### **Supporting Information**

## Trehalose-functionalized block copolymers form serum-stable micelles

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**Materials:** All chemicals were purchased from Sigma Aldrich Co and used as acquired with the exception of: anhydrous D-trehalose (99%, Acros Organics), acetic anhydride (99.6%, Fisher), dry pyridine (99.8%, Sigma Aldrich), sodium chloride (Fisher), silica gel (Sorbent technologies, porosity 60Å size 40-60µm), chlorotrimethylsilane TMSCI (Fisher, 98%), triethylamine (TEA) (Acros Organics, 99.7%), and HCl 1.25M in methanol (Fluka). All solvents were obtained from Fisher excluding hexanes (Macron Chemical, ACS grade) and used as received unless otherwise specified. Tetrahydrofuran (THF, HPLC grade) and N,N-dimethylformamide (DMF, HPLC grade) were dried using a solvent purification system purchased from MBRAUN. N,N-dimethylacrylamide (DMA) and methyl acrylate (MA) was purified by passing through basic alumina to remove trace amounts of monoethyl ether hydroquinone stabilizer. All polymers were characterized using Varian Inova 500 NMR Spectrometer.

#### Synthesis of 6-methacrylamido-6-deoxy trimethylsilyloxy trehalose (TMAT):

Synthesis of TMAT was achieved by a published procedure reported in the literature (NMR, Figure S1).<sup>1</sup>

#### Synthesis of poly(ethyelene-alt-polypropylene)-chain transfer agent (PEP-CTA):

Synthesis of PEP-OH was achieved by anionic polymerization, following a published procedure.<sup>2</sup> Next the PEP-CTA was also created by an established procedure.<sup>3, 4</sup> Briefly, the carboxy terminated CTA *S*-1-dodecyl-*S*'-( $\alpha,\alpha$ '-dimethyl- $\alpha$ ''-acetic acid) trithiocarbonate was reacted with oxalyl chloride to yield a more reactive acyl chloride derivative. The PEP-OH was then dissolved in DCM (dichloromethane) and treated with the acyl chloride derivative of the trithiocarbonate CTA yielding the PEP-CTA. The final PEP-CTA structure was purified by precipitation into ice cold and dry methanol five times from DCM.

 $M_n = 3.6 \text{ kg/mol by }^{1}\text{H} \text{ NMR in CDCl}_3 \text{ and } D = 1.08 \text{ (Figure S2)}.$ 

#### **Reactivity ratio study of DMA and TMS-MAT:**

The reactivity ratios of DMA and TMS-MAT were determined by completing a free radical polymerization study with both monomers using azobisisobutyronitrile (AIBN) as the initiator at 70 °C in toluene. The feed composition of DMA in terms of mole fraction added to the reaction was varied from 0.10 to 0.90. The conditions were the same as used for the synthesis of the PT diblock terpolymers (described below) with the exception that the total concentration of the monomers (DMA and TMS-MAT) was 0.5 M. Solutions (1200 $\mu$ L of a 0.5 M solution) of DMA and TMS-MAT were created in deuterated toluene.

Example: In an NMR tube, 300  $\mu$ L (1.5 × 10<sup>-4</sup> mol) of DMA and 50  $\mu$ L (2.5 × 10<sup>-5</sup> mol) of TMS-MAT were added. AIBN initiator was then added (3.5 × 10<sup>-7</sup> to 1.75 × 10<sup>-5</sup> mol) to the solutions. Nitrogen was bubbled through the solution in the NMR tube using a long syringe for 20 min before the NMR tube was placed into the preheated variable temperature NMR at 70 °C. <sup>1</sup>H NMR spectra were recorded at room temperature prior to varying the temperature. <sup>1</sup>H NMR was recorded at various time points (60 s for the first 15 min and 600s for next 1 hour). The

spectra were analyzed by Mestronova (Version 6.2.1) for the feed ratio and mole fraction of monomers in the copolymers. The  $F_1$  value, which is the mole fraction of TMS-MAT in copolymer, was calculated based on integration of the vinyl protons of TMS-MAT. Linear (Finemann and Ross) and non-linear least squares fitting of the data was performed to calculate reactivity ratios.

**Scheme S1.** Reactivity ratios of TMS-MAT (monomer 1) and DMA (monomer 2) were determined by varying the feed ratio of each monomer in free radical polymerizations.



**Conditions:** AIBN : monomer =500-1000 : 1 mole fraction, total conc. = 0.5 M, the conversion was kept below 15 mole %, temp = 70 °C.

**Table S1.** Details of the conversion of TMS-MAT and DMA used to determine the reactivity ratios for the copolymerization of TMS-MAT and DMA via free radical polymerization in toluene at 70 °C

Run	$f_{I}{}^{a}$	Conv. of TMS-MAT <sup>b</sup>	Conv. of DMA <sup>b</sup>	$F_1^c$
1	0.11	7.5	12.5	0.07
2	0.21	9.5	15.6	0.14
3	0.33	7	18	0.17
4	0.47	8.7	21	0.27
5	0.64	5.36	14.3	0.4
6	0.79	5.25	20.5	0.49
7	0.91	1.9	12	0.62

<sup>a</sup>TMS-MAT mole fraction in the feed determined by <sup>1</sup>H NMR spectroscopy, <sup>b</sup>Conversion of each monomer determined <sup>1</sup>H NMR spectroscopy, <sup>c</sup>Mole fraction of TMS-MAT in the copolymer calculated from the feed mole fraction of TMS-MAT and the conversion. Remark: The conversion of each monomer was restricted below 20 mol % to keep the instantaneous feed composition the same during the time course of the polymerization.

**Table S2:** Analysis of the reactivity ratios by the Finemann-Ross method for TMS-MAT (monomer 1) and DMA (monomer 2). The mole fractions of TMS-MAT and DMA,  $f_1$  and  $f_2$  respectively, in the feed were calculated by <sup>1</sup>H NMR.  $F_1$  and  $F_2$  are the mole fractions of TMS-MAT and DMA in the copolymer.

Fineman-Ross					
Run	$f_1$	$f_2$	$F_1$	$F_2$	
1	0.111	0.889	0.067	0.933	
2	0.209	0.791	0.138	0.862	
3	0.333	0.667	0.171	0.829	
4	0.467	0.533	0.27	0.73	
5	0.638	0.362	0.4	0.6	
6	0.787	0.213	0.491	0.509	
7	0.909	0.091	0.62	0.38	

**Table S3:** Calculations for determining the reactivity ratios  $(r_1 \text{ and } r_2)$  using the Fineman and Ross linear fitting model.

TMS-MAT (Monomer 1)						
<b>f</b> <sub>12</sub>	<i>F</i> <sub>12</sub>	$f_{12}^2/F_{12}$	$f_{12}(1-F_{12})/F_{12}$			
0.12	0.07	0.22	1.62			
0.26	0.16	0.44	1.39			
0.50	0.21	1.21	1.92			
0.88	0.37	2.08	1.49			
1.76	0.67	4.66	0.88			
3.69	0.96	14.2	0.14			
9.99	1.63	61.2	-3.87			

Where  $f_{12} = f_{TMAT}/f_{DMA}$  i.e. ratio of monomer TMAT to DMA

 $F_{12} = F_{TMAT}/F_{DMA}$  i.e. ratio of monomer TMAT to DMA in the copolymer



**Figure S6.** Linear fitting data using the Finemann-Ross method yielding reactivity ratios of TMS-MAT and DMA in free radical polymerization.

Where,  $r_1 = k_{11}/k_{12}$ 

$$r_2 = k_{22}/k_{21}$$

 $k_{11}$  = Reaction rate of monomer TMS-MAT with monomer TMS-MAT

 $k_{12}$  = Reaction rate of monomer TMS-MAT with monomer DMA

 $k_{22}$  = Reaction rate of monomer DMA with monomer DMA

 $k_{21}$  = Reaction rate of monomer DMA with monomer TMS-MAT

# Synthesis of poly(ethyelene-alt-polypropylene)-poly(DMA-grad-MAT) diblock (PT) terpolymers<sup>4</sup>:

Synthesis of the PT diblock terpolymers were achieved by copolymerization of DMA and TMS-MAT using RAFT polymerization (at 70 °C, AIBN initiator) in toluene with the PEP-CTA as the macromolecular chain transfer agent (MacroCTA). The feed composition for DMA and TMS-MAT is listed in Table 1. After polymerization, the TMS groups were deprotected in a 1.25 M HCl solution in methanol. A representative procedure is described in detail below.

For example: <u>PT (3.6-24.5-0.11)</u>: To a single neck reaction flask charged with 0.3 g of PEP-CTA ( $8.3 \times 10^{-3}$  mol), AIBN (0.68 mg, 4.41 × 10<sup>-6</sup> mol), DMA (that was filtered through basic alumina) (1.48 gm, 1.49 × 10<sup>-2</sup> mol), and TMS-MAT (1.52 gm, 1.66 × 10<sup>-3</sup> mol) were added along with 8.3 ml of toluene. The reaction mixture was degassed for 45 min (N<sub>2(g)</sub>) before placing in an oil bath preheated to 70 °C, and the mixture was stirred at 700 rpm for 10 hours. The polymerization was quenched by cooling the mixture to 0 °C in an ice bath and opening it to air. Conversion by <sup>1</sup>H NMR (Figure S2): DMA = 99.5% and TMS-MAT = 91.5%, *M*<sub>n</sub> by <sup>1</sup>H NMR = 38.1 kg/mol, *D* = 1.23 by GPC (Figure S3, chloroform).

Conversion by <sup>1</sup>H NMR (Figure S2) for PT (3.6-21.1-0.05): DMA = 92% and TMS-MAT = 92%,  $M_n$  by <sup>1</sup>H NMR = 29.3 kg/mol, D = 1.19 by GPC (Figure S3, chloroform).

Conversion by <sup>1</sup>H NMR (Figure S2) for PT (3.6-26.4-0.14): DMA = 93% and TMS-MAT =87%,  $M_n$  by <sup>1</sup>H NMR = 43.2 kg/mol, D = 1.28 by GPC (Figure S3, chloroform).

Next, the solvent was removed under vacuum and the reaction mixture redissolved in THF (20 ml), which was then sonicated. To this mixture, 0.5 ml of 1.25 M HCl in methanol was added (mixture turned cloudy) and 3 mL of methanol was added (resulting in a clear solution) to remove the TMS moieties from the trehalose groups. The solvent was then removed and the solid was redissolved in THF : MeOH (20:3) and precipitated into pentanes (2x) yielding a yellowish powder, 2.3g, 0.082 mmol, 93 % yield. <sup>1</sup>H NMR  $\geq$  99%.  $M_n = 28.1$  kg/mol by <sup>1</sup>H NMR (Figure

S2). For PT (3.6-21.1-0.05) = 1.8 g, 0.073 mmol, 67 % yield,  $M_n = 24.7$  kg/mol. For PT (3.6-26.4-0.14) = 1.6g, 0.053 mmol, 89 % yield,  $M_n = 30$  kg/mol.

Removal of the trithiocarbonate chain portion from the PT diblock was attained through subsequent aminolysis followed by Micheal addition as described below.<sup>5</sup>

<u>PT (3.6-24.5-0.11)</u><sup>4</sup>: 2.3 g (8.2 × 10<sup>-5</sup> mol) of PT (3.6-24.5-0.11) was dissolved in 20 ml (4:1 v/v) THF : MeOH. The reaction mixture was degassed by bubbling nitrogen through the reaction mixture for 45 min. After bubbling, *n*-butyl amine (178  $\mu$ L, 1.81 × 10<sup>-3</sup> mol) and tris(2carboxyethyl)phosphine hydrochloride (TCEP, 8.214 × 10<sup>-5</sup> mol, 21 mg) were added and stirred for 24 hours at 25 °C (it was noticed that the bright yellow color of the reaction mixture disappeared). Next, methyl acrylate (MA, 2.87 × 10<sup>-3</sup> mol, 260  $\mu$ L) was added and stirred for another 24 hours at 25 °C. The solvent was removed and the final product (1.8 g) was precipitated into pentane (3x) (UV-Vis curve is shown in Figure S4) yield = 78%, 0.06 mmol, 1.8 g.

PT  $(3.6-21.1-0.05) = 1.5 \text{ g}, 0.061 \text{ mmol}, 83 \% \text{ yield}, M_n = 24.7 \text{ kg/mol}.$ 

PT (3.6-26.4-0.14) = 1.2 g, 0.04 mmol, 75 % yield,  $M_n = 30$  kg/mol.

<u>PD (3.6-23.5)</u> was achieved according to the previously published method.<sup>4</sup> Details of polymerization and conversion are listed in Table 1.



**Figure S1.** <sup>1</sup>H NMR spectrum of TMS-MAT monomer in CD<sub>2</sub>Cl<sub>2</sub> at 21°C at 500 MHz.

The integration values below the peak are relative to proton 2. \* indicates the residual solvent peak.



**Figure S2**. <sup>1</sup>H NMR spectrum of the PEP-CTA, PD (3.6-23.5) and PT (3.6-24.5-0.11). <sup>1</sup>H NMR spectra were recorded in CDCl<sub>3</sub> with the exception of the spectrum for PT (3.6-24.5-0.11) (2 drops of deuterated methanol was added to help dissolve the sample).



**Figure S3:** SEC chromatograms of the PEP-CTA, PD (3.6-23.5) and the TMS protected PT polymers: PT (3.6-21.1-0.05), PT (3.6-24.5-0.11), and PT (3.6-26.4-0.14) immediately following polymerization. Chromatograms were recorded on a chloroform SEC equipped with three Jordi polydivinylbenzene columns with pore sizes of 10000, 1000, and 500 Å, respectively, and a Hewlett-Packard 1047A refractive index detector. The flow rate was set at 1.0 mL/min and the chromatography was run at 35 °C. The calibration curve was based on PS standards.

D = 1.07 for PEP-CTA, 1.12 for PEP-DMA and 1.23 for TMS protected PT (3.6-24.5-0.11).



**Figure S4:** UV-Vis curve of PEP-CTA shows absorption at 309 nm. This characteristic peak of the trithiocarbonate moiety is absent in PD(3.6 - 23.5), PT(3.6-21.1-0.05), PT(3.6-24.5-0.11) and PT (3.6-26.4-0.14) signifying the amminolysis reaction and removal of the endgroup. THF was used as the solvent for the PEP-CTA and PD (3.6-23.5). For the polymers PT(3.6-21.1-0.05), PT(3.6-24.5-0.11) and PT (3.6-26.4-0.14), a mixture of THF:methanol (20:3 v/v) was used as the solvent.



**Figure S5:** DSC thermograms of PD (3.6-23.5), PT (3.6-21.1-0.05), PT (3.6-24.5-0.11) and PT (3.6-26.4-0.14). Conditions: for each sample, between 5-8 miligrams of each polymer sample was placed in a hermetically-sealed Tzero aluminium pans and analyzed on a Discovery DSC. The heating rate was 10°C /min starting at 20 °C and heated to 200°C. The second cycle of heating is shown above. TRIOS software is used to calculate glass transition temperature. The DSC thermograms showed increase in  $T_g$  with an increase in MAT content.

Dynamic light scattering (DLS): All micellar dispersions were filtered through a 0.2µm filter to remove large scatters before loading into dust free glass tubes, which were then sealed with several layers of parafilm. Measurements were recorded over 5 different angles ranging from 60° to 150° at increments of 15°. For studies examining the serum stability of the micelles, 0.1 ml of each micellar dispersion (formed by nanoprecipitation) was diluted with 0.5 ml of biological media and filtered through a 0.2  $\mu$ m filter into dust free tubes. The data were recorded at time 0 and 14 hours to determine micelle stability in different media at 90°. Scattering measurements were performed on a Mini L-30 Laser (Brookhaven Instruments) equipped with a red laser source ( $\lambda = 637$  nm), BI-APD avalanche photo diode detector to determine scattering intensity, and a Brookhaven BI-9000 correlator. The samples were placed in a decalin oil bath at  $25.0 \pm 0.5$ °C. The intensity correlation functions  $(g_2(t))$  were measured over five different scattering angles between 60° and 120° and converted into field correlation,  $(g_1(t))$  using the Siegert relation  $g_2(t) = 1 + g_1(t)^2$ . Later, we used the cumulant (Equation 1) function for micellar dispersions with monomodal expansion and the double exponential function (Equation 2) for dispersion with multimodal expansion to extract information about the decay rate ( $\Gamma$ ).

$$g1(t) = A \exp(-\Gamma t) \left(1 + \frac{\mu^2}{2!} t^2 - \frac{\mu^3}{3!} t^3\right) + bkgd$$
 (Equation 1)  
$$g1(t) = A1 \exp(-\Gamma 1t) + A2 \exp(-\Gamma 2t) + bkgd$$
 (Equation 2)

Where:

Equation 1:  $\mu_2$ ,  $\mu_3$  are the second and third cumulant values

Equation 2:  $\Gamma_1$  and  $\Gamma_2$  are the fast and slow decay modes, respectively

Furthermore, the particle size distribution was determined by the second cumulant  $(\mu_2/\Gamma^2)$ , a measure of the width of the decay rate distribution. The translational diffusion coefficient (D) can be obtained by the relation  $\Gamma = q2D$  by performing linear regression using Origin software, where q is the scattering vector. Lastly, the apparent hydrodynamic radii ( $R_h$ ) were determined by using the Stoke-Einstein equation (Equation 3).

$$Rh = (kBT)/6\pi\eta D$$
 (Equation 3)

where, (k<sub>B</sub>: Boltzmann constant, T: temperature, and  $\eta$ : viscosity of the medium). We used literature values of refractive index (n) and viscosity of the specific media at 25 °C when analyzing samples prepared in the 5 different media.<sup>4</sup>



**Figure S7.** Linear regression of  $\Gamma$  vs.  $q^2$  over 5 different angles varying from 60° to 120° of the two decay modes in water with PD (3.6-23.5) (micelles formed via nanoprecipitation). The correlation function  $g_1(t)$  was fit using the cumulant expansion function. Micelles with radii of 14.7±0.2 nm were determined and  $\mu/\Gamma^2$  value at 90° scattering angle is 0.07.



**Figure S8.** Linear regression of  $\Gamma$  vs.  $q^2$  over 5 different angles varying from 60° to 120° of the two decay modes in water with PT (3.6-21.1-0.05) (micelles formed via nanoprecipitation). The correlation function  $g_1(t)$  was fit using cumulant expansion function. Micelles with radii of 14.4±0.4 nm were determined  $\mu/\Gamma^2$  value at 90° scattering angle is 0.152.



**Figure S9.** Linear regression of  $\Gamma$  vs.  $q^2$  over 5 different angles varying from 60° to 120° of the two decay modes in water with PT (3.6-24.5-0.11) (micelles formed via nanoprecipitation). The correlation function  $g_1(t)$  was fit using cumulant expansion function. Micelles with radii of 14.3±0.2 nm were determined  $\mu/\Gamma^2$  value at 90° scattering angle is 0.124.



**Figure S10.** Linear regression of  $\Gamma$  vs.  $q^2$  over 5 different angles varying from 60° to 120° of the two decay modes in water with PT (3.6-21.1-0.05) (micelles formed by direct dissolution). The correlation function  $g_1(t)$  was fit using double exponential function. Micelles with radii of 13.6±0.3 nm and 55±1.3 nm were determined.



**Figure S11.** Linear regression of  $\Gamma$  vs.  $q^2$  over 5 different angles varying from 60° to 120° of the two decay modes in water with PT (3.6-24.5-0.11) (micelles formed by direct dissolution). The correlation function  $g_1(t)$  was fit using double exponential function. Micelles with radii of 21.3±0.7 nm and 129.7±3.7 nm were determined.



**Figure S12.** Linear regression of  $\Gamma$  vs.  $q^2$  over 5 different angles varying from 60° to 120° of the

two decay modes in water with PT (3.6-26.4-0.14) (micelles formed by direct dissolution). The

correlation function  $g_1(t)$  was fit using double exponential function. Micelles with radii of

 $16.7\pm0.4$  nm and  $173.9\pm7.2$  nm were determined.

#### **References:**

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