Supporting Information to

Temperature-Triggered Protein Adsorption on Polymer-Coated Nanoparticles in Serum

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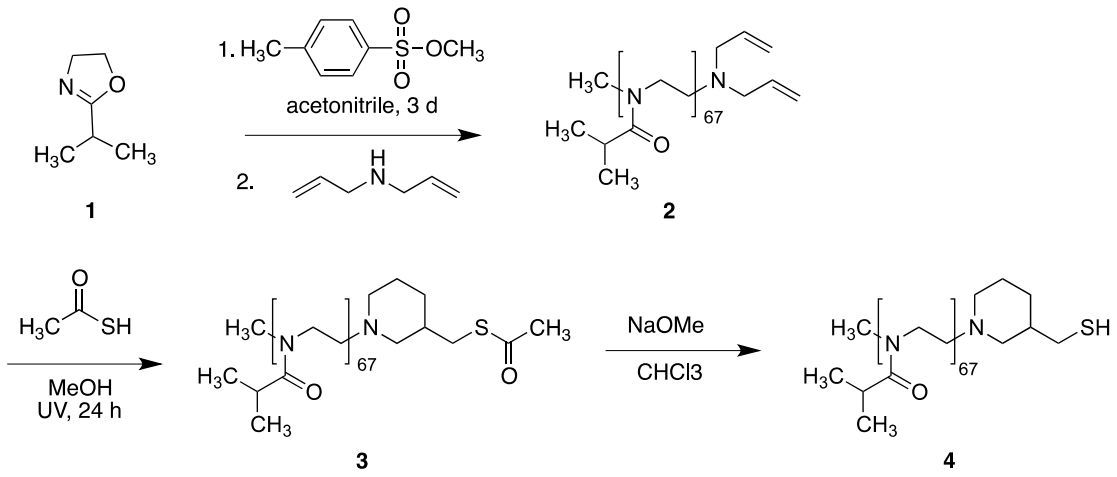
Synthesis and characterization of thiol-terminated poly(2-isopropyl-2-oxazoline).

Scheme S1. Synthesis of thiol-functionalized PiPOx.



^{1}H NMR measurements were carried out at room temperature using a Bruker DPX-400 spectrometer operating at 400.1 MHz. As solvent, $CDCl_3$ from Deutero GmbH, Germany was used. A mercury medium-pressure UV lamp TQ 150, Heraeus, 150 W, was used as light source for thiol-ene addition.

Polymerization. Diallyl-terminated poly(2-isopropyl-2-oxazoline) (**2**) was synthesized by cationic polymerization according to a procedure described elsewhere (Diehl, C. *Functional Microspheres through Crystallization of Thermoresponsive Poly(2-Oxazoline)s*. PhD thesis, University of Potsdam, September 2009). Briefly, a solution of 5.7 g 2-isopropyl-2-oxazoline (50 mmol, 100 equiv) in 10 mL dry acetonitrile was prepared under inert conditions. After addition of 0.09 mg methyl *p*-toluensulfonate (0.5 mmol, 1 equiv) the polymerization was allowed to proceed for three days at 70 °C under argon atmosphere. Afterwards, 0.2 mL diallyl amine were added and the reaction mixture was stirred over night. The polymer was purified by dialysis and freeze drying.

^{1}H NMR (400 MHz, *$CDCl_3$*) δ [ppm]: 1.1 (bs, 6H, *CH_3-isopropyl*), 2.5-3.0 (m, 1H, *CH*-$(CH_3)_2$ (*isopropyl*)), 3.0-3.2 (m, 7H, *H_3C*-N + N-*CH_2-allyl*), 3.4 (bs, 4H, N-*CH_2-CH_2*-N), 5.05-5.22 (m, 4H, *CH_2-allyl*), 5.7-5.9 (m, 2H, *CH-allyl*).

Thiol-ene photoaddition. 1.0 g of PiPOx-diallyl (**2**) were redissolved in methanol and degassed. 36 µL (0.5 mmol) thioacetic acid were added to reaction mixture under argon and the mixture was irradiated with UV for 24 h. The resulting product was purified by dialysis in methanol and dried under vacuum yielding 0.9 g thioacetic acid adduct of PiPOx (**3**).

^{1}H NMR (400 MHz, *$CDCl_3$*) δ [ppm]: 1.1 (bs, 6H, *CH_3* (*isopropyl*)), 1.4 (s, 2H, $N(CH_2)_2$-*CH_2*-CH (*piperidine*)), 2.05-2.13 (m, 3H, NCH_2-*CH_2*-CH_2 (*piperidine*)+*CH*-CH_2S), 2.3-2.4 (m, 3H, *CH_3*-CS=O), 2.5-3.0 (m, 1H, *CH*-$(CH_3)_2$ (*isopropyl*)), 3.0-3.2 (m, 7H, *H_3C*-N+N-*CH_2* (*piperidine*)), 3.4 (bs, 4H, N-*CH_2-CH_2*-N), 3.7 (bs, 2H, S-*CH_2*-piperidine).

Deprotection of thiol group. 0.8 g of PiPOx **3** were stirred with 2 mL methanolic sodium methanolate solution in 20 mL chloroform for 2 h. After removal of solvent under reduced pressure the crude product was redispersed in chloroform and purified by dialysis. After drying under vacuum 0.7 g of thiol-terminated PiPOx **4** were obtained.

^{1}H NMR (400 MHz, *$CDCl_3$*) δ [ppm]: 1.1 (bs, 6H, *CH_3* (*isopropyl*)), 1.4 (s, 2H, $N(CH_2)_2$-*CH_2*-CH (*piperidine*)), 2.0-2.2 (m, 3H, NCH_2-*CH_2*-CH_2 (*piperidine*)+*CH*-CH_2S), 2.5-3.0 (m, 1H, *CH*-$(CH_3)_2$ (*isopropyl*)), 3.0-3.2 (m, 7H, *H_3C*-N+N-*CH_2* (*piperidine*)), 3.4 (bs, 6H, N-*CH_2-CH_2*-N + S-*CH_2*-piperidine)

MALDI-ToF-MS: M_n = 7770 g/mol, M_w = 7810 g/mol

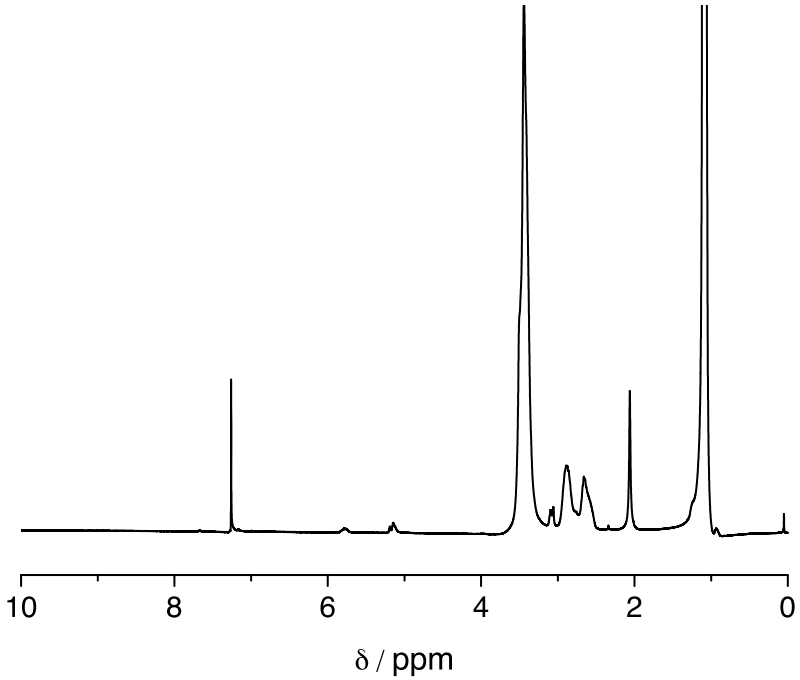


Figure S1. ^{1}H-NMR spectrum of PiPOx-diallyl **2**, $CDCl_3$, 400 MHz.

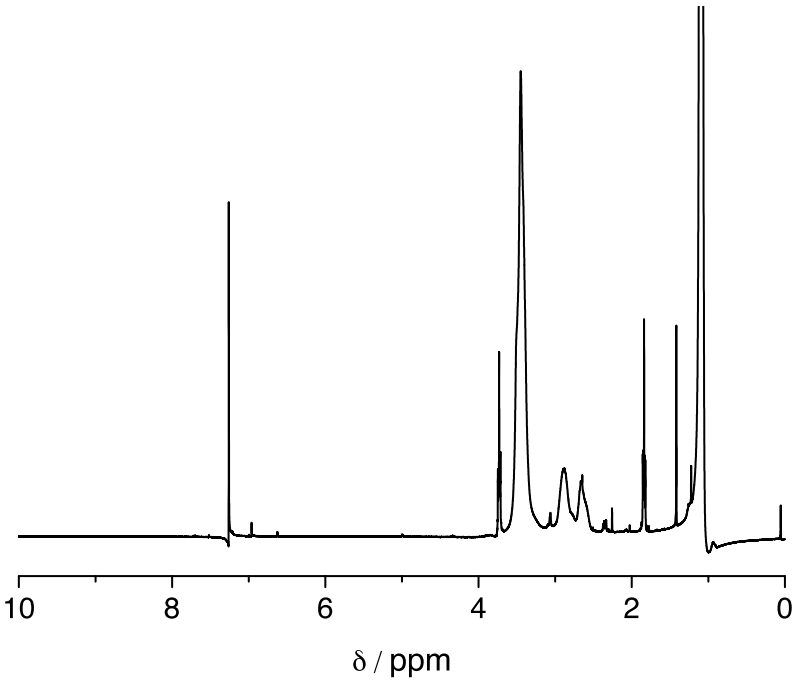


Figure S2. ^{1}H-NMR spectrum of thioacetic acid adduct of PiPOx **3**, $CDCl_3$, 400 MHz.

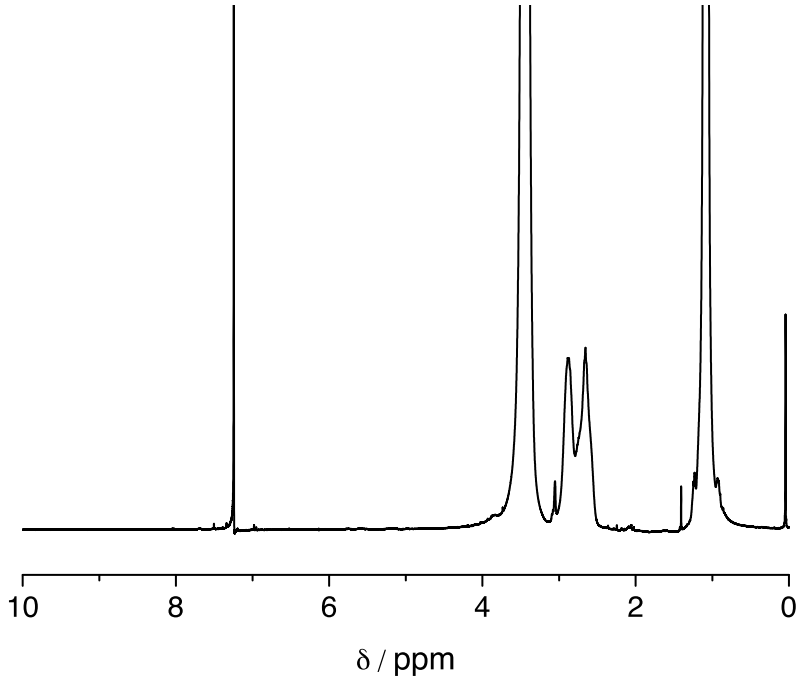


Figure S3. ^{1}H-NMR spectrum of PiPOx-thiol **4**, $CDCl_3$, 400 MHz.

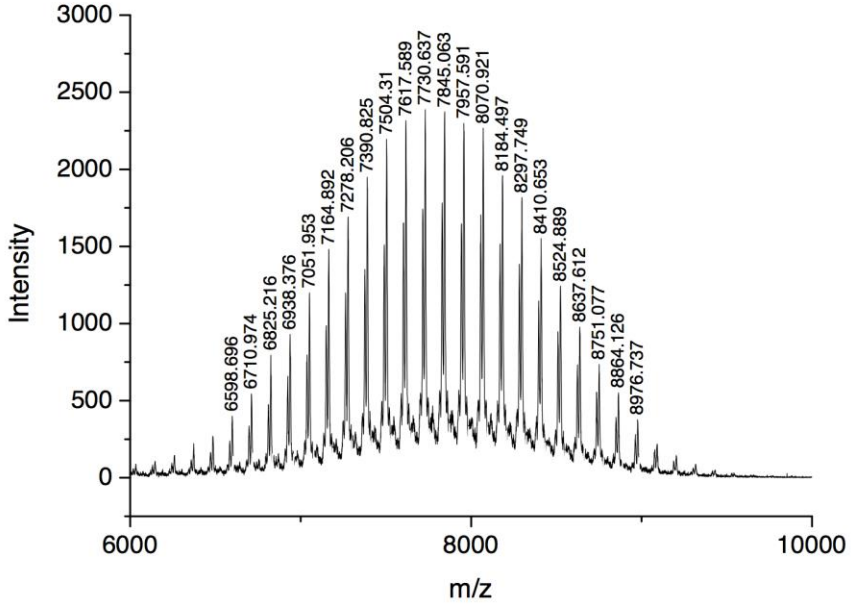
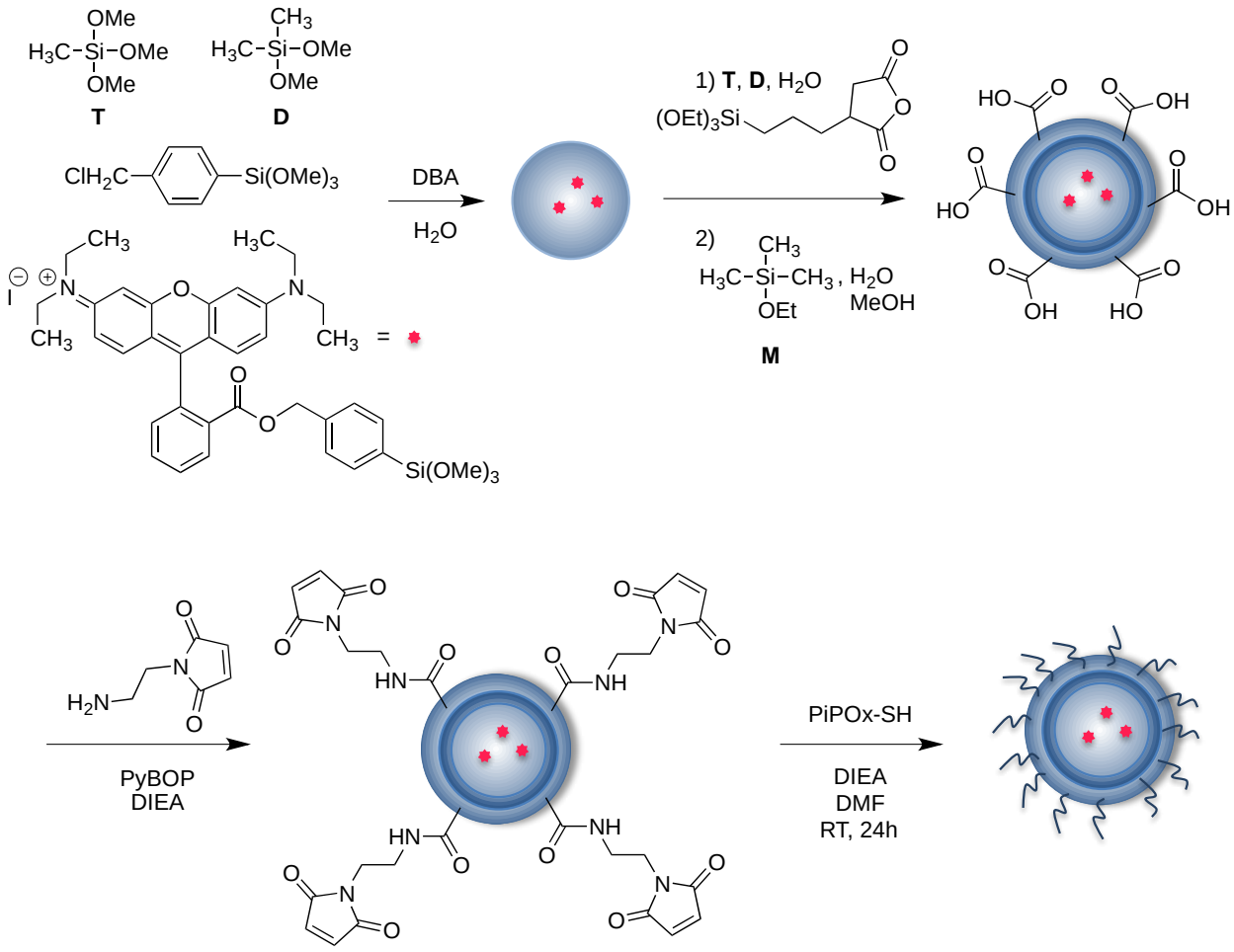


Figure S4. MALDI-ToF mass spectrum of thiol-functionalized PiPOx **4**.

Synthesis and characterization of PiPOx-coated nanoparticles.

Scheme S2. Synthesis of PiPOxylated nanoparticles



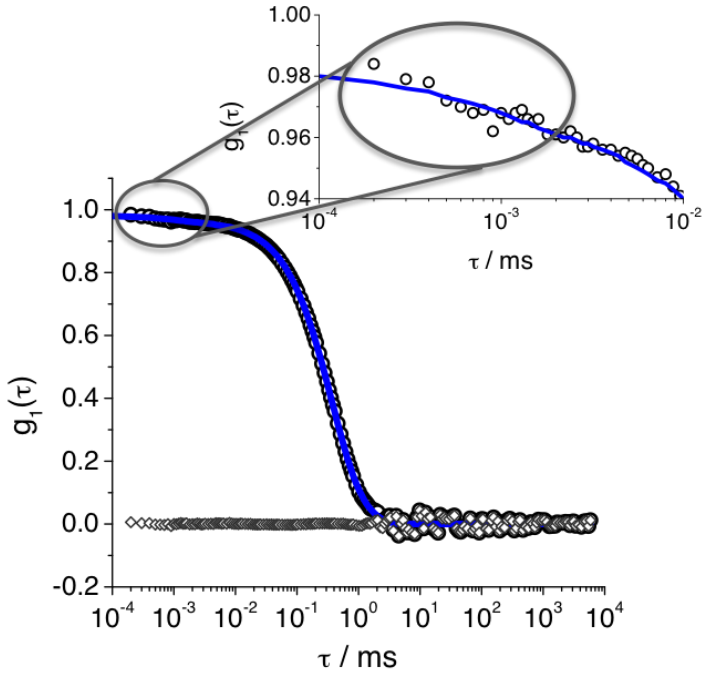


Figure S5. Autocorrelation function of PiPOxylated nanoparticles at $\theta = 90°$ (black circles) with triexponential fit function (blue line) and residuum (grey squares). The enlarged region indicates the presence of free polymer. $c = 0.25$ mg/mL, $T = 296$ K.

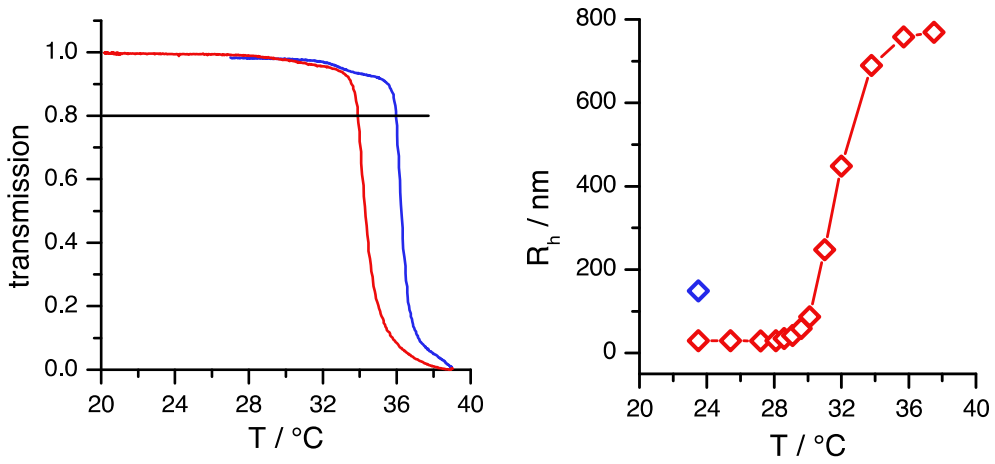


Figure S6. Determination of the cloud point of nanoparticles at c = 0.25 mg/mL. Left: turbidimetry (red line: heating; blue line: cooling). The temperature at which transmission deceased to 80%, here 34 °C, is considered as the phase separation or cloud point temperature. Right: DLS (red line-dot: heating; blue square: radius after cooling).

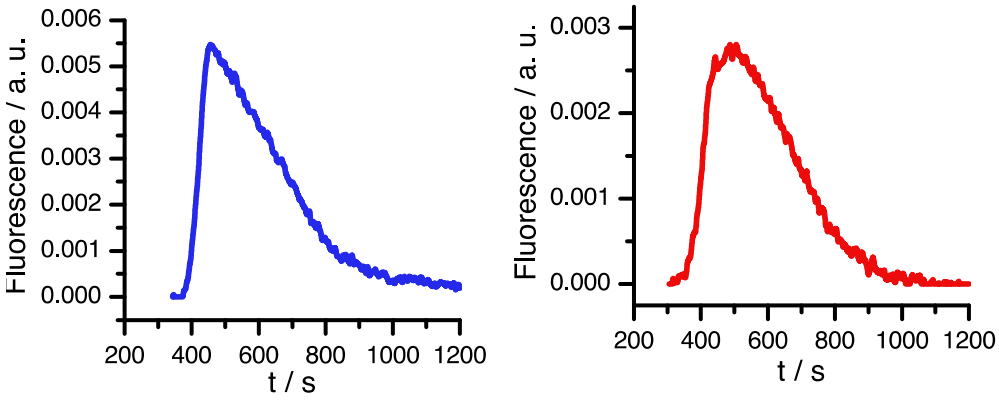


Figure S7. AF-FFF elution profiles of nanoparticles in water before (left) and after (right) heating to 37 °C. The approximate radius of the main fraction is 27 nm at room temperature and after incubation at 37 °C and cooling back to room temperature.

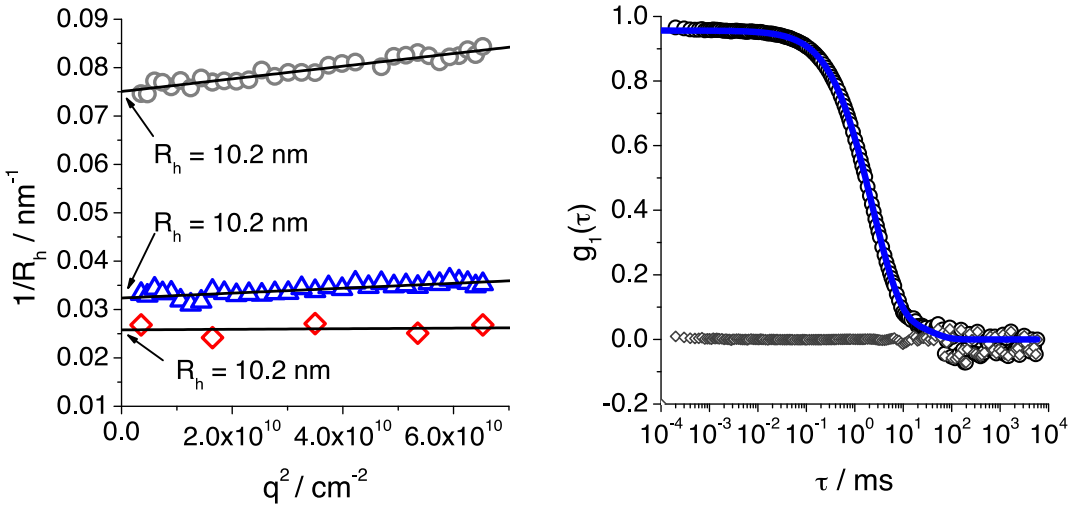


Figure S8. DLS analysis of nanoparticles. Right: Angular dependency of inverse hydrodynamic radius of FCS without nanoparticles (grey circles), nanoparticles in RPMI medium (blue triangles), nanoparticles in RPMI with 5% FCS (blue triangles). Right: Autocorrelation function of nanoparticles at $\theta = 26°$ (black circles) with fit according to multicomponent analysis (blue line) and residuum (grey squares). c(NP) = 0.25 mg/mL, T = 296 K.

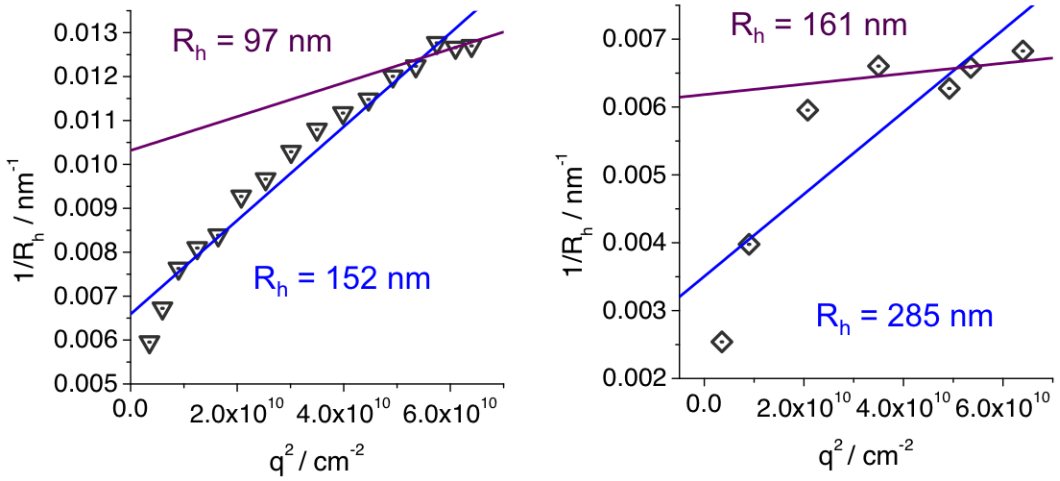


Figure S9. Angular dependency of inverse hydrodynamic radius in serum-free (left) and serum-containing (right) medium after cooling to 296 K and linear fits over all angles (blue line) or only large angles (purple line). c(NP) = 0.25 mg/mL.

Table **S1**. Determination of phase transition temperature in serum-free and serum-containing medium by DLS.

RPMI 1640		RPMI 1640 + 5% FCS	
T / °C	R_h / nm	T / °C	R_h / nm[a]
23.5	33	23.5	16
26.3	32	26.3	16
28.2	32	28.2	16
29.1	33	29.1	16
30.1	34	30.1	18
31.0	**42**	30.6	22
31.5	52	**31.0**	**43**
32.0	74	31.5	490
32.4	138	32.0	909
33.8	549	33.8	1076
35.7	840	35.7	n.a.[b]
37.6	1030	37.3	n.a.[b]

[a]The fiting of autocorrelation function in serum containing medium was done with a triexponential function. The resulting radii are average values of nanoparticles and serum proteins with $R_h \approx 13$ nm. The sizes bellow the phase transition temperature are, therefore, smaller than sizes obtained by evaluation with multicomponent analysis, where the scattering component of proteins is separated.

[b]A clear evaluation of autocorrelation function was not possible due to high polydispersity, approximate radius > 1000 nm.