Supporting Information for
A Nanoscale Metal-organic Framework for Highly Effective Photodynamic Therapy of
Resistant Head and Neck Cancer

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1. Materials and cell lines

All of the starting materials were purchased from Sigma-Aldrich and Fisher (USA), unless otherwise noted, and used without further purification.

The human head and neck cancer cell line SQ20B (cisplatin-resistant) was kindly provided by Dr. Stephen J. Kron (Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, USA). The cells were cultured in DMEM/F12 (1:1) medium (Gibco, Grand Island, NY, USA) containing 20% fetal bovine serum (FBS, Hyclone, Utah, USA).

Athymic female nude mice (6 weeks, 20-22 g) were provided by Harlan Laboratories, Inc (USA). The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Chicago.
2. Synthesis of 5, 15-di(p-benzoato)porphyrin (H₂DBP)

Dipyrrylmethane was synthesized based on a modified literature procedure.¹ To a 1-liter flask 500 mL of distilled pyrrole (7.2 mol) was added. To the flask paraformaldehyde (1.74 g, 58 mmol by formaldehyde) was added and the mixture was degassed for 15 minutes. The mixture was then heated at 60 °C to dissolve most of the solid. After cooling to room temperature, 0.53 mL of trifluoroacetic acid (TFA) was added slowly to the solution. The reaction mixture was stirred for an hour before the addition of 812 mg of sodium hydroxide, then the mixture was stirred for another 45 minutes. Pyrrole was distilled off under vacuum and the remaining solid was extracted with dichloromethane from water and washed with water twice. The crude product was purified by silica gel column chromatography with chloroform as eluent to afford the off-white product. Yield: 4.94 g, 33.8 mmol (58%).

¹H-NMR (500 MHz, chloroform-D, ppm): δ=7.72 (s, 2H), 6.61 (d, 2H), 6.15 (d, 2H), 6.03 (s,

Scheme S1. Synthesis of the 5, 15-di(p-benzoato)porphyrin ligand.
4-(Methoxycarbonyl)benzaldehyde (1.20 g, 7.3 mmol) and dipyrrylmethane (1.07 g, 7.3 mmol) were added to a round bottom flask. To the flask 1 L of anhydrous dichloromethane (DCM) was added. Trifluoroacetic acid (0.34 mL, 4.4 mmol) was added dropwise via a syringe. The mixture was stirred at room temperature for 4 hours. To the reaction mixture, 2.49 g 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 11.0 mmol) was then added and the mixture was stirred for another hour. Triethylamine was added to neutralize the reaction mixture. The solvent was removed with a rotary evaporator, and the 5, 15-di(p-methyl-benzoato)porphyrin (Me2DBP) product was purified by column chromatography with chloroform as the eluent. Yield: 810 mg, 1.40 mmol (38%). $^1$H-NMR (500 MHz, chloroform-D, ppm): $\delta$=10.38 (s, 2H), 9.45 (d, 4H), 9.06 (d, 4H), 8.52 (d, 4H), 8.39 (d, 4H), 4.16 (s, 6H), -3.12 (s, 2H).

Figure S1 $^1$H NMR spectrum of dipyrrylmethane in chloroform-D.
The aforementioned Me$_2$DBP (399 mg, 0.69 mmol) was dissolved in a mixture of tetrahydrofuran (THF) and methanol (90 mL, 1:1 vol/vol). A potassium hydroxide aqueous solution (14 mL, 2 M) was then added. The solution was heated to reflux under nitrogen protection overnight. Half of the solvent was removed with a rotary evaporator before the solution was neutralized to pH=3 with trifluoroacetic acid. The dark purple product was collected by centrifugation and washed with water and ether. The solid residue was dried under vacuum to give the pure H$_2$DBP product in 95% yield (362 mg, 0.66 mmol). $^1$H-NMR (500 MHz, DMSO-D$_6$, ppm): $\delta$=13.35 (s, 2H), 10.71 (s, 2H), 9.71 (d, 4H), 9.08 (d, 4H), 8.45 (m, 8H), -3.26 (s, 2H). $^{13}$C-NMR (125 MHz, DMSO-D$_6$, ppm):$\delta$= 168.05 (a), 145.36 (f),
135.35, 133.46, 131.22, 130.78 (b-e), 128.67 (g, j), 118.19 (k), 106.62 (h, i). ESI-MS for [H₂DBP+H]⁺: 551.1 calc; 551.2 found.

**Figure S3** Top, \(^1\)H NMR spectrum of 5, 15-di(p-benzoato)porphyrin in DMSO-\(D_6\). Bottom, an expanded view of a portion of the spectrum.
Figure S4 $^{13}$C NMR spectrum of 5, 15-di(p-benzoato)porphyrin in DMSO-D$_6$.

Figure S5 Mass spectrum of 5, 15-di(p-benzoato)porphyrin. The sample was prepared in DMSO as a 40 mg/L solution and was delivered by methanol.
3. Synthesis and characterization of the DBP-UiO NMOF

To a 20-mL glass vial was added 3 mL of HfCl$_4$ solution [2 mg/mL in N,N-dimethylformamide (DMF), 0.018 mmol], 3 mL of the H$_2$DBP solution (3.5 mg/mL in DMF, 0.018 mmol), and 0.45 mL of acetic acid (7.9 mmol). The reaction mixture was kept in a 90 °C oven for 3 days. The dark red powder was collected by centrifugation and washed with DMF, triethylamine/ethanol (1:20 vol/vol) and ethanol.

The powder X-ray diffraction pattern of DBP-UiO matches that of the Zn-DPDBP-UiO MOF [DPDBP refers to 10, 20-diphenyl-5, 15-di(p-benzoato)porphyrin]. The Zn-DPDBP-UiO MOF adopts a UiO structure with a framework formula of Zr$_6$O$_4$(OH)$_4$(Zn-DPDBP)$_6$. Chemical structure of Zn-DPDBP and crystallographic information of Zn-DPDBP-UiO are listed in Table S1 and the structure is depicted in Figure S7.

Nitrogen adsorption of the NMOF was tested on Autosorb-1 surface area and pore size analyzer (Quantachrome Instruments) at 77K (Figure S8). The BET surface area was calculated to be 558 m$^2$/g.

Thermogravimetric analysis on DBP-UiO NMOF was carried out on Shimadzu TGA-50 thermogravimetric analyzer. Heating speed was set to 3 °C/min and the sample was heated to 600 °C in air. The weight percentage was plotted against temperature (Figure S9). The normalized percent weight loss from 200 °C to 600 °C was 77%, which corresponded well to the calculated DBP ligand weight loss based on the MOF formula (74%).

A plate-like morphology of DBP-UiO NMOF was confirmed by transmission electron microscopy (TEM, Tecnai F30 and Tecnai Spirit, FEI, USA) as shown in Figure S6. The distances between SBUs are measured as shown in Figure S10. The particles display a plate-like morphology with thickness of about 10 nm and plate diameter of less than 100 nm. Particle sizes of DBP-UiO NMOFs were determined to be 76.3 nm (PDI=0.103) by dynamic light scattering (DLS, Nano-ZS, Malvern, UK; Figure S11).
**Table S1** Chemical structure of Zn-DPDBP and crystal structure information of Zn-DPDBP-UiO.

Zn-DPDBP =

| Property                          | Value               |
|----------------------------------|---------------------|
| Formula                          | \( \text{Zr}_6(\text{O})_4(\text{OH})_4(\text{Zn-DPDBP})_6 \) |
| Absorption coeff. (mm\(^{-1}\)) | 0.223               |
| \( M_r \)                        | 5263.82             |
| \( \text{F}(000) \)              | 10640.0             |
| Temperature (K)                  | 293                 |
| \( \theta \) range data collection | 1.01 – 13.00      |
| Wavelength (Å)                   | 0.41328             |
| Crystal system                   | cubic               |
| Space group                      | \( \text{Fm} \text{3m} \) |
| \( a \), Å                       | 38.758(2)           |
| \( b \), Å                       | 38.758(2)           |
| \( c \), Å                       | 38.758(2)           |
| \( \alpha \), °                  | 90                  |
| \( \beta \), °                   | 90                  |
| \( \gamma \), °                  | 90                  |
| \( V \), Å\(^3\)                | 58224(5)            |
| \( Z \)                          | 4                   |
| Density (calcd. g/cm\(^3\))     | 0.600               |

**Figure S6** Single-crystal X-ray structure of Zn-DPDBP-UiO. (a) The structure viewed from the [110] direction; (b) the octahedral cavity in the structure.
Figure S7 TEM images of DBP-UiO at different resolutions.
Figure S8 Nitrogen adsorption isotherm of DBP-UiO at 77K.

Figure S9 Thermogravimetric analysis of DBP-UiO.
Figure S10 TEM image (top) of DBP-UiO and the zoomed-in image (bottom) showing the distances between SBUs.
Figure S11 DLS plot showing the particle size of DBP-UiO.

4. DBP-UiO stability

To test the stability of DBP-UiO in physiological environments, the DBP-UiO particles were incubated in RPMI 1640 cell culture medium for 12 h. TEM images displayed an unchanged morphology of NMOFs after incubation (Figure S12).

Figure S12 TEM images of DBP-UiO before and after incubation in cell medium. (a) DBP-UiO before incubation; (b) and (c) DBP-UiO after incubation for 12 hours; (d) fast Fourier transform of TEM image (c).
5. *Photochemical properties of H$_2$DBP and DBP-UiO*

The UV-visible absorption spectra of H$_2$DBP and DBP-UiO were acquired with a UV-vis spectrophotometer (UV-2401PC, Shimadzu, Japan). The H$_2$DBP solution and DBP-UiO NMOF suspension were prepared in 0.67 mM phosphate buffer saline (PBS). The absorption of standard solutions of H$_2$DBP at concentrations of 0.2, 0.4, 0.6, 0.8, 1, 1.5, 4 and 8 mg/L were acquired and the standard curve was plotted by linear fitting of the absorbance at 402 nm (Figure S13, S14). The extinction coefficients of H$_2$DBP at 402 nm and 619 nm are $2.2 \times 10^5$ and $1.7 \times 10^3$ M$^{-1}$cm$^{-1}$, respectively.

The fluorescence spectra of H$_2$DBP ligand and DBP-UiO NMOF were taken on a spectrofluorophotometer (RF-5301PC, Shimadzu, Japan, Figure S15). The ligand fluorescence appears at 630 nm (strong) and 690 nm (weak), while DBP-UiO NMOF shows negligible fluorescence.

Time-domain lifetimes were measured on a ChronosBH lifetime fluorimeter (ISS, Inc.) using Time-Correlated Single Photon Counting (TCSPC) methods (Figure S16). The fluorimeter contained Becker-Hickl SPC-130 detection electronics and an HPM-100-40 Hybrid PMT detector. Tunable picosecond pulsed excitation was provided by a Fianium SC400-2 supercontinuum laser source with integrated pulse picker and AOTF. Emission wavelengths were selected with bandpass filters (Semrock and Chroma). The Instrument Response Function (IRF) was measured to be approximately 120ps FWHM in a 1% scattering solution of Ludox LS colloidal silica. Lifetimes were fit via a forward convolution method in the Vinci control and analysis software. The fitted lifetimes are listed in Table S2.

![Figure S13 UV-vis absorption spectra of H$_2$DBP at different concentrations.](image)
Figure S14 Linear fit of $H_2$DBP absorbance at 402 nm.

Figure S15 Fluorescence of $H_2$DBP and DBP-UiO in PBS (excitation: 405 nm). The expanded view for the DBP-UiO is shown in the inset. $H_2$DBP fluorescence is ~250 times higher than that of DBP-UiO.
Figure S16 Time-resolved fluorescence decay traces of H$_2$DBP (top) and DBP-UiO (bottom) in different media, together with instrument response function (excitation/emission 403/640 nm).
Table S2 Lifetimes of H₂DBP and DBP-UiO fluorescence in different medium, fitted by software.

| sample          | \(\tau_1\) (ns) | \(\tau_2\) (ns) | \(\bar{\tau}\) (ns) |
|-----------------|------------------|-----------------|---------------------|
| IRF             | 0.0074           | N/A             | N/A                 |
| H₂DBP_DMF       | 11.3             | N/A             | N/A                 |
| H₂DBP_aq        | 12.4             | 7.86            | 10.9                |
| DBP-UiO_DMF     | 0.44             | 1.31            | 0.54                |
| DBP-UiO_aq      | 0.21             | 0.70            | 0.26                |

6. Singlet oxygen generation of H₂DBP and DBP-UiO

A light-emitting diode (LED) array with peak emission at 640 nm (Figure S17) was used as the light source of singlet oxygen generation. The irradiance of LED is 100 mW/cm². Singlet oxygen sensor green (SOSG) reagent (Life Technologies) was employed for the detection of singlet oxygen. H₂DBP and DBP-UiO samples were prepared in 5 \(\mu\)M solutions/suspensions in HBSS buffer (for DBP-UiO samples, the concentration was calculated as ligand equivalents). To 2 mL each of these solutions/suspensions, SOSG stock solution (5 \(\mu\)L at 5 mM) was added (final concentration=12.5 \(\mu\)M) before fluorescence measurement.

For a typical measurement, fluorescence intensity was acquired on a spectrofluorophotometer (RF-5301PC, Shimadzu, Japan) with excitation at 504 nm and emission at 525 nm (slit width 1.5 nm/3 nm for ex/em). Fluorescence was measured after irradiation by LED for 0 (as background), 10 s, 20 s, 30 s, 1 min, 1.5 min, 2 min, 2.5 min, 3 min, 3.5 min, 4 min, 4.5 min, 5 min, 6 min and 7 min.

![Figure S17 Emission spectrum of LED.](image)

The photobleaching of the NMOF was tested by irradiation of LED light on 5 \(\mu\)M DBP-UiO sample (base on ligand concentration) for 0, 1, 2, 5 and 10 minutes (Figure S18). The NMOF concentration was tracked by UV-vis absorption. After 10 min irradiation, 98% of absorbance was preserved, proving insignificant photobleaching of the DBP-UiO NMOF.
As the light intensity and photosensitizer concentration are fixed, for the photoreaction, we can assume that \([PS^*]\) (the concentration of the excited state of the photosensitizer) is a constant. Therefore, we have singlet oxygen generation rate equation:

\[
\frac{d[1O_2]}{dt} = -\frac{d[O_2]}{dt} = k[PS^*][O_2] = k^*[O_2]
\]

Where \(k^* = k[PS^*]\). Here we have a coupled reaction of SOSG to consume singlet oxygen:

\[
\frac{d[SOSG^*]}{dt} = k_2[1O_2][SOSG]
\]

Where \([SOSG^*]\) is the concentration of reacted form of SOSG. Applying steady state assumption on \([1O_2]\), we have

\[
\frac{d[1O_2]}{dt} = k^*[O_2] - k_d\left[1O_2\right] - k_2\left[1O_2\right][SOSG] = 0
\]

\[
\left[1O_2\right] \approx \frac{k^*[O_2]}{k_d + k_2[SOSG]}
\]

Where \(k_d\) is the rate constant of singlet oxygen loss (due to relaxation or quenched by other species in the solution). When \(k_2[SOSG] \ll k_d\) and \([O_2]\) is high,

\[
\left[1O_2\right] \approx \frac{k^*[O_2]}{k_d} = \text{constant}
\]

\[
\frac{dI_F}{dt} \propto \frac{d[SOSG^*]}{dt} = k_1[SOSG]
\]

Where \(k_1 = k_2k^*[O_2]/k_d\), and the fluorescence intensity is proportional to \([SOSG^*]\):

\[
I_F = I_0\phi_F\varepsilon_b[SOSG^*]
\]

\(S17\)
Where $I_0$ is the incident light intensity, $\varphi_f$ is the fluorescence quantum yield of SOSG*, $\varepsilon_S$ is the extinction coefficient of SOSG*, and $b$ is the light path length. We can integrate the equation to obtain the correlation of fluorescence intensity $I_F$ and irradiation time $t$:

$$\ln \frac{[\text{SOSG}]}{c_0(\text{SOSG})} = -kt$$

$$I_F = A[1 - e^{-kt}]$$

Where $A$ and $k$ are fitting parameters,

$$A = \varphi_f I_0 \varepsilon_S b c_0(\text{SOSG})$$

$$k = \varphi_A N_{ir} \varepsilon_{PS} b c(PS) k_2/k_d$$

Where $c_0(\text{SOSG})$ is the initial SOSG concentration; $\varphi_A$ is the quantum yield of singlet oxygen generation, $N_{ir}$ is the irradiation light intensity by photons per second, $\varepsilon_{PS}$ is the extinction coefficient of photosensitizer at LED emission wavelength, $c(PS)$ is the photosensitizer concentration. Linear approximations are applied in above equations.

By non-linear regression, we obtained a series of fit curves with the aforementioned function.

Note that for $\text{H}_2\text{DBP}+\text{HfCl}_4$ sample, singlet oxygen generation rate is very low, SOSG consumption becomes negligible. Then the equation could be simplified to

$$I_F = Ak t$$

Thus we can compare the linear regression slope to product of Ak in the other groups. The summary of all fitting results are included in Table S3.

**Table S3** Fitting parameters of singlet oxygen generation rate.

|       | A   | $k$ (s$^{-1}$) | $r^2$ | Ak   |
|-------|-----|----------------|-------|------|
| $\text{H}_2\text{DBP}$ | 68  | 1.5×10$^{-3}$  | 0.998 | 0.102|
| $\text{DBP-UiO}$   | 88  | 3.3×10$^{-3}$  | 0.999 | 0.290|
| $\text{H}_2\text{DBP}+\text{HfCl}_4$ |     |                | 0.97  | 0.0062|

To further prove the validity of the rate equations, we tested the influence of SOSG concentration on fluorescence growth rate. To 5µM DBP-UiO solutions 5 µL or 2.5 µL of SOSG stock solution was added (final SOSG concentrations are 12.5 µM and 6.25 µM respectively). The fluorescence growth rate reduced to half when half amount of SOSG was added to the solution (Figure S19 and Table S4). This supports the kinetics we proposed here.
Figure S19 Comparison of fluorescence intensity growth with different SOSG concentrations.

Table S4 Fitting parameters of fluorescence growth rates with different concentrations of SOSG.

|            | A  | k (s⁻¹)  | r²  | Ak   |
|------------|----|----------|-----|------|
| SOSG 5 µL  | 52 | 4.7×10⁻³ | 0.997 | 0.244 |
| SOSG 2.5 µL | 31 | 4.3×10⁻³ | 0.998 | 0.133 |

7. Cellular uptake of DBP-UiO

SQ20B cells were seeded on 6-well plates at 5×10⁵ cells/well and further incubated for 24 h. The DBP-UiO samples were added to the cells at a concentration of 30 mg/L. After incubating for 4 h and 12 h, the cells were collected and the cell numbers were counted by hemocytometer. The cells were digested with concentrated nitric acid and subjected to ICP-MS for the determination of the Hf concentration. The cellular uptake amounts were expressed as Hf (ng) per 10⁵ of cells (Figure S20). Due to its favorable size (~100 nm), DBP-UiO NMOFs can be efficiently internalized into the cells with an uptake percent of 29.4±0.2% and 31.5±2.1% at 4 h and 12 h, respectively.
Figure S20 Cellular uptake of NMOF after 4 or 12 hour incubation.

8. Cytotoxicity

The cytotoxicity of DBP-Uio, H$_2$DBP, and PpIX was evaluated in human head and neck cancer cells SQ20B which are resistant to cisplatin and conventional radiotherapy. SQ20B cells were seeded on 96-well plates at 2000 cells/well. The cells were treated with DBP-Uio and H$_2$DBP at various ligand concentrations (5, 10, 20, 50 and 100 µM base on ligand concentrations) after a 24-h incubation. A further incubation of 4 h was allowed, followed by replacing the culture medium with 100 µL of fresh DMEM/F12 medium. The cells were irradiated with LED light (640 nm) at 100 mW/cm$^2$ for 15 min (total light dose 90 J/cm$^2$) or 30 min (total light dose 180 J/cm$^2$), respectively. The cells without irradiation treatment served as controls. The cells were further incubated to achieve a total incubation time of 72 h with DBP-Uio, H$_2$DBP or PpIX. The cell viability was detected by (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS) assay (Promega, USA).

Cells incubated with PBS after irradiation for 15 min and 30 min did not show appreciable viability changes (95.2±2.2% for 15 min and 96.1±5.2% for 30 min) compared with cells incubated with PBS and received no irradiation, suggesting that irradiation alone will not induce any cytotoxicity. As shown in Fig. 2d, no cytotoxicity was noticed in cells treated with H$_2$DBP or DBP-Uio without irradiation. This result indicated that H$_2$DBP and DBP-Uio alone are non-toxic to cells at tested concentrations up to 100 µM. Collectively, the cytotoxicity observed after PDT for H$_2$DBP, DBP-Uio, and PpIX was not the artifact from light irradiation or toxicity of H$_2$DBP, DBP-Uio, and PpIX.

9. In vivo efficacy

The PDT efficacy of DBP-Uio was investigated using SQ20B subcutaneous xenograft murine models. Tumor bearing mice were established by subcutaneous inoculation of SQ20B
cell suspension (5×10⁶ cells per mouse) into the right flank region of 6-week athymic female nude mice. Three groups were included for comparison: PBS as control, H₂DBP, and DBP-UiO. When tumors reached 100 mm³, PBS, H₂DBP, and DBP-UiO were intratumorally injected to animals at a DBP dose of 3.5 mg/kg. At 12 h post-injection, mice were anesthetized with 2% (v/v) isoflurane and tumors were irradiated with a 640 nm LED for 30 min. The light intensity was measured as 100 mW/cm², and the total light dose was 180 J/cm². Both injection and PDT were performed once.

To evaluate the therapeutic efficacy, tumor growth and body weight evolution were monitored. The tumor size was measured with a digital caliper every day. Tumor volumes were calculated as follows: (width² × length)/2. Finally, all mice were sacrificed on Day 8, and the excised tumors were photographed and weighed. Tumors were fixed with formalin. Paraffin-embedded 5 µm tumor sections were stained with hematoxylin and eosin (H&E) and observed with light microscopy (Pannoramic Scan Whole Slide Scanner, Perkin Elmer, USA).

The histologies of tumor slices of all three groups were observed after PDT treatment (Figure S21). The dominant normal tumor cells are observed in control and ligand treated groups. Prevailing apoptosis/necrosis of tumor cells were observed in tumor slices from NMOF group and massive inflammatory cells indicated the immunoresponse after PDT. The blood vessels in tumor tissue in NMOF treated group are destroyed after PDT while not disturbed in control and ligand treated groups.
Figure S21 Histology of tumor slices of mice in control group (top), ligand treated group (middle) and NMOF treated group (bottom) after PDT treatment.

10. References

(1) Wang, Q. M.; Bruce, D. W. Synlett 1995, 1995, 1267.