## Toroidal structures from brush amphiphiles

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# **Supporting Information**

#### **Experimental Section**

#### Materials

All reagents were commercially available and used as received unless otherwise noted. Glycidyl methacrylate (GMA, Aldrich 97%) was passed through a short basic alumina column to remove the inhibitor. 2,2'-Azobis(isobutyronitrile) (AIBN) and D,L-lactide were recrystallized from methanol and anhydrous ethyl acetate, respectively. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) was kept over molecular sieves (3 Å) overnight. Deionized water was purified in a Barnstead Nanopure system to a final resistance of 18.2 m $\Omega$ ; it will be referred to as Nanopure water.

### Characterization

Gel permeation chromatography (GPC) was performed on a Waters 1515 Isocratic HPLC equipped with two Styragel® columns (HR4, HR3, 300 mm x 7.8 mm) connected in series, a differential refractive index detector (Waters 2414) and a UV-visible detector (Waters 2489). HPLC grade tetrahydrofuran was used as the eluent, at a flow rate of 1 mL/min. Molecular weights are reported referenced to polystyrene standards (Shodex SL-105). <sup>1</sup>H NMR spectra were recorded on a Bruker AV 400 MHz spectrometer in either CDCl<sub>3</sub> or d<sub>6</sub>-DMSO. Spectra were referenced to CHCl<sub>3</sub> (7.26 ppm) or DMSO (2.50 ppm). Dynamic Light Scattering experiments were conducted on a Malvern Instruments Nano-ZS ZetaSizer equipped with a 4 mW He-Ne laser operating at 633 nm. All measurements were performed at 25 °C at a scattering angle of 173°. Bright-field transmission electron microscopy (TEM) was performed on a FEI Tecnai 12 TWIN transmission electron microscope operated at an acceleration voltage of 100 kV. TEM images were recorded by a SIS Megaview III wide-angle CCD camera. Carbon-coated copper grids (Electron Microscopy, Hatfield, PA) were ionized under plasma before sample preparation. Grids were placed on a drop of aggregate suspension (20  $\mu$ L). After 5 min, the grid was washed under five drops of doubly distilled water and placed onto a drop (20 µL) of a 2 wt% aqueous uranyl acetate solution for 30 s. Grids were then blotted with filter paper and samples were allowed to dry at room temperature prior to imaging.

#### Methods

#### Differential refraction index measurement

Measurements were performed on an Optilab-rEX refractive index detector (Wyatt Technology) at a wavelength of 658 nm. Flow cell temperature was set at 25 oC. Sample and solvent solutions were pumped with a syringe pump (New Era Pump System, NE-1000) at 0.2 mL/min through a 0.45 um PVDF syringe filter (Thermo Scientific) prior to measurements. Five concentrations of each sample were prepared in anhydrous dimethylformamide: 0.2, 0.5, 1.0, 1.5, and 2.0 mg/mL. Aggregate solutions were prepared in Nanopure water at the following concentrations: 0.01, 0.02, 0.04, 0.06, 0.12 and 0.21 mg/mL. dn/dc values were analyzed using an Astra 6.1 software.

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#### Static light scattering

Static light scattering (SLS) was performed on a Dawn Heleos II (Wyatt Technology) with a 120 mW GaAs linearly polarized laser operating at 658 nm. Sample and solvent solutions were pumped with a syringe pump (New Era Pump System, NE-1000) at 0.2 mL/min through a 0.45 um PVDF syringe filter (Thermo Scientific) prior to measurements. Polymer and aggregate concentrations were the same as above. Measurements of molecular brushes and micelles were normalized using polystyrene (20 kDa, Fluka; 5 mg/mL solution in dimethylformamide) and dextran (9 kDa-11 kDa, Aldrich; 5 mg/mL solution in Nanopure water), respectively. dn/dc values were applied to determine the absolute molecular weights and  $M_w$  was extracted from Zimm plots using Astra 6.1 software and the Debye model.

#### Self-assembly of amphiphilic molecular brushes.

#### a) Under a rapid change in solvent quality

Amphiphilic molecular brushes were dissolved in tetrahydrofuran (THF, 2.5 mg/mL unless otherwise specified) and sonicated. A lower concentration (0.5 mg/mL) was also examined for PGMA<sub>500</sub>-g-PEG<sub>16</sub>/PLA<sub>26</sub>. Rapid self-assembly was carried out in a four-stream vortex mixer. A detailed description and characterization of the mixer are provided elsewhere.<sup>1</sup> Nanopure water was charged into three 50 mL syringes (Hamilton, NJ) and the organic solution into a 10 mL syringe, and mounted on two separate syringe drivers (PHD Ultra, Harvard Apparatus). The THF:water volumetric ratio (solvent jump) used was 1:9 with mixing speeds of 12 mL/min and 108 mL/min (36 mL/min per stream) for the organic and aqueous phases, respectively. Other solvent jumps used were 1:3 and 1:12. For the 1:3 jump, the velocities of each stream were 30 mL/min (aqueous and organic). For the 1:12 jump, the velocity of the aqueous stream was 6 mL/min, while for the aqueous phases they were 36 mL/min. Samples were collected in clean scintillation vials and dialyzed (6-8 kDa MWCO, Spectrapor) against Nanopure water for 24 h. Water was replenished every 4 h throughout the dialysis process. Suspensions were stored in clean scintillation vials at 4 °C for further use.

#### b) By dialysis

For the dialysis method, 2.5 mg/mL of polymer solution in THF was transferred to a dialysis membrane (6-8 kDa MWCO, Spectrapor) and dialyzed against water under mild stirring for 24 h. Approximately 5 mL of the polymer solution (in THF) was loaded into a dialysis membrane and placed into a 1 L beaker containing 1 L of Nanopure water (final resistance of 18.2 m $\Omega$ ). The contents of the beaker were stirred with a magnetic stir bar at ~200 rpm. The dialysis bag did not touch the bottom of the beaker to prevent interference with stirring. Dialysis took place for 24 h at room temperature. During the first 12 h, water was replenished three times (approximately every 3 hours). The suspension was left in the water used for the fourth replenishment overnight. The contents of the dialysis bag were then carefully transferred into clean and dry scintillation vials for storage.

#### Synthesis

#### PGMA backbone

Synthesis of the PGMA backbone is described in detail elsewhere.<sup>2</sup> Briefly, GMA (3 mL, 0.022 mol), 2cyano-2-propyl benzodithioate (CPB) (4.3  $\mu$ L, 0.022 mmol,) and AIBN (0.7452 mg, 0.0045 mmol) were added to a clean and dry round bottom flask equipped with a magnetic stir bar and a septum. Reagents were dissolved in 3 mL of benzene, and the mixture was bubbled with nitrogen for 1 h. The reaction was conducted at 60 °C for 16 h. Polymerization was stopped by immersing the flask in an ice bath and opening it to atmosphere. Aliquots were taken to evaluate monomer conversion. Samples were diluted with dichloromethane and precipitated in methanol. The resulting precipitate was dried under vacuum. Yield: 46.7%. GPC: M<sub>n</sub>=71.06 kDa, M<sub>w</sub>/M<sub>n</sub>=1.16 (Figure SI1). <sup>1</sup>H NMR peaks assignments is shown in Figure SI2. Conversion was evaluated based on the ratio of the signal from polymer (e) and from unreacted monomer (e', Figure SI1 inset), according to  $I_e/I_e + I_{e'}$ . Conversion was calculated to be 50%, and the degree of polymerization (DP) is  $\sim$ 500. DP was estimated by multiplying conversion and the initial monomer/chain transfer agent molar ratio.

#### Synthesis of PGMA<sub>500</sub>-g-N<sub>3</sub>.

PGMA (500 mg, 3.52 mmol epoxy groups) was added to a round bottom flask and dissolved in 20 mL of *N*,*N*-dimethylformamide (DMF). Sodium azide (700 mg, 10.77 mmol) and ammonium chloride (575 mg, 10.75 mmol) were added once the polymer was completely dissolved. Azidolysis was conducted at 50 °C under reflux for 24 h. The resulting solution was cooled to room temperature and diluted with THF. Salts were filtered off and the solution was concentrated before precipitation into Nanopure water. Solids were freeze-dried. Yield: 64.2%. <sup>1</sup>H NMR peaks assignments are shown in Figure SI3; signals indicate complete conversion.

#### *Synthesis of PGMA*<sub>500</sub>-g-PLA<sub>x</sub>.

The protocol for this synthesis is adapted from Huang *et al.*<sup>2</sup> Known amounts of PGMA<sub>500</sub>-*g*-N<sub>3</sub> and D,L-lactide were loaded into a round bottom flask, and placed under high vacuum for ~5 h. After backfilling with nitrogen, anhydrous DMF was added to dissolve the reagents. DBU was then injected and the reaction was allowed to proceed for 1.5 h under nitrogen at room temperature. Polymerization was quenched by addition of benzoic acid. DMF was removed under vacuum and the polymer was redissolved in THF, followed by precipitation into a mixture of water and methanol (1:1, vol). Solids were lyophilized. <sup>1</sup>H NMR of the resulting grafted polymer is shown in Figure SI4. DP of PLA side chain was calculated by the ratio of signal from the terminal unit of PLA (g) and signal e from the PLA chain  $(I_e/I_e + 1).^2$ 

Table S1	. Summarv	of the sy	vntheses	of PGMA <sub>500</sub>	-g-PLA <sub>x</sub>	copolymers	with	different PL	A lengths.
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copolymer	PGMA <sub>500</sub> -g-N <sub>3</sub> (mg, mmol of OH)	D,L-lactide (g, mmol)	DBU (µL, mmol)	DMF (mL)	Benzoic acid (mg, mmol)	Yield
PGMA500-g-PLA11	130, 0.7	0.562, 3.9	21.7, 0.145	20	45, 0.368	72.9%
PGMA <sub>500</sub> -g-PLA <sub>26</sub>	50, 0.27	0.571, 3.96	21.9, 0.146	10	45, 0.368	84.1%
PGMA <sub>500</sub> -g-PLA <sub>56</sub>	100, 0.54	2.8, 19.43	108, 0.722	35	220, 1.801	68%

#### Alkynyl-terminated PEG.

Monomethoxypoly(ethylene glycol) (mPEG, 10.13 g, 13.5 mmol) was transferred to a round bottom flask and placed under high vacuum for ~5 h. After backfilling the flask with nitrogen, 5-hexynoic acid (1789  $\mu$ L, 16.21 mmol) and 4-(dimethylamino)pyridine (DMAP) (676.49 mg, 5.54 mmol) were added and purged with nitrogen for 30 min. Then, 30 mL of anhydrous dichloromethane (DCM) were added to dissolve the reagents. *N*,*N*'-dicyclohexylcarbodiimide (DCC) (5.57 g, 27 mmol) was dissolved in 5 mL of anhydrous DCM and added to the reaction mixture dropwise *via* syringe. The raw product was filtered and extracted with 1 M HCl solution 10 times, with DI water 3 times, and with a 1 M NaOH solution 5 times. The solution was then dried over anhydrous magnesium sulfate. After filtration, the solvent was removed under vacuum and the product was dried in the vacuum oven. Yield: 63.9%. <sup>1</sup>H NMR (Figure S5) indicated quantitative conversion.

#### $PGMA_{500}$ -g- $PEG_m/PLA_n$ .

The protocol for this synthesis was adapted from Chen, *et al.*<sup>3</sup> 'Click' reaction was catalyzed by CuSO<sub>4</sub>•5H<sub>2</sub>O/ascorbic acid. In all cases, a molar ratio of N<sub>3</sub>, alkynyl groups, CuSO<sub>4</sub>•5H<sub>2</sub>O and ascorbic acid was kept at 1:1:0.2:1 (details in Table SI2). PGMA<sub>500</sub>-g-PLA<sub>x</sub> was mixed with an equimolar amount of alkynyl-PEG in a round bottomed flask. After complete dissolution in DMF, ascorbic acid was added and the solution was bubbled with nitrogen for 30 min. CuSO<sub>4</sub>•5H<sub>2</sub>O was then added under nitrogen and

the reaction proceeded for 12 h. The solvent was evaporated, and the raw product re-dissolved in THF. Polymers were purified by dialysis against Nanopure water for 4 days and lyophilized. <sup>1</sup>H NMR spectra are shown in Figure SI6.

Table S2.	Summary of the syntheses	of PGMA500-g-PEG16/PLA	x amphiphilic bloc	k copolymers.
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copolymer	PGMA <sub>500</sub> -g-PLA <sub>n</sub> (mg, mmol of N <sub>3</sub> )	Alkynl-PEG (mg, mmol)	CuSO <sub>4</sub> • 5H <sub>2</sub> O (mg, mmol)	Ascorbic acid (mg, mmol)	DMF (mL)	Yield
PGMA500-g-PEG16/PLA11	200, 0.21	174.2, 0.21	9.78, 0.04	36.1, 0.21	15	86.1%
PGMA500-g-PEG16/PLA26	202, 0.098	86, 0.10	5.23, 0.02	17.75, 0.10	10	89.2%
PGMA500-g-PEG16/PLA56	500, 0.12	92.97, 0.11	5.22, 0.02	18.38	15	76.5%



Figure S1. Chromatogram of PGMA<sub>500</sub> backbone.



Figure S2. <sup>1</sup>H NMR spectra of purified PGMA<sub>500</sub> and unpurified material containing monomer (inset).



Figure S3. <sup>1</sup>H NMR spectrum of PGMA<sub>500</sub>-N<sub>3</sub>.



Figure S4. <sup>1</sup>H NMR spectra of PGMA<sub>500</sub>-g-PLA<sub>x</sub>. From bottom to top x=11, 26, and 56.



Figure S5. <sup>1</sup>H NMR spectrum of 5-hexynoic acid functionalized alkynyl-PEG.



Figure S6. <sup>1</sup>H NMR spectra of PGMA<sub>500</sub>-g-(PEG<sub>16</sub>/PLA<sub>x</sub>). From bottom to top x=11, 26, and 56.

### *Calculation of Reynolds' number in the Multi-Inlet Vortex Mixer (MIVM)* The Reynolds number was calculated based on the velocities of all streams according to

$$\operatorname{Re} = \sum_{i=1,N} \frac{V_i}{V_i} D$$

where *D* is the chamber diameter,  $V_i$  and  $v_i$  are the velocity and kinematic viscosity of the *n*-th inlet stream, and *N* the number of inlet streams.<sup>1</sup>

Table S3. Results from GPC and <sup>1</sup>H NMR analysis of amphiphiles used in this study.

	Mn <sup>a</sup> (kDa)	Mn <sup>b</sup> (kDa)	Mw <sup>b</sup> (kDa)	<b>Đ</b> <sup>b</sup>	PEG grafting efficiency <sup>a</sup> (%)	WPEG <sup>a</sup>	<i>R<sub>g</sub></i> <sup><i>c</i></sup> (nm)
PGMA <sub>500</sub>	71.0	53.8	62.6	1.16			
PGMA500-g-(PEG16/PLA11)	896		1,723	1.92	96	0.48	22.3
PGMA500-g-(PEG16/PLA26)	1,438		2,425	1.69	97	0.28	25.0
PGMA500-g-(PEG16/PLA56)	2,491		4,063	1.63	90	0.14	31.4

<sup>*a*</sup> estimated from <sup>1</sup>H NMR; <sup>*b*</sup> determined by gel permeation chromatography with polystyrene standards; <sup>*c*</sup> determined by static light scattering from DMF.



**Figure S7.** Particle size distributions of spherical particles from  $PGMA_{500}$ -*g*-( $PEG_{16}/PLA_{11}$ ) (**A**) and vesicles from  $PGMA_{500}$ -*g*-( $PEG_{16}/PLA_{56}$ ) (**B**), formed under conditions of rapid self-assembly and dialysis. Aggregates prepared under kinetically-arrested conditions generally exhibit narrower distributions and better control over size compared to those where processes leading to thermodynamic equilibration occur.



**Figure S8.** Network structure formed by the self-assembly of PGMA<sub>500</sub>-*g*-(PEG<sub>16</sub>/PLA<sub>56</sub>) under dialysis showing the effect of stirring on aggregate morphology.



Re=8196 H<sub>2</sub>O : THF= 9:1

**Figure S9.** Transmission electron micrograph of aggregate morphologies of PGMA<sub>500</sub>-*g*-(PEG<sub>16</sub>/PLA<sub>26</sub>), prepared by a rapid mixing process at an initial polymer concentration of 0.5 mg/mL. Reynolds number (Re) and final solvent quality (H<sub>2</sub>O:THF) are specified.

#### References

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- 3. P. Zhao, Y. C. Yan, X. Q. Feng, L. X. Liu, C. Wang and Y. M. Chen, Polymer, 2012, 53, 1992-2000.