# Electronic Supplementary Information

## Functional pH-responsive polymers containing dynamic enaminone linkages for the release of active organic amines

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## Contents

# 1 Experimental

## 1.1 Materials

2-(Acetoacetoxy)ethyl methacrylate (AEMA, 95%, passed through a column of activated basic alumina prior use), methyl α-bromophenylacetate (MBPA, 97%), propylamine (PR, 98%), α-bromophenyl acetic acid (98%), copper(II) bromide ($Cu(II)Br_2$, 99%), benzocaine (BNZ, HPLC, ≥ 99%), *p*-Toluenesulfonic acid monohydrate (*p*-TsOH ACS reagent, ≥ 98.5%), 2-(hydroxy)ethyl methacrylate (HEMA, 98%) and Celite® S were purchased from Sigma-Aldrich. Poly(ethylene glycol) monomethyl ether ($mPEG_{113}$-OH, $M_n$ = 5,000 g.mol$^{-1}$) was also purchased from Sigma-Aldrich. Poly(ethylene glycol) monomethyl ether ($mPEG_{43}$-OH, $M_n$ = 1,900 g.mol$^{-1}$), *N, N'*-dicyclohexylcarbodiimide (DCC, 99%), and dimethyl sulfoxide were purchased from Alpha Aesar by Thermo Fisher Scientific. 4-(Dimethylamino)pyridine (DMAP, ≥ 99%) was purchased from ReagentPlus® while *tris*(2-(dimethylamino)ethyl)amine ($Me_6$TREN) was synthesised according to previously reported literature.[1] Regenerated cellulose dialysis membranes were purchased from Specta/Por® with a MWCO cut-off of 2 kDa and 1 kDa. Phosphate buffers 0.2 M pH 4.4, 3.5 and 6.6 were purchased from Alfa Aesar. All materials were used as received unless otherwise stated.

## 1.2 Instrumentation

**Size exclusion chromatography (SEC)** analysis was performed on an Agilent Infinity II MDS instrument equipped with differential refractive index (DRI) and dual ultraviolet (UV) detectors. The mobile phase was DMF + 5mM $NH_4BF_4$ at 50 °C with a flow rate of 1 mL min$^{-1}$ and an injection volume of 100 µL, while poly(methyl methacrylate) (PMMA) standards (purchased from Agilent EasyVials) were used as calibrants. The instrument was equipped with 2 x PolarGel Mixed D columns (300 x 7.5 mm) and a PLgel 5 µm guard column. Polymers were dissolved in DMF and filtered through a GVHP nylon membrane (0.22 µm pore size) before analysis. Experimental molar mass ($M_{n,\,SEC}$) and dispersities ($Đ$) were calculated using Agilent GPC/SEC software.

**$^1$H-NMR, $^{13}$C-NMR, and DOSY-NMR** spectra were recorded in a Bruker DPX-400 or 500 $MH_z$ instrument using deuterated dimethyl sulfoxide (DMSO-$d_6$) as a solvent. Chemical shifts are given as $\delta$ in parts per million (ppm). For the kinetic experiments, the monomer percent conversions were determined using the above equation:

$$\textbf{Conv. (\%)} = (1 - \int I_{6.04ppm}) \times 100$$

Where $\int I_{6.04ppm}$ corresponds to the integral of a monomer vinyl proton after referencing the -$CH_2CH_2$ of the side chain as $\int I_{4.00-4,50ppm} = 4$.
All spectra were analysed using ACD/NMR Processor software.

**Infrared spectroscopy** was performed in a Bruker ALPHA II Fourier transform infrared (FTIR) spectrometer fitted with a crystal plate and a pressure tower running at 65 scans per sample with a speed of 0.5 cm $s^{-1}$.

**Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-ToF MS)** was performed on a Bruker Autoflex Speed MALDI-ToF mass spectrometer, equipped with a nitrogen laser delivering 2 ns laser pulses at 337 nm with positive ion ToF detection with an accelerating voltage of 25 kV. Solutions were prepared as follows: *trans*-2-[3-(4-*tert*-Butylphenyl)-2-methyl-2-propenylidene] malononitrile as the matrix (40 mg $mL^{-1}$) and the polymer sample (10 mg $mL^{-1}$) were each dissolved in THF containing sodium iodide as a cationizing agent (1 mg $mL^{-1}$). 20 µL of matrix and sample were mixed and 0.5 µL of the final solution was applied on the target plate. Spectra recording was made in reflective mode calibrating with PEG 5,000 Da.

**Differential scanning calorimetry (DSC)** was carried on a TA DSC 2500 instrument under nitrogen flow (50 mL $min^{-1}$) with a heating rate of 10 $^{o}C$ $min^{-1}$. Samples were loaded in 40 µL aluminum pans and heated for two cycles in total between -100 and 200 °C. All presented data concern the 2$^{nd}$ cycle thermograms.

**UV-Vis spectra** were recorded on an Agilent Technologies Cary 60 UV-Vis spectrometer in the range of 200-500 nm using HPLC graded water as a solvent. A glass cuvette (from HELLMA) with a 10 mm optical length was used for all measurements.

**Gas chromatography flame ionization detection (GC-FID)** was performed on a Shimadzu GC2014 equipped with a Shimadzu AO20i autosampler. The instrument had a polar Stabilwax-DA column (length 30 m, 0.32 mm ID and 0.25 µm film thickness) with hydrogen as the carrier

gas. An injection volume of 1 µL was used with a 39-split ratio. The injection temperature was 250 °C and the flame temperature was 300 °C. The heating profile was 60 – 200 °C at a rate of 10 °C $min^{-1}$ and 200-240 °C at a rate of 15 °C $min^{-1}$ held for 3 min. Samples were prepared by dissolving few drops in $CHCl_3$.

**Transmission Electron Microscopy (TEM)** images were obtained using a Jeol 2100 plus microscope, operated at 200 kV, and fitted with a Gatan Orius 11 megapixel digital camera. Samples were prepared by drop-casting a few milliliters of sample dispersions after ultra-sonication onto holey carbon grids, allowing the solvent to evaporate and leaving the sample to rest for 24 h at ambient temperature.

**Dynamic Light Scattering (DLS)** was used to determine the hydrodynamic diameter ($D_h$) and the size distribution (PD) of the nano-assemblies using an Anton-Paar Litesizer 500 equipped with a 40 mW - 658 nm laser diode source. Experiments were conducted at 25 °C with a 175 ° measurement angle (backscatter), an equilibration time of 12 min, and polystyrene latex as the reference material (RI = 1.585, Abs. coefficient = 0.0010). All determinations were carried out in triplicates with a maximum number of 60 runs per measurement. Solvents were filtered through a 0.45 µm *Fisher brand* nylon syringe filter prior use to remove undesirable dust particles.

**Zeta Potential Analysis** took place on an Anton-Paar Litesizer 500 at room temperature using distilled water (pH = 7) as the analysis media while applying a voltage of 10 V. The reported zeta potential data were an average of three runs with 40 measurements recorded per run.

**UV photo polymerisations:** Small scale photo polymerisations were conducted under a *Mylee* UV nail gel curing lamp equipped with four 9 W bulbs emitting at $\lambda_{max}$ ~ 365 nm. For bigger scale reactions, a custom-made UV box with $\lambda_{max}$ ~ 360 nm was used.

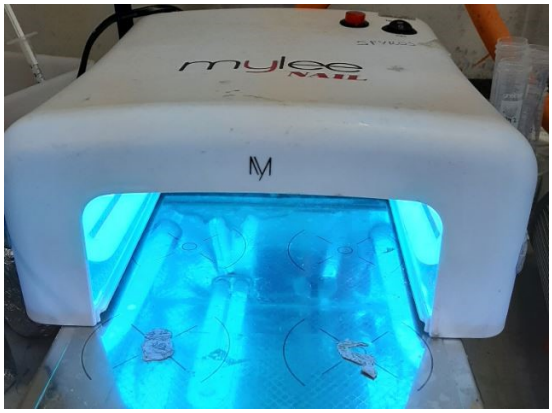



**Figure S1.** UV curing lamp equipped with four 9 W bulbs emitting at $\lambda_{max}$ ~ 365 nm for small scale

photo polymerisations.

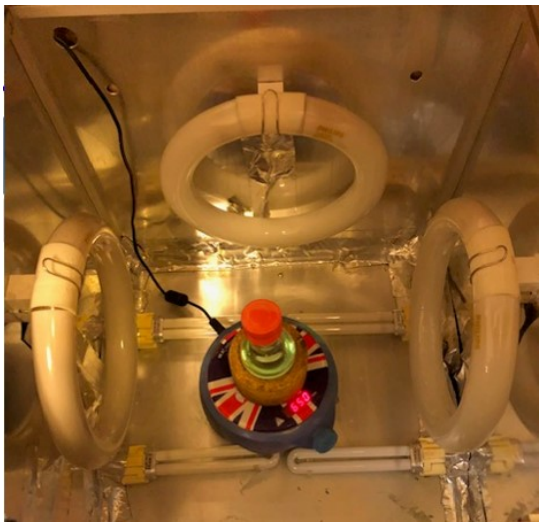
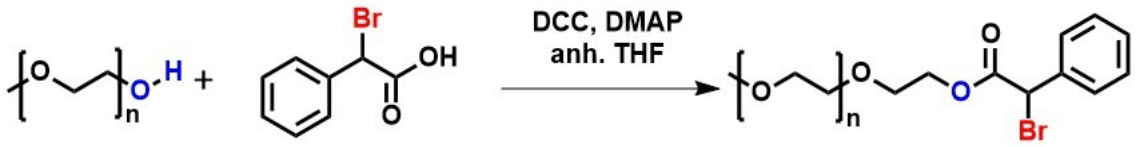


**Figure S2.** Custom-made UV box set up with $\lambda_{max}$ ~ 360 nm used for higher scale photo polymerisations.

## 1.3 Experimental Procedures

**Poly(ethylene glycol) monomethyl ether-2-bromo-2-phenylacetate macroinitiator synthesis by DCC coupling**




$mPEG_{113}$ -OH (20.0 g, 4.0 mmol, 1 eq.) was dissolved in anhydrous THF (150 mL) in a dry two-neck round bottom flask equipped with a magnetic stir bar. After complete dissolution of the polymer flakes, the flask was immersed in an ice bath and sealed with a rubber septum under nitrogen flow. DMAP (0.1 g, 0.8 mmol, 0.2 eq.), DCC (1.65 g, 8.0 mmol, 2 eq.) and α-bromophenyl acetic acid (3.4 g, 16.0 mmol, 4 eq.) were added successively. The reaction was let at 0 °C for 2 h and at room temperature for another 24 h until a second addition of DMAP (0.65 g, 5.3 mmol, 1 eq.) and DCC (2.19 g, 10.6 mmol, 2 eq.) was followed by cannulating a degassed mixture of them in anhydrous THF (6 mL) leaving the reaction for additional 24 h. The resulting mixture was filtered to remove most insoluble impurities and concentrated at a lower volume. The concentrated solution was left at -20 °C for at least 2 h and filtered ( × 2)

through Celite® S to receive a clear yellow solution. The resulting solution was finally concentrated and precipitated ( × 2) in cold diethyl ether. Solids were filtered and dried under vacuum for 24 h to receive a pale-yellow solid (70 % yield) referred as $mPEG_{113}$-BPA. The modification efficiency was found to be > 99% based on $^1$H-NMR (400 MHz, DMSO-$d_6$) integrations in combination with the complete disappearance of the –O**H** peaks at $\delta$ = 4.56 ppm.

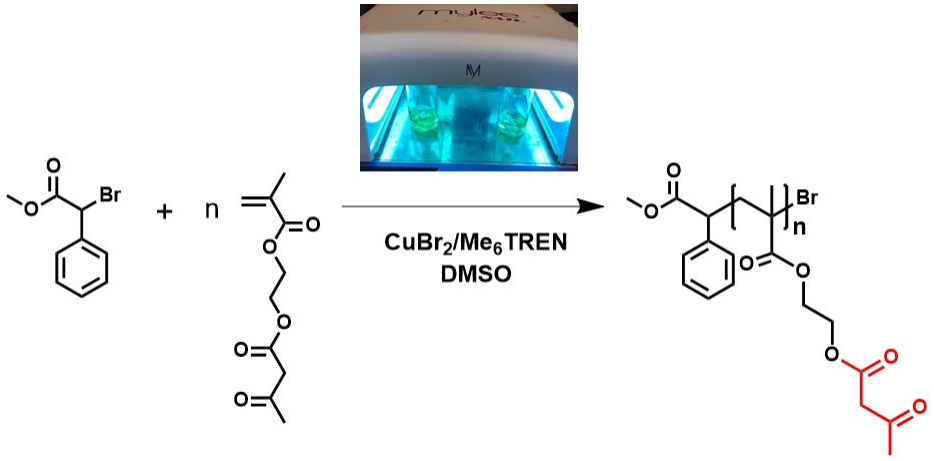
**$^1$H-NMR** (400 MHz, DMSO-$d_6$), $\delta$ (ppm): 3.25 (s, 3H, -O-C**H**$_3$), 3.51 (s, 168H, -O-C**H**$_2$-C**H**$_2$), 4.27 (m, 2H, -CH$_2$-O-CH$_2$-C**H**$_2$-O-CO), 5.95 (s, 1H, C**H**-Br), 7.40 (m, 3H, aromatic ring) and 7.57 (d, 2H, J = 6.72, aromatic ring).

**$^{13}$C-NMR** (400 MHz, DMSO-$d_6$), $\delta$ (ppm): 47.3 (**C**-Br), 58.5 (**C**H$_3$-O-), 65.9 (CH$_2$-**C**H$_2$-O-CO), 68.4 (**C**H$_2$-CH$_2$-O-CO), 70.3 (-O-**C**H$_2$-**C**H$_2$), 71.8 (CH$_3$-O-**C**H$_2$-), 127.1 (=CH-**C**H=CH-), 129.2 (=**C**H-CH=**C**H-), 129.6 (=**C**H-C-(CBr)=**C**H-), 136.7 (=CH-**C**-(CBr)=CH-) and 168.5 (O-**C**O-CBr-).

**FT-IR** ($\nu$, cm$^{-1}$): 698 (C-Br stretch), 1,750 (C=O)

**$mPEG_{113}$-BPA :** $\boldsymbol{M}_{n,\,SEC}$ = 8,800 g.mol$^{-1}$ ($\boldsymbol{Đ}$ = 1.05) and **$mPEG_{43}$-BPA :** $\boldsymbol{M}_{n,\,SEC}$ = 4,400 g.mol$^{-1}$ ($\boldsymbol{Đ}$ = 1.06)

**Synthesis of pAEMA homopolymers *via* photoinduced Cu-RDRP**



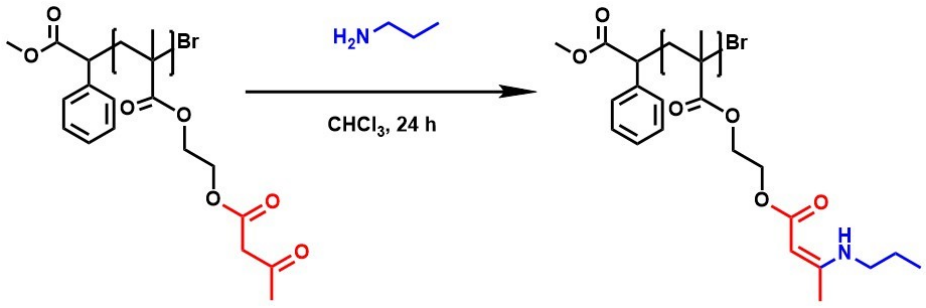

For a targeted $DP_{n,\,target}$ = 80, an aluminum foiled vial was charged with Cu(II)Br$_2$ (1.5 mg, 0.05 eq.), Me$_6$TREN (12.6 µL, 0.36 eq.) and DMSO (2 mL) following sonication for ~ 1 min until complete dissolution of the copper complex. AEMA (2 mL, 0.02 mol, 80 eq.), MBPA (20.6 µL, 1 eq.) along with a magnetic stir bar were added and the vial was sealed with a rubber septum and degassed for 20 min with nitrogen. The aluminum foil was removed and the degassed reaction mixture was immediately exposed to UV light ($\lambda_{max}$ ~ 365 nm) for the polymerisation to commence. The reaction was UV irradiated for 12 h to reach > 99 % conversion based on $^1$H-NMR. After the pass of polymerisation, the mixture was diluted with

THF and passed through an activated alumina column to remove copper. Finally, polymers were precipitated ( × 3) in a mixture of cold $H_2O$: MeOH (2:1) to remove potential unreacted substances following freeze-drying to yield purified **pAEMA$_{80}$** homopolymers. Products were finally analysed by NMR and SEC to determine the degree of polymerisation ($DP_n$) and dispersity ($Đ$).

**Synthesis of mPEG$_x$-*b*-pAEMA$_y$ block copolymers *via* photoinduced Cu-RDRP**

For a chain extension using mPEG$_{43}$-BPA macroinitiator targeting $DP_{n,\ target}$ = 80, Cu(II)Br$_2$ (1.5 mg, 0.05 eq.), $Me_6$TREN (12.6 µL, 0.36 eq.), DMSO (0.5 mL) and a magnetic stir bar were charged in an aluminum foiled vial which was sonicated for ~ 1 min until complete dissolution of the copper complex. In a second vial, synthesised mPEG$_{43}$-BPA macroinitiator ($M_{n,\ NMR}$ = 2,097 g mol$^{-1}$, 0.26 g, 1 eq.) was dissolved in DMSO (1.5 mL) following addition of AEMA (2 mL, 0.01 mol, 80 eq.). The mixture was carefully transferred to the first vial which was sealed with a rubber septum and degassed for 20 min with nitrogen. The polymerisation mixture was immediately exposed to UV light ($\lambda_{max}$ ~ 365 nm) for 12 h reaching > 99 % conversion. The reaction was diluted with THF and passed through activated alumina to remove the remaining copper salts. Polymers were then precipitated ( × 3) in a mixture of cold EtOH: MeOH (2:1) to remove any unreacted monomer and unconsumed macroinitiator. The blocks were finally dried under vacuum to yield purified **mPEG$_{43}$-*b*-pAEMA$_y$** block copolymers. The same protocol was followed using mPEG$_{113}$-BPA macroinitiator with the difference of using a [Cu(II)Br$_2$] : [$Me_6$TREN] = [0.1] : [0.72] ratio yielding **mPEG$_{113}$-*b*-pAEMA$_y$** block copolymers.

**Functionalisation of pAEMA$_{42}$ homopolymers with propylamine**

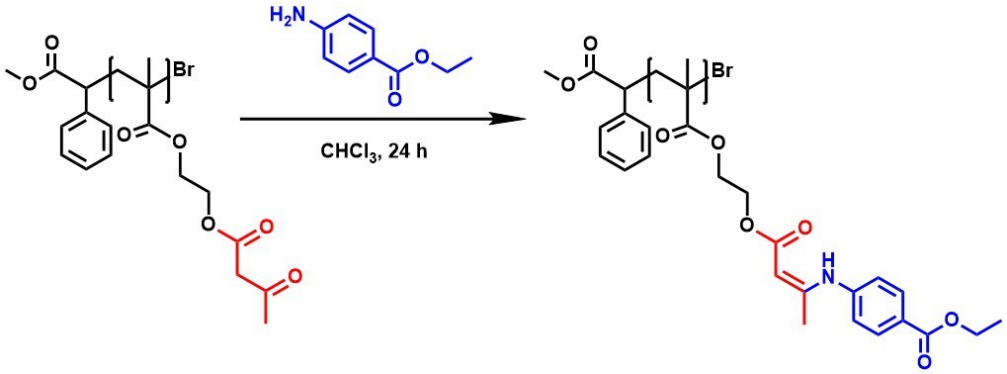


In a typical modification procedure, purified pAEMA$_{42}$ (0.1 g, $n_{pol.}$ = 0.01 mmol, $M_{n,\ NMR}$ = 9,200 g mol$^{-1}$, $Đ$ = 1.33, $n_{-acet.}$ = 0.42 mmol, 1 eq.) was charged in a vial and dissolved in $CHCl_3$ (4.0 mL). Propylamine (69 µL, 2 eq. to the moles of acetoacetate groups ($n_{-acet}$) was added and the reaction was let for 24 h at ambient temperature. The reaction mixture was concentrated under suppressed air and precipitated once in cold MeOH. The precipitate was collected after centrifugation and dried under vacuum at 40 °C for 24 h to remove the remaining solvents and the excess propylamine (b.p. = 48 °C). The modified **pAEMA$_{42}$/PrA** was analysed by NMR and FT-IR to determine the modification efficiency (%).

**Acid hydrolysis of pAEMA$_{42}$/PrA modified homopolymers**

Modified **pAEMA$_{42}$/PrA** (0.1 g, $n_{-PR.}$ = 0.36 mmol, $M_{n,\ NMR}$ = [$MW_{MBPA}$ + ($DP_{acet.}$ × $MW_{AEMA}$)] + [$DP_{PR}$ × ($MW_{AEMA}$ + $MW_{PR}$ – 18)] = 10,800 g mol$^{-1}$, 93 % modification based on $^1$H-NMR) was dissolved in 4 mL of THF. Excess of $H_3PO_4$ 85 wt% (38 µL, 0.72 mmol, 2 eq.) was added and the hydrolysis was let for 24 h. After 24 h, white propylamine salt appeared and removed by centrifugation while the supernatant was dialyzed against THF (MWCO = 2 kDa dialysis membrane) to remove any excess acid. Finally, the solvent was removed *in vacuo* and the hydrolysed polymers were dried under vacuum at 40 °C.

**Functionalisation protocol of homopolymers and block copolymers with benzocaine**




In a typical modification, purified pAEMA$_{42}$ (0.5 g, $n_{pol.}$ = 0.05 mmol, $M_{n,\ NMR}$ = 9,200 g mol$^{-1}$, $Đ$ = 1.33, $n_{-acet.}$ = 2.1 mmol, 1.0 eq.) was dissolved in ~ 7.0 mL of $CHCl_3$. Benzocaine (2.1 g, 12.6 mmol, 6 eq. to the moles of acetoacetate groups) and a stir bar were added and the reaction was let for 24 h at 40 °C. The modified polymers were precipitated (× 3) in cold diethyl ether under fast stirring and dried under vacuum at 25 °C to yield purified **pAEMA$_{42}$/BNZ**

**(modification efficiency %)** modified polymers. A similar protocol was followed for the modification of $mPEG_x$-*b*-$pAEMA_y$ block copolymers. The temperature, solvent, and eq. of BNZ were altered depending on the desired degree of modification while p-TsOH was included in the recipe to boost modification when required. Finally, polymers were characterised by NMR and FT-IR to determine the modification efficiency (%).

**Nanoparticles formation *via* the direct dilution method**

2 mg of $mPEG_x$-*b*-$pAEMA_y$ block copolymer were dissolved in 4 mL of previously filtered DI water at ambient temperature. This solution was sonicated for 10 min until complete dissolution of the blocks leading to a 0.5 mg $mL^{-1}$ opaque solution of nanoparticles. A similar procedure was used for the benzocaine modified blocks. Nanoparticles were characterised by DLS and TEM.

**Stock and working solutions of benzocaine**

A 200 µM stock solution of benzocaine was initially prepared by transferring 3.3 mg of BNZ in a 100 mL volumetric flask and dissolving in 1 mL of absolute ethanol. Then, the volume was made up to 100 mL with Milli-Q® water following 5 min sonication. Working solutions of different concentrations were then prepared (between 0.5 and 16.5 µg $mL^{-1}$) by diluting the stock solution appropriately.

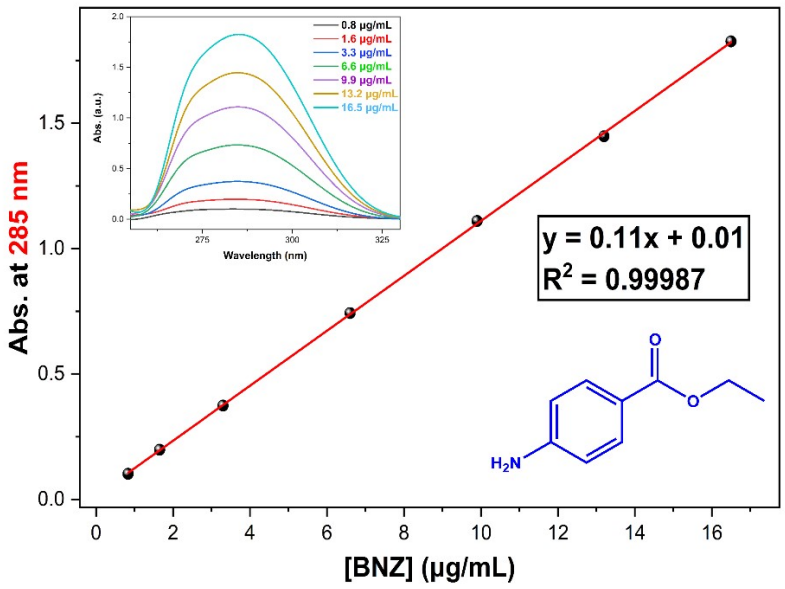



**Figure S3.** Calibration curve of benzocaine molecule in water recorded at $\lambda_{max}$ = 285 nm.

***In vitro* release study of benzocaine modified mPEG$_{113}$-*b*-pAEMA$_{28}$/BNZ(70 %) NPs**

A more accurate determination of benzocaine's dosage (μg) in the studied NPs was conducted using $^1$H-NMR spectroscopy with a known amount of DCM as the internal standard. It was found that 2.5 mg of block copolymers contained 700 μg of benzocaine. A 100 mg mL$^{-1}$ stock solution of mPEG$_{113}$-*b*-pAEMA$_{28}$/BNZ(70 %) NPs was prepared in DMF keeping it in the freezer to avoid amine release. As an example, 25 μL from the stock were injected into 5 mL of DI water giving a solution of 0.5 mg mL$^{-1}$ (containing 0.5 % of DMF). The solution of NPs was transferred to a 1 kDa dialysis bag, immersed in a 50 mL falcon tube containing 40 mL of the desired phosphate buffer (pH = 3.5, 4.4, and 6.6) and incubated under gentle agitation at 37 °C. At designated time points, 2 mL of buffer solution were collected and replaced with 2 mL of fresh benzocaine-free buffer. The collected samples were analysed by UV-Vis and the concentration of benzocaine at it each time point was calculated by fitting with benzocaine's calibration curve recorded at $\lambda_{max}$ = 285 nm, Figure S3. The cumulative percent release was determined using the formula :[2]

$$Cumulative\ Release\ \% = \frac{V_{total} \times C_j + V_{sample} \times \sum_{n=1}^{n-1} C_n}{m_{drug}}$$

where $V_{total}$ stands for the total volume of buffer (40 mL), $V_{sample}$ the sampling volume (2 mL), $m_{drug}$ the initial mass of benzocaine in the NPs (μg) and $C_n$ and $C_j$ the benzocaine concentration at j and n sampling times (μg mL$^{-1}$).

**Stability study of amphiphilic block copolymers in water**

In three separate vials, 10 mg of mPEG$_{43}$-b-pAEMA$_{25}$ were loaded along with 2 mL of DI $H_2O$ creating micellar solutions of 5 mg mL$^{-1}$. The first vial was left for 1 day, the second for 5 days and the third for 14 days. Water was removed by freeze drying and a $^1$H-NMR spectra of the remaining block copolymers was attained monitoring the -$CH_2$ peak of HEMA unit at δ = 3.91 ppm.

# 2 Supplementary analysis and experimental data

## 2.1 Characterisation of synthesised mPEG$_x$-BPA (x = 43 or 113) macroinitiators

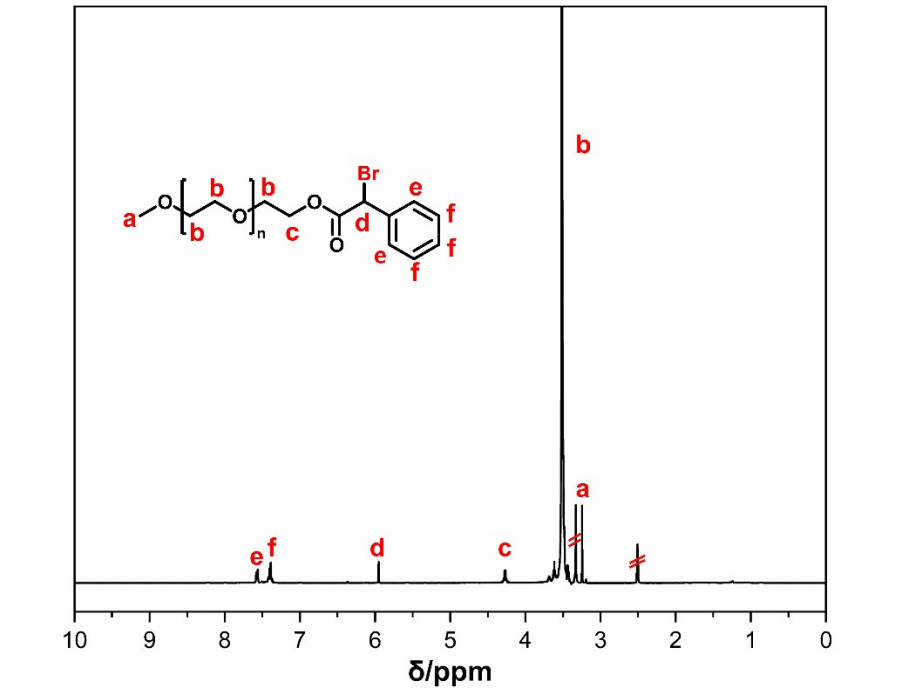



**Figure S4.** $^1$H-NMR (400 MHz, DMSO-d$_6$) spectrum of mPEG$_{43}$-BPA macroinitiator.

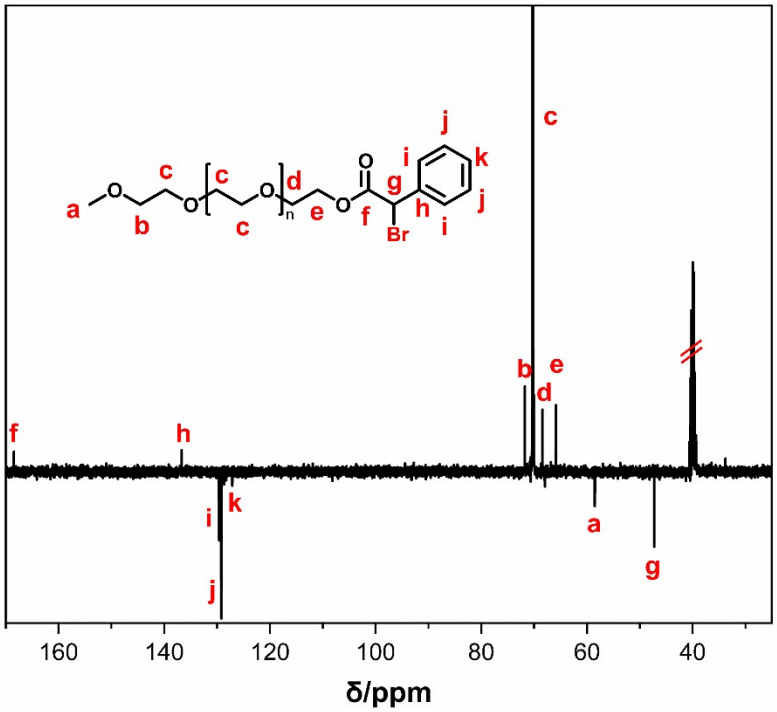



**Figure S5.** $^{13}$C-NMR (400 MHz, DMSO-$d_6$) spectrum of the synthesised mPEG$_{43}$-BPA macroinitiator.

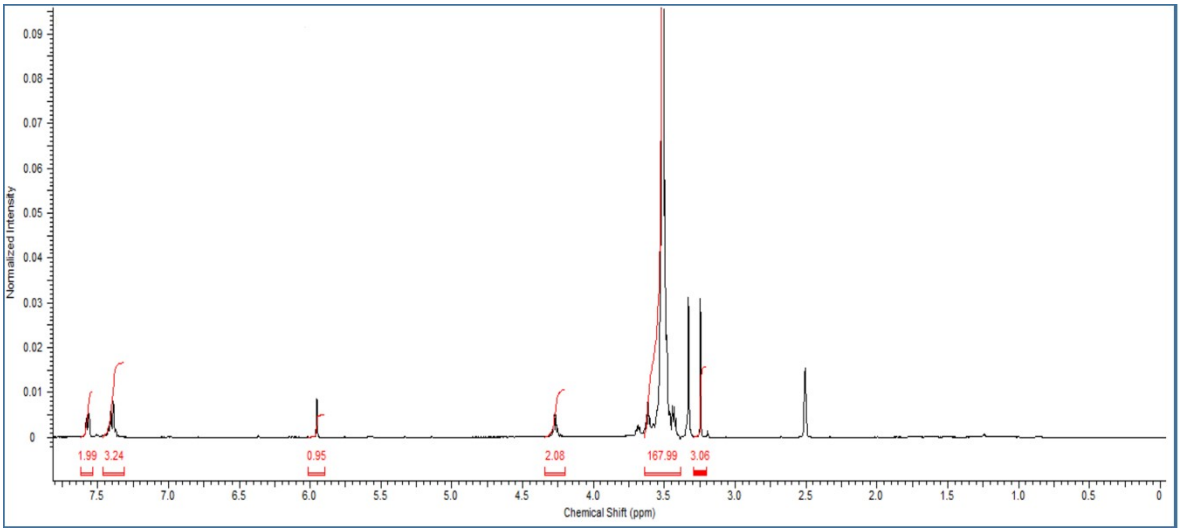



**Figure S6.** Integrated $^{1}$H-NMR (400 MHz, DMSO-$d_6$) peaks of mPEG$_{43}$-BPA synthesised macroinitiator.

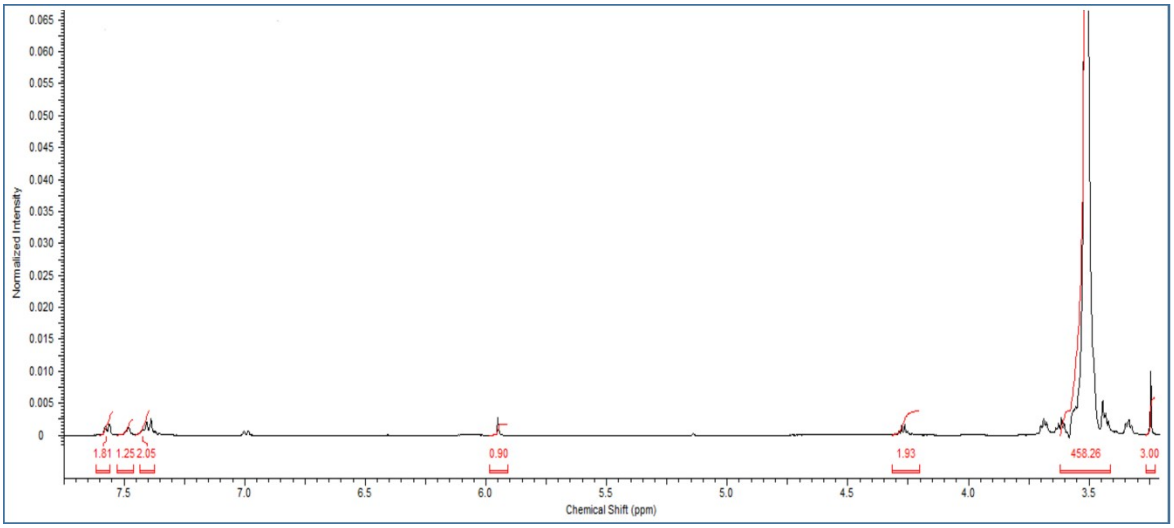



**Figure S7.** Integrated $^{1}$H-NMR (400 MHz, DMSO-$d_6$) peaks of mPEG$_{113}$-BPA synthesised macroinitiator.

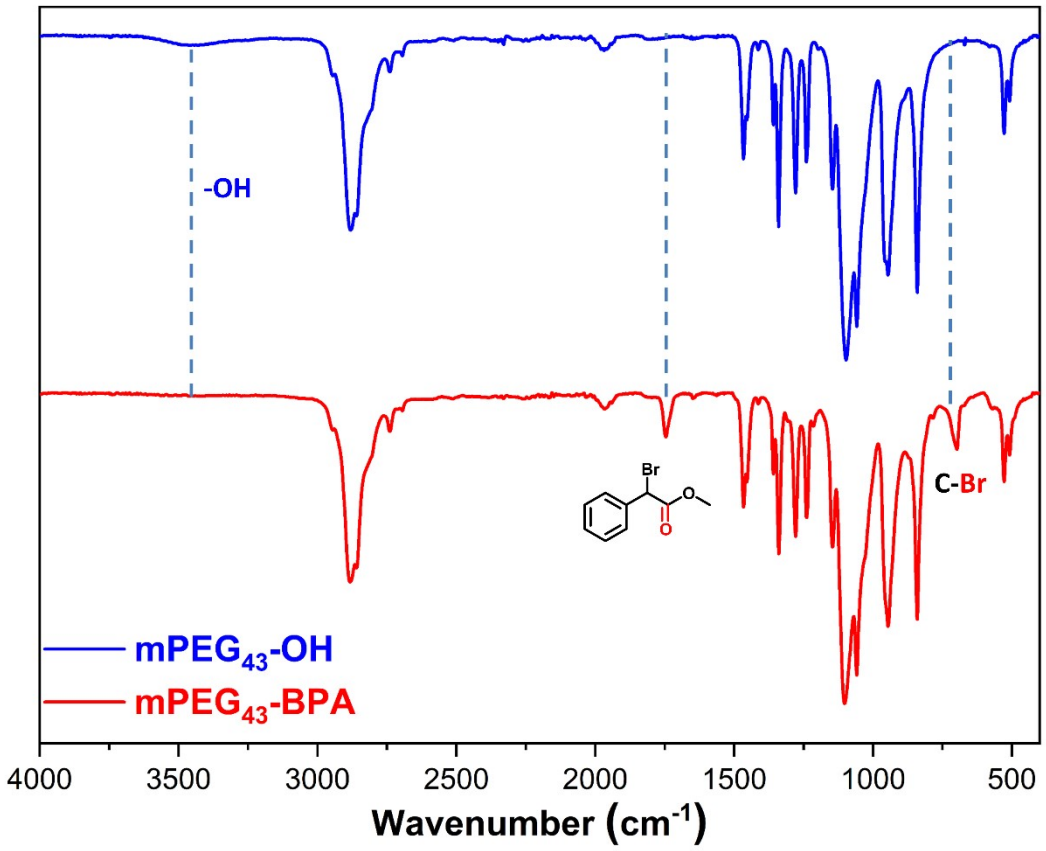



**Figure S8.** FT-IR spectrum of the synthesised mPEG$_{43}$-BPA macroinitiator using mPEG$_{43}$-OH as a precursor.

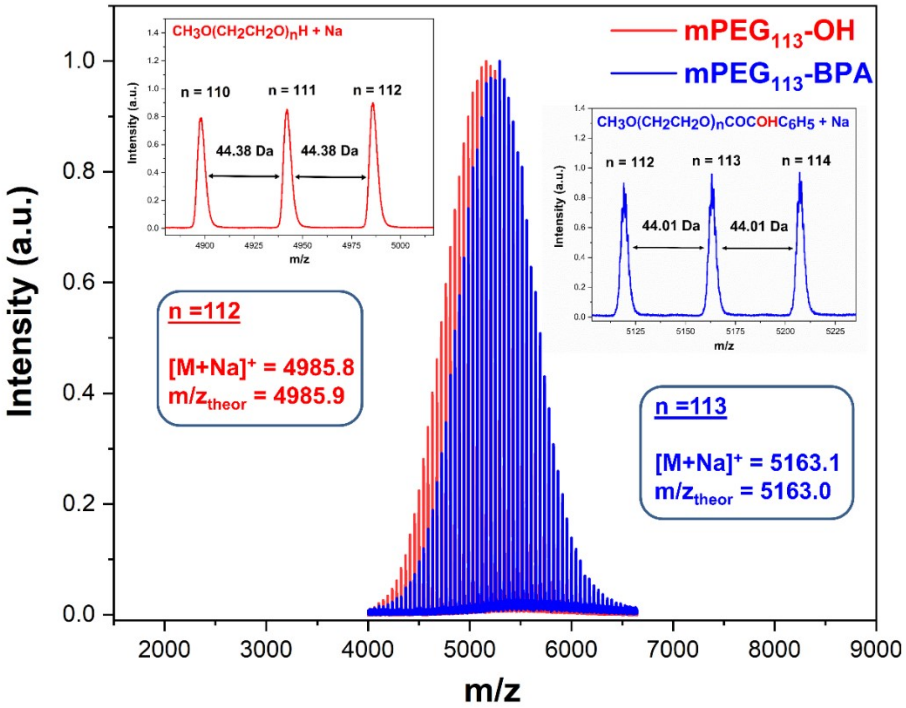



**Figure S9.** MALDI-ToF MS spectra comparison of $mPEG_{113}$-OH and $mPEG_{113}$-BPA macroinitiator.

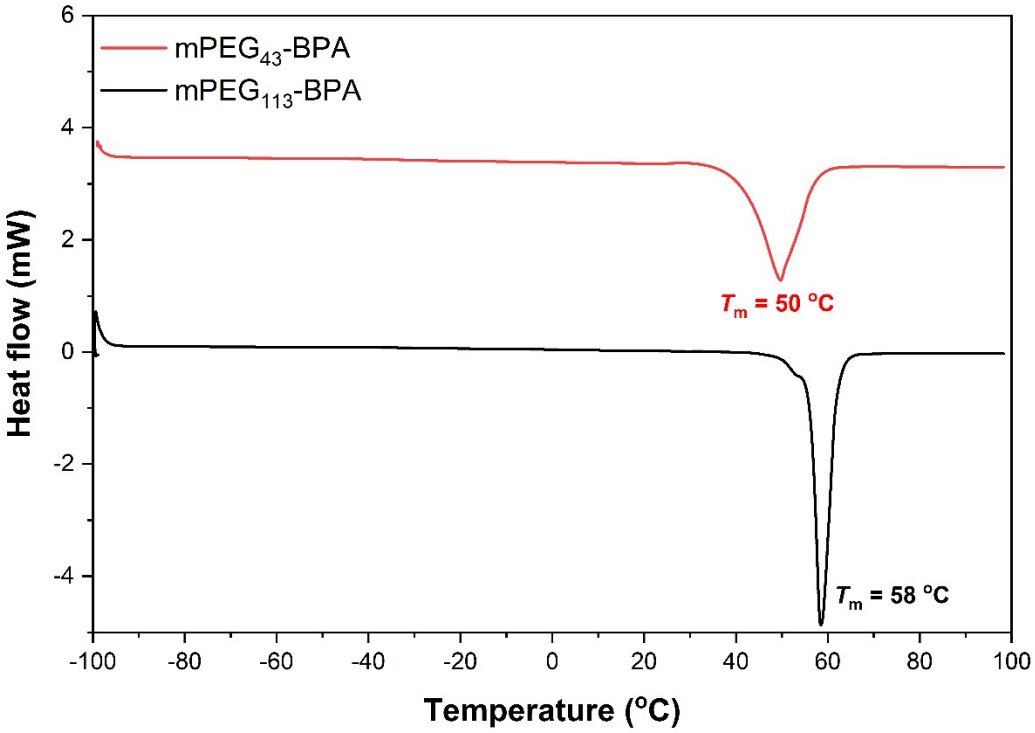



**Figure S10.** DSC analysis of $mPEG_x$-BPA (x = 43 or 113) synthesised macroinitiators.

## 2.2 Optimisation experiments for the synthesis of pAEMA homopolymers by photoinduced Cu(II)-mediated RDRP

**Table 1.** Polymerisation data from the optimisation reactions of the photoinduced Cu(II)-mediated RDRP of AEMA in DMSO using as I : MBPA and L : $Me_6$TREN. Polymerisation time for all reactions was 12 h.

| Entry | [AEMA]:[I]:[$Cu^{II}$]:[L] | Conv. (%) | $Đ$ | $M_{n,SEC}$ (g $mol^{-1}$) | $M_{n,th}$ (g $mol^{-1}$) | $M_{n,NMR}$ (g $mol^{-1}$) | $DP_{n,\ NMR}$ |
|---|---|---|---|---|---|---|---|
| 1 | 40:1:**0.10:0.18** | 20 | 2.16 | 10,260 | 8,800 | 1,900 | 8 |
| 2 | 40:1:**0.03:0.21** | 1 | - | - | 8,800 | - | - |
| 3 | 40:1:**0.04:0.29** | 87 | 1.44 | 17,900 | 8,800 | 9,800 | 45 |

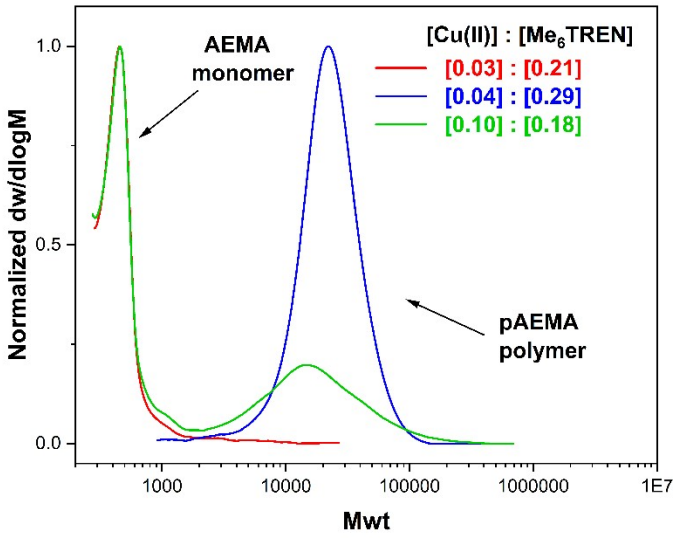



**Figure S11.** Molecular weight distribution traces from the crude optimisation reactions of AEMA using photoinduced Cu(II)-RDRP as measured by SEC in DMF.

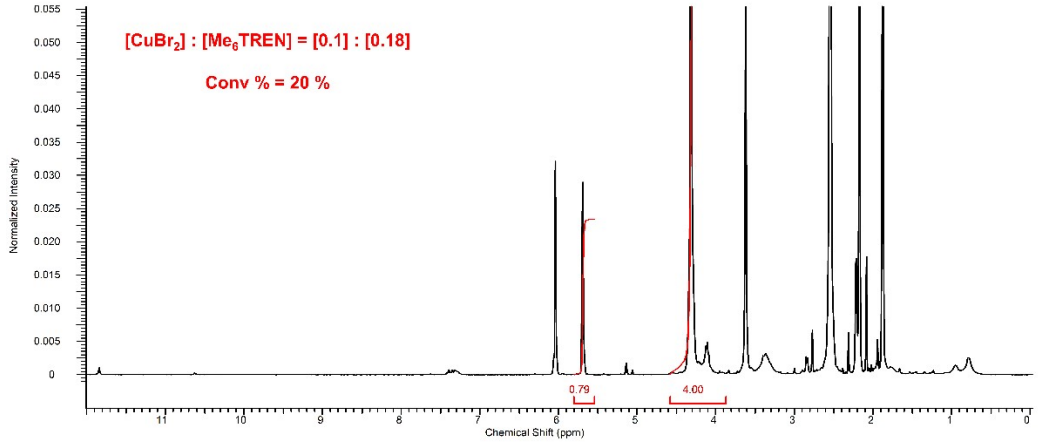



**Figure S12 .** $^{1}$H-NMR (400 MHz, DMSO-$d_6$) spectra of the crude reaction after the photoinduced Cu(II)-RDRP polymerisation of AEMA using a ratio of [$CuBr_2$] : [$Me_6$TREN] = [0.1] : [0.18] after 12 h.

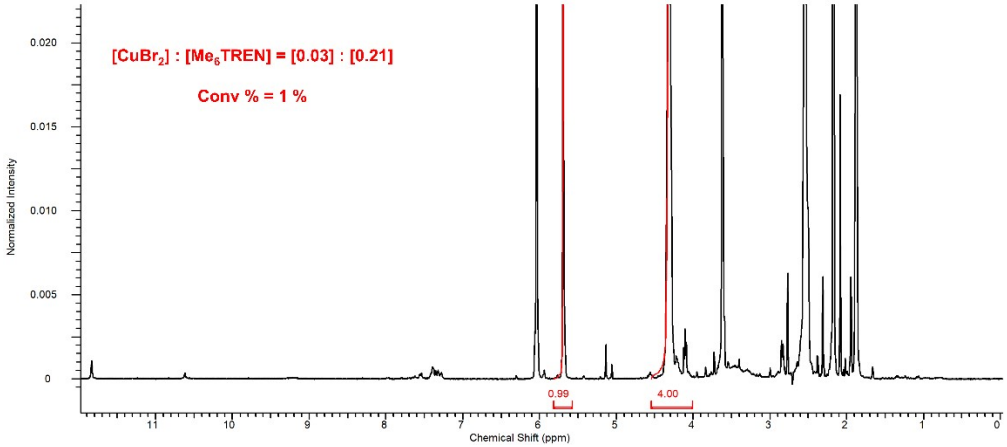



**Figure S13 .** $^{1}$H-NMR (400 MHz, DMSO-$d_6$) spectra of the crude reaction after the photoinduced Cu(II)-RDRP polymerization of AEMA using a ratio of [$CuBr_2$] : [$Me_6$TREN] = [0.03] : [0.21] after 12 h.

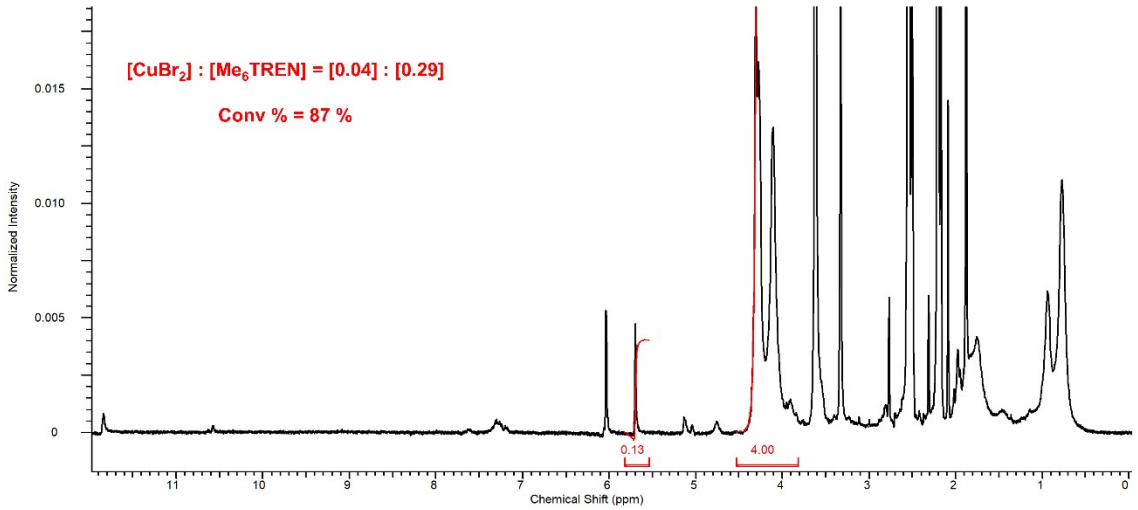



**Figure S14 .** $^{1}$H-NMR (400 MHz, DMSO-$d_6$) spectra of the crude reaction after the photoinduced Cu(II)-RDRP polymerisation of AEMA using a ratio of [$CuBr_2$] : [$Me_6$TREN] = [0.04] : [0.29] after 12 h.

## 2.3 Characterisation of pAEMA homopolymers synthesised by photoinduced Cu(II)-RDRP

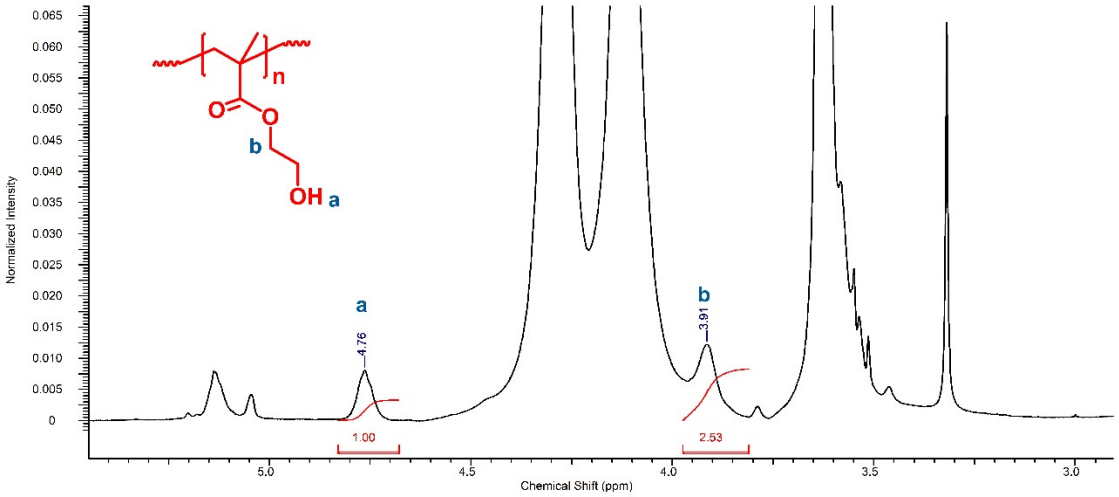



**Figure S15 .** $^{1}$H-NMR (400 MHz, DMSO-$d_6$) spectra of a synthesised pAEMA$_{38}$ homopolymer proving the existence of pHEMA units.

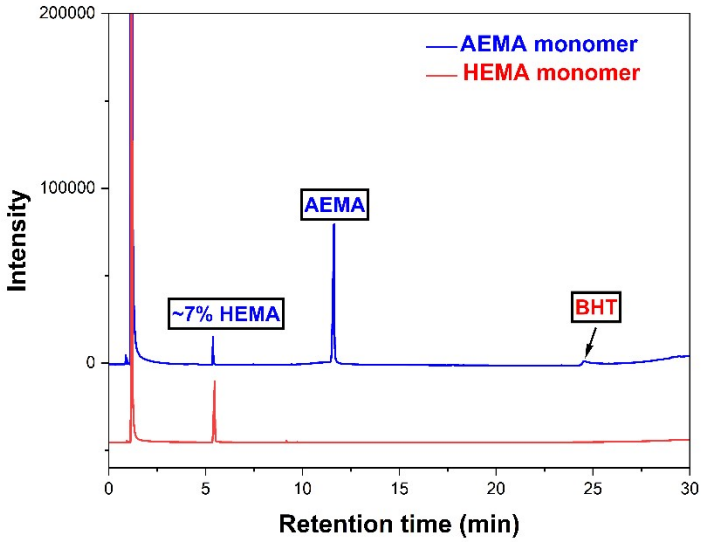



**Figure S16 .** GC-FID analysis of AEMA commercial monomer in $CHCl_3$ and comparison with HEMA monomer.

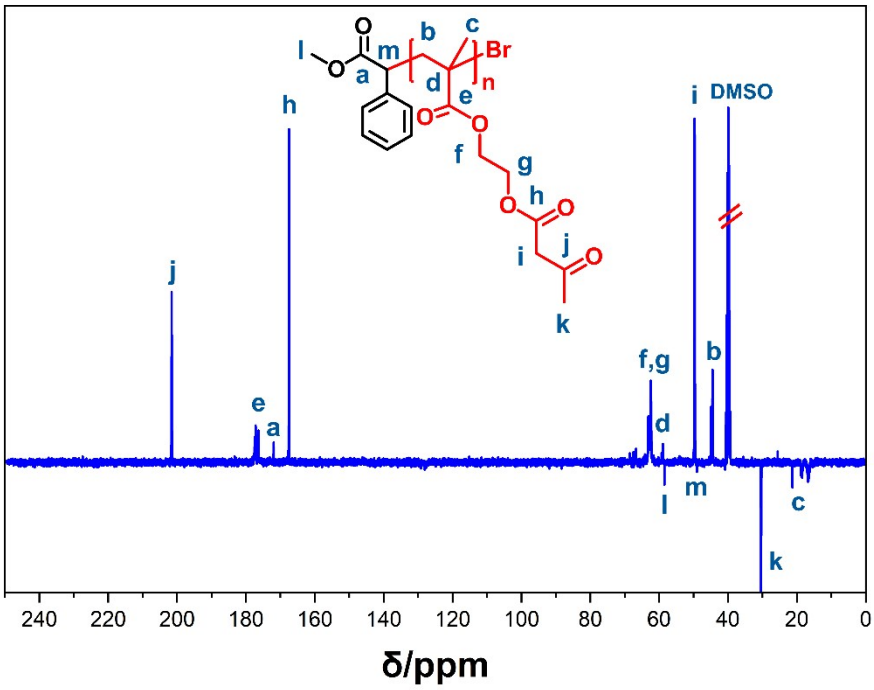



**Figure S17.** $^{13}$C-NMR (500 MHz, DMSO-$d_6$) spectra of a synthesised pAEMA$_{38}$ homopolymer.

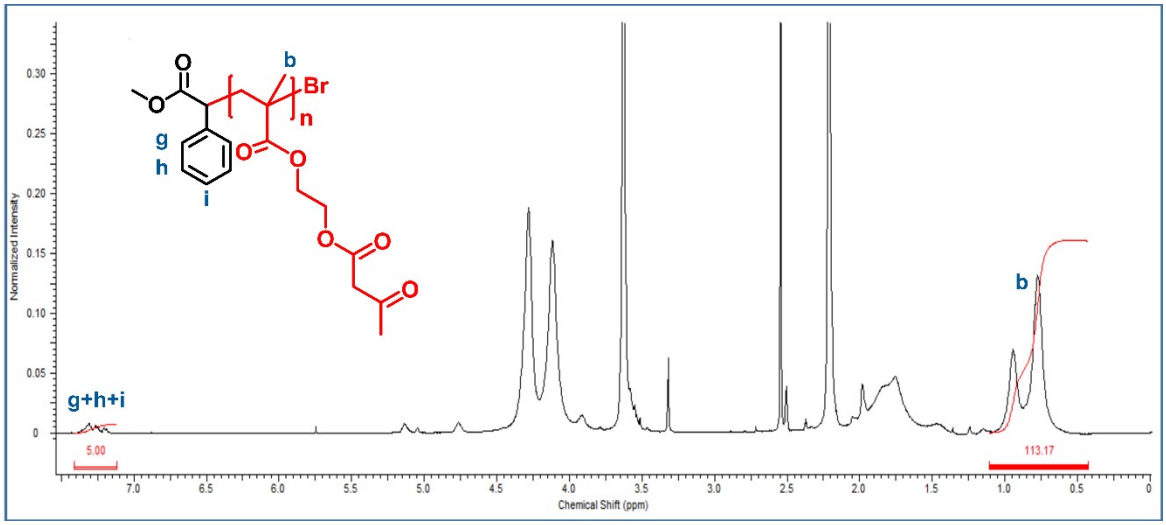



**Figure S18.** Integrated $^{1}$H-NMR (400 MHz, DMSO-$d_6$) peaks of purified pAEMA$_{38}$ after targeting $DP_{target}$ = 40.

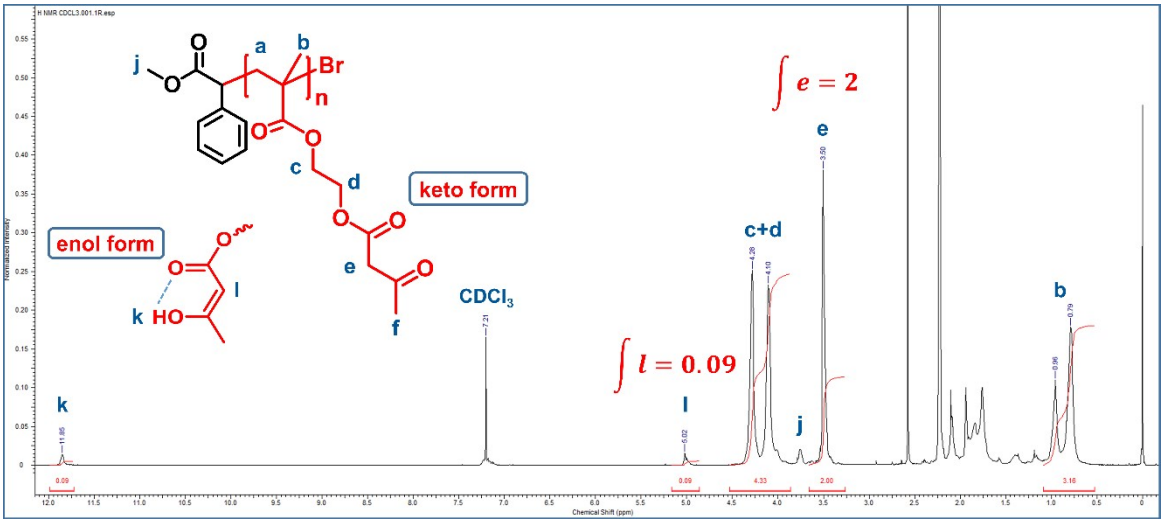



**Figure S19.** Integrated $^1$H-NMR (400 MHz, $CDCl_3$) spectra of purified $pAEMA_{38}$ showing the percent ratio composition between keto and enol form. Based on the integrals, 92% of units exist as a keto form.

## 2.4 Characterisation of $mPEG_x$-*b*-$pAEMA_y$ block copolymers synthesised by photoinduced Cu(II)-RDRP

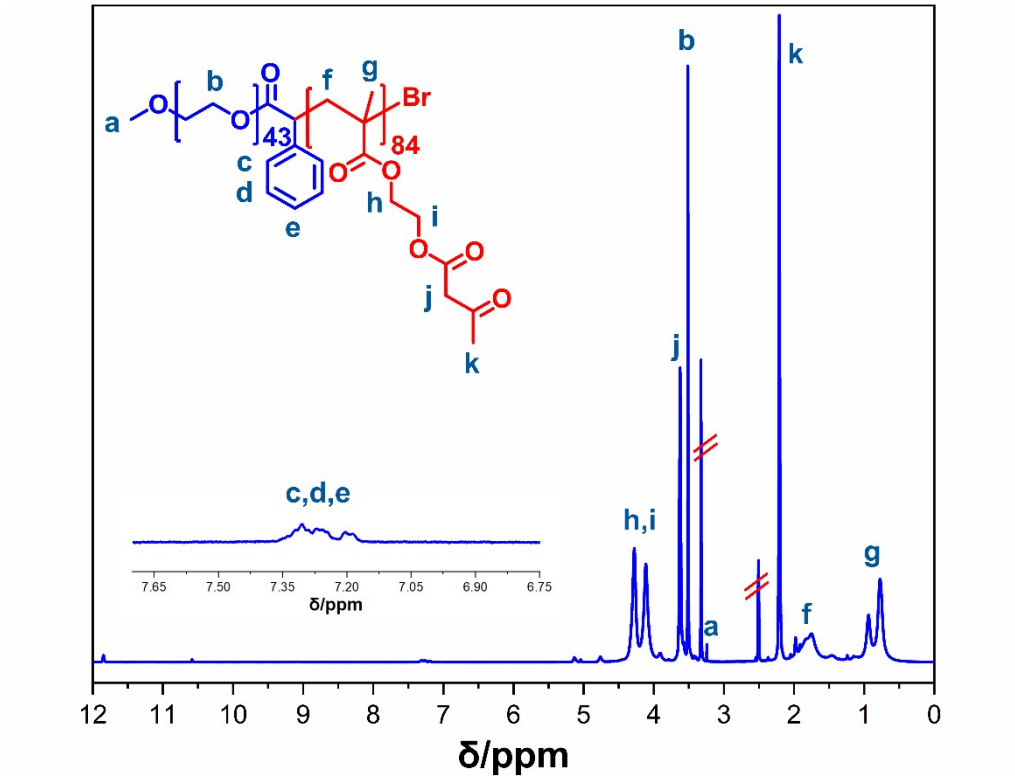



**Figure S20.** $^{1}$H-NMR (400 MHz, DMSO-$d_6$) spectra of a synthesised mPEG$_{43}$-*b*-pAEMA$_{84}$ block copolymer.

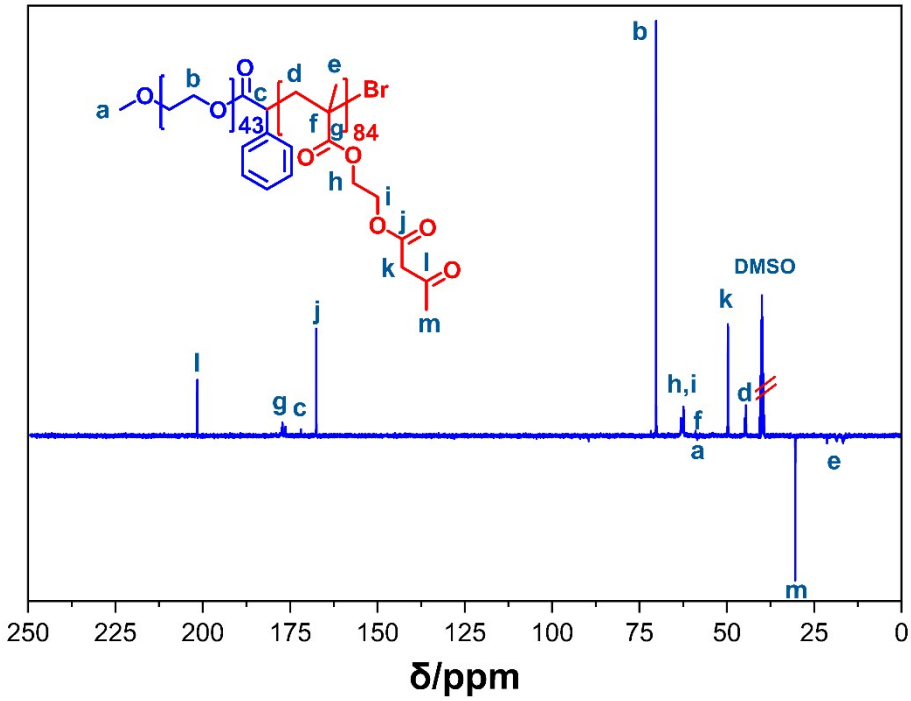



**Figure S21.** $^{13}$C-NMR (500 MHz, DMSO-$d_6$) spectra of a synthesised mPEG$_{43}$-*b*-pAEMA$_{84}$ block copolymer.

## 2.5 Polymerisation Kinetic studies

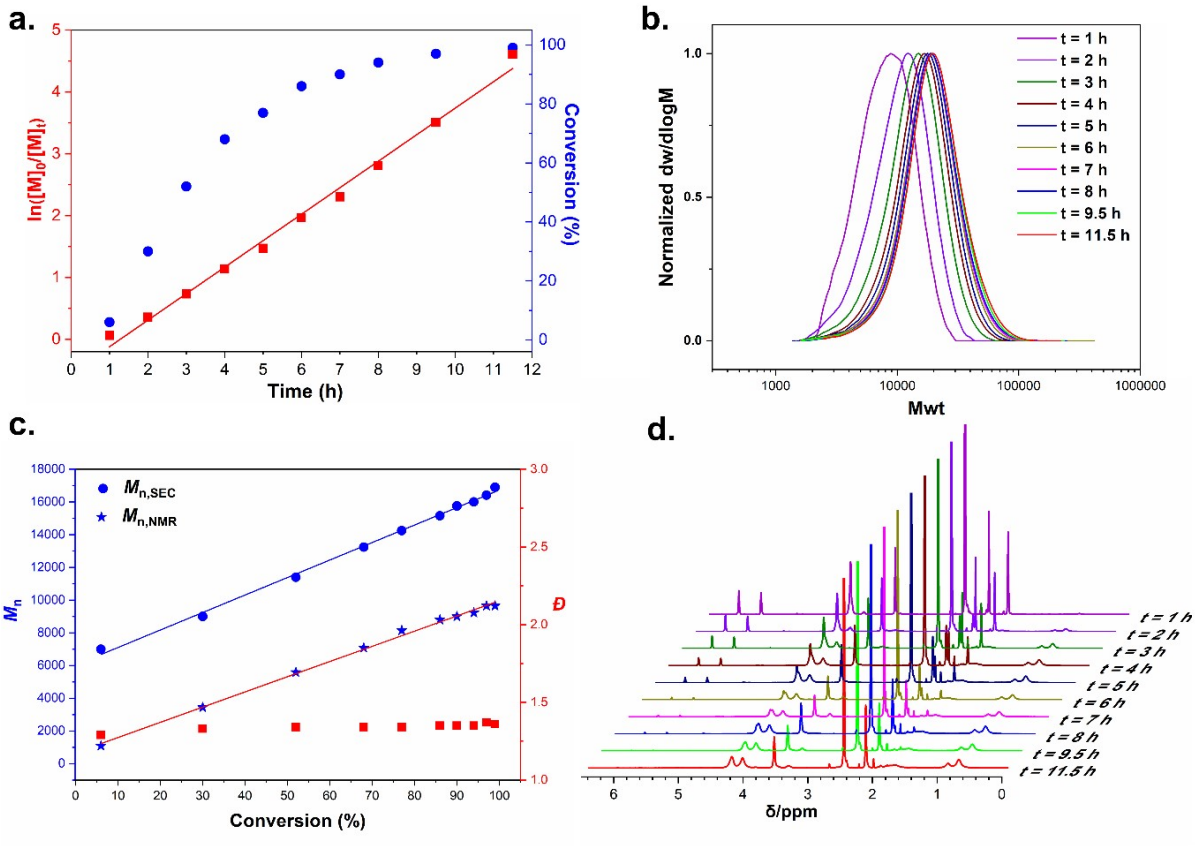



**Figure S22.** Polymerisation kinetics of the homopolymerisation of AEMA monomer *via* photo Cu-RDRP using MBPA initiator (a) kinetic plots of conversion and $\ln([M]_0/[M]_t)$ vs time (b) SEC traces at different times intervals (c) molecular weight evolution and dispersity against time (d) respective $^1$H-NMR spectra in DMSO-$d_6$ at different time intervals. Conditions: [AEMA]:[Cu(II)$Br_2$]:[$Me_6$TREN] = [40]:[0.05]:[0.36].

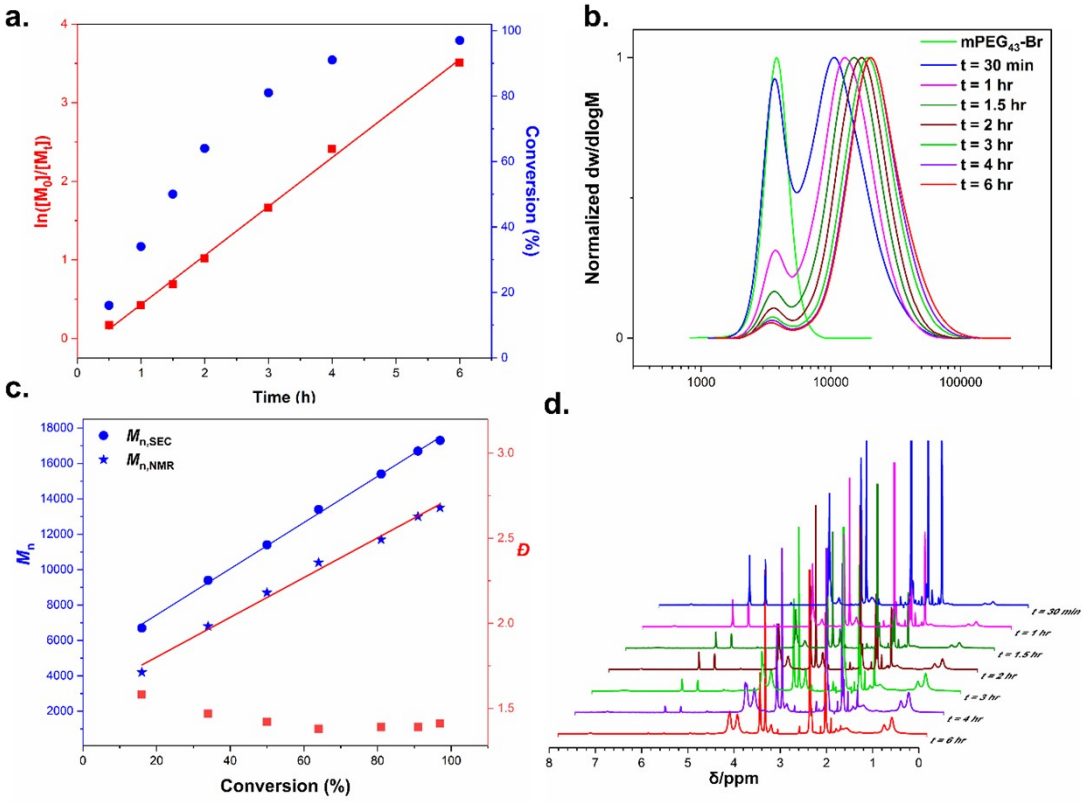



**Figure S23.** Polymerisation kinetics of the chain extension of AEMA with $mPEG_{43}$-BPA macroinitiator *via* photo Cu-RDRP (a) kinetic plots of conversion and $\ln([M]_0/[M]_t)$ vs time (b) SEC traces at different times intervals (c) molecular weight evolution and dispersity against time (d) respective $^1$H-NMR spectra in DMSO-$d_6$ at different time intervals. Conditions: [AEMA]:[Cu(II)$Br_2$]:[$Me_6$TREN] = [55]:[0.05]:[0.36].

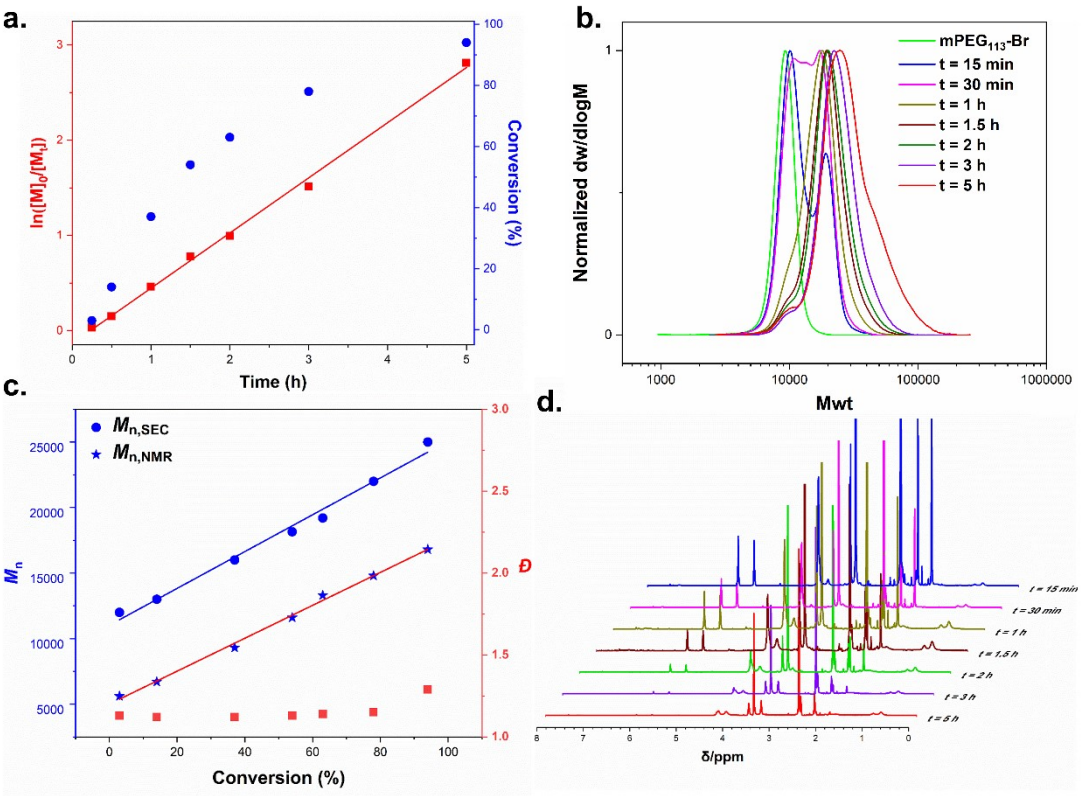



**Figure S24.** Polymerisation kinetics of the chain extension of AEMA with $mPEG_{113}$-BPA macroinitiator *via* photo Cu-RDRP (a) kinetic plots of conversion and $ln([M]_0/[M]_t)$ vs time (b) SEC traces at different times intervals (c) molecular weight evolution and dispersity against time (d) respective $^1$H-NMR spectra in DMSO-$d_6$ at different time intervals. Conditions: [AEMA]:[Cu(II)$Br_2$]:[$Me_6$TREN] = [55]:[0.1]:[0.72].

## 2.6 Modification results of pAEMA homopolymers with propylamine

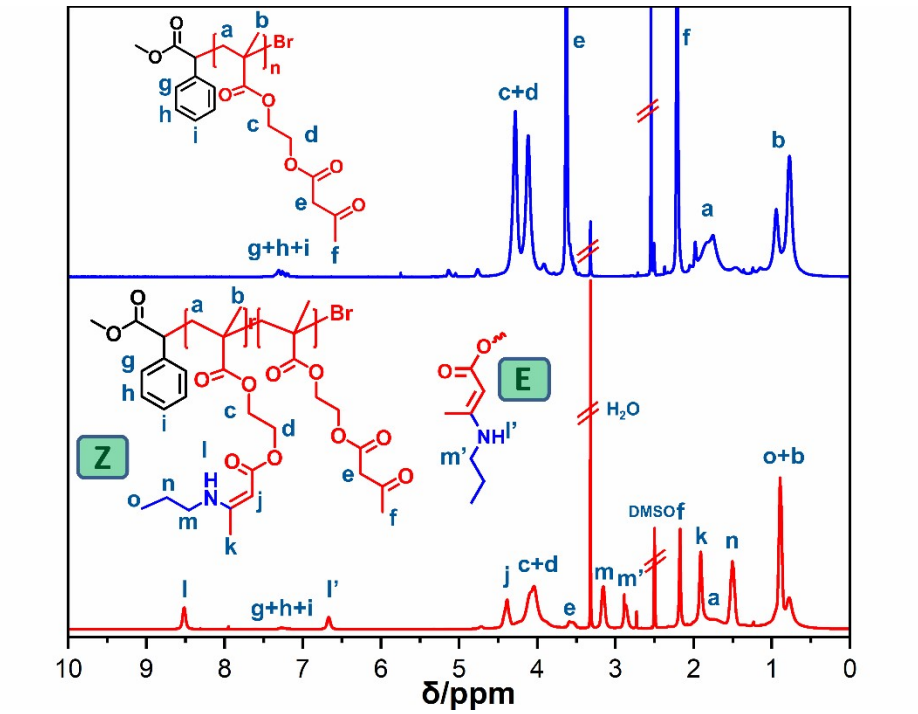



**Figure S25.** Assigned $^{1}$H-NMR (400 MHz, DMSO-$d_6$) spectra of purified pAEMA$_{42}$/PrA with 93% modification efficiency.

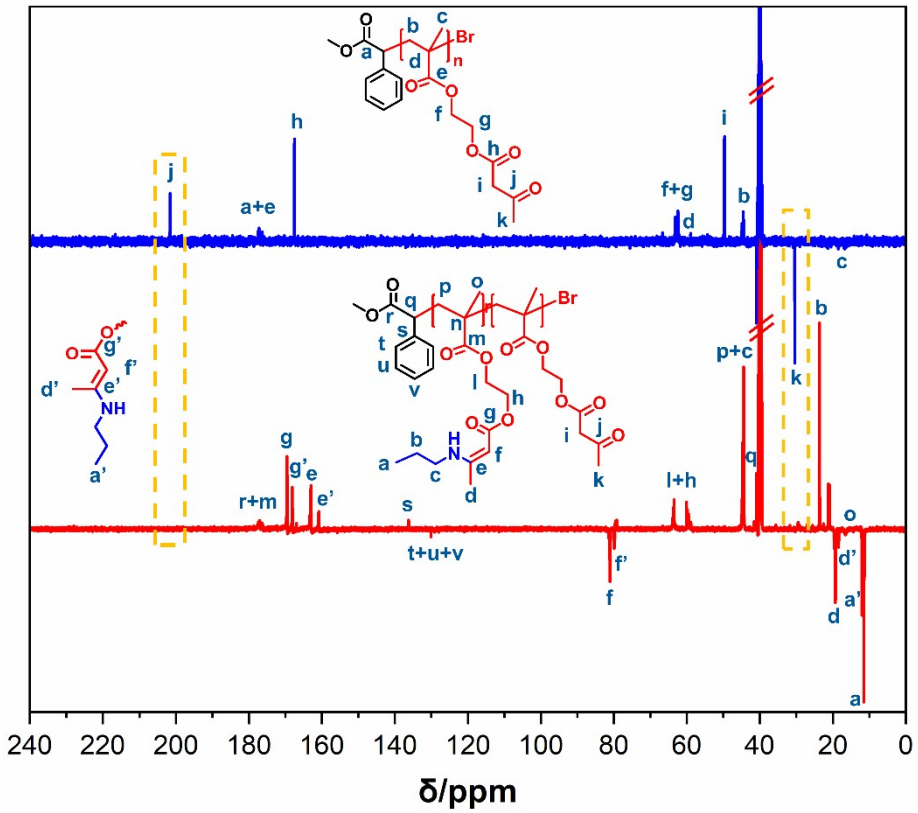



**Figure S26.** Assigned $^{13}$C-NMR (500 MHz, DMSO-$d_6$) spectra of purified pAEMA$_{42}$/PrA with 93% modification efficiency.

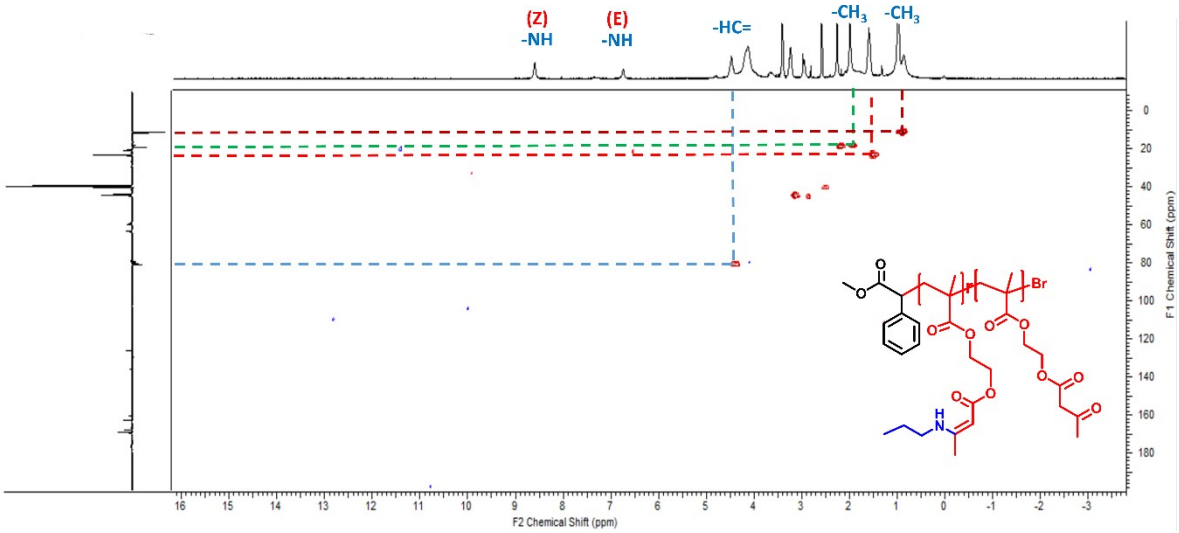



**Figure S27.** HSQC (400 MHz, DMSO-$d_6$) spectra of purified $pAEMA_{42}$/PrA with 93% modification efficiency.

## 2.7 Hydrolysis experiment and proposed mechanism

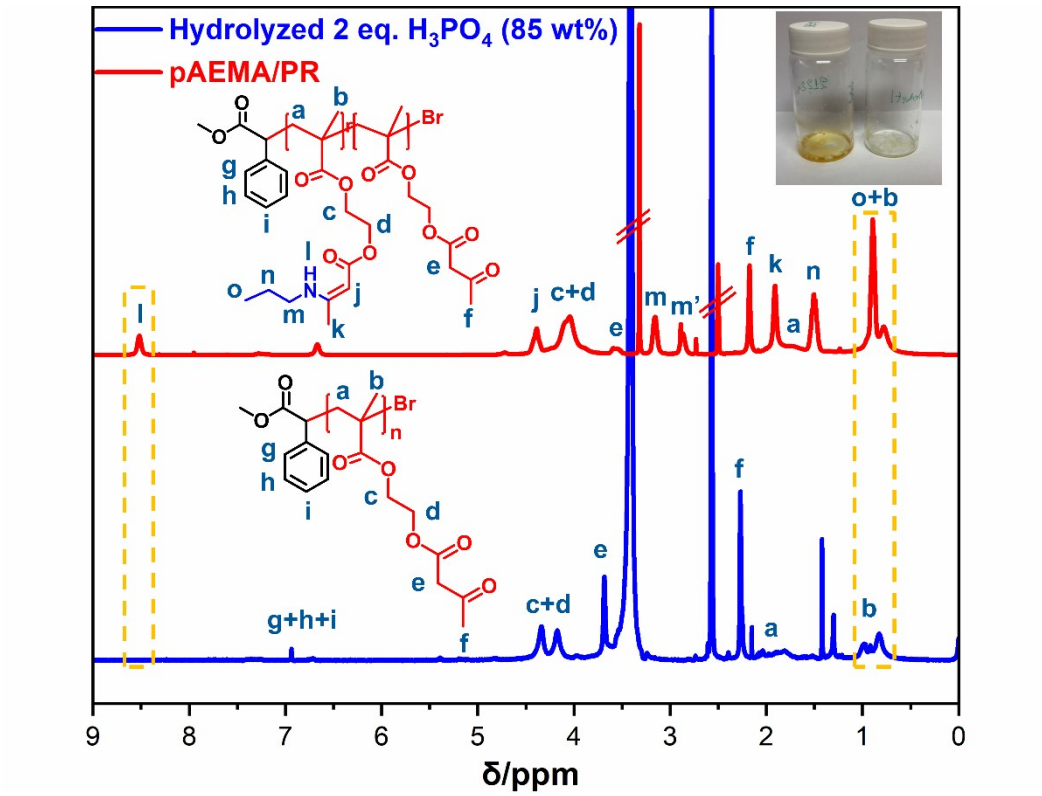



**Figure S28.** $^1$H-NMR (400 MHz, DMSO-$d_6$) comparison before and after hydrolysis of the enaminone bond by 2 eq. of $H_3PO_4$ (85 wt %).

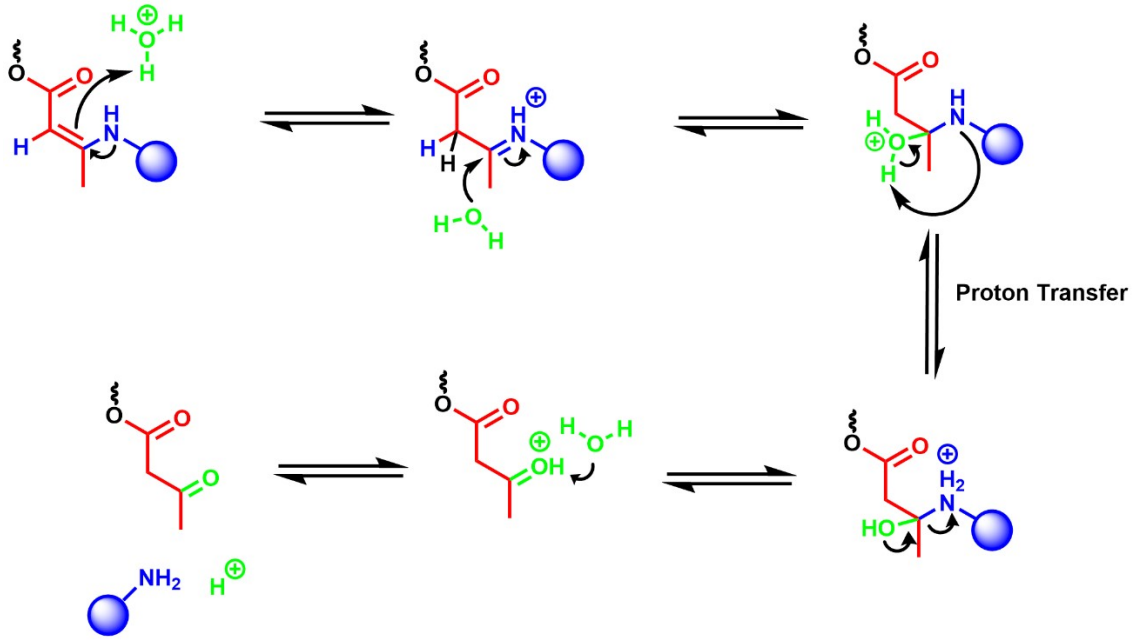



**Figure S29.** Proposed mechanism of enaminone hydrolysis under acidic conditions.[3]

## 2.8 Modification results of $pAEMA_x$ homopolymers and $mPEG_x$-*b*-$pAEMA_y$ block copolymers with benzocaine

**Table S2.** Tabulated results from the modifications of $pAEMA_x$ (where x denotes the degree of polymerisation) with benzocaine.

| Entry[1] | $pAEMA_x$ | Benzocaine (eq.) | T (°C) | $DP_{BNZ,NMR}$ | Mod (%) |
|---|---|---|---|---|---|
| 1 | 42 | 0.5 | 25 | ~ 5 | 13 |
| 2 | 42 | 1.0 | 25 | ~ 6 | 15 |
| 3 | 42 | 2.0 | 25 | ~ 20 | 50 |
| 4 | 42 | 6.0 | 25 | ~ 22 | 55 |
| 5 | 42 | 2.0 | 30 | ~ 25 | 63 |
| 6 | 42 | 6.0 | 30 | ~ 33 | 83 |
| 7 | 42 | 2.0 | 40 | ~ 31 | 78 |
| 8 | 42 | 6.0 | 40 | ~ 36 | 85 |
| 9 | 61 | 6.0 | 40 | ~ 52 | 87 |

[1]All modifications were conducted in $CHCl_3$ at a solid content of 0.22.

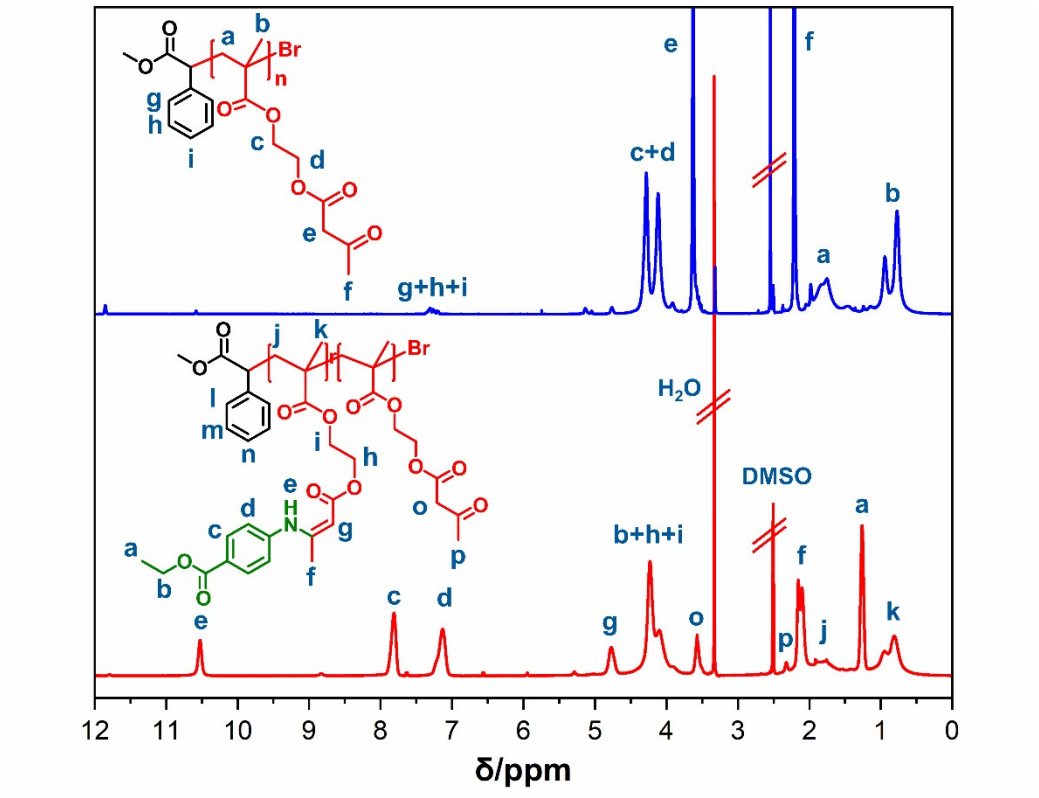



**Figure S30.** [1]H-NMR (400 MHz, DMSO-$d_6$) spectroscopy comparison of $pAEMA_{61}$ homopolymer against the modified $pAEMA_{61}$/BNZ(87%).

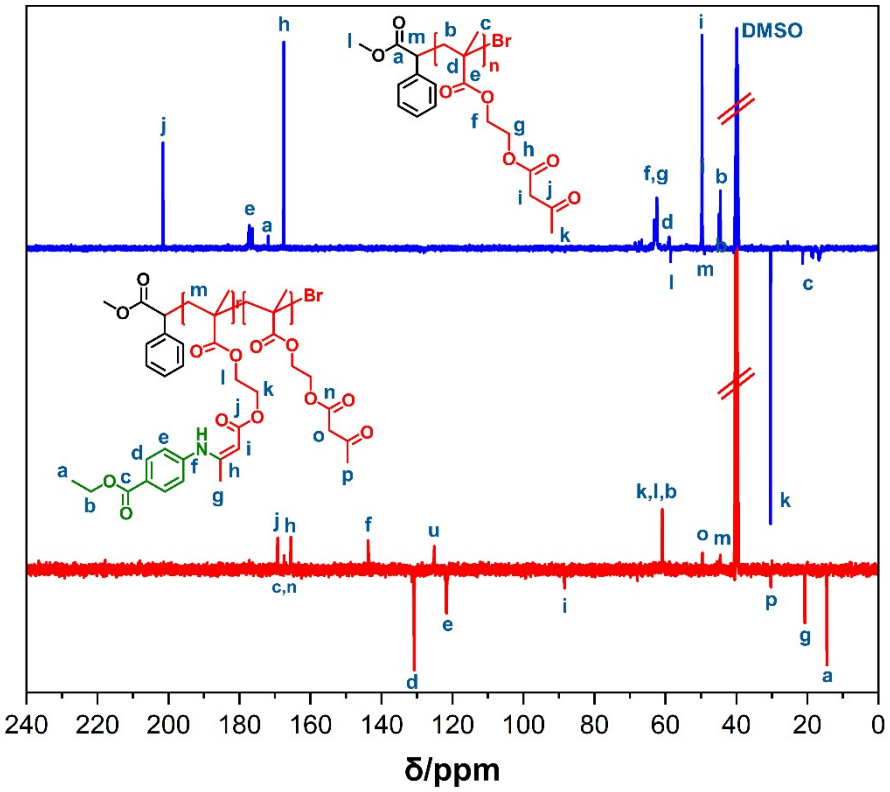



**Figure S31.** [13]C-NMR (400 MHz, DMSO-$d_6$) spectroscopy comparison of $pAEMA_{61}$ homopolymer against the modified $pAEMA_{61}$/BNZ(87%).

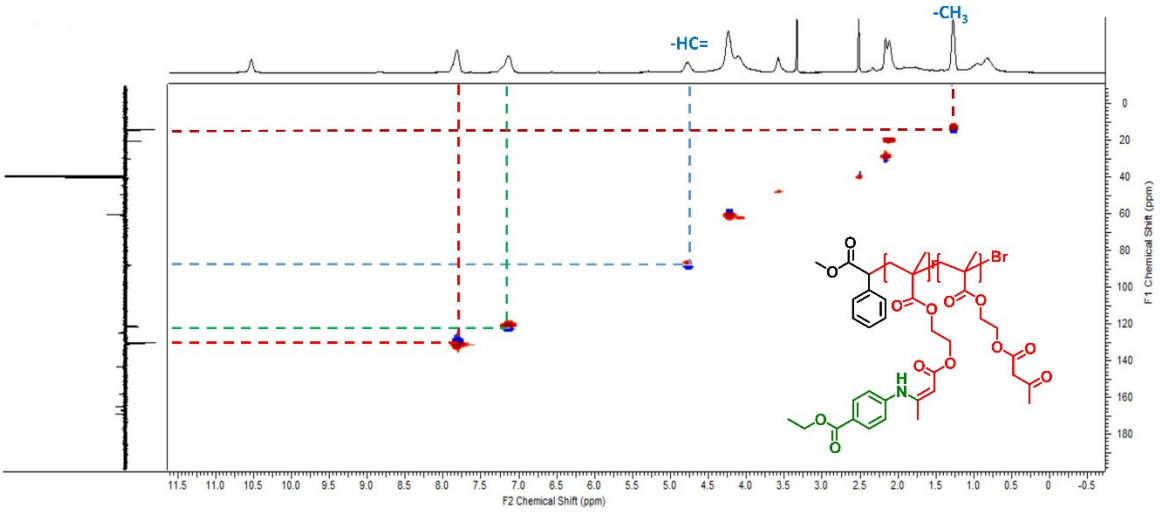



**Figure S32.** HSQC (400 MHz, DMSO-$d_6$) spectra of the modified pAEMA$_{61}$/BNZ(87%).

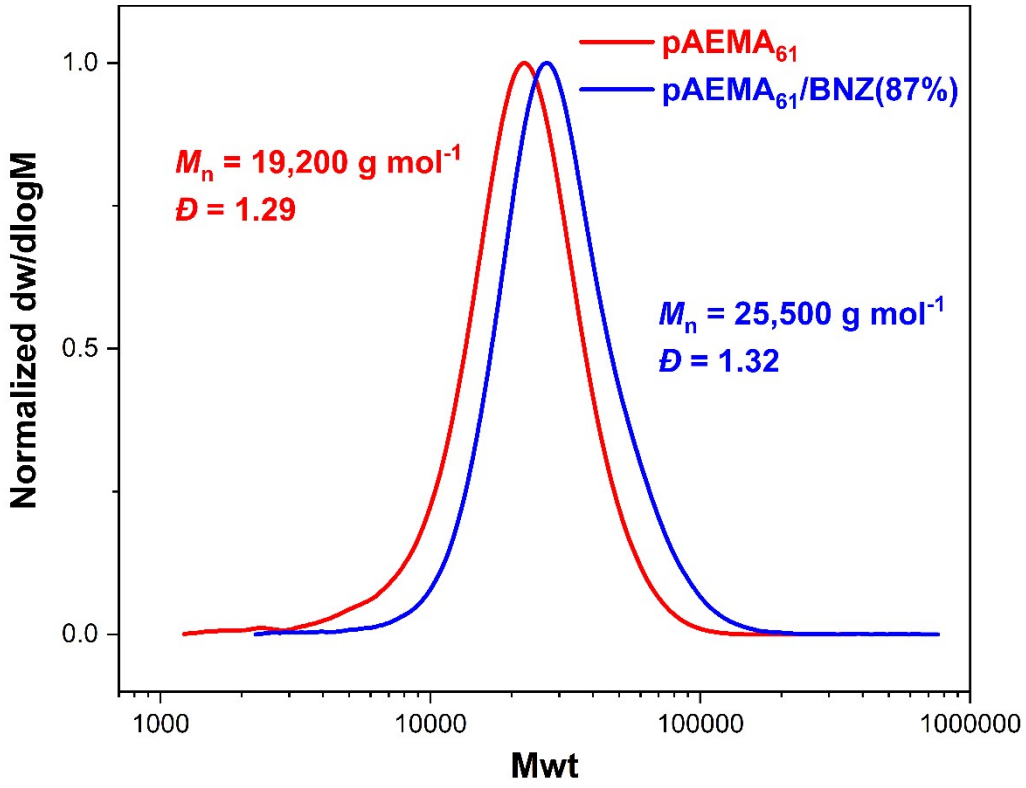



**Figure S33.** Molecular weight distribution traces before and after modification of pAEMA$_{61}$ homopolymers with benzocaine yielding pAEMA$_{61}$/BNZ(87%) *via* SEC in DMF.

**Table S3.** Tabulated results from the modifications of $mPEG_x$-*b*-$pAEMA_y$ (x =43 or 113) block copolymers with benzocaine.

| Blocks | Benzocaine (eq.) | *p*-TsOH (mol %) | Solvent | T (°C) | $DP_{BNZ,NMR}$ | Mod (%) |
|---|---|---|---|---|---|---|
| $mPEG_{43}$-*b*-$pAEMA_{84}$ | 1 | - | $CHCl_3$ | 25 | ~ 7 | 8 |
| $mPEG_{43}$-*b*-$pAEMA_{84}$ | 2 | - | $CHCl_3$ | 25 | ~ 13 | 15 |
| $mPEG_{113}$-*b*-$pAEMA_{52}$ | 2 | - | $CHCl_3$ | 40 | - | - |
| $mPEG_{113}$-*b*-$pAEMA_{52}$ | 6 | - | $CHCl_3$ | 40 | - | - |
| $mPEG_{113}$-*b*-$pAEMA_{52}$ | 6 | - | THF | 60* | - | - |
| $mPEG_{113}$-*b*-$pAEMA_{52}$ | 6 | 5 | THF | 40 | ~ 20 | 38 |
| $mPEG_{113}$-*b*-$pAEMA_{52}$ | 6 | 5 | $CHCl_3$ | 40 | ~ 24 | 47 |
| $mPEG_{113}$-*b*-$pAEMA_{52}$ | 6 | 5 | DMSO | 40 | ~ 30 | 58 |
| $mPEG_{113}$-*b*-$pAEMA_{28}$ | 3 | - | $CHCl_3$ | 40 | - | - |
| $mPEG_{113}$-*b*-$pAEMA_{28}$ | 6 | - | $CHCl_3$ | 40 | - | - |
| $mPEG_{113}$-*b*-$pAEMA_{28}$ | 6 | 5 | DMSO | 40 | ~ 20 | 70 |

* Performed under reflux conditions

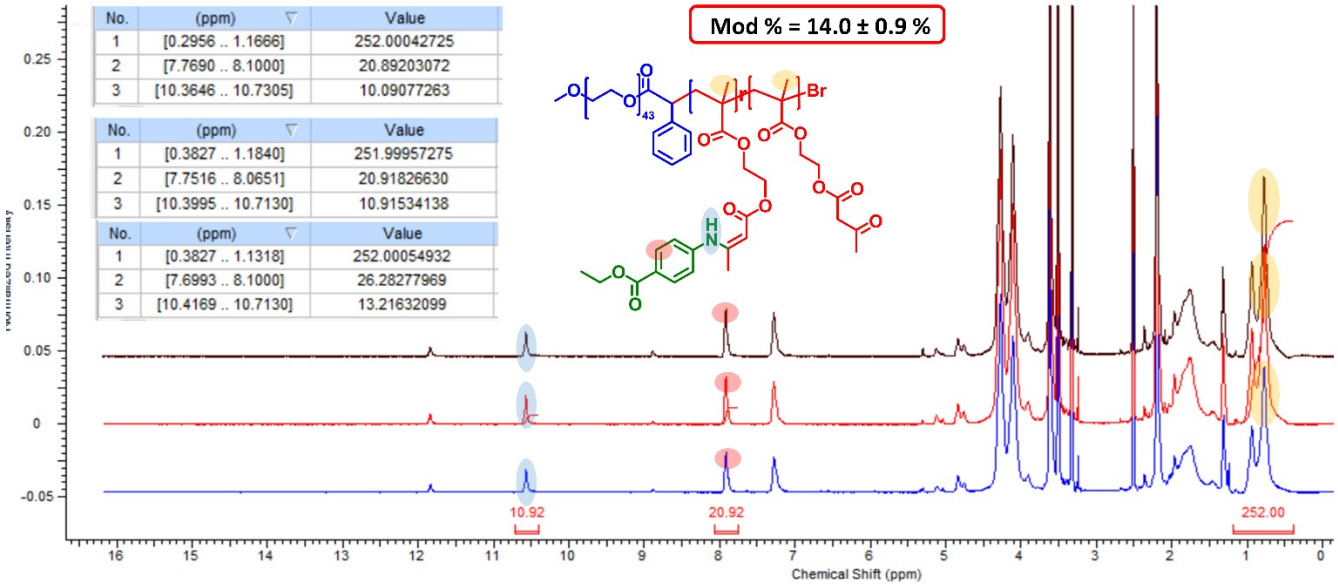



**Figure S34.** Combined $^1$H-NMR spectra from the reproducibility study of the block copolymer modification with benzocaine along with the appropriate integrations. Data are presented as mean ± SD (n =3 ).

## 2.9 Thermal Characterisation of synthesised products

**Table S4.** DSC tabulated results from the synthesised homopolymers, block copolymers and their benzocaine modified analogues as determined from their 2nd thermal cycle.

| Entry | Polymers | $T_{g,onset}$ (°C) | $T_{g,midpoint}$ (°C) |
|---|---|---|---|
| 1 | $pAEMA_{22}$ | - 3 | 7 |
| 2 | $pAEMA_{42}$ | - 4 | 6 |
| 3 | $pAEMA_{61}$ | - 3 | 6 |
| 4 | $pAEMA_{42}$/BNZ(55%) | 31 | 36 |
| 5 | $pAEMA_{42}$/BNZ(85%) | 48 | 54 |
| 8 | $mPEG_{43}$-*b*-$pAEMA_{84}$ | - 4 | 2 |
| 9 | $mPEG_{43}$-*b*-$pAEMA_{84}$/BNZ(15%) | 4 | 9 |
| 10 | $mPEG_{113}$-*b*-$pAEMA_{28}$ | - 27 | - 18 |
| 11 | $mPEG_{113}$-*b*-$pAEMA_{52}$ | - 21 | - 15 |
| 12 | $mPEG_{113}$-*b*-$pAEMA_{110}$ | - 13 | - 7 |
| 13 | $mPEG_{113}$-*b*-$pAEMA_{28}$/BNZ(70%) | - 33 | -21 |
| 14 | $mPEG_{113}$-*b*-$pAEMA_{52}$/BNZ(38%) | 11 | 20 |
| 15 | $mPEG_{113}$-*b*-$pAEMA_{52}$/BNZ(58%) | 11 | 25 |

## 2.10 Stability studies, DLS and Zeta potential data of block copolymers

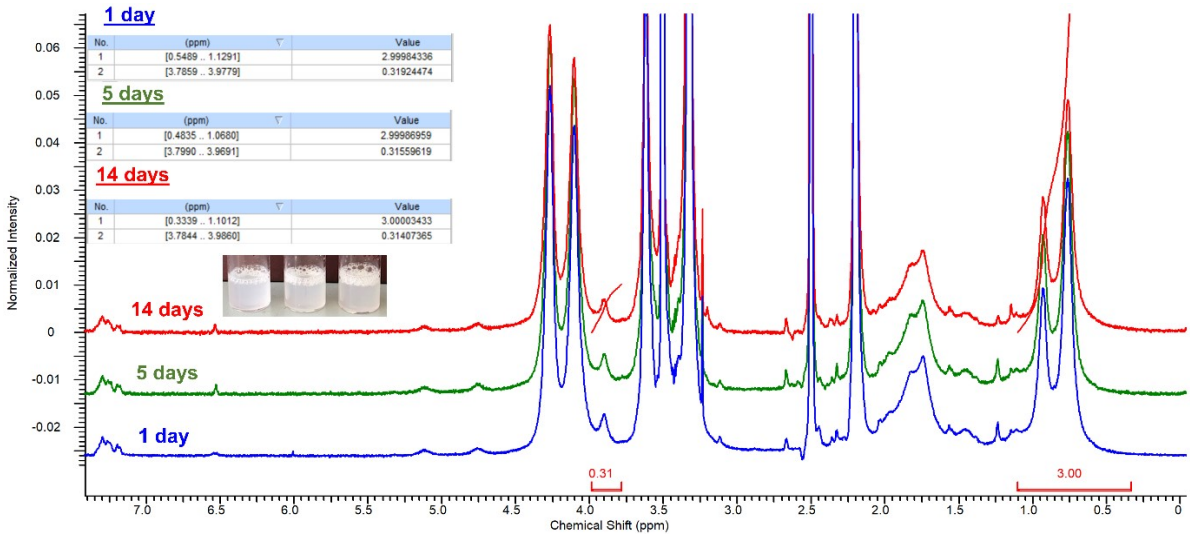



**Figure S35.** Overlayed $^1$H-NMR (400 MHz, DMSO-$d_6$) spectra from the stability study performed on $mPEG_{43}$-b-$pAEMA_{25}$ micelles in $H_2O$ (5 mg $mL^{-1}$) over a period of two weeks.

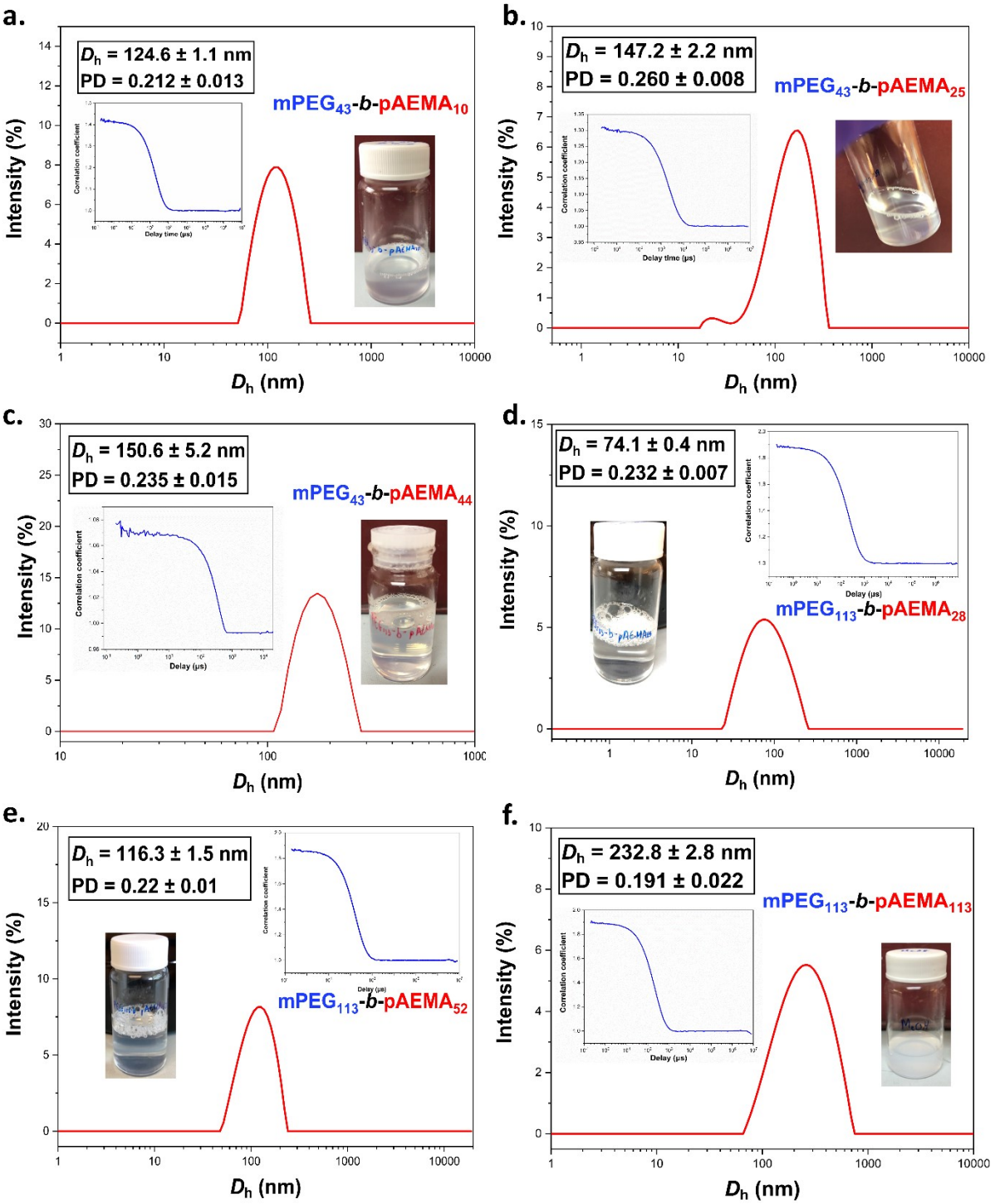



**Figure S36.** Intensity-weighted size distributions along with their collerograms obtained by DLS at a concentration of 0.5 mg mL$^{-1}$ for a) mPEG$_{43}$-*b*-pAEMA$_{10}$ in water b) mPEG$_{43}$-*b*-pAEMA$_{25}$ in water c) mPEG$_{43}$-*b*-pAEMA$_{44}$ in methanol d) mPEG$_{113}$-*b*-pAEMA$_{28}$ in water e) mPEG$_{113}$-*b*-pAEMA$_{52}$ in water and f) mPEG$_{113}$-b-pAEMA$_{113}$ in methanol.

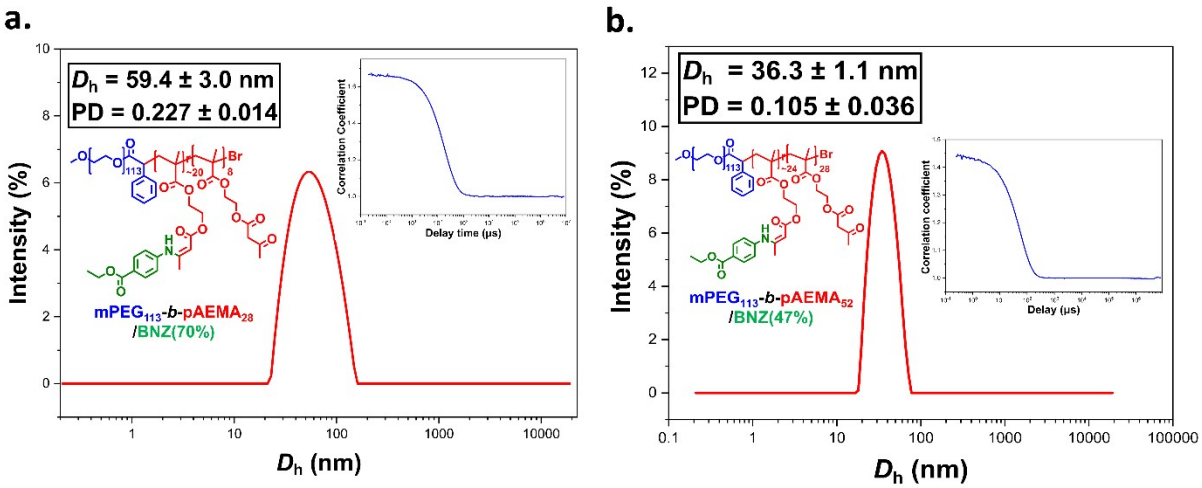
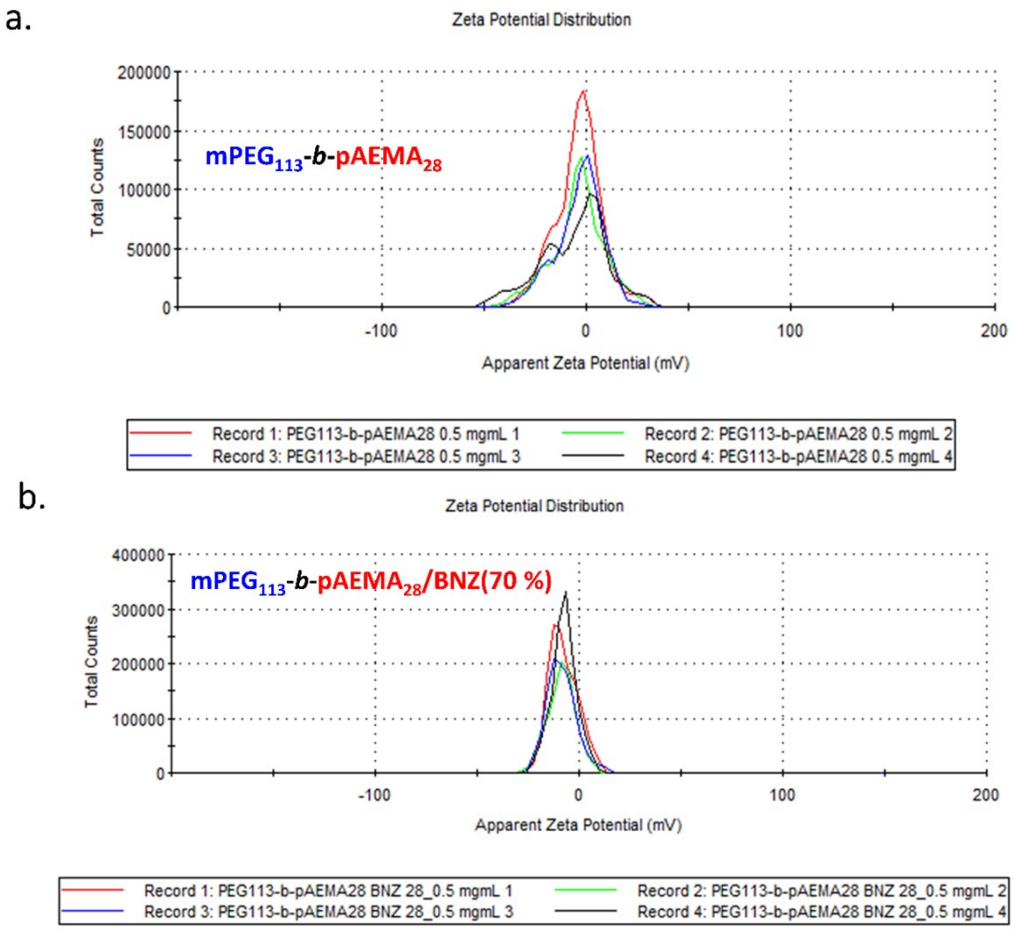



**Figure S37.** Intensity-weighted size distributions along with their collerograms obtained by DLS at a concentration of 0.5 mg mL$^{-1}$ in water for a) mPEG$_{113}$-*b*-pAEMA$_{28}$/BNZ(70%) and b) mPEG$_{113}$-*b*-pAEMA$_{52}$/BNZ(47%).



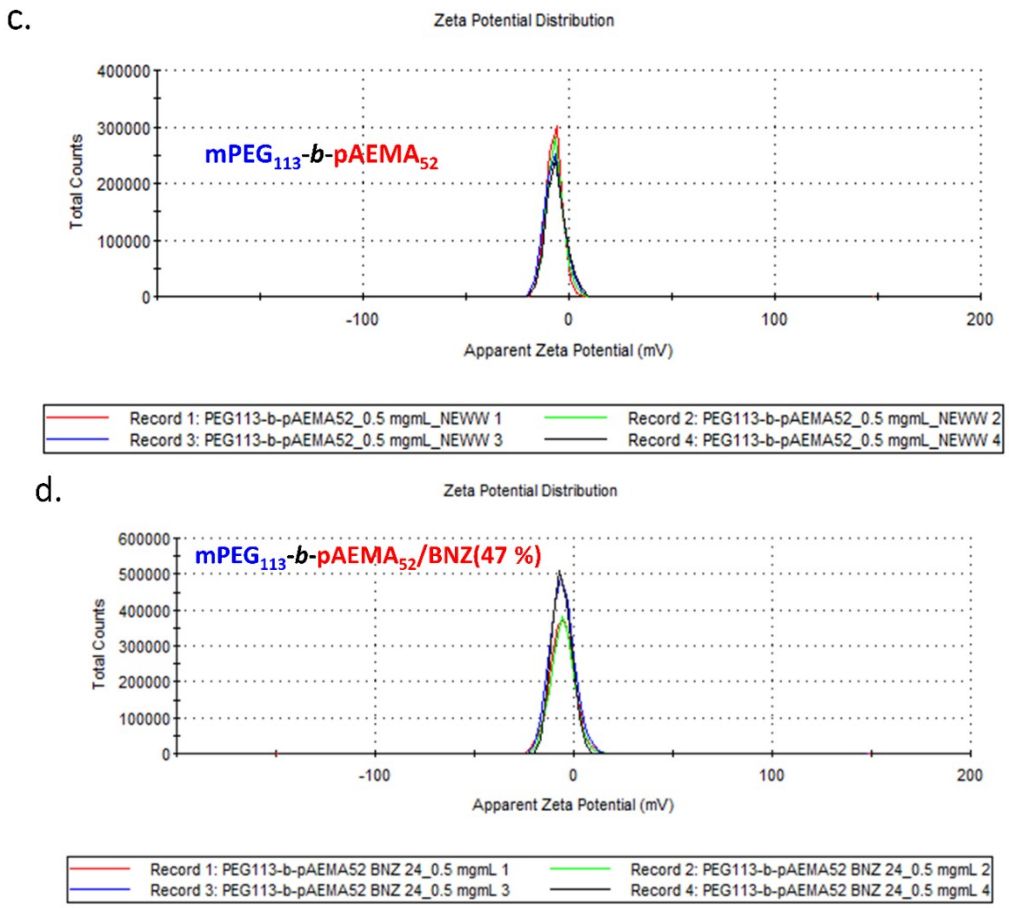



**Figure S38.** Zeta potential analysis of pristine and modified with benzocaine block copolymer assemblies in DI water (pH = 7) at a concentration of 0.5 mg mL$^{-1}$ for a) mPEG$_{113}$-*b*-pAEMA$_{28}$ b) mPEG$_{113}$-*b*-pAEMA$_{28}$/BNZ(70%) c) mPEG$_{113}$-*b*-pAEMA$_{52}$ and d) mPEG$_{113}$-*b*-pAEMA$_{52}$/BNZ(47%).

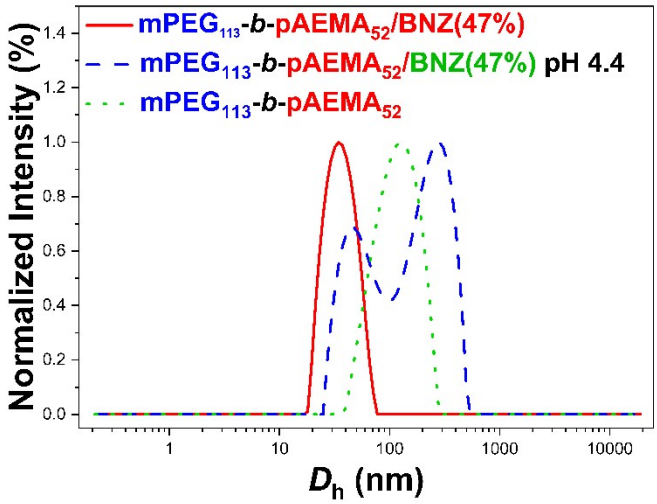



**Figure S39.** DLS comparison of **mPEG$_{113}$-*b*-pAEMA$_{28}$/BNZ(47%)** NPs after hydrolysis for 2 days in pH = 4.4.

## 2.11 Benzocaine release UV data at different pH environments

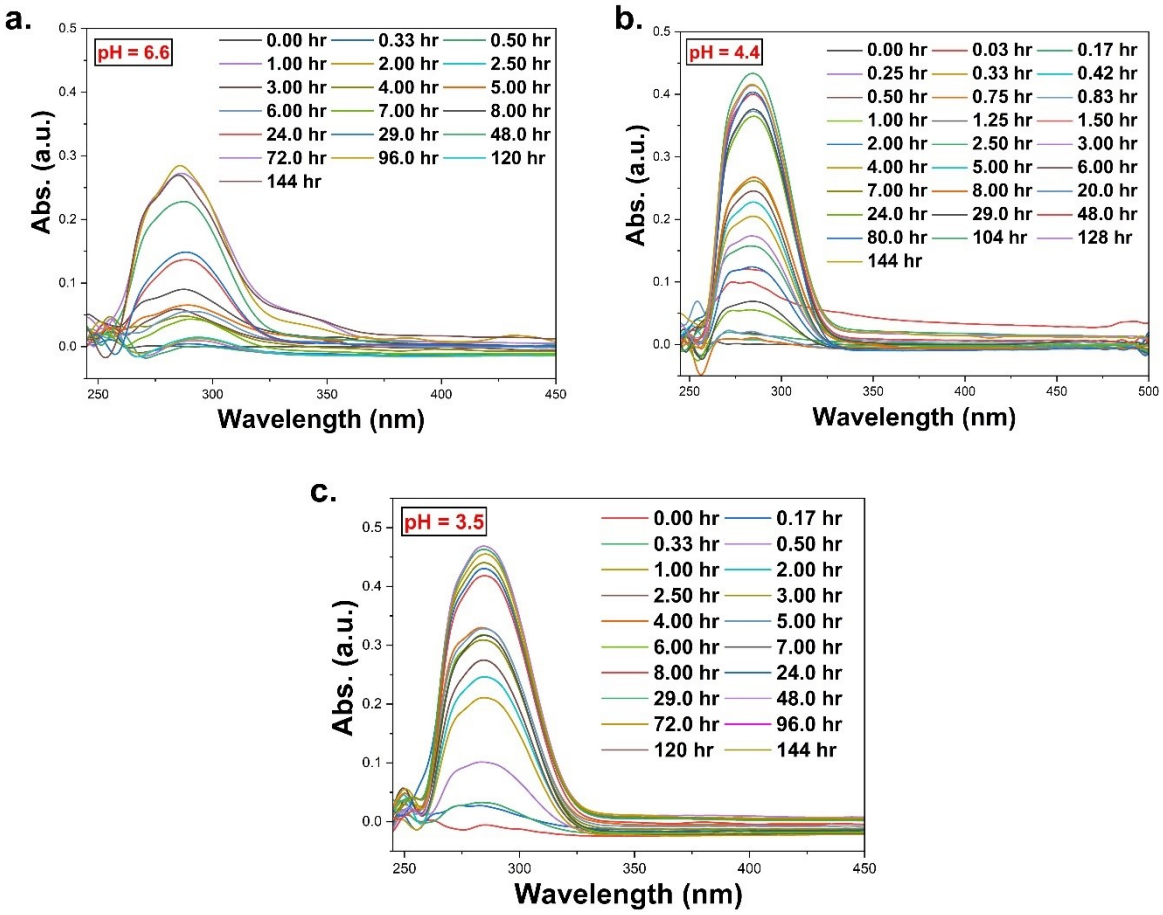



**Figure S40.** UV spectra during the release of BNZ at different time intervals at a) pH 6.6 (initial dose : 1400 µg BNZ), b) pH 4.4 (initial dose : 700 µg BNZ ) and c) pH 3.5 (initial dose : 500 µg BNZ).

# 3 References


1. M. Ciampolini and N. Nardi, *Inorg. Chem.*, 1966, **5**, 41-44.
2. A. M. Eissa, A. Abdulkarim, G. J. Sharples and N. R. Cameron, *Biomacromolecules*, 2016, **17**, 2672-2679.
3. P. Y. Sollenberger and R. B. Martin, *J. Am. Chem. Soc.*, 1970, **92**, 4261-4270.