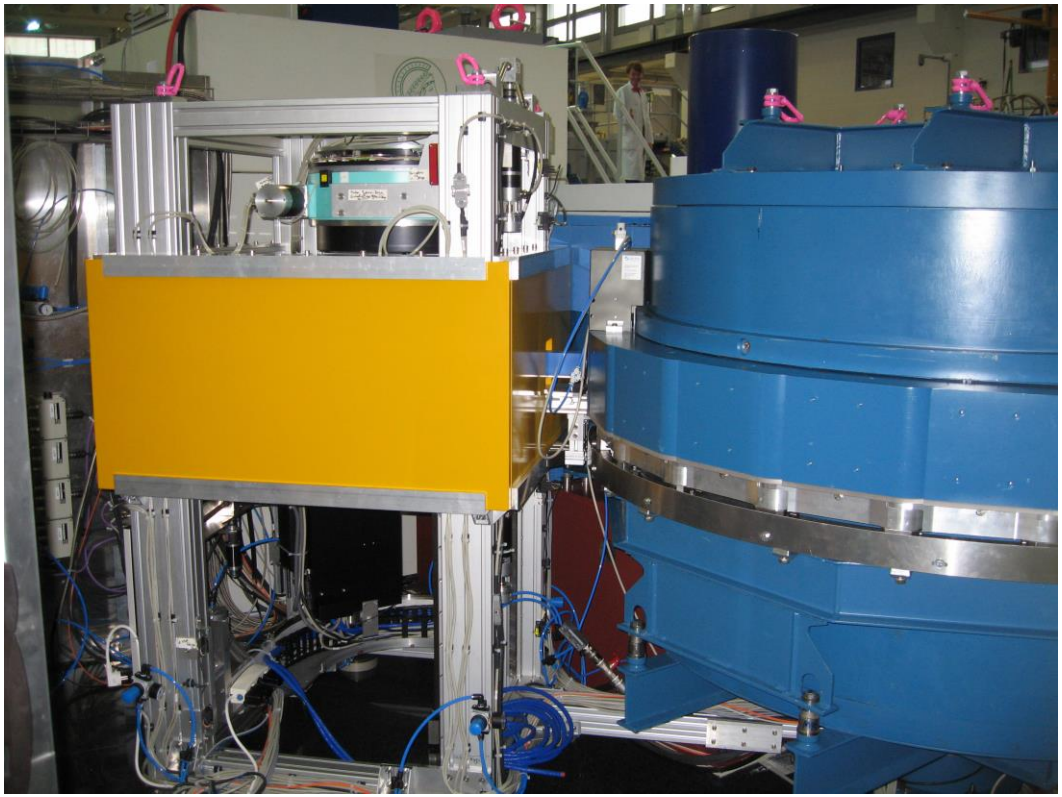
Combined neutron and light scattering analysis on the crystallization of the model protein Lysozyme

28.05.2015

Tobias E. Schrader

Motivation: For neutron protein crystallography large crystals are required



Necessary crysal size:
At least 0.5 mm^3

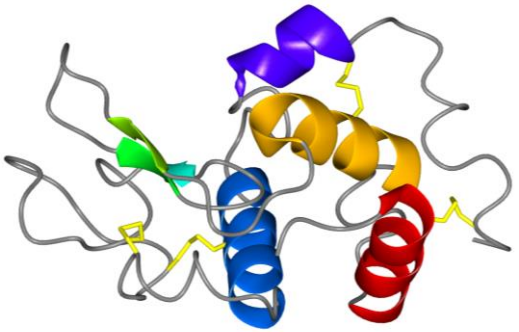
- Deeper understanding of the undelying crystallization mechanism is required

Chosen crystallization conditions

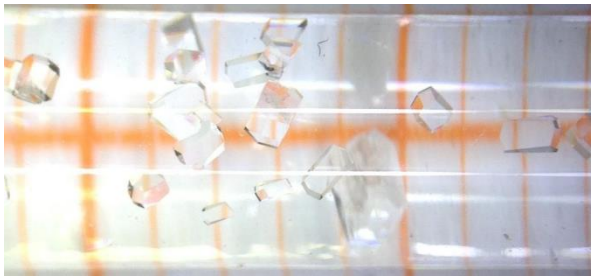
- Lysozyme 60 mg/ml in D_2O, pH adjusted with 1M NaAc 0,02 µm filtered
- NaCl 6wt% in D_2O Puffer 10mM NaAc HAc 0,02 µm filtered

- 1:1 mixture:

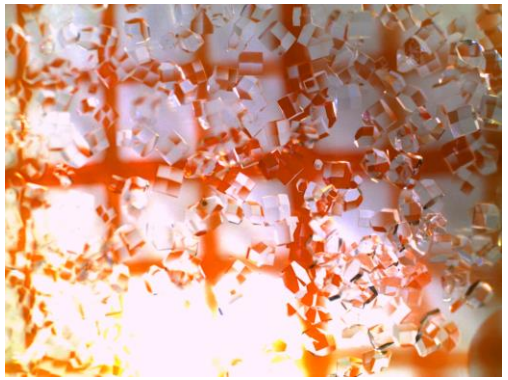
Lysozyme 30 mg/ml + NaCl 3 wt% in D_2O buffer @ pH 4.35



Monomer size: r = 1.9 nm



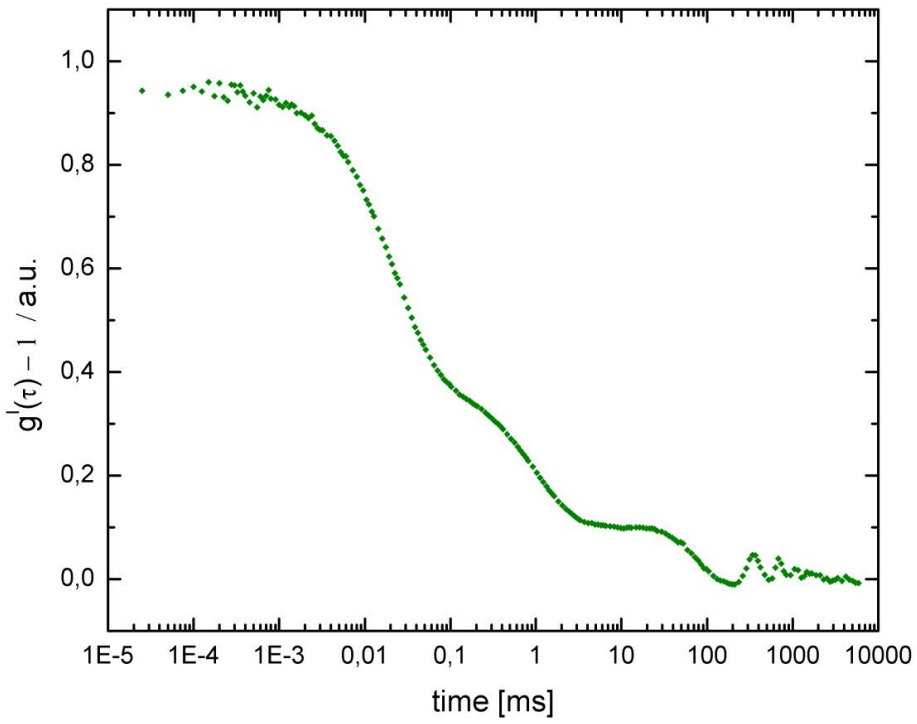
crystals ca. 1 mm at T = 298 K



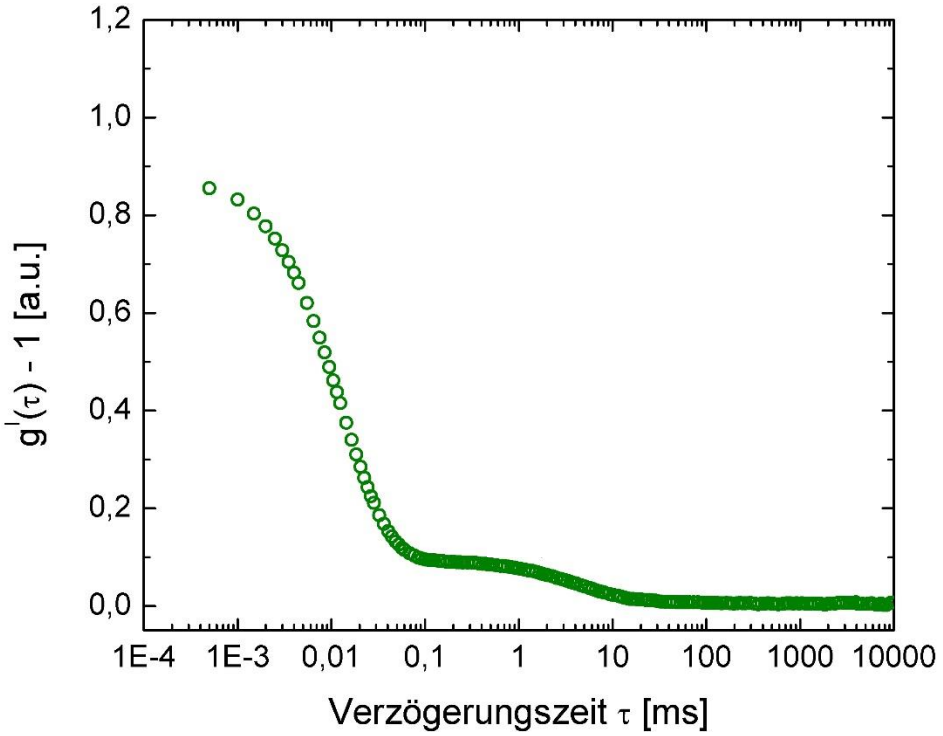
crystals ca. 0.2 mm at T = 294.5 K

Dynamic light scattering gives the number of particle sizes present

T= 294,5 K

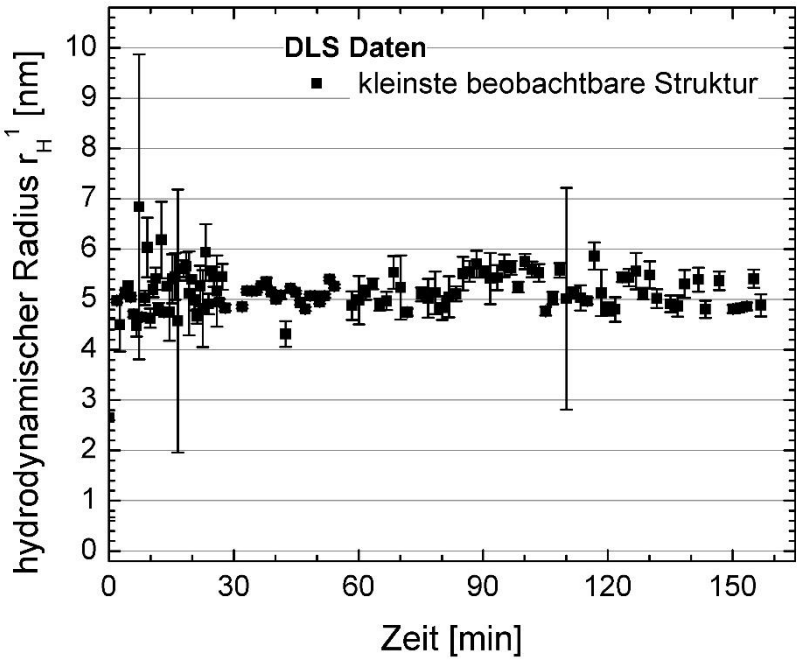


T= 298 K

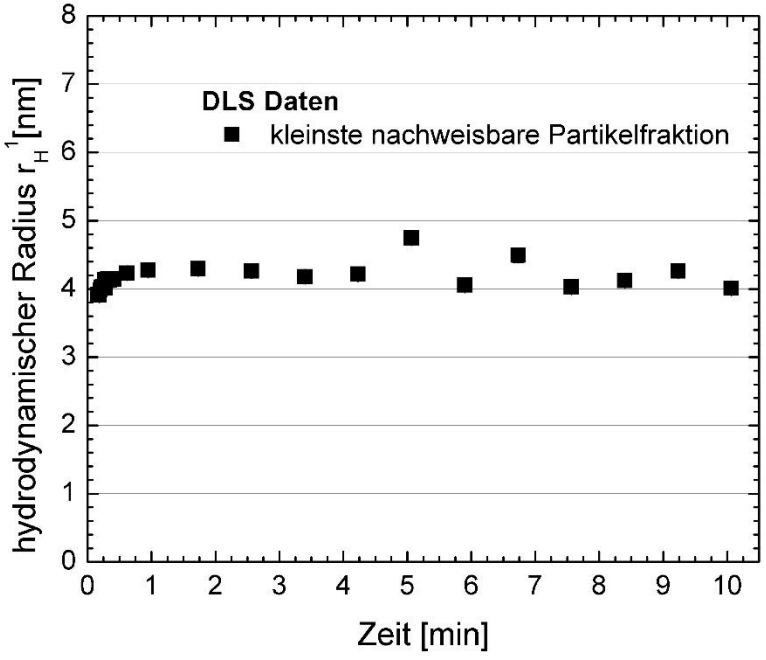


Pre-characterisation of the crystallisation speed with DLS

T= 294,5 K



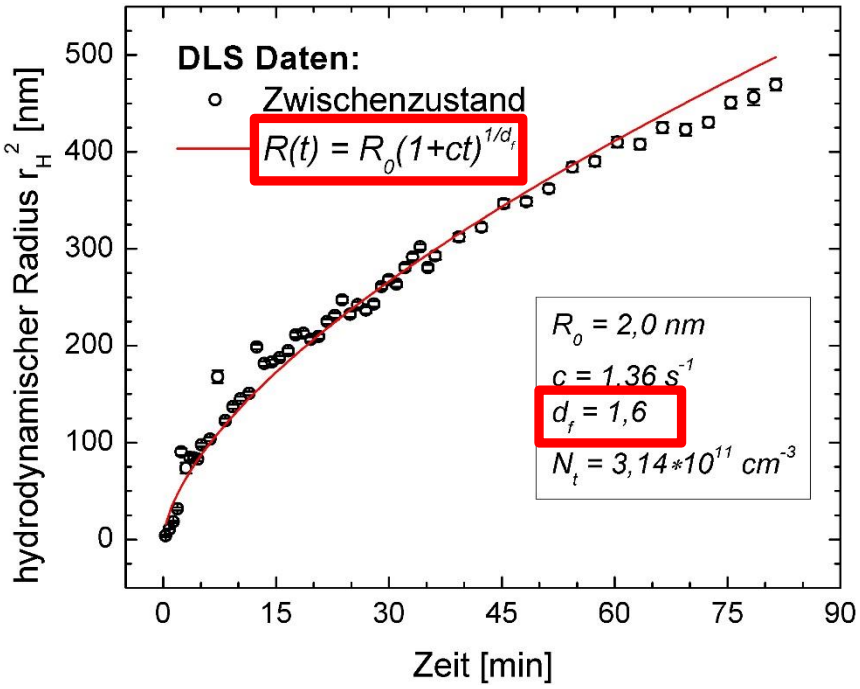
T= 298 K



- Constant radius of the dimer fraction in both cases

Comparision with the literature

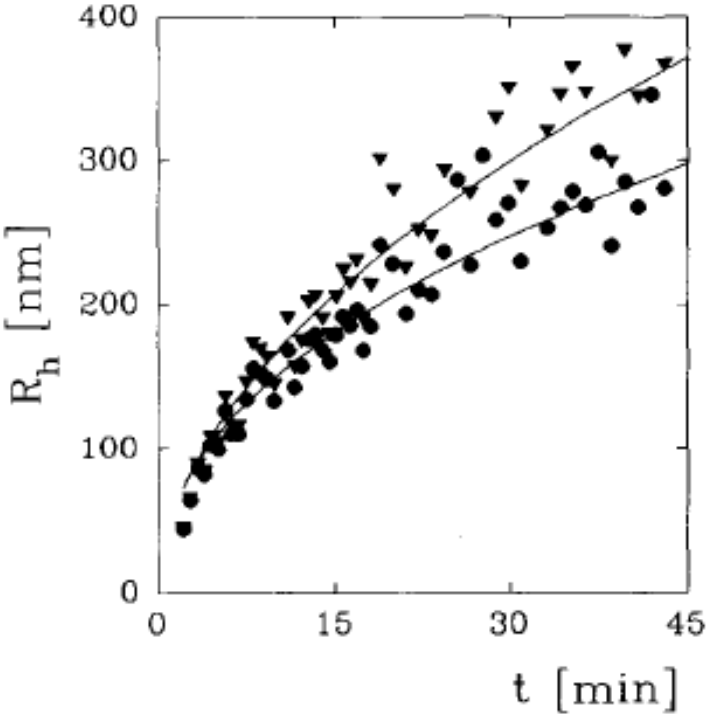
T= 294,5 K



DLS with 60mg/ml Lysozyme mixed with 6wt% in D_2O Puffer

pH 4.35; T = 294.5 K; scattering anglel 174°

Y. Georgalis, A. Zouni, W. Eberstein, W. Saenger, Crystal Growth 126, 245-260



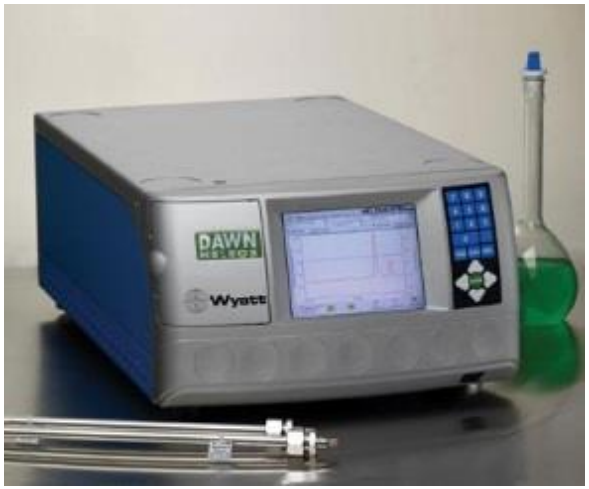
DLS with 61.3 mg/ml Lysozyme mixed with 7.2wt% NaCl in H_2O Puffer

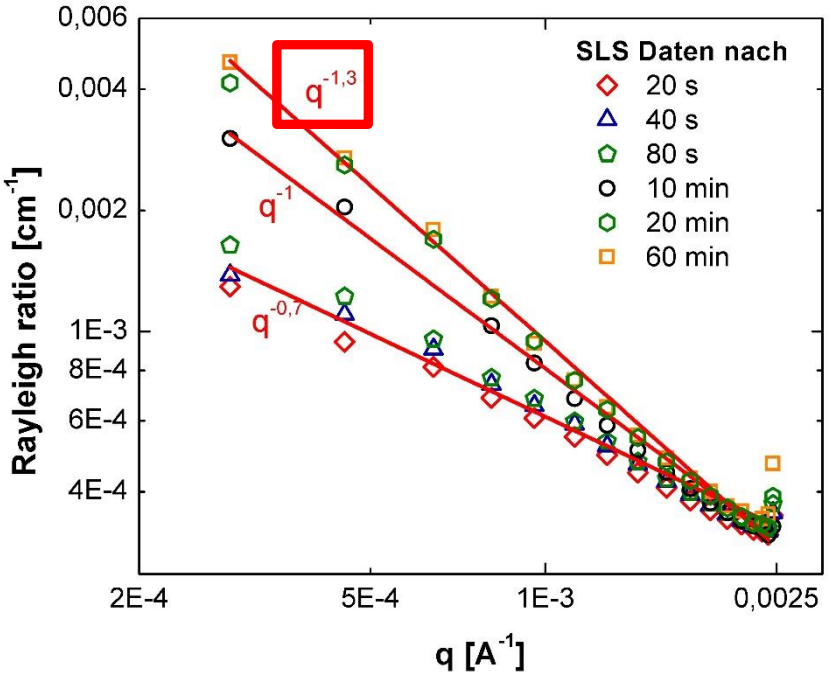
pH 4.2; T = 293 K; scattering angle 20°

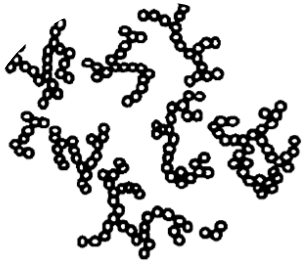
Change in fractal demension observed at T=294.5 K

T= 294.5 K

$d_f = 1,3$





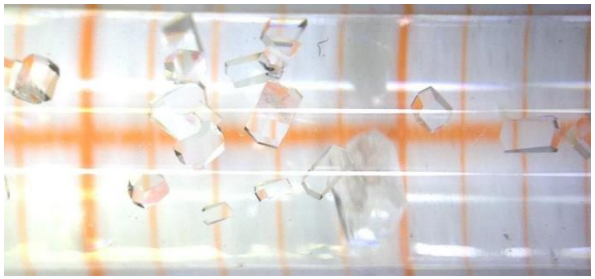


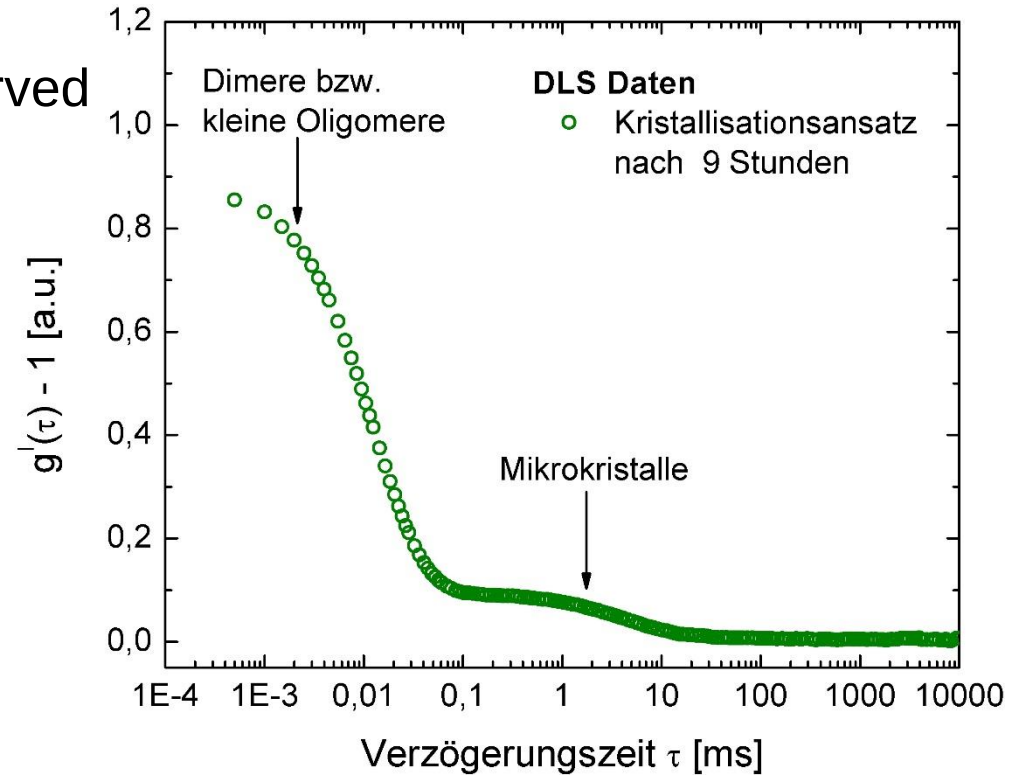
Fractals form!

Dynamic light scattering to characterize the sample system

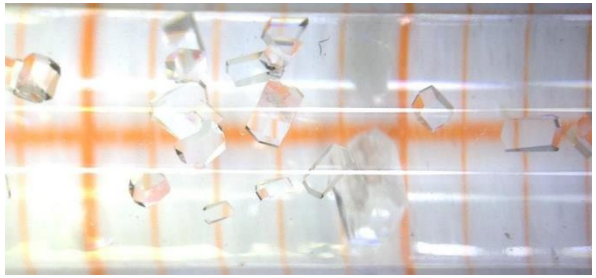
T= 298 K

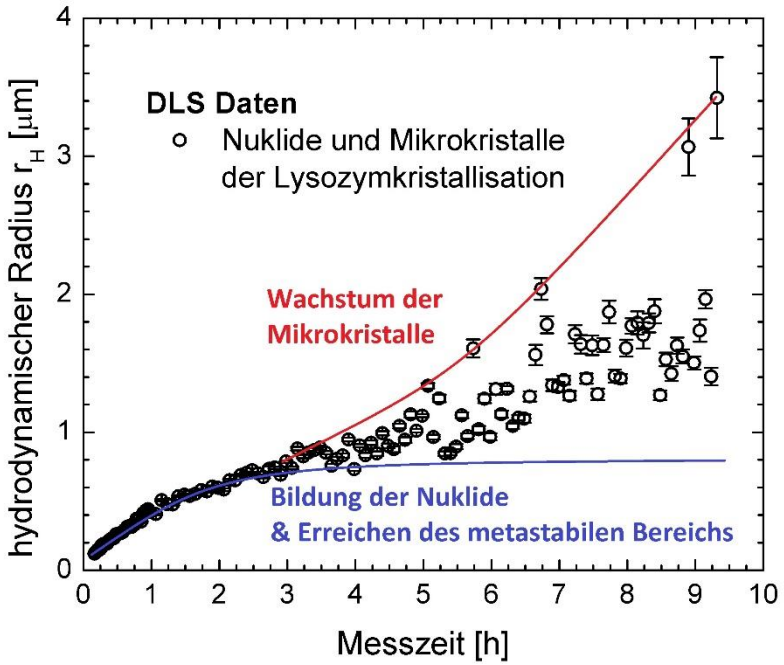
- No third particle fraction observed
- Crystals grow larger in size as at 294.5 K





Long term observation of the crystallisation process with DLS





T= 298 K

- In the beginning we have two particle fractions
- After three hours the sample is not ergodic any more: Large size fluctuations in the larger size fraction is observed

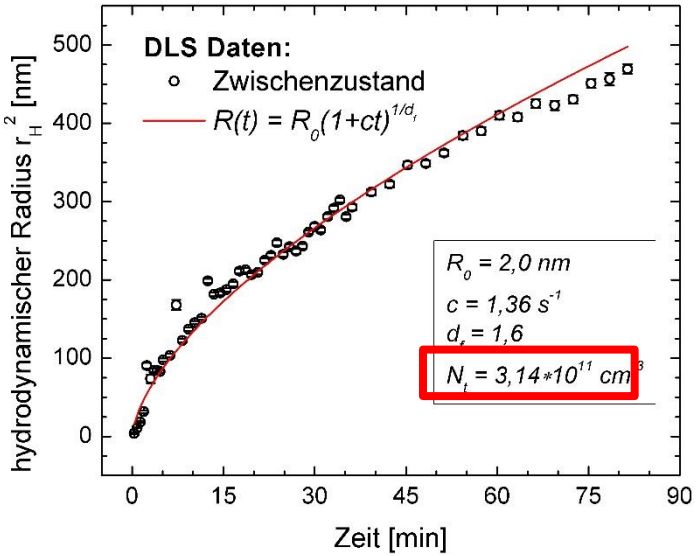
- Interpretation: Small crystals diffuse through the observation volume

Small angle scattering signal can be calculated using a model fit of the DLS data

Volume of the crystal nucleus

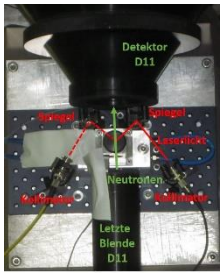
$$\frac{d\Sigma}{d\Omega}(q) = \frac{N_t}{V} * (\Delta\rho)^2 * V_p^2$$

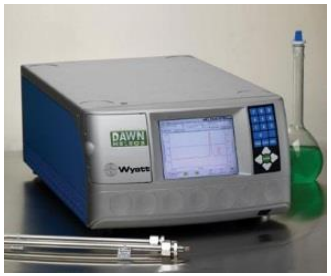
Scattering contrast of lysozyme



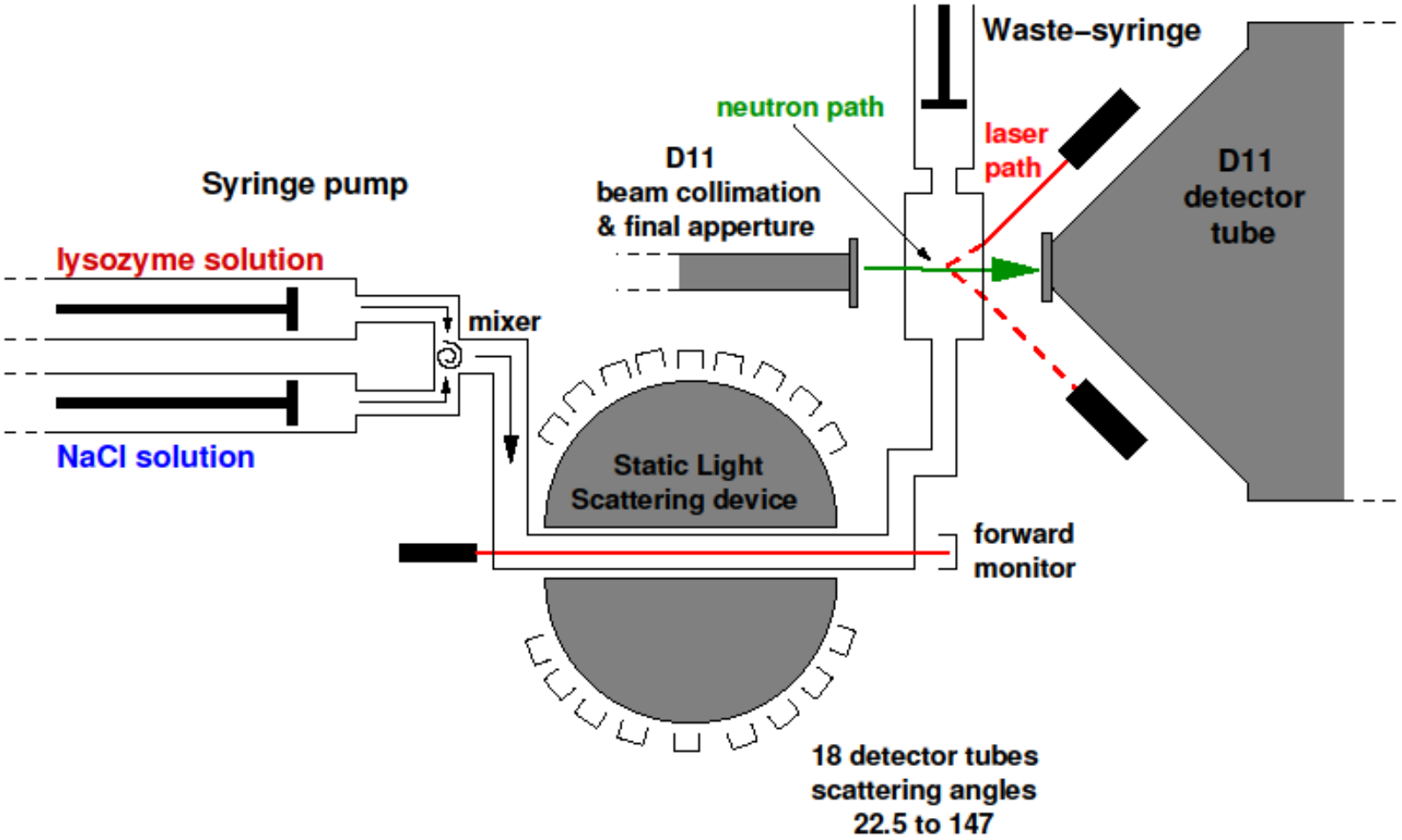
In-situ experiments at the instrument D11

Time resolved structural information on the Lysozyme crystallization: In-situ DLS and quasi-in-situ SLS together with mit Small angle neutron scattering (SANS)

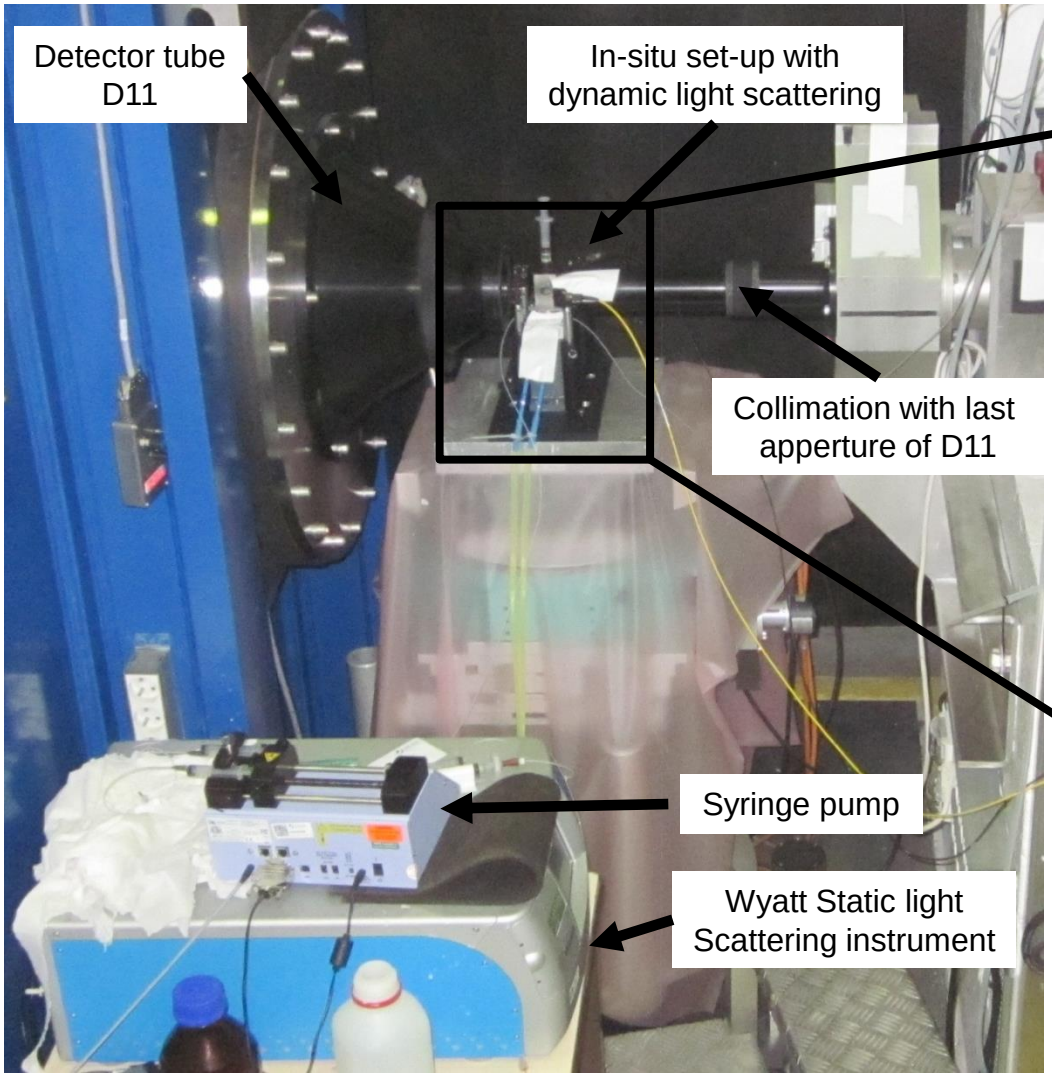


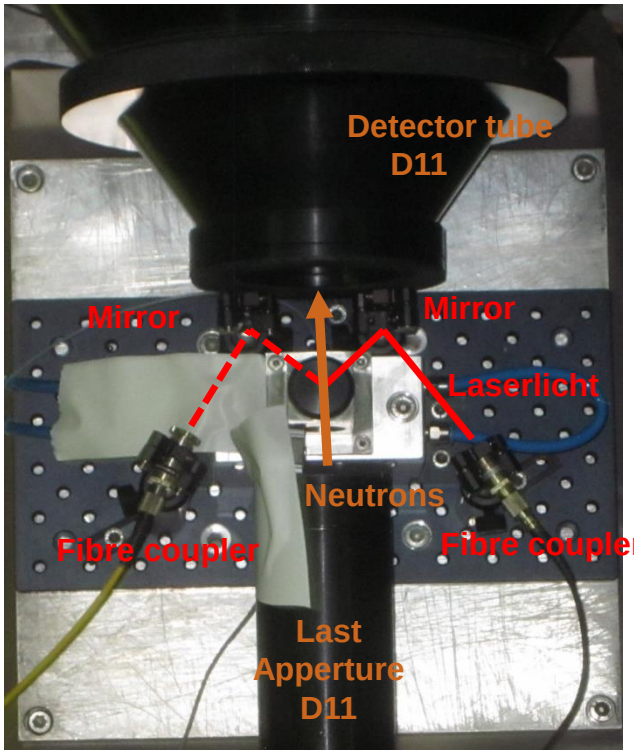


Scheme of the set-up



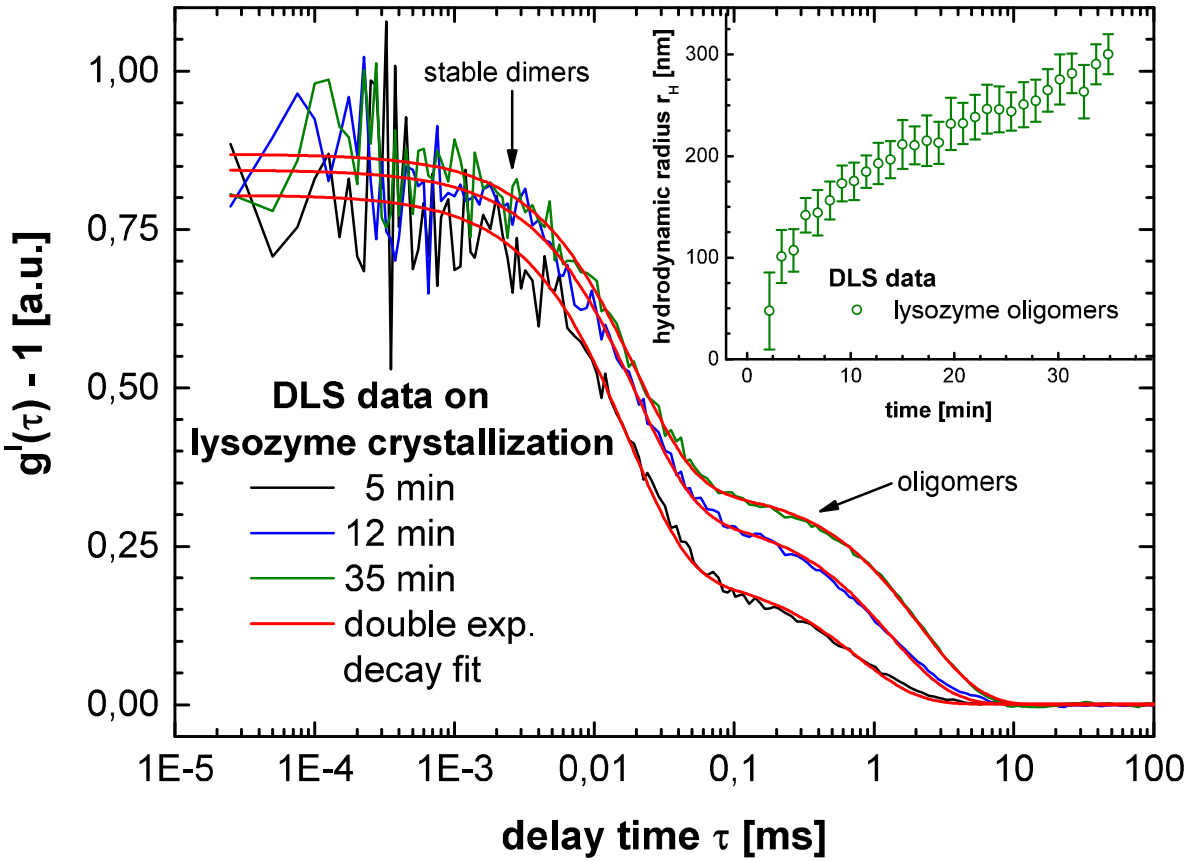
Picture of the set-up at D11





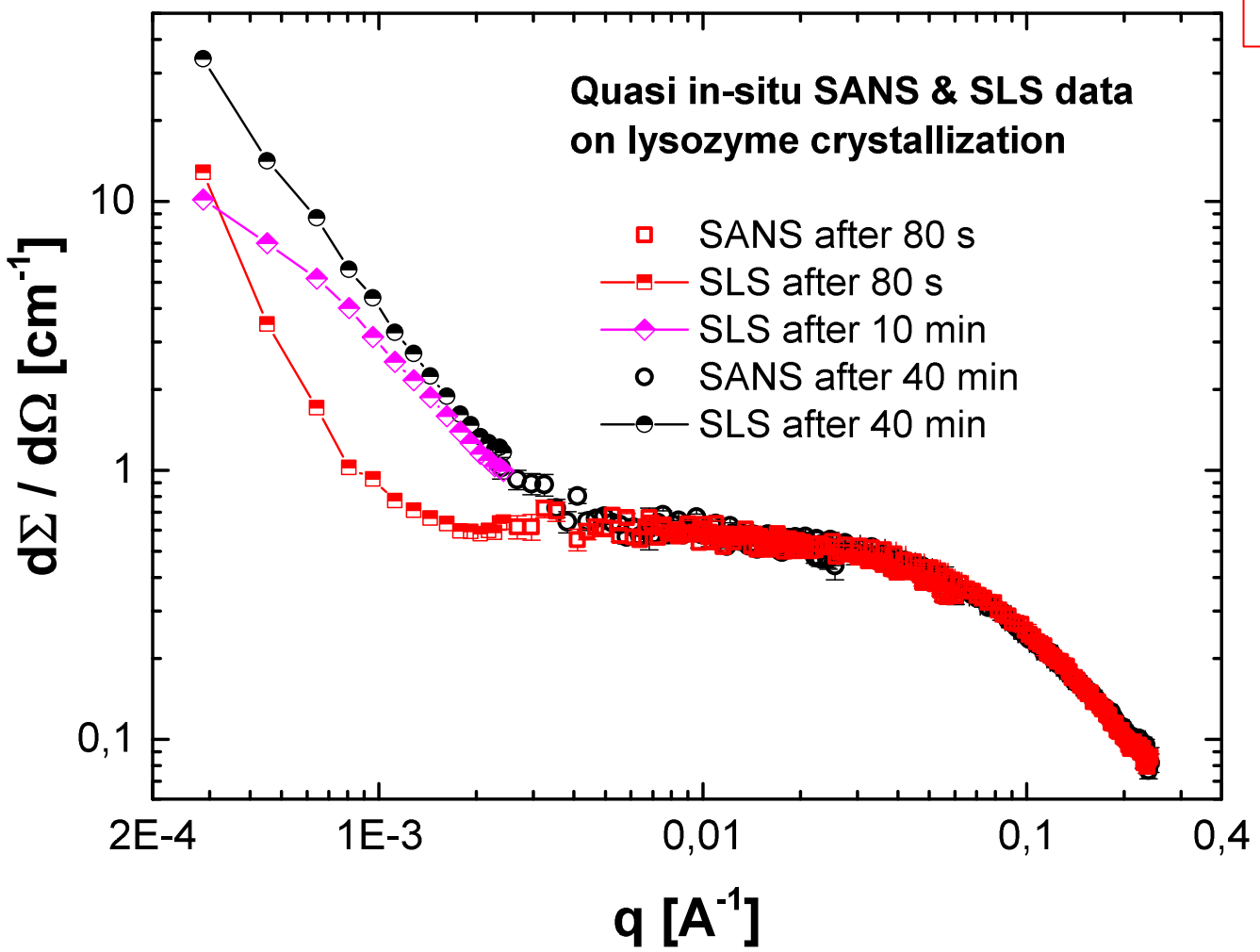
DLS-data recorded in-situ at D11

T= 298 K

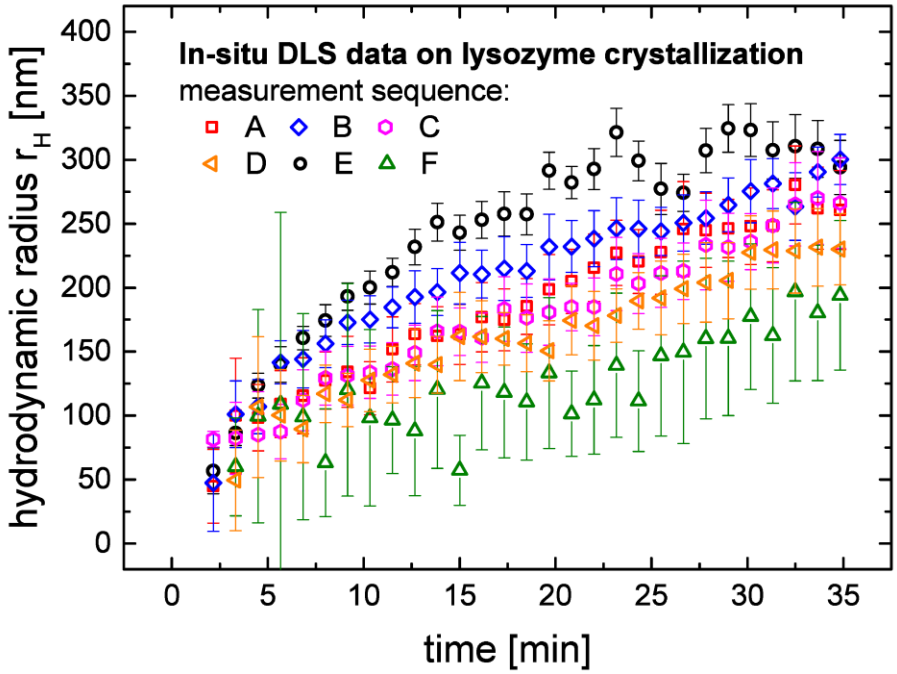


SANS + SLS

T= 298 K



On the reproducibility of the crystallisation runs



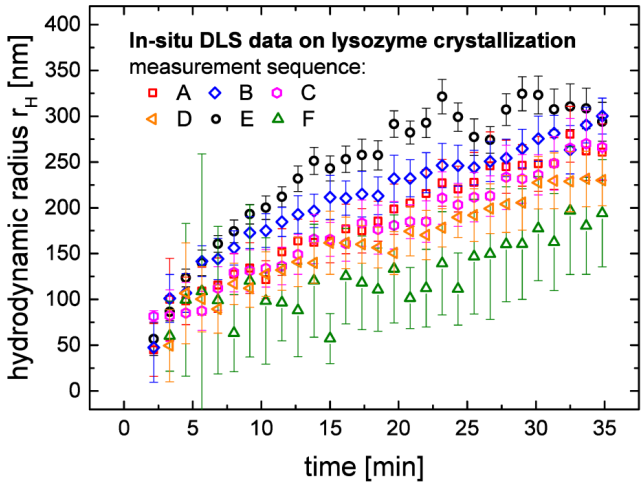
Differences in the speed of the Crystallisation process:

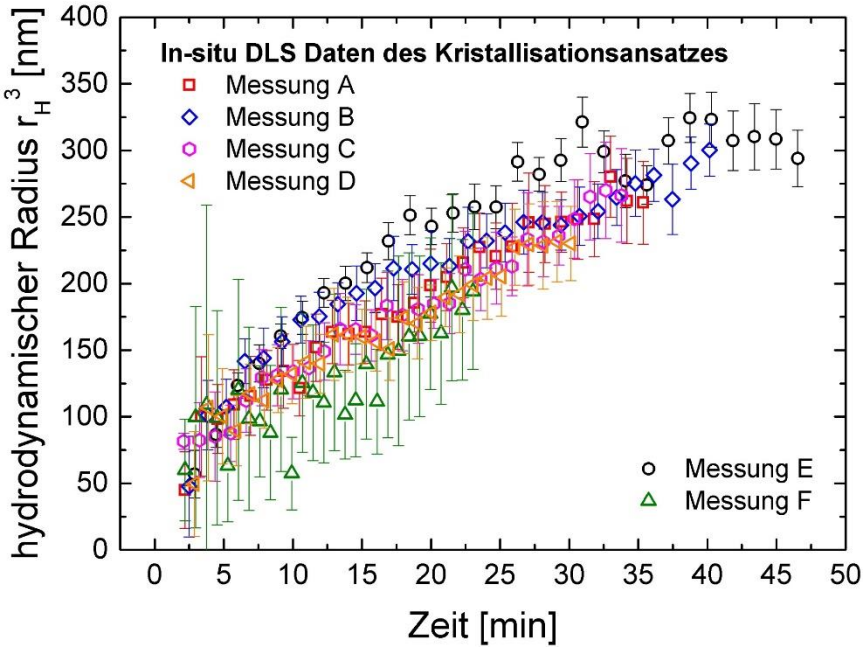
- Possible reasons are fluctuations of the temperature in the vicinity of the sample cell

➢ Scaling factor necessary to account for the differences

T= 298 K

Reproducibility of the results



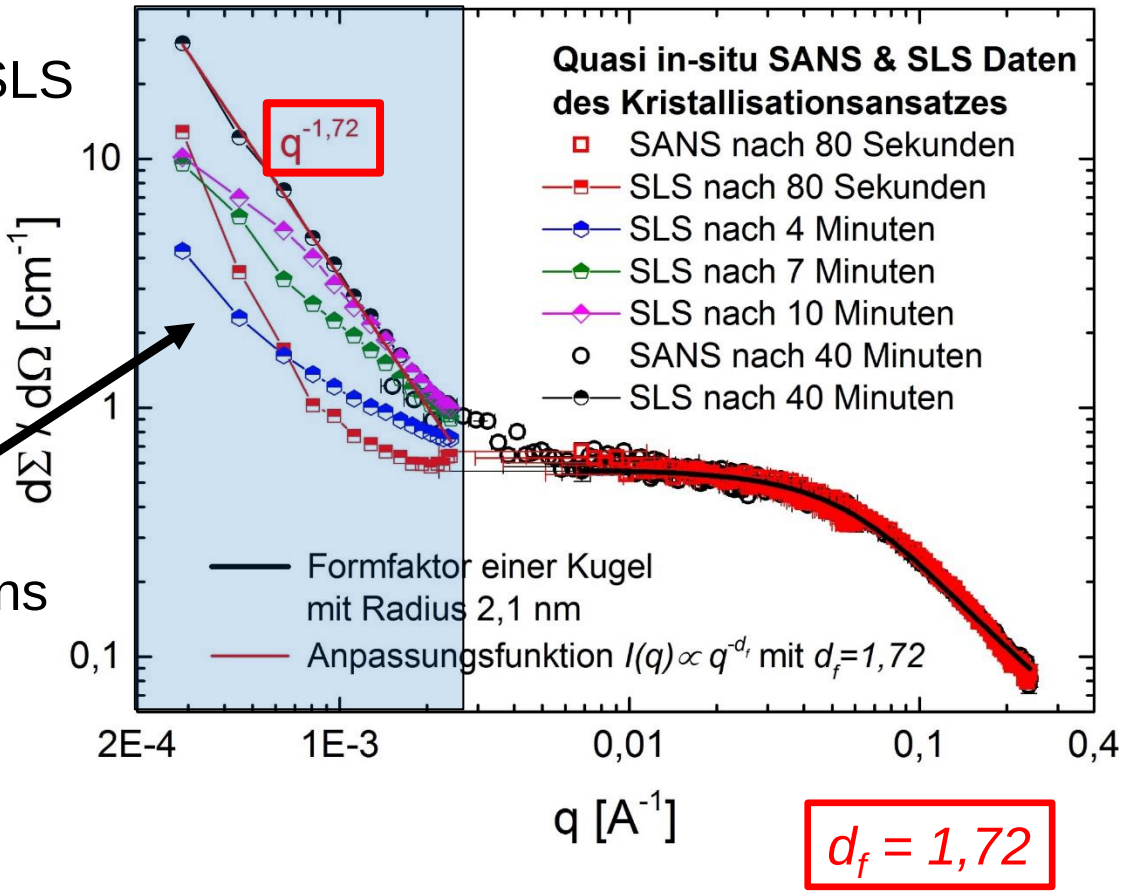


- A scaling factor can be determined to correct for tiny differences in crystallisation speed

T= 298 K

Results of the SANS and SLS measurements at 298 K

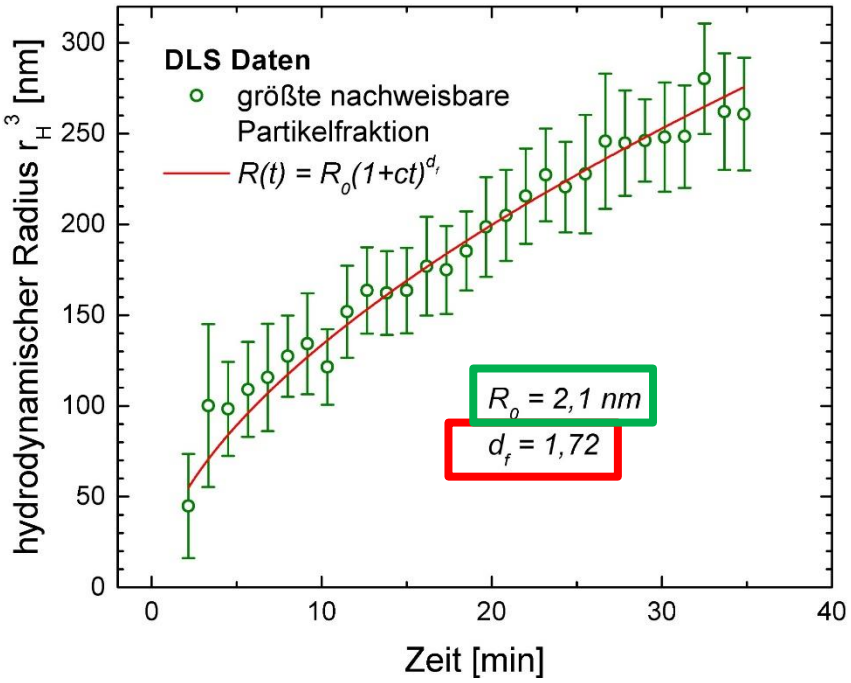
- Extended q-range due to SLS
- temporal evolution of the structure of the lysozyme nuclei can be followed
- Change of fractal dimensions observed

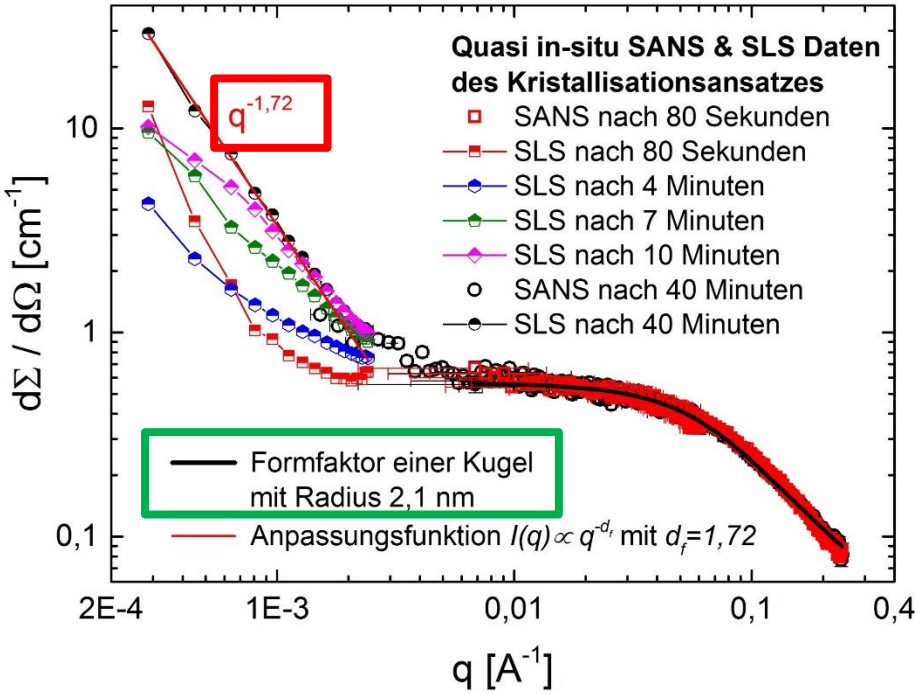


$d_f = 1,72$

T= 298 K

Agreement of SLS/SANS data with in-situ DLS data at 298 K



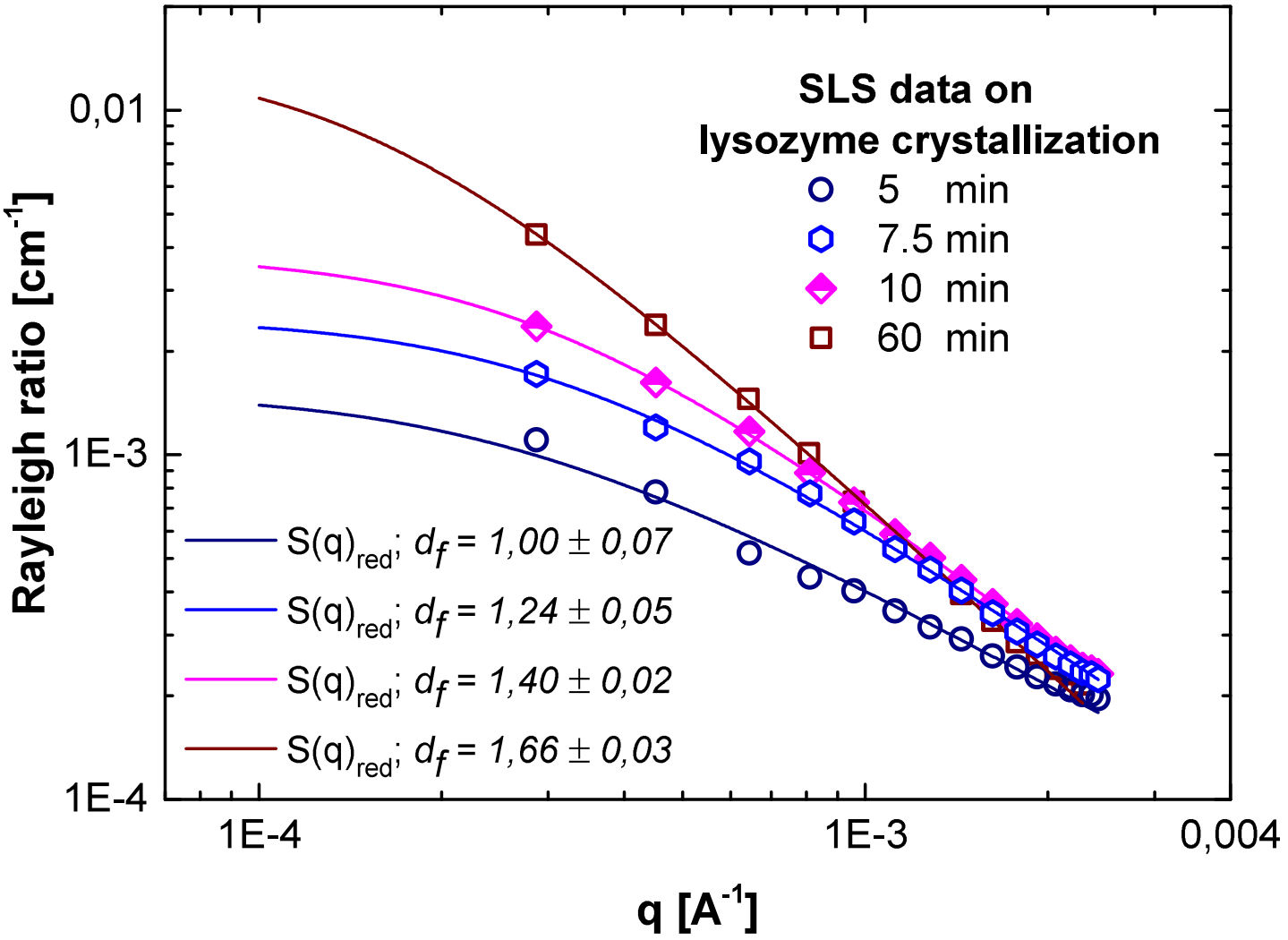


- Agreement of fractal dimension at 40 min. d_f
- *Fixed parameter* R_0 from SANS used for the model fit of the DLS data
- Verification of the diffusion limited aggregation model

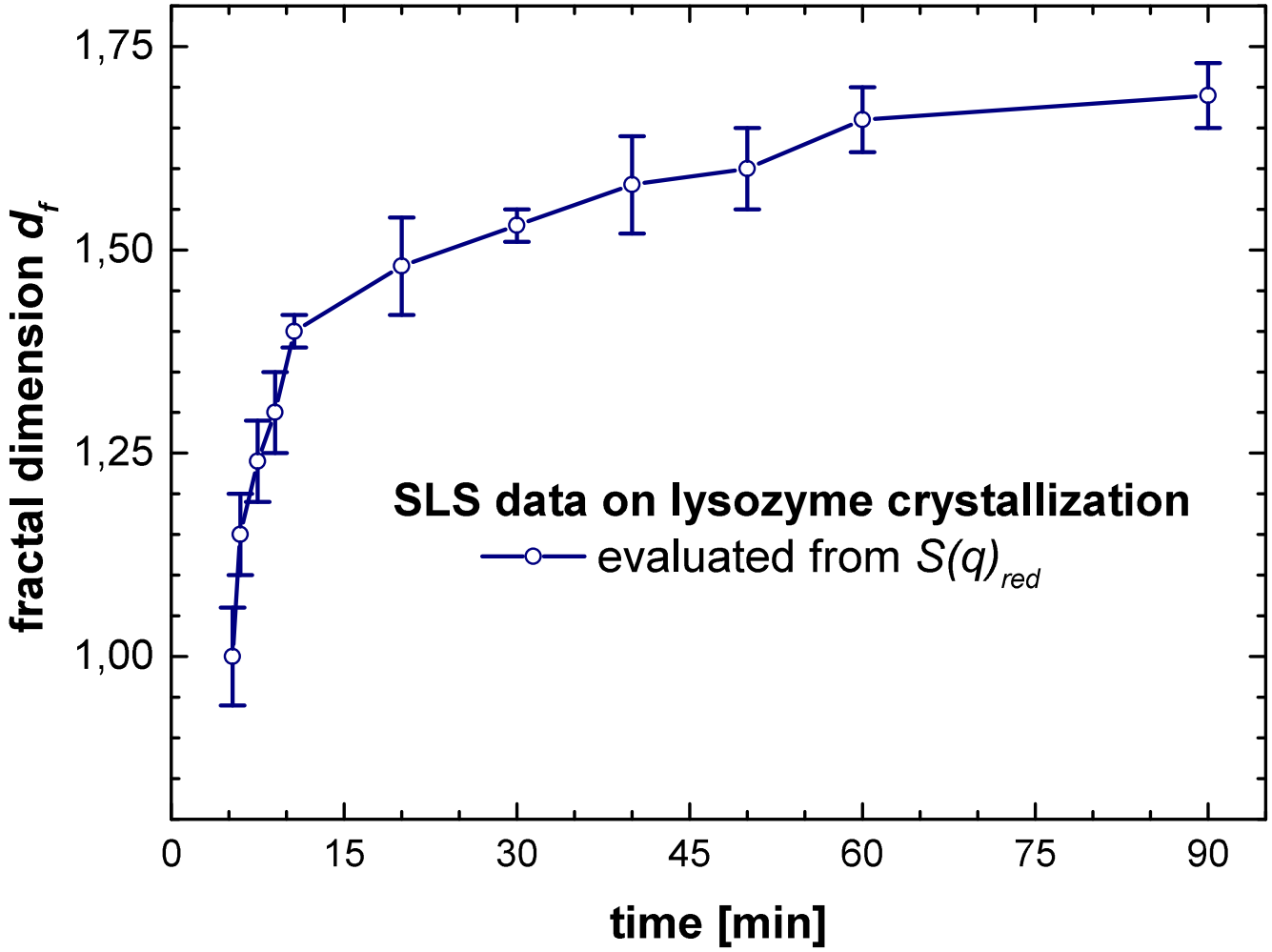
d_f = 1,72

T= 298 K

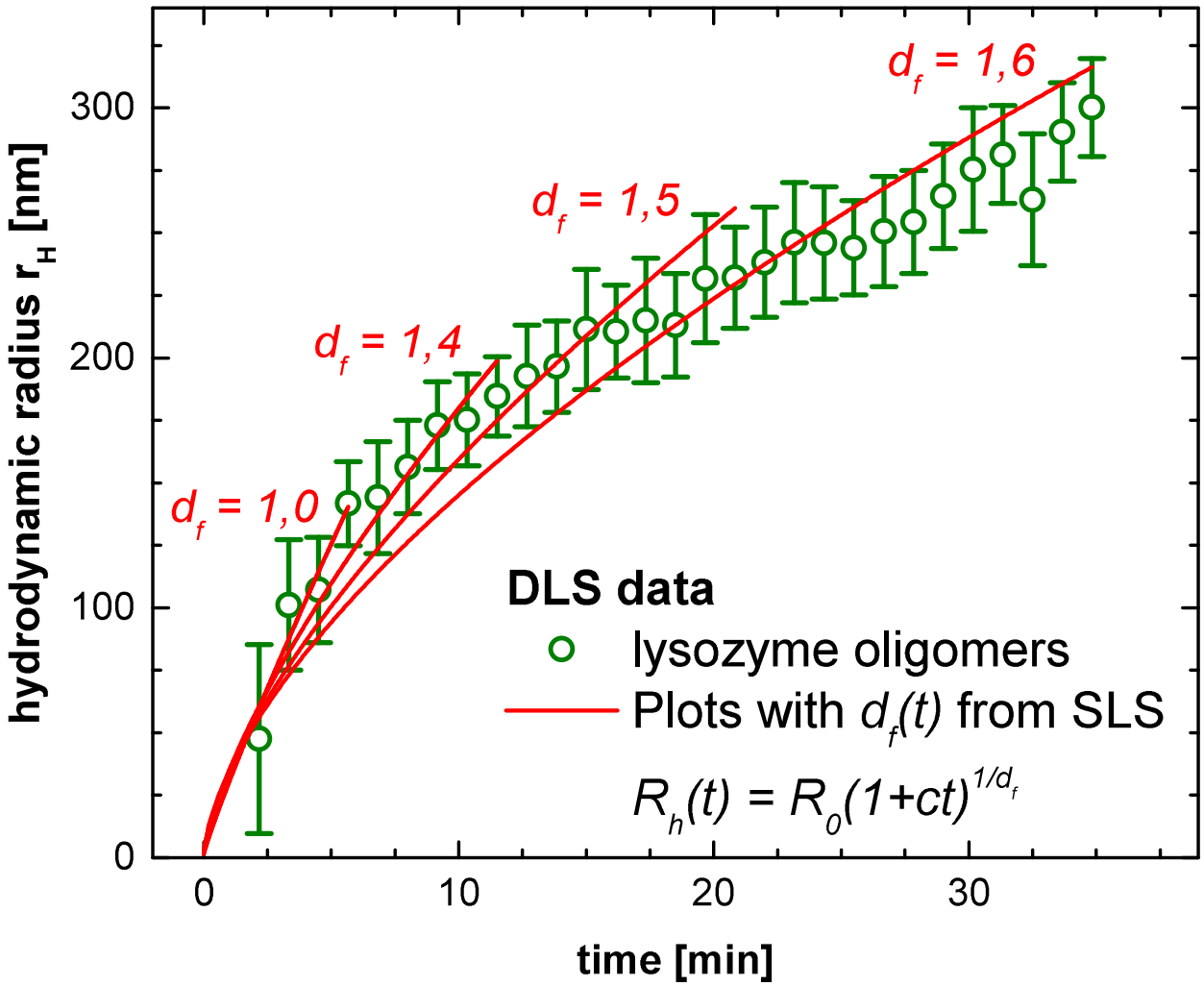
Just the SLS data is needed for fitting the fractal dimension



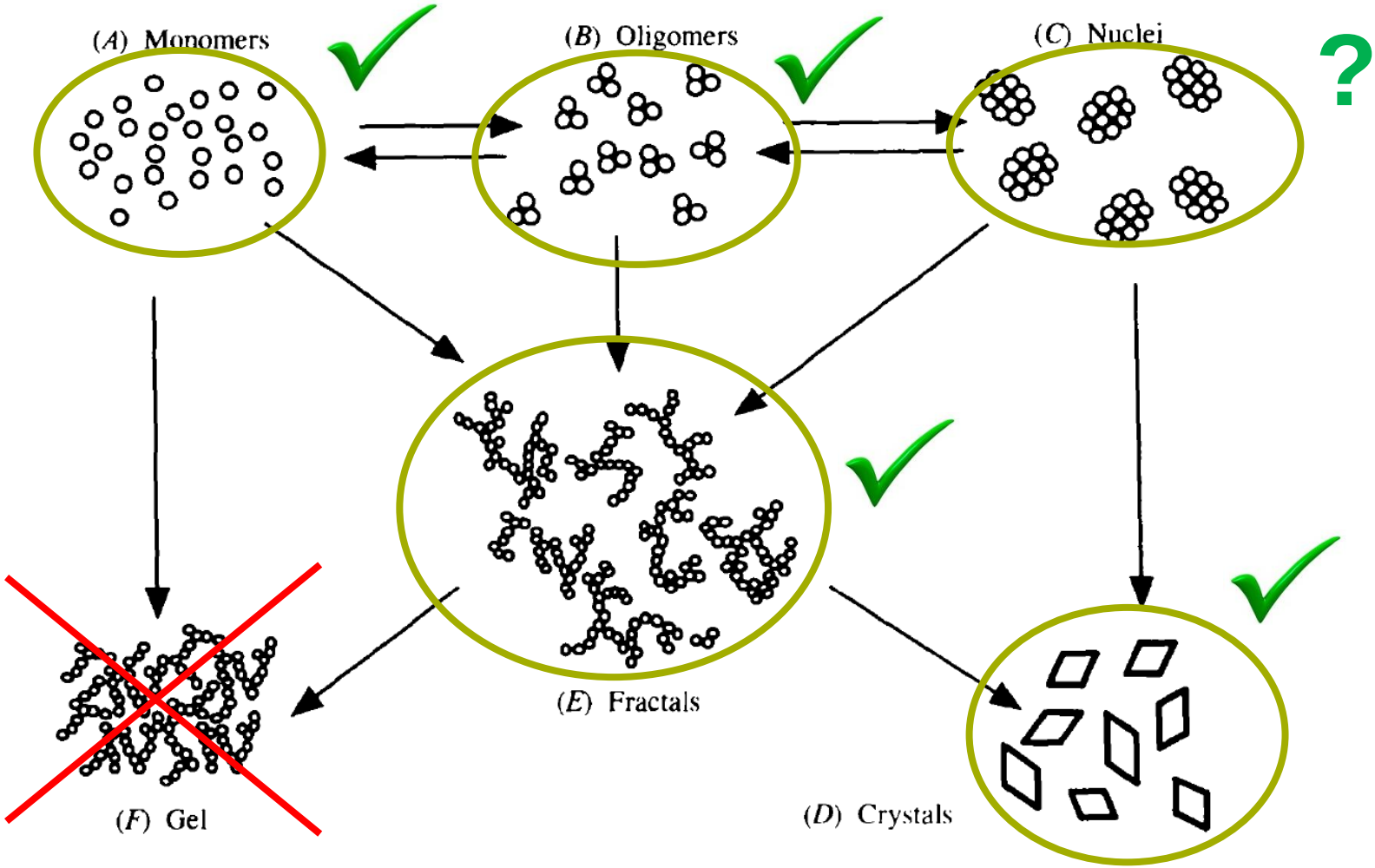
Change of fractal dimension



Agreement of the changing fractal dimension with the DLS data

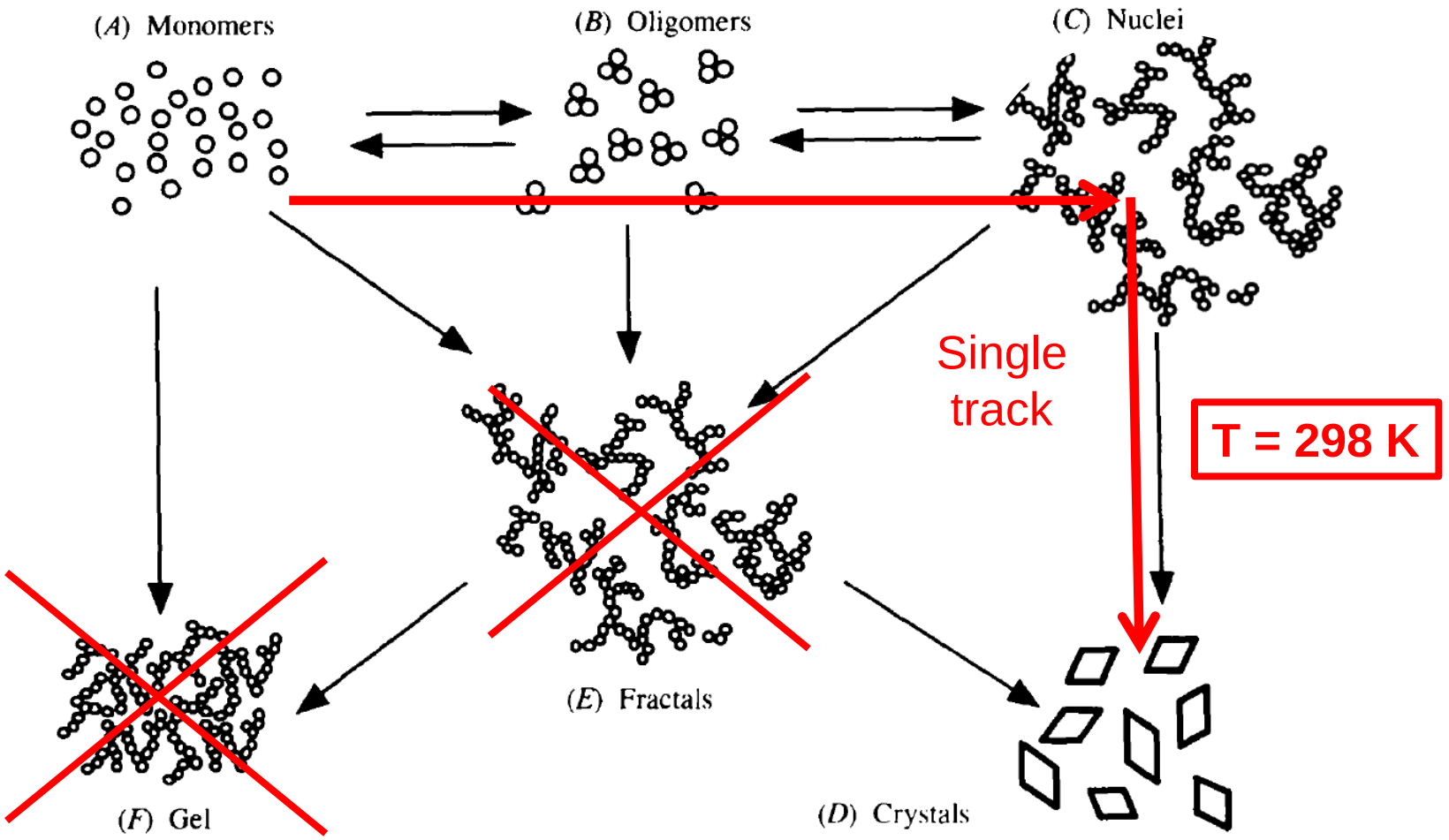


Model for the crysallization process



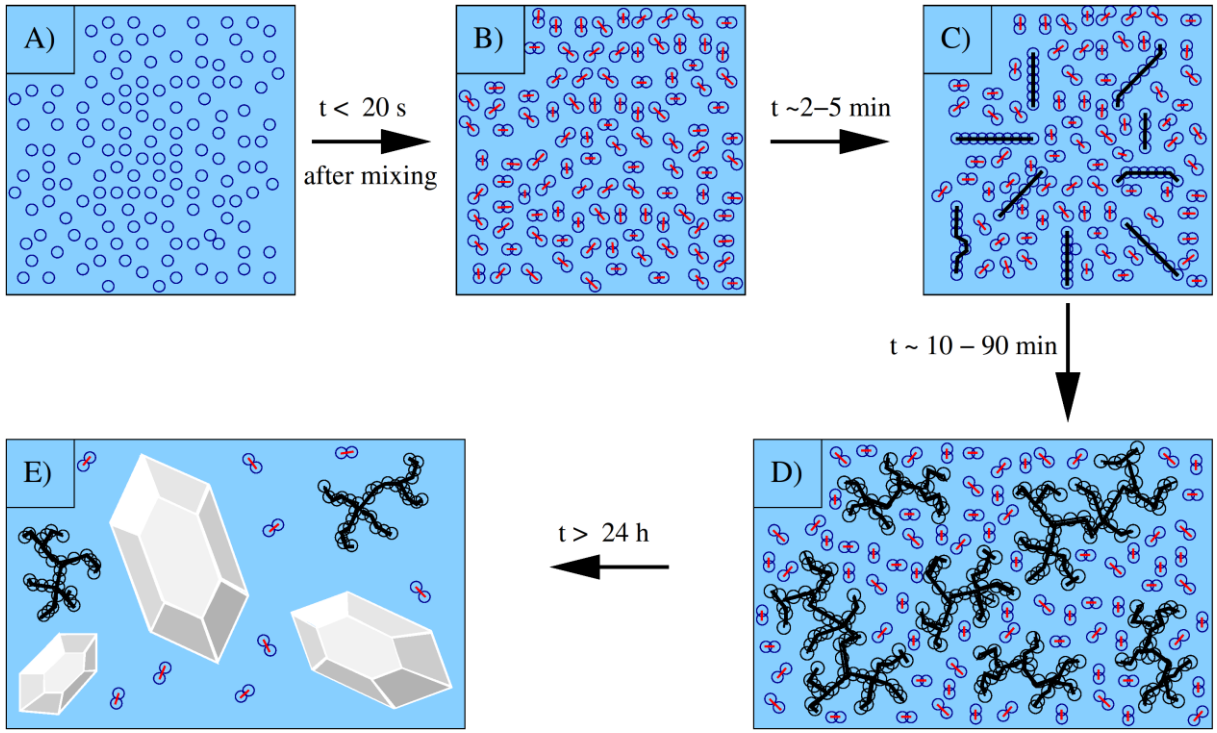
Y. Georgalis, P. Umbach, J. Raptis and Wolfram Saenger, Acta Cryst. 53 (1997) 703-712

Model for the crysallization process



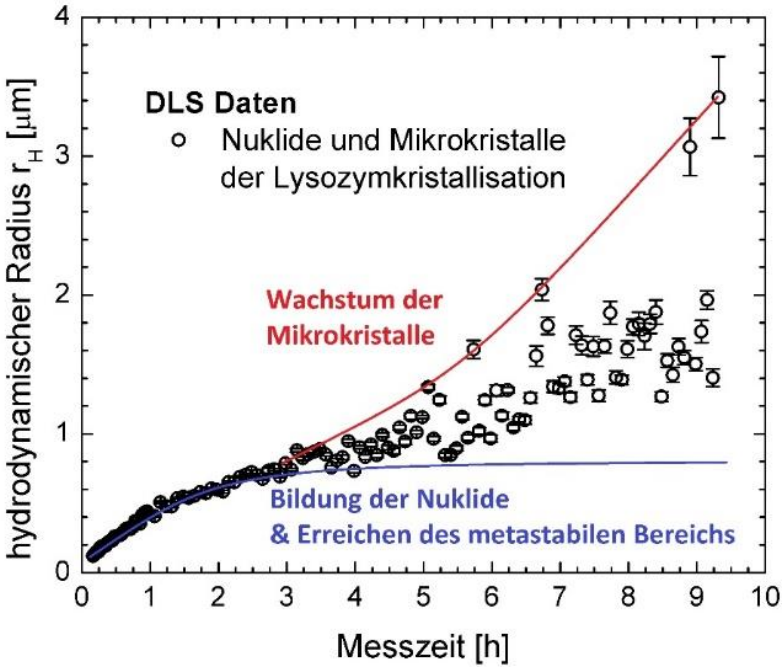
Y. Georgalis, P. Umbach, J. Raptis and Wolfram Saenger, Acta Cryst. 53 (1997) 703-712

Crystallisation at 298 K



Summary

- Lysozym dimers/ small Oligomers
  - Size constant in time
  - Concentration decreases (consumption due to crystal growth)
- Lysozyme oligomers
  - Fractal Strukture
  - Involved in crystal growth
  - Are not present at T=298 K
- Crystals
  - Growth at surfaces
  - Nucleation observed at T = 298 K
  - At the beginning: Fractal dimension with changing exponent



New Results

Differences in previous observations on the number of particle sizes resolved

- Temperature is the key parameter for different number of particle sizes observed
- The chosen method of observation also makes a difference

Successful observation of the nucleation phase with strutural information

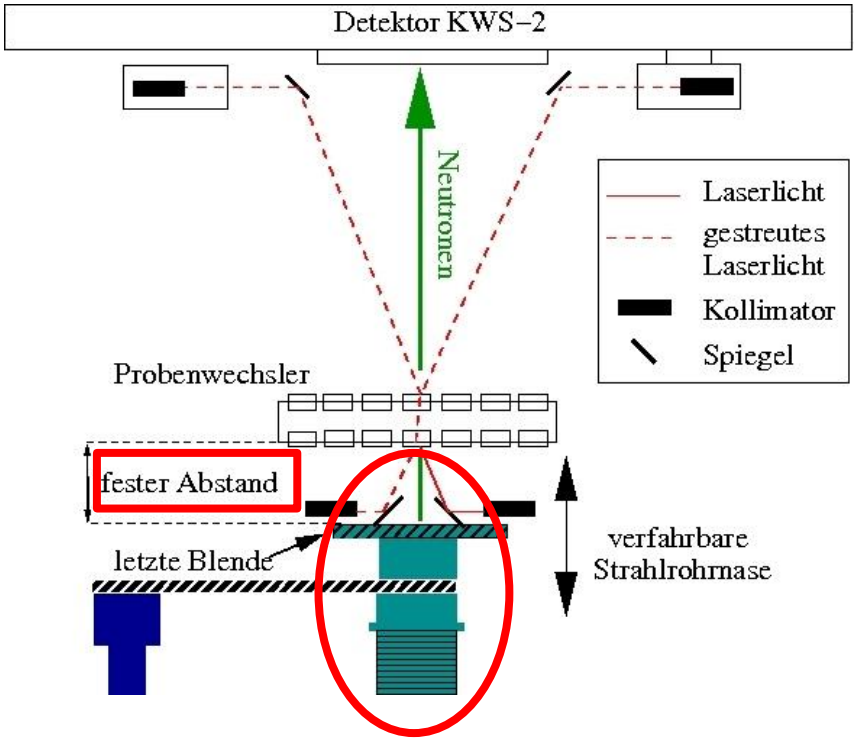
- Analysis of the speed of the nucleation process
- Not only size but also structural information gained

First successful and necessary application of the in-situ light scattering method

- With enlarged q-range
- Reproducibility checked

Outlook

- In-situ DLS at KWS-2
  - Additional scattering angles
  - Moving final apperture



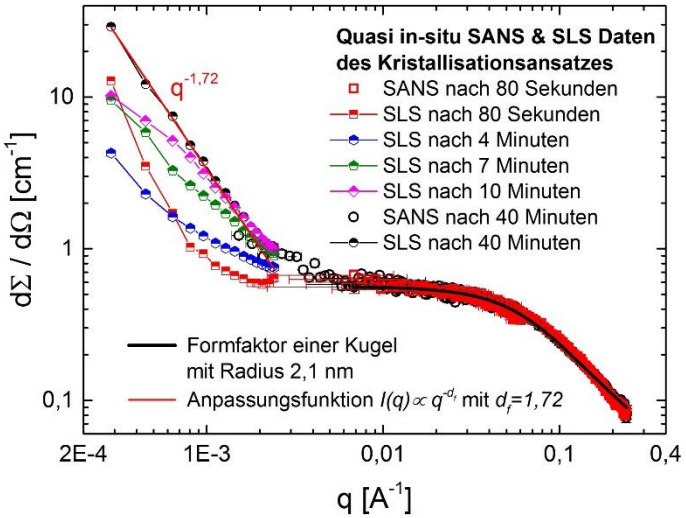
Outlook

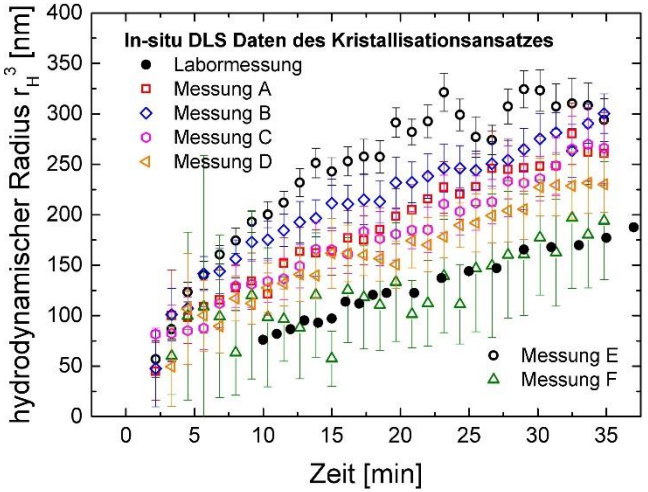
- In-situ DLS Versuche an KWS-2
  - Zusätzliche Streuwinkel
  - Verfahrbare Strahlrohrnase

- Protein crystallisation
  - Methods to increase the size of the crystals
  - Study of the nucleation process des

- Open questions

  - Informationen on the early times using a scaling factor to align the measurements, improved averaging of the neutron data
  - Kinetic model





Acknowledgements

Many thanks to... ... The D11 team:

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- David Hess
- Emanuel Kenzinger

Thank you for your attention!