Supporting information

Novel iridium(III) complex for HSA sensitive phosphorescent staining in proteome research†

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Contents

Experimental section

Materials and instruments

$IrCl_3 \cdot nH_2O$, HSA, BSA and other proteins were purchased from Sigma-Aldrich. 2-mercapto-1-methylimidazole and Coomassie blue G-250 were bought from Alfa Aesar. The concentration of HSA was measured by absorption method ([HSA] = A_{280}/36850, 1 cm curve). The dimer of iridium complex $[(2pq)_4Ir_2Cl_2]$ was prepared according to literature method.[1]

^1H NMR spectrum was recorded on a Bruker AV-400 spectrometer. Elemental analysis was performed by Exeter Analytical using a CHN/O/S Elemental Analyser (CE440). Positive ion ESI-MS spectrum was obtained using an Agilent 6130B single quad coupled to an automated sample delivery system (isocratic Agilent 1100 HPLC without column). UV-visible absorption spectra were recorded on a Varian Cary 300 UV-vis spectrophotometer. The fluorescent spectra were measured on JASCO FP-6500 Fluorimeter. The images of the gel were taken by Bio-Rad Gel Doc XR+ system.

Synthesis of iridium complex (Ir1)

The iridium dimer $[(2pq)_4Ir_2Cl_2]$ (0.05 mmol, 1eq), 2-mercapto-1-methylimidazole (0.3 mmol, 1.5 eq) were placed in a round bottom flask and DCM and methanol (2:1, 30 mL) was added. The mixture was heated at 65 °C for 3 h. After cooling the reaction mixture at room temperature, reduced the solvents to 3 mL, NH_4PF_6 water solution was added, the yellow solid filtered and washed with water and diethyl ether. The solid was further purified by silica column chromatography (elute, CH_2Cl_2:CH_3OH = 98:8) obtaining the pure complex. Yield: 86%. ESI-MS: 715.1$[M+H]^+$; ^1H NMR (400 MHz, DMSO-d^6): 8.55 (d, J = 6.0 Hz, 2H), 8.47 (d, J = 5.0 Hz, 2H), 8.37 (d, J = 5.2 Hz, 2H), 8.08 – 7.89 (m, 6H), 7.64 (t, J = 5.0 Hz, 2H), 7.52 (d, J = 6.0 Hz, 2H), 6.91 (d, J = 5.0 Hz, 2H), 6.59 (t, J = 5.2 Hz, 2H), 6.41 (d, J = 4.0 Hz, 1H), 6.24 (d, J = 4.0 Hz, 1H), 2.90 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d^6): δ 178.92, 171.60, 163.97, 161.46, 154.25, 152.41, 150.33, 143.00, 137.66, 135.90, 127.81, 124.20, 124.00, 122.84, 121.13, 118.10, 117.92, 117.38, 34.47. Anal. Calcd for $C_{34}H_{25}IrN_4S$: C, 57.20%; H, 3.53%; N, 7.85%; Found: C, 57.17%; H, 3.55%; N, 7.88%.

X-ray crystal structure

Single crystals of the iridium complex were grown from DMSO and ethanol. A suitable crystal was selected and mounted on a glass fibre with Fromblin oil and placed on an Xcalibur Gemini diffractometer with a Ruby CCD area detector. The crystal was kept at 150(2) K during data collection. Using Olex2[2], the structure was solved with the ShelXT structure solution program using Direct Methods and refined with the ShelXL refinement package using Least Squares minimisation. The asymmetric unit contains the complex, two molecules of DMSO and an ethanol at modeled at half occupancy. X-ray crystallographic data for the complex have been deposited in the Cambridge Crystallographic Data Centre under the accession number CCDC 1559254.

Circular dichroism (CD) measurement

The solution of HSA in the presence of the iridium complex was prepared in water. All experiments were carried out at 293 K and spectra were recorded using a JASCO-J-1500CD spectrometer and a quartz cuvette of path length of 1 mm. The scan range was 180 nm to 300 nm and each spectrum was the average of 8 scans. To avoid instrumental baseline drift between measurements, the background for water was subtracted for each individual sample measurement.

Docking simulation computation

The docking experiment was studied by standard GOLD 5.3.0 tool[3]. Two binding sites were defined as the residues with at least one heavy atom within 6 Å (standard default) from the Ir601 placement (PDB ID: 5IFO[4]). No water was present in any binding site. The default docking protocol was applied (1.0×auto settings, 10 GA) and the best pose saved. Each experiment was then repeated 3 times. The protocol was utilized for docking in which ChemPLP was used as the fitness function.

Emission measurement in urine sample

100 μL of non-pretreated urine samples collected from three healthy volunteers was mixed with 400 μL Milli-Q water to yield a 5-fold diluted urine sample. The iridium complex (5 μM) and indicated concentrations of HSA were added into the 5-fold diluted urine solutions. The mixtures were allowed to equilibrate at room temperature for 2 min. Emission spectra were recorded in the 520-680 nm range using an excitation wavelength of 465 nm.

Gel electrophoresis experiments

The glass plate sandwich of the electrophoresis apparatus is assembled according to the featured instructions (Biorad) using two clean glass plates and gasket. The stacking gel mixture is prepared and pipetted over the top. A 1 mm Teflon comb is inserted into the gel solution in between the two glass plates and the gel was allowed to polymerize. After completion of polymerization, the comb is carefully removed without tearing the edges of the wells. Wells are rinsed with the electrophoresis tank buffer. The proteins samples are carefully applied as a thin layer at the bottom of the well. The system is covered and connected to power supply at 15 mA constant current until the tracking dye reaches the bottom of the separating gel. The gel is incubated in Coomassie staining solution (100 mg/L Coomassie blue G250) for 1 h. Then the gel is taken out carefully and immersed in destaining solution (12% methanol, 7% glacial acetic acid and 81% H_2O) overnight. In case of staining with **Ir1**, the gel is incubated in respective concentrations of **Ir1** in the PBS solution and observed over UV transilluminator. No destaining is required.

Tables

Table S1. Crystal data and structure refinement for **Ir1**.

Empirical formula	$C_{34}H_{25}IrN_4S·2DMSO·1/2Ethanol$
Formula weight	893.13
Temperature/K	150(2)
Crystal system	triclinic
Space group	P-1
a/Å	12.17572(19)
b/Å	12.5686(2)
c/Å	12.97302(16)
α/°	97.9685(12)
β/°	95.6471(11)
γ/°	101.1031(14)
Volume/Å^3	1913.27(5)
Z	2
ρ_{calc}g/cm^3	1.550
μ/mm^{-1}	3.693
F(000)	894.0
Crystal size/mm^3	0.828 × 0.388 × 0.325 orange
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	5.274 to 75.318
Index ranges	-20 ≤ h ≤ 20, -21 ≤ k ≤ 21, -21 ≤ l ≤ 22
Reflections collected	64436
Independent reflections	19457 [R_{int} = 0.0330, R_{sigma} = 0.0352]
Data/restraints/parameters	19457/0/457
Goodness-of-fit on F^2	1.103
Final R indexes [I>=2σ (I)]	R_1 = 0.0384, wR_2 = 0.0976
Final R indexes [all data]	R_1 = 0.0475, wR_2 = 0.1021
Largest diff. peak/hole / e Å^{-3}	4.01/-1.82

Table S2. Selected bond lengths and angles for **Ir1**.

	Length (Å) or Angle (°)
Ir1-N5	2.196(3)
Ir1-S7	2.6196(7)
Ir1-N8	2.079(2)
Ir1-C23	1.985(3)
Ir1-N24	2.078(2)
Ir1-C39	1.992(3)
N5-Ir1-S7	65.35(7)
C23-Ir1-N8	80.20(10)
C39-Ir1-N24	80.04(10)
N8-Ir1-N5	83.91(9)
C23-Ir1-C39	92.92(11)
N24-Ir1-S7	85.47(6)
N8-Ir1-S7	100.82(7)
C23-Ir1-N5	97.31(10)
C23-Ir1-S7	162.22(8)
C23-Ir1-N24	94.82(10)
N24-Ir1-N5	101.82(9)
N24-Ir1-N8	172.92(8)
C39-Ir1-N5	169.39(10)
C39-Ir1-S7	104.61(8)
C39-Ir1-N8	95.11(10)

Table S3. Photophysical properties of **Ir1** in the absence and presence of HSA in air-saturated PBS solution (pH 7.4) at 298 K. The standard used was $[Ru(bpy)_3]^{2+}$ (Φ_{em} = 0.028).

Compounds	Emission	
	Lifetime τ/ns	Quantum yield Φ
Ir1	52	0.002
Ir1 + HSA	101	0.048

Table S4. Determination of HSA concentrations in healthy human urine.

Samples	HSA added (μg/mL)	Proposed method	
		Found (μg/mL)	Recovery (%)
1	2	2.1	100.5
2	5	4.7	94.0
3	10	10.3	103.0
4	30	29.8	99.3
5	100	101.5	101.5

Figures

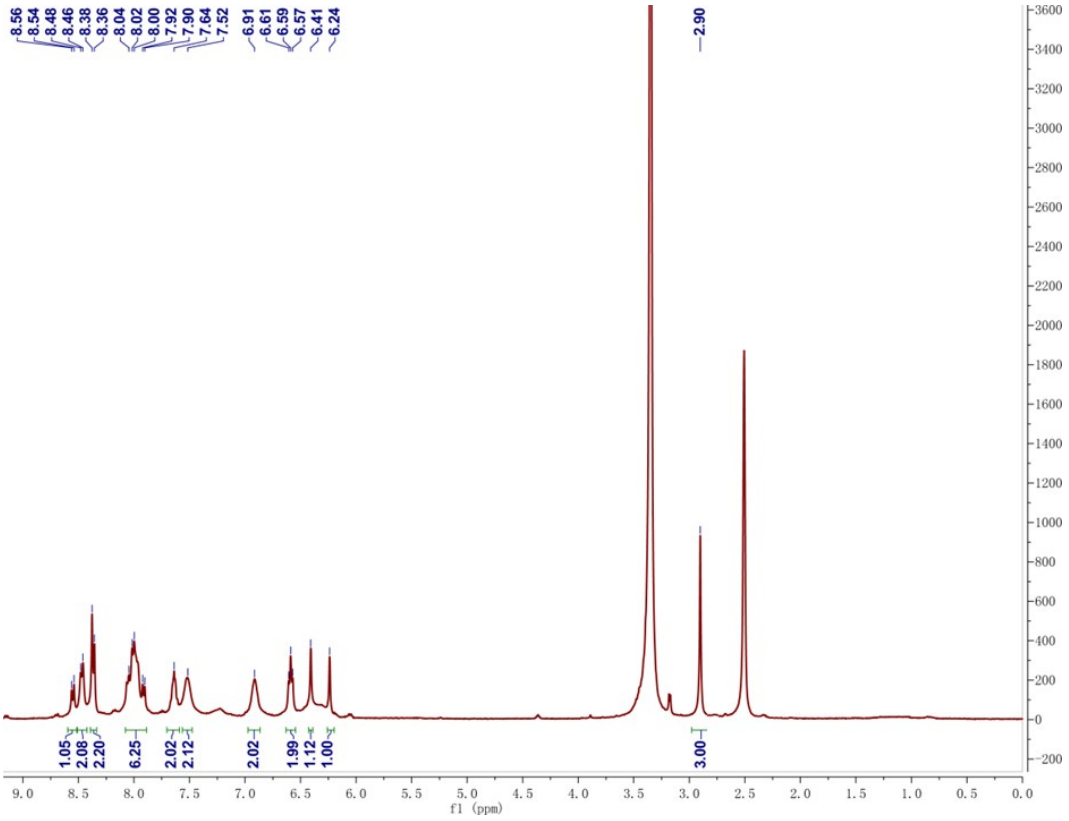

Fig. S1. The 400 MHz ^{1}H NMR spectrum of **Ir1** in the DMSO-d^{6} solution.

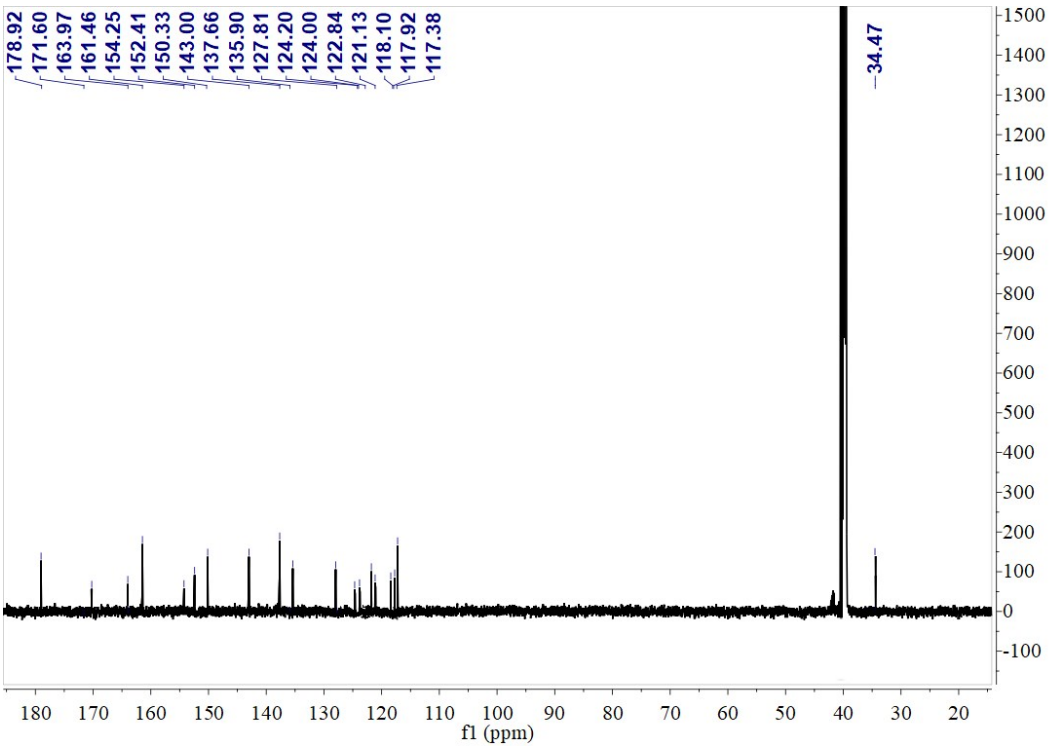

Fig. S2. The 126 MHz ^{13}C NMR spectrum of **Ir1** in the DMSO-d^{6} solution.

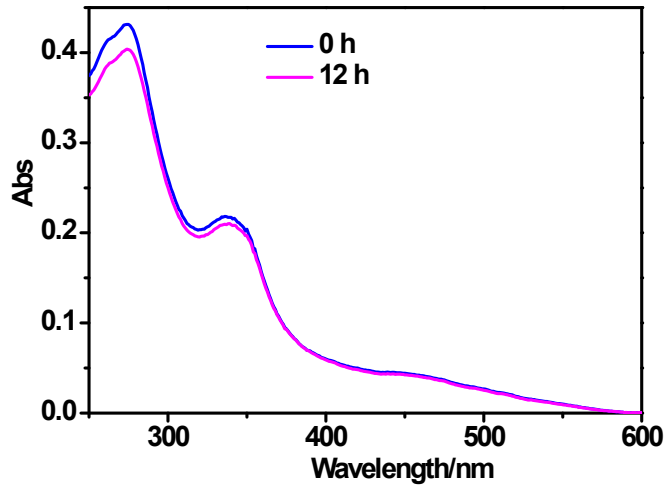

Fig. S3. The stability of **Ir1** in PBS solution (with 1% DMSO) for 12 h via UV-vis spectrophotometer.

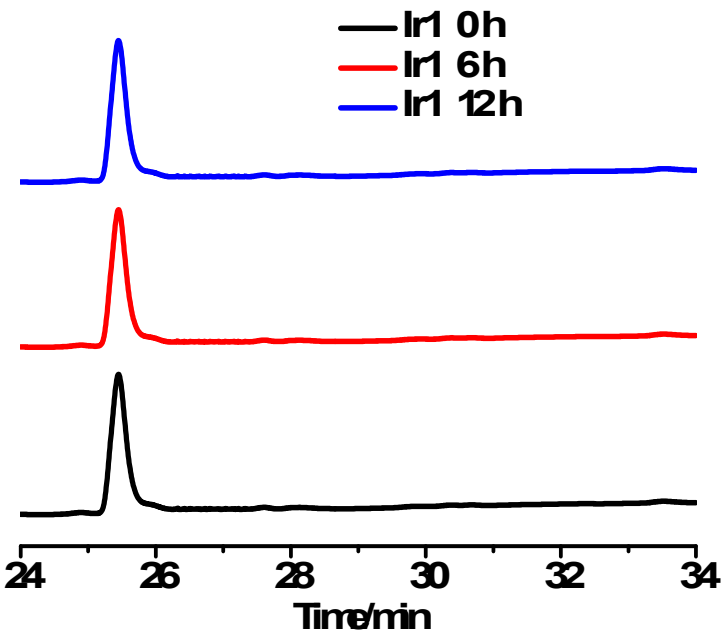

Fig. S4. The stability of **Ir1** in the water (with 1% DMSO) for 12 h via HPLC.

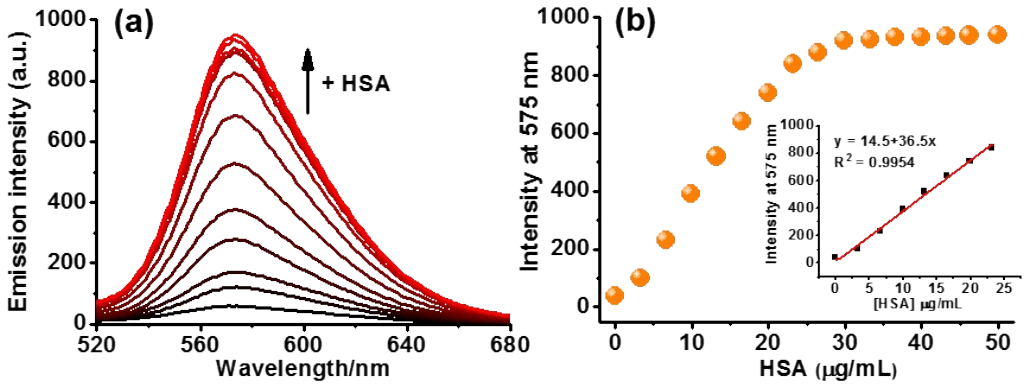

Fig. S5. (a) The emission spectra of **Ir1** (5 μM) in the presence of increasing concentrations of HSA (0-50 μg/mL) in the PBS solution. (b) The phosphorescence response of the system at 575 nm vs. HSA concentration. Inset: linear plot of the change in phosphorescence intensity at 575 nm vs. HSA concentration. The wavelength of the excitation is 465 nm.

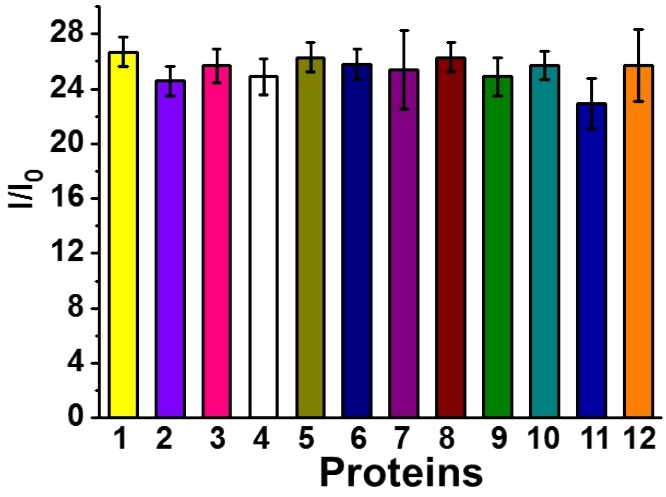

Fig. S6. The phosphorescence intensities of **Ir1** with HSA in the presence of other proteins (200 μg/mL); 1: **Ir1**+HSA; 2: **Ir1**+HSA+Hemin; 3: **Ir1**+HSA+Trypsin; 4: **Ir1**+HSA+Lysozyme; 5: **Ir1**+HSA+Apo-transferrin; 6: **Ir1**+HSA+Catalase; 7: **Ir1**+HSA+BSA; 8: **Ir1**+HSA+Hemoglobin; 9: **Ir1**+HSA+Methemoglibin; 10: **Ir1**+HSA+Holo-transferrin; 11: **Ir1**+HSA+Mytoglobin; 12: **Ir1**+HSA+Transferrin. The excitation wavelength is 465 nm.

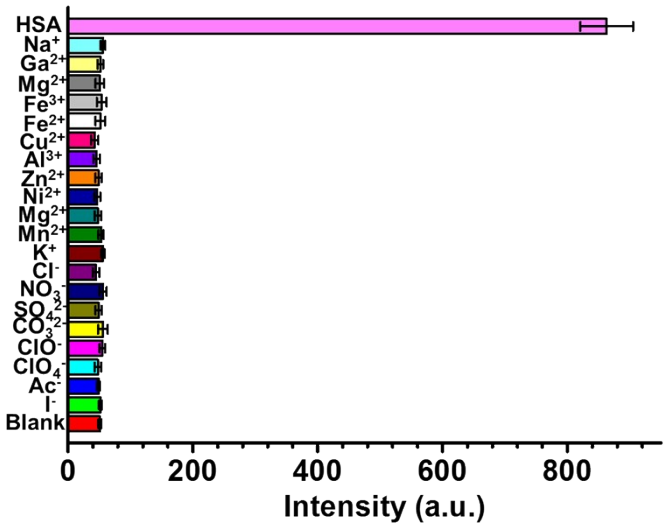

Fig. S7. The phosphorescence responses of **Ir1** (5 μM) to HSA (50 μg/mL) and various cations/anions (500 μM) at 575 nm in the PBS solution. λ_{ex} = 465 nm; Error bars represent the standard deviations of the results from three independent experiments.

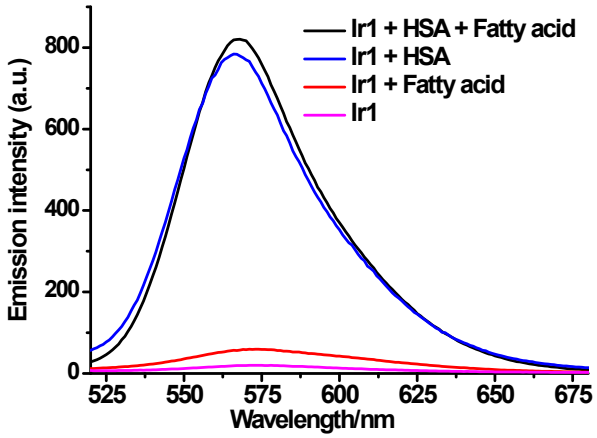

Fig. S8. The effect of fatty acid (isovaleric acid, 200 μg/mL) on phosphorescence emission spectra of **Ir1** in the presence of HSA (50 μg/mL). The excitation wavelength is 465 nm.

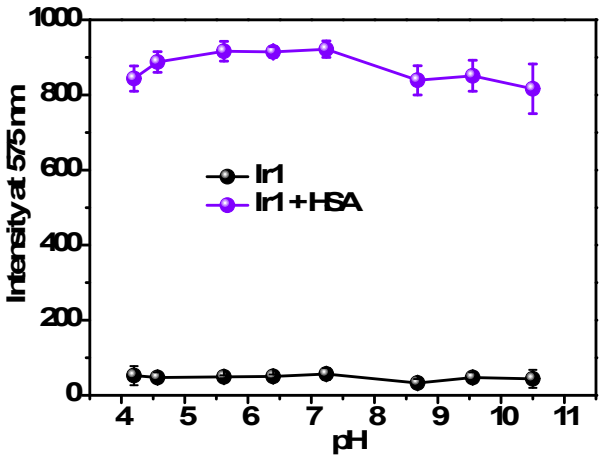

Fig. S9. The phosphorescence signal at 575 nm of **Ir1** (5 μM) with or without HSA (50 μg/mL) in the different values of pH at 298 K. The excitation wavelength is 465 nm.

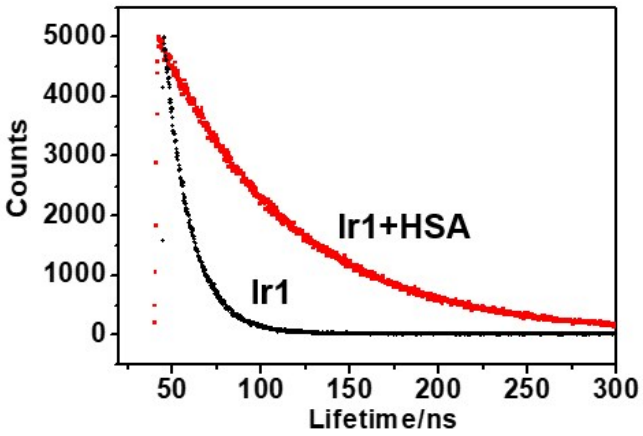

Fig. S10. The phosphorescence lifetimes of **Ir1** in the PBS solution (with 1% DMSO) in the absence and presence of HSA.

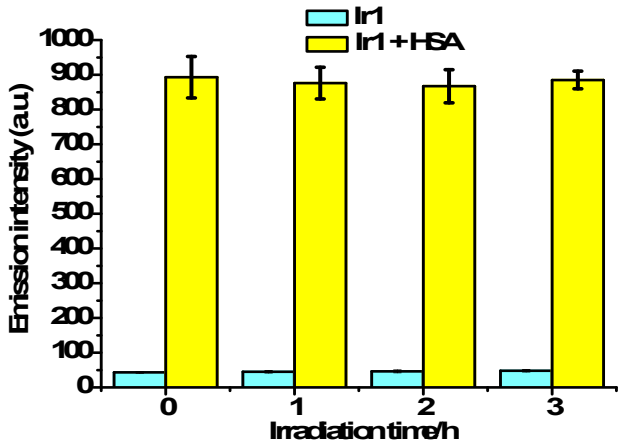

Fig. S11. The emission intensities of **Ir1** and **Ir1** + HSA at 575 nm upon 465 nm light irradiation for 0, 1, 2, 3 h.

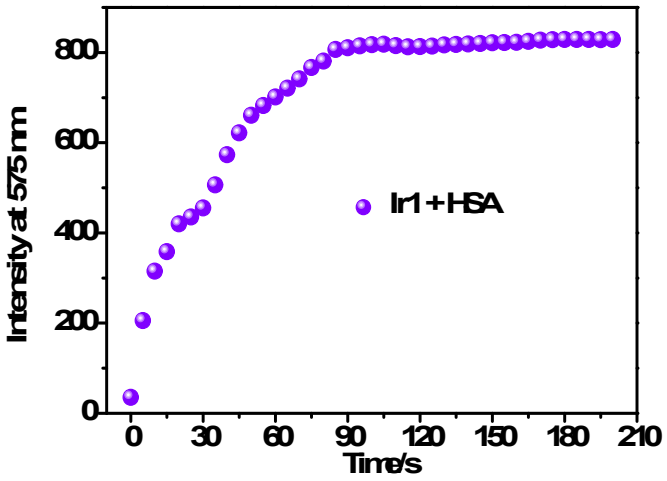

Fig. S12. Time course of phosphorescence signal at 575 nm of **Ir1** (5 μM) upon addition of HSA (50 μg/mL) in the PBS solution at 298K. The excitation wavelength is 465 nm.

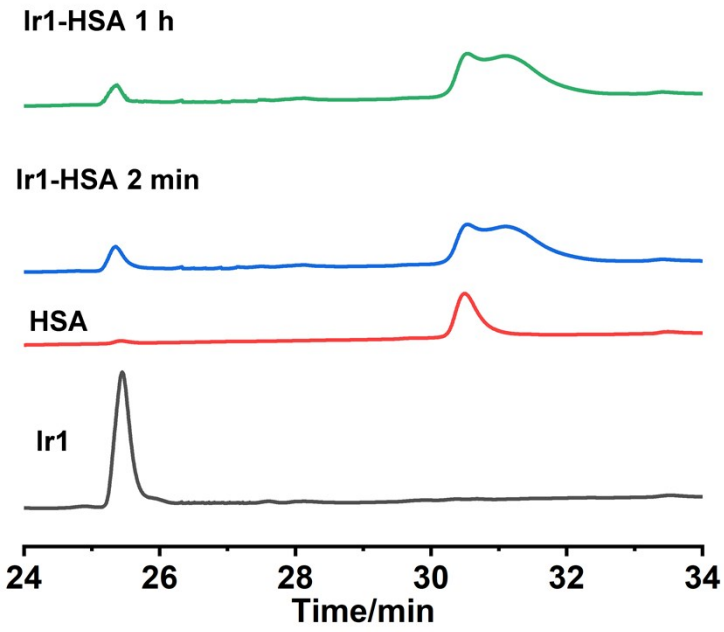

Fig. S13. Binding study of **Ir1** (5 μM) with 50 μg/mL HSA in the water using RP-HPLC.

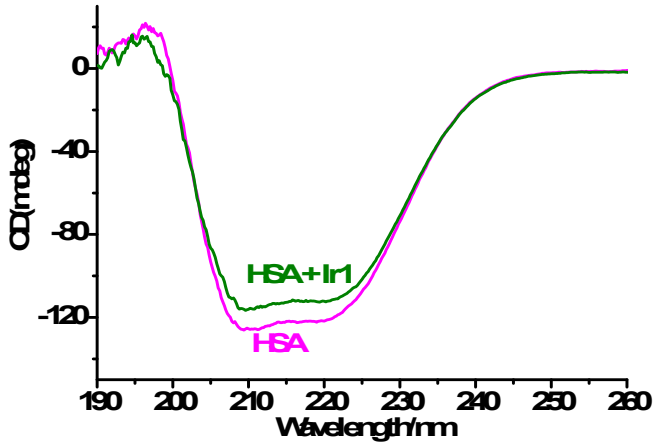

Fig. S14. Circular dichroism data of HSA (50 μg/mL) in the absence and presence of **Ir1** (5 μM).

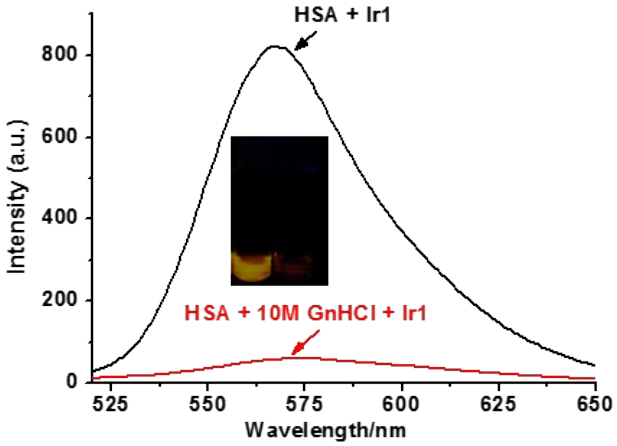

Fig. S15. The emission spectra of **Ir1** (5 μM) and HSA (50 μg/mL) in the presence or the absence of 10 M guanidine hydrochloride (GnHCl, a HSA unfolding agent) in HPLC water. The GnHCl and HSA mixed together for 2 h at 277 K before adding to **Ir1** solution. **Ir1** reacted with HSA alone or HSA-GnHCl mixture for 10 min at 298 K. The excitation wavelength is 465 nm.

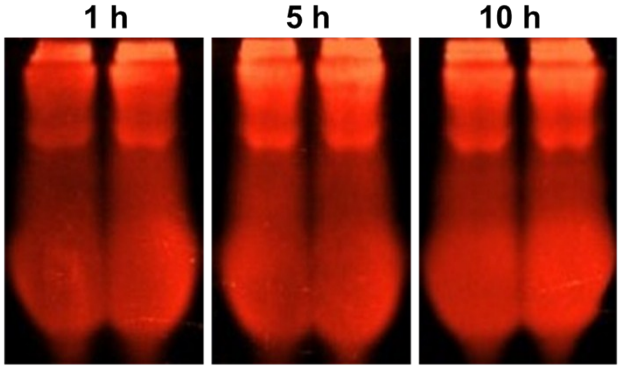

Fig. S16. The gel of 10 μg HSA per lane after separation treated with **Ir1** and then irradiated under UV lamp for 1 h, 5 h, 10 h. Twice in parallel with each experiment.

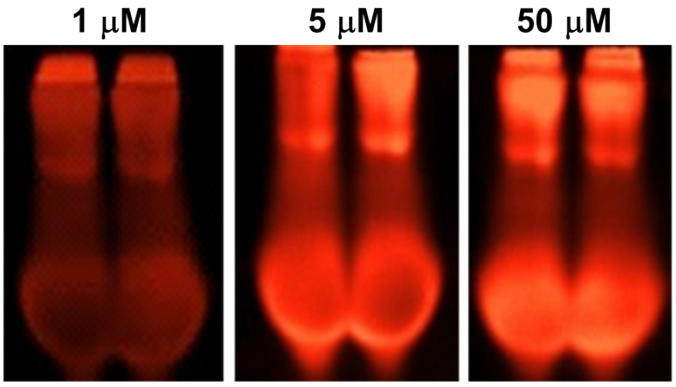

Fig. S17. The gel of HSA with an uniform concentration of 10 μg HSA per lane and gel treated with varying concentrations (1, 5, 50 μM) of **Ir1** in the PBS solution after separation. Twice in parallel with each experiment.

References

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