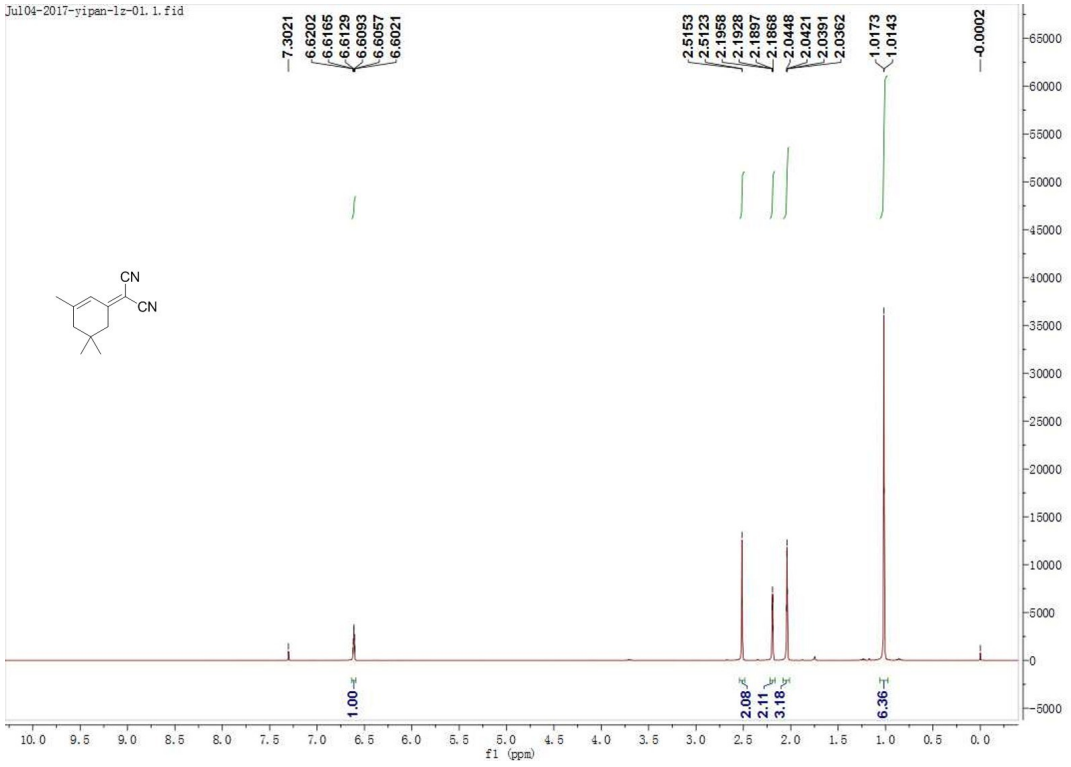

Fig.S13. ^{1}H NMR of compound **2** (400MHz, in $CDCl_3$)

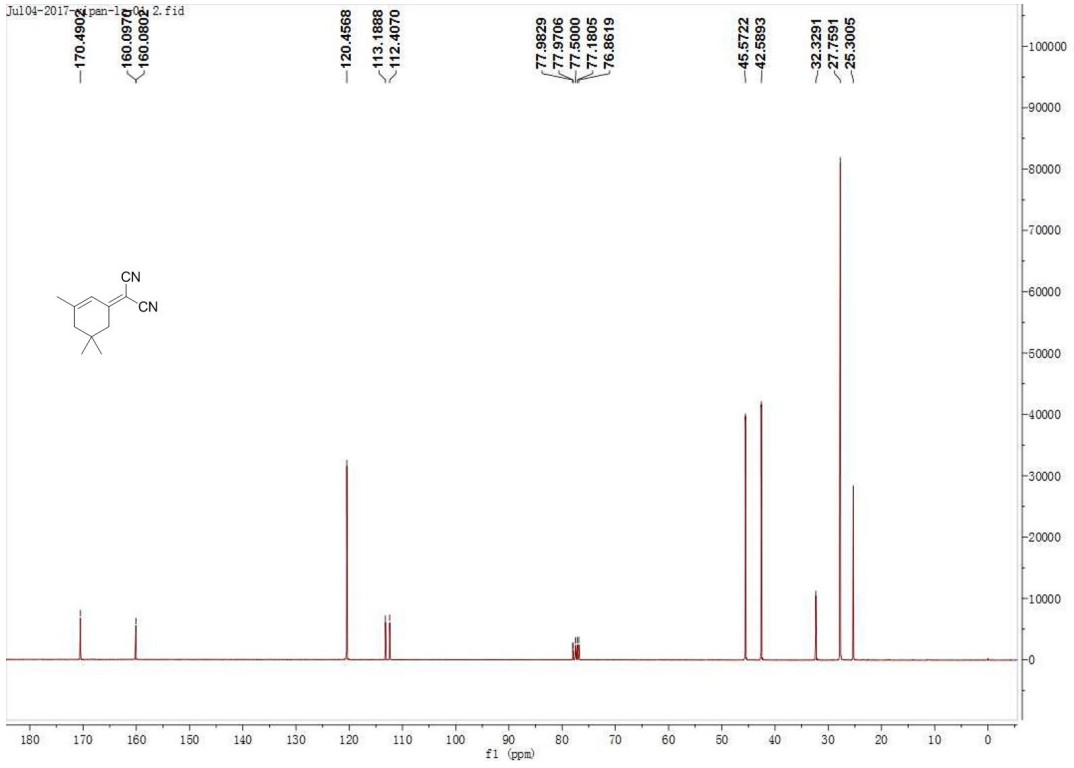

Fig.S14. ^{13}C NMR of compound **2** (100 MHz, in $CDCl_3$)

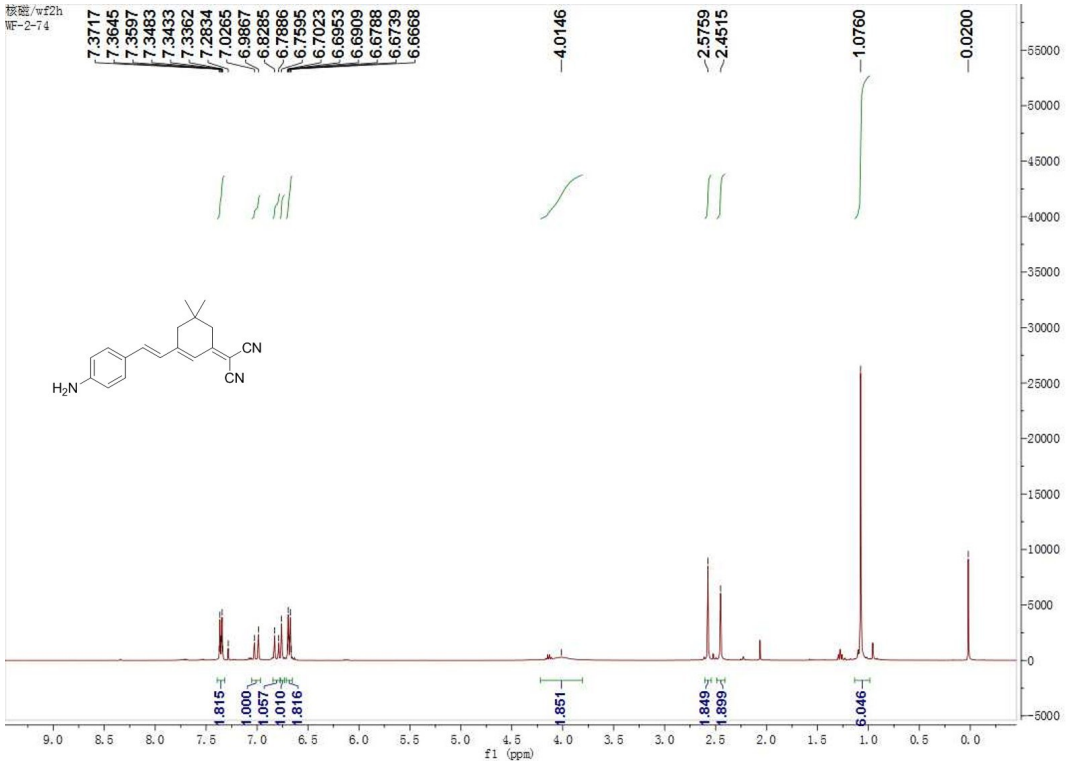

Fig.S15. ^{1}H NMR of compound **3** (400 MHz, in $CDCl_3$)

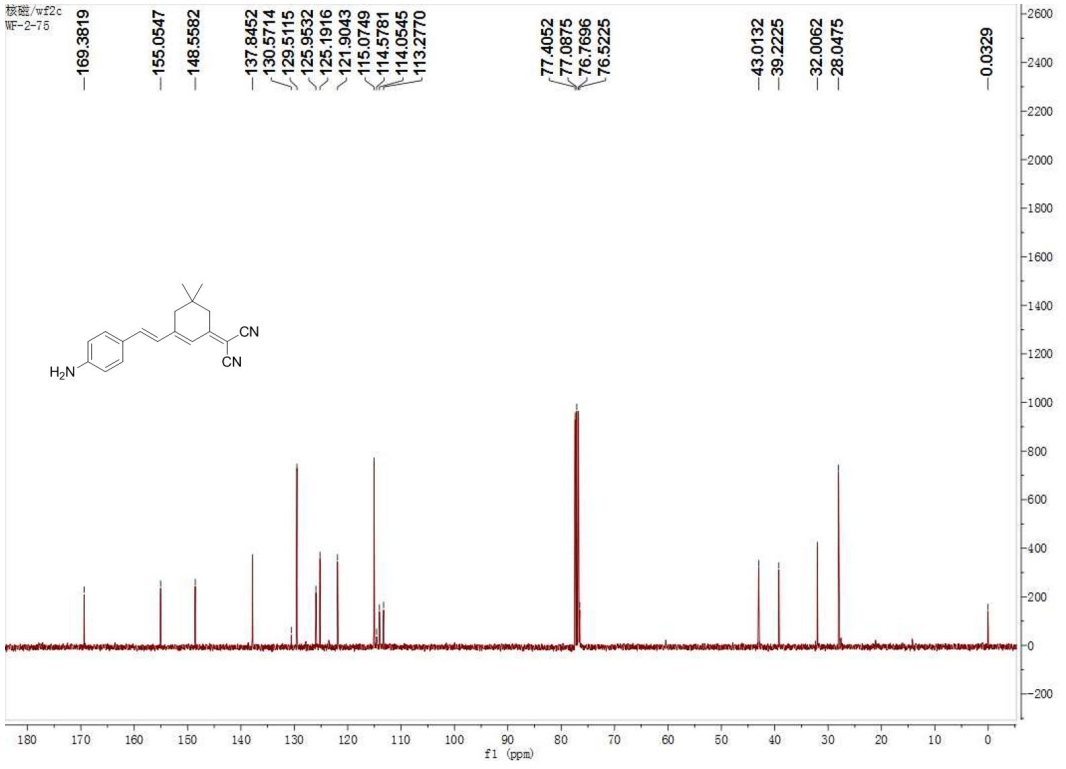

Fig.S16. ^{13}C NMR of compound **3** (100 MHz, in $CDCl_3$)

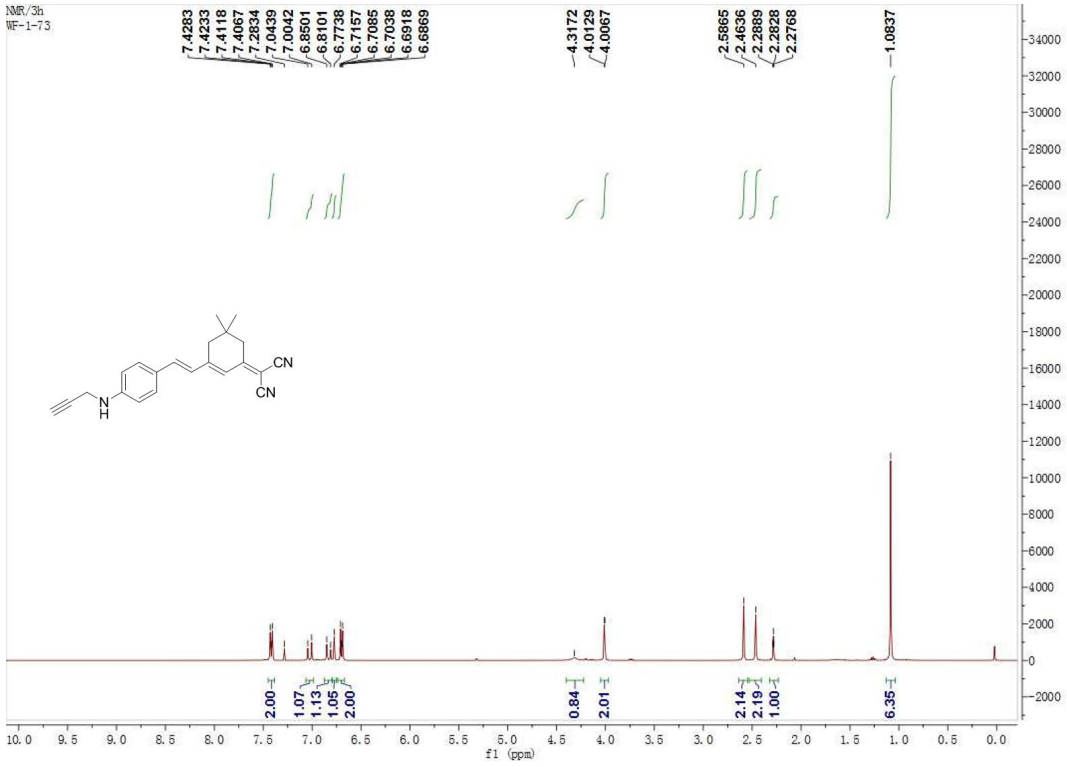

Fig.S17. ^{1}H NMR of **AuP** (400 MHz, in $CDCl_3$)

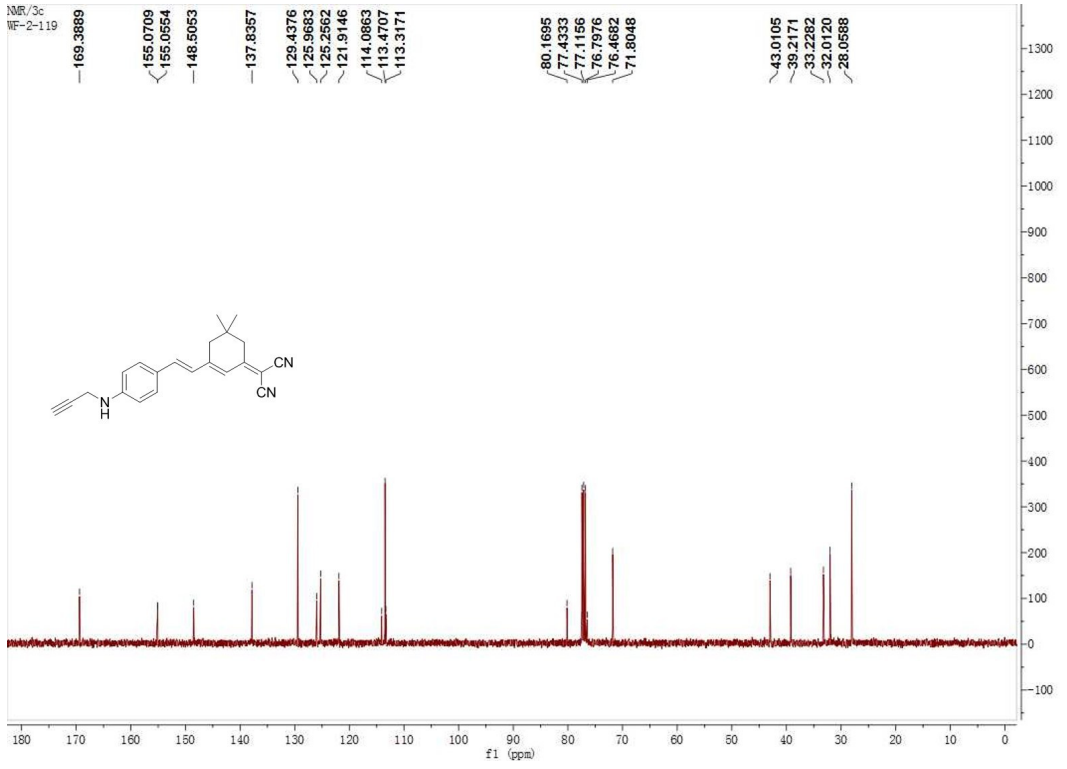

Fig.S18. ^{13}C NMR of **AuP** (100 MHz, in $CDCl_3$)