Supporting Information for

Aggregation Behavior of Doubly Thermoresponsive Polysulfobetaine-*b*-poly(N-isopropylacrylamide) Diblock Copolymers

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Dynamic Light Scattering (DLS).

DLS measurements were carried out using a Zetasizer Nano ZS (Malvern) laser particle size analyzer equipped with a 633 nm laser at an angle of 173° (backscattering detection). The maximum particle radius which can be resolved is 1000 nm. The diblock copolymer $PSPP_{430}$-*b*-$PNIPAM_{200}$ was measured in salt-free D_2O. 0.5 mL of sample was mounted in a plastic microcuvette (Malvern). A polymer solution in D_2O was prepared at 10 g L^{-1} at room temperature and was equilibrated for about 2 days in a thermoshaker at 25 °C, i.e. in regime II. Measurements were performed during heating from 20 °C to 60 °C in steps of 5 °C and were repeated 3 times at each temperature. The equilibration time was chosen at 15 min, and the measurement time at 3 min. For data evaluation, the DTS (Nano) software (Malvern) was used, which provides the hydrodynamic radius, R_h, and the volume fraction of each species (1 or 2) for each of the 3 measurements. The results were averaged.

Characterization of the unimers and aggregates using DLS

The temperature-dependent self-assembly behavior of the diblock copolymer in D_2O was explored in temperature-resolved DLS measurements. DLS in backscattering geometry was applied in order to minimize the effects of multiple light scattering in the turbid solutions. Moreover, the concentration of $PSPP_{430}$-*b*-$PNIPAM_{200}$ in salt-free D_2O was set to 10 g L^{-1} to minimize multiple scattering. Figure S1 presents the DLS data obtained in a heating run from 10 to 60 ºC. The measurement step size of 5 ºC is large enough to prevent the distortion of the results by hysteresis of the cloud points, which is less than 2 ºC.[1,2]

The autocorrelation curves show very slow decays in regime I (which could not be analyzed), fast decays in regime II and intermediate decays in regime III (Figure S1a). Thus, temperature markedly affects the aggregation behavior. Analysis of the curves reveals the presence of 2 types of aggregates in regime II, but only 1 type in regime III. Their hydrodynamic radii and volume fractions are shown in Figure S1b and c.

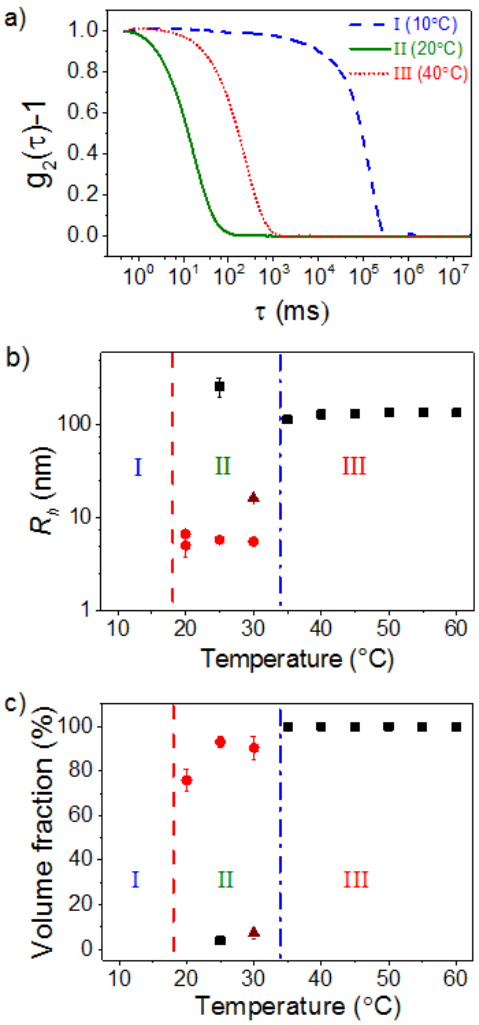

Figure S1. Results from DLS on a salt-free solution of $PSPP_{430}$-*b*-$PNIPAM_{200}$ in D_2O at 10 g L^{-1}, obtained in a heating run. (a) Normalized intensity autocorrelation functions. Temperatures are indicated in the graph. (b) Hydrodynamic radii and (c) volume fractions of the large aggregates (black squares), micelles (wine triangles) and unimers (red spheres) in dependence on

temperature. The dashed and dash-dotted lines in (b, c) indicate the CP_{UCST} (red) and CP_{LCST} (blue) values from turbidimetry, respectively. The regimes I, II and III are indicated.

At temperatures below 19 ºC, the size of the large aggregates in regime I could not be quantified because they are larger than the resolution. Thus, we assume the formation of very large aggregates with an average hydrodynamic radius R_h > 1000 nm. Between 20 and 35 °C, i.e. in regime II, R_h of the majority component (volume fraction 90-96%) is 5.5 ± 0.2 nm, which presumably corresponds to unimers; the minority component (remaining 4-10 %) are particles with R_h = 260 ± 60 nm at 25 ºC and R_h = 16.2 ± 0.2 nm at 30 ºC. The latter may tentatively be ascribed to micelles. Above 30 °C in regime III, only one component with R_h = 133 ± 8 nm is observed. The aggregates observed in regime III are considerably smaller than those in regime I.

Thus, the results from temperature-resolved DLS measurements in backscattering geometry are in good agreement with those from turbidity measurements: In regime I, the solution is completely turbid with very large aggregates, R_h > 1000 nm; in regime II, the solution is clear containing mostly unimers having R_h = 5.5 ± 0.2 nm; and in regime III, the solution is cloudy (partially turbid) with aggregates having R_h values of ca. 133 nm.

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[2] Hildebrand, V.; Laschewsky, A.; Zehm, D. *J. Biomater. Sci., Polym. Ed.* **2014**, *25*, 1602-1618.