Electronic Supplementary Information (ESI):

Water-soluble phosphorescent iridium(III) complexes as multicolor probes for imaging of homocysteine and cysteine in living cells

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1. ^{1}H NMR, ^{13}C NMR and MS (MALDI-TOF) date

[Ir(dfppy)$_2$cpa]$^+$PF$_6^-$ (Ir1'): Yield 63% ^{1}H NMR (400 MHz, $CDCl_3$, 298K): 9.32 (s, 1H), 8.88(d, 1H), 8.57 (d, 1H), 8.54 (s, 1H), 8.33 (d, 1H), 8.29 (d, 2H), 8.25 (d, 1H), 7.90-7.94 (dd, 1H), 7.74-7.81(m,3H), 7.29-7.33 (t, 2H), 6.99-7.03(t, 1H), 6.91-6.94(t, 1H), 6.59-6.66 (m, 2H), 5.76-5.79(m,2H), 4.40 (s, 2H). MS (MALDI-TOF) [m/e]: 772.2 (**Ir1'**−PF_6)$^+$.

[Ir(*t*-buppy)$_2$cpa]$^+$PF$_6^-$ (Ir2'): Yield 66% ^{1}H NMR (400 MHz, $CDCl_3$, 298K): 9.12 (s, 1H), 8.82(d, 1H), 8.54 (s, 1H), 8.48 (d, 1H), 8.20 (d, 1H), 8.16 (d, 1H), 7.83-7.88 (m, 3H), 7.69-7.74 (m, 3H), 7.60-7.63(dd,2H), 7.32-7.36 (dd, 2H), 7.09-7.13(m, 2H), 6.84-6.92 (m, 2H), 6.38-6.40 (dd, 2H), 4.40 (s, 2H), 1.11 (d, 18H). MS (MALDI-TOF) [m/e]: 884.2 [**Ir2'**−PF_6]$^+$.

[Ir(thq)$_2$cpa]$^+$PF$_6^-$ (Ir3'): Yield 58% ^{1}H NMR (400 MHz, $CDCl_3$, 298K): 9.16 (s, 1H), 8.9(d, 1H), 8.48 (s, 1H), 8.39 (d, 1H), 8.36 (d, 1H), 8.32 (d, 1H),8.02-8.09 (dd, 2H), 7.93-7.97 (dd, 1H), 7.60 (d, 3H), 7.55 (d, 1H), 7.40-7.43(t, 3H), 7.1.8-7.22 (t, 2H), 6.85-6.93(m, 2H), 6.74-6.82(m, 2H), 6.36-6.39 (t, 2H), 4.39 (s, 2H). MS (MALDI-TOF) [m/e]: 884.3 [**Ir3'**−PF_6]$^+$.

[Ir(pba)$_2$cpa]$^+$PF$_6^-$ (Ir4'): Yield 69% ^{1}H NMR (400 MHz, $CDCl_3$, 298K): 9.76 (d, 2H), 9.24(s, 1H), 8.80 (d, 1H), 8.52-8.55 (d, 2H), 8.23 (d, 1H), 8.19 (d, 1H), 8.04-8.09 (dd, 2H), 7.84-7.91 (m, 4H), 7.74-7.77 (dd, 1H), 7.58-7.61 (t, 2H), 7.43-7.46(t, 2H), 7.11-7.13 (t, 1H), 7.11-7.13 (t, 1H), 7.05-7.07 (t, 1H), 6.80-6.83 (d,2H), 4.40 (d, 2H). MS (MALDI-TOF) [m/e]: 828.2 [**Ir4'**−PF_6, Cl]$^+$.

[Ir(dfppy)$_2$pto]$^+$PF$_6^-$ (Ir1): Yield 19% ^{1}H NMR (400 MHz, $CDCl_3$, 298K): 9.62 (d, 1H), 8.74(s, 1H), 8.59 (d, 1H), 8.31 (d, 2H), 8.24 (d, 1H), 8.14 (d, 1H), 7.91-7.94 (t, 1H), 7.75-7.78 (t, 3H), 7.28-7.30 (t,2H), 6.90-6.96 (m, 2H), 6.57-6.63(t, 2H), 5.76-5.79(t, 2H), 3.66-3.72 (dd, 6H), 1.45 (t, 9H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 167.93, 167.87, 164.40, 150.36, 148.56, 148.36, 147.18, 143.67, 143.61, 138.09, 138.05, 131.90, 130.87, 130.78, 124.87, 124.79, 123.17, 122.78, 122.70, 119.65, 119.59, 54.73, 8.11ppm. MS (MALDI-TOF) [m/e]: 909.5 [**Ir1**−PF_6, Cl]$^+$.

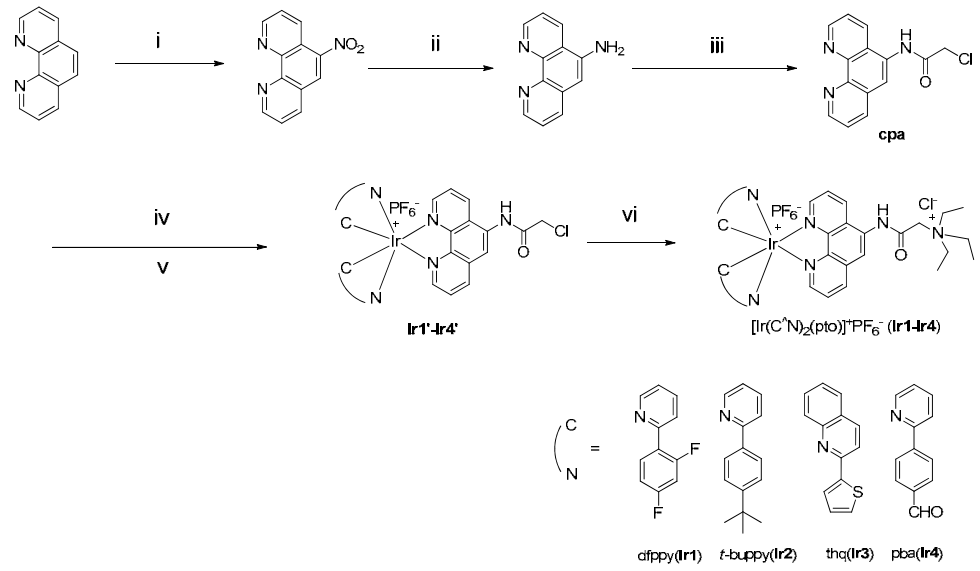
[Ir(*t*-buppy)$_2$pto]$^+$PF$_6^-$ (Ir2): Yield 36% ^{1}H NMR (400 MHz, $CDCl_3$, 298K): 9.61 (d, 1H), 8.73 (s, 1H), 8.60 (d, 1H), 8.15 (d, 1H), 8.08 (d, 1H), 7.84-7.90 (m, 3H),

7.67-7.74 (m, 3H), 7.61 (d, 2H), 7.30(d, 2H), 7.08 (d, 2H), 6.82-6.87(dd, 2H), 6.36-6.39 (d, 2H), 4.9 (d, 2H), 3.74-3.79 (dd, 6H), 1.48-1.51 (t, 9H),1.09 (t, 18H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 168.19, 164.05, 153.98, 150.59, 148.47, 148.33, 147.17, 140.96, 140.92, 137.90, 131.15, 128.07, 126.59, 126.52, 124.44, 124.37, 122.49, 120.11, 119.16, 55.27, 53.82, 53.45, 34.64, 31.75. 31.15, 29.38, 29.26, 8.23ppm. MS (MALDI-TOF) [m/e]: 949.5 [**Ir2**−PF_6, Cl]$^+$.

[Ir(thq)$_2$pto]$^+$PF$_6^-$ (Ir3): Yield 17% ^{1}H NMR (400 MHz, $CDCl_3$, δ): 9.74 (d, 1H), 8.8 (s, 1H), 8.66 (d, 1H), 8.23 (d, 1H), 8.17 (d, 1H), 7.87-7.91 (m, 3H), 7.78-7.80 (dd, 1H), 7.69-7.72 (m, 5H), 7.25 (d, 1H), 7.03-7.07(t, 2H), 6.89-6.95(m, 2H), 6.84-6.87 (dd, 2H), 6.34-6.37(t,2H), 5.00 (s, 2H), 3.81-3.87 (dd, 6H), 1.5-1.53 (t, 9H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 167.93, 167.87, 164.40, 150.36, 149.90, 148.56, 148.36, 147.18, 143.67, 143.61, 138.09, 138.05, 131.90, 130.87, 130.78, 126.16, 124.87, 124.79, 123.23, 123.17, 122.78, 122.70, 119.65, 119.59, 54.73, 8.11ppm. MS (MALDI-TOF) [m/e]: 949.7 [**Ir3**−PF_6, Cl]$^+$.

[Ir(pba)$_2$pto]$^+$PF$_6^-$ (Ir4): Yield 37% ^{1}H NMR (400 MHz, $CDCl_3$, 298K): 9.76 (s, 2H), 8.75 (s, 1H), 8.55 (d, 1H), 8.19 (d, 1H), 8.06-8.09 (t, 3H), 7.84-7.91 (m, 5H), 7.68-7.72 (dd, 1H), 7.58-7.60 (m, 2H), 7.42 (d, 1H), 7.38 (d, 1H),7.03-7.07(t, 2H), 7.02-7.10(m, 2H), 6.81-6.84 (dd, 2H), 3.66-3.72 (dd, 6H), 1.46-1.49 (t, 9H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 192.47, 166.08, 150.54, 149.87, 159.74, 149.93, 149.04, 148.97, 147.02, 146.88, 144.31, 138.90, 137.00, 136.98, 132.54, 132.42, 131.59, 126.59, 125.12, 121.23 53.43, 8.00ppm. MS (MALDI-TOF) [m/e]: 893.7 [**Ir4**−PF_6, Cl]$^+$.

2. Synthetic Procedure of the Complexes Ir1-Ir4



Scheme S1. Synthetic procedure of the complexes **Ir1**-**Ir4.** Reaction conditions: (i)

H_2SO_4, HNO_3, 160 °C−170 °C, 2h; (ii) 5% Pd/C, hydrazine hydrate, EtOH, reflux, 5h;

(iii) chloroacetyl chloride, triethylamine, R.T., overnight; (iv) $[Ir(C^N)_2Cl_2]_2$, CH_2Cl_2, MeOH, reflux, 4h; (v) KPF_6, R.T., 1h; (vi) DMF, triethylamine, 110 °C, 3h.

3. Low-temperature (77K) normalized photoluminescence spectra

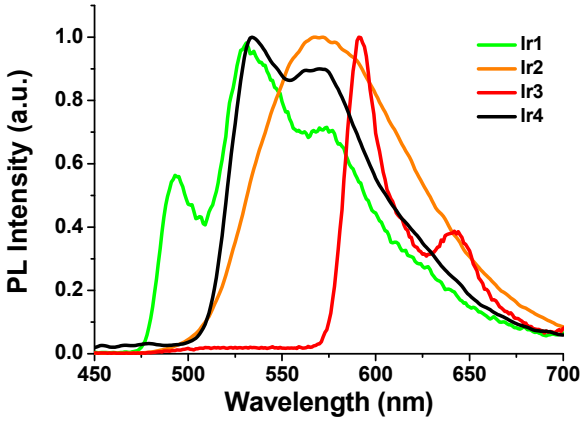


Fig. S1. Low-temperature (77K) normalized photoluminescence spectra of **Ir1-Ir4** in CH_2Cl_2.

4. Photoluminescence spectra and luminescent photographs of Ir4 in different solvents

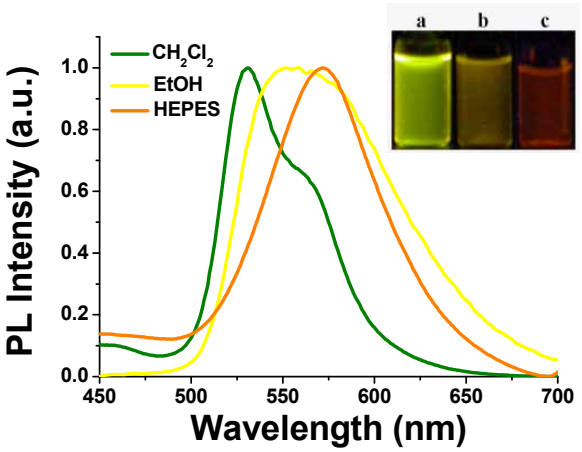


Fig. S2. Normalized photoluminescence spectra and luminescent photographs of **Ir4** in (a) CH_2Cl_2, (b) EtOH, (c) HEPES.

5. Z-scan confocal luminescence image

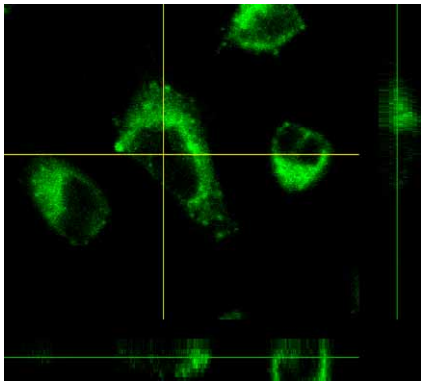


Fig. S3. The overlap Z-scan confocal luminescence image of live KB cells incubated with 20 μM **Ir1** in PBS (pH 7.0) for 30 min at 37 °C.

6. Flow cytometric results of Ir4 in KB cells.

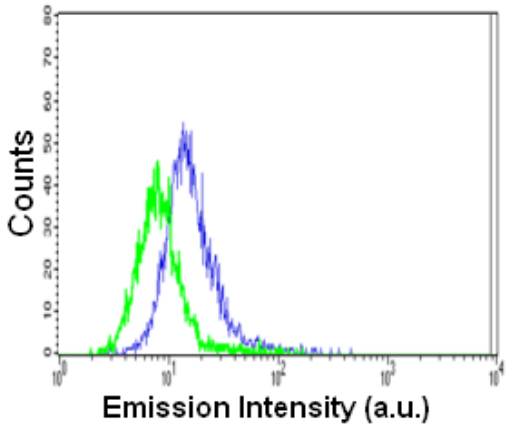
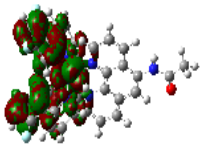
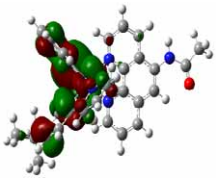
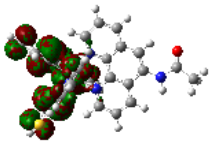
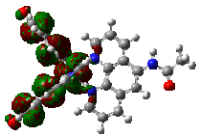
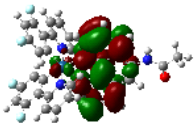
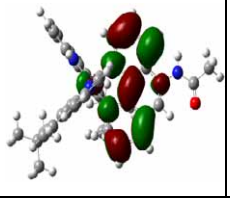
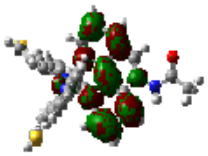
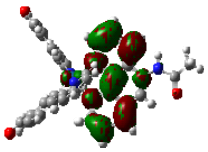


Fig. S4. Flow cytometric results of **Ir4** in KB cells. KB cells incubated with 200 μM N-ethylmaleimide for 30 min and then further incubatedwith 20 μM **Ir4** for 30min (green). KB cells incubated with 20 μM **Ir4** for 30 min (blue).

7. HOMO and LUMO Distributions of Complexes Ir1-Ir4

Table S1. HOMO and LUMO Distributions of Complexes **Ir1-Ir4**

	Ir1	Ir2	Ir3	Ir4
HOMO				
LUMO				

8. Calculated Excited Triplet States and Character of the Transitions for the Complexes

Table S2 Calculated Excited Triplet States and Character of the Transitions for the Complexes

	λ_{cal}, nm	E_{cal}, eV	excitation	Character
Ir1	550	2.25	HOMO→LUMO (0.44) HOMO-2→LUMO (0.38)	$^3ML_N\text{^}_NCT+^3LC_C\text{^}_N$
Ir2	691	1.79	HOMO→LUMO (0.70)	$^3ML_C\text{^}_NCT+^3LC_C\text{^}_N$
Ir3	725	1.71	HOMO→LUMO (0.70)	$^3ML_C\text{^}_NCT+^3LC_C\text{^}_N$
Ir4	559	2.22	HOMO→LUMO (0.66)	$^3ML_N\text{^}_NCT+^3LC_C\text{^}_N$

9. Lipophilicity (log $P_{o/w}$) of the complexes

Table S3. Lipophilicity (log $P_{o/w}$) of the complexes

complex	**1**	**2**	**3**	**4**
log$P_{o/w}$	0.29	0.31	0.19	0.05

10. Original ^{1}H NMR, ^{13}C NMR and MS spectra of the final compounds