# Electronic Supplementary Information

# Functional hydrophobic and hetero-grafted block comb polymers via a combination of spontaneous zwitterionic copolymerisation and redox-initiated RAFT polymerisation

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## Experimental Details

### Materials

Acrylic acid (AA, 99%, anhydrous, Sigma-Aldrich), acetonitrile (ACN, 99.8%, Sigma-Aldrich), 4-methoxyphenol (MEHQ, 99%, Sigma-Aldrich), petroleum ether (60-80 °C, Sigma-Aldrich), 1,4-dioxane (>99%, Sigma-Aldrich), Luperox® TBH70X tert-butyl hydroperoxide solution (tBuOOH, 70% wt.% in $H_2O$, Sigma-Aldrich), L-ascorbic acid (AsAc, Sigma Aldrich), 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate (DMTMM, 97%, Sigma-Aldrich) and D,L-dithiothreitol (DTT, > 99%, Sigma-Aldrich) were used as received. 2-(pyridyldithio)-ethylamine hydrochloride (PDA) was obtained from Speed Chemical, China. 2-Ethyl-2-oxazoline (EtOx, >99%, Sigma-Aldrich) and 2-methyl-2-oxazoline (MeOx, 98%, Sigma-Aldrich) were distilled to dryness over barium oxide (BaO) and stored in a nitrogen atmosphere. 2-Propyl-2-oxazoline and 2-butyl-2-oxazoline,[1] the chain transfer agent (CTA) 2-(((butylthio)-carbonothioyl)thio)propanoic acid,[2] and the amine-terminated poly(2-ethyl-2-oxazoline) ($PEtOx_{10}NH_2$)[3] were prepared according to literature procedures.

### Instrument and analysis

$^1$H Nuclear Magnetic Resonance ($^1$H NMR) spectroscopy of all samples was carried out using a Bruker AVANCE III HD 400 MHz spectrometer using deuterated solvents obtained from Sigma-Aldrich. Analyses of polymer solutions were performed using a Shimadzu modular system comprising a DGU-12A degasser, an SIL-20AD automatic injector, a 5.0 μm bead-size guard column (50 x 7.8 mm) followed by three KF-805L columns (300 x 8 mm, bead size: 10 μm, pore size maximum: 5000 Å), a SPD-20A ultraviolet detector, and an RID-10A differential refractive index detector. A CTO-20A oven was used to maintain the columns at 40 °C. *N*, *N*-dimethylacetamide (DMAc) with 0.03% w/v LiBr was used as the eluent where samples were run isocratically at 1 mL $min^{-1}$. Polystyrene standards (0.5 to 2000 kg $mol^{-1}$) were used for calibration. Analyte samples were filtered through 0.45 μm PTFE filters before injection. Molar mass ($M_{n,SEC}$) and dispersity (Đ) values of samples were determined on Shimadzu LabSolutions software. DSC spectra were recorded on a

Mettler Toledo DSC1. Dynamic light scattering (DLS) analysis was carried out using a Malvern Zetasizer Nano ZS. All measurements were carried out at 25 °C using filtered polymer solutions (MilliQ or PBS) of 2 mg $mL^{-1}$. TEM imaging was performed using a Tecnai F20 transmission electron microscope at an accelerating voltage of 200 kV at ambient temperature. An aliquot (5 μL) of 0.2 wt% particle solution was deposited on a Formvar coated copper grid (GSCu100F-50, Proscitech) and was allowed to dry overnight in air and at ambient temperature.

## Synthesis of macromonomers and polymers

### General procedure for SZWIP of PropOx and ButOx and AA

PropOx or ButOx (0.025 mol), AA (0.025 mol), ACN (2.5 mL) and MEHQ ($8.06 \times 10^{-6}$ mol) were added under nitrogen to a dried Schlenk flask, which was placed in an oil bath set at 70 °C for 24 h. Subsequently, the polymer solution was allowed to cool to room temperature and poured into ice-cold petroleum ether. The precipitate was separated by centrifugation. This process was repeated in total three times. Drying under vacuum yielded the products oligo(PropOx-*alt*-AA)$_n$A (**M**$_{PropOx/AA}$) or oligo(ButOx-*alt*-AA)$_n$A (**M**$_{ButOx/AA}$). The repeating units and molar mass of the oligomers were calculated by $^1$H NMR, by the ratio of the integrals of the vinyl end group and the ring-opened 2-oxazoline and AA repeating unit signals (see Table S1, Fig S1).

### General procedure for SZWIP of MeOx and AA

MeOx (0.025 mol), AA (0.05 mol), ACN (2.5 mL) and MEHQ ($8.06 \times 10^{-6}$ mol) were added under nitrogen to a dried Schlenk flask, which was placed in an oil bath set at 70 °C for 24 h. Subsequently, the polymer solution was allowed to cool to room temperature and poured into ice-cold petroleum ether. The precipitate was separated by centrifugation. This process was repeated in total three times. Drying under vacuum yielded the product oligo(MeOx-*alt*-AA)$_n$A (**M**$_{MeOx/AA}$) as a slightly yellowish oil. The repeating units and molar mass of the oligomer were calculated by $^1$H NMR, by the ratio of the integrals of the vinyl end group and the ring-opened 2-oxazoline and AA repeating unit signals (see Table S1).

**Table S1** Characterization of the macromonomers used in this study: oligo(MeOx-*alt*-AA)$_n$A (**M**$_{MeOx/AA}$), oligo(PropOx-*alt*-AA)$_n$A (**M**$_{PropOx/AA}$), and oligo(ButOx-*alt*-AA)$_n$A (**M**$_{ButOx/AA}$).

| Code | DP[a] (Ox/AA) | $M_{n,\ NMR}$[b] [g mol$^{-1}$] | $M_{n,\ SEC}$[c] [g mol$^{-1}$] | Đ[c] |
|---|---|---|---|---|
| $M_{MeOx/AA}$ | 2/2 | 386 | 1130 | 1.16 |
| $M_{PropOx/AA}$ | 2/2 | 442 | 1370 | 1.22 |
| $M_{ButOx/AA}$ | 2/2 | 470 | 1400 | 1.18 |

[a]Calculated by $^1$H NMR ($CDCl_3$, 400 MHz). [b]Calculated from DPs and molar masses of the monomers. [c]Determined by SEC (eluent: DMAc + LiBr, standard: PS).

**Typical procedure for the synthesis of homo comb polymers by RRAFT**

CTA, macromonomer and AsAc were added to a sample vial equipped with a magnetic stir bar and dissolved in deionized $H_2O$ and 1,4-dioxane. The mixture was deoxygenated by bubbling with nitrogen for 15 min. In parallel, an aqueous stock solution of tBuOOH was deoxygenated. An aliquot of the latter was added to the sample vial via a nitrogen-purged syringe. The sample vial was placed in a thermostated water bath set at 25 °C for 24 h. Subsequently, $^1$H NMR and SEC samples were taken to determine the conversion of the polymerization. The comb polymers were purified by dialysis (MWCO = 3500 g mol$^{-1}$) against MeOH and eventually deionized water for three days. Amounts and concentrations of the individual RRAFT polymerizations are provided in Table S2.

**Table S2** RRAFT conditions used to prepare homo comb polymers of different degree of polymerization (DP) in $H_2O$/1,4-dioxane at 25 °C using tBuOOH/AsAc as redox initiator. All polymerizations are performed in 300 mg scales.

| code | DP | $[M]_0$ [mmol $L^{-1}$] | [CTA] [mmol $L^{-1}$] | [AsAc] [mmol $L^{-1}$] | [tBuOOH] [mmol $L^{-1}$] | $H_2O$/ 1,4-dioxane (v%/v%) |
|---|---|---|---|---|---|---|
| **P1a** | 50 | 0.68 | 0.0136 | 0.0034 | 0.0068 | 75/25 |
| **P1b** | 100 | 0.68 | 0.0068 | 0.0017 | 0.0034 | 75/25 |
| **P3a** | 50 | 0.64 | 0.0128 | 0.0032 | 0.0064 | 42/58 |
| **P3b** | 100 | 0.64 | 0.0064 | 0.0016 | 0.0032 | 42/58 |
| $\mathbf{P}_{MeOx/AA}$ | 100 | 0.78 | 0.0078 | 0.0019 | 0.0039 | 75/25 |

**Typical procedure for the synthesis of hetero-grafted block comb polymers by RRAFT**

The macromonomer was dissolved in dioxane and added to a vial containing $\mathbf{P}_{MeOx/AA}$ macroCTA and AsAc dissolved in deionized $H_2O$. The mixture was deoxygenated by bubbling with nitrogen for 15 min. In parallel, an aqueous stock solution of tBuOOH was deoxygenated. An aliquot of the latter was added to the sample vial via a nitrogen-purged syringe. The sample vial was placed in a thermostated water bath set at 25 °C for 24 h. Subsequently, $^1$H NMR and SEC samples were taken to determine the conversion of the polymerization. The comb polymers were purified by dialysis (MWCO = 3500 g $mol^{-1}$) against deionized water for three days. Amounts and concentrations of the individual RRAFT polymerizations are provided in Table S3.

**Table S3** RRAFT conditions used to prepare hetero-grafted block comb polymers in $H_2O$/1,4-dioxane at 25 °C using tBuOOH/AsAc as redox initiator.

| code | DP | $[M]_0$ [mmol $L^{-1}$] | [CTA] [mmol $L^{-1}$] | [AsAc] [mmol $L^{-1}$] | [tBuOOH] [mmol $L^{-1}$] | $H_2O$/ 1,4-dioxane (v%/v%) |
|---|---|---|---|---|---|---|
| **P2** | 50 | 0.32 | 0.0064 | 0.0016 | 0.0032 | 50/50 |
| **P4** | 50 | 0.34 | 0.0068 | 0.0017 | 0.0034 | 50/50 |

**Post polymerisation modification of poly(oligo(ButOx-*alt*-AA)$_n$A) (P3b)**

**P3b-PEtOx**: 30 mg **P3b** and 32 mg DMTMM were dissolved in 1 mL dry DMF and stirred for 15 min at room temperature prior to the addition of 98 mg $PEtOx_{10}$-$NH_2$. Stirring was continued for 20 h. DMF was removed under airflow, the residual was re-dissolved in MilliQ and the polymer solution

was dialysed (MWCO = 3500 g $mol^{-1}$) against MilliQ for 3 days. Lyophilisation provided **P3b-PEtOx** as a slightly yellowish compound. Degree of functionalisation: 35%.

**P3b-PDA**: 30 mg **P3b** and 32 mg DMTMM were dissolved in 1 mL dry DMF and stirred for 15 min at room temperature prior to the addition of 13 mg PDA. Stirring was continued for 19 h. DMF was removed under airflow, the residual was re-dissolved in MilliQ and the polymer solution was dialysed (MWCO = 1000 g $mol^{-1}$) against MeOH and MilliQ for 3 days. Lyophilisation provided **P3b-PDA** as a slightly yellowish compound. Degree of functionalisation: 3%.

**Particle preparation**

Homo and block comb polymer particles were obtained by nanoprecipitation and direct dissolution, respectively. In case of the nanoprecipitation the polymer was dissolved in MeOH (5 mg $mL^{-1}$) and 1 mL was added dropwise to 2.5 mL MilliQ. After evaporation of the MeOH for 48 h at room temperature particle solution of a total concentration of 2 mg $mL^{-1}$ were obtained. Block comb polymer particles were obtained by stirring 2 mg of polymer in 2 mL MilliQ for 24 h.

**Crosslinking of P3b-PDA based particles**

0.035 mg DTT in 0.01 mL MilliQ were added to 4 mL of pre-assembled **P3b-PDA** particles (1 mg $mL^{-1}$). The particles were stirred for 5 h and subsequently dialysed (MWCO = 3500 g $mol^{-1}$) against MilliQ overnight. The disassembly of the particles was triggered by the addition of an excess DTT to a PBS particle solution.

**CAC determination**

A pyrene stock solution ($c = 5 \times 10^{-6}$ M) was prepared in acetone and 100 µL aliquots were divided over 10-12 vials. After evaporation of the acetone 1 mL aqueous polymer solutions were added to each vial and stirred for two days at room temperature. Different polymer concentrations (0.5 to 0.000001 mg $mL^{-1}$) were accomplished by diluting a polymer stock solution with the respective amount of MilliQ water. The fluorescence spectra were recorded from 360 to 410 nm using an excitation wavelength of 336 nm. The emission intensities measured at 373 nm ($I_1$) and 384 nm ($I_3$) were used to calculate the ratio $I_3/I_1$.

**Cell Culture**

NIH3T3 cells (purchased from ATCC) were used in this study and tested and cleared for mycoplasma. NIH3T3 were maintained in Dulbecco's modified Eagles Medium (DMEM) Glutamax$^{TM}$ supplemented 1mM sodium pyruvate and 10% v/v (A549) and 20% v/v (NIH3T3) fetal bovine serum. Cells were cultured at 37 ºC in a humidified incubator with 5% atmospheric $CO_2$. Cell counting for passaging was done by adding 0.4% Trypan Blue solution to the cells in medium and using a haemocytometer.

**Alamar Blue Cell Viability Assay**

NIH3T3 cells were treated with serial dilutions of polymeric samples (from 1 mg $mL^{-1}$ to 0.06 mg $mL^{-1}$) and cultured for 24 h at 37 ºC in a humidified incubator with 5% atmospheric $CO_2$. Each sample was run as a triplicate at each concentration to obtain representative cell viability values. Thereafter, the old medium was removed and a 10% v/v solution of Alamar Blue in Dulbecco's modified Eagles Medium (DMEM) Glutamax$^{TM}$ supplemented 1mM sodium pyruvate and 20% v/v fetal bovine serum was added to the cells. Afterwards, the cells were incubated for 6 h at 37 ºC in a humidified incubator with 5% atmospheric $CO_2$. The fluorescence was then measured by using an excitation wavelength of 540-570 nm (peak excitation at 570 nm) and reading the fluorescence emission at 580-610 nm (peak emission at 585 nm). Culture medium without cells was used to calibrate the zero absorbance (blank). Cells in control samples were treated with PBS.

## Figures/Schemes

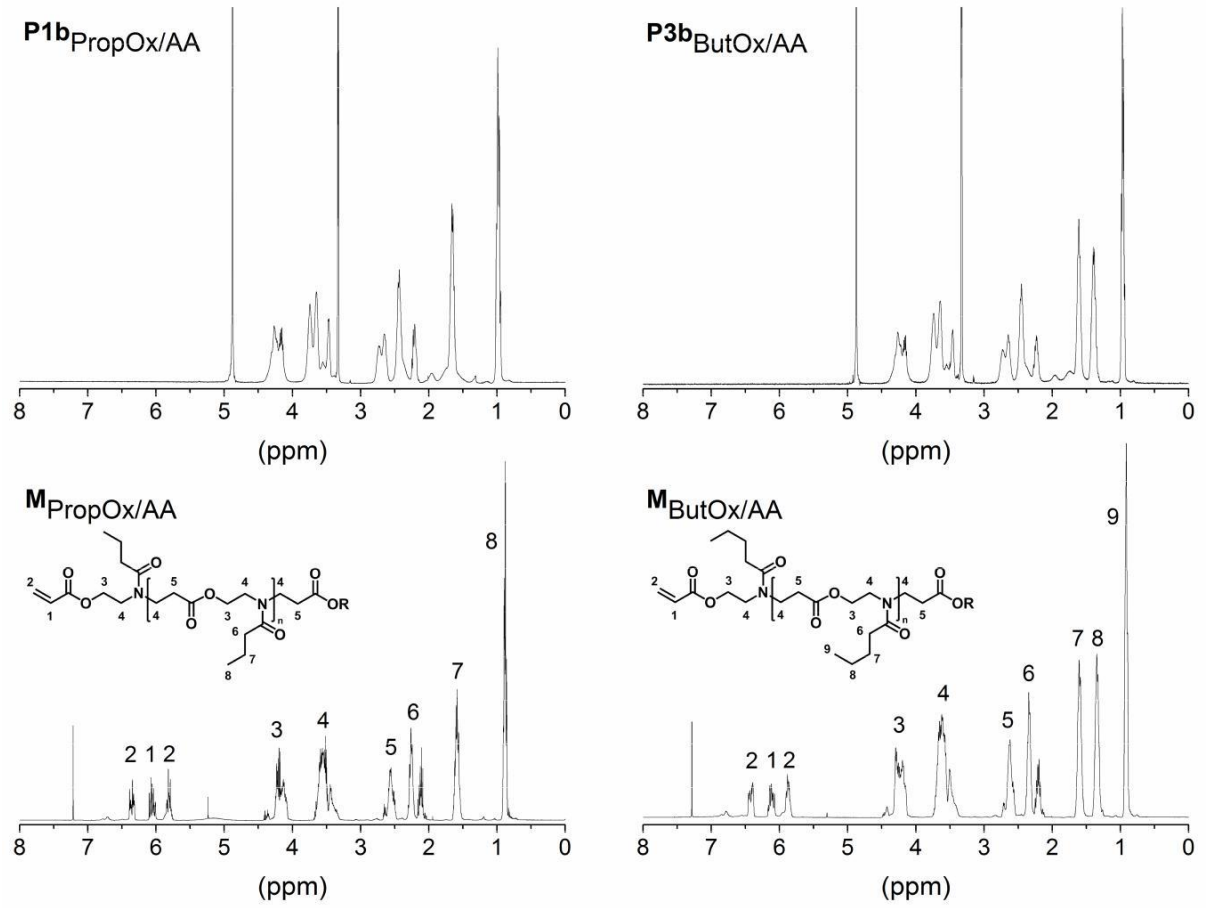


**Fig. S1** $^{1}$H NMR spectra (400 MHz, MeOD, $CDCl_3$) of **M**$_{PropOx/AA}$ and **M**$_{ButOx/AA}$ and their resulting comb polymers **P1b**$_{PropOx/AA}$ and **P3b**$_{ButOx/AA}$, respectively.

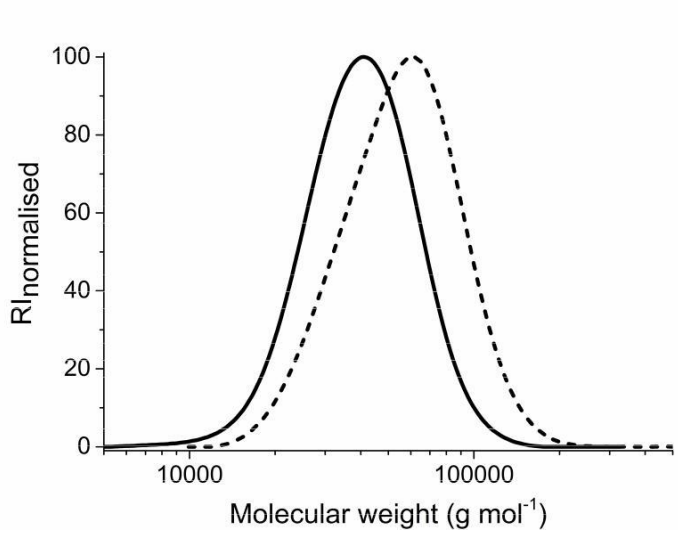


**Fig. S2** SEC spectra (DMAc) of homo comb polymers **P1b**$_{PropOx/AA}$ (--) and **P3b**$_{ButOx/AA}$ (—).

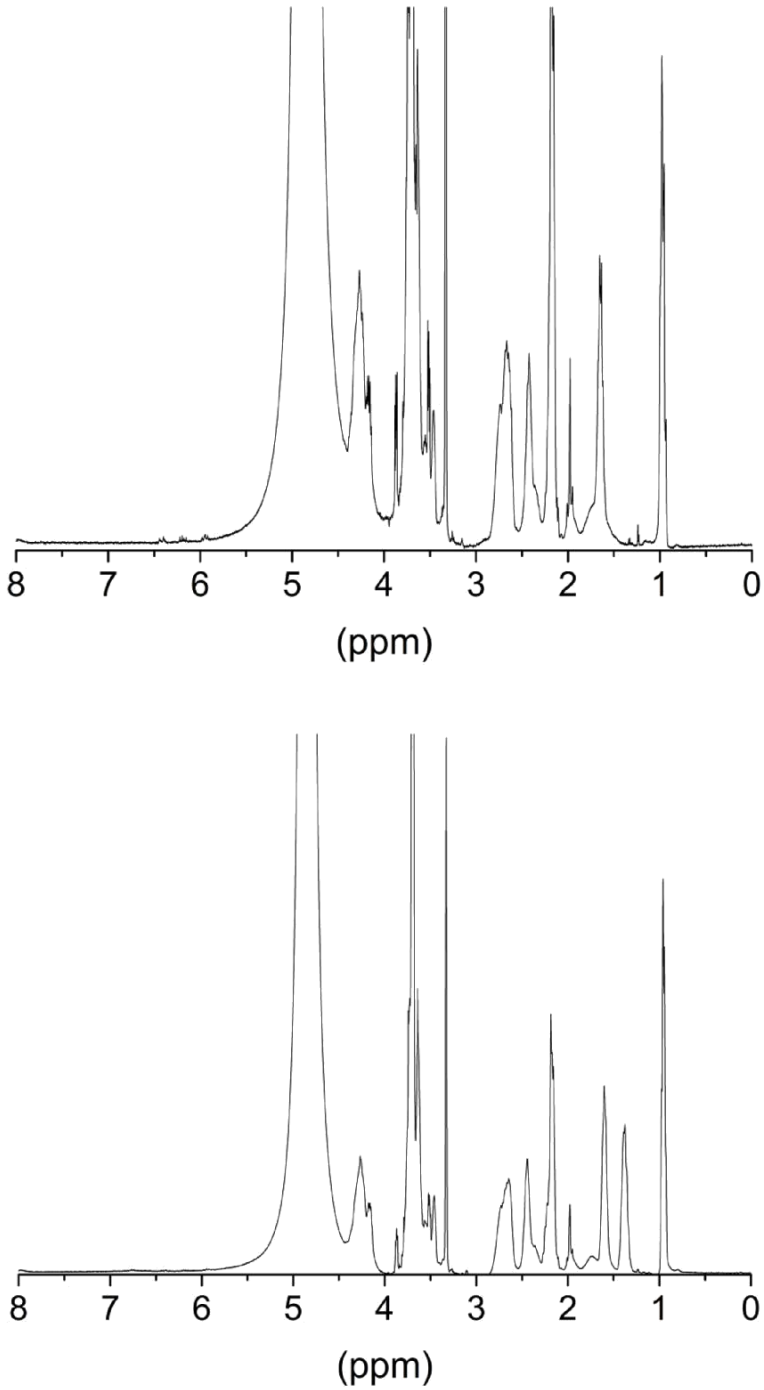


**Fig. S3** $^{1}$H NMR spectra (400 MHz, MeOD) of crude **P2**$_{MeOx/PropOx/AA}$ (top) and **P4**$_{MeOx/ButOx/AA}$ (bottom).

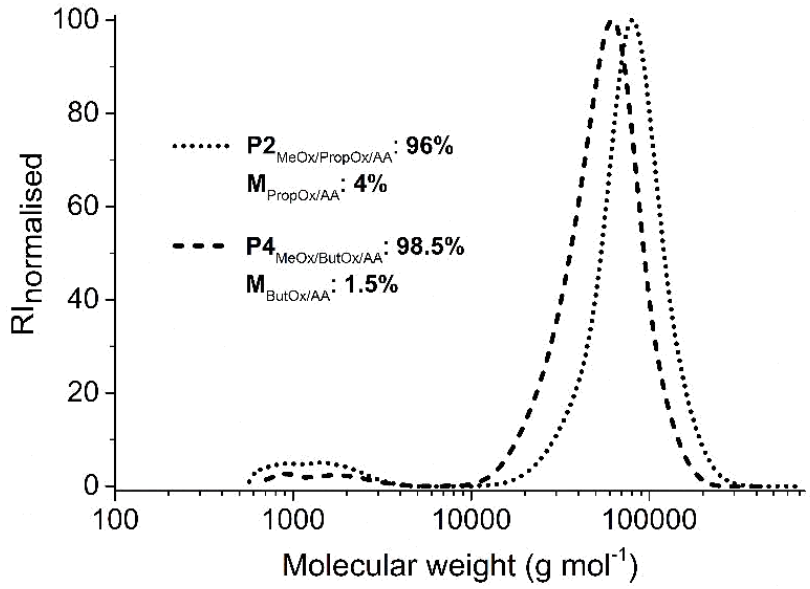


**Fig. S4** SEC spectra (DMAc) of crude polymer solutions, taken after 24 h reaction time (percentages refer to the area ratios of the macromonomers (**M**$_{PropOx/AA}$ or **M**$_{ButOx/AA}$) and block comb polymers (**P2** and **P4**)).

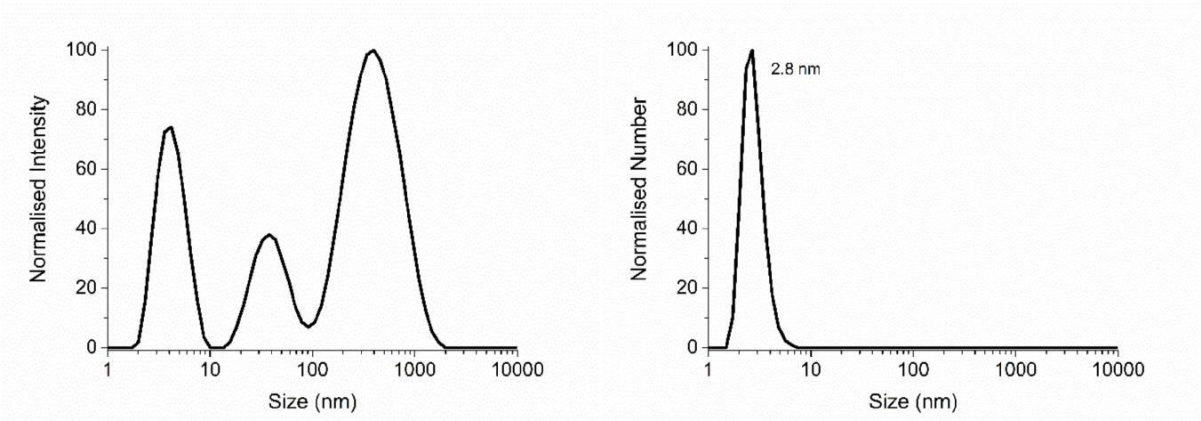


**Fig. S5** DLS spectrum of **P1b**$_{PropOx/AA}$ in MilliQ pH 7.4, intensity distribution (left) and number distribution (right).

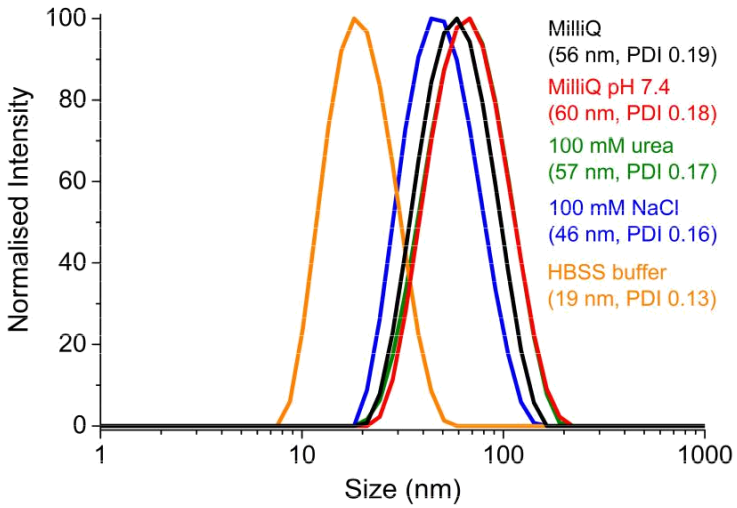


**Fig. S6** DLS spectra of **P3b**$_{PropOx/AA}$ in different aqueous media (HBSS: Hank's Balanced Salt Solution; values in parentheses are the diameters of the aggregates and the dispersity of the size distribution).

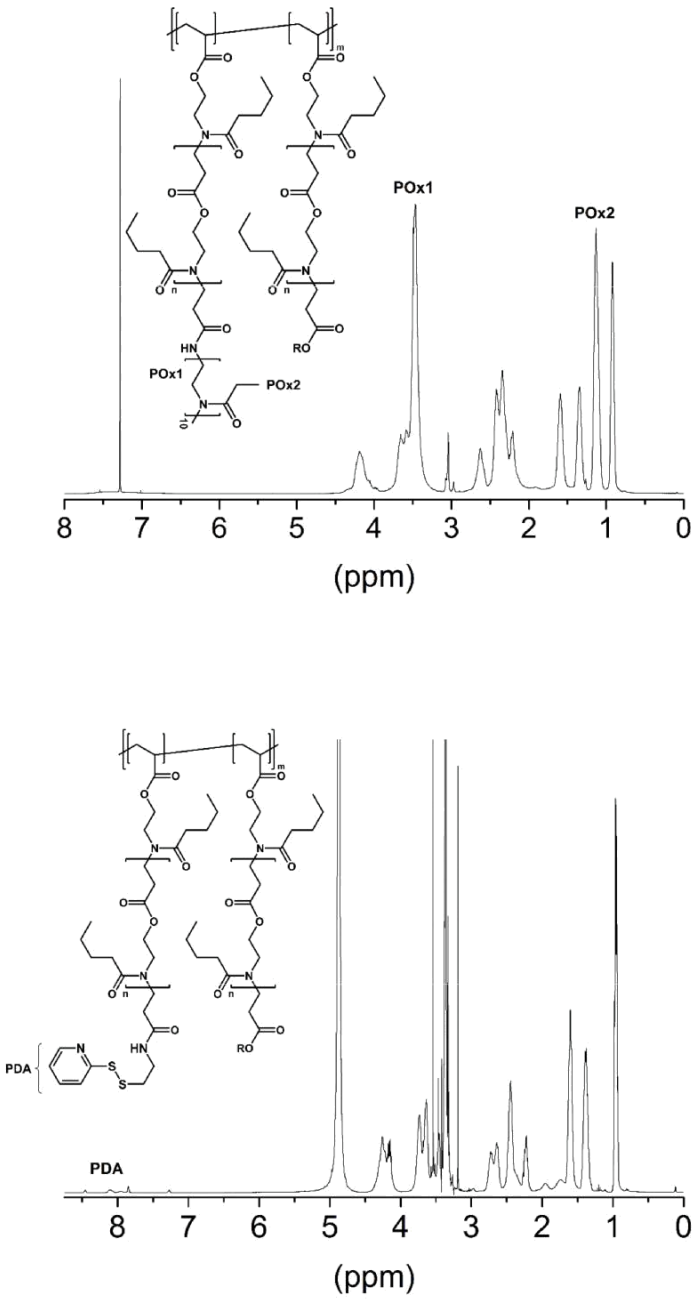


**Fig. S7** $^{1}$H NMR spectra (400 MHz, $CDCl_3$, MeOD) of **P3b-PEtOx** (top) and **P3b-PDA** (bottom).

## References


1. K. Kempe, M. Lobert, R. Hoogenboom and U. S. Schubert, *J. Comb. Chem.*, 2009, 11, 274-280.
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