Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information for

A macromolecule cyclometalated gold(III) amphiphile displays long-lived emissive excited state in water: self-assembly and *in vitro* photo-toxicity

Table of Contents

1. General experimental section

All chemicals, unless otherwise noted, were purchased from commercial sources. All solvents for synthesis were of HPLC grade.

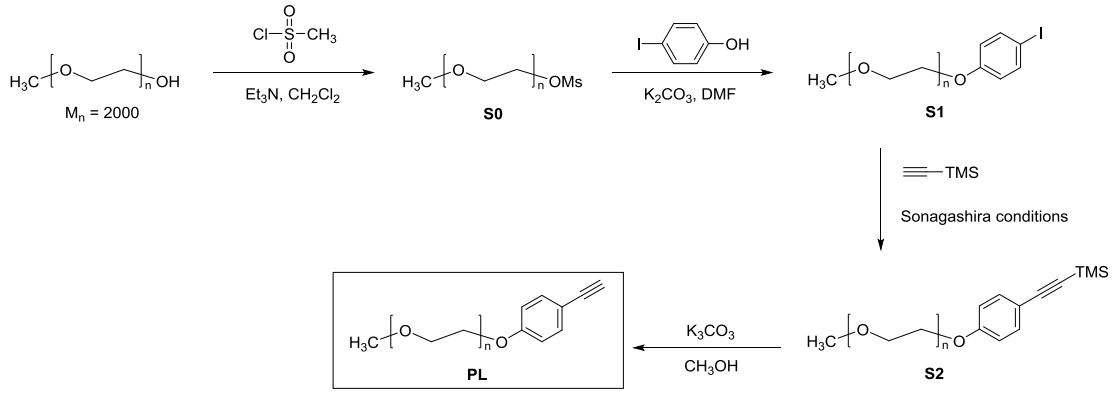
The nuclear magnetic resonance spectra were recorded on DPX-400 or DPX-300 Bruker FT-NMR spectrometer with chemical shift (in ppm) relative to tetramethylsilane (for $CDCl_3$). Inductively coupled plasma-atomic emission spectrometry and the electron spin resonance (ESR) spectra (Bruker E500) were performed at the Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing.

UV-vis absorption spectra were recorded on a Hewlett-Packard 8542A diode array spectrophotometer. Steady-state emission spectra were recorded on a SPEX 1681 Fluorolog-3 spectrophotometer. The emission lifetime measurements were performed on a Quantua Ray GCR 150-10 pulsed Nd:YAG laser system. Solutions for photophysical studies were degassed by using a high vacuum line in a two-compartment cell with five freeze-pump-thaw cycles. The emission quantum yield was determined by using $[Ru(bpy)_3](PF_6)_2$ (Φ = 0.062 in CH_3CN) as reference.

The dynamic light scattering measurement was recorded on a Malvern Zetasizer instrument employing a 4Mw He-Ne laser (λ = 632.8 nm) and equipped with a thermostatic sample chamber. The SEM images were recorded on a Philips XL-30 FEG.

2. Synthesis and characterization

(a) Synthesis of ligand **PL**



S0: To a solution of poly(ethylene glycol) methyl ether (M_n = *ca.* 2000) (5.0 g) and triethylamine (4.0 mL) in dry CH_2Cl_2 (50 mL) was added a CH_2Cl_2 (30 mL) solution of methanesulfonyl chloride (1.43 g, *ca.* 5.0 equiv.) dropwise at 0 °C. The mixture was stirred at 0 °C for 5 hours and room temperature for 24 hours. The reaction solution was washed with 50 mL of brine and 20 mL of water successively. The organic phase was collected and dried over anhydrous Mg_2SO_4, filtered. The solvent was removed under reduced pressure. The residue was dissolved in 20 mL of CH_3OH and a large volume of diethyl ether was added. The crude product was precipitated at 0 °C. The product as white powder solid was obtained by filtration. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.37 (t, *J* = 4.5 Hz, 2H), 3.86 (t, *J* = 5.0 Hz, 2H) 3.72–3.44 (polyethylene glycol peak), 3.37 (s, 3H), 3.08 (s, 3H). Yield: 85%.

S1: A mixture of **S0** (800 mg, 1.0 equiv. average molecular weight = *ca.* 2000 [1]), 4-iodophenol (264 mg, 3.0 equiv.), and K_2CO_3 (276 mg, 5.0 equiv.) in DMF (10 mL) was refluxed under N_2 for 48 hours. The mixture was filtered and the solvent was removed under reduced pressure. The crude product was dissolved in CH_2Cl_2 (100 mL) and washed with brine (25 mL × 10). The organic layer was extracted and dried over anhydrous $MgSO_4$, filtrated, then the filtrate was evaporated. The residue was dissolved in CH_2Cl_2 (10 mL) and a large volume

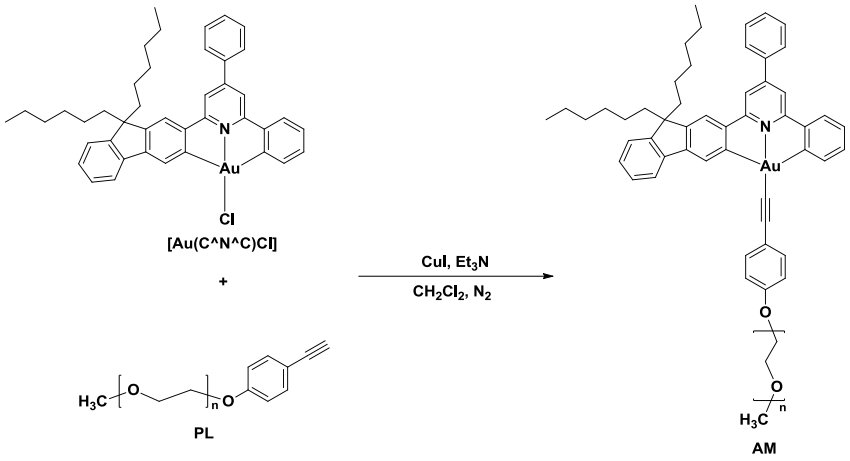
of Et_2O was added to precipitate the product at 0 °C. This procedure was repeated for 5 times to obtain pure product as white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (d, *J* = 8.9 Hz, 2H), 6.66 (d, *J* = 8.9 Hz, 2H), 4.05 (t, 2H, MeO-PEG-CH_2CH_2OPhI), 3.80 (t, 2H, MeO-PEG-CH_2CH_2OPhI) 3.67–3.39 (polyethylene glycol peak), 3.34 (s, 3H, CH_3O-PEG). Yield: 56%.

S2: A mixture of **S1** (400 mg, 1.0 equiv.), trimethylsilylacetylene (98 mg, 5.0 equiv.), $Pd(PPh_3)_2Cl_2$ (10 mg), CuI (5 mg), and Et_3N (5 mL) in 1,4-dioxane (15 mL) was refluxed under N_2 for 48 hours. The mixture was filtered and the solvent was removed under reduced pressure. The crude product was dissolved in CH_2Cl_2 (30 mL) and washed with brine (10 mL × 3) and water (10 mL). The extract was dried over anhydrous $MgSO_4$, filtered, and the filtrate was evaporated. The residue was dissolved in CH_2Cl_2 and a large volume of Et_2O was added to precipitate the product at 0 °C. This procedure was repeated for 5 times to obtain pure product as white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30 (d, *J* = 8.3 Hz, 2H), 6.75 (d, *J* = 8.2 Hz, 2H), 4.04 (t, *J* = 4.6 Hz, 2H, MeO-PEG-CH_2CH_2OC≡CTMS), 3.87–3.32 (polyethylene glycol peak), 3.30 (s, 3H, CH_3O-PEG), 0.15 (s, 9H, MeO-PEG-CH_2CH_2OC≡CSi(CH_3)$_3$). Yield: 60%.

PL: A mixture of **S2** (200 mg, 1.0 equiv.) and K_2CO_3 (69 mg, 5.0 equiv.) in CH_3OH (15 mL) was stirred at room temperature for 12 hours. The mixture was filtered and the solvent was removed under reduced pressure. The crude product was dissolved in CH_2Cl_2 (30 mL) and washed with brine (10 mL × 3) and water (10 mL). The extract was dried over anhydrous $MgSO_4$, filtrated, and the filtrate was evaporated. The residue was dissolved in CH_2Cl_2 and a large volume of Et_2O was added to precipitate the product at 0 °C. This procedure was repeated for 5 times to obtain pure product as light brown solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33 (d, *J* = 8.7 Hz, 2H), 6.78 (d, *J* = 8.7 Hz, 2H), 4.06 (t, 2H, MeO-PEG-CH_2CH_2OC≡CH), 3.86–3.36 (polyethylene glycol

peak), 3.31 (s, 3H, C*H*$_3$O-PEG), 2.96 (s, 1H, MeO-PEG-CH$_2$C*H*$_2$OC≡C*H*). Yield: 60%.

(b) Synthesis of **AM**



The complex **[Au(C^N^C)Cl]** was prepared by our group and reported previously.[2]

AM: A mixture of **PL** (120 mg, 1.0 equiv.), **[Au(C^N^C)Cl]** (95 mg, 2.0 equiv.), CuI (20 mg) and Et_3N (3 mL) in CH_2Cl_2 (15 mL) under N_2 was stirred at room temperature for 5 hours. The mixture was filtered and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and a large volume of Et_2O was added to precipitate the product at 0 °C. This procedure was repeated for 5 times. The precipitates were collected and dissolved in water, filtered (filter: 0.2 μm). The product as yellow power solid was obtained by freeze-drying the filtrate. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.47 (s, 1H), 8.13 (d, *J* = 7.2 Hz, 1H), 7.79 (d, *J* = 6.6 Hz, 3H), 7.66 (d, *J* = 8.7 Hz, 2H), 7.64–7.51 (m, 8H), 7.39 (t, *J* = 7.3 Hz, 1H), 7.33 (s, 3H), 6.93 (d, *J* = 8.5 Hz, 2H), 4.18 (t, *J* = 4.7 Hz, 2H), 3.95–3.41 (polyethylene glycol peak), 3.37 (s, 3H), 2.00 (s, 4H), 1.10–0.99 (m, 12H), 0.73 (t, *J* = 7.0 Hz, 6H), 0.62 (s, 4H). Yield: 52%.

3. DLS measurement results

Table S1 DLS measurement of AM aqueous solution at different concentrations.

Concentration (mg/mL)	Diameter (nm)	Intensity (%)
1.00	102	83
0.50	99	81
0.25	98	81
0.06	92	81

4. $^1O_2$ quantum yield measurement

The $^1O_2$ quantum yield was determined by using the chemical method. Here, Na_2-ADPA and Rose Bengal (RB) were used as the $^1O_2$-trapping agent and standard photosensitizer, respectively. An aqueous solution of Na_2-ADPA (1 mg/mL, 60 μL) was added to 1.5 mL of **AM** aqueous solution. The white light was employed as the light source. To eliminate the inner-filter effect, the absorption maxima of RB and **AM** were adjusted to *ca.* 0.2 OD. The absorption of Na_2-ADPA at 378 nm was recorded at certain irradiation time to obtain decomposition rate constant. The $^1O_2$ quantum yield of **AM** in water was calculated using the following equation:

$$\Phi_{AM} = \frac{\Phi_{RB} \times K_{AM} \times A_{RB}}{K_{RB} \times A_{AM}}$$

Where K_{AM} and K_{RB} are the decomposition rate constants under irradiation; A_{AM} and A_{RB} are light absorbed by **AM** and RB, respectively, which are determined by integration of the optical absorption bands in the wavelength range of 400-700 nm. Φ_{RB} = 0.75 is the $^1O_2$ quantum yield of RB in water.

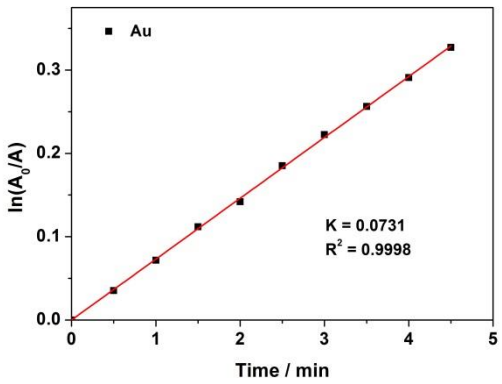


Fig. S1 The linear fitting of absorption change at 387 nm of aqueous solution containing **AM** and Na_2-ADPA in the course of irradiation under white light.

5. *In vitro* cytotoxicity

The dark cytotoxicity and phototoxicity of **AM** were evaluated in A549 cells using the standard MTT assay. A549 cells were obtained from the ATCC. They were cultured in DMEM/F12 medium supplemented with 10% fetal bovine serum (FBS), 50 unit/mL penicillin, and 50 μg/mL of streptomycin at 37 ^{o}C in a humidified incubator containing 5% CO_2. A549 cells were seeded in a 96-well plate at a density of 2×10^3 cells per well in culture medium and incubator for 12 hours. Then, the culture medium was removed and the cells were incubated in fresh culture media containing **AM** of different concentrations (0~0.2 mg/mL) for 4 hours before being irradiated with an intensity of 72 mW/cm^2 by using a 500 W Xe lamp as the light source for 0, 5 and 10 min. The cells were then incubated for 24 hours. 200 μL of the new culture medium (without FBS) containing MTT (20 μL, 5 mg/mL) was added, followed by incubation for another 4 hours to allow the formation of formazan crystals. Finally, the culture medium was discarded and 200 μL dimethylsulfoxide (DMSO) was added. Absorbance of the solution was measured at 570 nm, and the cell viability values were determined according to the following formula: cell viability (%) = (the absorbance of experimental group/the absorbance of control group) × 100%. The cell fluorescence imaging was studied by laser scanning confocal microscope (NIKON).

6. Photostability measurements of AM in water

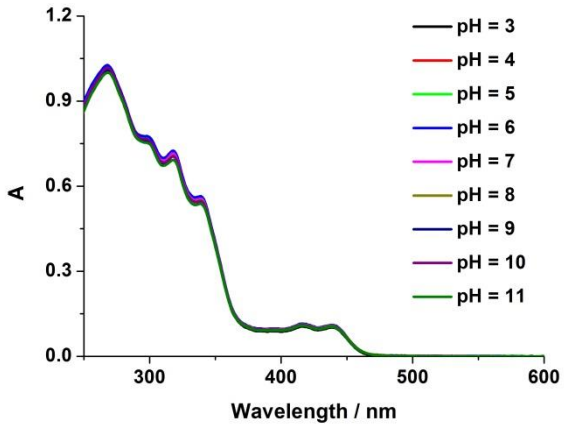


Fig. S2 UV-vis absorption spectra of **AM** in aqueous solution at different pH value.

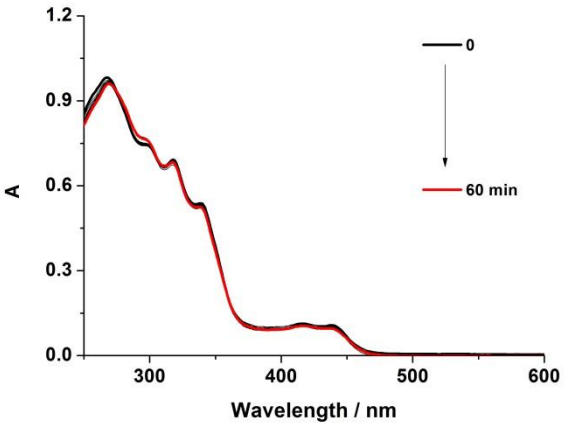


Fig. S3 The UV-vis absorption spectra of **AM** in aqueous solution under white light irradiation for 1 hour.

7. Fluorescence imaging *in vivo*

Animal experiments were approved by the China Committee for Research and Animal Ethics in compliance with the law on experimental animals. In the intravenous injection experiments, a subcutaneous tumor was established by injecting a suspension of 2×10^6 4T1 breast cancer cells in PBS (60 μL) into the buttock of each female nude mouse (4-week-old, 15−20 g) and was allowed to grow for 8–10 d when the tumor size reached approximately 25 mm^3. **AM** (2.0 mg/mL in water, 150 μL) was intravenously injected into the mice (~15 mg **AM**/kg body weight). After 24 h post-injection, the tumor-bearing mice were sacrificed and the tumor and major organs were harvested. The fluorescent scans were performed using a Maestro 2 Multispectral Small-animal Imaging System.

8. References

(1) In the synthesis, the average molecular weights of **S0**, **S1**, **S2**, and **PL** are unified regarded as 2000 for calculation of equivalent.

(2) W.-P. To, K. T. Chan, G. S. M. Tong, C. Ma, W.-M. Kwok, X. Guan, K.-H. Low and C.-M. Che, *Angew. Chem. Int. Ed.*, 2013, **52**, 6648.