Electronic Supplementary Material (ESI) for Inorganic Chemistry Frontiers.

Supporting Information

Confined in-situ polymerization in nanoscale porphyrinic metal-organic framework for fluorescence imaging-guided synergistic phototherapy

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Instrument. UV−visible absorption spectra were recorded on a UV-265 spectrophotometer. Fluorescence measurements were carried out on a Hitachi F-7000 fluorescence spectrophotometer. SEM were detected by REGULUS8230*. The ^{1}H-NMR spectra recorded on at 25°C, using Bruker 400/600 Ultrashield spectrometer were reported as parts per million (ppm) from TMS (δ). ESI Mass Spectrometer was recorded using LTQ Orbitrap XL. TEM were carried on a JEM-2100. XRD were recorded on SmartLab 9KW. IR spectra (4000-400 cm^{-1}), in KBr pressed pellets, were recorded on a Nicolet FT-IR-870SX spectrophotometer. Brookhaven BI-200 SM dynamic light scattering (DLS). Confocal microscopy was acquired with a Leica SP8 confocal microscopy and 100/63×oil-immersion objective lens. NIR light was supported by Merry Change MC-XF300. Visible light was supported by LED light (400-700 nm, 40 mW cm^{-2}). PTT experiments used a femtosecond laser pulse and a Ti: sapphire system (680-1080 nm, 80 MHz, 140 fs) as the light source.

Measurement of Photothermal Performance. PPy@MOF-525@HA aqueous solution (1 mL) with different concentrations (150, 200 and 250 μg mL^{-1}) and water as control were placed under the 960 nm laser irradiation for 10 min, respectively. Subsequently, **PPy@MOF-525@HA** aqueous solution (200 μg mL^{-1}) with different laser power intensity (0.8, 1 and 1.2 W cm^{-2}) were placed under the 960 nm laser irradiation for 10 min, respectively. The temperature changes in the above process are recorded by the NIR camera.

The photothermal conversion efficiency (η) was calculated by the following equation (1):

$$\eta = \frac{hS(T_{max} - T_{Surr}) - Q_{Dis}}{I(1-10^{-A_{960}})} \quad (1)$$

Where h indexes the heat transfer coefficient; S is the surface area of the used contanier; T_{max} and T_{surr} are the equilibrium temperature and room temperature, respectively; Q_{Dis} is the heat dissipation from the light loss of the quartz sample; I is laser intensity (1 W cm^{-2}), and A_{960} represents the absorbance of **PPy@MOF-525@HA** NPs at 960 nm. The hS was calculated by the following equation (2):

$$\tau_s = \frac{m_D C_D}{hS} \quad (2)$$

Where m_D and C_D are the mass (1 g) and heat capacity (4.2 J g^{-1}) of deionized water used as the solvent, respectively. τ_s is calculated to be 127.47 s.

Singlet Oxygen ($^1O_2$) Detection. ABDA was employed to detect the singlet oxygen ($^1O_2$). 200 μL of ABDA in DMF (1.0 mM) was mixed with 2 mL **PPy@MOF-525@HA** in aqueous solution (25 μg mL^{-1}). Then, the solution was placed under the LED light for different time at the power densities of 40 mW cm^{-2}. The absorptions of ABDA were recorded to analyze singlet oxygen generated from the **PPy@MOF-525@HA**.

Reactive Oxygen Species (ROS) Detection. DCF-DA was employed to detect the reactive oxygen species (ROS). 1 mL **PPy@MOF-525@HA** in aqueous solution (25 μg mL^{-1}) was mixed with 1 mL DCF-DA (10.0 μM) aqueous solution. Subsequently, the mixture solution was irradiated with LED light for different time.

The emission of DCF-DA at 525 nm (Excitation wavelength 488 nm) was recorded to reflect the production of ROS.

Electron spin resonance (ESR) assay. ESR was used to assess the generation of $^1O_2$ by **PPy@MOF-525@HA**. The spin traps 2,2,6,6-tetramethylpiperidine (TEMP for trapping $^1O_2$, 20 μL) was used to verify the species of reactive oxygen species (ROS) generated by **PPy@MOF-525@HA** (50 μg mL^{-1}). The ESR signals of the **PPy@MOF-525@HA** before and after LED light irradiation were recorded.

Cell Culture. The HepG2 cells were cultured in 25 cm^2 DMEM, supplemented with fetal bovine serum (10%), penicillin (100 units mL^{-1}) and streptomycin (50 units mL^{-1}) at 37 °C 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 and were allowed to grow when the cells reached more than 70% confluence.

Hemolysis Assay. The mouse red blood cells (RBCs) were obtained by removing serum from the blood by centrifugation and washing. Then RBCs were suspended in phosphate-buffered saline (PBS) was mixed with an equal volume of **PPy@MOF-525@HA** solution at a final **PPy@MOF-525@HA** concentration of 2, 5, 10, 20, 50 and 100 μg mL^{-1}. RBCs in PBS solution and 1% Triton X-100 solution were set as the negative control and the positive control, respectively. After the mixtures were incubated at 37 °C for 2 h and centrifugated for 5 min, the absorbance at 450 nm of these supernatants was measured using a microplate reader. The hemolysis percentage was calculated using the following formula:

$$\text{Hemolysis}\% = \frac{(I_{\text{sample}} - I_{\text{nergative control}})}{(I_{\text{positive control}} - I_{\text{negative control}})} * 100\%$$

where I represent the absorbance at 450 nm.

Cell uptake analysis. HepG2 cells (CD44-positive cells) were seeded onto corresponding cell culture dishes and grown to about 70% confluency before used. HepG2 cells were treated with **PPy@MOF-525@HA** (100 μg mL^{-1}). And after 5 h incubation, the cellular uptake ability of **PPy@MOF-525@HA** was analyzed using CLSM.

ROS generation in vitro. The ROS production in living cells was also assessed. HepG2 cells were seeded in Petri dishes and incubated for 24 h. **PPy@MOF-525@HA** (100 μg mL^{-1}) was added and incubated with the cells for 5 h. Then, the cells were washed with PBS solution and incubated with SOSG (5 M) for 30 min, after which the cells were incubated shielded from laser or irradiated for 5 min and 10 min (514 nm laser). And then the cells were observed by CLSM.

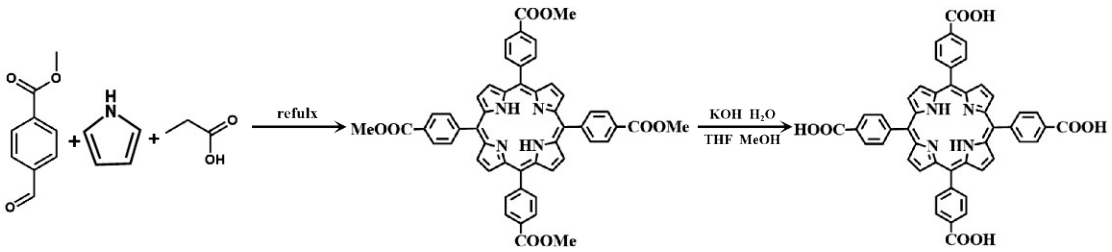
Phototoxicity Evaluation. HepG2 cells were seeded on 96-well plate incubation overnight for phototoxicity testing. Next, HepG2 cells were disposed with different concentrations **PPy@MOF-525@HA** (0, 50, 100, 150 and 200 μg mL^{-1}) for 12 h and then irradiated with visible light and NIR light. The cell viability was determined by standard MTT assay.

Annexin V-FITC and PI assay. HepG2 cells were incubated with 100 μg mL^{-1} **PPy@MOF-525@HA** for 5 h at 37 ºC. The HepG2 cells were stained with Annexin

V-FITC/PI. Then, the cells were exposed to a laser (514 nm, 100 mW cm^{-2}) for 5 min and a laser (960 nm, 100 mW cm^{-2}) for 10 min, respectively. And then the cells were observed by CLSM.

Flow Cytometry Study. Cells seeded into the 6-well plates were cultured for 24 h. Next, the medium was replaced with medium containing **PPy@MOF-525@HA** (100 μg mL^{-1}), at 37 °C for 5 hours. After irradiated by visible light and NIR light, the cells were collected by centrifugation and resuspended in binding buffer containing Propidium Iodide (PI, 10 μL) and Annexin-V FITC (5 μL) for 15 min in darkness. The signal was collected by a BD FACS Calibur flow cytometer (Beckaman/Gallios).

Culture of 3D multicellular tumor spheroids (3D MCTs). 5 mL Poly HEMA solution was added to 25 mL cell culture flask, the ethanol was evaporated at 37 °C, and then sterilized under ultraviolet lamp for 3-5 h. The culture flask was washed twice with PBS, and then 1 mL mother liquor of tumor cells was added. When the cell mass density was relatively high, the flask treatment was conducted, and the cells were further cultured for 3-5 days, 3D multicellular spheroids could be formed with appropriate diameter. 3D MCTs were incubated with **PPy@MOF-525@HA** (100 μg mL^{-1}) for 5 h. Then, 3D MCTs stained with Calcein AM and PI for 15 min, then washed with PBS solution and analyzed by confocal laser scanning microscope (CLSM).



Scheme S1. Synthetic procedures for **H_4TCPP**.

Table S1. The surface area and pore volume of **MOF-525** and **PPy@MOF-525.**

	Surface Area (m^2/g)	**Total pore volume (cm^3/g)**	**Micropore volume (cm^3/g)**	**Mesopore and macropore volume (cm^3/g)**
MOF-525	2673.215	2.669	0.918	1.751
PPy@MOF-525	92.112	0.564	0.009	0.555

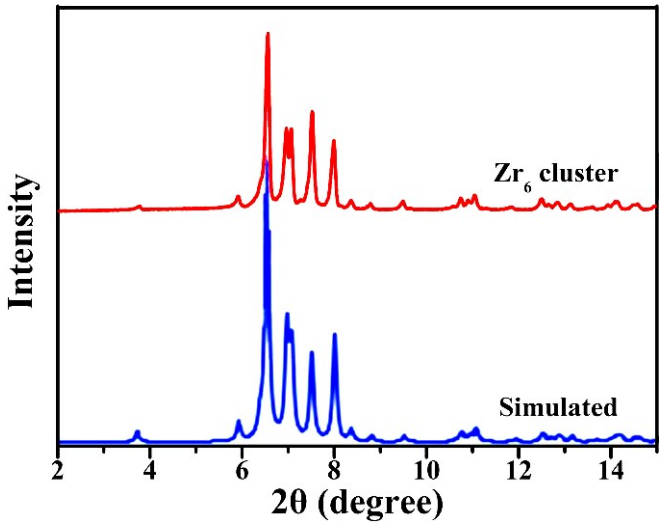


Figure S1. PXRD pattern of Zr_6 cluster.[1-3]

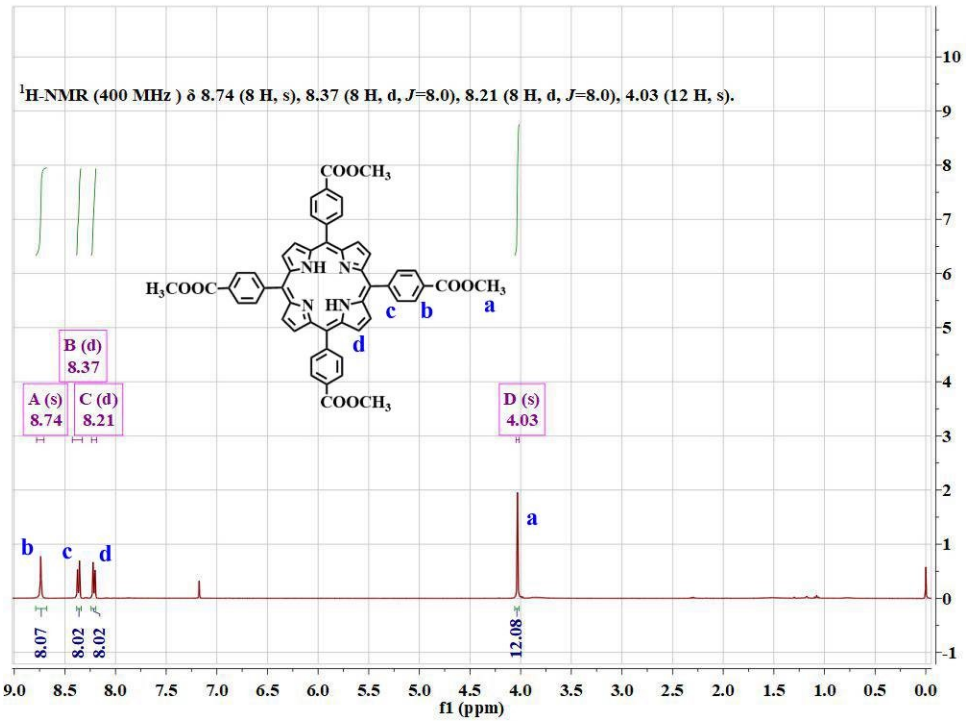


Figure S2. ^{1}H NMR spectrum of **TCPP-OME** (*d*-$CDCl_3$).

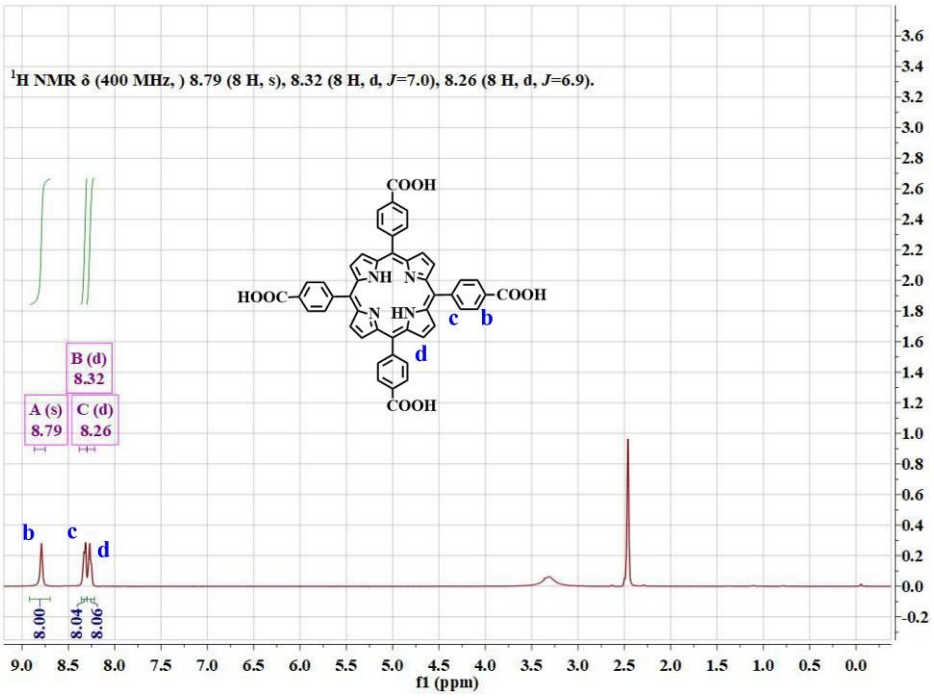


Figure S3. ^{1}H NMR spectrum of **H_4TCPP** (*d_6*-DMSO).

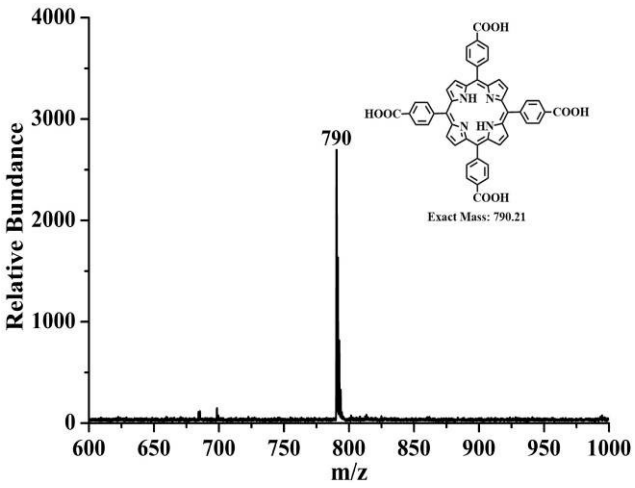


Figure S4. Mass spectra of **H_4TCPP** ligand.

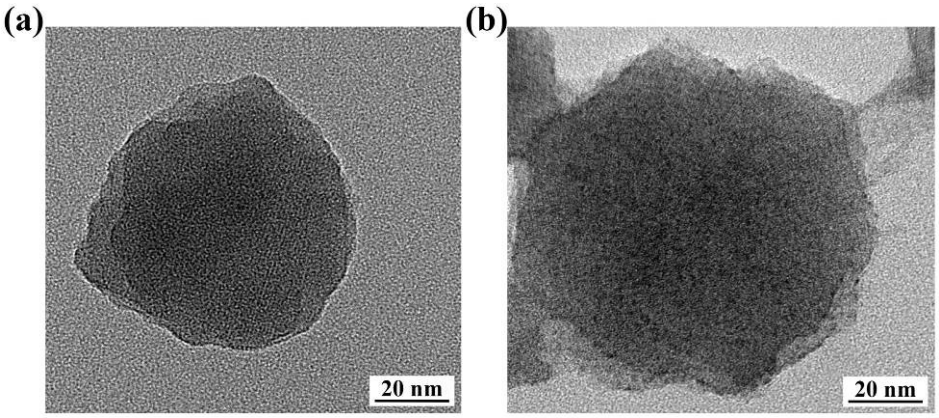


Figure S5. HRTEM images of (a) **MOF-525**, (b) **PPy@MOF-525**.

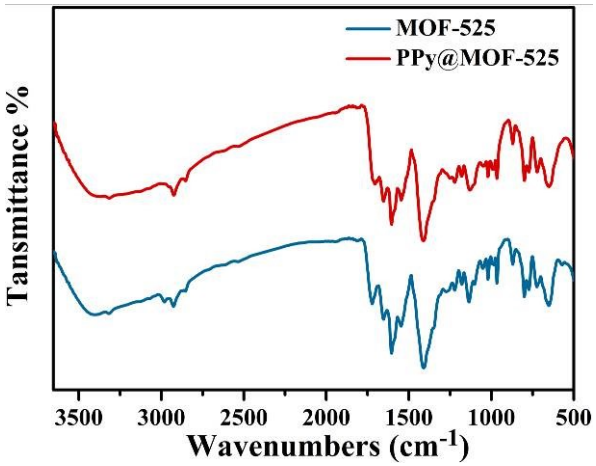


Figure S6. FT-IR spectra of **MOF-525** and **PPy@MOF-525**.

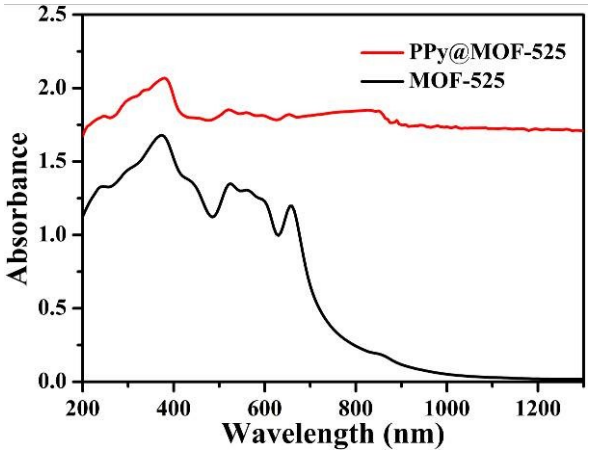


Figure S7. Solid-state UV−visible absorption spectra of **MOF-525** and **PPy@MOF-525**.

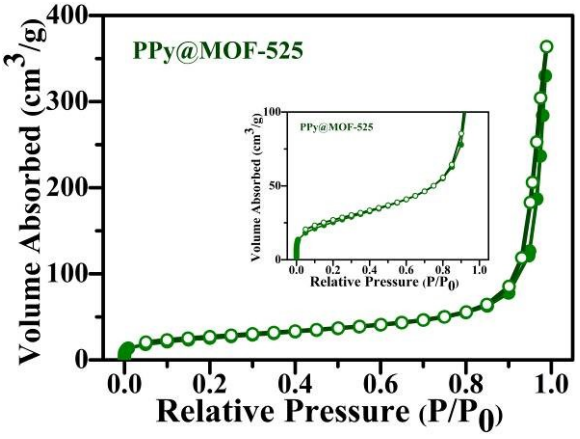


Figure S8. BET of **PPy@MOF-525**.

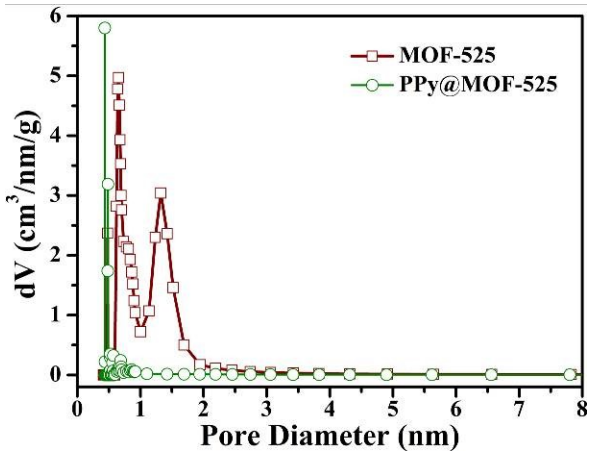


Figure S9. Pore size distribution of **MOF-525** and **PPy@MOF-525**.

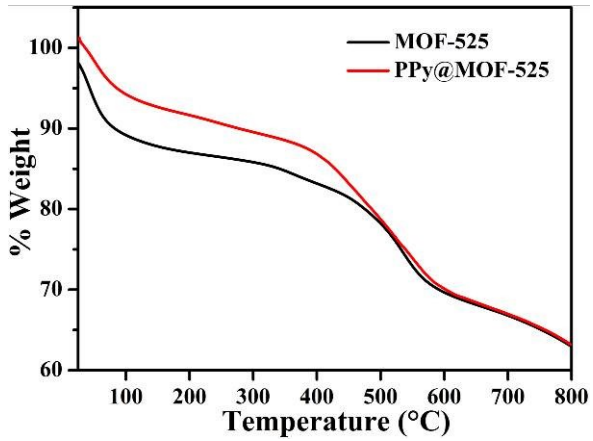


Figure S10. Thermogravimetric analysis (TGA) of **MOF-525** and **PPy@MOF-525**.

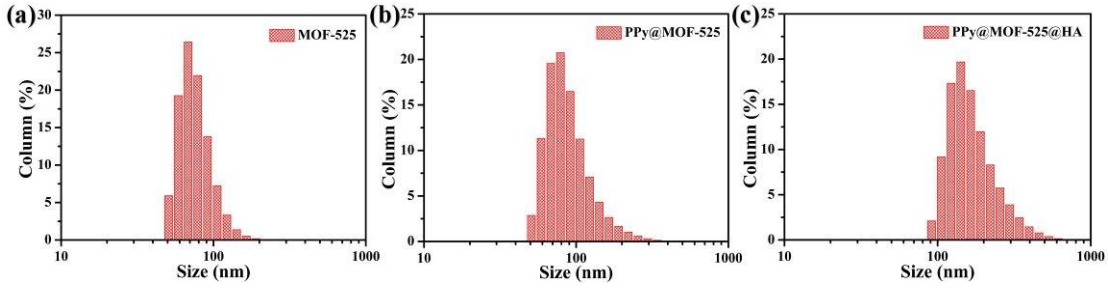


Figure S11. DLS of **MOF-525**, **PPy@MOF-525** and **PPy@MOF-525@HA**.

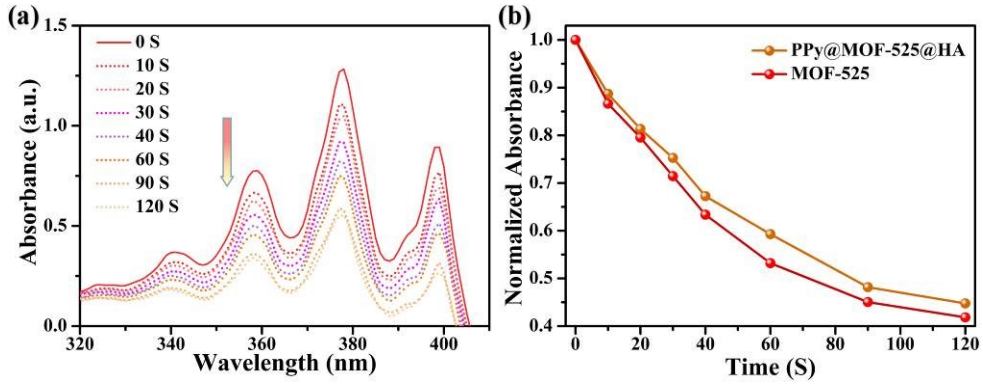


Figure S12. (a) UV-Vis absorption spectra of the mixed solution of **MOF-525** (25 μg mL^{-1}) and ABDA under visible light irradiation over time. (b) Normalized absorption of ABDA mixed with **MOF-525** (25 μg mL^{-1}) and **PPy@MOF-525@HA** (25 μg mL^{-1}) under visible light irradiation over time, respectively.

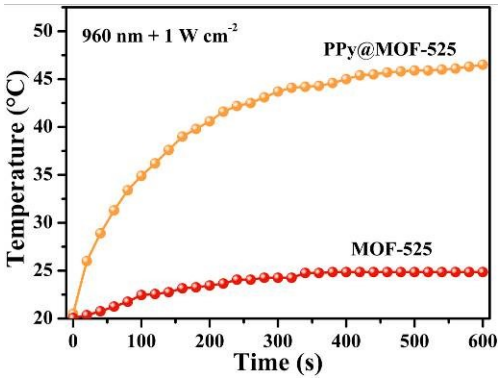


Figure S13. Temperature changes of **MOF-525** and **PPy@MOF-525** under NIR light irradiation (960 nm, 1.0 W cm^{-2}) over time.

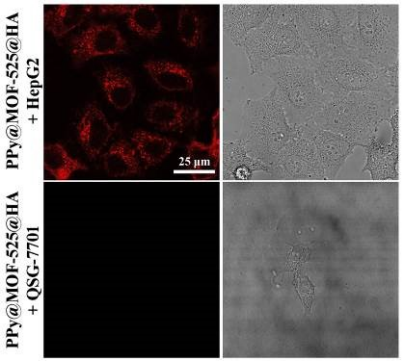


Figure S14. Confocal images to check the cell uptake of **PPy@MOF-525@HA**.

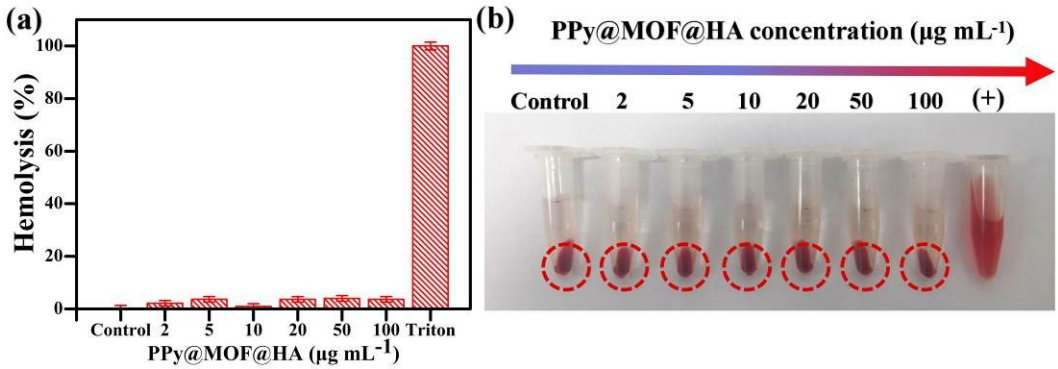


Figure S15. (a) Hemolysis ratio of red blood cells incubated with **PPy@MOF-525@HA** (2, 5, 10, 20, 50 and 100 μg mL^{-1}). (b) Image of red blood cells centrifugation and treatment with different concentrations of **PPy@MOF-525@HA**.

References

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