Electronic Supplementary Information

Temperature-responsive telechelic dipalmitoylgllycerl poly(N-isopropylacrylamides) vesicles: real-time morphology observation in aqueous suspension and in the presence of giant liposomes

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**Materials.** Methacryloyloxyethyl isocyanate was a gift of Showa Denko K. K. (Tokyo, Japan). Diethylene glycol di[2-(1-isobutyl)sulfanylthiocarbonylsulfanyl-2-methylpropionate] was synthesized as described previously.\[^{22}\] Azobisisobutyronitrile (AIBN) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and recrystallized from methanol prior to use. N-Isopropylacrylamide (NIPAM) was obtained from TCI Chemicals (Tokyo, Japan) and recrystallized from an acetone/hexanes (4/6, v/v) mixture. Other reagents were purchased from Sigma-Aldrich Co. LLC (St. Louis, MO) and used without further purification. MilliQ water (EMD Millipore, Billerica, MA) was used for all aqueous solution.

**Synthesis of 3-(2-methacryloyloxyethyl carbamoyloxy)propane-1,2-diyl dipalmitate (DPG-MA)** (see Scheme S1) Methacryloyloxyethyl isocyanate (169 mg, 1.0 mmol) was added to a solution of dipalmitoyl glycerol (206 mg, 0.36 mmol) in dry chloroform (7 mL) placed in a flame-dried flask. The mixture was warmed to 50 ºC and stirred at this temperature for 105 h. It was cooled to room temperature. The solvent was removed by evaporation in a flask. The residual product was purified by chromatography over a silica gel column eluted with an ethyl acetate/hexane gradient (1:1 v:v to 5:1 v:v). Yield: 226 mg, 84.6 %. \[^{1}\] H NMR (CDCl\(_3\)) ppm, \(\delta\): 0.87 (t, -(CH\(_2\))\(_{13}\)CH\(_2\)C\(_3\)H), 1.18 (s, NHCH(C\(_3\)H\(_2\))), 1.26 (s, -(CH\(_2\))\(_{11}\)CH\(_2\)CH\(_3\))), 1.50 - 2.60 (multiplets, polymer backbone protons), 3.99 (s, -NHC\(_3\)H). The molecular weight data

**Synthesis of α,ω-di(dipalmitoyl glyceroyl poly(N-isopropylacrylamide), (tDPG-PNIPAM)** (see Scheme S1) α,ω-Dimercapto poly(NIPAM) samples (M\(_n\) 11,700, 21,000 and 28,800 g/mol) were prepared as reported previously.\[^{25}\] They were converted to tDPG-PNIPAM following the procedure described here in the case of α,ω-dimercapto poly(NIPAM) (M\(_n\) : 21,000 g/mol). The polymer (234.5 mg, 2.07 mmol NIPAM unit) was dissolved in dimethylformamide (4 mL) containing a small amount of Tris(2-carboxyethyl)phosphine Hydrochloride (TCEP). DPG-MA (92.3 mg, 0.128 mmol) was added to the mixture and the mixture was stirred overnight at room temperature. The resulting polymer was precipitated into diethyl ether polymer was purified by two consecutive precipitations from dimethylformamide into diethyl ether, followed by dialysis (MWCO = 3,500) against water. \(^{1}\)H NMR (CDCl\(_3\)) ppm, \(\delta\): 0.87 (t, -(CH\(_2\))\(_{13}\)CH\(_2\)CH\(_3\)), 1.18 (s, NHCH(CH\(_2\))\(_2\)), 1.26 (s, -(CH\(_2\))\(_{11}\)CH\(_2\)CH\(_3\))), 1.50-2.60 (multiplets, polymer backbone protons), 3.99 (s, -NHCH). The molecular weight data
listed in Table 1 are those of the PNIPAM precursors (tTTC-PNIPAM in Scheme S1) of each sample. They were determined by gel permeation chromatography (Agilent 1100 isocratic pump, Agilent Technologies, CA) connected to multangle laser light scattering detector at 690 nm (Dawn EOS, Wyatt Technology, CA). (Eluent: dimethylformamide, Column: TSK-gel R-M + TSK-gel R-3000, Temperature: 40 °C, Flow rate 0.5 mL/min). The dn/dc value of PNIPAM was determined to be 0.0738 mL/g at 690 nm in DMF at 40 ºC (Optilab DSP interferometric refractometer, Wyatt Technology). UV-visible spectrophotometry (CHCl₃; λ_{max} = 310 nm, ε = 11,400 L mol⁻¹ cm⁻¹) and ¹H NMR spectroscopy by comparison of integrals of the peaks derived from DPG (δ: 0.87) and PNIPAM (δ: 3.98).

**Preparation of tDPG-PNIPAM polymersomes**

A solution of DPG-PNIPAM in chloroform (1 mg/mL) was placed in a round-bottom flask. Subsequently, the chloroform was evaporated at 40°C to form a thin film on the surface of the flask. The film was kept under high vacuum overnight at room temperature to remove traces of chloroform. Water or a 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPES) buffer (10 mM including 10 mM MgCl₂, pH 7.5, 1 mL) was poured in the flask to rehydrate the film. The polymer thin film peeled of the glass surface upon treatment with a vortex mixer (3 min). The resulting mixture was subjected to ultrasonication with a sonicator probe (UD-201, TOMY SEIKO Co. ltd. Tokyo, Japan) at 40 W for 15 min. The solution was filtered through a 0.45 µm PVDF membrane filter to remove large polymer aggregates and dusts. The recovered filtrate was used without further treatment.

**Characterization of tDPG-PNIPAM polymersomes in solution**

The size of tDPG-PNIPAM polymersomes obtained by sonication in water as described above was evaluated by dynamic light scattering (DLS) measurements using a SZ-100 system (HORIBA, Ltd, Kyoto, Japan) equipped with a Peltier temperature controller. The DLS measurements were performed on polymer solutions with a concentration of 1 g/L at a wavelength of 632.8 nm and a 173° detection angle. The cloud point of DPG-PNIPAM polymersomes suspensions was evaluated by measuring the temperature-induced changes in transmittance at 600 nm of polymer solutions (1 mg/mL) using a UV-Vis spectrophotometer (V-630, JASCO co., Tokyo, Japan) equipped with a Peltier
temperature controller. The polymer solutions were heated from 10 °C to 45 °C at a heating rate of 0.1 °C/min. Fluorescence anisotropy (r) measurements were performed on a Fluorolog-2 spectrometer (SPEX, Edison, NJ, USA) equipped with a Glan-Thompson polarizer for excitation and a polarizing filter for emission in the L-format configuration. The temperature of the sample fluid was controlled using a water-jacketed cell holder connected to a circulating water bath. It was measured with a thermocouple immersed in a water-filled cell placed in one of the four cell holders in the sample compartment. The slit settings were 1.47 nm for both excitation and emission. The excitation and emission wavelengths were set at 340 and 426 nm, respectively. Fluorescent anisotropy was measured by using diphenyl hexatriene (DPH) as a fluorescent probe. The DPH (Final concentration: 1.0 µM) was dissolved in chloroform together with t-DPG-PNIPAM when preparing polymersome. The polymersome/DPH suspension was excited at a wavelength of 355 nm with the bandpass of 2.5 nm. The fluorescence anisotropy was calculated from the relationship:

\[ r = \frac{I_{VV} - G \cdot I_{VH}}{I_{VV} + 2G \cdot I_{VH}} \]

where G = I_{VH}/I_{HH} is an instrumental correction factor and I_{VV}, I_{VH}, I_{HV} and I_{HH} refer to the emission intensities at 426 nm polarized in the vertical and horizontal planes with excitation using vertically-polarized light and those with excitation using horizontally-polarized light, respectively (second subindex).

**Microscopic observation of polymersomes suspensions upon temperature jump from 24 to 50 °C.** The observations were performed on a microscopic observation system equipped with a fluid chip temperature controller described in detail previously[1] and shown schematically in Figure S7. The fluid chip consists of a hand-made small chamber (18 x 18 x 1 mm) with a flow path from a reservoir held at 20°C or 50°C and placed in contact with the sample via the objective immersion oil acting as heat transfer agent. The sample temperature was controlled by the water temperature and the flow rate adjusted by varying the height of the reservoir. Polymer solutions were subjected to temperature jumps from 24 °C to 50 °C or from 50 °C to 24 °C within 20 s and monitored as a function of time for up to 50 min. After this time, the experiment was discontinued since the system became unstable, particularly at high
temperature, possibly due to slow evaporation of the water. The behavior of the tDPG-PNIPAM polymersomes was recorded by a CCD video camera (WAT-232S, Watec, Yamagata, Japan) and analyzed using Image J ver. 1.42q software.

**Interactions between tDPG-PNIPAM polymersomes and giant DOPC liposomes.** A solution of 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC) and N-(Texas Red sulfonyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (Texas Red-DHPE) in chloroform was placed in a round-bottom flask. Subsequently, the chloroform was evaporated at 40 °C to form a thin film on the surface of the flask. The film was kept under high vacuum overnight at room temperature to remove traces of chloroform. The thin membrane was hydrated with HEPES buffer (10 mM including 10 mM MgCl₂, pH 7.5) at 30 °C for 1 h. The final concentration of DOPC and Texas Red-DHPE were 0.1 mM and 0.1 µM, respectively. The liposome suspension was added in a 1/1 v/v ratio to a tDPG-PNIPAM polymersome solution (1.0 mg/mL in HEPES buffer) kept at room temperature. The mixture was observed by bright field and fluorescence microscopy below and above the LCST of the tDPG-PNIPAM polymersome suspension.

**LS Measurements.**

For detailed and more accurate analysis of the structures formed at room temperature, solutions with a polymer concentration of 0.2 g/L were subjected, first, to sonication (Branson 1210) in a cooled bath during 30 min. Subsequently, they were filtered through 0.45 and 0.22 µm PVDF membrane filters to remove small amounts of large polymeric fragments and metallic fragments formed during the sonication. Two filters were used to facilitate filtration, since membranes of 0.22 µm used alone rapidly clog up due to the adsorption of the metallic fragments. The SLS and DLS measurements were carried out at 20 °C in the angular range from 35 to 135 ° to obtain $R_g$ and $R_h$ values, correspondingly. Estimated ratio of these sizes $R_g/R_h$ gave information about vesicular structures of particles. The results for two the most stable samples are provided in Figure S5.

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**Table S1** Synthetic results of tTTC-PNIPAM

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Time (h)</th>
<th>[NIPAM]/[DIGDIM] (mol/mol)</th>
<th>(M_n \times 10^3) (g mol(^{-1}))</th>
<th>Yield (%)</th>
<th>UV</th>
<th>NMR</th>
<th>SEC-MALS (Mw/Mn)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tTTC-PNIPAM8K</td>
<td>6</td>
<td>100</td>
<td>8.1</td>
<td>1.06</td>
<td>8.1</td>
<td>7.9</td>
<td>7.6</td>
</tr>
<tr>
<td>tTTC-PNIPAM10k</td>
<td>5.5</td>
<td>100</td>
<td>10.8</td>
<td>1.10</td>
<td>10.8</td>
<td>10.8</td>
<td>11.7</td>
</tr>
<tr>
<td>tTTC-PNIPAM20k</td>
<td>6</td>
<td>200</td>
<td>19.5</td>
<td>1.05</td>
<td>19.5</td>
<td>18.7</td>
<td>21.0</td>
</tr>
<tr>
<td>tTTC-PNIPAM30k</td>
<td>6</td>
<td>300</td>
<td>30.0</td>
<td>1.08</td>
<td>30.0</td>
<td>30.5</td>
<td>28.8</td>
</tr>
</tbody>
</table>

* [DIGDIM]/[AIBN]=5/1

**Scheme S1** Synthetic scheme for the preparation of tDPG-PNIPAM
**Figure S1** $^1$H-NMR spectrum of t'TTC-PNIPAM10k (CDCl$_3$)

**Figure S2** $^1$H-NMR spectrum of DPG-MA (CDCl$_3$)
**Figure S3** UV spectra telechelic PNIPAM-10K before and after aminolysis of the trithiocarbonate groups (CHCl₃).

**Figure S4** $^1$H-NMR spectrum of tDPG-PNIPAM10k (CDCl₃)
Figure S5  Determination of radius of gyration $R_g$ (left) and hydrodynamic radius $R_h$ (right) for tDPG-PNIPAM20k (A) and tDPG-PNIPAM30k (B) at 20 °C.

Figure S6  Concentration dependence of turbidity of DPG-PNIPAM10K
Figure S7  Microscopic observation system equipped with rapid temperature control unit

Figure S8  Microscopic observation of the dissociation of micrometer-scale tDPG-PNIPAM10K polymersomes by cooling a tDPG-PNIPAM10K aqueous suspension from 50 °C to 25 °C. Time 0 sec is the start of cooling and inset is monitoring temperature using system illustrated in Figure S7.
Figure S9  Phase-contrast and fluorescence images of micrometer-scale tDPG-PNIPAM10K polymersome and DOPE giant liposome (including 0.1 mol% of Texas Red-DHPE) at 50°C. The time point is after Figure 3 (j).
Figure S10  Fluorescence (a), (j) and optical (b) – (h) microscopic observation of the dissociation of micrometer-scale tDPG-PNIPAM10K polymersomes by cooling a tDPG-PNIPAM10K aqueous suspension from ~50 °C to 24 °C in the presence of DOPE giant liposome (including 0.1 mol% of Texas Red-DHPE). (a) is the start of cooling to (j). The inset is monitoring temperature using system illustrated in Figure S7.