Electronic supplementary information (ESI)

Rational design of luminescent nanoprobe for hypoxia imaging *in vivo* via ratiometric and photoluminescence lifetime imaging microscopy

Qi Yu,[a] Tianci Huang,[a] Yipeng Li,[a] Huanjie Wei,[a] Shujuan Liu,*[a] Wei Huang,*[a,b] Jing Du,[a] and Qiang Zhao*[a]

[a]*Key Laboratory for Organic Electronics and Information Displays and Institute of Advanced Materials (IAM), Jiangsu National Synergetic Innovation Center for Advanced Materials (SICAM), Nanjing University of Posts and Telecommunications (NUPT), Nanjing 210023, P. R. China.*
[b]*Key Laboratory of Flexible Electronics (KLOFE) and Institute of Advanced Materials (IAM), Jiangsu National Synergetic Innovation Center for Advanced Materials (SICAM), Nanjing Tech University (NanjingTech), Nanjing 211816, P. R. China.*

Experimental Section

General Experimental Information: All reagents and chemicals were purchased from commercial sources and used without further purification. $HAuCl_4 \bullet 3H_2O$ was purchased from Sigma-Aldrich company. 11-Mercaptoundecanoic acid (MUA), tetrakis(hydroxymethyl)phosphonium chlorid (THPC), 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC), and *N*-hydroxysuccinimide (NHS) were purchased from J&K company. All solvents were of analytical grade and directly used without purification. Cell culture reagents and fetal bovine serum (FBS) were purchased from KeyGEN BioTECH company. Transmission electron microscopy (TEM) images were obtained from a JEM-2010 transmission electron microscope at an acceleration voltage of 200 kV. Dynamic light scattering (DLS) experiment was conducted on Zetasizer Nanoseries (Nano ZS90). X-ray photoelectron spectroscopy (XPS) spectra were measured on PHI 500 ESCA System with Al/Mg X-ray source ($h\upsilon$ = 93.9 eV). The UV-visible absorption spectra were obtained with a Shimadzu UV-2600/2700 spectrophotometer. Photoluminescence spectra and luminescence lifetime were obtained from an Edinburgh FL 920 spectrophotometer. Confocal luminescence and photoluminescence lifetime images were measured from an Olympus IX81 laser scanning confocal microscope that was equipped with a photoluminescence lifetime setup. The luminescence lifetime was calculated with professional software provided by PicoQuant Company. A diode laser was employed as an excitation source at 405 nm. The green and red channels in the confocal luminescence images were collected at 500 ± 20 and 600 ± 20 nm. The luminescence lifetimes from two channels were

respectively collected through 520 ± 20 nm and 600 ± 10 nm bypasses.

Synthesis of MUA Capped Au NCs: MUA (13.1 mg) and NaOH (8 mg) were added into 20 mL H_2O. $HAuCl_4$ (510 μL, 30 mM) and THPC (445 μL) solution were added into the mixture. The THPC solution was diluted by adding THPC (12 μL) to water (1 mL). The mixture was stirred for 2 h. Au NCs@MUA were obtained by adding NaCl (10 mL, 500 mM) and centrifugation with water at 15000 rpm for 20 min for twice. The obtained Au NCs was dispersed in 2 mL water.

Synthesis of the nanoprobe: EDC (16 mg) and NHS (23 mg) were mixed with Au NCs solution. The pH value was adjusted to 4 and the mixture was stirred for 4 h. After 4 h, the mixture was purified by centrifugation with water at 15000 rpm for 20 min for twice to obtain the functionalized Au NCs. The functionalized Au NCs and THF solution of iridium(III) complex **1** (1 mg/mL) were mixed and stirred for 24 h. The mixture was separated by centrifuging at 15000 rpm for 20 min. The precipitation was washed with THF for 3 times to remove the unconjugated complex. The obtained nanoprobe was dispersed in 5 mL water.

*Cell Culture and Cytotoxicity***:** HeLa cells were grown in Dulbecco's Modified Eagle Medium supplemented with 10% FBS at 37 °C and 5% CO_2. Cells (5×10^8/L) were placed on 18 mm glass coverslips and allowed to adhere for 24 h. The cytotoxicity of the nanoprobe towards HeLa cells was calculated by the methyl thiazolyl tetrazolium (MTT) assay. Before incubation at 37 °C and 5% CO_2 atmosphere for 24 h, HeLa cells in log phase were seeded into a 96-well cell-culture plate at 1×10^4/well. The Au NCs-Cy1 at concentrations of 5, 10, 20, 50, 100 μg mL^{-1} was added to the wells of the

treatment group, and MTT containing 0.2% DMSO to the negative control group. The cells incubated at 37 °C and 5% CO_2 atmosphere for 24 h. 20 mL MTT solution (5 mg mL^{-1}) was added to each well of the 96-well assay plate, and the solution was incubated for another 3 h under the same condition. A Tecan Infinite M200 monochromator based multifunction microplate reader was used for measuring the OD570 (absorbance value) of each well referenced at 690 nm. The following formula was used to calculate the viability of cell growth: viability (%) = [(mean of absorbance value of treatment group)/(mean absorbance value of control)]×100%.

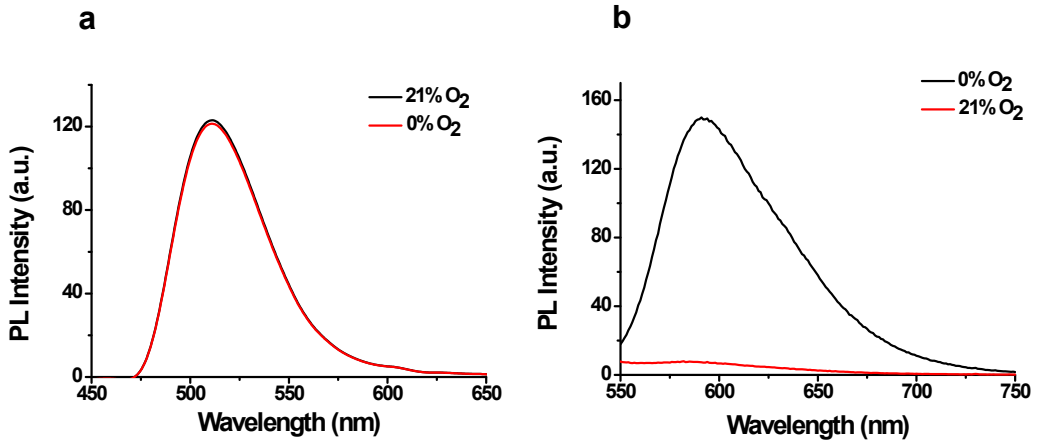

Fig. S1 (a) The photoluminescence spectra of Au NCs in PBS buffer (pH = 7.4) under 21% and 0% O_2 degree; (b) The photoluminescence spectra of iridium(II) complex **1** in DMSO under 21% and 0% O_2 degree.

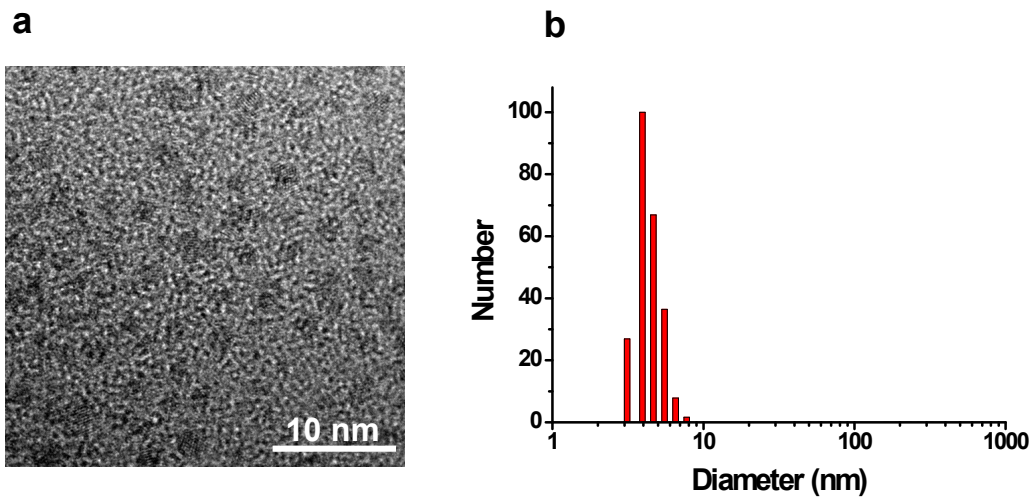

Fig. S2 (a) TEM images of Au NCs; (b) DLS results of Au NCs.

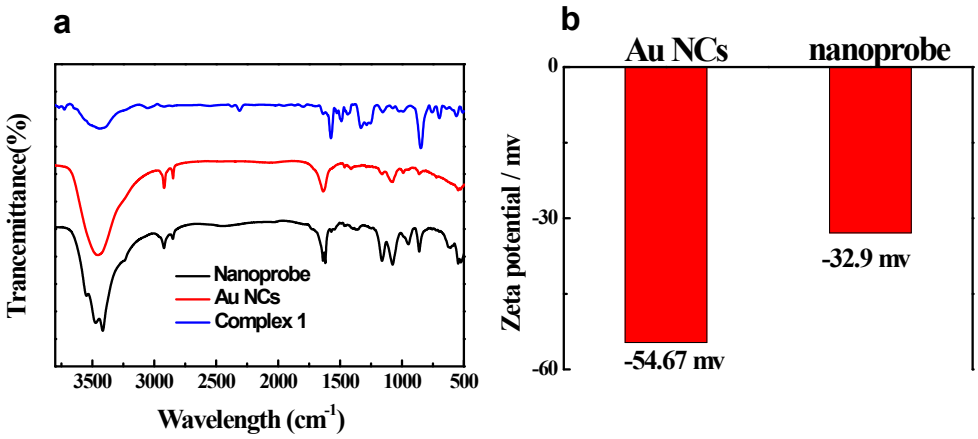

Fig. S3 (a) Fourier transform infrared spectra of Au NCs, iridium(III) complex **1** and nanoprobe; (b) Zeta potentials of Au NCs and nanoprobe.

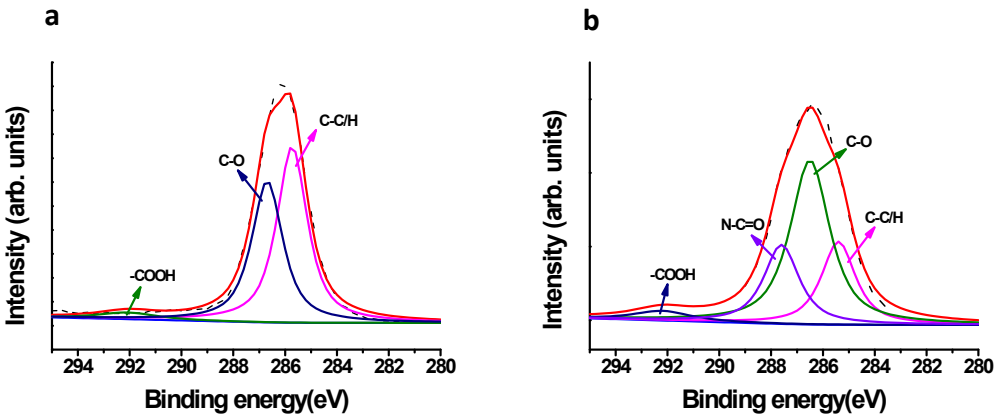

Fig. S4 The measured C(1s) core level XPS spectra of (a) Au NCs and (b) nanoprobe. The broad peak centered at ca 288 eV in (b) can be related to the nitrogen in the amide bond of the nanoprobe.

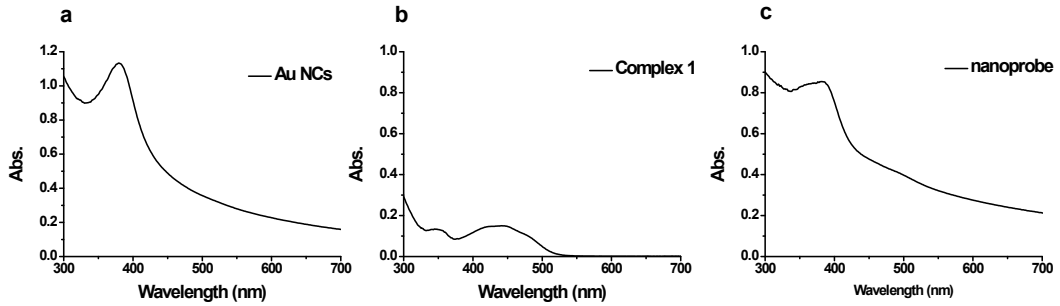

Fig. S5 UV/vis absorption spectra of Au NCs (a), complex **1** (b) and nanoprobe (c).

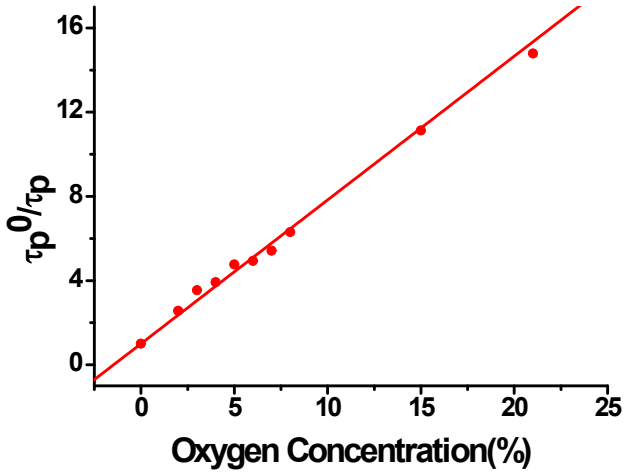

Fig. S6 The corresponding Stem–Volmer plot of τ_p^0 / τ_p against the partial pressure of oxygen. The ratios between the phosphorescence lifetime of complex **1** and the lifetime of Au NCs in the absence and presence of O_2 are defined as τ_p^0 and τ_p. The K_{sv} was calculated to be $0.68\%^{-1}$, which was in accordance to the results obtained from ratio.

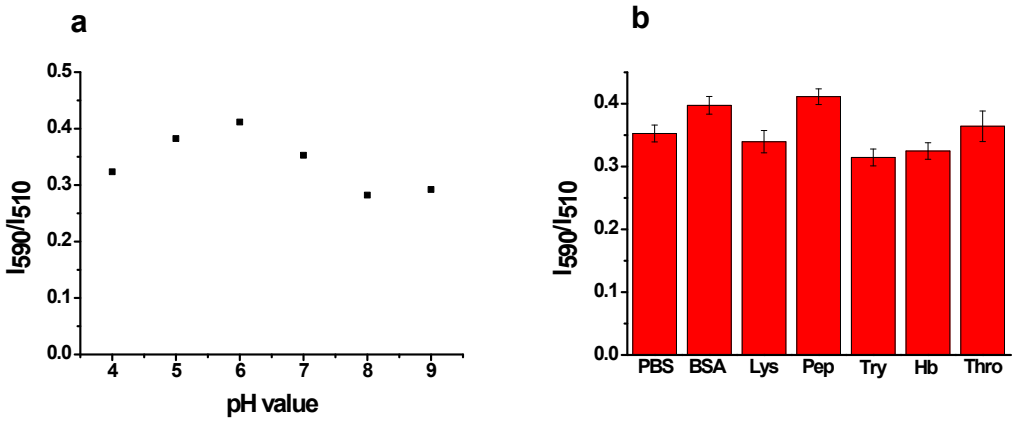

Fig. S7 (a) The luminescence ratio changes of the nanoprobe in PBS buffer solution with different pH value; (b) The luminescence ratio changes of the nanoprobe in different proteins solution.

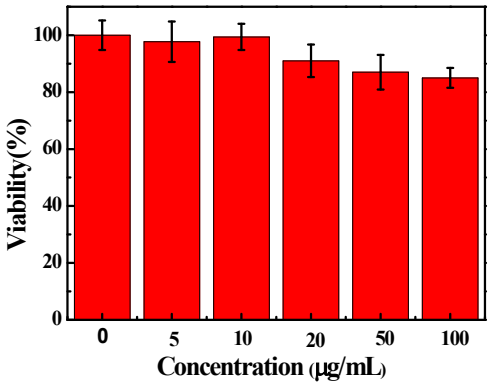

Fig. S8 The viability of HeLa cells loaded with the nanoprobe at different concentrations at 37 °C for 24 h.

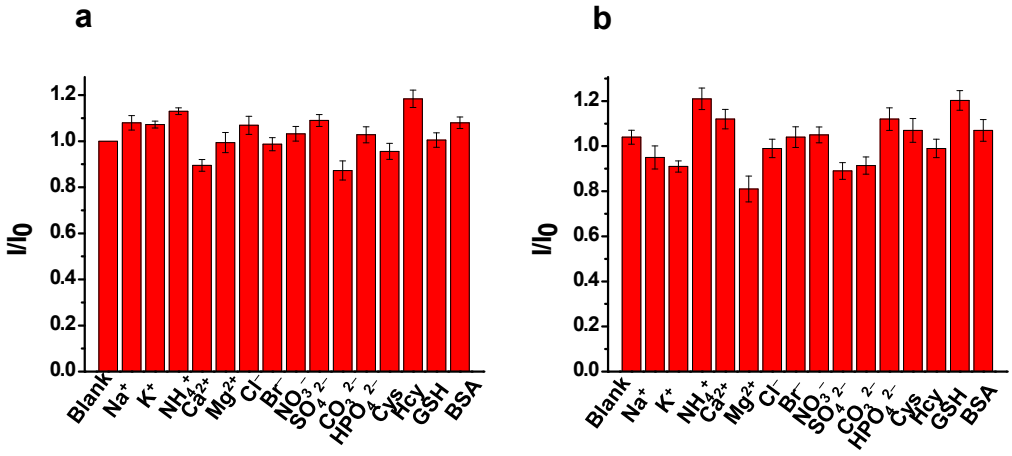

Fig. S9 The photoluminescence spectra of the iridium(III) complex (a) and gold nanoclusters (b) in buffer solutions containing different ions, amino acid and protein. The luminescence intensity of the iridium(III) complex **1** and Au NCs exhibit small fluctuation between 0.8 to 1.2 when different ions, amino acid and proteins are added.

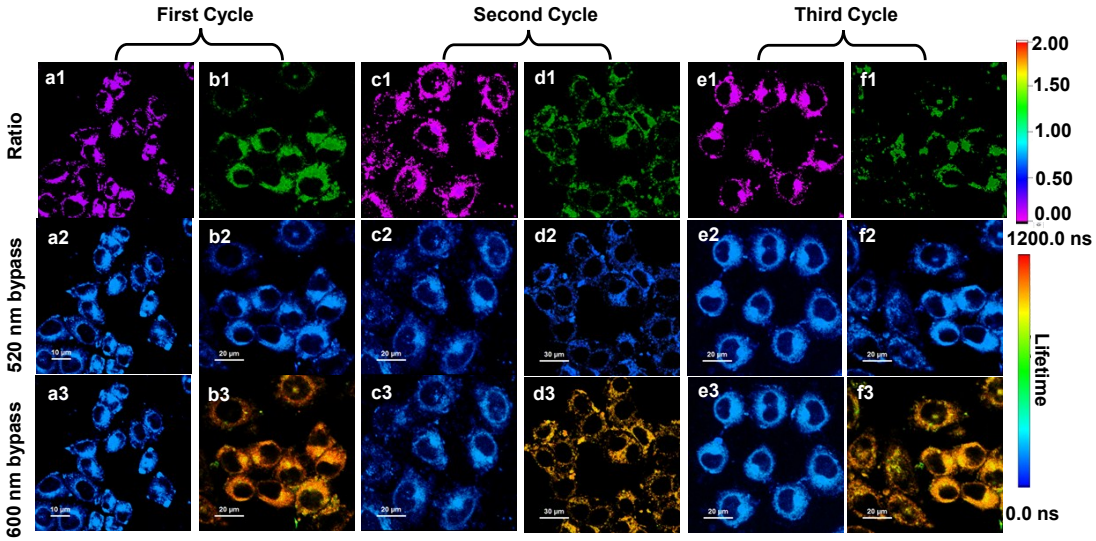

Fig. S10 Ratiometric luminescence images of HeLa cells incubated with 15 μg/mL nanoprobe (a1-f1); Photoluminescence lifetime images of HeLa cells incubated with 15 μg/mL nanoprobe through 520 nm bypass (a2-f2) and 600 nm bypass (a3-f3). Excitation wavelength is 405 nm. Cells were exposed to cycles of normoxia (21%) (a, c, e) and hypoxia (2.5%) (b, d, f).

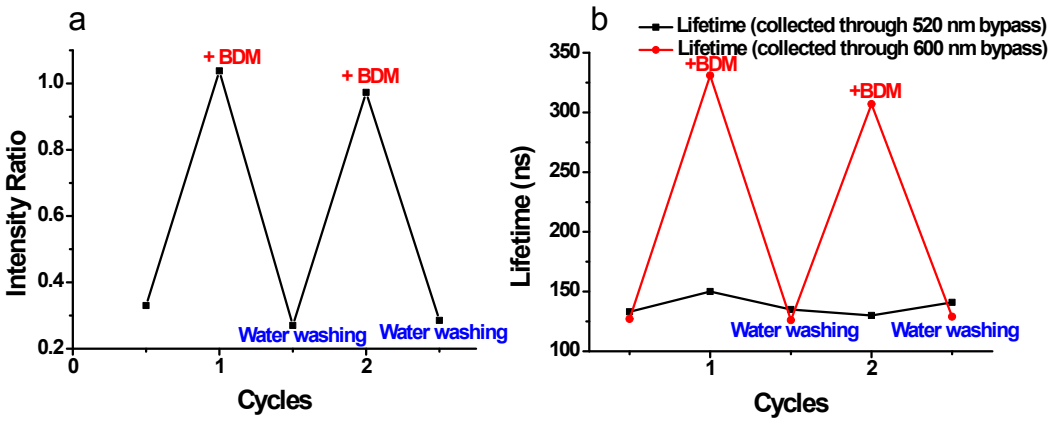

Fig. S11 The ratio (a) and lifetime (b) changes of zebrafish injected with the nanoprobe repeatedly washed with BDM and water.