Change of Fractal Dimension during the early stages of Lysozyme Crystallization

18.06.2015

Tobias E. Schrader
Motivation: For neutron protein crystallography large crystals are required

Necessary crystal size: At least 0.5 mm$^3$

• Deeper understanding of the underlying crystallization mechanism is required
Chosen crystallization conditions

- Lysozyme 60 mg/ml in D$_2$O, pH adjusted with 1M NaAc 0,02 µm filtered
- NaCl 6wt% in D$_2$O Puffer 10mM NaAc HAc 0,02 µm filtered

1:1 mixture:
Lysozyme 30 mg/ml + NaCl 3 wt% in D$_2$O 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 to characterize the sample system

- No third particle fraction observed
- Crystals grow larger in size as at 294.5 K
Long term observation of the crystallisation process with DLS

- 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.

\[
\frac{d\Sigma}{d\Omega} (q) = \frac{N_t}{V} \ast (\Delta \rho)^2 \ast V_p^2
\]

Scattering contrast of lysozyme

Volume of the crystal nucleus

DLS Daten:
- Zwischenzustand
- \( R(t) = R_0 (1 + ct)^{1/2} \)

- \( R_0 = 2,0 \text{ nm} \)
- \( c = 1,36 \text{ s}^{-1} \)
- \( d_i = 1,6 \)

- \( N_i = 3,14 \times 10^{11} \text{ cm}^{-3} \)
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

Syringe pump
lysozyme solution
NaCl solution

Static Light Scattering device

D11 beam collimation & final aperture
neutron path
laser path

Waste-syringe
D11 detector tube

forward monitor

18 detector tubes scattering angles 22.5 to 147
In-situ Messungen

Detector tube D11

In-situ set-up with dynamic light scattering

Collimation with last apperture of D11

Syringe pump

Wyatt Static light Scattering instrument

Detector tube D11

Mirror

Fibre coupler

Neutrons

Mirror

Laserlight

Fibre coupler

Last Apperture D11

Detector tube D11
DLS-data recorded in-situ at D11

\[ T = 298 \text{ K} \]
SANS + SLS

Quasi in-situ SANS & SLS data on lysozyme crystallization

- SANS after 80 s
- SLS after 80 s
- SLS after 10 min
- SANS after 40 min
- SLS after 40 min

$T = 298 \text{ K}$
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

\[ T = 298 \, \text{K} \]
Reproducibility of the results

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

\[ q^{-1,72} \]

\[ d_f = 1,72 \]

\[ T = 298 \text{ K} \]
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

$R_0 = 2.1 \text{ nm}$

$\boxed{d_f = 1.72}$

$T = 298 \text{ K}$
Just the SLS data is needed for fitting the fractal dimension
Change of fractal dimension

SLS data on lysozyme crystallization

fractal dimension $d_f$

time [min]

evaluated from $S(q)_{red}$
Agreement of the changing fractal dimension with the DLS data

\[ R_h(t) = R_0 (1+ct)^{1/d_f} \]

DLS data
- lysozyme oligomers
- Plots with \( d_f(t) \) from SLS

\( d_f = 1.0 \)
\( d_f = 1.4 \)
\( d_f = 1.5 \)
\( d_f = 1.6 \)
Model for the crystallization process

(A) Monomers

(B) Oligomers

(C) Nuclei

(D) Crystals

(E) Fractals

(F) Gel

Model for the crystallization process

Crystallisation at 298 K

A) t < 20 s after mixing

B) t ~2–5 min

C) t ~ 10 – 90 min

E) t > 24 h

D)
Summary

- Lysozym dimers/ small Oligomers
  - Size constant in time
  - Concentration decreases (consumption due to crystal growth)

- Lysozyme oligomers
  - Fractal Strukture
  - Involved in crystal growth

- Crystals
  - Growth at surfaces
  - Nucleation observed at T = 298 K
  - At the beginning: Fractal dimension with changing exponent
In-situ DLS at KWS-2

- Additional scattering angles
- Moving final apperture
Acknowledgements

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

- Raimund Heigl
- Dieter Richter
- Simon Staringer
- Ralf Biehl
- Aurel Radulescu
- Jörg Stellbrink
- Andreas Ostermann

- Ralf Schweins
- David Bowyer
- David Hess
- Emanuel Kenzinger

Thank you for your attention!