Supporting Information

Template-free synthesis and metalation of hierarchical covalent organic framework spheres for photothermal therapy

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Experimental Procedures

Materials

Paraphenylenediamine (Pa, AR, 97.0%, Aladdin), Dichloromethane (CH_2Cl_2, AR, Beijing Chemical Works), Trichloromethane ($CHCl_3$, AR, Beijing Chemical Works), Ferric chloride ($FeCl_3$, AR, Aladdin), Pyridium Chlorochromate (98%, Aladdin), Hexamethylenetetramine (HMT, 99%, Tianjin Fuchen Chemical Reagents Factory), Phloroglucinol Anhydrous (HPLC, 99%, Aladdin), Hydrochloric Acid (HCl, AR, Beijing Chemical Works), Trifluoroacetic Acid (TFA, 99%, Aladdin), Acetic acid (99%, Aladdin), Ethanol (AR, Beijing Chemical Works), Celite (Aladdin). 1,3,5-Triformylphloroglucinol (Tp) was synthesized following the published procedure (*Org. Lett.* **2003**, *5*, 3823). All of the chemicals and solvents were used as received without further purification.

Synthesis of HCOF. Tp (2 mg, 0.009517 mmol), Pa (2 mg, 0.01849 mmol) and 0.015 mL acetic acid were dissolved in 1.5 mL CH_2Cl_2 and 0.5 mL $CHCl_3$, and the mixture was aging at room temperature for 48 h. The products were harvested by centrifugation and washed with CH_2Cl_2 for twice.

Synthesis of Fe-HCOF. The as-synthesized HCOF (1 mg) were mixed with 0.1 mL $FeCl_3$ (0.308 M) in 2 mL DI water, and stirred for 10 h at room temperature. The products were harvested by centrifugation and washed with DI water.

Photothermal heating experiments on Fe-HCOF. Aqueous solutions of Fe-HCOF particles with different concentrations (0-1200 μg/mL) were suspended in different wells of a 96-well plate, and irradiated by 808 nm laser with a power density of 1.5 W/cm^2 for different times. The temperatures were carefully measured by a digital thermometer with a thermocouple probe. To a certain Fe-HCOF concentration (800 μg mL^{-1}), the heating and cooling (ON/OFF) cycle for photostability was evaluated for more than five times upon laser irradiation (808 nm, 1.9 W cm^{-2}). In each heating-cooling cycle, laser irradiation lasted for 10 min followed by a 16 min cooling period until it reached room temperature again. The *in vivo* photothermal effect was conducted by intratumoral injection of Fe-HCOF (2 mg/mL) into the tumor on a Balb/C mouse. All of the temperatures were recorded every 15 s by a photothermal camera.

Calculation of photothermal conversion efficiency: First, the temperature change of Fe-HCOF in aqueous solutions was measured for a prolonged irradiation time (808 nm; 1.9 W/cm^2 for 10

min). Then the heated aqueous dispersion was cooled naturally and the temperatures during cooling were also carefully monitored every 15 s by a thermometer with a thermocouple probe. The photothermal conversion efficiency (η) was calculated by the following equation:

$$\eta = \frac{hS\Delta T_{max} - Q_{Dis}}{I(1 - 10^{-A_{808}})}$$

where h is the heat transfer coefficient, S is the surface area of the container, T_{max} is the equilibrium temperature after 10 min irradiation, T_{Sur} is ambient temperature, Q_{Dis} expresses the heat dissipation by the test cell, I is 808 nm laser power (1.9 W/cm^2), and $A808$ is the absorbance of the Fe-HCOF solution at 808 nm. The value of hS is determined according to the following equation:

$$hS = \frac{m_d C_d}{\tau_s}$$

Where m_d is the mass (0.4 g) and C_d is the heat capacity (4.2 J/g) of the aqueous solvent, τ_s is the sample system time constant, and is defined as the ratio of ΔT and $\Delta Tmax$.

$$t = -\tau_s(\ln\theta)$$

***In vitro* cytotoxicity of Fe-HCOF.** The biocompatibility of Fe-HCOF was evaluated using a standard [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) test. The HeLa cells were seeded in a 96-well plate (8000 cells per well) and incubated in a humidified atmosphere of 5% CO_2 overnight to ensure that the cells had attached to the wells. After that, Fe-HCOF with different concentrations (8.12, 16.2, 37.5, 75, 150, 300, 600 and 1200 μg/mL) were added into each well, and incubated for another 24 h. MTT solution (20 μL, 5 mg/mL) was added into each well, and incubated for 4 h; then 150 μL of DMSO were added after removing the original culture medium. The final fraction surviving of HeLa cells was measured by a microplate reader at the wavelength of 490 nm. In vitro cytotoxicity of PEG-2000-NH_2-modified MCOP was assayed against HeLa cells under similar experimental conditions as described above. For the photothermal heating experiments, after incubation for 4 h, the Fe-HCOF were removed by rinsing three times with PBS, fresh culture medium was then added into the wells. The cells were exposed to NIR light (1.8 W/cm^2) for 5 min to conduct the photothermal treatment, and then continued incubated at 37 ℃ in 5% CO_2 for 24 h. While for the control group, it was incubated

with cells for 24 h without additional treatment. Finally, the viability of the HeLa cells was evaluated using a microplate reader at 490 nm.

Cellular uptake of the nanoparticles: 10 mg of EDC, 3 mg of NHS and 5 mg of RhB were added into the aqueous of Fe-HCOF, the mixture was stirred at room temperature overnight, and then the products were harvested by centrifugation (4000 r/min) and washed several times with ethanol until the supernatant was colorless. The HeLa cells were seeded into 6 well at a density 8 x 10^5 per plate, and then incubated with Fe-HCOF-RhB for 0, 2 and 4 h, respectively. After that, washed with PBS two times, fixed with 4% paraformaldehyde for 10 min in the dark and then stained with DAPI. The cells uptake process was monitored by intracellular fluorescence microscope.

***In vivo* antitumor efficacy of Fe-HCOF.** Female Balb/C mice (about 18 g) were purchased from the Center of Experimental Animals, Jilin University (Changchun, China), and the animal experiments agreed with the criterions of The National Regulation of China for Care and Use of Laboratory Animals. The tumors were established by subcutaneous injection of U14 (murine cervical carcinoma) cancer cells in the leg of the mice. The tumor bearing mice were randomly divided into four groups (n = 4, each group) after the size of the tumors reached 60-100 mm^3, and treated with PBS (control), PBS + NIR, Fe-HCOF and Fe-HCOF + NIR by intratumoral injection, respectively. The mice were treated on day 1 and accompanied with the NIR laser irradiation (1.5 W/cm^2, 10 min). The body weight and tumor volume of each mouse were monitored every day, and after two weeks treatment, the tumors were dissected and weighed to evaluate the therapeutic efficacy. In a typical calculation, the tumor volume was calculated by V = 4/3 x Length x $width^2$/8. The relative tumor volume was calculated as V/V_0, where V_0 was the tumor volume before the treatment. Tumor growth inhibition rate was determined as (C – T)/C × 100%, in which C was the average tumor weight of the control group, while T is the average tumor weight of each treated group. Finally, the major organs of mice, such as liver, spleen, heart, lung, and kidney, were removed and fixed in 4 % paraformaldehyde solution for histological examination in order to further investigate the biocompatibility of Fe-HCOF.

Characterization

Powder X-ray diffraction (PXRD) measurements were performed on Rigaku MiniFlex 600 at a scanning rate of 10°/min in the 2θ range from 3 to 40°, with graphite monochromatized Cu Kα

radiation (λ= 0.15405 nm). Thermogravimetric analysis (TGA) data were recorded with Thermal Analysis Instrument (SDT 2960, TA Instruments, New Castle, DE) with a heating rate of 10 °/min in a nitrogen flow of 100 mL/min. The morphology of the samples was characterized by using a field-emission scanning electron microscope (FE-SEM, S-4800, Hitachi) equipped with an energy-dispersive X-ray (EDX) spectrometer. Gas sorption isotherm measurements were carried out on Quantachrome Instrument Autosorb IQ. The samples were fully exchanged with ethanol, and degassed at 120 ^{o}C for 10 h before the gas sorption measurement. Transmission electron microscopy (TEM) images were obtained on a FEI Tecnai G2 S-Twin with a field emission gun operating at 200 kV. Fourier transform infrared spectra were measured on a Vertex PerkinElmer 580BIR spectrophotometer (Bruker) with KBr pellet technique. The UV-vis adsorption spectra were measured on a Hitachi U-3100 spectrophotometer. Inductively Coupled Plasma (ICP) was taken on an iCAP 6300 of Thermo scientific. The X-ray photoelectron spectra (XPS) were taken on a VG ESCALAB MK II electron energy spectrometer using Mg KR (1253.6 eV) as the X-ray excitation source. MTT experiments were carried out using a microplate reader (Thermo Multiskan MK3). The electron paramagnetic resonance (EPR) spectrum was obtained on a Bruker EMX Nano spectrometer.

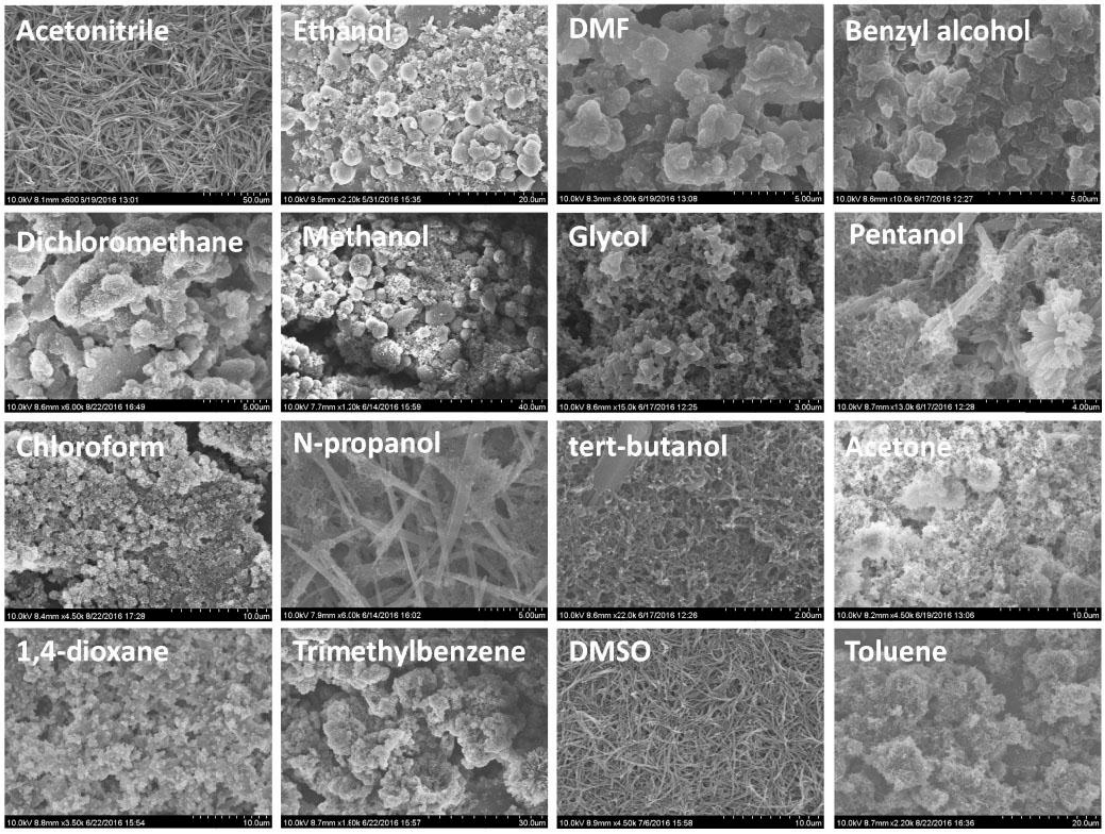

Fig. S1 SEM images of TpPa-COF prepared in different solvents.

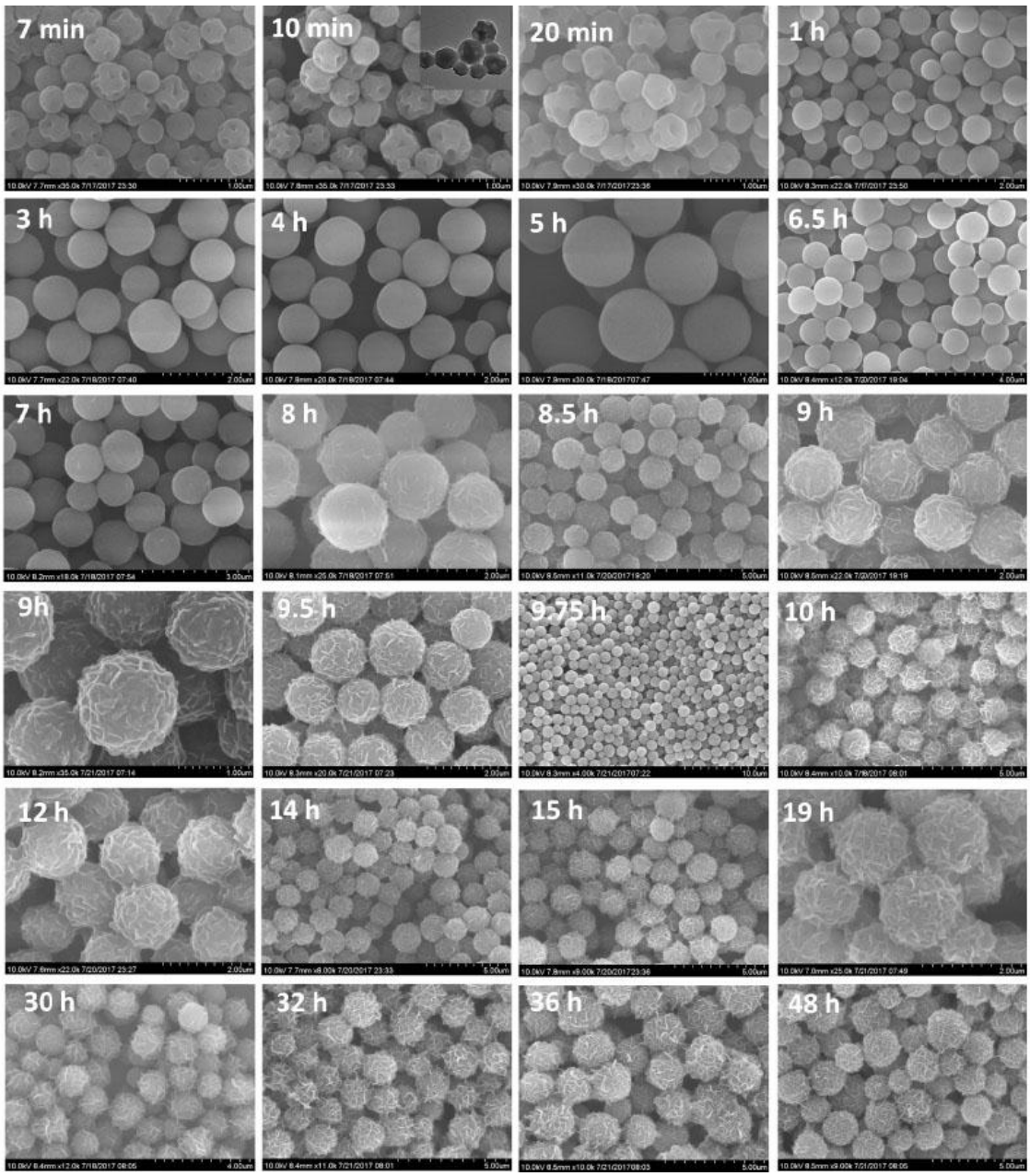

Fig. S2 Additional SEM images of products prepared at different times.

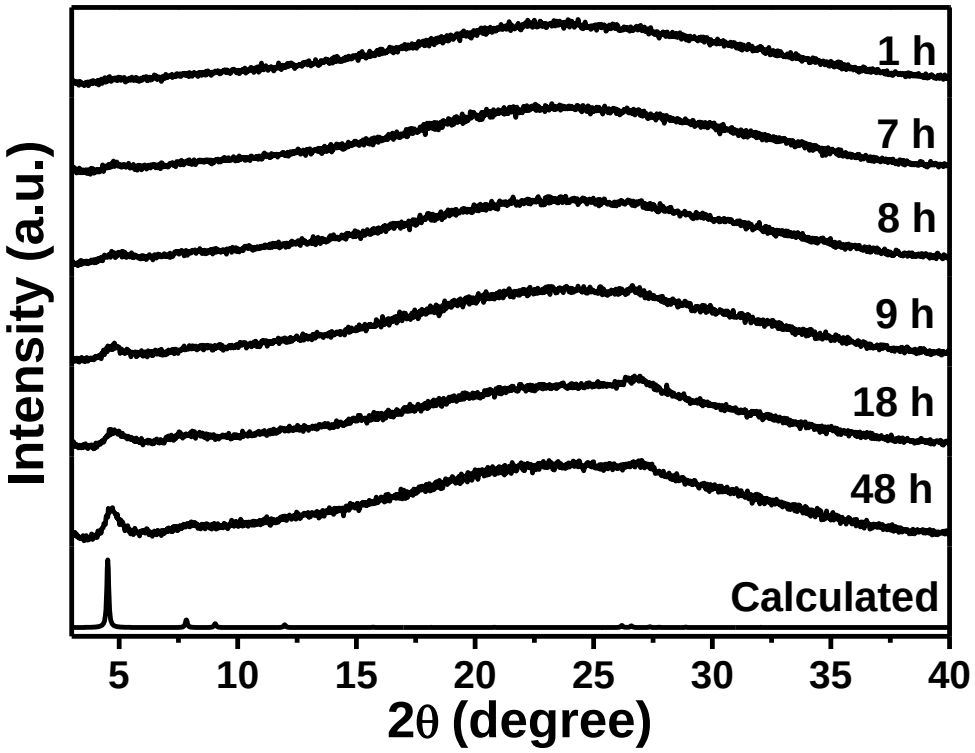

Fig. S3 PXRD patterns of HCOF at different aging times.

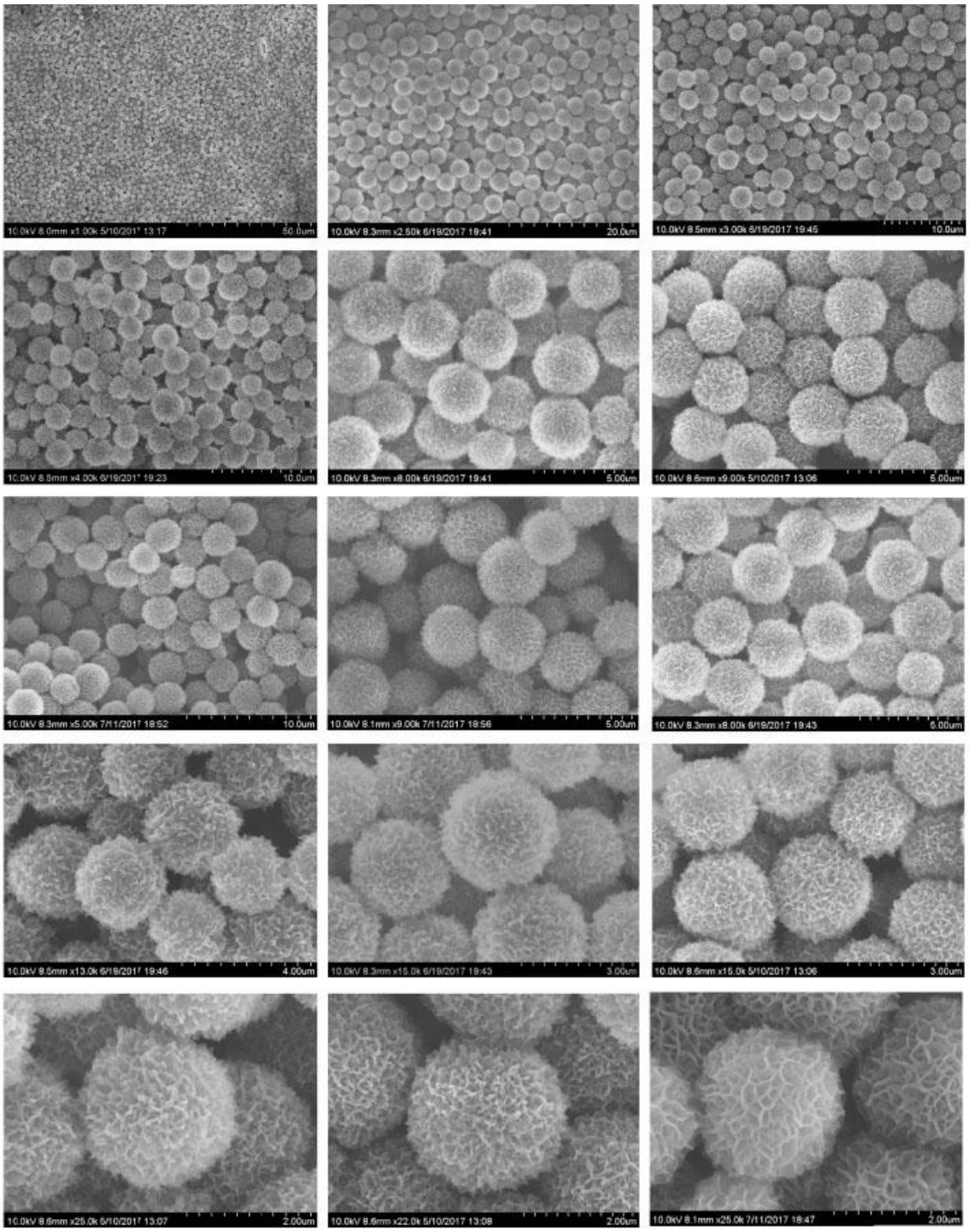

Fig. S4 Additional SEM images of HCOF.

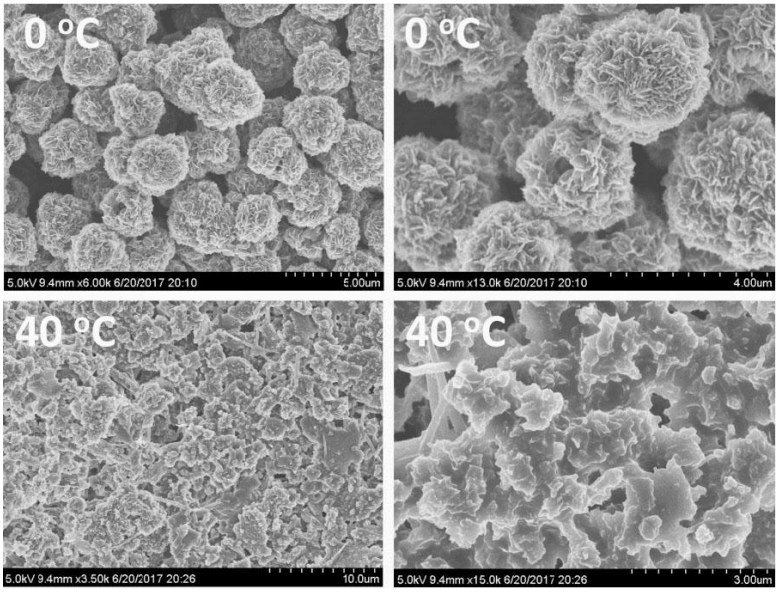

Fig. S5 SEM images of HCOFs prepared at different temperatures.

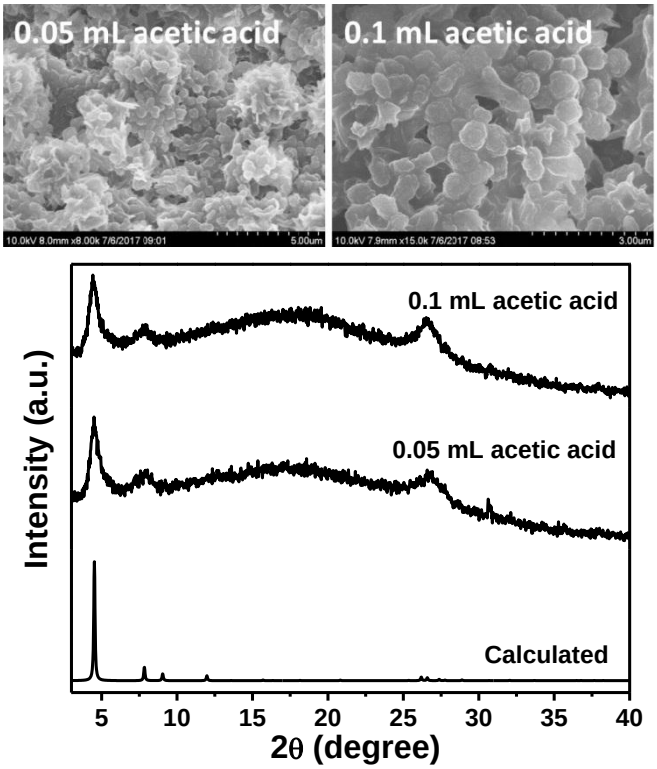

Fig. S6 SEM and XRD patterns of the products prepared by adding different amounts of acetic acid.

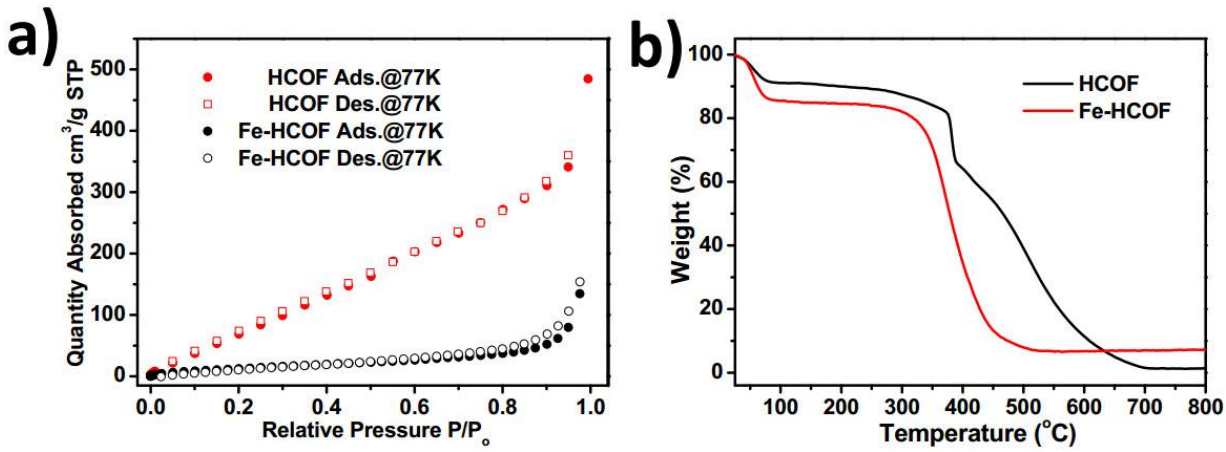

Fig. S7 (a) Nitrogen adsorption-desorption isotherms and (b) TGA curves of HCOF and Fe-HCOF.

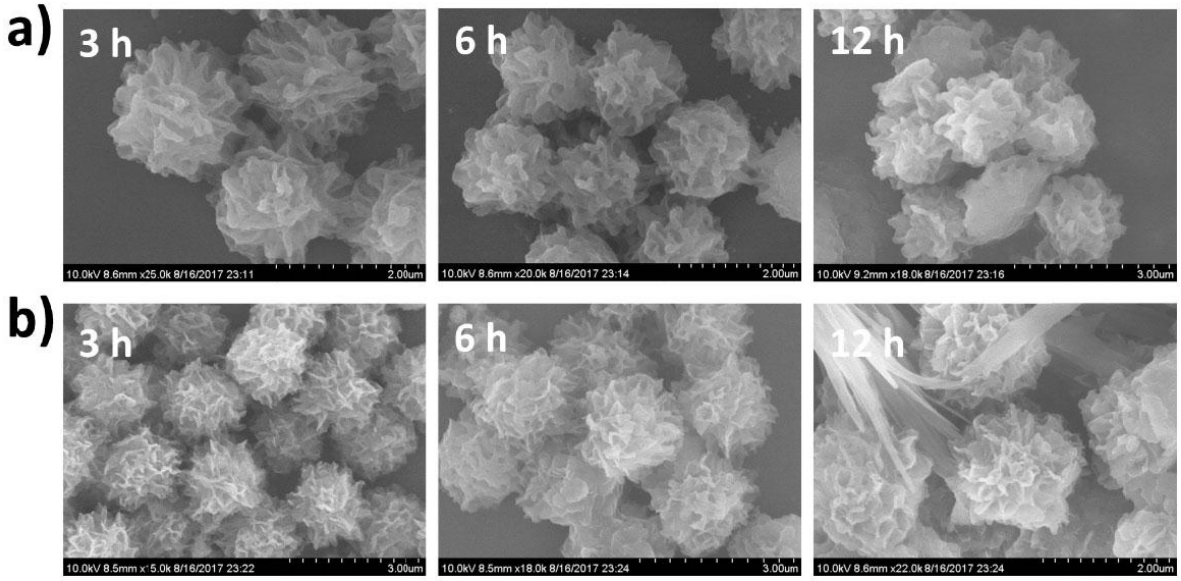

Fig. S8 SEM images of as-synthesized HCOF in the presence of (a) 6 M HCl and (b) 6 M NaOH.

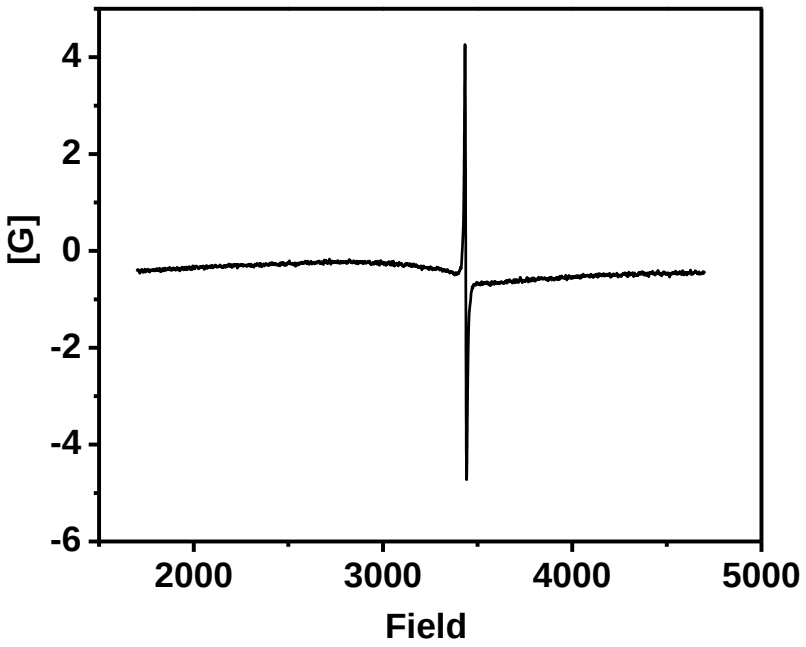

Fig. S9 EPR spectrum of Fe-HCOF.

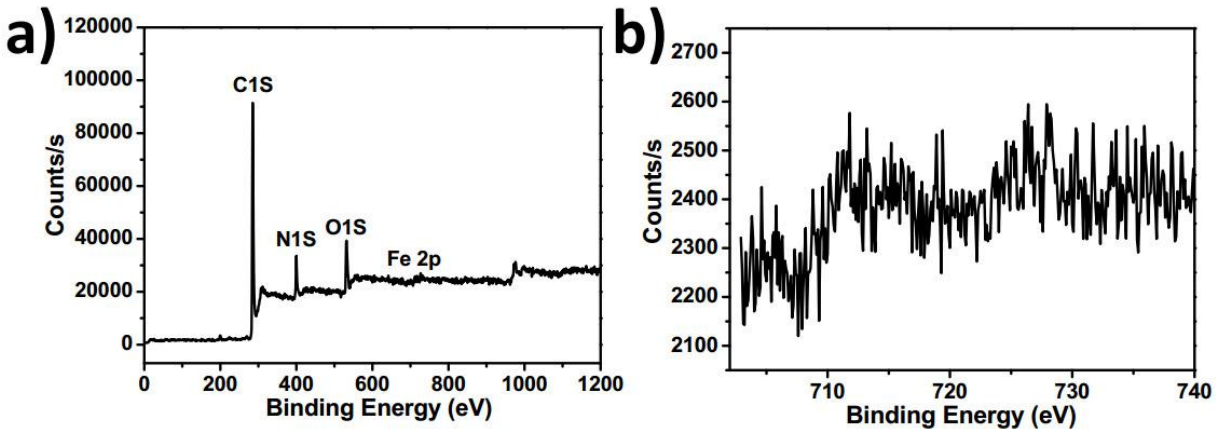

Fig. S10 XPS spectra of Fe-HCOF.

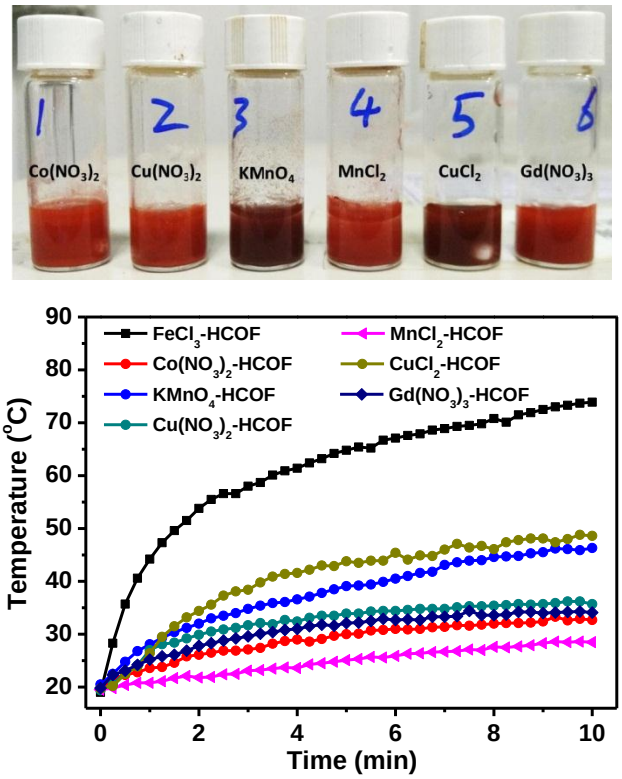

Fig. S11 Metalation of HCOFs with different metal salts and the corresponding temperature variation curves under 808 nm laser irradiation (1.8 W/cm^2).

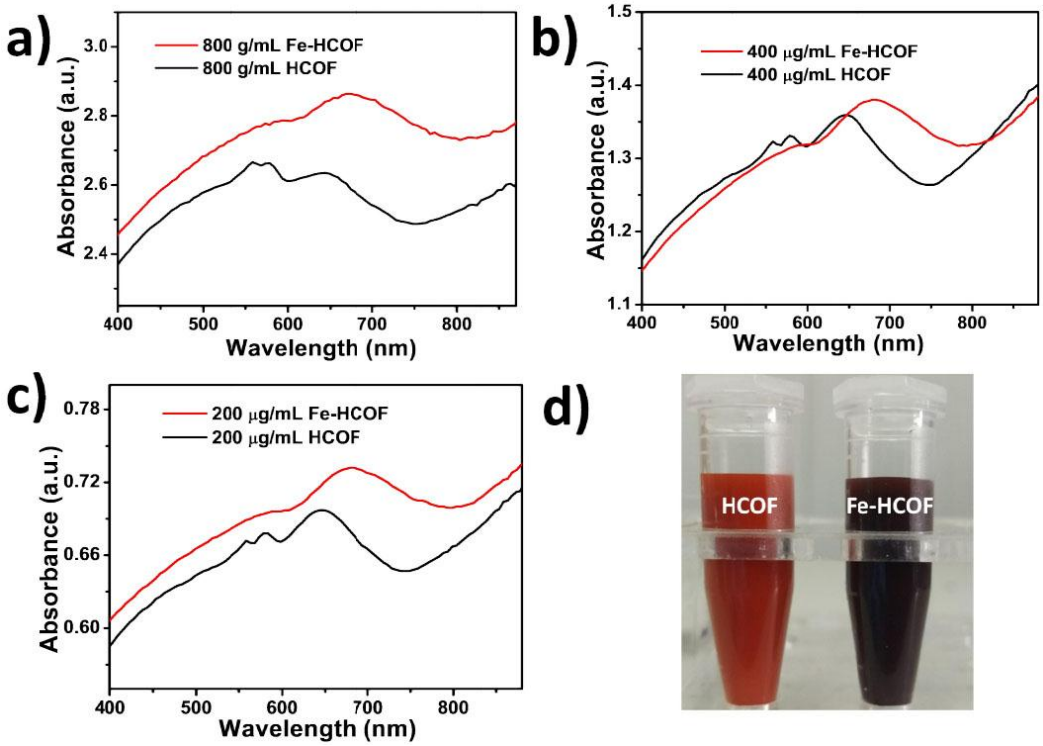

Fig. S12 SEM images of Fe-HCOF prepared with different amounts of $FeCl_3$ solution (50 mg/mL in water).

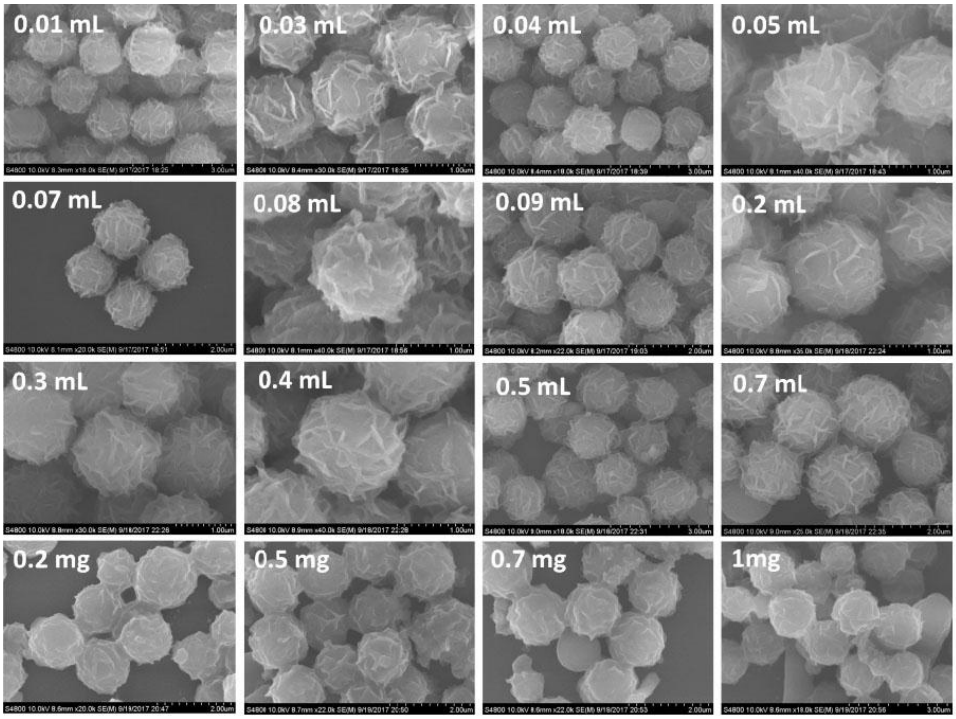

Fig. S13 SEM images of Fe-HCOF prepared with different amounts of $FeCl_3$ solution (50 mg/mL in water).

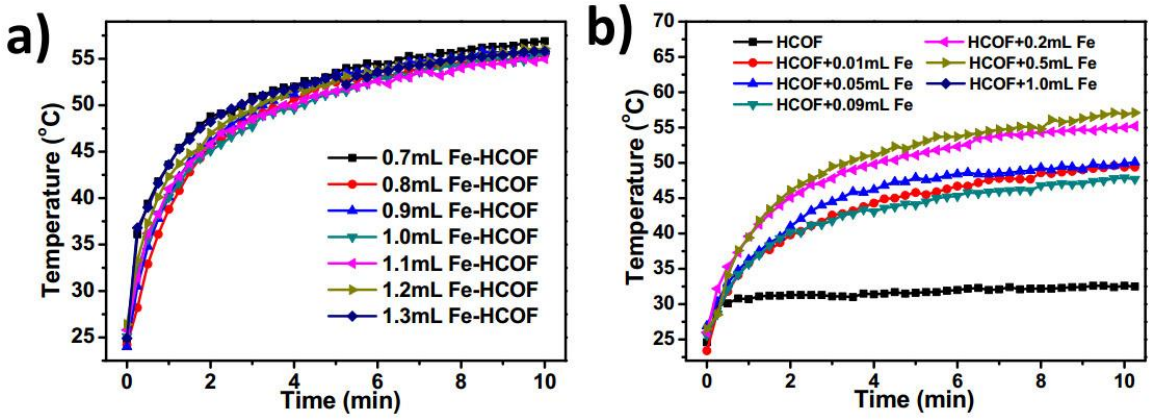

Fig. S14 Photothermal effect of Fe-HCOF with (a) 0.7-1.3 mL of $FeCl_3$ (50 mg/mL), and (b) 0.01-1 mL of $FeCl_3$ (500 mg/mL).

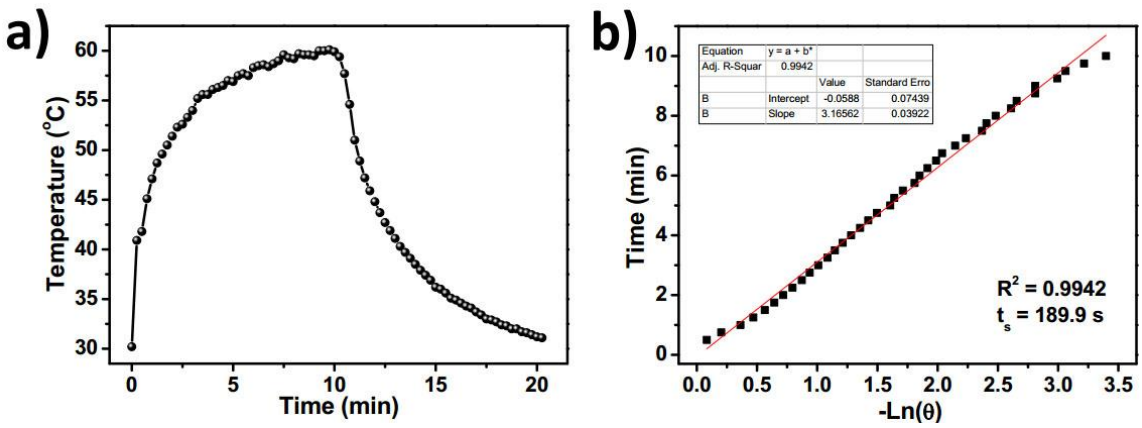

Fig. S15 (a) Photothermal effect of Fe-HCOF aqueous solution irradiated by an 808 nm laser (1.9 W/cm^2) for 10 min. The laser was turned off after irradiation. (b) Plot of cooling time versus negative natural logarithm of the temperature difference, obtained from the cooling stage as shown in (a).

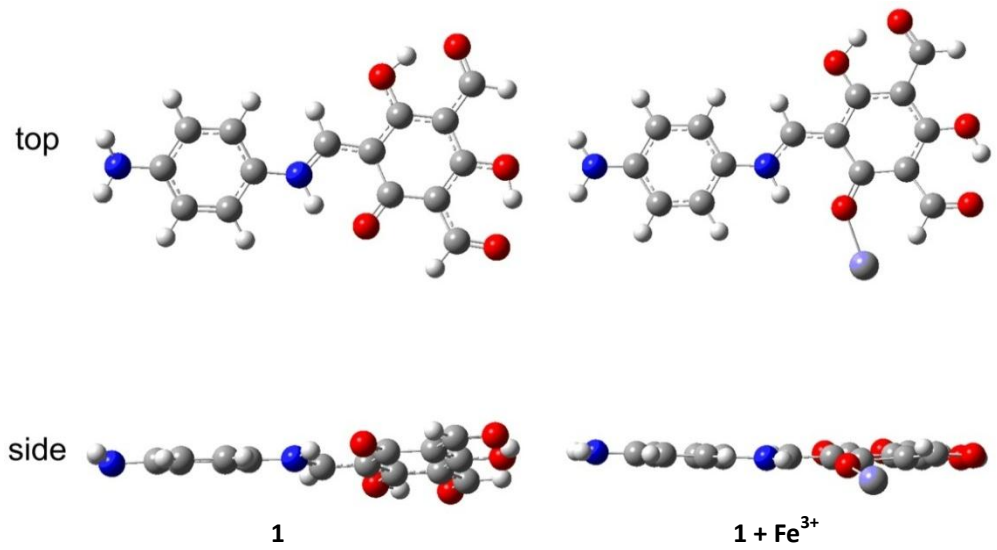

Fig. S16 The optimized geometry of **1** and **1+Fe^{3+}** from top and side view.

Table S1 Electron transition configurations, excitation energies, oscillator strengths (f), and assignment for main absorption band of 1 and **1+Fe^{3+}**.

	state	Major contrib. [a]	Energy(nm/eV)	*f*	assignment
1	S_1	H→L (70.3%) H-1→L (26.5%)	430/2.88	0.4466	Pa+Tp→Tp+Pa
	S_2	H–1→L (67.2%) H→L (28.5%)	387/3.20	0.3309	Pa+Tp→Tp+Pa
1+Fe^{3+}	S_8	αH→αL (41.9%) [b] αH–2→αL (24.7%) αH–4→αL (8.1%) αH–1→αL (8.1%)	1303/0.95	0.0395	Pa+Fe
	S_{14}	αH–7→αL (64.6%) αH–8→αL (17.0%)	721/1.72	0.0158	Pa+Fe
	S_{20}	αH→αL+1 (51.0%) αH–2→αL+1 (9.2%) αH–1→αL+1 (8.6%)	575/2.15	0.0367	Pa+Fe→Tp+Fe
	S_{24}	βH→βL (42.3%) βH-1→βL (27.3%)	498/2.49	0.0911	Fe→Tp+Pa
	S_{28}	βH-2→βL (51.3%) αH–8→αL+1 (15.9%) αH–2→αL+1 (9.4%)	442/2.80	0.3688	Pa+Tp+Fe→Tp+Pa+Fe
	S_{30}	αH–8→αL (31.2%) αH–3→αL+2 (22.5%) αH–9→αL (14.2%)	426/2.91	0.0709	Tp→Pa

[a] 'H' means HOMO and 'L' means LUMO. [b] 'α' and 'β' manifest the orbitals occupied by spin-up and spin-down electrons, respectively.

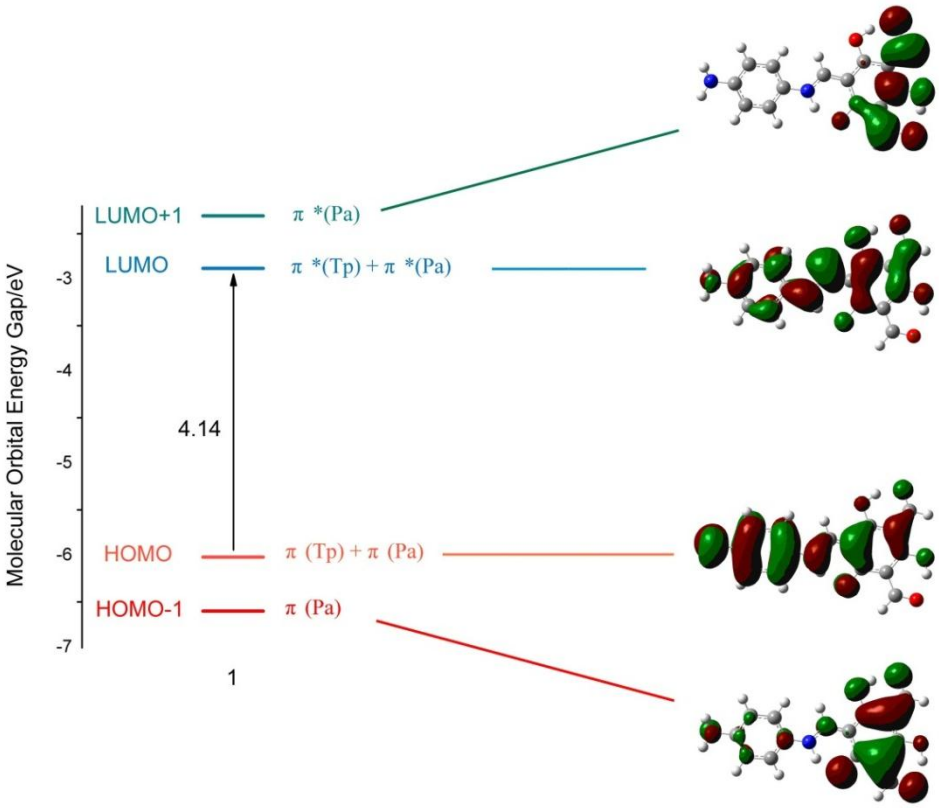

Fig. S17 The energy level and spatial molecular orbital distribution of **1**.

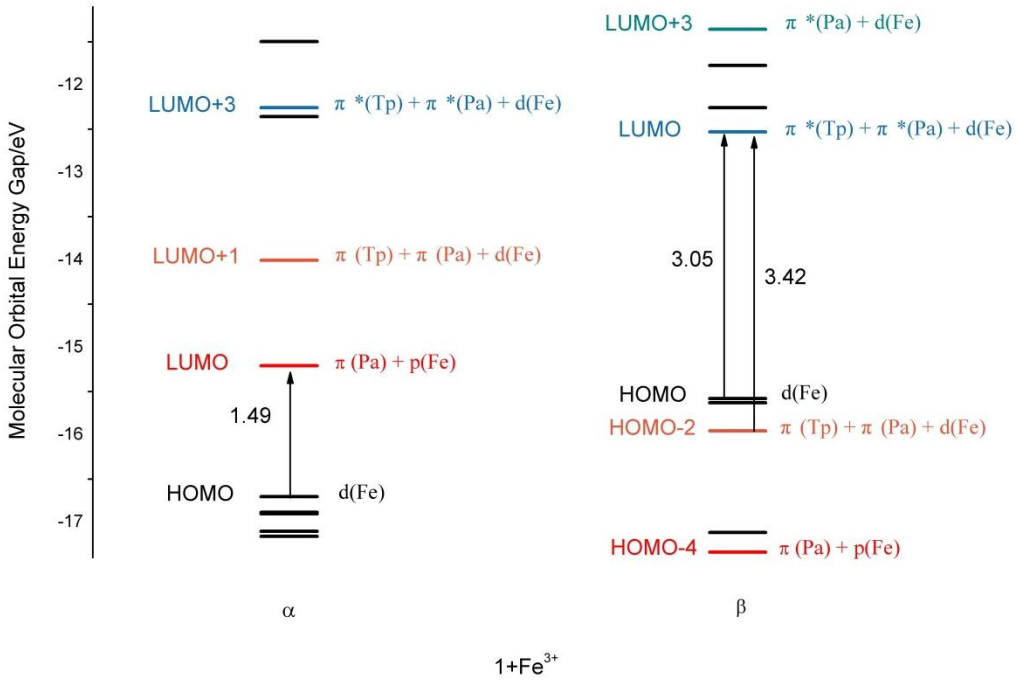

Fig. S18 The energy levels of spin–up(α) and –down(β) modes as well as molecular orbitals in **1+Fe^{3+}**.

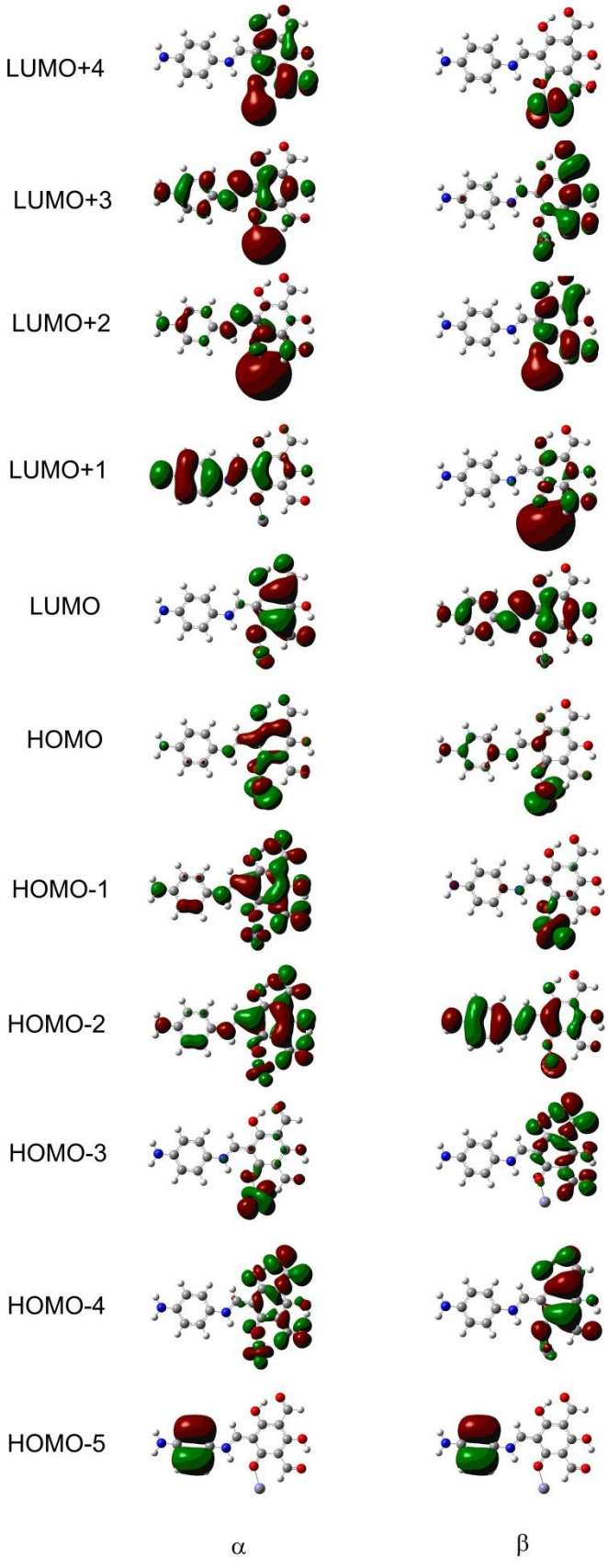

Fig. S19 The spatial molecular orbital distribution of **1**+**Fe**$^{3+}$.

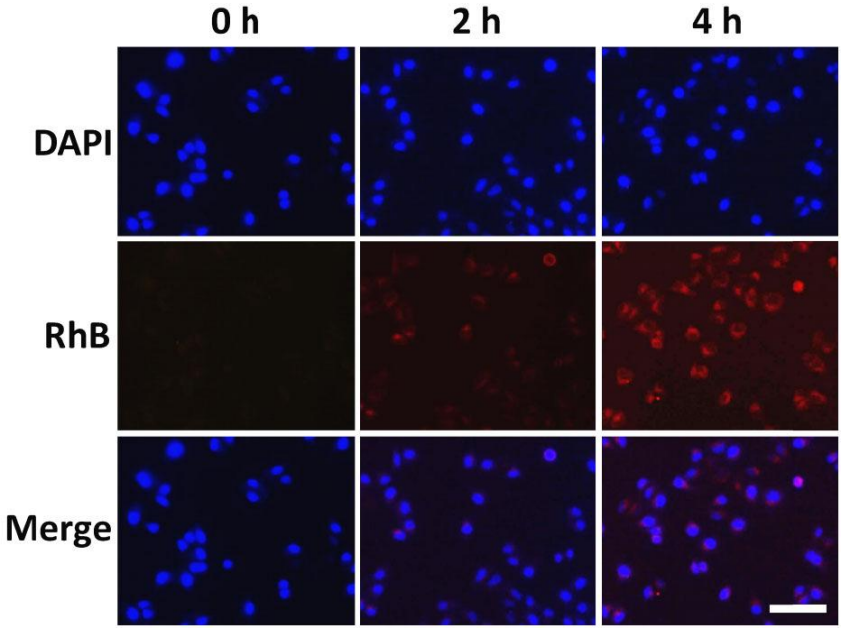

Fig. S20 Fluorescence images of HeLa cells after incubation with Fe-HCOF nanoparticles for different times. Scale bar is 40 μm.

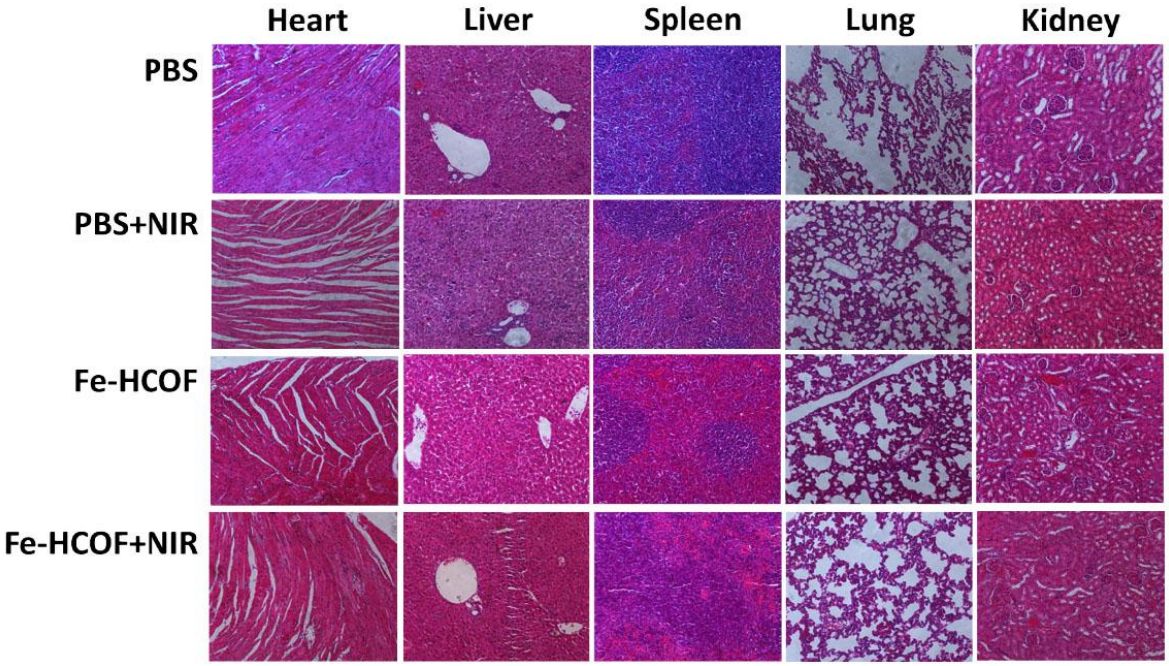

Fig. S21 Hematoxylin and eosin (H&E) stained images of major organs (Heart, Liver, Spleen, Lung, Kidney) for all groups.