COMMUNICATION

Electronic Supplementary Material (ESI)

Materials and sample preparation

Dextran 10 kDa was purchased from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Polyethylene glycol (PEG) 4.6 kDa was obtained from Sigma-Aldrich (Steinheim, Germany). Dextran 10 kDa labeled with rhodamine B was purchased from Invitrogen (Thermo Fischer Scientific). As described by Baltierra-Jasso *et al.*,[1] the DNA oligonucleotides were synthesized and fluorescently labelled by IBA life solutions GmbH (Goettingen, Germany). The sequences of the two DNA strands used to prepare the DNA hairpin are: H2 strand: 5'-TGG CGA CGG CAG CGA GGC TTA GCG GCA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AGC CGC X-3' (here, X is T-Atto 550); A2 strand: 5'-GCC TCG CXG CCG TCG CCA-3' (here, X is T-Atto 647N). The oligonucleotide strands H2 and A2 are mixed in a 1:1 ratio in a buffer containing 20 mM Tris-HCl, 50 mM NaCl, at pH 7.5. For annealing, the nucleic acid mixture is heated at 95 °C for 5 min and then gradually cooled to room temperature at a rate of -0.5 °C/min using a thermocycler. The buffer solution used in the measurements contains 20 mM TRIS (pH 7.4) and 15 mM NaCl and was filtered by a 0.45 µm sterile Whatman Puradisc 30 syringe filter.

Characterization of the ATPS

The ATPS system used in our study consists of PEG 4.6 kDa (polyethylene glycol) and dextran 10 kDa, a glucose polymer, and has been characterized in detail und used for various biochemical applications by Keating *et al.*[2] The phase diagram of the ATPS was determined by cloudpoint titration (Figure SI 1). To this end, solutions with different concentrations of dextran in buffer were titrated against a 20 wt% PEG buffer solution until turbidity was clearly visible. After determination of the binodal for PEG (4.6 kDa) and dextran (10 kDa) in our buffer used, the composition of the ATPS chosen for the smFRET measurements was set to 11 wt% PEG and 11 wt% dextran.

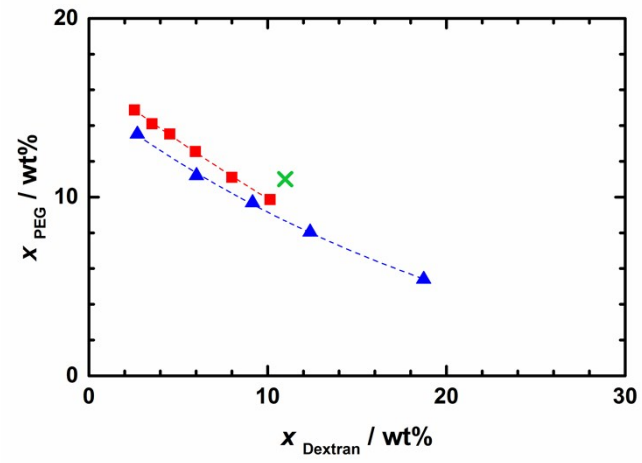

Figure SI 1: Phase diagram of aqueous two-phase systems of PEG 4.6 kDa and dextran 10 kDa. Blue triangles: in H_2O at 25 °C (data replotted from reference 2); red squares: in buffer at 22 °C (this work). Green cross: composition of the ATPS used in this work. The dashed lines represent the respective binodal coexistence curves. Compositions above the binodal curve form two-phase systems.

Microscopy

Light microscopy images were recorded using a Nikon inverted microscope (ECLIPSE TE2000-U) equipped with a digital camera. Fluorescence images were recorded with a Nikon Eclipse TE-300 inverted microscope equipped with a CCD camera (Andor iXon Ultra, Acal BFi, Germany) and a mercury-vapor lamp (Hg 100 W, Nikon, Tokyo, Japan). ATPS samples were first thoroughly stirred and then sucked into a square-shaped flexible fused silica microcapillary with an outer diameter of 360 µm and an inner diameter of 50 µm. The equilibrium of the phase separation inside the capillary was reached after 15 min.

Single-molecule Förster Resonance Energy Transfer (smFRET)

The smFRET measurements were performed on a commercial time-resolved confocal fluorescence microscope (PicoQuant, MicroTime 200) using the pulsed interleaved excitation FRET (PIE-FRET) technique and time-correlated single photon counting (TCSPC) detection. Detailed information of our setup and analysis methods can be found in references 3-5. High pressure smFRET measurements were carried out under freely diffusing conditions in the same capillary. The optical window thickness of the capillary is 150 µm. A detailed description of the high-pressure cell including its optimization and optical characterization was published by us in a recent report.[4] The capillary is glued into a pressure plug which is connected to a translation table that allows movements in *x*-, *y*-, and *z*-direction as well as rotations with high precision. Thereby, we were able to adjust the confocal plane of the microscope to the inside of a droplet of the ATPS.

Additional figures

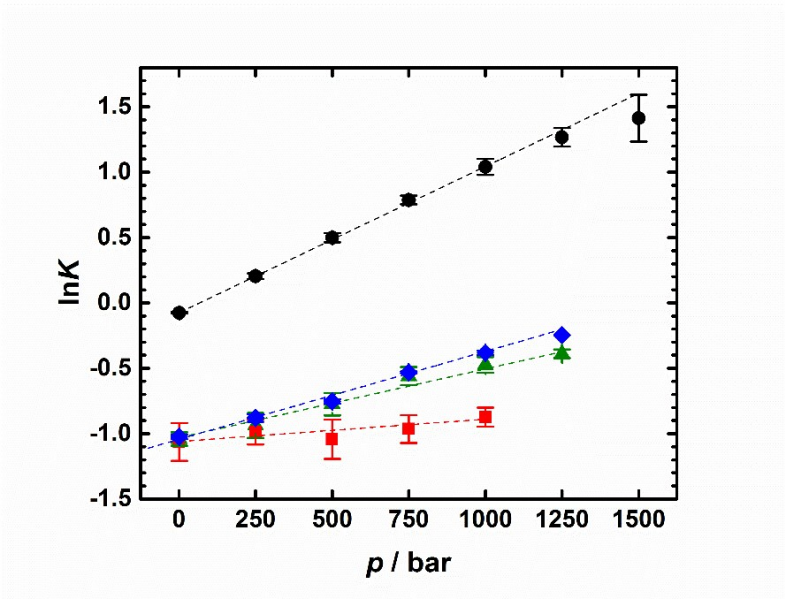

Figure SI 2: Pressure dependence of ln*K* of the unfolding equilibrium of the DNA hp. $K = f_{open}/f_{closed}$, with f_{open} and f_{closed} representing the fraction of the open and closed conformation, respectively. Black dots: neat buffer; red squares: ATPS; green triangles: 11 wt% dextran; blue rhomb: 11 wt% PEG. Data points represent mean ± sd of three independent measurements.

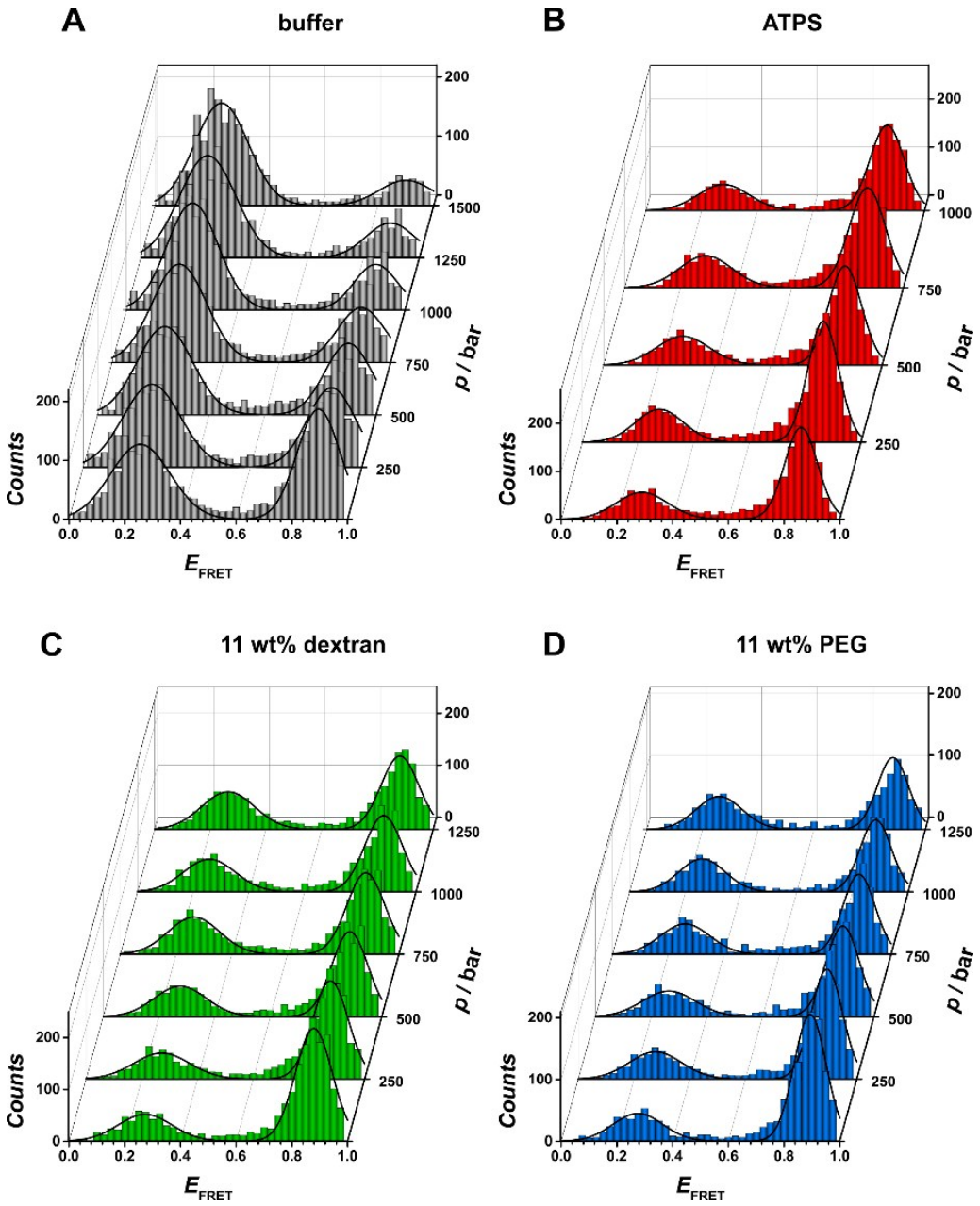

Figure SI 3: Pressure dependent smFRET histograms of the DNA hp in different media: (A) neat buffer, (B) ATPS consisting of 11 wt% PEG, 11 wt% dextran and buffer, (C) 11 wt dextran in buffer, (D) 11 wt PEG in buffer. The upper pressure limit varies due to experimental restrictions (the sealing of the pressure plug varies in quality).

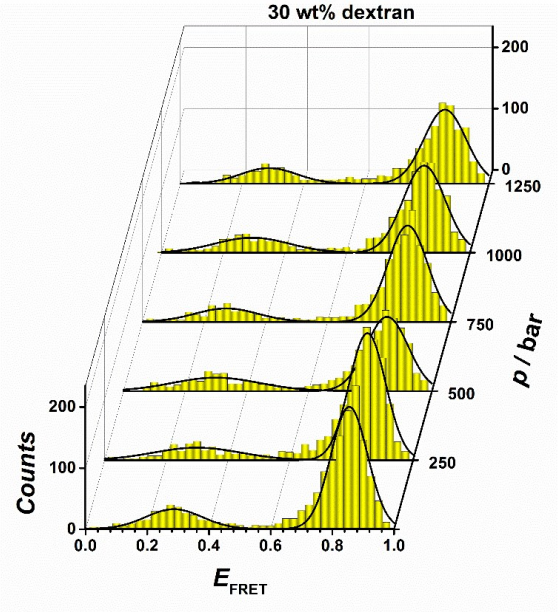

Figure SI 4: Pressure dependent smFRET histograms of the DNA hp in 30 wt dextran in buffer. Owing to the strong excluded volume effect of the macromolecular crowding agent dextran, the conformational equilibrium between the open and the closed state is not effected by the increasing pressure.

References

1 L. E. Baltierra-Jasso, M. J. Morten, L. Laflör, S. D. Quinn, and S. W. Magennis, *J. Am. Chem. Soc.*, 2015, **137**, 16020–16023.
2 M. S. Long, C. D. Jones, M. R. Helfrich, L. K. Mangeney-Slavin, and C. D. Keating, *PNAS*, 2005, **102**, 5920-5925.
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4 S. Patra, C. Anders, P. H. Schummel, and R. Winter, *Phys. Chem. Chem. Phys.*, 2018, **20**, 13159–13170.
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