Mitochondria Targeting by a Nonionic and Emissive Push-Pull Type Chromophore or its Polymer Conjugate

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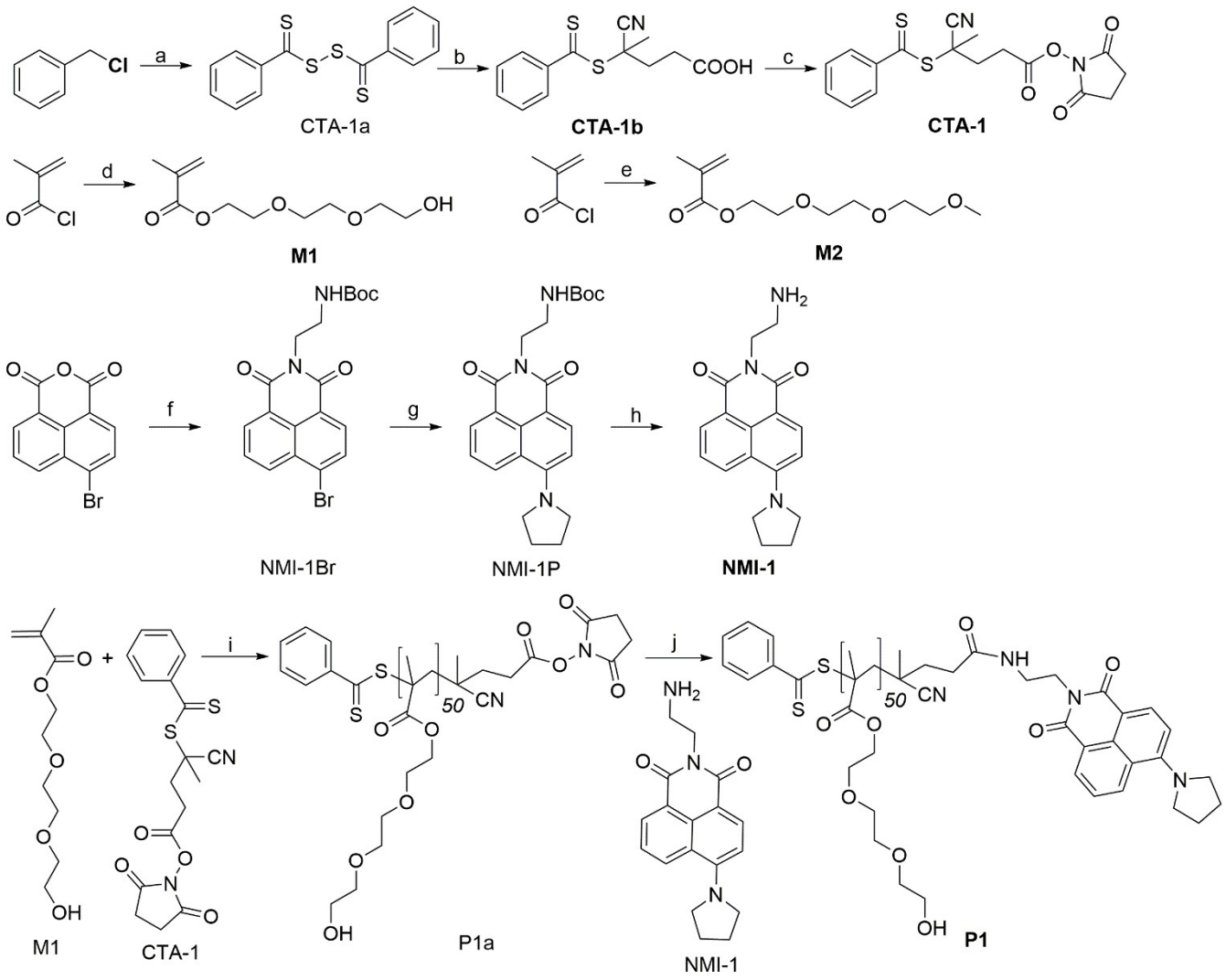
Materials and methods:

Solvents and reagents were purchased from commercial sources and purified following reported protocols.[1] 2-mercapto propanoic acid, methacryloyl chloride, triethylene glycol, triethylene glycol monomethyl ether, 4-Bromo-1,8-naphthalic anhydride and ethylene diamine were purchased from Sigma-Aldrich. Spectroscopic grade solvents were used for all physical studies. ^{1}H NMR spectra were recorded in a Bruker DPX-600 MHz, 500 MHz, 400 MHz or 300 MHz spectrometer and the peak positions were calibrated using TMS as an internal standard. Molecular weights of the polymers were estimated by size exclusion chromatography (SEC) in THF (2.0 mg/ mL) at 35 °C with respect to poly(methyl methacrylate) (PMMA) standards in a Waters machine equipped with Acquity APC XT 200 2.5 µm, Acquity APC XT 450 2.5 µm and 4.6 x 150 mm columns, connected in a series. THF was used as the eluent with a flow rate of 0.6 mL/ min at 35˚C. UV/Vis experiments were done in a Perkin Elmer Lambda 25 spectrometer. Fluorescence studies were carried out in a FluoroMax-3 spectrophotometer from HORIBA Jobin Yvon. Dynamic light scattering (DLS) measurements were done in Malvern instrument. Fluorescence microscopy images were captured by a Carl Zeiss LSM880 confocal microscope (Zeiss, Germany) equipped with Zen Blue software. Cryo-TEM images were captured in JEOL JEM 2100 PLUS Cryo-TEM with cryogen cooled pole piece. One drop of the aqueous solution of P1/P1/P3 (c= 0.1 mg/ml) was syringed out and mounted on 300 mesh carbon coated Cu grid hung on to GATAN cryo-plunger which was then immediately transferred to a cryogen cooled specially designed GATAN sample holder and examined under 120 kV electron beam. Nucleus staining dye, Hoechst 33342 was purchased from Sigma-Aldrich, whereas the Mitotochondria staining dye MitoTracker Red CMXRos was obtained from Thermo Fischer Scientific. Dulbecco's Modified Eagle's Medium (DMEM) was used for cell culture studies and was purchased from Thermo Fisher Scientific. The absorbance of MTT was measured by the microplate reader (VARIOSKAN, Thermo Fisher).



Reagent and conditions: (a) i) NaOMe, S, MeOH, 67 ^{o}C**,** 12 h; ii) Potassium ferricyanide, H_2O, 2 h; (b) (E)-4,4'-(diazene-1,2-diyl)bis(4-cyanopentanoic acid), EtOAc, reflux, 12 h; (c) N-hydroxysuccinimide, DCC, 4-DMAP, DCM, 0 ^{o}C-r.t., 48 h; (d) triethylene glycol, triethylamine, DCM, 0 ^{o}C-r.t., 12 h; (e) triethylene glycol monomethyl ether, triethylamine, DCM, 0 ^{o}C-r.t., 12 h; (f) mono Boc-protected ethylene diamine, EtOH, 80 ^{o}C, reflux, 12 h; (g) pyrrolidine, DMSO, 80 ^{o}C, reflux, 12 h; (h) 10% TFA, DCM, 3 h; (i) AIBN, anisole, 75 ^{o}C**,** 12 h; (j) Et_3N, DMF, r.t., 12 h.

Synthetic Procedure

Scheme S1. Synthetic scheme of **P1**.

Synthesis of CTA-1a: Sodium methoxide (30% solution in methanol) was added to a dry three-necked round-bottomed flask equipped with a magnetic stir bar, addition funnel (250 mL), thermometer, and rubber septum for liquid transfers. Anhydrous methanol (250 mL) was added to the flask via a cannula, followed by rapid addition of elemental sulfur (32 g, 1.0 mol). Benzyl chloride (63 g, 0.5 mol) was then added dropwise via the addition funnel over a period of 1 h at room temperature under a dry nitrogen

atmosphere. The reaction mixture was heated in an oil bath at 67 °C for 10 h. After this time, the reaction mixture was cooled to 0 °C using an ice bath. The precipitated salt was removed by filtration, and the solvent was removed in vacuum. Deionized water (500 mL) was then added to the residue. The solution was filtered a second time and transferred to an extraction funnel. The crude sodium dithiobenzoate solution was washed with diethyl ether (3 × 200mL). Diethyl ether (200 mL) and 1.0 N HCl (500 mL) were added, and dithiobenzoic acid was extracted into the ether layer. Deionized water (300 mL) and 1.0 N NaOH (600 mL) were added, and sodium dithiobenzoate was extracted to the aqueous layer. This washing process was repeated two more times to finally yield a solution of sodium dithiobenzoate. Potassium ferricyanide (III) (32.93 g, 0.1 mol) was dissolved in deionized water (500 mL). Sodium dithiobenzoate solution (350 mL) was transferred to a conical flask equipped with a magnetic stir bar. Potassium ferricyanide solution was added dropwise to the sodium dithiobenzoate via an addition funnel over a period of 1 h under vigorous stirring. The red precipitate was filtered and washed with deionized water until the washings became colourless. The solid was dried in vacuum at room temperature overnight. Crude product was taken to the next step without any purification.

Synthesis of CTA-1b: In a 100 mL round bottom flask, 4-azobis(4-cyanopentanoic acid) (1.639 g, 9.75 mmol) and CTA-1a (1.2 g, 6.54 mmol) were taken with 20 mL distilled ethyl acetate and refluxed at 75°C for 18 h. After 18 h, the solvent was removed under vacuum, and the product was purified by column chromatography using 30% EtOAc/hexane as eluent (yield 82%). ^{1}H-NMR ($CDCl_3$, 400 MHz, TMS): δ (ppm) = 7.90 (d, 2H); 7.56 (dd, 1H); 7.40 (dd, 2H); 2.78-2.59 (m, 2H); 2.51-2.44 (m, 2H); 1.95 (s, 3H).

Synthesis of CTA-1: CTA-1b (500 mg, 1.79 mmol) and 4-DMAP (22 mg, 0.179 mmol) were taken in a round bottomed flask in dry DCM and cooled to 0^{o}C. Then N-hydroxysuccinimide (824 mg, 7.16 mmol) and DCC (406 mg, 1.969 mmol) were added to it and stirred at room temperature under Ar atmosphere overnight. The solvent was then evaporated and ethyle acetate was added. The obtained white precipitate was filtered off. The filtrate was evaporated, and the product was purified by column chromatography

using 20% EtOAc/hexane as eluent (yield 74%). ^{1}H-NMR ($CDCl_3$, 400 MHz, TMS): δ (ppm) = 7.94 (d, 2H); 7.58 (dd, 1H); 7.42 (dd, 2H); 3.01 (m, 2H); 2.86 (s, 4H); 2.51-2.44 (m, 2H); 1.96 (s, 3H).

Synthesis of M1: Triethylene glycol (3.69 g, 24.46 mmol) was dissolved in dry DCM in a round bottomed flask and cooled to 0^{o}C and triethyl amine (2.69 g, 3.74 mmol) was added to it. Methacryloyl chloride (2.14 g, 20.47 mmol) was added to it dropwise and the mixture was stirred at room temperature under Ar atmosphere for 12 h. The crude product was extracted by 0.1 N HCl followed by $NaHCO_3$ solution and brine. The product was purified by column chromatography using 50% EtOAc/hexane as eluent (yield 80%). ^{1}H-NMR ($CDCl_3$, 300 MHz, TMS): δ (ppm) = 6.11 (s, 1H); 5.55 (s, 2H); 4.28 (t, 2H); 3.74-3.56 (m, 10H); 2.85 (t, 2H); 1.92 (s, 3H).

Synthesis of M2: Triethylene glycol monomethyl ether (3.69 g, 24.46 mmol) was dissolved in dry DCM in a round bottomed flask and cooled to 0^{o}C and triethyl amine (2.69 g, 3.74 mmol) was added to it. Methacryloyl chloride (2.14 g, 20.47 mmol) was added to it dropwise and the mixture was stirred at room temperature under Ar atmosphere for 12 h. The crude product was extracted by 0.1 N HCl followed by $NaHCO_3$ solution and brine. The product was purified by column chromatography using 50% EtOAc/hexane as eluent (yield 80%). ^{1}H-NMR ($CDCl_3$, 300 MHz, TMS): δ (ppm) = 6.11 (s, 1H); 5.55 (s, 2H); 4.28 (t, 2H); 3.74-3.56 (m, 10H); 3.36 (s, 3H); 2.85 (t, 2H); 1.92 (s, 3H).

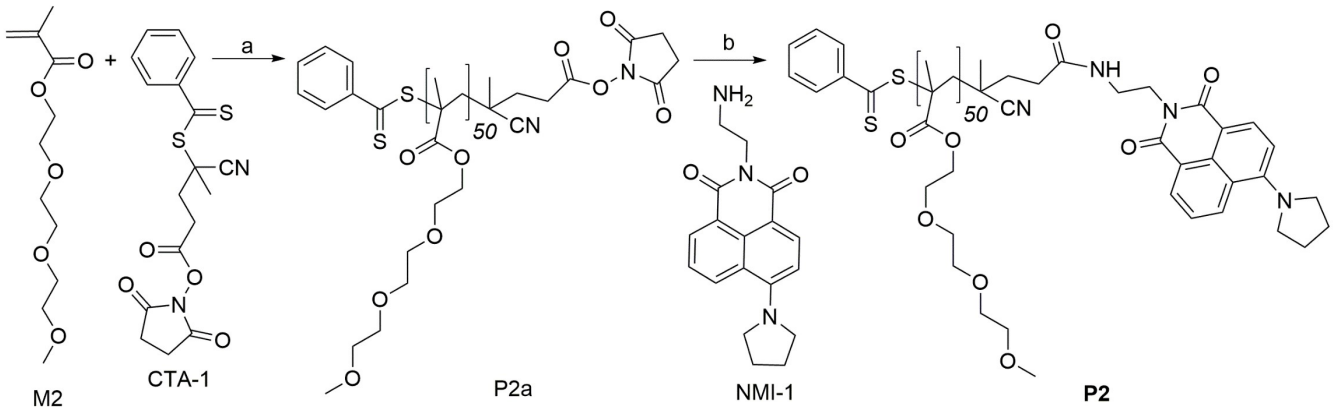
Synthesis of NMI-1Br: 4-Bromo-1,8-naphthalic anhydride (471 mg, 1.70 mmol) and mono-Boc protected ethylene diamine (300 mg, 1.87 mmol) were taken in a round bottomed flask in ethanol and refluxed at 65 ^{o}C for 12 h. The reaction mixture was then cooled to room temperature and 20 mL ethanol was added. The obtained precipitate was filtered and washed with ethanol to get the product (yield 70%). ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ (ppm) = 8.66 (d, 1H); 8.60 (d, 1H); 8.42 (d, 1H); 8.04 (d, 1H); 7.85 (t, 1H); 4.90 (b, 1H); 4.36 (t, 2H); 3.53 (t, 2H); 1.27 (s, 9H).

Synthesis of NMI-1P: NMI-1Br (500 mg, 1.19 mmol) was taken in a round bottomed flask and 5 mL DMSO was added to dissolve under Ar atmosphere. Pyrrolidine (250 mg, 3.56 mmol) was added to it and

the solution was stirred at 85°C for 12 h. The reaction mixture was cooled to room temperature and 50 mL water was added. The product was extracted using EtOAc and washed with brine solution. The combined organic layer was collected and dried over anhydrous Na_2SO_4, and solvent was evaporated to get the product (yield 80%). ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ (ppm) = 8.58 (q, 2H); 8.42 (d, 1H); 7.53 (t, 1H); 6.80 (d, 1H); 5.15 (b, 1H); 4.37 (t, 2H); 3.78 (t, 4H); 3.51 (t, 2H); 2.10 (t, 4H); 1.34 (s, 9H).

Synthesis of NMI-1: Boc deprotection of NMI-1P was done by dissolving it in DCM followed by dropwise addition of 10% TFA. The solvent and excess TFA was evaporated after stirring for 3 h. The product obtained was used in the next step without any further purification.

Synthesis of P1a: M1 (500 mg, 2.29 mmol) was taken in a polymerization tube along with CTA-2 (11 mg, 0.029 mmol) in dry anisole. The solution was degassed with Ar for 15 minutes. AIBN (1.5 mg, 0.009 mmol) was added to it and the solution was stirred at 75 °C under Ar atmosphere for 12 h. The polymer was precipitated from cold diethyl ether and dried under vacuum (yield 60%). ^{1}H-NMR ($CDCl_3$, 300MHz, TMS): δ (ppm) = 4.11 (broad), 3.70 (multiplate), 2.84 (broad), 1.04 (broad), 0.88 (broad) (Figure S1). Molecular weight was determined to be 10600 gmol^{-1} by end group analysis using H_a and H_b protons.



Reagent and conditions: (a) AIBN, anisole, 75 °C, 12 h; (b) Et_3N, DMF, r.t., 12 h.

SEC traces obtained using THF as solvent revealed M_n ~10000 gmol^{-1} (Đ = 1.2) (Figure S4).

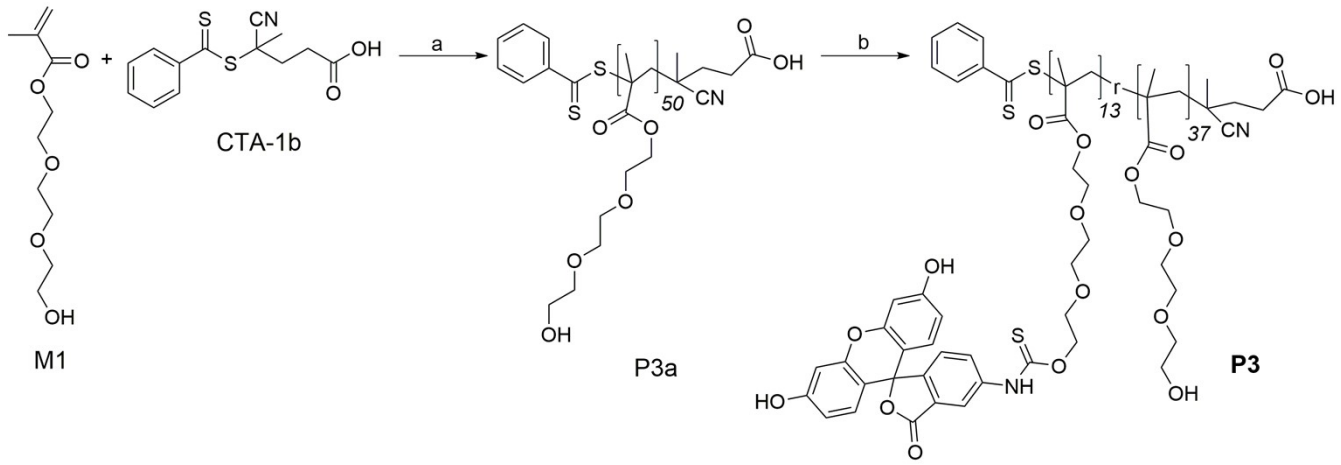
Scheme S2. Synthetic scheme of **P2**.

Synthesis of P1: P1a (100 mg, 0.0018 mmol) was taken in a polymerization tube in dry DMF. To it a solution of NMI-1 (11 mg, 0.0036 mmol) and triethyl amine (3.6 mg, 0.0036 mmol) in dry DMF was added and stirred at room temperature under Ar atmosphere for 12 h. The polymer was precipitated from cold diethyl ether and dried under vacuum (yield 80%). ^{1}H-NMR ($CDCl_3$, 300 MHz, TMS): δ (ppm) = 4.12 (broad), 3.67 (multiplet), 1.24 (broad), 1.05 (broad), 0.88 (broad) (Figure S2). Molecular weight was determined to be 11500 gmol^{-1} by end group analysis using H_a and H_g protons. SEC in THF could not be performed due to insolubility in THF. Molecular weight was determined using the molar extinction coefficient of NMI-1 in water and was calculated to be 9400 gmol^{-1}.

Synthesis of P2a: P2a was synthesized in a similar protocol used for P1a. Here MR-2 was used as the monomer instead of M-1. M-2 (500 mg, 2.15 mmol) was taken in a polymerization tube along with CTA-1 (10 mg, 0.029 mmol) in dry anisole. The solution was degassed with Ar for 15 minutes. AIBN (1.5 mg, 0.009 mmol) was added to it and the solution was stirred at 75 ^{o}C under Ar atmosphere for 12 h. The polymer was precipitated from cold diethyl ether and dried under vacuum (yield 63%). ^{1}H-NMR ($CDCl_3$, 400 MHz, TMS): δ (ppm)= 4.09 (b, 2H); 3.65-3.55 (m, 10H); 3.38 (s, 3H); 2.84 (b, 4H); 1.03 (b, 2H); 0.87 (b, 3H) (Figure S8). Molecular weight was determined to be 13400 gmol^{-1} by end group analysis using H_a and H_b protons. SEC traces obtained using THF as solvent revealed M_n ~12000 gmol^{-1} (Đ =1.12) (Figure S4).

Synthesis of P2: P2 was synthesized in a similar protocol used for P1. P2a (100 mg, 0.0018 mmol) was taken in a polymerization tube in dry DMF. To it a solution of NMI-1 (11 mg, 0.0036 mmol) and triethyl amine (3.6 mg, 0.0036 mmol) in dry DMF was added and stirred at room temperature under Ar atmosphere for 12 h. The polymer was precipitated from cold diethyl ether and dried under vacuum (yield 80%). ^{1}H-NMR ($CDCl_3$, 400 MHz, TMS): δ (ppm) = 8.57 (t, 2H); 8.42 (d, 1H); 7.52 (t, 1H); 6.80 (d, 1H); 4.09 (b, 2H); 3.65-3.55 (m, 10H); 3.38 (s, 3H); 1.03 (b, 2H); 0.87 (b, 3H) (Figure S9). Molecular weight was determined to be 14000 gmol^{-1} by end group analysis using H_a and H_g protons. SEC in THF could

not be performed due to insolubility in THF. Molecular weight was determined using the molar extinction coefficient of NMI-1 in water and was calculated to be 20000 $gmol^{-1}$.



Reagent and conditions: (a) AIBN, dry and degassed anisole, 75 °C, 12 h; (b) FITC, DMF, 80 °C, 12 h.

Scheme S3. Synthetic scheme of **P3**.

Synthesis of P3a: P3a was synthesized in a similar protocol used for P1a. Here CTA-1 was used as the chain transfer agent instead of CTA-2. MR-1 (200 mg, 0.9169 mmol) was taken in a polymerization tube along with CTA-1a (3.6 mg, 0.0115 mmol) in dry anisole. The solution was degassed with Ar for 15 minutes. AIBN (0.5 mg, 0.003 mmol) was added to it and the solution was stirred at 75 °C under Ar atmosphere for 12 h. The polymer was precipitated from cold diethyl ether and dried under vacuum (yield 63%). ^{1}H-NMR ($CDCl_3$, 400 MHz, TMS): δ (ppm) = 4.11 (b, 2H); 3.73-3.61 (m, 10H); 1.84 (t, 2H); 1.66 (t, 2H); 1.05 (b, 2H); 0.88 (b, 3H) (Figure S13). Molecular weight was determined to be 11000 gmol^{-1} by end group analysis using H_a and H_b protons. SEC traces obtained using THF as solvent revealed M_n ~7500 gmol^{-1} (Đ = 1.2) (Figure S4).

Synthesis of P3: P3a (100 mg, 0.458 mmol) was taken in a polymerization tube in dry DMF and a solution of FITC (9 mg, 0.023 mmol) in DMF was added to it. The reaction mixture was then stirred at 90 °C for 12 h. The polymer was purified by dialysis against water (yield 82%). ^{1}H-NMR (DMSO-D_6, 400 MHz, TMS): δ (ppm) = 10.12 (b, 1H); 7.95 (d, 2H); 6.67 (d, 2H); 6.58 (b, 2H); 4.55 (b, 2H); 4.02 (b, 2H); 3.61-3.44 (m, 10H); 1.75 (b, 3H); 0.95 (b, 2H); 0.79 (b, 3H) (Figure S14). Molecular weight was determined to be 16000 gmol^{-1} by end group analysis using H_b and H_c protons. SEC in THF could not be performed due to insolubility in THF.

Experimental Details

UV/Vis and PL studies: Samples for UV/Vis and PL studies were prepared by directly dissolving the polymers in water at a concentration of 1.0 mg/mL and absorbance spectra were recorded using a cuvette of 1.0 cm path length. PL studies were performed using the entry and exit slits at 3. To calculate the molecular weight of the polymers P1 and P2, first the molar extinction coefficient of NMI-1 was estimated from the slope of the concentration vs. absorbance plot of the dye in water, and it was calculated to be 5×10^3 $M^{-1}cm^{-1}$. This value was used in the Lambert-Beer's law to calculate the concentration of the polymer in molar unit which was then converted to molecular weight. To calculate the extent of PyDs conjugation in P1, a similar experiment was performed where the molar extinction coefficient of MR-3 was calculated using the absorbance vs. concentration plot of PyDS-1 in water. The value obtained was 3.6×10^3 $M^{-1}cm^{-1}$ (Figure S18) which was used in Lambert-Beer's law to get the amount of PyDs conjugation which was found to be ~18%.

DLS and cryo-TEM studies: The polymers were dissolved in water at a concentration of 1 mg/mL and diluted to 0.1 mg/mL. These solutions were used for dynamic light scattering experiments. Cryo-TEM of the samples were performed at a concentration of 0.1 mg/mL.

Cell Culture Condition: Human cervical cancer (HeLa) and cells were used for cellular uptake studies described in this chapter. The cells were seeded in a high glucose Dulbecco's Modified Eagle Medium (DMEM) which was supplemented with 10% fetal bovine serum (FBS) and 1% L-Penicillin-Glutamine-Streptomycin (PSG). Cells were maintained by passaging them at ~ 80% confluency at 37 °C in presence of 5% CO_2 in an incubator provided with a humidified environment.

Cell Viability study by MTT assay: The cell viability of the HeLa cells was checked in the presence of all the polymers under study by MTT assay. Approximately, 10^4 cells per well were seeded in a 96-well plate in DMEM and left overnight for the cells to adhere. The next day, the spent media was replaced with fresh DMEM containing polymers at various concentrations (20, 50, 100, 200 μg mL^{-1}) and incubated for another 24 h. After 24h, the media containing polymers was again removed and 100 μL of

fresh media was added followed by 50 µL of 5 mg mL^{-1} MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) salt per well and incubated for another 4 h at 37 °C. After 4h the media was carefully removed without disturbing the formazan crystals formed at the base of each well. Dimethyl sulfoxide (DMSO) (100 µL per well) was added to dissolve the purple formazan crystals and incubated for another 30 min before the absorbance of these treated cells was recorded at 570 nm using a plate reader (VARIOSKAN, ThermoFisher). MTT added to the untreated cells were considered as the positive control. The cell viability (% percentage of cell death) was calculated as:

$$\frac{O.D\ of\ the\ polymer\ treated\ cells}{O.D\ of\ the\ untreated\ cells} \times 100$$

Flow Cytometry to study the mechanism of cellular internalization: Approximately 10^5 cells (HeLa cell) were seeded in 35x10 mm cell culture plates in complete DMEM and incubated overnight for the cells to adhere. The next day the spent media was removed, and fresh media was added containing 100 µg/ml of each of the polymers and cells were further incubated at 37 ºC or 4 ºC for predetermined time (1 h) period. After this the media was removed and cells were treated with trypsin EDTA and cell pellet collected after centrifugation. After the removal of supernatant, the cells were re-suspended in DMEM and transferred to FACS tube (12 x 75 mm polystyrene round bottom style). Data for 10000 events of live cells were collected and analyzed in BD FACSAriaTM III.

Confocal imaging: 10^5 cells were seeded in confocal imaging dishes (dimension 35 X 10 mm purchased from Genetix, Biotech Asia Pvt. Ltd) and were incubated overnight for the cells to get adhered to the dish. The next day the media was removed and replaced with fresh media containing polymers (c = 100 µg/ml) followed by incubation for the desired time. For co-localization experiments cells, which were pre-incubated with the polymer solution of (c = 100µg/mL) at 37 ºC for 1h, were again incubated with Hoechst 33342 and Mitotracker red for 10 min each. After this the cells were washed thrice with fresh media and imaged. The red channel was excited at 543 nm, whereas for green channel and blue channel, 488 nm and 405 nm respectively, was used for excitation. Images were captured using a confocal laser-scanning

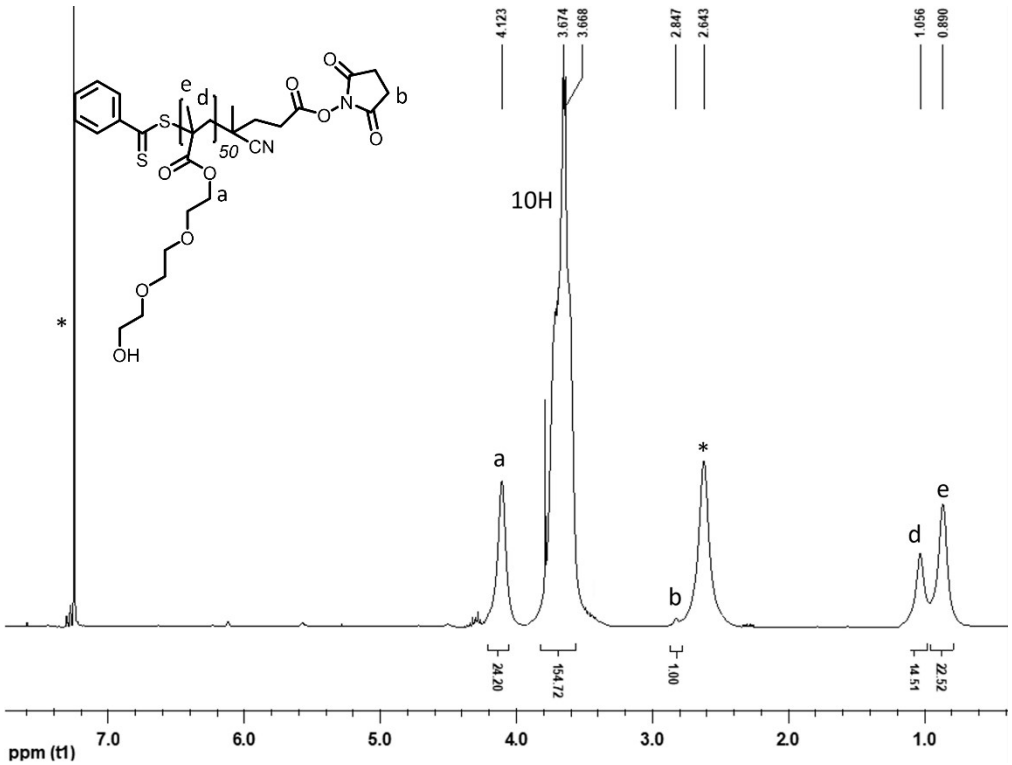
Eclipse Ti-E microscope equipped with a plan apochromatic VC 60×/1.4 oil objective, and Digital Sight DS-Qi1MC monochromatic camera with NIS-AR software (Nikon, Tokyo, Japan).

Mitochondrial extraction by Differential Centrifugation: Cells were grown on a 60 cm^2 culture plate at 37 °C and 5 % CO_2 till 80 % confluent. To this plate 0.1 mg/ml of P1b or NMI-1 was added and the plate was incubated for another 4h. After this the plate was taken out of incubator and incubated in ice-cold 1X PBS for 10 minutes. Thereafter the cells were scrapped off the plate and collected in microcentrifuge tube followed by centrifugation at 500*g* for 5 minutes at 4 °C. The obtained cell pellet was given osmotic shock by resuspended it in 500 µl buffer (containing 10 mM NaCl, 1.5 mM $CaCl_2$ and 10 mM Tris HCl) of pH 7.5. The resuspended cells were kept undisturbed for 5 minutes at 25 °C. The cell lysate was then homogenized by passing the lysate through 27-gauge needle few times in presence of one -sixth volume of stabilizing buffer (2M sucrose, 35 mM EDTA, 50 mM Tris- HCl pH 7.5) at 25 °C. This cell lysate was again centrifuged at 750*g* for 5 minutes at 4 °C to pellet down the cell debris. The supernatant was collected and centrifuged at 2000*g* for 10 minutes at 4 °C to pellet down the nucleus. The supernatant from the previous step was again centrifuged twice at 10000g for 20 minutes at 4 °C and the final pellet was dissolved in MT buffer (250 mM sucrose, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 5 mM DTT, 20 mM Tris-HCL pH 7.4) at 25 °C and stored at -80 °C.

Western Blot: Isolated mitochondrial fraction concentration was measured using Bradford Protein Assay method. Mitochondrial fraction was boiled in 2X Laemmli-SDS sample buffer for 10 minutes in 1:1 ratio and resolved in SDS-PAGE gel with 25 µg sample in each well. The resolved sample was transferred to PVDF membrane and after blocking (in 0.5% BSA-PBST solution for 6 hrs) blot was incubated overnight (at 4°C) with antibody (Sigma) against SAM50. After washing with PBST blots were then incubated for 2hrs (at room temperature) with HRP tagged secondary antibodies (Sigma). This was followed by another wash with PBST and chemiluminescence signals were captured by Bio-Rad ChemiDoc system. 10% resolving gel [2.05 ml H2O, 1.67 ml of 30% Polyacrylamide solution, 1.25 ml resolving buffer(pH=8.8),

50 µl of 10% Sodium Dodecyl Sulfate, 3 µL TEMED, 30 µL Ammonium Persulfate] and 4% stacking gel [3 ml H2O, 0.66 ml of 30% Polyacrylamide solution, 1.25 ml stacking buffer(pH=6.8), 50 µl of 10% Sodium Dodecyl Sulfate, 6 µL TEMED, 30 µL Ammonium Persulfate] were used in this experiment.

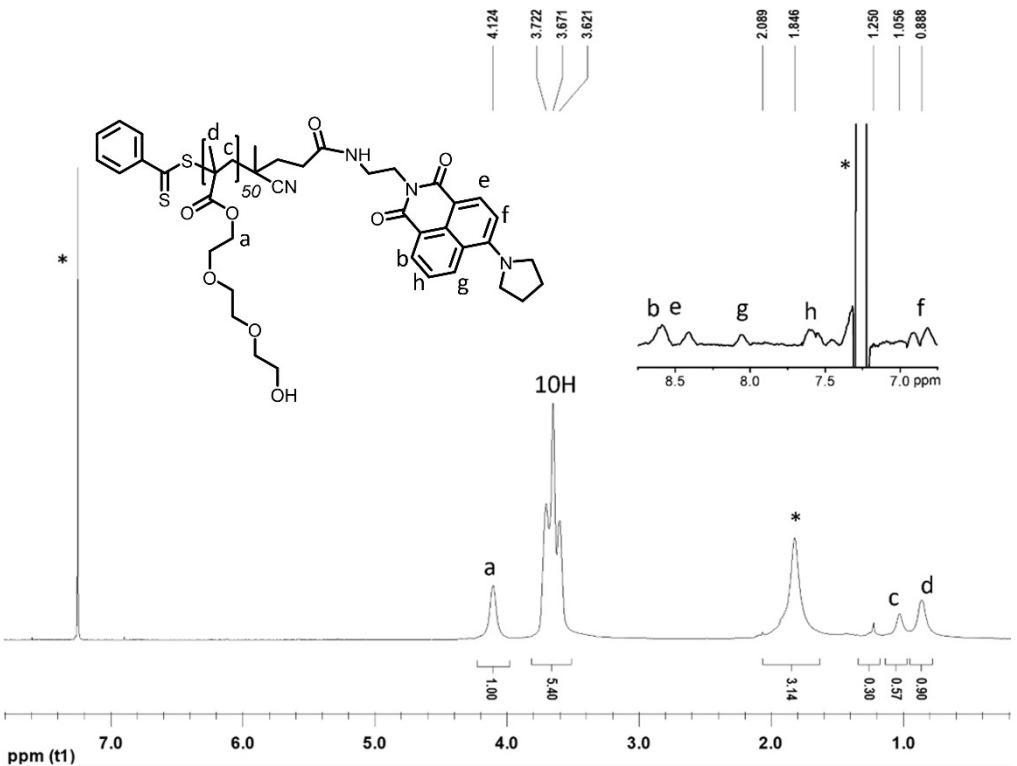
Immunofluorence microscopy: HeLa cells was grown on coverslip and washed with PBS then fixed with 4% Para-Formaldehyde solution for 15 minutes. The coverslips were again washed with PBS and treated with 0.1% TritonX-100 to permeabilise the lipid membrane. After washing, 3% BSA-PBS solution was used for 1 hour for blocking. Then washed again and incubated overnight (at 4°C) with antibody against SAM50 followed by secondary antibodies tagged with Alexa 647 for 45 minutes (at room temperature). DAPI was used to stain nucleus for 2 minutes. To mount coverslip on glass slide antifade mounting medium was applied. Images were captured using Leica STELLARIS microscope with confocal (LIGHTNING) mode in 63X zoom.



4. Additional Figures

Figure S1. ^{1}H-NMR of **P1a** in $CDCl_3$ (* indicates residual solvent peak).

Figure S2. ^{1}H-NMR of **P1** in $CDCl_3$ (* indicates residual solvent peak).



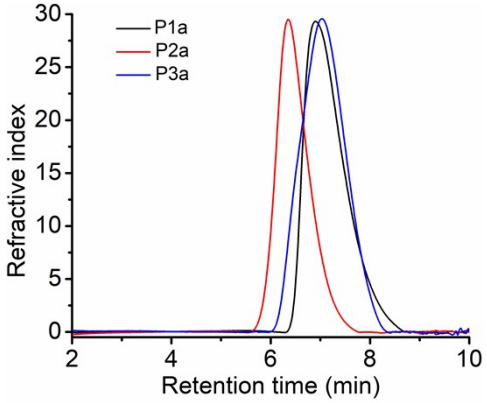


Figure S3. Size exclusion chromatogram of the polymers **P1a**, **P2a** and **P3a** in THF.

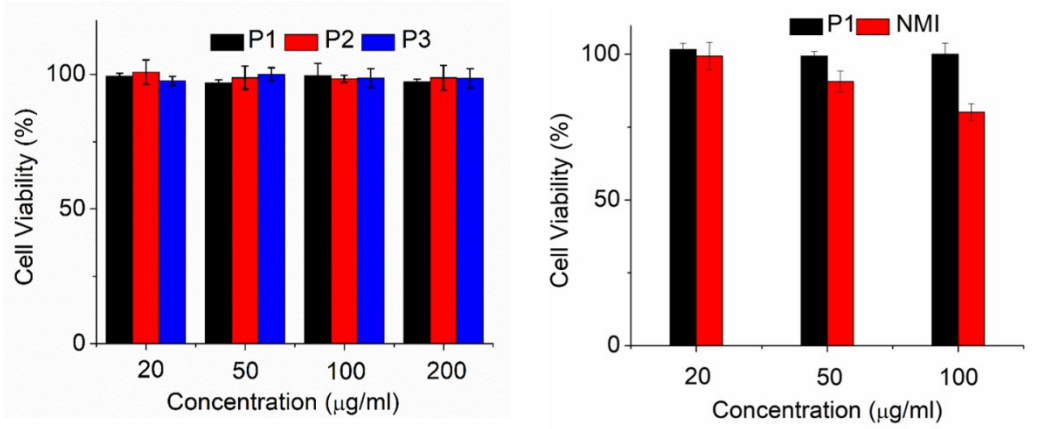


Figure S4. Cell viability of HeLa cells (left) in presence of the polymers and (right) NMI-1 after 24 h.

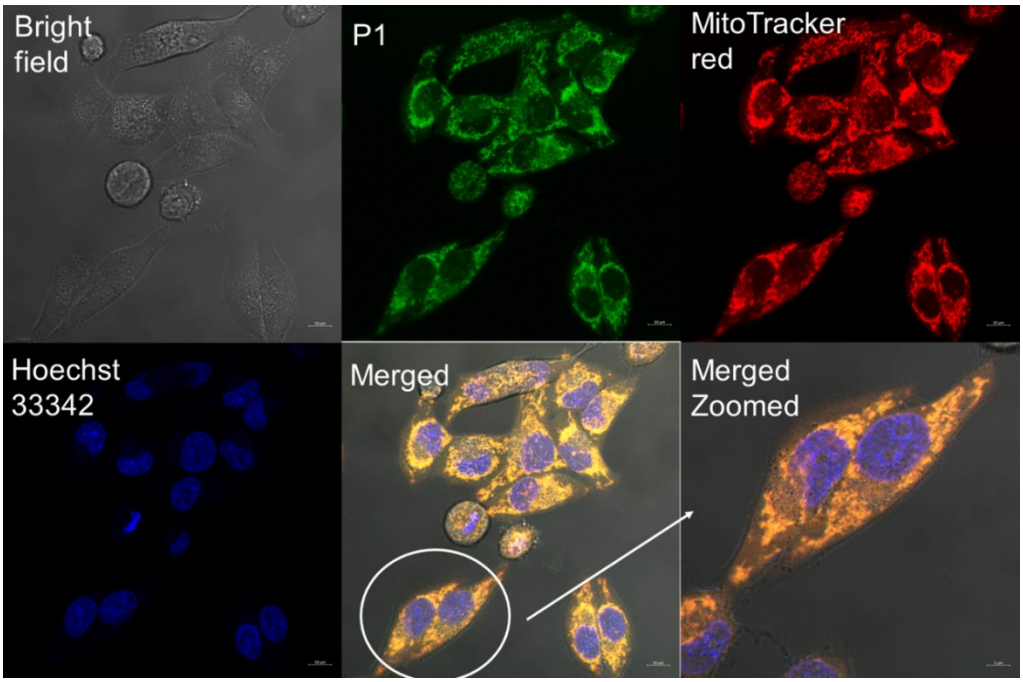


Figure S5. CLSM images showing mitochondria targeting of **P1** in HeLa cells (c = 0.1 mg/mL) after 1h of incubation. Pearson's coefficient = 0.91. Scale bar (shown in bottom left panel) = 10 μm.

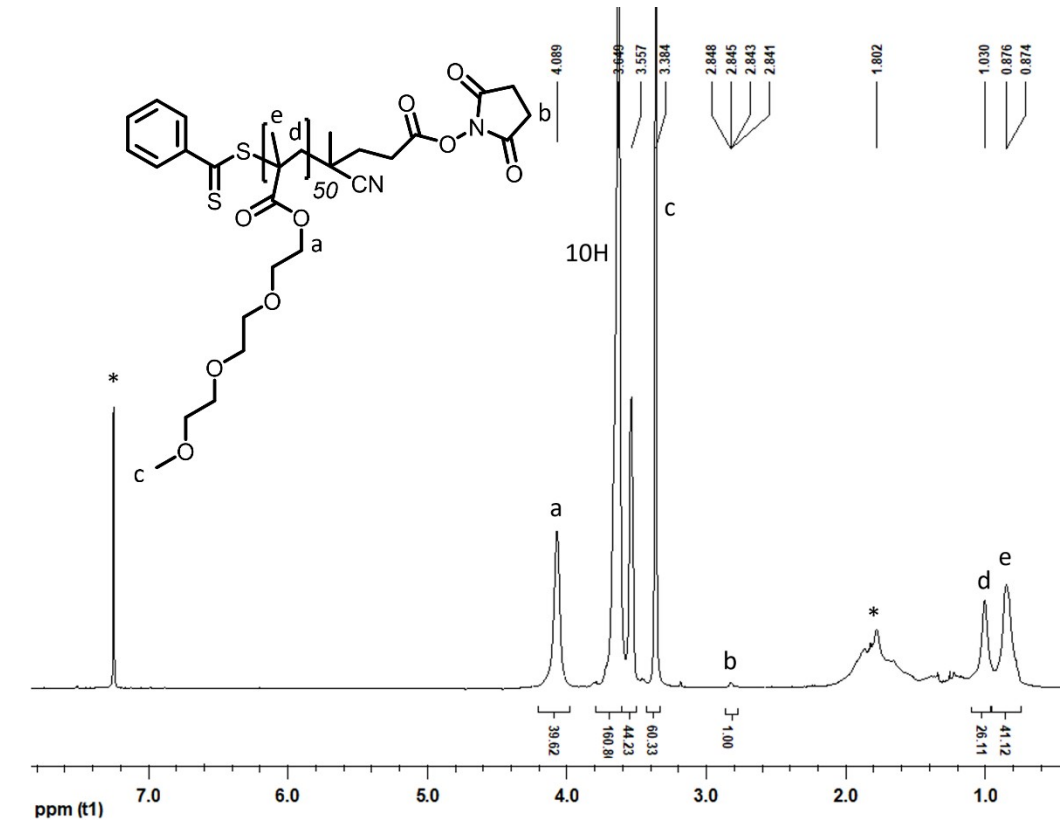


Figure S6. ^{1}H-NMR of **P2a** in $CDCl_3$ (* indicates residual solvent peak).

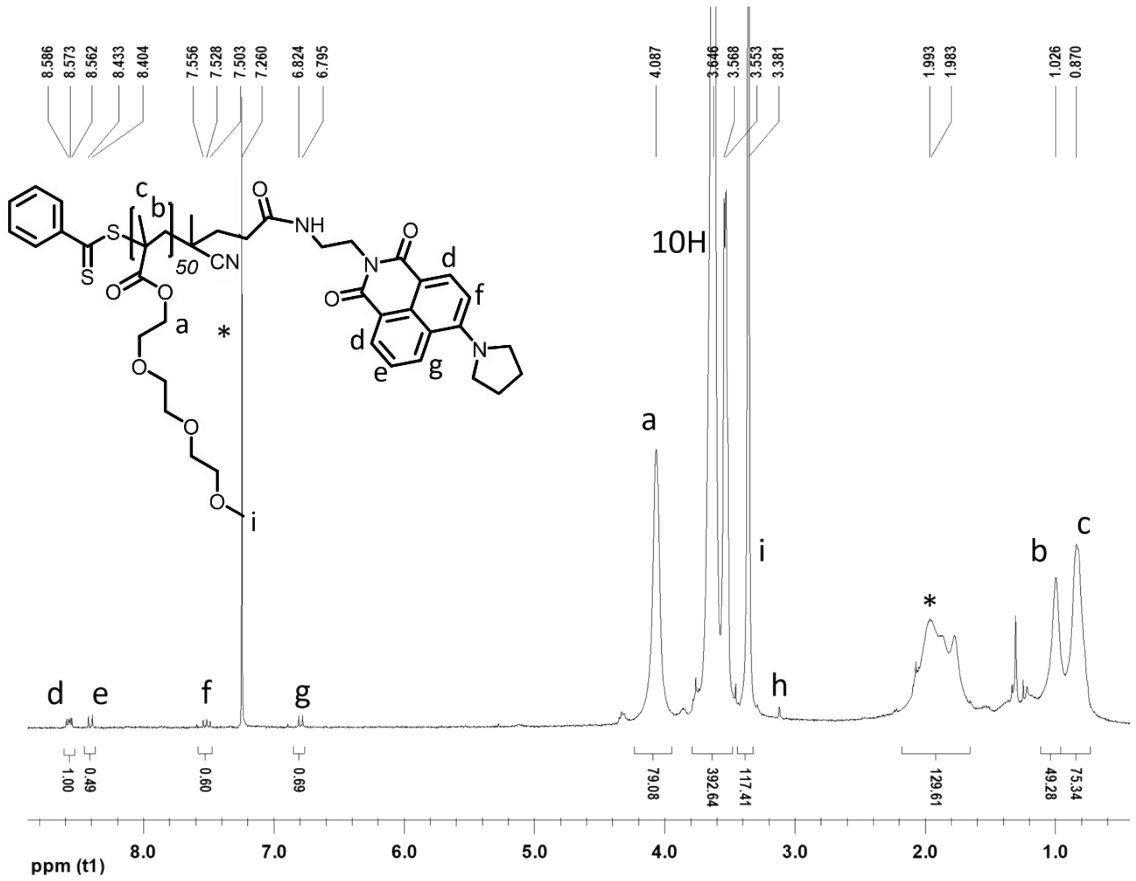


Figure S7. ^{1}H-NMR of **P2** in $CDCl_3$ (* indicates residual solvent peak).

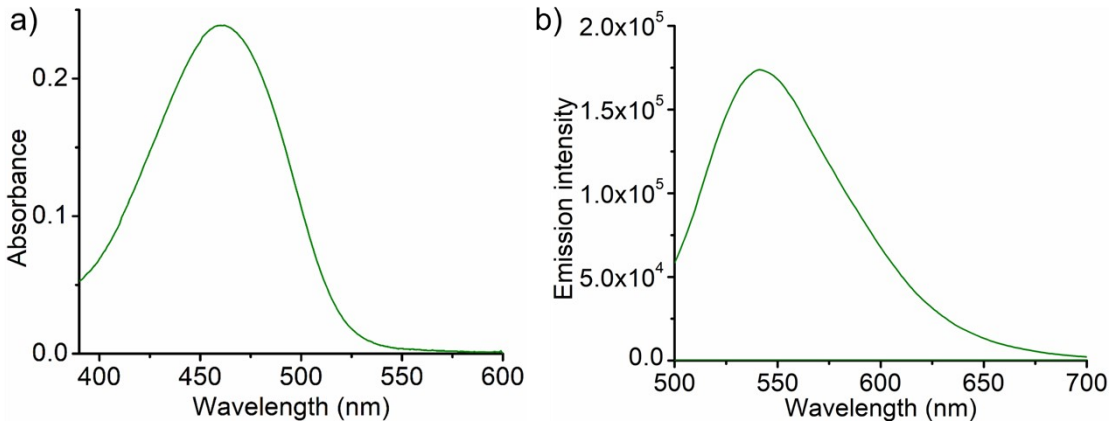


Figure S8. (a) UV/Vis spectra of **P2** in water (c = 1 mg/mL, l = 1 cm); (b) emission spectra of **P2** in water (c = 0.1 mg/mL, λ_{ex} = 460 nm, slit = 3/3).

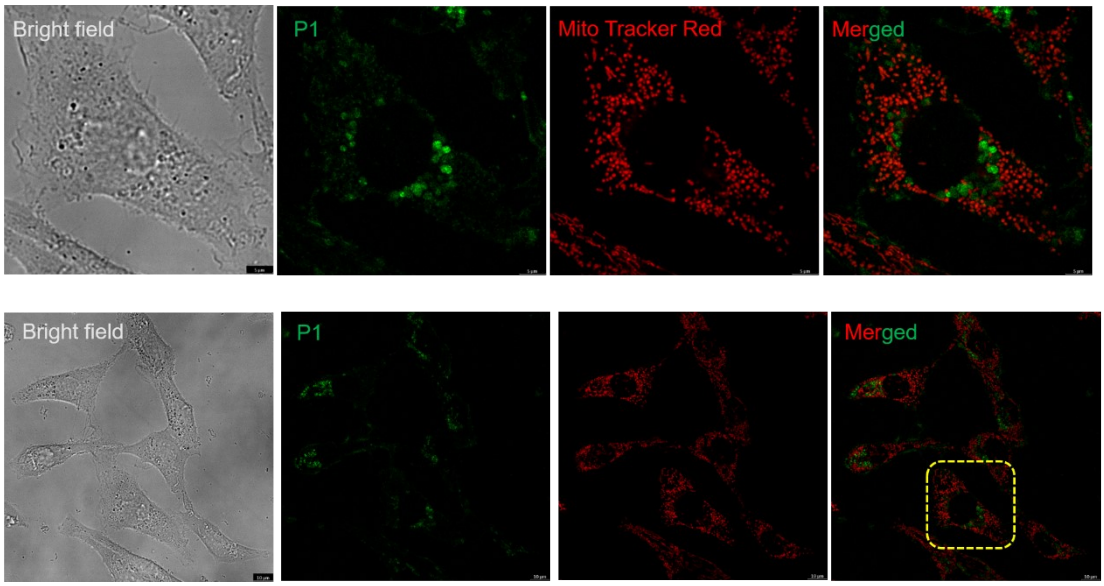


Figure S9. CLSM images showing escape of **P1** (c = 0.1 mg/mL) from mitochondria after 4h. Scale bar = 10 μm. Top panels show enlarged view of the cell marked with yellow box.

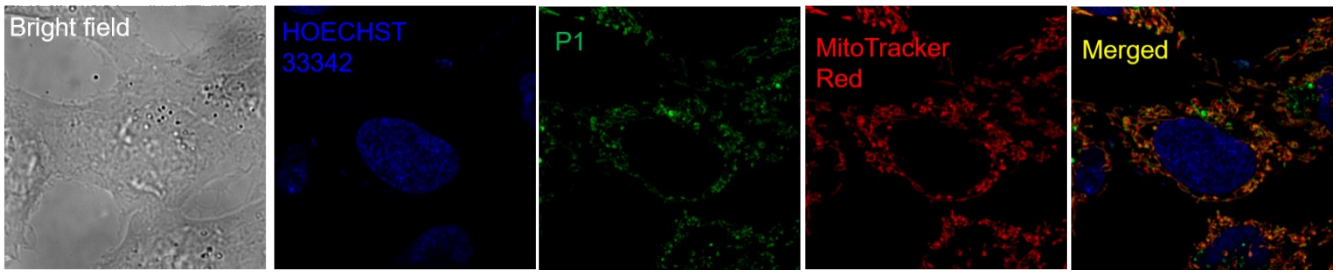


Figure S10. CLSM images showing mitochondria targeting of **P1** in MDA-MB 468 cells (c = 0.1 mg/mL) after 1h of incubation. Pearson's coefficient = 0.81.

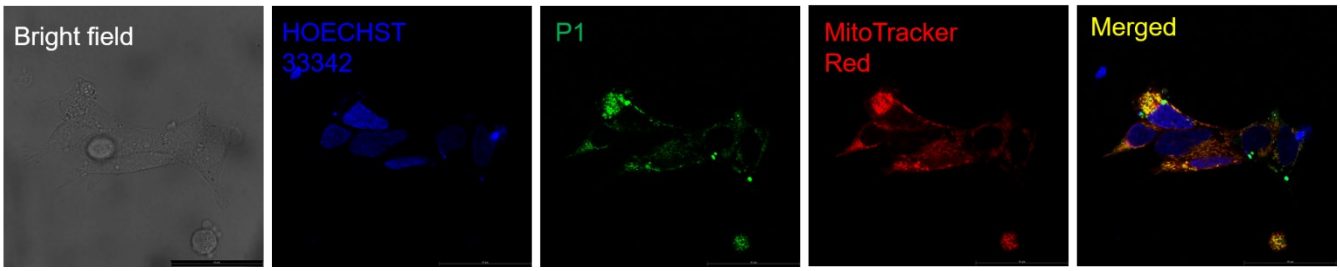


Figure S11. CLSM images showing mitochondria targeting of **P1** in HEK 293 cells (c = 0.1 mg/mL) after 1h of incubation. Pearson's coefficient = 0.79.

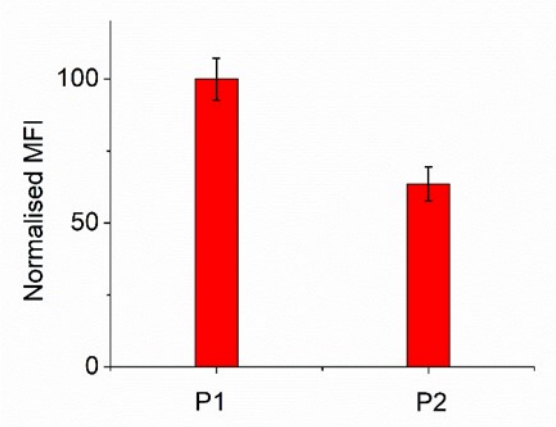


Figure S12. MFI showing the relative internalization of **P2** and **P1** (c = 0.1 mg/mL) by HeLa cells after 1 h. Normalized (with respect to the intrinsic difference of the NMI-1 content in **P1** and **P2**) emission is used for the quantification of internalization.

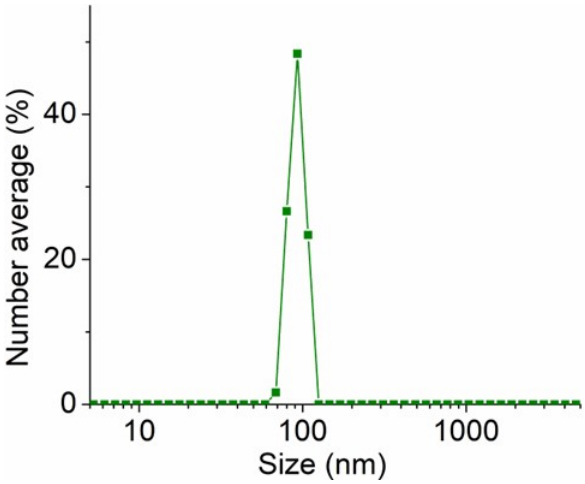


Figure S13. DLS of **P2** in water (c = 0.1 mg/mL).

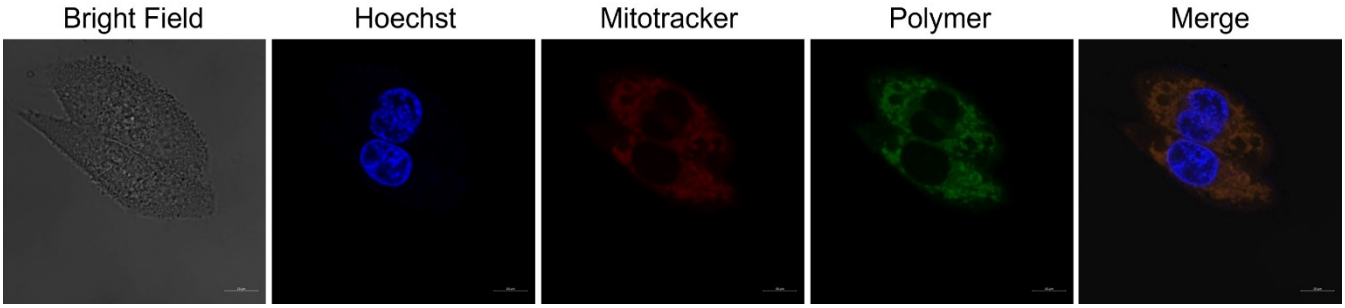


Figure S14. CLSM images showing internalization of **P2** by HeLa cells (c = 0.1 mg/mL) at 1 h of incubation with Hoechst and Mito-tracker red.

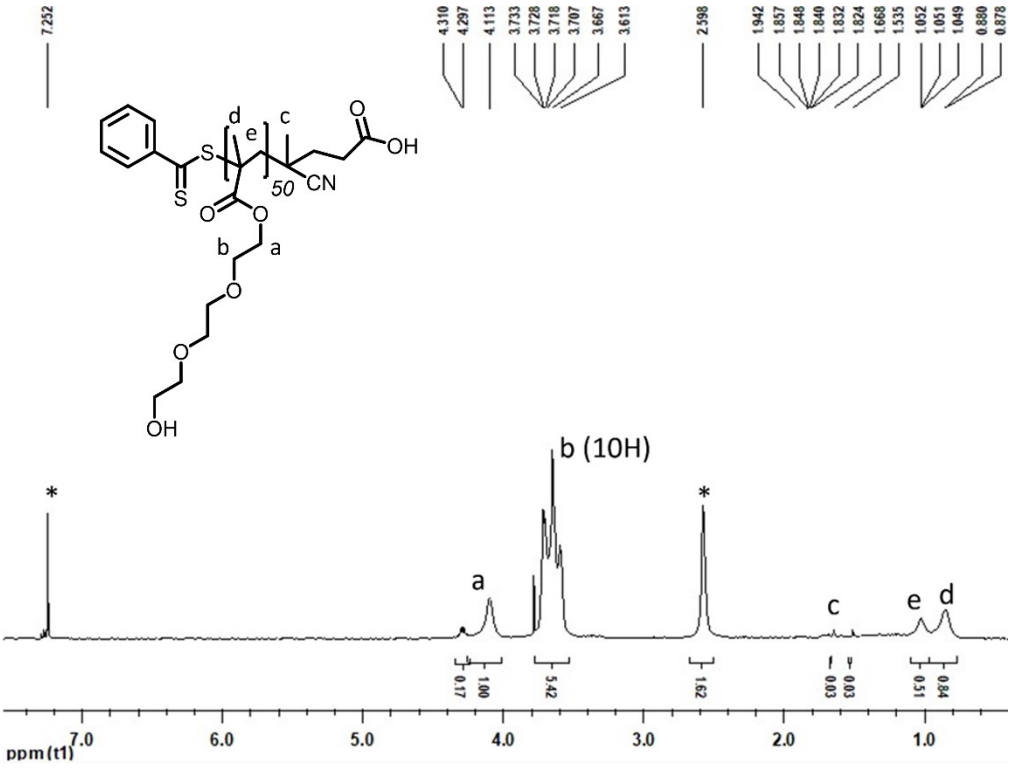


Figure S15. ^{1}H-NMR of **P3a** in $CDCl_3$ (* indicates residual solvent peak)

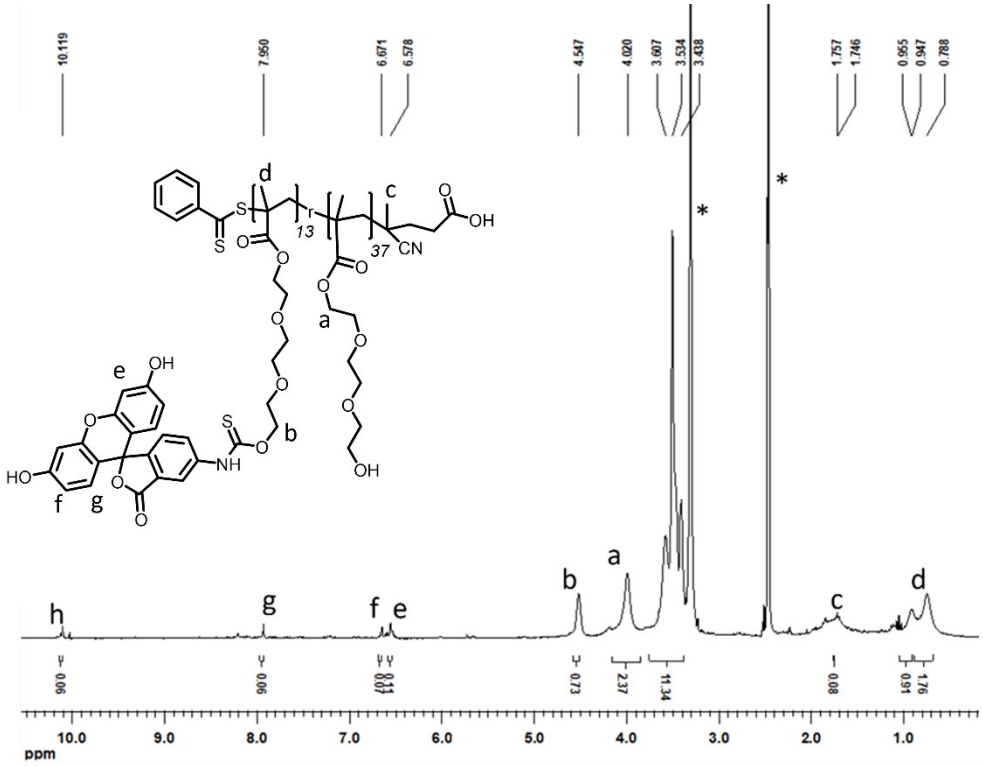


Figure S16. ^{1}H-NMR of **P3** in DMSO-D_6 (* indicates residual solvent peak).

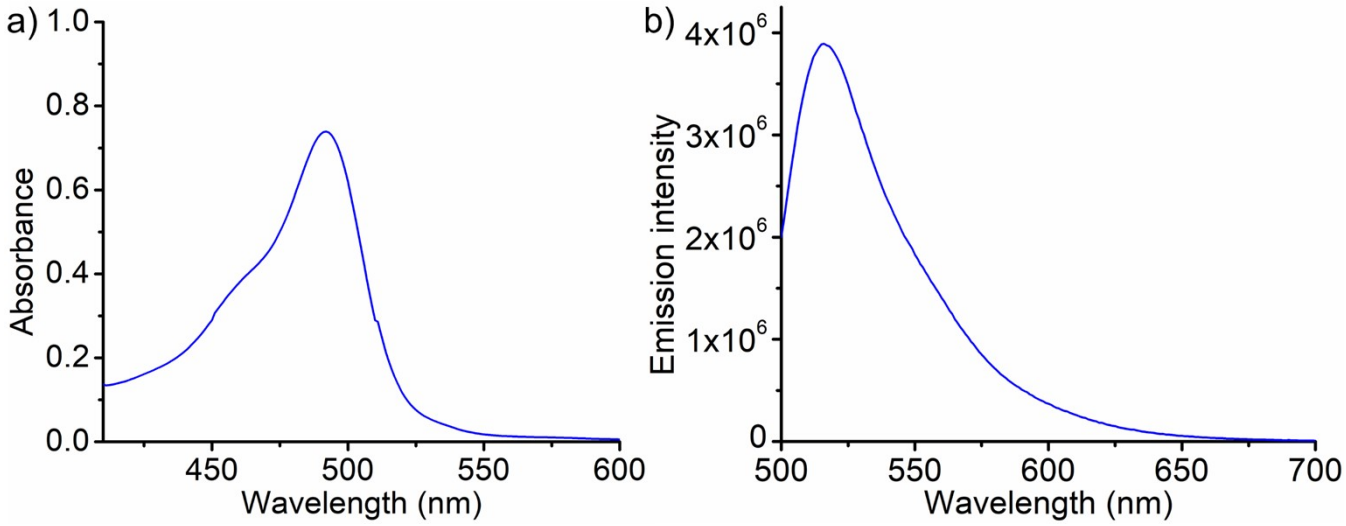
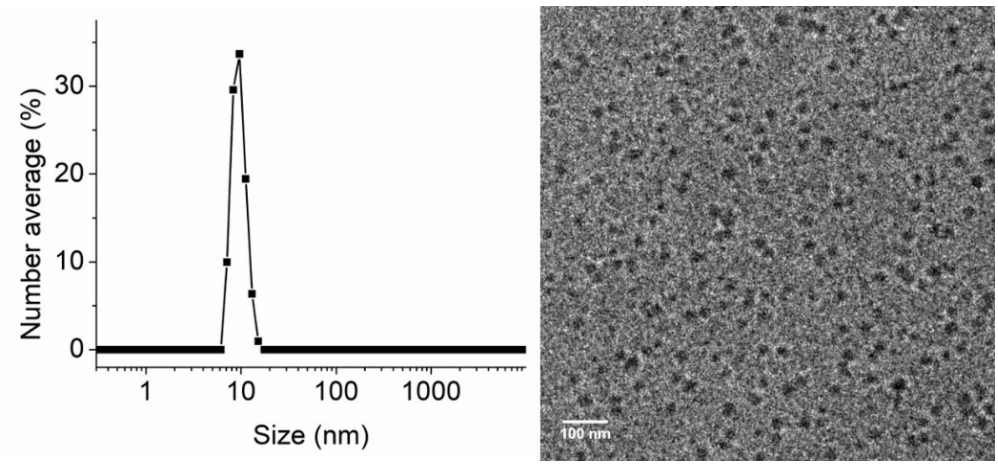


Figure S17. (a) UV/Vis spectra of **P3** and **P4** in water (*c* = 1 mg/mL, *l* = 0.1 cm); (b) emission spectra of **P3** and **P4** in water (*c* = 1 mg/mL, λ_{ex} = 460 nm, slit = 3/3).



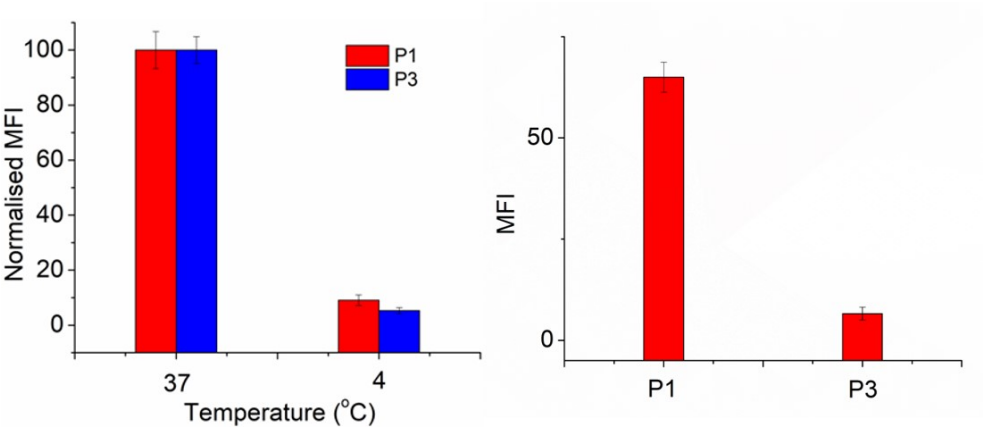


Figure S18. DLS and Cryo-TEM image of **P3** in water (c = 0.1 mg/mL).

Figure S19: MFI as a function of temperature showing the energy dependent uptake of P3 and also kinetics of internalization of **P1** and **P3** (c = 0.1 mg/mL) by HeLa cells.

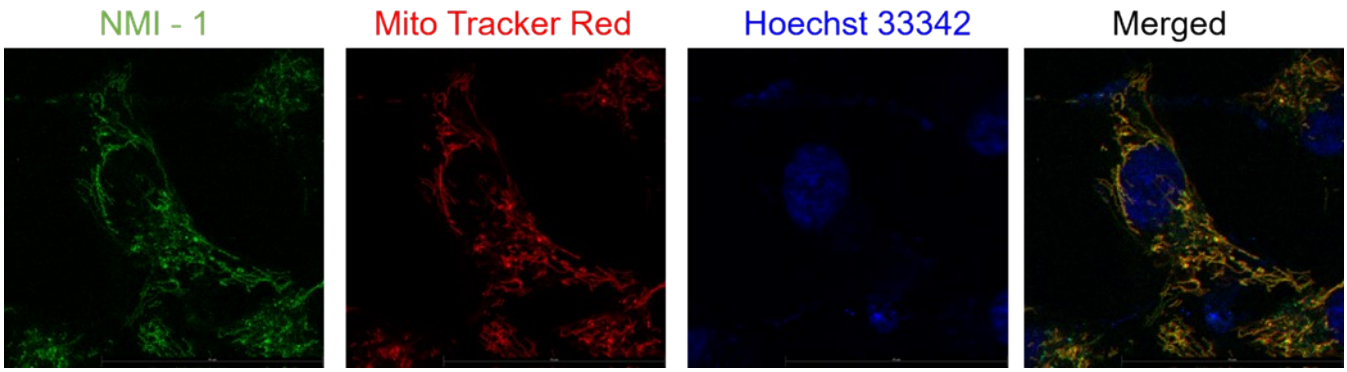


Figure S20. CLSM images showing internalization of **NMI** by HeLa cells (c = 0.03 mg/mL) at 1 h of incubation with Hoechst and Mito-tracker red. Scale bar (shown in bottom left panel) = 50 μm.

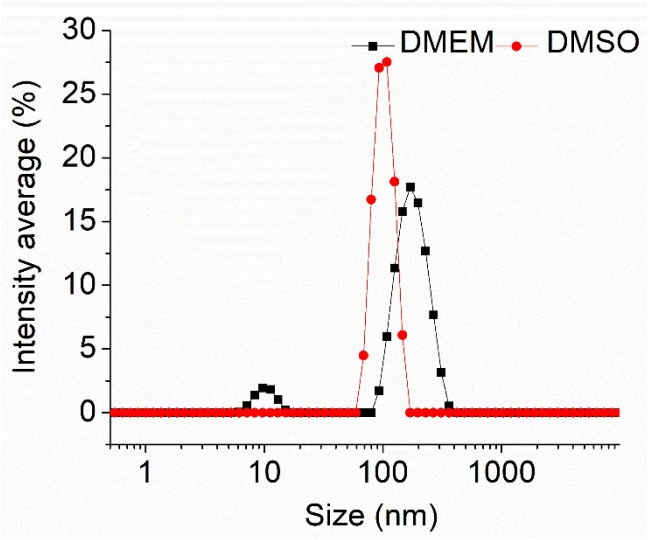


Figure S21. DLS of NMI-1 in DMSO and DMEM media. Small peak at ~ 10 nm is due to DMEM media (Nanoscale, 2025,17, 5732-5742).

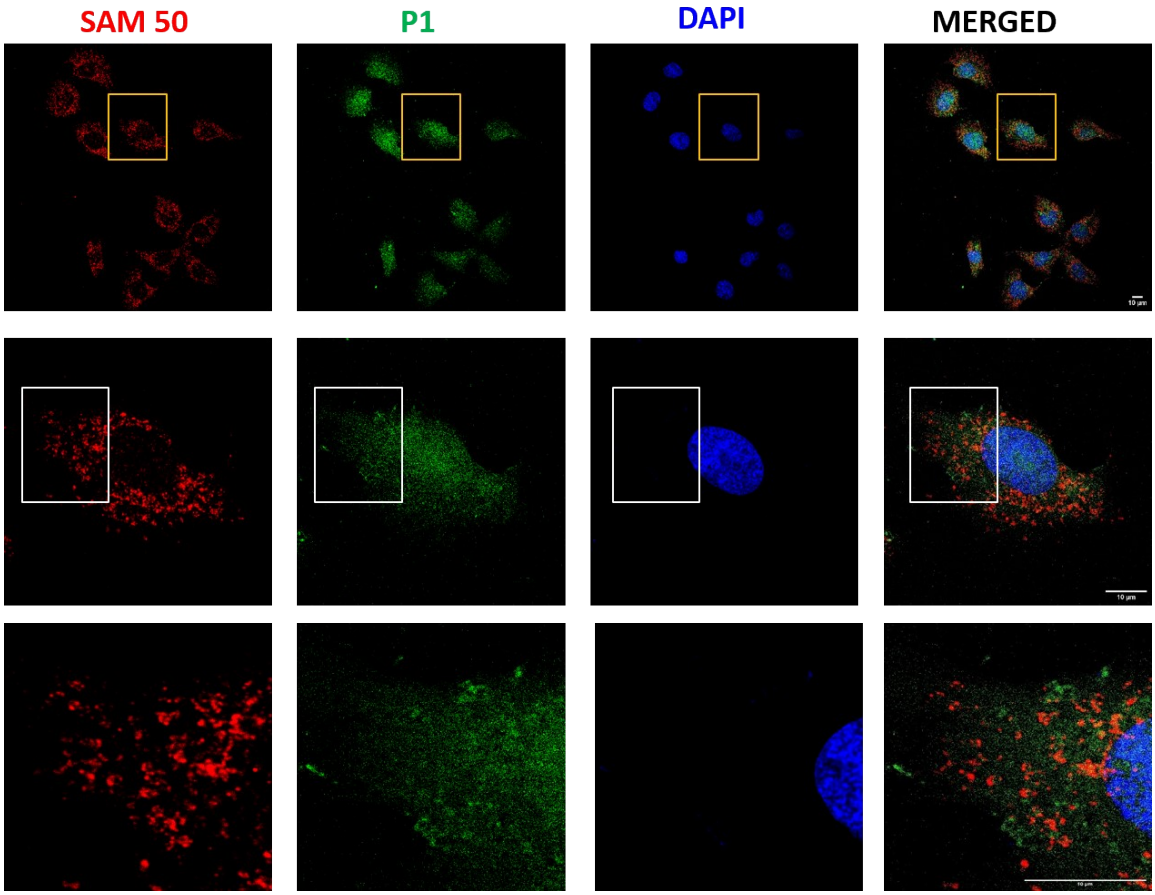


Figure S22: HeLa cells were treated with 2 µM FCCP drug, 15 minutes before P1 treatment followed by P1 for 1 hour. It was then fixed and immuno-stained for SAM50 (red) and DAPI (blue) stain was used for nucleus. Zoomed regions are shown in bottom row. Scale bar, 10 µm

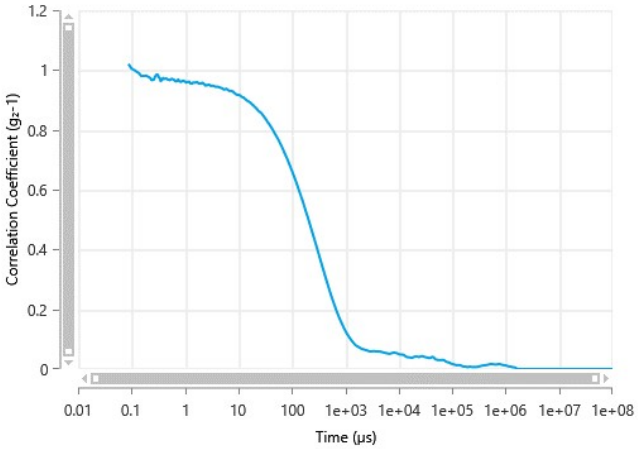


Figure S23. Correlation curve of size distribution of aqueous solutions of P1 in DLS (c = 0.1 mg/mL).

Figure S24. Uncropped image of different fractions of cell lysate after mitochondrial extraction from isolated cells treated with P1, P1 + FCCP, NMI or NMI + FCCP subjected to immunoblot analyses with antibodies against SAM50, a mitochondrial marker protein. Selected region of the image indicated by the red dotted line is shown in the main manuscript (Figure 4b) along with labels for each lane.

Table S1. Molecular weight of the polymers.

Polymer	Molecular weight (NMR) ($gmol^{-1}$)	Molecular weight (Absorption Spectra) ($gmol^{-1}$)	M_n ($gmol^{-1}$) (SEC)	$Đ$
P1a	10600	---	10000	1.2
P1	11500	9400	---	---
P2a	13400	---	12000	1.12
P2	14000	20000	---	---
P3a	11000	---	7500	1.2
P3	16000	---	---	---

Note: Difference in molecular weight values depending on the method of estimation is due to intrinsic limitation of SEC technique which only provides relative values as the calibration curves are made of standards, which may not have identical hydrodynamic volume compared to the sample polymers. Therefore for low molecular weight sysems, such as these polymers, end group analysis provides more accurate numbers. Among NMR and UV, NMR estimated values may be more accurate as the absoprtion intensity of the dye may be affected by the possibility of aggregation or polymer conjugtaion

References

1. A. P. G. Kieboom, D.D. Perrin and W. L. F. Armarego. Purification of Laboratory Chemicals, 3rd Edition. Pergamon Press, Oxford, 1988, **107**, 685.