

*Supplementary Information*

Real-time self-tracking of anticancer small molecule nanodrug based on  
colorful fluorescence variations

Siteng Wang,<sup>a</sup> Hongping Deng,<sup>a</sup> Ping Huang,<sup>a</sup> Pei Sun,<sup>a</sup> Xiaohua Huang,<sup>b</sup> Yue Su,<sup>a</sup> Xinyuan Zhu,<sup>\*a</sup> Jian Shen,<sup>\*b</sup> and Deyue Yan<sup>a</sup>

<sup>a</sup> *School of Chemistry and Chemical Engineering, State Key Laboratory of Metal Matrix Composites, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, P. R. China. Fax: +86-21-54741297; Tel: +86-21-34203400; E-mail: xyzhu@sjtu.edu.cn*

<sup>b</sup> *Jiangsu Collaborative Innovation Center of Biomedical Functional Materials, Jiangsu Key Laboratory of Biomedical Materials, College of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210046, P. R. China. E-mail: shenjianbio@hotmail.com*

## 1. Synthesis

### 1.1 Synthesis of Ir-NPC

NPC (0.5 g, 2.48 mmol) was dissolved in dried  $\text{CH}_2\text{Cl}_2$  (20 mL). After stirring uniformly, pyridine (200  $\mu\text{L}$ ) was added to the solution. Then, a solution of Ir (0.25 g, 0.43 mmol) in dried  $\text{CH}_2\text{Cl}_2$  (5 mL) was added dropwise to the reaction mixture and stirred for 24 h at room temperature in the dark. Then the reaction mixture was evaporated under reduced pressure to remove  $\text{CH}_2\text{Cl}_2$ . The crude product was purified by column chromatograph using  $\text{CH}_2\text{Cl}_2$  and dichloromethane/methanol ( $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$ , 10:1 v/v) as the eluent. The product was collected and the solvent was removed by rotary evaporation to give a yellowy solid (265 mg, 82%).

### 1.2 Synthesis of Ir-DOX conjugate

Ir-NPC (100 mg, 0.133 mmol) was dissolved in dried DMF (2 mL). After stirring uniformly, a solution of DOX•HCl (82 mg, 0.141 mmol) in dried DMF (2 mL) was added slowly to the reaction mixture. Then TEA (25  $\mu\text{L}$ ) was added dropwise to the solution and stirred for 24 h at room temperature in the dark. Then the reaction mixture was concentrated under vacuum to remove DMF. The crude product was purified by column chromatograph using  $\text{CH}_2\text{Cl}_2$  and  $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$  (20:1 v/v) as the eluent. The product was collected and the solvent was removed by rotary evaporation to give a deep red solid (95.3 mg, 62%).

## 2. $^1\text{H}$ NMR, $^{13}\text{C}$ NMR spectra and Elemental analyses

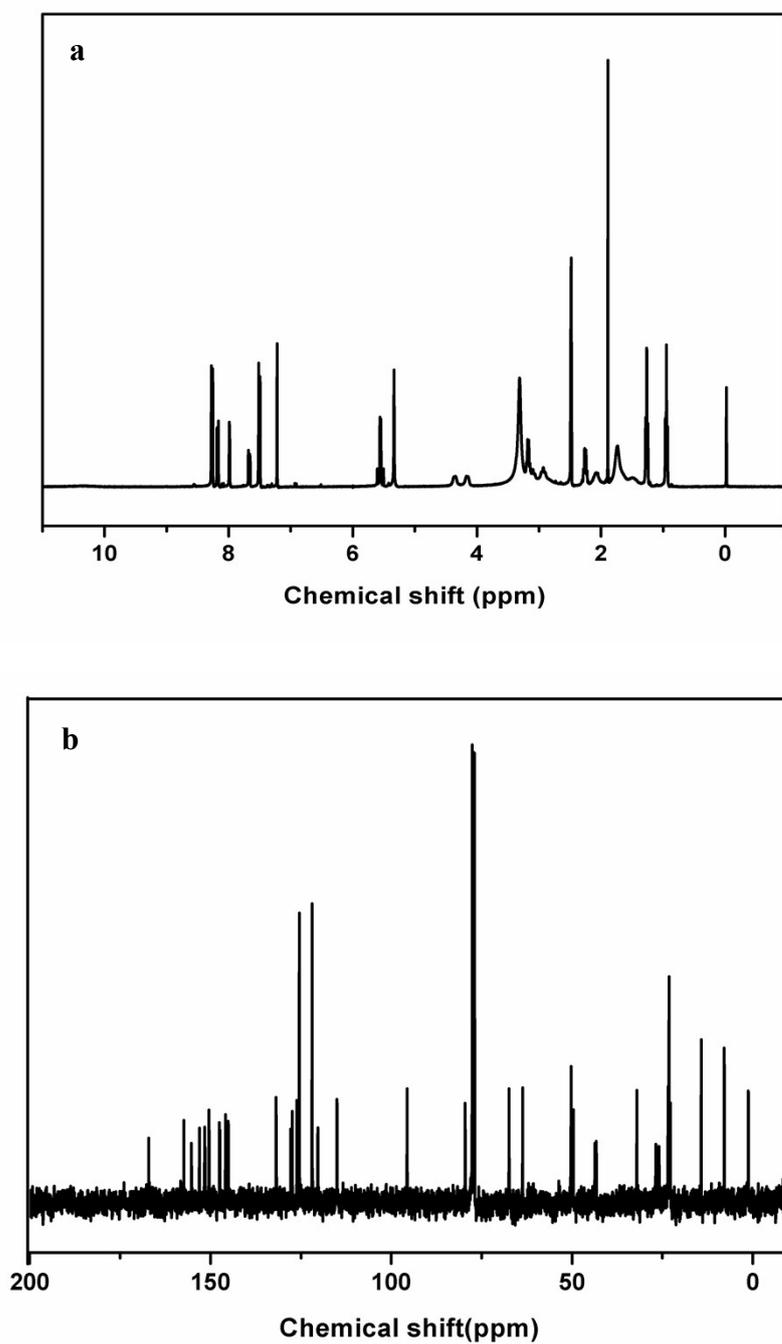
### 2.1 Ir-NPC

Yellow solid, yield 82%.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 8.45 – 8.22 (m, 2H), 8.17 (d,  $J = 9.2$  Hz, 1H), 7.99 (d,  $J = 2.5$  Hz, 1H), 7.67 (dd,  $J = 9.2$  Hz, 2.5 Hz, 1H), 7.61 – 7.46 (m, 2H), 7.22 (s, 1H), 5.55 (q,  $J = 16.9$  Hz, 2H), 5.34 (s, 2H), 4.35 (m, 1H), 4.17 (m, 1H), 3.26 (m, 3H), 3.15 (m, 3H), 2.93 (m, 3H), 2.22 (m, 2H), 1.98 – 1.71 (m, 9H), 1.48 (m, 1H), 1.25 (t,  $J = 7.4$  Hz, 3H), 0.95 (t,  $J = 7.4$  Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 167.1, 157.4, 155.3, 153.1, 151.6, 151.4, 150.4, 147.5, 147.4, 145.9, 145.7, 145.1, 131.9, 127.8, 127.4, 126.1, 125.5, 121.9, 120.3, 115.0, 95.6, 79.5, 77.6, 77.3, 77.0, 67.4, 63.7, 50.3, 49.7, 43.8, 43.5, 32.1, 26.8, 25.9, 23.7, 23.3, 22.8, 14.3, 7.9. Elemental analyses Calcd for  $\text{C}_{40}\text{H}_{41}\text{N}_5\text{O}_{10}$ : C, 63.91; H, 5.50; N, 9.32. Found: C, 63.69; H, 5.14; N, 9.14.

### 2.1 Ir-DOX conjugate

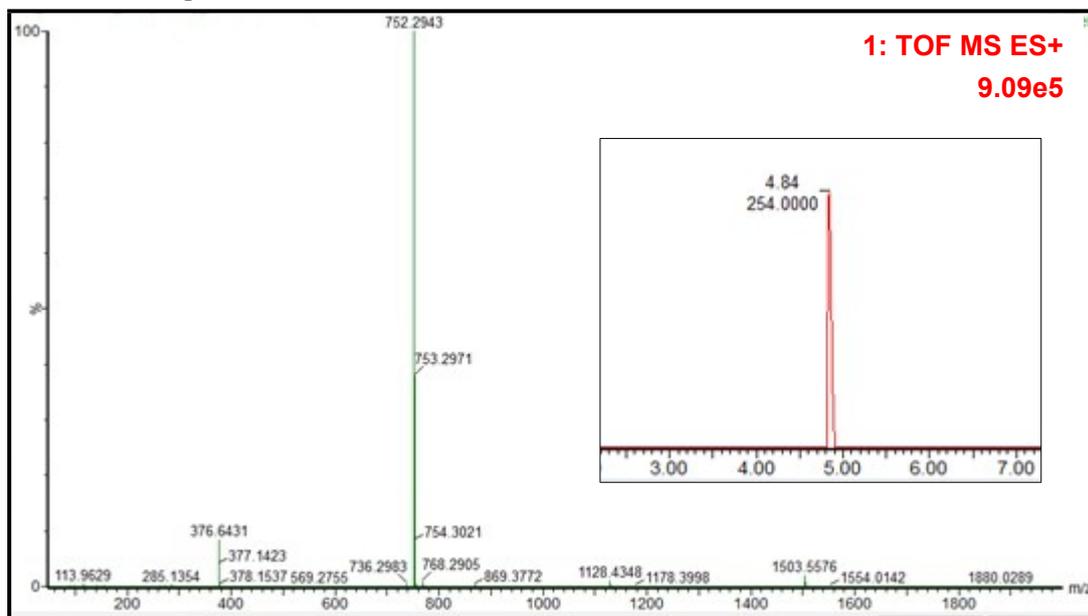
Red solid, yield 62%.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 8.05 – 7.97 (m, 1H), 7.73 (dd,  $J = 104.5$  Hz,  $J = 50.6$  Hz, 5H), 7.56 (s, 2H), 7.56 (s, 2H), 7.47 (s, 1H), 7.29 (s, 1H), 7.20 (s, 1H), 6.93 (s, 1H), 6.84 (s, 1H), 5.74 (s, 2H), 5.48 – 5.26 (m, 2H), 5.22 (s, 1H), 5.19 (d,  $J = 25.8$  Hz, 2H), 4.98 (d,  $J = 15.2$  Hz, 1H), 4.95 (s, 1H), 4.91 – 4.62 (m, 4H), 4.51 (d,  $J = 4.7$  Hz, 2H), 4.38 – 3.91 (m, 4H), 3.91 – 3.82 (m, 1H), 3.64 (s, 4H), 3.19 – 3.11 (m, 1H), 3.07 (s, 1H), 2.90 (s, 1H), 2.88 – 2.48 (m, 7H), 2.14 – 1.87 (m, 6H), 1.54 – 1.18 (m, 4H), 1.13 (d,  $J = 6.5$  Hz, 3H), 0.77 (t,  $J = 7.4$  Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 214.2, 185.9, 168.6, 160.7, 157.0, 156.0, 154.7, 153.0, 151.6, 150.1, 146.4, 145.2, 136.2, 135.7, 134.3, 134.1, 131.1, 128.1, 127.0, 125.8, 120.1, 119.5, 119.3, 119.0, 114.7, 110.6, 110.4, 100.4, 95.4, 75.9, 75.1, 69.3, 69.0, 67.9, 67.2, 64.5, 62.7, 56.7, 55.6, 49.7, 48.2, 43.8, 40.9, 40.5, 32.7, 31.8, 30.4, 26.2, 23.0, 17.7, 14.1, 8.0. Elemental analyses Calcd for  $\text{C}_{61}\text{H}_{65}\text{N}_5\text{O}_{18}$ : C, 63.37; H, 5.67; N, 6.06. Found: C, 62.87; H, 5.10;

N, 5.59.

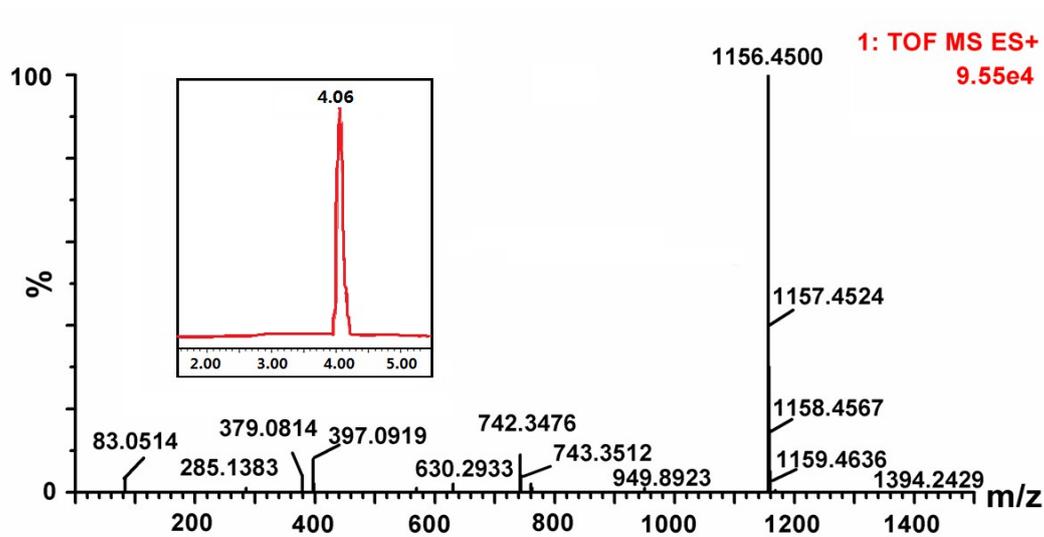


**Figure S1.** (a) <sup>1</sup>H NMR and (b) <sup>13</sup>C NMR spectra of Ir-NPC in DMSO-*d*<sub>6</sub>.

### 3. LC-HRMS spectra

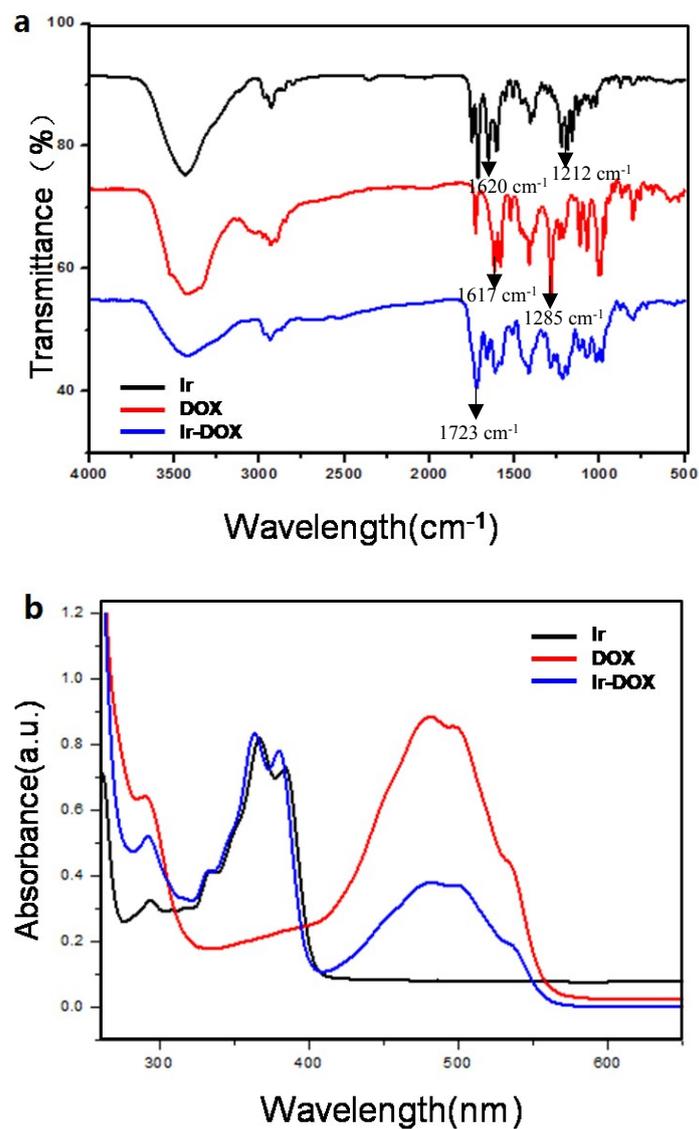


**Figure S2.** Mass spectrum of Ir-NPC. Inset: The LC profile of Ir-NPC. ESI-MS  $m/z$  ( $M+H^+$ ) calcd 752.7810, found 752.2943 ( $M+H^+$ ).



**Figure S3.** Mass spectrum of Ir-DOX. Inset: The LC profile of Ir-DOX. ESI-MS  $m/z$  ( $M+H^+$ ) calcd 1157.1915, found 1156.4500 ( $M+H^+$ ).

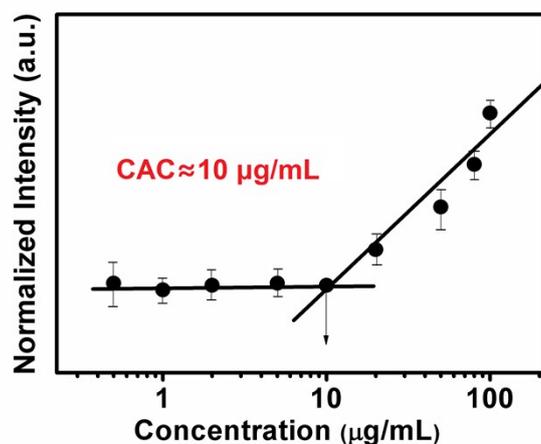
#### 4. FTIR and UV-Vis spectra



**Figure S4.** (a) FTIR spectra of Ir, DOX, and Ir-DOX. (b) UV-Vis spectra of Ir, DOX, and Ir-DOX.

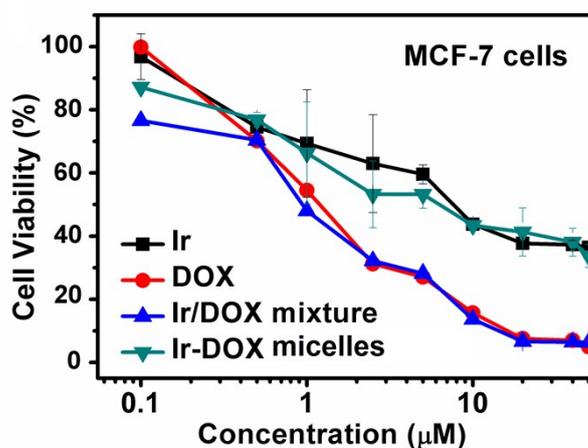
Ir-DOX IR (KBr): 3425, 2973, 2940, 2872, 2647, 2532, 1723, 1659, 1617, 1576, 1510, 1415, 1322, 1286, 1219, 1117, 1077, 1021, 986, 878, 810, 724, 525 cm<sup>-1</sup>.

## 5. The CAC value of Ir-DOX micelles



**Figure S5.** The CAC value of Ir-DOX micelles is about 10  $\mu\text{g/mL}$ . Values are presented as average standard error ( $n = 3$ )

## 6. MTT assay of MCF-7 cells



**Figure S6.** Cell viability of MCF-7 cells incubated with Ir, DOX, Ir/DOX mixture and Ir-DOX micelles after 72 h at various concentrations determined by MTT assay. Values are presented as average standard error ( $n = 3$ )

**Table S1.** IC<sub>50</sub> values of the different compounds in MCF-7 and MCF-7/ADR cell lines.

	Ir	DOX	Ir/DOX mixture	Ir-DOX micelles
MCF-7/ADR cells	27.31	36.10	25.02	7.40
MCF-7 cells	8.17	1.24	1.12	6.10