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Supporting information for article:

Concentrated protein solutions investigated using acoustic levitation and small-angle X-ray scattering

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S1. Data collection

Table S1 Sample overview of all samples presented in Figure 7, giving the buffer, the initial concentration and volume and the acquisition time and delay.

Buffer	Initial rHSA Concentration (mg/mL)	Acquisition time (s)	Delay between exposure (s)	Initial volume (µL)
145 mM NaCl, 8 mM octanoate, 0.05 mg/mL polysorbate 80	5	10	50	1.3
	20	10	50	2.4
	20	30	30	2.0
	50	10	50	2.4
	100	30	10	2.9
	100	10	50	2.7
25 mM NaH_2PO_4, 215 mM NaCl, pH 6.5	0	30	30	2.5
	0	10	50	2.4
	5	30	30	1.2
	5	30	30	1.4
	20	30	30	2.5
	20	10	50	2.3
	50	10	50	1.3
	100	10	50	3.0

Table S2 Beamline and instrument specifications

Levitation

Instrument	I911-SAXS, MAXIV, Lund, Sweden
Detector	Pilatus 1M
Wavelength (Å)	0.91
q range ($Å^{-1}$)	0.0098-0.52
Sample – detector distance (m)	1.9285
Beam size at sample	300 × 300 μm^2

Flow cell

Instrument	I911-SAXS, MAXIV, Lund, Sweden
Detector	Pilatus 1M
Wavelength (Å)	0.91
q range ($Å^{-1}$)	0.00825-0.5
Sample – detector distance (m)	1.9725

S2. Determination of droplet volume

SAXS data acquisition and droplet monitoring was synchronized manually and the photos of the droplet size from the USB-microscope camera was analyzed in MATLAB to determine the protein concentration. The two horizontal dimensions *a* and *b* were assumed to be equal, as illustrated in Figure S1. The decrease in droplet dimensions were used to determine the *in-situ* protein concentration in the droplet.

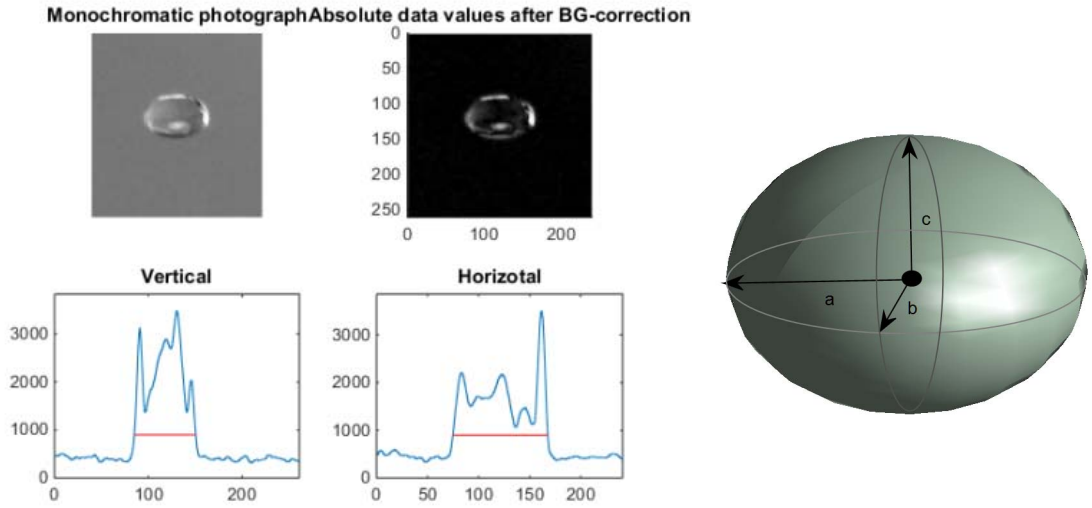

Figure S1 Top: Photos of droplet from the USB-microscope camera. Left bottom: Determination of vertical and horizontal droplet dimensions. Right: Ellipsoid dimensions, describing the droplet size.

S3. Scaling using Eq. 5

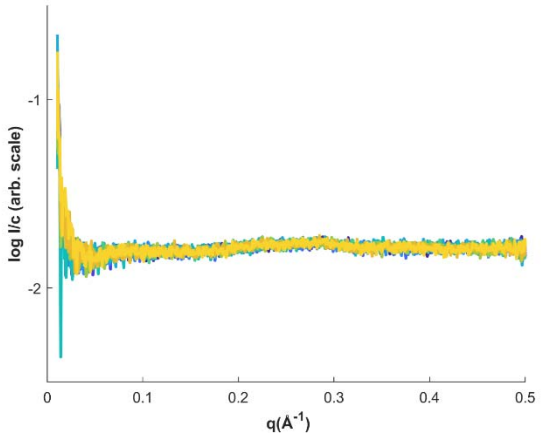

Figure S2 A complete set of levitation measurements from a water droplet during approximately 10 minutes. After scaling by Eq.5 all water scattering data are seen to overlap.

S4. Droplet alignment in beam

The droplet was initially aligned both vertically and horizontally in the beam. This was done for each experiment. During the experiment the droplet did shrink in an asymmetrically way (see Figure S3), which eventually resulted in bad alignment and surface reflection. The change in droplet position during an experiment is illustrated in Figure S3. Surface reflections due to misalignment were easy to detect and these data were excluded from analysis.

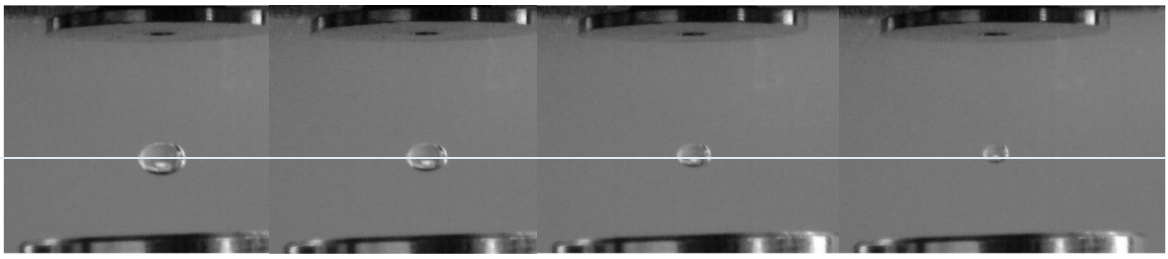

Figure S3 Droplet position in the beam over time. Beam high position is indicated with a white line.

S5. Scattering profiles

SAXS intensity curves, I(q), for all samples described in Table 2 are shown in Figure S4.

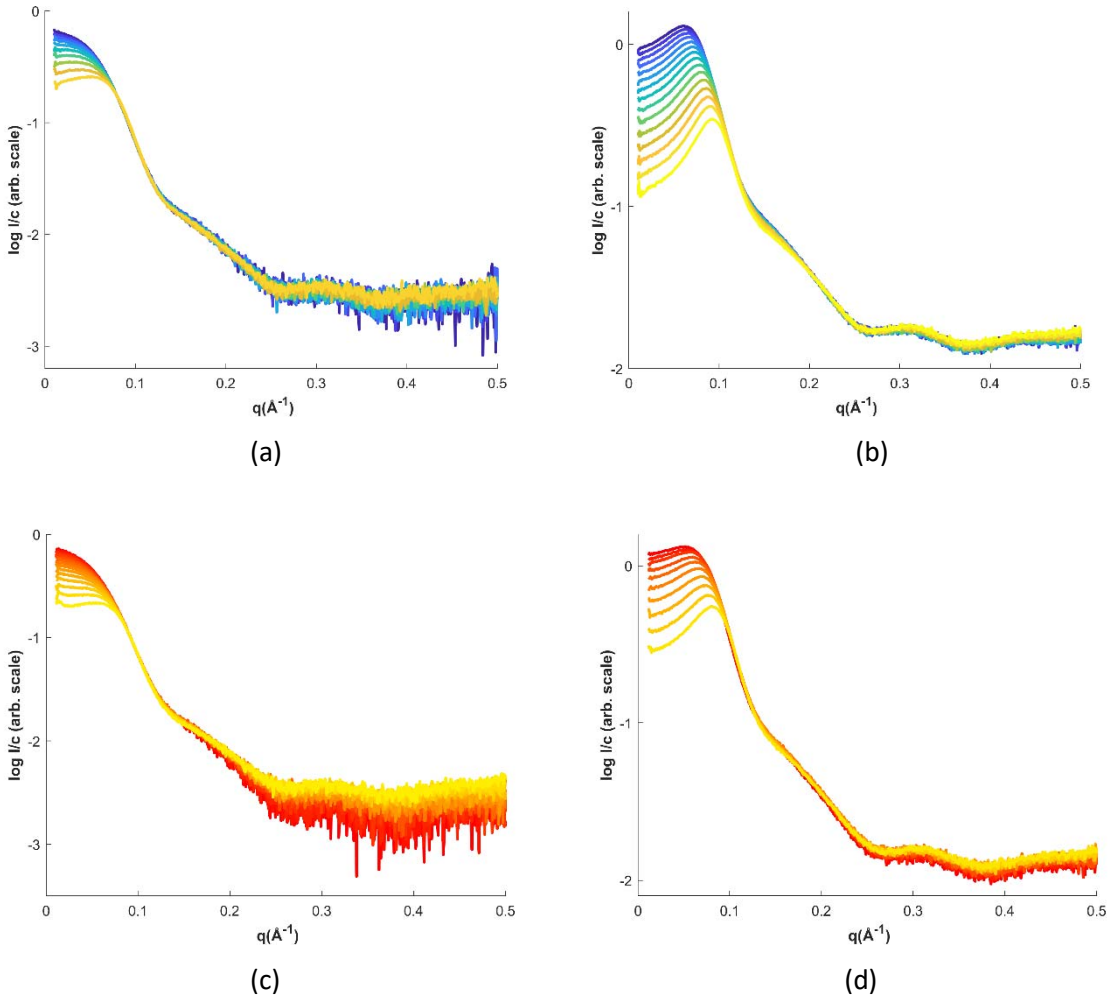

Figure S4 Scattering curves used to derive structure factors. a) Initial rHSA concentration 20 mg/mL, buffer: 145 mM NaCl, 8 mM octanoate, 0.05 mg/mL Tween 80; b) Initial rHSA concentration 100 mg/mL, buffer: 145 mM NaCl, 8 mM octanoate, 0.05 mg/mL Tween 80; c) Initial rHSA concentration 20 mg/mL, buffer: 25 mM NaH_2PO_4, 215 mM NaCl; d) Initial rHSA concentration 100 mg/mL, buffer: 25 mM NaH_2PO_4, 215 mM NaCl.