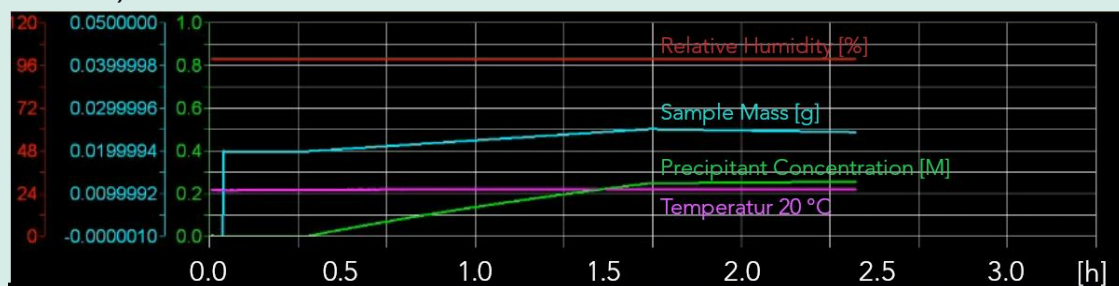


Drop manipulation to obtain ~1.2 mm Crystals

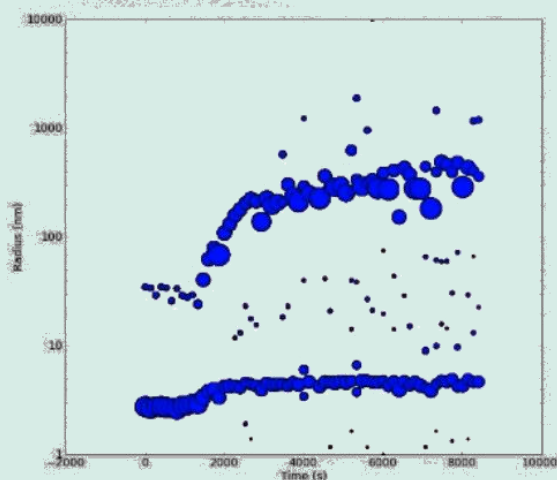
Thaumatococcus (8 mg/ml) was used in this case study because of its well known crystallization properties. The experiment was carried out in a classical vapor diffusion experiment with precipitant addition in one step of a duration of ~1h to a 0.27 M concentration, followed by controlled evaporation over several hours.

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



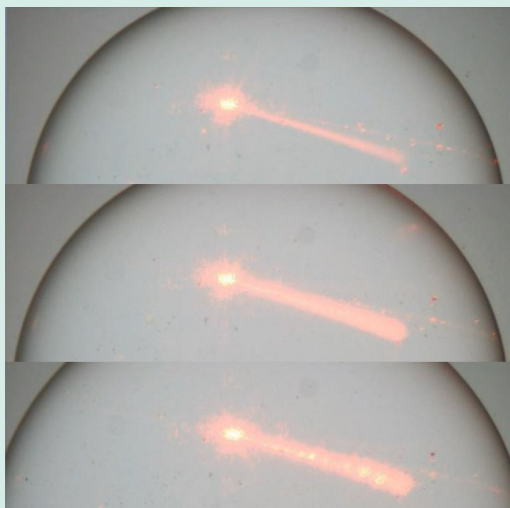
Precipitant Addition For

Thaumatococcus crystallization Sodium tartrate is the precipitant of choice. When added, thaumatococcus soon shows clustering induced when 0.05 M Na-tartrate was exceeded. Cluster formation was indicated via DLS as a pronounced second peak starting at 20 nm and grow to 500 nm.

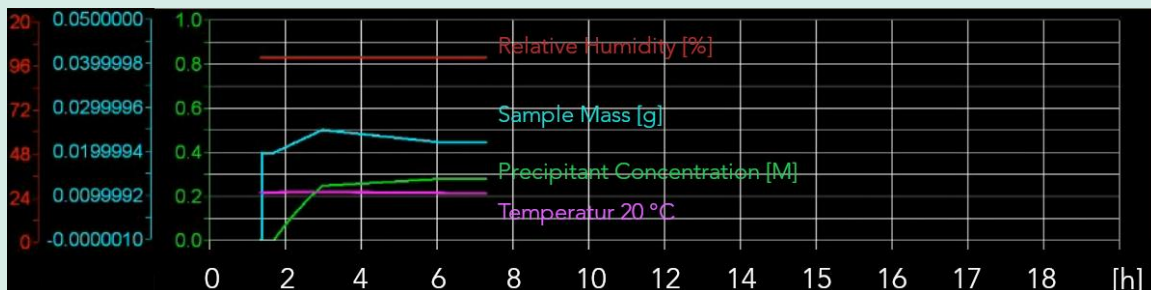


Visualizing Cluster Formation

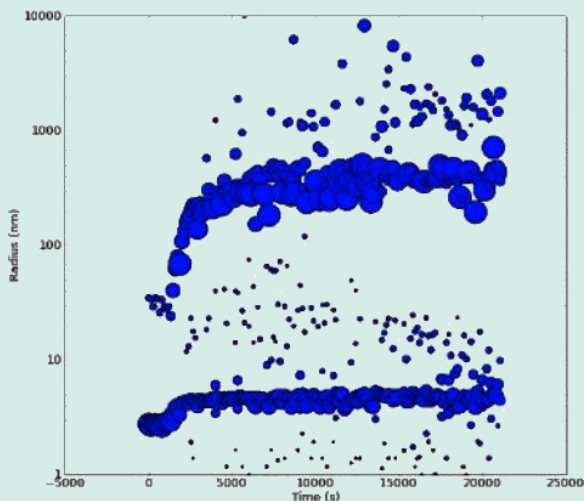
Clustering can also be observed by the camera since the scattered light intensity increases drastically when clusters are formed. The reason for that is the dependency of the scattered light from the particle size. Discrete particles were not visible by illumination with laser light at this point. This changes over time in course of cluster ripening.



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



Cluster Maturation Cluster's early growing of ends at around 500 nm in size. This plateau stays stable even at much higher precipitant concentration. However, after several hours, the particle size distribution becomes more complex. This kind of triple or quadruple formation. Further evaporation of solvent forces the protein to form crystals.



Controlled Solvent Evaporation and Ostwald Ripening After completing the precipitant addition the sample drop was stabilized for 10 min. before a controlled evaporation started 20% solvent removal in 3 h. The sample develops furthermore now mainly by Ostwald ripening resulting in a few crystals of considerable dimensions, while the cluster peak amplitude was reduced.

