Supporting Information

Metal Node Engineering in Metal-Organic Frameworks for Enhanced High-Order Multiphoton Excited Fluorescence

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1. Experimental methods

1.1 Materials

All raw materials are commercially purchased and unpurified. $ZrCl_4$ and $HfCl_4$ were purchased from Aladdin Co., Ltd. Benzoic acid, Potassium hydroxide, N,N-dimethylformamide (DMF), Methyl 4-formylbenzoate, Dithioacetamide, tetrahydrofuran (THF), Methanol were purchased from Macklin Co., Ltd. Hydrochloric acid was purchased from Sinopharm Chemical Reagent Co., Ltd.

1.2 Characterization

UV-vis-NIR absorption spectra were recorded on a Perking Elmer Lambda 1050 + UV/vis/NIR spectrophotometer. Zeta potentials were acquired using a zeta potential analyzer (Malvern, UK). Powder X-ray diffraction (XRD) patterns were recorded on Bruker D2 PHASER X-ray diffractometer. SEM were detected by REGULUS8230*. UV-vis absorption spectra were recorded on a UV-265 spectrophotometer. Fluorescence measurements were carried out on a Hitachi F-7000 fluorescence spectrophotometer. Multiphoton fluorescence lifetime was determined by FLUORMAX-4P. Two-photon spectra, multiphoton spectra and z-scan data was performed using Coherent Monaco 1035: 80-60 System which equipped with POLAND OPERA-F light conversion. Confocal laser scanning microscope imaging data acquisition and processing were performed using LEICA STELLARIS 8 which equipped with one photon excitation source (405 nm), white laser and femtosecond laser (680 - 1300 nm, 80 MHz,140 fs).

1.3 Synthesis of Tc

Measure 30 mL of DMF and add it to a 100 mL Schlenk flask. After purging with N_2 for 1 h, successively add 1.96 g of methyl 4-formylbenzoate (12 mmol) and 0.6 g of dithioacetamide (5 mmol), stir and reflux for 6 h. After the reaction, cool to room temperature, filter the reaction mixture to obtain a pale-yellow solid product, and wash it with DMF three times. Then, dissolve 0.2 g (0.48 mmol) of the pale-yellow product in a mixed solvent (8 mL of tetrahydrofuran and 8 mL of methanol), add 8 mL of a potassium hydroxide (0.08 g, 14.4 mmol) aqueous solution, and reflux for 12 h. After

the reaction, cool to room temperature, remove tetrahydrofuran and methanol by rotary evaporation, and add an appropriate amount of water. Gently heat until the solid dissolves, then acidify the above solution with diluted hydrochloric acid until no precipitate forms (pH = 2 - 3). Filter off the yellow solid with water and vacuum dry to obtain pure Tc. ^{1}H NMR (400 MHz, d_6-DMSO, r.t.) δ 13.05 (s, 2H), 8.01 (d, J = 8.0 Hz, 4H), 7.82 (d, 4H).

1.4 Synthesis of $Zr_xHf_{1-x}Tc$

Thirty milliliters of DMF solutions containing Tc (0.014 mol/L) and $ZrCl_4$ (0.01 mol/L); Tc (0.014 mol/L), $ZrCl_4$ (0.008 mol/L) and $HfCl_4$ (0.002 mol/L); Tc (0.014 mol/L), $ZrCl_4$ (0.006 mol/L) and $HfCl_4$ (0.004 mol/L); Tc (0.014 mol/L), $ZrCl_4$ (0.004 mol/L) and $HfCl_4$ (0.006 mol/L); Tc (0.014 mol/L), $ZrCl_4$ (0.002 mol/L) and $HfCl_4$ (0.008 mol/L); as well as Tc (0.014 mol/L), $HfCl_4$ (0.002 mol/L) and benzoic acid (12.2 mg) were respectively added into 50 mL Teflon-lined stainless steel autoclaves. The autoclaves were sealed and maintained at 120 ℃ for 48 h. After cooling to room temperature, the resulting solids were collected by centrifugation, washed with DMF and ethanol three times respectively, and then dried under vacuum at 60 ℃.

1.5 Synthesis of $Zr_xHf_{1-x}Tc$@FA

Folic acid (FA, 10 mg) was dispersed in 10 mL DMF under ultrasonic treatment. Then, **$Zr_xHf_{1-x}Tc$** (10 mg) was added to the above solution, and the mixture was stirred overnight at 30 ℃. The products were washed with DMF and ethanol three times respectively, and dried in vacuum at 60 ℃.

1.6 Two-photon excited fluorescence (2PEF) spectroscopy

2PEF spectra were obtained by the two-photon excited fluorescence (2PEF) method with a femtosecond laser pulse and a Ti: sapphire system (680 - 1080 nm, 80 MHz, 140 fs) as the light source. The concentration of **$Zr_xHf_{1-x}Tc$** was 200 μg/mL in DMF.

1.7 Multiphoton excited fluorescence (3PEF, 4PEF) spectroscopy

MPEF spectra were obtained by the multiphoton excited fluorescence method with

Coherent Astrella + TOPAS Prime (1150 - 2600 nm, 1 kHz, 120 fs) as the light source. The concentration of $\mathbf{Zr_xHf_{1-x}Tc}$ was 200 μg/mL in DMF.

1.8 Z-scan measurements

To verify the high-order nonlinear optical property of $\mathbf{Zr_xHf_{1-x}Tc}$, their three-photon absorption properties were confirmed by an open aperture Z-scan technique using a Coherent Monaco 1035: 80-60 System which equipped with POLAND OPERA-F light conversion as the light source. The concentration of $\mathbf{Zr_xHf_{1-x}Tc}$ was 200 μg/mL in DMF and at same pulse energy (100 mW) upon 1250 nm laser excitation and all measurements were carried out at room temperature.

1.9 Photoelectrochemical characterization

The electrochemical measurement was carried out by the electrochemical workstation with the model of CHI 760. The test was carried out in a three-electrode system, in which the Pt sheet was used as the counter electrode, the Ag/AgCl electrode was used as the reference electrode, and the prepared powder sample was used as the working electrode. In addition, 0.5 M Na_2SO_4 was utilized as the electrolyte solution, and a xenon lamp equipped with a filter ($\lambda > 400$ nm) was utilized as the visible light source. 2 mg of $\mathbf{Zr_xHf_{1-x}Tc}$ and 20 μL of Nafion solution were dissolved in 1 mL of anhydrous ethanol solution, sonicated for 30 min to make them evenly mixed, and then drop onto the ITO glass (1 cm × 2 cm) to make the working electrode.

1.10 DFT calculation

The molecular models for density functional theory (DFT) are periodic fragments taken from $\mathbf{Zr_xHf_{1-x}Tc}$. Geometry optimization is calculated at B3LYP hybrid functional with 6-31G(d) basis sets in GAUSSIAN 16 package.[1-2] The NLO static is calculated by the sum-over-states (SOS) method using time-dependent density functional theory (TDDFT).[3] Multiwfn 3.8 is used to analyze molecular orbitals (MOs) and hyper polarizability and VMD 193 software for molecular visualization.[4-5]

1.11 Cell culture

The Hela cells and HEK 293T cells were cultured in 25 cm^2 culture flasks in DMEM, supplemented with fetal bovine serum (10%), penicillin (100 units/mL) and streptomycin (50 units/mL) at 37 ℃ in a CO_2 incubator (95% relative humidity, 5% CO_2). Cells were seeded in 35 mm cell culture dishes at a density of 1×10^5 cells/dish and were allowed to grow when the cells reached more than 70% confluence.

1.12 Stability experiment of $Zr_xHf_{1-x}Tc$@FA

$Zr_xHf_{1-x}Tc$@FA was dispersed in phosphate buffer saline solution (PBS, pH = 7.4) and serum-containing dulbecco's modified eagle's medium (DMEM), respectively, then particle size of **$Zr_xHf_{1-x}Tc$@FA** in different solutions were collected within 7 days.

1.13 Cell uptake experiment

HeLa cells (human cervical cancer cells) and HEK 293T cells (human embryonic kidney cells) were seeded onto respective cell culture dishes and grown to approximately 70% confluency. Subsequently, HeLa cells were treated with $Zr_{0.4}Hf_{0.6}Tc$ (30 μg/mL) and **$Zr_{0.4}Hf_{0.6}Tc$@FA** (30 μg/mL), respectively, while HEK 293T cells were treated only with **$Zr_{0.4}Hf_{0.6}Tc$@FA** (30 μg/mL). After 12 h of incubation following seeding, the uptake of **$Zr_{0.4}Hf_{0.6}Tc$@FA** in folate-overexpressing cancer cells was analyzed using CLSM. Then, the cells were planted into six-well plates and cultured for 24 h to allow adherence. The treated HeLa cells (treated with **$Zr_{0.4}Hf_{0.6}Tc$** and **$Zr_{0.4}Hf_{0.6}Tc$@FA**, respectively) and HEK 293T cells treated with **$Zr_{0.4}Hf_{0.6}Tc$@FA** were further incubated for 12 h. Subsequently, the cell suspension was washed with PBS solution (pH = 7.4), digested with nitric acid (65-68%), diluted, and the element contents were detected by ICP-MS.

1.14 Cytotoxicity Assay

$Zr_{0.4}Hf_{0.6}Tc$@FA stock solutions were diluted by fresh medium in to desired concentration (0, 10, 30, 50, 70, 90, 110, 130 μg/mL). Hela cells were cultured in a 96-well plates for 24 h before experiments. The DMEM cell medium was then exchanged

by different concentrations of **$Zr_{0.4}Hf_{0.6}$Tc@FA** solutions. They were incubated at 37 ℃ in 5% CO_2 for 12 h before cell viability was measured by the MTT assay. The cell medium solutions were exchanged by 100 μL of fresh DMEM, followed by the addition of 20 μL (5 mg/mL) MTT aqueous solution to each well. The cell plates were then incubated at 37 ℃ in 5% CO_2 for 4 h. After MTT medium removal, the formazan crystals were dissolved in DMSO (100 μL/well) and the absorbance was measured at 490 nm using a microplate reader. The duplicated experiments have been tested.

1.15 One/Two/three-photon fluorescence imaging

All the animal procedures were approved by the Institutional Animal Care and Use Committee of Anhui University (serial number: 2024-017) based on the National Standard of China GB/T35892-2018 guidelines for Ethical Review of Experimental Animal Welfare. We have taken great efforts to reduce the number of animals used in these studies and taken effort to reduce animal suffering from pain and discomfort. LEICA STELLARIS 8 which equipped with one photon excitation source (405 nm), white laser and femtosecond laser (680 - 1300 nm, 80 MHz, 140 fs) was employed to achieve one/two/three-photon fluorescence imaging. Hela cells treated with **ZrTc@FA** and **$Zr_{0.4}Hf_{0.6}$Tc@FA** (30 μg/mL) were selected for cell fluorescence imaging, respectively. The tissue slices were prepared from myocardial tissue from Balb/c mouse model. The tissue sections were cut to 300 μm thickness. The tissue sections were incubated with **$Zr_{0.4}Hf_{0.6}$Tc@FA** for 60 min. The one-photon, two-photon and three-photon 3D fluorescence imaging was observed upon excitation at 458 nm, 800 nm and 1250 nm (100 mW), respectively.

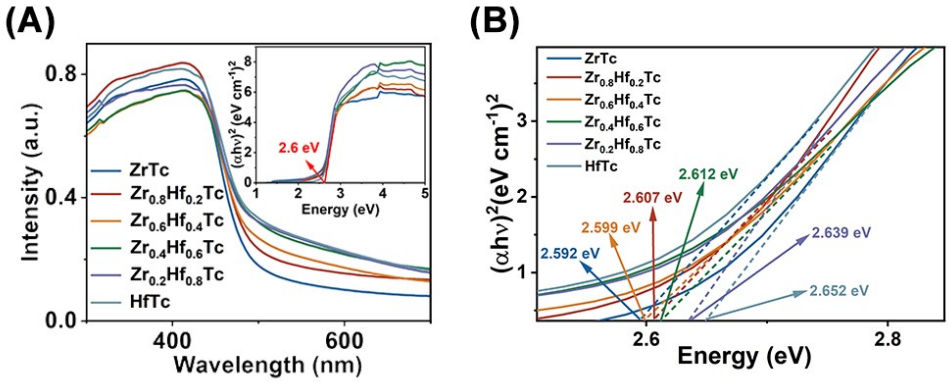

Fig. S1. (A) Solid UV-vis absorption spectra of $\mathbf{Zr_XHf_{1-X}Tc}$. Inset: Tauc plots of $\mathbf{Zr_XHf_{1-X}Tc}$. (B) Locally magnified tauc plot of $\mathbf{Zr_XHf_{1-X}Tc}$.

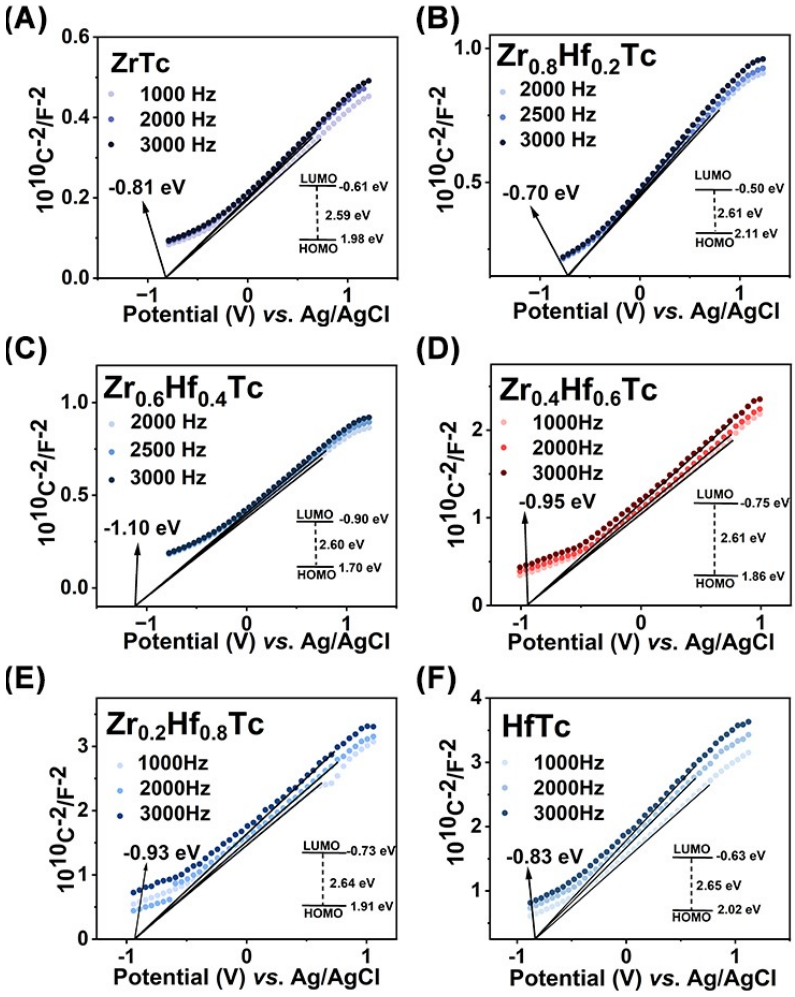

Fig. S2. Mott-Schottky plot of $\mathbf{Zr_xHf_{1-x}Tc}$.

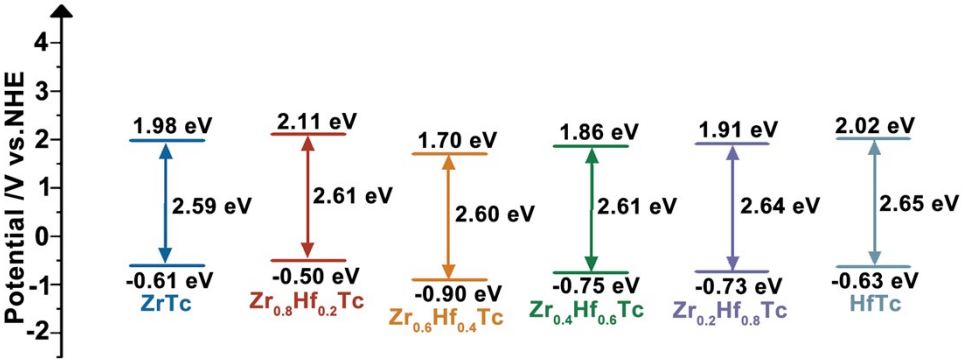

Fig. S3. Energy level diagrams of $\mathbf{Zr_xHf_{1-x}Tc}$.

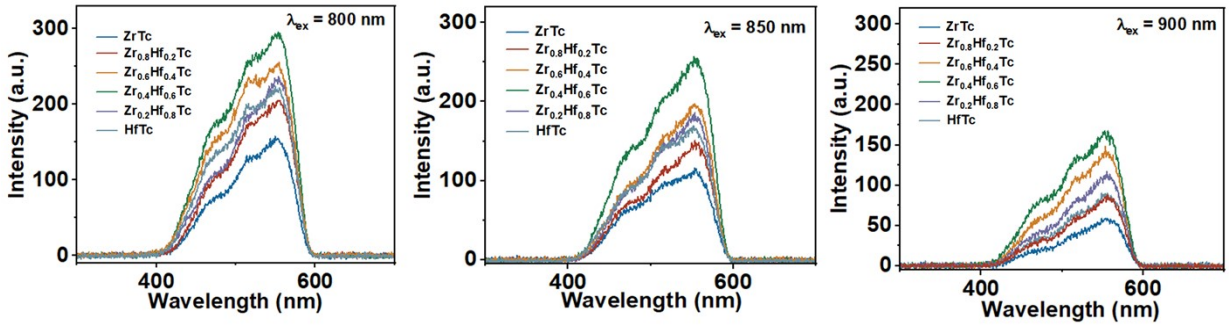

Fig. S4. Two-photon excited fluorescence spectra of $\mathbf{Zr_xHf_{1-x}Tc}$ (200 μg/mL, fixed power: 400 mW, λ_{ex} = 800, 850 and 900 nm) in DMF.

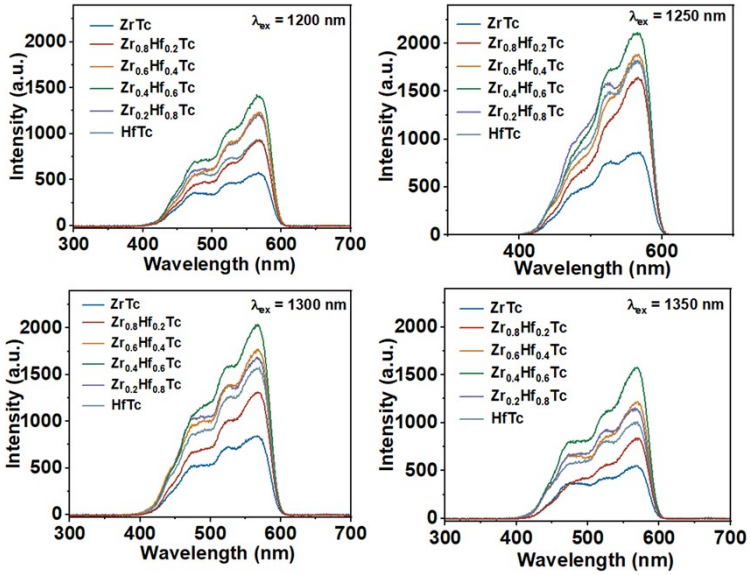

Fig. S5. Three-photon excited fluorescence spectra of $\mathbf{Zr_xHf_{1-x}Tc}$ (200 μg/mL, fixed power: 400 mW, λ_{ex} = 1200, 1250, 1300 and 1350 nm) in DMF.

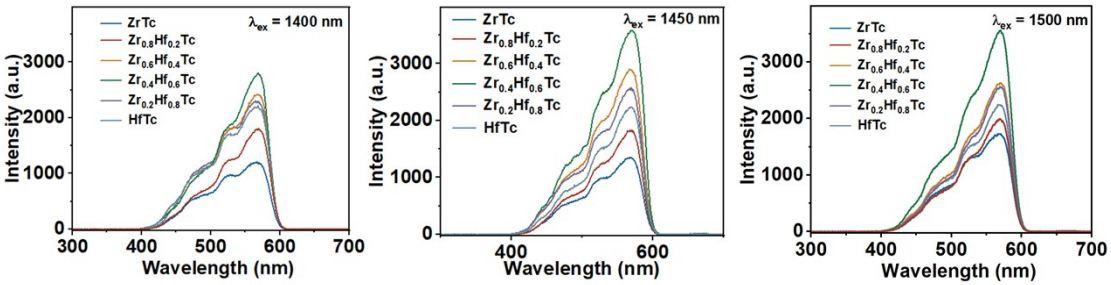

Fig. S6. Four-photon excited fluorescence spectra of $\mathbf{Zr_xHf_{1-x}Tc}$ (200 μg/mL, fixed power: 400 mW, λ_{ex} = 1400, 1450 and 1500 nm) in DMF.

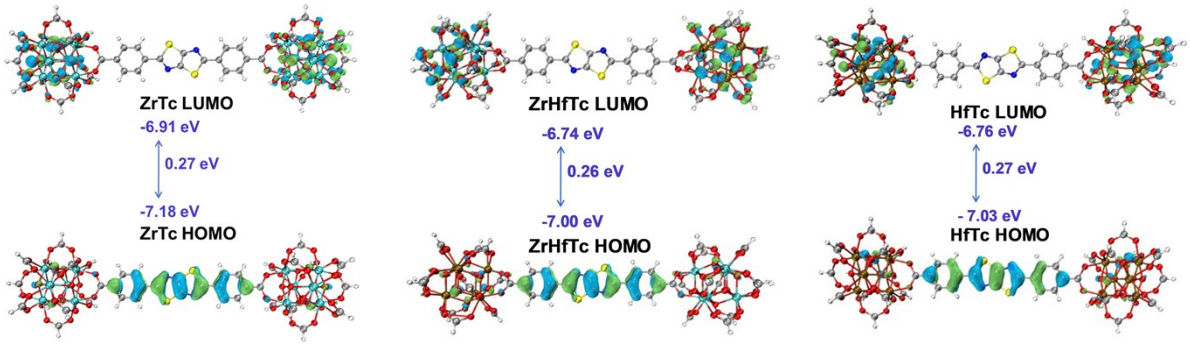

Fig. S7. The frontier molecular orbitals diagram of the highest occupied molecular orbital and the lowest unoccupied molecular orbital for **ZrTc, ZrHfTc** and **HfTc**.

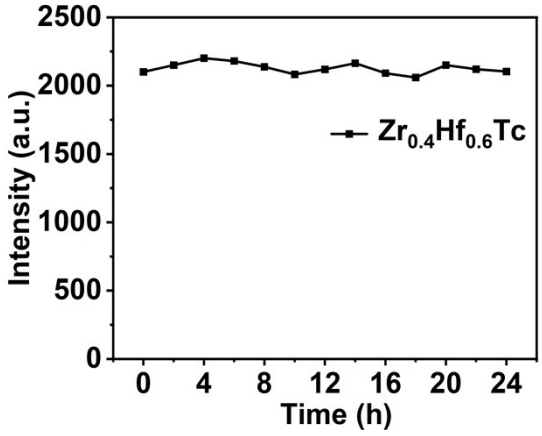

Fig. S8. Three-photon excited fluorescence intensity of $\mathbf{Zr_{0.4}Hf_{0.6}Tc}$ under 1250 nm excitation at different time during 24 h.

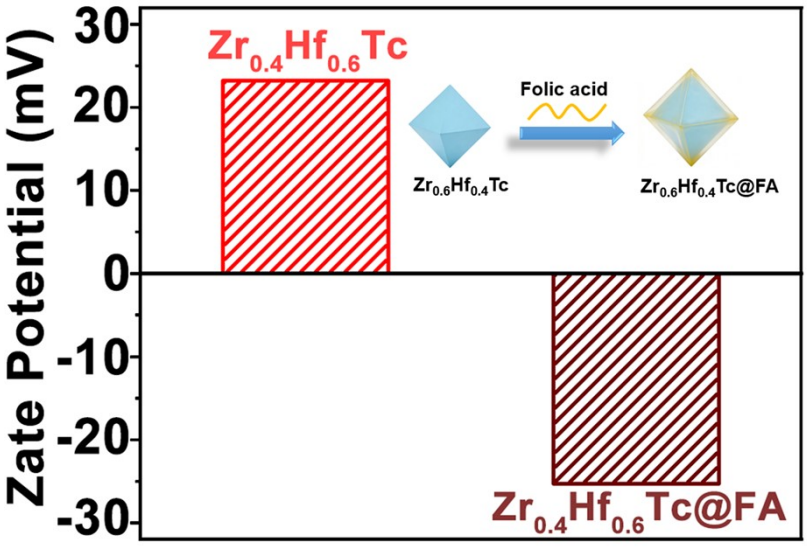

Fig. S9. Zeta potentials of **$Zr_{0.4}Hf_{0.6}Tc$**, **$Zr_{0.4}Hf_{0.6}Tc$@FA** in deionized water.

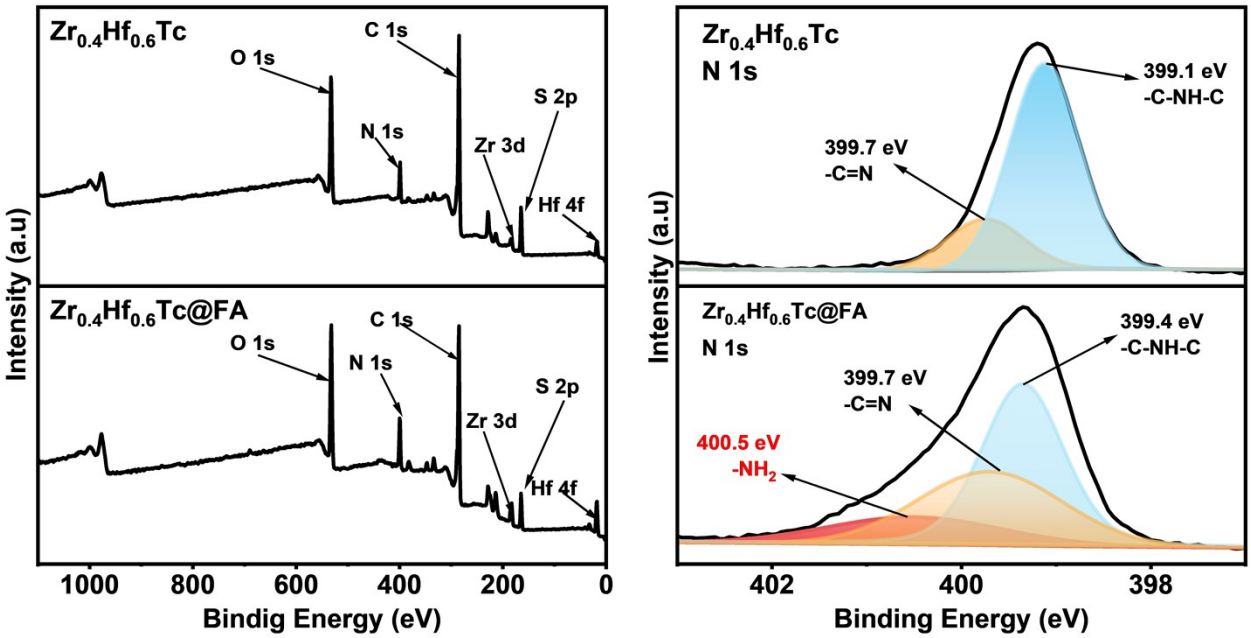

Fig. S10. X-ray photoelectron survey spectra and high resolution N 1s spectra of **$Zr_{0.4}Hf_{0.6}Tc$** and **$Zr_{0.4}Hf_{0.6}Tc$@FA.**

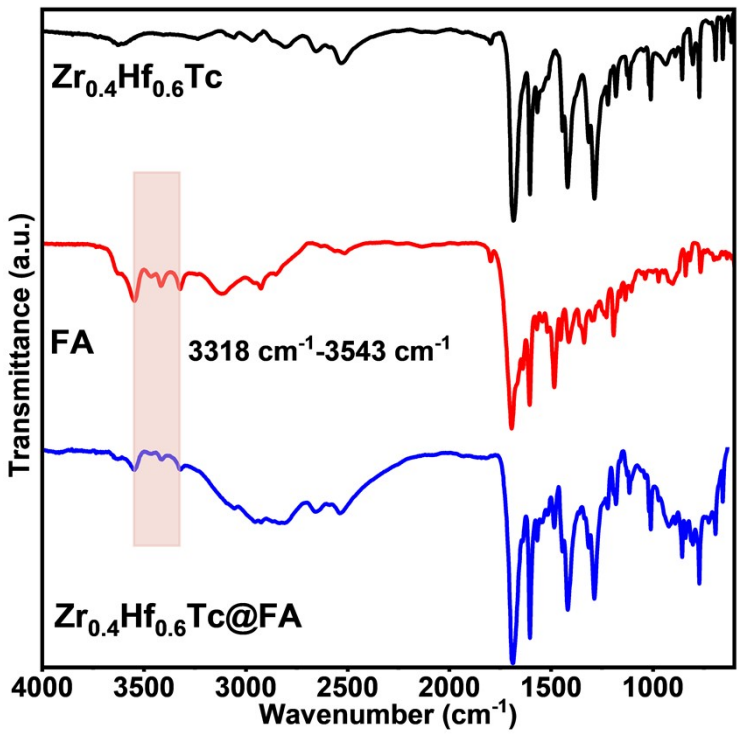

Fig. S11. Fourier transform infrared spectra of **$Zr_{0.4}Hf_{0.6}Tc$**, **FA** and **$Zr_{0.4}Hf_{0.6}Tc$@FA**.

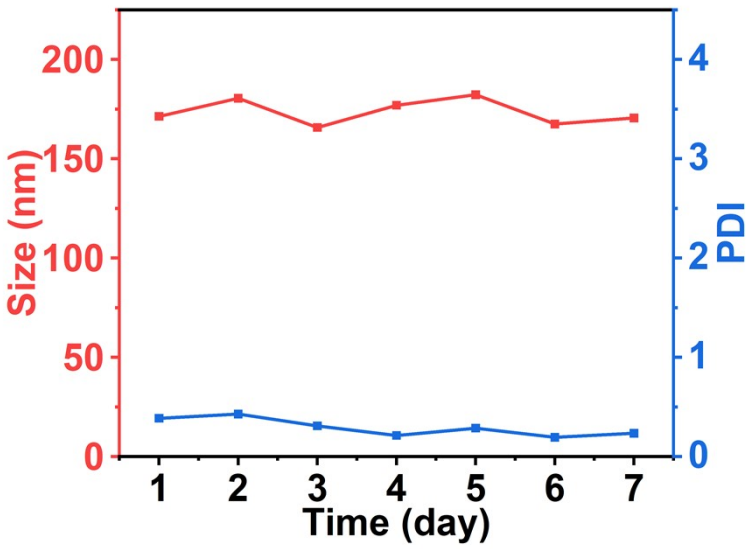

Fig. S12. Particle size distribution and PDI changes of **$Zr_{0.4}Hf_{0.6}Tc$@FA** in PBS solution (pH = 7.4) during 7 days.

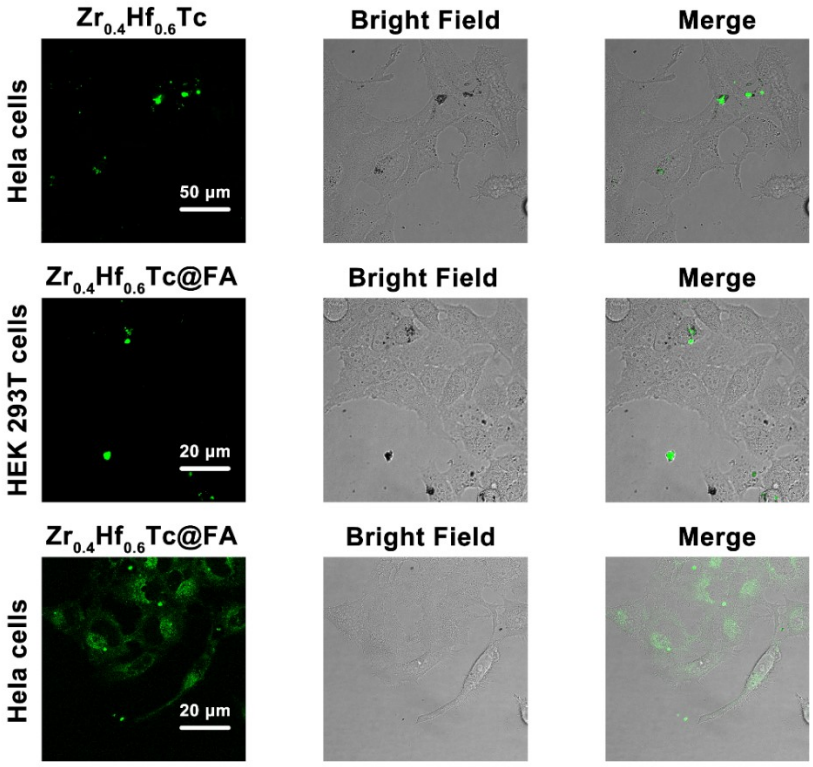

Fig. S13. Confocal images to check the HeLa cells uptake of **$Zr_{0.4}Hf_{0.6}Tc$** and HEK 293T cells of **$Zr_{0.4}Hf_{0.6}Tc$@FA**.

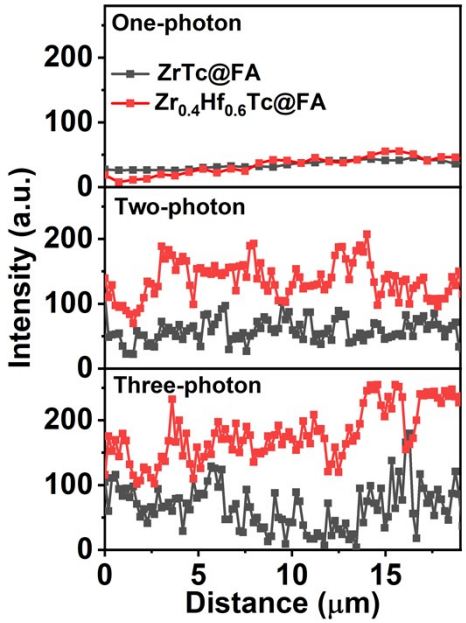

Fig. S14. One-/two-/three-photon CLSM images intensity of HeLa cell treatment with **ZrTc@FA** (30 μg/mL) and **$Zr_{0.4}Hf_{0.6}Tc$@FA** (30 μg/mL) under 458 nm, 800 nm, 1250 nm excitation (input laser power: 100 mW), respectively (Scale bar: 50 μm).

Table S1 Elemental analysis of **$Zr_{0.4}Hf_{0.6}Tc$** and **$Zr_{0.4}Hf_{0.6}Tc$@FA**.

Sample	C (%)	N (%)	S (%)	H (%)
$Zr_{0.4}Hf_{0.6}Tc$	42.9	5.22	5.48	2.51
$Zr_{0.4}Hf_{0.6}Tc$@FA	46.08	13.74	3.42	3.90

Table S2. Zr content determined by ICP-MS.

Sample	Concentration of Zr (μg/mL)
Hela cells treated with **$Zr_{0.4}Hf_{0.6}Tc$**	3.7
Hela cells treated with **$Zr_{0.4}Hf_{0.6}Tc$@FA**	103.9
HEK 293T cells treated with **$Zr_{0.4}Hf_{0.6}Tc$@FA**	5.4

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