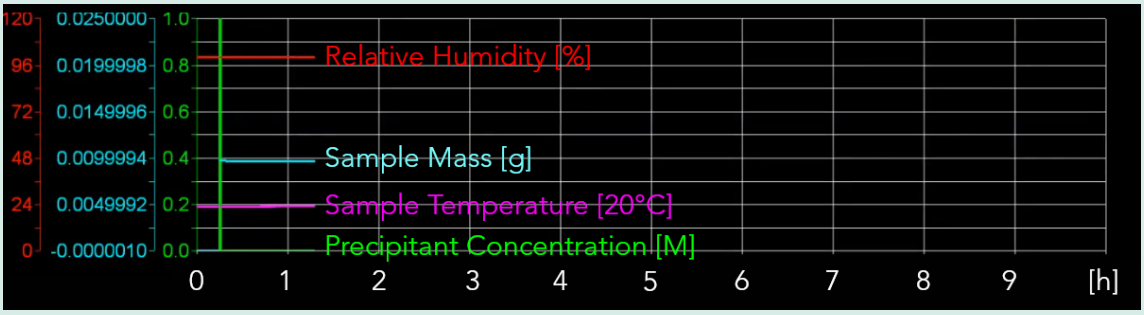
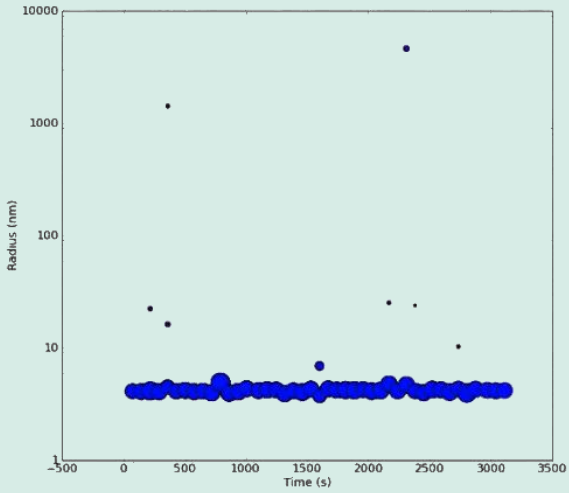
Drop Manipulation to obtain Nanocrystals

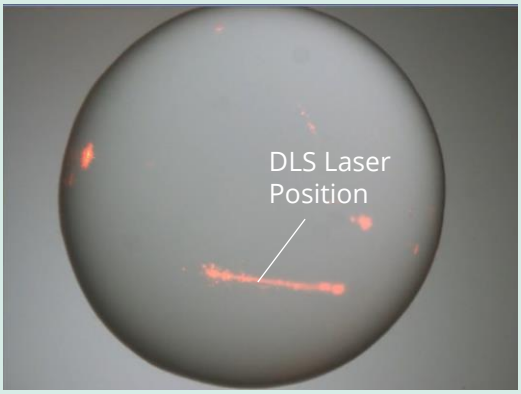
The protein used in this experiments was Mistletoe Lectin I (ML1) (*Viscum album*) a 58 kD glyco-protein heterodimer. Obtained crystals of nanometer dimensions were distributed evenly throughout the sample volume. They do not settle even after many days and cannot be seen by classic optical microscopy. Therefore, sample drops appear to be clear throughout the entire experiment. Nanocrystal formation is indicated by a certain size distribution signature and was later confirmed as uranyl acetate negative stained samples via TEM.



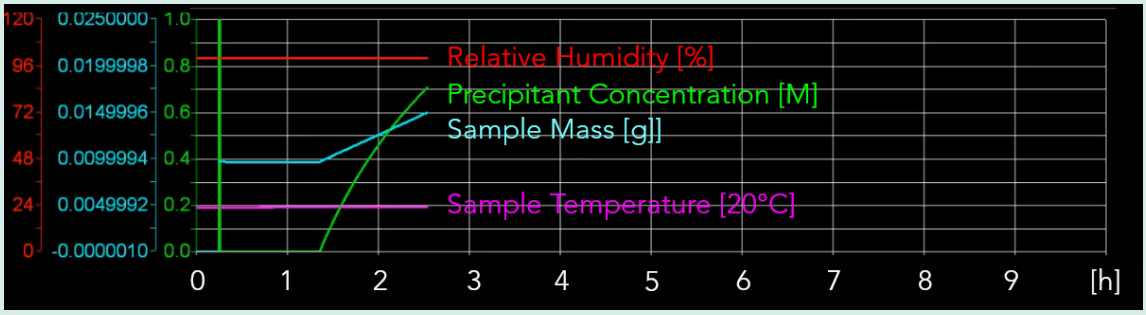
Starting conditions
Mistletoe Lectin I (6.8 mg/ml), dissolved in 0.2 M glycine buffer pH 2.5 was used. The sample was stabilized by water addition for 60 min. before adding the precipitant (stock solution 2 M Ammonium sulphate [AS]). DLS has been measured permanently and revealed a R_h of 5.1 nm.



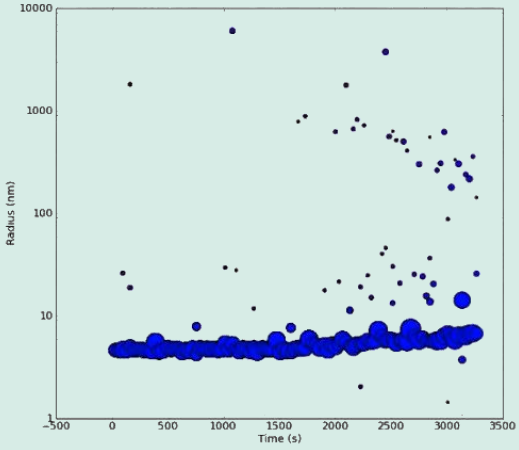
Particle uniformity should be ensured before precipitant addition in order to be able to detect homogenous nucleation. Although this sample was not perfectly free of colloidal impurities, they are less in numbers, so they won't be misinterpreted as nuclei or clusters. The nucleation concentration of the precipitant Ammonium sulphate was identified to be 0.75 – 0.78 M in an previously conducted experiment.

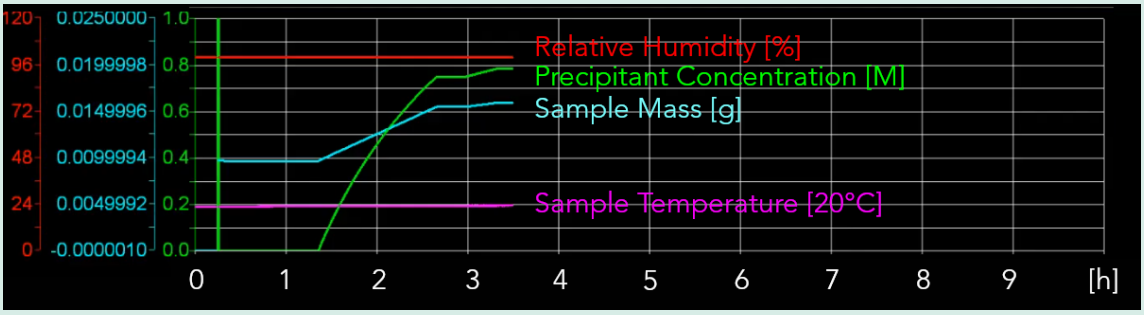


Over a time period of 1h, the precipitant has been added in two steps to a pre-nucleation concentration of 0.75 M.

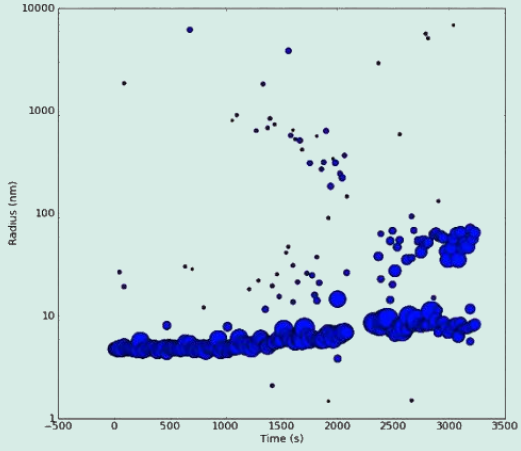


Equilibration at pre-nucleation precipitant concentration Precipitant has been added in one step of 1 h duration for reaching 0.75 M AS. At this concentration, ML1 is very close to its nucleation zone. Now, precipitant addition was paused for ~30 min. in order to achieve an equilibration of precipitant concentration throughout the entire sample volume.

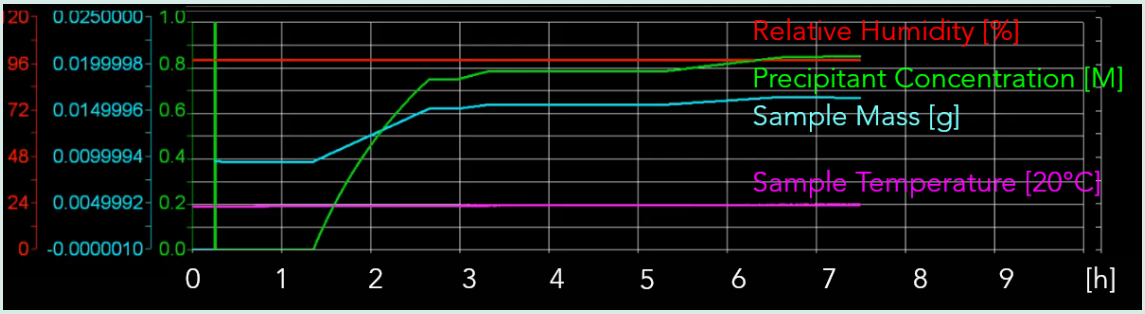




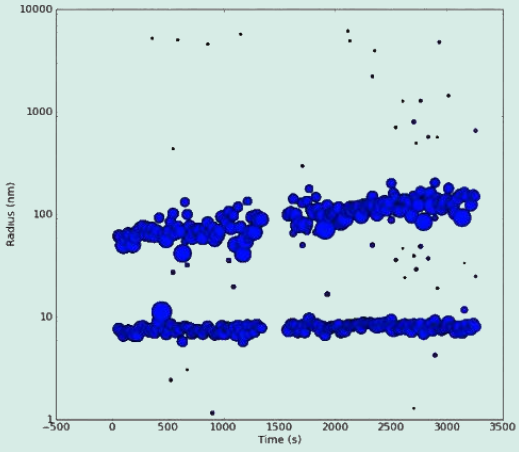
Induction of cluster formation
After an equilibration of ~30 min. cluster formation was induced by a slight addition of precipitant, raising its concentration from 0.75 M to 0.78 M AS. An immediate clustering of the protein was detected. Furthermore, the size distribution revealed a remarkable gap between the 58 kDa ML1 and the clusters, a hint of the critical radius. Protein aggregation smaller than this critical radius are instable and therefore very low in concentration.



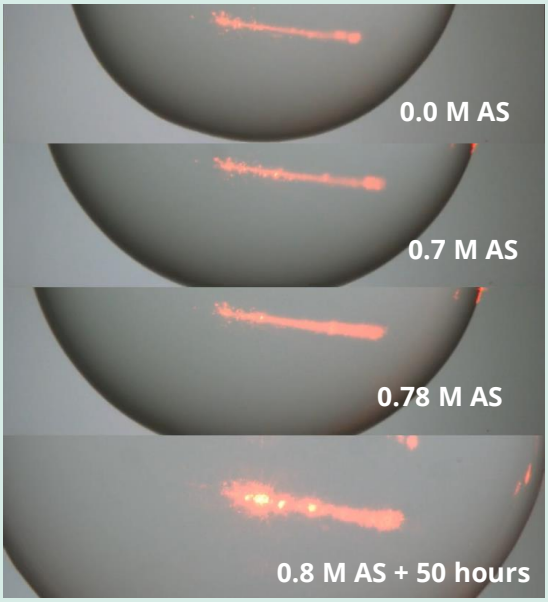
Within a few minutes, the precipitant has been added in one step to an intermediate concentration of 0.78 M and incubated for ~3h.



Liquid dense clusters are likely still amorphous at this step. Aliquots from the sample of an analogue experiment were taken and investigated via negative stain TEM. The first aliquot was taken ~4 h after precipitant addition. At this phase, spherical particles were found on the grids (see also page 4).

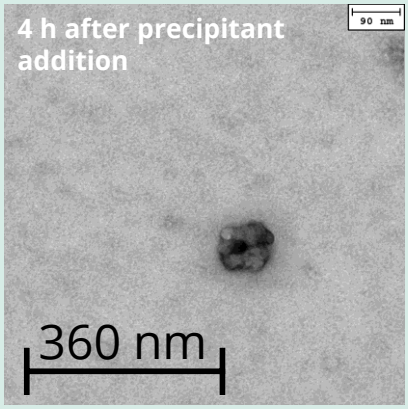


More distinct particles became visible Illumination by the laser beam indicates uniformity of the particles. Even after achieving a precipitant concentration of 0.78 M, the beam became much brighter but no distinct particles were visible as bright spots. After incubation of ~50 h, some particles became visible as bright spots, indicating an advancing transformation of the particles (clusters), likely to a nanocrystal population as later confirmed by negative stained TEM (see also page 4).

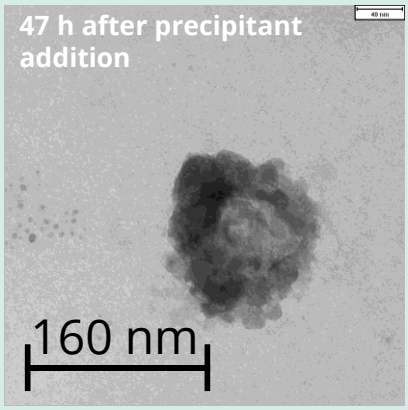


Investigation of particles have been carried out by applying uranyl-acetate stained samples on copper grids and imaged via TEM after grid drying for 24 h.

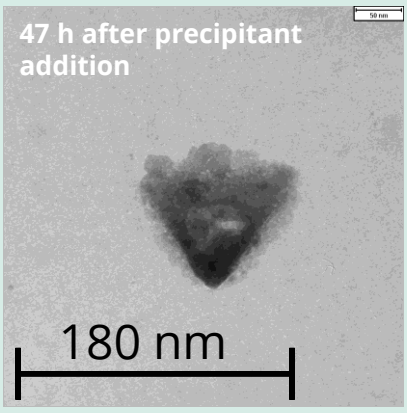
Aliquot taken after 4 h (grid 1) a single image from a grid is often not representative for all objects found on the grid. However, particles of similar shape and size were frequently observed on this grid. This is one of such images, giving an example of the particle shape and size.



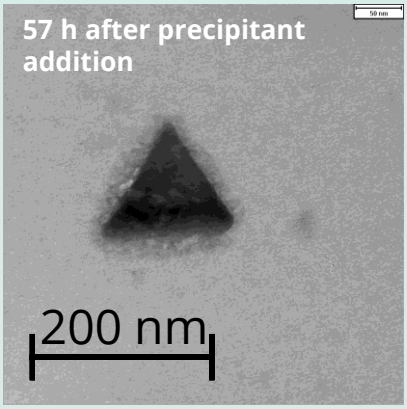
Aliquot taken after 47 h (grid 2, image 1) On this grid (grid 2), particles had a comparable size to grid 1. Some appeared to be still spherical, while others started to develop a distinct geometrical shape (see also Image 2).



Aliquot taken after 47 h (grid 2, image 2) A reordering of the material to more geometrically shaped particles could be observed as well.



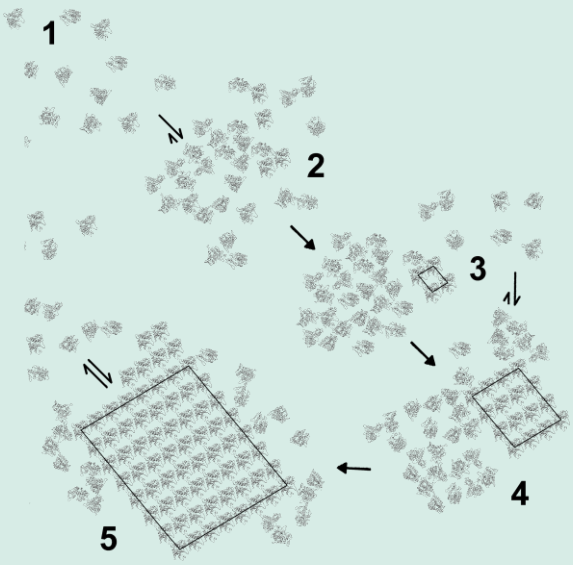
Aliquot taken after 57 h (grid 3) the tetrahedral geometry was now visible throughout the particle, thus the internal reordering towards nanocrystals was completed at this step (Space group of ML1 crystals is $P6_522$).



DLS data of various nano crystallization experiments share a remarkable size distribution signature.

Model of cluster maturation

1 macromolecules exist in a monodisperse distribution, they move randomly and independently from each other. **2** After precipitant addition, a lack of solvent forces the macromolecules to interact with each other, forming amorphous clusters. **3** At the right precipitant concentration, such clusters are densely packed but relative orientations of the macromolecules still change permanently. There's a most stable relative orientation, where surface interactions are strong enough to preserve this orientation permanently. **4** Once this most stable orientation has formed, other molecules get attached on the provided surface, taking the same orientation and thus forming a periodically repeating lattice. **5** Finally, most subunits of former clusters are rearranged into this (crystal) lattice and a cluster to crystal transformation is complete. Crystal dimensions are limited by former cluster dimensions and previously assembled subunits. Uniformity in size prevents subsequent Ostwald ripening.



Interpretation of DLS revealed particle size distribution although DLS can't reveal the chemical nature of a particle or tell the internal order state, one remarkable phenomenon can be exploited to tell, whether reordering processes take place or not. Since the energy difference between an attached particle and a still dissolved particle is very low, the particles exist in a steady state. Therefore the monomer peak is always present throughout the entire experiment. Cluster maturation cannot be directly detected but it seems to be indicated by a triple or quadruple-peak distribution. When occurred, nanocrystals could be identified via TEM.