

Supporting Information

The Hydrolytic Behavior of *N,N'*- (dimethylamino)ethyl Acrylate Functionalized Polymeric Stars

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Contents

1. Materials and Methods.....	2
Synthetic Procedures.....	2
Hydrolysis Analysis.....	4
2. SEC and ¹ H NMR spectroscopic analysis of short armed PEGA ₉₈ particles.....	4
Figure S1. SEC analysis and ¹ H NMR spectrum of PEGA ₉₈ arms (1).....	4
Figure S2. Triple detection SEC analysis of PEGA ₉₈ armed particles 2-20 , 2-15 , and 2-10 with the Mark-Houwink curve overlaid on the molecular weight distribution.....	5
3. Size analysis of short armed PEGA ₉₈ particles.....	5
Figure S3. Size distribution of 2-15	5
Table S1. Size distribution analysis.....	6
4. SEC and ¹ H NMR spectroscopic analysis of PHEA ₁₀₄	6
Figure S4. SEC analysis and ¹ H NMR spectrum of PHEA ₁₀₄ arms (7).....	6
5. Analysis of HEA ₁₀₄ armed particles.....	7
Figure S5. Triple detection SEC analysis of PHEA ₁₀₄ armed particles 8-20 , 8-15 , and 8-10 with the Mark-Houwink curve overlaid on the molecular weight distribution.....	7
Figure S6. Size distribution of 8-20 , 8-15 and 8-10	7
6. Hydrolysis analysis of PEGA armed particles by DLS.....	8
Figure S7. Size distribution of 2-20 at 25 °C and 50 °C before hydrolysis, and before and after hydrolysis at 50 °C.....	8
7. SEC analysis of medium and long armed PEGA particles.....	8
Figure S8. SEC analysis of medium and long PEGA homopolymers (3 and 5 respectively) and their corresponding stars (4 and 6 respectively).....	8

8. Size analysis of medium and long armed PEGA particles.....	9
Figure S9. Size distribution of 4 and 6	9
9. Hydrolysis analysis of PHEA ₁₀₄ armed particles by ¹ H NMR spectroscopy	9
Figure S10. Hydrolysis analysis of PHEA armed particles 8-20 , 8-15 and 8-10 , hydrolyzed in D ₂ O at 25 °C	9
10. Enzymatic determination of [DMAE].....	9
Figure S11. Calibration curve	10
Equation S2: Calibration for the initial rate of rise at $\lambda = 405$ nm vs. [DMAE]	10

1. Materials and Methods

Synthetic Procedures

The PEGA arms and subsequent star polymers were synthesized according to the procedure outlined in the main paper. Characterization data for polymers **1**, **2-20**, **7** and **8-20** is reported in the main paper.

PEGA₁₄₈ (**3**)

¹H NMR (CDCl₃): δ (ppm) 4.16 (br s, OCH₂CH₂), 3.47-3.89 (m, O(CH₂CH₂)₈ and SCH₂(CH₂)₉), 3.38 (s, CH₂OCH₃), 1.25-2.29 (m, CH₂ backbone, CH₂(CH₂)₁₀ CTA), 0.88 (t, 3H, (CH₂)₁₀CH₃, ³J_{H-H} = 6.0 Hz). $M_{n, SEC} = 46.2$ kDa, $D_M = 1.64$.

PEGA₂₈₈ (**5**)

¹H NMR (CDCl₃): δ (ppm) 4.17 (br s, OCH₂CH₂), 3.47-3.82 (m, O(CH₂CH₂)₈ and SCH₂(CH₂)₉), 3.38 (s, CH₂OCH₃), 1.26-2.67 (m, CH₂ backbone, CH₂(CH₂)₁₀ CTA), 0.88 (t, 3H, (CH₂)₁₀CH₃, ³J_{H-H} = 6.1 Hz). $M_{n, SEC} = 47.0$ kDa, $D_M = 1.52$.

15% Crosslinked Polymer (2-15)

¹H NMR (CDCl₃): δ (ppm) 4.17 (br s, OCH₂CH₂O, OCH₂CH₂O(CH₂CH₂O)₇, and CH₂CH₂N), 3.47-3.70 (m, O(CH₂CH₂)₈, SCH₂(CH₂)₉ and OCH₂CH₂O), 3.32 (s, CH₂OCH₃), 2.54 (br s, CH₂N), 2.27 (br s, N(CH₃)₂), 1.25-2.03 (m, CH₂ backbone, CH₂(CH₂)₁₀ CTA), 0.88 (t, 3H, (CH₂)₁₀CH₃, ³J_{H-H} = 6.2 Hz). $M_{n, SEC} = 39.9$ kDa, $D_M = 2.88$. $D_h = 9$ nm.

10% Crosslinked Polymer (2-10)

^1H NMR (CDCl_3): δ (ppm) 4.18 (br s, $\text{OCH}_2\text{CH}_2\text{O}$, $\text{OCH}_2\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_7$ and $\text{CH}_2\text{CH}_2\text{N}$), 3.45-3.80 (m, $\text{O}(\text{CH}_2\text{CH}_2)_8$, $\text{SCH}_2(\text{CH}_2)_9$ and $\text{OCH}_2\text{CH}_2\text{O}$), 3.32 (s, CH_2OCH_3), 2.62 (br s, CH_2N), 2.23 (br s, $\text{N}(\text{CH}_3)_2$), 1.29-2.06 (m, CH_2 backbone, $\text{CH}_2(\text{CH}_2)_{10}$ CTA), 0.90 (t, 3H, $(\text{CH}_2)_{10}\text{CH}_3$, $^3J_{\text{H-H}} = 6.0$ Hz). $M_{\text{n, SEC}} = 43.8$ kDa, $D_{\text{M}} = 2.25$. $D_{\text{h}} = 10$ nm.

20% Crosslinked Polymer (4)

^1H NMR (CDCl_3): δ (ppm) 4.16 (br s, OCH_2CH_2 , $\text{OCH}_2\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_7$ and $\text{CH}_2\text{CH}_2\text{N}$), 3.47-3.82 (m, $\text{O}(\text{CH}_2\text{CH}_2)_8$, $\text{SCH}_2(\text{CH}_2)_9$ and $\text{OCH}_2\text{CH}_2\text{O}$), 3.38 (s, CH_2OCH_3), 2.54 (br s, CH_2N), 2.27 (br s, $\text{N}(\text{CH}_3)_2$), 1.25-2.10 (m, CH_2 backbone, $\text{CH}_2(\text{CH}_2)_{10}$ CTA), 0.88 (t, 3H, $(\text{CH}_2)_{10}\text{CH}_3$, $^3J_{\text{H-H}} = 6.2$ Hz). $M_{\text{n, SEC}} = 66.3$ kDa, $D_{\text{M}} = 1.73$. $D_{\text{h}} = 11$ nm.

20% Crosslinked Polymer (6)

^1H NMR (CDCl_3): δ (ppm) 4.12 (br s, OCH_2CH_2 , $\text{OCH}_2\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_7$ and $\text{CH}_2\text{CH}_2\text{N}$), 3.41-3.73 (m, $\text{O}(\text{CH}_2\text{CH}_2)_8$, $\text{SCH}_2(\text{CH}_2)_9$ and $\text{OCH}_2\text{CH}_2\text{O}$), 3.24 (s, CH_2OCH_3), 2.57 (br s, CH_2N), 2.15 (br s, $\text{N}(\text{CH}_3)_2$), 1.13-2.00 (m, CH_2 backbone, $\text{CH}_2(\text{CH}_2)_{10}$ CTA), 0.78 (t, 3H, $(\text{CH}_2)_{10}\text{CH}_3$, $^3J_{\text{H-H}} = 6.2$ Hz). $M_{\text{n, SEC}} = 43.6$ kDa, $D_{\text{M}} = 1.81$. $D_{\text{h}} = 11$ nm.

The HEA armed star polymers were synthesized according to the procedure outlined in the paper.

15% Crosslinked Polymer (8-15)

^1H NMR (DMSO-d_6): δ (ppm) 4.73 (br s, CH_2OH), 4.01 (br s, $\text{OCH}_2\text{CH}_2\text{OH}$ and $\text{OCH}_2\text{CH}_2\text{O}$), 3.31 (br s, $\text{OCH}_2\text{CH}_2\text{OH}$, $\text{SCH}_2(\text{CH}_2)_9$ and $\text{OCH}_2\text{CH}_2\text{O}$), 2.49 (br s, CH_2N), 2.15 (br s, $\text{N}(\text{CH}_3)_2$), 1.58-1.79 (m, CH_2 backbone, $\text{CH}_2(\text{CH}_2)_{10}$ CTA),), 0.87 (br s, 3H, $(\text{CH}_2)_{10}\text{CH}_3$). $M_{\text{n, SEC}} = 27.8$ kDa, $D_{\text{M}} = 1.25$. $D_{\text{h}} = 14$ nm.

10% Crosslinked Polymer (8-10)

^1H NMR (DMSO-d_6): δ (ppm) 4.73 (br s, CH_2OH), 4.00 (br s, $\text{OCH}_2\text{CH}_2\text{OH}$ and $\text{OCH}_2\text{CH}_2\text{O}$), 3.34 (br s, $\text{OCH}_2\text{CH}_2\text{OH}$, $\text{SCH}_2(\text{CH}_2)_9$ and $\text{OCH}_2\text{CH}_2\text{O}$), 2.44 (br s, CH_2N), 2.25 (br s, $\text{N}(\text{CH}_3)_2$), 1.39-1.90 (m, CH_2 backbone, $\text{CH}_2(\text{CH}_2)_{10}$ CTA),), 0.88 (br s, 3H, $(\text{CH}_2)_{10}\text{CH}_3$). $M_{\text{n, SEC}} = 24.9$ kDa, $D_{\text{M}} = 1.31$. $D_{\text{h}} = 25$ nm.

Typical procedure for the chain extension of PEGA with DMAEA and MA

PEGA macro-CTA (1.0 eq.), DMAEA (200 eq.) and MA (40 eq.) were dissolved in 1,4-dioxane together with radical initiator AIBN (0.2 eq.). Following four freeze-pump-thaw cycles the ampule was refilled with nitrogen and the mixture heated to 70 °C for 24 hours (67% conversion). The reaction was quenched by immersion in liquid nitrogen and purified by precipitation into 5:1 hexane/ diethyl ether, affording a viscous pale yellow liquid (57%). ¹H NMR (CDCl₃): δ (ppm) 4.15 (br s, OCH₂CH₂), 3.55-3.65 (m, OCH₃, O(CH₂CH₂)₈, and SCH₂(CH₂)₉), 3.38 (s, CH₂OCH₃), 2.54 (br s, CH₂N), 2.27 (br s, N(CH₃)₂), 1.25-1.89 (m, CH₂ backbone, CH₂(CH₂)₁₀ CTA), 0.87 (t, 3H, (CH₂)₁₀CH₃, ³J_{H-H} = 6.1 Hz). *M*_{n,SEC} = 33.5 kDa, *D*_M = 1.70.

Hydrolysis Analysis

Polymers (30 mg.) were dissolved in D₂O (0.6 mL) and stirred for 5 minutes. The solution was transferred to an NMR tube. Measurements were taken at various time intervals at both 25 °C and 50 °C. The percentage hydrolysis was calculated according to Equation S1, using the integrals for the CH₂N of the dimethylethanolamine and the corresponding polymer peak at 3.25 and 3.05 ppm for PEGA armed particles, and 2.85 and 2.64 ppm for HEA particles.

Equation S1: Hydrolysis Determination (shown for PEGA particles)

$$\% \text{ hydrolysis} = \frac{I_{3.25 \text{ ppm}}}{I_{3.25 \text{ ppm}} + I_{3.05 \text{ ppm}}}$$

2. SEC and ¹H NMR spectroscopic analysis of short armed PEGA₉₈ particles

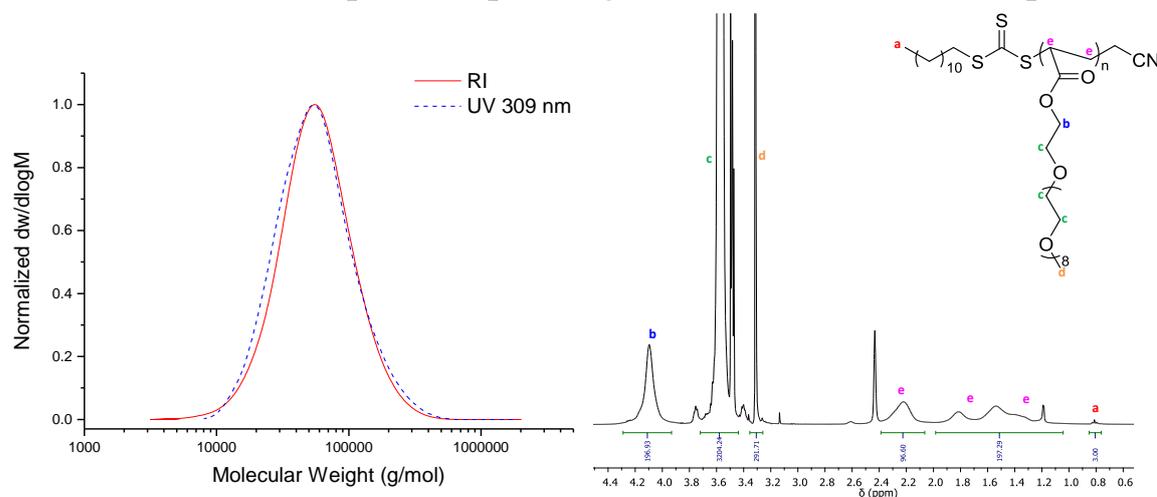


Figure S1. SEC analysis (DMF with 5 mM NH₄BF₄ eluent, with PMMA standards) and ¹H NMR spectrum (400 MHz, CDCl₃) of PEGA₉₈ arms (**1**), polymerized in 1,4-dioxane.

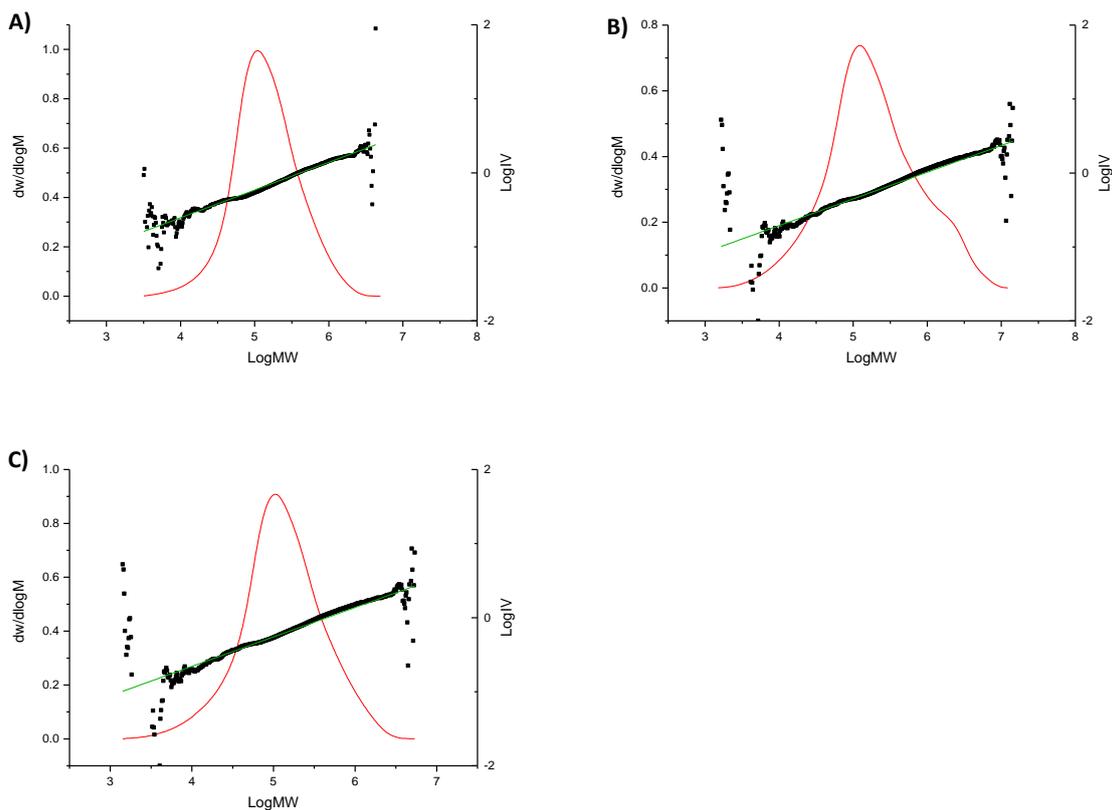


Figure S2. Triple detection SEC analysis of PEGA₉₈ armed particles **2-20** (a), **2-15** (b), and **2-10** (c) with the Mark-Houwink curve overlaid on the molecular weight distribution (DMF with 5 mM NH₄BF₄ eluent, with PMMA standards). Red line is the molecular weight distribution, black data points are the Mark-Houwink plot and the green line is the linear fit of the Mark-Houwink plot.

3. Size analysis of short armed PEGA₉₈ particles

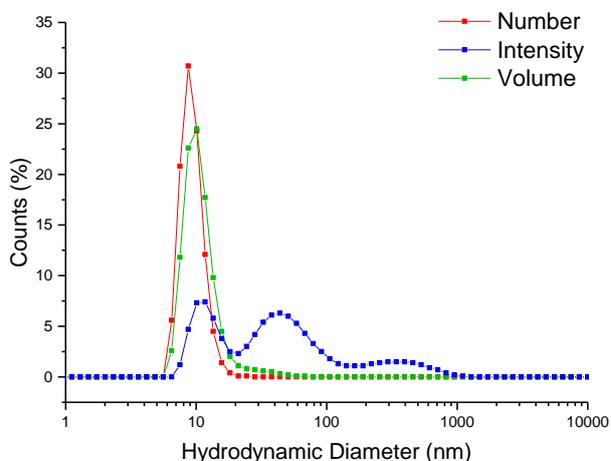


Figure S3. Size distribution, by number, intensity and volume of **2-15**, in chloroform at 5 mg /mL at 25 °C, obtained by DLS (detection angle = 173 °).

Polymer	D_h at 25 °C (nm)			D_{hat} 50 °C (nm)		
	By Number	By Volume	PD	By Number	By Volume	PD
2-20	11	14	0.347	12	15	0.383
2-15	12	12	0.460	12	14	0.327
2-10	9	12	0.278	12	13	0.309

Table S1. Size distribution analysis, by number and volume, obtained by DLS (detection angle = 173 °) in chloroform at 5 mg /mL. PD is given in brackets.

4. SEC and ^1H NMR spectroscopic analysis of PHEA₁₀₄

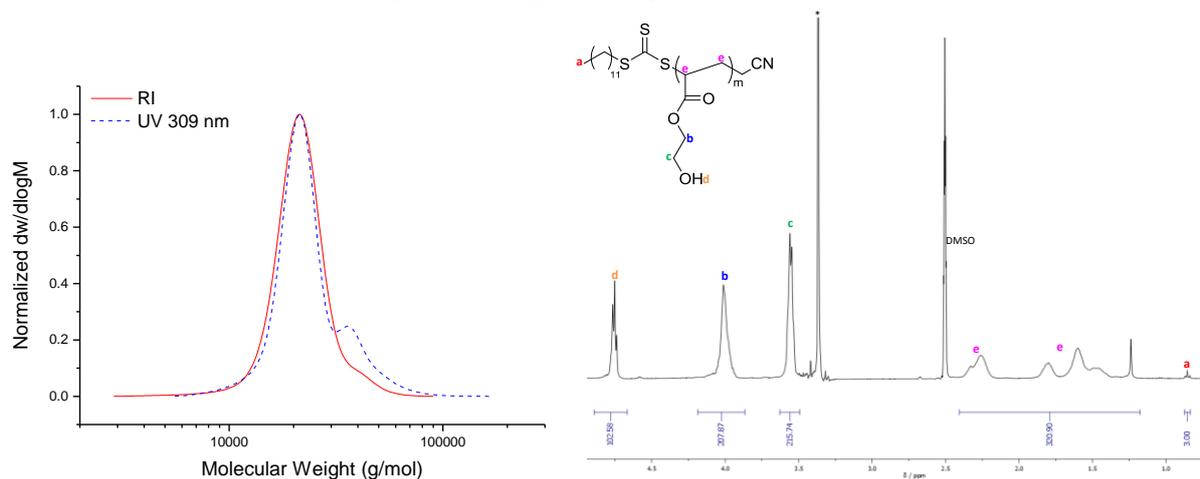


Figure S4. SEC analysis (DMF with 5 mM NH_4BF_4 eluent, with PMMA standards) and ^1H NMR spectrum (400 MHz, CDCl_3) of PHEA₁₀₄ arms (**7**), polymerized in 1,4-dioxane.

5. Analysis of PHEA₁₀₄ armed particles

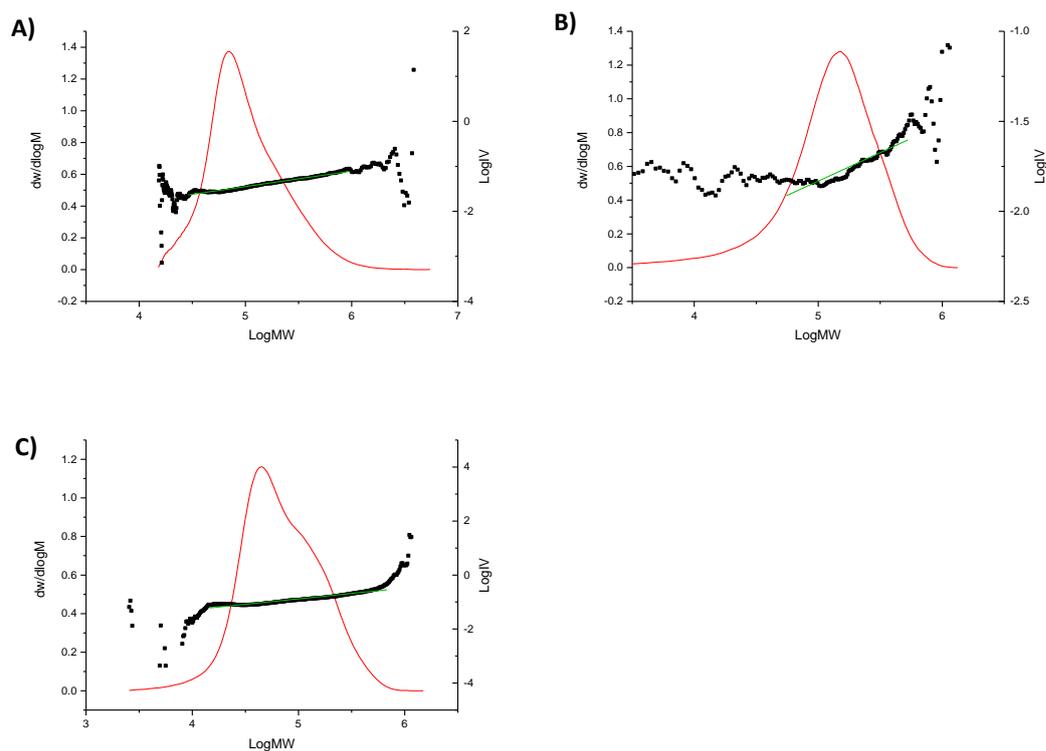


Figure S5. Triple detection SEC analysis of PHEA₁₀₄ armed particles **8-20** (a), **8-15** (b), and **8-10** (c) with the Mark-Houwink curve overlaid on the molecular weight distribution (DMF with 5 mM NH₄BF₄ eluent, with PMMA standards). Red line is the molecular weight distribution, black data points are the Mark-Houwink plot and the green line is the linear fit of the Mark-Houwink plot.

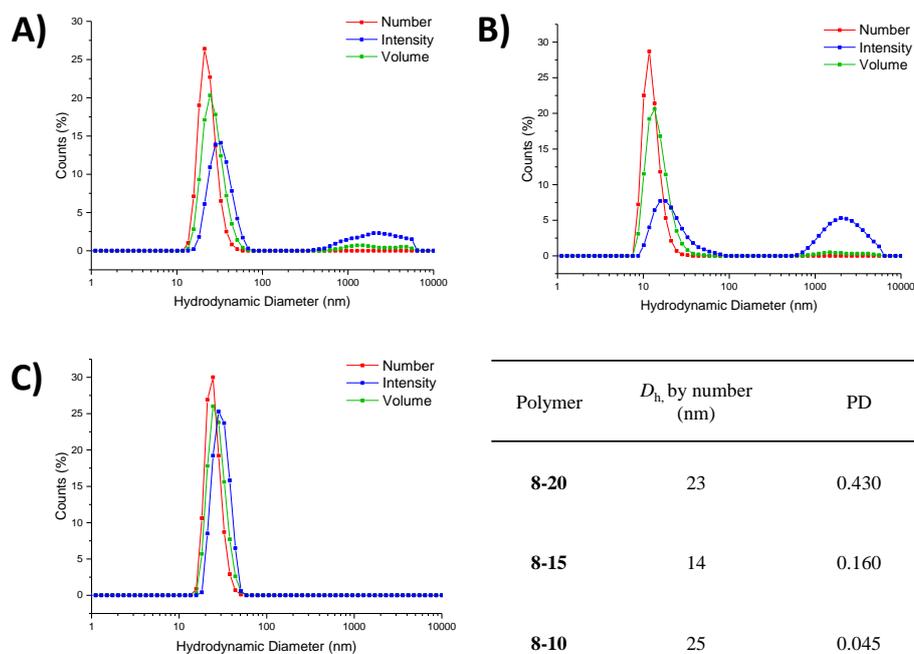


Figure S6. Size distribution of **8-20** (a), **8-15** (b) and **8-10** (c) in DMSO at 5 mg /mL at 25 °C, obtained by DLS (detection angle = 173 °).

6. Hydrolysis analysis of PEGA₉₈ armed particles by DLS

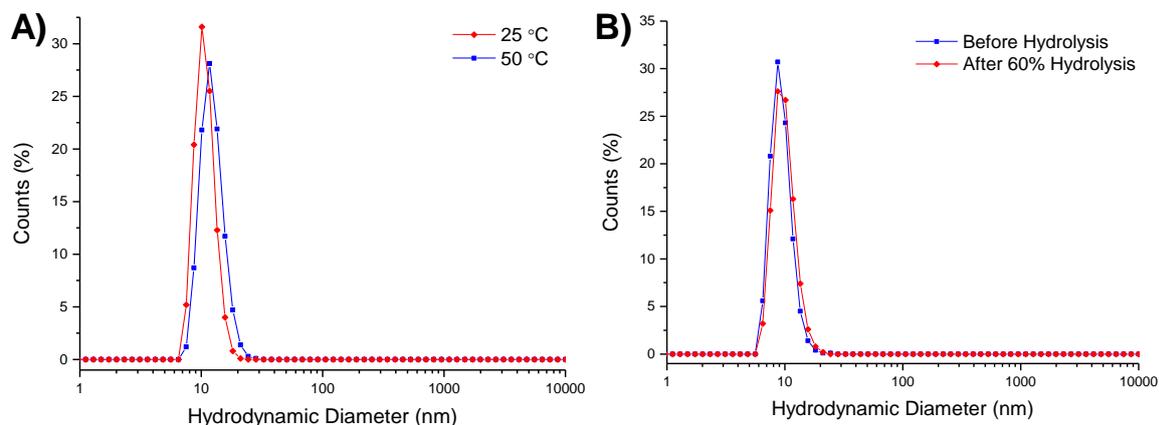


Figure S7. Size distribution, by number, of **2-20** at 5 mg /mL in chloroform. (a) at 25 °C and 50 °C before hydrolysis, and (b) before and after hydrolysis at 50 °C, obtained by DLS (detection angle = 173 °).

7. SEC analysis of medium and long armed PEGA particles

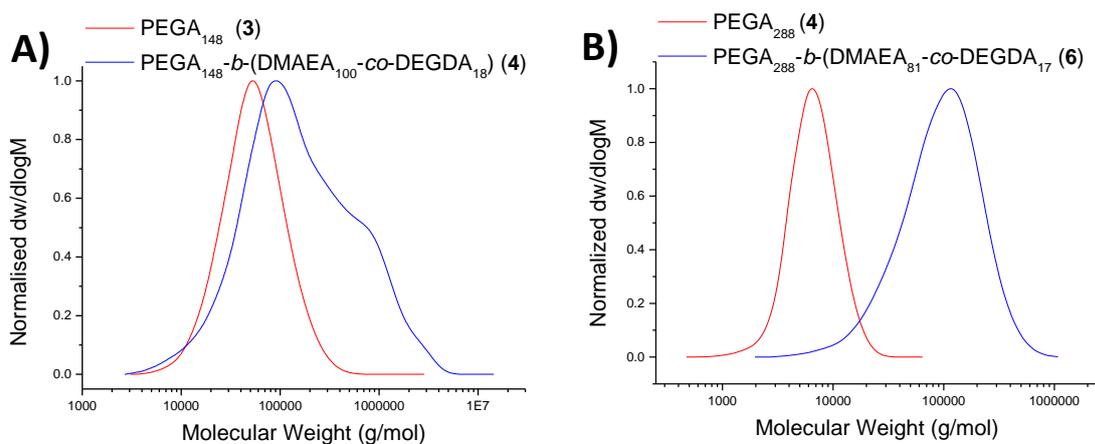


Figure S8. SEC analysis (DMF with 5 mM NH₄BF₄ eluent, with PMMA standards) of medium and long PEGA homopolymers (**3** and **5** respectively) and their corresponding stars (**4** and **6** respectively).

8. Size analysis of medium and long armed PEGA particles

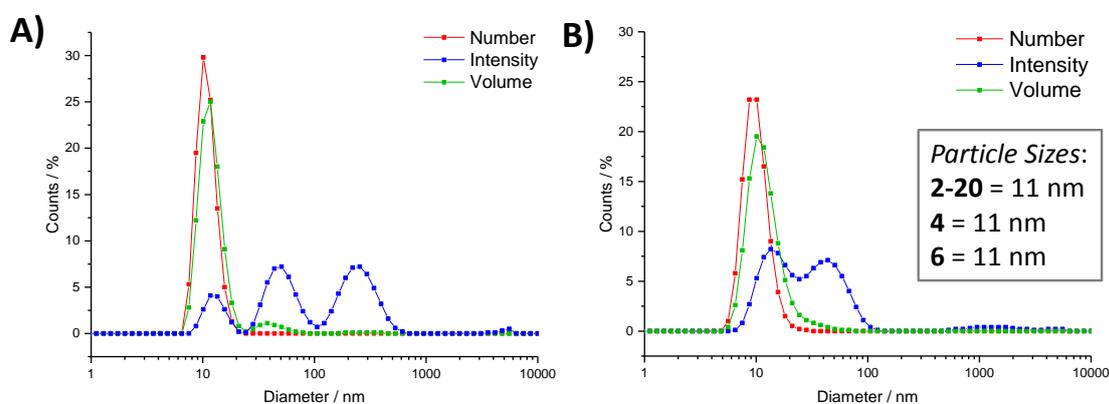


Figure S9. Size distribution of **4** (a) and **6** (b) in chloroform at 5 mg /mL at 25 °C, obtained by DLS (detection angle = 173 °).

9. Hydrolysis analysis of PHEA₁₀₄ armed particles by ¹H NMR spectroscopy

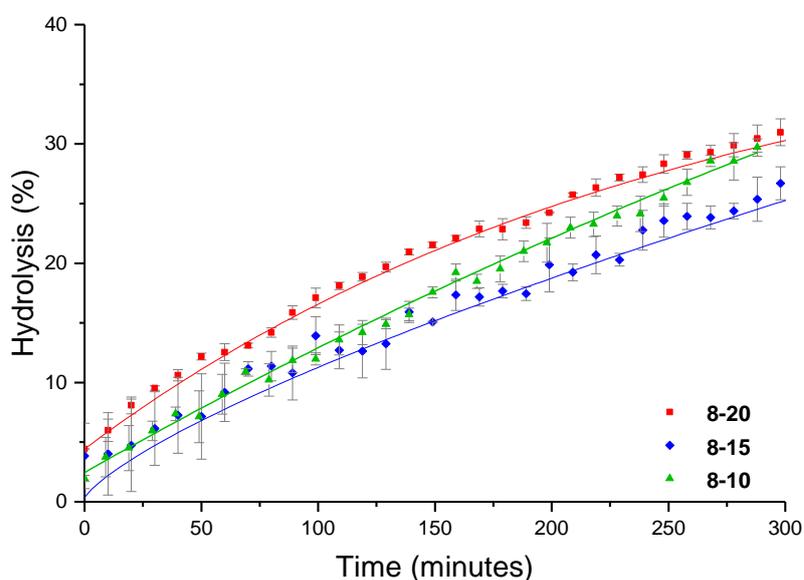


Figure S10. Hydrolysis analysis of PHEA₁₀₄ armed particles **8-20**, **8-15** and **8-10**, hydrolyzed in D₂O at 25 °C. Error bars produced from the standard deviation of 2 repeats.

10. Enzymatic determination of [DMAE]

For the production of the calibration curve, solutions of DMAE of varying concentrations (0-150 μ M in 18.2 M Ω .cm water) were produced. 50 μ L samples of each concentration (3 repeats) were added to a 96 well plate, and *p*-NPBA (4 mM in DMSO) and choline oxidase (7.5 μ M in Tris-HCl pH8 buffer) were added and the assay

analyzed for 10 hours, monitoring the initial rate of rise for the UV absorbance at 405 nm, attributed to the *p*-nitrophenol produced. Fitting of the resultant data produced a calibration of:

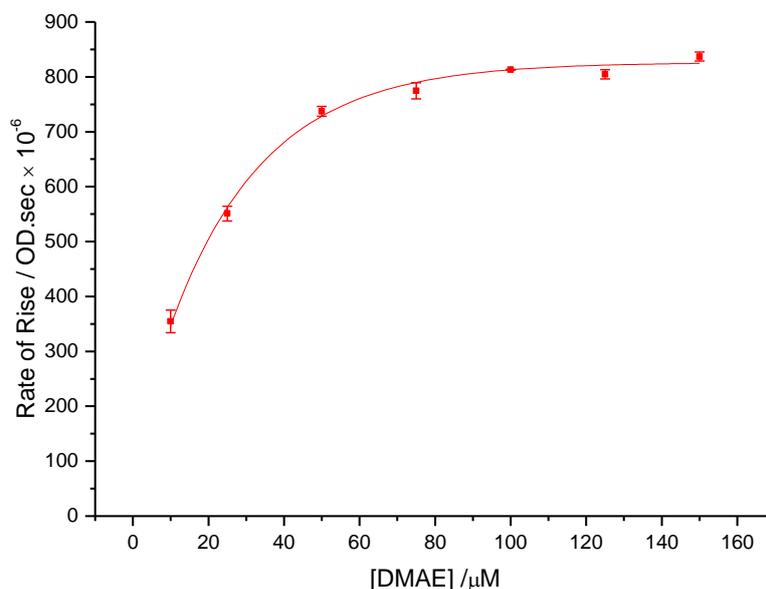


Figure S11. Calibration curve for the initial rate of rise at $\lambda = 405$ nm vs. concentration of DMAE

Equation S2: Calibration for the initial rate of rise at 405 nm vs. [DMAE]

$$\text{Rate of Rise} = 826.41 + (-712.02e^{(-0.04x)})$$

For analysis of polymer samples, preparation of the 96 well plate was as follows: at each time point a 250 μ L aliquot of the polymer solution (50 mg/ml in 18.2 M Ω .cm water) was purified twice through removal of the polymer (Corning Spin-X UF concentrators, 500 μ L, 5kDa MWCO) and the resultant supernatant stored in the freezer. Upon completion of sampling, 50 μ L samples of each time point (3 repeats) were added to a 96 well plate, and *p*-NPBA(4 mM in DMSO) was added. Following addition of all the samples and *p*-NPBA solution, choline oxidase (7.5 μ M in Tris-HCl pH8 buffer) was added and the plate analyzed for 10 hours.