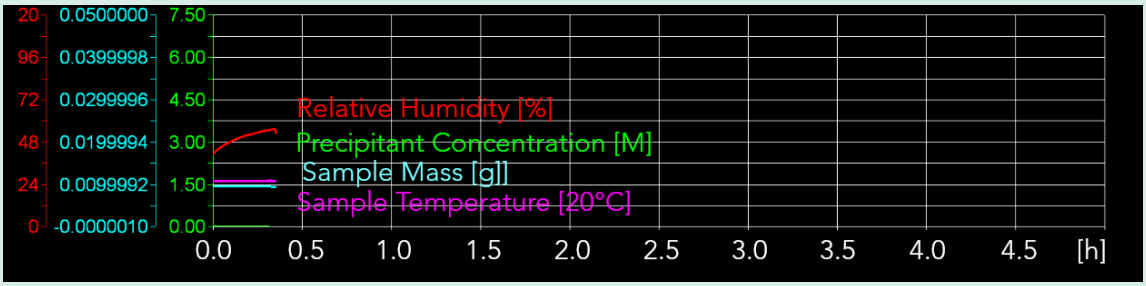
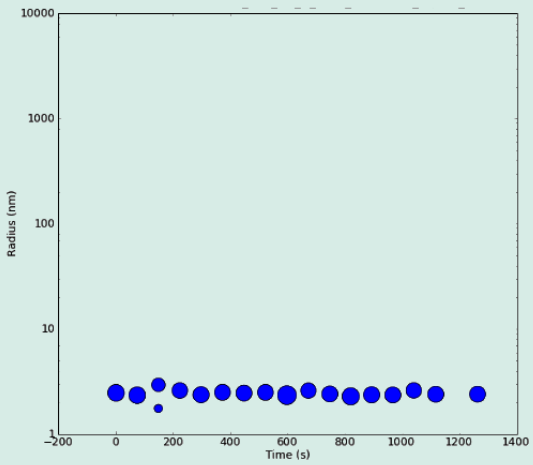
Drop manipulation to obtain ~25 µm Crystals

Proteinase K (10 mg/ml) was used for this Experiment. The precipitant solution was 4 M $NaNO_3$ The experiment was carried out as an precipitant addition experiment, where vapor diffusion is neglectable. With precipitant addition in two steps to 0.05 M $NaNO_3$ ~in ~5 min and to a 2.25 M final concentration, followed by a phase of keeping the sample constant in weight for several hours where crystal growth came to a standstill.

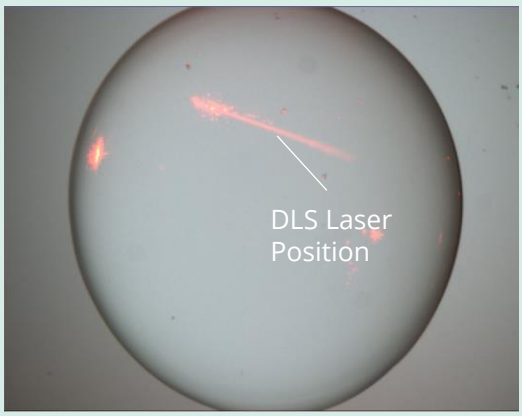
Drop parameters are permanently monitored Before addition of the precipitant (green curve at 0 M), the protein is measured in its buffer used as a size distribution reference (ca. 20 min).



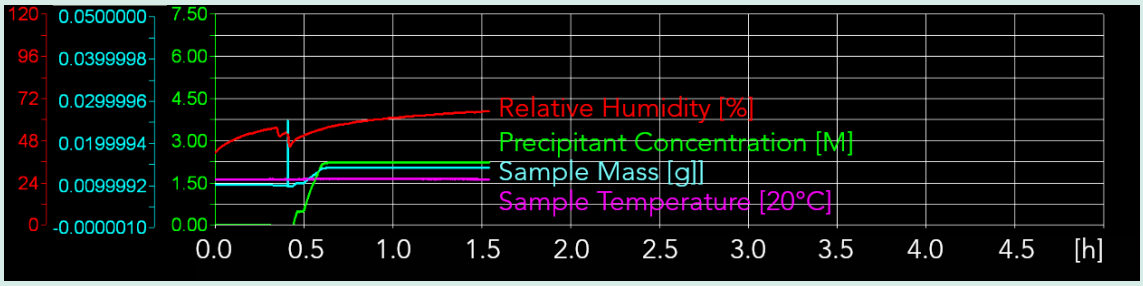
Size distribution before precipitant addition
Proteinase K is a 29 kDa protein possessing a hydrodynamic radius of ~2.7 nm before precipitant was added. The sample was dust free and any aggregates of the protein were detected.



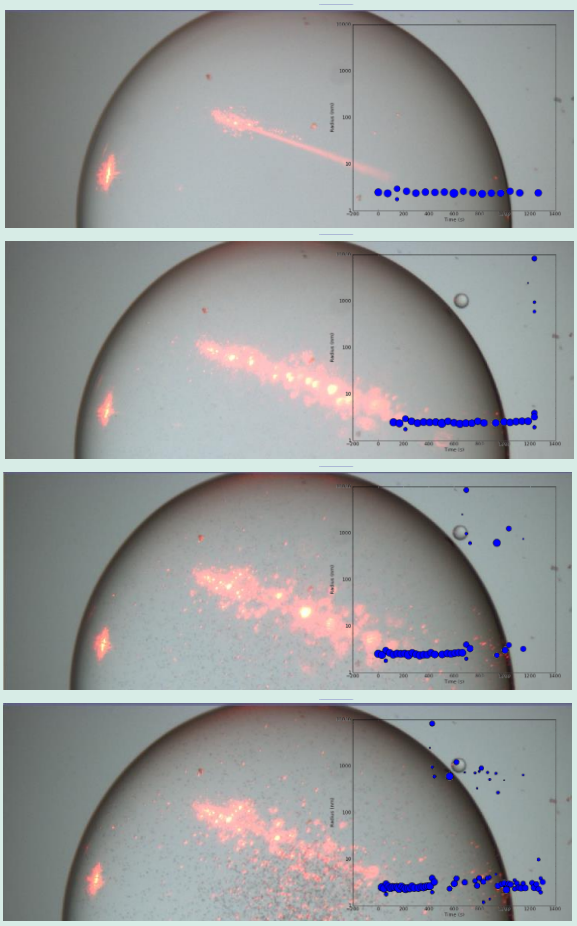
Visualizing the absence of larger particles an optical inspection of the laser beam indicated the absence of larger particles. Such impurities become visible as red dots when illuminated by laser light. Over a period of 20 min. no larger object could be observed.



Within a few minutes, the precipitant has been added in two steps to a final concentration of 0.75 - 2.25 M.

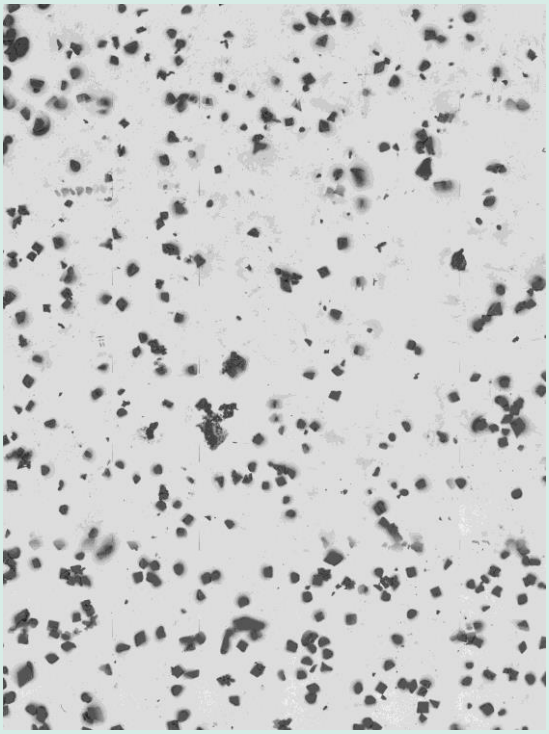


Crystal formation without intermediate clustering a remarkable observation in Proteinase K micro-crystallization was the absence of a pronounced second peak usually interpreted as liquid dense cluster formation. This was observed in all crystallization experiments of Proteinase K. A similar observation was made by crystallizing Clucose Isomerase.

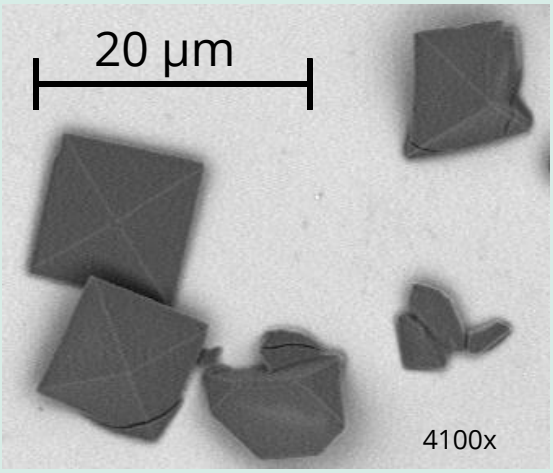


Particles emerged everywhere immediately after the first precipitant addition. When they diffuse into the laser beam, they got illuminated and become visible for the camera. The overall concentration is much lower than the concentration of liquid dense clusters. Only occasionally they pass the DLS detection volume and therefore measured in size which is around 1 µm. Their size didn't change much, but after some time, a layer of micro crystals became visible at the bottom.

Controlled evaporation over 3 hours and stabilization for more than 12 h imitate a classical vapor diffusion experiment.



REM-Imaging at low magnification revealed the crystalline nature of the obtained particles with the typical octahedral morphology of Proteinase K crystals. Uniformity in size were observed as well despite the partial destruction of the crystals in course of the sample preparation.



Micro Crystals of 10 to 15 µm were obtained after a few hours of growth without evaporation. Crystal dimensions might be controlled by varying the starting conditions e.g. slower addition of precipitant with an equilibration break in between etc.