Electronic Supplementary Information

Green Light Responsive Metal-Organic Frameworks for Colorectal Cancer Treatment

Hannah D. Cornell,[a] Yumeng Zhu,[a] Stefan Ilic,[a] Naomei E. Lidman,[a] Xiaozhou Yang,[a] John B. Matson,[a] and Amanda J. Morris*[a]

[a]*Department of Chemistry, Virginia Tech, Blacksburg, VA 24061*
**E-mail: ajmorris@vt.edu*

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I. Materials

The following chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA): sodium iodide, zirconium chloride (≥99%), and poly(ethylene glycol) bis(3-aminopropyl) terminated (M_n ~1,500). Ethyl 4-amino-3,5-difluorobenzoate (≥95%) was purchased from Synthonix (Wake Forest, NC). Formic acid, potassium hydroxide, and dimethyl sulfoxide were obtained from Oakwood Chemical (Estill, SC, USA). 5-fluorouracil (≥99%), ethanol, glacial acetic acid, hydrochloric acid, tetrahydrofuran (THF, ACS grade), ethyl acetate (ACS grade), dichloromethane (DCM, ACS grade) and N,N-dimethylformamide (DMF, ≥99.8%) were obtained from Fisher Scientific (Hampton, NH, USA). 4,4′-azobenzenedicarboxylic acid was prepared according to literature procedures.[1]

II. Characterization Methods

Nuclear Magnetic Resonance (NMR) Spectroscopy

All ^{1}H and ^{19}F NMR experiments were performed using an Agilent U4-DD2 (400 MHz) instrument. For MOF digestion samples, 2 drops of H_2SO_4 was added to 2–5 mg of particles suspended in d_6-DMSO (650 μL) in order to fully degrade the particles for quantitative assessment. For quantifying polymer coating, the relaxation delay was increased to 10 s. For ^{19}F quantitative NMR (5-fluorouracil content/release), 64 scans and a relaxation delay of 15 s was used.

Powder X-Ray Diffraction (PXRD)

PXRD measurements were performed using a Rigaku Miniflex diffractometer (Cu Kα radiation λ= 1.5418 Å). Powder samples were loaded on a Rigaku Si510 sample holder disc and analyzed at a 0.05° resolution and a 5.0°/min continuous scanning mode over 2θ = 2−50°.

UV-Vis Spectroscopy

Absorbance measurements were taken using a Cary 5000 UV-Vis-NIR spectrometer controlled with Cary WinUV software. The Scan application was used to collect spectra from 250-700 nm to characterize 4,4'-(diazene-1,2-diyl)bis(3,5-difluorobenzoic acid) and measure degradation of UiO-AZB-F upon green light irradiation.

Thermogravimetric Analysis (TGA)

Thermogravimetric Analysis was performed with a TGA 550 thermal analyzer (TA Instruments, New Castle, DE, USA). In a typical experiment, samples were heated at a ramp rate of 10 °C/min under nitrogen from 25–800 °C. All data was analyzed in TRIOS software (TA Instruments, New Castle, DE, USA) using the weight change function. A mass loss for drug cargo and MOF were obtained and used to calculate experimental drug loadings. The temperatures used for these calculations are included beneath each TGA plot.

N_2 Adsorption Isotherms (BET Surface Area Analysis)

N_2 adsorption and desorption isotherms were collected using a Micrometrics 3-Flex surface analyzer (Micromeritics, Norcross, GA, USA). To access permanent porosity, samples were

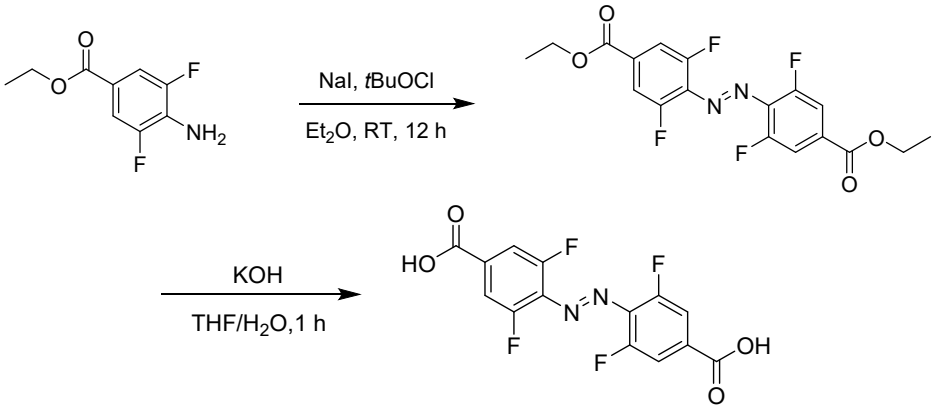
solvent exchanged in acetone for 3 days. The solvent was refreshed each day. Prior to analysis, samples were placed in sample cells and activated under vacuum (0.1 mbar) for 24 h at 120 °C. After analysis, PXRD was performed on each of the samples. PXRD data confirms that crystallinity was maintained throughout the activation/adsorption process.

Dynamic Light Scattering (DLS)
The size distribution of the UiO-AZB-F nanoparticles was measured using a Malvern ZetasizerNano-ZS, with three measurements taken per trial. Nanoparticles were suspended in ethanol via sonication and passed through a 0.45 µm filter prior to measurement. Particle size and zeta potential values represent the average of 3 distinct samples (2+ replicates per sample).

Scanning Electron Microscopy (SEM)
Silicon wafers were adhered to SEM stages *via* double-sided copper tape. MOF samples were suspended in ethanol solution and drop-cast onto the wafer and allowed to evaporate in air. SEM stages were coated with a 7 nm thick Pt and Pd layer prior to analysis. SEM images were collected with a LEO 1550 field-emission scanning electron microscope (Car Zeiss, Oberkochen, Germany) at 5.0 kV and a 7.0 mm working distance.

Transmission Electron Microscopy (TEM)
MOF samples were suspended in ethanol solution and drop-cast onto a lacey carbon TEM grid (300 mesh, 150 micron) and allowed to evaporate in air. TEM images were collected with a JEOL 2100 electron microscope at 200 kV.

III. Synthesis/Characterization of AZB-F



Scheme 1. Synthesis of 4,4'-(diazene-1,2-diyl)bis(3,5-difluorobenzoic acid)

Synthesis of diethyl 4,4'-(diazene-1,2-diyl)-bis(3,5-difluorobenzoate)[2]

In a 100 mL round-bottom flask, ethyl 4-amino-3,5-difluorobenzoate (3.996 g, 19.9 mmol), NaI (5.97 g , 39.8 mmol), and Et_2O (60 mL) were combined. The flask was sealed and placed under argon atmosphere. Next, *tert*-butyl hypochlorite (4.5 mL, 39.7 mmol) was added dropwise over a period of 10 min. The reaction was stirred under argon flow for 30 min, then removed and stirred for an additional 12 h at room temperature. The reaction was quenched with a 1.0 M aqueous

sodium thiosulfate solution (200 mL) and extracted with DCM (2 × 100 mL). The organic layers were collected, dried over calcium chloride and evaporated to obtain a reddish-brown solid (3.01 g crude yield). The solid was dissolved in EtOAc (70 mL) and, after being placed in a freezer overnight, a red-orange precipitate was collected. The product was further purified using a silica plug (solvent system: DCM/pentane 50/50), yielding the desired product as a dark red solid (1.89 g 48% yield).

R_f value (DCM/hexane 1:1): 0.11. **Isomeric ratio:** 91:9 **^{1}H NMR** ($CDCl_3$, 400 MHz): δ major isomer: 7.73 (dd, 4H), 4.42 (q, 4H), 1.42 (t, 6H). minor isomer: 7.55 (dd, 4H), 4.35 (q, 4H), 1.37 (t, 6H). **^{13}C NMR** ($CDCl_3$, 126 MHz): δ 163.7, 156.0, 154.0, 133.7, 114.0, 62.2, 14.2. **HRMS** (ESI +): 399.0926 $(M+H)^+$, 416.1229 $(M+NH_4)^+$, 421.0759 $(M+Na)^+$

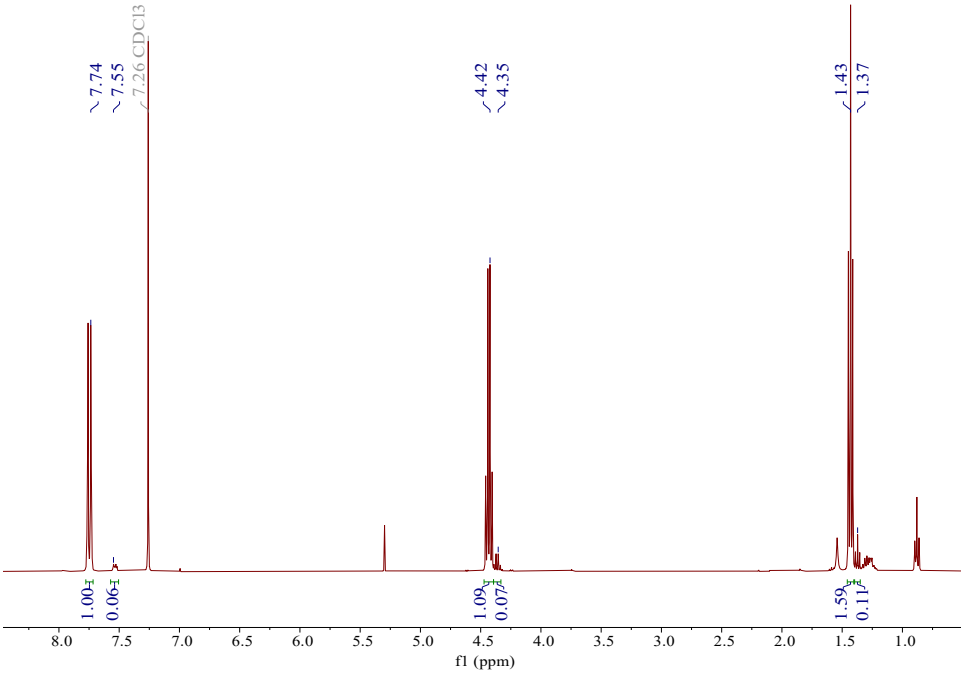


Figure S1. ^{1}H NMR of diethyl 4,4'-(diazene-1,2-diyl)-bis(3,5-difluorobenzoate)

Synthesis of 4,4'-(diazene-1,2-diyl)bis(3,5-difluorobenzoic acid)[3]

Diethyl 4,4'-(diazene-1,2-diyl)bis(3,5-difluorobenzoate) (0.4994 g, 1.25 mmol) was dissolved in THF (25 mL). In a separate flask, KOH (0.32 g, 17.77 mmol) was dissolved in H_2O (13 mL). The solutions were combined and stirred for 1 h. After this time, the reaction was cooled to room temperature. Next, 1.0 M HCl (20 mL) was added to precipitate out a solid. The light orange solid collected via centrifugation and washed with methanol. (0.42 g, 98% yield)

Isomeric ratio: 91:9 **^{1}H NMR** (d_6-DMSO, 400 MHz): δ major isomer: 13.85 (br s, 2H), 7.78 (dd, 4H) **^{13}C NMR** ($CDCl_3$, 126 MHz): δ 163.7, 156.0, 154.0, 133.7, 114.0, 62.2, 14.2. **^{19}F NMR** (d_6-DMSO, 400 MHz): δ -120.08

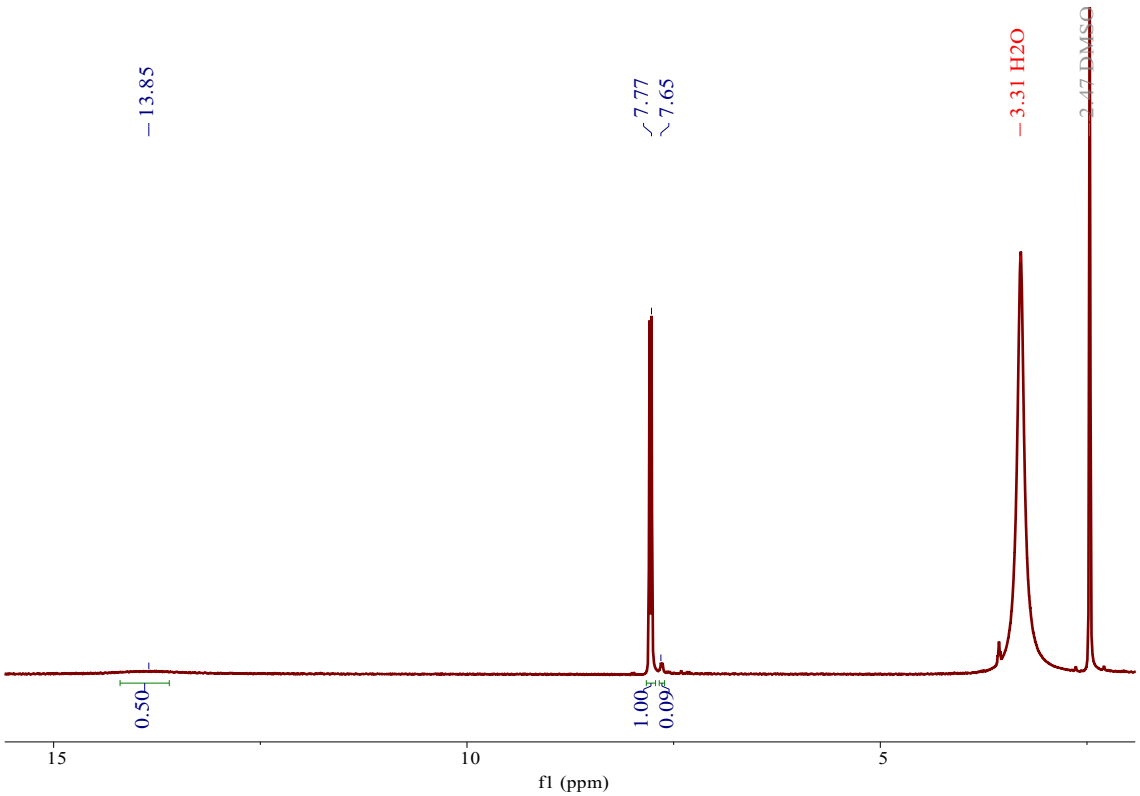


Figure S2. ^{1}H NMR of 4,4'-(diazene-1,2-diyl)bis(3,5-difluorobenzoic acid)

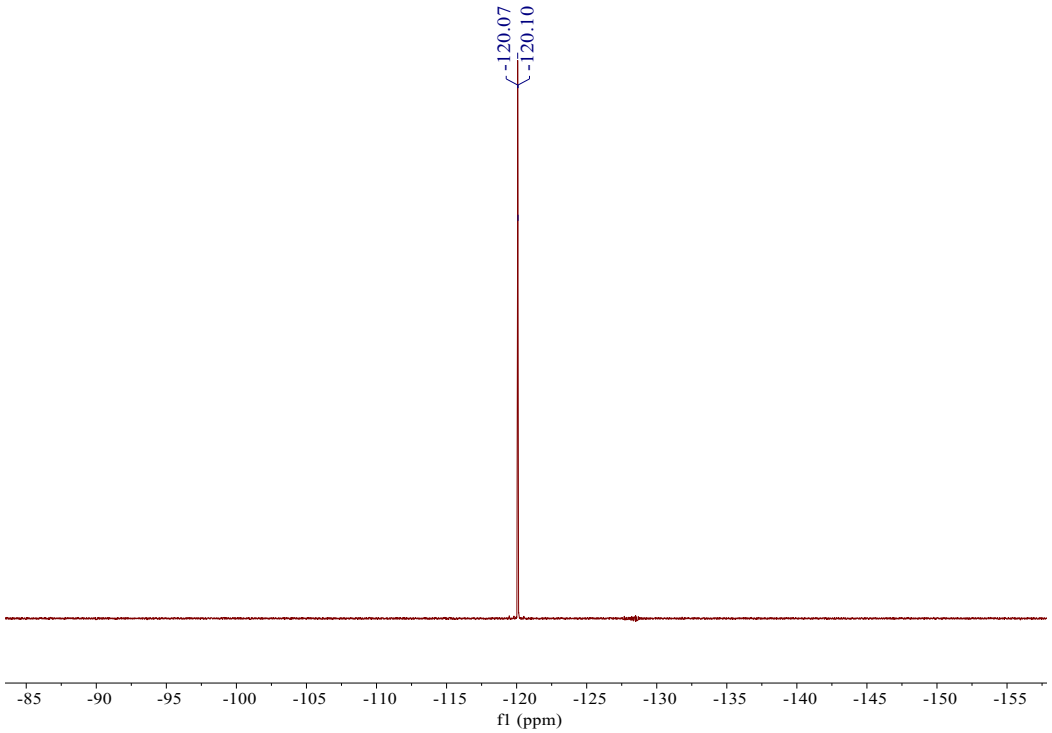


Figure S3. ^{19}F NMR of 4,4'-(diazene-1,2-diyl)bis(3,5-difluorobenzoic acid)

IV. Experimental/Simulated UV-Vis Data

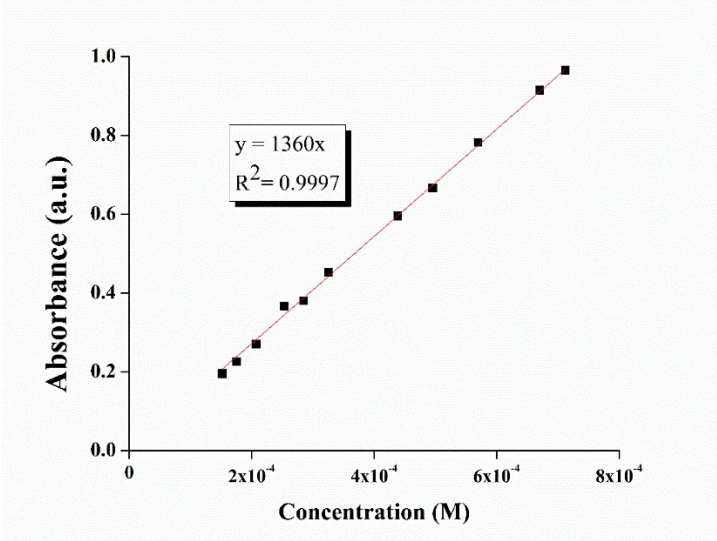


Figure S4. Calibration curve used to calculate molar extinction coefficient of AZB-F at 455 nm

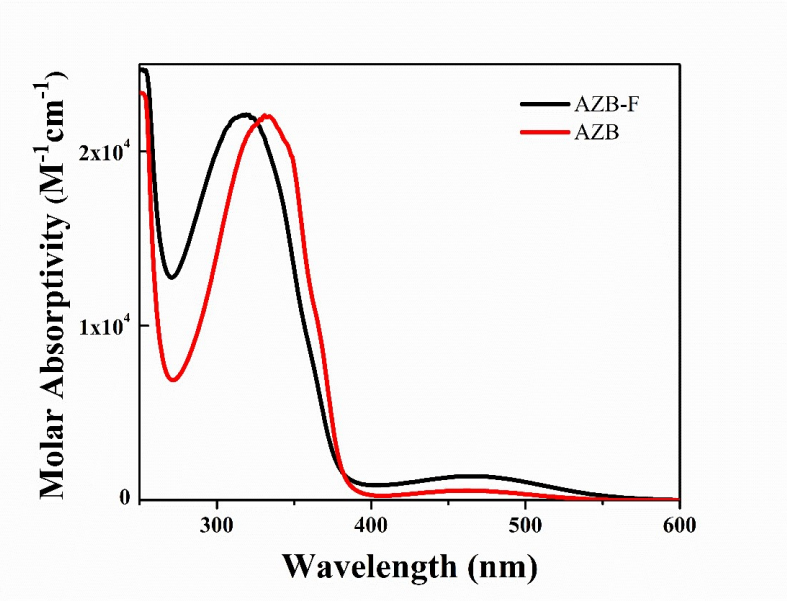


Figure S5. Comparison of molar absorptivity of AZB-F (black) and AZB (red) derivatives

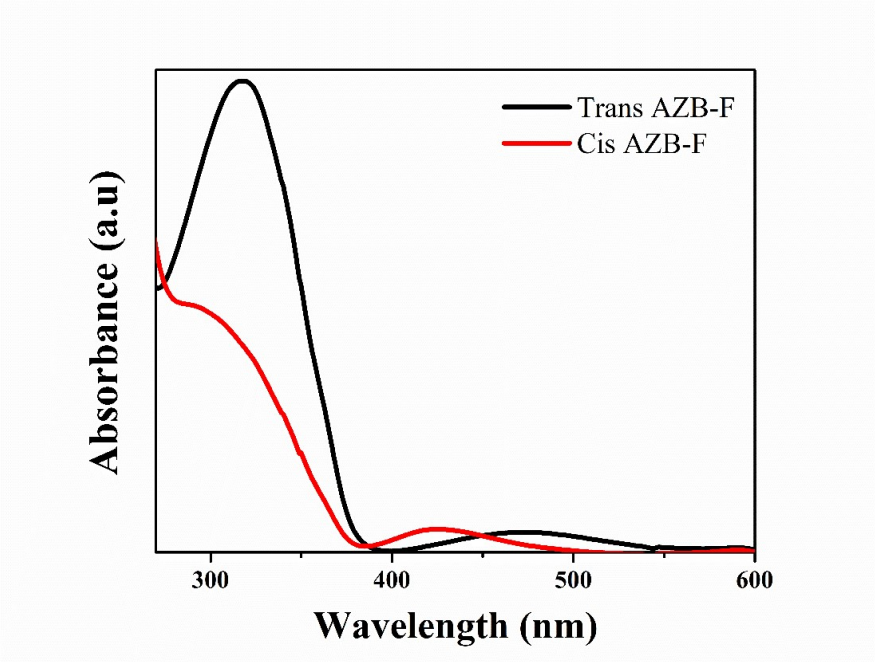


Figure S6. Experimental UV-Vis absorbance of AZB-F in trans (red) and cis (black) configurations (DMSO as solvent)

All calculations were performed using Gaussian 09[4] with the resources available at the Advance Research Computing at Virginia Tech. The geometries were optimized with the ωB97XD functional[5] and the 6-311G(d) basis set.[6] Solvation effects of DMSO were simulated using an implicit polarized continuum solvation model (CPCM).[7,8] The frequency calculations were performed at the same level of theory to confirm the absence of imaginary frequencies. The spectroscopic parameters were obtained using time-dependent DFT using the same functional, basis set, and solvation model as the ground-state optimization. Molecular orbital surfaces for HOMOs and LUMOs were visualized using GaussView 5.0.

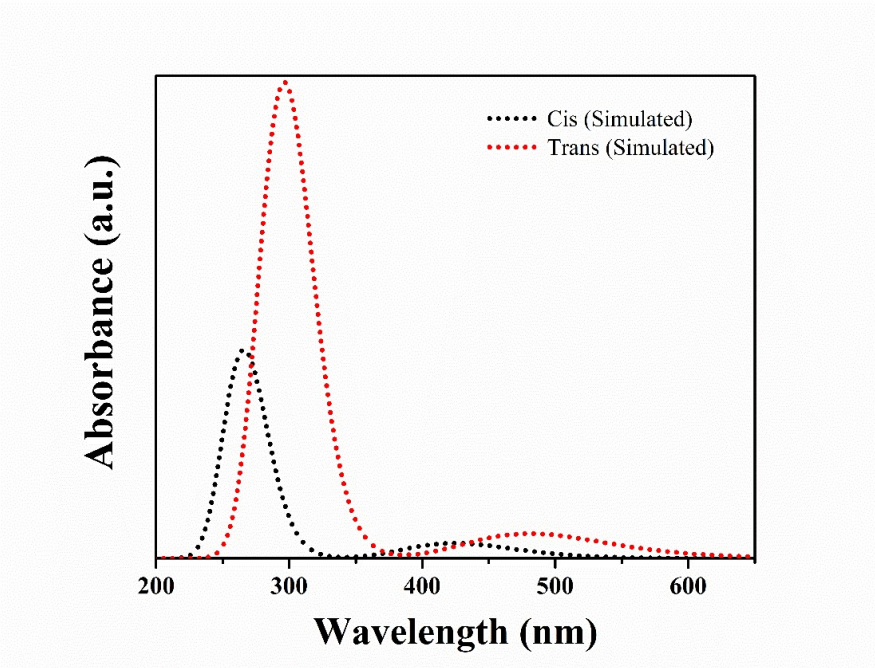


Figure S7. Simulated UV-Vis absorbance of AZB-F in trans (red) and cis (black) configurations

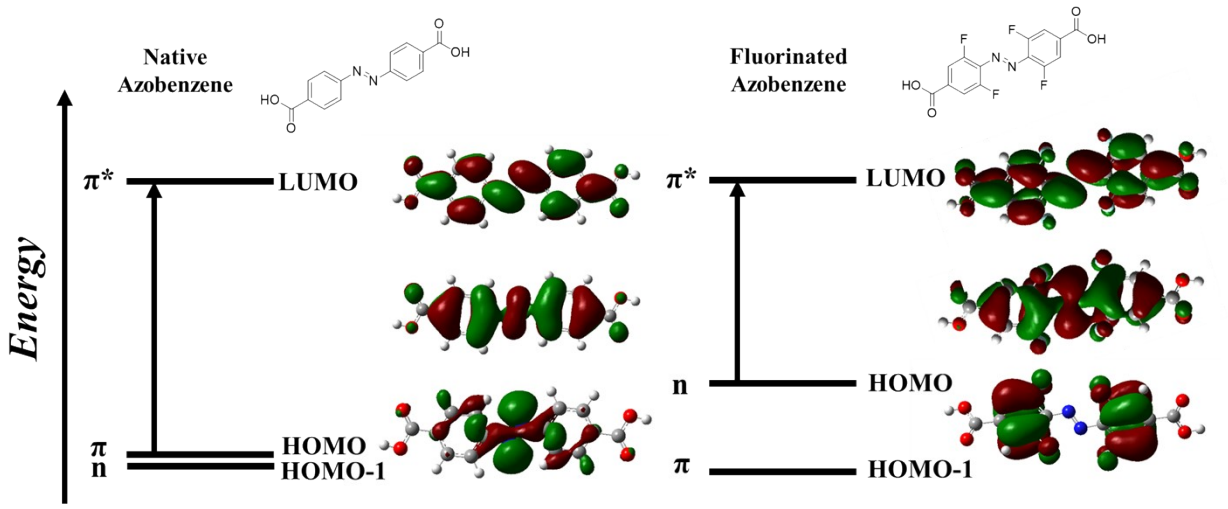


Figure S8. Depiction of frontier molecular orbitals for AZB (left) and AZB-F (right)

Table S1. Relative HOMO/LUMO energies (from calculations)

	HOMO	**LUMO**
4,4′-azobenzenedicarboxylic acid (AZB)	-0.326	0.051
4,4'-(diazene-1,2-diyl)bis(3,5-difluorobenzoic acid) (AZB-F)	-0.326	-0.056

V. Synthesis/Characterization of UiO-AZB-F

Traditional UiO-AZB-F Synthesis

In a typical synthesis, $ZrCl_4$ (23.7 mg) and DMF (1.5 mL) were combined in a 6-dram vial. Next, formic acid* (75.5 µL) was added, and the mixture was sonicated for 5 min. 4,4'-(diazene-1,2-diyl)bis(3,5-difluorobenzoic acid) (34.9 mg) was added, followed by H_2O (75 uL), and the mixture was sonicated again. The vial was placed on a stir plate for 1-2 min to ensure the starting materials were fully dissolved, then placed in a 120 °C oven for 15 min. Next, the vials were cooled to RT and the contents were transferred to 15 mL centrifuge tubes. The particles were collected via centrifugation (5 min) and subsequently washed with DMF (x1) and acetone (x3). After solvent exchange, particles were collected via centrifugation and dried under vacuum for 1 day at 60 °C.

**Optimized conditions (formic acid, 20 eq. to Zr) are described. For the modulator screening, conditions were kept the same, but other modulators (acetic acid 30-50 eq., hydrochloric acid 10-30 eq., and difluoroacetic acid 30-50 eq. to Zr) were used instead of formic acid.*

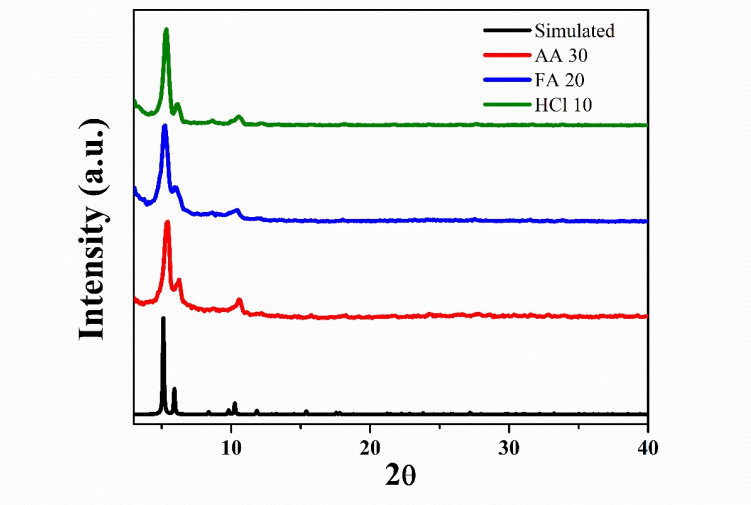


Figure S9. Experimental PXRD patterns for UiO-AZB-F under select modulated conditions: acetic acid, 30 equiv. (red), formic acid, 20 equiv. (blue), and HCl, 10 equiv. (green). The experimental patterns match that of the simulated pattern (black) for UiO-AZB (CCD#:889532).[9]

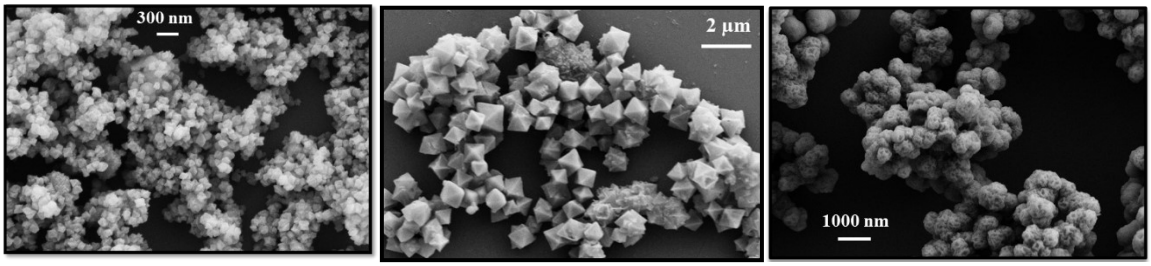


Figure S10. SEM Images of UiO-AZB-F under select modulated conditions: (from left to right) formic acid (20 eq.), acetic acid (30 eq.), and HCl (10 eq.)

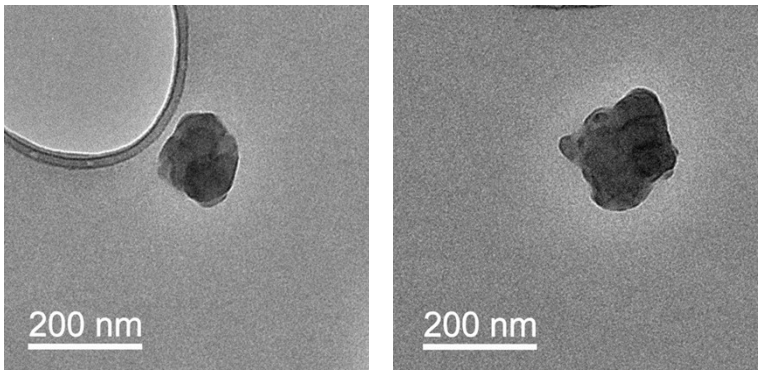


Figure S11. TEM Images of individual UiO-AZB-F (FA 20) particles

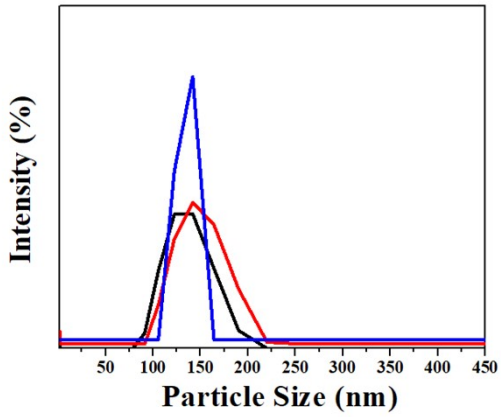


Figure S12. DLS analysis of UiO-AZB-F (Intensity weighted distribution)

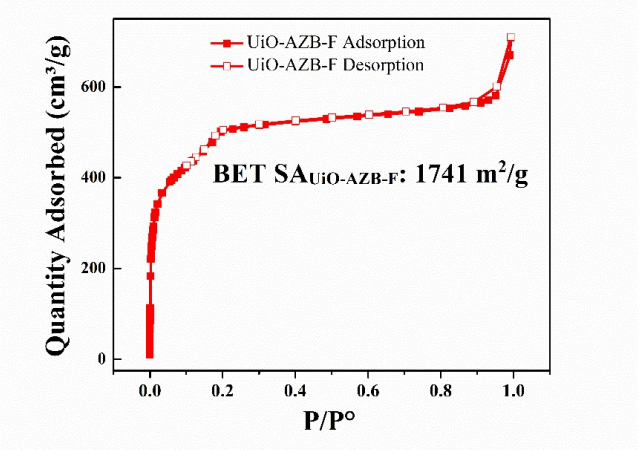


Figure S13. N_2 adsorption isotherm for UiO-AZB-F (formic acid, 20 eq.)

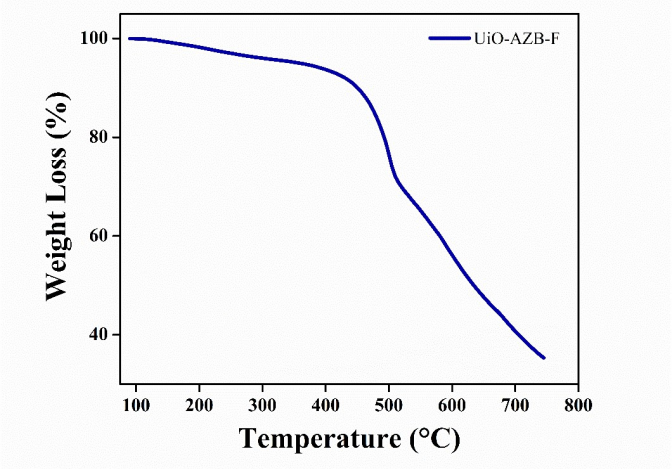


Figure S14. Thermogravimetric analysis (TGA) of UiO-AZB-F MOF under N_2. The initial weight loss between 100–200 °C is attributed to loss of residual modulator (formic acid) and the second weight loss occurring from 450 °C–700 °C corresponds to degradation of the linker/framework

VI. Degradation Studies of UiO-AZB-F

UiO-AZB-F Degradation Studies

For degradation studies, 2-3 mg of MOF was placed in a 24/40 joint cuvette and layered with 3 mL of DMSO. DMSO was selected as the solvent for these experiments due to higher solubility of linker in solution (AZB-F is insoluble in aqueous solution at pH=7). The sample was capped with a septum, covered, and left for 2 h to ensure particles were fully settled to the bottom of the solution. Next, samples were placed in a green light LED box and irradiated for 8 h. The linker release was monitored by collecting UV-Vis spectra and monitoring the change in absorbance at 455 nm (the isosbestic point of 4,4'-(diazene-1,2-diyl)bis(3,5-difluorobenzoic acid)) every hour. For control experiments, this procedure was repeated, but instead of irradiating samples, cuvettes were wrapped in aluminum foil and heated at 37 °C for 8 h. The total concentration of MOF in the cuvette can be calculated based on the mass of MOF added (m), the molecular weight of UiO-AZB-F (MW=2721 g/mol), and volume of DMSO added (V).

$$C_{Total} = \frac{6\,m}{(MW_{UiOAZB-F})V}$$

The concentration of degraded MOF present in solution was calculated according to the equation below, where Abs_t is the measured absorbance @ 455 nm at a given timepoint (t=0–8h).

$$C_{Degraded} = \frac{Abs_t}{\varepsilon_{455\,nm}}$$

Using these values, the percent degradation of MOF is calculated as:

$$\%\,Degradation = \frac{C_{Degraded}}{C_{Total}} \times 100$$

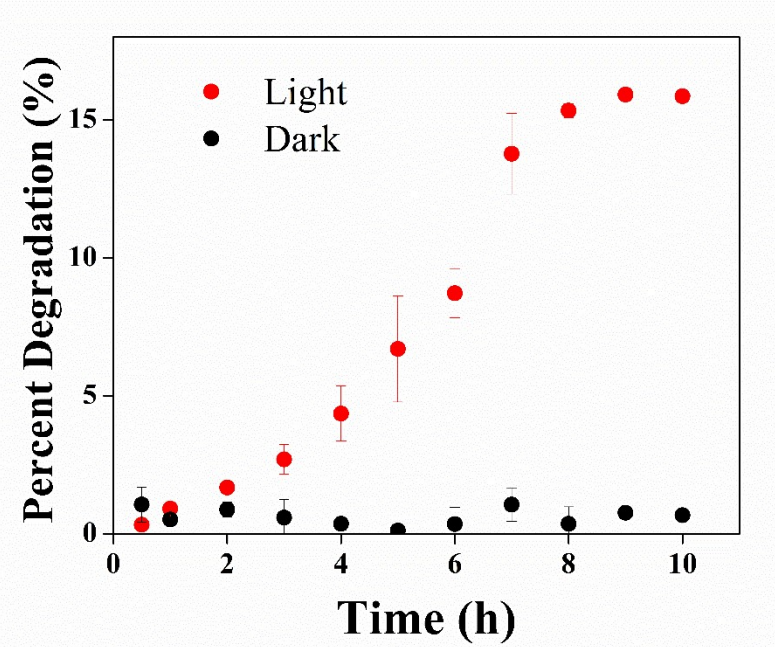
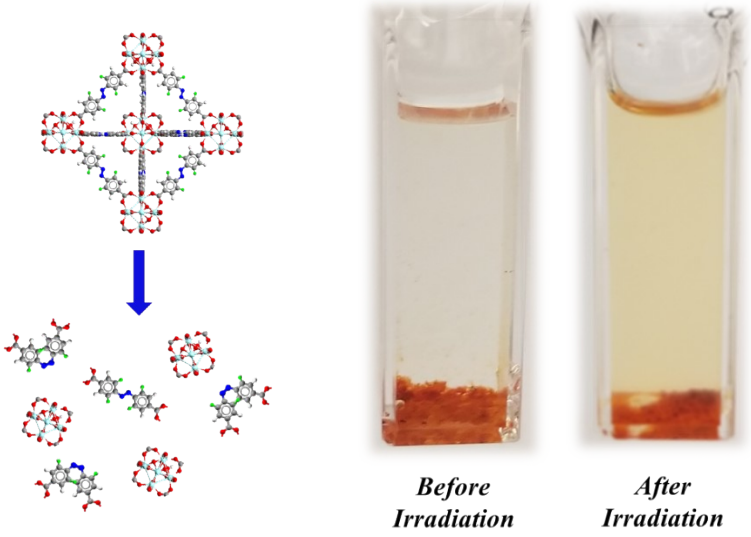


Figure S15. Calculated percent degradation of UiO-AZB-F when irradiated with green light (red) or heated at 37 °C in the dark (black) for 10 h. After 8h, the red profile plateaus due to reaching the solubility limit of AZB-F in solution. During the 8 h period, irradiated samples show up to 15% degradation, while heated samples show less than 1% degradation on average.



Scheme 2. Visual demonstration of MOF degradation (left) and visual color change observed after the irradiation period (right)

VII. Preparation of $PEGNH_2$-UiO-AZB-F+5FU

UiO-AZB-F Drug Loading (Post-synthetic)

In a flask, 400 mg of 5-fluorouracil was added to 120 mL EtOH. The mixture was stirred vigorously at 70 °C until the drug was fully dissolved (45 min). The solution was removed from heat. Next, UiO-AZB (F) (100 mg) was added, and the flask was sonicated for 15 min to suspend the MOF particles. After sonication, the suspension was stirred at 60 °C for 5 days. The particles were collected via centrifugation, washed with EtOH to remove surface-bound drug, and dried under vacuum for 1 day at 60 °C.

UiO-AZB-F Drug Loading (With sonication)

In a vial, 300 mg of 5-fluorouracil was added to 25 mL MeOH. The mixture was stirred vigorously at 70 °C until the drug was fully dissolved (45 min). Next, UiO-AZB (F) (100 mg) was added. The suspension was then sonicated with a tip sonicator for 20 h. The particles were collected via centrifugation, washed with EtOH to remove surface-bound drug, and dried under vacuum for 1 day at 60 °C.

UiO-AZB-F Drug Loading (In -situ)

In a typical synthesis, $ZrCl_4$ (23.7 mg) and DMF (1.5 mL) were combined in a 6-dram vial. Next, formic acid (75.5 µL) was added, and the mixture was sonicated for 5 min. 4,4'-(diazene-1,2-diyl)bis(3,5-difluorobenzoic acid) (34.9 mg) was added and sonicated briefly. Next, 5-fluorouracil (500 mg) and H_2O (75 µL) were added to the vial, and the mixture was sonicated again. The vial was heated to 120 °C on a hotplate and stirred for 15–20 min. Next, the vials were cooled to RT and the contents were transferred to 15 mL centrifuge tubes. The particles were collected via centrifugation (5 min) and washed with DMF (x1) and acetone (x3).

PEG-NH_2 Polymer Coating

Particles are resuspended in EtOH (5 mL) and transferred to a vial and sonicated. PEG-NH_2 (100 mg) is added to the solution and the vial is sonicated until contents are fully suspended (~15 min). The solution was stirred at room temperature for 24 h in the dark. After, the particles were collected via centrifugation and washed with EtOH (x1). After washing, the particles were collected via centrifugation and dried under vacuum for 1 day at 60 °C.

For the purposes of this paper, drug loading is calculated as:

$$Weight\ \% = \frac{Mass\ of\ drug}{Mass\ of\ MOF} \times 100$$

Drug loading was determined using quantitative ^{19}F NMR. First, the molar ratio of drug:linker was calculated according to the equation below:

$$\frac{mol\ 5FU}{mol\ linker} = \frac{Intergration\ of\ 5FU\ peak}{\#\ of\ F\ in\ 5FU} \times \frac{\#\ of\ F\ in\ Linker}{Integration\ of\ linker\ peak}$$

The value was then converted to molar ratio of drug:MOF using the molecular formula (6 mol AZB-F linker : 1 mol UiO-AZB-F). Finally, the weight percent was calculated using the molar masses of drug and MOF (130.08 and 2289 g/mol respectively).

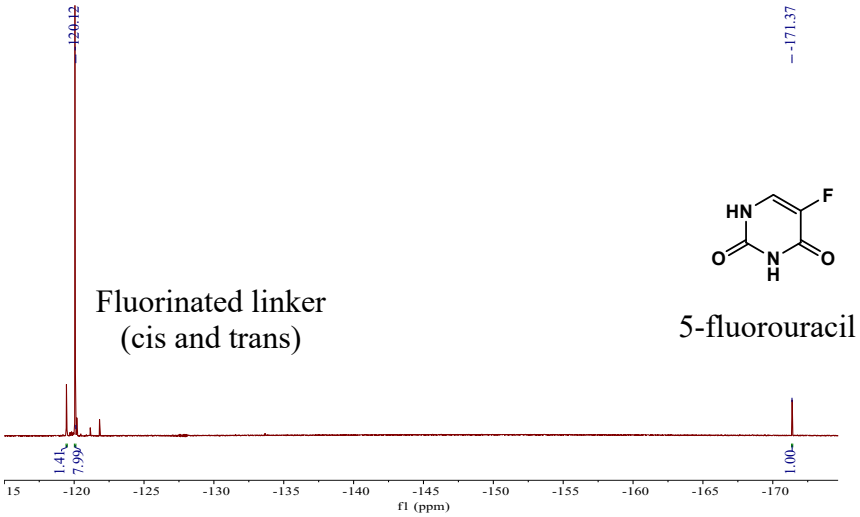


Figure S16. Quantitative ^{19}F NMR on digested MOF sample to quantify 5FU loading

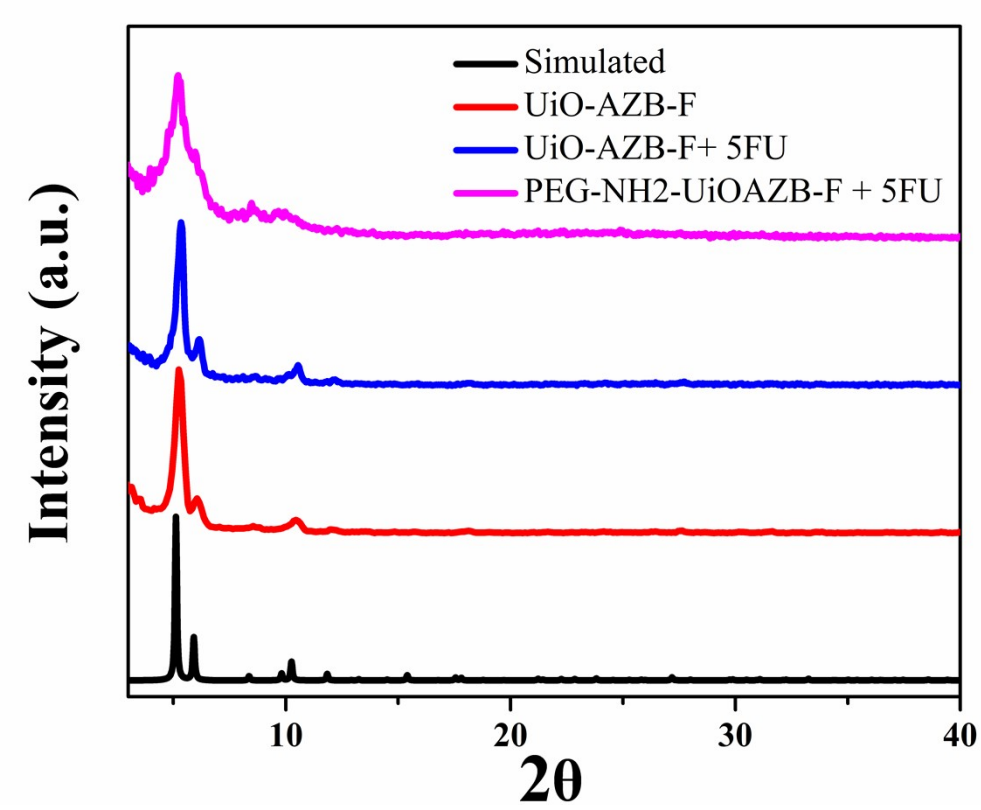


Figure S17. PXRD of $PEGNH_2$-UiO-AZB-F+5FU

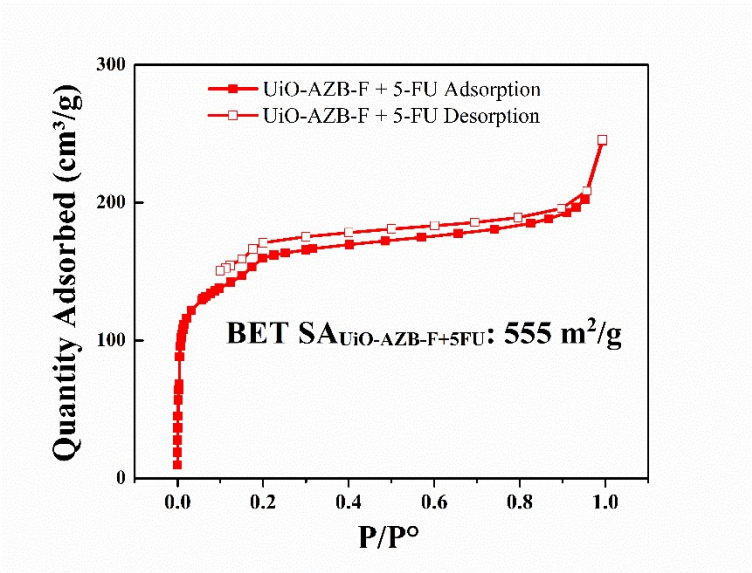


Figure S18. BET Data of UiO-AZB-F+5FU. The BET SA is reduced after drug loading, indicating incorporation of drug within MOF pores.

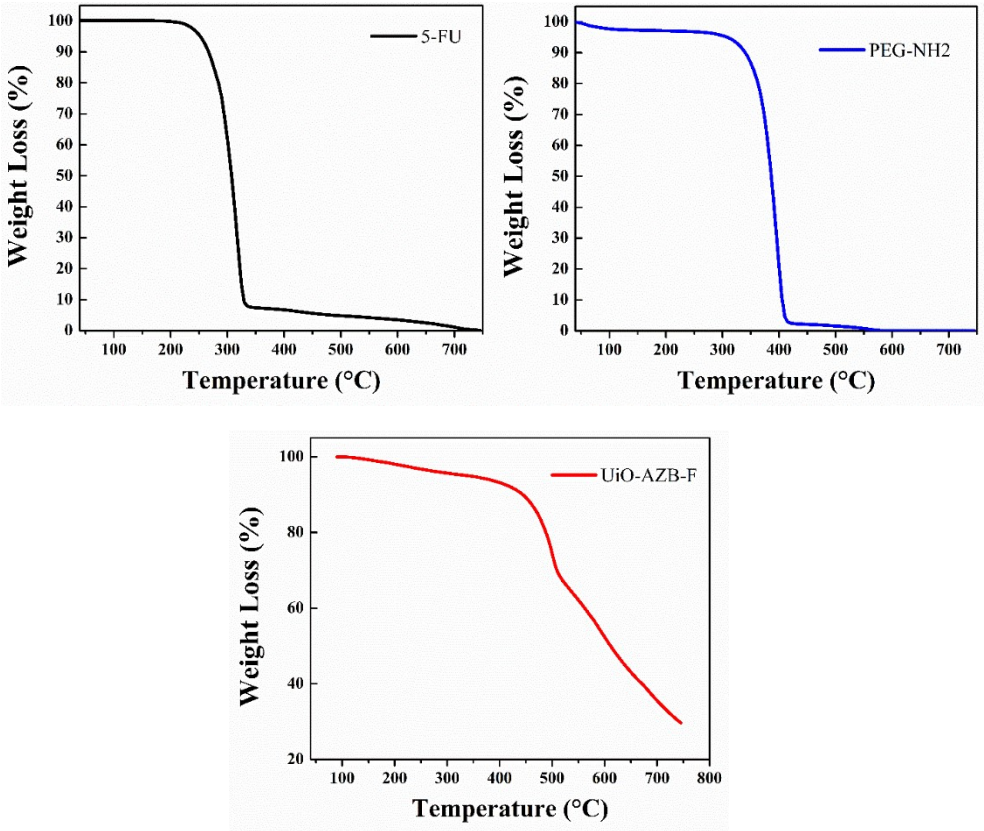


Figure S19. TGA of starting materials: 5-FU (black), PEG-NH_2 (blue), and UiO-AZB-F (red).

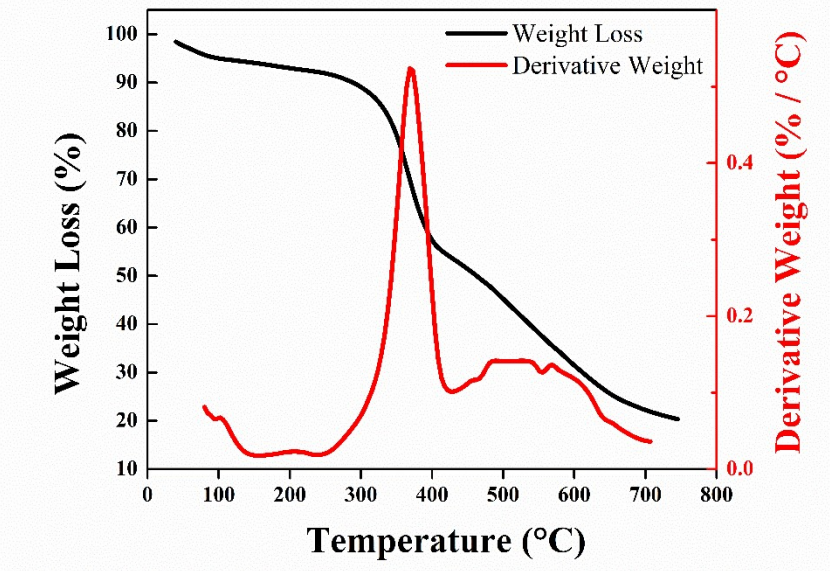


Figure S20. TGA analysis of PEG-NH_2-UiO-AZB-F+5-FU. The first major weight loss occurring from 225–410 °C is attributed to loss of drug and polymer. The second major weight loss occurring from 450–750 °C is due to degradation of the organic linker.

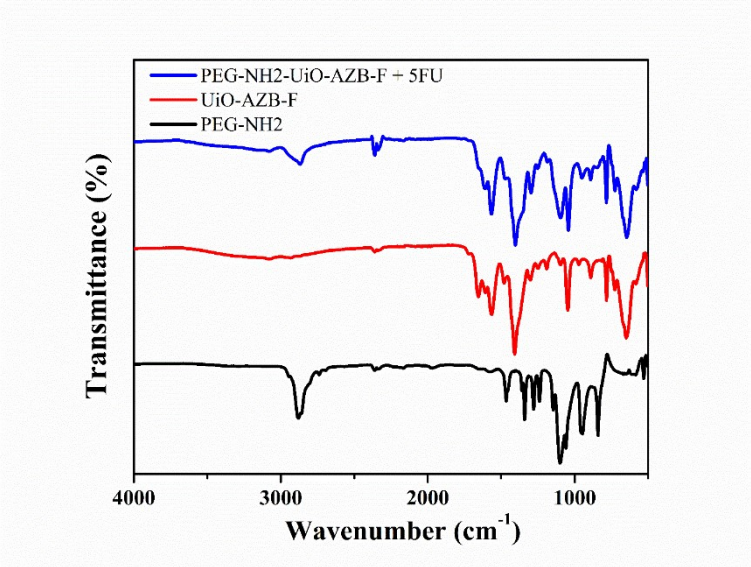
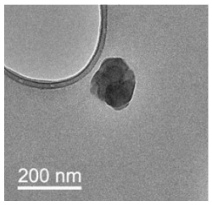
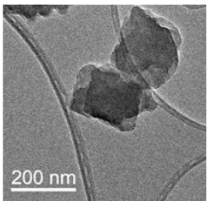
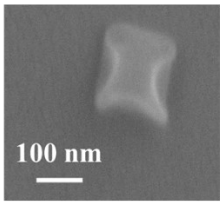
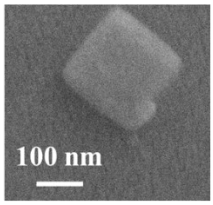


Figure S21. IR analysis of PEG-NH_2 (black), UiO-AZB-F (red), and PEG-NH_2-UiO-AZB-F+5FU (blue). The PEG-NH_2-UiO-AZB-F+5FU shows enhanced signal at 2300 cm^{-1} and a new signal at 2800 cm^{-1} (corresponding to NH_2 stretch).

Table S2. Morphology of UiO-AZB-F before and after 5FU loading

	PEGNH2-UiO-AZB-F	*PEGNH2-UiO-AZB-F+5FU*
TEM		
SEM		
Average Particle Diameter	150 ± 10 nm	141 ± 3 nm
PDI	0.24	0.25
Zeta Potential	-7 ± 6 mV	-26 ± 8 mV

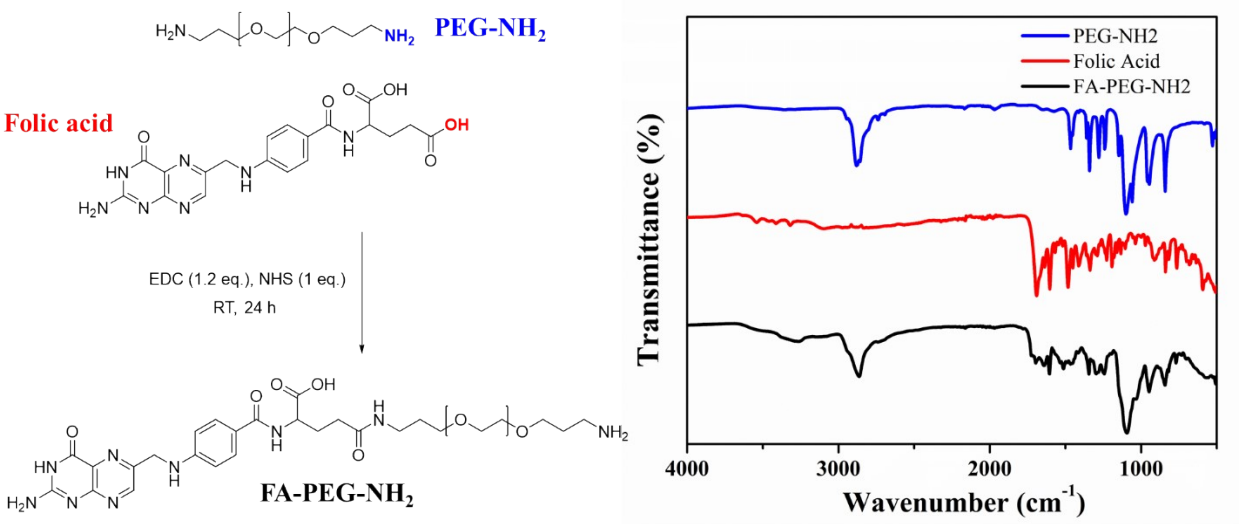


Figure S22. IR analysis confirming attachment of folic acid (red) to PEG-NH_2 (blue) scaffold.

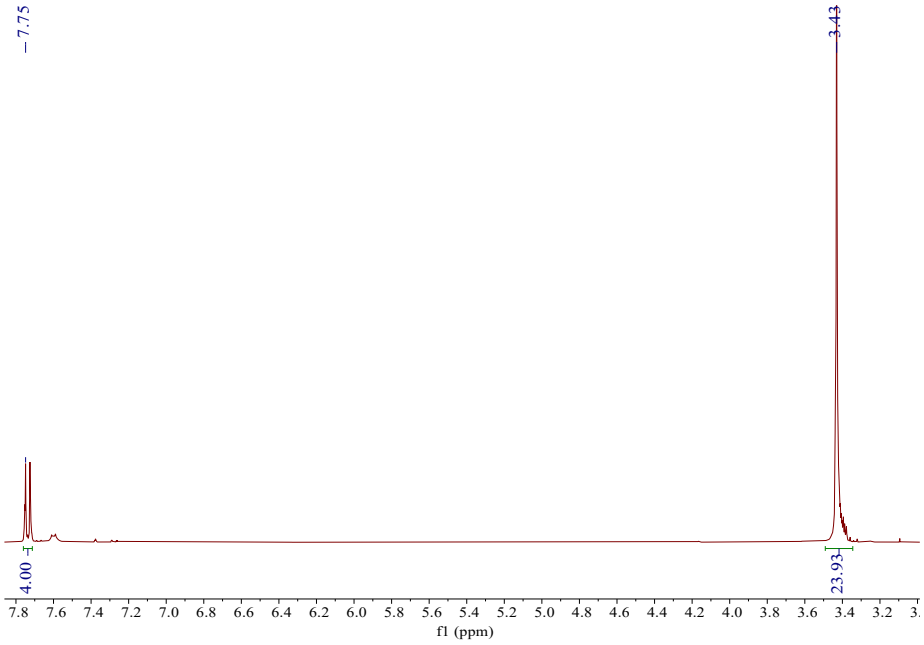


Figure S23. ^{1}H NMR Digestion of PEG-NH_2-UiO-AZB-F+5FU

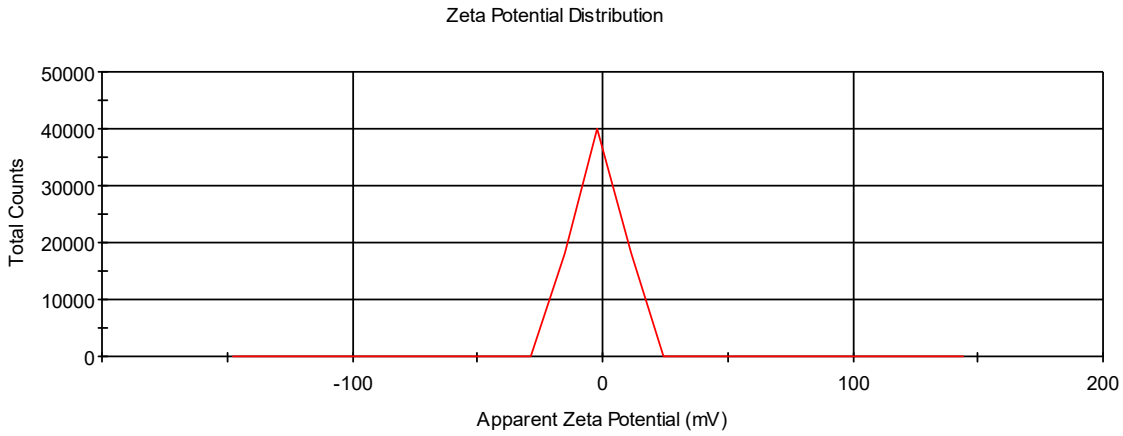


Figure S24. Sample zeta potential measurement for PEGNH_2-UiO-AZB-F (average: -2 mV)

VIII. 5FU Release Studies

5-fluorouracil Release Studies

To quantify 5-fluorouracil release via ^{19}F NMR, an internal standard was used. A stock solution of internal standard (2,6-difluorobenzoic acid, IS) and D_2O was prepared, and pH adjusted to 7.2. In a 24 well plate, between 2-4 mg of drug loaded material was added to each well. Next, 700 µL of IS solution was added to each well. The well plate was placed an LED irradiation chamber, and irradiated for 15, 30, 60, 90, or 120 min. After the designated time, the sample was filtered through a 0.45 µm syringe filter to remove MOF particles and transferred to an NMR tube for analysis. For the dark control experiments, sample preparation and workup is the same, but the well plate was wrapped in aluminum foil. (*Note*: the linker itself does not show up in these spectra because it is only soluble in aqueous solution at very acidic or basic pH).

The molar ratio of 5FU:IS was calculated according to the equation below:

$$\frac{mol\ 5FU}{mol\ IS} = \frac{Intergration\ of\ 5FU\ peak}{\#\ of\ F\ in\ 5FU} \times \frac{\#\ of\ F\ in\ IS}{Integration\ of\ IS\ peak}$$

To account for slight differences in initial sample mass/loading efficiency across trials, percent of release from the framework is calculated. The total amount of 5FU in the MOF is calculated by multiplying the mass of MOF added by the wt% loading (calculated previously). The absolute mass of 5FU released is estimated from ^{19}F NMR.

$$\%\ 5FU\ Release = \frac{Mass\ of\ 5FU\ (from\ 19F\ NMR)}{Total\ Mass\ of\ 5FU\ in\ MOF} \times 100$$

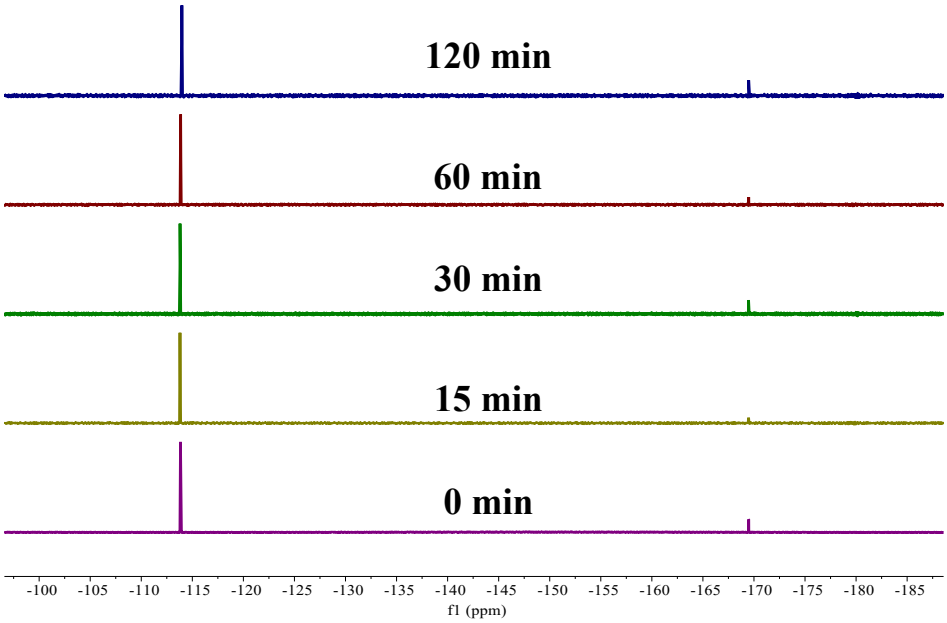


Figure S25. Quantitative ^{19}F NMR of 5-FU release from PEG-NH_2-UiO-AZB-F in the dark. The signals for the internal standard 2,6-difluorobenzoic acid and 5-fluorouracil are visible at -114 and -169.5 ppm respectively. The intensity of the 5-FU peak remains the same throughout the time trial, indicating that the carrier shows no release after the original "burst".

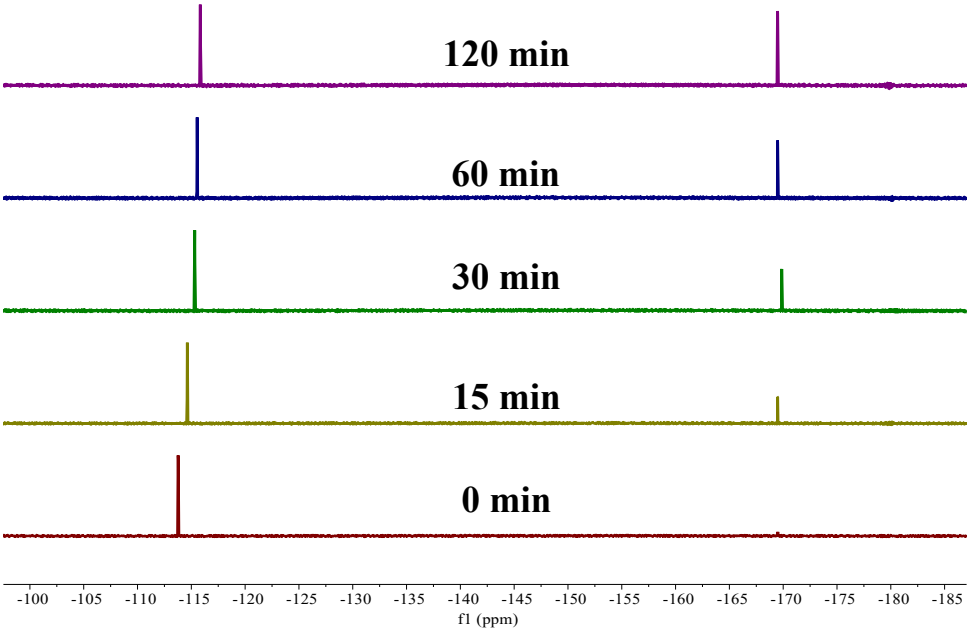
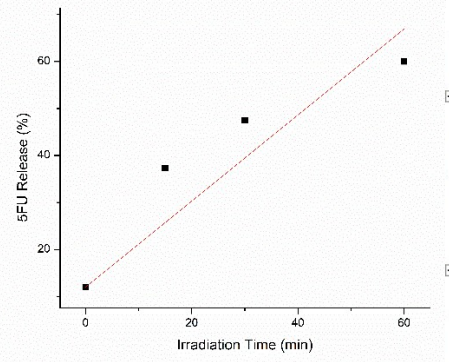


Figure S26. Quantitative ^{19}F NMR of 5-FU release from PEG-NH_2-UiO-AZB-F under green light irradiation. The signals for the internal standard 2,6-difluorobenzoic acid and 5-fluorouracil are visible at -114 and -169.5 ppm respectively. The position of the internal standard peak shifts slightly, likely due to the increasing amount of 5-fluorouracil present in solution.



		Value	Standard Error
5FU Release	Intercept	12	--
	Slope	0.9154	0.13215

Statistics

	5FU Release
Number of Points	4
Degrees of Freedom	3
Residual Sum of Squares	247.55238
Pearson's r	0.94485
Adj. R-Square	0.95537

Summary

	Intercept		Slope		Statistics
	Value	Standard Error	Value	Standard Error	Adj. R-Square
5FU Release	12	--	0.9154	0.13215	0.95537

Figure S27. Rate analysis of 5FU release from PEGNH_2-UiO-AZB-F. A regression was fit to the linear portion of the release profile (time points 0–60 min).

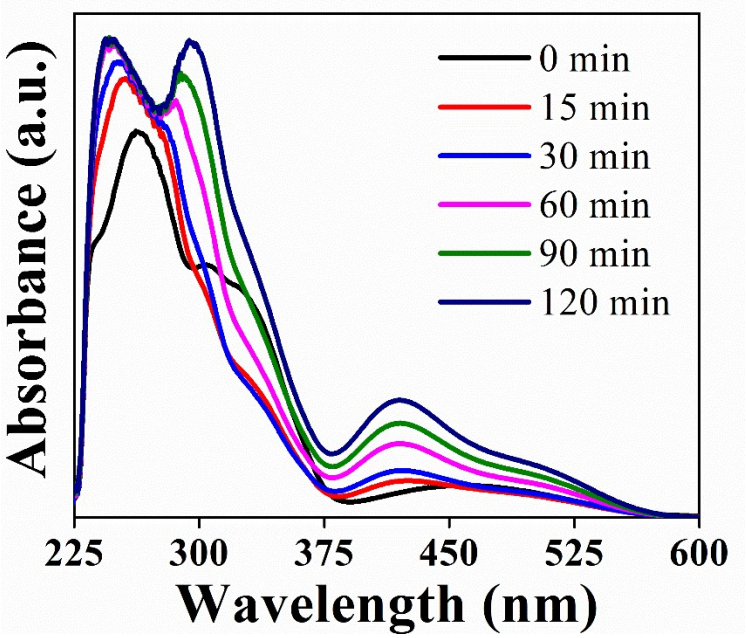


Figure S28. UVVis spectra showing synchronized particle degradation (AZB-F quantified between 400-525 nm) and 5FU release (increasing peak @ 320 nm).

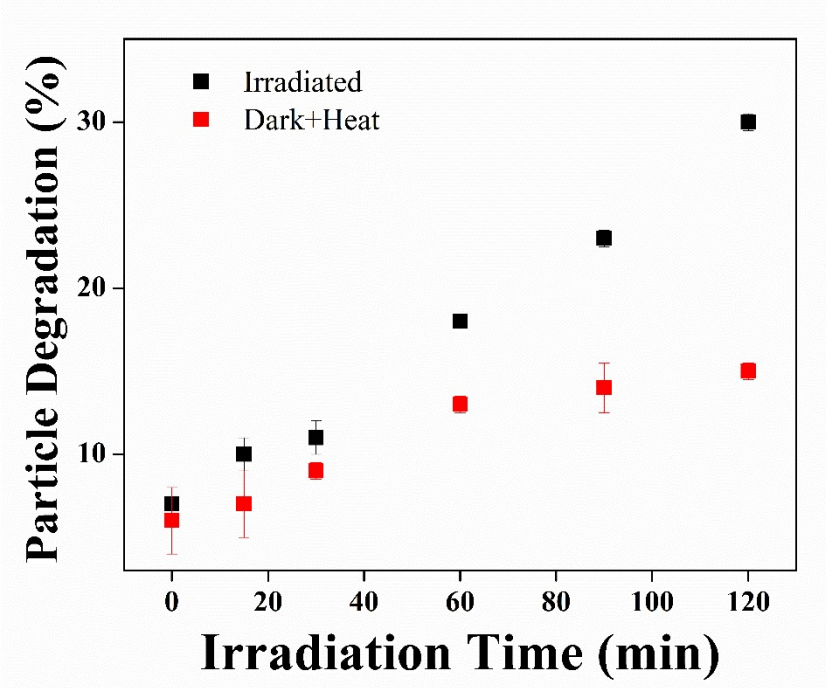


Figure S29. Particle degradation in PBS/FBS/McCoys media under irradiation (black) and dark + heat (red)

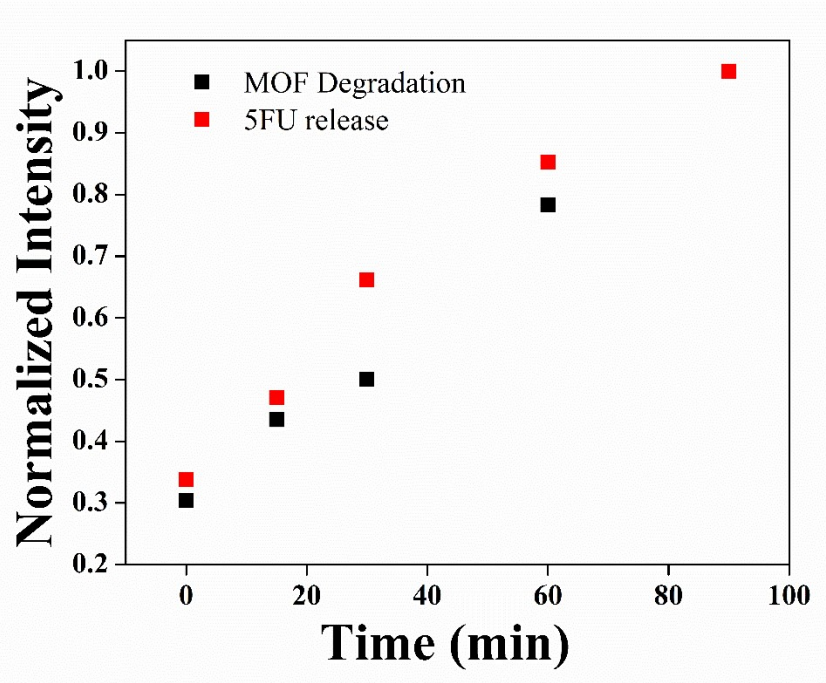


Figure S30. Normalization and overlay of MOF degradation (black) and 5FU release (red)

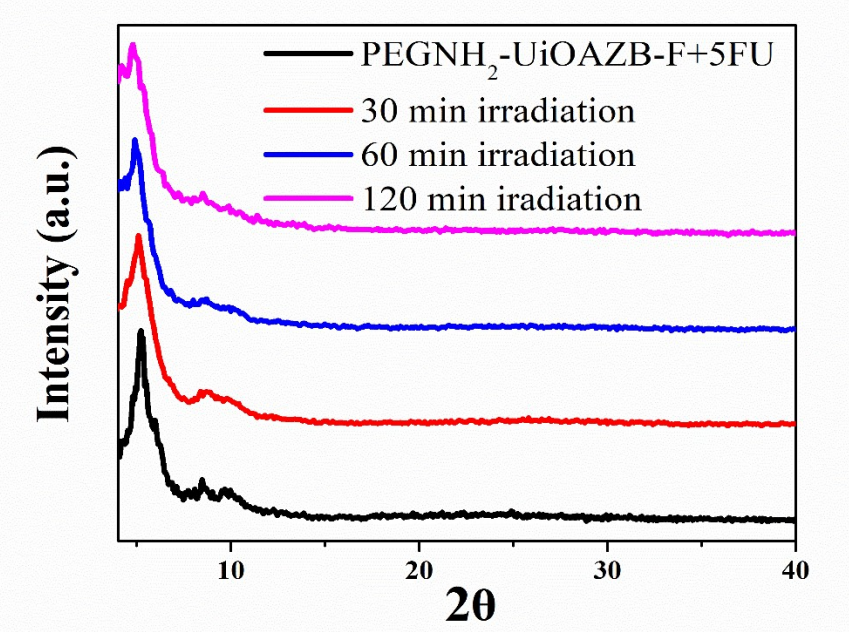


Figure S31. PXRD patterns of MOF particles at various points throughout the irradiation treatment, supporting the photo-degradation of particles over time

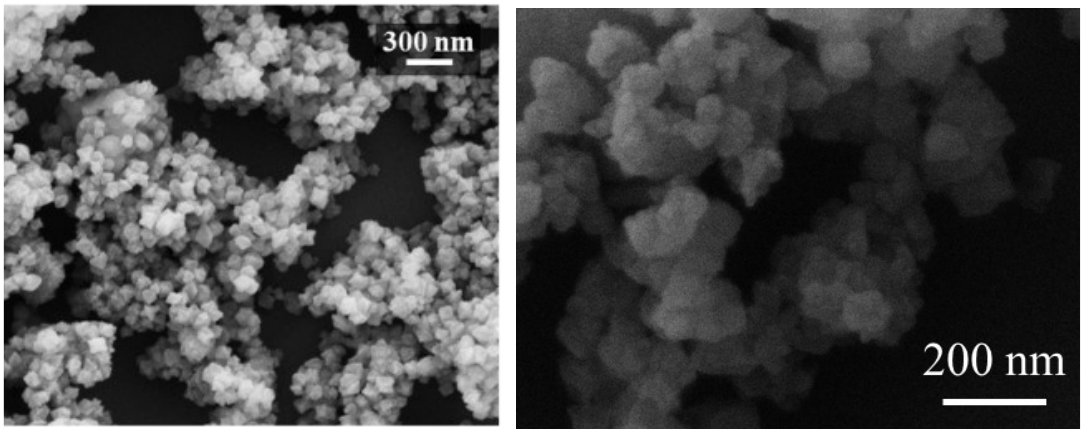


Figure S32. SEM images of MOF particles before (left) and after two hours of irradiation treatment (right), showing a decrease in average particle size.

IX. Cell Studies

Materials

Trypsin (0.25%)−EDTA was purchased from VWR (Radnor, PA, USA). Human colorectal carcinoma cells (HCT116) were continuously cultured in our laboratory. McCoy's 5A Medium was purchased from HyClone™ Cytiva (Marlborough, MA, USA). Dulbecco's Phosphate-Buffered Saline (DPBS) was purchased from Gibco (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Fetal bovine serum (FBS) was purchased from VWR (Radnor, PA, USA). 50 IU/mL penicillin and 50 µg/mL streptomycin were purchased from MP Biomedicals (Santa Ana, California, USA). All other reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA) or VWR (Radnor, PA, USA), unless otherwise stated.

Cell Culture

Cell studies were conducted using a human colorectal carcinoma cell line (HCT116). Cultures were grown in McCoy's 5A medium (HyClone™ Cytiva, Marlborough, MA), supplemented with 10% fetal bovine serum (FBS, VWR, Radnor, PA), 50 IU/mL penicillin, and 50 µg/mL streptomycin (MP Biomedicals). Cells were cultured at 37 °C in 5% CO_2-air. The media was changed every other day. The cultures were passaged after 70–80 % confluence was achieved. Cells were rinsed with 1X PBS solution three times, and then released with 0.25 % trypsin-EDTA solution (VWR, Radnor, PA). The suspension of released cells was centrifuged at 1000 rpm for 5 min before counting and plating for experiments.

Cell Viability Assays

HCT116 cells were plated in a 96-well plate at a density of 5000 cells per well in 200 µL complete McCoy's 5A medium per well. After culturing for 24 h, the media was discarded and the cells

were washed with 1X PBS three times before 180 µL serum containing McCoy's 5A medium was added. Next, 20 µL of either PBS, 5FU (100 µg/mL), $PEGNH_2$-UiO-AZB-F (500 µg/mL, 1 mg/mL, 2 mg/mL, suspended in PBS), or $PEGNH_2$-UiO-AZB-F+5FU (20 wt% 5-FU loading, 500 µg/mL, 1 mg/mL, 2 mg/mL, suspended in PBS) was added separately to 5 wells for each treatment group. The final concentrations of MOF (with or without 5-FU) ranged from 50 to 200 µg/mL. Two identical plates were prepared: One plate was placed inside the light box that was inside a biosafety hood (which had been sterilized with 70% alcohol followed by overnight UV light treatment) for 2 h light treatment (15 min light-on, then 15 min light-off); the other plate was covered with aluminum foil and placed in the same biosafety hood as the control plate but not in the light box. After 2 h treatment, both plates were placed in the incubator. The light treatment was repeated at 24 h and 48 h by removing both plates from the incubator, treating the treatment plate with light and covering the other with foil, then returning both plates to the incubator after the 2 h treatment. After incubation for 72 h, cells were washed three times with 1X PBS and then treated with serum-free ECGM media (100 µL) and 10 µL Cell counting kit 8 solution (CCK-8, Dojindo, Rockville, MD). After incubation for another 3 h to allow for development of the CCK8 dye, absorbance was recorded at 450 and 750 nm using a BioTek Synergy Mx plate reader (BioTek, Winooski, VT). Worked-up data (absorbance at 750 nm subtracted from absorbance at 450 nm) were graphed using GraphPad InStat, version 3 (GraphPad Software, Inc., San Diego, CA). Mean values are reported together with the standard error of mean (SEM) representing the combination of 3 different experimental runs with five replicates per experiment.

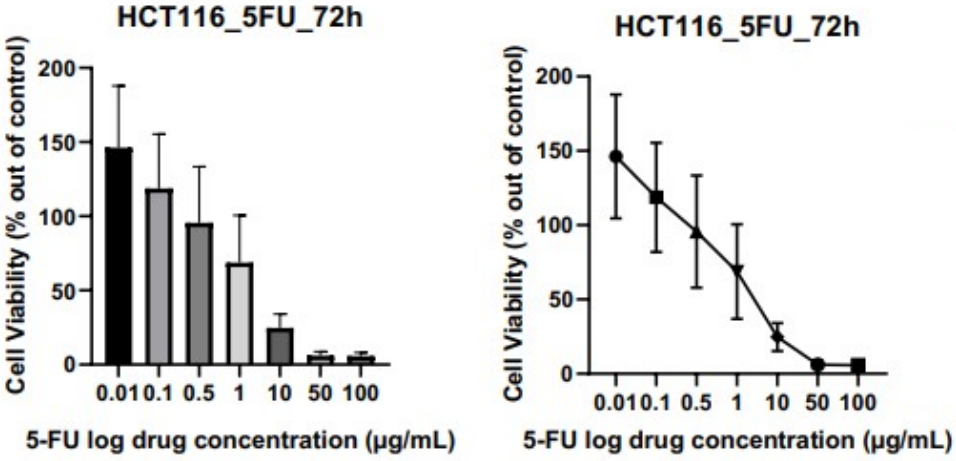


Figure S33. CCK-8 Results for 5FU incubation (72 h). IC_{50} for HCT-116 cell line was determined to be 3±2 µg/mL

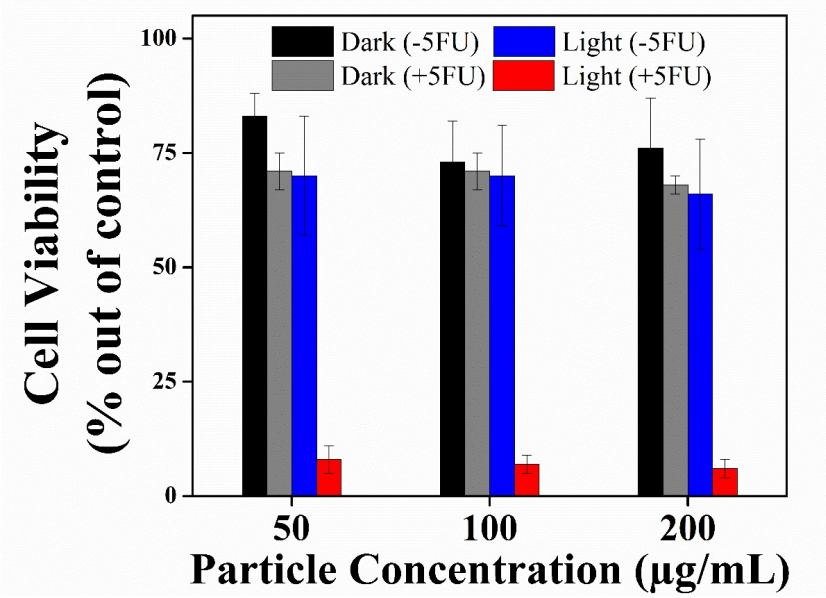


Figure S34. CCK-8 cell viability data after treatment with 50, 100, and 200 µg/mL of $PEGNH_2$-UiO-AZB-F MOF (72 h incubation). Error bars represent the standard deviation of 15 replicates.

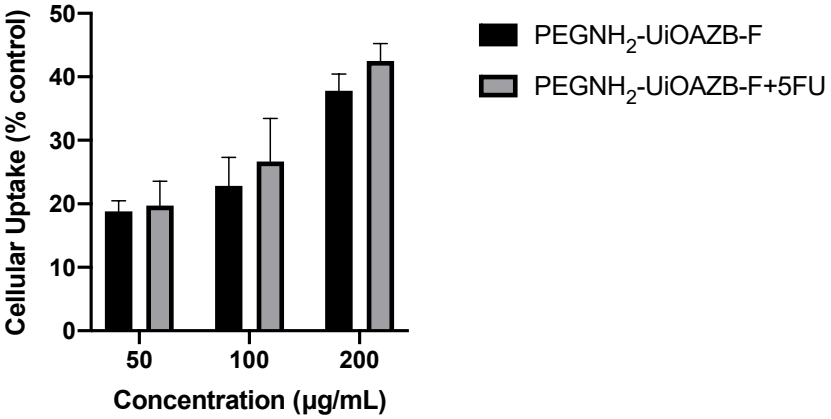


Figure S35. Determination of cellular uptake of $PEGNH_2$-UiOAZB-F and $PEGNH_2$-UiOAZB-F+5FU in HCT116 at various concentrations (50, 100, 200 µg/mL) at 72 h incubation time. The data was analyzed by ICP-MS. The total amount of zirconium was then measured using ICP-MS and compared to the amount of zirconium in untreated cells. As the concentration of MOF increases, the cellular uptake increases as well. There is no difference between $PEGNH_2$-UiOAZB-F and $PEGNH_2$-UiOAZB-F+5FU at the same concentration. Additionally, the cellular uptake of 200 µg/mL is around 2-fold higher than 50 µg/mL. These results verify that the MOFs are entering the cells.

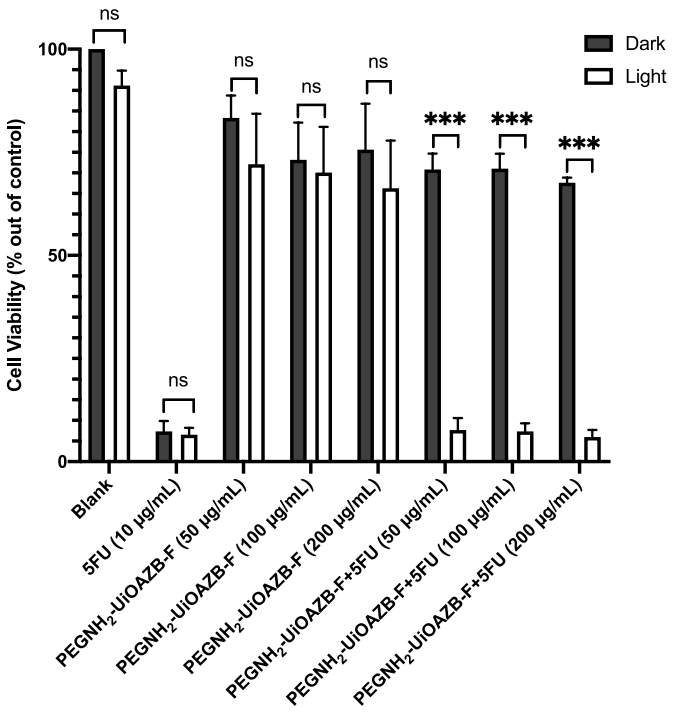


Figure S36. CCK-8 cell viability data after treatment with 50, 100, and 200 μg/mL of $PEGNH_2$-UiO-AZB-F and $PEGNH_2$-UiO-AZB-F +5FU with or without irradiation at 72 h incubation. Error bars represent the standard deviation over three independent experiments with five replicates per experiment (n = 3). ns = no statistical significance, *** indicates $p < 0.001$ among indicated treatment groups. Error bars indicate standard deviation of three separate experiments with five replicates per experiment. Group comparisons are indicated as determined by a one-way analysis of variance (ANOVA) with a Student−Newman−Keuls comparisons post hoc test.

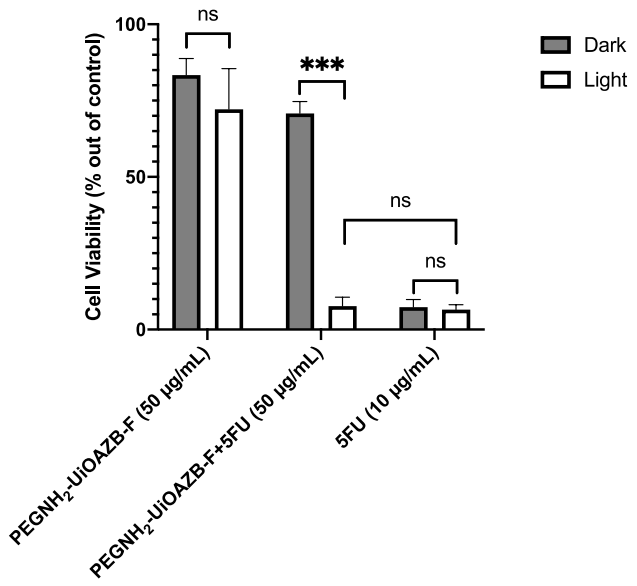


Figure S37. Cell viability data for HCT-116 cell line after treatment with PEGNH2-UiO-AZB-F, PEGNH2-UiO-AZB-F+5FU, or 5FU under dark (grey) or irradiative (white) conditions at 72 h incubation time. Error bars represent the standard deviation over three independent experiments with five replicates per experiment (n = 3). ns = no statistical significance, *** indicates $p < 0.001$ among indicated treatment groups. Error bars indicate standard deviation of three separate experiments with five replicates per experiment. Group comparisons are indicated as determined by a one-way analysis of variance (ANOVA) with a Student−Newman−Keuls comparisons post hoc test.

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