

**The Critical Importance of Size on Thermoresponsive Nanoparticle Transition
Temperatures: Hard and Soft Nanoparticles**

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Experimental

General

All chemicals were used as supplied unless otherwise stated. Dioxane and diethyl ether were all purchased from Fischer Scientific at laboratory reagent grade. The dioxane used in the synthesis of PVPip homopolymers and PVPip-*b*-PVAc diblock copolymers was dried over CaH₂ and distilled under reduced pressure prior to use. 2,2'-Azobis(2-methylpropionitrile) (AIBN, Molekular) was recrystallized from methanol and stored at 5 °C in the dark. *N*-vinylpiperidone (VPip) was donated by BASF, stored in a desiccator, and sublimed before use. Vinyl acetate was dried over CaH₂, distilled under static vacuum, and then stored at 5 °C in the dark. PVPip, P1 and P4 were synthesized according to the literature procedures.¹ Deuterated chloroform (99.9 atom % D), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (≥ 97%), 4,4'-azobis(4-cyanovaleric acid) (≥ 98.0%), oligo(ethyleneglycol)methacrylates (97.0%), tetrachloroauric acid and trisodium citrate (>99 %) were all purchased from Sigma-Aldrich. Ultrahigh quality water with a resistance of 18.2 MΩ.cm (at 25 °C) was obtained from a Millipore Milli-Q gradient machine fitted with a 0.22 μm filter.

Analytical and Physical Methods

¹H and ¹³C NMR spectra were recorded on Bruker DPX-300 and DPX-400 spectrometers using deuterated solvents obtained from Sigma-Aldrich. SEC was conducted on a Varian 390-LC system in DMF (1 g/L LiBr) at 50 °C, equipped with refractive index and viscometry detectors, 2 x PLgel 5 μm mixed-D columns (300 x 7.5 mm), 1 x PLgel 5 μm guard column (50 x 7.5 mm) and autosampler. Data was analysed using Cirrus 3.2 software. Molecular weight was determined relative to narrow poly(methyl methacrylate)

standards. Lower critical solution temperatures were evaluated using OptiMelt MPA100 system from Stanford Research Systems. The cloud point of POEGMA nanoparticles was determined by normalising the turbidimetry curve such that the values were in the range of 0 to 1, and the transition temperature was defined as being the temperature corresponding to a normalised absorbance of 0.5. A constant heating rate of 2 °C.min⁻¹ was used in all experiments. Cloud point measurements of PVPip micelles were analyzed using a Perkin-Elmer UV/vis Spectrometer (Lambda 35) equipped with a Peltier temperature controller at 500 nm with a heating/cooling rate of 1 °C/min. An average of at least two cycles were recorded for each sample. Dynamic light scattering was conducted using a Nano-Zs from Malvern Instruments, UK. Scattered light was detected at 173° and the observed count rates recorded. Hydrodynamic radii (where appropriate) were determined using the manufacturer's software. Transmission electron microscopy (TEM) samples were prepared by drop deposition onto copper/carbon grids that had been treated with oxygen plasma to increase the surface hydrophilicity. The particles were stained using a dilute 3% solution of uranyl acetate. Micrographs were collected at magnifications varying from 12 to 100 K and calibrated digitally. Histograms of number-average particle diameters (D_{av}) and standard deviations were generated from the analysis of a minimum of 100 particles from at least three different micrographs using Image J software.

Procedures

Synthesis of Poly[oligo(ethyleneglycol methyl ether-methacrylate)]. As an example the polymerisation using di(ethyleneglycol) methacrylate is given. Diethyleneglycol methacrylate (2 g, 10 mmol) was added to a Schlenk tube and 2 mL of dioxane added. Next, 0.67 mL of a dioxane solution containing 0.16 M 2-cyano-2-propyl benzodithiolate and 24 mM of

azobisisobutyronitrile (AIBN) was added to the Schlenk tube by syringe giving [monomer]:[initiator]:[chain transfer agent] = 100:0.2:1. The solution was degassed by 4 freeze-pump-thaw cycles and back-filled with nitrogen gas. The flask was then immersed into a thermostated oil bath at 70 °C for 180 minutes. After this time, a 25 μL sample was removed and diluted with CDCl_3 for NMR analysis. The remainder was rapidly cooled in an ice-water bath and precipitated into diethyl ether (35 mL). The polymer was re-precipitated from THF to diethyl ether twice to yield a waxy pink polymer. Isolated yield: 1.05 g, 53 %; Conversion (NMR): 70 %; M_n (theo): 20700 $\text{g}\cdot\text{mol}^{-1}$; M_n (SEC): 9200 $\text{g}\cdot\text{mol}^{-1}$; $M_w/M_n = 1.15$ (SEC).

^1H NMR (300 MHz, CDCl_3) δ_{ppm} : 1.41 (3H, backbone- CH_3) 1.80 - 2.00 (2H, backbone- CH_2), 3.35 (3H, CH_3 -PEG), 3.40 –3.80 (16H, $\text{CH}_2\text{CH}_2\text{O}$), 4.09 (2H, $\text{CH}_2\text{OC}(=\text{O})$), 7.42 (o-Ar, end-group), 7.61 (p-Ar, end-group), 7.85 (m-Ar, end-group).

Synthesis of citrate-stabilized gold nanoparticles. As a typical example is described. First, 350 mL of a 0.83 $\text{mmol}\cdot\text{L}^{-1}$ (0.33 $\text{mg}\cdot\text{mL}^{-1}$) aqueous solution of HAuCl_4 was heated to reflux in a scratch-free round bottomed flask. After that, 10.2 mL of a 0.1 $\text{mol}\cdot\text{L}^{-1}$ aqueous solution of sodium citrate was added in a single portion to the HAuCl_4 solution to give an Au:citrate ratio 1 : 3.5. The temperature was maintained at reflux for 30 minutes, during which time a deep red coloration formed. The reaction mixture was then allowed to cool to room temperature over a period of 3 hours. Gold nanoparticles with different diameters were prepared using the same protocol but by adjusting the concentration of the sodium citrate solution. Assuming complete reduction of the HAuCl_4 into the particles, the total gold concentration in the final solution was 0.83 $\text{mmol}\cdot\text{L}^{-1}$ (0.16 $\text{mg}\cdot\text{mL}^{-1}$).

Synthesis of 5 nm citrate-coated gold nanoparticles. 240 mL of a 0.21 $\text{mmol}\cdot\text{L}^{-1}$, (0.08 $\text{mg}\cdot\text{mL}^{-1}$) aqueous solution of HAuCl_4 was prepared at room temperature. To this was added

13.8 mg (0.05 mmol) of trisodium citrate to give a final citrate concentration of 0.21 mmol.L^{-1} . 5 mL of an ice cold 0.1 M (0.5 mmol, 18.5 mg) solution of NaBH_4 was added to the gold/citrate solution and stirred at room temperature overnight. A 0.5 mL sample was reserved for UV-Visible and dynamic light scattering analysis. Assuming complete reduction of the HAuCl_4 to gold particles, the total gold concentration in the final solution was 0.21 mmol.L^{-1} (0.04 mg.mL). Further concentration by centrifugation was conducted as necessary.

General procedure for the synthesis of polymer-coated gold nanoparticles.

Approximately 10 mg of the desired thiol-terminated polymer was added to a falcon tube, and dissolved in 1 mL of high-purity water. To this tube was added 12 mL of the citrate-stabilized gold nanoparticle solution (0.83 mmol.L^{-1} total gold concentration), which was then agitated overnight in the absence of light. To remove excess polymer, the particles were centrifuged for 3 hours at 8000 rpm. Following careful decantation of the supernatant, the particles were then re-dispersed in 10 mL of high-quality water and the centrifugation-resuspension process repeated for a total of 3 cycles. After the final cycle the particles were dispersed in 2 mL of high-quality water for future use. Assuming complete incorporation of the citrate coated gold particles into the final polymer coated particles the total concentration of gold in the final solution was 5.0 mmol.L^{-1} , 0.98 mg.mL^{-1} .

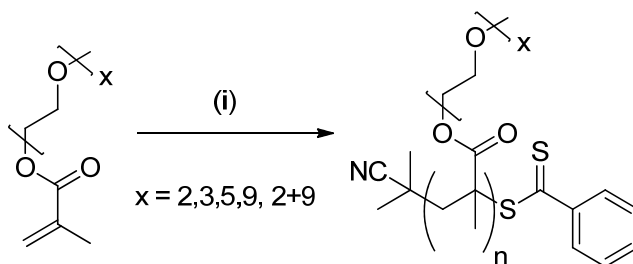
General procedure for the synthesis of poly(*N*-vinylpiperidone)-*block*-poly(vinylacetate)

As a representative example, PVPip₆₂ (M_n^{GPC} (DMF) = 2.9 kDa, M_w/M_n (DMF GPC) = 1.25, 0.10 g, 0.01 mmol), VAc (0.33g, 0.0141 mol), and AIBN (0.021 mL of 2 mg.mL^{-1} solution in 1,4-dioxane, 0.0026 mmol) were dissolved in 1,4-dioxane (3.87 mL) in a Schlenk tube equipped with a Young's tap. The resulting solution was then freeze-pump-thawed at least six

times. The polymerization was then carried out at 60 °C for 120 h, after which an aliquot was withdrawn from the reaction mixture for ^1H NMR conversion analysis—determined by comparing the integrations of the vinyl ($\delta = 7.25$ ppm) and the $-\text{CH}_3$ ($\delta = 2.11$ ppm) proton signals of the VAc monomer with those of the $-\text{CH}_3$ proton peaks ($\delta = 2.00$ ppm) of the resulting polymer. The polymerization was quenched with liquid nitrogen, thawed and subsequently precipitated into diethyl ether twice, giving an off-white fluffy material. This was centrifuged to give an off-white residue with a clear colourless supernatant which was then carefully decanted. **P3** was recovered as a white powder after drying under high vacuum for 2 days. Characterization of **P3**: ^1H NMR (400 MHz, CDCl_3): $\delta = 4.84$ ($-\text{CH}-$ of PVAc, br), 4.54 ($-\text{NCH}-$ of PVPip, br), 4.11 ($-\text{OCH}_2\text{CH}_3$, end group), 3.02 ($-\text{NCH}_2-$, br, PVPip), 2.34–2.18 ($\text{O}=\text{C}-\text{CH}_2-$, br, PVPip), 2.00 ($-\text{CH}_3$, br, PVAc), 1.83–1.40 ($-\text{CH}_2-$ on PVPip heterocycle, $-\text{CH}_2-$ on PVPip and PVAc polymer backbone, br). $M_n^{\text{GPC}}(\text{DMF}) = 5.8$ kDa, $M_w/M_n = 1.58$.

Characterisation

Poly[oligo(ethyleneglycol methyl ether-methacrylate)s



Scheme S1. Conditions. [Monomer]:[CTA]:[Initiator] = 100:1:0.2/ 70 °C/ 90 mins. CTA = 2-Cyano-2-propyl benzodithioate. Initiator = AIBN

Code	Composition ^(a) (-)	Conversion ^(b) (-)	M _{n,(Theo)} ^(c) (g.mol ⁻¹)	M _{n,(SEC)} ^(d) (g.mol ⁻¹)	M _w /M _n ^(d)
P1	OEGMA _{9-co} -OEGMA ₂ ^(e)	70 %	20700	9200	1.15
P2	OEGMA ₅	71 %	20700	16000	1.21
P3	OEGMA ₉	77 %	36000	23300	1.19

OEGMA_{xxx} = oligo(ethyleneglycol)methacrylate where average number of ethylene glycol repeat units in the side chain is indicated by xxx. (a) Indicates the monomer(s) used in the polymerisation; (b) Determined by ¹H NMR; (c) Determined from the [monomer]:[CTA] ratio and the degree of conversion; (d) Determined by SEC in DMF using PMMA standards; (e) ratio of monomers used in polymerisation: PEG₉MA:PEG₂MA = 8:92.

Poly[oligo(ethyleneglycol methyl ether-methacrylate) coated gold nanoparticles

Citrate-coated gold nanoparticles were first synthesised and characterised by DLS and UV-Vis, Table S1. Following this, the particles were coated with RAFT-derived POEGMAs and characterisation information is given in Table S2.

Table S1. Citrate-stabilized nanoparticles used in this study.

Particle ^(a)	Citrate: Au ratio	NaBH ₄ : Au Ratio ^(b)	SPR (nm)	Particle diameter (nm)	
				DLS	UV ^(c)
Au ₅	1	10	506	5	3.5
Au ₁₂	3.5	--	523	12	18.5
Au ₃₀	2.5	--	519	30	18

(a) Au_x: X = diameter of gold nanoparticle as determined by DLS; (b) NaBH₄ used as reducing agent to obtain NP with diameter < 10 nm; (c) Determined using the method of Haiss *et al.*².

Table S2. Polymer-coated nanoparticles used in this study

Particle ^(a)	P1		P2		P3	
	SPR (nm)	d _{DLS} (nm)	SPR (nm)	d _{DLS} (nm)	SPR (nm)	d _{DLS} (nm)
Au ₅	510	16	512	18	510	15
Au ₁₂	532	38	520	25	525	35

Au₃₀	533	45	528	40	526	41
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(a) Au_x: X = diameter of gold nanoparticle as determined by DLS;

Demonstration of Cloud Point Depression

Poly(OEGMA) with a cloud point of 26 °C, added to 12 nm AuNP at 20 °C. Within 20 minutes the colour has changed to blue (central image) indicating aggregation of the particles. This shows that the cloud of the particle is below that of the free polymer, as a stable unaggregated gold dispersion is red in colour. If this was allowed to stand for 30 minutes at 20 °C full precipitation occurred and all colour from solution has been removed. Conversely, if this sample is cooled to 5 °C, resolubilisation occurs demonstrating reversibility.



Figure S1. Photographs demonstrating the aggregation of POEGMA-coated gold nanoparticles in response to temperature changes.

References

1. N. S. Jeong, M. Redhead, C. Bosquillon, C. Alexander, M. Kelland and R. K. O'Reilly, *Macromolecules*, 2011, **44**, 886 - 893.
2. W. Haiss, N. T. K. Thanh, J. Aveyard and D. G. Fernig, *Anal. Chem.*, 2007, **79**, 4215-4221.