Supplementary Information

DNA-Engineered Metal-Organic-Framework Nanocarrier as a General Platform for Activatable Photodynamic Cancer Cell Ablation

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Supplementary Experimental Section

General Methods, Materials, and Instruments:

TPAAQ was prepared following our previous work,[S1] and all other materials for organic synthesis were purchased from Sigma-Aldrich. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under dry nitrogen immediately prior to use. DNase I was purchased from New England Biolabs Inc. (Beverly, MA, USA). The DBCO-functionalized oligonucleotides were synthesized, standard desalting purified, and freeze-dried by Integrated DNA Technologies, Inc. The detailed sequence of the nucleic acid used in this study was as follows: 5′-DBCO-amide-CCTCCGCTACCTGGGGGAGTATTGCGGAGGAAGGTA-3′. All other chemicals were purchased from Sigma-Aldrich and used as received. Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation, Bedford, USA). ^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance 400 spectrometer using tetramethylsilane (TMS; $\delta = 0$ ppm) as an internal standard. Nanoscale images were captured under TEM (JEOL-JEM 2010F). UV-vis and photoluminescence spectra were recorded using Shimadzu UV-1700 and Perkin-Elmer LS 55 spectrometer, respectively. Hydrodynamic diameter and size distribution were measured by laser light scattering (LLS) with Zetasizer Nano S (Malvern Instruments Ltd, Worcestershire, UK) at room temperature. PXRD data were collected at ambient temperature on a Bruker D8 Advance diffractometer at 40 kV, 40 mA for Cu Ka (l = 1.5418 A) at a scan rate of 0.01

deg s^{-1}. TGA was performed under air atmosphere using a Shimadzu DTG-60AH instrument. The N_2 and O_2 sorption isotherms were obtained using a Micromeritics ASAP 2020 physisorption analyzer. In vivo fluorescence imaging was performed by using an IVIS Lumina XRMS in vivo Imaging System (PerkinElmer Inc, Waltham, Massachusetts, USA).

Synthesis of Amine-Modified MIL-101(Fe):

MIL-101-NH_2(Fe) was synthesized according to a published procedure with modification.[S2] MIL-101-NH_2(Fe) was obtained by 18 h solvothermal reaction at 110 °C of the mixture 1.24 mM BDC-NH_2, 2.5 mM $FeCl_3 \cdot 6H_2O$ and 10 μM CTAB. Activation of MIL-101-NH_2(Fe) was performed by extraction with ethanol for 12 h and then with DMF for 24 h in a Soxhlet extractor, washing with methanol several times, and then storing in anhydrous ethanol at room temperature.

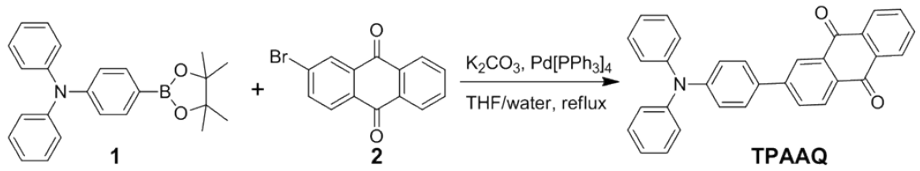
Synthesis of Azide-Modified MIL-101(Fe):

The amine-functionalized MIL-101(Fe) (30 mg in 1 mL anhydrous ethanol) was dispersed in 6 mL of THF. Then, 1.8 mL of the tBuONO and 1.6 mL of the $TMSN_3$ were added, respectively, according to a reported approach.[S3] The mixture was incubated and stirred at room temperature overnight to produce the azide-modified MIL-101(Fe).

Synthesis of Compound TPAAQ:

A mixture of compound 1 (222.7 mg, 0.60 mmol), 2 (143.6 mg, 0.50 mmol), potassium carbonate (680 mg, 5.0 mmol), 15 mL of THF, 5 mL of water and $Pd(PPh_3)_4$ (3 mol%) were carefully degassed and charged with nitrogen. Then the reaction mixture was stirred at 60 °C for 24 h. After cooling to ambient temperature, the reaction was stopped by the addition of water, extracted with dichloromethane, and washed with brine. The organic layer was dried over anhydrous magnesium sulfate and purified by column chromatography using n-hexane/dichloromethane (1/2, v/v) as eluent to afford TPAAQ (183.1 mg, 81.2 %) as red solid. NMR spectrums of TPAAQ are shown in Figures S1

and S2. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K), δ (TMS, ppm): 8.49 (d, J = 2.0 Hz, 1H, ArH), 8.30 (m, 3H, ArH), 7.97 (m, 1H, ArH), 7.79 (m, 2H, ArH), 7.61 (d, J = 8.8 Hz, 2H, ArH), 7.31 (t, J = 7.2 Hz, 4H, ArH), 7.17 (d, J = 7.6 Hz, 6H, ArH), 7.09 (t, J = 7.2 Hz, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$, 298 K), δ (ppm): 183.3, 182.8, 148.8, 147.3, 146.3, 134.1, 133.9, 133.7, 133.6, 131.9, 131.5, 131.5, 129.5, 128.1, 128.0, 127.2, 127.2, 125.0, 124.7, 123.7, 122.9.



PS Loading in Azide-Modified MIL-101(Fe):

PSs were dissolved in benign solvents (MB and TPAAQ in THF), then soaked with azide-modified MIL-101(Fe) (MIL-101-N_3) suspended in ethanol, with a final concentration of 2 and 1 mg mL^{-1} for PSs and MIL-101-N_3, respectively. The soaking mixtures were centrifuged to take supernatants for real-time analyses of loading capacity via absorption of PSs. The loading capacity of MIL-101-N_3 was calculated using the following equation:

$$wt\% = (1 - \frac{A_{PSs\ in\ the\ supernatant}}{A_{PSs\ before\ loading}}) \times \frac{C_{PSs}}{C_{MIL-101-N3}}$$

Fabrication of DNA-Engineered and PS-loaded MIL-101(Fe):

MIL-101-N_3 (2 mg mL^{-1}, 2 mL) were reacted with DBCO-functionalized nucleic acid (100×10^{-6} M, 0.8 mL), respectively. The resulting mixture solutions were stirred at 40 °C for 72 h. The DNA-functionalized MIL-101(Fe) was washed with PBS solution several times to remove unreacted nucleic acids.

Cell Culture:

Human carcinoma HeLa cells were purchased from Perkin Elmer Inc. and maintained in our lab. They were cultured on 96-well plates in DMEM (with 10% FBS, and 1% penicillin/streptomycin). The cells were maintained in an atmosphere of 5% CO_2 and

95% humidified air at 37 °C.

Cell Imaging:

HeLa cells were seeded and cultured in a glass-bottom 8-well chamber for 12 h. After reaching 80% confluence, various PS agents (5 μg mL^{-1} based on PSs) were then added into the medium and incubated with cancer cells for 24 h. The cells were further washed by PBS and cultured with DCFDA (20 μM) and Hoechst (10 μM) containing fresh culture medium for 30 min before being treated upon light irradiation for 5 min. Thereafter, the cells were washed with PBS and imaged by CLSM. The fluorescent signal of TPAAQ within the cells was captured by confocal laser scanning microscopy (CLSM, Leica TSC SP8, Germany) with excitation at 480 nm and signal collection from 550 nm to 800 nm. The fluorescent signal of MB was captured with excitation at 650 nm and signal collection from 670 nm to 800 nm. The fluorescent signal of DCFDA was captured with excitation at 488 nm and signal collection from 505 nm to 525 nm. The fluorescent signal of Hoechst was captured with excitation at 400 nm and signal collection from 420 nm to 450 nm.

Cell Viability Test:

HeLa cancer cells were seeded in 96 well plates at a density of 3000 cells in 200 μL per well for 12 h. Various agents at different concentrations were added into the cell culture medium separately. The cells were further incubated with different NPs for 24 h without or with H_2O_2 treatment, followed by fresh medium washing. With or without light treatment for 5 min, MTT (40 μL, 1 mg mL^{-1}) was added into a medium for 3 h. The media was then removed, and DMSO (100 μL) was added into each well and gently shaken for 10 min at room temperature. The absorbance of MTT at 550 nm was measured by using a SpectraMax M5 Microplate Reader. Cell viability was measured by the ratio of the absorbance of the cells incubated with different nanoparticles to that of the cells incubated with normal culture medium.

Tumor Mouse Model:

All animal studies were performed in compliance with the guidelines set by Beijing Committee of Use and Care of Laboratory Animals and the overall project protocols were approved by the Animal Ethics Committee of Beijing Institute of Technology. BALB/c mice were purchased from Charles River Experimental Animal Co., Ltd. and kept in the Animal Laboratory of Beijing Institute of Technology. The xenograft tumor model was generated by subcutaneous injection of 4T1 cancer cells (1×10^6) suspended in 30 μL of saline into the right shoulder of the mouse. BALB/c mice bearing 4T1 tumors were randomly divided into 3 groups (n = 3) when the tumors grew to around 300-400 mm^3. Tumor volumes were estimated using the spherical tumor volume formula $V=\pi/6\times ab^2$, where a represents length (mm) and b represents width (mm).

In Vivo Mouse-Based Fluorescence Imaging and Tumor Therapy

The mice were treated by intravenous injection of PBS, DNA-MB@MIL-101, or DNA-TPAAQ@MIL-101 (injection volume = 100 μL, [MB or TPAAQ] = 0.5 mg mL^{-1}) at Day 0. The mice injected with DNA-MB@MIL-101 or DNA-TPAAQ@MIL-101 were exposed to white light irradiation at 8 h post-injection at 400 mW cm^{-2} for 5 min. In vivo fluorescence imaging was performed at different time points post injection using an IVIS Lumina XRMS in vivo Imaging System (PerkinElmer Inc, Waltham, Massachusetts, USA). The mice were anesthetized with isoflurane and imaged at the indicated time points. For DNA-MB@MIL-101-treated mice, the excitation wavelength was set at 405 nm, and the emission wavelength was chosen from 660 to 720 nm. For DNA-TPAAQ@MIL-101-treated mice, the excitation wavelength was set at 450 nm, and the emission wavelength was chosen from 630 to 700 nm. The tumor size of mice was recorded every 3 days for 7 times. At 1-day and 7-day post injection, three mice in different groups were sacrificed. The normal organs (kidney, spleen, and liver) were excised and washed with PBS (1×) for inductively coupled plasma mass spectrometry (ICP-MS) analysis.

Histological Studies

In the histological assay, the paraffin-embedded tumor or organ samples were cut into

5 mm thick sections, then dewaxed and rehydrated. The samples were stained with hematoxylin and eosin (H&E) for microscopic observation. Apoptosis of the tumor cells or normal organs was determined by the H&E staining method according to the manufacturer's instructions.

Statistical Analysis*:*

Quantitative data were expressed as mean standard deviation. ANOVA analysis and Student's T-test were utilized for statistical contrast. $P < 0.05$ was figured statistically significant.

References

S1 Y. Wang, W. Wu, J. Liu, P. N. Manghnani, F. Hu, D. Ma, C. Teh, B. Wang and B. Liu, *ACS Nano*, 2019, **13**, 6879-6890.

S2 T. Čendak, E. Žunkovič, T. U. Godec, M. Mazaj, N. Z. Logar and G. Mali, *J. Phys. Chem. C*, 2014, **118**, 6140-6150.

S3 W.-H. Chen, G.-F. Luo, Y. S. Sohn, R. Nechushtai and I. Willner, *Adv. Funct. Mater.*, 2019, **29**, 1805341.

Supplementary Experimental Section

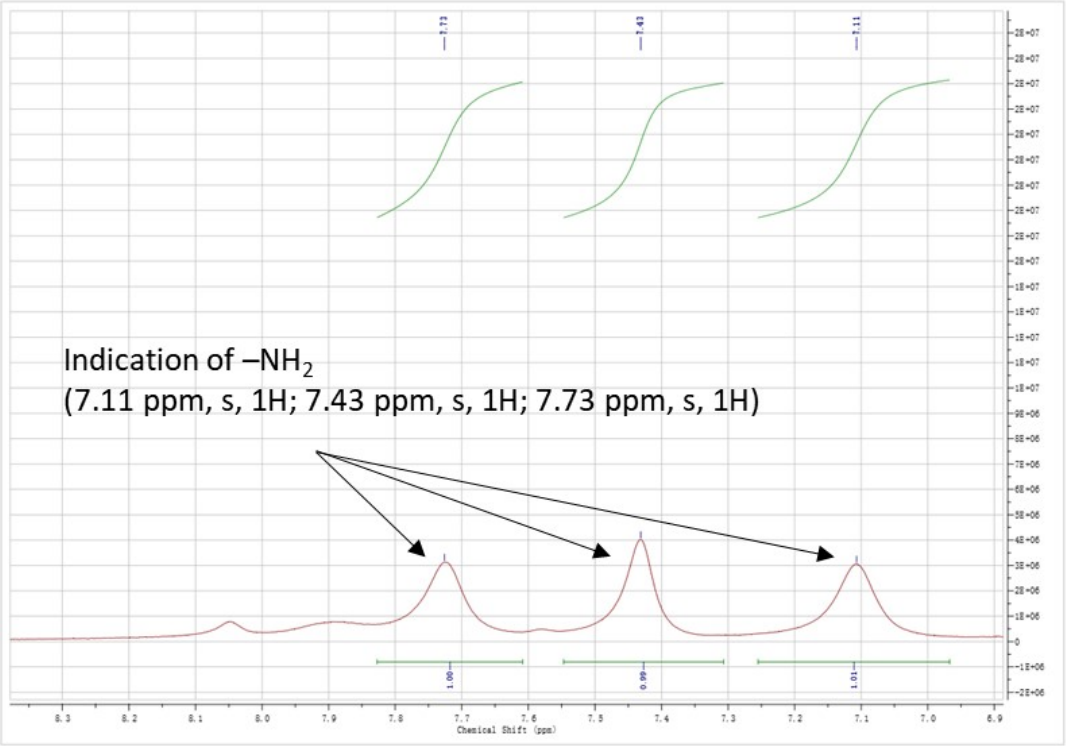


Fig. S1 NMR spectrum of MIL-101-NH_2 digested by deuterium chloride.

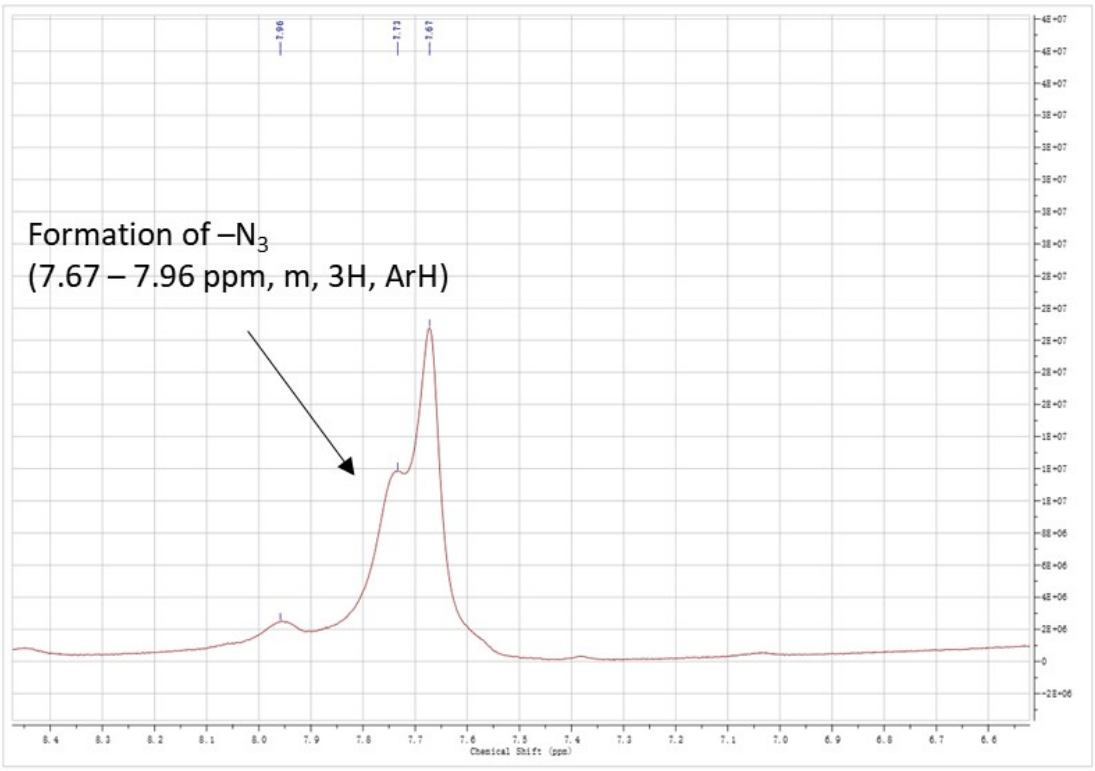


Fig. S2 NMR spectrum of MIL-101-N_3 digested by deuterium chloride.

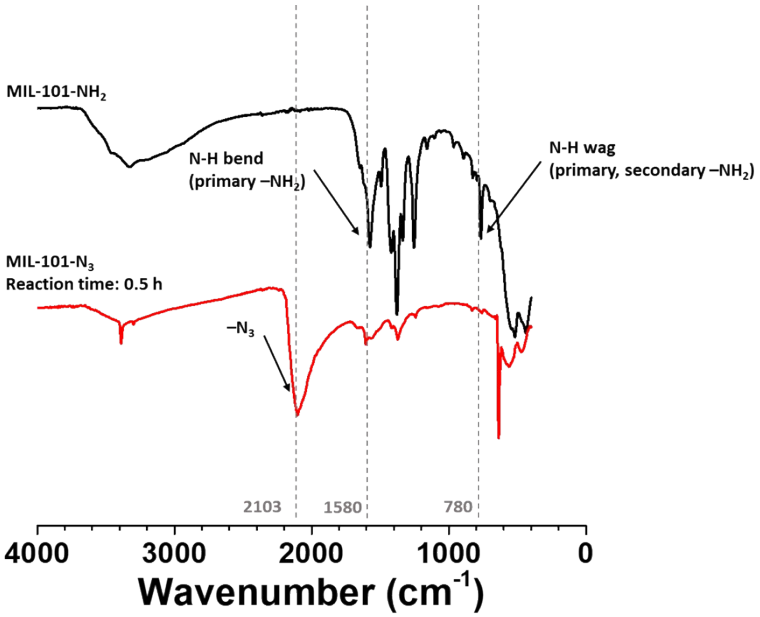


Fig. S3 FT-IR spectra of MIL-101-NH_2 and MIL-101-N_3.

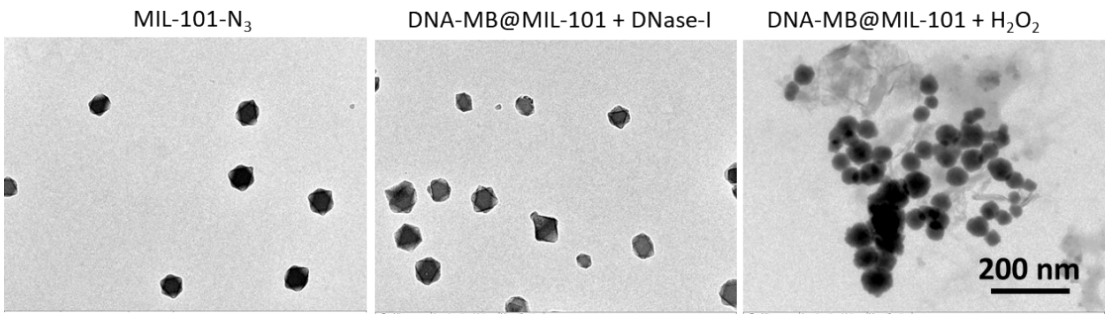


Fig. S4 TEM images of MIL-101-N_3 and DNA-MB@MIL-101 after DNase-1 or H_2O_2 treatment for 4 h. All the images share the same scale bar.

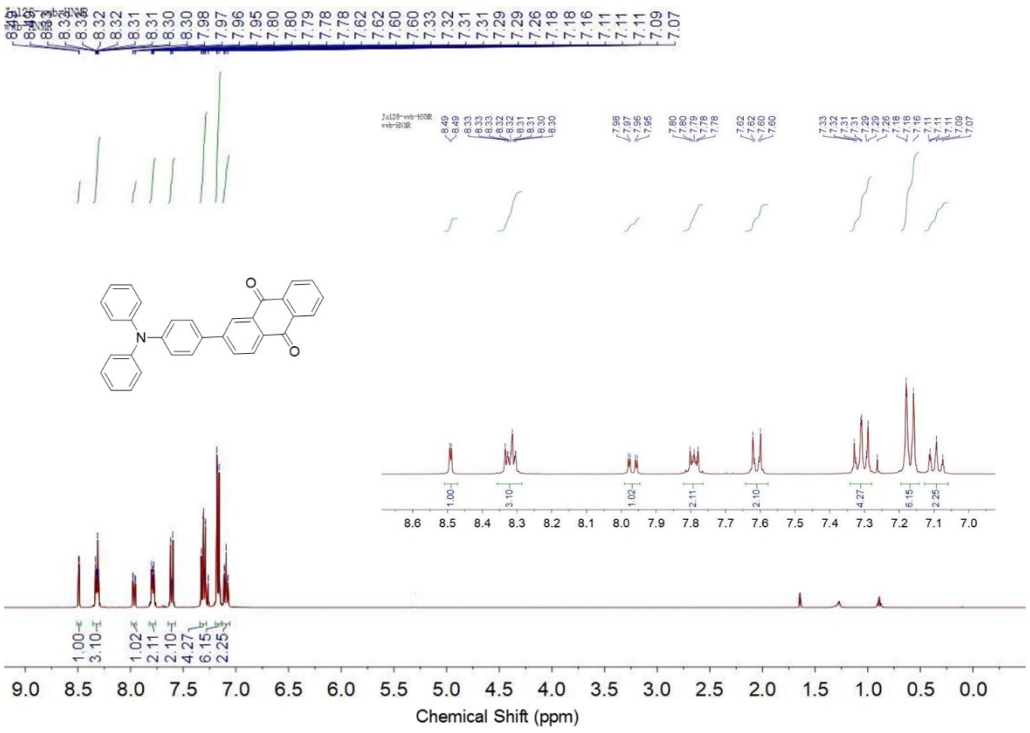


Fig. S5 ^{1}H NMR spectrum of TPAAQ.

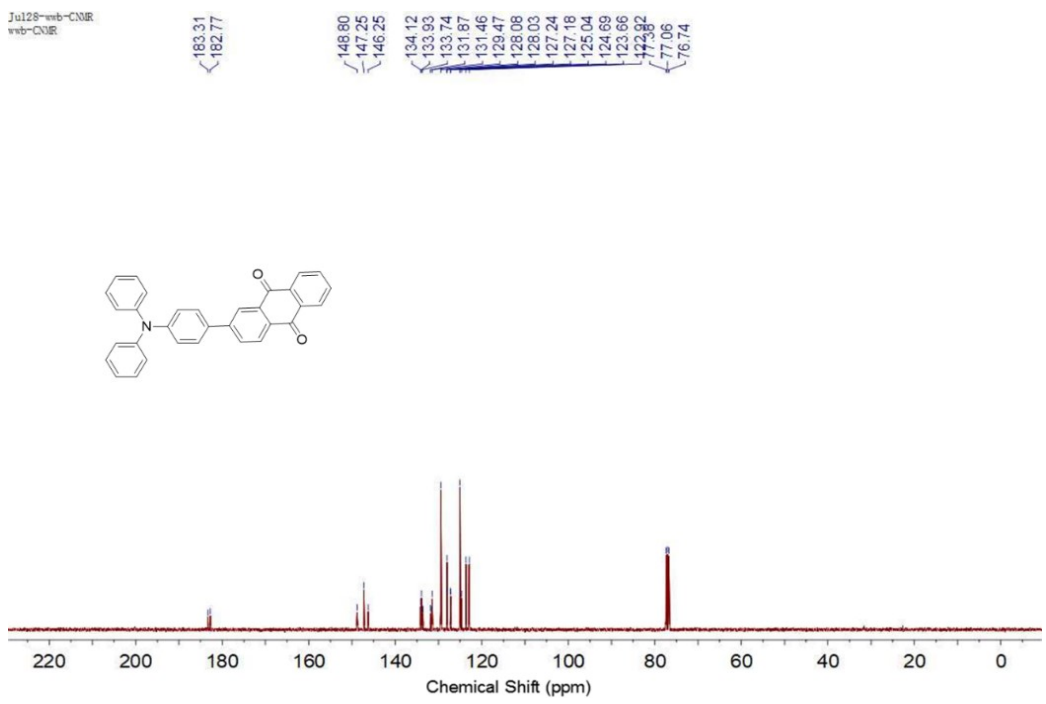


Fig. S6 ^{13}C NMR spectrum of TPAAQ.

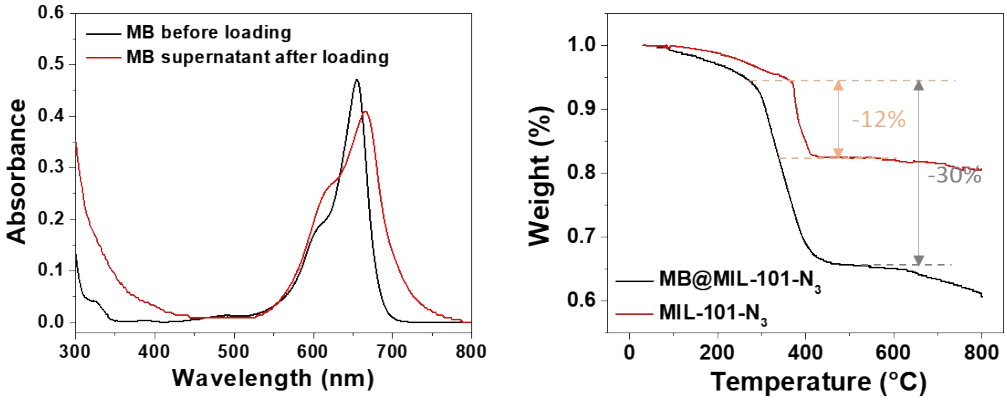


Fig. S7 UV-vis absorption spectra of MB before and after encapsulation into MIL-101-N_3 during its synthesis and TGA results of MIL-101-N_3 and MB@ MIL-101-N_3. For absorption measurement, [MB] = 1 μg mL^{-1}. For loading process, [MB] = 2 mg mL^{-1}, [MIL-101-N_3] = 1 mg mL^{-1}.

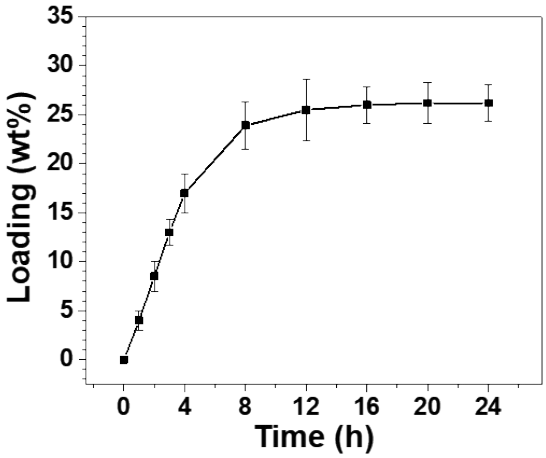


Fig. S8 Loading kinetics of MB into MIL-101-N_3.

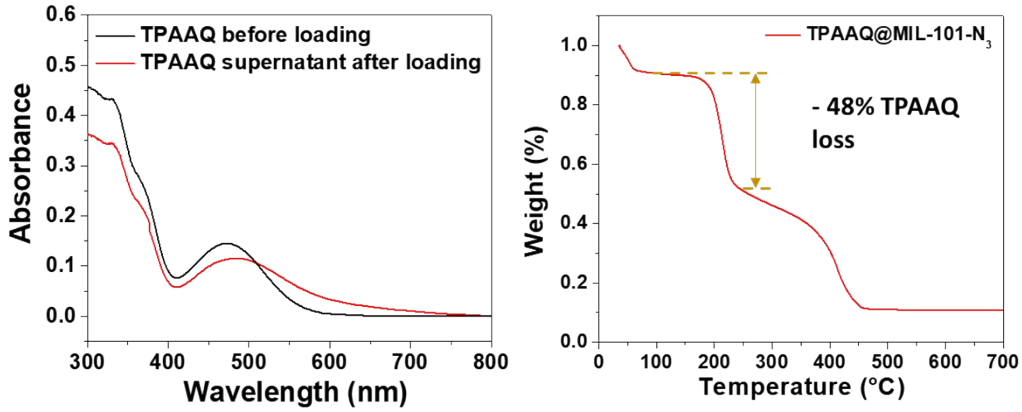


Fig. S9 UV-vis absorption spectra of TPAAQ before and after encapsulation into MIL-101-N_3 during its synthesis and TGA results of MIL-101-N_3 and TPAAQ@ MIL-101-N_3. For absorption measurement, [TPAAQ] = 10 μg mL^{-1}. For loading process, [TPAAQ] = 2 mg mL^{-1}, [MIL-101-N_3] = 1 mg mL^{-1}.

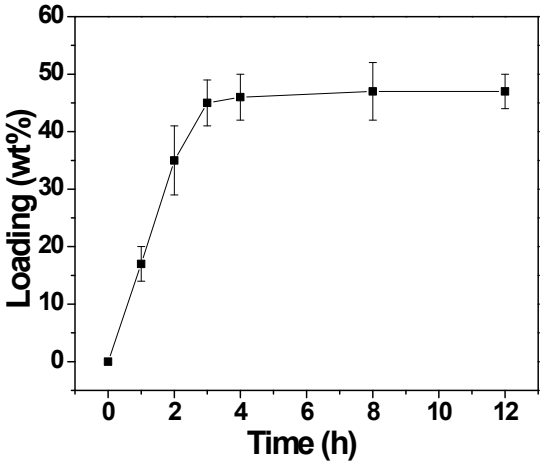


Fig. S10 Loading kinetics of TPAAQ into MIL-101-N_3.

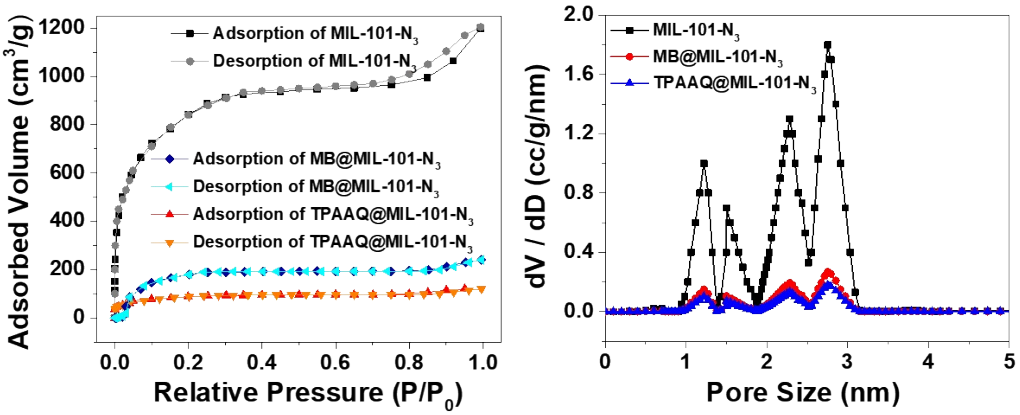


Fig. S11 N_2 adsorption and desorption isotherms and pore size distribution of MIL-101-N_3, MB@MIL-101-N_3, and TPAAQ@ MIL-101-N_3 at 77 K.

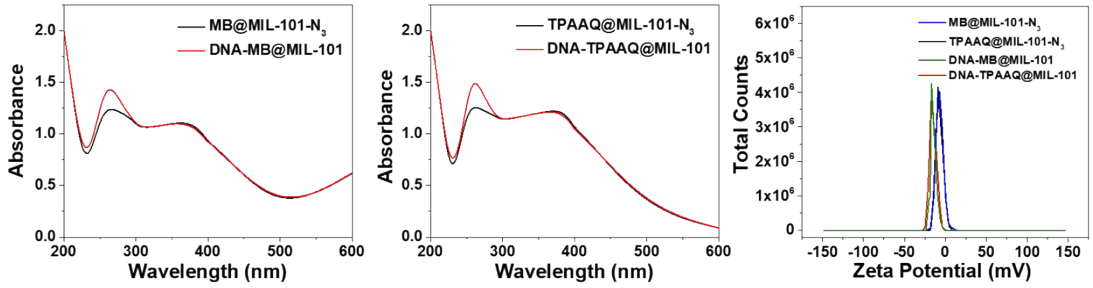


Fig. S12 UV-vis absorption spectra and zeta-potentials of MB@MIL-101-N_3, DNA-MB@MIL-101, TPAAQ@MIL-101-N_3, and DNA-TPAAQ@MIL-101.

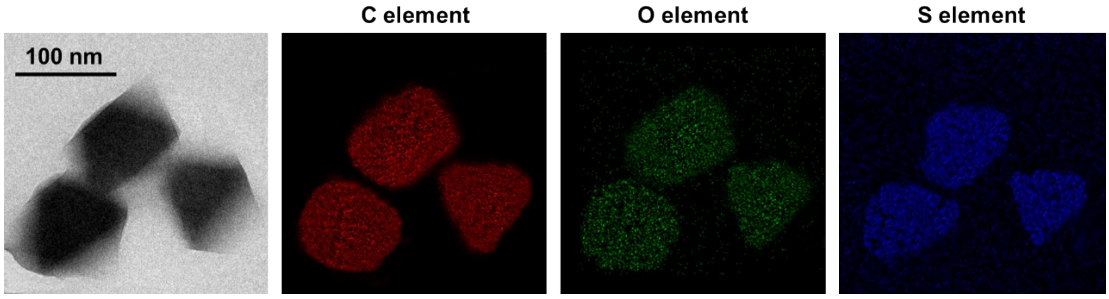


Fig. S13 A TEM image and energy dispersive X-ray spectroscopy in scanning transmission electron microscopy (STEM-EDS) mapping of DNA-MB@MIL-101.

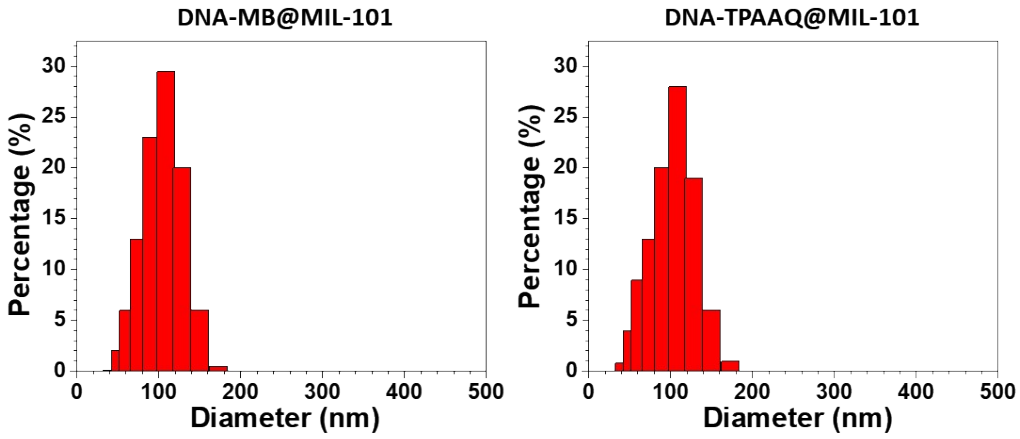


Fig. S14 Dynamic light scattering (DLS) results of DNA-MB@MIL-101 and DNA-TPAAQ@MIL-101.

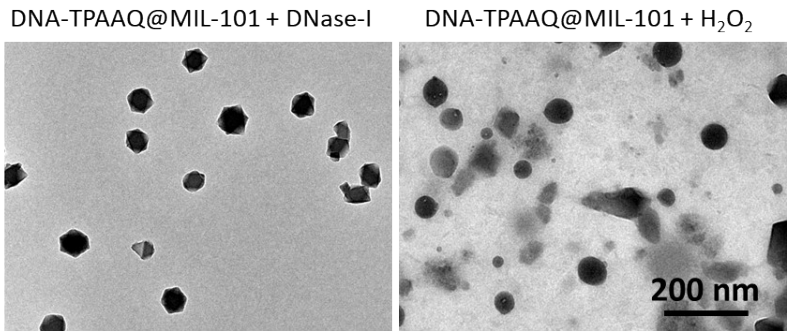


Fig. S15 TEM images of TPAAQ@MIL-101-N_3 and DNA-TPAAQ@MIL-101 after DNase-1 or H_2O_2 treatment for 4 h. Both images share the same scale bar.

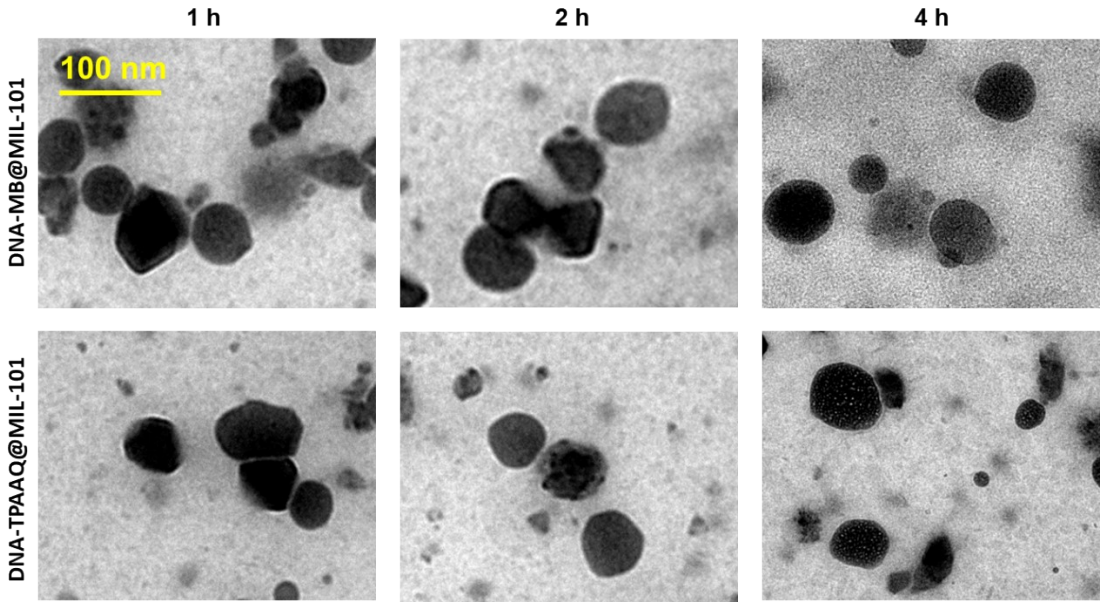


Fig. S16 TEM images of DNA-MB@MIL-101 and DNA-TPAAQ@MIL-101 after H_2O_2 treatment for different time. All the images share the same scale bar.

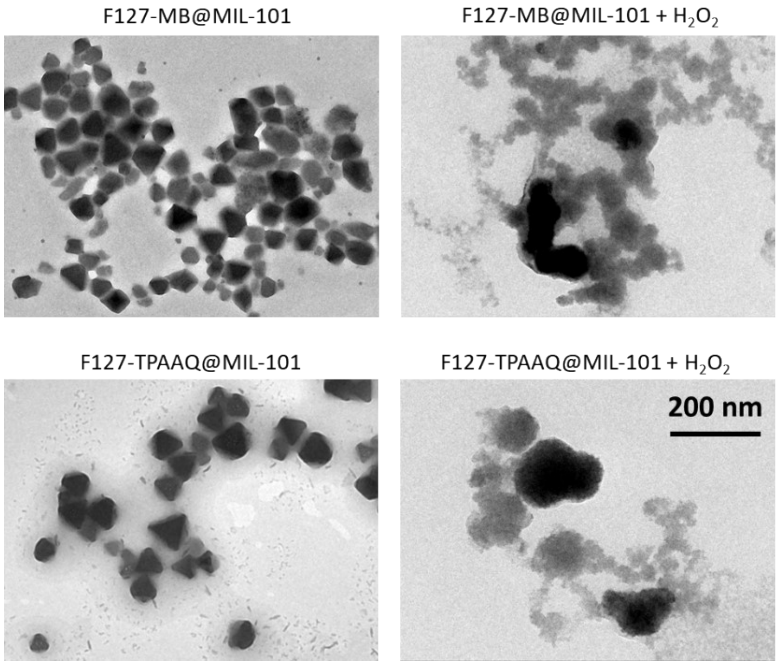


Fig. S17 TEM images of F127-MB@MIL-101 and F127-TPAAQ@MIL-101 before or after H_2O_2 treatment for 4 h. All the images share the same scale bar.

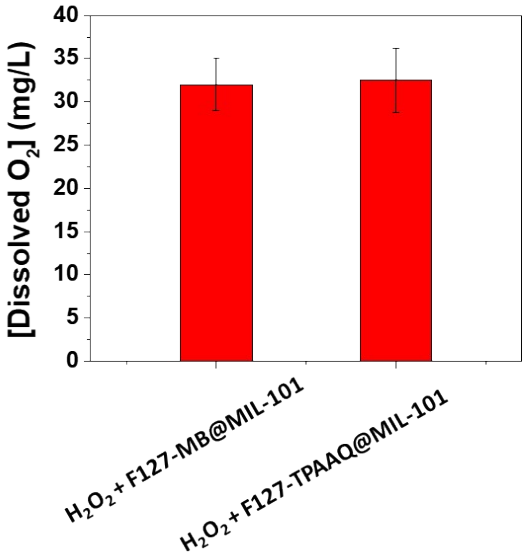


Fig. S18 Concentrations of dissolved O_2 in the F127-MB@MIL-101 or F127-TPAAQ@MIL-101 solutions treated by H_2O_2.

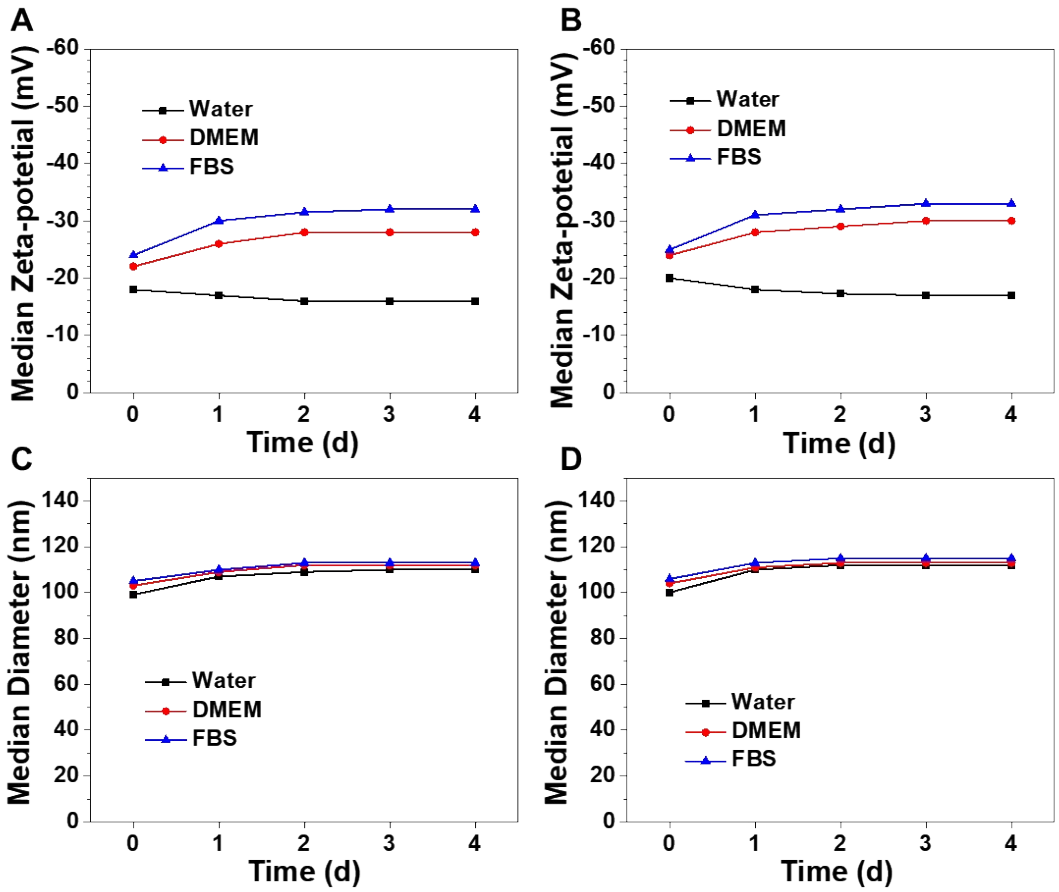


Fig. S19 Zeta-potentials and DLS results of DNA-MB@MIL-101 A) C) and DNA-TPAAQ@MIL-101 B) D) (1 mg mL^{-1} based on MIL-101) after immersion in different biological media for 0, 1, 2, 3, or 4 days.

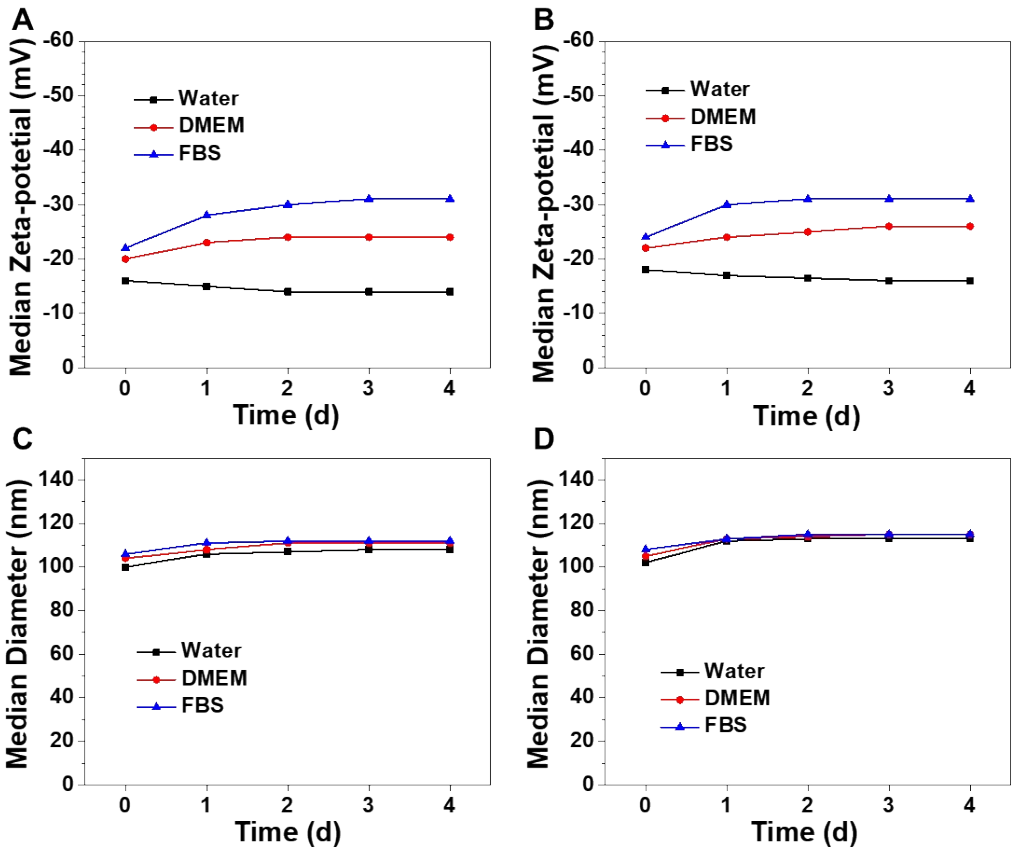


Fig. S20 Zeta-potentials and DLS results of F127-MB@MIL-101 A) C) and F127-TPAAQ@MIL-101 B) D) (1 mg mL^{-1} based on MIL-101) after immersion in different biological media for 0, 1, 2, 3, or 4 days.

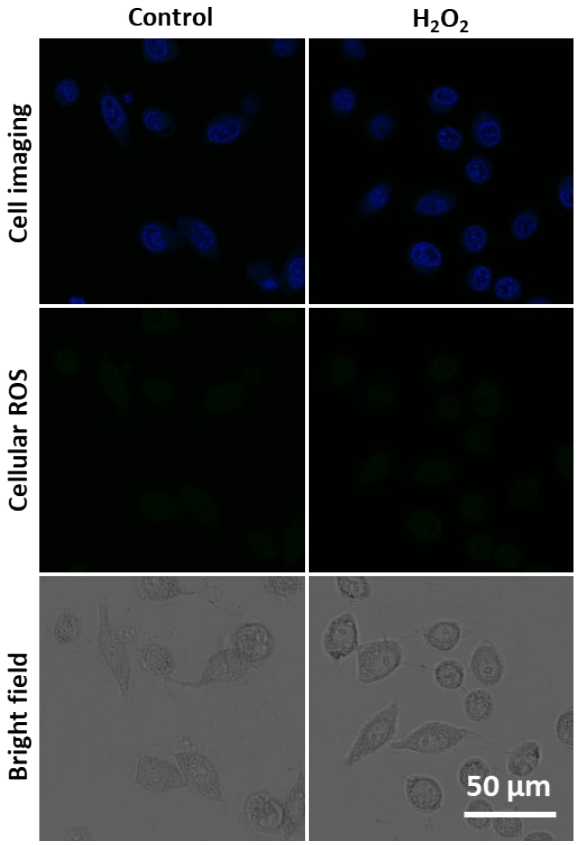


Fig. S21 CLSM images of HeLa cells with or without H_2O_2 treatment before being stained with Hochest and DCFDA. All the images share the same scale bar.

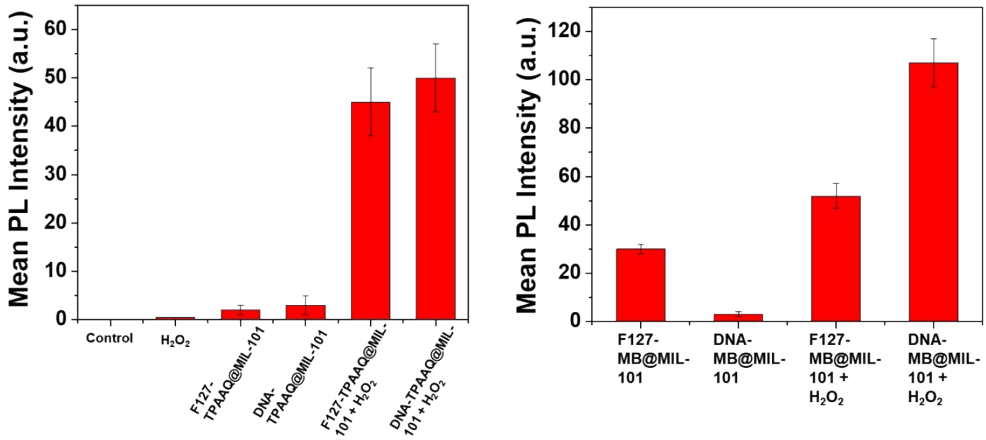


Fig. S22 Mean fluorescence intensities of DCFDA inside HeLa cells treated with different agents for 24 h.

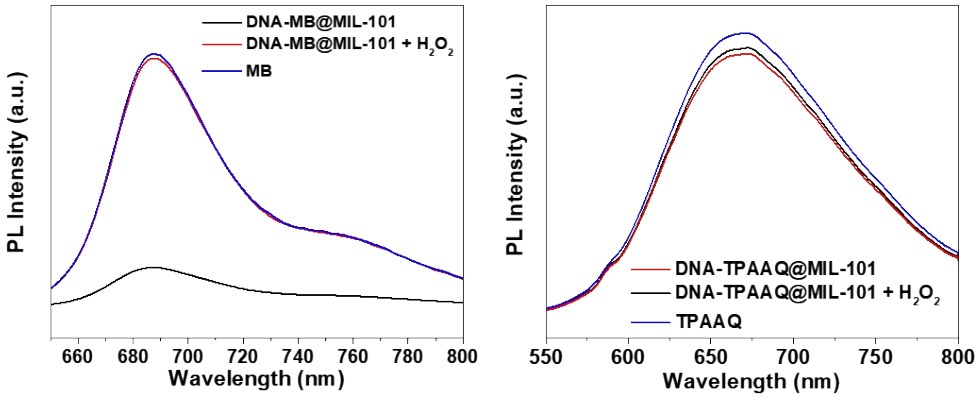


Fig. S23 Photoluminescence spectra of MB, TPAAQ, DNA-MB@MIL-101, and DNA-TPAAQ@MIL-101 before or after H_2O_2 treatment.

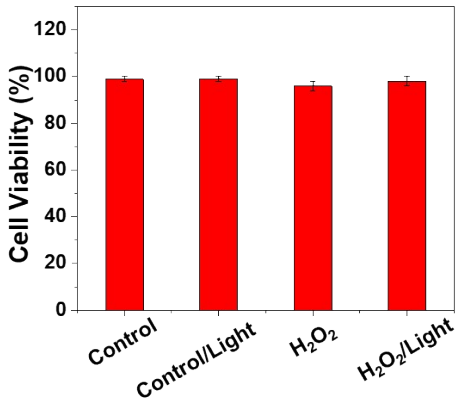


Fig. S24 Viability of HeLa cells with or without light and H_2O_2 (100 μM) treatments. Illumination intensity, time, and wavelength are 100 mW cm^{-2}, 5 min, and 400-700 nm, respectively.

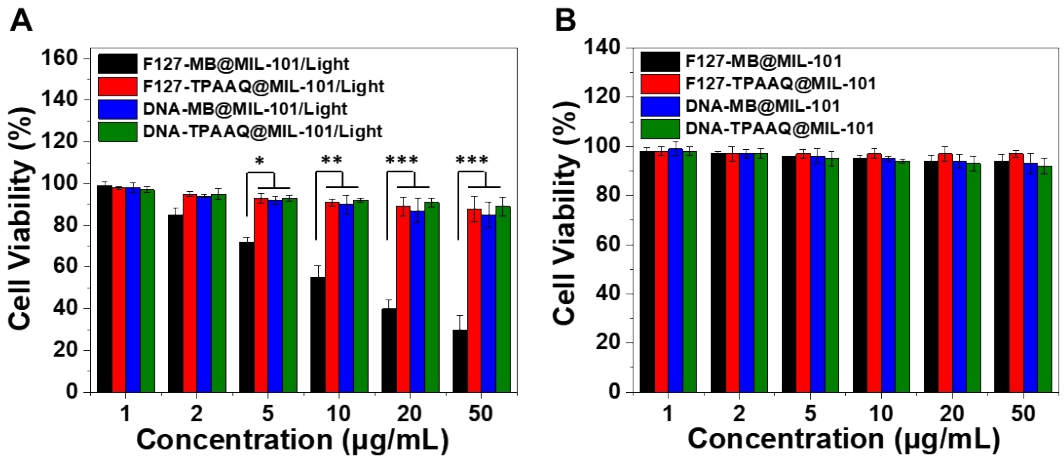


Fig. S25 Viability of 3T3 cells upon incubation with F127-MB@MIL-101, F127-TPAAQ@MIL-101, DNA-MB@MIL-101, or DNA-TPAAQ@MIL-101 under white light A) or dark B). Illumination intensity, time, and wavelength are 100 mW cm^{-2}, 5 min, and 400-700 nm, respectively. Concentrations are based on PSs. $n = 4$ per group, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

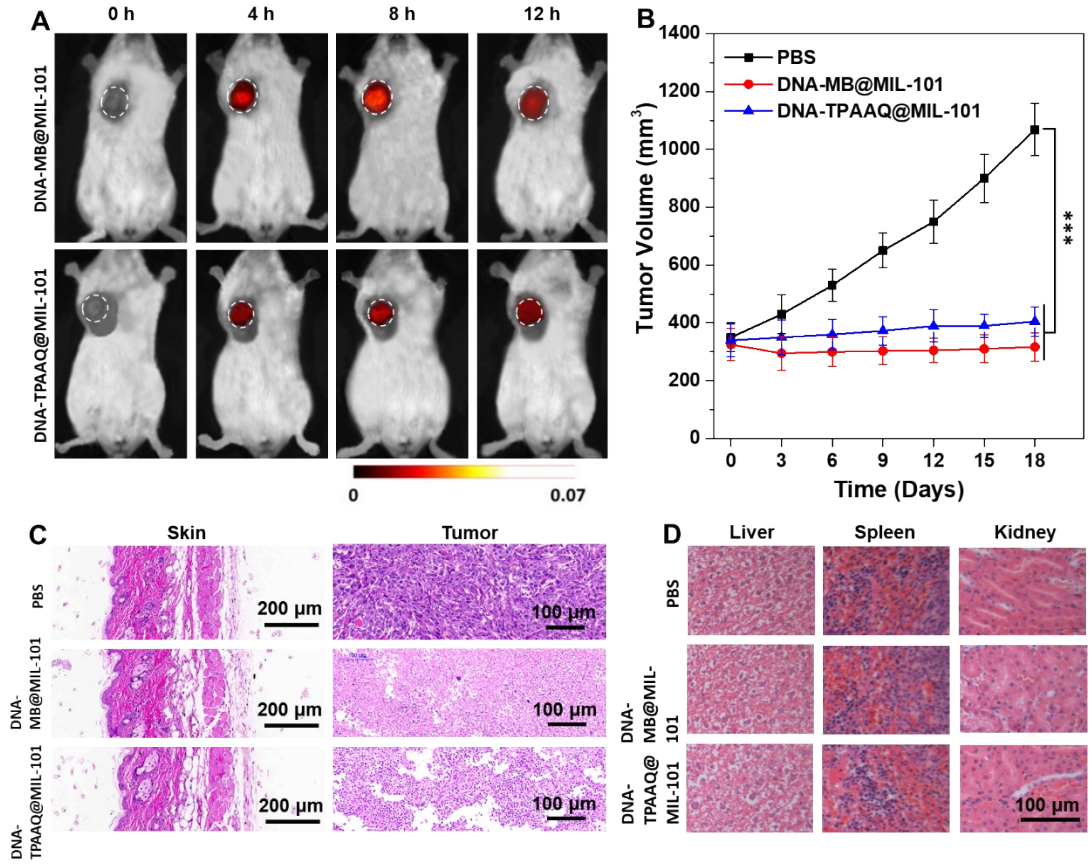


Fig. S26 A) In vivo imaging of tumor-bearing mice before and after intravenous injection of DNA-MB@MIL-101 or DNA-TPAAQ@MIL-101 at different time points. B) Tumor volumes of mice injected with PBS, DNA-MB@MIL-101, or DNA-TPAAQ@MIL-101 at different time points. C) H&E staining performed on the tumor and skin slices of 4T1 tumor-bearing mice 18 days after intravenous administration with PBS, DNA-MB@MIL-101, or DNA-TPAAQ@MIL-101 upon white light irradiation. Prior to the preparation of skin slices, DNA-MB@MIL-101 or DNA-TPAAQ@MIL-101 was subcutaneously injected into normal skin, followed by the same light illumination. D) H&E staining of different mouse organ cross sections 18 days post-treatment. All the images share the same scale bar of 100 μm. White light (400 mW cm^{-2}, 400-700 nm, 5 min) was adopted to irradiate the tumor region at 8 h post-injection. Injection volume = 100 μL, [DNA-MB@MIL-101] = [DNA-TPAAQ@MIL-101] = 0.5 mg mL^{-1} (based on MB or TPAAQ, respectively). Data are mean ± SEM, n = 3 per group, *** $p < 0.001$.

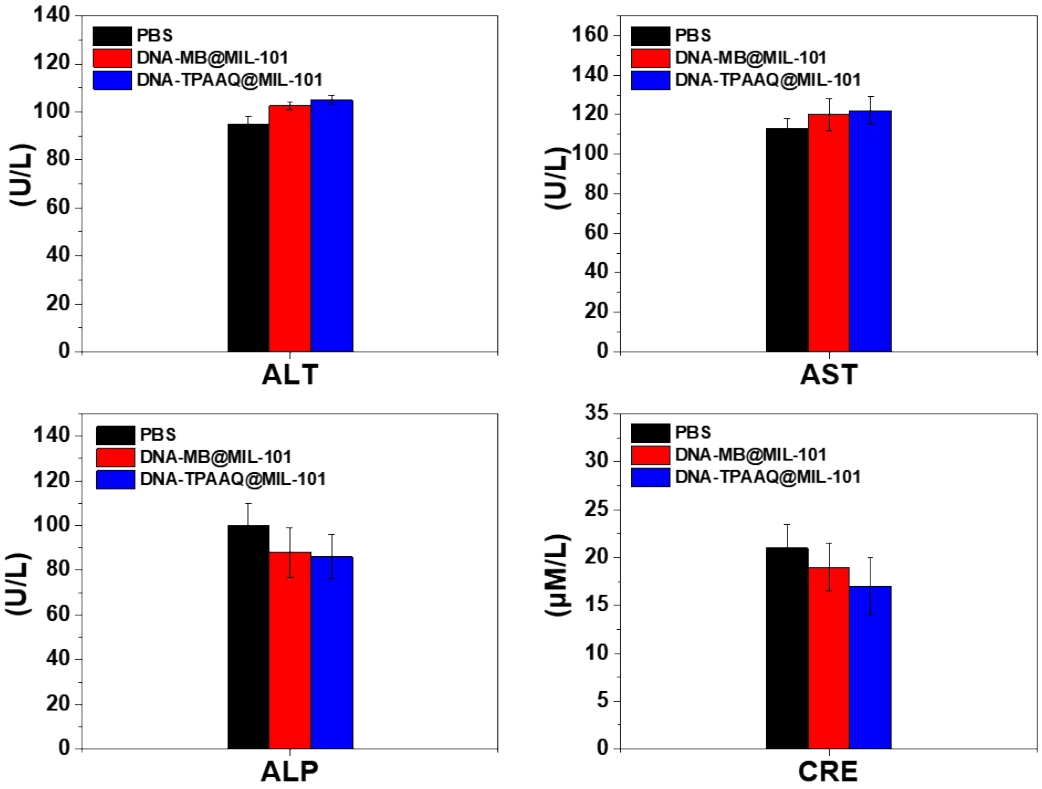


Fig. S27 Blood chemistry data of mice 18 days after intravenous injection. ALT is short for alanine transferase; AST is short for aspartate transaminase; ALP is short for alkaline phosphatase; CRE is short for creatinine, n = 3 per group.

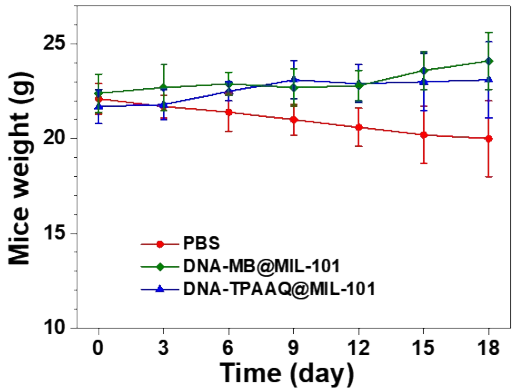


Fig. S28 Body weight changes of mice receiving different treatments (n = 3 mice per group).

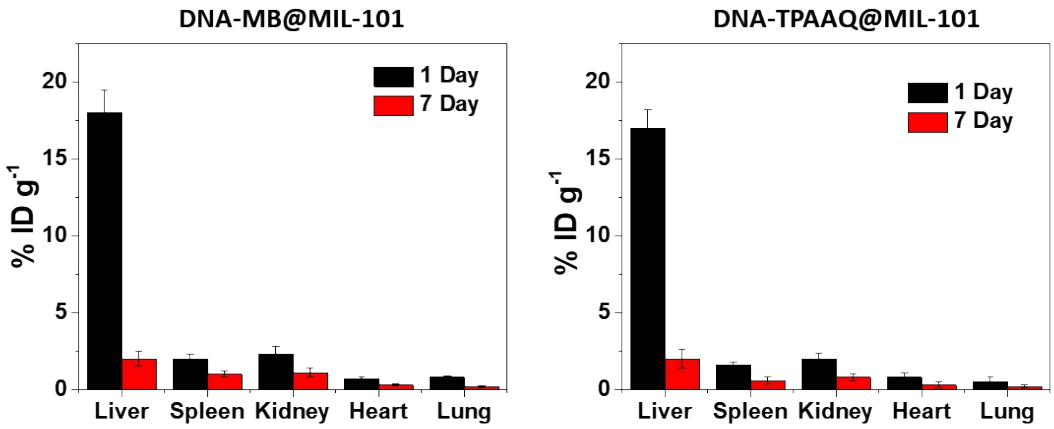


Fig. S29 Biodistribution of DNA-MB@MIL-101 or DNA-TPAAQ@MIL-101 at 1 day and 7 days after intravenous administration based on ICP-MS analysis (data expressed as percent injected dose per gram of tissue (% ID/g tissue), n = 3 mice per group.