Supplementary Information

A Photo-switchable Surfactant Possessing a Spiropyran-moiety in its Backbone – Unravelling the Structure of Micelles with Small-Angle Neutron Scattering (SANS) and Transmission Electron Microscopy (TEM)

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Experimental section

1 Instrumentation

1.1 Small Angle Neutron Scattering

For measuring the SANS spectra, we used commercial quartz cuvettes with a circular shape (Helma Analytics) exhibiting a cuvette diameter of 2 cm and thickness of 2mm. The neutral sample was measured at a concentration of 10 mM in D_2O (TCI, 99.8 % D). The acidic sample was measured at a concentration of 20 mM with a pH value of ~ 4 that was adjusted by the addition of trifluoroacetic acid (Sigma Aldrich, 99 %). Small-angle neutron scattering experiments were performed on the SANS-I instrument at the Swiss Spallation Neutron Source SINQ, Paul Scherrer Institute, Switzerland. Measurements were performed at a wavelength λ of 5 Å and at sample to detector distances of 1.6 and 8 m covering a range of the magnitude of the scattering vector $q = \frac{4\pi}{\lambda} sin\frac{\theta}{2}$ with scattering angle θ from 0.1 to 4.9 nm^{-1}. Raw data were reduced using the software package BERSans[2] and the incoherent background was subtracted as a constant. The curves were analysed using the software SASfit.[3] The scattering length density of the surfactant of $1.19 \cdot 10^{-4}$ nm^{-2} was calculated using the molecular volume of 638 $Å^3$ determined by density measurements using an Anton Paar DMA 4500 M density meter. For illuminating the sample inside the sample holder, a blue light LED source with a wavelength of 455 nm [Thorlabs GmbH (455 nm (M455L4)] was collimated perpendicularly to the sample, where the entire sample was illuminated by the light source. The light intensity is directly measured at the sample position prior to each measurement with a commercial S170C power meter (Thorlabs) and was set to a value of 1 mW cm^{-2}.

1.2 SANS-Data Processing

The volume fraction ϕ obtained from invariant analysis $Q = \int q^2\, I(q)dq = 2\pi(\Delta SLD)^2\phi(1-\phi)$ where ΔSLD refers to the difference in scattering length density between solvent and micelles, is in good agreement with the expected value for a 10 mM sample. Given the q^{-1} slope of the curves we decided to fit them using the form factor of cylindrical rods with polydispersity in the radius:

$$I(\mathrm{q}) = {}^{1}\mathrm{N}\Delta SLD^2 \int f\,(R)V(R,L)^2 P(q,R,L)dR, \qquad \text{S1}$$

where ${}^{1}\mathrm{N} = \phi/\int f(R)V\,(R,L)dR$ is the particle number density, V(R,L) is the volume of a cylinder with radius R and length L. For the distribution of radii f(R) we used a log-normal distribution. And the form factor of a cylindrical rod reads

$$P(q,R,L) = \int \left(2\frac{J_1\left(qR\sqrt{1-x^2}\right)}{qR\sqrt{1-x^2}}\frac{sin(qLx/2)}{qLx/2}\right)^2 dx, \qquad \text{S2}$$

with the Bessel function of the first kind of order 1 J_1. For fitting, the mean radius was fixed at 1.8 nm based on the position of the smeared-out form factor minimum slightly below 2 nm^{-1} and its relative standard deviation was set to 0.2 which matches the experimentally observed level of smearing of the form factor oscillations and is a mixture of instrumental smearing, polydispersity and a slightly smeared out contrast profile. The volume fractions (0.031 and 0.043) were taken from the invariant, leaving the length as the only fitting parameter and values of 20, 21 and (20 ± 2) nm were obtained for the neutral samples in dark, under blue illumination and the acidic sample under blue illumination respectively, which results in Aggregation numbers of 320, 340 and 320±30.

2 Surfactant measurements

2.1 Synthesis

The surfactant was prepared as described in our previous work.[1]

2.2 Critical micellar determination

The data shown in Figure 2 has been reproduced from our previous work.[1]

Briefly, we measured the surface tension using the pendant-drop method. We recorded the contact angle with the hardware (dataphysics OCA-15, dataphysics, Germany), and we analyzed the data with the dataphysics SCA202 software. The measurements were performed at a pH of approx. 10, buffering with a NaOAc/NaOH (c ~ 10^{-4} M) aqueous solution, in MilliQ water, and at a pH of 4, where the surfactant was protonated by adding one equivalent of TFA and dissolved in a TFA solution (c = 10^{-4} M). These solutions were measured either in their thermodynamically relaxed state (water bath at 60°C for 15 minutes, afterward cooling to room temperature) and under photo-illumination using the aforementioned LED lamp with 455 nm excitation wavelength for 10-20 minutes prior to the measurements and during the measurement, whereby the pending drop was irradiated for further 2 min. Each measurement was conducted as a triplicate and is represented as mean ± standard deviation.

2.3 Isothermal titration calorimetry (ITC)

Isothermal titration calorimetry (ITC) measurements were conducted at 25 °C using a MicroCal PEAQ-ITC calorimeter from Malvern Panalytical. For SP demicellization a 5 mM SP aqueous solution (MilliQ) with an injection volume of 2 µL was titrated into MilliQ water with 300 s spacing and a reference power of 42 µJ s^{-1}. In the case of MCH^+ demicellization a 40 mM MCH^+ in a trifluoroacetic acid (TFA) solution with the first six injection volumina 0,5 µL, subsequently six times 1 µL followed by 34 injections à 2 µL was titrated into a TFA solution with 300 s spacing and a reference power of 4,2 µJ s^{-1}. Finally, for the titration of MC with TFA a 5 mM TFA solution with an injection volume of 0,5 µL was titrated into a

0.5 mM MC solution at initially pH 7 with 150 s spacing and a reference power of 42 µJ s^{-1}. The measurements were conducted as triplicates. The isotherms of the demicellization experiments were fitted with a generic sigmoidal function by a nonlinear least-squares fitting routine of OriginLab OriginPro 2021 software to obtain the heats of demicellization.

2.3 Cryo-TEM measurements

Samples were cryo-fixed under dim-light conditions in liquid ethane on Quantifoil holey carbon grids (type 2/2 (acidic sample), type 1.2/1.3 (neutral sample), Quantifoil Micro Tools GmbH, Großlöbichau, Germany) at a concentration of 10 mM using a Grid Plunger (Leica EM GP 2) with the following parameters: 75%-80% humidity, 10 °C, 3.5 µL sample volume, pre-blotting incubation 45 s and 10 s blotting time. Images were recorded at a Talos F200C G2 microscope (Thermo Scientific) with a magnification of 120,000x and a dose of 54 e^{-} $Å^{-2}$ on a Falcon III direct electron detector in 33 frames. The neutral sample was measured at a concentration of 10 mM. The acidic sample was measured at a concentration of 20 mM with a pH value of ~4 that was adjusted by the addition of trifluoroacetic acid. All samples were measured in solutions of MilliQ water.

2.4 ^{1}H NMR measurements

^{1}H NMR spectroscopic measurements were recorded on a Bruker Avance III-HD 400 spectrometer operating at 400 MHz for ^{1}H NMR spectroscopy at room temperature. All chemical shifts (δ) are reported in parts per million regarding solvent residual signals of the deuterated solvent (D_2O) as the internal reference. The samples were measured at a concentration of 5 mM and 20 mM, respectively, whose pH values were adjusted to ~ 4 with trifluoroacetic acid.

3 Supplementary results

3.1 Additional TEM images

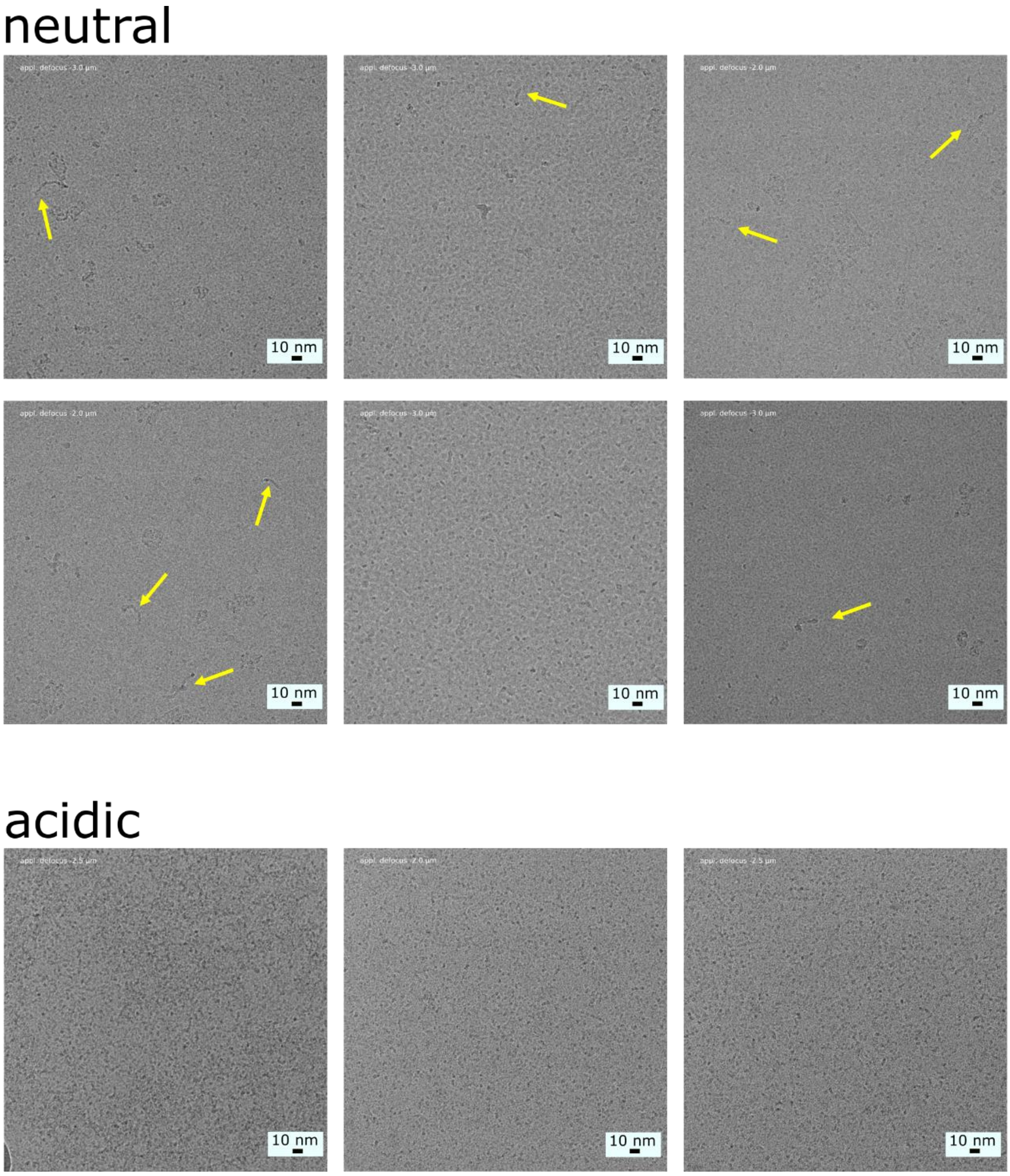

Fig. S1. Additional TEM micrographs of the surfactant in neutral (10 mM concentration) and under acidic (20 mM concentration) conditions.

3.2 ^{1}H NMR data interpretation

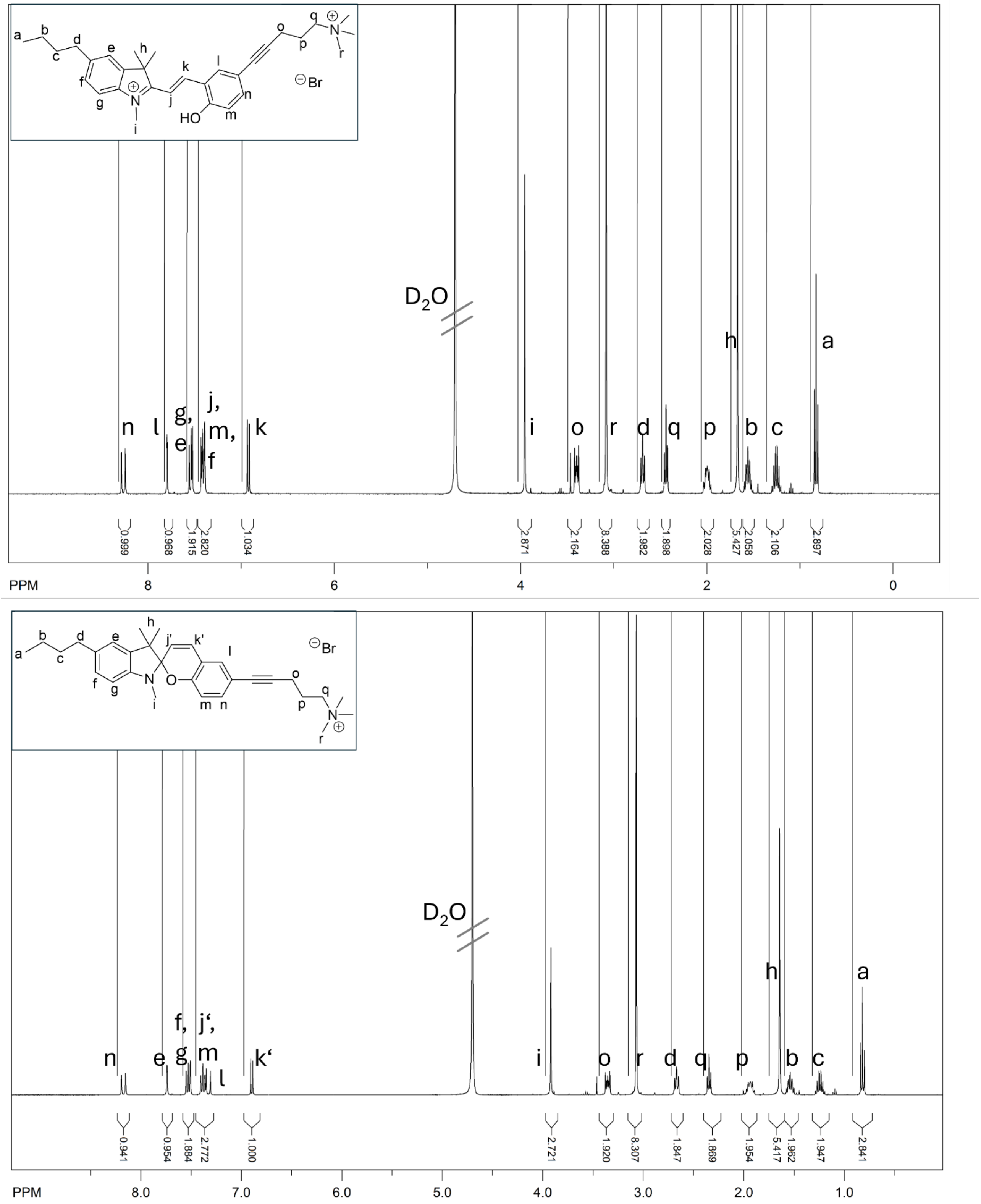

Figure S2. ^{1}H NMR spectrum of the molecule under acidic conditions (400 MHz, D_2O, pH ~ 4) with peak assignment. Top: Concentration ~ 5 mM, bottom: ~20 mM.

3.1 Isothermal titration calorimetry (ITC)

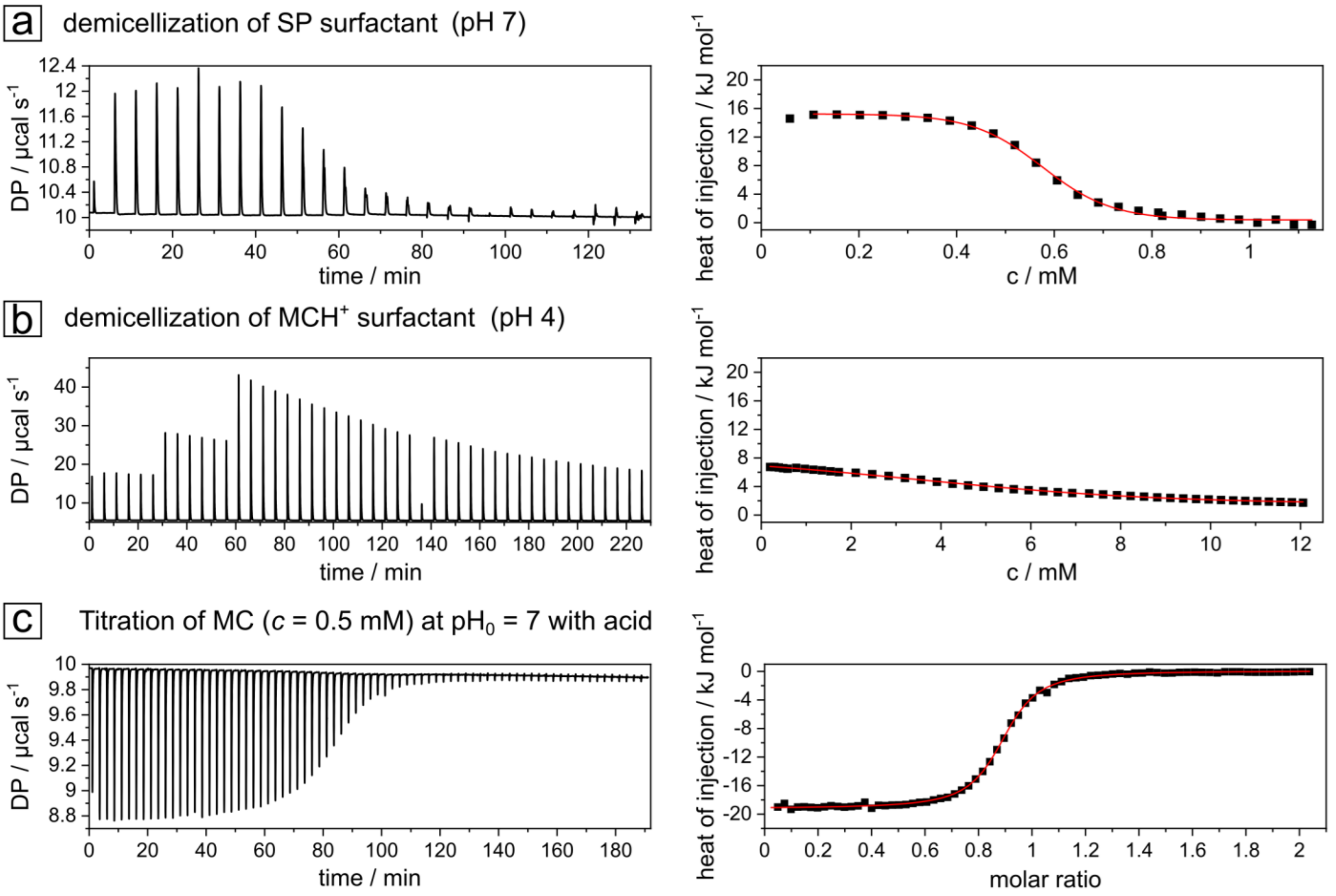

Figure S3. Isothermal titration calorimetry. Demicellization of the surfactant a) in MilliQ water and b) at an acidic pH value. c) Titration MC + H_3O^+ → MCH^+.

4 Supporting Information references

1 M. Reifarth, M. Bekir, A. M. Bapolisi, E. Titov, F. Nußhardt, J. Nowaczyk, D. Grigoriev, A. Sharma, P. Saalfrank, S. Santer, M. Hartlieb and A. Böker, *Angew. Chem. Int. Ed.*, 2022, **61**, e202114687.

2 U. Keiderling, *Appl. Phys. A*, 2002, **74**, s1455–s1457.

3 I. Bressler, J. Kohlbrecher and A. F. Thunemann, *J. Appl. Crystallogr.*, 2015, **48**, 1587–1598.