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Supporting Information

# Reaction-based phosphorescent nanosensor for ratiometric and time-resolved luminescence imaging of fluoride in live cells

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## Materials

All starting materials and reagents, unless otherwise specified, were purchased from commercial suppliers and used without further purification. Complex **Ir1** and **Ir2** were synthesized according to the previous report.[1]

## Characterization

The $^{1}H$ and $^{13}C$ spectra were measured at room temperature by using a Bruker Ultra Shield Plus 400 MHz spectrometer. Phosphorescence spectral and lifetime measurements were carried out by using an Edinburgh LFS-920 spectrometer. Electronic absorption spectra were recorded with Shimadzu UV-3600 UV-VISNIR spectrophotometers. Powder small-angle Xray diffraction (XRD) measurements were carried out on a Bruker Smart APEX CCD diffractometer at 40 kV and 20 mA using Cu-Kα radiation (λ = 1.54 Å). Transmission electron microscopy (TEM) observations were carried out on a JEOL JEM-2100 transmission electron microscope at an acceleration voltage of 200 kV. Nitrogen adsorption–desorption measurements were performed on a V-Sorb 2800 surface area analyzer using the volumetric method and samples were degassed. Particle size was measured with a nanoparticle size analyzer Brookhaven 90Plus. Pore size distributions were estimated from adsorption branches of the isotherms by using the Barrett–Joyner–Halenda (BJH) method.

## Cell imaging experiments

The cell lines HepG-2 were provided by the Institute of Biochemistry and Cell Biology, SIBS, CAS (China). The HepG-2 liver cancer cells were grown in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) at 37 °C with 5% $CO_2$. HepG-2 liver cancer cells were planted on confocal petri dish and allowed to adhere for 24 h before confocal imaging measurement.

HepG-2 liver cancer cells were treated with nanosensor for 3 h, and then incubated with NaF ($1.5 \times 10^{-5}$ M) for 1 h to investigate its intracellular sensing behaviour toward $F^{-}$. Confocal luminescence imaging was carried out on an Olympus IX81 laser scanning confocal microscope equipped with a 40 immersion objective lens. A 405 nm semiconductor laser was served as excitation of the HepG-2 liver cancer cells

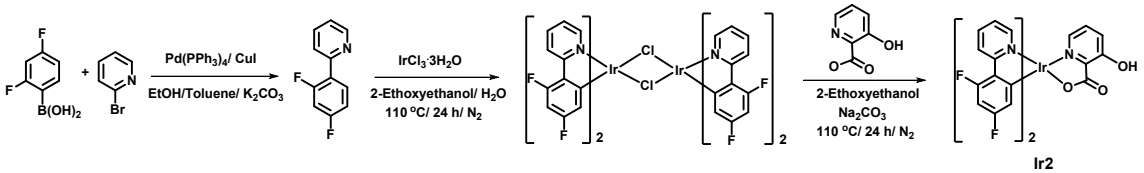
incubated with nanosensor. The one-photon emission was collected at 440–490 and 500–600 nm, respectively.

The lifetime imaging setup is integrated with Olympus IX81 laser scanning confocal microscope. The luminescence signal was detected by the system of the confocal microscope and correlative calculation of the data was performed by professional software which was provided by PicoQuant Company. The light from the pulse diode laser head (PicoQuant, PDL 800-D) with excitation wavelength of 405 nm and frequency of 0.5 MHz was focused onto the sample with a 40x/NA 0.95 objective lens for single-photon excitation. HepG-2 cells attached onto a glass slide were covered with a thin glass cover slip, onto which an excitation beam was focused.

The in vitro cytotoxicity was measured using a standard methyl thiazolyl tetrazolium (MTT, Sigma Aldrich) assay in HepG-2 cell lines. Briefly, cells growing in log phase were seeded into 96-well cell culture plate at $1 \times 10^4$/well. Nanosensor was added to the wells of the treatment group at concentrations of 100, 200, 300 and 400 μg/mL. Cells were incubated for 24 h at 37 °C under 5 % $CO_2$. The combined MTT/PBS solution was added to each well of the 96-well assay plate and incubated for an additional 4 h. An enzymelinked immunosorbent assay (ELISA) reader was used to measure the OD570 (absorbance value) of each well referenced at 490 nm. The following formula was used to calculate the viability of cell growth: Viability (%) = (mean of absorbance value of treatment group / mean of absorbance value of control) ×100.

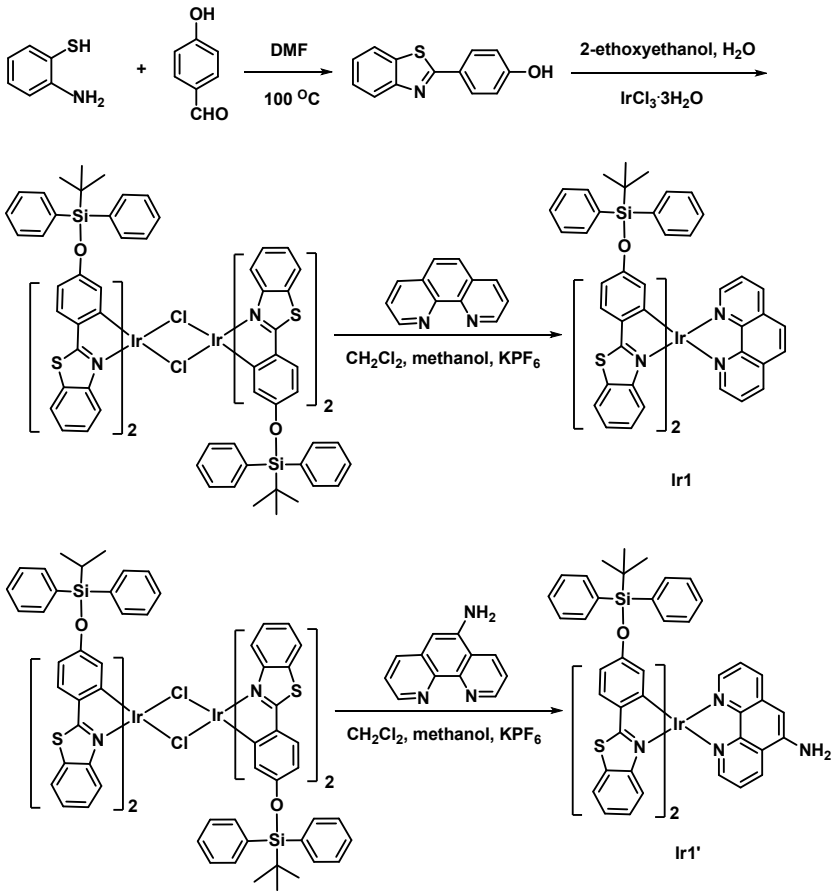
## Synthesis of Ir2

**Ir2**, **Ir1** and **Ir1'** was prepared according to literature methods.[1]




**Scheme S1**. Synthetic route of **Ir2**.

**Synthesis of Ir1 and Ir1'**



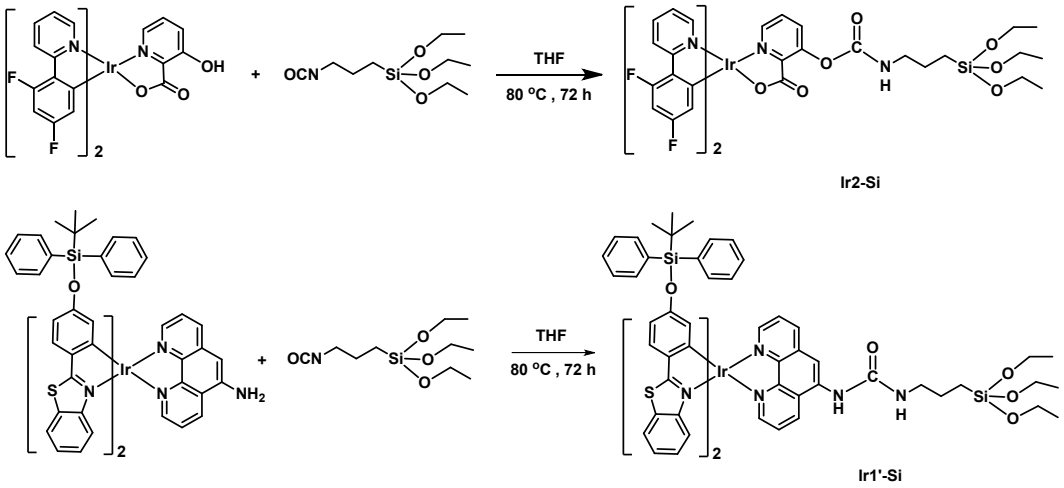

**Scheme S2.** Synthetic route of **Ir1** and **Ir1'**.

**Ir1:** $^{1}$H NMR (400 MHz, $CDCl_3$, $\delta$): 8.66 (d, 2H), 8.10 (s, 2H), 7.89 (d, 2H), 7.72 (dd, 2H), 7.59 (d, 2H), 7.35-7.46 (br, 6H), 7.30 (d, 6H), 7.15-7.25 (br, 8H), 7.00 (m, 3H), 6.78 (d, 2H), 6.54 (t, 2H), 5.73 (s, 2H), 5.03 (d, 2H). MS (MALDI-TOF -MS) [m/z]: 1300.437 (**Ir1**-$PF_6^+$).

**Ir1':** $^{1}$H NMR (400 MHz, $CDCl_3$, $\delta$): 8.66 (d, 2H), 8.10 (s, 2H), 7.89 (d, 2H), 7.72 (dd, 2H), 7.59 (d, 2H), 7.35-7.46 (br, 6H), 7.30 (d, 6H), 7.15-7.25 (br, 8H), 7.00 (m, 4H), 6.78 (d, 2H), 6.54 (t, 2H), 5.73 (s, 1H), 5.03 (d, 2H). $^{13}$C NMR (100 MHz, DMSO-$d_6$, $\delta$):180.14, 180.03, 158.24, 158.17, 148.44, 148.39, 148.13, 145.60, 140.86, 135.86, 135.14, 134.70，134.16, 134.09, 131.79, 131.56, 130.76, 130.74, 130.67, 130.48, 128.28, 128.23, 101.85, 55.38, 40.60, 40.41, 40.18, 39.98, 39.57, 39.36, 26.41, 19.10 MS (MALDI-TOF -MS) [m/z]: 1315.952 (**Ir1'**-$PF_6^+$).

**Synthesis of Ir2-Si and Ir1'-Si**

Ir2-Si and Ir1'-Si were prepared according to literature methods.[2]




**Scheme S3.** Synthetic route of Ir2-Si and Ir1'-Si.

**Synthesis of Ir2-functionalized MSN (Ir2-MSN)**

Ir2-MSN was prepared by the organosiloxane/siloxane co-condensation method. Hexadecyl trimethyl ammonium chloride (CTAB, 0.5 g) and triethanolamine (TEA) were dissolved in turn in 30 mL water at 95 °C under intensive stirring. After 20 min, the mixture of 0.5 mL of tetraethyl orthosilicate (TEOS) and Ir2-Si was added dropwise and the resulting mixture was stirred for 2 h. The products were collected by centrifugation and washed for several times with ethanol to remove the residual reactants. The collected products are obtained as colloidal suspensions featuring long-term stability after template removal by ion exchange with an ethanolic solution of ammonium nitrate.

**Synthesis of Ir2-MSN-Ir1'**

20 mg of the as-prepared Ir2-MSN was suspended in 30 mL of anhydrous THF, and it was dispersed through ultrasound for 30 min before adding Ir1'-Si. The mixture was stirred overnight under nitrogen atmosphere at room temperature. The products were washed several times with THF by repetitive ultrasonic dispersion/centrifugation cycles to remove unreacted phosphorescent complex.

**Mass spectra of Ir1 before and after adding $F^-$**

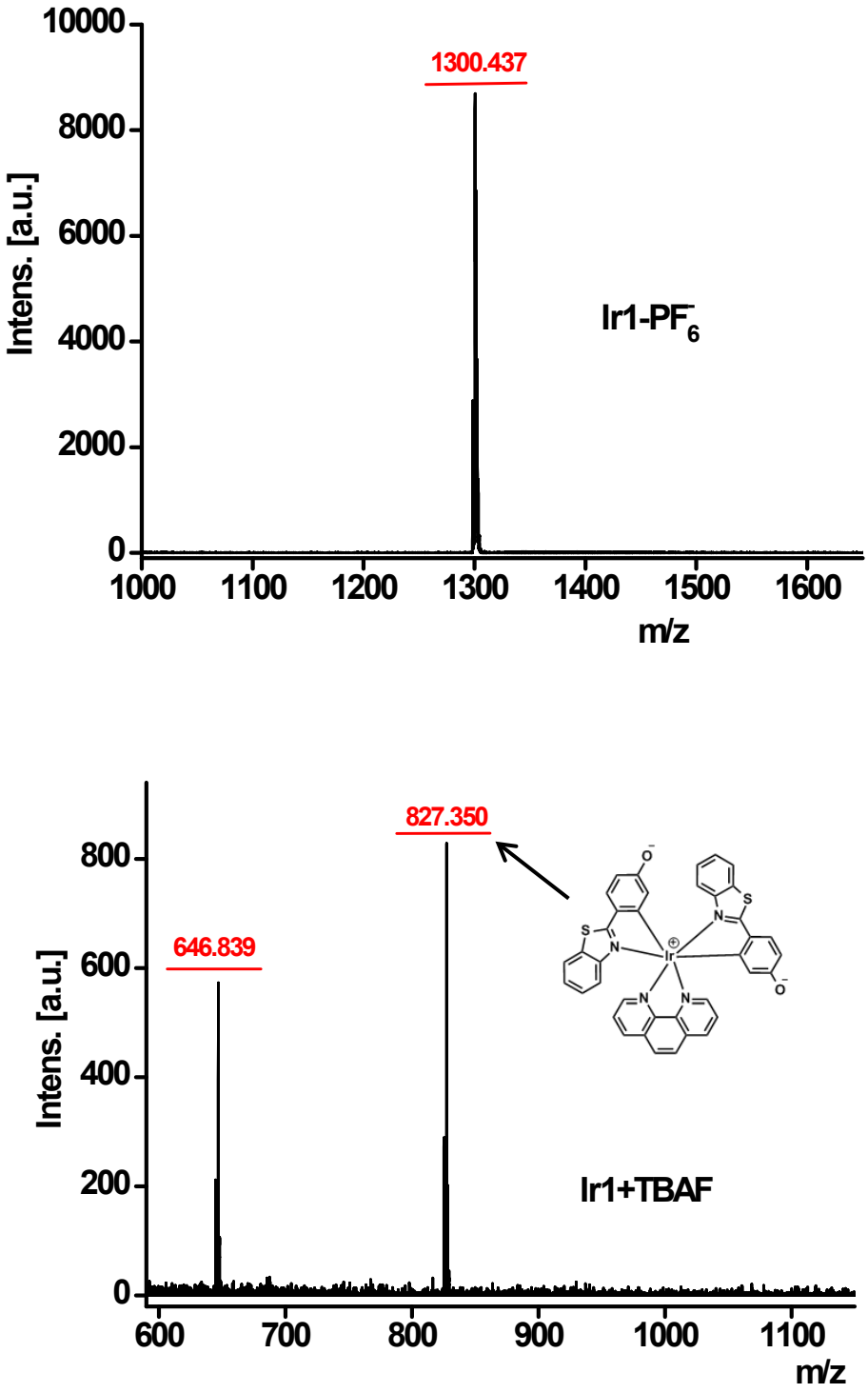



**Fig. S1.** Mass spectral data of **Ir1** and the corresponding products after adding excessive fluoride ion.

## $^1$H NMR titration

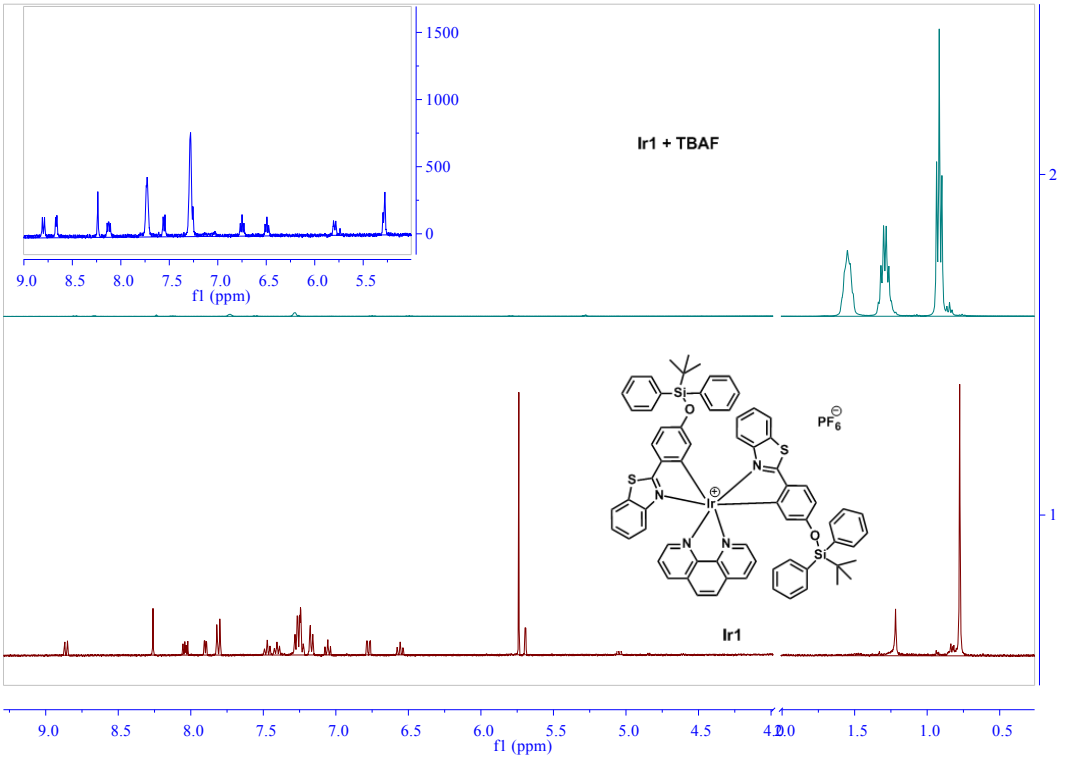



**Fig. S2.** $^1$H NMR titration of **Ir1** with $F^-$.

## Emission spectra of Ir1 solution upon titration with TBAF

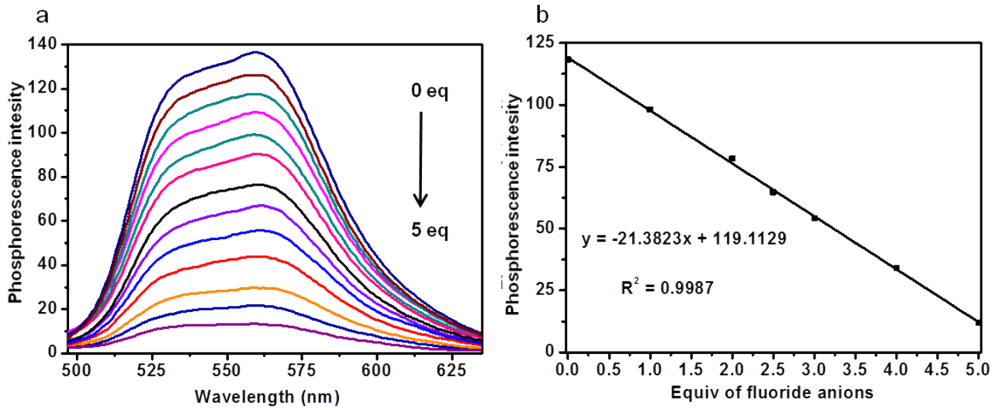



**Fig. S3.** Emission spectra of of **Ir1** upon titration with TBAF in THF ([**Ir1**] = 15 μM, [$F^-$] = 5 mM in methanol, 0-5 equiv (0.0, 0.5, 1.0, 1.2, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 4.8, 5.0) (a). Phosphorescence intensities at 526 nm versus the equivalents of $F^-$ (b).

## DLS measurements

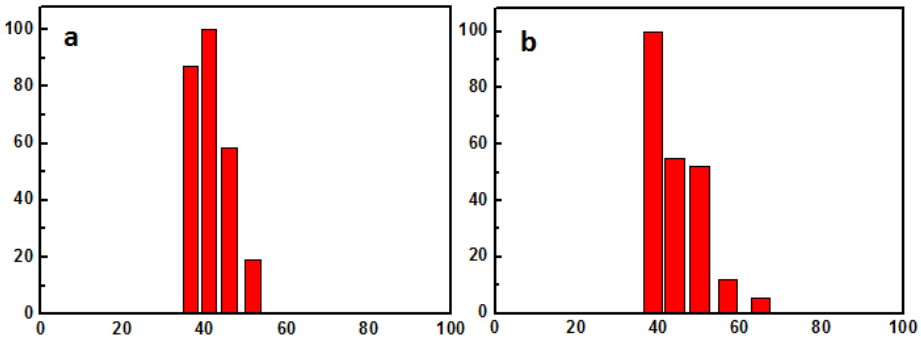



**Fig. S4.** DLS measurements of Ir2-MSNs (a) and Ir2-MSNs-Ir1' (b).

## Small-angle XRD

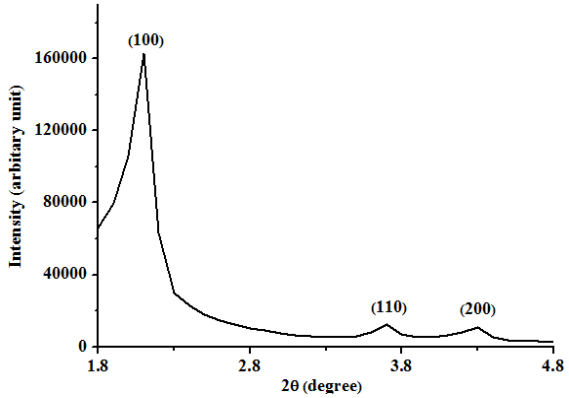



**Fig. S5.** Low-angle XRD patterns of Ir2-MSN.

## Zeta potential

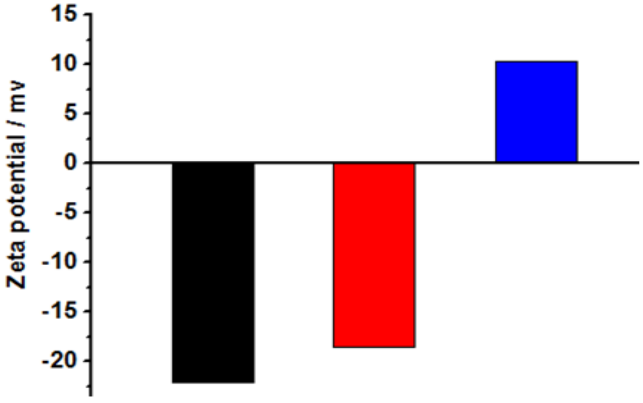



**Fig. S6.** The zeta potential of MSN (black bar ), Ir2-MSN (red bar ) and Ir2-MSN-Ir1'

(blue bar).

**FT-IR spectra**

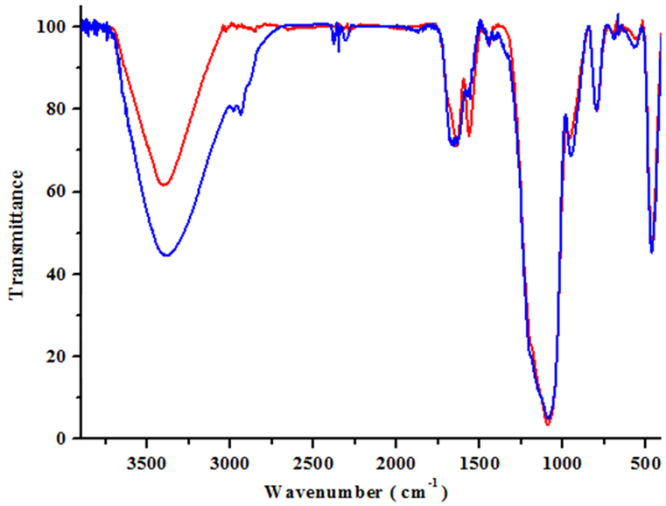



**Fig. S7.** FT-IR spectra of MSNs before (blue) and after (red) removal of surfactant CTAB.

**UV-Vis spectra**

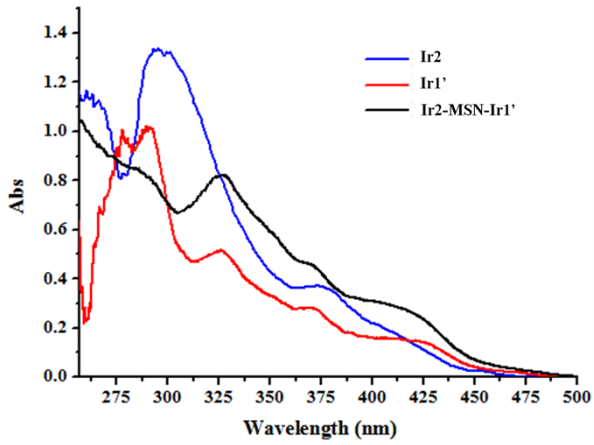



**Fig. S8.** UV-vis absorption spectra of **Ir2** (4.0 μM), **Ir1'** (2.0 μM) and Ir2-MSN-Ir1' (1 mg/mL).

**Cytotoxicity assays of Ir2-MSN-Ir1'**

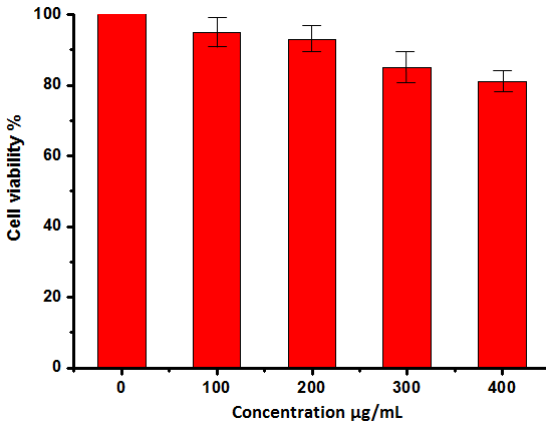



**Fig. S9.** HepG-2 cells viabilities after 24 h incubation with Ir2-MSN-Ir1' at different concentrations.

**Intracellular average luminescence intensity in selected points before and after adding $F^-$.**

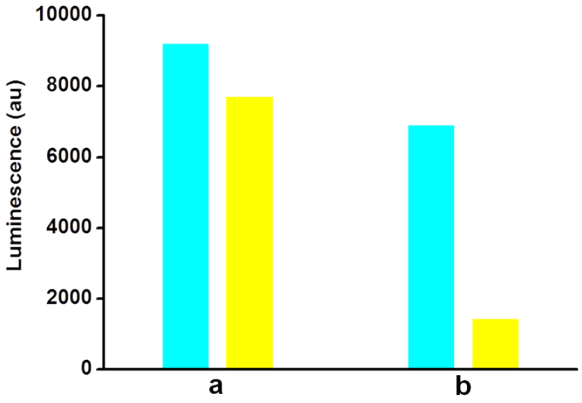



**Fig. S10.** Intracellular average luminescence intensity of blue and yellow channel in selected points before (a) and after (b) adding of $F^-$.

**Intracellular luminescence intensity ratio analysis**

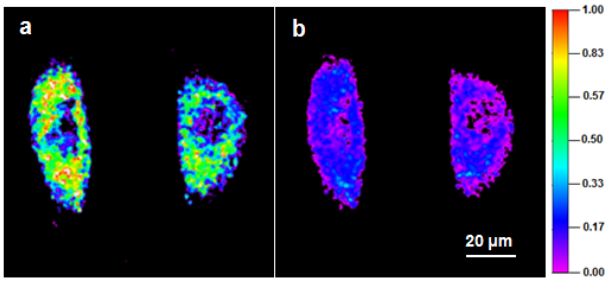



**Fig. S11.** Intracellular luminescence intensity ratio of blue to yellow channel before (a) and after (b) adding of $F^-$.

**References**

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2 X. M. Liu, N. Xi, S. J. Liu, Y. Ma, H. R. Yang, H. R. Li, J. H. He, Q. Zhao, F. Y. Li, W. Huang, *J. Mater. Chem*., 2012, **22**, 7894.