Supporting Information

Triple-Function Porphyrin in Glycopolymeric Photosensitizer: From PhotoATRP to Targeted PDT

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Experimental Section

Materials. Nitrotetrazolium blue chloride (NBT) was purchased from Meryer (Shanghai) Chemical Technology Co., Ltd. Copper(II) bromide ($CuBr_2$) and N',N'-bis[2-(dimethylamino)ethyl]-N,N-dimethylethane-1,2-diamine (Me_6TREN) were brought from Bidepharm (Shanghai, China). Oligo(ethylenglyco)-methylether methacrylate (OEGMA, 300 g/mol), obtained from Sigma-Aldrich, underwent purification through a basic alumina column in order to remove any inhibitor. L929 and MCF-7 cells required for the experiments were all obtained from the National collection of Authenticated Cell Cultures (Shanghai, China). Fetal bovine serum (FBS) and cell counting kit-8 (CCK-8) were purchased from Beyotime (Shanghai, China). Dulbecco's Modified Eagle Medium (DMEM) form Vivacell (Shanghai, China) was used as received. Other reagents were purchased from Adamas (Shanghai, China) and used without further purification, unless otherwise specified. Reagent grade DMSO was purified by means of distillation with calcium hydride under reduced pressure. Milli-Q water was used throughout the experiments. The 1-O-methacryloyl-2,3:4,5-di-O-isopropylidene-*β*-D-fructopyranose (MIF),[1] 1-O-acryloyl-2,3:4,5-di-O-isopropylidene-*β*-D-fructopyranose (AIF)[2], 1-O-p-vinylbenzoate-2,3:4,5-di-O-isopropylidene-*β*-D-fructopyranose (VIF)[3, 4], porphyrin-based ATRP initiator (ZnTPPC6Br)[1] and pentaerythritol tetrakis(2-bromoisobutyrate) (PETB)[5] were prepared according to previous reports (Supporting Information).

Characterization. The ^1H and ^{13}C NMR spectra of the monomers, starting compounds and star-shaped copolymers, dissolved in $CDCl_3$ or DMSO-d_6, were obtained using a Bruker AVANCE III spectrometer. Polymerizations were irradiated under yellow light LEDs (560-580 nm, 15 mW/cm^2) purchased from Xuzhou Ai Jia electronic technology Co. Ltd. For size exclusion chromatography (SEC), a Waters Breeze system was utilized, which included a 717 plus autosampler, a 1525 binary

HPLC pump, a 2410 refractive index detector, and three Waters Styragel columns (HR3, HR4, and HR6) maintained at 45°C. Tetrahydrofuran (THF) served as the eluent, filtered through 0.2 μm nylon Millipore filters, at a flow rate of 0.5 mL/min. Calibration involved polystyrene standards ranging from 2500 to 608000 g/mol. The hydrodynamic diameter was measured using a Malvern Zetasizer Nano ZS with a He-Ne laser ($\lambda = 633$ nm) and noninvasive backscatter detection at 173°. Fluorescence analysis, with excitation wavelengths of 420 nm, was performed on a PTI QM-TM fluorescence spectrophotometer. The UV-visible spectra were recorded on a SHIMADZU UV2600. Fluorescence microscopy was conducted using an OLYMPUS CKX41, and flow cytometry data was obtained using a BD FACSCalibur flow cytometer.

General procedure for photoATRP. To a 100 mL reaction vial, a stock solution of $CuBr_2$ (145 mg, 0.645 mmol), PMDETA (1.35 mL, 6.45 mmol) in DMF (3.75 mL), and a stock solution of ZnTPPC6Br (1.125 g, 0.645 mmol) in DMF/DMSO (4/6 v/v, 75 ml) were added followed by addition of AIF (19.08 mL, 51.6 mmol). The flask was irradiated under yellow light (560-580 nm, 15 mW cm^{-2}) for 15 h to yield ZnTPP-P(AIF_{20})$_4$. Partial mixtures were periodically collected and analyzed using ^{1}H NMR and SEC to assess monomer conversion and polymerization kinetics. By varying the type and amount of glycomonomers and the irradiation duration, diverse star-shaped glycopolymer PSs were synthesized, with each arms containing approximately 20 glycomonomers. The polymers were precipitated in cold methanol to obtain the precursors, which were then deprotected in trifluoroacetic acid/water (8/2, v/v) for 3 h at room temperature. Subsequently, the final glycopolymers underwent a three-day dialysis process followed by freeze-drying.

Chain extension. To investigate the reactivity of the polymer terminal, ZnTPP-P(MIF_{20})$_4$-*b*-P($OEGMA_{20}$)$_4$ block copolymer was synthesized through sequential photoATRP of OEGMA,

employing ZnTPP-P(MIF_{20})$_4$ as the macroinitiator. The polymerization mixture with a composition of [OEGMA]/[ZnTPP-P(MIF_{20})$_4$]/[$CuBr_2$]/[PMDETA] = 80/1/1/10 was exposed to yellow LEDs (560-580 nm, 15 mW cm^{-2}) for 4 h. Partial samples were periodically withdrawn and analyzed using ^{1}H NMR and SEC to ascertain the conversion and polymer kinetics. Subsequently, by substituting OEGMA with MIF, ZnTPP-P(MIF_{20})$_4$-*b*-P(MIF_{20})$_4$ was prepared following a similar procedure.

Porphyrin content in glycopolymers, **singlet oxygen detection**, **fluorescence quantum yields and singlet oxygen quantum yields in DMF, *in vitro* cytotoxicity**, **intracellular ROS detection**, **cell uptake, and inhibition assay.** They were performed according to our previous report.[6, 7]

Statistical analysis. The data were expressed as the mean ± standard deviation (SD) derived from a minimum of three independent measurements. Statistical evaluations were conducted utilizing Origin 2021 software. A *p*-value less than 0.05 was considered to indicate statistically significant differences.

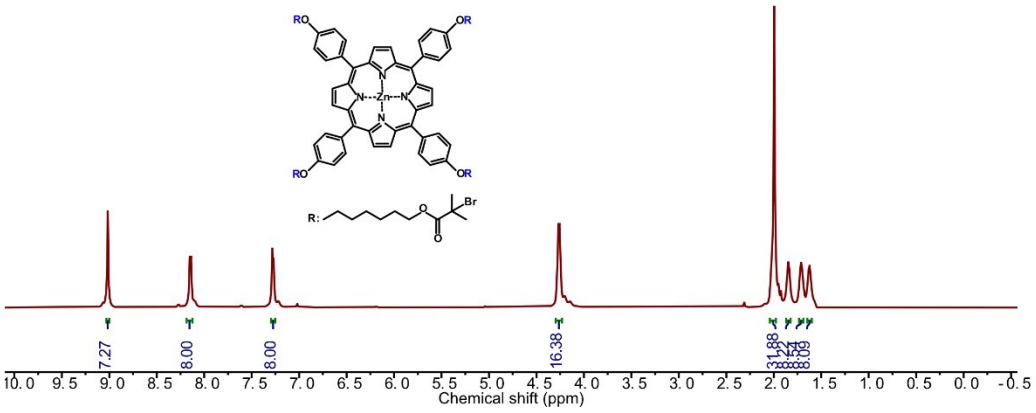


Fig. S1 The structure and ^{1}H NMR of ZnTPPC6Br.

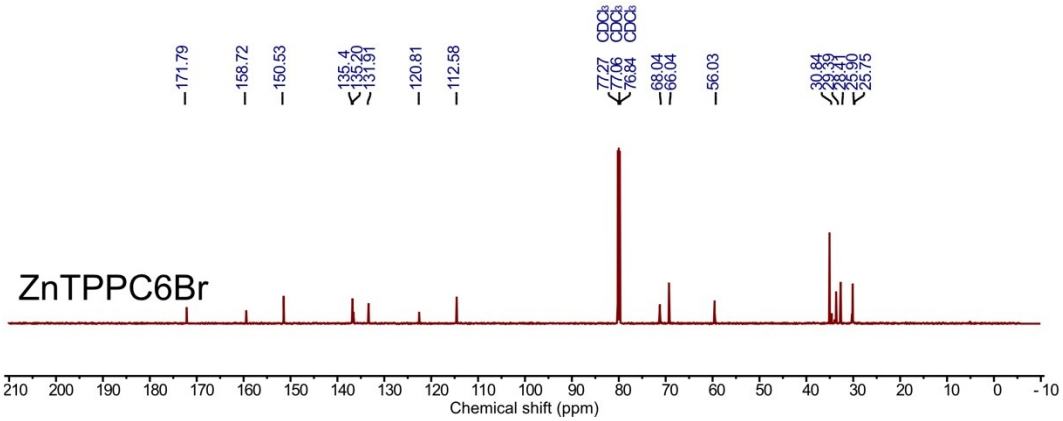


Fig. S2 The ^{13}C NMR of ZnTPPC6Br.

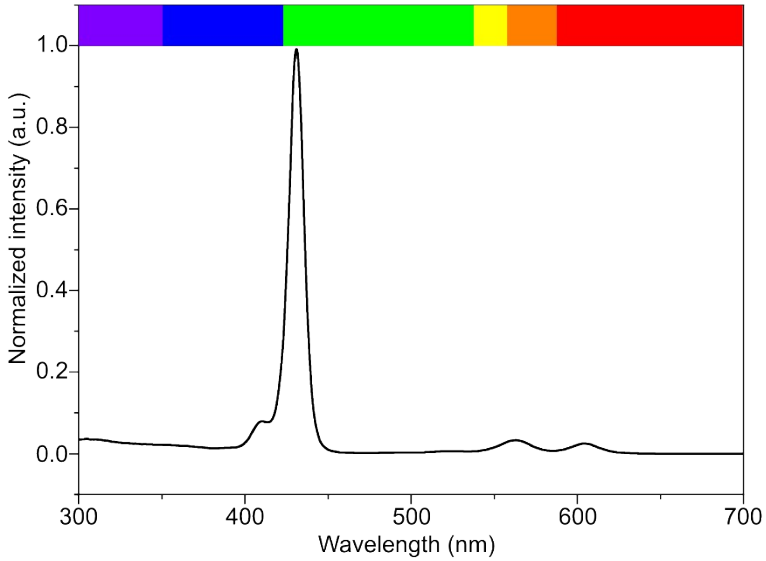


Fig. S3 The UV-vis spectrum of ZnTPPC6Br.

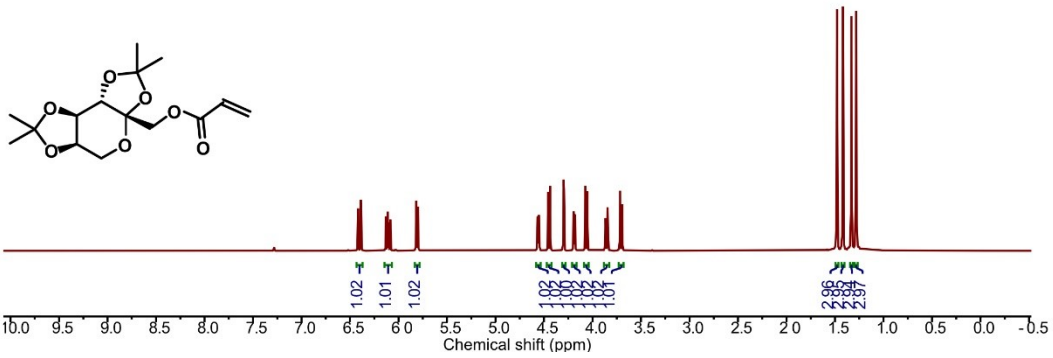


Fig. S4 The structure and ^{1}H NMR of AIF.

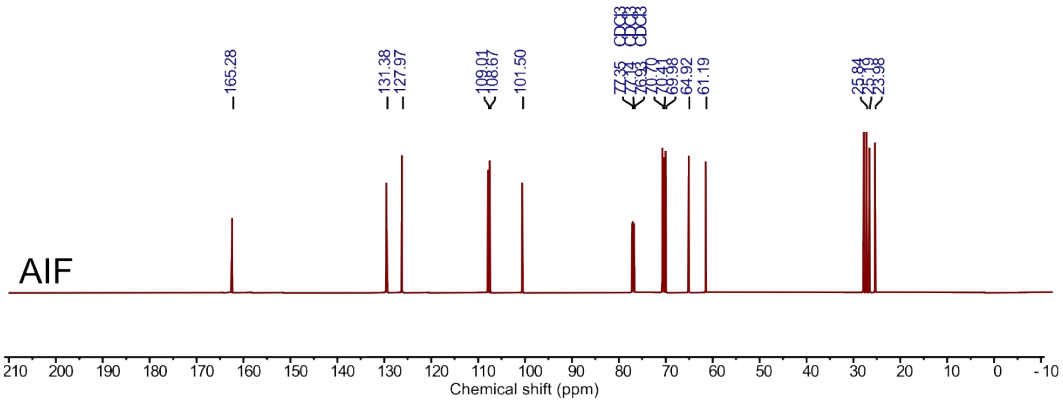


Fig. S5 The ^{13}C NMR of AIF.

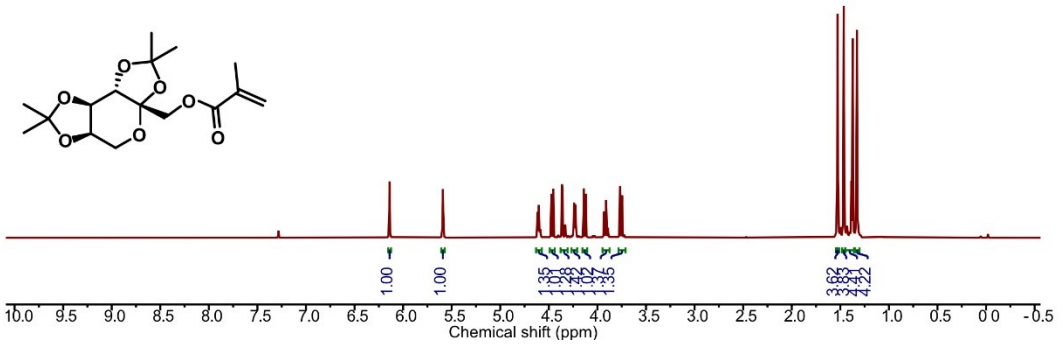


Fig. S6 The structure and ^{1}H NMR of MIF.

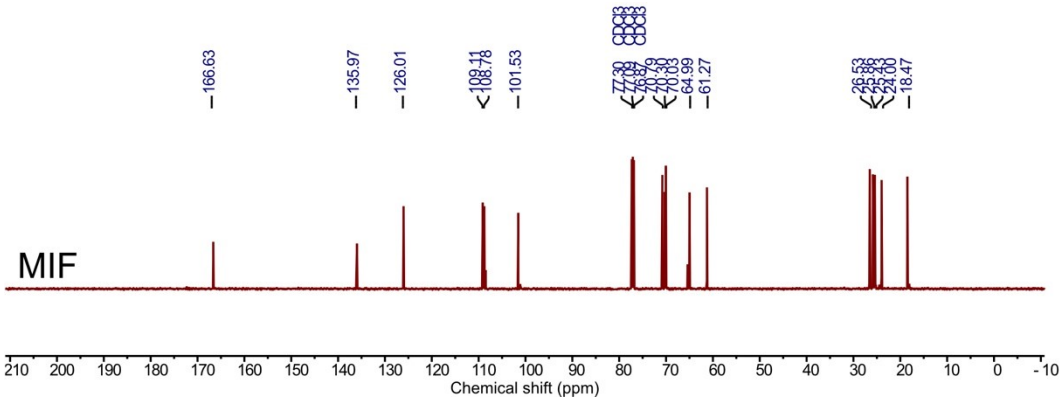


Fig. S7 The ^{13}C NMR of MIF.

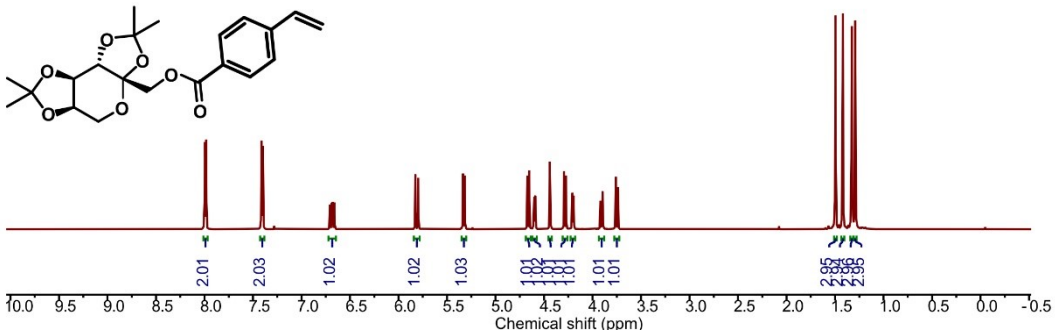


Fig. S8 The structure and ^{1}H NMR of VIF.

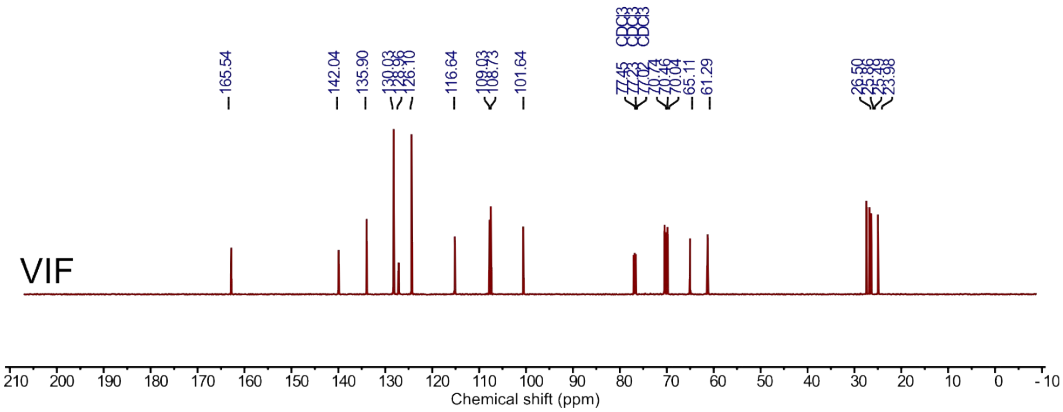


Fig. S9 The ^{13}C NMR of VIF.

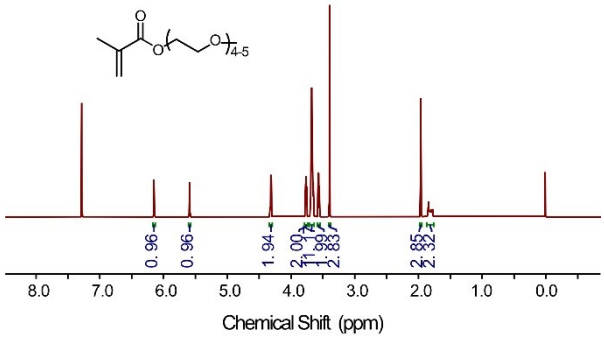


Fig. S10 The structure and ^{1}H NMR of OEGMA.

Table S1 Results of oxygen-tolerance photoATRP of AIF in the presence of TEA/TEOA under yellow light irradiation[a]

entry	reducing agents	*conv*. (%)[b]	$M_{n,th}$ (g/mol)[b]	M_n (g/mol)[c]	$Đ$[c]
1[d]	TEA	58	38 600	12 000	1.37
2[d]	TEOA	86	55 700	19 100	1.45
3[e]	TEA	62	40 700	10 300	1.36
4[e]	TEOA	84	54 500	14 300	1.47

[a]The reaction was carried out in a ratio of 200/1/1/10 for [AIF]/[ZnTPPC6Br]/[$CuBr_2$]/[L], irradiated under yellow LEDs (560-580 nm, 15 mW cm^{-2}) for 10 hours in 4 mL vials, using a 4:6 DMF:DMSO mixture as the solvent. [b]Calculated by ^{1}H NMR spectra. [c]Measured by SEC. [d]Reaction vials sealed with a stopper without deoxygenation. [e]Reaction vials without stoppers and deoxygenation.

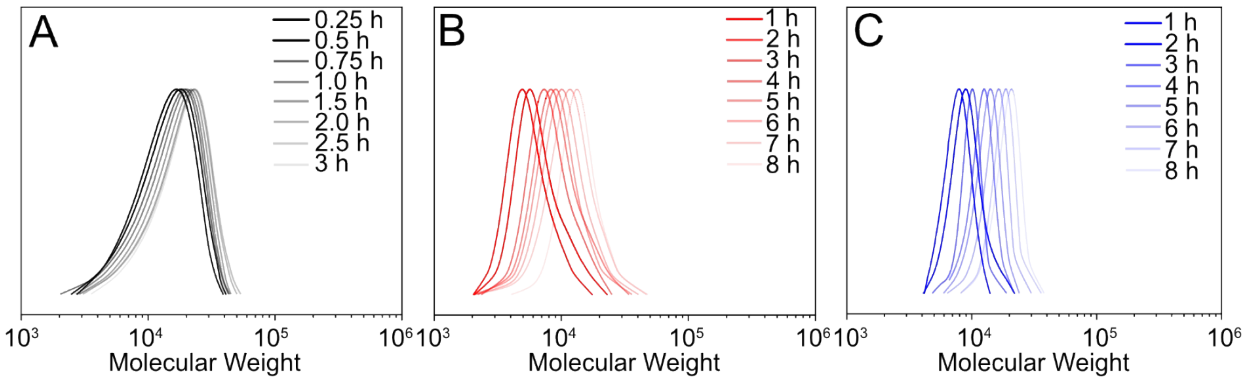


Fig. S11 SEC traces of MIF(A), AIF(B) and VIF (C) evolution with time.

Table S2. Results of photoATRP of monomers in the presence of different monomer feed ratios[a]

entry	monomer feed ratio	monomer	*conv.* (%)[b]	$M_{n,th}$ (g/mol)[b]	M_n (g/mol)[c]	$Đ$[c]
1	40	AIF	51	8 100	6 600	1.19
2	80	AIF	49	14 000	9 900	1.19
3	100	AIF	57	19 600	17 500	1.19
4	200	AIF	52	34 400	21 800	1.20
5	40	MIF	95	14 200	8 200	1.23
6	80	MIF	96	26 900	12 900	1.22
7	100	MIF	94	32 600	16 900	1.25
8	200	MIF	94	63 500	19 200	1.21
9	40	VIF	75	13 400	7 400	1.08
10	80	VIF	68	23 000	14 000	1.07
11	100	VIF	70	29 100	18 400	1.03
12	200	VIF	73	58 700	22 400	1.06

[a]Reaction conditions: [Monomer]/[ZnTPPC6Br]/[$CuBr_2$]/[L]=x/1/1/10, irradiated for 10 h (MIF for 4 h) under yellow LEDs (560-580 nm, 15 mW cm^{-2}) in a sealed 4 mL vial containing 4 mL solvent (DMF:DMSO=4:6). [b]Calculated by ^{1}H NMR spectra. [c]Measured by SEC.

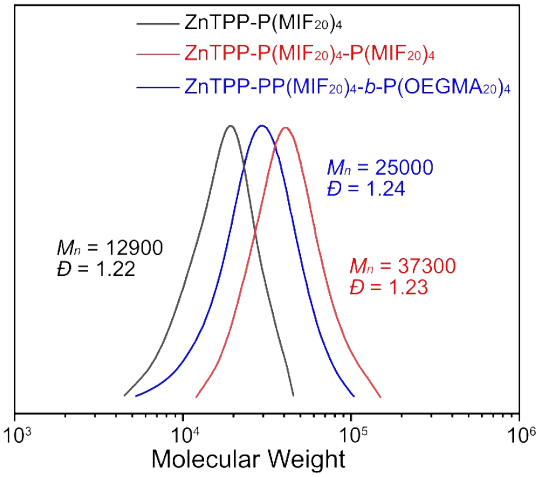


Fig. S12 Chain extension of ZnTPP-P(MIF_{20})$_4$ macroinitiator with MIF (red) and OEGMA (blue).

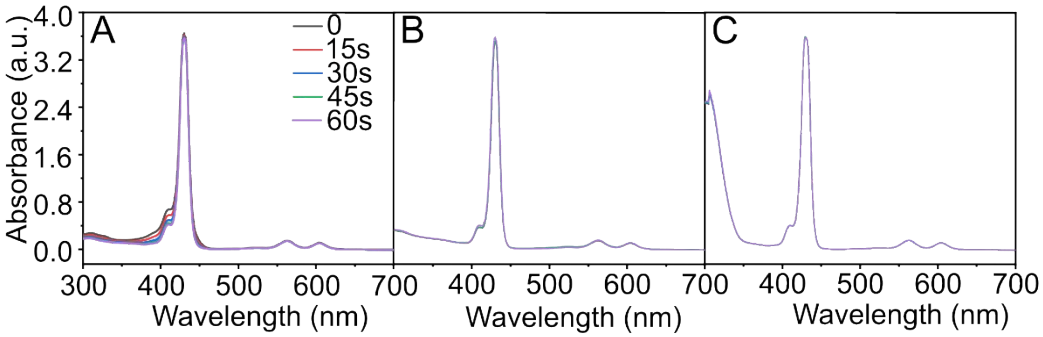


Fig. S13 ROS species detection by (A) DPBF, (B) NBT, and (C) TMB.

Fig. S14 Scale-up equipment used for photoATRP of MIF (left), AIF (middle) and VIF (right).

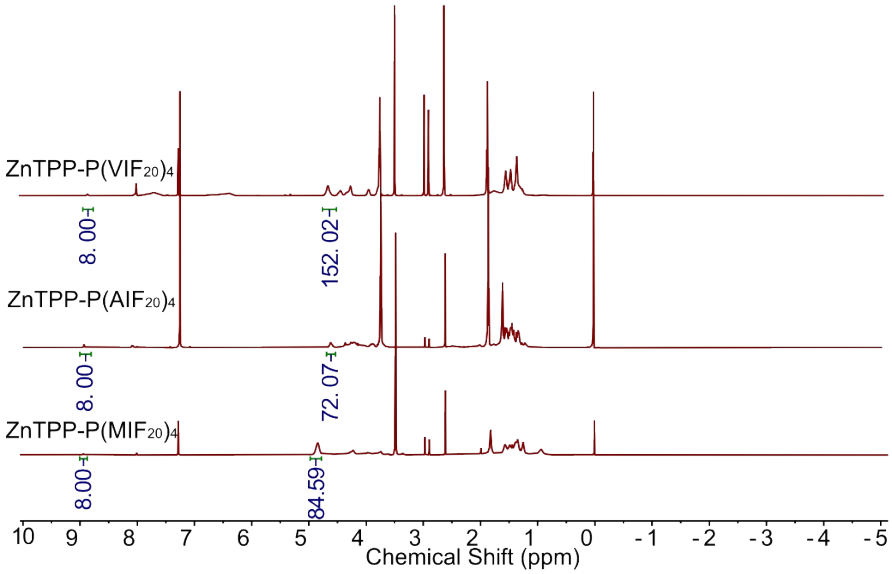


Fig. S15 ^{1}H NMR of prepolymers with isopropylidene protection in $CDCl_3$.

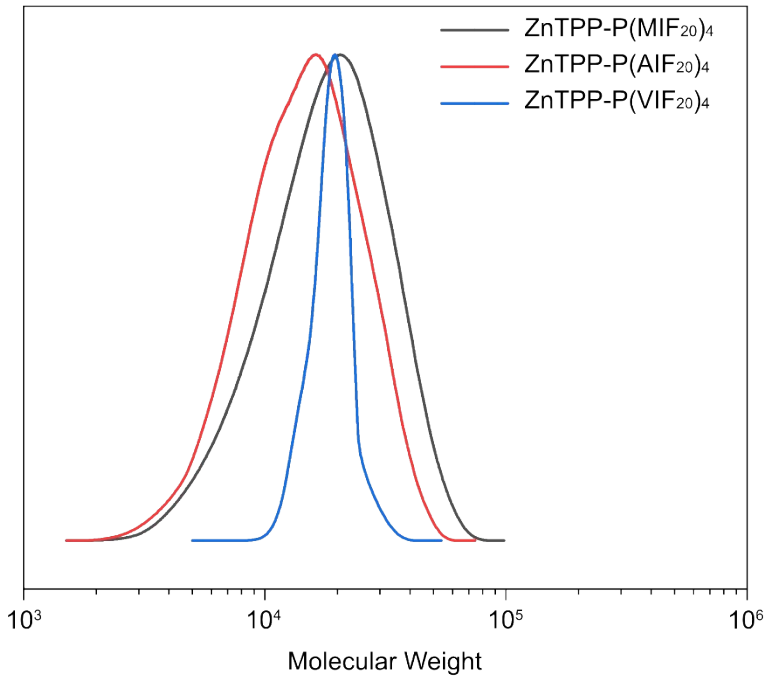


Fig. S16 SEC traces of the star-shaped copolymers with protection groups in THF.

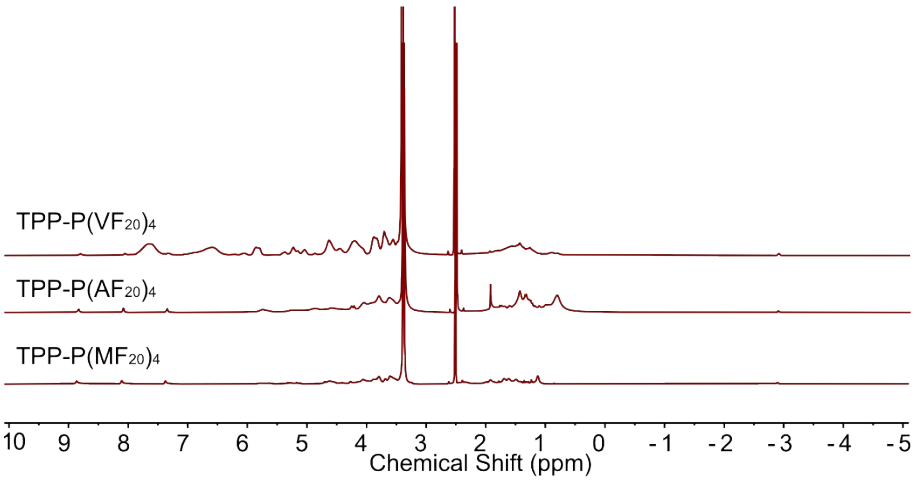


Fig. S17 ^{1}H NMR of final glycopolymers in DMSO-d_6.

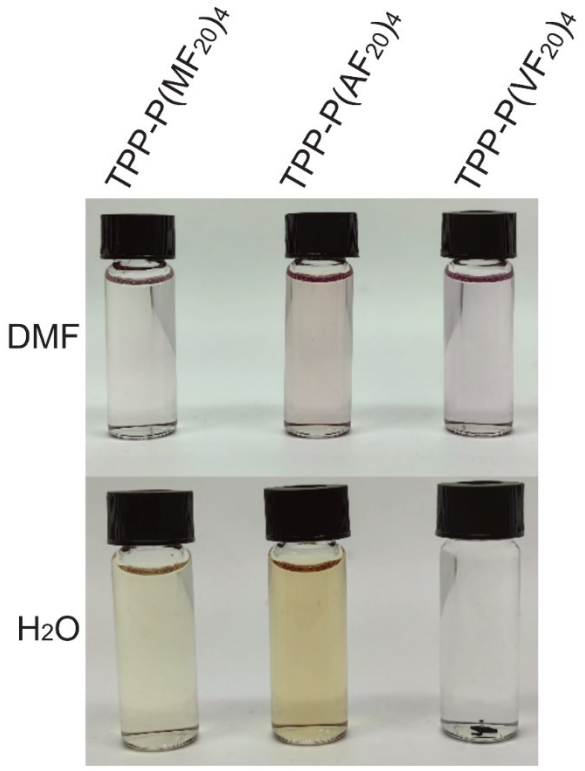


Fig. S18 Solubility of star-shaped copolymers in water and DMF (0.1 mg/ml).

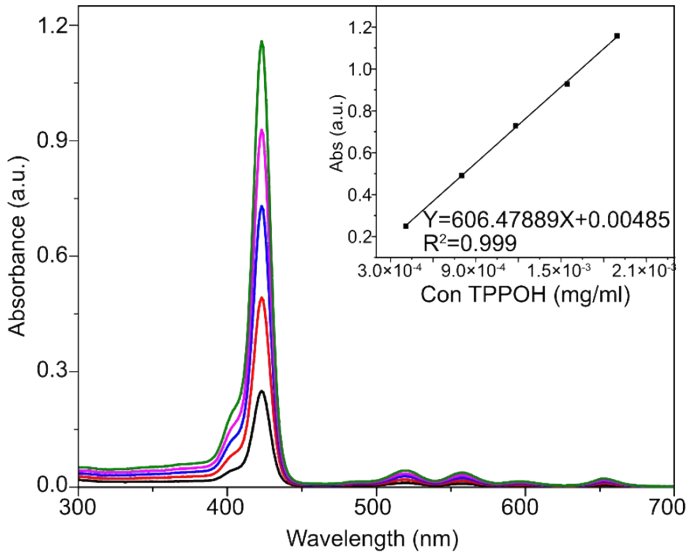


Fig. S19 Calibration curve of TPPOH in DMF obtained from UV-vis spectra.

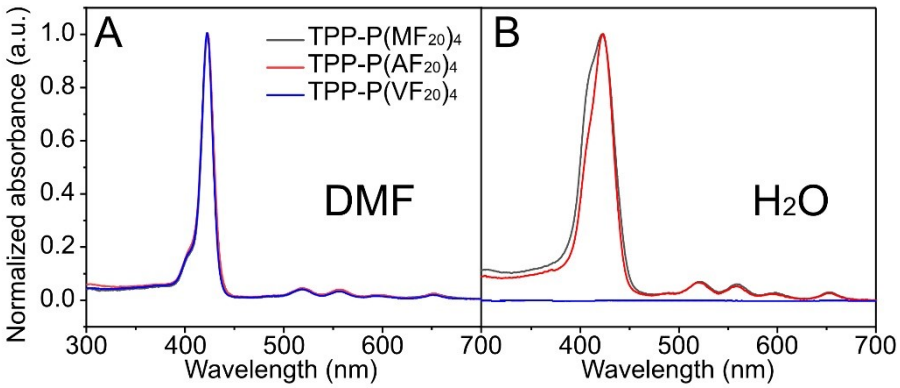


Fig. S20 UV-vis spectra of star-shaped glycopolymers in DMF (A) and water (B).

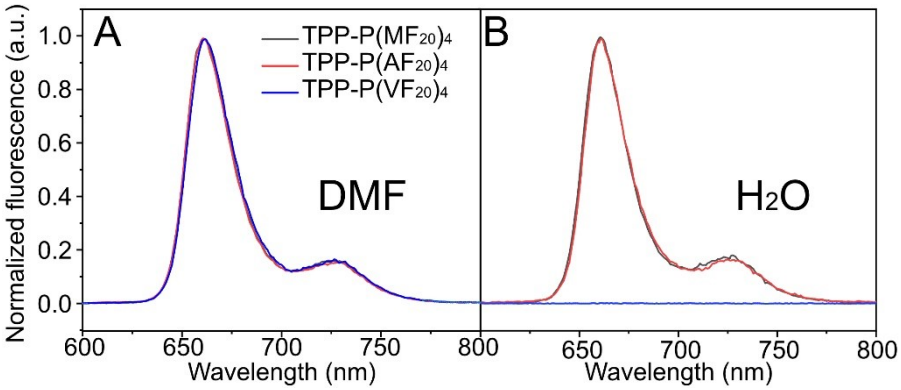
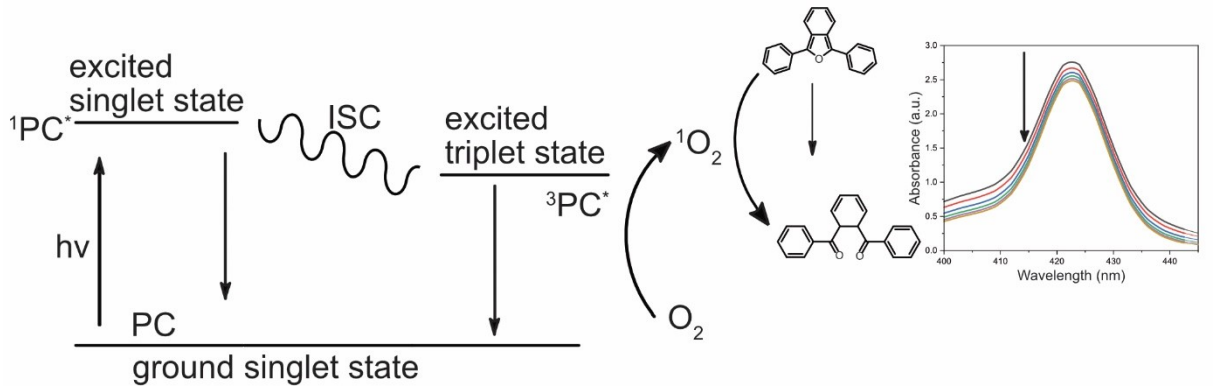


Fig. S21 Fluorescence excitation spectra of star-shaped glycopolymers in DMF (A) and water (B).

Scheme S1. Generation of $^1O_2$ by the photosensitizer and its reaction with DPBF leading to a decrease in UV absorbance at 414 nm.



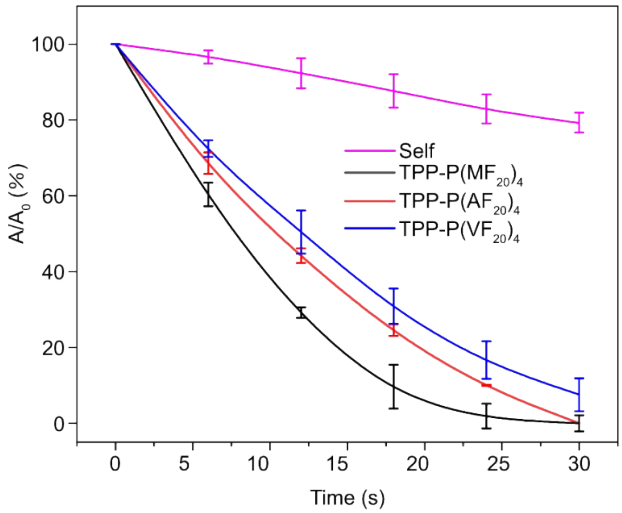


Fig. S22 Photobleaching of DPBF in DMF by $^1O_2$ in the presence of polymers under yellow light irradiation (25 mW cm^{-2}).

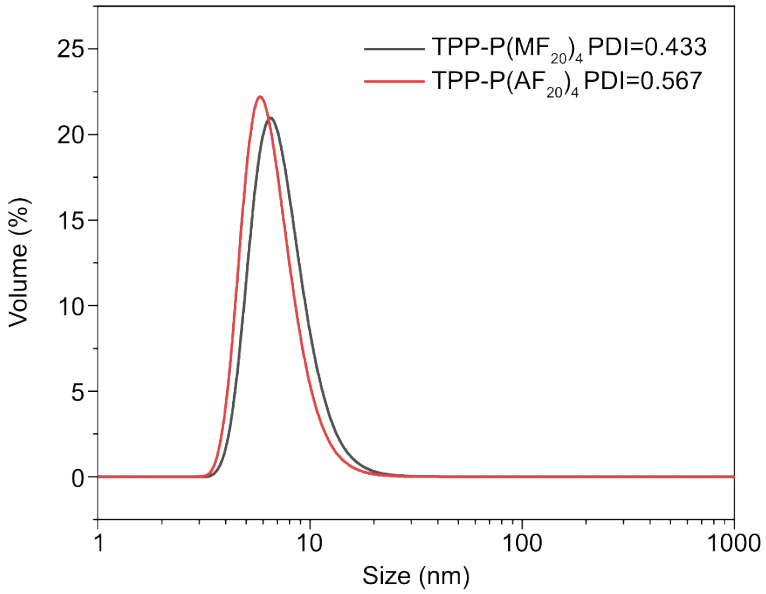


Fig. S23 Volume-average size distribution of the polymers in water (0.25 g/L).

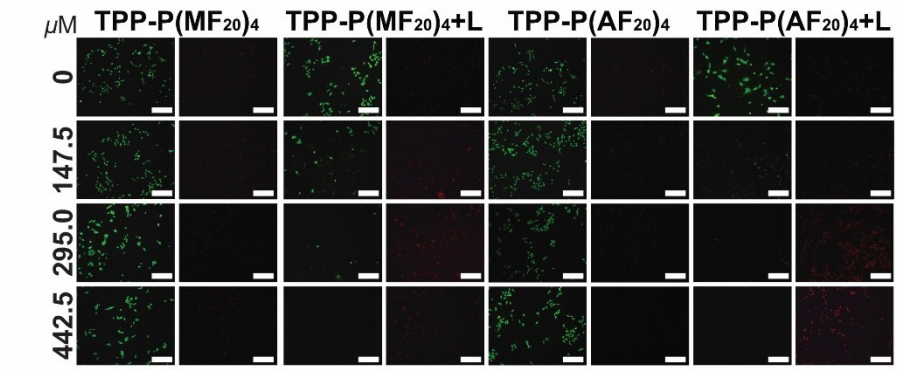


Fig. S24 Live/dead cell staining of L929 cells treated with copolymers under different porphyrin concentrations.

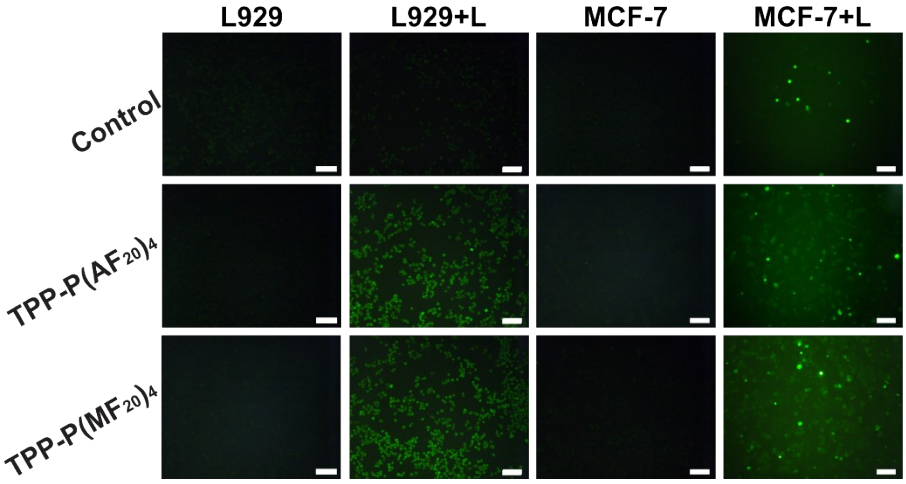


Fig. S25 Intracellular ROS generation following treatment with various glycopolymers, with or without light irradiation, for L929 and MCF-7 cells, assessed using DCFH-DA as a probe. Scale bar: 200 μm.

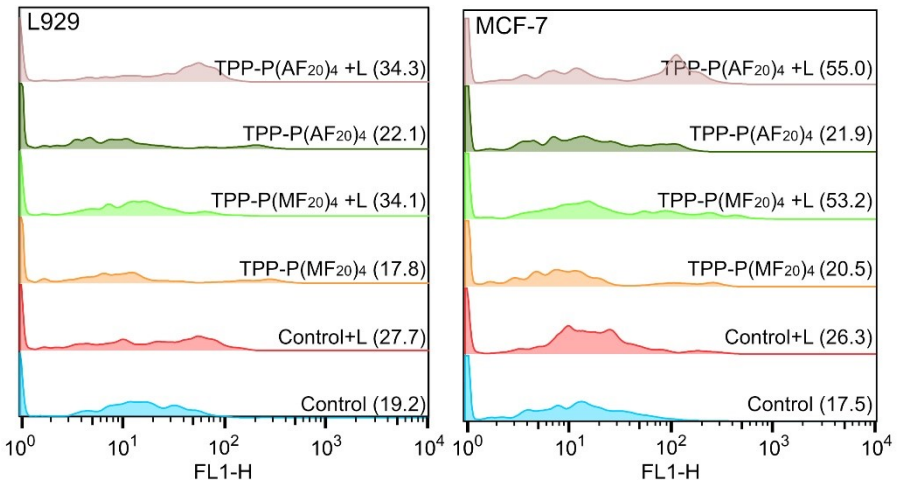


Fig. S26 Quantification of ROS generation analyzed by flowcytometry, with MFI values indicated in brackets.

References

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