

Electronic Supplementary Information for:

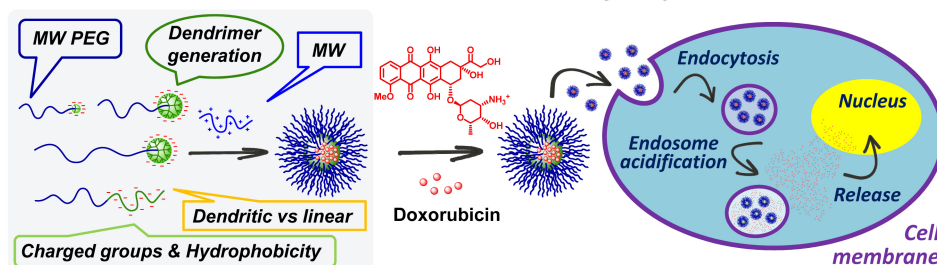
# A Dendrimer-Hydrophobic Interaction Synergy Improves the Stability of Polyion Complex Micelles

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## PIC Micelle Stabilization with Dendrimers & Hydrophobic Interactions



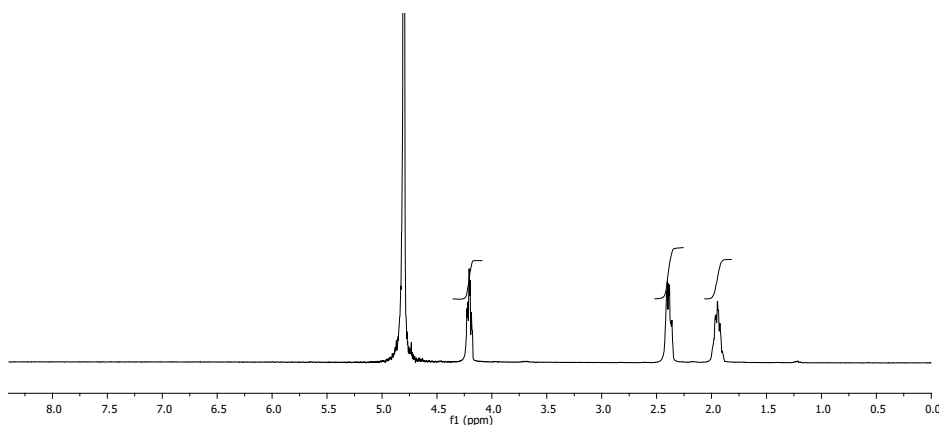
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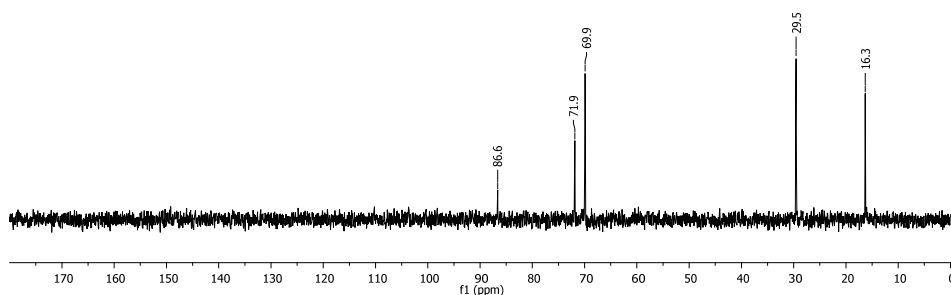
## 1. Synthesis and Characterization of New Compounds

**Sodium 4-pentyn-1-sulfate.** ClSO<sub>3</sub>H (0.40 mL, 5.94 mmol) was added dropwise to a solution of pyridine (0.98 mL, 13.1 mmol) in CHCl<sub>3</sub> (5.4 mL) at 0 °C. After 30 min of stirring, 4-pentyn-1-ol (0.55 mL, 5.94 mmol) was added, and the reaction was left at 0 °C for 4 h. Then, reaction was allowed to reach rt, and after 12 h of stirring, it was extracted with H<sub>2</sub>O (3 x 15 mL). The combined aqueous phase was evaporated to half volume, and then sat Na<sub>2</sub>CO<sub>3</sub> added till no CO<sub>2</sub> evolution was observed. The resulting mixture was concentrated, and then triturated with hot EtOH (80 mL), and filtered. The filtrate was evaporated to give sodium 4-pentyn-1-sulfate as a white powder (1.04 g, 95%).

<sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O) δ: 4.20 (dt, *J* = 2.8, 6.1 Hz, 2H), 2.52-2.25 (m, 3H), 2.07-1.81 (m, 2H). <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O) δ: 86.6, 71.9, 69.9, 29.5, 16.3.



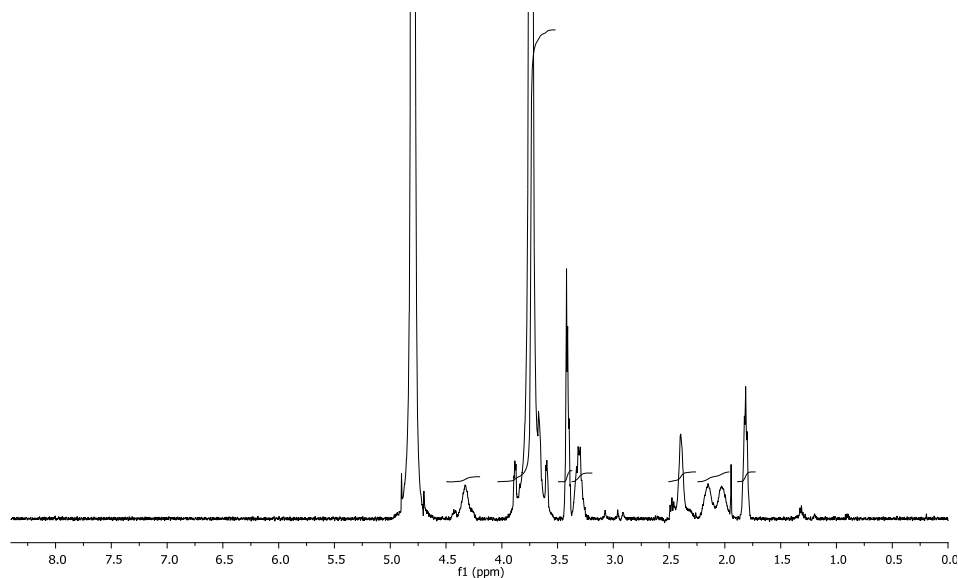
<sup>1</sup>H NMR spectrum of sodium 4-pentyn-1-sulfate



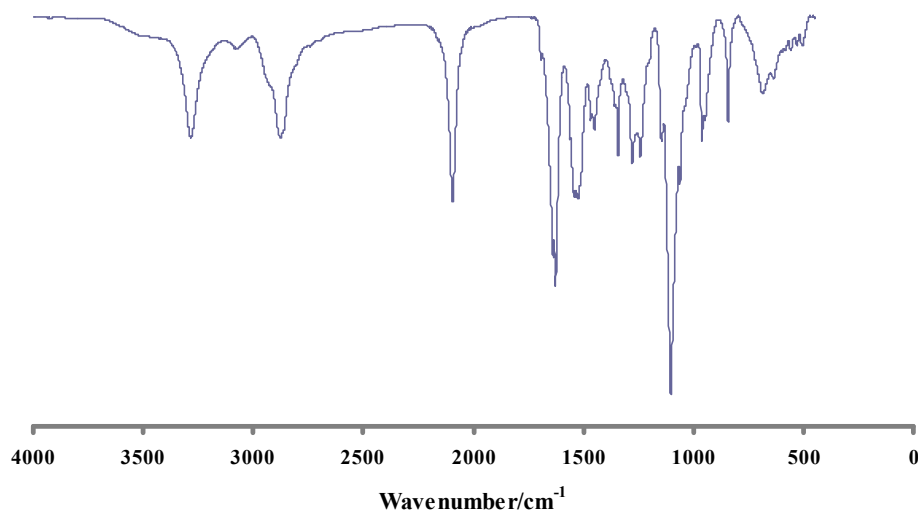
<sup>13</sup>C NMR spectrum of sodium 4-pentyn-1-sulfate

**PEG<sub>5000</sub>-PGA<sub>25</sub>-N<sub>3</sub>.** PEG<sub>5000</sub>-PGA<sub>25</sub> (10 mg, 1.14  $\mu\text{mol}$ , DP 23) and 1-azido-3-aminopropane (4.3 mg, 42.8  $\mu\text{mol}$ , 1.5 eq per carboxylate group) were dissolved in DMF (2.84 mL, final concentration of carboxylate groups 0.01 M) under Ar. HOBt (5.8 mg, 42.8  $\mu\text{mol}$ , 1.5 eq per carboxylate) and EDC (8.2 mg, 42.8  $\mu\text{mol}$ , 1.5 eq per carboxylate) were added. After 24 h of stirring at rt, the reaction mixture was poured into H<sub>2</sub>O and purified by ultrafiltration (YM1) washing with H<sub>2</sub>O (6 x 30 mL) to give PEG<sub>5000</sub>-PGA<sub>25</sub>-N<sub>3</sub> as a white powder (9.6 mg, 82%).

<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O)  $\delta$ : 4.48-4.19 (m, 23H), 3.93-3.51 (m, ~472H), 3.46-3.38 (m, 49H), 3.37-3.21 (m, 46H), 2.51-2.27 (m, 46H), 2.25-1.96 (m, 46H), 1.89-1.72 (m, 46H). IR (KBr, cm<sup>-1</sup>): 3283, 2875, 2095, 1624, 1541, 1102.



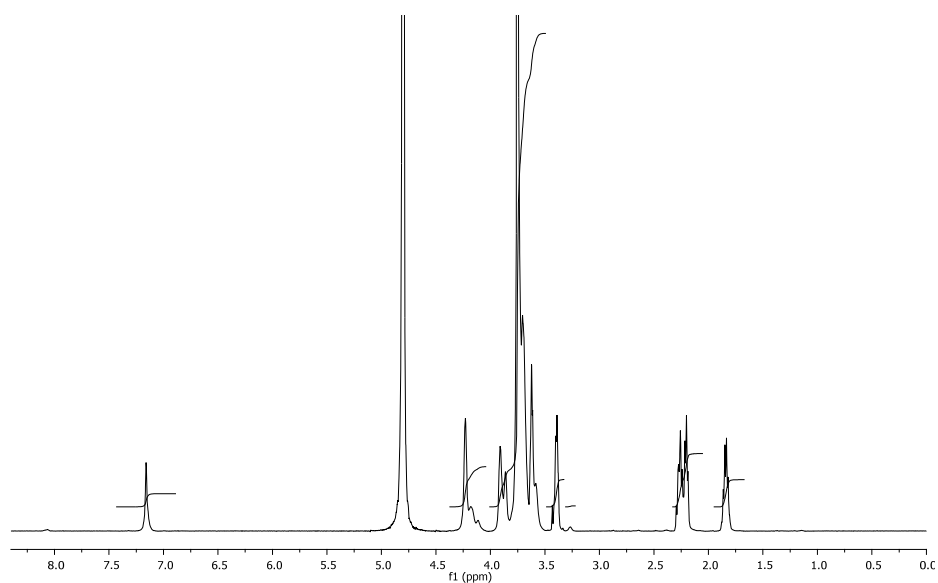
<sup>1</sup>H NMR spectrum of PEG<sub>5000</sub>-PGA<sub>25</sub>-N<sub>3</sub>



IR spectrum of PEG<sub>5000</sub>-PGA<sub>25</sub>-N<sub>3</sub>

**PEG<sub>5000</sub>-[G3]-A-CO<sub>2</sub><sup>-</sup>.** PEG<sub>5000</sub>-[G3]-NH<sub>2</sub>·HCl (20.0 mg, 1.5 μmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (0.41 mL, final concentration of amine groups 0.1 M) under Ar. Then, DIPEA (35 μL, 0.203 mmol, 5 eq per amine) and glutaric anhydride (23.2 mg, 0.203 mmol, 5 eq per amine) were added. After stirring overnight, the reaction mixture was concentrated, dissolved in MeOH and poured into diluted aq. NaHCO<sub>3</sub>. The resulting mixture was purified by ultrafiltration (YM3) washing with H<sub>2</sub>O (5 x 30 mL) to give PEG<sub>5000</sub>-[G3]-A-CO<sub>2</sub>Na as a white foam after freeze-drying (22.5 mg, 97%).

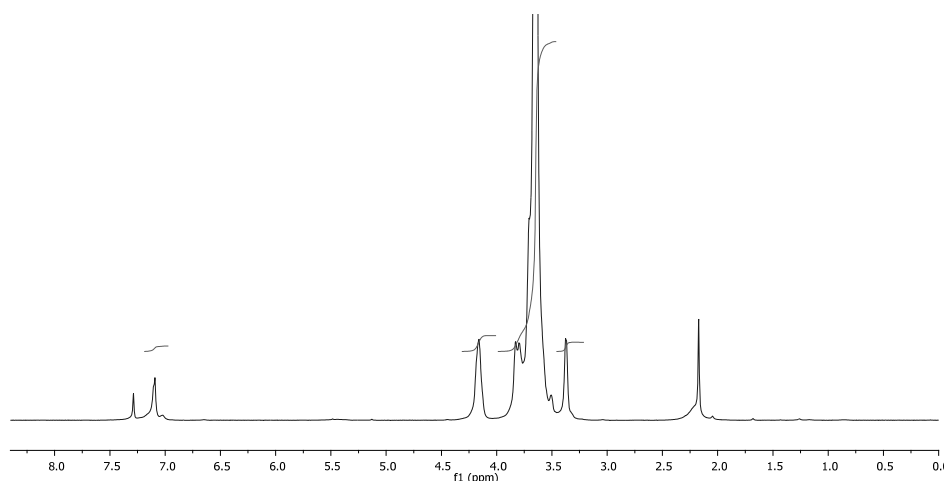
<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ: 7.16 (s, 26H), 4.38-4.04 (m, 80H), 4.01-3.51 (m, ~796H), 3.49-3.32 (m, 57H), 3.26 (br s, 2H), 2.34-2.09 (m, 108H), 1.94-1.72 (m, 54H).



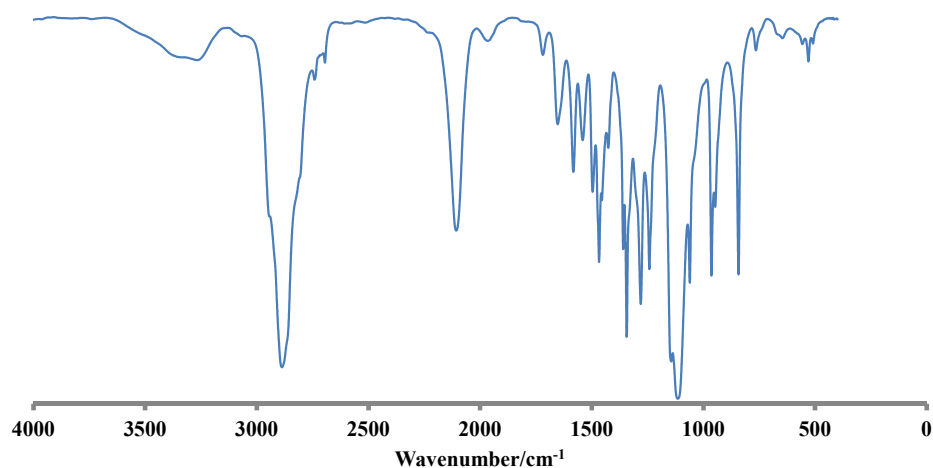
<sup>1</sup>H NMR spectrum of PEG<sub>5000</sub>-[G3]-A-CO<sub>2</sub><sup>-</sup>

**PEG<sub>10000</sub>-[G3]-N<sub>3</sub>.** PEG<sub>10000</sub>-[G2]-NH<sub>2</sub>·HCl (123 mg, 9.82 μmol), GATG repeating unit (73.7 mg, 115 μmol, 1.3 eq per amine) and Et<sub>3</sub>N (24.5 μL, 177 μmol, 1.5 eq per amine) were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (0.83 mL, final concentration of amine groups 0.1 M) under Ar. Then, HOBt (15.5 mg, 115 μmol, 1.3 eq per amine) and EDC·HCl (21.9 mg, 115 μmol, 1.3 eq per amine) were added. After 24 h of stirring at rt, the reaction mixture was evaporated under reduced pressure and purified by precipitation (MeOH/iPrOH) to afford PEG<sub>10000</sub>-[G3]-N<sub>3</sub> (158 mg, 90%) as a white powder.

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ: 7.19-6.97 (m, 26H), 4.27-4.05 (m, 80H), 3.97-3.45 (m, ~1240H), 3.44-3.26 (m, 59H). IR (KBr, cm<sup>-1</sup>): 3267, 2887, 2107, 1115.



<sup>1</sup>H NMR spectrum of PEG<sub>10000</sub>-[G3]-N<sub>3</sub>

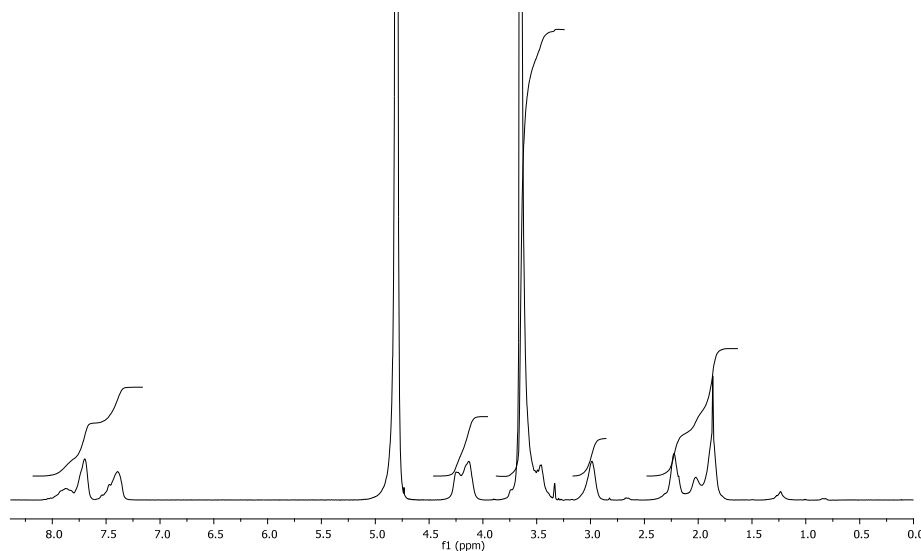


IR spectrum of PEG<sub>10000</sub>-[G3]-N<sub>3</sub>

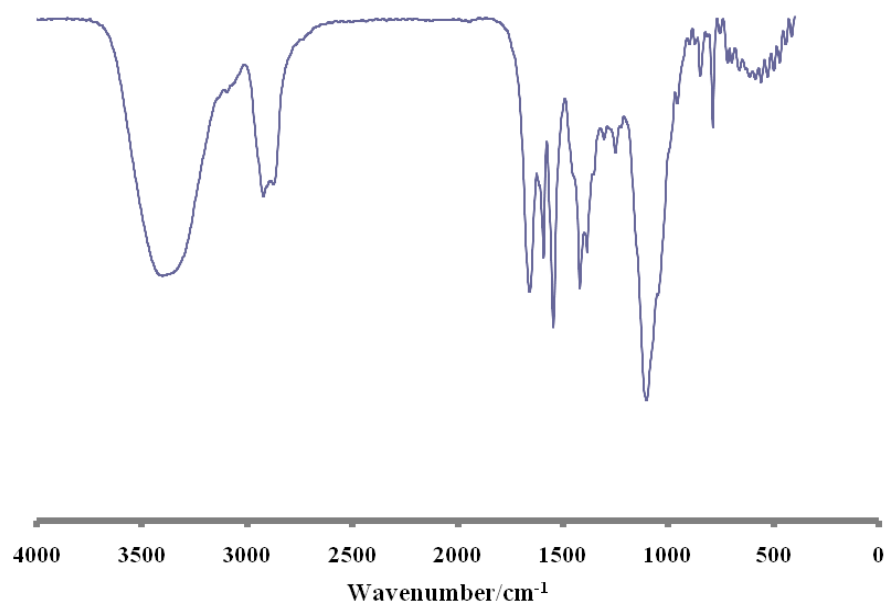
**General Procedure for CuAAC Reactions.** Azide containing block copolymers and alkynes (200 mol % per terminal azide groups) were dissolved in DMF/H<sub>2</sub>O or *t*-BuOH/H<sub>2</sub>O mixtures. Then, freshly prepared aqueous solutions of CuSO<sub>4</sub> (5 mol % per azide, 0.1 M) and sodium ascorbate (25 mol % per azide, 0.5 M) were added (final concentration of terminal azides 0.1 M). After 24 h of stirring at rt protected from light, reaction mixtures were purified by ultrafiltration (YM3) washing with aq 0.1 M EDTA pH6 (2 x 30 mL), sat NaHCO<sub>3</sub> (1 x 30 mL), and H<sub>2</sub>O (5 x 30 mL) to afford the desired products after freeze-drying.

**PEG<sub>5000</sub>-PGA<sub>25</sub>-PhCO<sub>2</sub><sup>-</sup>.** Starting from PEG<sub>5000</sub>-PGA<sub>25</sub>-N<sub>3</sub> (9.4 mg, 0.91 μmol), 4-ethynyl benzoic acid (6.7 mg, 45.6 μmol), NaHCO<sub>3</sub> (7.7 mg, 91.2 μmol), CuSO<sub>4</sub> (22.8 μL, 1.14 μmol, 0.05 M) and sodium ascorbate (28.5 μL, 5.7 μmol, 0.2 M) dissolved in *t*-BuOH (0.11 mL)/H<sub>2</sub>O (0.06 mL) and following the general procedure for CuAAC reactions, PEG<sub>5000</sub>-PGA<sub>25</sub>-PhCO<sub>2</sub>Na (10.2 mg, 77%; ultrafiltration with YM1) was obtained as a pale yellow foam.

<sup>1</sup>H NMR (750 MHz, D<sub>2</sub>O) δ: 8.18-7.16 (m, 115H), 4.46-3.95 (m, 69H), 3.88-3.24 (m, ~475H), 2.99 (br s, 46H), 2.48-1.63 (m, 138H). IR (KBr, cm<sup>-1</sup>): 3402, 2924, 1657, 1545, 1420, 1101.



<sup>1</sup>H NMR spectrum of PEG<sub>5000</sub>-PGA<sub>25</sub>-PhCO<sub>2</sub><sup>-</sup>

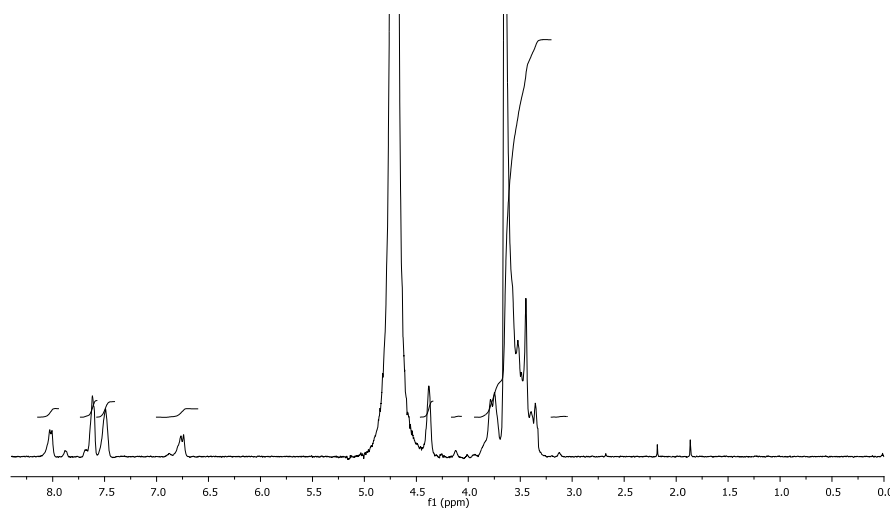


IR spectrum of PEG<sub>5000</sub>-PGA<sub>25</sub>-PhCO<sub>2</sub><sup>-</sup>

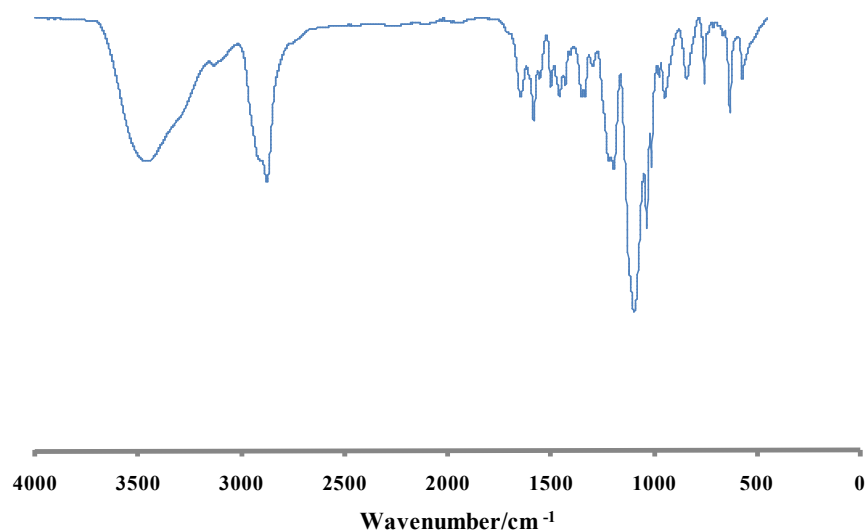


**PEG<sub>5000</sub>-[G3]-PhSO<sub>3</sub><sup>-</sup>.** Starting from PEG<sub>5000</sub>-[G3]-N<sub>3</sub> (15 mg, 1.15 μmol), ammonium 4-ethynyl benzene sulfonate (12.4 mg, 62.2 μmol), CuSO<sub>4</sub> (15.6 μL, 1.56 μmol, 0.1 M) and sodium ascorbate (15.6 μL, 7.8 μmol, 0.5 M) dissolved in DMF (0.16 mL)/H<sub>2</sub>O (0.12 mL) and following the general procedure for CuAAC reactions, PEG<sub>5000</sub>-[G3]-PhSO<sub>3</sub>Na (18.7 mg, 87%) was obtained as a pale yellow foam.

<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ: 8.15-7.94 (m, 27H), 7.62 (br s, 54H), 7.49 (br s, 54H), 6.96-6.63 (m, 26H), 4.38 (br s, 54H), 4.12 (br s, 2H), 3.94-3.21 (m, ~877H), 3.12 (br s, 2H). IR (ATR, cm<sup>-1</sup>): 3454, 2873, 1194, 1095.



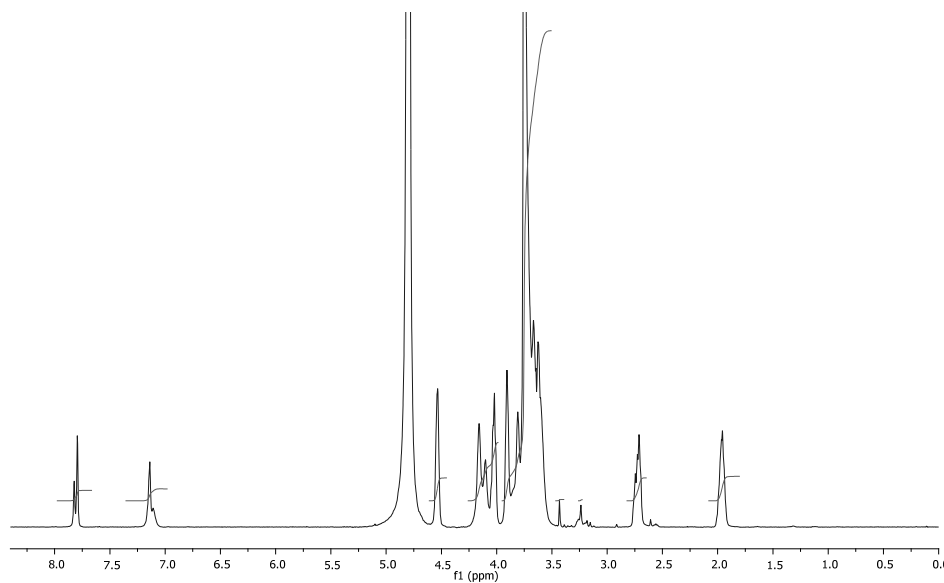
<sup>1</sup>H NMR spectrum of PEG<sub>5000</sub>-[G3]-PhSO<sub>3</sub><sup>-</sup>



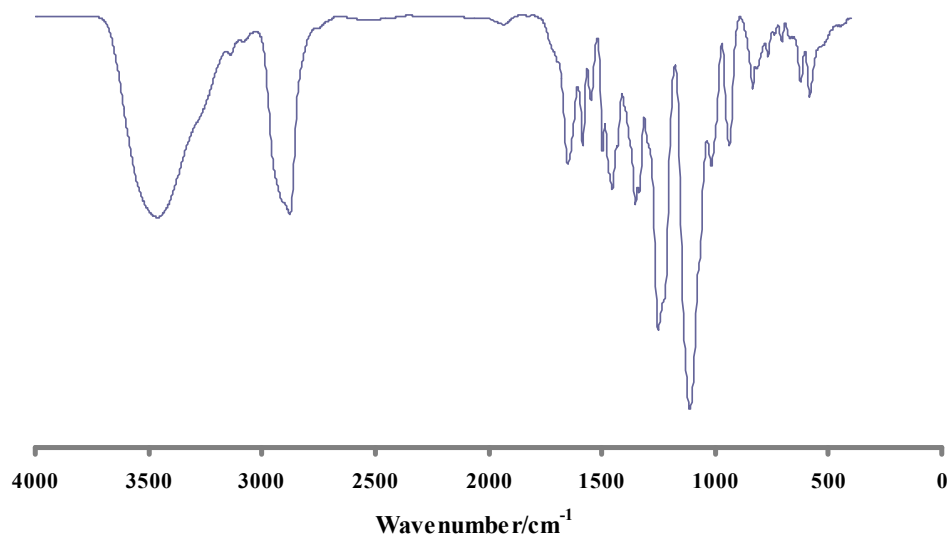
IR spectrum of PEG<sub>5000</sub>-[G3]-PhSO<sub>3</sub><sup>-</sup>

**PEG<sub>5000</sub>-[G3]-OSO<sub>3</sub><sup>-</sup>.** Starting from PEG<sub>5000</sub>-[G3]-N<sub>3</sub> (30 mg, 2.31 μmol), sodium 4-pentyn-1-sulfate (23.1 mg, 125 μmol), CuSO<sub>4</sub> (31.2 μL, 3.12 μmol, 0.1 M) and sodium ascorbate (31.2 μL, 15.6 μmol, 0.5 M) dissolved in DMF (0.31 mL)/H<sub>2</sub>O (0.25 mL) and following the general procedure for CuAAC reactions, PEG<sub>5000</sub>-[G3]-OSO<sub>3</sub>Na (39.8 mg, 96%) was obtained as a pale yellow foam.

<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ: 7.98-7.66 (m, 27H), 7.36-6.98 (m, 26H), 4.54 (br s, 54H), 4.26-3.98 (m, 134H), 3.95-3.50 (m, ~794H), 3.43 (s, 3H), 3.24 (br s, 2H), 2.82-2.64 (m, 54H), 2.08-1.85 (m, 54H). IR (KBr, cm<sup>-1</sup>): 3458, 2878, 1249, 1113.

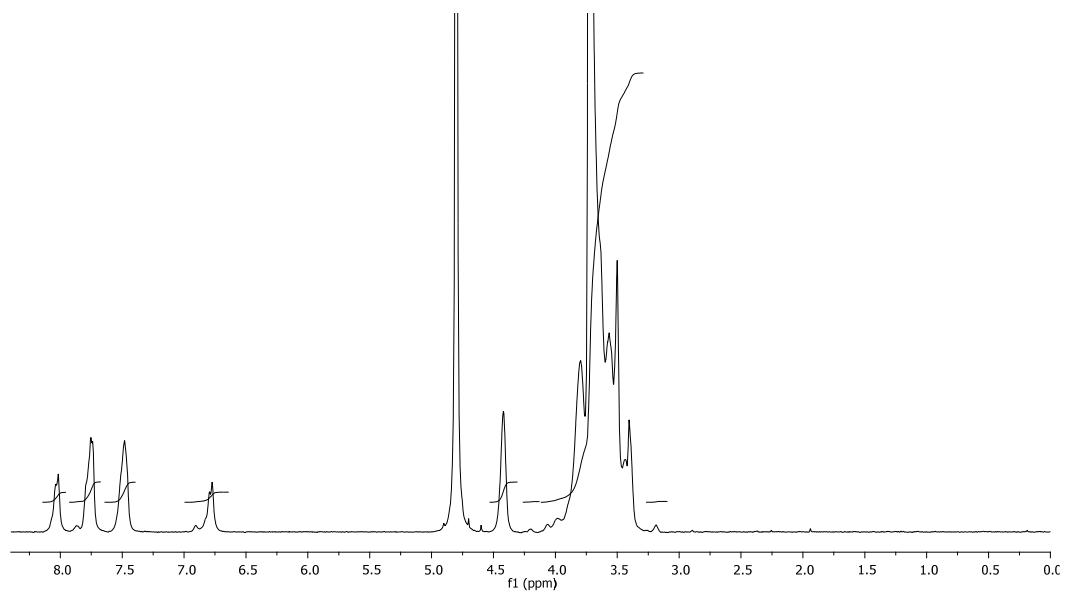


<sup>1</sup>H NMR spectrum of PEG<sub>5000</sub>-[G3]-OSO<sub>3</sub><sup>-</sup>

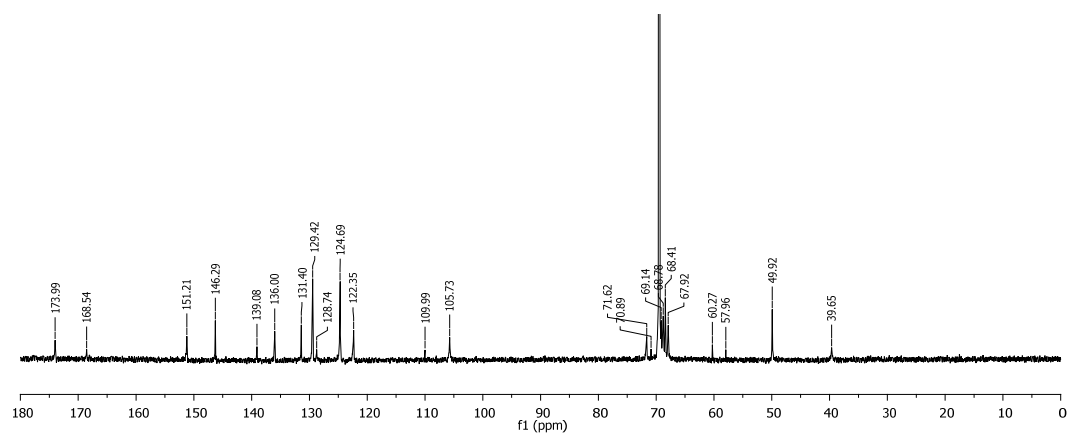


IR spectrum of PEG<sub>5000</sub>-[G3]-OSO<sub>3</sub><sup>-</sup>

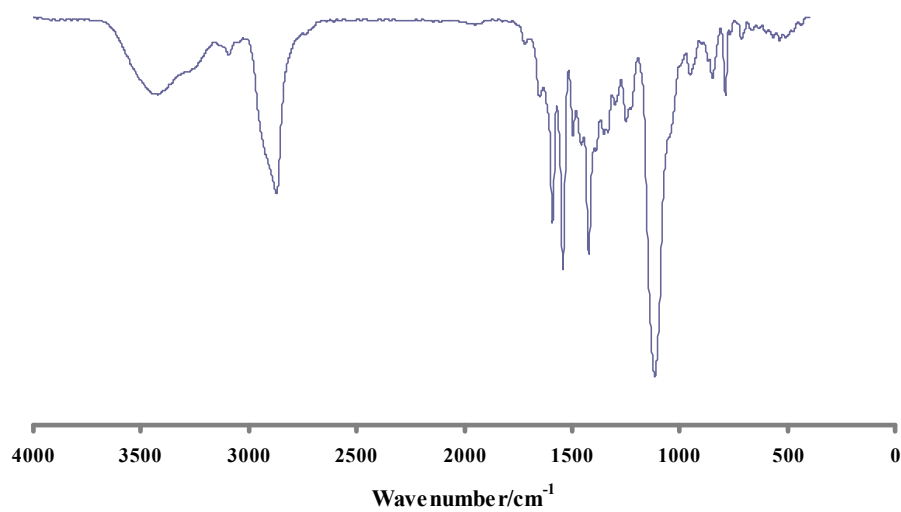
**PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup>**



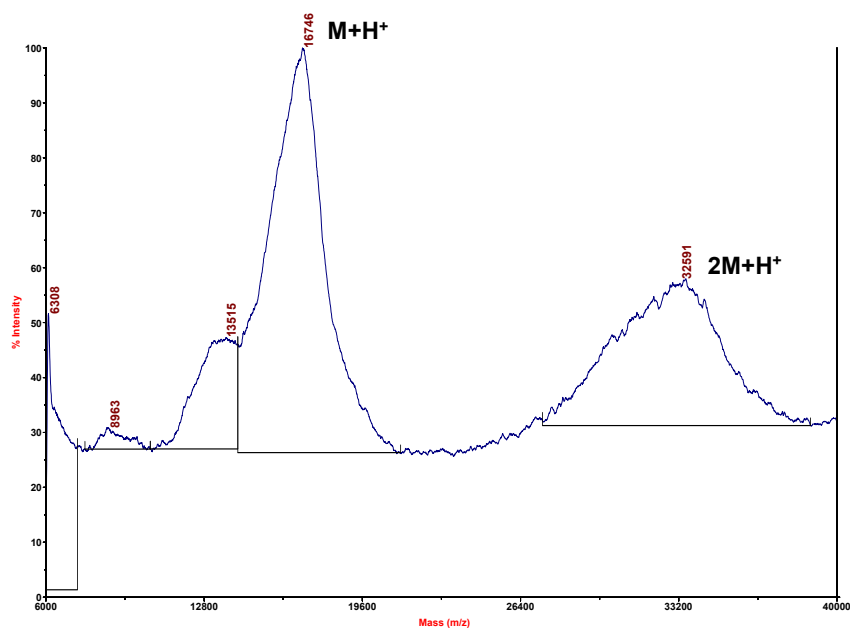
<sup>1</sup>H NMR spectrum of PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup>



<sup>13</sup>C NMR spectrum of PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup>



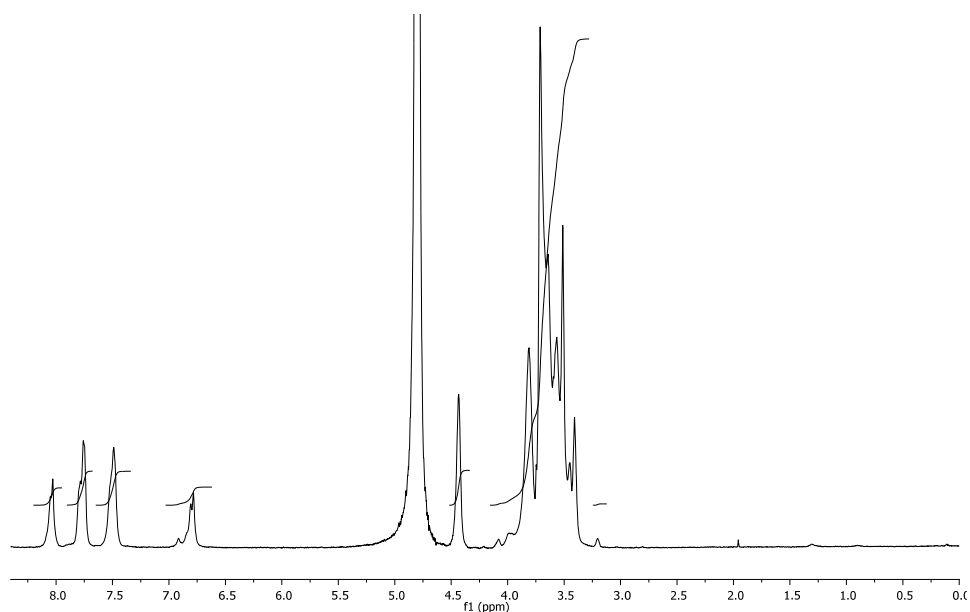
IR spectrum of PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup>



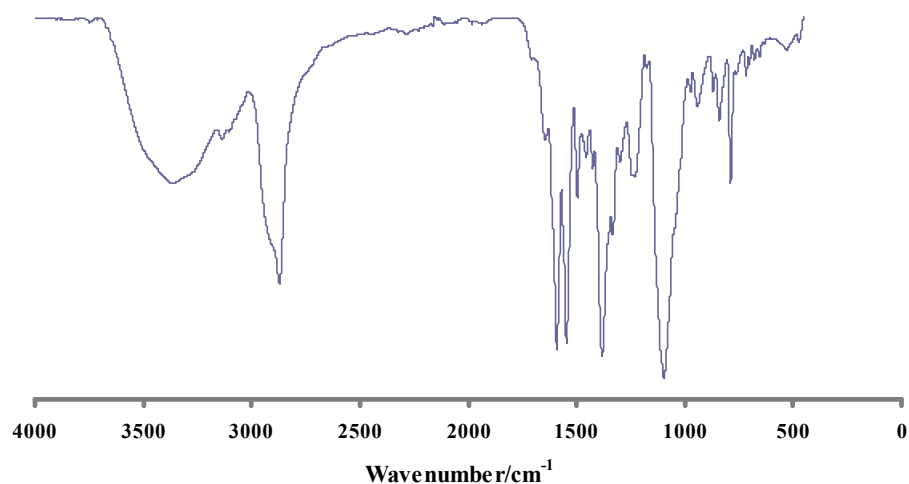
MALDI-TOF MS of PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup>

**PEG<sub>2000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup>.** Starting from PEG<sub>2000</sub>-[G3]-N<sub>3</sub> (21.0 mg, 21.0 μmol), 4-ethynyl benzoic acid (19.1 mg, 113 μmol), NaHCO<sub>3</sub> (19.1 mg, 227 μmol), CuSO<sub>4</sub> (28.4 μL, 2.84 μmol, 0.1 M) and sodium ascorbate (28.4 μL, 14.2 μmol, 0.5 M) dissolved in DMF (0.28 mL)/H<sub>2</sub>O (0.23 mL) and following the general procedure for CuAAC reactions, PEG<sub>2000</sub>-[G3]-PhCO<sub>2</sub>Na (25.3 mg, 83%) was obtained as a pale yellow foam.

<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ: 8.20-7.94 (m, 27H), 7.90-7.67 (m, 54H), 7.49 (br s, 54H), 7.03-6.62 (m, 26H), 4.44 (br s, 54H), 4.17-3.28 (m, ~607H), 3.20 (br s, 2H). IR (ATR, cm<sup>-1</sup>): 3366, 2871, 1590, 1546, 1382, 1096.



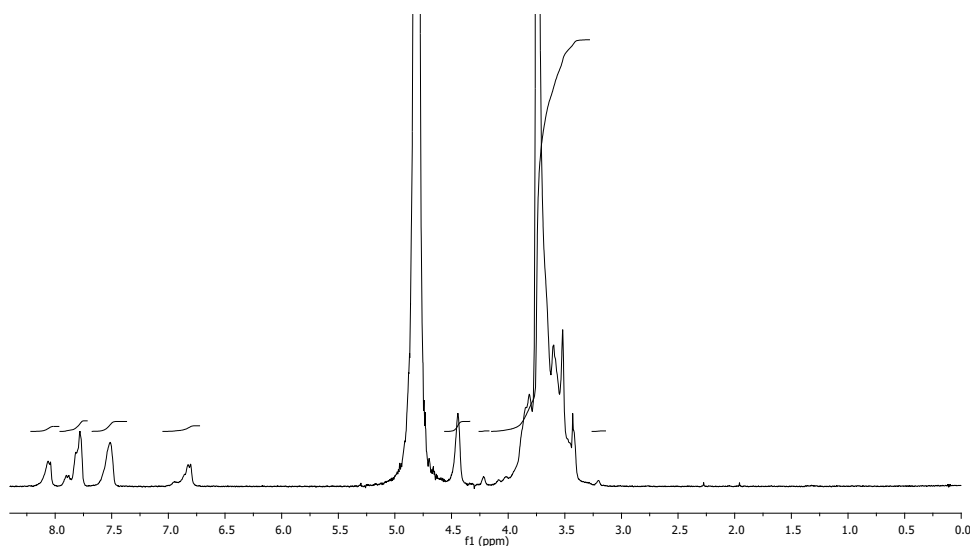
<sup>1</sup>H NMR spectrum of PEG<sub>2000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup>



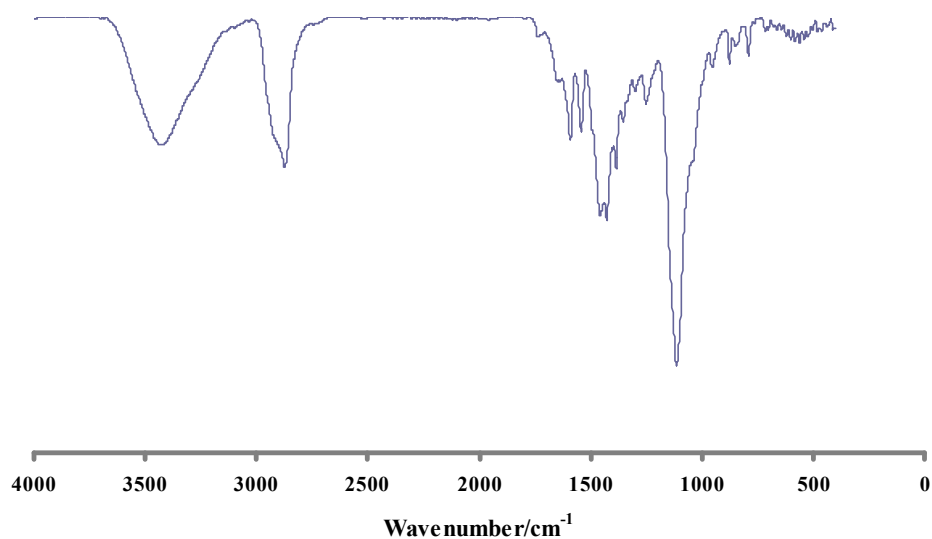
IR spectrum of PEG<sub>2000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup>

**PEG<sub>10000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup>.** Starting from PEG<sub>10000</sub>-[G3]-N<sub>3</sub> (20.0 mg, 1.11 μmol), 4-ethynyl benzoic acid (8.8 mg, 60.2 μmol), NaHCO<sub>3</sub> (10.1 mg, 120 μmol), CuSO<sub>4</sub> (15.1 μL, 1.51 μmol, 0.1 M) and sodium ascorbate (15.1 μL, 7.55 μmol, 0.5 M) dissolved in DMF (0.15 mL)/H<sub>2</sub>O (0.12 mL) and following the general procedure for CuAAC reactions, PEG<sub>10000</sub>-[G3]-PhCO<sub>2</sub>Na (24.9 mg, 99%) was obtained as a pale yellow foam.

<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ: 8.22-7.97 (m, 27H), 7.96-7.70 (m, 54H), 7.68-7.36 (m, 54H), 7.03-6.69 (m, 26H), 4.45 (br s, 54H), 4.22 (br s, 2H), 4.15-3.28 (m, ~1321H), 3.20 (br s, 2H). IR (KBr, cm<sup>-1</sup>): 3424, 2872, 1589, 1454, 1113.



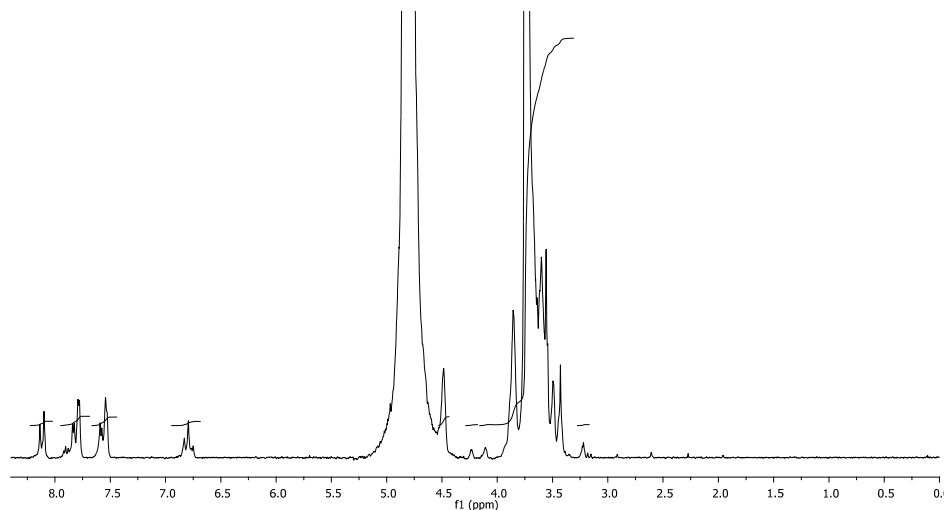
<sup>1</sup>H NMR spectrum of PEG<sub>10000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup>



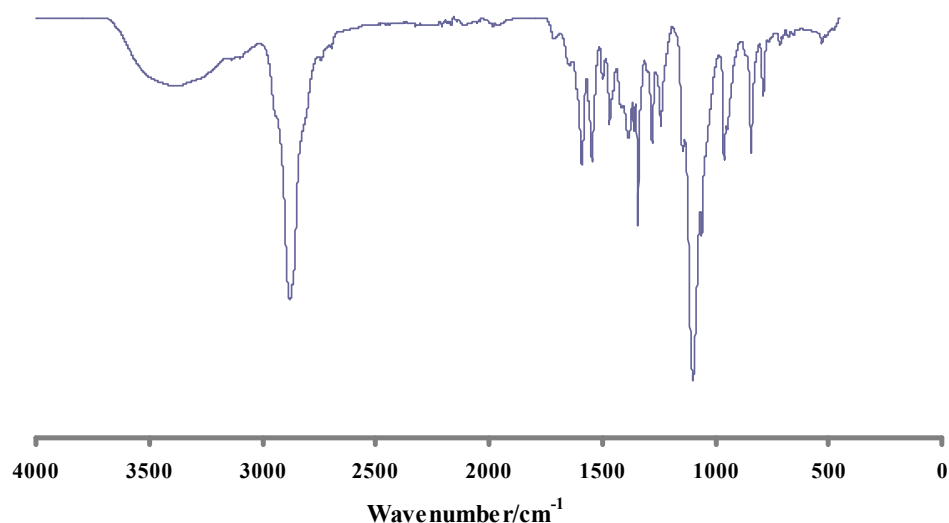
IR spectrum of PEG<sub>10000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup>

**PEG<sub>5000</sub>-[G2]-PhCO<sub>2</sub><sup>-</sup>.** Starting from PEG<sub>5000</sub>-[G2]-N<sub>3</sub> (16.4 mg, 2.1 μmol), 4-ethynyl benzoic acid (6.5 mg, 38.6 μmol), NaHCO<sub>3</sub> (6.5 mg, 77.6 μmol), CuSO<sub>4</sub> (19.3 μL, 0.97 μmol, 0.05 M) and sodium ascorbate (24.2 μL, 4.8 μmol, 0.2 M) dissolved in DMF (0.10 mL)/H<sub>2</sub>O (0.05 mL) and following the general procedure for CuAAC reactions, PEG<sub>5000</sub>-[G2]-PhCO<sub>2</sub><sup>-</sup> (16.8 mg, 85%; ultrafiltration with YM1) obtained as a pale yellow foam.

<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ: 8.23-8.02 (m, 9H), 7.95-7.68 (m, 18H), 7.66-7.47 (m, 18H), 6.95-6.68 (m, 8H), 4.48 (br s, 18H), 4.23 (br s, 2H), 4.16-3.31 (m, ~589H), 3.22 (br s, 2H). IR (ATR, cm<sup>-1</sup>): 3373, 2879, 1589, 1544, 1342, 1143.



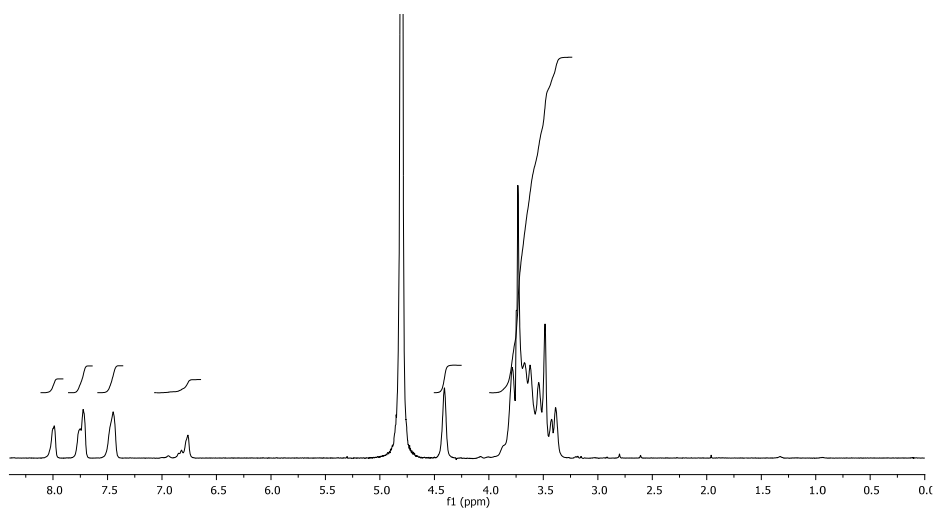
<sup>1</sup>H NMR spectrum of PEG<sub>5000</sub>-[G2]-PhCO<sub>2</sub><sup>-</sup>



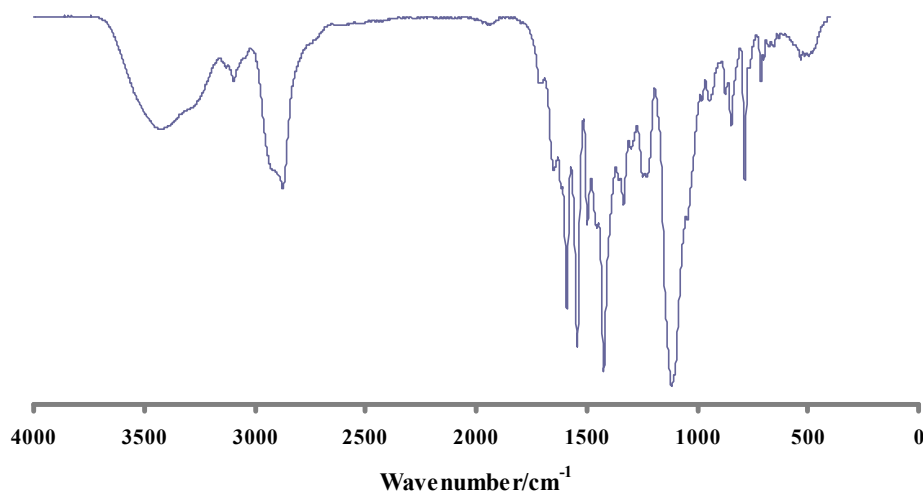
IR spectrum of PEG<sub>5000</sub>-[G2]-PhCO<sub>2</sub><sup>-</sup>

**PEG<sub>5000</sub>-[G4]-PhCO<sub>2</sub><sup>-</sup>.** Starting from PEG<sub>5000</sub>-[G4]-N<sub>3</sub> (20.0 mg, 0.69 μmol), 4-ethynyl benzoic acid (16.2 mg, 111 μmol), NaHCO<sub>3</sub> (18.7 mg, 222 μmol), CuSO<sub>4</sub> (27.8 μL, 2.78 μmol, 0.1 M) and sodium ascorbate (27.8 μL, 13.9 μmol, 0.5 M) dissolved in DMF (0.28 mL)/H<sub>2</sub>O (0.22 mL) and following the general procedure for CuAAC reactions, PEG<sub>5000</sub>-[G4]-PhCO<sub>2</sub>Na (22.2 mg, 79%) was obtained as yellow foam.

<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ: 8.00 (br s, 81H), 7.84-7.67 (m, 162H), 7.45 (br s, 162H), 6.99-6.67 (m, 80H), 4.41 (br s, 162H), 4.12-3.27 (m, ~1745H). IR (KBr, cm<sup>-1</sup>): 3416, 2874, 1589, 1541, 1421, 1115.



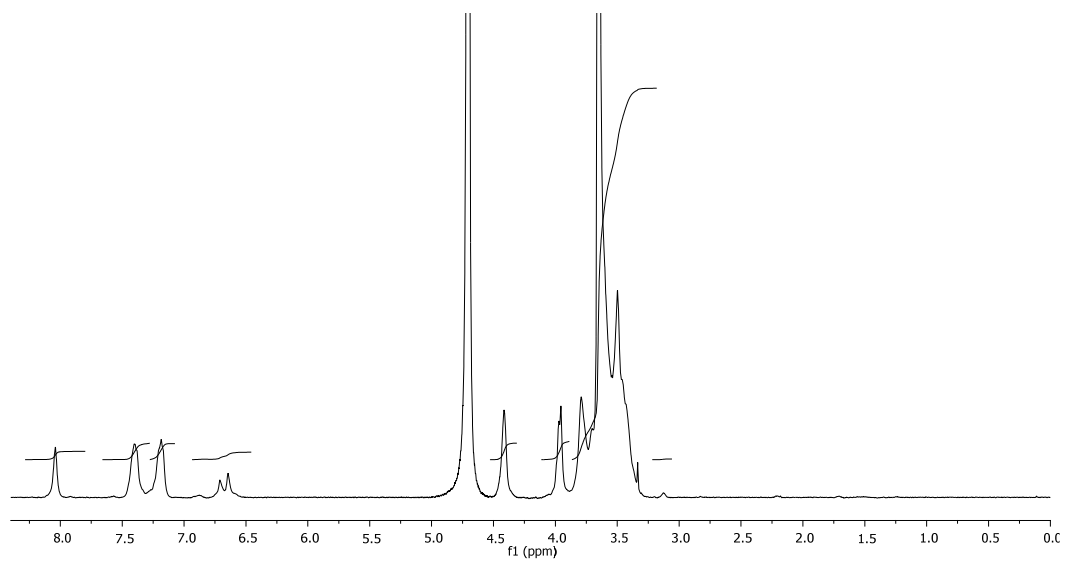
<sup>1</sup>H NMR spectrum of PEG<sub>5000</sub>-[G4]-PhCO<sub>2</sub><sup>-</sup>



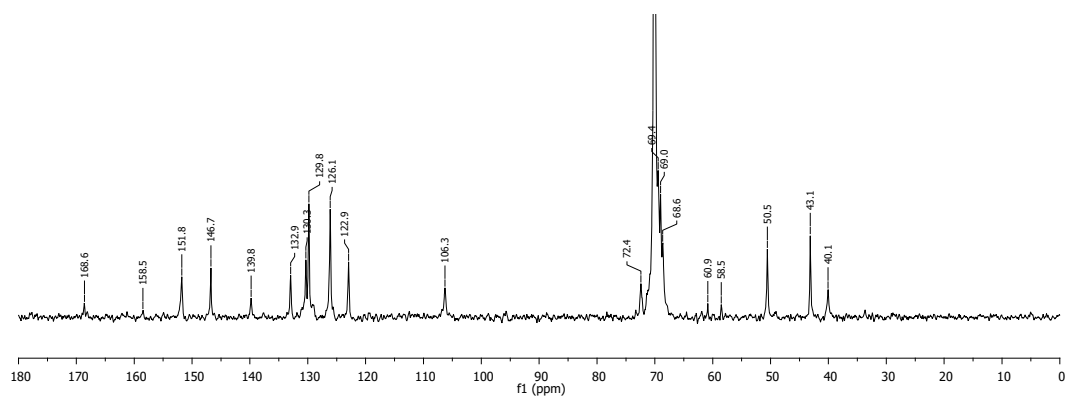
IR spectrum of PEG<sub>5000</sub>-[G4]-PhCO<sub>2</sub><sup>-</sup>



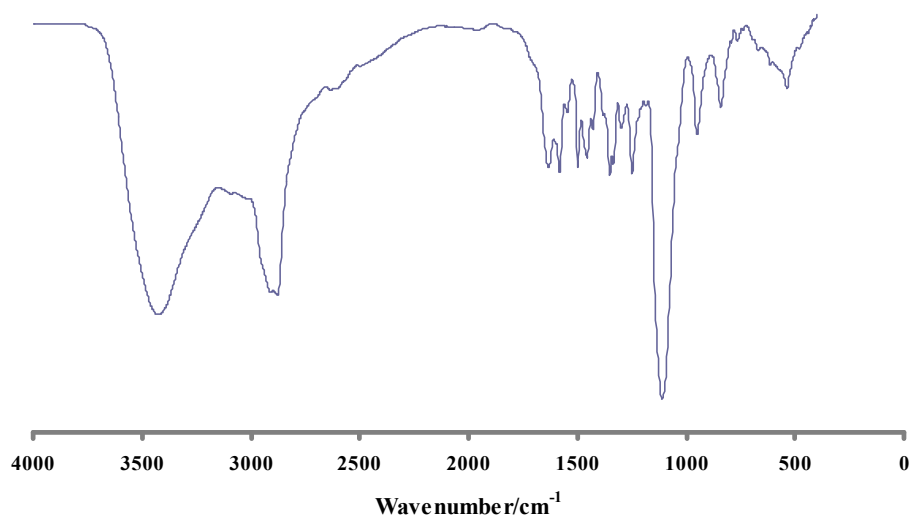
**PEG<sub>5000</sub>-[G3]-BnNH<sub>3</sub><sup>+</sup>**



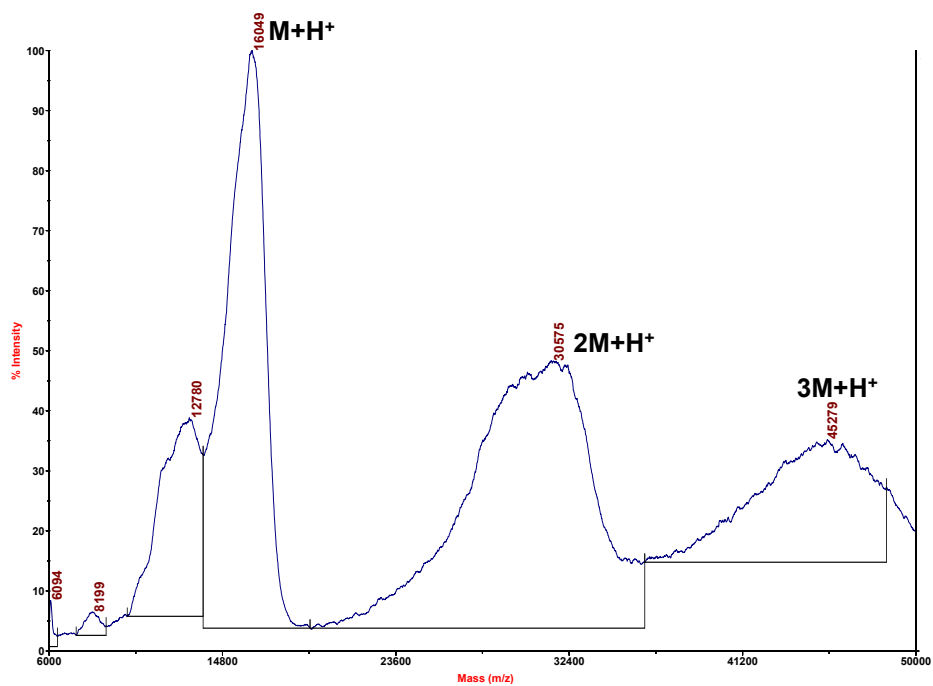
<sup>1</sup>H NMR spectrum of PEG<sub>5000</sub>-[G3]-BnNH<sub>3</sub><sup>+</sup>



<sup>13</sup>C NMR spectrum of PEG<sub>5000</sub>-[G3]-BnNH<sub>3</sub><sup>+</sup>

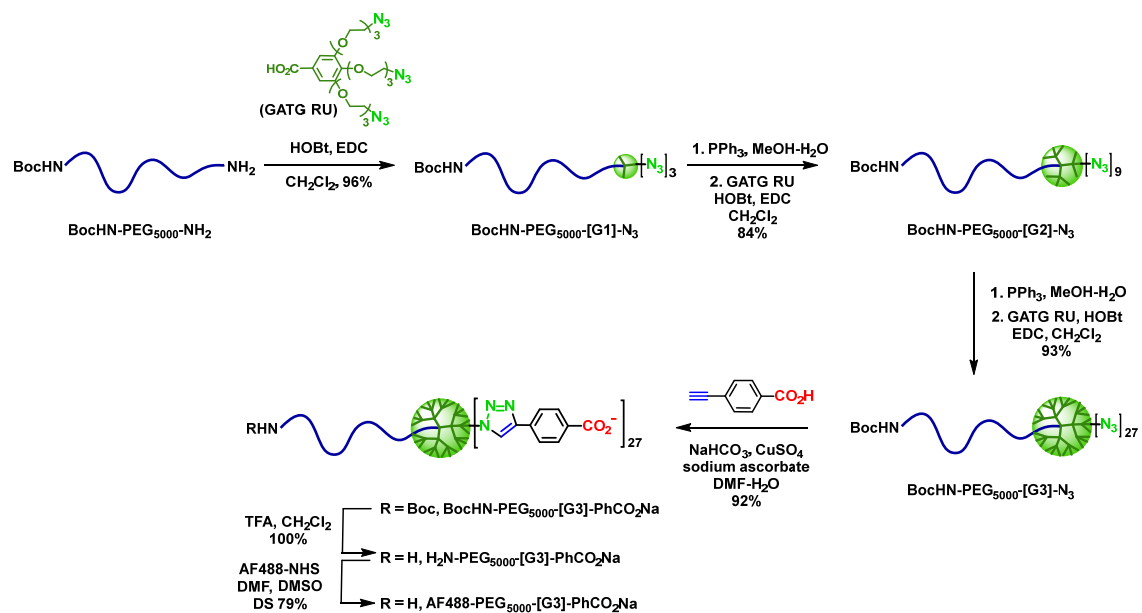


IR spectrum of PEG<sub>5000</sub>-[G3]-BnNH<sub>3</sub><sup>+</sup>



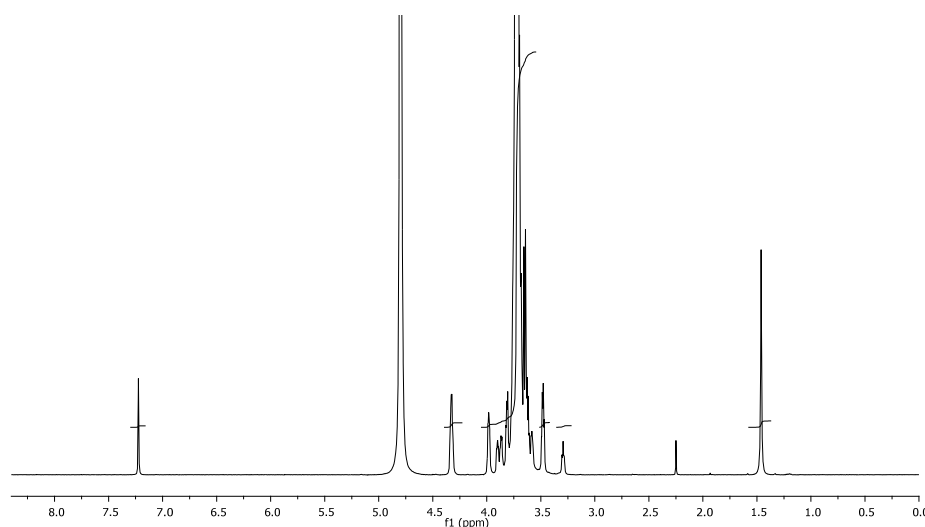
MALDI-TOF MS of PEG<sub>5000</sub>-[G3]-BnNH<sub>3</sub><sup>+</sup>

## 2. Synthesis of Fluorescently Labeled PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup> with Alexa Fluor 488

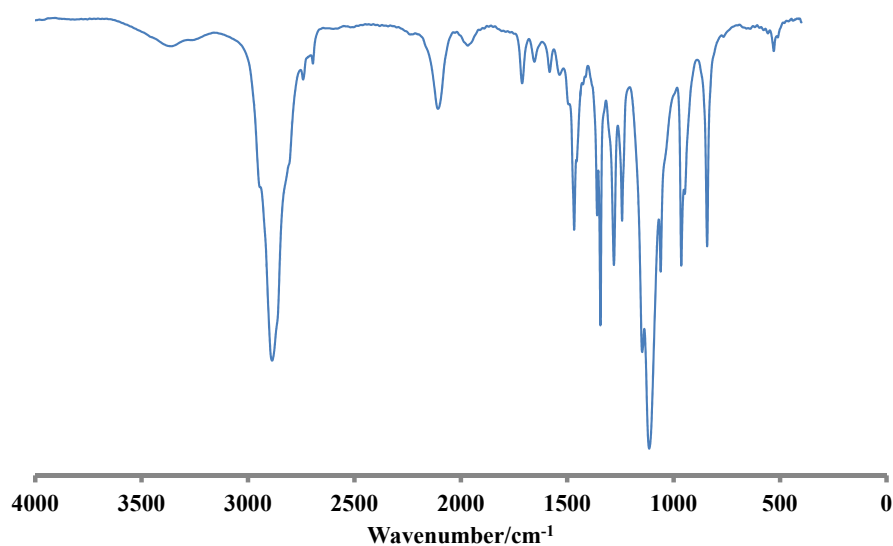


**BocHN-PEG<sub>5000</sub>-[G1]-N<sub>3</sub>.** BocHN-PEG<sub>5000</sub>-NH<sub>2</sub> (200 mg, 39.9 μmol) and GATG repeating unit (51.2 mg, 79.8 μmol, 2 eq per amine) were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (4.0 mL, final concentration of amine groups 0.1 M) under Ar. Then, HOBt (16.2 mg, 119 μmol, 3 eq per amine) and EDC·HCl (22.9 mg, 119 μmol, 3 eq per amine) were added. After stirring for 24 h at rt, the reaction mixture was evaporated under reduced pressure and purified by precipitation (MeOH/iPrOH) to afford BocHN-PEG<sub>5000</sub>-[G1]-N<sub>3</sub> (216 mg, 96%) as a white solid.

<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ: 7.22 (s, 2H), 4.40-4.22 (m, 6H), 4.05-3.54 (m, ~462H), 3.51-3.42 (m, 6H), 3.29 (t, *J* = 5.2 Hz, 2H), 1.46 (s, 9H). IR (KBr, cm<sup>-1</sup>): 3360, 2887, 2108, 1115.



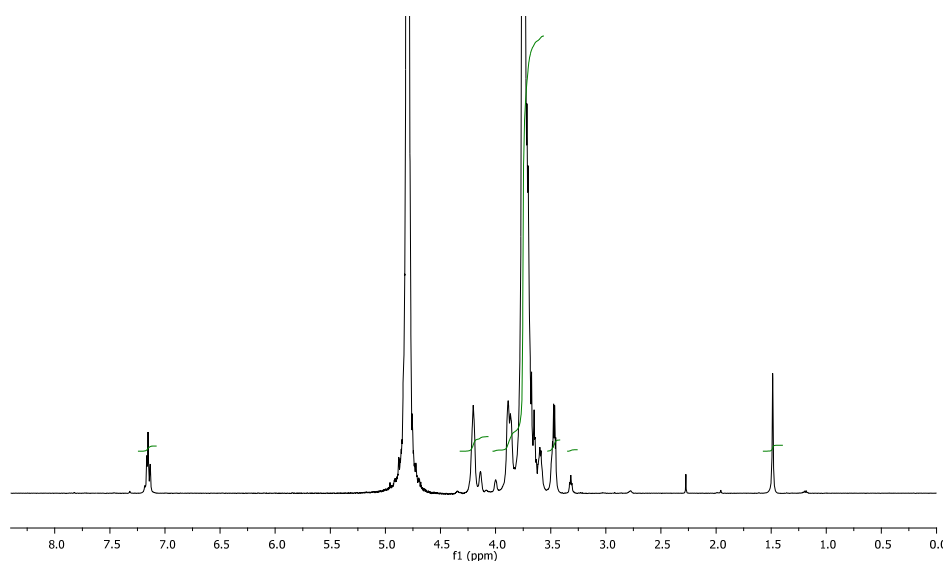
<sup>1</sup>H NMR spectrum of BocHN-PEG<sub>5000</sub>-[G1]-N<sub>3</sub>



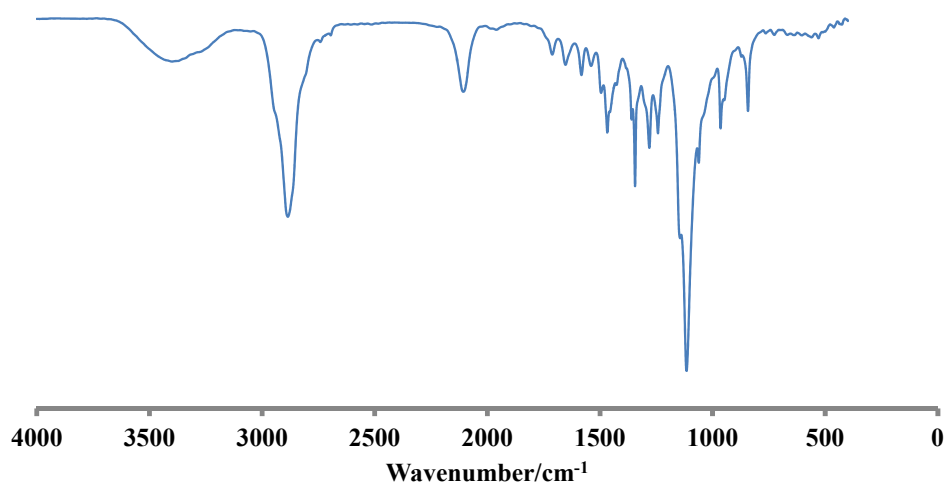
IR spectrum of BocHN-PEG<sub>5000</sub>-[G1]-N<sub>3</sub>

**BocHN-PEG<sub>5000</sub>-[G2]-N<sub>3</sub>.** BocHN-PEG<sub>5000</sub>-[G1]-N<sub>3</sub> (117 mg, 20.7 μmol) was dissolved in a mixture of MeOH (1.4 mL) and H<sub>2</sub>O (70 μL, 5% v/v). Then, PPh<sub>3</sub> (18.8 mg, 71.4 μmol, 1.15 eq per azide) was added. After stirring for 12 h at rt, the reaction mixture was evaporated under reduced pressure and purified by precipitation (CH<sub>2</sub>Cl<sub>2</sub>/Et<sub>2</sub>O) to give BocHN-PEG<sub>5000</sub>-[G1]-NH<sub>2</sub> (108 mg, 94%). This polymer and GATG repeating unit (74.8 mg, 116 μmol, 2 eq per amine) were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (0.63 mL, final concentration of amine groups 0.1 M) under Ar. Then, HOBt (15.7 mg, 116 μmol, 2 eq per amine) and EDC·HCl (22.3 mg, 116 μmol, 2 eq per amine) were added. After stirring for 24 h at rt, the reaction mixture was evaporated under reduced pressure and purified by precipitation (MeOH/iPrOH) to afford BocHN-PEG<sub>5000</sub>-[G2]-N<sub>3</sub> (127 mg, 89%) as a white solid.

<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ: 7.24-7.08 (m, 8H), 4.33-4.06 (m, 24H), 4.03-3.56 (m, ~540H), 3.53-3.41 (m, 18H), 3.32 (t, *J* = 5.2 Hz, 2H), 1.49 (s, 9H). IR (KBr, cm<sup>-1</sup>): 3400, 2885, 2106, 1115.



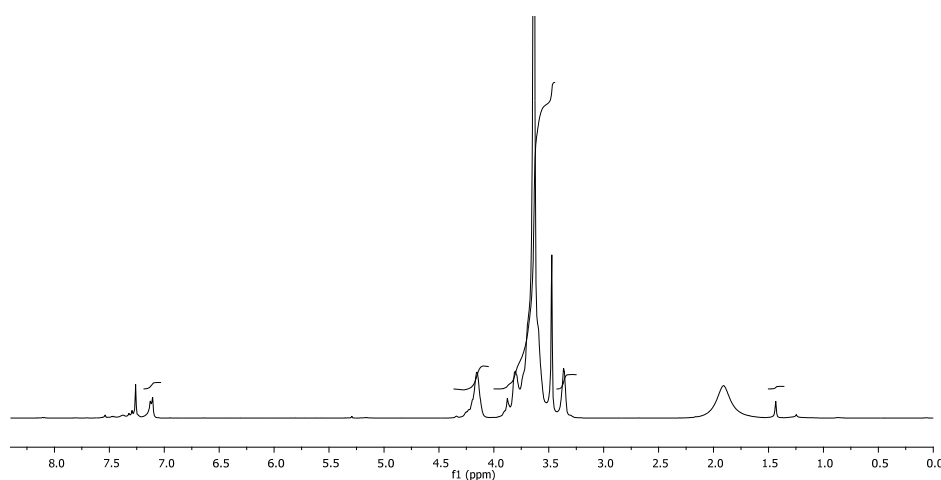
<sup>1</sup>H NMR spectrum of BocHN-PEG<sub>5000</sub>-[G2]-N<sub>3</sub>



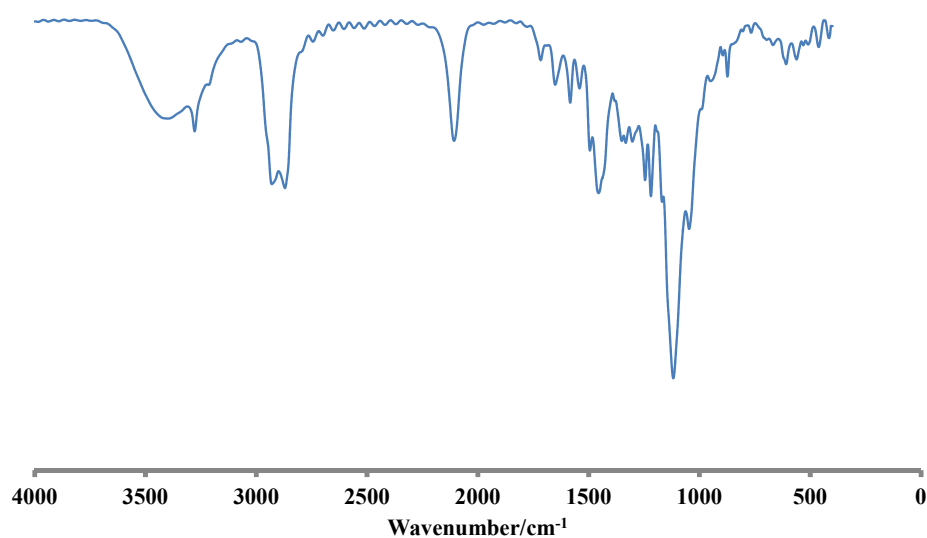
IR spectrum of BocHN-PEG<sub>5000</sub>-[G2]-N<sub>3</sub>

**BocHN-PEG<sub>5000</sub>-[G3]-N<sub>3</sub>.** BocHN-PEG<sub>5000</sub>-[G2]-N<sub>3</sub> (58.7 mg, 7.91  $\mu\text{mol}$ ) was dissolved in a mixture of MeOH (0.49 mL) and H<sub>2</sub>O (25  $\mu\text{L}$ , 5% v/v). Then, PPh<sub>3</sub> (21.5 mg, 81.8  $\mu\text{mol}$ , 1.15 eq per azide) was added. After stirring for 12 h at rt, the reaction mixture was evaporated under reduced pressure and purified by precipitation (CH<sub>2</sub>Cl<sub>2</sub>/Et<sub>2</sub>O) to give BocHN-PEG<sub>5000</sub>-[G2]-NH<sub>2</sub> (55.8 mg, 98%). This polymer and GATG repeating unit (88.3 mg, 138  $\mu\text{mol}$ , 2 eq per amine) were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (0.72 mL, final concentration of amine groups 0.1 M) under Ar. Then, HOBt (18.7 mg, 138  $\mu\text{mol}$ , 2 eq per amine) and EDC·HCl (26.4 mg, 138  $\mu\text{mol}$ , 2 eq per amine) were added. After stirring for 24 h at rt, the reaction mixture was evaporated under reduced pressure and purified by ultrafiltration (YM1), washing with MeOH:H<sub>2</sub>O (1:1, 3 x 30 mL) and H<sub>2</sub>O (3 x 30 mL), to afford BocHN-PEG<sub>5000</sub>-[G3]-N<sub>3</sub> (94.5 mg, 95%) as a pale yellow foam after freeze-drying.

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.19-7.04 (m, 26H), 4.37-4.05 (m, 78H), 4.00-3.44 (m, ~774H), 3.43-3.25 (m, 56H), 1.43 (s, 9H). IR (KBr, cm<sup>-1</sup>): 3402, 2870, 2108, 1456, 1119.



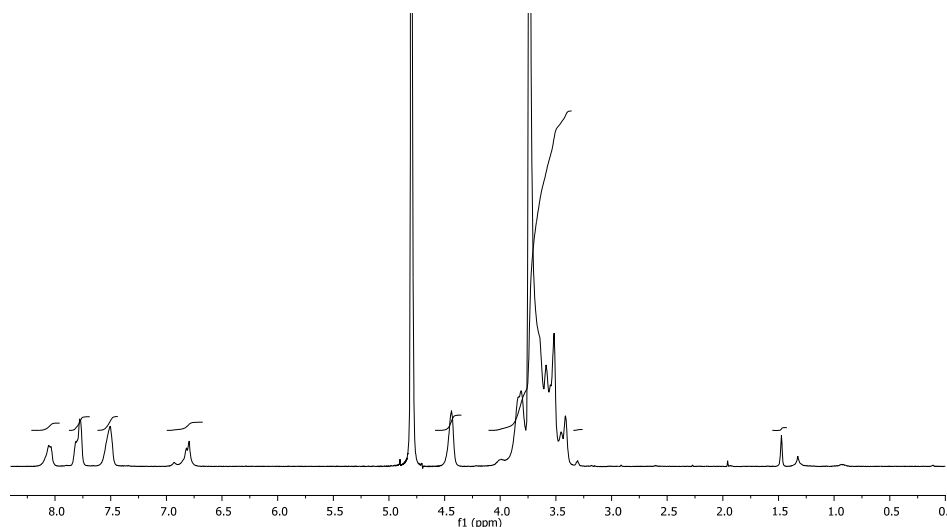
<sup>1</sup>H NMR spectrum of BocHN-PEG<sub>5000</sub>-[G3]-N<sub>3</sub>



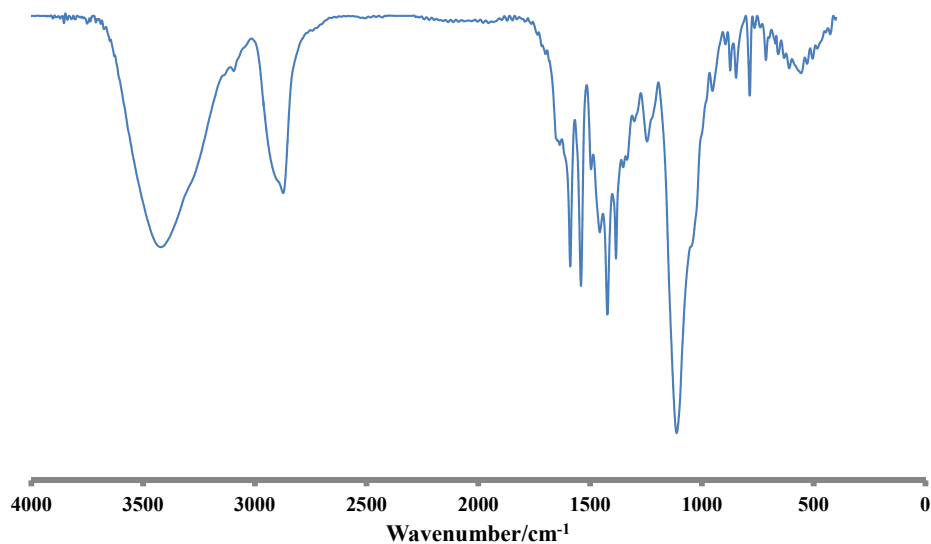
IR spectrum of BocHN-PEG<sub>5000</sub>-[G3]-N<sub>3</sub>

**BocHN-PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup>.** Starting from BocHN-PEG<sub>5000</sub>-[G3]-N<sub>3</sub> (17.0 mg, 1.33 μmol), 4-ethynyl benzoic acid (10.5 mg, 71.7 μmol), NaHCO<sub>3</sub> (12.0 mg, 143 μmol), CuSO<sub>4</sub> (17.9 μL, 1.79 μmol, 0.1 M) and sodium ascorbate (17.9 μL, 8.95 μmol, 0.5 M) dissolved in DMF (0.18 mL)/H<sub>2</sub>O (0.14 mL) and following the general procedure for CuAAC reactions, BocHN-PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub>Na (20.5 mg, 92%) was obtained as a pale yellow foam.

<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ: 8.21-7.96 (m, 27H), 7.87-7.69 (m, 54H), 7.62-7.44 (m, 54H), 7.00-6.67 (m, 26H), 4.44 (br s, 54H), 4.11-3.36 (m, ~852H), 3.30 (br s, 2H), 1.47 (s, 9H). IR (KBr, cm<sup>-1</sup>): 3421, 2873, 1589, 1541, 1423, 1113.



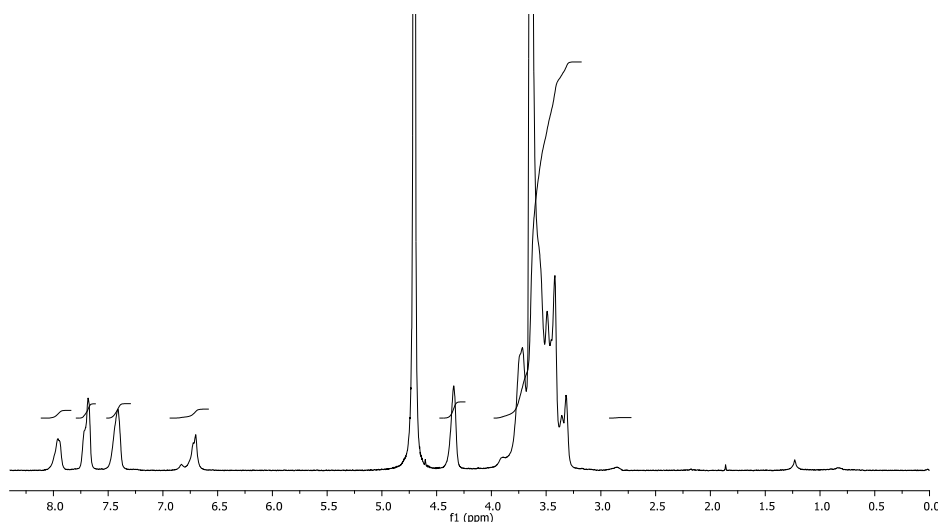
<sup>1</sup>H NMR spectrum of BocHN-PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup>



IR spectrum of BocHN-PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup>

**H<sub>2</sub>N-PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup>.** BocHN-PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup> (17 mg, 1.01 μmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (0.50 mL) and TFA (0.17 mL). After 15 min of stirring at rt, the reaction mixture was evaporated, dissolved in sat NaHCO<sub>3</sub> and purified by ultrafiltration (YM3) washing with H<sub>2</sub>O. After freeze-drying, H<sub>2</sub>N-PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub>Na was obtained (17 mg, 100%) as a pale yellow foam.

<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ: 8.27-7.97 (m, 27H), 7.94-7.69 (m, 54H), 7.65-7.41 (m, 54H), 7.06-6.63 (m, 26H), 4.44 (br s, 54H), 4.12-3.28 (m, ~852H), 2.96 (br s, 2H).



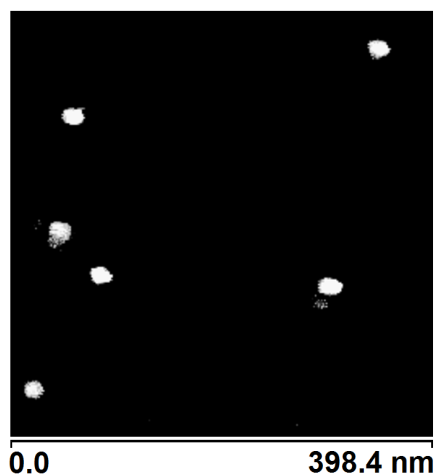
<sup>1</sup>H NMR spectrum of H<sub>2</sub>N-PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup>

**AF488-PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup>.** H<sub>2</sub>N-PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub>Na (1.0 mg, 57 nmol) was dissolved in DMF (0.20 mL). Then, Alexa Fluor 488 carboxylic acid, succinimidyl ester (0.4 mg) dissolved in dry DMSO (0.10 mL) was added and the resulting solution was stirred at rt overnight in the dark. The reaction mixture was purified by ultrafiltration (YM3) washing with sat NaHCO<sub>3</sub> and H<sub>2</sub>O. After freeze-drying, AF488-PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub>Na (0.8 mg, 79%) was obtained. A degree of functionalization of 79% in Alexa Fluor 488 was determined by absorbance at 494 nm ( $\lambda_{494}$ : 73000 cm<sup>-1</sup>M<sup>-1</sup> as provided by supplier).



### 3. Atomic Force Microscopy (AFM)

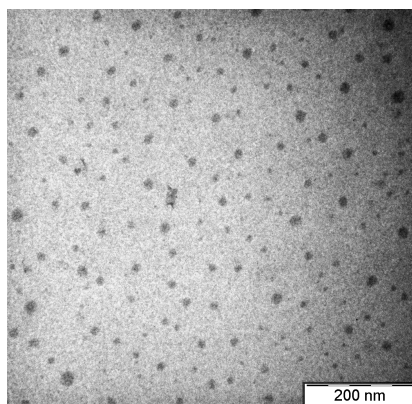
Samples for AFM imaging were prepared by depositing aqueous solutions of PIC micelles (0.025-0.05 mg/mL) onto Si wafers. AFM measurements were performed at an atomic force microscope MFP-3D (Asylum Research). Average diameters of  $30\pm 8$  nm for PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup>/PLL<sub>108</sub> micelles and  $27\pm 5$  nm for PEG<sub>5000</sub>-[G3]-BnNH<sub>3</sub><sup>+</sup>/PGA<sub>136</sub> were determined using NanoScope Analysis software (Veeco).



**Figure S1.** AFM image of PIC micelles prepared from PEG<sub>5000</sub>-[G3]-BnNH<sub>3</sub><sup>+</sup> and PGA<sub>136</sub>.

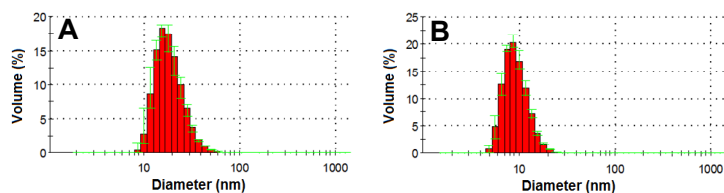
### 4. Transmission Electron Microscopy (TEM)

TEM measurements were performed on a Philips CM-12 or JEOL JEM1010 operated at 80 kV, electron microscopes. All samples were ultrafiltered against Milli-Q water (Amicon, MWCO 3000) to remove salts. A drop of a solution of PIC micelles was settled on Formvar precoated or carbon films on copper grids, and allowed to dry at rt for 24 h. Negative staining was performed by using a droplet of 1% phosphotungstic acid or 2% uranyl acetate following standard procedures. An average diameter of *ca.*  $24\pm 4$  nm was determined by measuring the size of about 35 micelles using ImageJ software.

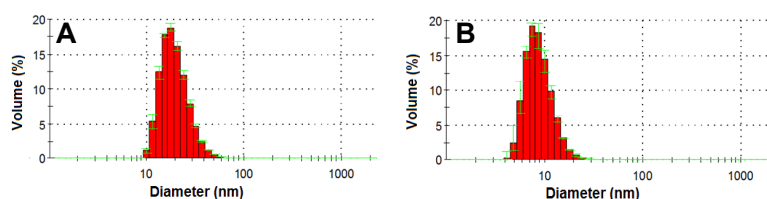


**Figure S2.** TEM image of PIC micelles prepared from PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup> and PLL<sub>108</sub>.

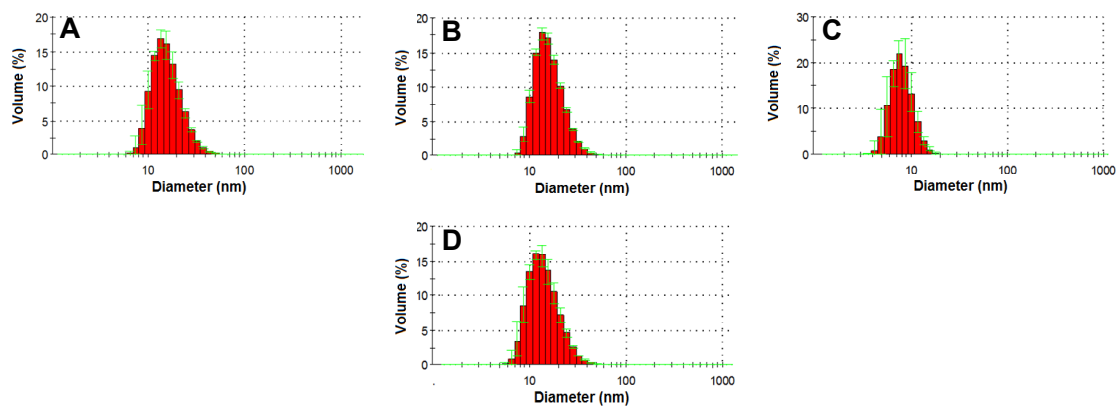
## 5. Stability of PIC Micelles - Dynamic Light Scattering (DLS)



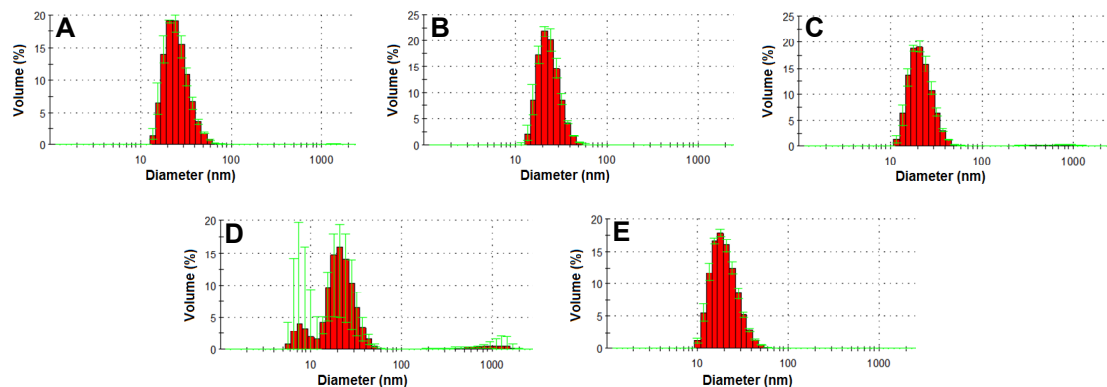
**Figure S3.** PIC micelles prepared from PEG<sub>5000</sub>-[G3]-A-CO<sub>2</sub><sup>-</sup> and PLL<sub>108</sub> in 10 mM PB, pH 7.4, 25 °C. DLS histograms upon formation (A) and 1 h after the addition of 150 mM NaCl (B).



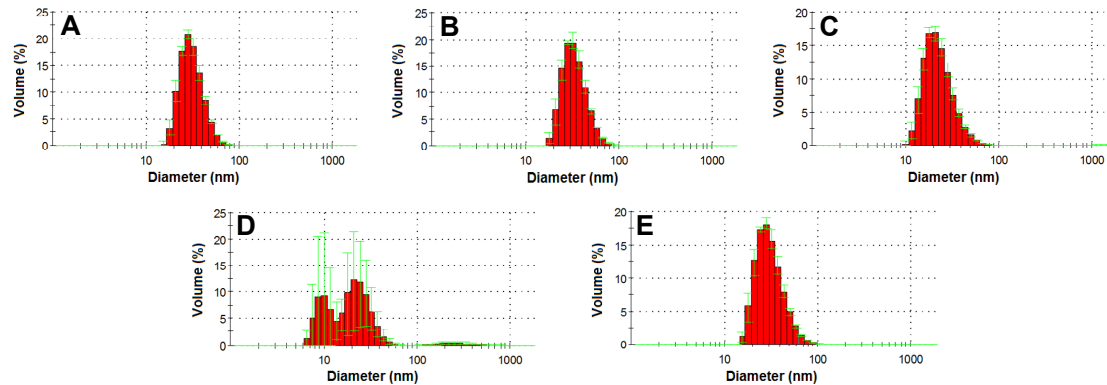
**Figure S4.** PIC micelles prepared from PEG<sub>5000</sub>-[G3]-CO<sub>2</sub><sup>-</sup> and PLL<sub>108</sub> in 10 mM PB, pH 7.4, 25 °C. DLS histograms upon formation (A) and 1 h after the addition of 150 mM NaCl (B).



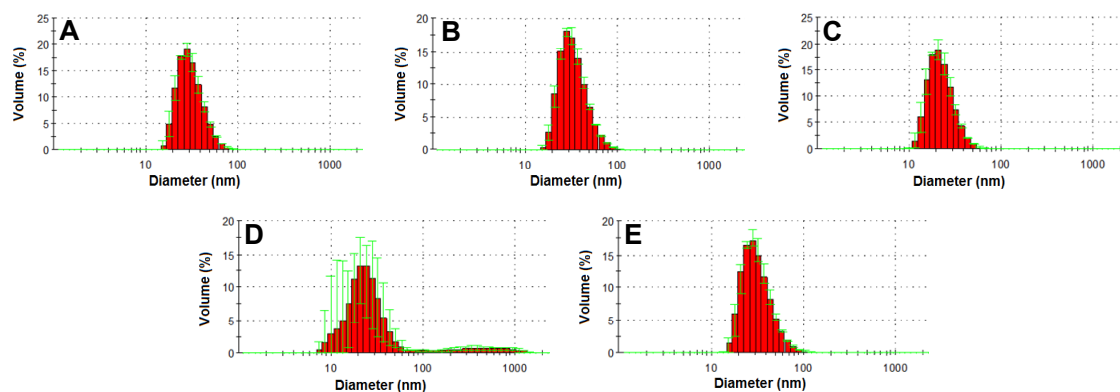
**Figure S5.** PIC micelles prepared from PEG<sub>5000</sub>-[G3]-OSO<sub>3</sub><sup>-</sup> and PLL<sub>108</sub> in 10 mM PB, pH 7.4, 25 °C. DLS histograms upon formation (A), and 1 h after the addition of 150 mM NaCl (B) or 300 mM NaCl (C). DLS histogram of the micelles in 150 mM NaCl after 12 h at 37 °C (D).



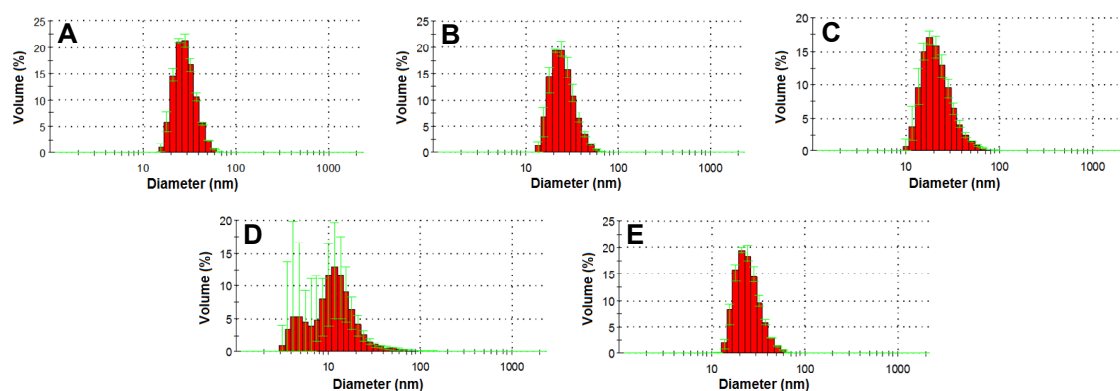
**Figure S6.** PIC micelles prepared from PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup> and PLL<sub>64</sub> in 10 mM PB, pH 7.4, 25 °C. DLS histograms upon formation (A), and 1 h after the addition of 150 mM NaCl (B), 450 mM NaCl (C) or 600 mM NaCl (D). DLS histogram of the micelles in 150 mM NaCl after 12 h at 37 °C (E).



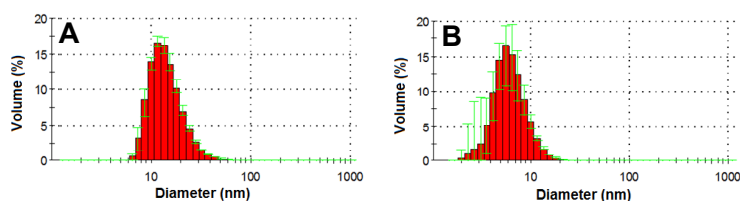
**Figure S7.** PIC micelles prepared from PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup> and PLL<sub>108</sub> in 10 mM PB, pH 7.4, 25 °C. DLS histograms upon formation (A), and 1 h after the addition of 150 mM NaCl (B), 600 mM NaCl (C) or 750 mM NaCl (D). DLS histogram of the micelles in 150 mM NaCl after 12 h at 37 °C (E).



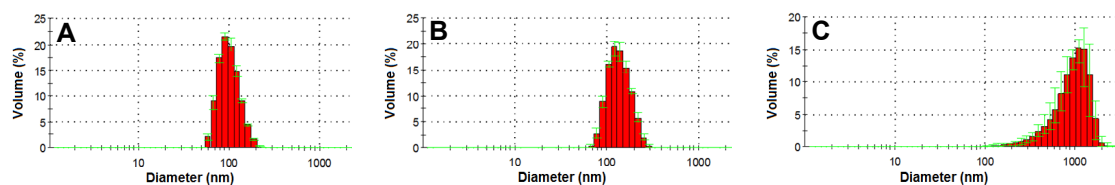
**Figure S8.** PIC micelles prepared from PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup> and PLL<sub>157</sub> in 10 mM PB, pH 7.4, 25 °C. DLS histograms upon formation (A), and 1 h after the addition of 150 mM NaCl (B), 600 mM NaCl (C) or 750 mM NaCl (D). DLS histogram of the micelles in 150 mM NaCl after 12 h at 37 °C (E).



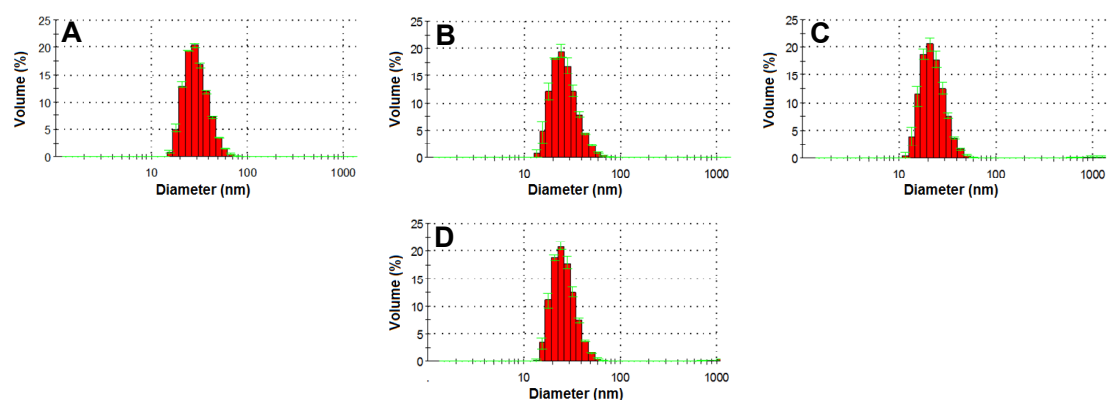
**Figure S9.** PIC micelles prepared from PEG<sub>10000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup> and PLL<sub>108</sub> in 10 mM PB, pH 7.4, 25 °C. DLS histograms upon formation (A), and 1 h after the addition of 150 mM NaCl (B), 600 mM NaCl (C) or 750 mM NaCl (D). DLS histogram of the micelles in 150 mM NaCl after 12 h at 37 °C (E).



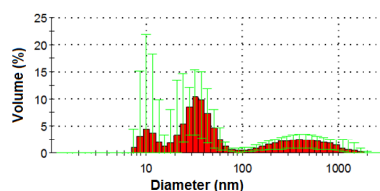
**Figure S10.** PIC micelles prepared from PEG<sub>5000</sub>-[G2]-PhCO<sub>2</sub><sup>-</sup> and PLL<sub>108</sub> in 10 mM PB, pH 7.4, 25 °C. DLS histograms upon formation (A) and 1 h after the addition of 150 mM NaCl (B).



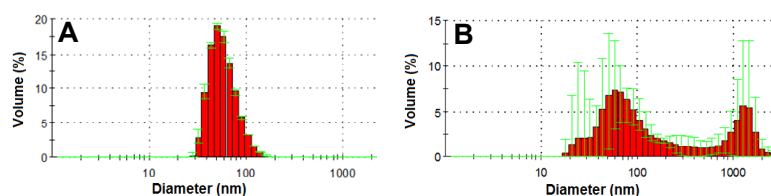
**Figure S11.** PIC micelles prepared from PEG<sub>5000</sub>-[G4]-PhCO<sub>2</sub><sup>-</sup> and PLL<sub>108</sub> in 10 mM PB, pH 7.4, 25 °C. DLS histograms upon formation (A) and 1 h after the addition of 150 mM NaCl (B). DLS histogram in 150 mM NaCl after 12 h at 37 °C (C).



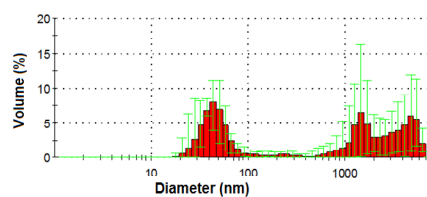
**Figure S12.** PIC micelles prepared from PEG<sub>5000</sub>-[G3]-PhSO<sub>3</sub><sup>-</sup> and PLL<sub>108</sub> in 10 mM PB, pH 7.4, 25 °C. DLS histograms upon formation (A), and 1 h after the addition of 150 mM NaCl (B) or 1.05 M NaCl (C). DLS histogram of the micelles in 150 mM NaCl after 12 h at 37 °C (D).



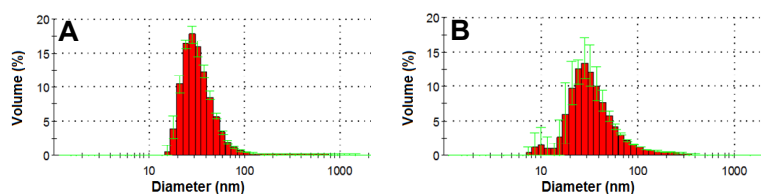
**Figure S13.** DLS histogram of the mixture PEG<sub>5000</sub>-PGA<sub>25</sub> and PLL<sub>108</sub> in 10 mM PB, pH 7.4, 25 °C.



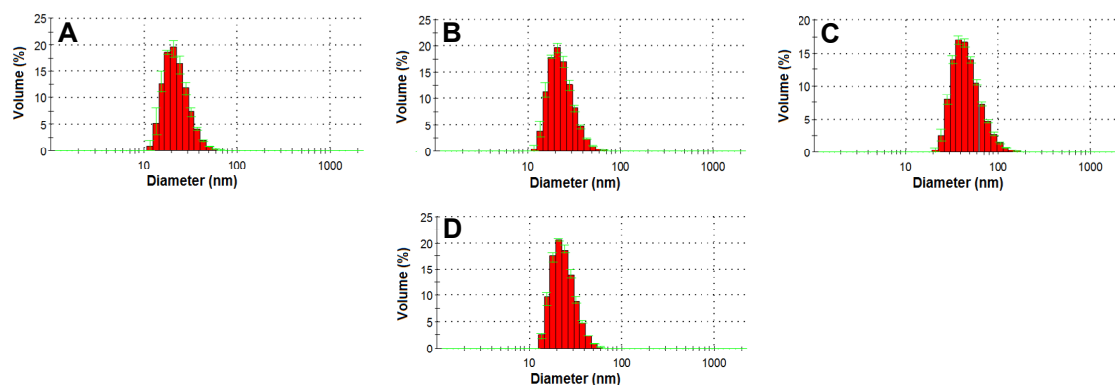
**Figure S14.** PIC micelles prepared from PEG<sub>5000</sub>-PGA<sub>50</sub> and PLL<sub>108</sub> in 10 mM PB, pH 7.4, 25 °C. DLS histograms upon formation (A) and 1 h after the addition of 150 mM NaCl (B).



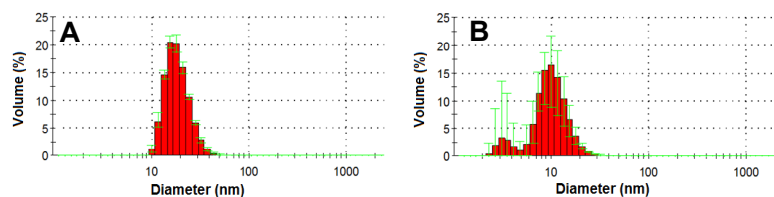
**Figure S15.** DLS histogram of the mixture PEG<sub>5000</sub>-PGA<sub>100</sub> and PLL<sub>108</sub> in 10 mM PB, pH 7.4, 25 °C.



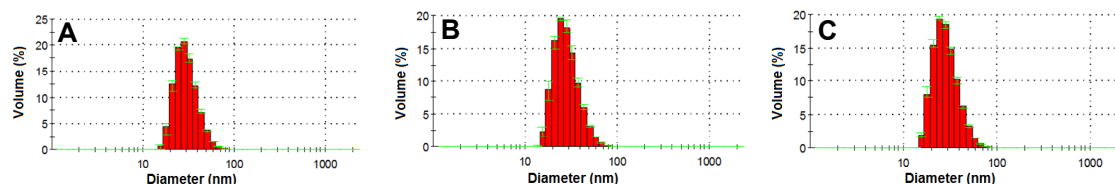
**Figure S16.** PIC micelles prepared from PEG<sub>5000</sub>-PGA<sub>25</sub>-PhCO<sub>2</sub><sup>-</sup> and PLL<sub>108</sub> in 10 mM PB, pH 7.4, 25 °C. DLS histograms upon formation (A) and 1 h after the addition of 150 mM NaCl (B).



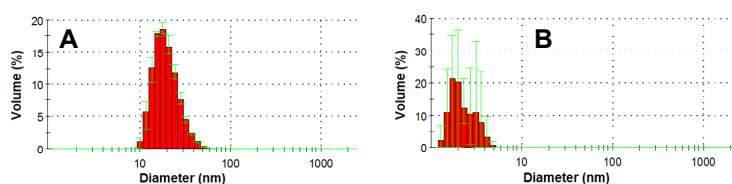
**Figure S17.** PIC micelles prepared from PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup> and PEG<sub>5000</sub>-[G3]-BnNH<sub>3</sub><sup>+</sup> in 10 mM PB, pH 7.4, 25 °C. DLS histograms upon formation (A), and 1 h after the addition of 150 mM NaCl (B) or 3 M NaCl (C). DLS histogram of the micelles in 150 mM NaCl after 12 h at 37 °C (D).



**Figure S18.** PIC micelles prepared from PEG<sub>5000</sub>-[G3]-NH<sub>3</sub><sup>+</sup> and PGA<sub>136</sub> in 10 mM PB, pH 7.4, 25 °C. DLS histograms upon formation (A) and 1 h after the addition of 150 mM NaCl (B).



**Figure S19.** PIC micelles prepared from PEG<sub>5000</sub>-[G3]-BnNH<sub>3</sub><sup>+</sup> and PGA<sub>136</sub> in 10 mM PB, pH 7.4, 25 °C. DLS histograms upon formation (A) and 1 h after the addition of 150 mM NaCl (B). DLS histogram of the micelles in 150 mM NaCl after 12 h at 37 °C (C).



**Figure S20.** DLS histograms of: PEG<sub>5000</sub>-[G3]-OSO<sub>3</sub><sup>-</sup>/PLL<sub>108</sub> micelles after 4.5 days of dialysis at 37 °C against 50 mM acetate buffer, pH 5.0 (A), and PEG<sub>5000</sub>-[G3]-CO<sub>2</sub><sup>-</sup>/PLL<sub>108</sub> micelles after 1 h at 25 °C in 50 mM acetate buffer, pH 5.0 (B).

## 6. Tables S1 and S2. Summary of the Stability of PIC micelles by DLS

**Table S1.** Stability of PIC micelles upon formation and towards increasing concentrations of NaCl and heating at 37 °C (+: stable, -: not stable)

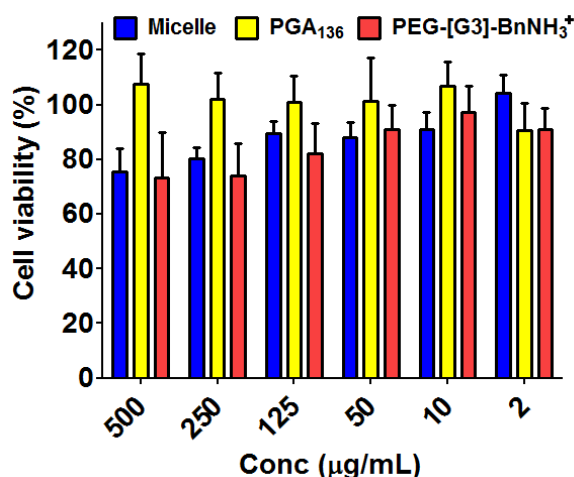
PIC	PLL (DP)	Upon formation PB 7.4 25 °C	PB 7.4 25 °C 150 mM NaCl	PB 7.4 25 °C 300 mM NaCl	PB 7.4 25 °C 450 mM NaCl	PB 7.4 25 °C 600 mM NaCl	PB 7.4 25 °C 750 mM NaCl	PB 7.4 25 °C 900 mM NaCl	PB 7.4 25 °C > 1 M NaCl
PEG <sub>5000</sub> -[G3]-A-CO <sub>2</sub> <sup>-</sup>	108	+	-	-	-	-	-	-	-
PEG <sub>5000</sub> -[G3]-CO <sub>2</sub> <sup>-</sup>	108	+	-	-	-	-	-	-	-
PEG <sub>5000</sub> -[G3]-OSO <sub>3</sub> <sup>-</sup>	108	+	+	-	-	-	-	-	-
PEG <sub>5000</sub> -[G3]-PhCO <sub>2</sub> <sup>-</sup>	64	+	+	+	+	-	-	-	-
PEG <sub>5000</sub> -[G3]-PhCO <sub>2</sub> <sup>-</sup>	108	+	+	+	+	+	-	-	-
PEG <sub>5000</sub> -[G3]-PhCO <sub>2</sub> <sup>-</sup>	157	+	+	+	+	+	-	-	-
PEG <sub>2000</sub> -[G3]-PhCO <sub>2</sub> <sup>-</sup>	108	-	-	-	-	-	-	-	-
PEG <sub>10000</sub> -[G3]-PhCO <sub>2</sub> <sup>-</sup>	108	+	+	+	+	+	-	-	-
PEG <sub>5000</sub> -[G2]-PhCO <sub>2</sub> <sup>-</sup>	108	+	-	-	-	-	-	-	-
PEG <sub>5000</sub> -[G4]-PhCO <sub>2</sub> <sup>-</sup>	108	+	+	-	-	-	-	-	-
PEG <sub>5000</sub> -[G3]-PhSO <sub>3</sub> <sup>-</sup>	108	+	+	+	+	+	+	+	+
PEG <sub>5000</sub> -PGA <sub>25</sub>	108	-	-	-	-	-	-	-	-
PEG <sub>5000</sub> -PGA <sub>50</sub>	108	+/-	-	-	-	-	-	-	-
PEG <sub>5000</sub> -PGA <sub>100</sub>	108	-	-	-	-	-	-	-	-
PEG <sub>5000</sub> -PGA <sub>25</sub> -PhCO <sub>2</sub> <sup>-</sup>	108	+	-	-	-	-	-	-	-
PEG <sub>5000</sub> -[G3]-PhCO <sub>2</sub> <sup>-</sup>	PEG <sub>5000</sub> -[G3]-BnNH <sub>3</sub> <sup>+</sup>	+	+	+	+	+	+	+	+



**Table S2.** Hydrodynamic diameters and PDI (in brackets) of PIC micelles upon formation and after the addition of NaCl and heating at 37 °C

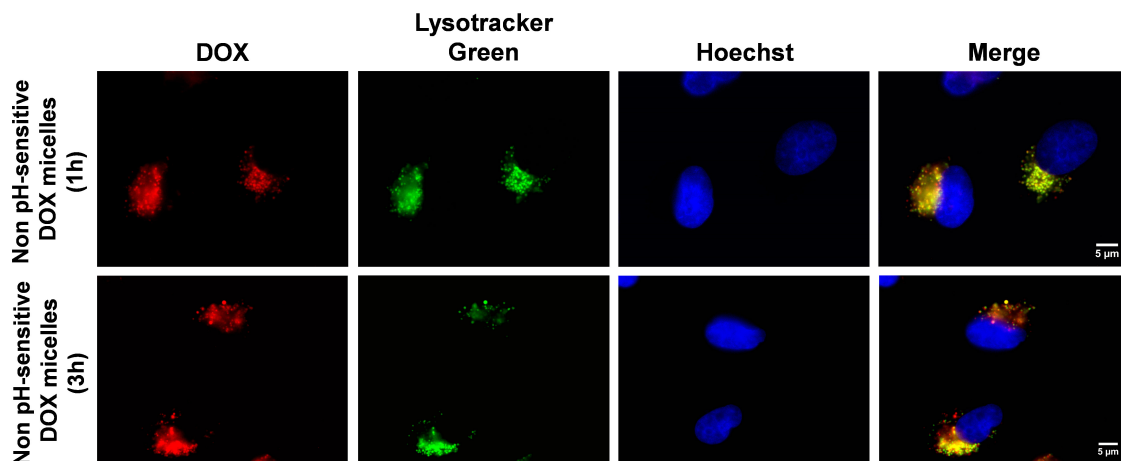
PIC	PLL/PGA (DP)	Upon formation PB 7.4 25 °C	PB 7.4 25 °C 150 mM NaCl	PB 7.4 37 °C 150 mM NaCl, 12 h
PEG <sub>5000</sub> -[G3]-A-CO <sub>2</sub> <sup>-</sup>	PLL(108)	19.4 (0.179)	–	–
PEG <sub>5000</sub> -[G3]-CO <sub>2</sub> <sup>-</sup>	PLL(108)	20.4 (0.115)	–	–
PEG <sub>5000</sub> -[G3]-OSO <sub>3</sub> <sup>-</sup>	PLL(108)	16.5 (0.292)	17.8 (0.261)	14.8 (0.334)
PEG <sub>5000</sub> -[G3]-PhCO <sub>2</sub> <sup>-</sup>	PLL(64)	26.8 (0.046)	23.8 (0.051)	20.8 (0.128)
PEG <sub>5000</sub> -[G3]-PhCO <sub>2</sub> <sup>-</sup>	PLL(108)	31.8 (0.070)	34.4 (0.082)	31.9 (0.151)
PEG <sub>5000</sub> -[G3]-PhCO <sub>2</sub> <sup>-</sup>	PLL(157)	31.9 (0.137)	34.8 (0.158)	32.9 (0.203)
PEG <sub>2000</sub> -[G3]-PhCO <sub>2</sub> <sup>-</sup>	PLL(108)	–	–	–
PEG <sub>10000</sub> -[G3]-PhCO <sub>2</sub> <sup>-</sup>	PLL(108)	26.7 (0.140)	24.8 (0.172)	25.1 (0.166)
PEG <sub>5000</sub> -[G2]-PhCO <sub>2</sub> <sup>-</sup>	PLL(108)	15.1 (0.373)	–	–
PEG <sub>5000</sub> -[G4]-PhCO <sub>2</sub> <sup>-</sup>	PLL(108)	103 (0.036)	141 (0.030)	–
PEG <sub>5000</sub> -[G3]-PhSO <sub>3</sub> <sup>-</sup>	PLL(108)	30.4 (0.078)	27.8 (0.165)	26.0 (0.174)
PEG <sub>5000</sub> -PGA <sub>25</sub>	PLL(108)	–	–	–
PEG <sub>5000</sub> -PGA <sub>50</sub>	PLL(108)	54.1 (0.196)	–	–
PEG <sub>5000</sub> -PGA <sub>100</sub>	PLL(108)	–	–	–
PEG <sub>5000</sub> -PGA <sub>25</sub> -PhCO <sub>2</sub> <sup>-</sup>	PLL(108)	35.1 (0.321)	–	–
PEG <sub>5000</sub> -[G3]-PhCO <sub>2</sub> <sup>-</sup>	PEG <sub>5000</sub> -[G3]-BnNH <sub>3</sub> <sup>+</sup>	23.2 (0.164)	23.8 (0.143)	24.2 (0.224)
PEG <sub>5000</sub> -[G3]-NH <sub>3</sub> <sup>+</sup>	PGA(136)	19.0 (0.202)	–	–
PEG <sub>5000</sub> -[G3]-BnNH <sub>3</sub> <sup>+</sup>	PGA(136)	30.6 (0.150)	29.1 (0.129)	29.4 (0.176)

## 7. Cell Studies



**Figure S21.** Cell viability (MTT) of A549 cells at 24 h in the presence of PIC micelles prepared from PEG<sub>5000</sub>-[G3]-BnNH<sub>3</sub><sup>+</sup> and PGA<sub>136</sub> (PEG<sub>5000</sub>-[G3]-BnNH<sub>3</sub><sup>+</sup> and PGA<sub>136</sub> at the same concentrations as in micelle were used as controls).

**Non pH-Sensitive DOX-loaded Control Micelles.** PIC micelles from PEG<sub>5000</sub>-[G3]-PhCO<sub>2</sub><sup>-</sup> and PLL<sub>108</sub> were prepared as described. Then, a freshly prepared solution of EDC (0.1 g/mL in H<sub>2</sub>O, 10 eq per carboxylate group in the micelle) was added. The resulting solution was allowed to stir overnight at rt. Cross-linked micelles were dialyzed against 10 mM PB pH 7.4, 24 h (Spectrum Labs, Spectra/Por<sup>®</sup> 6 membrane, MWCO 1 KDa) before being loaded with DOX following identical procedure as for non-crosslinked micelles.



**Figure S22.** Fluorescence microscopy images of A549 cells treated with control non pH-sensitive DOX-loaded micelles. Cells were incubated for 1 h and then were washed with PBS and imaged immediately (upper row) or after 2 h (lower row). Images from left to right show DOX fluorescence in cells (red), lysosomes stained by Lysotracker Green (green), cell nuclei stained by Hoechst (blue), and merged images.