Supporting Information

A Light-driven Molecular Motor-Polypeptide Conjugate Supports Controlled Cell Uptake

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1. Materials

All reactions involving oxygen-sensitive reagents were performed under a nitrogen atmosphere. Reagents purchased from Sigma-Aldrich, Acros Organics, or TCI Europe were used as received. Reagent-grade solvents were used without prior water removal unless otherwise indicated. Maleimidocaproyl-L-valine-L-citrulline-p-aminobenzyl alcohol p-nitrophenyl carbonate (VC linker) was purchased from MedChemExpress, pyridylthio-cysteamine hydrochloride (PD-NH2) from ABCR, and triethylamine and tris(2-carboxyethyl)phosphine from Thermo Fisher. N_3-PEG_3-PGA_{50} was kindly provided by Curapath. Diblock copolymer polyornithine-polyproline (PLO_6-PLP_{22}, **PP**) was synthesized as previously reported[14,15]. Anhydrous solvents were obtained using either an MBraun SPS-800 solvent purification system or from Acros Organics. Solvents were degassed by purging with nitrogen for at least 30 min/three freeze-pump-thaw cycles. Flash column chromatography used silica gels (Merck, type 9385, 230 to 400 mesh) or a Büchi Reveleris purification system using silica cartridges. Thin-layer chromatography used aluminum sheets coated with silica gel 60F254 (Merck). Compounds were visualized with a UV lamp and/or by staining with $KMnO_4$, cerium ammonium molybdate, or vanillin. ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury Plus 400 or a Bruker AVANCE 600 NMR spectrometer at 298 K unless otherwise indicated. Photostationary state (PSS) studies were performed on a Varian Unity Plus 500 NMR spectrometer. Chemical shifts are shown as parts per million relative to the residual solvent signal ($CDCl_3$ = δ 7.26 for ^1H, δ 77.2 for ^{13}C; CD_2Cl_2 = δ 5.32 for ^1H, δ 53.8 for ^{13}C). Multiplets in ^1H NMR spectra are designated as s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet), or br (broad). High-resolution mass spectrometry used an LTQ Orbitrap XL spectrometer.

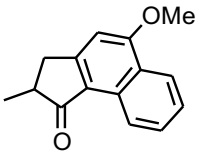
Vivaspin® 2000 and 3000 Da molecular weight cut-off (MWCO) and PD-10 desalting columns packed with Sephadex G-25 resin were purchased from Sartorius and Cytiva. VC and PD-NH_2 content were quantified by UV-vis (JASCO V-630 spectrophotometer). ^1H NMR spectra were acquired at 27°C (300K) on a 500 MHz Bruker NMR spectrometer. Cyanine5 amine (Cy5) was purchased from Lumiprobe.

Further details on N_3-PEG_3-PGA_{50}-VC-PP (**P**) physicochemical and biological characterization can be found in [14,15].

2. Preparation and characterization of the molecular motor

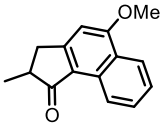
2.1. Synthetic procedure for preparing the upper half of MM

5-methoxy-2-methyl-2,3-dihydro-1H-cyclopenta[a]naphthalen-1-one (**S1**)

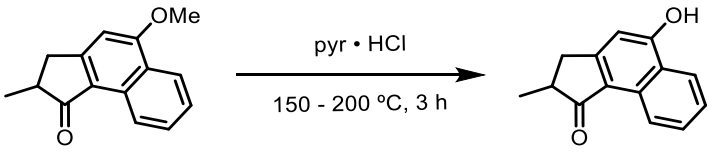


S1

Ketone **S1** was synthesized according to a previously described procedure[16].



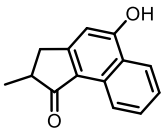
^{1}H NMR (600 MHz, $CDCl_3$) δ 9.13 (d, J = 8.3 Hz, 1H), 8.25 (d, J = 8.4 Hz, 1H), 7.70 – 7.63 (m, 1H), 7.52 (t, J = 7.7 Hz, 1H), 6.77 (s, 1H), 4.08 (s, 3H), 3.47 – 3.37 (m, 1H), 2.82 – 2.72 (m, 2H), 1.36 (d, J = 7.3 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 211.0, 164.3, 161.8, 133.3, 131.9, 128.6, 127.8, 126.5, 126.1, 125.1, 104.1, 80.0, 79.7, 79.3, 58.6, 44.8, 38.5, 19.6.



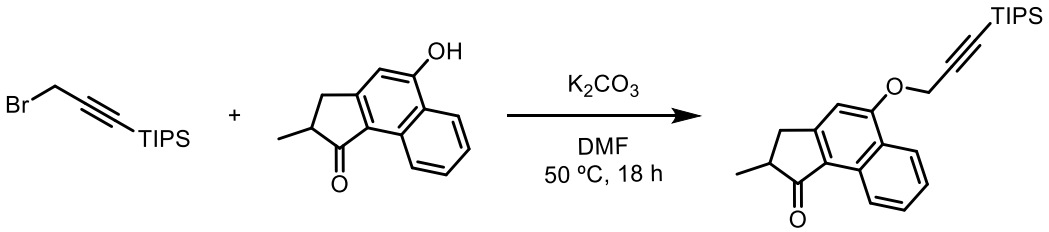
S1 **S2**

5-hydroxy-2-methyl-2,3-dihydro-1H-cyclopenta[a]naphthalen-1-one (**S2**).

Pyridine hydrochloride (14.700 g, 0.127 mol, 30.0 eq) was melted in a vial at 150°C before addition to an oven-dried vial charged with **S1** (0.963 g, 0.004 mol, 1.0 eq). The mixture was heated to 200°C for 3 h and then cooled to room temperature. The solid was partitioned between water and EtOAc. The organic layer was washed with aq. 0.5 M HCl, water, and brine before drying over $MgSO_4$, followed by undergoing filtration and finally the samples concentrated. The residue was purified by flash column chromatography to produce a light orange solid (0.765 g, 0.004 mol, 85%). NMR data in accordance with those in previous reports[17].



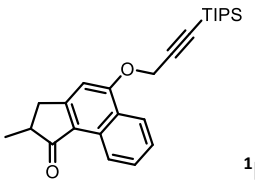
^{1}H NMR (400 MHz, DMSO-d6) δ 11.41 (s, 1H), 8.96 (d, J = 8.3 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H), 7.67 (t, J = 7.5 Hz, 1H), 7.53 (t, J = 7.7 Hz, 1H), 6.92 (s, 1H), 3.37 (dd, J = 18.4, 8.3 Hz, 1H), 2.80 – 2.61 (m, 2H), 1.21 (d, J = 7.2 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d6) δ 207.0, 160.4, 159.8, 130.5, 129.1, 125.4, 124.1, 122.9, 122.8, 121.1, 105.6, 41.4, 34.9, 16.6.



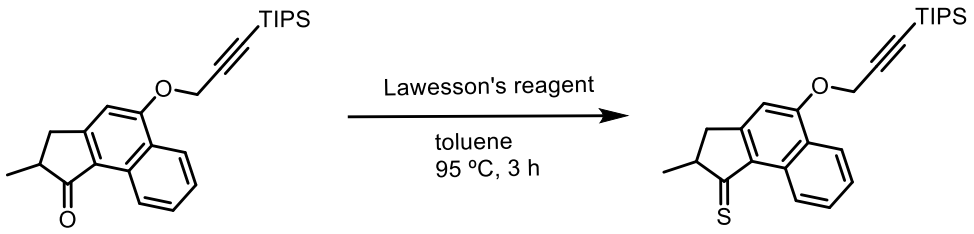
S3 **S2** **S4**

2-methyl-5-((3-(triisopropylsilyl)prop-2-yn-1-yl)oxy)-2,3-dihydro-1H-cyclopenta[a]naphthalen-1-one (**S5**).

An oven-dried vial was charged with **S2** (0.544 g, 2.563 mmol, 1.0 eq.) and K_2CO_3 (1.416 g, 10.249 mmol, 4.0 eq.). Dimethylformamide (DMF) (30 mL, 0.085 M) was then added, followed by 3-bromoprop-1-yn-1-yl)triisopropylsilane (**S3**) addition (0.919 g, 3.154 mmol, 1.2 eq.). **S3** was synthesized following a previously described procedure[17]. The resulting mixture was heated to 50°C for 18 h and then cooled to room temperature, and water and dichloromethane (DCM) were added. The mixture was extracted with DCM, and the combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Flash column chromatography (SiO_2, dry load on celite, 5% EtOAc in pentane) gave **S4** (0.763 g, 1.805 mmol, 70%) as a light-yellow oil.



^{1}H NMR (400 MHz, $CDCl_3$) δ 9.13 (d, J = 8.4 Hz, 1H), 8.27 (d, J = 8.4 Hz, 1H), 7.67 (t, J = 8.3 Hz, 1H), 7.58 – 7.50 (m, 1H), 7.03 (s, 1H), 5.02 (s, 2H), 3.46 – 3.36 (m, 1H), 2.81 – 2.72 (m, 2H), 1.36 (d, J = 7.3 Hz, 3H), 1.04 (s, 22H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 208.6, 159.6, 158.8, 130.8, 129.4, 126.1, 125.4, 124.0, 124.0, 122.7, 103.7, 100.9, 90.9, 57.4, 42.4, 36.0, 18.6, 17.1, 11.2. **HRMS** (ESI pos) m/z calcd for $C_{26}H_{35}O_2Si$ $[M+H]^+$ 407.24008, found 407.24036.

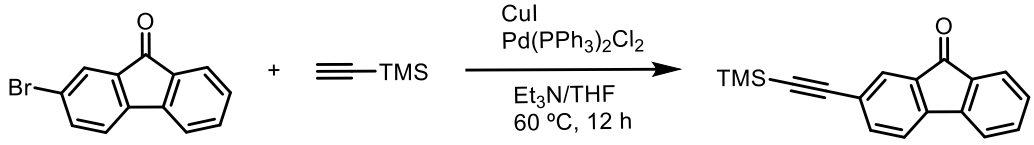


S4 **S5**

2-methyl-5-((3-(triisopropylsilyl)prop-2-yn-1-yl)oxy)-2,3-dihydro-1H-cyclopenta[a]naphthalene-1-thione (**S5**).

A mixture of **S4** (0.793 g, 0.002 mol, 1.0 eq.) and Lawesson's reagent (0.485 g, 1.200 mmol, 6.0 eq.) in anhydrous toluene (41 mL, 0.06 M) was heated at 95°C. After 6 h, toluene was removed in vacuo, and the resulting thioketone intermediate **S5** was purified by flash column chromatography (SiO_2, 10% DCM in pentane) and used without further isolation.

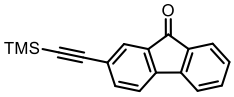
2.2. Synthetic procedure for preparing the bottom half of the MM



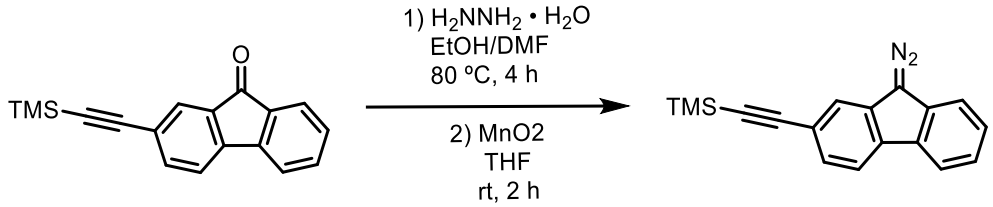
S6 **S7**

2-((trimethylsilyl)ethynyl)-9H-fluoren-9-one (**S7**).

The corresponding aryl halide **S6** (2-bromo-9H-fluoren-9-one, 0.500 g, 1.930 mmol, 1.0 eq.) was obtained commercially and loaded into a glass vial together with CuI (0.018 g, 0.096 mmol, 0.05 eq.) and $Pd(PPh_3)_2Cl_2$ (0.067 g, 0.096 mmol, 0.05 eq.). Tetramethylsilane (TMS, 0.284 g, 2.895 mmol, 1.5 eq.) was mixed with tetrahydrofuran (THF, 16 mL, 0.12 M) and triethylamine (Et_3N, 3 mL, 0.64 M) in another vial and degassed by nitrogen bubbling for 15 min. After degassing, the resulting mixture was added to the solids and the mixture stirred at 60°C overnight. After 12 h, the product solution was cooled to room temperature, filtered over a silica plug, which was flushed with DCM. The remaining solvent was evaporated, and the residue was purified by flash column chromatography to yield **S7** as an off-white solid (0.498 g, 1.802 mmol, 93%).



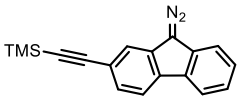
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.54 (d, J = 7.3 Hz, 1H), 7.48 (d, J = 7.7 Hz, 1H), 7.41 – 7.34 (m, 2H), 7.31 (d, J = 7.7 Hz, 1H), 7.20 (t, J = 7.9 Hz, 1H), 0.25 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 192.9, 143.8, 137.9, 134.8, 134.3, 134.0, 129.3, 127.7, 124.4, 124.0, 120.6, 120.1, 104.0, 96.2, -0.0. **HRMS** (ESI pos) m/z calcd for $C_{18}H_{16}OSi$ $[M+H]^+$ 277.10000, found 277.18349.



S7 **S8**

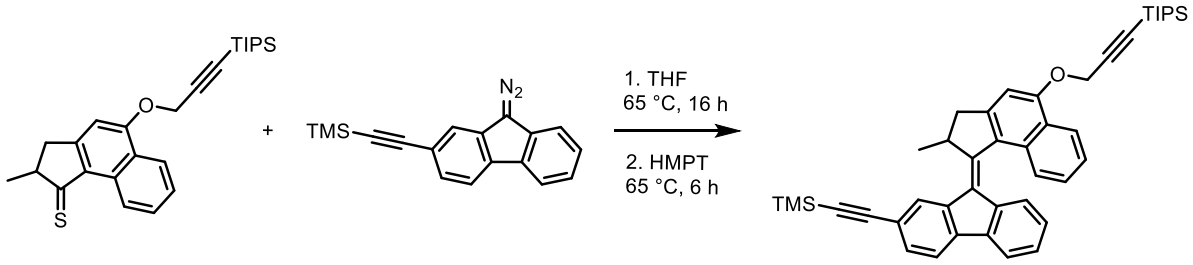
((9-diazo-9H-fluoren-2-yl)ethynyl)trimethylsilane (**S8**).

Hydrazine monohydrate (50-60% in H_2O, 1.6 mL, 32.411 mmol, 18.0 eq.) was added to a mixture of **S7** (0.500 g, 1.809 mmol, 1.0 eq) and EtOH (21 mL, 0.086 M) and the resulting suspension was heated at 80°C. After 4 h, the mixture was allowed to cool to room temperature, and H_2O was added. A precipitate appeared almost immediately and was placed in the freezer at -18°C to crystallize overnight. The resultant solid was collected the following day, yielding a pale-yellow solid being the corresponding hydrazone (0.393 g, 1.353 mmol, 75%). The crude hydrazone was dissolved in anhydrous THF (22.4 mL, 0.06 M), and MnO_2 (2.712 g, 31.200 mmol, 23.0 eq.) was added. The resultant black solution was stirred at room temperature for 2 h and filtered over a celite plug. The product solution was concentrated in vacuo to yield a yellow solid (0.210 g, 0.728 mmol, 54%), which was used immediately in the next step.



^{1}H NMR (400 MHz, $CDCl_3$) δ 10.22 – 10.14 (m, 1H), 8.33 (dd, J = 8.4, 1.4 Hz, 1H), 7.74 (ddd, J = 8.5, 6.9, 1.4 Hz, 1H), 7.56 (ddd, J = 8.3, 6.9, 1.3 Hz, 1H), 7.18 (d, J = 2.8 Hz, 1H), 7.08 (s, 1H), 7.02 – 6.90 (m, 1H), 1.05 (s, 9H).

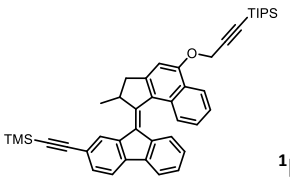
2.3. Synthetic procedure for preparing the final MM



S5 **S8** **S9**

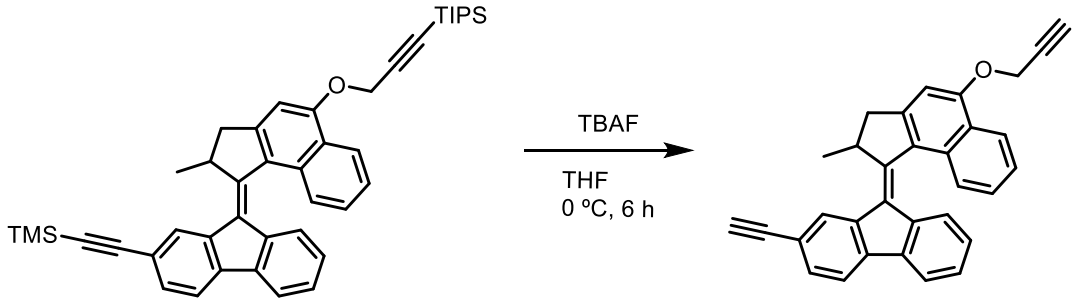
(E)-Triisopropyl(3-((2-methyl-1-(2-((trimethylsilyl)ethynyl)-9H-fluoren-9-ylidene)-2,3-dihydro-1H-cyclopenta[a]naphthalen-5-yl)oxy)prop-1-yn-1-yl)silane (**S9**).

An oven-dried vial was charged with **S6** (830 mg, 1.964 mmol, 1.1 eq.) and **S7** (510 mg, 1.768 mmol, 1.0 eq.), and oxygen was removed. THF (15 mL) was added, and the mixture was stirred overnight at 65°C. After 16 h, hexamethylphosphoramide (HMPT, 1.8 mL) was added, and the mixture was stirred for another 6 h at 65°C. The resulting crude product was concentrated in vacuo. Flash column chromatography (SiO_2, dry load on celite, 1% DCM in pentane) provided **S9** as a yellow solid (226 mg, 0.347 mmol, 20%). The product was isolated as a mixture of the two isomers, *E* could be isolated as a small amount (> 2 mg) using high-performance liquid chromatography (HPLC) (0-0.5% DCM in heptane); however, due to the technical difficulties involved in the separation process, the procedure was continued with a racemic mixture of the compound.



^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (d, J = 8.7 Hz, 1H), 7.98 (d, J = 7.9 Hz, 1H), 7.86 (d, J = 7.4 Hz, 1H), 7.72 (d, J = 7.7 Hz, 1H), 7.60 (d, J = 8.7 Hz, 1H), 7.48 (d, J = 10.6 Hz, 2H), 7.43 – 7.40 (m, 2H), 7.32 (d, J = 8.1 Hz, 1H), 7.05 (s, 1H), 6.75 (s, 1H), 4.30 (s, 1H), 3.50 (d, J = 9.8 Hz, 1H), 2.72 (d, J = 15.3 Hz, 1H), 1.20 (s, 2H), 1.02 (d, J = 6.8 Hz, 22H), 0.07 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 155.8, 152.6, 149.0, 140.0, 138.7, 138.1, 130.3, 130.0, 129.8, 129.4, 127.3, 127.1, 127.0, 126.7, 126.2, 126.1, 125.5, 124.7, 124.6, 123.5, 122.7, 119.7, 119.2, 104.6, 104.5, 90.0, 77.2, 76.8, 76.5, 75.7, 57.0, 50.3,

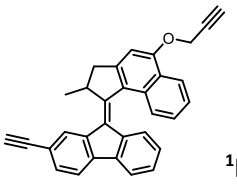
45.0, 42.2, 19.4, 19.3, 18.3, 10.8, 0.2, 0.4. **HRMS** (ESI pos) m/z calcd for $C_{44}H_{50}OSi_2$ $[M+H]^+$ 651.34000, found 650.99802.



S9 **MM**

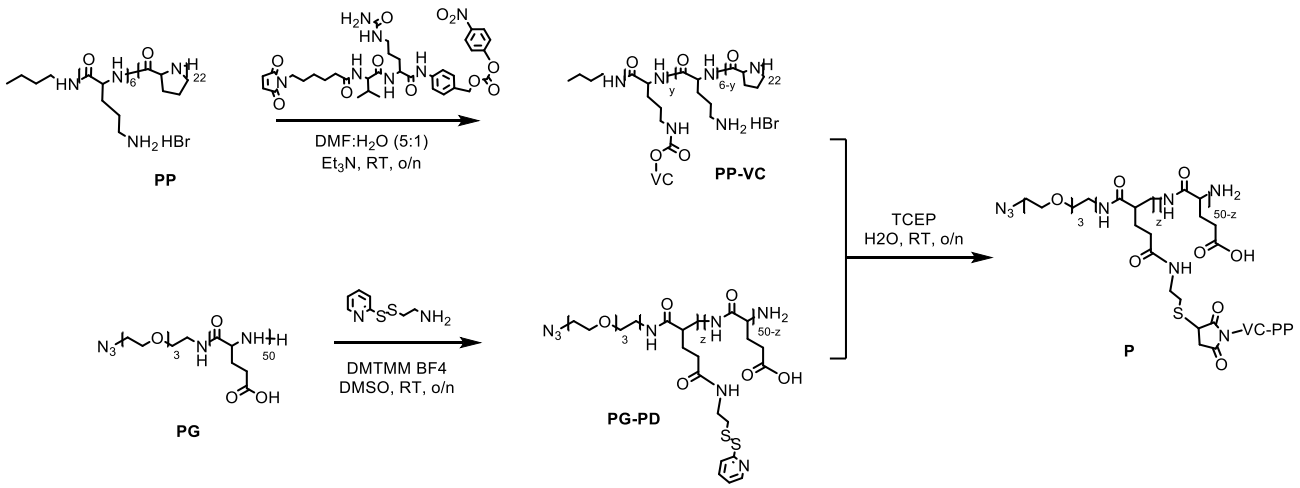
2-ethynyl-9-(2-methyl-5-(prop-2-yn-1-yloxy)-2,3-dihydro-1H-cyclopenta[a]naphthalen-1-ylidene)-9H-fluorene (**MM**).

In a Schlenk tube under a nitrogen atmosphere, tetra-n-butylammonium fluoride (TBAF, 21.1 mg, 0.081 mmol, 2.1 eq.) was added dropwise to a solution of **S9** (25 mg, 0.038 mmol, 1.0 eq.) in thiourea (THI, 1.7 mL) at 0°C. The mixture was left to stir for 6 h, quenched with ammonium chloride (1 M, 0.5 ml), and subsequently separated between water and DCM. The aqueous layer was extracted with DCM, and the combined organic layers were dried over Mg_2SO_4 and concentrated in vacuo. Flash column chromatography (SiO_2, dry load on celite, 5% DCM in pentane) gave the final molecular motor (**MM**) as a yellow solid. The product was isolated as a mixture of the two isomers, and all attempts to separate them by HPLC failed due to the low polarity of the compound.



^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 – 8.53 (m, 1H), 7.92 (d, J = 1.9 Hz, 1H), 7.83 (s, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.57 (ddd, J = 8.2, 6.8, 1.2 Hz, 1H), 7.53 – 7.52 (m, 1H), 7.38 (ddd, J = 8.3, 6.7, 1.3 Hz, 1H), 7.31 (d, J = 1.2 Hz, 1H), 6.92 (dd, J = 8.5, 1.9 Hz, 1H), 6.53 (d, J = 8.5 Hz, 1H), 5.30 (s, 2H), 4.24 (p, J = 6.6 Hz, 1H), 3.59 – 3.53 (m, 1H), 2.78 (d, J = 15.0 Hz, 1H), 1.38 (d, J = 6.7 Hz, 4H), 1.26 (s, 3H), 0.89 – 0.86 (m, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 152.7, 148.7, 147.4, 141.0, 140.5, 138.9, 136.5, 136.2, 133.1, 130.7, 129.8, 129.6, 128.1, 127.7, 126.7, 125.7, 125.5, 124.7, 124.1, 123.5, 122.7, 122.5, 121.4, 116.0, 97.6, 87.7, 67.5, 67.4, 53.8, 45.6, 42.0, 19.7. **HRMS** (ESI pos) m/z calcd for $C_{32}H_{22}O$ $[M+H]^+$ 423.17000, found 423.17022.

3. Preparation and characterization of the polypeptide-based nanocarrier, P



Scheme S1. The general convergent synthetic procedure for synthesizing compound N_3-PEG_3-PGA_{50}-VC-PP (polypeptide, **P**) initially involved the synthesis of PP-VC and PG-PD separately. Adapted from Pegoraro et al.[14].

The detailed synthesis of N_3-PEG_3-PGA_{50}-VC-PP (polypeptide, **P**) is described in Pegoraro et al.[14] (reported as compound 16). Briefly, the convergent synthesis involved the synthesis of PP-VC and PG-PD separately. For the synthesis of PP-VC (described in Pegoraro et al.[14] as 6b), a reaction between PP, dissolved in 5:1 DMF:H2O (1 eq, 10 mg/mL), and a VC linker (i.e., 0.03 eq for 3% mol VC modification), dissolved in DMF, followed by Et_3N, was left stirring 24 h. The product was purified by PD-10 columns, yielding a 70% product with over 90% conjugation efficiency. VC functionalization was quantified using UV-vis spectroscopy. Separately, PG (described in e Pegorarot al.[14] as 17) was dissolved in anhydrous DMSO (1 eq., 10 mg/mL), activated with 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate (DMTMM BF4 i.e., 0.1 eq for 5% mol PD-NH2 modification), and reacted with PD-NH2 (i.e., 0.05 eq. for 5% mol modification). After pH adjustment, the reaction proceeded for 48 h, and the product was purified by Vivaspin®, 3000 MWCO Da (after conversion to its salt form), achieving a 60% yield and 90% conjugation efficiency. PD functionalization was determined using both ^{1}H-NMR and UV-vis spectroscopy. Finally, PG-PD (1 eq., 10 mg/ml) was dissolved in water and reacted with PP-VC (i.e., 0.02 eq. for 2% mol PP-VC modification) followed by the reducing agent tris(2-carboxyethyl)phosphine (TCEP, i.e., 0.0025 eq. for 2% mol PP-VC modification). **P** was purified using PD-10 columns, resulting in a 50% yield and 80-90% conjugation efficiency. The functionalization of PP-VC was indirectly calculated by comparing the % wt of PD-NH_2 in PG-PD and P, using UV-vis analysis, resulting in 3.3 % wt of PP-VC.

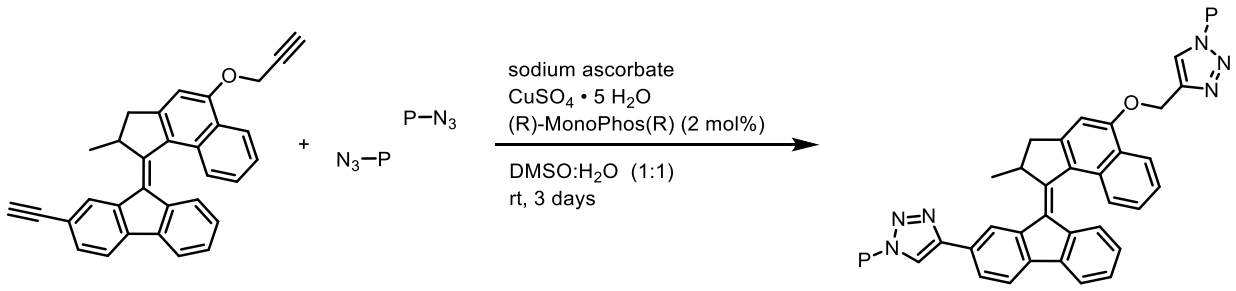
PP-VC: ^{1}H NMR (500 MHz, D_2O, 25°C) δ 7.6-7.3 (m, 4H, p, q, r, s), 6.8 (s, 2H, a), 4.77 – 4.71 (m, 1H, Cα of PLP; 1H g covered with D_2O), 4.50 – 4.20 (m, 1H, Cα of PLO; 1H l; 2H t), 3.96 – 3.30 (m, 2H, Cδ of PLP; CH_2 of $CH_3CH_2CH_2CH_2NH$ and 2H b), 3.24 – 3.06 (m, 2H, Cδ of PLO and 2H o), 2.51 – 1.23 (m, 4H, $CH_2CH_2CH_3$, 4H Cβ + Cγ of PLP; 4H Cβ + Cγ of PLO; 13H c, d, e, f, h, m, n), 0.9 (s, 6H, i), 0.82 (t, J = 7.3 Hz, 3H, $CH_2CH_2CH_3$).

PG-PD: 1H NMR (500 MHz, D_2O, 25°C) δ 8.40 (d, 1H, u), 7.80 (m, 2H, v, x), 7.25 (d, 1H, y), 4.40 – 4.20 (m, 1H, Cα of poly-L-glutamic acid (PGA)), 3.80 – 3.30 (m, 12H, $N_3(CH_2CH_2)_3O$; 4H OCH_2CH_2NH), 3.17 - 2.66 (m, 4H, k, j), 2.51 – 1.66 (m, 4H, Cβ + Cγ of PGA).

P: ^{1}H NMR (500 MHz, D_2O, 25°C) δ 8.40 (d, 1H, u), 7.80 (m, 2H, v, x), 7.25 (d, 1H, y), 4.77 – 4.71 (m, 1H, Cα of PLP; 1H g covered with D_2O), 4.40 – 4.20 (m, 1H, Cα of PGA, 1H, Cα of PLO; 1H l; 2H t), 3.83 – 3.30 (12H, $N_3(CH_2CH_2)_3O$, 4H OCH_2CH_2NH, 2H Cδ of PLP; CH_2 of $CH_3CH_2CH_2CH_2NH$ and 2H b), 3.20 – 2.66 (2H, Cδ of PLO, 2H o and 4H k, j), 2.51 – 1.66 (4H Cβ + Cγ of PGA, 4H $CH_2CH_2CH_3$, 4H Cβ + Cγ of PLP; 4H Cβ + Cγ of PLO; 13H c, d, e, f, h, m, n), 0.9 (s, 6H, i), 0.82 (t, J = 7.3 Hz, 3H, $CH_2CH_2CH_3$).

The detailed physicochemical and biological characterization of **P** is provided in Pegoraro et al. [14]. Briefly, **P** revealed a branched structure with a compact core (5.0 ± 0.3 nm) as determined by small-angle x-ray scattering (SAXS), similar to parental polymer PP. Due to PGA, Zeta potential measurements revealed a negatively charged surface (-15.7 ± 0.2 mV). **P** demonstrated excellent biocompatibility with over 80% cell viability across all tested concentrations. Flow cytometry analysis showed significant cell internalization via an energy-independent mechanism due to its small size and flexible structure. Importantly, **P** maintained its mitochondrial colocalization capability, with a high Pearson r value (>0.6) after 30 min of incubation in MDA-MB-231 cells. In membrane interaction studies, **P** formed selective patches in cardiolipin-rich supported lipid bilayers, confirming cardiolipin-specific interaction while preserving membrane physical properties. Activity studies verified a safer biological profile than PP, with no significant alterations in reactive oxygen species (ROS) production, mitochondrial morphology, or respiration.

4. Preparation of the polypeptide-molecular motor conjugate



MM **P-MM**

Scheme S2. Synthetic procedure for **P-MM** preparation.

An oven-dried vial was charged with **MM** (1.2 mg, 2.8 µmol, 0.5 eq.) and **P** (40 mg, 5.6 µmol, 1.0 eq.) in a mixture of DMSO:H_2O (1:1). Sodium ascorbate (40 mg, 5.5 µmol, 5.0 eq.), copper sulfate pentahydrate (1.4 mg, 5.6 µmol, 1.0 eq, and ligand (*R*)-MonoPhos(*R*) (0.04 mg, 0.112 µmol, 0.02 eq.) were added to the mixture. The reaction mixture was stirred for three days at room temperature. After DMSO elimination using a rotavapor and freeze-drying, the product was purified by PD-10 desalting columns packed with Sephadex G-25 resin.

*Note on characterization of **P-MM**: The final compound could not be characterized by NMR spectroscopy due to the low prevalence of the molecular motor peaks in the peptide signal. Proofs of the obtain compound come from the change in color of the product; **MM** as an intense yellow, **P** as a soft white solid, gave **P-MM** as a soft solid with an intense yellow color after purification. Raman spectroscopy (see section below), confirmed the complete disappearance of alkyne signals (around 2120 cm^{-1}) present in **MM** after conjugation to **P**, confirming that no free alkynes are present in the resulting structure. IR spectroscopy (see section below) further corroborated the conjugation via the disappearance of P's azide peaks (around 2110 cm^{-1}). Changes in the supramolecular conformation of **P-MM** after light irradiation (see Cryo-TEM section) also give undirect proof of molecular motor attachment. Attempts with MALDI-TOF spectroscopy were not successful.*

5. Raman spectroscopy

Steady-state non-resonant Raman spectra of **P, MM,** and **P-MM** were recorded using an excitation laser at 785 nm. The Raman data was baseline corrected using Spectragryph.

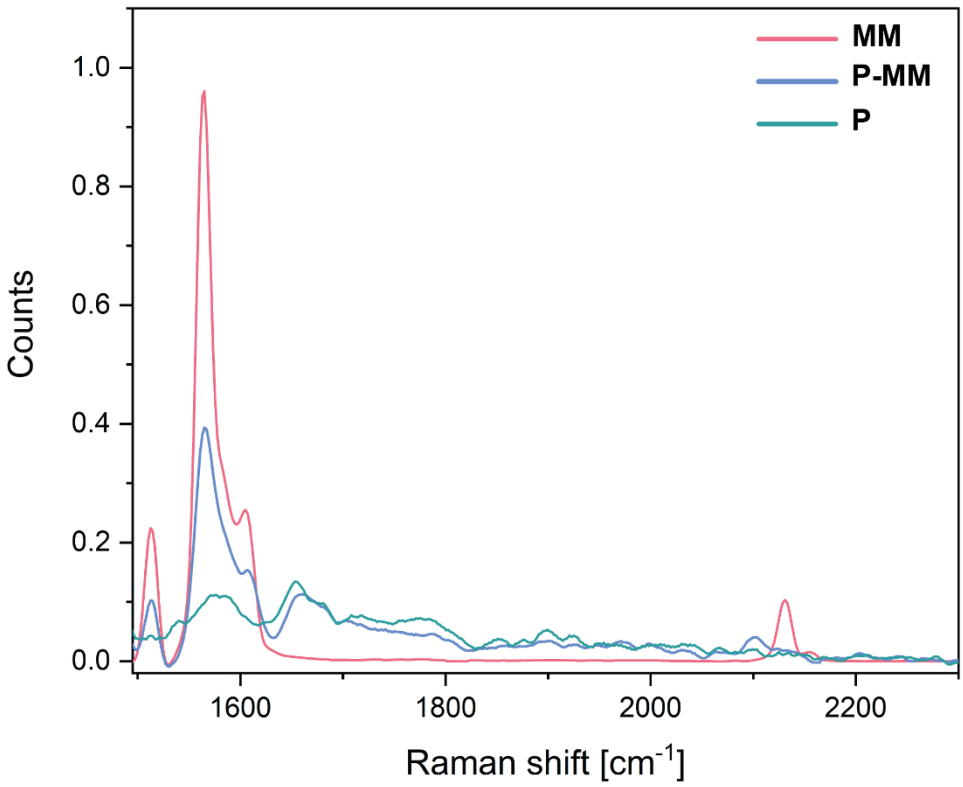


Figure S1. Raman spectra of **MM** (red), **P-MM** (blue) and **P** (green) superposed.

6. InfraRed spectroscopy

Infrared spectrometry (IR) of **P** and **P-MM** was carried out with Spectrum Two FT_IR Spectrometer (PerkinElmer) in the solid state.

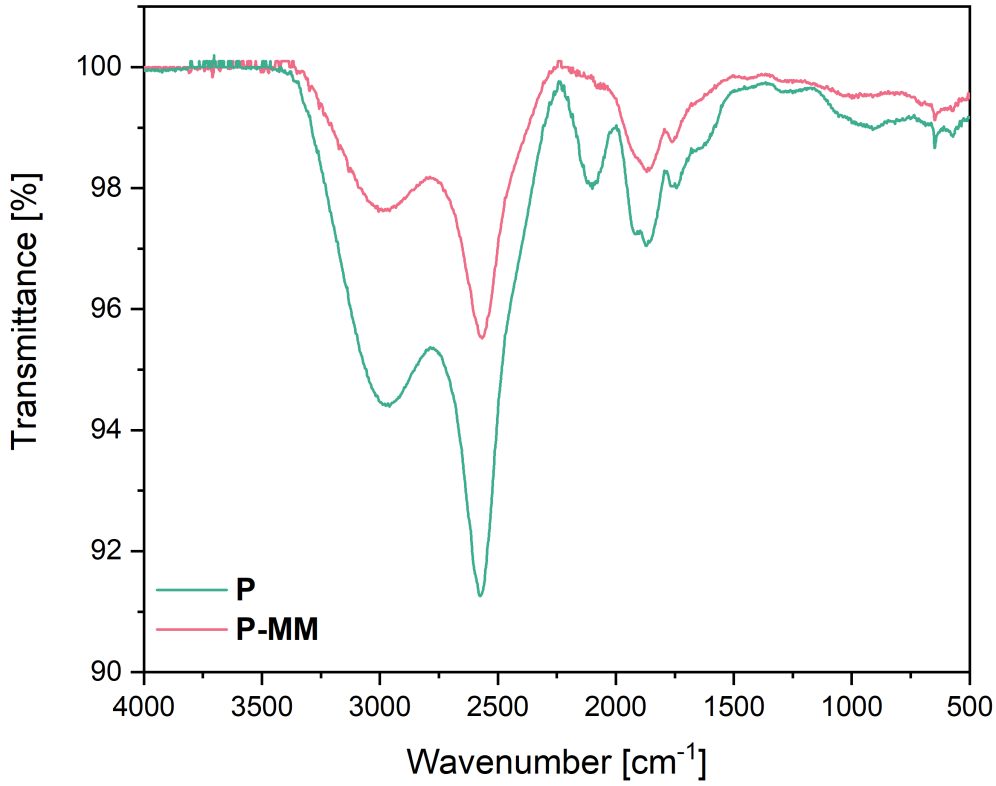
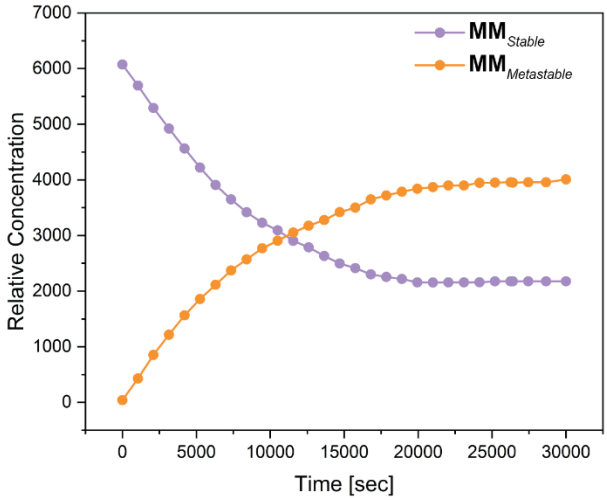


Figure S2. InfraRed spectra of **P** (green) and **P-MM** (red) superposed.

7. NMR studies of 'MM's rotation cycle

A 1 mM solution of **MM** in its stable form (**MM**$_{\text{Stable}}$) was prepared in $CDCl_3$ and transferred into an NMR tube fitted with a glass optic fiber for *in situ* irradiation studies. The sample was placed in a Varian Unity Plus 500MHz NMR and cooled to -15°C. ^{1}H NMR spectra were recorded before irradiation while irradiating at 405 nm until the PSS was reached and during the THI step until completion.

A



B

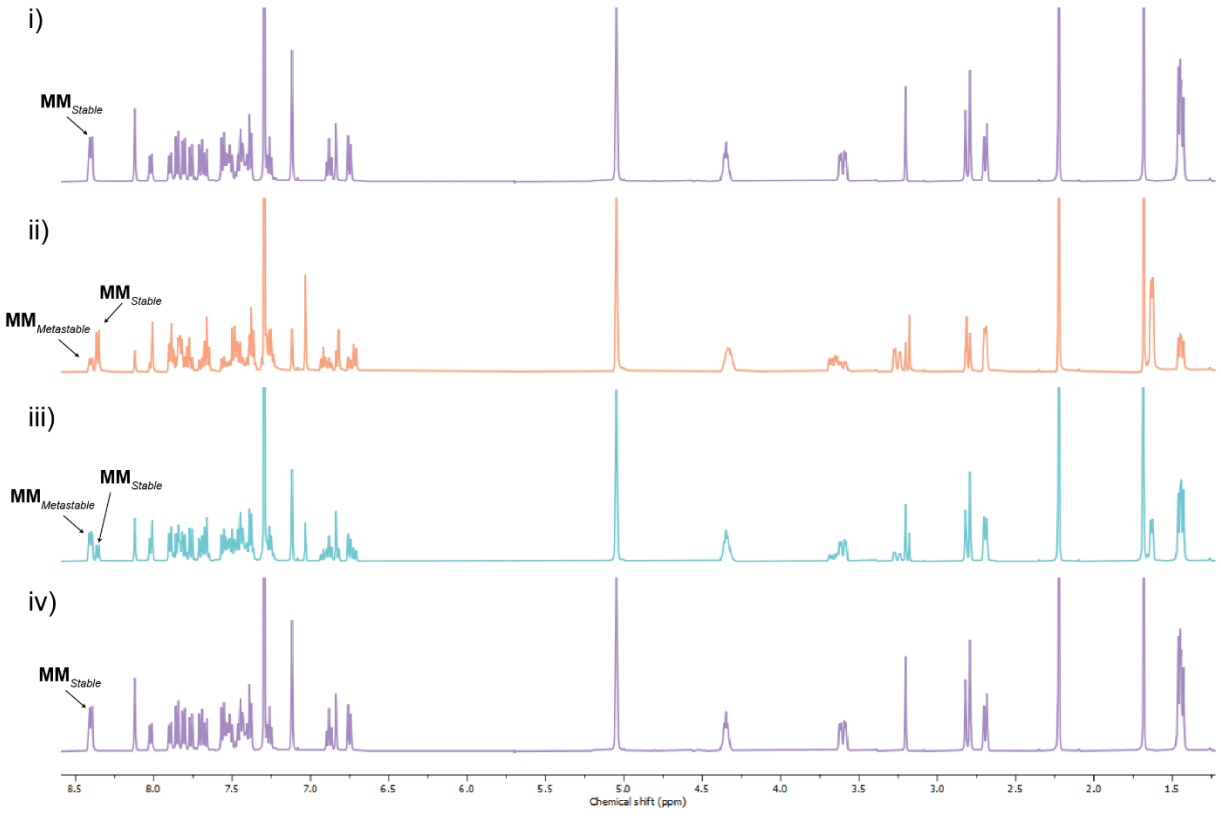


Figure S3. A) ^{1}H-NMR irradiation studies of **MM** in its stable form in $CDCl_3$ (c = 5 mM) at 5°C. **B**) Kinetics of distinct species of **MM**$_{Stable}$ ^{1}H-NMR spectra change under irradiation. i) **MM**$_{Stable}$ before irradiation, ii) after 1 h of irradiation at 405 nm, iii) 7 h of irradiation reaching PSS, iv) no irradiation THI, after 12 h in the dark.

8. UV-vis

UV–vis spectroscopy was used to evaluate **MM** and **P-MM** isomerization and for thermodynamic studies using an Agilent 8453 UV–vis Diode Array System equipped with a Quantum Northwest Peltier controller. Unless specified, irradiations were conducted using a built-in setup coupled to an LED. Solutions were prepared and measured using a 10 mm quartz cuvette. The same procedure was used for UV-Vis controls on **P**.

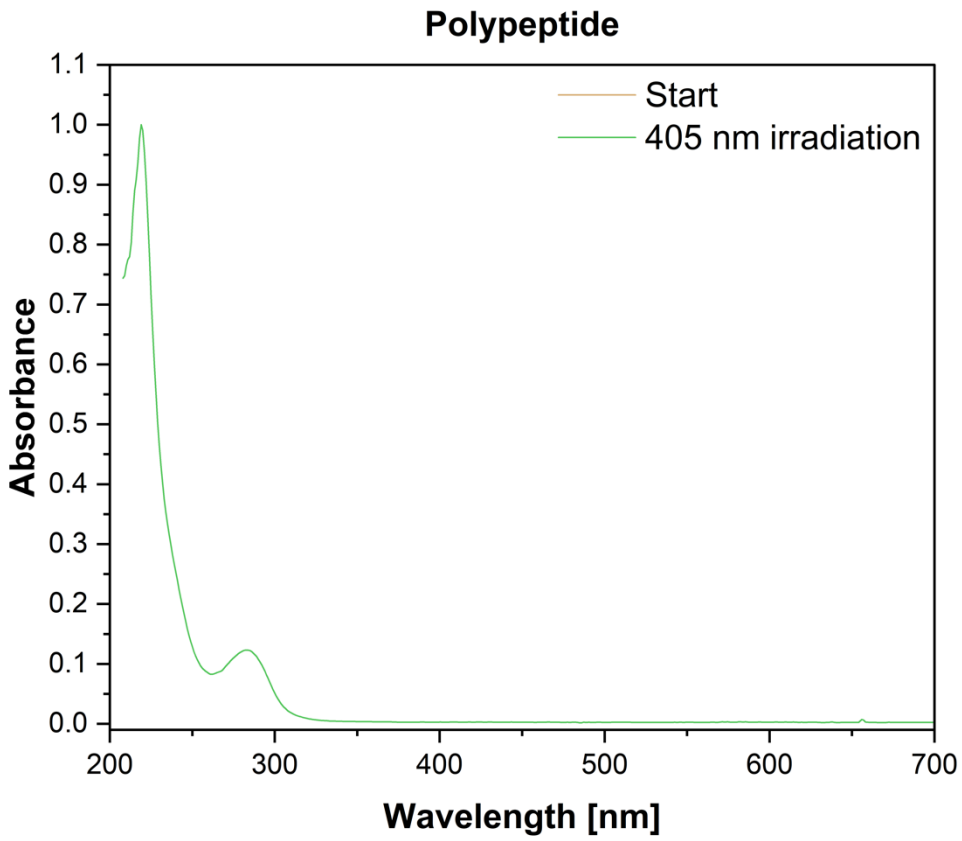


Figure S4. Absorption spectra of **P** under light irradiation (in PBS, 37°C). The orange line is not visible due to the complete overlap of the two spectra.

9. Thermodynamics - Eyring analysis

50 µM solutions of **MM** and **P-MM** were prepared in a 10 mm quartz cuvette and irradiated with 405 nm light until the PSS was reached. An Eyring plot analysis of the thermal isomerization processes in different environments was performed by monitoring the decrease in absorption of a UV-Vis sample over time at five different temperatures. Rate constants, (k), as changes of absorbance over time were

determined by fitting a first-order decay and plotted to determine the thermodynamic parameters of the thermal helix inversions using Origin 2023 software and a least squares analysis was performed on the Eyring equation to retrieve the ΔG ‡. The activation parameters at 25°C for the metastable → stable process can be determined and are displayed in **Table S1**. Eyring plots and half-live times at 25°C for the studied environments are shown in **Figure S4**.

Table S1. Activation parameters of **MM** and **P-MM** at 37°C.

Compound	$\Delta G^{\ddagger}$ $[kcal/mol]$	$\Delta H^{\ddagger}$ $[kcal/mol]$	$\Delta S^{\ddagger}$ $[kcal/mol]$	$t_{1/2}$ $[sec]$
MM	18.953±0.027	18.944	-0.0272	2.43
P-MM	22.136±0.051	7.822	-46.151	425.38

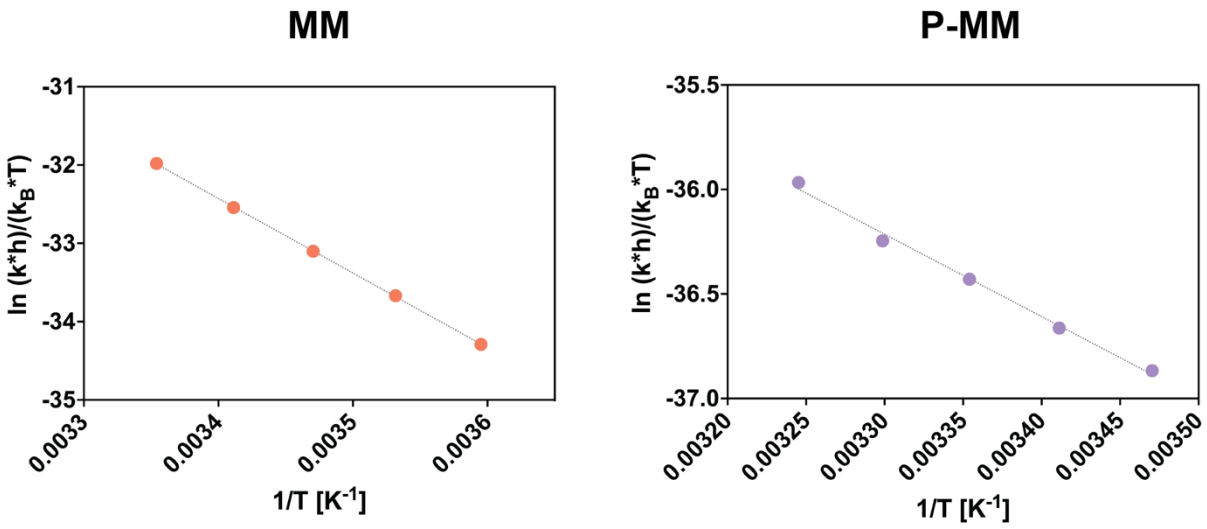


Figure S5. Eyring plot analysis of the **MM** and **P-MM** monitoring the decrease in absorption at 425 nm at five different temperatures.

10. Circular Dichroism

Circular dichroism (CD) spectroscopy was conducted utilizing a J-815 CD Spectrometer from JASCO Corporation, incorporating a Peltier thermostated cell holder (PTC-423, JASCO Corporation) equipped with a recirculating cooler (JULABO F250, JASCO Corporation). A nitrogen flow of approximately 1.5 L/min was directed through the spectrometer and regulated using a nitrogen flow monitor from Afriso Euro-Index. The samples were prepared in PBS buffer at 0.25 mg/mL. Each sample was analyzed three times (n=3) in a quartz cuvette with a 1 mm diameter.

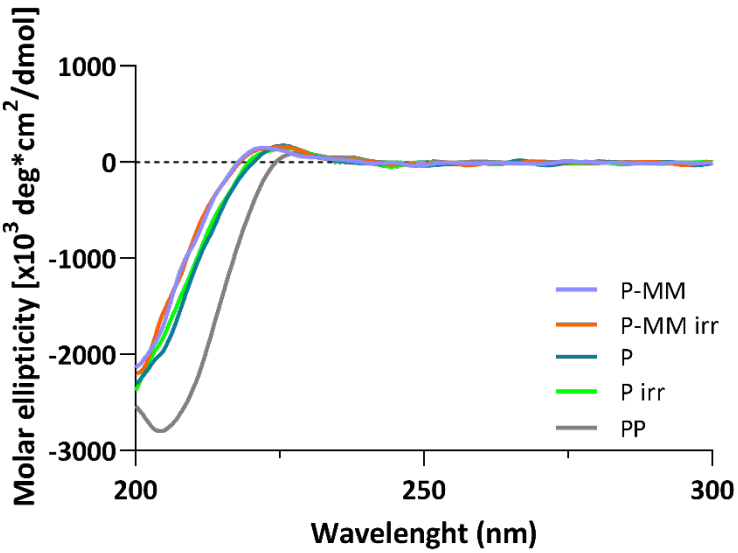


Figure S6. Circular dichroism spectra of irradiated (irr) and non-irradiated **P-MM** and **P** (random coil) compared to the diblock copolymer PP (PPII).

11. Critical Aggregation Concentration Studies

The differential self-assembly of the **P-MM** after irradiation was assessed by incorporation of the hydrophobic solvatochromic probe Nile Red (9-diethylamino-5-benzo[α]phenoxazinone), which exhibits a blue shift emission upon inclusion in hydrophobic environments. Starting from a 2 mg/ml **P-MM** solution, 1:2 dilutions were prepared in PBS. Nile Red (0.5 mM in ethanol) was added to each **P-MM** solution to a final concentration of 375 nM. Using a JASCO FP6200 fluorometer, the solutions were excited at 550 nm, and the spectra were recorded over a wavelength range of 580–750 nm. After 5-10 min of irradiation at 405 nm of each solution, the measurements were repeated under the same conditions. Blue shifts were calculated by subtracting the emission wavelength of Nile Red in buffer solutions (PBS) from the emission wavelength of the sample. Blue shifts were plotted against concentrations (log. Conc in ug/ml) to determine the critical aggregation concentration (CAC) value.

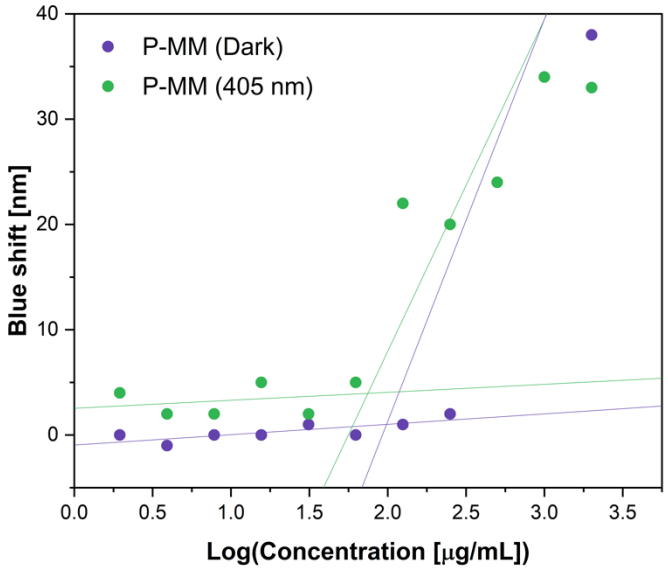


Figure S7. Blue shift in Nile Red maxima absorbance peak with increasing concentrations of non-irradiated (purple) and irradiated (green) **P-MM** in aqueous solution.

Table S2. CAC values obtained for **P-MM** with/without irradiation at 405 nm.

P-MM	Before irradiation	**59.4 μM**
	5 min at 405 nm	**25.9 μM**

12. Cryo-TEM

2.5 µL (2 mM) samples were placed on a glow-discharged hollow carbon-coated grid (Quantifoil 3.5/1, QUANTIFOIL Micro Tools GmbH). After blotting, the corresponding grid was rapidly frozen in liquid ethane (Vitrobot, FEI) and kept in liquid nitrogen until measurement. The grids were observed with a Gatan model 626 cryostage in a Tecnai T20 Field Electron and Ion Company (FEI) cryo-electron microscope operating at 200 keV. Cryo-TEM images were recorded on a slow-scan charge-coupled device (CCD) camera under low-dose conditions. All processes were performed in the dark. The same procedure was used with a quartz cuvette for irradiated samples immediately before freezing.

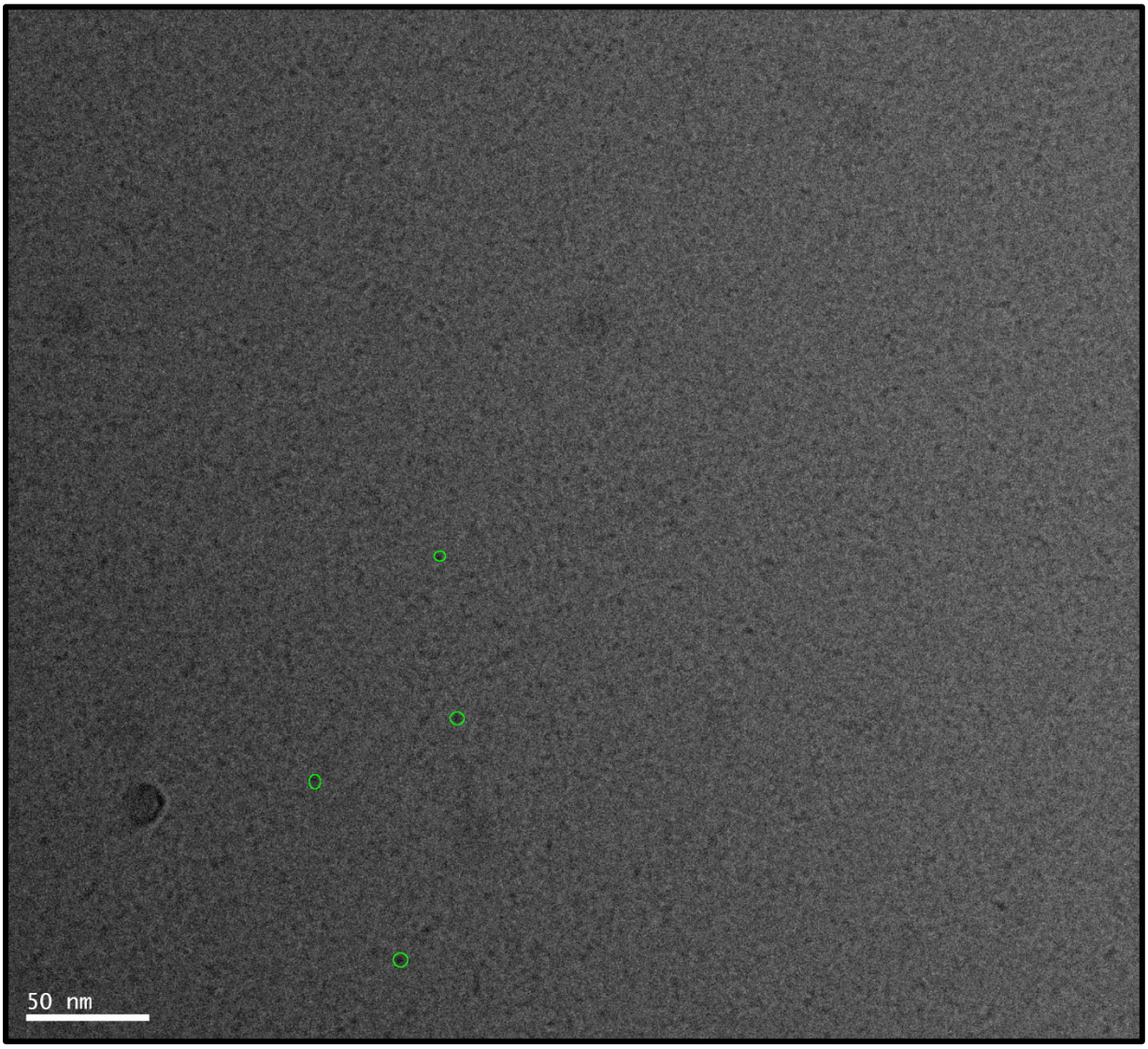


Figure S8. Cryo_EM picture of P-MM (in PBS solution) before irradiation.

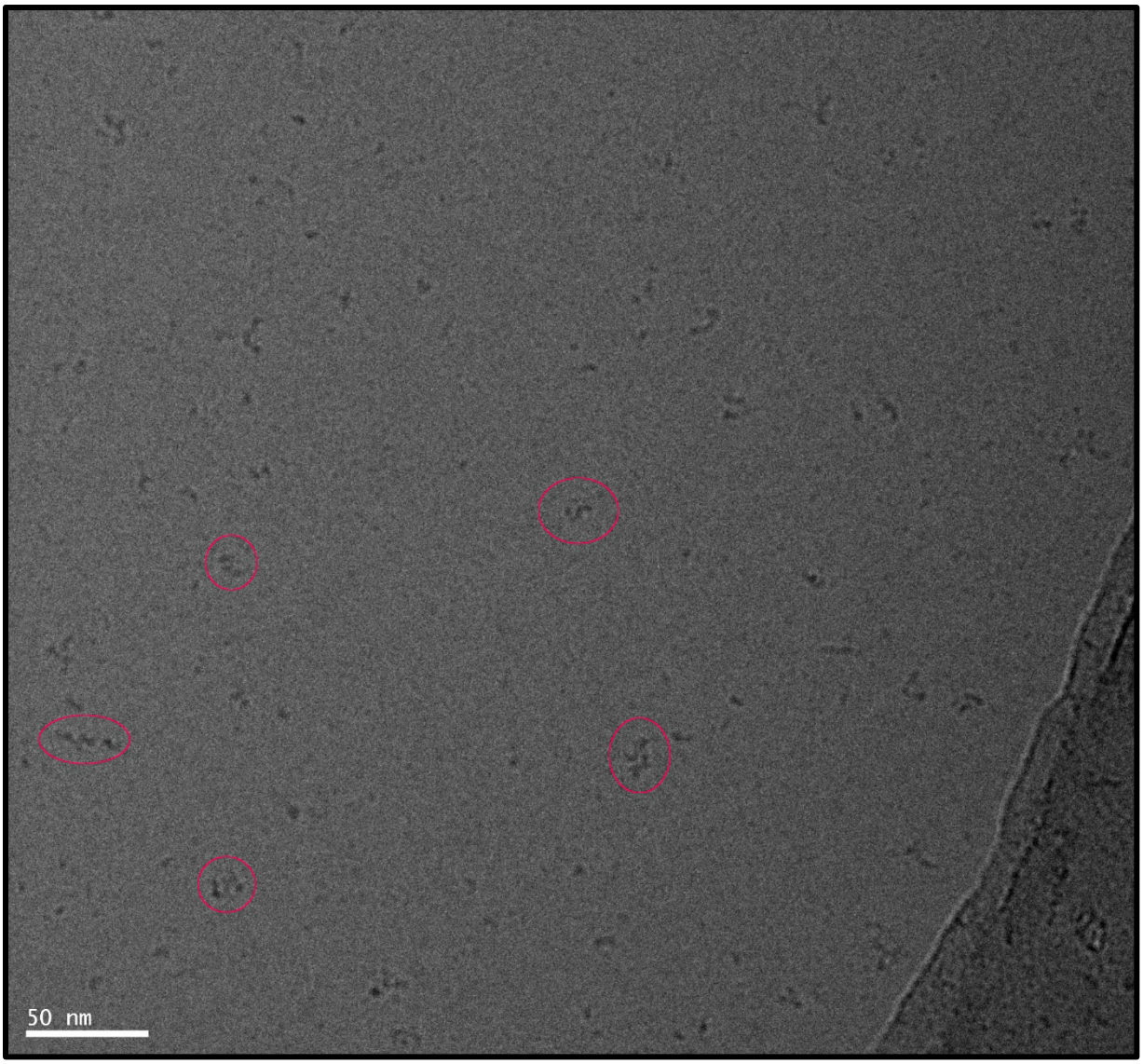


Figure S9. Cryo-EM picture of **P-MM** after irradiation (5 min, 405 nm LED, PBS solution).

13. Cell viability studies

Cell viability was assessed after treatment with the **P** and **P-MM**. MDA-MB-231 cells were seeded in 96-well microlitre plates (5,000 cells/well, 0.05 ml per well) and incubated for 24 h at 37° and 5% CO^2. Concentrated stock solutions of P and P-MM were prepared in PBS buffer and diluted in DMEM/F-12 medium (with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S). P and P-MM solutions were sterilized using a 0.2 µm filter and added to give a final concentration of 0-1 mg/ml (dilution factor 1.5). After 24/72 h of incubation, the CellTiter-Glo® 2.0 Cell Viability Assay was performed to determine cell viability. Following 20 min of incubation, ATP luminescence was measured

spectrophotometrically using a CLARIOstar plate reader. The luminescence values were expressed as a percentage of cell viability, with 100% representing the cell viability of untreated control cells. Value's represent mean ± SD (n=3).

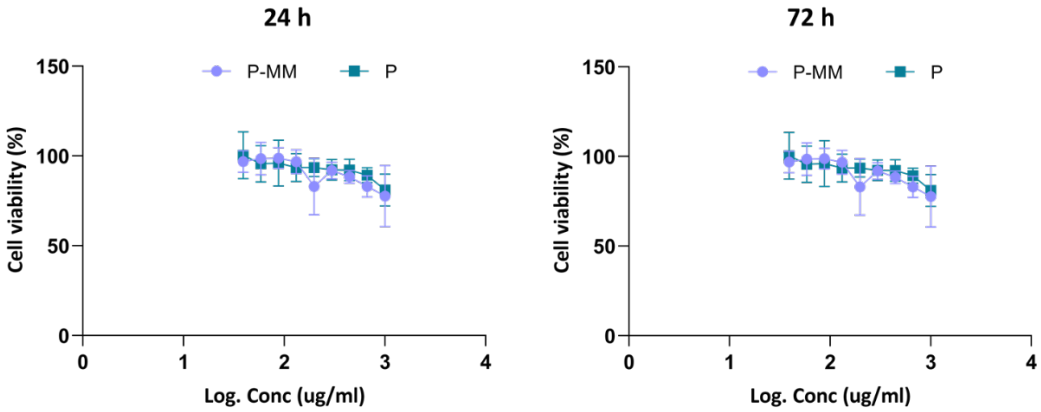


Figure S10. MDA-MB 231 cell viability CellTiter-Glo® 2.0 Cell Viability Assay in response to **P-MM** and **P** treatment (24 and 72 h, n = 3, mean ± SEM).

Cell viability studies were performed using MTS assays at various exposure times to determine the optimal non-toxic irradiation time at 405 nm for further biological investigations. MDA-MB-231 cells were seeded in 96-well microlitre plates (5,000 cells/well, 0.05 ml per well) and incubated for 24 h at 37° and 5% CO_2. The cells were then irradiated using a Lumidox® II 96-Well LED Arrays (lens mat/solid base) (UV wavelength of 405 nm) powered by the Lumidox® II LED Controller (LUM2CON) with customizable power light/well and irradiation time control. After irradiation, the CellTiter-Glo® 2.0 Cell Viability Assay was performed to determine cell viability. Following 20 min of incubation, ATP luminescence was measured spectrophotometrically using a CLARIOstar plate reader. The luminescence values were expressed as a percentage of cell viability, with 100% representing the cell viability of untreated control cells. Values represent mean ± SD (n=3). Among the tested exposure times, 30 s was selected as the optimal condition for further experiments.

Table S3. Laser power per well at varying irradiation times at 405 nm

Irradiation 405nm - 65mW/well (355 mW/cm^2 = 0.355 J/cm^2 x sec)	
Time (sec)	Laser Power (J/cm^2)
0	0
30	10.65
60	21.3
120	42.6

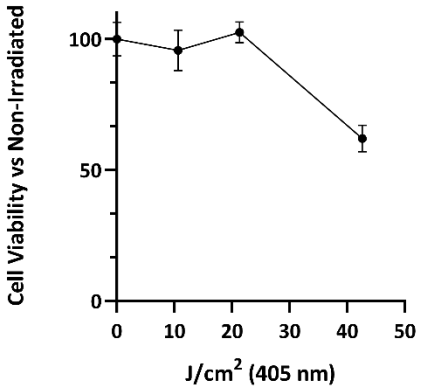
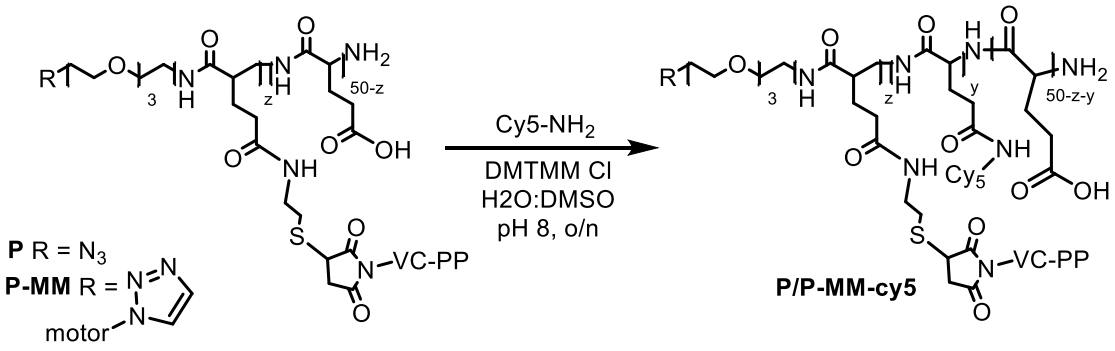


Figure S11. MDA-MB 231 cell viability CellTiter-Glo® 2.0 Cell Viability Assay in response to different irradiation times at 405nm (n = 3, mean ± SEM).

Cell viability was then assessed after treatment with the **P** and **P-MM** after 30 s of irradiation at 405 nm. MDA-MB-231 cells were seeded in 96-well microlitre plates (5,000 cells/well, 0.05 ml per well) and incubated for 24 h at 37° and 5% CO_2. Concentrated stock solutions of **P** and **P-MM** were prepared in PBS and diluted in DMEM/F-12 medium (with 10% FBS and 1% P/S). **P** and **P-MM** solutions were then sterilized using a 0.2 µm filter and added to give a final concentration of 0-1 mg/ml (dilution factor 1.5). Immediately after addition, cells were irradiated using a Lumidox® II 96-Well LED Arrays (lens mat/solid base) (UV wavelength of 405 nm) powered by the Lumidox® II LED Controller (LUM2CON) for 30 sec. After 3/24 h, CellTiter-Glo® 2.0 Cell Viability Assay was performed to determine cell viability. Following 20 min of incubation, ATP luminescence was measured spectrophotometrically using a CLARIOstar plate reader. The luminescence values were expressed as a percentage of cell viability, with 100% representing the cell viability of untreated control cells. Values represent mean ± SD (n=3).

14. Fluorescence labeling of P and P-MM with Cy5



Scheme S3. Labeling of **P** and **P-MM** with Cy5-NH_2.

In a one-neck bottom flask, **P** or **P-MM** was dissolved in Milli-Q water (1 eq., 10 mg/mL), and the carboxylic group was activated with DMTMM Chloride (i.e., 0.03 eq. for 1.5% mol Cy5-NH_2) dissolved in 0.5 mL of Milli-Q water. In a glass vial protected by light, Cy5-NH_2 was dissolved in DMSO and added to the solution (0.015 eq., 1.5% mol). The pH was adjusted with sodium bicarbonate (1M, pH 8). The reaction was allowed to proceed overnight, the mixture protected from light, and evaluated by thin-layer chromatography (MeOH as mobile phase). After DMSO elimination on the rotavapor and by freeze-drying, the product was purified using PD-10 desalting columns packed with Sephadex G-25 resin. Dye loading was calculated by fluorescence using the fluorescent plate reader CLARIOstar. First, the corresponding free dye (DMF:H2O) calibration curve was prepared in dark 96-well multiwell plates. Fluorescence was measured at λex: 632 nm λem: 684 nm for Cy5-NH2. Yields: 80%. Conjugation efficiency 60-100%.

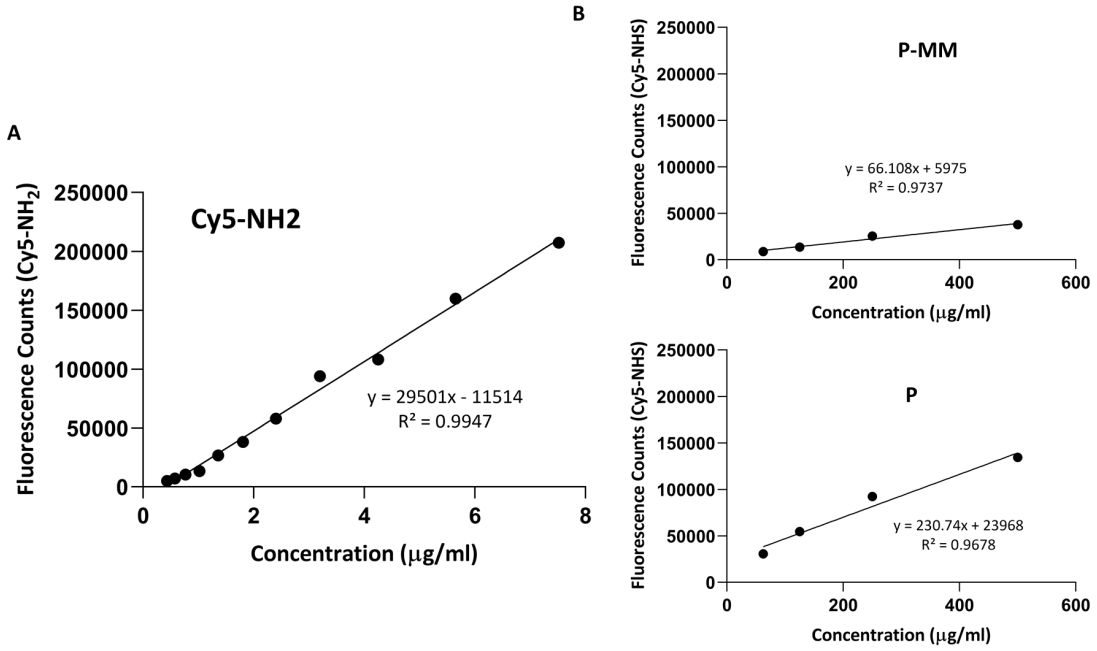


Figure S12. Fluorescence calibration curves of **(A)** Cy5-NH2 and **(B)** P-MM (**upper**) and P (**lower**) in DMF:H_2O for quantifications. % wt **P-MM** = 0.5 and **P** = 1.8.

15. Cell Uptake studies

Cell uptake of **P** and **P-MM** with/without irradiation at 405 nm was quantitatively analyzed using the high-throughput In Cell Analyzer 2200 (Cytiva, UK) system with four fluorescent channels and a 40x objective. The experiments, conducted under controlled CO_2 and temperature conditions, were performed using specific fluorescent markers: Invitrogen™ CellTracker™ Red CMTPX Dye (cytosol, 0.5 μM, ex. 575/25 em. 620/30), Helix NP™ Green (nuclei, 50 nM, ex. 475/28 em. 525/48), and Cyanine5 (Cy5, **P** and **P-MM**, 0.2 mg/ml, ex. 632/22 em. 684/25). MDA-MB-231 cells (5,000 cells/well, 0.05 ml per well) were seeded in a 96-well black optical-bottom plate and incubated for 24 h at 37°C with 5% CO_2. DMEM/F-12 medium (with 10% FBS and 1% P/S) was removed and substituted with DMEM/F-12 medium (1% P/S, without FBS), followed by a 1-h incubation with Invitrogen™ CellTracker™ Red CMTPX Dye. **P** and **P-MM** were added (0.2 mg/ml) to reach a final volume of 100 μl per well, and the plate was rapidly irradiated using a Lumidox® II 96-Well LED Arrays (lens mat/solid base) (UV wavelength of 405 nm) powered by the Lumidox® II LED Controller (LUM2CON) for 30 s. Non-irradiated treated cells with **P** and **P-MM**, control cells (to establish autofluorescence levels), and control conditions (Invitrogen™ CellTracker™ Red CMTPX Dye (cytosol, 0.5 μM) and Helix NP™ Green) were also analyzed. For each condition (n=3), 7 regions of interest (ROIs) (~ 60 live cells/ROI) were selected, and the fluorescence

intensity was quantified for each channel every 20 min for 6 h.

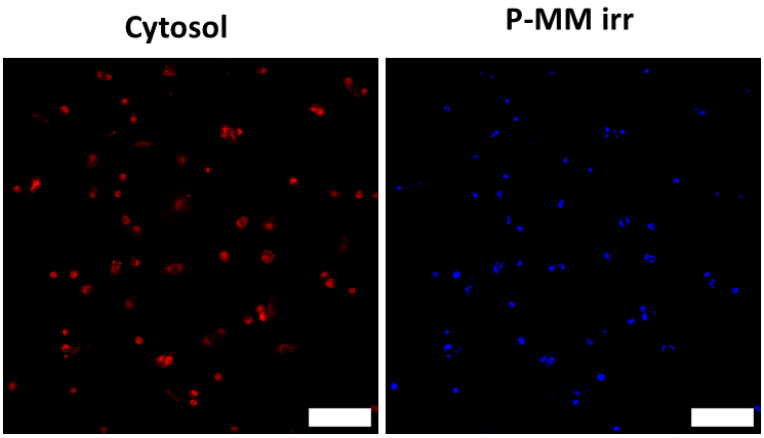


Figure S13. Representative ROI of irradiated (irrr) **P-MM** 20 min post-irradiation at 405 nm (right). CellTracker™ Red CMTPX Dye used to label the cell cytosol (left). For each condition (n=3), 7 ROI (~ 60 live cells/ROI) were analyzed using the InCell Analyzer 2200. Scale bar: 200 μm.

For the data analysis, the mean fluorescence intensity of the **P** and **P-MM**-treated wells was normalized to the % of Cy5 for each treatment. The Cy5 fluorescence intensity was adjusted to account for the quenching effect caused by irradiation at 405 nm for 30 s. To determine the quenching conversion factors, the fluorescence intensity of **P** and **P-MM** was monitored at 20-min intervals over 6 h in both irradiated and non-irradiated states using a CLARIOstar plate reader, replicating the conditions of an InCell experiment without cells. The quenching conversion factors, calculated as the ratio of Cy5 fluorescence intensity between non-irradiated and irradiated samples, were 2.3 (± 0.03) for **P-MM** and 2.15 (± 0.02) for **P**. The Cy5 fluorescence intensity was determined in CellTracker™ Red CMTPX Dye positive cells. Values represent mean ± SD (n=3).

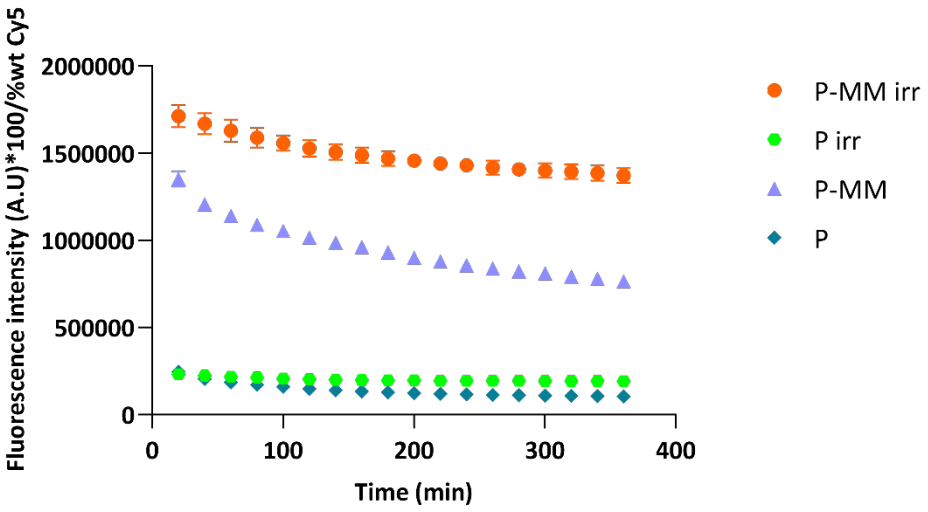


Figure S14. Cy5 fluorescence intensity of **P and P-MM** (non-irradiated and irradiated [irr] at 405 nm for 30 s) after cell segmentation every 20 min for 6 h. The slight decrease in Cy5 fluorescence intensity over time for **P-MM** is due to the quenching effect of **MM**, which acts as an effective Förster resonance energy transfer (FRET) acceptor when they are close to molecular dyes such as Cy5. The same effect was not observed for **P**.

The cellular uptake of **P-MM** with/without irradiation at 405 nm was qualitatively analyzed by confocal fluorescence microscopy (live-cell imaging) using a Leica TCS SP8 confocal microscope with a 63x 1.4NA CS2 oil immersion objective. Excitation at 488 nm, 561 nm, and 638 nm was employed, along with four fluorescent photomultiplier tubes (PMTs) and two hybrid Detectors (HyD). The experiments were conducted under controlled CO_2 and temperature conditions. Pulse-chase studies (n=3) were performed using specific fluorescent markers: MitoTracker™ Red CM-H2Xros M7513 (mitochondria, 100nM), LysoTracker™ Green DND-26 (lysosomes, 100nM), and Cyanine5 (P-MM). MDA-MB-231 cells (1,500 cells/well, 0.03 ml per well) were seeded in a 384-well black optical-bottom plate and incubated for 24 h at 37°C with 5% CO_2. For pulse-chase studies, **P-MM** was added at different time points (20, 40, 80,120, 160, and 200 min), immediately followed by irradiation (in case of irradiated conditions) using a Lumidox® II 96-Well LED Arrays (lens mat/solid base) (UV wavelength of 405 nm) powered by the Lumidox® II LED Controller (LUM2CON) for 30 s. Mitotracker and Lysotracker were added 1 h before analysis. Control cells with similar incubation times were also analyzed to establish autofluorescence levels. Image processing was conducted using Leica Application Suite X (LAS X) and the Image J-JACoP plugin for Pearson r calculation.

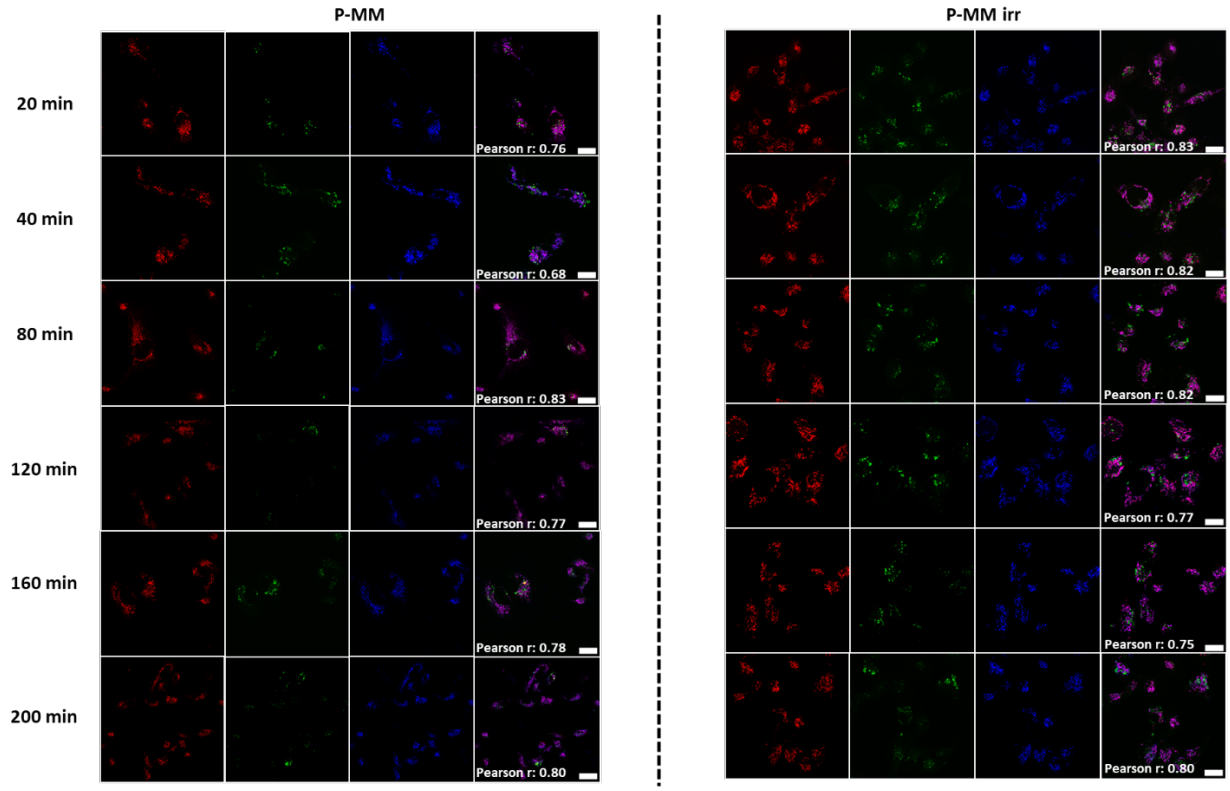


Figure S15. Confocal images of **P-MM** uptake at different times post-treatment and irradiation (405 nm, 30 s for P-MM irr) in MDA-MB-231 cells following a pulse-chase experiment (Figure SXX) (Red – MitoTracker™ Red CM-H2Xros for mitochondria; Green – LysoTracker® Green for lysosomes; Blue – Cy5 for P-MM and P-MM irr). Colocalization of **P-MM and P-MM irr** with Mitotracker Red shown in the merged image (in purple). The Pearson r value was calculated using the ImageJ – JACoP plugin. Scale bar: 10 μm.

16. Reactive oxygen species production

MDA-MB-231 cells were seeded into a 6-well plate (150,000/well, 2 ml per well) and allowed to adhere for 24 h at 37°C and 5% CO_2. P-MM and P (labeled with Cy5) were added to give a final concentration of 0.2 mg/ml (same concentration used for the uptake studies), immediately followed by irradiation (in case of irradiated conditions) using a Lumidox® II 96-Well LED Arrays (lens mat/solid base) (UV wavelength of 405 nm) powered by the Lumidox® II LED Controller (LUM2CON) for 30 s. After 3/24 h, cells were washed with PBS, trypsinized, and centrifuged to obtain cell pellets resuspended in fresh DMEM/F-12 medium (with 10% FBS and 1% P/S). Cells were then incubated with 5 μg/ml of 2,7-dichlorodihydrofluorescein (DCFH) for 30 min at 37°C and 5% CO_2. Subsequently, propidium iodide (2 μg/ml), a marker for cell viability, was added, and the resulting samples were analyzed using flow

cytometry (CytoFLEX S, Beckman-Coulter, CA, USA). Data collection involved 10,000 counts per sample, and data were analyzed using CytExpert version 2.3 software. Data were normalized to the control set as 100%. Control cells with the same irradiation times (with/without **P** and **P-MM**) were also analyzed to establish autofluorescence levels. Values represent mean ± SD (n=3).

17. NMR spectra

The ^{1}H and ^{13}C NMR spectra of newly reported compounds are reported here.

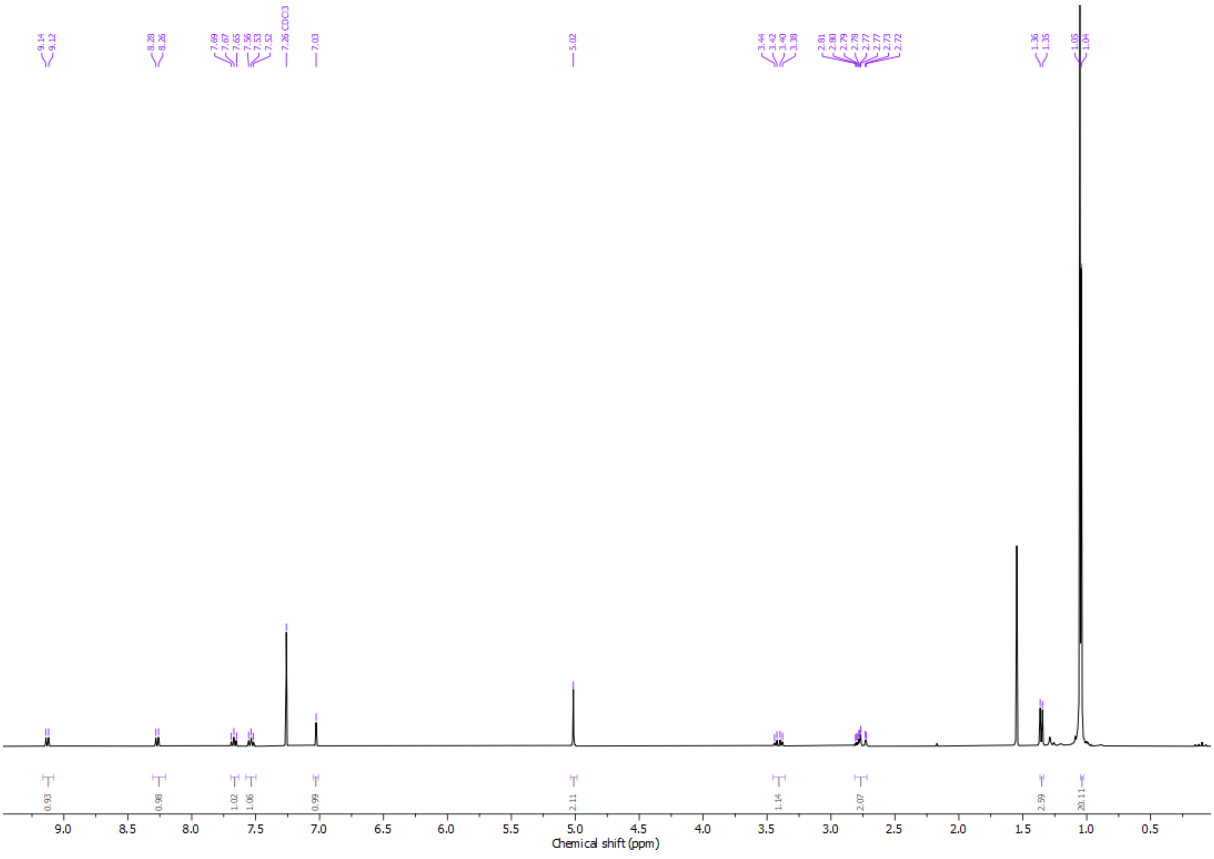


Figure S16. ^{1}H-NMR spectra of **S5**.

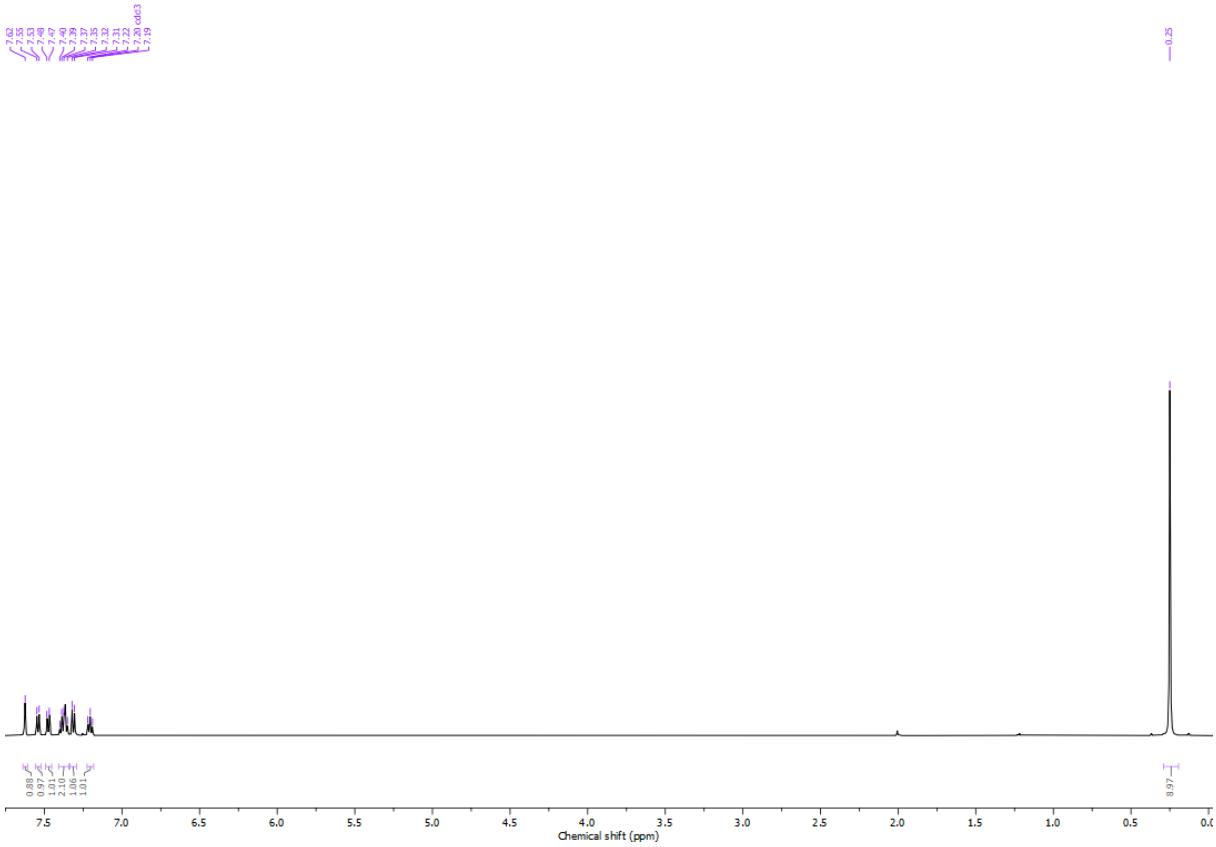


Figure S17. ^{1}H-NMR spectra of **S6**.

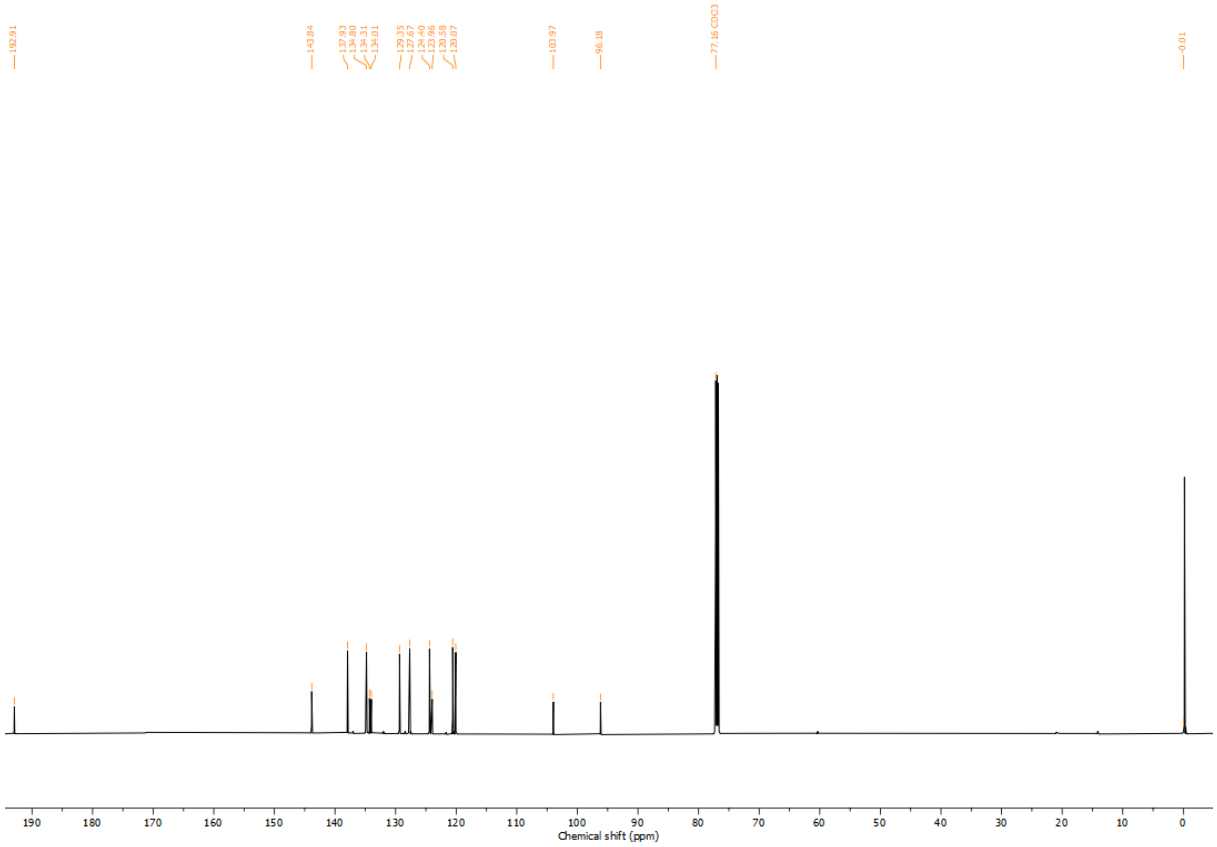


Figure S18. ^{13}C-NMR spectra of **S6**.

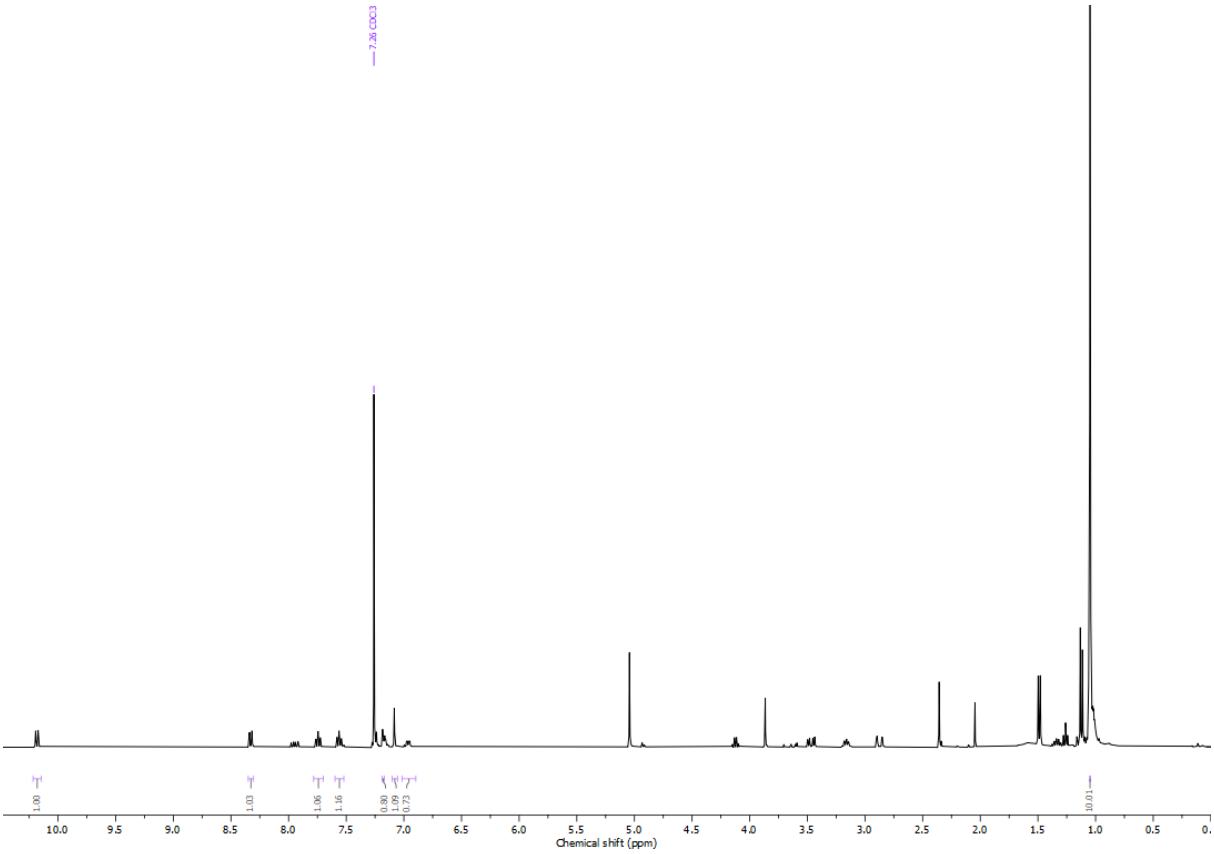


Figure S19. ^{1}H-NMR spectra of **S7**.

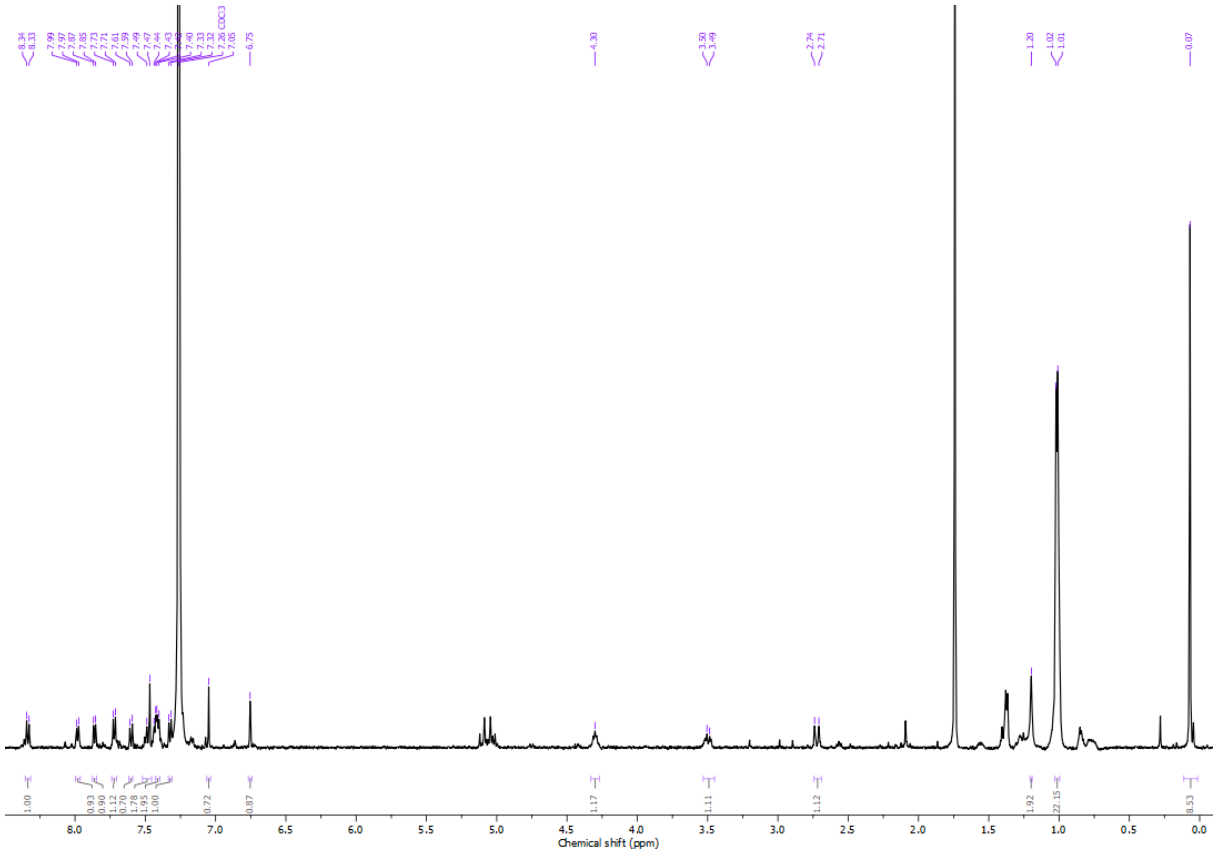


Figure S20. ^{1}H-NMR spectra of **S9**.

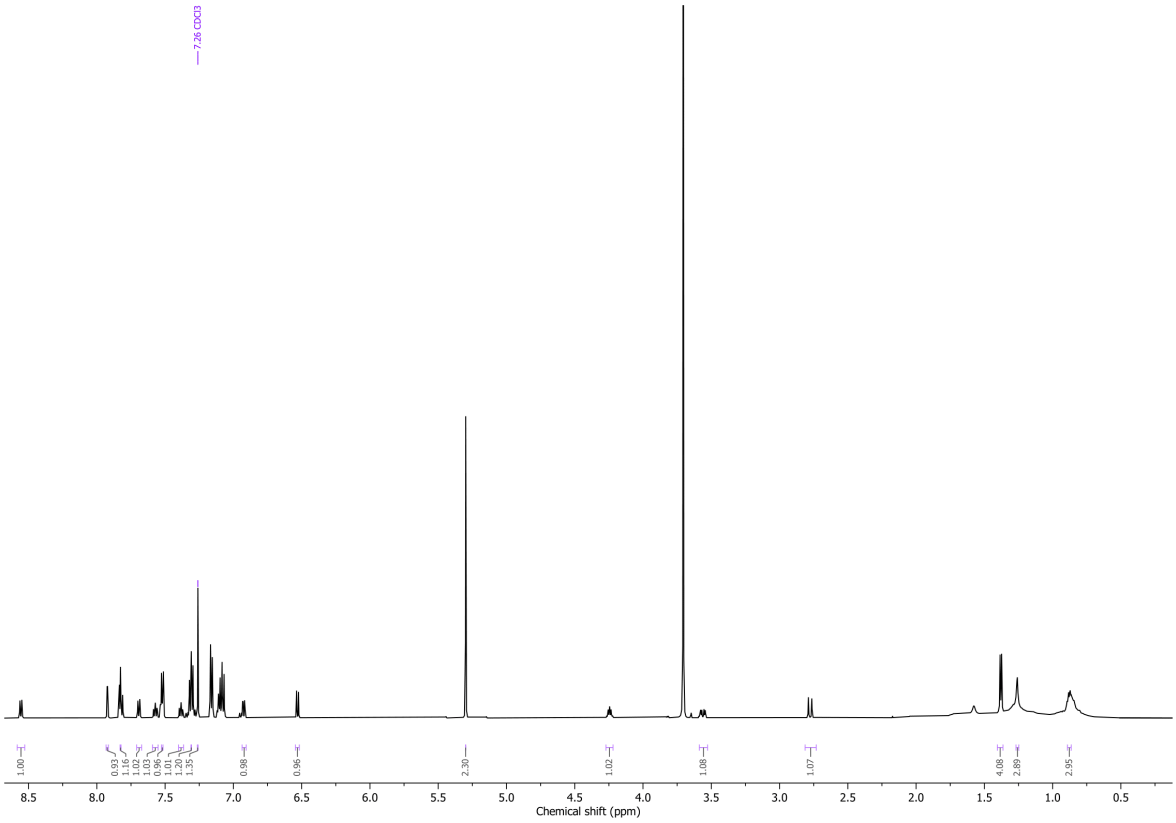


Figure S21. ^{1}H-NMR spectra of **MM**.

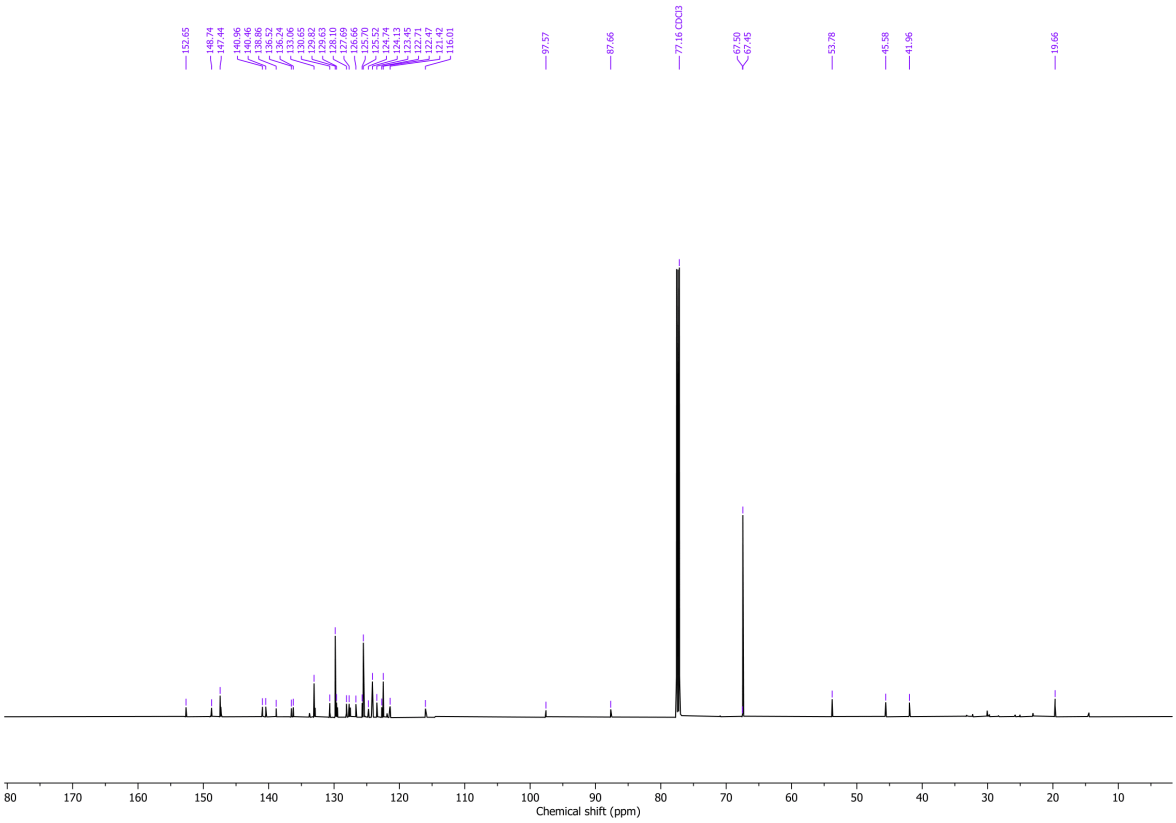


Figure S22. ^{13}C-NMR spectra of **MM**.

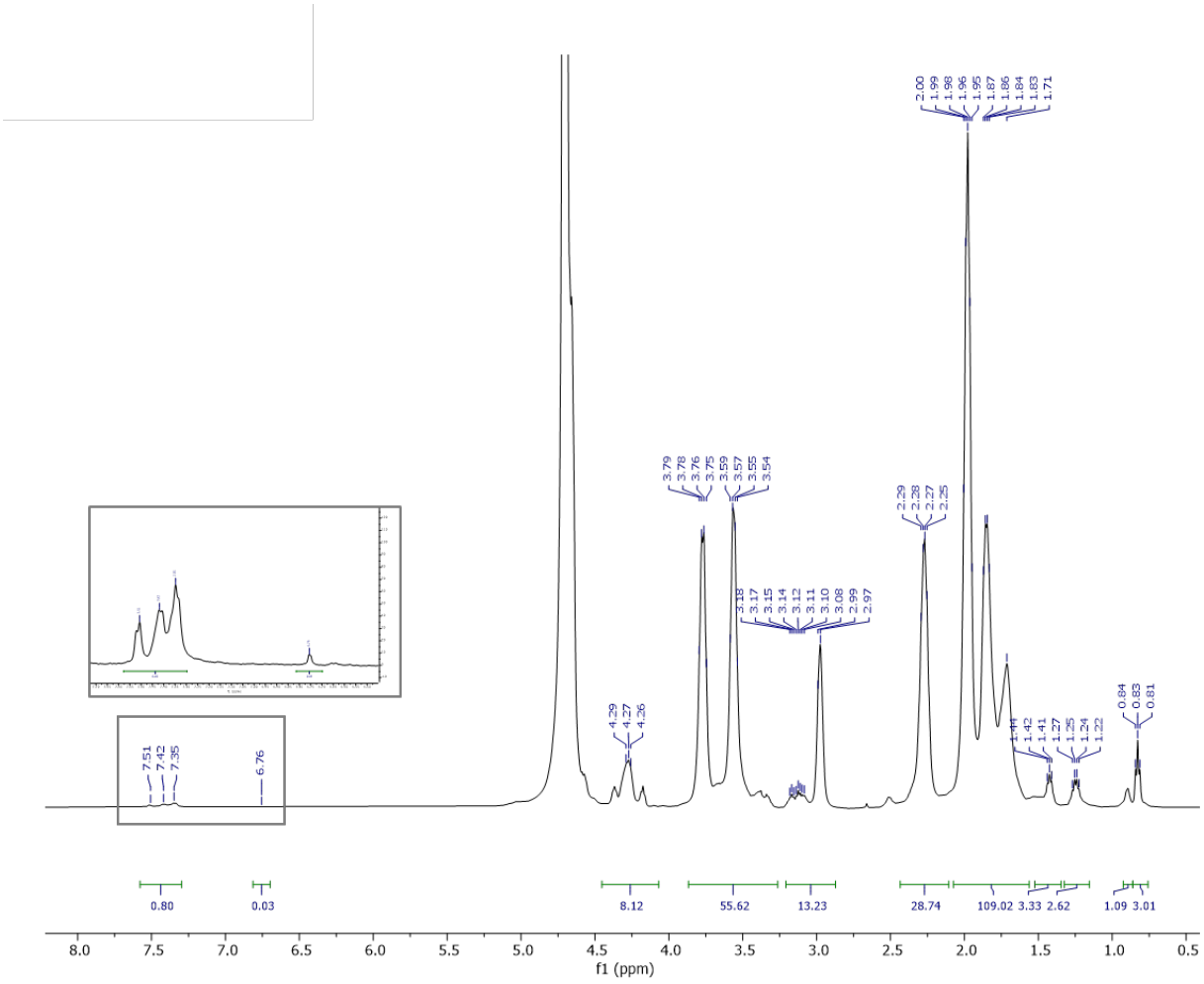


Figure S23. ^{1}H-NMR spectra of **PP-VC.** Adapted from Pegoraro et al. [14].

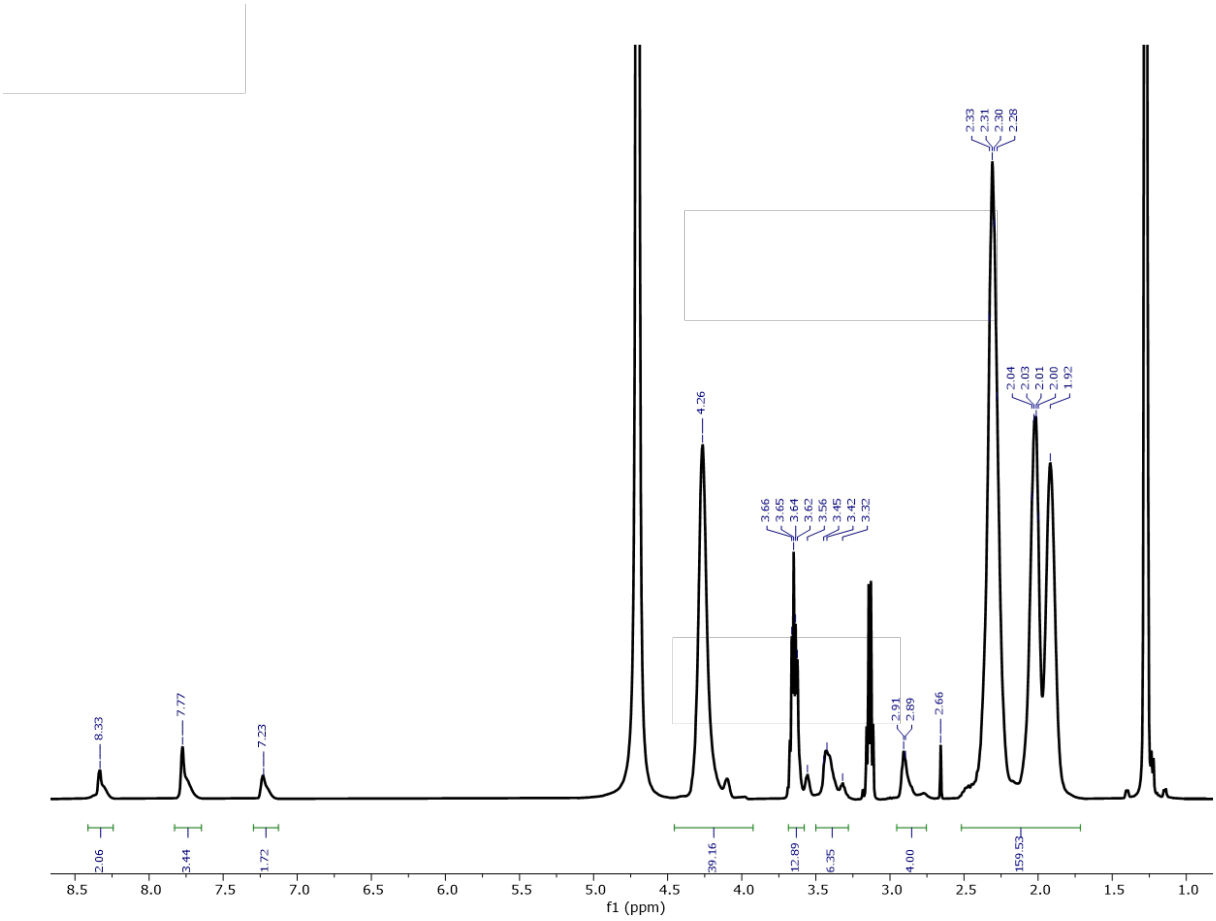


Figure S24. ^{1}H-NMR spectra of **PG-PD.** Adapted from Pegoraro et al. [14].

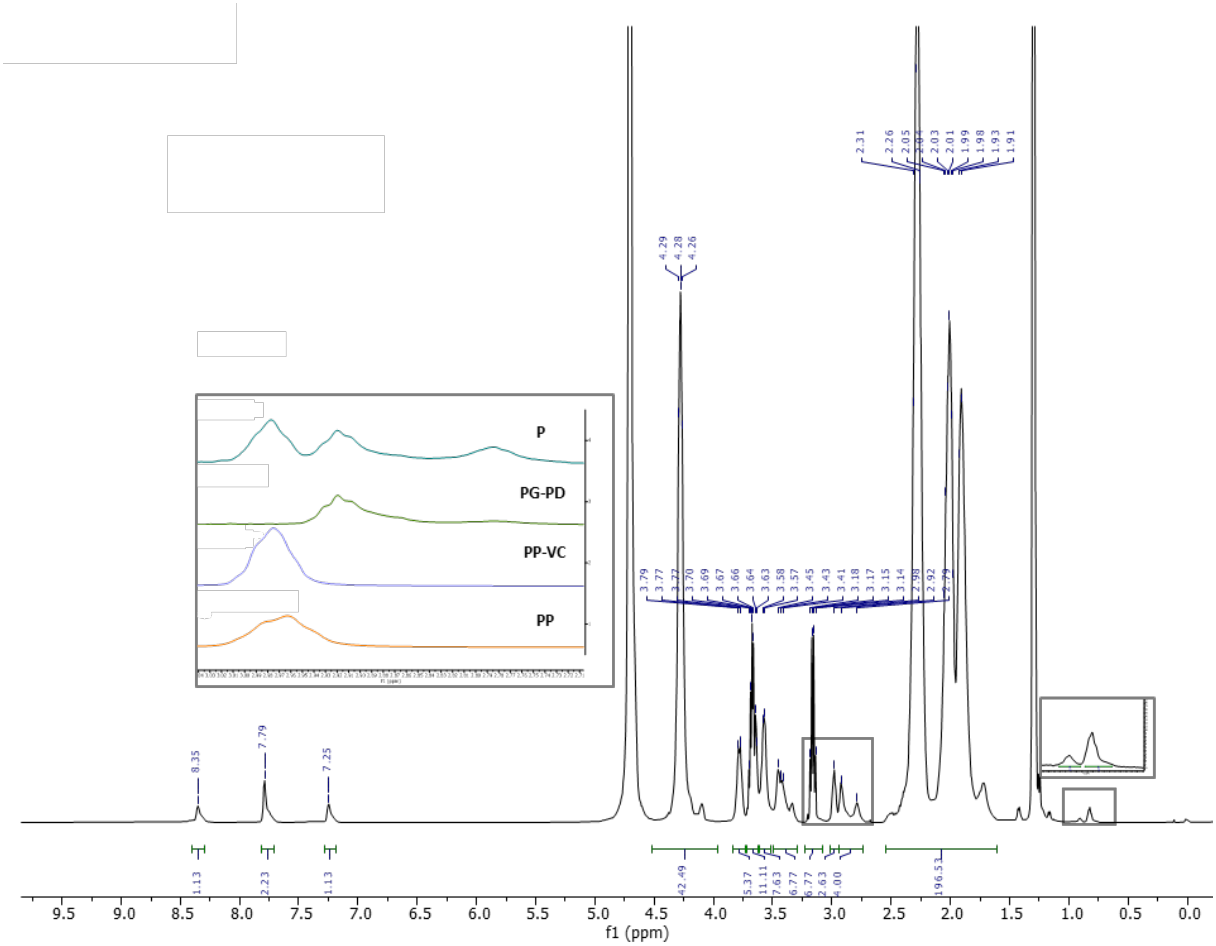


Figure S25. ^{1}H-NMR spectra of **P.** Adapted from Pegoraro et al. [14].

18. Statistical Analysis

Data from the experiments was analyzed using a t-test. We considered differences significant in all cases when p****<0.0001; p***<0.001; p**<0.01; p*<0.05; ns: not significant.