Electronic Supplementary Material (ESI) for Lab on a Chip

Iterative operations on microdroplets and continuous monitoring of processes within them; determination of solubility diagrams of proteins

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Electronic Supporting Information

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Fabrication of the microfluidic chip. We fabricated the chips via direct milling in polycarbonate (PC) sheets (Macroclear, Bayer, Germany) of various thicknesses using a CNC milling machine (Ergwind, Poland). The CNC machine has a reproducibility of positioning of 5 μm, and allows for using milling bits as small as 100 5 μm in diameter. We bounded the milled microchip with a flat slab of polycarbonate using a method described by *Ogonczyk et al* [1]. We have modified the surface with dodecylamine to prevent wetting of buffer as described by *Jankowski et al* [2]. Because polycarbonate absorbs wavelengths below 400 nm we have placed the fiber waveguides in contact with the continuous phase using microfluidic channels connecting to the main channel. Before operating the chip we inserted and sealed the waveguides to prevent leaking of the continuous phase. Figures S1 shows the layout of the microfluidic chip.

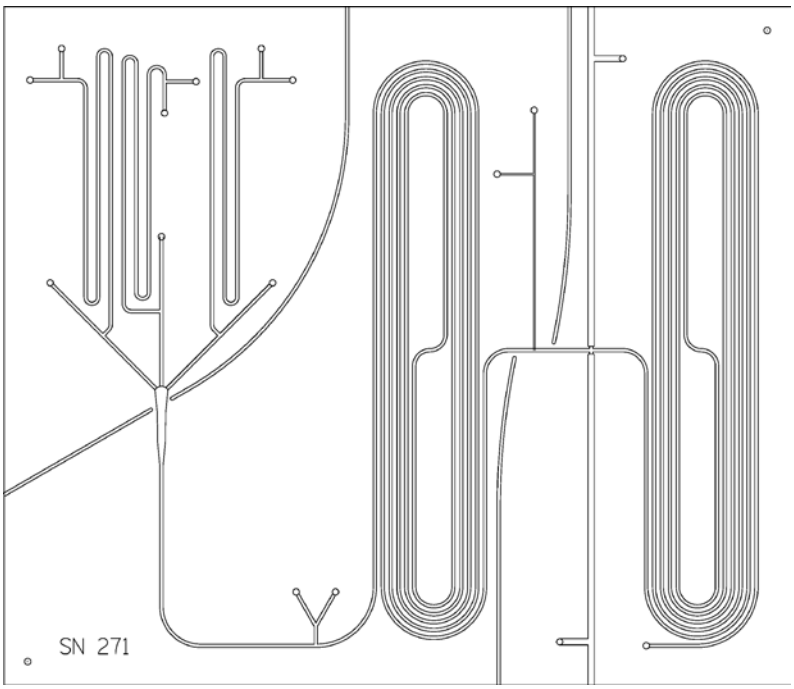

Figure S1. Layout of the microfluidic chip.

Liquids and protein solubility assays Hen egg white lysozyme (Sigma, L6876) was dissolved in 100 mM sodium acetate trihydrate buffer at pH 4.5 to obtain a protein stock solution of concentration of 110 mg/mL, and filtered with a 0,22 μm Millipore/Whatman syringe filter. The initial concentration of the precipitant solution was 2.2 M sodium chloride (Sigma) in 100 mM, pH 4.5 sodium acetate trihydrate buffer. Once the solutions of buffer, protein and precipitant were deposited onto the chip, the LabView code controlled the operation of the valves to generate droplets with specific concentration of protein ranging from 15 to 50 mg/mL, and sodium chloride concentration ranging from 0,3 to 1,5 M. Ribonuclease A (RNase A, R550, Sigma) from bovine pancreas was dissolved in 100 mM sodium acetate trihydrate buffer at pH 4.5 to obtain a protein stock solution of concentration of 900 mg/mL with an initial precipitant concentration of NaCl at 4.5 M. In all experiments we used paraffin oil of dynamic viscosity 6 cSt in 20°C (DTHC, Poland) as the continuous phase.

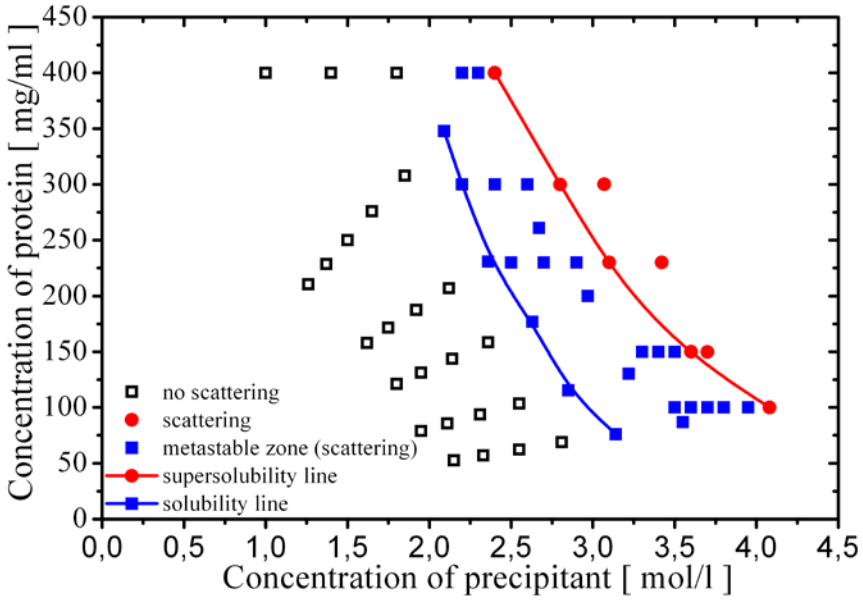

Figure S2. Solubility diagram of Ribonuclease A spanned by the protein concentration (C_{RNAse}) and concentration of precipitant (C_{NaCl}). The diagram shows the solubility and supersolubility boundaries obtained with an automated protocol executed at 24 °C.

Dynamic light scattering and spectrophotometric measurements In order to verify our detection methodology we have first performed experiments with standard spectrophotometer and dynamic light scattering technique. We prepared solution of protein and precipitant at the concentrations that represent nucleation zone. 1 ml stock solutions of 60 mg/ml lysozyme and 2,2 M sodium chloride were prepared and passed through a 0,22 μm syringe filter. Sodium chloride was added slowly to the lysozyme solution to avoid rapid precipitation of the protein. By mixing equal

volumes of both solutions the final lysozyme concentration was 30 mg/ml and 1,1M sodium chloride which according to the phase diagram that was previously determined promotes nucleation/crystallization.

The UV-Vis spectra (see Fig. S3) indicate raise of the baseline similarly as we observe in the droplets, although the raise is not as uniform across wavelengths. We attribute this difference to easily noticeable sedimentation of precipitates in the couvette. The intensity of scattered light measured with dynamic light scattering (BI 200SM Brookhaven Instruments, Fig. S3) on an identical sample show a monotonic increase of the intensity of scattered light.

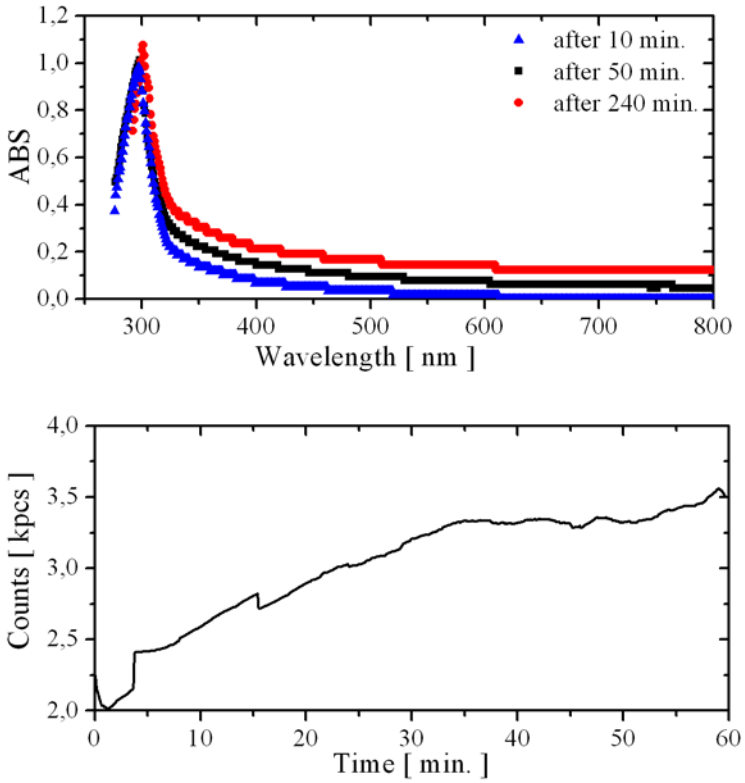

Figure S3. (top) Traditional Spectrophotometry in cuvette of crystallization process and (bottom) Dynamic Light Scattering measurements

Rate of evaporation of droplets. Maintenance of droplet composition is crucial in micro-batch crystalization techniques. Therefore, we have performed tests in order to measure the change in droplet volume during 15 hours. We measured the volume of droplets composed of buffer (0.1M sodium acetate buffer) circulating in paraffin oil back and forth using same or similar parameters (size of channel, size of droplet, rate of flow, temperature etc.) to the ones used in the determination of phase diagrams of proteins. The volume of the droplets is estimated via image analysis. The volume of static droplets (no flow) has not changed when measured every 20 minutes during 15 hours (Fig

S4top). Separate experiment has been performed to observe non-stationary conditions in which droplets flew. Negligible change of volume (~0.1%) has been detected during 15 hours of observation (Fig S4 bottom).

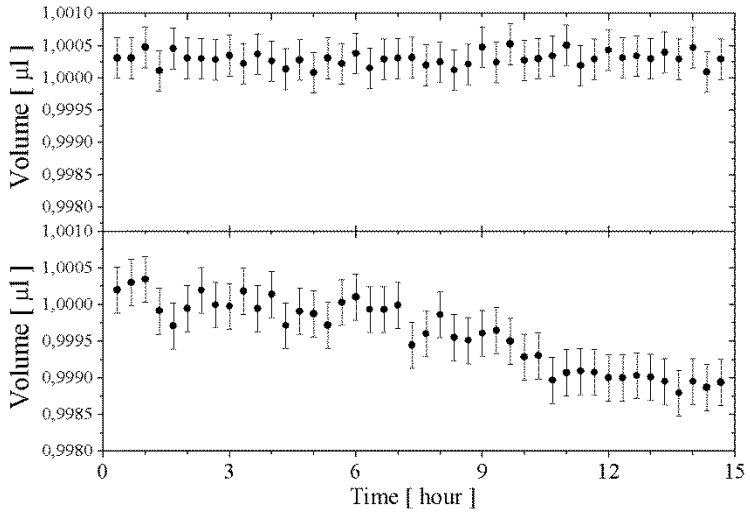

Fig S4. Volume of droplet as a function of time. a) Stationary droplet, and b) droplet circulating back and forth in the system under similar conditions to the ones used in the determination of phase diagrams of proteins.

REFERENCES

(1) Ogonczyk, D; Wegrzyn, J; Jankowski, P; Garstecki, P. Lab on a chip **2010**, 10, 10, 1324-1327

(2) Jankowski, P; Ogonczyk, D; Kosinski, A; Lisowski, W; Garstecki, P. Lab on a chip **2011**, 11, 4, 748-752