Supporting Information

Temperature-switchable Agglomeration of Magnetic Particles Designed for Continuous Separation Processes in Biotechnology

*Anja S. Paulus *, Raphael Heinzler, Huey Wen Ooi, Matthias Franzreb*

Karlsruhe Institute of Technology (KIT), Institute of Functional Interfaces (IFG),

Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany

Corresponding Author

Anja S. Paulus, anja.paulus@kit.edu, Phone: +49 721 608-22984

This Supporting Information file contains additional chapters, figures and tables with respect to the following topics:

- M-PVA Particle Characterization
- PNIPAM Polymer Characterization
- M-PNIPAM Particles
- End-group Modified M-PNIPAM Particles
- Two-Block Copolymer PNIPAM Particles
- Three-Block Copolymer PNIPAM Particles

M-PVA Particle Characterization

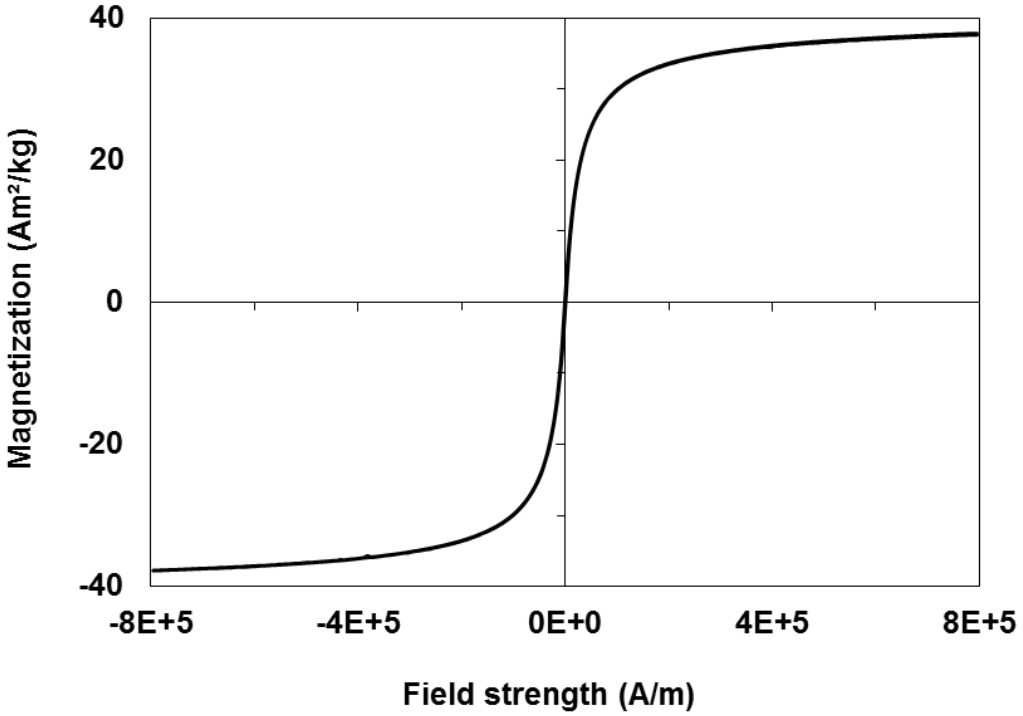

Figure S1 **Magnetization curve of magnetic micro beads (M-PVA 012, Chemagen) recorded on an alternating gradient magnetometer (2900 AGM, Princeton Measurement Corporation, USA). The particles have a very low remanence (<0.5 Am²/kg) and a high saturation magnetization (~38 Am²/kg).**

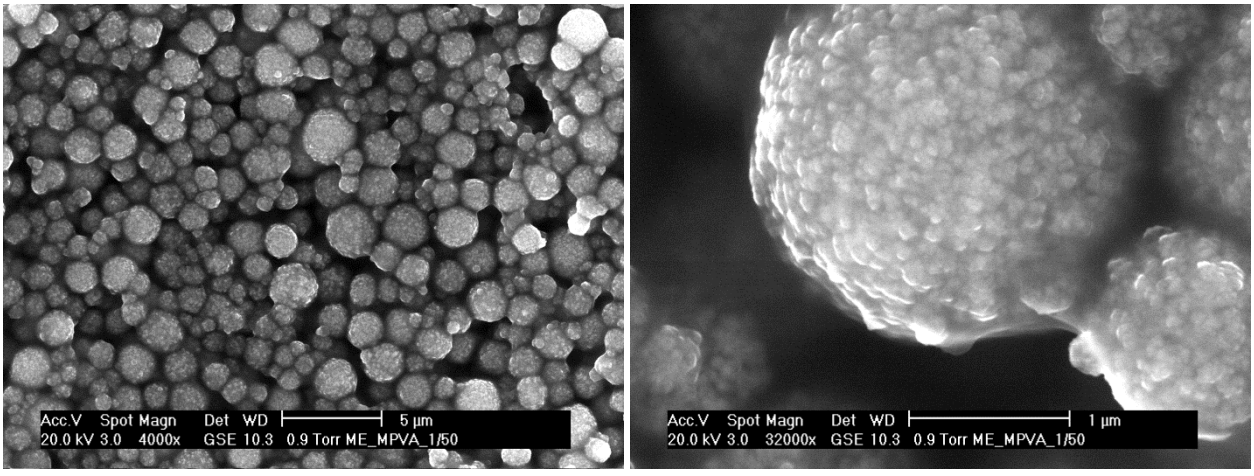

Figure S2 **Microscopic images (environmental scanning electron microscope) of M-PVA 012 magnetic particles at 4,000 x (left) and 32,000 x (right) magnification.**

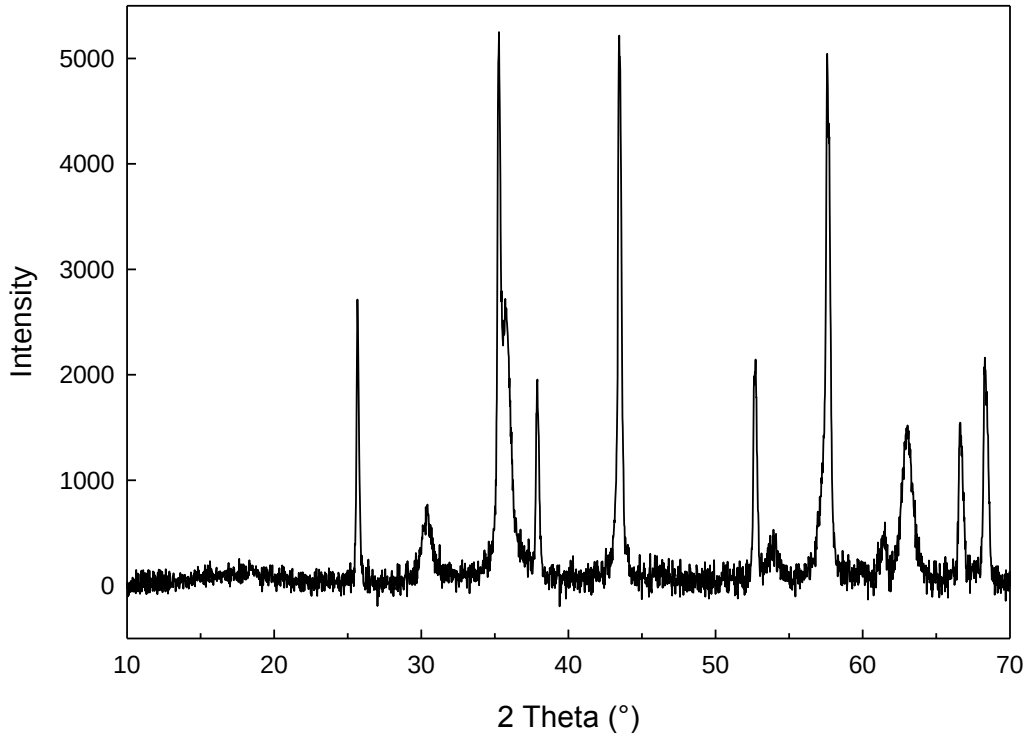

Figure S3 **X-ray diffraction patterns of magnetic particles (M-PVA 012, Chemagen) using Al_2O_3 as a reference. The results indicate a cubic structure, space group: Fd-3m, and a lattice constant a = 8.35. The oxidation degree of the material was calculated to 72%, which means that the iron oxide nano-crystals are mainly maghemite instead of magnetite.**

Table S1 **Summary of Important Properties of M-PVA 012 Particles (PerkinElmer Chemagen Technologie GmbH, Baesweiler, Germany).**

Property	
Size, shape	1-3 µm, spherical
BET surface area	25 $m^2 \cdot g^{-1}$
Magnetite content	50-60% [a]
Saturation magnetization	38 $Am^2 \cdot kg^{-1}$
Remanent magnetization	< 0.5 $Am^2 \cdot kg$
Crystalline structure	cubic, space group Fd-3m

a) According to XRD measurements, the magnetite is 72% oxidized to maghemite which is still magnetic, however.

PNIPAM Polymer Characterization

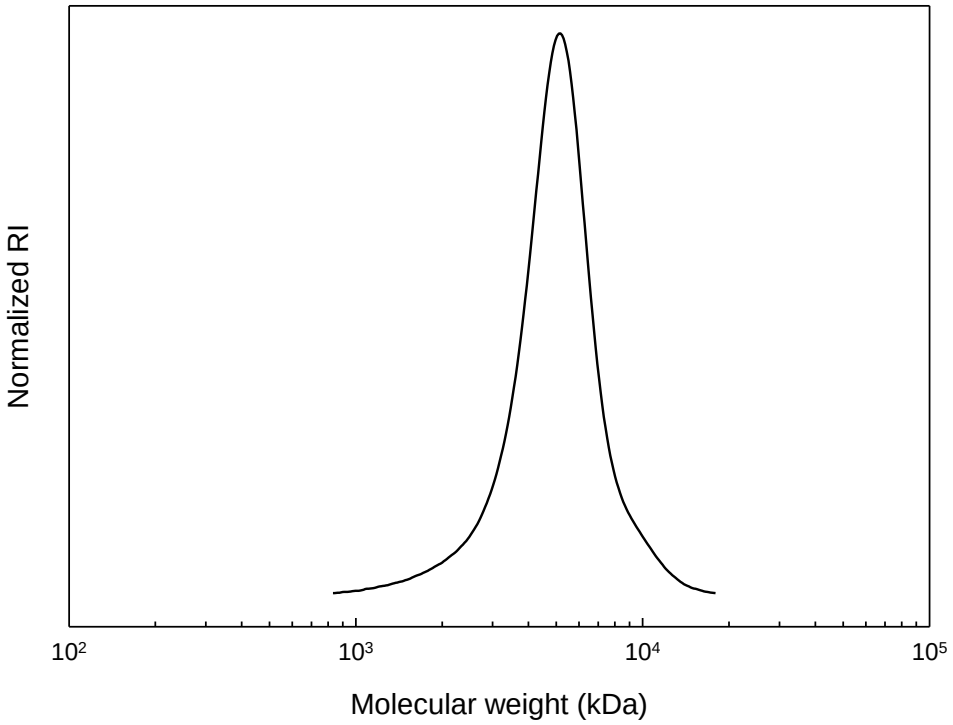

Figure S4 **Molecular weight distribution of PNIPAM 17 kDa determined via gel permeation chromatography. The average molecular weight is 45.5 kDa and the PDI is 1.15. The sample was taken from the supernatant of the M-PNIPAM 17 kDa RAFT synthesis.**

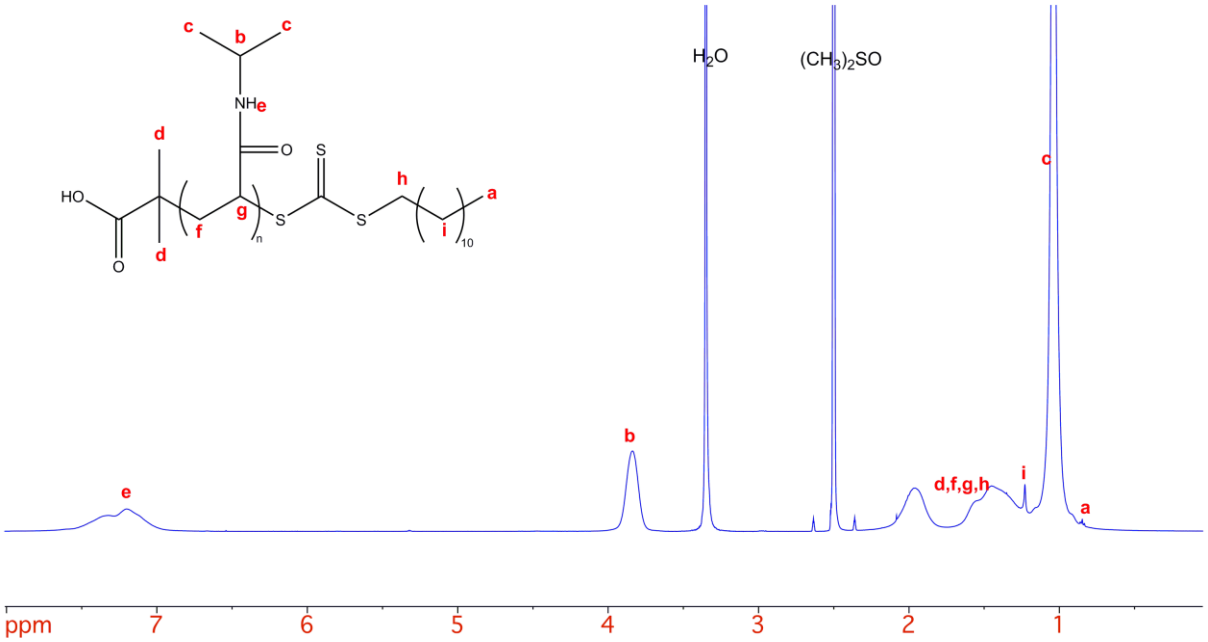

Figure S5 **NMR spectrum of PNIPAM 17 kDa: The sample was taken from the supernatant of the M-PNIPAM 17 kDa RAFT synthesis.**

M-PNIPAM Particles

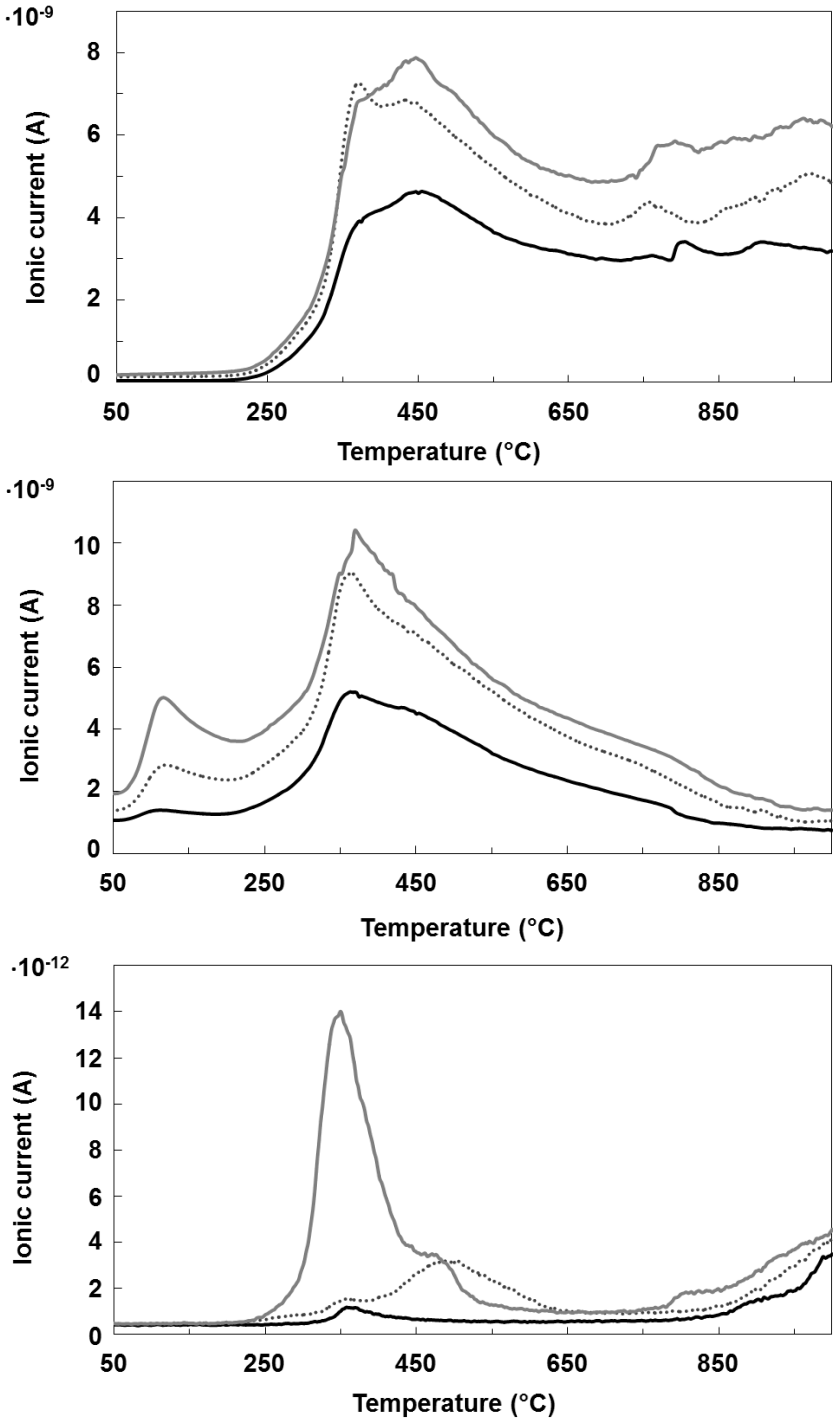

Figure S6 **Mass spectroscopy data of CO_2 (top), H_2O (middle), and SO_2 (bottom) corresponding to the thermal analysis curves in Figure 2 (right), for M-PVA (solid black line), M-PNIPAM (dotted black line), M-P(NIPAM-SPA) (solid grey line) magnetic particles. A QMS 403 C (Netzsch, Germany) was used for analysis of the volatile decomposition products during TGA.**

Effect of Particle Concentration on Particle Agglomeration

First observations of M-PNIPAM particles showed that particle concentration has a dominating influence on the agglomeration behavior. While agglomeration was not visible at low particle concentrations, the effect seemed to increase at high particle concentrations. This can be explained by an increased number of particle-particle contacts and therefore higher agglomeration rates [1]. Figure S7 shows recorded transmission values of M-PVA (A) and M-PNIPAM (B) suspensions over time at 40°C for particle concentrations of 1-4 g/L. For M-PVA suspensions, transmission values are nearly constant for all concentrations. For M-PNIPAM 17 kDa suspensions, the transmission increases over time and the transmission increase is larger at high concentrations. The highest transmission is observed at 4 g/L after three minutes of settling. At 40°C, the polymer chains of the M-PNIPAM particles are collapsed and hydrophobic side chains are exposed. Therefore, the observed effect can be attributed to an increase in particle-particle contacts at high concentrations, which lead to agglomeration and subsequently to faster sedimentation and higher transmission. At low particle concentrations, collisions between particles are less likely to occur hence reducing agglomeration. The sedimentation of these single particles is slower and the well dispersed suspension has a lower transmission. It can be concluded, that the observed effect of faster sedimentation of M-PNIPAM particles is a direct result of the surface modification.

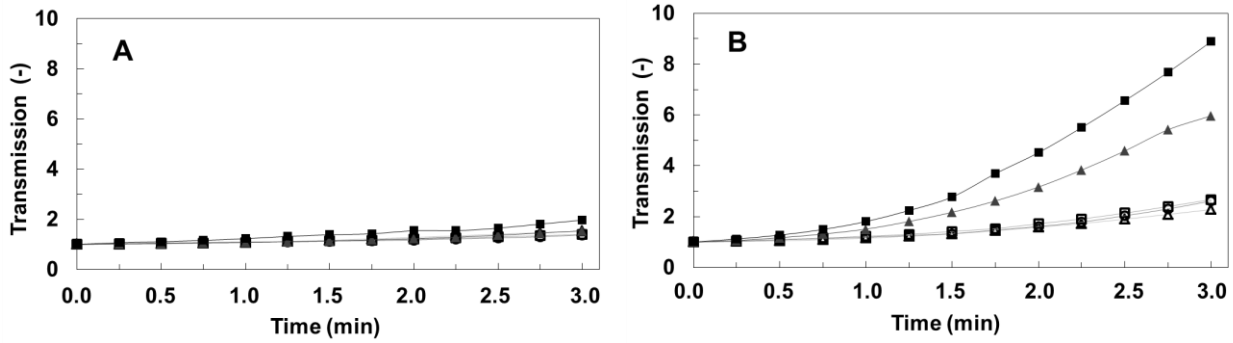

Figure S7 **Normalized transmission of (A) M-PVA and (B) M-PNIPAM 17 kDa particle suspensions recorded over time at 500 nm. Testing was done at pH 7 in 0.1 M phosphate buffer at 40°C and the particle concentration was varied (filled squares, 4 g/L; filled triangles, 3 g/L; empty circles, 2 g/L; empty squares, 1.5 g/L; empty triangles, 1 g/L).**

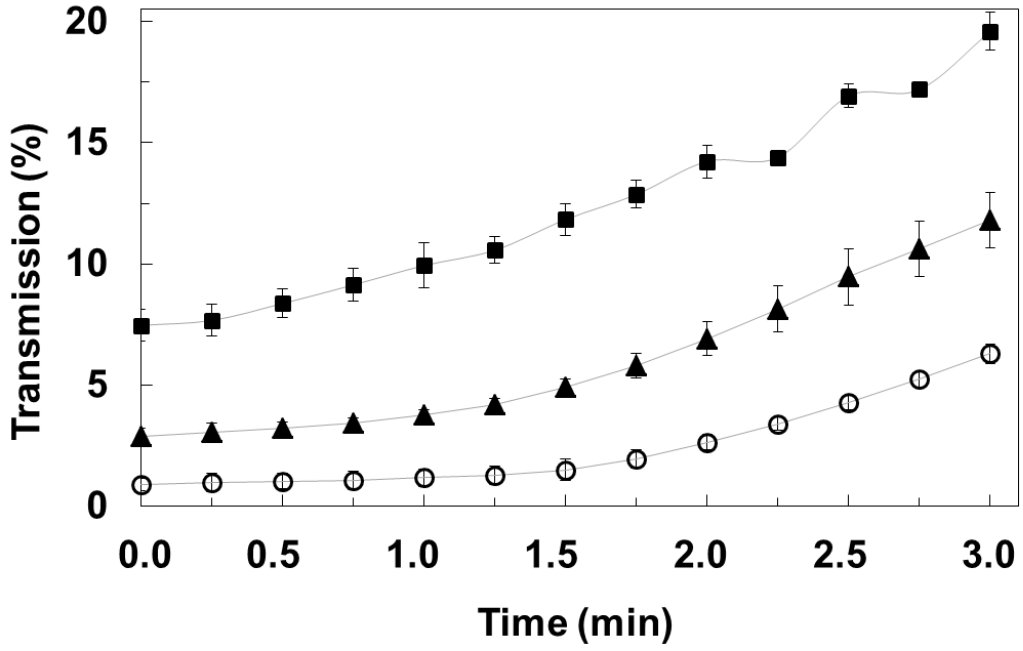

Figure S8 **Transmission of M-PNIPAM 30 kDa particle suspension recorded over time at 500 nm. The temperature (40°C) and particle concentration (4 g/L) was kept constant. The pH of the suspension (0.1 M phosphate buffer) was varied (square, pH 4; triangle, pH 7; circle, pH 10).**

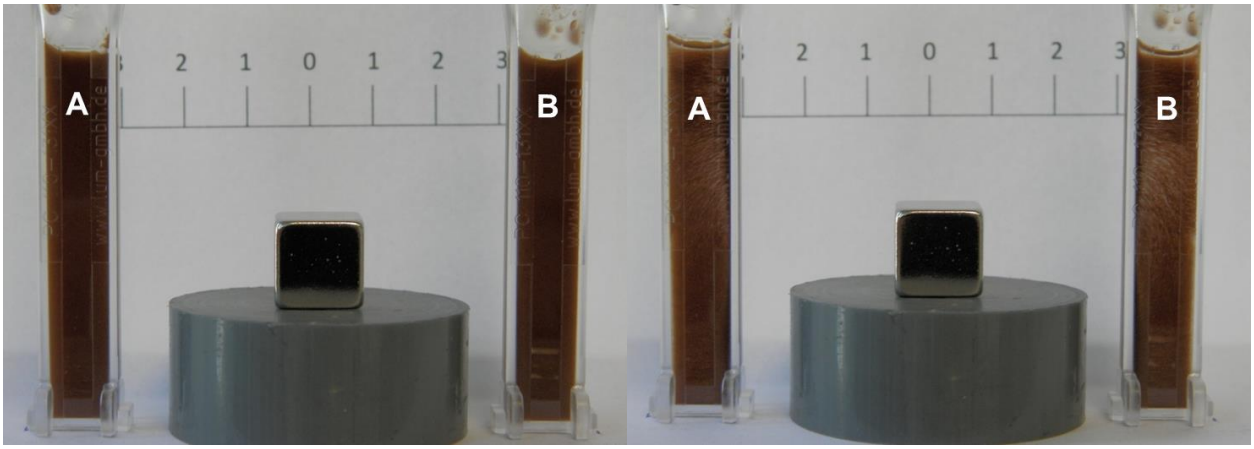

Figure S9 **Images of the magnetic separation of M-PVA particles, recorded 5 s (left) and 30 s (right) after placement next to a small permanent magnet block. The temperature of the suspensions was previously set to 20°C (A) and 35°C (B). The particle concentration was 4 g/L in 0.1 M acetate buffer, pH 4.**

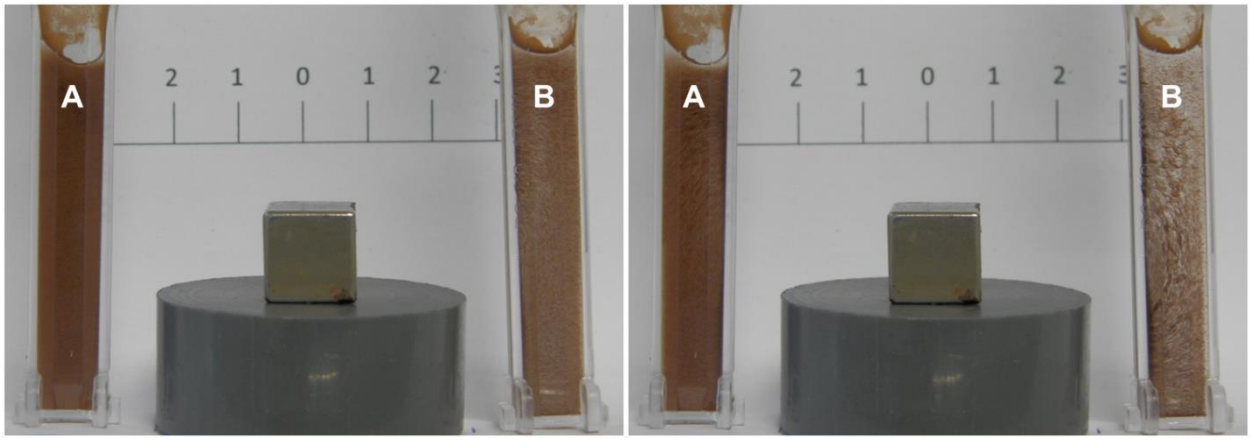

Figure S10 **Images of the magnetic separation of M-PNIPAM 17 kDa particles, recorded 5 s (left) and 30 s (right) after placement next to a small permanent magnet block. The temperature of the suspensions was previously set to 20°C (A) and 35°C (B). The particle concentration was 4 g/L in 0.1 M acetate buffer, pH 4.**

End-group Modified M-PNIPAM Particles [M-PNIPAM-CEA and M-PNIPAM-SPA]

The end groups of the M-PNIPAM 17 kDa particles were converted from alkane chains ($-C_{12}H_{25}$) into ion exchange groups. Figure S11 shows the reaction scheme for this end-group functionalization. Two acrylates, SPA and CEA, were used as cation exchange molecules. The analysis of protein loading capacity via UV/Vis adsorption measurements indicated very low protein adsorption. However, the lower detection limit of this technique is approx. 5 $mg \cdot g^{-1}$. Therefore, fluorescence microscopy was employed to show adsorption of GFP. M-PNIPAM particles showed no adsorption (Figure S12). M-PNIPAM-CEA (Figure S13) and M-PNIPAM-SPA (Figure S14) end-group modified particles showed presence of a small amount of GFP bound to the surface. This indicates that a single ionic group per PNIPAM chain was insufficient to achieve targeted adsorption capacity, therefore ionic block copolymers were integrated as described in Section 3.2.

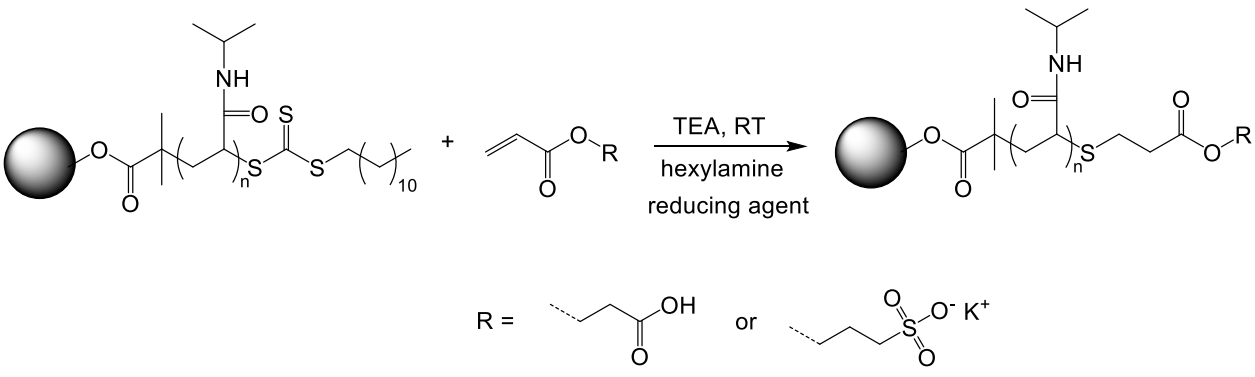

Figure S11 **Reaction scheme for end-group functionalization of M-PNIPAM particles with acrylates CEA and SPA. 'n' is the number of NIPAM monomers.**

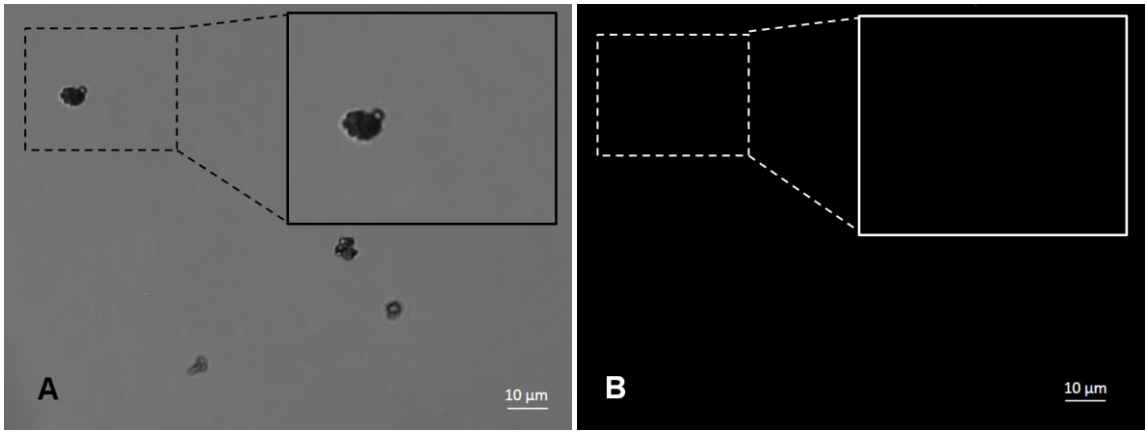

Figure S12 **Microscopic images of M-PNIPAM particles after 30 min adsorption with GFP at 18°C. The images were taken in bright field (A) and with GFP specific filter (B). Inset shows enlarged image.**

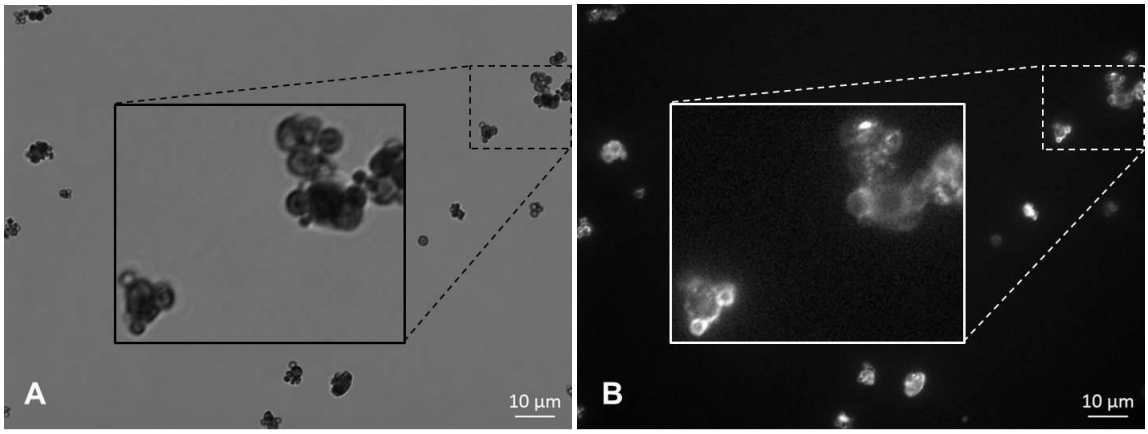

Figure S13 **Microscopic images of M-PNIPAM-CEA particles after 30 min adsorption with GFP at 18°C. The images were taken in bright field (A) and with GFP specific filter (B). Inset with enlarged image.**

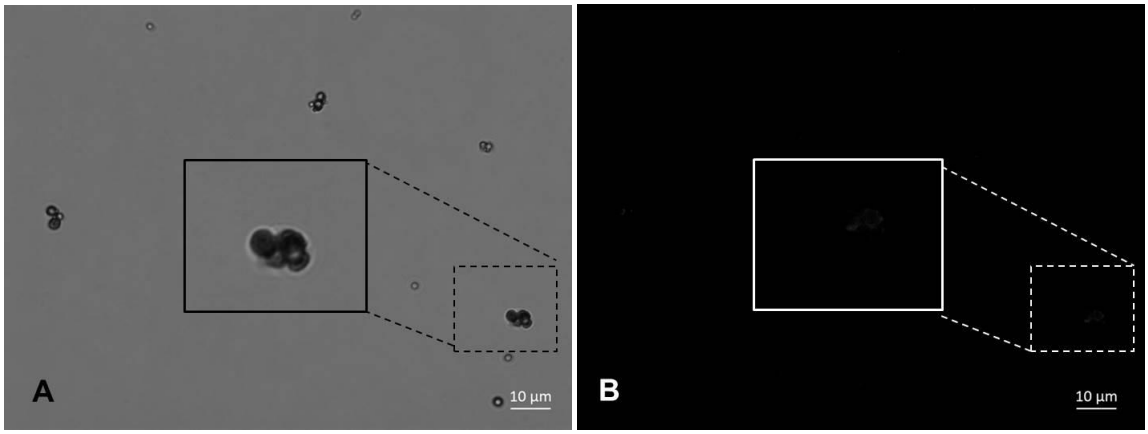

Figure S14 **Microscopic images of M-PNIPAM-SPA particles after 30 min adsorption with GFP at 18°C. The images were taken in bright field (A) and with GFP specific filter (B). Inset shows enlarged image.**

Two-block Copolymer PNIPAM Particles

Table S2 Bi-Langmuir Fitting Parameters for the Adsorption Isotherms of Lactoferrin to Magnetic Particles given in Figure 9 [a]

Particle type	Temperature (°C)	a (mg g^{-1})	b (g L^{-1})	c (mg g^{-1})	d (g L^{-1})
M-PVA	20	32	1.35	0	0
M-PVA	40	59	5.76	0	0
M-P(NIPAM-SPA)	20	110	1	70	< 0.005 [b]
M-P(NIPAM-SPA)	40	106	1	72	< 0.005 [b]

a) Calculated using eq 6.
b) The particles show a very high affinity, but the exact value cannot be determined from the experimental data.

Three-block Copolymer PNIPAM Particles

The reaction scheme for the three-block synthesis procedure is shown in Figure 11. Binding and elution of lysozyme, BSA, and lactoferrin was studied for the three-block copolymer particles (see Table S3). For all three proteins, the protein loading is nearly constant at both temperatures. A slight decrease of protein loading is visible at 40°C which may be attributed to collapse of the PNIPAM chains and increased steric hindrance. The desorption values are fairly high ranging from 86-107%. This indicates that binding is still mainly caused by ionic interactions between particle surface and proteins and not by hydrophobic interaction with collapsed PNIPAM. The lowest desorption values are achieved for lysozyme (20°C and 40°C) and BSA (40°C), because these proteins are more hydrophobic in nature. Compared to the two-block copolymer particles, the three-block particles have somewhat lower binding capacities. While the third, PNIPAM block shields the ionic copolymer blocks, the proteins can still access the binding sites, because the polymer chains are sufficiently far apart.

Table S3 Protein Loading Capacity of M-P(NIPAM-SPA-NIPAM) Particles [a]

Protein	pH	Temperature (°C)	Protein loading [b] (mg·g^{-1} particle)	Desorption [c] (%)
Lysozyme	7	20	52 ± 2	86 ± 6
		40	50 ± 3	90 ± 7
BSA	4	20	37 ± 2	103 ± 10
		40	34 ± 2	88 ± 7
Lactoferrin	7	20	112 ± 2	107 ± 1
		40	107 ± 7	107 ± 6

a) A protein concentration of 2 g/L and a particle concentration of 5 mg/mL were used.
b) Calculated using eq 4.
c) Relative desorption values were obtained by elution with 1 M NaCl.

The adsorption isotherm for M-P(NIPAM-SPA-NIPAM) particles for lysozyme is shown in Figure S15. Here, it is evident that only a small decrease in loading capacity takes place when the temperature is increased from 20°C to 40°C.

The temperature-switchable agglomeration was again observed for particles with adsorbed lysozyme and BSA. However, particles without bound protein did not show agglomeration. It is assumed that the ionic polymer block prevents agglomeration even though it is capped by PNIPAM block. This corresponds well with the only small decrease of protein loading capacity.

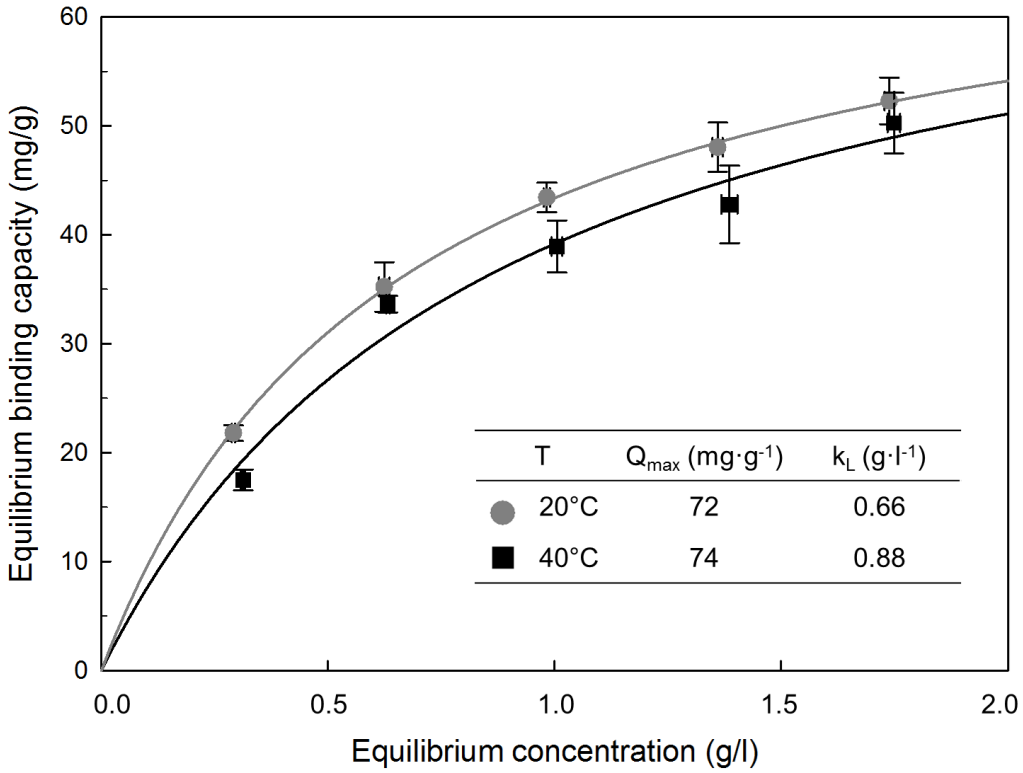

Figure S15 **Isotherm of M-P(NIPAM-SPA-NIPAM) particles for lysozyme adsorption at 20°C (grey circles) and 40°C (black diamonds), with the respective Langmuir isotherm fit. The Langmuir parameters are given in the table inset.**

Reference

1. **Allouni, Z. E.; Cimpan, M. R.; Høl, P. J.; Skodvin, T.; Gjerdet, N. R., Agglomeration and Sedimentation of TiO2 Nanoparticles in Cell Culture Medium. *Colloids Surf., B.* 2009, *68* (1), 83-87.**