The Temperature-Induced Phase Separation of well-defined Cyclic Poly(N-isopropylacrylamides) in Aqueous Solution

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SUPPORTING INFORMATION

Materials. All chemicals were purchased from Sigma-Aldrich Chemicals Co. unless otherwise specified. Azobisisobutyronitrile (AIBN, 98%) was recrystallized from methanol prior to use. N-Isopropylacrylamide (NIPAM, 99%) was obtained from Acros Organics and recrystallized from an acetone/hexanes (4/6, v/v) mixture. Propargyl acrylate (98%), 2-(2-chloroethoxy)ethanol (99%), 2-methyl-1-propanethiol (92%), carbon disulfide (99.9%), sodium hydroxide (97%+), triethylamine (99.5%) and tricaprylylmethylammonium chloride were used as received. 1,4-Dioxane and tetrahydrofuran (THF) were purified by a solvent purification system with two packed columns of activated alumina provided by Innovative Technology Inc. All other solvents were of reagent grade and used as received. The ion-exchange resins AG 501-X8 (D) was obtained from BioRad Laboratories. Water was deionized using a Millipore MilliQ system.

Instrumentation. ¹H NMR spectra were recorded on a Bruker AMX-400 (400 MHz) spectrometer. FTIR spectra were recorded on a Bruker Vector-22 spectrometer. Mass spectra were recorded on a Micromass AutoSpec TOF instrument equipped with a LSIM source (Centre Regional de Spectrometrie de Mass, Universite de Montreal, Montreal, QC, Canada). Gel permeation chromatography (GPC) was performed on a GPC-MALLS system consisting of an Agilent 1100 isocratic pump, a set of TSK-gel α-M (particle size 13 μ, exclusion limit 1_10⁷ Da for polystyrene in DMF) and a TSK-gel α-3000 (particle size 7 μ, exclusion limit 1_10⁵ Da for polystyrene in DMF) (Tosoh Biosep) columns, a Dawn EOS multi-angle laser light scattering detector λ = 690 nm (Wyatt Technology Co.) and an Optilab DSP interferometric refractometer λ = 690 nm (Wyatt Technology Co.) under the following conditions: injection volume, 100 μL; flow rate, 0.5 mL/min; eluent, DMF; temperature, 40 °C. The dn/dc value of PNIPAM was determined to be 0.0738 mL/g at 690 nm in DMF at 40 °C using an Optilab DSP interferometric refractometer (Wyatt Technology Corp).

Synthesis of 2-(2-azidoethoxy) ethanol¹. 2-(2-Chloroethoxy)ethanol (6.2 g, 50 mmol, 1.0 equiv) and sodium azide (6.5 g, 100 mmol, 2.0 equiv) were dissolved in 25 mL of deionized (DI) water. The solution was heated to 80 °C and stirred under N₂ atmosphere for 12 hrs. After cooling to room temperature, the solution was extracted with diethyl ether (Et₂O, 5_20 mL). The organic phases were combined and dried over anhydrous Na₂SO₄. The solvent was evaporated to yield a light yellow liquid product. Yield 6.2 g,
UV/Vis (methanol): S1 → OC 1.96 → CH was removed end methyl was equiv)

Yield solid Buchner aqueous (5.6 mL, 5.6 mmol) was added in one portion, followed by dropwise addition of an aqueous NaOH solution (13 mL, 50 wt%, 163 mmol). The resulting reaction mixture was stirred overnight at room temperature. Water (65 mL) was added, followed by concentrated HCl (33 mL) to acidify the reaction mixture to pH = 1 – 2. The remaining aceton was removed by purging with nitrogen. The yellow solid was collected with a Buchner funnel, washed with distilled water to eliminate the excess of salt. The orange solid was recrystallized from acetone/hexanes (1:10) to yield a bright yellow crystalline. Yield 6.8 g, 61%. MS (M+) found: 280.0617 m/z, calc. for C_{11}H_{20}O_{5}S_{2}: 280.47 m/z. \( ^1H \) NMR (CDCl \textsubscript{3}) ppm, δ 1.01 (d, 6H, -CH(CH \textsubscript{3})\textsubscript{2}), 1.69 (s, 6H, -SC(CH\textsubscript{3})\textsubscript{2}(C=O)), 1.96 (septet, 1H, -CH(CH\textsubscript{3})\textsubscript{2}), 3.19 (d, 2H, -SCH\textsubscript{2}).

2-(2-Azidoethoxy) ethyl 2-(1-isobutyl)sulfanylthiocarbonylsulfanyl-2-methyl propionate (AIP). Oxaly chloride (1.8 mL, 15 mmol, 3 equiv) was added while stirring to 2-(1-isobutyl)sulfanylthiocarbonylsulfanyl-2-methyl propionic acid (1.27 g, 5 mmol, 1 equiv) in 5 mL of dry CH\textsubscript{2}Cl\textsubscript{2} kept under nitrogen at room temperature. After the addition was completed, the mixture was warmed up to 40 – 50 °C for 1 – 2 hr resulting in the formation of a dark redish solution. The excess oxaly chloride and CH\textsubscript{2}Cl\textsubscript{2} were removed in vacuo to yield 2-(1-isobutyl)sulfanylthiocarbonylsulfanyl-2-methyl propionyl chloride. A solution of 2-(2-azidoethoxy)ethanol (1.0 g, 7.5 mmol) in dry CH\textsubscript{2}Cl\textsubscript{2} (5 mL) was added dropwise to the flask containing 2-(1-isobutyl)sulfanylthiocarbonylsulfanyl-2-methyl propionyl chloride. The resulting solution was kept at 40 – 50 °C for 6 hr. At the end of the reaction, the solution was cooled to room temperature and the solvent was removed in vacuo. The red oily liquid was redissolved in Et\textsubscript{2}O (50 mL). The Et\textsubscript{2}O phase was washed in sequence with saturated NaHCO\textsubscript{3}, brine, water and dried over anhydrous MgSO\textsubscript{4}. The Et\textsubscript{2}O was removed in vacuo and the product was dissolved in 10 mL of CH\textsubscript{2}Cl\textsubscript{2} and purified through a silica gel column using CH\textsubscript{2}Cl\textsubscript{2}/hexanes (7:3, v/v) as eluent. Yield 0.9 g, 50%. MS (M+) found: 365.0983 m/z, calc. for C\textsubscript{12}H\textsubscript{23}N\textsubscript{3}O\textsubscript{3}S\textsubscript{2}: 365.54 m/z. \( ^1H \) NMR (CDCl \textsubscript{3}) ppm, δ 1.02 (d, 6H, -CH(CH \textsubscript{3})\textsubscript{2}), 1.70 (s, 6H, -SC(CH\textsubscript{3})\textsubscript{2}(C=O)), 1.96 (septet, 1H, -CH(CH\textsubscript{3})\textsubscript{2}), 3.21 (d, 2H, -SCH\textsubscript{2}), 3.38 (t, 2H, -CH\textsubscript{2}N3), 3.68 (t, 2H, -OCH\textsubscript{2}CH\textsubscript{2}N3), 3.73 (t, 2H, -OCH\textsubscript{2}CH\textsubscript{2}O(C=O)-), 4.29 (t, 2H, -CH\textsubscript{2}O(C=O)-) (Figure S1); FTIR ν 2959, 2870, 2103, 1733, 1464, 1257, 1122, 1059, 813 cm\textsuperscript{-1} (Figure S2); UV/Vis (methanol): \( \lambda_{\text{max}} = 310 \text{ nm; } \varepsilon_{310} = 14,500 \text{ M}^{-1} \cdot \text{cm}^{-1} \).
Figure S1. $^1$H NMR spectrum of the chain transfer agent, 2-(2-azidoethoxy)ethyl 2-(1-isobutyl)sulfanylthiocarbonylsulfanyl-2-methyl propionate) (AIP), in CDCl$_3$.

Figure S2. FTIR spectrum of the chain transfer agent, AIP.
Polymerization. All RAFT polymerizations were performed in a septa-sealed round bottom flask with magnetic stirring. The typical procedure is exemplified with the preparation of the parent heterodifunctional telechelic polymer, \( \alpha-(2-(2-azidoethoxy)ethyl-2-methylpropionyl) \) _isobutylsulfonylthiocarbonylsulfonyl PNIPAM (p-PNIPAM-12k). A solution of the chain transfer agent (AIP, 73.1 mg, 0.2 mmol), the initiator (AIBN, 3.3 mg, 0.02 mmol) and the monomer (NIPAM, 2.26 g, 20 mmol) in 1,4-dioxane (10 mL) was placed in a round bottom flask with rubber septa. The solution was de-oxygenated by bubbling nitrogen for 30 min at room temperature. Then, the flask was placed in an oil bath preheated to 65 °C. The polymerization was allowed to proceed for 4 hrs under constant magnetic stirring. At the end of the polymerization, the solution was cooled to room temperature. The polymer was isolated by precipitation in diethyl ether. It was purified by two consecutive reprecipitations from THF into diethyl ether. Yield 1.30 g, 80%. \(^1\)H NMR (CDCl\(_3\)) ppm, \( \delta \) 1.02 (d, -CH\(_2\)CH(CH\(_3\))\(_2\)), 1.16 (s, -NHCH(CH\(_3\))\(_2\)), 1.20-2.40 (multiplets, polymer backbone protons), 2.31 (s, -C(=S)SC\(_3\)), 2.41 (t, -CH\(_2\)N\(_3\)), 2.71 (t, -CH\(_3\)OCH\(_2\)), 4.02 (s, -NHCH), 4.22 (s, -C(=O)OCH\(_2\)), 6.40 (bs, -C(=O)NH); FTIR \( \nu \) 3293, 2972, 1640, 1538, 1458, 1367, 1171, 1130, 736 cm\(^{-1}\).

Polymer end-group transformation by a one-pot aminolysis/Michael-addition sequence. The parent telechelic PNIPAM (p-PNIPAM-12k) (0.6 g, 0.05 mmol) was dissolved in THF (6 mL). The solution was degassed by bubbling N\(_2\) for 15 min. Propargyl acrylate (55 mg, 0.5 mmol, 10 equiv) and n-butyamine (37 mg, 0.5 mmol, 10 equiv) were added to the solution. The reaction mixture was stirred for 2 hours at room temperature under N\(_2\) atmosphere. The linear precursor, \( \alpha-(2-(2-azidoethoxy)ethyl-2-methylpropionyl) \) _propargyl acrylate PNIPAM (l-PNIPAM-12k) was recovered by precipitation in diethyl ether and purified by two consecutive reprecipitations from THF into diethyl ether. \(^1\)H NMR (CDCl\(_3\)) ppm, \( \delta \) 1.02 (d, -CH\(_2\)CH(CH\(_3\))\(_2\)), 1.16 (s, -NHCH(CH\(_3\))\(_2\)), 1.20-2.40 (multiplets, polymer backbone protons), 2.68 (s, -SCH\(_2\)CH\(_2\)C(=O)), 2.81 (s, -SCH\(_2\)CH\(_2\)C(=O)), 3.41 (t, -CH\(_2\)N\(_3\)), 3.71 (s, -CH\(_2\)OCH\(_2\)), 4.02 (s, -NHCH), 4.22 (s, -C(=O)OCH\(_2\)), 6.40 (bs, -C(=O)NH); FTIR \( \nu \) 3299, 2972, 2112, 1639, 1534, 1458, 1366, 1266, 1171, 1130, 736 cm\(^{-1}\).

Cyclization by “click” reaction. Deionized water (DI) (250 mL) was placed in a round bottom flask and degassed by bubbling N\(_2\) for 30 min. Sodium ascorbate (119 mg, 0.6 mmol, 75 equiv) was added to the aqueous solution followed by dropwise addition of CuSO\(_4\) (64 mg, 0.4 mmol, 50 equiv) in 1 mL of DI water, yielding a turbid brown suspension. A solution of l-PNIPAM-12k (100 mg, 0.008 mmol, 1 equiv) in deionized water (40 mL) was added to this suspension via a syringe pump at a rate of 2 mL/hr. The reaction was carried out at 30 °C under N\(_2\) atmosphere for 20 hrs. In mid of the reaction, another batch of catalyst consisting of sodium ascorbate (119 mg, 0.6 mmol, 75 equiv) and CuSO\(_4\) (64 mg, 0.4 mmol, 50 equiv), was added to the reaction mixture to compensate for the settled catalyst. At the end of the addition of aqueous l-PNIPAM-12k solution, the mixture was allowed to stir for another 60 min. The brown catalyst precipitate was removed by filtration. The filtrate was deionized through a column of ion-exchange resins AG 501-X8 (D) and dialyzed against DI water for 3 days. The cyclic polymer (c-PNIPAM-12k) was then recovered by freeze-drying. Yield 95 mg, 95%. \(^1\)H NMR (CDCl\(_3\)) ppm, \( \delta \) 1.02 (d, -CH\(_2\)CH(CH\(_3\))\(_2\)), 1.16 (s, -NHCH(CH\(_3\))\(_2\)), 1.20-2.40
(multipeaks, polymer backbone protons), 2.68 (s, -SCH$_2$CH$_2$C(=O)), 2.82 (s, -SCH$_2$CH$_2$C(=O)), 3.68 (t, -OCH$_2$CH$_2$OC(=O)\text{-}), 4.18 (t, -OCH$_2$CH$_2$OC(=O)\text{-}), 4.60 (t, =NCH$_2$CH$_2$O\text{-}), 5.25 (t, -C(=O)OCH$_2$C), 6.40 (bs, -C(=O)NH), 7.85 (s, H of triazole); FTIR ν 3302, 2970, 2112, 1639, 1537, 1458, 1367, 1267, 1172, 1130, 735 cm$^{-1}$.

Figure S3. $^1$H NMR spectra of p-PNIPAM-6k, l-PNIPAM-6k, and c-PNIPAM-6k in CDCl$_3$. 
Figure S4. FTIR spectra of l-PNIPAM-6k and c-PNIPAM-6k.
Figure S5. \(^1\)H NMR spectra of p-PNIPAM-12k, l-PNIPAM-12k, and c-PNIPAM-12k in CDCl\(_3\).

Figure S6. FTIR spectra of l-PNIPAM-12k and c-PNIPAM-12k
Figure S7. $^1$H NMR spectra of p-PNIPAM-19k, l-PNIPAM-19k, and c-PNIPAM-19k in CDCl$_3$.

Figure S8. FTIR spectra of l-PNIPAM-19k and c-PNIPAM-19k.
Figure S9. GPC traces of p-PNIPAM-6k, l-PNIPAM-6k, and c-PNIPAM-6k in DMF.

Figure S10. GPC traces of p-PNIPAM-19k, l-PNIPAM-19k, and c-PNIPAM-19k in DMF.
**Determination of the $M_n$ values by UV/Vis spectroscopy.** Since the parent telechelic PNIPAM (p-PNIPAM) chains are terminated with an isobutylsulfanylthiocarbonylsulfanyl moiety, the strong absorbance of this thiocarbonylthio group at 310 nm can be used to evaluate the $M_n$ of this polymer by UV/Vis spectroscopy (Figure S11). For a p-PNIPAM sample with accurately measured weight ($w$ in g), the $M_n$ can be calculated by the formula:

$$M_n = \frac{w}{c_{CTA}} \quad (1)$$

where $c_{CTA}$ is the amount of isobutylsulfanylthiocarbonylsulfanyl residues in the polymer sample (in mol) determined experimentally by application of Beer’s law, and using the molar extinction coefficient of AIP in methanol ($\varepsilon_{310} = 14,500 \text{ M}^{-1}\text{cm}^{-1}$). The $M_n$ values of p-PNIPAMs determined by UV/Vis and GPC-MALLS are listed in Table 1. The two $M_n$ values are very close to each other, implying that the isobutylsulfanylthiocarbonylsulfanyl end functionality on p-PNIPAM chains is nearly 100% in each of the parent telechelic polymers (p-PNIPAMs).

![Figure S11. UV/Vis spectra of p-PNIPAM-6k, l-PNIPAM-6k, and c-PNIPAM-6k in methanol at the concentration ca. 0.5 g/L.](image-url)
Table 1. Characterization of the parent telechelic poly(N-isopropylacrylamide)s by GPC and UV/Vis spectroscopy.

<table>
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<th>Sample Name</th>
<th>$M_n$ (kDa)</th>
<th>$M_w/M_n$ (GPC)</th>
<th>UV-Vis</th>
<th>GPC</th>
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<td>6.2</td>
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<td>11.8</td>
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<tr>
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<td>18.7</td>
<td>18.8</td>
<td>1.04</td>
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**High-Sensitivity Differential Scanning Calorimetry (HS-DSC).** HS-DSC measurements were performed on a VP-DSC microcalorimeter (MicroCal Inc.) at an external pressure of ca. 180kPa. The cell volume was 0.5204 mL. The heating rate was set at 1.0 °C/min. The experimental data were analyzed using the Origin based software supplied by the manufacturer. Typically, a polymer concentration of 1.0 g/L was used. The temperature of the phase transition ($T_m$) was taken at the maximum point of endotherm peak. The enthalpy of the transition ($\Delta H$) was determined from the area of the endotherm. At least three measurements were performed for each polymer solution.

Figure S12. Microthermograms of the aqueous solutions of l-PNIPAM-6k and c-PNIPAM-6k at the concentration of 1.0 g/L.
Figure S13. Microthermograms of the aqueous solutions of l-PNIPAM-19k and c-PNIPAM-19k at the concentration of 1.0 g/L.

**Cloud point determinations.** UV/Vis spectra and cloud points were measured using a Hewlett Packard 8452A photodiode array spectrophotometer equipped with a temperature controller. A 1 cm path length quartz cuvette was used for sample solution. Aqueous polymer solutions (1.0 g/L) were heated at the rate of 0.2 °C/min. The solution turbidity was monitored from the transmittance at 500 nm.
Figure S14. Turbidity curves of aqueous solutions of l-PNIPAM-6k and c-PNIPAM-6k at the concentration of 1.0 g/L.

Figure S15. Turbidity curves of aqueous solutions of l-PNIPAM-6k and c-PNIPAM-6k at the concentration of 1.0 g/L.