Supplementary Information: Synthesis and Structure of Deuterated Ultra-Low Cross-Linked poly(*N*-isopropylacrylamide) Microgels

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1. EXPERIMENTAL

1.1. Labels of the Samples

The polymerization products named pNIPAM, pNIPAM-SDS, pD3-NIPAM, pD3-NIPAM-SDS, pD7-NIPAM and pD7-NIPAM-SDS correspond to the samples MB-smallscale-ULCs-3-x. x corresponds to the numbers as depicted in figure S.1. Figure S.2 shows the results of the polymerzations labeled with MB-smallscale-ULCs-4.

Preliminary polymerization series are MB-smallscale-ULCs-1 and MB-smallscale-ULCs-2, which were meant to check the reproducibility of the syntheses.

1.2. Transmission Electron Microscopy

Transmission electron microscopy (TEM) was performed on a Carl Zeiss Libra 120 microscope operating at a voltage of 120 kV with a bottom mounted CCD camera. Zero-loss energy-filtered transmission electron microscopy was used. TEM and Cryo-TEM samples were transferred into the microscope and let to equilibrate before starting the measurements. The contrast of the micrographs was adjusted if necessary.

For conventional (dry state) TEM $4 \mu L$ of the microgel dispersion was transferred onto a carbon coated TEM grid and left to dry.

For cryogenic-TEM (Cryo-TEM), samples were prepared by vitrification of the microgel dispersions in liquid ethane. $4 \,\mu\text{L}$ of the dispersion was transferred in a vitrobot system onto a Lacey carbon coated TEM grid which has been hydrophilized in air plasma.

2. RESULTS AND DISCUSSION

2.1. Turbidity of the Reaction Solutions

Figure S.1 illustrates the turbidity of all samples (polymerization with and without SDS) above the lower critical solution temperature (LCST) of the monomers (immediately after the synthesis; *top picture*) and below the LCST (after cooling to room temperature; *bottom picture*). The turbidities of pNIPAM (vial 1) and pNIPAM-SDS (vial 2) as well as pD3-NIPAM (vial 3) and pD3-NIPAM-SDS (vial 4) sustain below the LCST of the polymer and thus, of the VPTT of the generated microgels. However, the turbidity of pD7-NIPAM (vial 5) and pD7-NIPAM-SDS (vial 6) above the LCST and its disappearance at low temperatures lead to the assumption that polymer chains were synthesized which collapse above the LCST.



FIG. S.1. *Top:* Reaction vials after the polymerizations above the LCST of pNIPAM(1), pNIPAM-SDS(2), pD3-NIPAM(3), pD3-NIPAM-SDS(4), pD7-NIPAM(5) and pD7-NIPAM-SDS(6) and *Bottom:* Reaction vials after cooling to room temperature.

2.2. Swelling/Deswelling Cycles

The polymerizations of all three monomers have been repeated to investigate the unpurified reaction products. After reaction, the polymerization solutions were kept in an oven above the LCST without cooling down. This ensures the investigation of the initial particle sizes in the native collapsed state.

The samples were probed without cooling down (cycle 0) and after several swelling/deswelling cycles (cycle 1 to 3) at 50 °C by dynamic light scattering. A cycle consists of a cooling (below LCST) and heating (above LCST) process. Figure S.2 summarizes the results of three swelling/deswelling cycles of the unpurified polymerization products of NI-PAM (black circles), D3-NIPAM (red diamonds) and D7-NIPAM (cyan triangles). It can clearly be observed that all particle sizes decrease after the first swelling/deswelling cycle. Further swelling/deswelling cycles do not considerably vary the collapsed radii. In the case of pNIPAM and pD3-NIPAM, the size differences (15 and 10 nm, respectively) resulting from the first swelling are rather small. This decrease of the collapsed radii is related to polymer chains that are located in the microgels at cycle 0, but not chemically bound to the network of the microgels. Upon swelling, these unbound chains diffuse out of the networks and thus, a repeated deswelling results in smaller microgels. The polymerization product of D7-NIPAM, however, undergoes a considerably stronger change upon first swelling. The size of the collapsed globules resulting from precipitation polymerization is about 100 nm. A swelling/deswelling cycle results in a drastic decrease of the hydrodynamic radius to about 28 nm. This strongly indicates that the collapsed globules formed during the polymerization consist of precipitated polymer chains, which are not cross-linked

lapsed globules formed during the polymerization consist of precipitated polymer chains, which are not cross-linked enough to form stable microgel networks. The globules disintegrate into fragments, which then again collapse into smaller globules.



FIG. S.2. Hydrodynamic radius at 50 °C probed by DLS as a function of swelling/deswelling cycles of the unpurified polymerization products of pNIPAM (black circles), pD3-NIPAM (red diamonds) and pD7-NIPAM (cyan triangles).

2.3. Zimm Plot Analysis

The molecular weights have been determined by Zimm plot analysis [1]. The pNIPAM-SDS and pD3-NIPAM-SDS microgels were chosen for molecular weight investigations as they possess equal deswollen sizes, which makes a comparison more reasonable. The Zimm plots (Figures S.3 to S.6) were obtained according to Zimm by probing different concentrations at different scattering angles at 20 °C in SLS. The molecular weights, M_w are obtained from the extrapolations to zero concentration and zero scattering vector.

Both pD7-NIPAM and pD7-NIPAM-SDS reveal rather low molecular weights of 565 ± 4 kg/mol and 486 ± 2 kg/mol, respectively. The pNIPAM-SDS and pD3-NIPAM-SDS microgels are with $M_w = (130 \pm 1) \cdot 10^3$ kg/mol and $M_w = (128 \pm 1) \cdot 10^3$ kg/mol much larger than the polymer fragments of pD7-NIPAM.



FIG. S.3. Zimm plot of the polymerization product of pD7-NIPAM probed by SLS.



FIG. S.4. Zimm plot of the polymerization product of pD7-NIPAM-SDS probed by SLS.



FIG. S.5. Zimm plot of the pNIPAM-SDS ULC microgels probed by SLS.



FIG. S.6. Zimm plot of the pD3-NIPAM-SDS ULC microgels probed by SLS.

2.4. Form Factor of pD3-NIPAM ULC Microgels at 20 $^\circ\mathrm{C}$

Figure S.7 illustrates the form factor of the D3-NIPAM microgels probed over the complete available q-range. The solid line represents the fit by the fuzzy sphere model by Stieger *et al.* [2]. The model deviates from the experimental data in the high q-region. See main article for discussion.



FIG. S.7. Form factor of pD3-NIPAM ULC microgels with fuzzy sphere fit probed at 20 $^\circ\mathrm{C}$ by SLS.

2.5. Density Profiles Obtained from Form Factor Analysis

Figure S.8 illustrates the radial density profiles of the pNIPAM (A) and pD3-NIPAM (B) ULC microgels at 20 °C (solid lines) and 40 °C (dashed lines) from form factor analysis. The form factors were fitted with the fuzzy sphere model by Stieger *et al.* [2]. The overall radii are in agreement with DLS analysis. At 40 °C, the typical box-like profiles with homogeneous polymer distribution are obtained for the fully deswollen ULC microgels. Below the VPTT, at 20 °C, the microgels are swollen and show an inhomogeneous polymer distribution with decaying density toward the peripheries.

As already discussed in the main article, the polymerization of D3-NIPAM results in a larger and more densely crosslinked network. This is also observable from the radial density profile: The relative volume fraction is greater for the pD3-NIPAM than for the pNIPAM microgels when compared to their respective collapsed state.



FIG. S.8. Radial density profiles of (A) pNIPAM and (B) pD3-NIPAM ULC microgels at 20 $^{\circ}$ C (dashed lines) and 40 $^{\circ}$ C (continuous lines) obtained from the SLS or SAXS form factor analysis with the fuzzy sphere model [2].

2.6. Transmission Electron Microscopy

Figure S.9 shows dry state and Cryo-TEM micrographs of pNIPAM and pD3-NIPAM. TEM images the dried samples on the substrate, whereas Cryo-TEM shows the hydrated, quick-frozen state. TEM was used to visualized the pD7-NIPAM polymerization product.

As already concluded from light scattering, here, we visualize that pNIPAM (figure S.9A and D) and pD3-NIPAM (figure S.9B and E) form microgels. From TEM (figure S.9; upper row), no conclusion on overall size or lateral deformation can be made as the microgels are randomly adsorbed, distributed and dried on the substrate. The pNIPAM and pD3-NIPAM microgels show distinct morphology which may result from the different cross-linking densities in the networks (see main article) and drying effects. In the hydrated state (figure S.9; bottom row), both pNIPAM and pD3-NIPAM ULC microgels unravel a spherical shape. However, no distinct boundaries can be determined due to the low contrast of these microgels. Thus, no information on the density or internal structure can be deduced. The pD7-NIPAM (figure S.9C) sample shows dried very loosely cross-linked or single polymer chains, which do not

form distinct microgels. These pD7-NIPAM chains do not have enough contrast to be visualized in the hydrated state as their density is considerably lower than of the properly cross-linked networks of microgels.



FIG. S.9. Top row: TEM micrograph of dried (A) pNIPAM, (B) pD3-NIPAM and (C) pD7-NIPAM. Bottom row: Cryo-TEM micrograph of hydrated (D) pNIPAM and (E) pD3-NIPAM.

^[1] B. H. Zimm, The Journal of Chemical Physics, 1948, 16, 1093–1099.

^[2] M. Stieger, W. Richtering, J. S. Pedersen and P. Lindner, J. Chem. Phys., 2004, 120, 6197–6206.