Well-defined Polymeric Vesicles with High Stability and Modulation of Cell Uptake by a Simple Coating Protocol.

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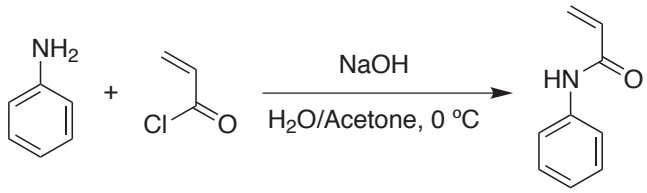
Monomer Synthesis

• Phenyl acrylamide (PAm)



PAm

To a solution of aniline (4.9 mL, 5.00 g, 53.4 mmol) in acetone (100 mL) and aqueous NaOH solution (2M, 100 mL) at 0 ºC, acryloyl chloride (10 mL, 11.14 g, 118.2 mmol, 2.2eq) was added dropwise over a period of 30 min and the mixture was left to react for 1 h at r.t. Acetone was removed in the rotavap, upon which a white solid appeared. The solid was filtered and washed with NaOH 2M (1 x) and H_2O (2 x). After recrystallization from hexane:EtOAc 3:1, 13.8 g (85%) of the title compound were isolated as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 10.13 (s, 1H, NH), 7.67 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.32 (t, *J* = 7.9 Hz, 2H, Ar-H), 7.07 (t, *J* = 7.4 Hz, 1H, Ar-H), 6.44 (dd, *J* = 17.0, 10.1 Hz, 1H, C<u>H</u>=C<u>H</u>$_2$), 6.26 (dd, *J* = 17.0, 2.0 Hz, 1H, C<u>H$_2$</u>=CH), 5.75 (dd, *J* = 10.1, 2.0 Hz, 1H, C<u>H$_2$</u>=CH).

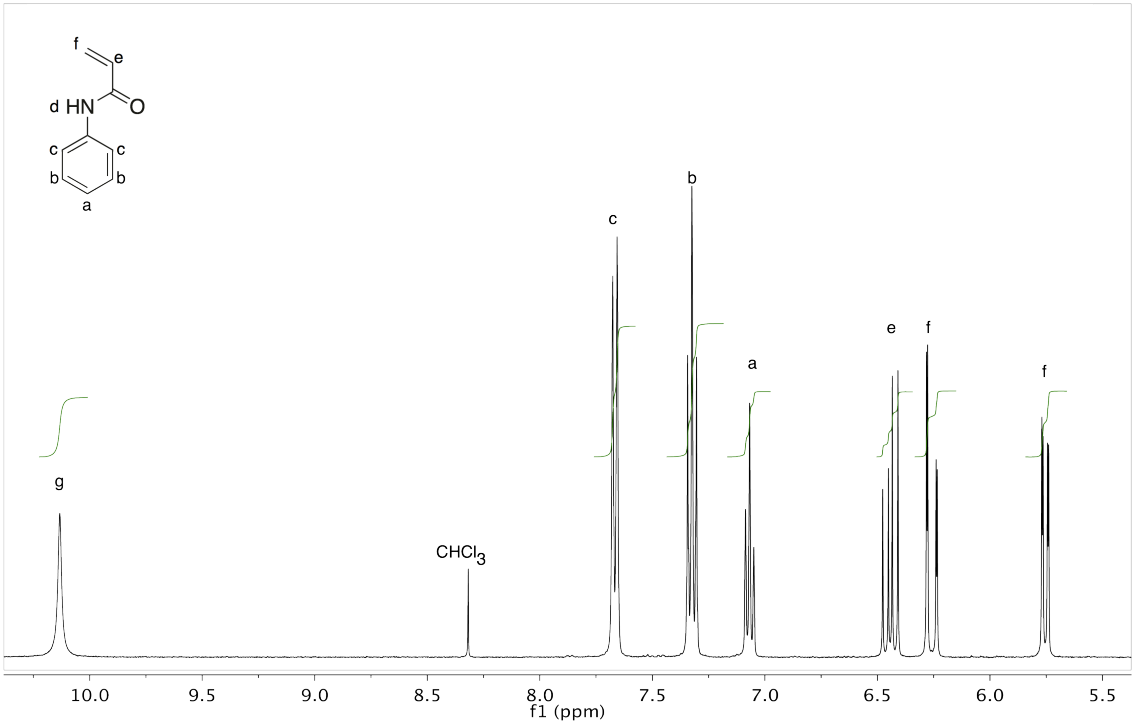
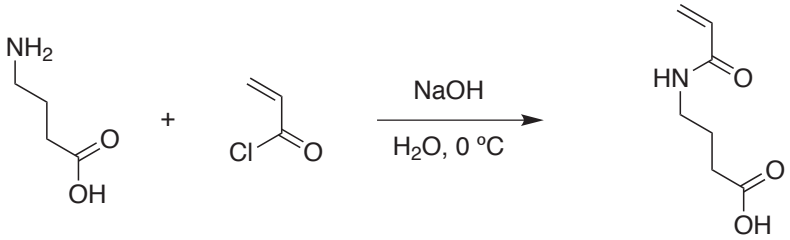


Figure S01: ^{1}H-NMR spectra of **PAm**

• N-ω-Acrylamidobutanoic acid (4AmBA)



4AmBA

To a solution of 4-aminobutanoic acid (5.12 g, 48.1 mmol) in an aqueous NaOH solution (2M, 126 mL) at 0 ºC, acryloyl chloride (13 mL, 14.48 g, 153.6 mmol, 2.3 eq) was added dropwise over a period of 30 min and the mixture was left to react for 1 h at r.t. The pH of the final solution was adjusted to 1 using an aqueous HCl solution (1M). The aqueous phase was saturated with NaCl, washed with $CHCl_3$ (3 x) and extracted with EtOAc (4 x). The combined EtOAc extracts were washed with NaCl (3 x) and H_2O (2 x). The organic phase was dried, and its volume reduced in the rotavap until a white precipitate appears. The solid was allowed to precipitate in the freezer overnight, filtered, washed with cold EtOAc and dried under vacuum, to yield 3.60 g (40%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 12.06 (s-broad, 1H, COOH), 8.08 (s-broad, 1H, NH), 6.19 (dd, *J* = 17.1, 10.0 Hz, 1H, C<u>H</u>=CH_2), 6.06 (dd, *J* = 17.1, 2.3 Hz, 1H, C<u>H$_2$</u>=CH), 5.56 (dd, *J* = 10.0, 2.3 Hz, 1H, C<u>H$_2$</u>=CH), 3.13 (dd, *J* = 12.8, 6.9 Hz, 2H, C<u>H$_2$</u>-NH), 2.23 (t, *J* = 7.4 Hz, 2H, C<u>H$_2$</u>-COOH), 1.65 (m, 2H, CH_2). HRMS (TOF-ESI) Calcd for $C_5H_6NO_3^-$ (M^-): 128.0353 found: 128.0091.

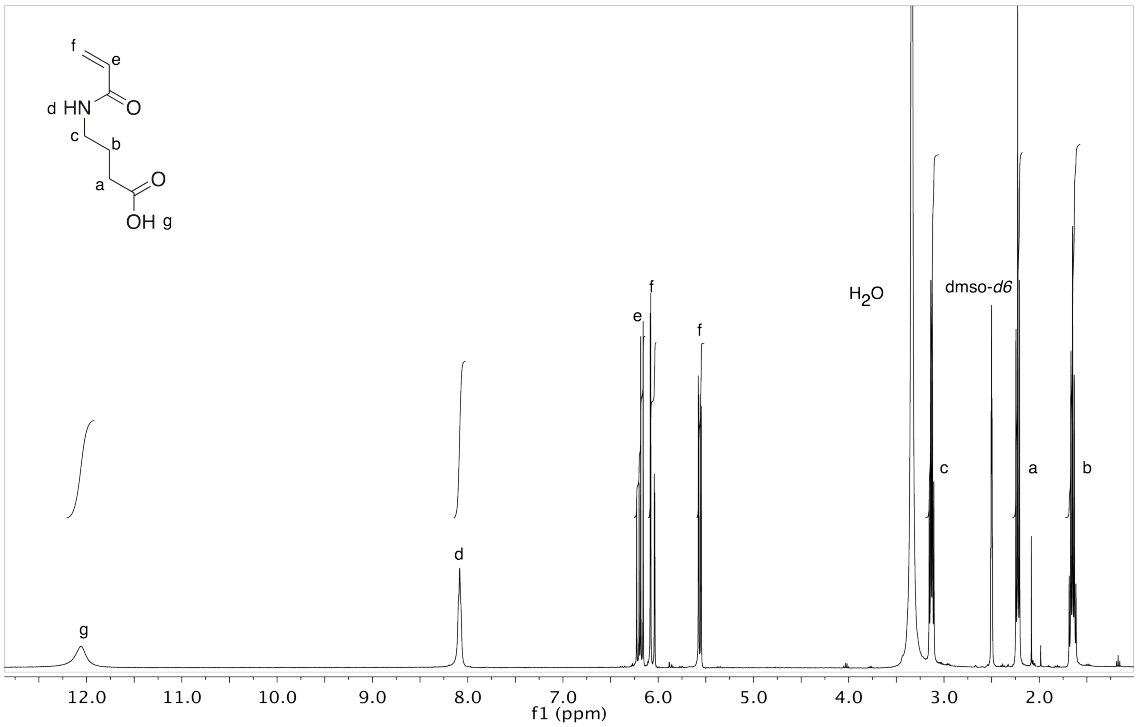


Figure S02: ^{1}H-NMR spectra of **4AmBA**

Polymer Characterization

• poly(N-Phenylacrylamide) (p(PAm)-R) (**P1**)

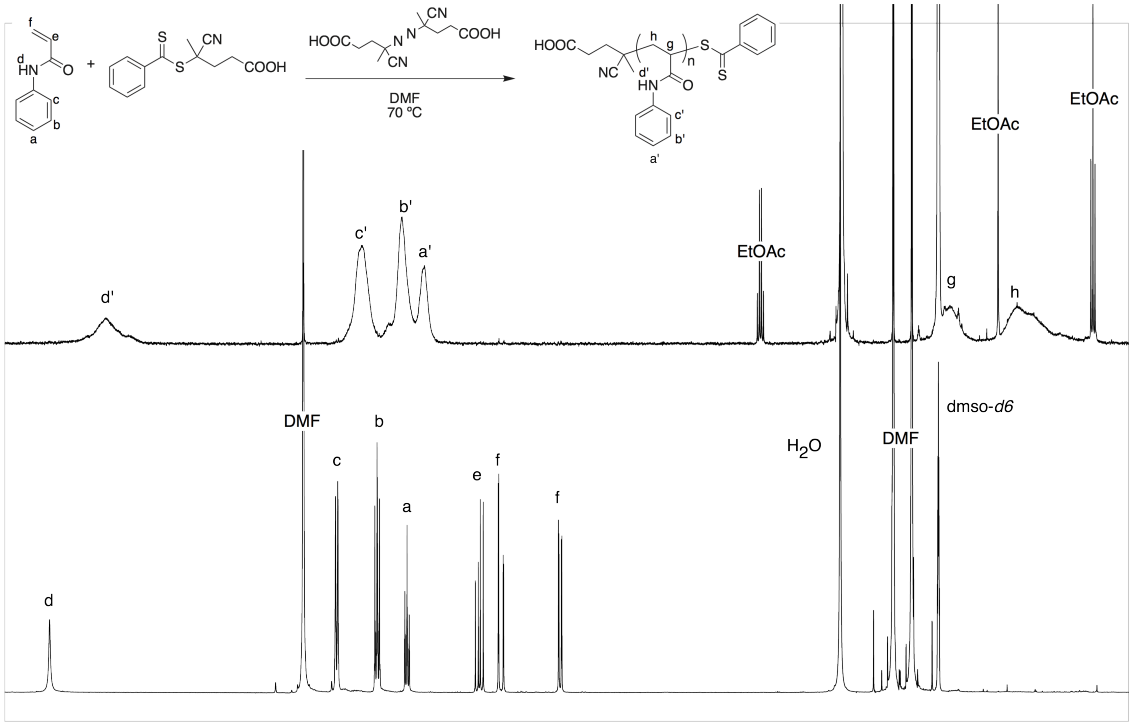


Figure S03: ^{1}H-NMR spectra for the polymerization of **PAm** to yield **P1**. Top: Purified polymer. Bottom: Reaction mixture at the beginning of the polymerization.

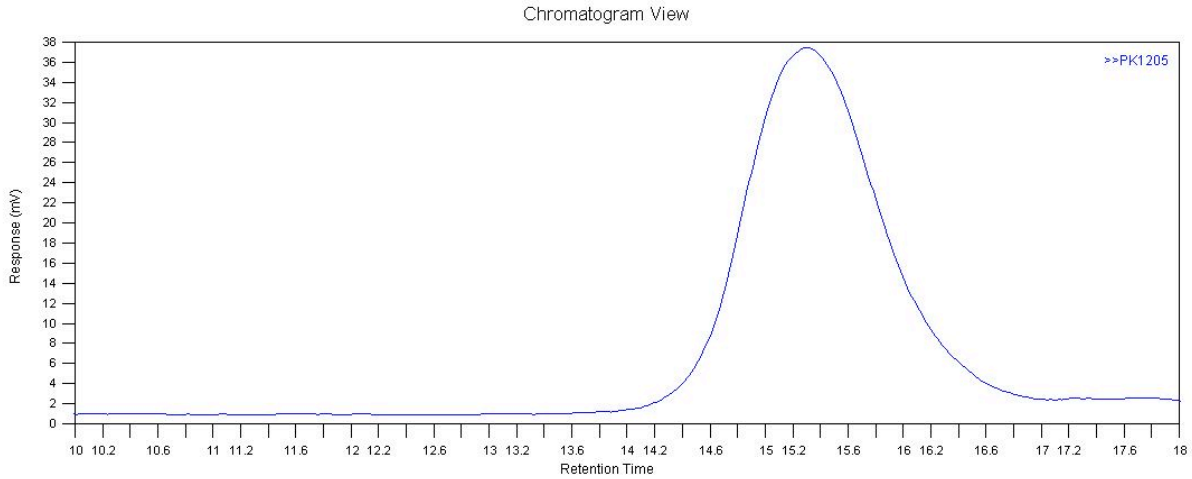


Figure S04: Representative GPC elugrams for **p(PAm)**

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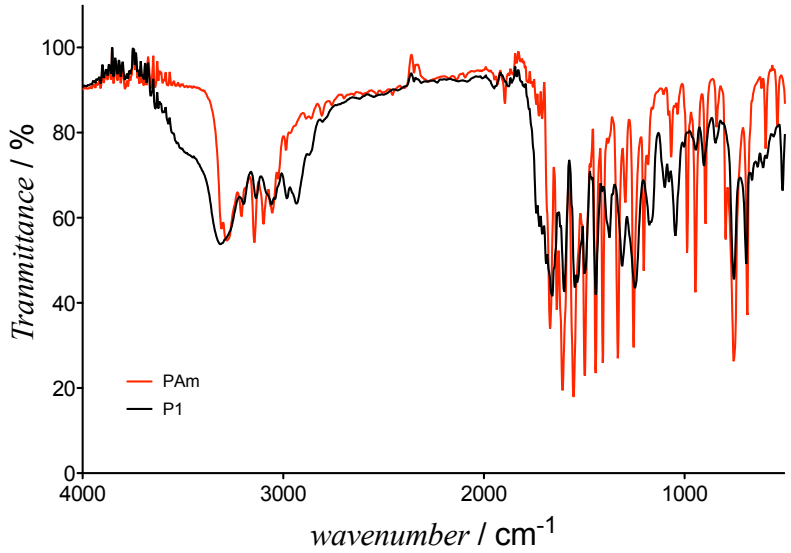


Figure S05: Representative FTIR for **P1**. Red: Starting monomer **PAm**. Bottom: Purified polymer.

• poly(4-Acrylamidobutanoic acid) (p(4AmBA)-R) (**P2**)

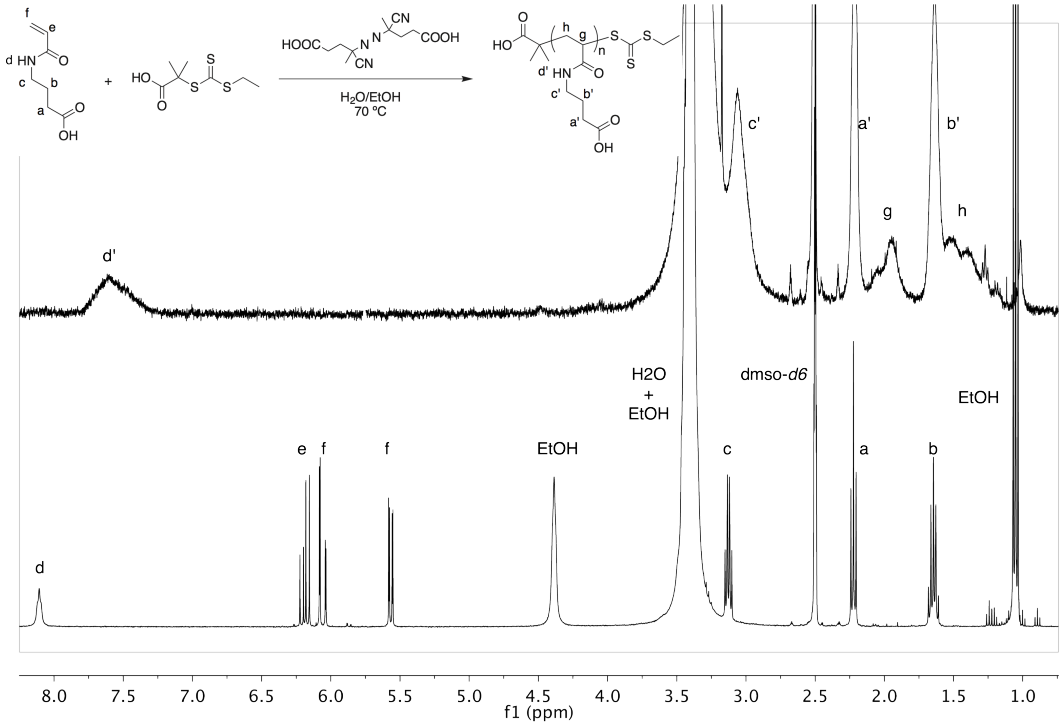


Figure S06: ^{1}H-NMR spectra for the polymerization of **4AmBA** to yield **P2**. Top: Purified polymer. Bottom: Reaction mixture at the beginning of the polymerization.

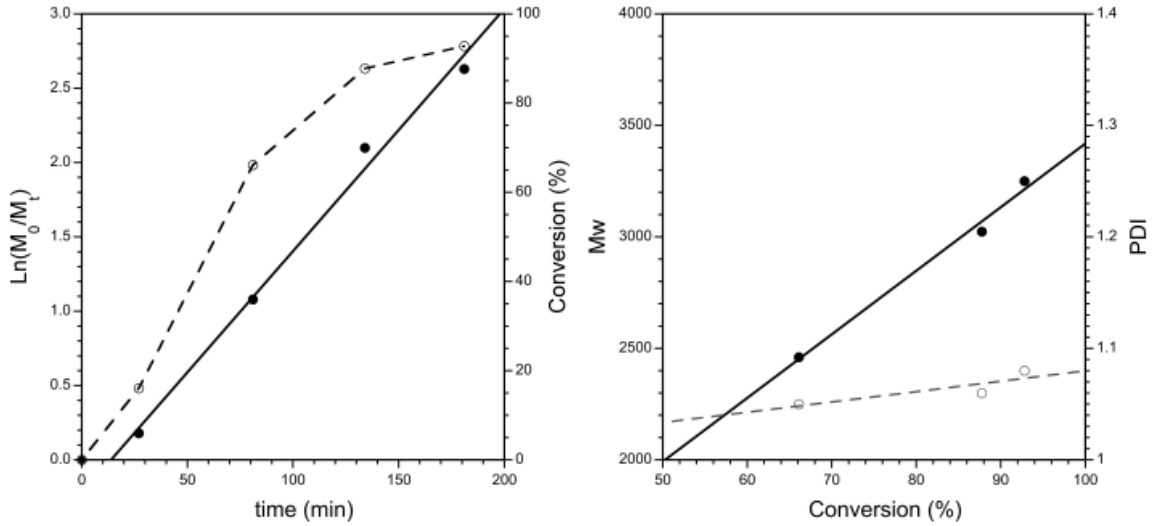


Figure S07: Left: Representative linear plot of Ln$[M]_0/[M]_t$ vs time (solid) and plot of conversion vs time (dashed). Right: Representative plot of calculated Mw vs conversion (solid) and PDI vs Conversion (dashed). Conditions: 70 °C; [**4AmBA**]=0.75 M; [**4AmBA**]/[**CTA3**]=30; [**CTA1**]/[**V-501**]=10; H_2O/EtOH.

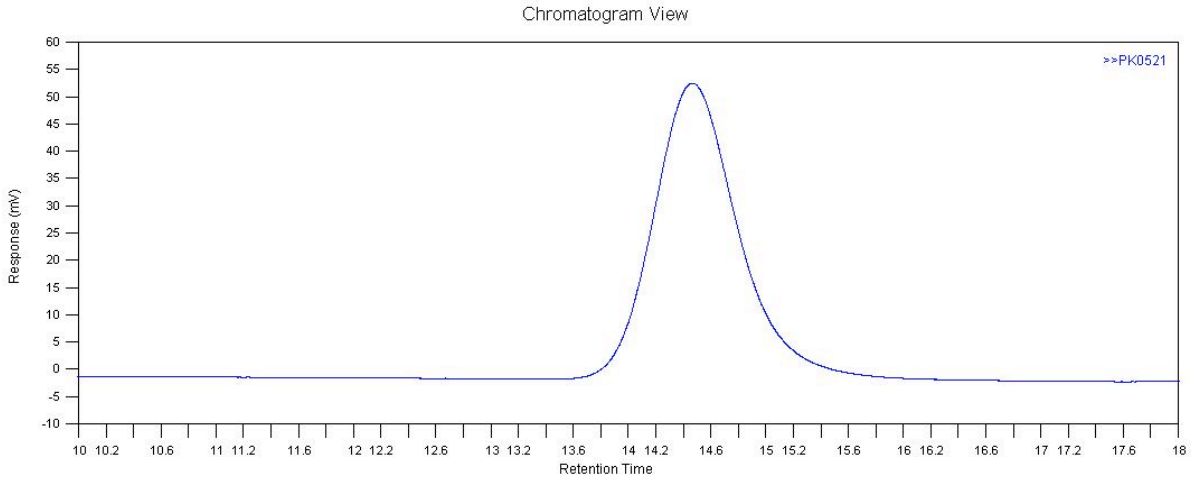


Figure S08: Representative GPC elugram for **p(4AmBA)**.

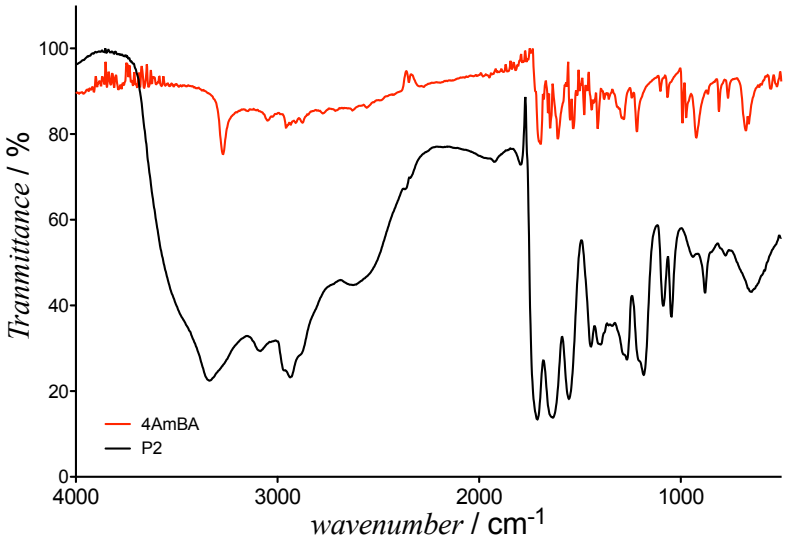


Figure S09: Representative FTIR for **P2**. Red: Starting monomer **4AmBA**. Bottom: Purified polymer.

- poly(4-Acrylamidobutanoic acid)-block-poly(N-Phenylacrylamide)-RAFT (**p(4AmBA)-b-p(PAm)-R**)

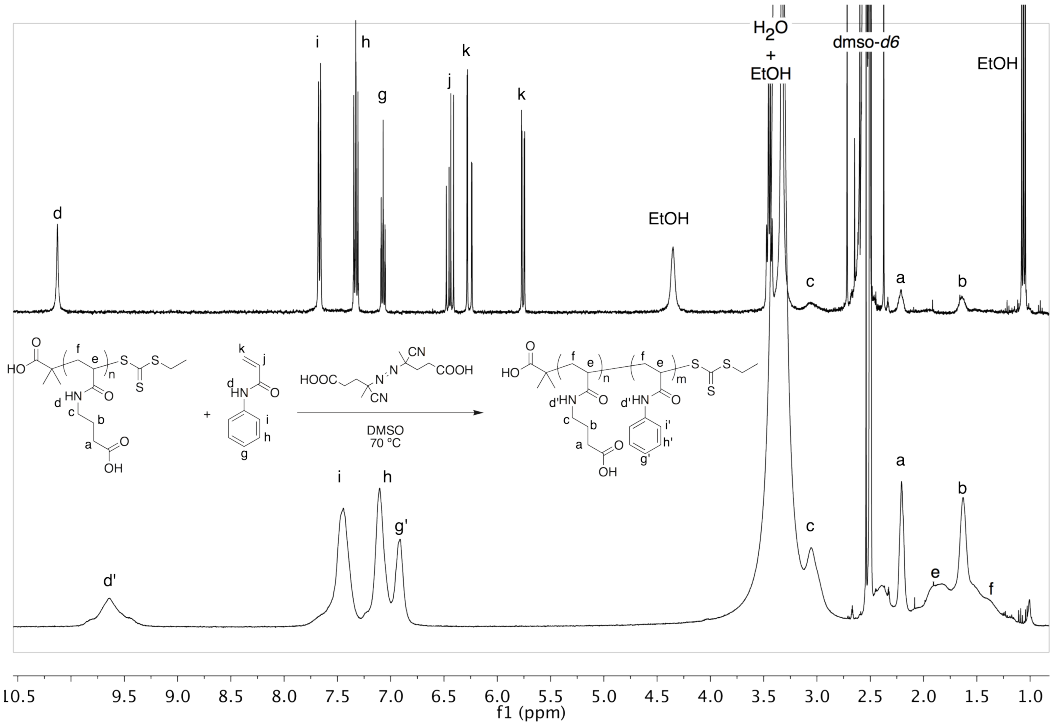


Figure S10: ^{1}H-NMR spectra for the polymerization of **PAm** to yield **(p(4AmBA)-b-p(PAm)-R)**. Top: Reaction mixture at the beginning of the polymerization. Bottom: Purified polymer.

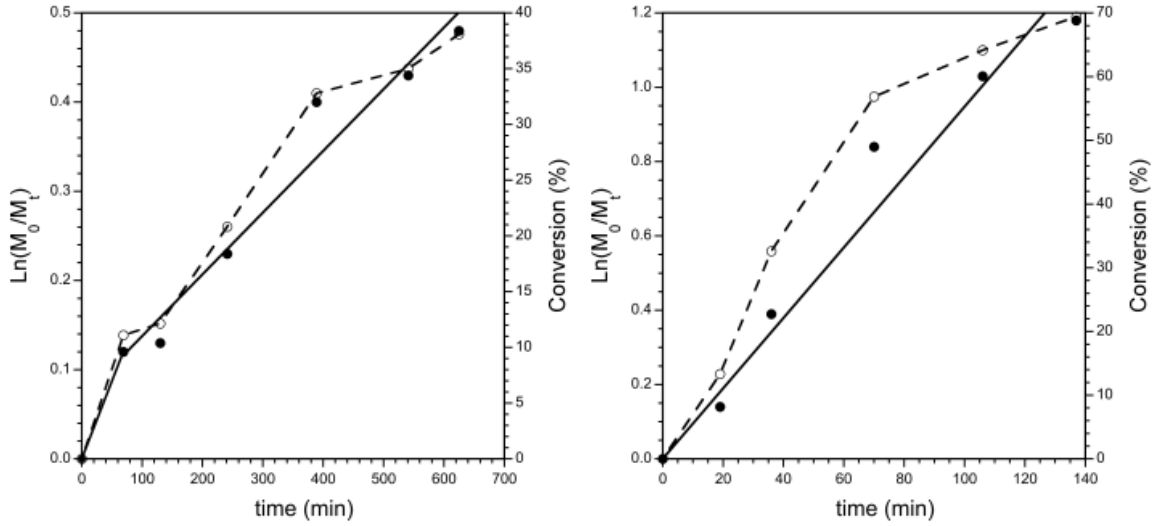


Figure S11: Left: Representative linear plot of Ln$[M]_0/[M]_t$ vs time (solid)and plot of conversion vs time (dashed) of the polymerisation of **4AmBA**. Conditions: 70 °C; [**4AmBA**]=1.6 M; [**P1c**]/[**4AmBA**]=36; [**P1c**]/[**V-501**]=3; DMF. Right: Representative linear plot of Ln$[M]_0/[M]_t$ vs time (solid) and plot of conversion vs time (dashed) of the polymerisation of **PAm**. Conditions: 70 °C; [**PAm**]=0.5 M; [**P2a**]/[**PAm**]=55; [**P2a**]/[**V-501**]=4; DMSO.

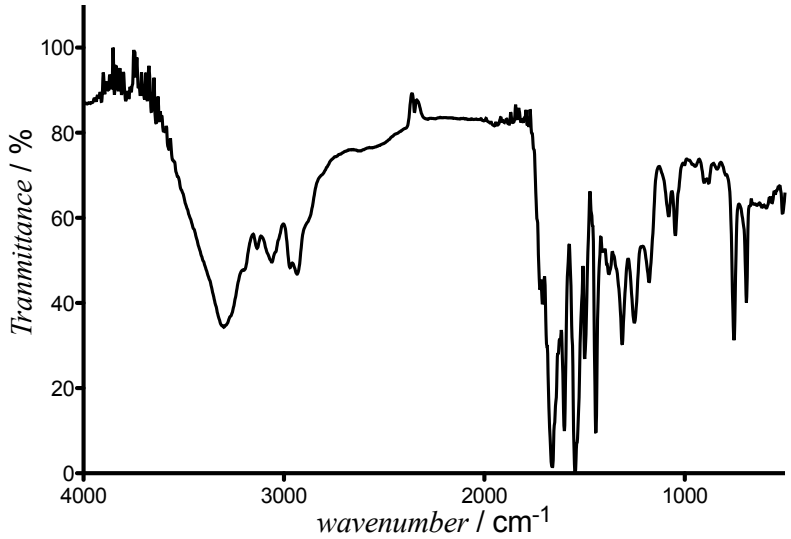
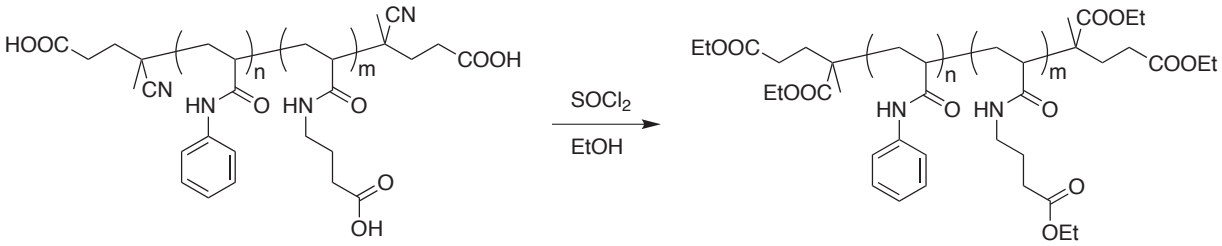


Figure S12: Representative FTIR for **P3**.

• General Protocol for the esterification of P3



In a typical experiment, to a solution of **P3** (50 mg, 2.3 µmol (62.8 µmol **4AmBA**), 50 mg/mL) in EtOH (1 mL), $SOCl_2$ (45 µl, 74 mg, 610 µmol) was added. The reaction was carried out overnight at room temperature. The title compound was purified by precipitation into Pet Ether:Et_2O 1:1 and recovered as a white powder (50 mg, quantitative yield) after freeze-drying from water (dark, 2 days).

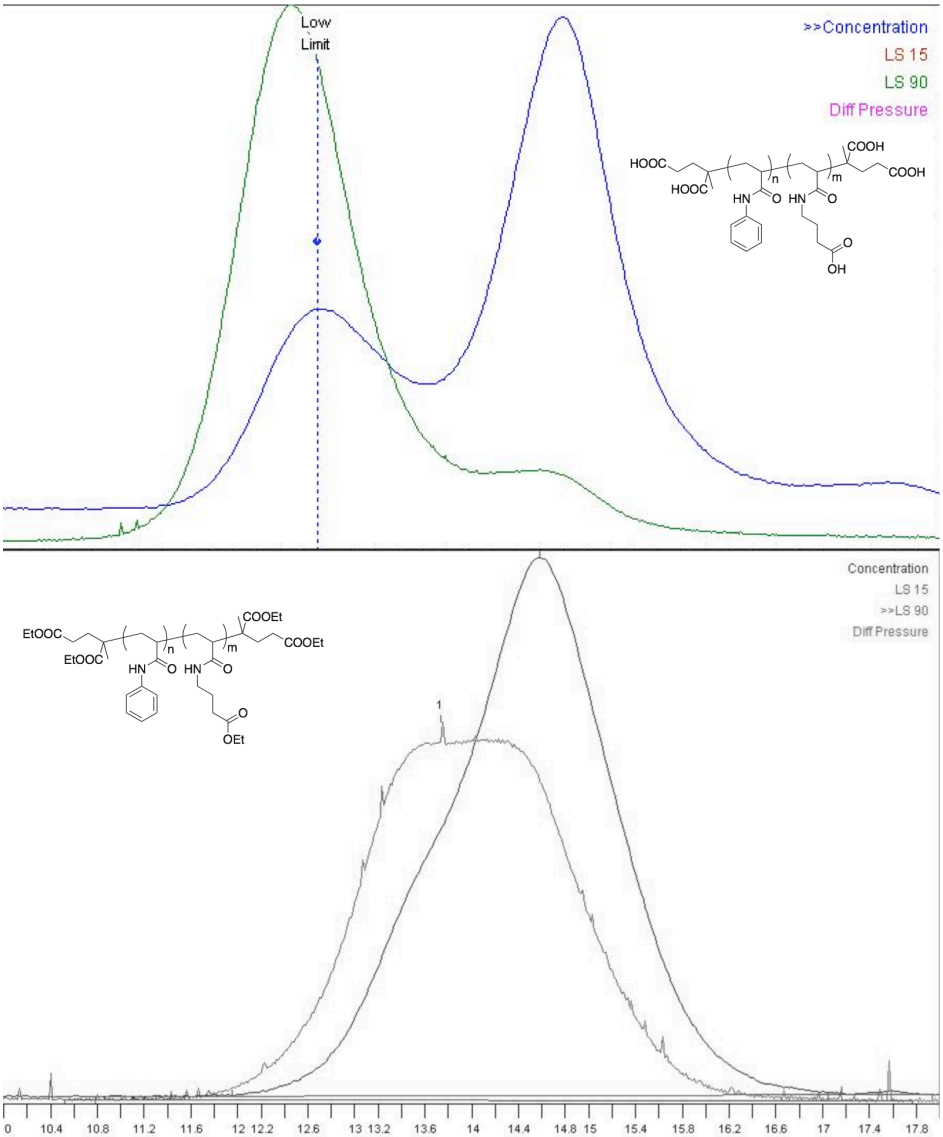


Figure S13: Representative comparison of GPC elugrams before and after esterification of the acid groups in **P3** (**P3b**). Multiangle detection was used in this case and both RI and LS traces are shown.

AFM

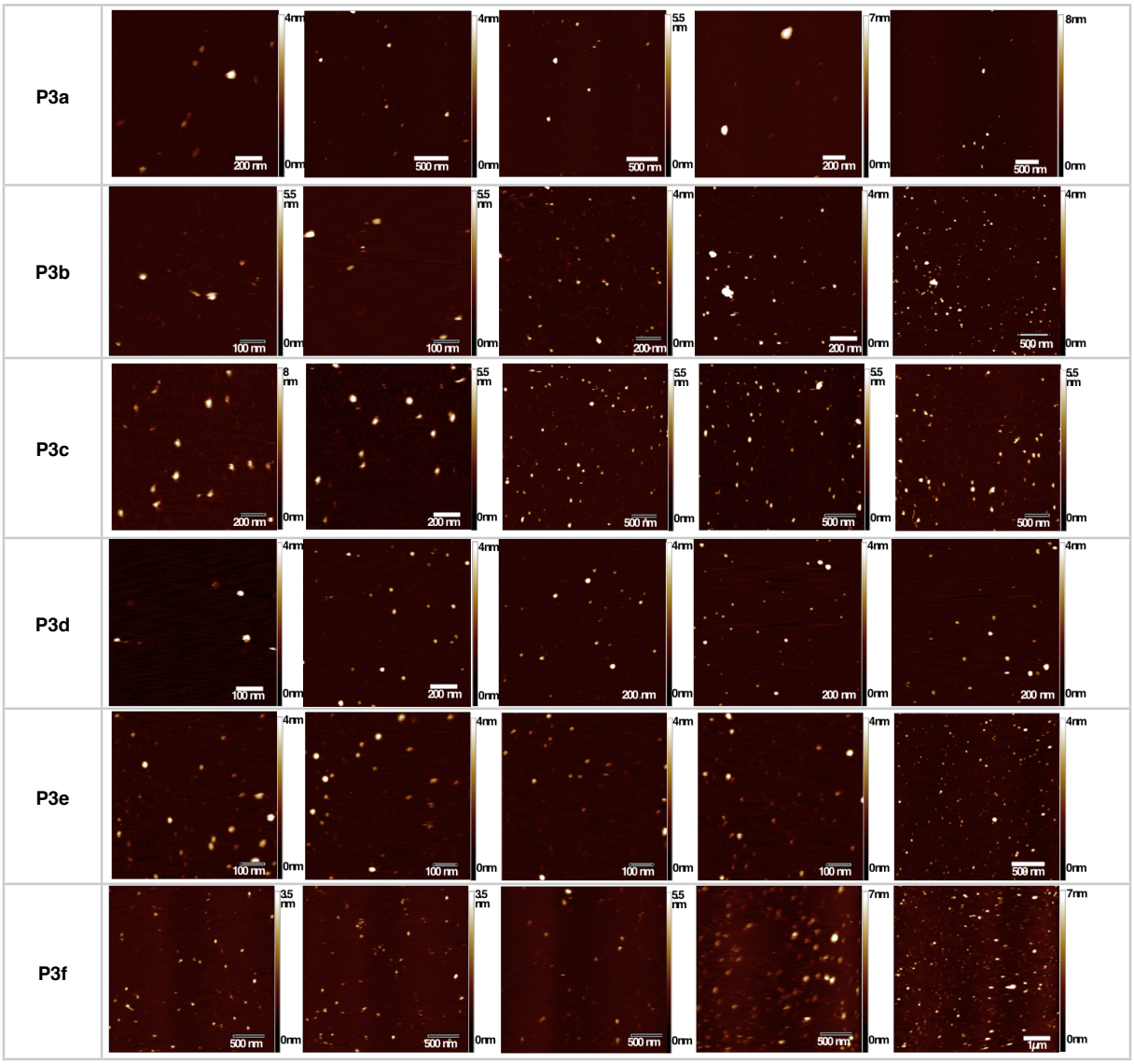


Figure S14: Representative AFM topography images for self-assembled structures of **P3**. 5 mg/mL of **P3** for vesicle preparation.

TEM

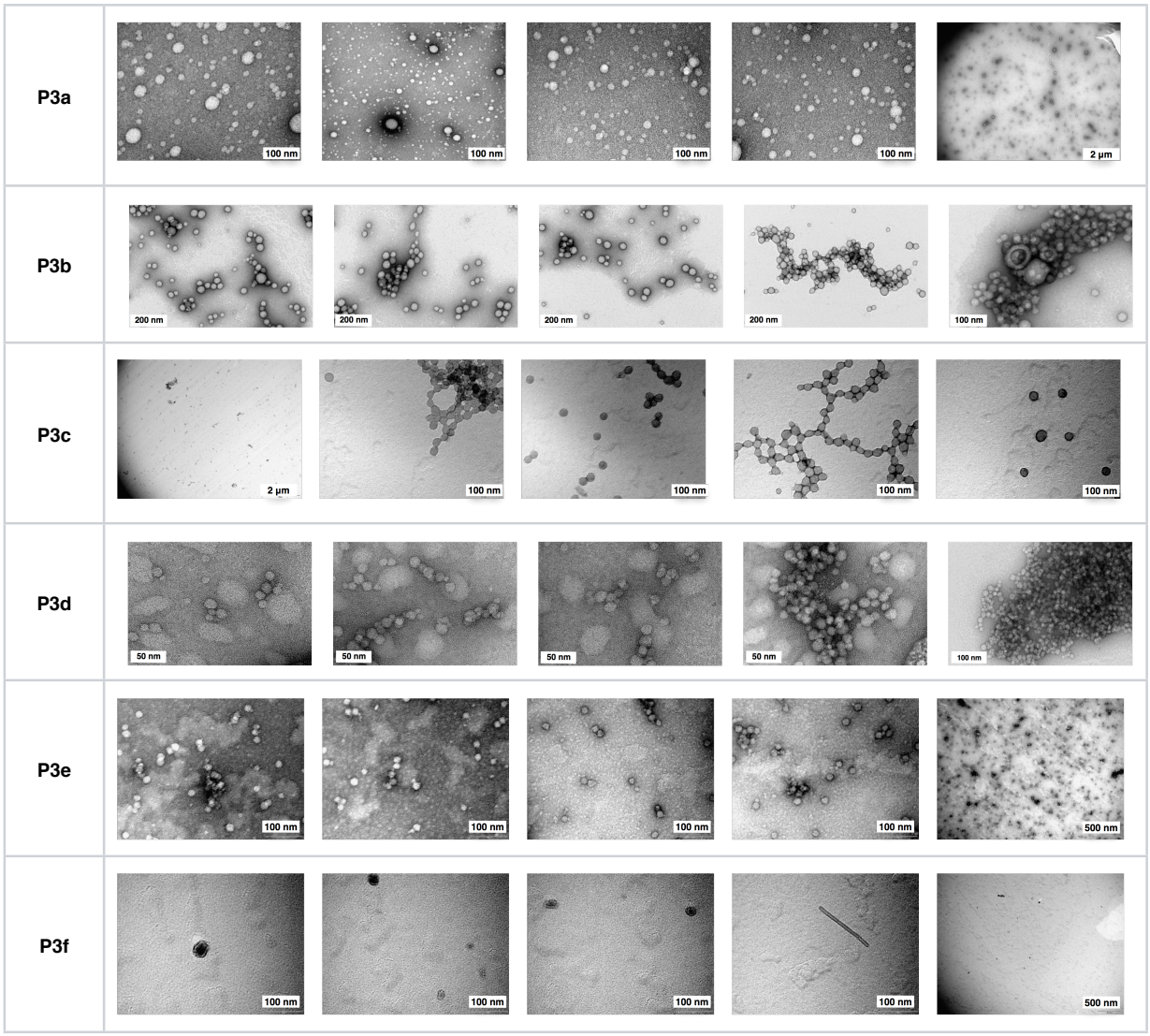


Figure S15: Representative TEM Micrographs for the self-assembled structures of **P3**. 5 mg/mL of **P3** for vesicle preparation.

Vesicle Stability

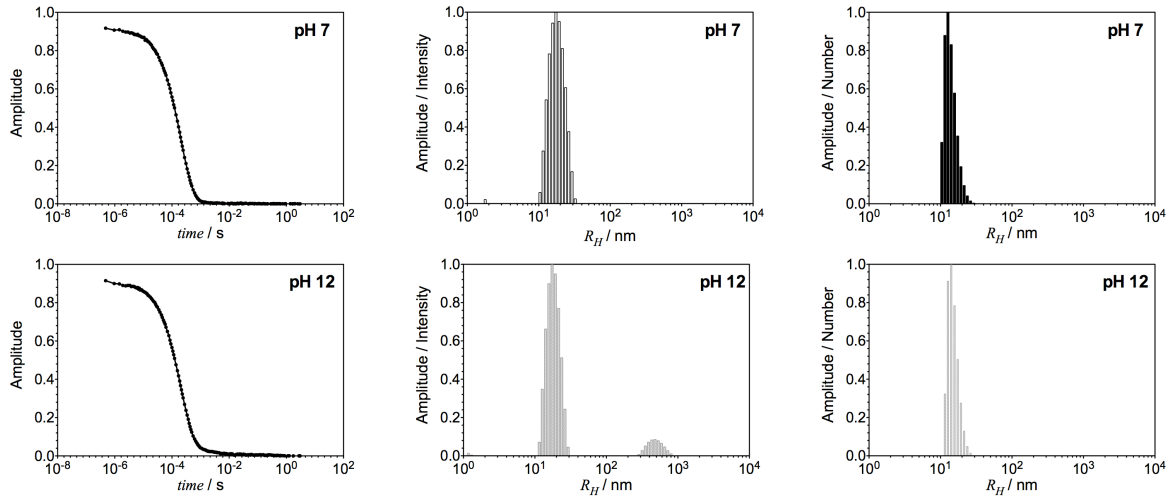


Figure S16: Representative DLS data for vesicle stability at different pH. Experiments carried out with **P3b** in 10 mM HEPES, 100 mM NaCl. 10 mg/mL of **P3** for vesicle preparation.

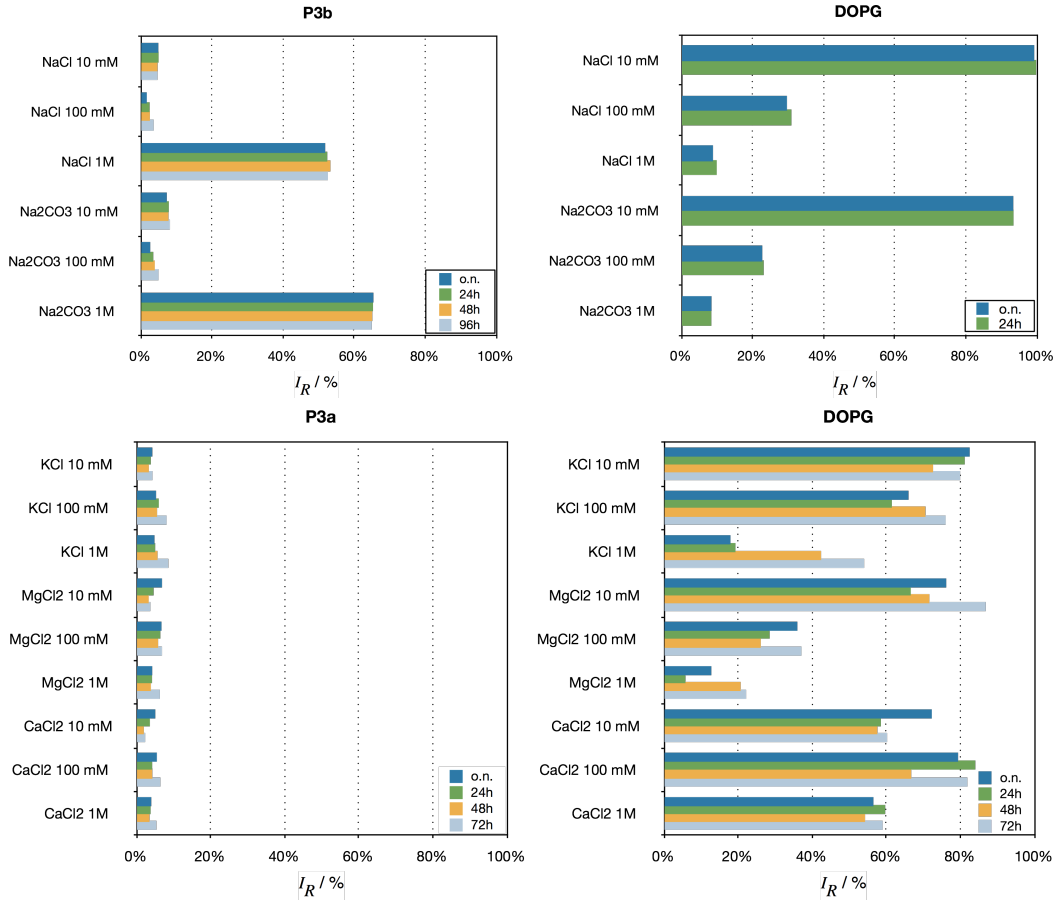


Figure S17: Percentage of CF released after incubating vesicles o.n. in the presence of different salts at different concentrations. Experiments carried out with **P3b** in 10 mM HEPES, 100 mM NaCl. 10 mg/mL of **P3** for vesicle preparation.

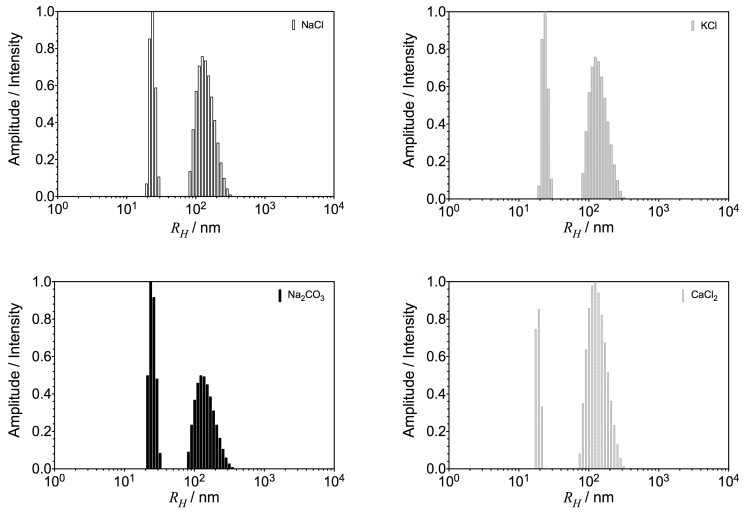


Figure S18: Representative DLS data for vesicle solution in the presence of 100 mM of the appropriate salt. Experiments carried out with **P3b** in 10 mM HEPES, 100 mM NaCl. 10 mg/mL of **P3b** for vesicle preparation.

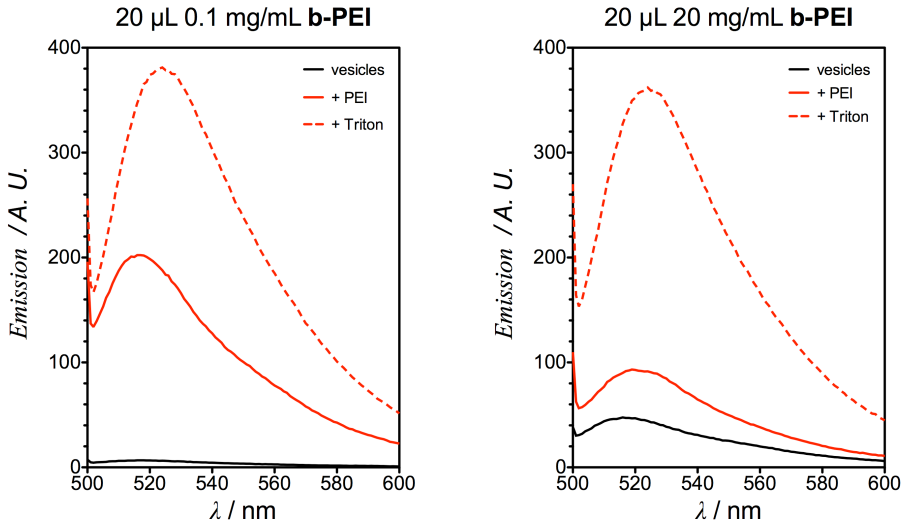


Figure S19: Effect of **b-PEI** concentration on the stability of polymersomes. Experiments carried out with **P3c**. 15 mg/mL of **P3c** for vesicle preparation.

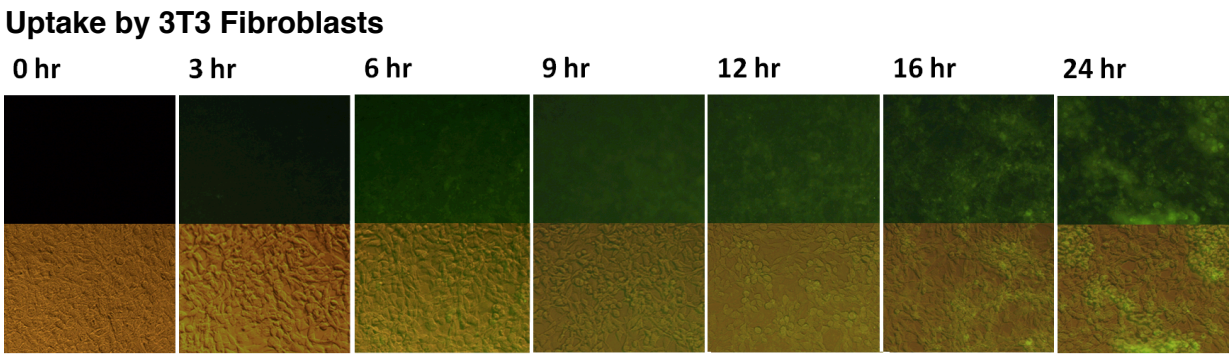


Figure S20: Representative fluorescent (top) and merged (bottom) micrographs of uptake of polymersomes by 3T3 fibroblasts.

Uptake by A549 cells

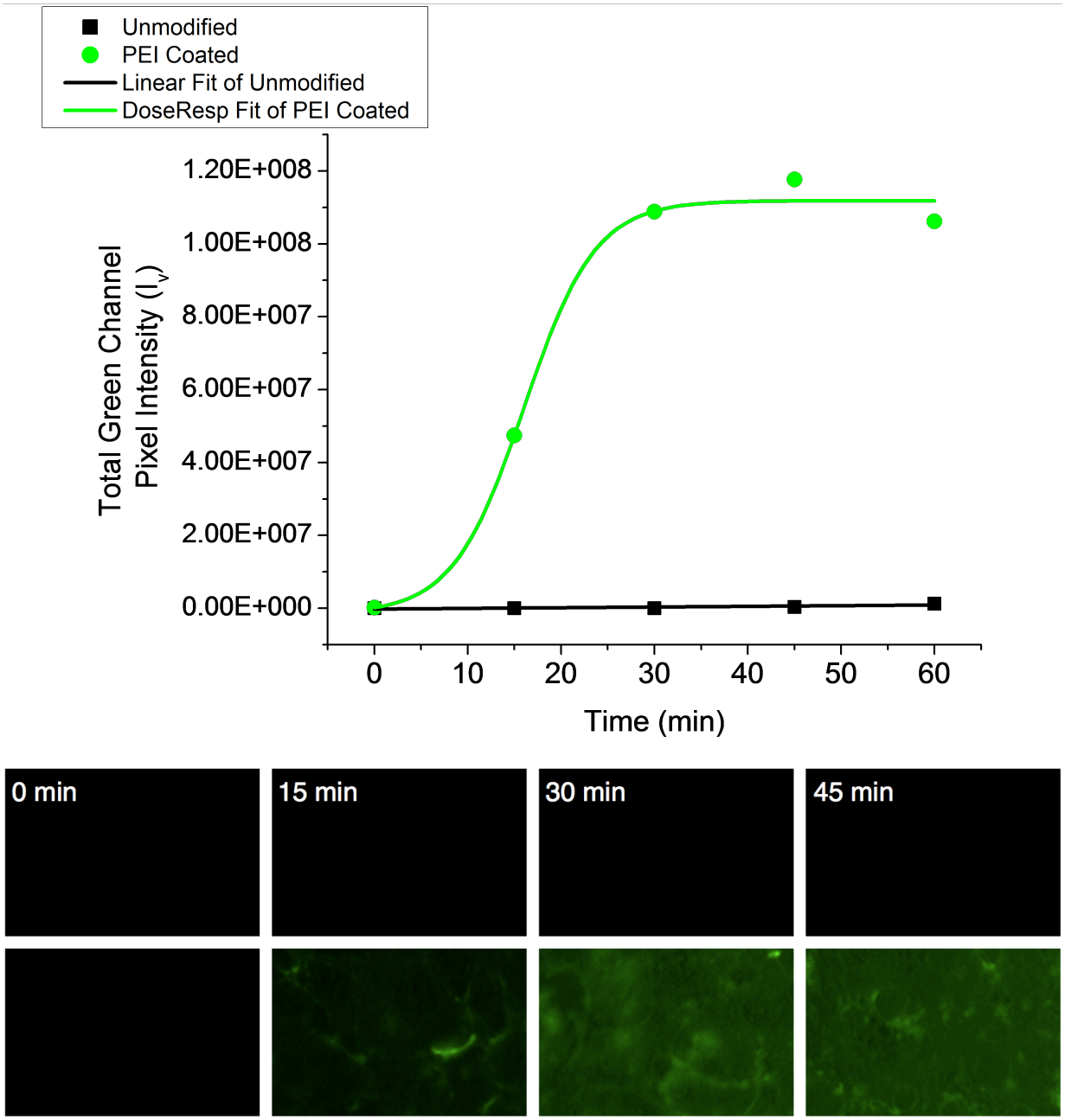


Figure S21: Uptake of polymersomes by A549 cells and representative fluorescent micrographs of unmodified (top) and **b-PEI** coated (bottom) polymersomes.

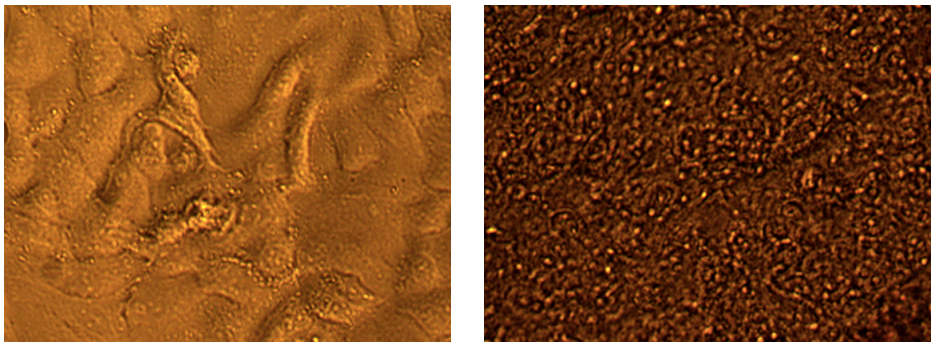


Figure S22: Brightfield images of A549 cells following a 60 min incubation in the absence (left) and presence (right) of **b-PEI** coated polymersomes. Experiments carried out with **P3c**.