

## Supplementary information for:

# Programmed Hydrolysis of Nanoassemblies by Electrostatic Interaction-mediated Enzymatic-degradation†

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### 1. Materials

Azobisisobutyronitrile (AIBN, 98%, Aldrich) was recrystallized from methanol before use. DL-lactide (98%, Alfa Aesar) was purified by recrystallization from ethyl acetate. Dimethylformamide (DMF), dichloromethane (DCM), 2-butanone, methanol, acetic acid, trifluoroacetic acid (TFA), porcine liver esterase (PLE) (activity = 154 U mg<sup>-1</sup>, concentration = 35.6 mg of protein mL<sup>-1</sup>), proteinase K (PK) (activity = 1362 U mL<sup>-1</sup>, concentration = 36 mg of protein mL<sup>-1</sup>) and 1,8-diazabicycloundec-7-ene (DBU) were used as received from Sigma-Aldrich Company, St. Louis, MO. Chain transfer agent (CTA) 5-hydroxypentyl 2-(((dodecylthio)carbonothioyl)thio)-2-methylpropanoate and *tert*-butyl (2-methacrylamidoethyl)carbamate monomer were synthesized as reported.<sup>[1]</sup> Spectro/Por® membranes (MWCO 12-14 kDa, Spectrum Medical Industries, Inc., Laguna Hills, CA) were used for dialysis. The lactate colorimetric assay kit (ab65331) was purchased from Abcam®.<sup>[2]</sup>

### 2. Instruments

<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded on Varian 500 MHz and Varian 300 MHz spectrometers. Chemical shifts were referenced to solvent resonance signals.

The DMF gel permeation chromatography (GPC) was conducted on a Waters Chromatography, Inc. (Milford, MA) system equipped with an isocratic pump model 1515, a differential refractometer model 2414, and a four-column set of 5 µm guard (50 x 7.5 mm), Styragel HR 45 µm DMF (300 x 7.5 mm), Styragel HR 4E 5 µm DMF (300 x 7.5 mm), and Styragel HR 2 5 µm DMF (300 x 7.5 mm). The system was equilibrated at 70 °C in prefiltered DMF containing 0.05 M LiBr, which served as polymer solvent and eluent (flow rate set to 1.00 mL min<sup>-1</sup>). Polymer solutions were prepared at a concentration of *ca.* 3 mg mL<sup>-1</sup>, and an injection volume of 200 µL was used. Data collection and analysis were performed with Empower 2 v. 6.10.01.00 software (Waters, Inc.). The system was calibrated with poly(ethylene glycol) standards (Polymer Laboratories, Amherst, MA).

Thermogravimetric analysis (TGA) was performed under N<sub>2</sub> atmosphere using a Mettler-Toledo model TGA/SDTA851e, with a heating rate of 10 °C min<sup>-1</sup> and cooling rate of 5 °C min<sup>-1</sup>. Measurements were analyzed using Mettler-Toledo Star<sup>e</sup> v. 7.01 software.

Glass transition temperatures ( $T_g$ ) were measured by differential scanning calorimetry (DSC) on a Mettler-Toledo DSC822® (Mettler-Toledo, Inc., Columbus, OH), with a heating rate of 10 °C min<sup>-1</sup>. Measurements were analyzed using Mettler-Toledo Star<sup>e</sup> v. 7.01 software. The  $T_g$  was taken as the midpoint of the inflection tangent, upon the third heating scan.

Dynamic light scattering (DLS) measurements were conducted using Delsa Nano C (Beckman Coulter, Inc., Fullerton, CA) equipped with a laser diode operating at 658 nm. Size measurements were made in nanopure water ( $n = 1.3329$ ,  $\eta = 0.890$  cP at  $25 \pm 1$  °C). Scattered light was detected at 165° angle and analyzed using a log correlator over 70 accumulations for a 3.0 mL sample in a glass sizing cell (4.0 mL capacity). The samples in the glass sizing cell were equilibrated for 30 minutes before measurements were made. The photomultiplier aperture and the attenuator were automatically adjusted to obtain a photon counting rate of *ca.* 10 kcps. Calculation of the particle size distribution and distribution averages was performed using CONTIN particle size distribution analysis routines. The peak averages of histograms from number distributions out of 70 accumulations were reported as the average diameters of the particles.

Transmission electron microscopy (TEM) images were collected on a JEOL 1200EX operating at 100 kV and micrographs were recorded at calibrated magnifications using a SIA-15C CCD camera. The samples as aqueous solutions (4  $\mu$ L) were deposited onto carbon-coated copper grids. Excess sample was wicked off using filter paper and the grids were allowed to dry in air for 2 min. Following that, the grids were stained with 4  $\mu$ L of a 2% uranyl acetate aqueous solution. Excess stain was wicked off using filter paper after 15 sec. The sample grids were dried under vacuum overnight before analyses.

A SpectraMax M5 microplate reader was used for the analysis of lactic acid by the colorimetric assay.

### 3. Synthesis of poly(DL-lactide)<sub>40</sub>-macro chain transfer agent (PDLLA<sub>40</sub>-macroCTA)

A 250 mL Schlenk flask equipped with a stir bar was flame dried under vacuum and cooled under nitrogen. The flask was then charged with hydroxyl-functionalized chain transfer agent (390 mg, 0.868 mmol), DL-lactide (5.01 g, 34.7 mmol) and DCM (100 mL), and stirred under nitrogen until complete dissolution of monomer. A stock solution of DBU in DCM (158 mg in 100  $\mu$ L, 1.04 mmol) was added under nitrogen flow and the yellow color solution was allowed to stir at room temperature. After 45 min >99% monomer to polymer conversion was achieved as measured by <sup>1</sup>H NMR, and the reaction mixture was quenched by the addition of acetic acid (1.04 g, 17.4 mmol) and poured into 150 mL of methanol to afford a yellow color sticky precipitation. The reaction mixture was concentrated by rotary evaporation of dichloromethane. The precipitate was isolated by filtration and dried under vacuum to yield 3.8 g (70% yield based on conversion) of yellow color powder. <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm):  $\delta$  5.25-5.08

(m, CH of PDLLA), 4.17-4.01 (m, CH<sub>2</sub>CH<sub>2</sub>O and COOCH<sub>2</sub> of CTA), 3.28-3.20 (t, CH<sub>2</sub>CH<sub>2</sub>S of CTA), 1.75-1.61 (m, CH<sub>3</sub>CCH<sub>3</sub> and OCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>O of CTA), 1.61-1.42 (m CH<sub>3</sub> of PDLLA), 1.42-1.16 (m, CH<sub>3</sub>(CH<sub>2</sub>)<sub>10</sub>CH<sub>2</sub>S of CTA), 0.88 (t, terminal CH<sub>3</sub> of CTA). <sup>13</sup>C NMR (CDCl<sub>3</sub>, ppm): δ 169.6, 69.2, 16.8. IR (cm<sup>-1</sup>): 2994-2947, 1746, 1452, 1381, 1265, 1182, 1082, 866.  $M_n^{\text{calc}} = 6200$  Da,  $M_n^{\text{DMF-GPC}} = 16100$  Da, PDI = 1.04. DSC:  $T_g = 41$  °C. TGA in N<sub>2</sub>: 260-385 °C, >92% mass loss.

#### 4. Synthesis of poly(*tert*-butyl acrylate)<sub>80</sub>-*block*-poly(DL-lactide)<sub>40</sub> (PtBA<sub>80</sub>-*b*-PDLLA<sub>40</sub>) diblock copolymer

To a 25 mL Schlenk flask with a stir bar and sealed by a rubber septum were charged with *t*BA (4.17 g, 32.6 mmol), PDLLA<sub>40</sub>-macroCTA (1.01 g, 0.163 mmol), AIBN (1.33 mg, 8.15 μmol, 5 mol %), and 5 mL of 2-butanone as the solvent. After three cycles of freeze-pump-thaw, the flask was placed in an oil bath at 56 °C. The polymerization was quenched after 4.5 h when the monomer conversion was measured to be 40% by <sup>1</sup>H NMR. The polymer solution was precipitated three times in methanol/H<sub>2</sub>O (2:1). The product was collected and dried under vacuum overnight at room temperature to afford PtBA<sub>80</sub>-*b*-PDLLA<sub>40</sub> as a pale yellow solid. Yield: 2.50 g (93% yield based on conversion). <sup>1</sup>H NMR (CDCl<sub>3</sub>, ppm): δ 5.23-5.10 (m, CH of PDLLA), 4.20-4.01 (m, CH<sub>2</sub>OC(O)), 3.32 (t, CH<sub>2</sub>SC(S)), 2.36-2.13 (br, CHC(O) of PtBA polymer backbone), 1.92-1.09 (br, alkyl chain of CTA, C(CH<sub>3</sub>)<sub>2</sub> of CTA, HOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O-, -CH<sub>2</sub>- of polymer backbone, *tert*-butyl group of *t*BA units, CH<sub>3</sub> of PDLLA), 0.87 (t, CH<sub>3</sub> of CTA chain end). <sup>13</sup>C NMR (CDCl<sub>3</sub>, ppm): δ 174.4-173.9, 169.8-169.4, 80.6-80.4, 69.3-67.0, 42.6-41.8, 37.6-36.0, 28.2, 16.8. IR (cm<sup>-1</sup>): 3070-2792, 1724, 1448, 1365, 1255, 1143, 844, 750.  $M_n^{\text{calc}} = 16400$  Da,  $M_n^{\text{DMF-GPC}} = 33800$  Da, PDI = 1.05. DSC:  $T_g = 42$  °C. TGA in N<sub>2</sub>: 190-225 °C, 29% mass loss; 225-310 °C, 18% mass loss, 310-360 °C, 28% mass loss; 360-395 °C, 8% mass loss, 17% mass remaining above 395 °C.

#### 5. Synthesis of poly(acrylic acid)<sub>80</sub>-*block*-poly(DL-lactide)<sub>40</sub> (PAA<sub>80</sub>-*b*-PDLLA<sub>40</sub>) diblock copolymer

A 250 mL round bottom flask equipped with a stir bar was charged with PtBA<sub>80</sub>-*b*-PDLLA<sub>40</sub> (2.00 g, 0.122 mmol). TFA (150 mL) was added to dissolve the polymer, and the reaction was allowed to stir for 2 h at room temperature, after which the TFA was removed under vacuum. The crude product was dissolved in 100 mL of DMF and transferred to a presoaked dialysis tubing (MWCO 12-14 kDa), and dialysis against nanopure water proceeded for 2 days. As partial precipitation was observed upon dialysis, the final mixture was lyophilized to yield a yellow solid. Yield: 1.35 g, 93%. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, ppm): δ 13.23-11.82 (br, COOH), 5.36-5.05 (m, CH of PDLLA), 4.28-4.15 (m, CH<sub>2</sub>OC(O)), 2.39-2.07 (br, CH of PAA polymer backbone), 1.89-1.64 (br, CH<sub>2</sub> of PAA polymer backbone) 1.64-1.14 (br, alkyl chain of CTA, C(CH<sub>3</sub>)<sub>2</sub> and HOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O of CTA, CH<sub>3</sub> of PDLLA), 0.87 (t, -CH<sub>3</sub> of CTA chain end). <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, ppm): δ 176.5-175.9, 169.8-169.2, 69.7-68.7, 42.1-41.0, 36.9-35.9, 16.9. IR (cm<sup>-1</sup>): 3550-2744, 1747, 1643, 1452, 1382, 1182, 1089, 1041, 908.

$M_n^{\text{calc}} = 11900$  Da. DSC:  $T_g(\text{PDLLA}) = 49$  °C,  $T_g(\text{PAA}) = 115$  °C. TGA in  $\text{N}_2$ : 195-220 °C, 40% mass loss; 220-370 °C, 30% mass loss.

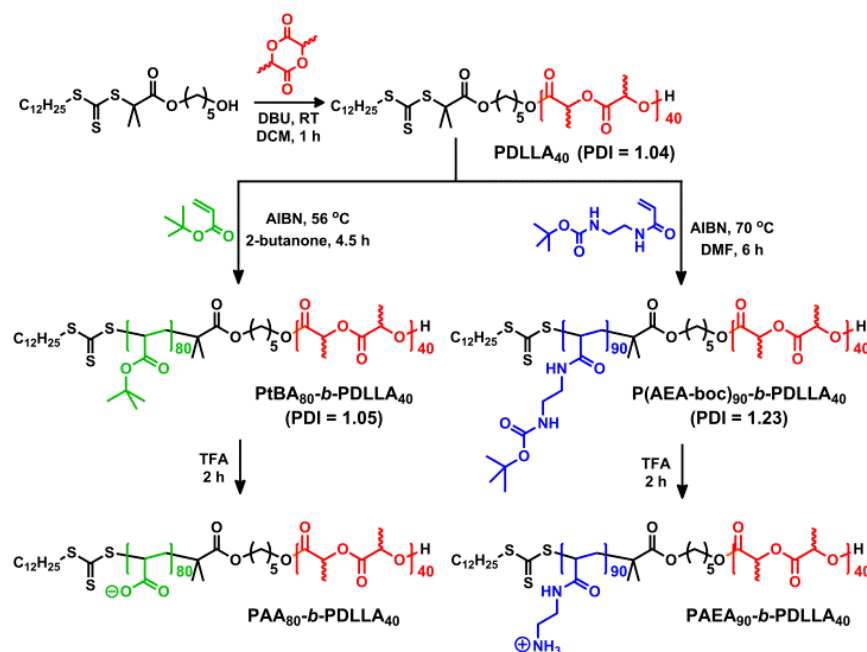
#### 6. Synthesis of poly(acrylamidoethylamine-boc)<sub>90</sub>-*block*-poly(DL-lactide)<sub>40</sub> (P(AEA-boc)<sub>90</sub>-*b*-PDLLA<sub>40</sub>) diblock copolymer

A 50 mL Schlenk flask equipped with a stir bar was flame dried under vacuum and charged with PDLLA<sub>40</sub>-macroCTA (1.50 g, 0.242 mmol), *tert*-butyl (2-methacrylamidoethyl)carbamate (5.17 g, 24.2 mmol), DMF (33 mL), and AIBN (7.94 mg, 48.4 μmol), and the mixture was allowed to stir under nitrogen. Upon complete dissolution of the starting material, the reaction mixture was subjected to three freeze-pump-thaw cycles. The reaction was allowed to stir for 6.5 h at 70 °C to afford 90% conversion. The product was precipitated into a 1:1 methanol: water mixture, isolated by filtration and dried under vacuum overnight to yield 4.4 g (73% yield) of pale yellow color solid. <sup>1</sup>H NMR (DMF-d<sub>7</sub>, ppm): δ 8.02-7.78 (br, boc-NH), 6.99-6.71 (br, CH<sub>2</sub>CH<sub>2</sub>NHCO), 5.32-5.24 (m, CH of PDLLA), 4.37-3.96 (br, CH<sub>2</sub>CH<sub>2</sub>O, COOCH<sub>2</sub> and CH<sub>2</sub>CH<sub>2</sub>S of CTA), 3.47-3.05 (br, NHCH<sub>2</sub>CH<sub>2</sub>NH), 2.40-2.00 (br, CH of PAEA-boc backbone), 1.86-1.63 (br, CH<sub>2</sub> of PAEA-boc backbone and, CH<sub>3</sub>CCH<sub>3</sub> and OCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>O of CTA), 1.61-1.51 (m CH<sub>3</sub> of PDLLA), 1.45-1.35 (br, CH<sub>3</sub> of boc groups), 1.35-1.20 (br, CH<sub>3</sub>(CH<sub>2</sub>)<sub>10</sub>CH<sub>2</sub>S of CTA), 0.88 (t, terminal CH<sub>3</sub> of CTA). <sup>13</sup>C NMR (DMF-d<sub>7</sub>, ppm): δ 175.4 (br), 170.1 (br), 156.4, 78.4, 69.6-69.1 (br), 45.6-39.0 (br), 28.8, 16.9. IR (cm<sup>-1</sup>): 3312, 3078-2976, 1755, 1690-1651, 1520, 1452, 1366, 1250, 1167, 1090, 1001.  $M_n^{\text{calc}} = 25500$  Da,  $M_n^{\text{DMF-GPC}} = 27000$  Da, PDI = 1.26. DSC:  $T_g = 48$  °C. TGA in  $\text{N}_2$ : 200 – 250 °C, 39% mass loss; 250 – 440 °C, 33% mass loss; 28% mass remaining above 440 °C.

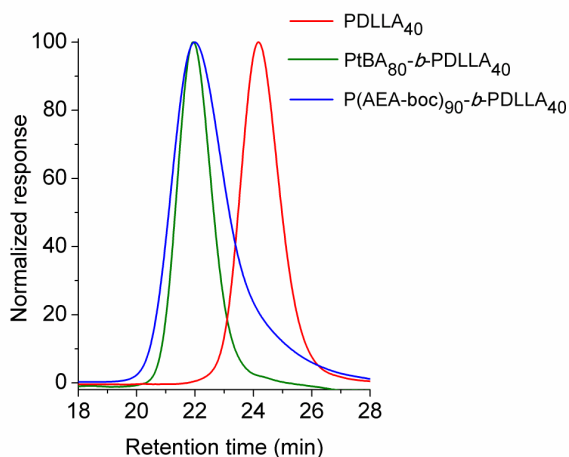
#### 7. Synthesis of poly(acrylamidoethylamine)<sub>90</sub>-*block*-poly(DL-lactide)<sub>40</sub> (PAEA<sub>90</sub>-*b*-PDLLA<sub>40</sub>) diblock copolymer

The diblock copolymer P(AEA-boc)<sub>90</sub>-*b*-PDLLA<sub>40</sub> (100 mg, 3.92 μmol) was dissolved in excess TFA (6.03 g, 52.9 mmol) in a 20 mL scintillation vial and allowed to stir for 2 h at room temperature. After this reaction period, TFA was removed under vacuum, to yield a light yellow solid. The product was dissolved in DMF (30 mL), transferred into a presoaked dialysis tube (12-14 kDa MWCO) and dialyzed against nanopure water for 2 d with frequent replacement of the dialysis medium with fresh nanopure water, to yield 84.6 mL of clear micelles in water (0.80 mg mL<sup>-1</sup>). The solution of micelles was divided into aliquots, lyophilized in 1.5 mL centrifugation tubes (0.5 mg of polymer per tube, based on final concentration) and stored at -4 °C. <sup>1</sup>H NMR (DMF-d<sub>7</sub>, ppm): δ 8.86-8.34 (br, NH), 6.61-4.48 (br, NH<sub>2</sub>), 5.32-5.24 (m, CH of PDLLA, overlapped with br NH<sub>2</sub> peaks), 4.37-3.96 (br, CH<sub>2</sub>CH<sub>2</sub>O, COOCH<sub>2</sub> and CH<sub>2</sub>CH<sub>2</sub>S of CTA, overlapped with br NH<sub>2</sub> peaks), 3.74-3.34 (br, NH<sub>2</sub>CH<sub>2</sub>), 3.31-3.07 (br, CH<sub>2</sub>NHCO), 2.44-2.09 (br, CH of PAEA backbone), 1.91-1.60 (br, CH<sub>2</sub> of PAEA backbone, CH<sub>3</sub>CCH<sub>3</sub> and OCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>O of CTA), 1.60-1.47 (m, CH<sub>3</sub> of PDLLA), 1.35-1.20 (br, CH<sub>3</sub>(CH<sub>2</sub>)<sub>10</sub>CH<sub>2</sub>S of CTA), 0.88 (t, terminal CH<sub>3</sub> of CTA). <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, ppm): δ 176.0-174.0 (br), 169.6, 80.2-78.6 (multiple overlapping peaks), 69.1, 43.8-41.2 (br), 17.1.

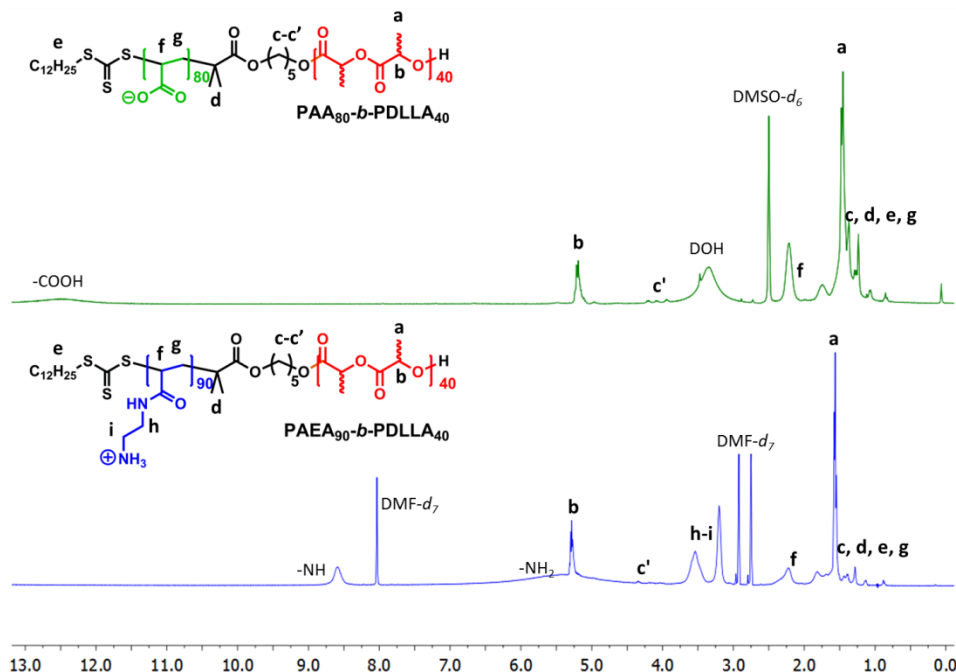
IR (cm<sup>-1</sup>): 3590-2502 (br), 1749, 1643, 1537, 1454, 1389, 1179, 1126, 835, 799.  $M_n^{\text{calc}} = 16500$  Da, DSC:  $T_g = 48$  °C. TGA in N<sub>2</sub>: 200 – 245 °C, 25% mass loss; 245 – 450 °C, 52% mass loss; 23% mass remaining above 450 °C.



**Scheme S1.** Synthesis of PDLLA<sub>40</sub> homopolymer and PtBA<sub>80</sub>-*b*-PDLLA<sub>40</sub> and P(AEA-boc)<sub>90</sub>-*b*-PDLLA<sub>40</sub> diblock copolymers by sequential ROP and RAFT polymerization, followed by acidolysis to afford the final amphiphilic diblock copolymers PAA<sub>80</sub>-*b*-PDLLA<sub>40</sub> and PAEA<sub>90</sub>-*b*-PDLLA<sub>40</sub>.



**Fig. S1.** GPC traces of homopolymer PDLLA<sub>40</sub>, and diblock copolymers PtBA<sub>80</sub>-*b*-PDLLA<sub>40</sub> and P(AEA-boc)<sub>90</sub>-*b*-PDLLA<sub>40</sub>, showing narrow molecular weight distributions.



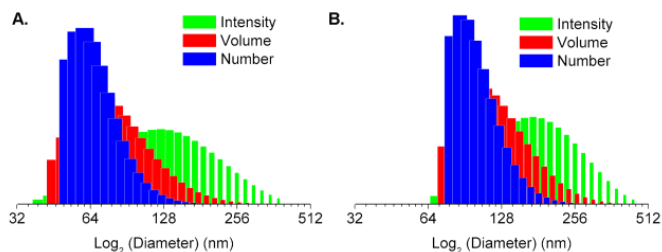
**Fig. S2.**  $^1\text{H}$  NMR spectra of diblock copolymers  $\text{PAA}_{80}\text{-}b\text{-PDLLA}_{40}$  and  $\text{PAEA}_{90}\text{-}b\text{-PDLLA}_{40}$ .

#### 8. Preparation of anionic micelles

Anionic micelles were prepared by a solvent displacement method. A 20 mL scintillation vial equipped with a stir bar was charged with 4 mL of 0.1 M Tris-HCl buffer containing 0.05% w/v  $\text{NaN}_3$  (pH 7.4). A stock solution of  $\text{PAA}_{80}\text{-}b\text{-PDLLA}_{40}$  in DMF (10 mg in 4.00 mL of DMF) was prepared and added dropwise to the solution of buffer under stir, and the mixture was allowed to stir for 1 h. The bluish solution of micelles was transferred into presoaked dialysis tubing (MWCO ca. 12-14 kDa) and dialyzed against 0.1 M Tris-HCl buffer containing 0.05% w/v  $\text{NaN}_3$  (pH 7.4) for 1 d. The final volume of micelles solution was measured to be 14.7 mL and the concentration was calculated to be  $0.68 \text{ mg mL}^{-1}$ .  $(D_h)_n$  (DLS) =  $65 \pm 18 \text{ nm}$ ,  $(D_h)_v$  (DLS) =  $87 \pm 35 \text{ nm}$ ,  $(D_h)_i$  =  $147 \pm 67 \text{ nm}$ , Zeta potential =  $-55 \text{ mV}$  in 0.1 M Tris-HCl buffer at pH 7.4.  $(D_{av})$  (TEM) =  $20 \pm 3 \text{ nm}$ .

#### 9. Preparation of cationic micelles

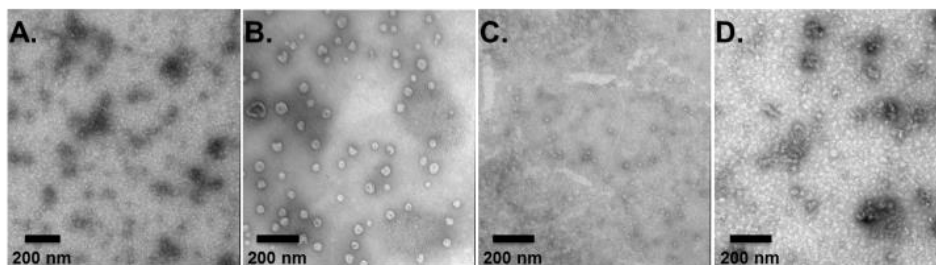
Cationic micelles were prepared by a direct-dissolution method. Lyophilized polymer  $\text{PAEA}_{90}\text{-}b\text{-PDLLA}_{40}$  was dissolved in 0.1 M Tris-HCl buffer containing 0.05% w/v  $\text{NaN}_3$  at pH 7.4 at a predetermined concentration of  $0.66 \text{ mg mL}^{-1}$ .  $(D_h)_n$  (DLS) =  $101 \pm 26 \text{ nm}$ ,  $(D_h)_v$  (DLS) =  $128 \pm 46 \text{ nm}$ ,  $(D_h)_i$  =  $191 \pm 74 \text{ nm}$ , Zeta potential =  $+26 \text{ mV}$  in 0.1 M Tris-HCl buffer at pH 7.4.  $(D_{av})$  (TEM) =  $44 \pm 7 \text{ nm}$ .



**Fig. S3.** Characterization by DLS showing histograms of number-, volume- and intensity-averaged hydrodynamic diameters of anionic micelles (A) and cationic micelles (B).

#### 10. Degradation of micelles

For the degradation experiments, the initial concentrations of the freshly prepared solutions of anionic and cationic micelles were adjusted to maintain equivalent PDLLA (substrate) concentrations. For anionic micelles, the solution was diluted to  $0.48 \text{ mg mL}^{-1}$  ( $0.040 \text{ mM}$  of PAA<sub>80</sub>-*b*-PDLLA<sub>40</sub>,  $3.2 \text{ mM}$  of LA) in  $0.1 \text{ M}$  Tris HCl buffer at pH 7.4 containing  $0.05\%$  w/v NaN<sub>3</sub>. For cationic micelles the initially prepared concentration of  $0.66 \text{ mg mL}^{-1}$  ( $0.040 \text{ mM}$  of PAEA<sub>90</sub>-*b*-PDLLA<sub>40</sub>,  $3.2 \text{ mM}$  LA) was carried forward. Each solution of micelles ( $0.5 \text{ mL}$ ) was placed in  $1.5 \text{ mL}$  centrifugation tubes. Two stock solutions of PLE and PK were prepared in the same buffer to maintain similar enzymatic activities (*ca.*  $1 \text{ U } \mu\text{L}^{-1}$ ). Solution of PLE ( $91 \text{ } \mu\text{L}$ ,  $5482 \text{ U mL}^{-1}$ ) was diluted in  $409 \text{ } \mu\text{L}$  of Tris-HCl buffer and solution of PK ( $367 \text{ } \mu\text{L}$ ,  $1362 \text{ U mL}^{-1}$ ) was diluted in  $133 \text{ } \mu\text{L}$  of Tris-HCl buffer. For the enzyme-catalyzed hydrolysis of micelles, stock solutions of enzyme ( $50 \text{ } \mu\text{L}$ ,  $1 \text{ U } \mu\text{L}^{-1}$ ) was added to each tube, mixed by the use of a vortex, and incubated at  $37 \text{ } ^\circ\text{C}$ , using an incubating shaker. To determine the hydrolytic degradation rates of the samples in the absence of enzyme catalysis,  $50 \text{ } \mu\text{L}$  of Tris-HCl buffer was added to each solution (to maintain identical polymer concentrations as the enzyme catalyzed experiment), and incubated at  $37 \text{ } ^\circ\text{C}$ . The lactate assays were performed following the standard protocol of ab65331 as described below. At time points 0, 1, 6, 12 and 24 h,  $50 \text{ } \mu\text{L}$  of sample was withdrawn from each tube, mixed with  $50 \text{ } \mu\text{L}$  of lactate enzyme assay mix in a  $100 \text{ } \mu\text{L}$  Falcon clear well, protected from light and incubated at room temperature for 30 min to produce color, and analyzed using a plate reader for absorption at  $450 \text{ nm}$ .<sup>[2]</sup> Each analysis was performed in triplicate and average absorbance values with standard deviations were reported. A calibration curve for DL-lactic acid was constructed by the use of serial dilutions produced from a standard solution of  $0.1 \text{ M}$  DL-lactic acid in  $0.1 \text{ M}$  Tris-HCl buffer (calibration range: 0-10 nmol of lactate), and the production of DL-lactic acid at each time point was quantified and reported as a percentage of the total theoretical DL-lactic acid present in each solution at the predetermined concentrations.



**Fig. S4.** TEM micrographs of micelles after incubation with enzymes for 24 h; (A) anionic micelles, partially aggregated and ill-defined after treatment with PK, upon *ca.* 35% core hydrolysis; (B) cationic micelles, mostly intact, yet relatively ill-defined and swollen after treatment with PK, upon *ca.* 15% core hydrolysis; (C) anionic micelles, fairly well-defined and intact after treatment with PLE, upon <3% core hydrolysis; (D) cationic micelles, mostly disassembled, ill-defined and aggregated after treatment with PLE, upon *ca.* 15% core hydrolysis. The core diameters of the anionic and cationic micelles before degradation were ~20 nm and ~44 nm, respectively (Fig. 1).

#### References for Supporting Information

- [1] a) A. Li, H. P. Luehmann, G. Sun, S. Samarajeewa, J. Zou, S. Zhang, F. Zhang, M. J. Welch, Y. Liu, K. L. Wooley, *ACS Nano* **2012**, *6*, 8970-8982; b) J. Zou, S. Zhang, R. Shrestha, K. Seetho, C. L. Donley, K. L. Wooley, *Polymer Chemistry* **2012**, *3*, 3146-3156.
- [2] Abcam Home Page. <http://www.abcam.com>. Lactate Assay Colorimetric Protocol. <http://www.abcam.com/ps/products/65/ab65331/documents/ab65331%20L%20Lactate%20Assay%20Kit%20Colorimetric%20%28Website%29.pdf>