Supporting Information for:

Size Dependent LCST Transitions of Polymer-Coated Gold Nanoparticles: Co-operative Aggregation and Surface Assembly

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Experimental

Materials. 4,4-Azobis(4-cyanovaleric acid) (> 97 %), gold(III)chloride trihydrate (H\text{AuCl}_4 > 49 \text{ %} \text{ Au, ACS grade}), 1,4 dioxane (> 99 %), poly(ethylene glycol) monomethylethermethacrylate (M_n = 475 \text{ g.mol}^{-1}), poly(ethylene glycol) monomethylethermethacrylate (M_n = 232 \text{ g.mol}^{-1}), 2-ethylhexyl methacrylate (> 98 %), 4,4'-dinonyl-2,2'-bipyridine 97 %, 1,4,8,11-tetraazacyclotetradecane (> 97 %) and CuBr (99.999 %) were purchased from Sigma-Aldrich. Citric acid trisodium salt (> 99.8 %) was purchased from Acros Organics. Inhibitor was removed from the monomers by passage through a column of basic alumina. 4-Cyanopentanoic acid dithiobenzoate,[1] the ATRP initiator (6’-dimethylchlorosilylhexyl)2-bromo-2-methylpropionate[2]) and pentafluorophenyl-methacrylate[3] were synthesized according to literature procedures. All other chemicals were used as received unless specified otherwise. Ultrahigh quality water with a resistance of 18.2 M\Omega.cm (at 25 °C) was obtained from a Millipore Milli-Q gradient machine fitted with a 0.22 µm filter. Pre-formulated, powdered, phosphate buffered saline was purchased from Sigma-Aldrich, and the desired solution made by addition of ultra high quality water to give [NaCl] = 0.138 M, [KCl] = 0.0027 M and pH = 7.4.

Analytical and Physical Methods. UV-Visible absorbance spectra were obtained on a Varian Cary 100 Bio spectrophotometer operating at 25 °C using 10 mm pathlength cuvettes with a total volume of 2.5 mL. Dynamic light scattering measurements were carried out on a Brookhaven Instruments Corporation system consisting of a BI-200SM goniometer and a BI-9000AT autocorrelator. A 100 mW Ar + ion laser (Lexel Lasers) operating at 488 nm was
used. All measurements were performed at a temperature of 25.0 (± 0.2) °C, which was
maintained by a filtered decalin bath. All experiments were performed at a scattering angle of
90 °. Borosilicate cuvettes were used with a minimum of 3 mL of the nanoparticle solution.
Hydrodynamic radii were determined by the CONTIN algorithm using the manufacturer’s
software. The values reported are an average from a minimum of 15 experiments. Brush
thicknesses were determined by use of a Philips Plasmos SD 2300 ellipsometer working with
a He-Ne laser (λ = 632.8 nm) at an angle of incidence of 70°. For ellipsometric measurements,
silicon wafers were used instead of glass slides. The method for calculation was based on a
three-layer silicon/polymer brush/ambient model, assuming the polymer brush to be isotropic
and homogeneous. A refractive index of 1.45 was used for the polymer layer. Contact angles
were measured on a Dataphysics OCA30 semi-automatic contact angle meter, using MilliQ
water. Size exclusion chromatography (SEC) was performed on a Waters Alliance GPCV
2000 system equipped with refractive index and differential viscometer detectors. Separation
was carried out at 60 °C with TSK-Gel Alpha 3000 + 4000 columns using DMF + 0.5 g.L⁻¹
LiBr as eluent and a flow rate of 0.6 mL.min⁻¹. Molecular weights were determined relative to
narrow polydispersity poly(methyl methacrylate) (PMMA) standards. Results were calculated
with the Empower Pro multidetection GPC software (V. 5.00). Plasma cleaning of glass and
silicon wafers was undertaken using a Teppla 300 plasma stripper.

Procedures

Synthesis of Poly(poly(ethylene glycol) monomethylethermethacrylate) (A):
Poly(ethylene glycol) monomethylethermethacrylate (Mₙ = 475 g.mol⁻¹ (2 g, 4.2 mmol) was
added to a Schlenk tube and 1.5 mL of dioxane added. Next, 1.9 mL of a dioxane solution
containing 0.03 M 4-cyanopentanoic acid dithiobenzoate and 7.2 mM of 4,4-azobis(4-
cyanovaleric acid) were added via a syringe, to give a [monomer]:[chain transfer
agent]:[initiator] ratio of 65:1:0.2. The solution was degassed by 4 freeze-pump-thaw cycles
and the Schlenk tube filled with nitrogen. The flask was then immersed into a thermostated oil bath at 90 °C for 180 minutes. After this time, a 25 µL sample was removed and diluted with CDCl₃ 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: 1.12 g, 55.9 %; Conversion: 61 % (¹H NMR); Mₙ (expected): 18800 g.mol⁻¹; Mₙ (SEC): 16800 g.mol⁻¹, Mₙ (NMR): 18900 g.mol⁻¹; Mₚ/Mₙ = 1.27 (SEC). ¹H NMR (400 MHz, CDCl₃) δ ppm: 1.38 (3H, backbone-CH₃) 1.80 - 2.00 (2H, backbone-CH₂), 3.39 (3H, CH₃PEG), 3.50 – 3.80 (16H, CH₂CH₂O), 4.09 (2H, CH₂OC=O), 7.42 (o-Ar, end-group), 7.61 (p-Ar, end-group), 7.85 (m-Ar, end-group).

Synthesis of poly(pentafluorophenyl methacrylate) (PPFMA): In a typical experiment pentafluorophenyl methacrylate (5 g, 0.02 mol) was added to a Schlenk tube and 8 mL of dioxane added. Then, 1.03 mL of a dioxane solution containing 0.16 M 4-cyanopentanoic acid dithiobenzoate and 0.075 M of 4,4-azobis(4-cyanovaleric acid) were added via a syringe to give a [monomer]:[chain transfer agent]:[initiator] ratio of 120:1:0.5. The solution was degassed by 4 freeze-pump-thaw cycles and the Schlenk tube filled with nitrogen. The flask was then immersed into a thermostated oil bath at 90 °C for 90 minutes. After this time, a 25 µL sample was removed and diluted with CDCl₃ for NMR analysis. The remainder was rapidly cooled in an ice-water bath and precipitated into pentane (35 mL). The polymer was re-precipitated from THF to pentane twice to yield a pink polymer product. Isolated yield: 2.47 g, 50 %; Conversion: 65 % (¹H NMR); Mₙ (expected): 19800 g.mol⁻¹; Mₙ (SEC): 8900 g.mol⁻¹, Mₙ (NMR): 23000 g.mol⁻¹. ¹H NMR (400 MHz, CDCl₃) δ ppm: 1.53 (3H, CH₃), 2.53 (2H, CH₂), 7.45 (o-Ar, end-group), 7.57 (p-Ar, end-group), 7.87 (m-Ar, end-group). ¹³C NMR (100 MHz, ¹H decoupled, CDCl₃) δ ppm: 25.6 (CH₃), 45.36 (CH₂), 124.1 (C-O), 136.1 – 141.7 (C-F), 172.0 (C=O).

Synthesis of poly(N-(2-hydroxypropyl)methacrylamide) (B): 200 mg (0.79 mmol pentafluorophenyl groups) of poly(pentafluorophenyl methacrylate) was dissolved in 3 mL of
dry DMF and stirred to dissolution under nitrogen. Then, 3.3 mL of a 0.5 M solution of 2-hydroxypropylamine (containing 0.5 M triethylamine) in DMF was added via a syringe (2 fold excess of amine to active ester). The reaction mixture was then stirred at 50 °C under nitrogen for 16 hours before being precipitated into diethyl ether. After 3 additional re-precipitations from DMF to diethyl ether and vacuum drying for 1 hour at 40 °C, the polymer was dissolved in 5 mL of MilliQ water and dialyzed for 3 days against MilliQ water using dialysis tubing with a molecular weight cut-off of 3500 g.mol\(^{-1}\). The final product was isolated as a white powder by freeze drying. Isolated yield, 76 mg, 70 %. \(M_n\) (theoretical): 10980 g.mol\(^{-1}\); \(M_n\) (SEC): 12400 g.mol\(^{-1}\). \(^1\)H NMR (400 MHz, D\(_2\)O) \(\delta_{ppm}\): 0.90-1.45 (backbone-C\(_3\)H\(_3\), C\(_3\)H\(_3\)CH(OH)), 3.12 (C\(_6\)H\(_2\)NH), 3.89 (C\(_6\)H\(_3\)OH).

**Synthesis of citrate-stabilized gold nanoparticles (10-50 nm diameter).** As a typical example, the synthesis of gold nanoparticles with a diameter of 17 nm is given here. First, 60 mL of a 0.83 mmol.L\(^{-1}\) (0.33 mg.mL\(^{-1}\)) aqueous solution of HAuCl\(_4\) was heated to reflux in a scratch-free round bottomed flask, then 1.71 mL of a 0.1 mol.L\(^{-1}\) aqueous solution of sodium citrate was added to the HAuCl\(_4\) solution in a single portion to give a Au:citrate ratio of 1:3.5. The temperature was maintained at reflux for 30 minutes, during which time a deep red coloration formed. This was then allowed to cool to room temperature over a period of 3 hours. To tune the size of the gold particles the concentration of the sodium citrate solution was varied to the appropriate Au:citrate ratio, which are summarized in Table 1 in the main text. A 0.5 mL aliquot of each 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 0.81 mmol.L\(^{-1}\) (0.16 mg.mL\(^{-1}\))

**Synthesis of citrate-stabilized gold nanoparticles (5 nm diameter).** 240 mL of a 0.21 mmol.L\(^{-1}\), (0.08 mg.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\(^{-1}\). Then, 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 for 1 hour. 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$^{-1}$)

**Synthesis of polymer B coated gold nanoparticles.** 20 mg of polymer B was dissolved in 0.5 mL of MilliQ water. This was then added to a 60 mL solution of citrate coated gold nanoparticles (total gold concentration 0.16 mg.mL$^{-1}$, 0.81 mmol.L$^{-1}$, diameter = 18 nm by DLS) and allowed to stir protected from light for 16 hours. Excess polymer was removed by at least 3 centrifugation (8500 rpm, 180 minutes)-resuspension cycles. After the final cycle, the particles were dispersed in 5 mL of high-quality water for future use. Assuming complete conversion of the citrate stabilised particles into the polymer coated particles, the final concentration of gold was 1.92 mg.mL$^{-1}$, 9.75 mmol.L$^{-1}$.

**Substrate Preparation.** The silicon or glass wafers were cleaned using a sonic bath in acetone, then ethanol and finally water for 5 minutes each, followed by washing with dichloromethane and dried under a stream of air. Subsequently, the slides were cleaned with oxygen plasma for 1 minute (500 W, flow 400 mL.min$^{-1}$) to remove organic residues and promote formation of silanol groups. The cleaned wafers were immersed in a 0.1 % (by volume) solution of the ATRP initiator in dry toluene overnight. Excess initiator was removed by washing with dichloromethane and methanol and dried under a flow of air.

**Surface-initiated ATRP of poly(ethylene glycol) monomethylethermethacrylate:** Glass or silicon wafers, functionalised with the ATRP initiator ((6’-dimethylchlorosilylhexyl)2-bromo-2-methylpropionate, see Supporting Information) were used to polymerize PEGMA$_{232}$ using a literature procedure.$^{[4]}$ The thickness determined by ellipsometry was 160 nm and the static water contact angle (room temperature) was 51 °.
Surface-initiated ATRP of 2-ethylhexyl methacrylate (EHMA). A solution containing 10 mL (0.044 mol) of EHMA and 10 mL of dry DMF was added to 9.4 mg (0.047 mmol) of 1,4,8,11-tetraazacyclotetradecane and 16.3 mg (0.040 mmol) 4,4′-dinonyl-2,2′-bipyridine in a Schlenk tube. After two freeze-pump-thaw cycles the mixture was transferred by cannulation to another nitrogen purged Schlenk tube containing 5.3 mg (0.037 mmol) of CuBr and the tube was placed into an oil bath thermostated to 50 °C. After the copper salt had dissolved, 4 µL (0.015 µmol) of ethyl α-bromoisobutyrate was added under nitrogen. The mixture was stirred for a further 2 minutes and transferred to a nitrogen purged reactor containing initiator-functionalized glass and silicon wafers and heated at 50 °C for 2 minutes. The slides were then removed and extensively washed with THF and dried under air. The obtained brush thickness was 56 nm according to ellipsometry and the static water contact angle was 93.9°.

Determination of the Lower Critical Solution Temperature (LCST) values. Solutions of the indicated nanoparticle were prepared in phosphate buffered saline at room temperature. The reported concentrations relate to the total mass of gold in solution. The solution temperature was increased at a rate of 1 °C.min⁻¹ and the total absorbance at 700 nm recorded at 1 °C intervals. The data was then treated such that the absorbance values were in the range of 0 (start of the experiment) to 1 (maximum turbidity). The LCST was defined as being the temperature when the normalized absorbance equal 0.5. The exact LCST for polymer A, however, was difficult to determine as there was no clear end point. For this sample, the LCST was taken as the point of maximum inflection, determined from the first derivative of the turbidimetry curve (d_Abs/d_T). In this way, an LCST of around 100 °C was determined, which is in good agreement with literature.^[5]

Citrate Stabilized Nanoparticle Characterization.

Figure S1 shows the normalized UV-Visible absorption spectra of the citrate coated nanoparticles. It can be clearly seen that the wavelength of maximum absorbance (the surface
plasmon resonance peak) shifts to longer wavelengths with increasing nanoparticle diameter. This also demonstrated that each particle in the series is unique in terms of its diameter.

**Figure S1.** UV-Visible absorption spectra of the (precursor) citrate-coated gold nanoparticles used in the synthesis of PPEGMA coated nanoparticles as shown in Table 1. Spectra have been normalized such that the absorbance at 700 nm = 0, and the maximum absorbance (SPR peak) = 1. Spectra were obtained in water using a total gold concentration ~ 0.02 mg.mL$^{-1}$.

**Determination of the LCST Transition of Nanoparticle Solutions.**

**Figure S2** shows the difference between a thermoresponsive nanoparticle (**A@Au$_{17}$, Figure S2A**) and a non-thermoresponsive nanoparticle (**B@Au$_{18}$, Figure S2B**) when 0.2 mg.mL$^{-1}$ (total gold concentration) solutions are heated in PBS solution. There was no change when B@Au$_{18}$ was used, but a significant shift in both the SPR peak location and total turbidity at 700 nm were observed when A@Au$_{17}$ was used. **Figure S3** shows the increase in turbidity at 700 nm for A@Au$_{19}$ during heating in PBS solution at various concentrations. The dotted line
indicates the observed LCST, which was defined as the temperature where the normalized turbidity was 0.5.

**Figure S2.** UV-Visible spectra showing the effect of heating 0.2 mg.mL\(^{-1}\) (total gold concentration) nanoparticle solutions in PBS from 25 to 85 °C: A) \(\text{A@Au}_17\) and B) \(\text{B@Au}_{18}\).

**Figure S3.** Determination of the LCST value (indicated by the intercept of the dotted line with the solid lines) of \(\text{A@Au}_{19}\) at 3 different concentrations in PBS.
Determination of Polymer Grafting Density on Nanoparticle.

3 Additional polymer coated nanoparticles were synthesized to determine the effect of gold core diameter on the grafting density of the polymer. The characteristics of these particles are summarized in Table S1. Figure S4 shows the thermogravimetric traces of these polymer A coated gold nanoparticles during heating from 25 to 700 °C. The mass fraction of polymer within the particle was taken as the relative loss of mass at 700 °C from the initial mass at 25 °C.

Figure S4. Thermogravimetric analysis of the polymer A coated gold nanoparticles listed in Table S1.

To calculate the grafting density ($\Gamma_{\text{pol}}$) of the polymer chains, the following equations were used: Equation 1 defines the mass fraction of the polymer ($F_{\text{pol}}$) within the whole nanoparticle, and is rearranged in Equation 2 to aid the later stages. Equation 3 equates the total mass of polymer on a particle to the grafting density, assuming a perfect sphere. Finally, Equation 4
was obtained by combining Equation 2 and 3. The results of the analysis are shown in Table S1.

\[ \Gamma_{\text{pol}} = \frac{N_{\text{pol}}}{N_{\text{pol}} + N_{\text{AuNP}}} \]  
Equation 1

\[ M_{\text{pol}} = \frac{M_{\text{AuNP}} \Gamma_{\text{pol}}}{(1 - \Gamma_{\text{pol}})} \]  
Equation 2

Also: \[ M_{\text{pol}} = \Gamma_{\text{pol}} \cdot \frac{4 \pi r^2}{M_N} \]  
Equation 3

Therefore: \[ \Gamma_{\text{pol}} = \frac{M_{\text{AuNP}} \Gamma_{\text{pol}}}{4 \pi r^2 M_N [1 - \Gamma_{\text{pol}}]} \]  
Equation 4

Abbreviations used: \( F_{\text{pol}} \) = Mass fraction of polymer within a single nanoparticle; \( M_{\text{pol}} \) = Total mass of polymer within a single nanoparticle; \( M_{\text{AuNP}} \) = Mass of single gold nanoparticle; \( \Gamma_{\text{pol}} \) = Grafting density of polymer on the nanoparticle surface; \( r \) = Radius of the precursor, citrate-coated nanoparticles determined by DLS; \( M_N \) = Number average molecular weight of a single polymer chain determined by GPC.

\( M_N \) of polymer A = \( 2.79 \times 10^{-20} \) g per chain. (Molar mass of polymer = 16800 g.mol\(^{-1}\))

\( M_{\text{AuNP}} \) = 1.0107x10\(^{-20}\).\((2r)^3 \) g per NP. This assumes that all the nanoparticles are perfect spheres and face centered cubic (fcc) packing of the gold atoms with a density of 19.3 g.cm\(^{-3}\) and the radius in nm\(^{[6]}\).

**Table S1.** Calculated grafting densities of polymer A coated gold nanoparticles used for TGA analysis.

<table>
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<th>Code</th>
<th>Citrate coated particle diameter (nm)</th>
<th>Mass Fraction Polymer</th>
<th>Grafting density (chains.nm(^{-2}))</th>
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In Figure 1 in the main text, a grafting density value of 0.3 chains.nm$^{-2}$ was assumed to allow the relative change in the polymer mass fraction as function of nanoparticle diameter to be displayed. This value was decided upon based on the data in Table S1, and thus underestimates the mass fraction of polymer at very low nanoparticle diameters.

**Cooperative Aggregation of Thermosensitive Nanoparticles onto Complementary Polymer Brush Surfaces.**

Photographs of glass wafers coated with poly((poly(ethylene glycol) monomethylether methacrylate), which had been incubated with either A@Au$_{17}$ or B@Au$_{18}$ were shown in Figure 2 of the main text. **Figure S6** shows the original photograph showing the two wafers side-by-side, demonstrating the increased absorption of the thermosensitive nanoparticles (indicated by the strong red/blue coloration)

![Figure S6](image)

**Figure S6.** Photograph of glass wafers coated with 160 nm poly((poly(ethylene glycol) monomethylethermethacrylate) which have been exposed to 0.1 mg.mL$^{-1}$ PBS solutions of (left) A@Au$_{17}$ or (right) B@Au$_{18}$ for 30 minutes

To rule out any changes in the nanoparticle solution during incubation with the polymer brush control experiments were undertaken. The total absorbance of the nanoparticle solutions at 530 nm (near the SPR peak) was measured over a period of 30 minutes while heating to
various temperatures. (i.e. the same conditions used for the assembly onto the wafers). Figure S7 shows the results of these experiments. Both the thermosensitive (A@Au$_{17}$, Figure S7A) and non-thermosensitive (B@Au$_{18}$, Figure S7B) nanoparticles show the same behavior in the temperature range tested suggesting there was no aggregation in solution.

**Figure S7.** Change in absorbance at 530 nm of 0.1 mg.mL$^{-1}$ solutions of (A), A@Au$_{17}$ and (B) B@Au$_{18}$ during heating at the indicated temperatures in PBS solution.

**Estimation of the LCST of Poly(poly(ethylene glycol) monomethylethermethacrylate)) Polymer Brushes on Silicon Wafers.**

The LCST of the poly((poly(ethylene glycol) monomethylethermethacrylate) brushes was estimated by static water contact angle measurements. **Figure S8** shows the observed water contact angle measurements as the temperature of the wafer was increased. At higher temperatures evaporation complicated the experiments, but a clear increase in the contact angle occurring between 50 and 60 °C. To ensure that the brush had passed through its LCST for the cooperative assembly experiments, temperatures above 60 °C were used.
Figure S8. Static water contact angles of a poly((polyethylene glycol)monomethylether methacrylate) polymer brush (160 nm thickness) as a function of temperature.

References


