VIDEO 1: Millimeter-scale production of optimized crystals of crotamine

The video, **crotamine3_milli.avi**, was constructed from screen images grabbed at roughly regular intervals over the course of the experiment.

Sample: 8µl droplet of crotamine (20 mg/ml in H_2O) isolated from *Crotalus durissus terrificus*, 4.9 kDa.

Nanodrop generator adding precipitant loaded with 1.9 M ammonium sulfate in 0.2 M sodium isothiocyanate, pH 6.1.

The video covers roughly the first half of the experiment, a span of 28h:45m from approx. 1pm 14 December 2009 to almost 5pm the next day, to show formation of crystals of millimeter scale. Crystals continue to grow under final conditions until experiment terminated.

Description of screen format:

Top half: Sample parameters shown as curves over time.

Ordinates are the sample parameter variables

red: relative humidity [%] inside the process chamber

magenta: air temperature [°C] inside the process chamber

turquoise: sample mass [g]

Abscissa is time (h:m:s), beginning at 12:56:00 and ending just after 16:42:00 the next day.

Video time (s), hereinafter VT, shown at lower right. Total video duration 75s

Lower left:

Microscope image of the protein droplet over course of the experiment.

A red laser beam passes through the sample, necessary for the *in situ* DLS measurements.

Lower mid: Radius profile from DLS over time. Initial DLS measurements show a R_H=1.0-1.5 nm

Lower right: Count rate of scattered photons over time.

Phases of crystal growth process:

Phase 1 **(**VT 0:00-0:02): Protein returns to steady state conditions after introduction of protein droplet. Besides crotamine at R_H = 1.0-1.5 nm, colloidal impurities or aggregates are detected at approx. 100nm.

The count rate of scattered light photons is about 30 kHz.

Phase 2 **(**VT 0:03-0:04): Addition of 4.5µl precipitant solution to droplet (vic. 13:58:00) produces increase in sample mass and blip in relative humidity.

Phase 3 **(**VT 0:04-33): Evaporation rate is set for a 50% sample mass reduction in 10800 s, shown as slow trend down in sample mass. This is accompanied by an increase in count rate of scattered light photons.

Phase 4 **(**VT 0:33-34): An anomaly in the count rate of the scattered light photons indicates the

beginning of reaction events. An increased presence of particles with an R_H of around 200 nm becomes visible (extreme right of lower mid).

Phase 5 **(**VT 0:40-48): The count rate decreases significantly despite of the decreasing sample mass. Later on first small crystals become visible and magnification of light image of drop increases in a single step.

Phase 6 **(**VT 0:49-1:16): The crotamine crystals grow fast over a period of 10 hours. DLS is switched off (ca. VT = 0:55) after the presence of crystals is detected. Crystals reach final diameters of approx 0.2 mm in all directions.

VIDEO 2: Production of micro-scale crystals of mistletoe lectin I (ML_I_microcrystallization.avi)

Top half: As before, sample parameters shown as curves over time.

Experiment spanned approx. 10 hours.

Ordinates are the sample parameter variables

red: relative humidity [%] inside the process chamber

magenta: air temperature [°C] inside the process chamber

turquoise: sample mass [g]

with the addition of

green: ammonium sulfate concentration [mol/L] of the sample

Below left: sample drop (10.0 µl MLI, 1.9 mg/ml, in 0.2 M glycine-HCl, pH 2.5)

Nano-drop generator 1: H_2O filtered and degassed

Nano-drop generator 2: precipitant, 2 M ammonium sulfate in 0.2 M glycine-HCl, pH 2.5, also filtered and degassed

Below right: Radius profile from DLS over time.

At the beginning, the measured particle size distribution of ML-I indicates the protein solution is almost mono-disperse, with particles of R_H of 5.7 nm.

At about 12:45, after 45 min, the addition of precipitant begins. The precipitant concentration is increased up to 0.75 mol/L.

After about 2.6 h, nucleation is detected (visible in the radius distribution screen, lower right).

At about 4 h, evaporation is induced in the drop. Drop mass goes down and precipitant concentration increases.

After about 11 h, evaporation is stopped.

VIDEO 3: Production of nano-scale crystals of mistletoe lectin I (ML_I_nano.avi).

Top half: As before, sample parameters shown as curves over time.

Experiment spanned approx. 10 hours.

Ordinates are the sample parameter variables

red: relative humidity [%] inside the process chamber

magenta: air temperature [°C] inside the process chamber

turquoise: sample mass [g]

green: ammonium sulfate concentration [mol/L] of the sample

Below left: sample drop (9.8 µl ML-I, 1.9 mg/ml, in 0.2 M glycine-HCl, pH 2.5)

Nano-drop generator 1: H_2O filtered and degassed

Nano-drop generator 2: precipitant, 2 M ammonium sulfate in 0.2 M glycine-HCl, pH 2.5, also filtered and degassed

Below right: Radius profile from DLS over time.

At the beginning, the measured particle size distribution of ML-I indicates the protein solution is almost mono-disperse, with particles of R_H of 5.7 nm.

After 1 h and 25 min, the addition of precipitant begins. The precipitant concentration is increased up to 0.75 mol/L, inducing nucleation (visible at the radius distribution screen) at approx. 2.6 h.

After 3 h, nano-crystals are obtained by increasing the precipitant concentration to 0.79 mol/L. At that precipitant concentration, an intensive nucleation signal becomes visible.

At approx. 5 h and 20 m, the precipitant concentration was increased to 0.85 mol/L in a third precipitant-addition step.

Aliquots G1, G2 and G3 (Figure 4a) were taken after 4.3 h, 47.2 h and 56 h and investigated via electron microscope. In aliquot G3, nano-crystals were found. The prior two grids showed nothing of interest. DLS indicated developments took place in the solution. The radius distribution changed from a double peak signal to a more complex radius distribution signal of three peaks of approximate radii 7-8 nm, 100-200 nm, and 2000 nm.

***a)* Further details of the instrument:**

The ultrasensitive balance has a resolution of 0.1 µg (Sartorius SE2, Satorius, Germany). The sample is constantly being weighed, thereby effectively monitoring evaporation losses of the drop or additions to the drop, and facilitating a recording of molarities. For visual inspection of the droplet and crystals therein, a microscope is used that allows stepwise variation of the magnification between 25 x and 250 x (Mikroskop Technik Rathenow GmbH, Germany). A CCD camera (DFK 41AF02, The Imaging Source Europe GmbH, Germany) with a resolution of 1280 x 960 pixels is adapted to the microscope to allow further image analysis, e.g. recording microscopic crystal growth.

The DLS system is a modification of a commercially available system (Spectrosize 302, Molecular Dimensions, UK). The laser beams are deflected by a gold-coated mirror (Edmund Optics, Germany), so that the intersection of the beams can be precisely positioned in the sample drop. The wavelength of the laser is 660 nm and the optical power is approx. 100 mW. The Xtal- Controller includes two nanodroplet generators systems (MD-K-130-010, Microdrop Technologies GmbH, Germany). The distance between the nozzles of the pumps and the drop is rather large (several cm) to reduce the speed of the nanodrops by air friction before they reach the crystallizing drop, to avoid causing turbulence in the crystallizing drop.

***b*) Optimized conditions for crystal production with the Xtal-Controller:**

Optimized conditions always refer to the outcome of crystallization experiments. Final conditions means the compositions of protein and precipitant solutions when crystals appeared and crystal growth was observed via microscope.

Crotamine:

Protein concentration: 20 mg/ml in H_2O

Precipitant (in droplet generator): 1.9 M ammonium sulfate in 0.2 M sodium isothiocyanate.

Note that nucleation was observed at 1.066 M ammonium sulfate in the sample drop. Continuous evaporation at 95% relative humidity leads to crystals.

Thaumatin:

Protein concentration: 40 mg/ml in 100 mM Tris HCl, pH 6.8

Precipitant (in droplet generator): 1.2 M Na tartrate in 100 mM Tris HCl, pH 6.8

Note that crystals were detected at 0.416 M Na tartrate in the sample drop.

ML-1:

Protein concentration: 1.9 mg/ml in 0.2 M glycine/HCl, pH 2.5

Precipitant (in droplet generator): 2 M ammonium sulfate in 0.2 M glycin/HCl, pH 2.5

Nucleation was observed at 0.79 M ammonium sulfate in the sample drop. Aliquot taking for electron microscopy was done at 0.88 M ammonium sulfate.