Supporting Information

A luminescent bimetallic iridium(Ⅲ) complex for ratiometric tracking intracellular viscosity

Fengyu Liu[a,b,*], Jia Wen[c], Su-Shing Chen[b,*] and Shiguo Sun [d,*]

[a]*State Key Laboratory of Fine Chemicals, School of Chemistry, Dalian University of Technology, No.2 linggong Road, Ganjingzi, District, Dalian 116023, China*
[b]*Systems Biology Laboratory, Department of Computer Information Science and Engineering, University of Florida, Gainesville, FL, United States*
[c]*Shaanxi Key Laboratory of Natural Products & Chemical Biology, College of Chemistry & Pharmacy, Northwest A&F University, Yangling, Shaanxi 712100, China*
[d]*Key Laboratory of Xinjiang Endemic Phytomedicine Resources, Ministry of Education, School of Pharmacy, Shihezi University, Shihezi 832000, China*

**Corresponding author:*
Fengyu Liu (liufengyu@dlut.edu.cn)
Su-Shing Chen (suchen@cise.ufl.edu)
Shiguo Sun (sunsg@nwsuaf.edu.cn)

Table of Contents

Materials and methods

Materials and instruments

All solvents and reagents used were reagent grade and used without further purification unless otherwise stated. **C10** was dissolved in DMSO at a concentration of 1 mM as the stock solution and stored in a refrigerator (2 ºC) for use. Phosphate buffer saline solution was mixed by 0.1 M sodium dihydrogen phosphate and disodium hydrogen phosphate solution, the pH of the mixed solution was measured with a pH meter to make that pH was 7.40. Deionized water was used throughout the experiment. Silica gel (100–200 mesh) was used for flash column chromatography for purifications. Etoposide (20 mg/mL in NS), Human Serum Albumin (HSA), Calf thymus DNA (ct-DNA) and 18 amino acids were purchased from Beijing Baoxi Di company. Cells were purchased from Institute of Basic Medical Sciences (IBMS) of the Chinese Academy of Medical Sciences.

NMR spectra were recorded on a Bruker Avance II 400 MHz spectrometer. Chemical shifts (δ) were reported as ppm (in $CDCl_3$, with TMS as the internal standard). Emission spectra were performed on a VAEIAN CARY Eclipse fluorescence spectrophotometer (Serial No. FL0812:M018). Excitation and emission slit widths were modified to adjust the fluorescence intensity to a suitable range. Absorption spectra were measured on a Perkin Elmer Lambda 35 UV/VIS spectrophotometer (Perkin Elmer). Mass spectrometric data were achieved with HP1100LC/MSD MS and an LC/Q-TOF-MS instruments. All pH measurements were performed using a Model PHS-3C meter. Electrochemical properties of compounds were performed on a CHI660D electrochemical workstation. Luminescent lifetime was performed on a HORIBA fluorescence lifetime meter. Quantum yield was accurately measured on a HAMAMATSU

C11347 quantum yield instrument. Cytotoxicity experiments were performed on a full-wavelength scanning fluoroscopy instrument (Varioskan FLash). Cell imaging was performed on an Olympus laser confocal microscope OLYMPUS FV1000-IX81.

Synthesis of intermediates and compound C10

The synthesis route of **C10** is shown in Scheme S1 in the Supporting Information.

Synthesis procedure of 6-(benzo[b]thiophen-2-yl)phenanthridine (btph)[1]

Tetrakis(triphenylphosphine)palladium (0.21 g, 0.18 mmol) was added to a mixture of 6-chlorophenanthridine (1.28 g, 6.0 mmol), benzo[b]thiophene-2-boronic acid (1.28 g, 7.2 mmol), toluene (10 mL), ethanol (5 mL), and a 2 M sodium carbonate aqueous solution (10 mL) under vigorous stirring. The mixture was refluxed for 5 h under N_2 atmosphere. After cooling to room temperature, the reaction mixture was poured into water (200 mL) and the product was extracted with toluene (50 mL × 3). The organic layer was washed with water (50 mL × 3), dried over anhydrous $MgSO_4$, filtered, and evaporated in vacuum. The product was purified by silica gel column chromatography using a dichloromethane/petroleum ether (1:1) mixture as the eluent to give a yellow powder (1.47 g, 79% yield).

Synthesis of $(btph)_2Ir(\mu\text{-}Cl)_2Ir(btph)_2$

$IrCl_3 \cdot 3H_2O$ (390 mg, 1.1 mmol) and water (10 mL) were added to a solution of 6-(benzo[b]thiophen-2-yl)phenanthridine (btph) (685 mg, 2.2 mmol) in 2-ethoxyethanol (30 mL). The mixture was refluxed overnight. After cooling, the precipitate was filtered off, washed with water, methanol and hexane, respectively, and then dried in vacuum to give a purple brown powder (900 mg, 96% yield). It was used without any further purification.

Synthesis of $(F_2ppy)_2Ir(\mu\text{-}Cl)_2Ir(F_2ppy)_2$

The synthesis of $(F_2ppy)_2Ir(\mu\text{-}Cl)_2Ir(F_2ppy)_2$ was similar with $(btph)_2Ir(\mu\text{-}Cl)_2Ir(btph)_2$. 1.73g, 83% yield.

*Synthesis of **Ir-A**, **Ir-B** and **C10***

Ir-A, Ir-B and **C10** were synthesized according to the literature procedure.[2] In short, $(F_2ppy)_2Ir(\mu\text{-}Cl)_2Ir(F_2ppy)_2$ (or $(btph)_2Ir(\mu\text{-}Cl)_2Ir(btph)_2$) and 4,4'-dimethyl-2,2'-bipyridine were used as reagents to synthesize **Ir-A (Ir-B)**. 4,4'-dimethyl-2,2′-bipyridine and dibromoctane were used as starting material for the synthesis of Mebpy-C10-Mebpy ligand, and then they were employed toward the complexation of binuclear iridium using $(F_2ppy)_2Ir(\mu\text{-}Cl)_2Ir(F_2ppy)_2$ and $(btph)_2Ir(\mu\text{-}Cl)_2Ir(btph)_2$ as precursor, leading to **C10** with high purity. yield: 40%.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.56 (s, 1H), 9.44 – 9.36 (m, 3H), 8.80 (s, 1H), 8.62 (dd, *J* = 17.6, 8.6 Hz, 3H), 8.35 – 8.22 (m, 7H), 8.02 – 7.93 (m, 5H), 7.83 (dd, *J* = 18.1, 8.6 Hz, 5H), 7.69 (dd, *J* = 13.9, 5.6 Hz, 2H), 7.47 (t, *J* = 6.5 Hz, 2H), 7.28 (d, *J* = 7.5 Hz, 3H), 7.25 – 7.02 (m, 11H), 6.78 – 6.61 (m, 7H), 6.53 (dd, *J* = 12.1, 9.2 Hz, 2H), 5.67 (d, *J* = 6.5 Hz, 2H), 2.95 (t, *J* = 7.5 Hz, 2H), 2.69 (s, 4H), 2.42 (s, 3H), 1.78 – 1.70 (m, 2H), 1.48 – 1.38 (m, 2H), 1.22 (m, 15H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 167.93, 164.31, 164.26, 162.73, 162.63, 162.51, 162.40, 159.80, 157.85, 157.44, 155.50, 155.26, 154.49, 153.47, 153.00, 149.13, 148.90, 148.57, 145.95, 145.76, 145.70, 143.76, 143.72, 143.61, 139.09, 138.56, 138.52, 133.76, 133.68, 133.36, 129.08, 128.79, 128.72, 128.24, 128.17, 127.99, 127.74, 127.50, 127.36, 127.06, 127.01, 126.95, 126.82, 126.76, 125.94, 125.60, 125.15, 124.79, 124.13, 123.73, 123.57, 122.89, 122.83, 122.72, 122.63, 122.05, 113.98, 113.85, 99.21, 98.99, 98.78, 35.41, 34.78, 29.99, 29.86, 29.19, 29.00, 28.61, 21.57, 21.17.

HRMS: $[M\text{-}2Cl^-]^{2+}$, calculated: *m/z* = 932.2336, found: *m/z* = 932.2330.

Fluorescence detection

The solvents were obtained by mixing deionized water–glycerol system in different proportions. Measurements were carried out with a NDJ-7 rotational viscometer, and each viscosity value was recorded. The solutions of **C10** in different viscosity were prepared by adding 50 μL stock solution (1.0 mM) to 5.0 mL of solvent mixture (water–glycerol solvent system) to obtain the final concentration of the dye (10 μM). These solutions were sonicated for 20 min to eliminate air bubbles. After standing for 25 min at a room temperature, the solutions were measured in a UV spectrophotometer and a fluorescence spectrophotometer. A luminescence lifetime measuring equipment (HORIBA) was used to obtain the luminescence lifetimes of **C10** under anaerobic condition, with the excitation wavelength at 450 nm and detection around 711 nm. For interference measurements, molecules such as HSA, ct-DNA and 18 amino acids were added in PBS buffer of **C10** (10 μM) respectively. The absorption and emission spectra of solutions were measured in a UV spectrophotometer and a fluorescence spectrophotometer.

Determination of the quantum yield

The luminescence quantum yields for compounds with Absolute PL Quantum Yield Spectrometer (HAMAMATSU C11347). Load a sample and press the start button to measure the photoluminescence quantum yields, excitation wavelength dependence, PL excitation spectrum and other properties in a short time. The PL Quantum Yield (Φ) is expressed as the ratio of the number of photons emitted from molecules (PN_{em}) to that absorbed by molecules (PN_{abs}). $\Phi = PN_{em}/PN_{abs}$

Cytotoxicity determined by MTT method

Measurement of cell viability was evaluated by reducing of MTT (3-(4,5)-dimethylthiahiazo(-2-yl)-3,5-diphenytetrazoliumromide) to formazan crystals using mitochondrial dehydrogenases. MCF-7 cells were seeded in 96-well microplates (Nunc, Denmark) at a density of 1×10^5 cells/mL

in 100 mL medium containing 10% fetal bovine serum (FBS, invitrogen). After 24 h of cell attachment, the plates were washed with 100 μL/well PBS. The cells were then cultured in medium with 1, 5, 10 and 20 μM of **C10** for 24 h. Cells in culture medium without **C10** were used as the control. Six replicate wells were used for each control and test concentration 10 μL of MTT (5 mg/mL) prepared in PBS was added to each well and the plates were incubated at 37 ºC for another 4 h in a 5% CO_2 humidified incubator. The medium was then carefully removed, and the purple crystals were lysed in 200 μL DMSO. Optical density was determined on a microplate reader (Thermo Fisher Scientific) at 570 nm (OD) with subtraction of the absorbance of the cell-free blank volume at 630 nm (OD_K). Cell viability was expressed as a percent of the control culture value, and it was calculated using the following equation:[3]

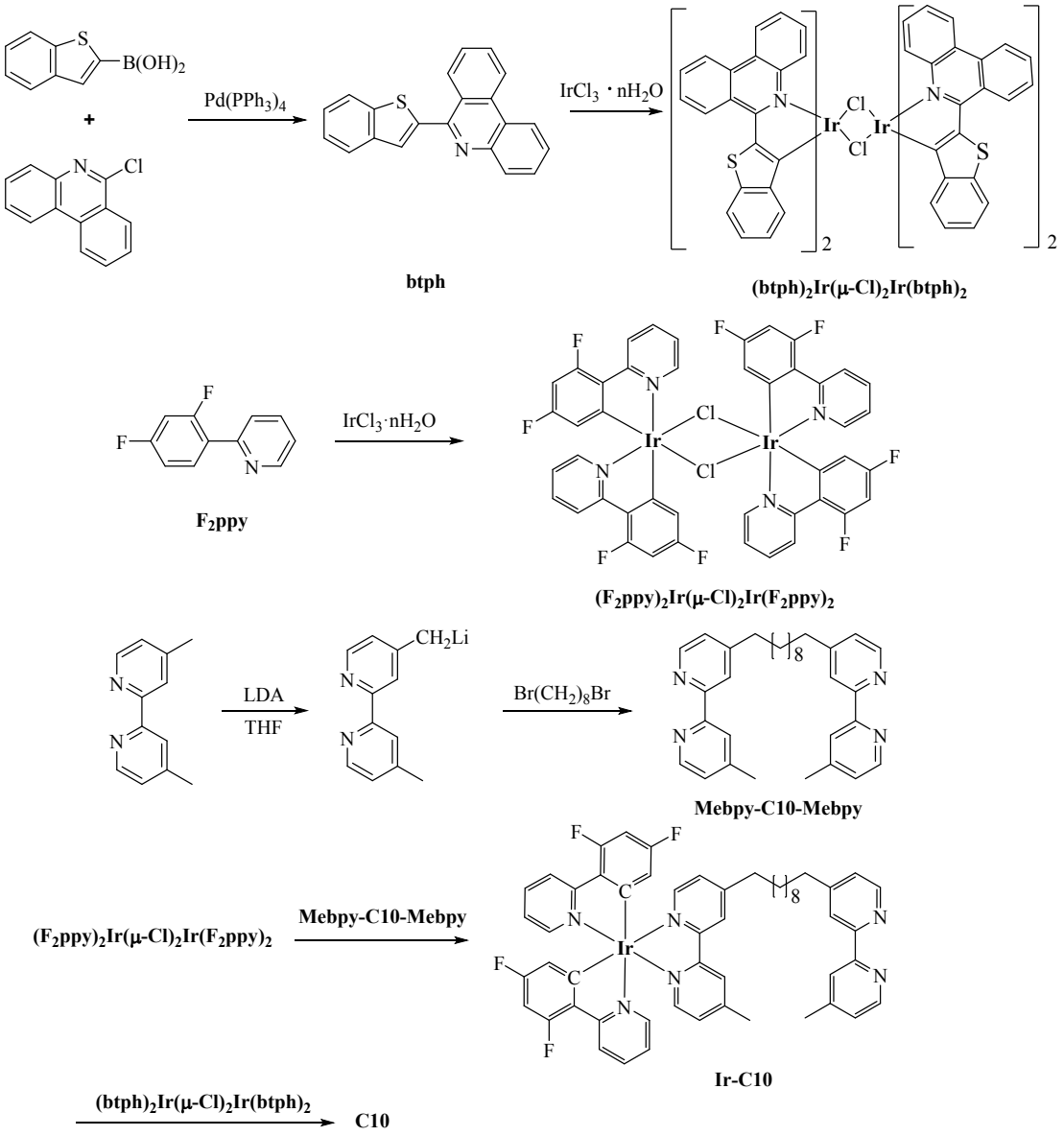
Cells viability (%) = $(OD_{dye} - OD_{Kdye}) / (OD_{control} - OD_{Kcontrol}) \times 100$.

Live cell imaging experiments

Human breast cancer cells MCF-7, human lung cancer cells A549 and human embryonic kidney cells 293T were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS) (Invitrogen), 1% penicillin/streptomycin (complete DMEM). Human hepatoma cells HepG-2 and human normal liver cells HL7702 were cultured in 1640 medium containing 10% FBS, 1% penicillin/streptomycin (complete 1640). The cells were seeded in 24-well flat-bottomed plates and then incubated for 24 h at 37ºC under 5% CO_2. HepG-2 and HL7702 cells were seeded in one culture dish at a seeding density of 1 x 10^5 cells.[4] Before imaging, the live cells were incubated with **C10** (10 μM) for different time and then washed with 0.1 M phosphate-buffered saline (PBS) three times. Fluorescence imaging was performed using an OLYMPUSFV-1000 inverted fluorescence microscope with a 60 × objective lens. Under the confocal fluorescence microscope, **C10** was excited at 405 nm and emission was

collected at 500-550 and 575-675 nm. For the detection of viscosity changes, MCF-7 cells were incubated with **C10** (10 μM) at 37 ºC for 1 h and in the process of taking cell images, 10 μL etoposide (20 mg/mL in NS) was added, and then got the other cell images in different time.

Scheme S1



Scheme S1 The synthetic routes of probe **C10**.

Fig. S1

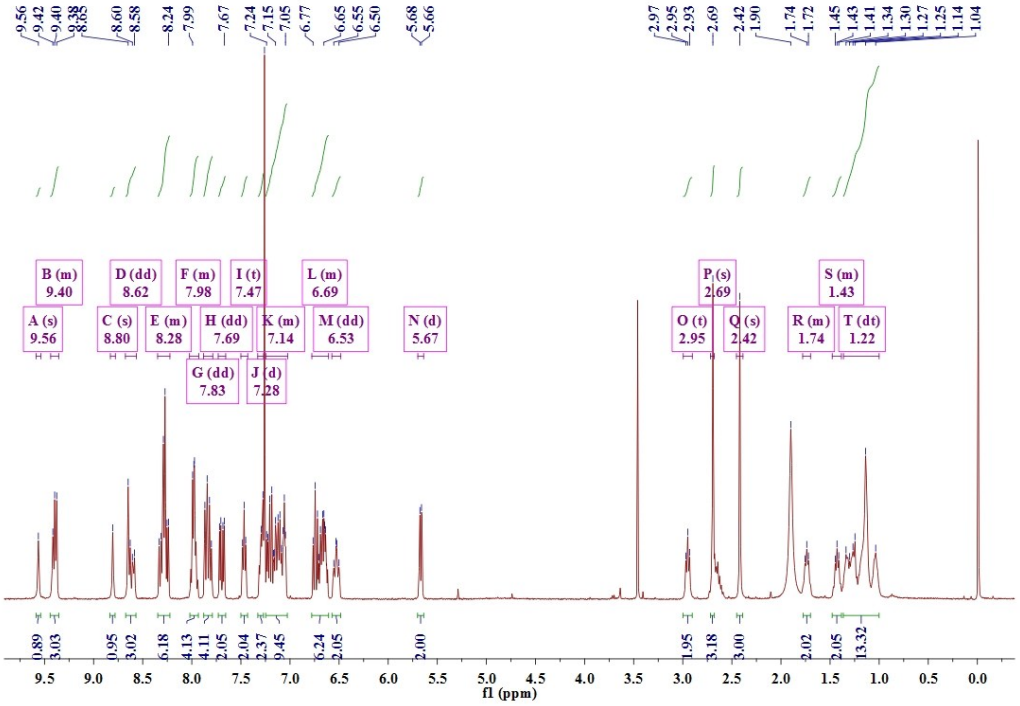


Fig. S1 ^{1}H NMR spectrum of **C10** in $CDCl_3$.

Fig. S2

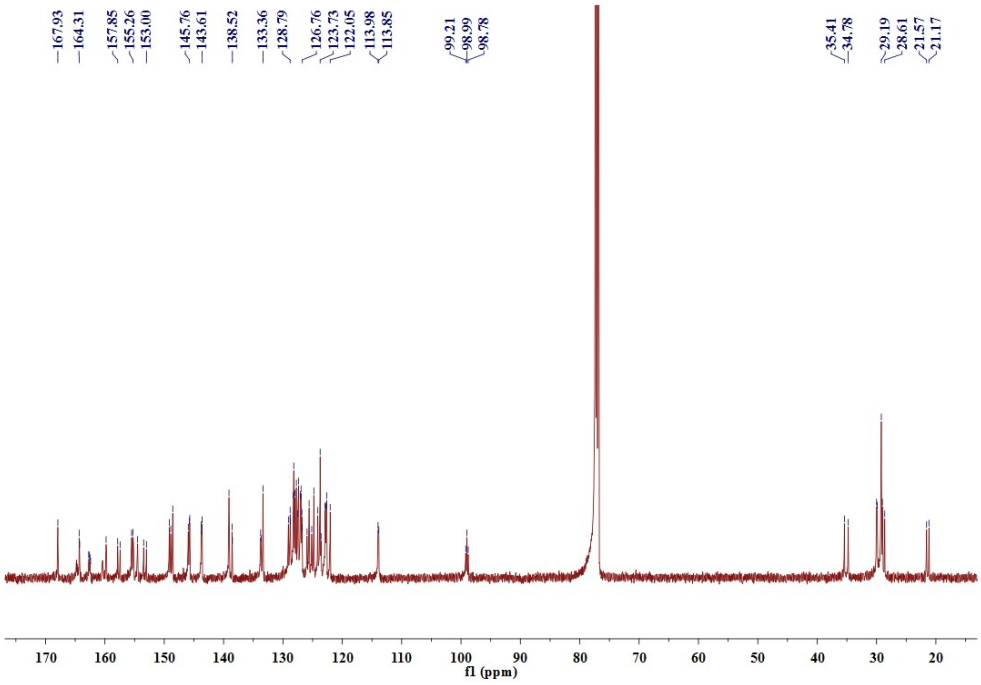


Fig. S2 ^{13}C NMR spectrum of **C10** in $CDCl_3$.

Fig. S3

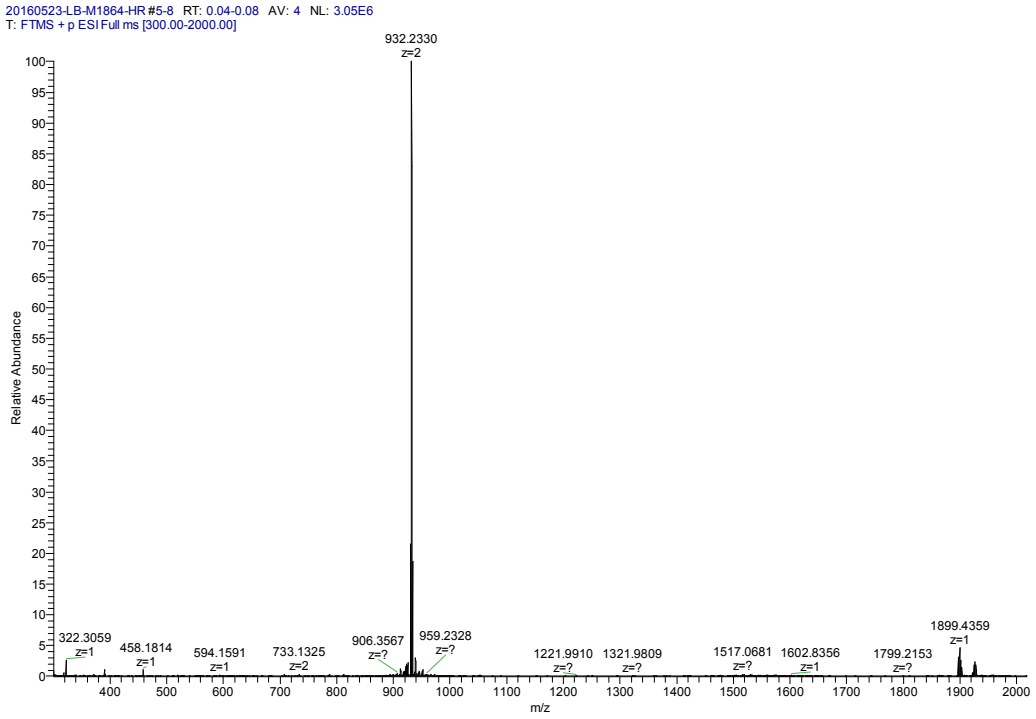


Fig. S3 HRMS of **C10**.

Fig. S4

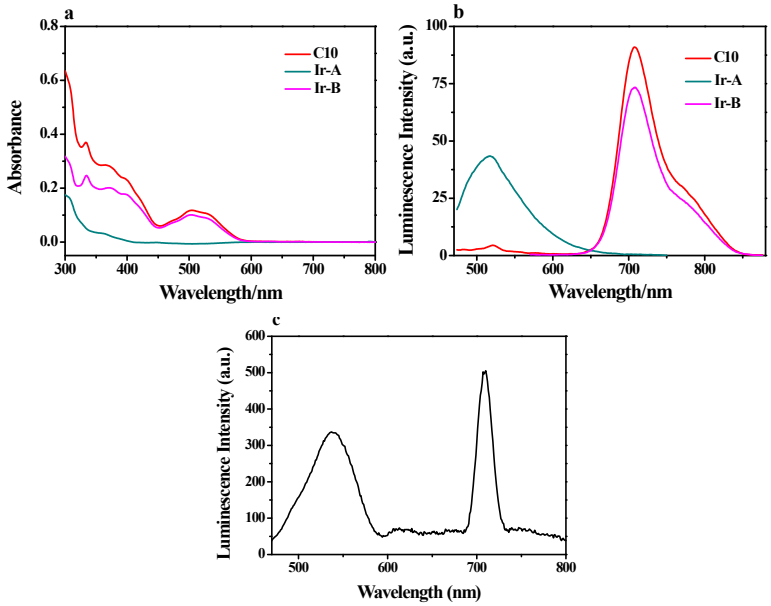


Fig. S4 (a) Absorbance and (b) emission spectra (λ_{ex}=450 nm) of **C10**, **Ir-A**, **Ir-B** and (c) excitation spectrum of **C10** in acetonitrile.

Fig. S5

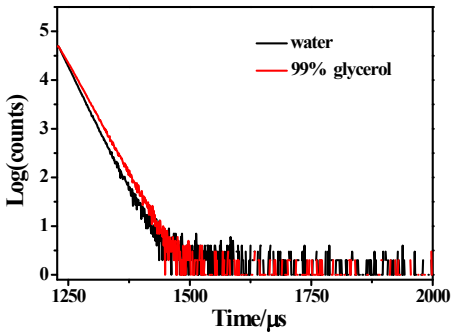


Fig. S5 Luminescent lifetime of probe **C10** in water and 99 % glycerol, excited at 450 nm.

Fig. S6

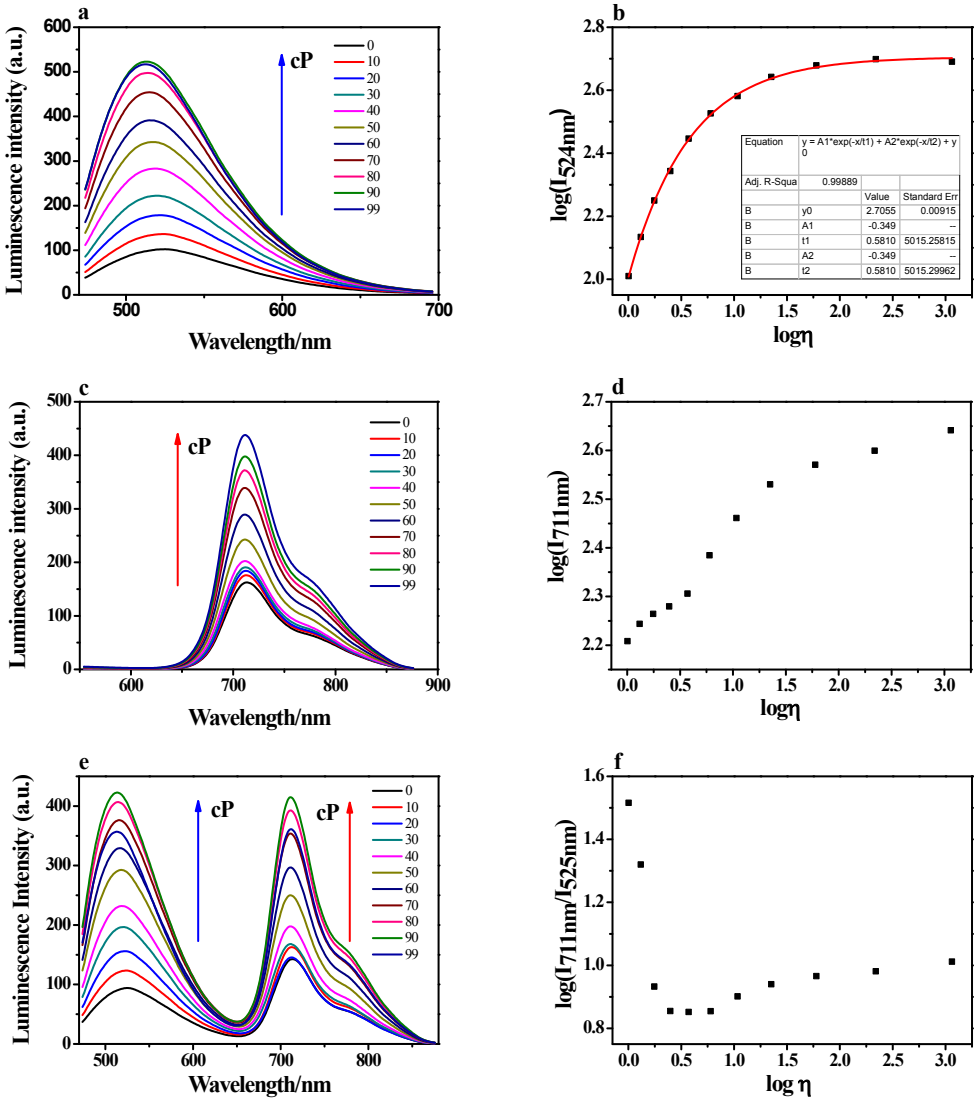


Fig. S6 Emission spectra of (a) **Ir-A** (c) **Ir-B** (e) **Ir-A**:**Ir-B**=1:1 (10 μM) in the mixed glycerol-water solvents, excited at 450 nm; (b) **Ir-A** (d) **Ir-B** (f) **Ir-A**:**Ir-B**=1:1 the non-linearity of log I versus log η plot.

Fig. S7

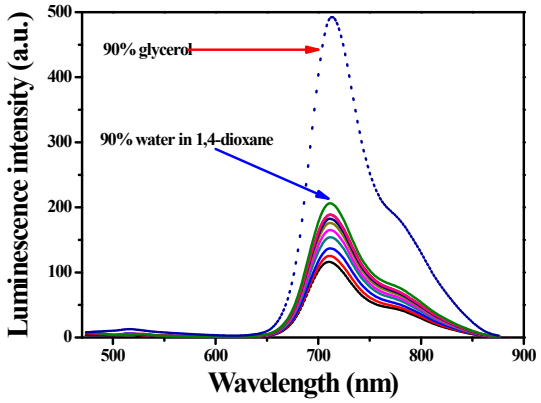


Fig. S7 Emission spectra of **C10** (10 μM) in different solvents. The solid lines represent the probe in different proportional water-1, 4-dioxane system; the dash and dot curve in the figure is the probe in glycerol-water (v/v: 90/10) at the same condition (all excited at 450 nm).

Fig. S8

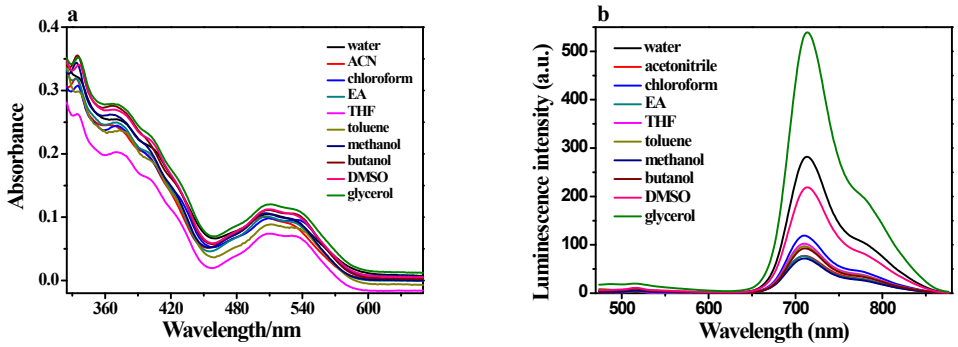


Fig. S8 (a) Absorbance and (b) emission spectra (λ_{ex}=450 nm) of probe **C10**（10 μM）in different solvents.

Fig. S9

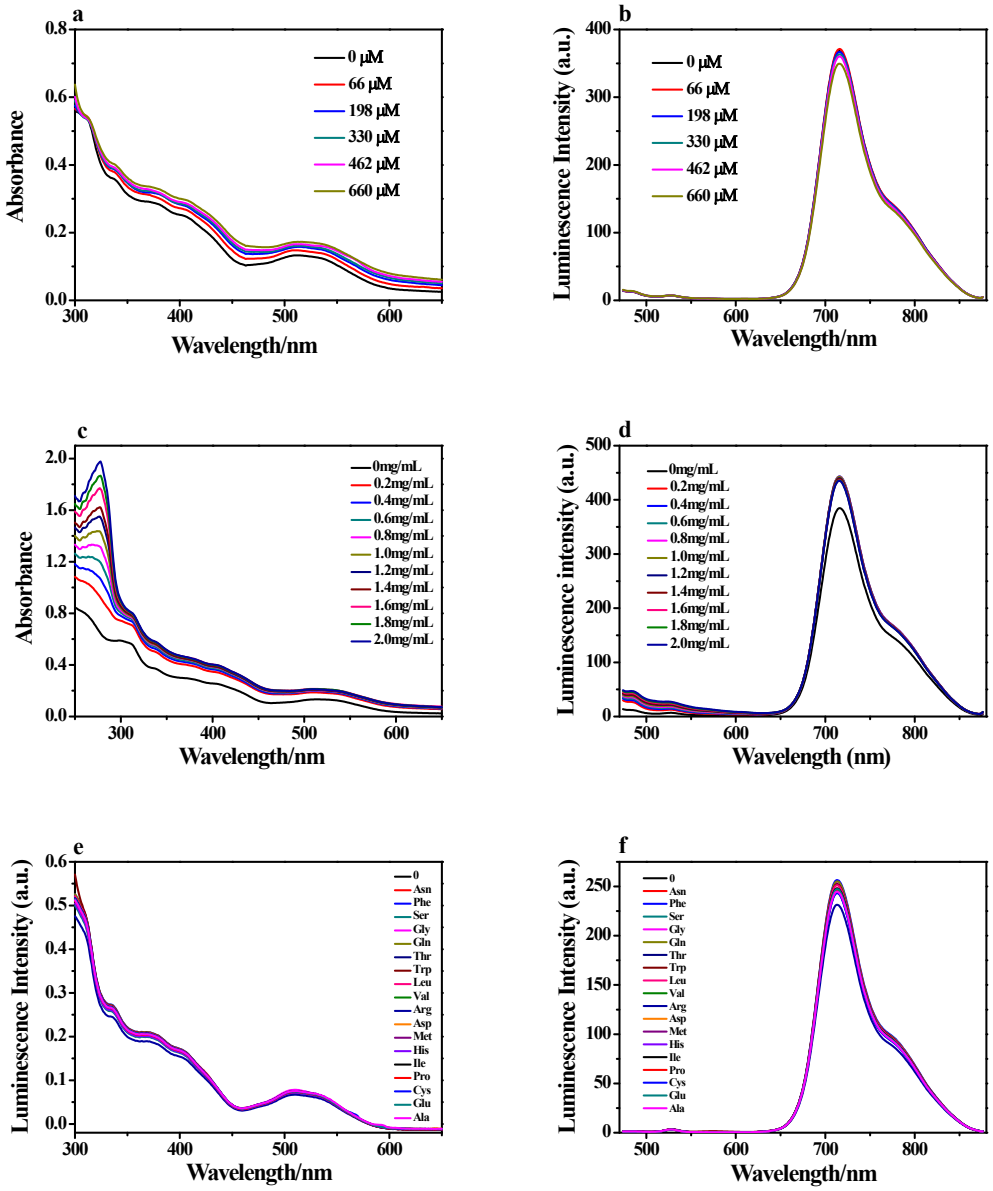


Fig. S9 Absorption (a) and emission (b) spectra of probe **C10** (10 μM) in PBS buffer (0.1 M, pH = 7.40) upon titration of calf thymus DNA solution. Absorption (c) and emission (d) spectra of probe **C10** (10 μM) in PBS buffer (0.1 M, pH = 7.40) upon titration of HSA solution. Absorption (e) and emission (f) spectra of probe **C10** (10 μM) in PBS buffer (0.1 M, pH = 7.40) with addition of 18 kinds of amino acids (100 μM). The excitation was at 450 nm.

Fig. S10

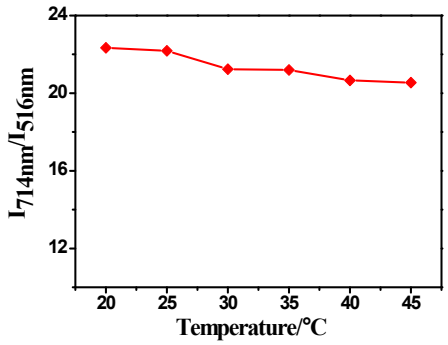


Fig. S10 Ratiometric luminescence intensity change of probe **C10** (10 μM) measured along with temperature changes in DMSO solvent, excited at 450 nm.

Reference

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2. W. Sun, S. Sun, N. Jiang, H. Wang and X. Peng, *Organometallics*, 2015, **34**, 3385-3389.
3. H. Li, Q. Yao, J. Fan, C. Hu, F. Xu, J. Du, J. Wang and X. Peng, *Ind. Eng. Chem. Res.*, 2016, **55**, 1477-1483.
4. A. P. Thomas, L. Palanikumar, M. T. Jeena, K. Kim and J.-H. Ryu, *Chem. Sci.*, 2017, **8**, 8351-8356.