Supplementary Figure 1. Synthesis procedure of Ir-PVP.
Supplementary Figure 2. Synthesis procedure of Ir-PVP-RhB.
Supplementary Figure 3. Synthesis procedure of Ir-PVP-N797.
Supplementary Figure 4. Synthesis procedure of Ir-OH.
**Supplementary Figure 5. Comparison between the oxygen sensitivity of Ir-PVP and commercial oxygen probe.** (a) Emission spectra of commercial oxygen probe (Mito-ID intracellular O$_2$ probe, Enzo Life Sciences) under increasing oxygen levels (0, 1, 2.5, 5, 10, 20, 40, 60, 80 and 100%). Excitation: 390 nm. (b) Comparison between the decay curves of peak phosphorescent intensity of commercial oxygen probe (black) and Ir-PVP (red) over the increase of oxygen level. The peak phosphorescent intensities are displayed as relative intensities compared to the value at 100% O$_2$. 
Supplementary Figure 6. Emission spectra of Ir-PVP under various pH conditions.
Supplementary Figure 7. Penetration depth assessment of Ir-PVP. A small piece of tumor (about 2 mm diameter) containing Ir-PVP was used as the signal source. Pork ham was used to represent the animal tissue and the slices of pork harm with the thickness of 1.5 mm were covered one by one upon the signal source. The images were repeatedly captured with the adding of the slices by Maestro EX fluorescence imaging system (Cambridge Research & Instrumentation, CRi, USA). A 595 nm light source is used for excitation and the emission is received from 670 to 800 nm.
Supplementary Figure 8. Immunostaining of 4T1 and HeLa tumor section. The frozen section of 4T1 tumor (a, b) and HeLa tumor (c, d) were stained with HIF-1α antibody (green fluorescence) and pimonidazole antibody (red fluorescence).
Supplementary Figure 9. Isothermal titration calorimetry (ITC) data. Ir-PVP (a) and Ir-OH (b) were titrated with injections of BSA solution, respectively. The black solid squares represent the integrated heats of each injection and the red lines show on-site binding fitting curves. Binding parameters including binding stoichiometries (N), binding constant (K), enthalpy change (ΔH) and entropy change (ΔS) are listed in the insert charts.
Supplementary Figure 10. Total percentage of cell early apoptosis, late apoptosis and necrosis at different concentrations of Ir-PVP. SH-SY5Y cells were incubated with Ir-PVP for 48 h at different concentrations (0.1 - 1.6 mg/mL). Then the cells were stained with annexin V-FITC and propidium iodide (PI) and analyzed by flow cytometry. The inserted charts show the ratios of early and late apoptosis and necrosis of the cells treated with PBS as control (left) and 1.6 mg/mL Ir-PVP (right).
Supplementary Figure 11. Phototoxicity test of Ir-PVP. Confocal luminescence image of ROS generation stained with DCFH-DA (a) and flow cytometric analysis of cell apoptosis and necrosis (b) in SH-SY5Y cells after irradiated by a 595 nm light source at the power of 40 J cm\(^{-2}\) under 21% O\(_2\). The cells were incubated with Ir-PVP (1.6 mg/mL) for 2 h before phototoxicity test. The cells incubated with PBS for 2 h were used as control.
Supplementary Figure 12. Blood coagulation results of Ir-PVP. (a) Fibrinogen (FIB), (b) prothrombin time (PT) and (c) activated partial thromboplastin time (APTT) were measured to evaluate the abnormality of coagulation factor I, extrinsic and intrinsic coagulation pathways, respectively. Ir-PVP was tested at various concentrations (0.1, 0.2, 0.4, 0.8 and 1.6 mg/mL). The results are presented as the mean ± SD (n = 5).
Supplementary Figure 13. Serum biochemistry test of Ir-PVP. (a) Serum biochemistry results for alanine aminotransferase (ALT), (b) Aspartate aminotransferase (AST), (c) Total bilirubin (TBIL), (d) blood urea nitrogen (BUN), (e) Creatinine (CREA) and (f) Urea (UREA) from ICR mice 1 d and 7 d after intravenous injection of Ir-PVP (40 mg/kg). ICR mice intravenously injected with PBS were used as control. For each group, five mice were tested. The results are presented as the mean ± SD (n = 5).
Supplementary Figure 14. Histological examination of heart, liver, kidney, spleen and lung stained with haematoxylin and eosin. All tissues were from ICR mice at 7 days after intravenous injection of PBS as control or Ir-PVP probe (40 mg/kg). Scale bar, 100 μm.
Supplementary Methods

**General materials and methods.** Azodiisobutyronitrile (AIBN) was recrystallized from 95% ethanol before use. Other commercially available chemicals were used as received. Human neuroblastoma cell line SH-SY5Y, murine hepatic cancer cell line H22 and other cell lines used in the research were obtained from Shanghai Institute of Cell Biology (Shanghai, China). Male ICR mice and BALB/c nude mice (6-8 weeks old and weighing 18-22 g) were provided by Animal Center of Drum Tower Hospital (Nanjing, China).

$^1$H NMR spectra were collected on a Bruker DRX-300, DRX-500 and DRX-600 spectrometer. ESI-MS spectrometric data were determined with a LCQ ESI-MS Therso Finnigan mass spectrometer. High resolution mass spectrometry (HRMS) data were determined with Agilent liquid chromatography-mass spectrometry (G6500). Gel permeation chromatography (GPC) analysis was conducted on a Waters 244 (Milford, MA, USA) with 0.03M LiBr-contained DMF as eluent. UV-visible spectra were recorded on a Shimadzu UV-2401 spectrophotometer. Steady-state emission spectra at room temperature were measured on a Horiba Jobin Yvon FluoroMax-4 NIR spectrofluorometer. Nitrogen-oxygen mixed gas regulated with a mass-flow meter was bubbled through the sample solution for 30 minutes before luminescence measurement. Luminescence quantum yield of the probe was determined with reference to Rhodamine B (0.69 in ethanol).

**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 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 hours under N₂ 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 MgSO₄, 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). ¹H NMR (300 MHz, CDCl₃): δ 8.72 (d, J = 8.3 Hz, 1H, 1-H phenanthridine), 8.66 (d, J = 8.2 Hz, 1H, 7-H phenanthridine), 8.60 (d, J = 8.1, 1H, 4-H phenanthridine), 8.26 (d, J = 8.2, 1H, 7-H benzo[b]thiophene), 7.97-7.89 (m, 3H, 3, 10-H phenanthridine, 4-H benzo[b]thiophene), 7.87 (s, 1H, 3-H benzo[b]thiophene), 7.80-7.67 (m, 3H, 2, 8, 9-H phenanthridine), 7.46-7.41 (m, 2H, 5, 6-H benzo[b]thiophene); ¹³C NMR (125 MHz, CDCl₃): δ 154.0 (C), 143.7 (C), 142.7 (C), 140.8 (C), 140.1 (C), 133.6 (C), 130.7 (CH), 130.4 (CH), 129.0 (CH), 128.0 (CH), 127.6 (CH), 127.4 (CH), 126.1 (CH), 125.2 (CH), 124.8 (CH), 124.6 (CH), 124.3 (CH), 123.7 (CH), 122.4 (C), 122.3 (CH), 122.0 (C); ESI-MS (m/z): [M]⁺ calcd. for C₂₁H₁₃NS, 312; found, 312; HRMS (m/z): [M]⁺ calcd. for C₂₁H₁₃NS, 312.0841; found, 312.0842.

Synthesis of the chloride-bridged Ir(III) dimer Ir₂(btph)₄μ-Cl₂ (2). IrCl₃·3H₂O
(390 mg, 1.1 mmol) and water (10 mL) were added to a solution of 1 (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. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.96 (d, $J = 8.0$ Hz, 4H, 1-H phenanthridine), 8.44 (d, $J = 8.1$ Hz, 4H, 4-H phenanthridine), 7.97 (m, 8H, 4-H benzo[b]thiophene, 7-H phenanthridine), 7.92 (t, $J = 7.5$ Hz, 4H, 3-H phenanthridine), 7.80 (t, $J = 7.5$ Hz, 4H, 2-H phenanthridine), 7.43 (d, $J = 7.9$ Hz, 4H, 7-H benzo[b]thiophene), 6.71 (t, $J = 7.4$ Hz, 4H, 9-H phenanthridine), 6.58 (t, $J = 7.4$ Hz, 4H, 8-H phenanthridine), 5.97 (t, $J = 7.6$ Hz, 4H, 6-H benzo[b]thiophene), 5.81 (t, $J = 7.7$ Hz, 4H, 5-H benzo[b]thiophene), 5.39 (d, $J = 8.2$ Hz, 4H, 10-H phenanthridine); IR: 1421 cm$^{-1}$; UV/Vis: $\lambda_{\text{max}}$ 332 nm.

**Synthesis procedure of 3, 6-dihydroxy-1-(4-methoxyphenyl)-2-hexen-1-one (mho-OH, 3).** Ethanol (0.2 mL) was added to a stirred suspension of sodium hydride (60% in paraffin oil, 10 g) in diethyl ether (200 mL) at 0 °C. To above mixture was then added $\gamma$-butyrolactone (12.9 g, 150 mmol), followed by dropwise addition of 4'-methoxyacetophenone (15 g, 100 mmol) in diethyl ether (50 mL). The mixture was allowed to stir for 96 h at room temperature. Ethanol (10 mL) was then added to destroy excess sodium hydride, followed by addition of aqueous solution of ammonium chloride (10 g in 100 mL water). Ether layer was separated, washed with brine (100 mL × 3) and dried over anhydrous MgSO$_4$. Then the solvent was removed.
under reduced pressure. The residue was purified by column chromatography on silica gel using petrol/ethyl acetate (1/1) as the eluent to provide a white solid (8.26 g, 35% yield). $^1H$ NMR (300 MHz, CDCl$_3$): $\delta$ 7.90 (d, $J = 8.7$ Hz, 2H, 2, 6-H PhCO), 6.92 (d, $J = 8.7$ Hz, 2H, 3, 5-H PhCO), 6.53 (s, 1H, COCH), 4.29 (t, $J = 7.1$ Hz, 2H, CH$_2$OH), 3.86 (s, 3H, OCH$_3$), 3.28 (t, $J = 7.7$ Hz, 2H, HOCH$_2$), 2.15 (m, 2H, CH$_2$CH$_2$OH); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 195.5 (C), 183.5 (C), 162.6 (C), 129.7 (CH), 129.1 (C), 113.6 (CH), 94.9 (CH), 61.9 (CH$_2$), 55.6 (CH$_3$), 31.4 (CH$_2$), 23.8 (CH$_2$); ESI-MS (m/z): [M]$^-$ calcd. for C$_{13}$H$_{16}$O$_4$, 235; found, 235; HRMS (m/z): [M]$^-$ calcd. for C$_{13}$H$_{16}$O$_4$, 235.0976; found, 235.0980.

Synthesis procedure of 4-hydroxy-6-(4-methoxyphenyl)-6-oxohex-4-enyl 2-(ethoxycarbonothioylthio)propanoate (mho-CTA, 4). 2-bromopropionyl bromide (3.43 g, 31.8 mmol) dissolved in anhydrous tetrahydrofuran (20 mL) was added slowly to a mixture of 3 (5 g, 21.2 mmol), pyridine (2.6 mL, 31.8 mmol) and anhydrous tetrahydrofuran (30 mL) under vigorous stirring at 0 °C. The mixture was allowed to stir at room temperature overnight and then concentrated to 15 mL under reduced pressure. The concentrated solution was poured into diluted hydrochloric acid (0.01 M, 300 mL) and extracted with dichloromethane (50 mL × 3). The dichloromethane layer was collected, washed with water (50 mL × 3) and dried over anhydrous MgSO$_4$. The solvent was evaporated under reduced pressure. Then the residue was dissolved in acetone (20 mL) and slowly added to the mixture of potassium O-ethyl xanthate (3.4 g, 21.2 mmol) and acetone (20 mL). The mixture was
stirred for 24 h at room temperature. The solvent was evaporated under vacuum. The residue was dissolved in dichloromethane (200 mL), washed with water (50 mL × 3), and dried over anhydrous MgSO₄. The solvent was evaporated under vacuum, and the product was purified by column chromatography on silica gel using petrol/ethyl acetate (1/1) as the eluent to provide a red liquid (2.97 g, 34% yield). ¹H NMR (300 MHz, CDCl₃): δ 7.87 (d, J = 8.8 Hz, 2H, 2, 6-H PhCO), 6.94 (d, J = 8.8 Hz, 2H, 3, 5-H PhCO), 6.12 (s, 1H, COCH), 4.64 (q, J = 7.1 Hz, 2H, CH₂CH₃), 4.40 (q, J = 7.4 Hz, 1H, CHCH₃), 4.23 (t, J = 5.9 Hz, 2H, CH₂OCO), 3.87 (s, 3H, OCH₃), 2.50 (t, J = 7.3 Hz, 2H, HOCH₂), 2.05 (m, 2H, CH₂CH₂OCO), 1.58 (d, J = 7.3 Hz, 3H, CHCH₃), 1.41 (t, J = 7.1 Hz, 3H, CH₂CH₃); ¹³C NMR (125 MHz, CDCl₃): δ 214.9 (C), 193.8 (C), 183.7 (C), 173.7 (C), 163.1 (C), 130.6 (CH), 129.1 (C), 114.0 (CH), 95.3 (CH), 69.7 (CH₂), 64.6 (CH₂), 55.5 (CH₃), 47.2 (CH), 35.2 (CH₂), 24.7 (CH₂), 21.0 (CH₃), 16.8 (CH₃); ESI-MS (m/z): [M + Na]⁺ calcd. for C₁₉H₂₄O₆S₂, 435; found, 435; HRMS (m/z): [M + Na]⁺ calcd. for C₁₉H₂₄O₆S₂, 435.0906; found, 435.0908.

**Synthesis procedure of PVP with mho-CTA as chain transfer agent (mho-PVP, 5).**

Compound 4 (0.083 g, 0.2 mmol), AIBN (3 mg, 0.018 mmol) and fresh distilled N-vinypyrrolidone (4 g, 36 mmol) were dissolved in anhydrous tetrahydrofuran (4 mL) and heated to 60 °C under Ar atmosphere for 48 hours. The mixture was dissolved in dichloromethane (4 mL), precipitated into diethyl ether (300 mL) and dried in vacuum to give a white powder (2.76 g, 69% monomer conversion). ¹H NMR (500 MHz, CDCl₃): δ 7.88 (d, J = 8.3 Hz, 2H, 2, 6-H PhCO), 6.96 (d, J = 8.4 Hz, 2H, 3, 5-H
PhCO), 6.16 (s, 1H, COCH), 4.66 (q, 7.1 Hz, 2H, CH₂CH₃), 3.70 (m, broad, 118H, PVP NCH), 3.19 (m, broad, 240H, PVP NCH₂), 2.68-1.39 (m, broad, 728H, PVP CHCH₂, COCH₂, NCH₂CH₂); ¹³C NMR (125 MHz, CDCl₃): δ 175.8 (C, PVP), 161.1 (C), 130.5 (CH), 129.3 (C), 113.9 (CH), 94.4 (CH, PVP), 44.9 (CH, PVP), 42.1 (CH₂, PVP), 34.9 (CH₂, PVP), 31.4 (CH₂, PVP), 18.3 (CH₂, PVP); Mn (NMR) = 13,700 Da, Mn (GPC) = 12,400 Da, PDI = 1.52; IR: 1652 cm⁻¹; UV/Vis: λ_max 284, 324 nm.

**Synthesis procedure of PVP-conjugated iridium (III) complex (Ir-PVP, 6).**

Ir₂(btph)₄μ-Cl₂ (136 mg, 0.08 mmol), Compound 5 (1.04 g, 0.08 mmol) and sodium carbonate (85 mg, 0.8 mmol) were refluxed under N₂ atmosphere in 2-ethoxyethanol (15 mL) for 48 hours. After cooling to room temperature, the mixture was filtered and precipitated into diethyl ether. The precipitate was washed with diethyl ether and dried under vacuum to give a purple-black powder (1.01 g, yield 86%). ¹H NMR (600 MHz, CDCl₃): δ 9.27 (d, J = 8.3 Hz, 1H, 1-H phenanthridine), 9.06 (d, J = 8.2 Hz, 1H, 1-H phenanthridine), 8.80 (d, J = 8.3 Hz, 1H, 4-H phenanthridine), 8.65 (d, J = 8.4 Hz, 1H, 4-H phenanthridine), 8.56 (d, J = 8.2 Hz, 1H, 4-H benzo[b]thiophene), 8.19 (d, J = 8.2 Hz, 1H, 4-H benzo[b]thiophene), 8.04-7.99 (m, 2H, 2, 6-H PhCO), 7.94 (t, J = 7.8 Hz, 2H, 3-H phenanthridine), 7.86 (t, J = 7.7 Hz, 1H, 2-H phenanthridine), 7.71 (d, J = 8.0 Hz, 1H, 7-H phenanthridine), 7.52 (t, J = 7.7 Hz, 1H, 2-H phenanthridine), 7.58 (d, J = 8.0 Hz, 1H, 7-H phenanthridine), 7.33 (m, 4H, 7-H benzo[b]thiophene, 9-H phenanthridine), 7.09-7.04 (m, 2H, 3, 5-H PhCO), 6.91 (m, 2H, 8-H phenanthridine), 6.55 (t, J = 7.8 Hz, 2H, 6-H benzo[b]thiophene), 6.42 (d, J = 8.3 Hz,
1H, 10-H phenanthridine), 6.33 (t, J = 7.7 Hz, 1H, 5-H benzo[b]thiophene), 6.30 (t, J
= 7.8 Hz, 1H, 5-H benzo[b]thiophene), 6.06 (d, J = 8.1 Hz, 1H, 10-H phenanthridine),
5.18 (s, 1H, COCH), 3.72 (m, broad, 125H, PVP NCH), 3.21 (m, broad, 248H, PVP
NCH₂), 2.36-1.41 (m, broad, 741H, PVP CHCH₂, COCH₂, NCH₂CH₂); 13C NMR
(150 MHz, CDCl₃): δ 188.2 (C), 175.4 (C, PVP), 161.2 (C), 132.3 (C), 131.9 (CH),
131.6 (CH), 129.3 (CH), 129.1 (C), 128.4 (CH), 127.7 (CH), 127.3 (CH), 126.6 (CH),
125.9 (CH), 125.2 (CH), 124.7 (CH), 123.9 (CH), 122.4 (CH), 121.7 (C), 112.9 (CH),
95.2 (CH), 44.9 (CH, PVP), 42.1 (CH₂, PVP), 35.5 (CH₂, PVP), 31.5 (CH₂, PVP),
18.3 (CH₂, PVP); Mn (NMR) = 14,100 Da, Mn (GPC) = 13,000 Da, PDI = 1.50; IR:
1652 cm⁻¹; UV/Vis: λ max 530, 560 nm.

Synthesis procedure of the iridium (III) complex without PVP chain (Ir-OH, 7).
Ir₂(btpb)₄μ-Cl₂ (136 mg, 0.08 mmol), Compound 3 (47 mg, 0.2 mmol) and sodium
carbonate (85 mg, 0.8 mmol) were refluxed under N₂ atmosphere in 2-ethoxyethanol
(10 mL) overnight. After cooling to room temperature, the mixture was precipitated
into diethyl ether. The precipitate was filtered, washed with diethyl ether and then
purified by column chromatography on silica gel using chloroform as the eluent to
provide a black powder (109 mg, yield 65%). ¹H NMR (300 MHz, DMSO-d₆): δ 9.22
(m, 2H, 1-H phenanthridine), 8.94 (m, 2H, 4-H phenanthridine), 8.65 (t, J = 7.7 Hz,
2H, 3-H phenanthridine), 8.07 (m, 4H, 4-H benzo[b]thiophene, 2, 6-H PhCO), 7.89 (t,
J = 7.7 Hz, 2H, 2-H phenanthridine), 7.79 (d, J = 8.4 Hz, 1H, 7-H phenanthridine),
7.72 (d, J = 8.4 Hz, 1H, 7-H phenanthridine), 7.48 (t, J = 7.6 Hz, 1H, 9-H
phenanthridine), 7.40 (d, J = 8.8 Hz, 2H, 7-H benzo[b]thiophene), 7.30 (m, 2H, 3, 5-H PhCO), 7.09 (t, J = 7.7 Hz, 2H, 8-H phenanthridine), 6.85 (t, J = 7.8 Hz, 1H, 9-H phenanthridine), 6.69 (d, J = 8.8 Hz, 2H, 10-H phenanthridine), 6.60 (t, J = 7.7 Hz, 2H, 6-H benzo[b]thiophene), 6.40 (m, 2H, 5-H benzo[b]thiophene), 5.29 (s, 1H, COCH), 3.65 (s, 3H, OCH₃), 1.77 (m, 2H, CH₂OH), 1.04 (m, 2H, HOCCH₂), 0.87 (m, 2H, CH₂CH₂OH); ¹³C NMR (125 MHz, CDCl₃): δ 188.1 (C), 178.1 (C), 168.1 (C), 161.6 (C), 146.6 (C), 145.1 (C), 143.1 (C), 133.8 (C), 131.9 (C), 129.1 (CH), 128.9 (C), 128.5 (CH), 128.2 (CH), 127.7 (CH), 127.3 (CH), 126.7 (CH), 126.5 (CH), 126.3 (CH), 126.2 (CH), 125.3 (CH), 124.7 (CH), 123.9 (CH), 122.4 (CH), 122.3 (C), 121.7 (CH), 121.5 (C), 113.0 (CH), 95.5 (CH), 61.8 (CH₂), 55.3 (CH₃), 37.7 (CH₂), 29.0 (CH₂); ESI-MS (m/z): [M + Na]⁺ calcd. for IrC₅₅H₃₉O₄NS, 1071; found, 1071; HRMS (m/z): [M + Na]⁺ calcd. for IrC₅₅H₃₉O₄NS, 1071.1872; found, 1071.1869; UV/Vis: λₘₐₓ 530, 560 nm.

**Linking Ir-PVP with Rhodamine B and NIR-797.** Ir-PVP labelled with Rhodamine B (Ir-PVP-RhB, 10) or NIR-797 (Ir-PVP-N797, 11) was prepared using a previously reported method¹. Briefly, mho-PVP (5) was transformed to mho-PVP-OH (8) by hydrolysis in 40 °C distilled water. After reaction, the 4.66 ppm peak in the ¹H NMR spectrum disappeared, revealing the transformation from xanthate to hydroxyl group. Then mho-PVP-OH (8) was allowed to react with Ir₂(btph)₄μ-Cl₂ to yield Ir-PVP-OH (9) in the same method as the preparation of Ir-PVP (6). Ir-PVP-OH (9) and Rhodamine B isothiocyanate (RhB-NCS, mole ratio of 10%) was dissolved in CH₂Cl₂
and the mixture was stirred for 24 h at room temperature. After reaction, the mixture was precipitated into diethyl ether, filtered and washed three times with diethyl ether. The crude product was dried in vacuum, dissolved in distilled water and dialyzed in excess distilled water for three times to remove unbonded dyes. Then the product Ir-PVP-RhB (10) was obtained as dry powder after freeze drying. The linking of Rhodamine B was confirmed by the 565 nm peak in the luminescence emission spectrum of the product upon excitation at 510 nm. Ir-PVP-N797 (11) was prepared in the same way as Ir-PVP-RhB and the linking of NIR-797 was confirmed by the 825 nm peak in the luminescence spectrum upon excitation at 750 nm.

**Flow cytometry measurement.** SH-SY5Y cells were plated in a 6-well plate at a density of $10^6$ cell/well for 12 h and co-incubated with a series doses of Ir-PVP (0.1-1.6 mg/mL) at 37 °C for 48 h. Then the cells were harvested, washed with cold PBS and tested with flow cytometry (BD FACS Aria II SORP, USA) by staining early/late apoptosis and necrosis with annexin V-FITC and PI according to the manufacturer’s protocol.

**Blood coagulation test of the probe.** Fresh blood from ICR mice anticoagulated with citric-acid-dextrose was centrifugated for 10 min at 3000 rpm to isolate the plasma. 500 μL plasma was incubated with 50 μL PBS solution with various concentrations of Ir-PVP (0, 0.1-1.6 mg/mL) for 5 min. After centrifugation at 4000 rpm for 2 min, the upper solutions were sucked out to measure FIB, PT and APTT values on an ACL
7000 fully-automatic blood coagulation analyzer using HemosIL kit (Instrumentation Laboratory Company, USA).

**Serum biochemistry and histopathology examination.** ICR mice (n=10) were i.v. injected with Ir-PVP (40 mg/kg). 1d and 7d later, blood samples were collected and serum biochemistry was examined by the analysis of ALT, AST, TBIL, BUN, CREA and UREA. On the 7th day after injection of Ir-PVP (40 mg/kg), the organs (heart, liver, spleen, kidney and lung) were dissected after the removal of blood, cleaned and then stained with haematoxylin and eosin (H&E). ICR mice (n=10) i.v. injected with PBS were examined in the same way as Ir-PVP and the results were as control.

**Phototoxicity examination of Ir-PVP.** SH-SY5Y cells were seeded on 6-well plates and incubated with Ir-PVP (1.6 mg/mL) for 2 h at 37 °C. Then the cells were irradiated by a 595 nm light source (40 J cm\(^2\)) from Maestro EX fluorescence imaging system. Afterward, the cells were stained with annexin V-FITC and PI, harvested, washed with PBS and subjected to flow cytometric assay. For the measurement of ROS production, the cells were further incubated with 10 μM DCFH-DA for 20 min after treatment of Ir-PVP (1.6 mg/mL for 2 h). Then the cells were irradiated by the 595 nm light source (40 J cm\(^2\)). The ROS production was observed by the luminescence signal of DCF with CLSM (Zeiss LSM-710 microscope)
**Isothermal titration calorimetry (ITC).** The thermodynamic quantities of the probes binding to BSA were determined by ITC (MicroGal ITC200 system, GE Healthcare). For Ir-PVP titration, the solutions of BSA and Ir-PVP were made in distilled water. For Ir-OH titration, the solutions of BSA and Ir-OH were made in distilled water containing 1 % DMSO. All solutions were filtered by 0.22 μm Millipore filters. After instrument stabilization at 25 °C, 40 μL of Ir-PVP (100 μM) or Ir-OH (100 μM) solution was titrated with successive injections of BSA solution (50 μM, 200μL). The interval between injections was 3 min. The solutions were stirred at 1000 rpm in the reaction cell during the experiments. Heats of dilutions were corrected by subtracting values for probe-free blank solutions. A one-site binding model was used to fit the binding isotherms.

**Penetration depth assessment of Ir-PVP.** An ICR mouse bearing subcutaneously implanted H22 tumor was i.v. injected with Ir-PVP (40 mg/kg). At 8 h after injection, the mouse was sacrificed and a small piece of tumor (about 2 mm diameter) was dissected, which was then used as the signal source. Then pork ham slices with the thickness of 1.5 mm and diameter of 2 cm were covered one by one upon the signal source. The images of the signal source were captured repeatedly with the adding of slices with Maestro EX fluorescence imaging system. A 595 nm light source is used for excitation and the emission is received from 670 to 800 nm.

**In vitro analysis of cell oxygen consumption with the probe.** The changes of
phosphorescence intensity of H22 cell suspensions incubated with Ir-PVP in vitro over time was monitored using a protocol reported in the literature.²,³ H22 cells were diluted with pre-warmed air-saturated (37 °C) medium (without phenol red) to the desired concentrations (5×10⁷, 5×10⁶, 5×10⁵, 5×10⁴, 5×10³, 5×10² cells/mL), and 200 µL/well was dispensed into a 96-well plate (black body). 10 µL of Ir-PVP (2 mg/mL in PBS) was transferred into each well at 37 °C. Then the wells were covered with 100 µL of pre-warmed (37 °C) mineral oil. Time-resolved measurements were carried out on a fluorescence microplate reader (Tecan, Safire, USA) using 560 nm excitation and 710 nm emission filters, reading every 5 minutes for 60 minutes. Other cell lines, 4T1, HeLa and murine hepatocytes, were tested in the same way.

The probe responses to the change of cancer cell oxygen consumption in vivo.

Rotenone, Oligomycin A, Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP) and Ryanodine were dissolved in DMSO to 2 mM, respectively. 20 µL of the solution was added to H22 cell suspension (2 mL, 5×10⁶ cells/mL) and incubated for 30 minutes at 37 °C. Another 2 mL of H22 cell suspension (5×10⁶ cells/mL) was treated with 20 µL DMSO and incubated in the same condition. After mixed with 10 µL of Ir-PVP (5 mg/mL in PBS), 200 µL of H22 suspension (5×10⁶ cells/mL) treated with the interfering agent or DMSO was injected into the right and left rear thigh muscle of the mice, respectively. The mice were scanned in the imaging system 1 h later.
**In vivo real-time imaging.** Luminescence images of whole mice bodies and isolated organs were obtained using the Maestro EX fluorescence imaging system (Cambridge Research & Instrumentation, CRi, USA). For Ir moiety imaging, the samples were excited at 595 nm and the signal was received at 650-770 nm. For NIR-797 moiety imaging, the samples were excited at 745 nm and the signal was received at 780-950 nm. Spectral fluorescence images consisting of autofluorescence spectra and the spectra of the probe were obtained and then de-mixed based on their spectral patterns using commercial software (Maestro software, CRi, Inc.). The intensities of probe signal were automatically calculated with de-mixed images by the Maestro software. The images captured at different time points were collected and re-processed by the Maestro software so that they could share the same scale bar.

**Biodistribution examination of probe in vivo.** ICR mice bearing subcutaneously implanted H22 tumors in the armpit were i.v. injected with Ir-PVP (5 mg/mL in PBS, 200μL for each mouse). At 1, 4, 8, 24, 48, 72, 96, 120 h after injection, the blood samples were collected, heparinized and centrifuged to obtain the plasma. Five mice were used at each time point. Then the mice were sacrificed and various organs were excised and weighed. The organs and plasma were suspended in dichloromethane and intensely homogenated. After filtration, the phosphorescence intensity of the air-equilibrated filtrate was measured with the spectrofluorometer (Horiba Jobin Yvon FluoroMax-4 NIR) at an excitation wavelength of 560 nm and emission wavelength of 710 nm. The concentrations of the probes in the blood and various organ samples
were determined according to standard curves. Five mice were used as blank group and the average values measured from the organs of blank group were served as background and deducted from the corresponding sample of the administrated mice. The blood circulation half-life of the probe was calculated using software kinetica 4.4.

References

