Supporting Information

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

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10. Enzymatic determination of [DMAE]

Figure S11. Calibration curve

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

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.

\textit{PEGA}_{148} (3)

\textsuperscript{1}H NMR (CDCl\textsubscript{3}): $\delta$ (ppm) 4.16 (br s, OCH\textsubscript{2}CH\textsubscript{2}), 3.47-3.89 (m, O(CH\textsubscript{2}CH\textsubscript{2})\textsubscript{8} and SCH\textsubscript{2}(CH\textsubscript{2})\textsubscript{9}), 3.38 (s, CH\textsubscript{2}OCH\textsubscript{3}), 1.25-2.29 (m, CH\textsubscript{2} backbone, CH\textsubscript{2}(CH\textsubscript{2})\textsubscript{10} CTA), 0.88 (t, 3H, (CH\textsubscript{2})\textsubscript{10}CH\textsubscript{3}, $^3$J\textsubscript{H-H} = 6.0 Hz). $M\text{\textsubscript{n, SEC}} =$ 46.2 kDa, $D_M =$ 1.64.

\textit{PEGA}_{288} (5)

\textsuperscript{1}H NMR (CDCl\textsubscript{3}): $\delta$ (ppm) 4.17 (br s, OCH\textsubscript{2}CH\textsubscript{2}), 3.47-3.82 (m, O(CH\textsubscript{2}CH\textsubscript{2})\textsubscript{8} and SCH\textsubscript{2}(CH\textsubscript{2})\textsubscript{9}), 3.38 (s, CH\textsubscript{2}OCH\textsubscript{3}), 1.26-2.67 (m, CH\textsubscript{2} backbone, CH\textsubscript{2}(CH\textsubscript{2})\textsubscript{10} CTA), 0.88 (t, 3H, (CH\textsubscript{2})\textsubscript{10}CH\textsubscript{3}, $^3$J\textsubscript{H-H} = 6.1 Hz). $M\text{\textsubscript{n, SEC}} =$ 47.0 kDa, $D_M =$ 1.52.

15\% Crosslinked Polymer (2-15)

\textsuperscript{1}H NMR (CDCl\textsubscript{3}): $\delta$ (ppm) 4.17 (br s, OCH\textsubscript{2}CH\textsubscript{2}O, OCH\textsubscript{2}CH\textsubscript{2}O(CH\textsubscript{2}CH\textsubscript{2}O)\textsubscript{7}, and CH\textsubscript{2}CH\textsubscript{2}N), 3.47-3.70 (m, O(CH\textsubscript{2}CH\textsubscript{2})\textsubscript{8}, SCH\textsubscript{2}(CH\textsubscript{2})\textsubscript{9} and OCH\textsubscript{2}CH\textsubscript{2}O), 3.32 (s, CH\textsubscript{2}OCH\textsubscript{3}), 2.54 (br s, CH\textsubscript{2}N), 2.27 (br s, N(CH\textsubscript{2})\textsubscript{2}), 1.25-2.03 (m, CH\textsubscript{2} backbone, CH\textsubscript{2}(CH\textsubscript{2})\textsubscript{10} CTA), 0.88 (t, 3H, (CH\textsubscript{2})\textsubscript{10}CH\textsubscript{3}, $^3$J\textsubscript{H-H} = 6.2 Hz). $M\text{\textsubscript{n, SEC}} =$ 39.9 kDa, $D_M =$ 2.88. $D_h =$ 9 nm.
10% Crosslinked Polymer (2-10)

$^1$H NMR (CDCl$_3$): $\delta$ (ppm) 4.18 (br s, OCH$_2$CH$_2$O, OCH$_2$CH$_2$O(CH$_2$CH$_2$O)$_7$ and CH$_2$CH$_2$N), 3.45-3.80 (m, O(CH$_2$CH$_2$)$_3$, SCH$_2$(CH$_2$)$_6$ and OCH$_2$CH$_2$O), 3.32 (s, CH$_2$OCH$_3$), 2.62 (br s, CH$_2$N), 2.23 (br s, N(CH$_3$)$_2$), 1.29-2.06 (m, CH$_2$ backbone, CH$_2$(CH$_2$)$_{10}$ CTA), 0.90 (t, 3H, (CH$_2$)$_3$CH, $^3$J$_{H,H}$ = 6.0 Hz). $M_n$,SEC = 43.8 kDa, $D_M$ = 2.25. $D_h$ = 10 nm.

20% Crosslinked Polymer (4)

$^1$H NMR (CDCl$_3$): $\delta$ (ppm) 4.16 (br s, OCH$_2$CH$_2$, OCH$_2$CH$_2$O(CH$_2$CH$_2$O)$_7$ and CH$_2$CH$_2$N), 3.47-3.82 (m, O(CH$_2$CH$_2$)$_3$, SCH$_2$(CH$_2$)$_6$ and OCH$_2$CH$_2$O), 3.38 (s, CH$_2$OCH$_3$), 2.54 (br s, CH$_2$N), 2.27 (br s, N(CH$_3$)$_2$), 1.25-2.10 (m, CH$_2$ backbone, CH$_2$(CH$_2$)$_{10}$ CTA), 0.88 (t, 3H, (CH$_2$)$_3$CH, $^3$J$_{H,H}$ = 6.2 Hz). $M_n$,SEC = 66.3 kDa, $D_M$ = 1.73. $D_h$ = 11 nm.

20% Crosslinked Polymer (6)

$^1$H NMR (CDCl$_3$): $\delta$ (ppm) 4.12 (br s, OCH$_2$CH$_2$, OCH$_2$CH$_2$O(CH$_2$CH$_2$O)$_7$ and CH$_2$CH$_2$N), 3.41-3.73 (m, O(CH$_2$CH$_2$)$_3$, SCH$_2$(CH$_2$)$_6$ and OCH$_2$CH$_2$O), 3.24 (s, CH$_2$OCH$_3$), 2.57 (br s, CH$_2$N), 2.15 (br s, N(CH$_3$)$_2$), 1.13-2.00 (m, CH$_2$ backbone, CH$_2$(CH$_2$)$_{10}$ CTA), 0.78 (t, 3H, (CH$_2$)$_3$CH, $^3$J$_{H,H}$ = 6.2 Hz). $M_n$,SEC = 43.6 kDa, $D_M$ = 1.81. $D_h$ = 11 nm.

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

15% Crosslinked Polymer (8-15)

$^1$H NMR (DMSO-d$_6$): $\delta$ (ppm) 4.73 (br s, CH$_2$OH), 4.01 (br s, OCH$_2$CH$_2$OH and OCH$_2$CH$_2$O), 3.31 (br s, OCH$_2$CH$_2$OH, SCH$_2$(CH$_2$)$_6$ and OCH$_2$CH$_2$O), 2.49 (br s, CH$_2$N), 2.15 (br s, N(CH$_3$)$_2$), 1.58-1.79 (m, CH$_2$ backbone, CH$_2$(CH$_2$)$_{10}$ CTA), 0.87 (br s, 3H, (CH$_2$)$_3$CH$_3$). $M_n$,SEC = 27.8 kDa, $D_M$ = 1.25. $D_h$ = 14 nm.

10% Crosslinked Polymer (8-10)

$^1$H NMR (DMSO-d$_6$): $\delta$ (ppm) 4.73 (br s, CH$_2$OH), 4.00 (br s, OCH$_2$CH$_2$OH and OCH$_2$CH$_2$O), 3.34 (br s, OCH$_2$CH$_2$OH, SCH$_2$(CH$_2$)$_6$ and OCH$_2$CH$_2$O), 2.44 (br s, CH$_2$N), 2.25 (br s, N(CH$_3$)$_2$), 1.39-1.90 (m, CH$_2$ backbone, CH$_2$(CH$_2$)$_{10}$ CTA), 0.88 (br s, 3H, (CH$_2$)$_3$CH$_3$). $M_n$,SEC = 24.9 kDa, $D_M$ = 1.31. $D_h$ = 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, 3JH-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 (I), polymerized in 1,4-dioxane.
Figure S2. Triple detection SEC analysis of PEGA$_{98}$ 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 NH$_4$BF$_4$ 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 PEGA$_{98}$ 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 °).
<table>
<thead>
<tr>
<th>Polymer</th>
<th>$D_h$ at 25 °C (nm)</th>
<th>$D_h$ at 50 °C (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>By Number</td>
<td>By Volume</td>
</tr>
<tr>
<td>2-20</td>
<td>11</td>
<td>14</td>
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<td>2-15</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>2-10</td>
<td>9</td>
<td>12</td>
</tr>
</tbody>
</table>

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 $^1$H NMR spectroscopic analysis of PHEA$_{104}$

![SEC and $^1$H NMR spectra](image)

Figure S4. SEC analysis (DMF with 5 mM NH$_4$BF$_4$ eluent, with PMMA standards) and $^1$H NMR spectrum (400 MHz, CDCl$_3$) of PHEA$_{104}$ arms (7), polymerized in 1,4-dioxane.
5. Analysis of PHEA\(_{104}\) armed particles

![Graph A](image)

**Figure S5.** Triple detection SEC analysis of PHEA\(_{104}\) 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 NH\(_4\)BF\(_4\) 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.

![Graph B](image)

![Graph C](image)

**Table:**

<table>
<thead>
<tr>
<th>Polymer</th>
<th>(D_h) by number (nm)</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-20</td>
<td>23</td>
<td>0.430</td>
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<tr>
<td>8-15</td>
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<td>0.160</td>
</tr>
<tr>
<td>8-10</td>
<td>25</td>
<td>0.045</td>
</tr>
</tbody>
</table>

**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 PEGA$_{98}$ 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 NH$_4$BF$_4$ 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 μM in 18.2 MΩ cm water) were produced. 50 μL 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 μM 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 \( \lambda = 405 \text{ nm} \) vs. concentration of DMAE

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

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

For analysis of polymer samples, preparation of the 96 well plate was as follows: at each time point a 250 \( \mu \)L aliquot of the polymer solution (50 mg/ml in 18.2 M\( \Omega \cdot \text{cm} \) water) was purified twice through removal of the polymer (Corning Spin-X UF concentrators, 500 \( \mu \)L, 5kDa MWCO) and the resultant supernatant stored in the freezer. Upon completion of sampling, 50 \( \mu \)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 \( \mu \)M in Tris-HCl pH8 buffer) was added and the plate analyzed for 10 hours.