

Supporting Information

UCST-Type Polymer Capsules Formed by Interfacial Complexation

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1. Materials

Poly(L-lysine) hydrobromide (**PLL**) was purchased from Sigma-Aldrich ($M_w = 15\text{-}30\text{ kg/mol}$) or Alamanda Polymers ($M_w = 52\text{ kg/mol}$). Poly(L-lysine)-graft-poly(ethylene glycol) (PLL-g-PEG) was purchased from SuSoS (NL) as a 20 kg/mol PLL chain grafted with on average 3.5 strands of 5 kg/mol PEG. O-[N-(3-Maleimidopropionyl)aminoethyl]-O'-[3-(N-succinimidyl)-3-oxopropyl]triethylene glycol (**Mal-PEG4-NHS**), 5(6)-carboxy-X-rhodamine N-hydroxysuccinimidyl ester (**rho-NHS**), 7-(diethylamino)coumarin-3-carboxylic acid N-succinimidyl ester (**coum-NHS**), potassium thiocyanate (**KSCN**), 8-anilino-1-naphthalenesulfonic acid (**ANS**), dextran-fluorescein $M_w 10\text{ kg/mol}$ (**DF**), tris(2-carboxyethyl)phosphine (**TCEP**), monoethanolamine (**MEA**) and Fluorinert FC-70 fluorocarbon oil (FCO) were purchased from Sigma-Aldrich. **Krytox** 157 FS(L) ($M_w = 2.5\text{ kg/mol}$) was purchased from Samaro. FluoSpheres™ NeutrAvidin™-Labeled Microspheres (**fluoNPs**, 40 nm diameter, 10 mg/mL in water) displaying yellow-green fluorescence (505/515 nm) were purchased from ThermoFisher Scientific. Econix™ gold nanoparticles coated with 40 kg/mol poly(vinyl pyrrolidone) (**AuNPs**, 50 nm diameter, 5 mg/mL in water) were purchased from nanoComposix. Unless otherwise noticed, all chemicals were used without further purification.

2. Synthesis

Synthesis of poly(acrylamide-co-acrylonitrile) chains (PAAMAN):

All reagents were purchased from Sigma-Aldrich. N-Acrylamide (AAM) was recrystallized in chloroform, acrylonitrile (AN) was purified in basic alumina column. The RAFT chain transfer agent, *S,S'*-bis(α,α' -dimethyl- α'' -acetic acid)trithiocarbonate (BTC), was synthesized according to J. Lai et al. *Macromolecules* **2002**, *35* (18), 6754-6756. To prepare the random copolymer, a mix of AAM (2.8 g), AN (760 mg), BTC (74.4 mg), AIBN (8.7 mg), and DMSO (12.3 mL) was degassed by freeze–vacuum–thaw (3 cycles), then heated to 70 °C for 2 hours. The final polymer was precipitated twice from DMSO into methanol, then dialyzed against deionized water for 3 days and recovered by lyophilization.

NMR shown in Fig. S1A, using the feed ratio AAM:AN of 73:27 mol/mol yielded a 66:34 mol/mol ratio in the copolymer. SEC analysis (Fig. S1B) gave a M_w of 15 kg/mol and dispersivity of 1.22.

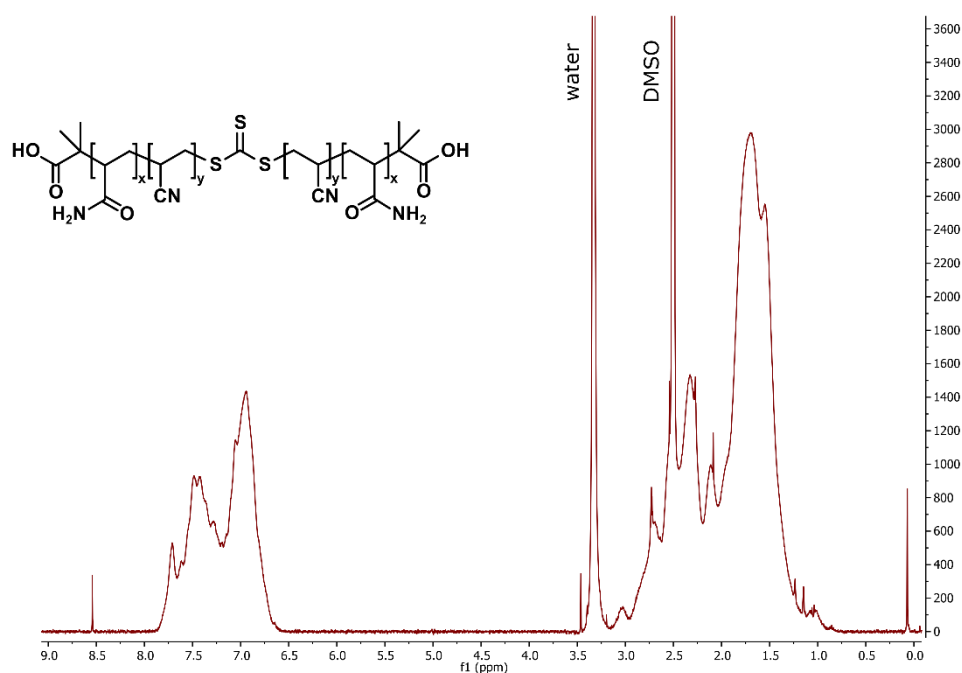


Figure S1A. ¹H NMR spectrum of PAAMAN with $x=0.66$ and $y=0.34$ at 400 MHz in d_6 -DMSO.

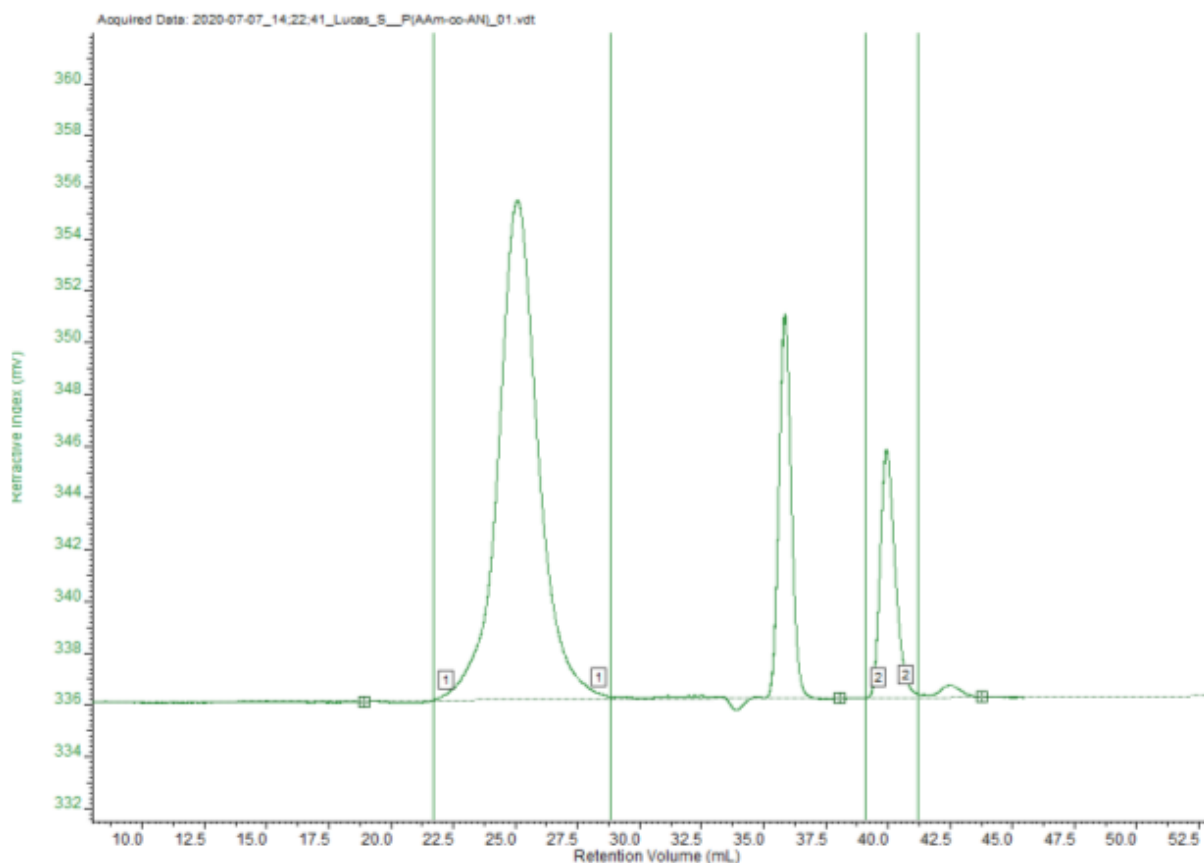


Figure S1B. GPC-SEC refractive index trace of PAAMAN eluted in DMSO in PSS-Gram columns. Peak 1 is the polymer, peak 2 the toluene marker used as reference.

Synthesis of poly(L-lysine)-g-poly(acrylamide-co-acrylonitrile) (PLL-g-PAAMAN):

In a typical synthesis of PLL-g-PAAMAN, PLL (52 kg/mol, 20 mg, 250eq lysine) and Mal-PEG4-NHS (2.0 mg, 10eq) were dissolved in 3 mL D₂O. The solution was stirred for 4 h at room temperature. A 300 μ L aliquot of the mixture was collected and diluted with 300 μ L D₂O for NMR analysis of the intermediate PLL-g-(Mal-PEG4). The solution was then heated up to 60 $^{\circ}$ C, and PAAMAN (17.2 mg, 3eq), MEA (4 μ L, 175eq) and TCEP (2.9 mg, 30eq) were introduced. After 4 h at 60 $^{\circ}$ C, the mix was cooled back to room temperature (below the cloud point), and polymer was pelleted by centrifugation at 4 $^{\circ}$ C (10 min at 15 000 rcf). Two additional dissolution/precipitation cycles were applied to the pellet by redispersion in DI water at 60 $^{\circ}$ C, cooling down to 4 $^{\circ}$ C and centrifugation. Eventually, the purified pellet was sonicated (Sonics Vibra-Cell VCX 750 equipped with a 3-mm microtip) to obtain a solution in DI water that was lyophilized overnight using a Labconco Freezone Plus 2.5 apparatus, yielding a pink powder.

Fluorescence labeling of PLL-g-PAAMAN (by rhodamine or coumarin) was achieved by adding the suitable reactive fluorophore (either rho-NHS or coum-NHS, at 1 g/L in DMSO) during the first step of the synthesis. The volume of the fluorophore solution was calculated to reach a fluorophore/lysine molar ratio of 1/125. The number of NHS-terminated strands was adjusted as a molar fraction of total hydrobromide lysine units ($M = 209$ g/mol) to reach the targeted theoretical grafting ratio τ (number of functionalized lysine units/total number of lysine units).

The experimental grafting ratio τ_{exp} of PLL by Mal-PEG4-NHS linkers was determined by comparing ^1H NMR integration values of protons characterizing grafted and non-grafted lysine units (e' and e respectively), with protons of PLL backbone (a) as reference protons: $\tau_{\text{exp}} = 0.15/2 \sim 0.07$.

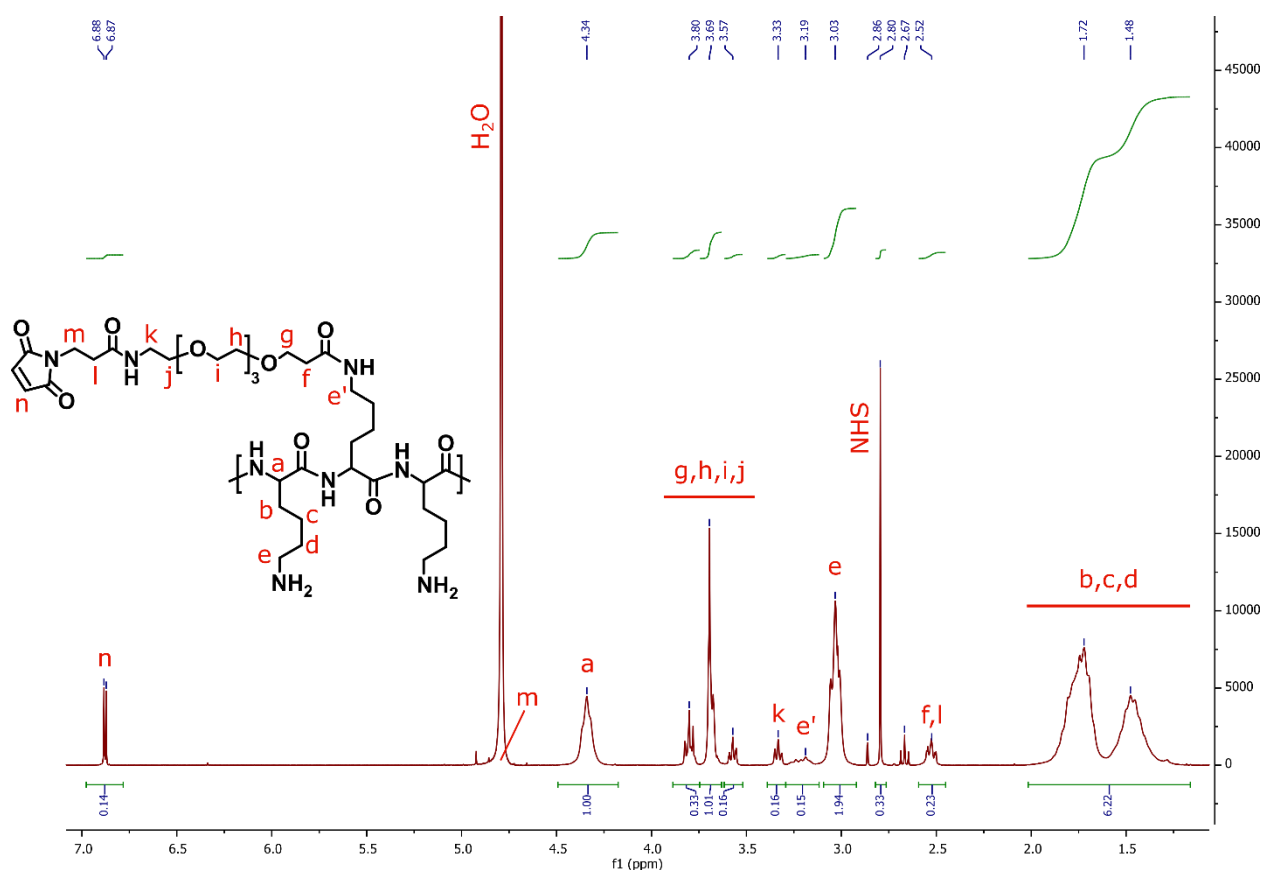


Figure S2. ^1H NMR spectrum of PLL grafted with Mal-PEG4-NHS (400 MHz, D_2O , δ): 1.5-1.7 (lysine [$\beta,\gamma,\delta\text{-CH}_2$], 6.22H), 2.5 (linker [$\text{CH}_2\text{-CONH}$], 0.23H), 2.8 (released NHS units, 0.33H), 3.0 (lysine [non-grafted $\alpha\text{-CH}_2$], 1.94H), 3.2 (lysine [grafted $\alpha\text{-CH}_2$], 0.15H), 3.3 (linker [CONH-CH_2], 0.16H), 3.6-3.8 (PEG backbone, 1.5H), 4.3 (lysine backbone, 1H), 6.9 (maleimide double-bond, 0.14H).

3. Methods

Turbidimetry by UV-Visible spectrophotometry: UV-Visible spectra were recorded on a single cell Thermo Scientific Evolution Array UV-Vis spectrophotometer equipped with a Peltier temperature-controlled cell holder (± 0.1 °C). Sample solutions were injected in a 150 μ L quartz micro-cuvette with an optical path length of 1 cm and submitted to an increase of temperature from 20 °C to 60 °C by steps of 2 °C. A spectrum was recorded at every step temperature after 3 min thermalization and the mean transmittance between 650 and 750 nm was used to plot the turbidimetry curve.

Zeta potential: Zeta potential measurements were performed in solutions of 50-nm PVP-coated AuNPs in the presence or absence of 52 kg/mol PLL (incubation in excess of polymer for 48h at ambient temperature) using DTS 1060C Zeta cells and the Zetasizer Nano-ZS (Malvern Instruments). The measurements were performed at 25 °C. For each sample, the average zeta distribution was obtained from the average of three measurements (22 runs each), separated by 2 min stabilization.

Emulsion formulation and observation: To prepare water-in-oil emulsions, 5 μ L of aqueous phase (containing the polymer at 10 g/L in 20 mM KSCN, pH 5) and 95 μ L of FC-70 oil phase (containing 0.5 g/L Krytox) were heated separately at 60 °C before being mixed in a 0.5 mL Eppendorf tube, and manually emulsified until the mixture became turbid. For microscopy experiments, a few μ L of emulsion were injected in a hollow rectangle capillary ($H \times W = 0.1 \times 1$ mm, CMSscientific) previously coated with PLL-g-PEG to prevent the breakage of water droplets at the contact with bare glass side. After injection, the capillary was sealed with Vitrex putty.

The internal sides of capillaries were coated as follows: capillaries were filled with an aqueous solution of PLL-g-PEG ($M_w = 2$ kg/mol for PEG strands, 1 g/L in water) and incubated for 30 min before evaporation on a heating plate at 60 °C. Then DI water was introduced by capillarity and evaporated at 60 °C.

Confocal Laser Scanning Microscopy: Confocal images were acquired on a Zeiss LSM 710 META Laser Scanning Microscope equipped with a Plan Apochromat x20 (0.8 NA) air objective and a temperature-controlled heating stage (25-50 °C). To ensure emulsion

thermalization, capillaries filled with the emulsion were placed on a pierced supporting copper slide that was fixed on the heating stage of the confocal microscope. Low temperatures (18-20 °C) were achieved by placing a glass petri dish full of ice on top of the supporting copper slide close to the capillary. The actual temperature of the supporting copper slide was measured with a REED ST-640B thermocouple. The 405 nm excitation of ANS or coumarin was performed using a 30 mW CW/pulsed diode laser. The 458 nm excitation of FluoSpheres™ NeutrAvidin™-Labeled Microspheres and the 514 nm excitation of rhodamine were performed using a 25 mW argon laser. Images were acquired using LSM ZEN 2009 software and processed with Fiji software (ImageJ).

Fluorescence Recovery After Photobleaching (FRAP): FRAP experiments were performed on coumarin-labeled PLL-g-PAAMAN shells at 18 °C and 48 °C on the Zeiss LSM 710 confocal microscope presented above. The sample was imaged using a 405-nm excitation at 0.15 mW (0.5 % of the maximum intensity) with a scanning speed of 0.39 $\mu\text{s}/\text{px}$. The photobleaching was performed inside a circle (region of interest, ROI) by 405-nm excitation at 30 mW (100 % of the maximum intensity) with a scanning speed of 6.30 $\mu\text{s}/\text{px}$. The acquisition rate was set to 1 image/second and the mean fluorescence level in the ROI was monitored for 10 s before bleaching and 120 s after.

Scanning Electron Microscopy (SEM): SEM imaging was performed on 5 μL emulsion dried on a glass coverslip for three days at ambient air. Prior to imaging, the dried capsules were metalized by deposition of a 30 nm-thin layer of Ag using an Emitech K675X Sputter Coater (Ag sputtering for 60 s at 120 mA). SEM images were recorded with a Hitachi TM3030 Tabletop Microscope in the EDX mode.

4. Cloud point measurements

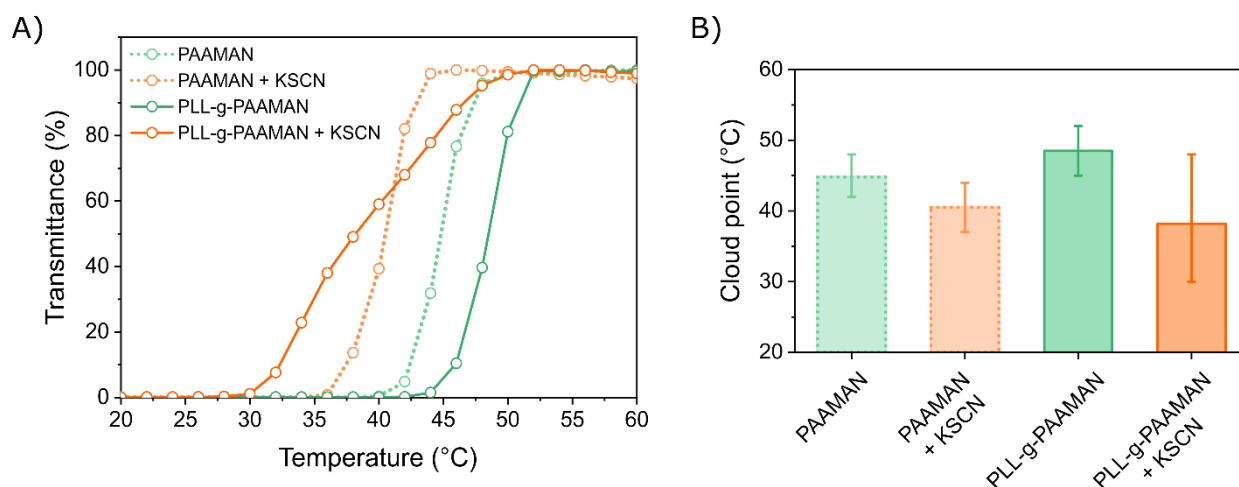


Figure S3. Cloud point of solutions of either poly(acrylamide-co-acrylonitrile) (PAAMAN, i.e. the non ionic chains not yet grafted on PLL) or PLL_{0.53}-g-PAAMAN_{0.47} (quoted PLL-g-PAAMAN) in the presence or absence of thiocyanate salt (KSCN). A) Turbidimetry heating curves of PAAMAN and PLL-g-PAAMAN solutions at 10 g/L in either pure water or 20 mM KSCN solution. B) Cloud points extracted from the turbidimetry curves at 50 % transmittance (the bars indicate the start and end of the thermal transition).

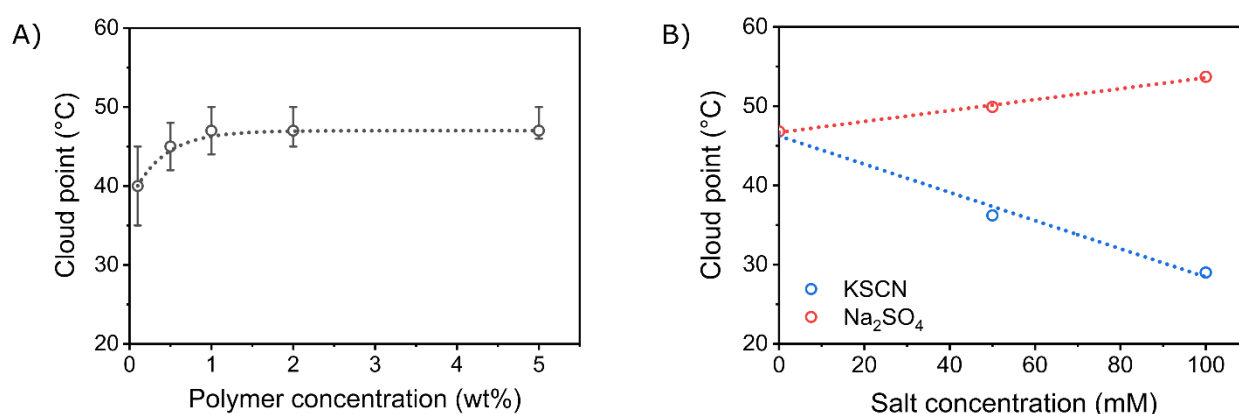


Figure S4. Cloud point of PAAMAN in water at increasing polymer concentration (A, note that 1 wt% = 10 g/L) or in aqueous salt-containing solutions and 10 g/L polymer (B).

5. Reversibility of the thermal transition.

To complement Fig. 2 in main text, the representative confocal fluorescence image of a droplet initially thermalized at 25 °C, then heated up to 48 °C and cooled back to 18 °C shows that a hydrophobic shell appeared again upon cooling.

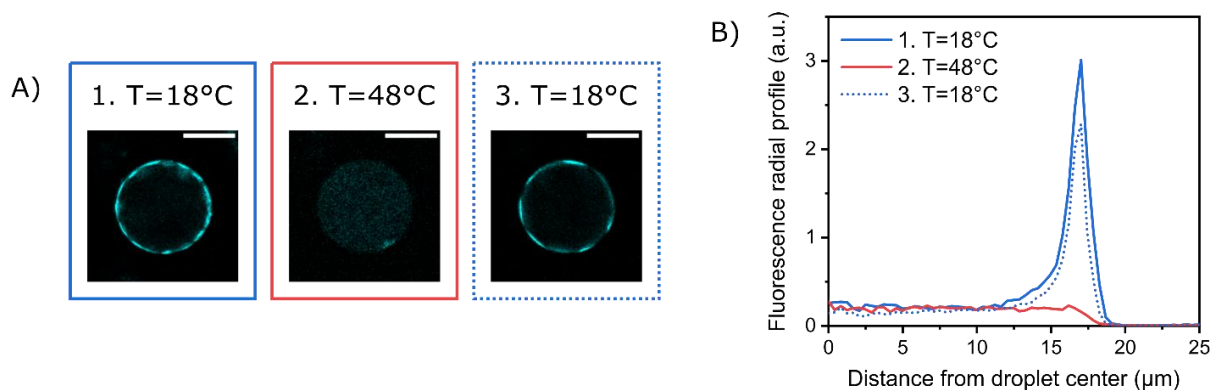


Figure S5. Confocal images and radial profile fluorescence of 100 μM 8-anilinonaphtalene-1-sulfonic acid present in droplets of 10 g/L PLL-g-PAAMAN, 20 mM KSCN solution dispersed in 0.5 g/L Krytox in FC-70 oil. The emulsion was initially below the cloud point, at 18 $^{\circ}\text{C}$, then equilibrated at 48 $^{\circ}\text{C}$, and eventually cooled back to 18 $^{\circ}\text{C}$ as quoted. Scale bars = 20 μm .

Using rhodamine-labeled PLL-g-PAAMAN in 20 mM KSCN, fluorescence measurements enabled to quantify the surface excess $S = ([\text{polymer}]_{\text{tot}} - [\text{polymer}]_{\text{bulk}})/A$ (with A the surface area of droplet, $[\text{polymer}]_{\text{tot}}$ the concentration of polymer introduced in the solution, and $[\text{polymer}]_{\text{bulk}}$ the polymer concentration measured at thermal equilibrium in the middle of the droplet) as described by Sixdenier, L. et al., in *Adv. Funct. Mater.* 2021, 2105490. Data obtained by randomly picking droplets in images are shown in Fig. S6 as a function of either the measured droplet diameter, or the concentration of polymer in the core of the droplets ($[\text{polymer}]_{\text{bulk}} / [\text{polymer}]_{\text{tot}}$).

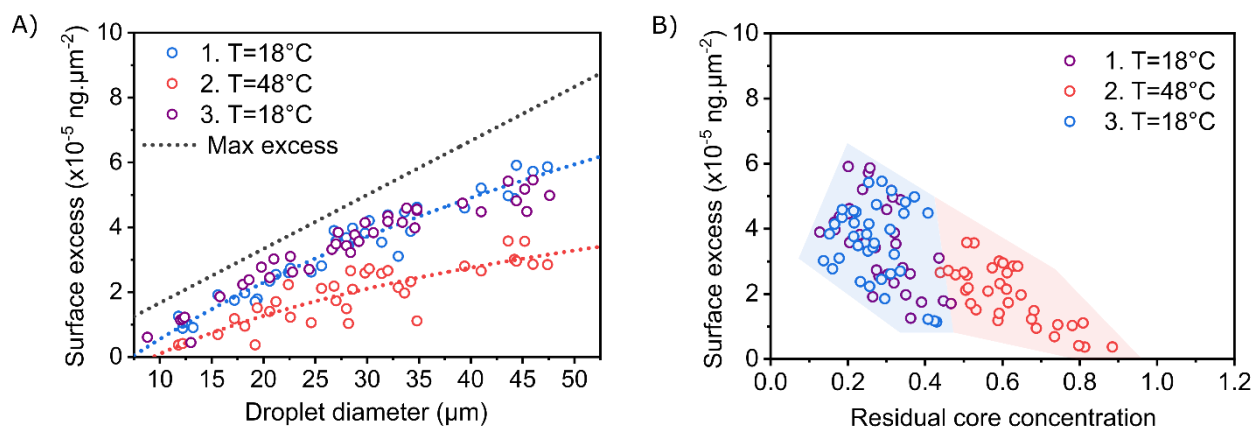


Figure S6. Surface excess of rhodamine-labeled PLL-g-PAAMAN (10 g/L in 20 mM KSCN) measured by confocal microscopy during a cycle of thermalization at 18 $^{\circ}\text{C}$, then 48 $^{\circ}\text{C}$ and finally cooling the sample back to 18 $^{\circ}\text{C}$. Dotted line indicates the maximum surface excess achievable, i.e. hypothetical condition of 100 % binding of the polymer in the outer layer.

6. Zeta potential of PVP-coated AU NPs.

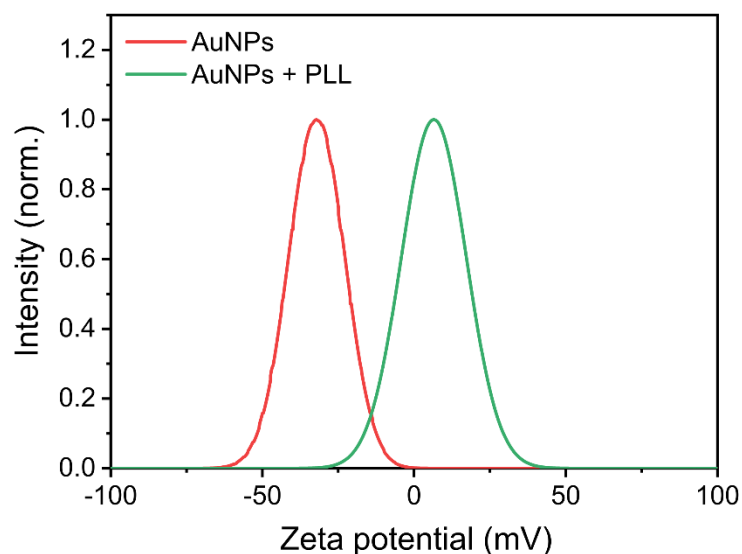


Figure S7. Zeta potential distributions of PVP-coated AuNPs (50-nm diameter, 0.5 g/L) before and after incubation with PLL (52 kg/mol, 1 g/L) in 7.7 mM phosphate buffer pH 7.3.

7. Qualitative observation of a thermoplasmonic effect

Light-triggered dissolution of PLL-g-PAAMAN shell induced by thermoplasmonic effects in the presence of co-encapsulated AuNPs was detected by phase contrast microscopy on water droplets prepared at 60 °C and containing 20 g/L of PLL-g-PAAMAN and 0.5 g/L of AuNPs (in 20 mM KSCN) dispersed in oil phase containing 1 g/L of Krytox. The emulsion was thermalized at 25 °C. The droplet was irradiated with a 532-nm continuous laser at 15 mW for 5 seconds. After turning off the laser, polymer granules appeared in the droplet core and progressively migrated towards the interface in a few minutes as illustrated in Fig. S8.

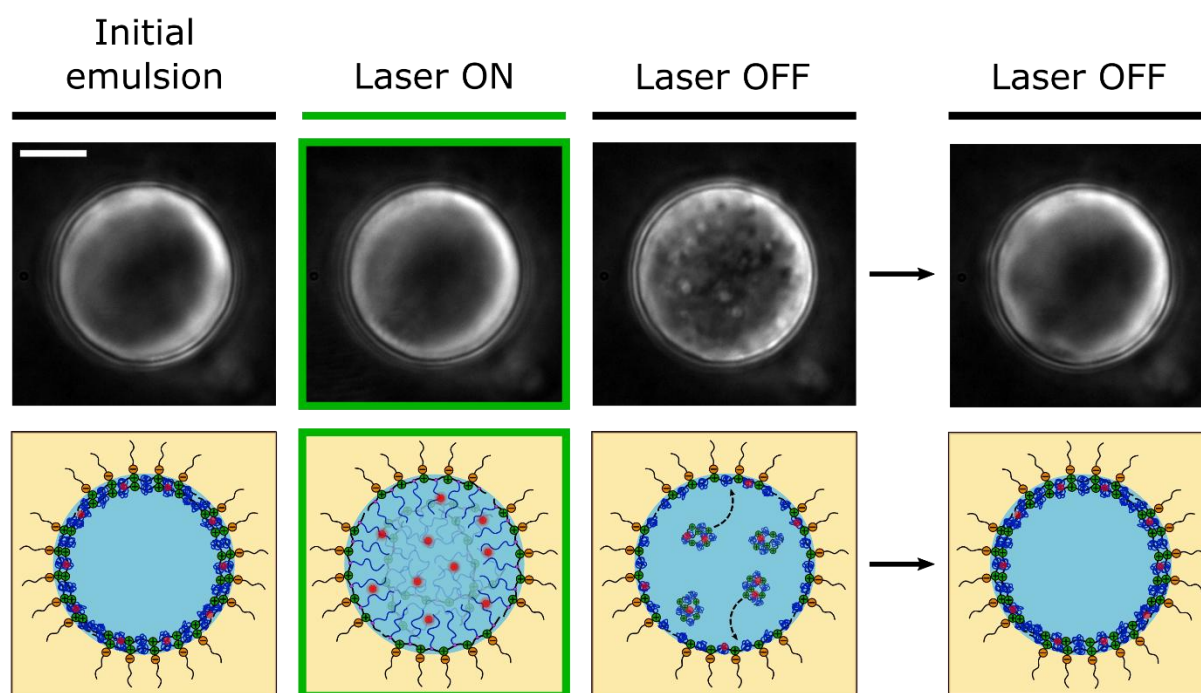


Figure S8. Light-triggered dissolution of PLL-g-PAAMAN shell induced by thermoplasmonic effects of co-encapsulated AuNPs. The phase contrast micrographs shows a water droplet containing 20 g/L of PLL-g-PAAMAN and 0.5 g/L of AuNPs (in 20 mM KSCN) dispersed in oil phase containing 1 g/L of Krytox, and thermalized at 25 °C (scale bar = 10 μ m). The droplet was irradiated with a 532-nm laser at 15 mW for 5 seconds. After turning off the laser, polymer granules spontaneously formed in the droplet core ("laser off", left) and progressively migrate towards the interface in a few minutes ("laser off", right).

8. Permeability assessment

A fluorescent FC-70 oil was prepared by first dispersing a 100 g/L solution of Dextran-fluorescein (DF) (Mw of 10 000 g/mol) into Krytox (to reach 10 % water in Krytox) that was mixed with FC-70 to obtain a dispersion at 1 g/L DF. The dispersion was sonicated for 1 min (Vibra Cell Bioblock Scientific) and centrifuged for 2 min (Eppendorf MiniSpin, at 7000 rpm) to cream out the largest droplets. The limpid bottom phase was collected and used as the continuous, DF-containing oil phase.

Emulsions were prepared as described above in method section, using 20 g/L polymer solutions in phosphate buffer (pH 7.3) dispersed in 0.5 g/L Krytox in FC-70. PLL-g-PEG (see materials, from SuSoS) was used as a reference grafted PLL copolymer that is hydrophilic irrespective of temperature. Emulsions with PLL-g-PEG (10 % water in oil) were prepared at room temperature by manual agitation and pipetting (see method section). Emulsions with PLL-g-PAAMAN at the same volume fraction as above were prepared at 60 °C, prior to be cooled down to room temperature. Eventually, an aliquot of the emulsion (that rapidly creamed on the top of the samples) was collected and mixed with the same volume of the DF-containing oil at room temperature. A microscope image of a representative PLL-g-PEG stabilized droplets is shown in Fig. S9.

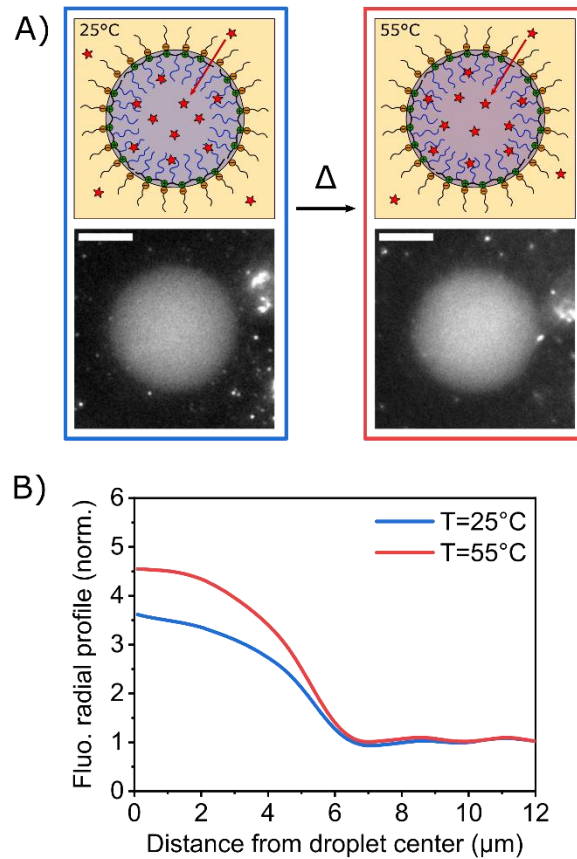


Figure S9. Permeability of PLL-g-PEG shells. A) Fluorescence micrographs of a representative droplet containing 20 g/L of PLL-g-PEG and prepared at 25°C in DF-free oil, prior to be mixed with DF-containing FC-70. Images were taken at 25 °C, and after heating up to 55 °C and cooling back to 25°C (quoted "55°C"). Scale bars = 10 μm. The fluorescence indicates that DF crossed the PLL-g-PEG shell and diffused in the droplet core. B) Fluorescence radial profiles of the droplets (normalized by the value in the oil phase).

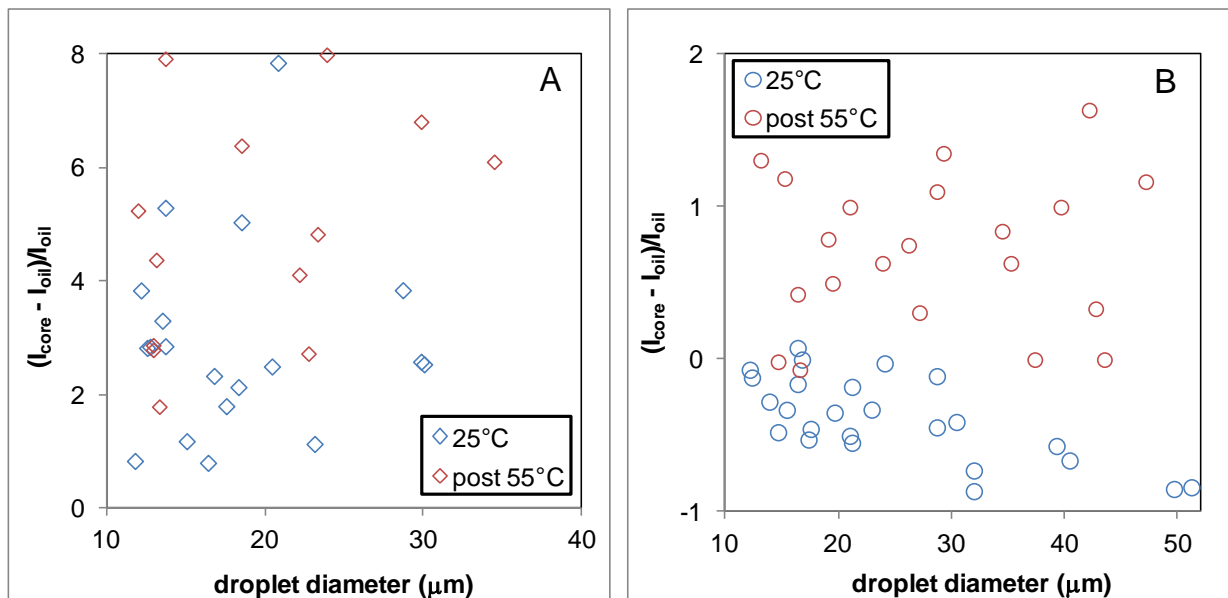


Figure S10. Permeability of droplets estimated from the fluorescence intensity in the core after mixing with DF-containing FC-70. I_{core} and I_{oil} are the fluorescence measured in the core and in the continuous oil phase respectively. Emulsion were prepared in 0.5 g/L Krytox in FC-70 (without DF) and 20 g/L (A) PLL-g-PEG or (B) PLL-g-PAAMAN. After 50:50 v/v mixing at room temperature with DF-containing oil, fluorescence was measured either at 25°C or after heating up to 55°C and cooling back to 25°C (quoted "post 55°C").