Pressure-Sensitive and Osmolyte-Modulated Liquid-Liquid Phase Separation of Eye-Lens Gamma-Crystallins

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Supplementary Information

Pressure dependent measurements of the intrinsic tryptophan fluorescence of γ-D-crystallin

To investigate whether γ-D-crystallin unfolds by pressure in the pressure range studied here, intrinsic tryptophan fluorescence spectra of 0.1 mg mL^{-1} γ-D-crystallin (50 mM TRIS, 150 mM NaCl, pH 7.4, *T* = 4 °C) were recorded. When a buried tryptophan residue of a protein exposes to water upon protein unfolding, the tryptophan fluorescence band shifts to larger wavelengths (red shift), indicating a conformational transition the protein. Figure SI 1 shows the pressure-dependent fluorescence band. No pressure-induced changes could be observed up to 2.4 kbar, i.e. the protein remains in the folded state in the whole pressure range covered.

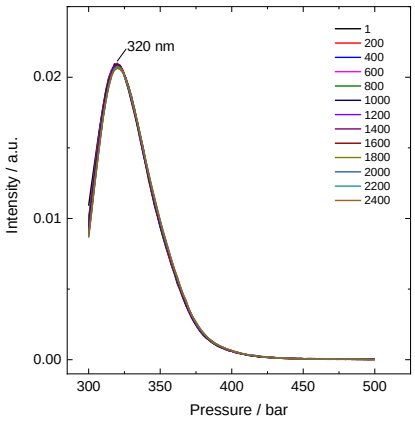

Figure SI 1. Pressure dependent measurements of intrinsic tryptophan fluorescence (0.1 mg mL^{-1} γ-D-crystallin, 50 mM TRIS, 150 mM NaCl, pH 7.4, *T* = 25 °C). Excitation wavelength: 295 nm, emission wavelength: 300-500 nm.

Pressure dependent FT-IR studies on γ-D-crystallin

Samples of human γD-Crystallin were prepared with pressure-stable 50 mM TRIS buffer (150 mM NaCl, D_2O, pD 7.4). All measurements were carried out using a diamond anvil cell with type IIa-diamonds and stainless steel with a thickness of 50 µm was used as a spacer between the diamonds. The sample temperature was held at 1 °C or 24 °C via a circulating water bath. FTIR spectra were recorded on a Nicolet 6700 FTIR spectrometer in a pressure range of 1 to 3000 bar. Each spectrum consisted of an average of 256 scans recorded with 2 cm^{-1} resolution. As pressure indicator, barium sulfate ($BaSO_4$) was used, which has a characteristic pressure-sensitive infrared peak at 983 cm^{-1}.[1]

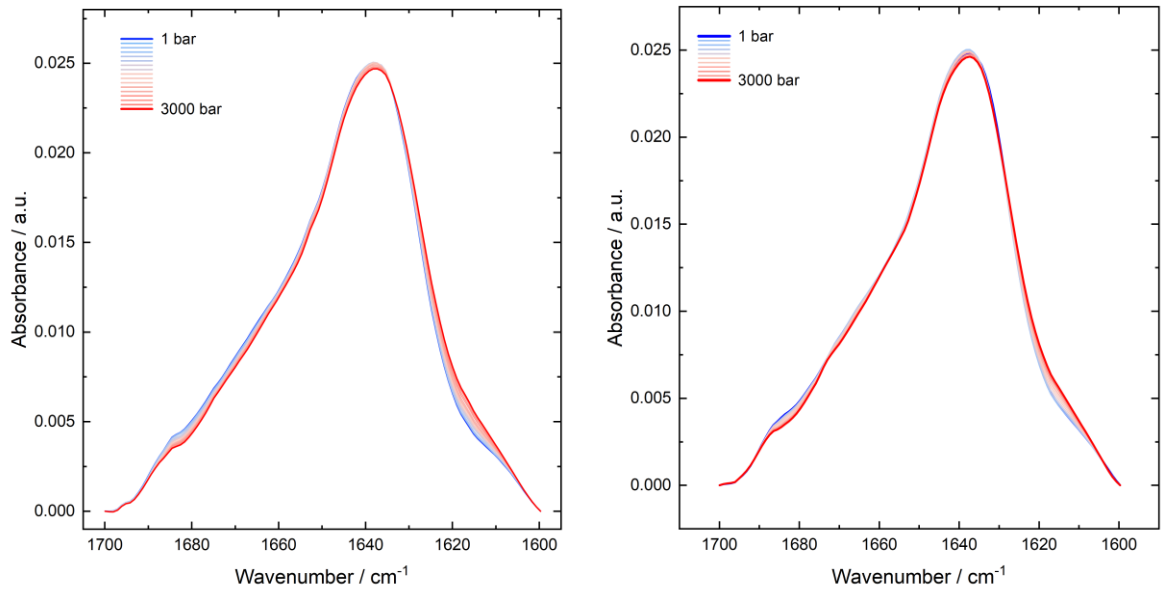

Figure SI 2. FT-IR absorption spectra of γ-D-crystallin in 50 mM TRIS buffer (150 mM NaCl, pD 7.4) as a function of pressure (1-3000 bar) at a concentration of 5 mg/mL at 24 °C (left) and at a concentration of 50 mg/mL at 1 °C (right).

Figure SI 2 shows the FT-IR absorption spectra of γD-Crystallin in 50 mM TRIS buffer as a function of pressure up to 3 kbar. The IR spectrum in the amide I' region (1700-1600 cm^{-1}) consists of a single broad band, with a maximum at ~1638 cm^{-1}, which is characteristic of the *β*-sheet rich structure of γ-D-crystallin, in agreement with literature data [2-3]. With increasing pressure up to 3000 bar, a slight pressure-induced elastic red shift of the absorption band is observed, only, no sign of pressure-induced unfolding is visible. There is no difference between the secondary structure of the protein in the dilute solution (left) and in the LLPS region (right).

[1] P. T. T. Wong, D. J. Moffat, *Applied Spectroscopy* **1989**, *43*, 1279-1281
[2] K. P Das, L.-P Choo-Smith, J. M. Petrash, W. K. Surewicz, *J. Biol. Chem*. **2002**, *274*, 33209–33212.
[3] K. Papanikolopoulou, I. Mills-Henry, S. L. Thol, Y. Wang, A. A. R. Gross, D. A. Kirschner, S. M. Decatur, J. King, *J. Phys. Chem. B*. **2013**, *117*, 15436–15443.

Temperature and pressure dependent turbidity studies on γ-D-crystallin

The temperature dependent UV/Vis measurements were performed using a Shimadzu UV-1800, with 2 nm resolution in the wavelength range 250-550 nm. Coacervation was examined by monitoring the turbidity (apparent absorption) through light scattering at 400 nm. The temperature of the sample cell was controlled by an external water thermostat. The pressure dependent measurements were carried out on a PerkinElmer Lambda 25 spectrophotometer with a home-built high-pressure optical cell. As window material, sapphire with a diameter of 20 mm and a thickness of 10 mm was used. Pressure was applied using a high-pressure hand pump and was measured by a pressure sensor (Burster Präzisionsmesstechnik, Gernsbach). The pressurizing medium was water.

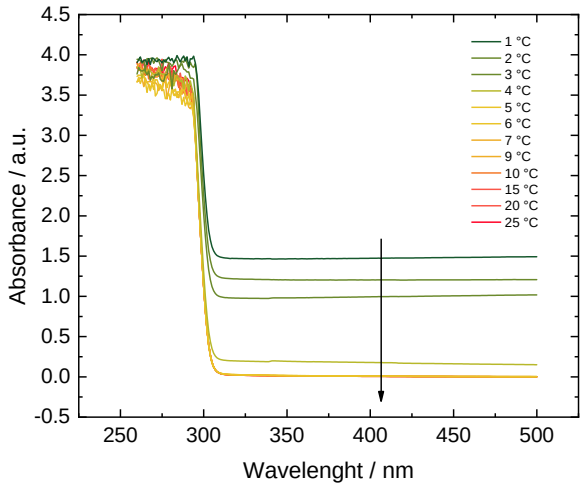

Figure SI 3. UV/Vis absorption spectrum of γ-D-crystallin (50 mM TRIS, 150 mM NaCl, pH 7.4) during cooling at atmospheric pressure in the temperature range from 25 to 1 °C. The arrow indicates temperature increase.

Figure SI 3 shows the absorption intensity of a 55 mg mL^{-1} solution of γ-D-crystallin (50 mM TRIS, 150 mM NaCl, pH 7.4) upon cooling at atmospheric pressure. At 275 nm, the spectrum shows a strong band (here saturated) due to absorption of the tyrosine residues of γ-D-crystallin. With decreasing temperature, the background intensity at higher wavelength increases. This is a consequence of increased light scattering due to the formation of coacervate.

Additional Figures and Movie

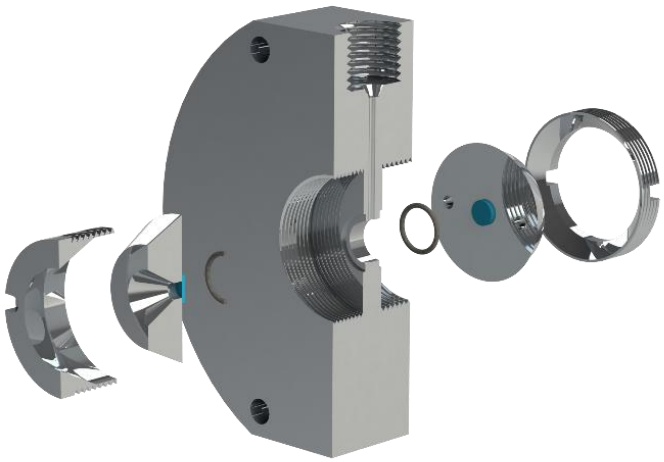

Figure SI 4. Schematic view of the home-built high-pressure microscopy cell. Pressure was generated hydrostatically by a high-pressure hand pump with water as pressure-transmitting fluid. Diamond windows were used as optical window material on both sides.

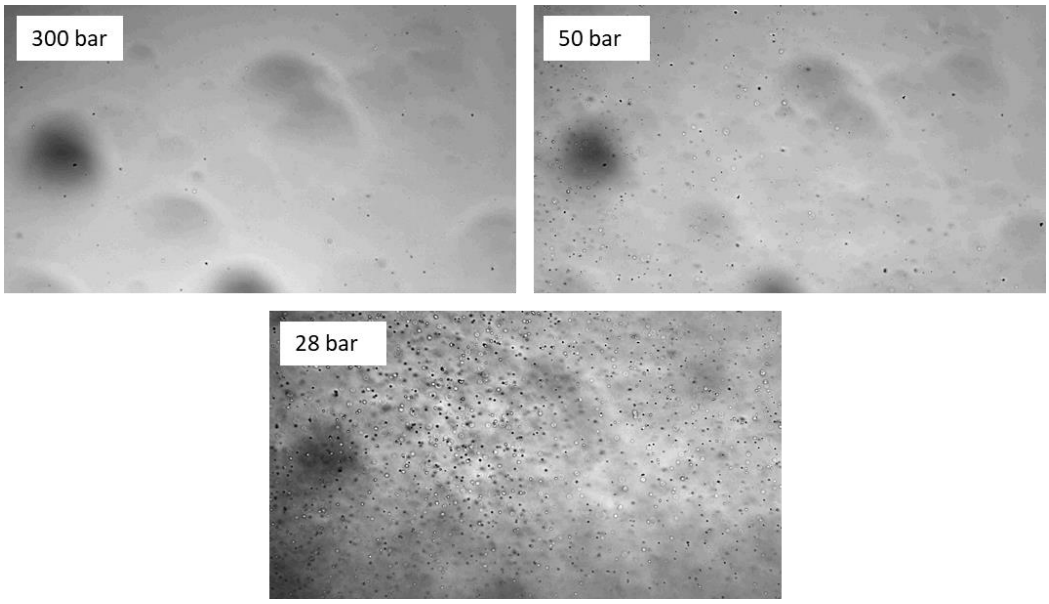

Figure SI 5. Pressure-dependent light microscopy measurements of 55 mg mL^{-1} human γ-D-crystallin (50 mM TRIS, 150 mM NaCl, pH 7.4, T = 4 °C) during pressure release in the bulk. Owing to hysteresis effects and slow kinetics of nucleation, the onset and visualization of droplet formation may vary in the pressurization and depressurization direction to some extent.

Movie 1: Light microscopy data in the bulk phase of 55 mg mL^{-1} human γ-D-crystallin (50 mM TRIS, 150 mM NaCl, pH 7.4, T = 4 °C) during pressure release.