# Change of Fractal Dimension during the early stages of Lysozyme Crystallization

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Motivation / chosen model system

The BioDiff instrument at MLZ in Garching:
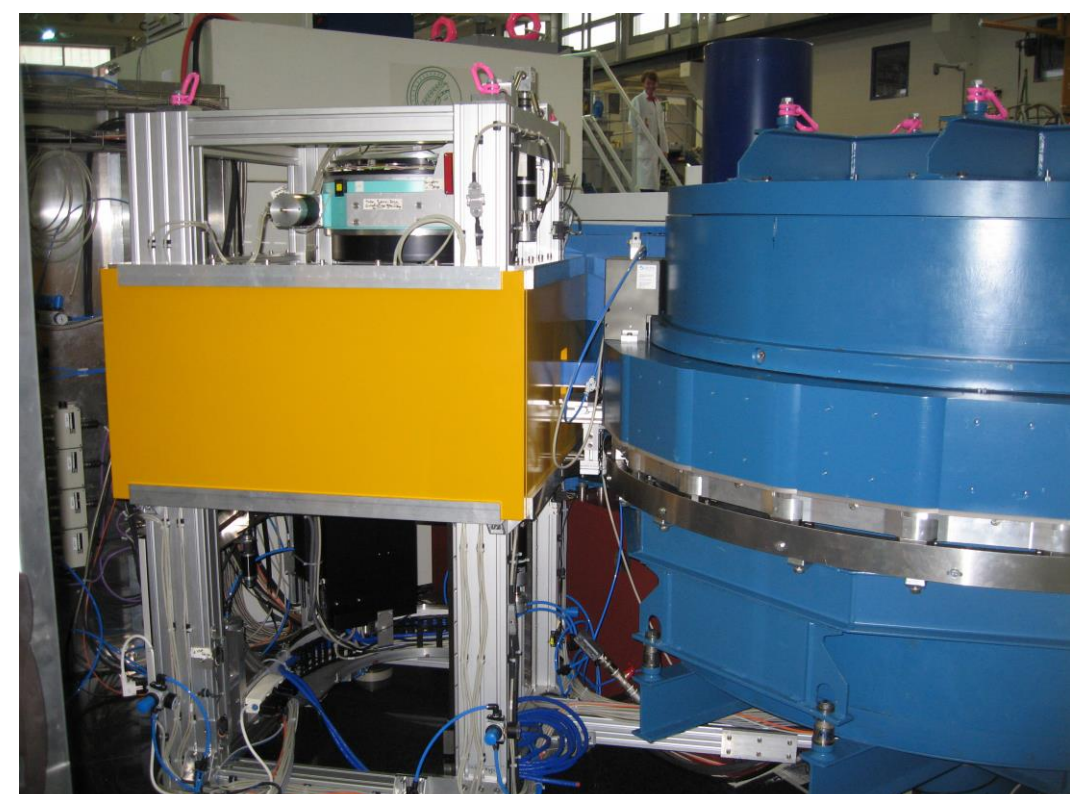
Necessary crysal size:
At least 0.5 mm^3

• Deeper understanding of the undelying crystallization mechanism is required

For a deeper understanding of the enzymatic action of some proteins, hydrogen atoms play a key role. Be it due to their involvement in a critical hydrogen bonding network or as part of a proton transport pathway. Often x-ray crystallography fails to reveal the atom positions of these important hydrogen atoms. Here, neutron protein crystallography as for example performed with the instrument BioDiff provides a solution, because neutrons scatter from nuclei and hydrogen has a similar scattering cross section as carbon or oxygen. The big drawback of this method is the need for a comparatively large protein crystal in order to obtain a decent signal. The aim of this study was to develop a microscopic picture of protein crystal growth. Especially the early phase of crystal growth decides whether many crystal seeds lead to the growth of many but small crystals or few but large crystals. The latter being disireable for neutron protein crystallography.
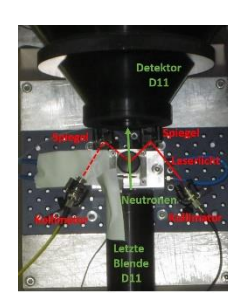
Chosen crystallization conditions

➢ 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:
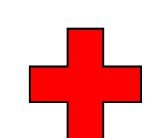Lysozyme 30 mg/ml + NaCl 3 wt% in D$_2$O buffer @ pH 4.35

Monomer size: r = 1.9 nm

crystals ca. 1 mm at T = 298 K

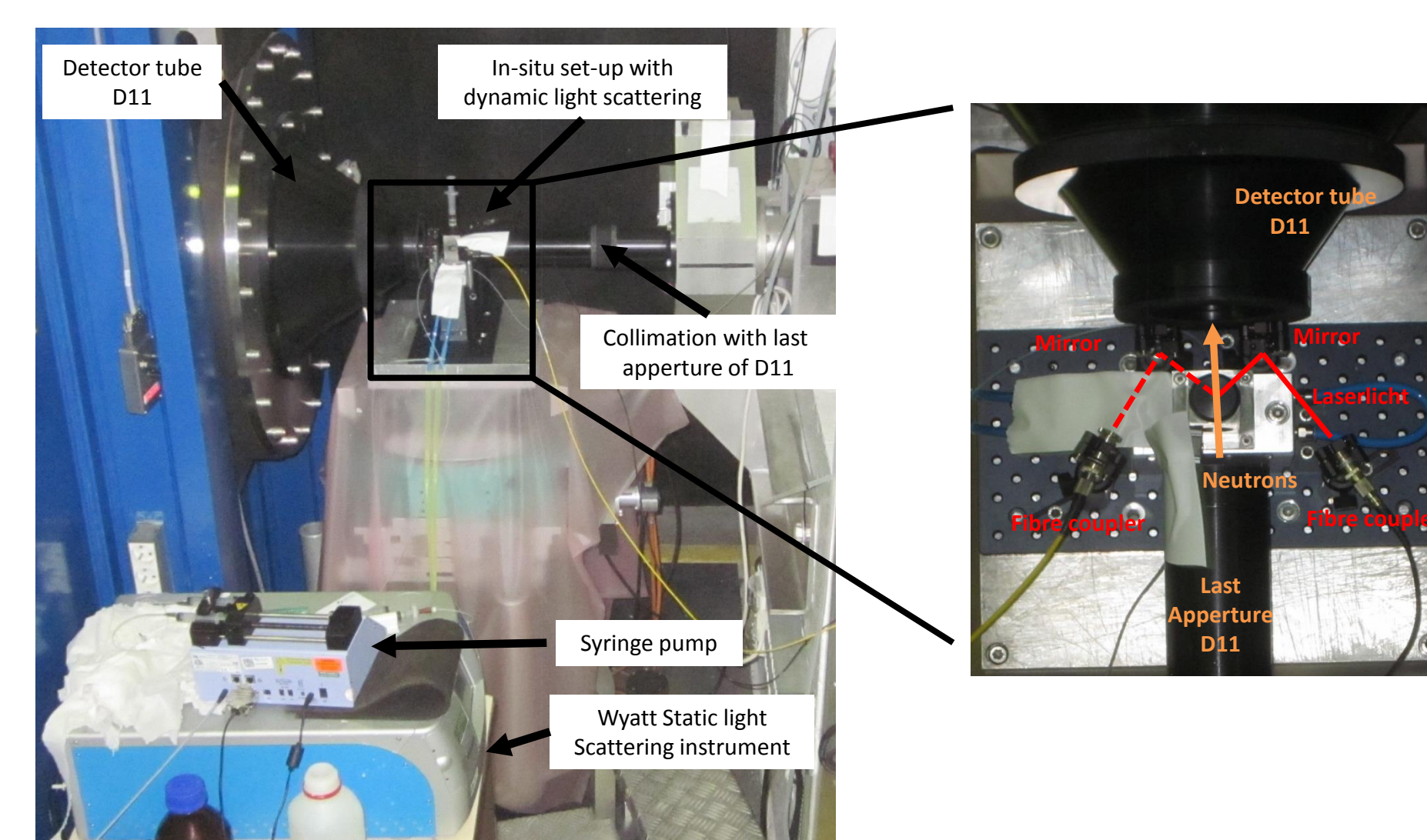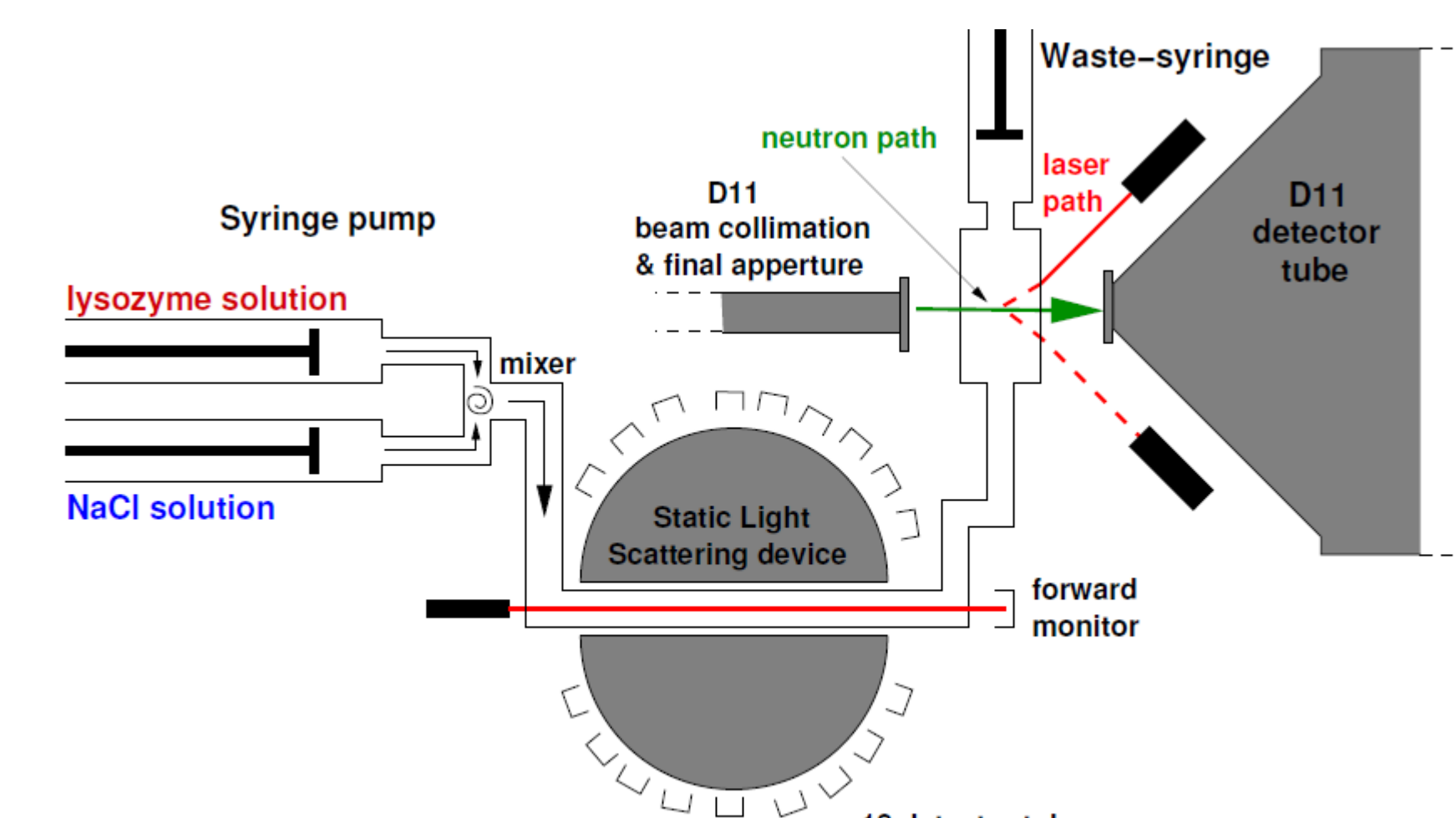crystals ca. 0.2 mm at T = 294.5 K
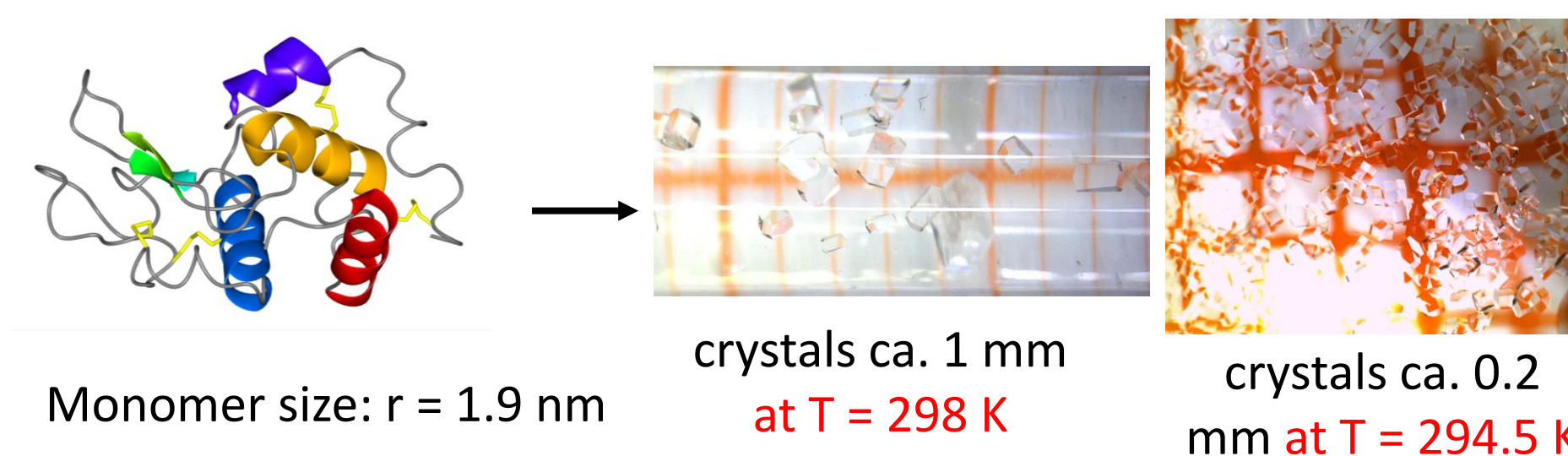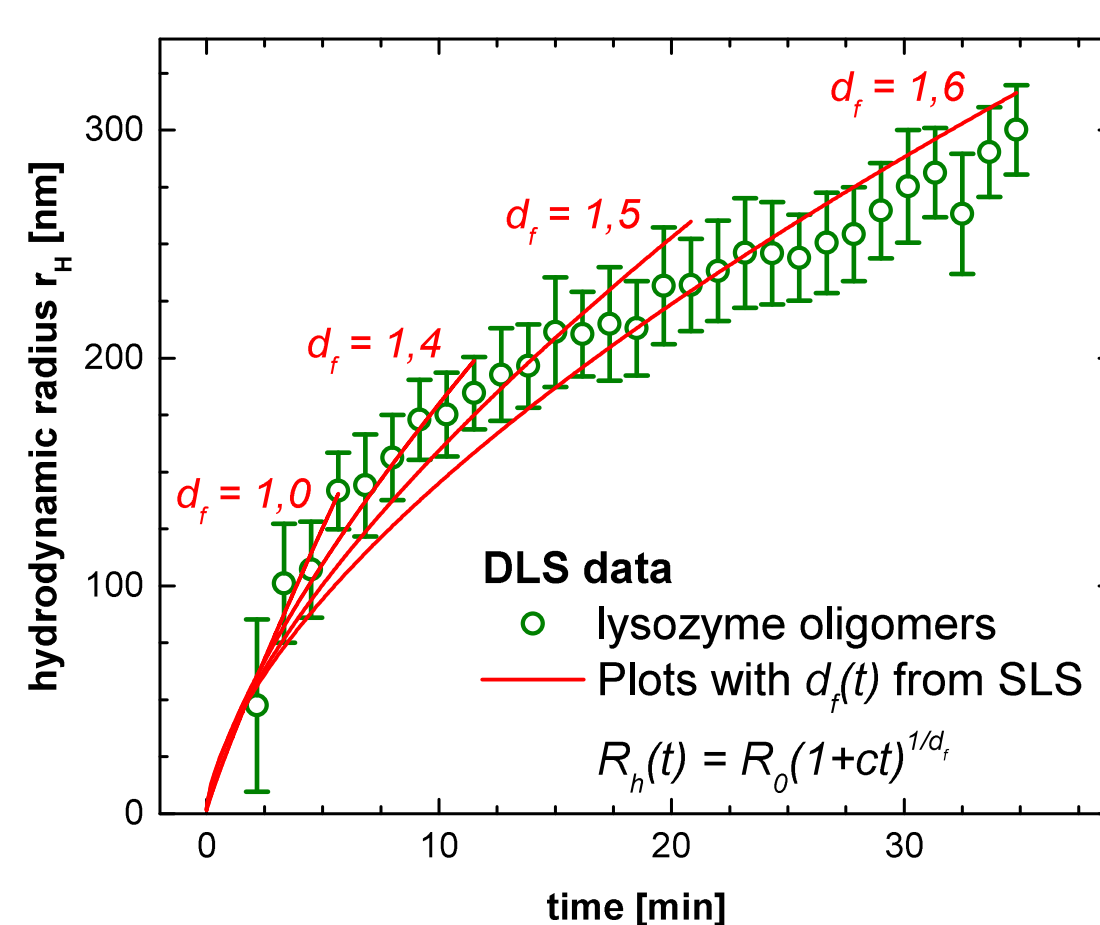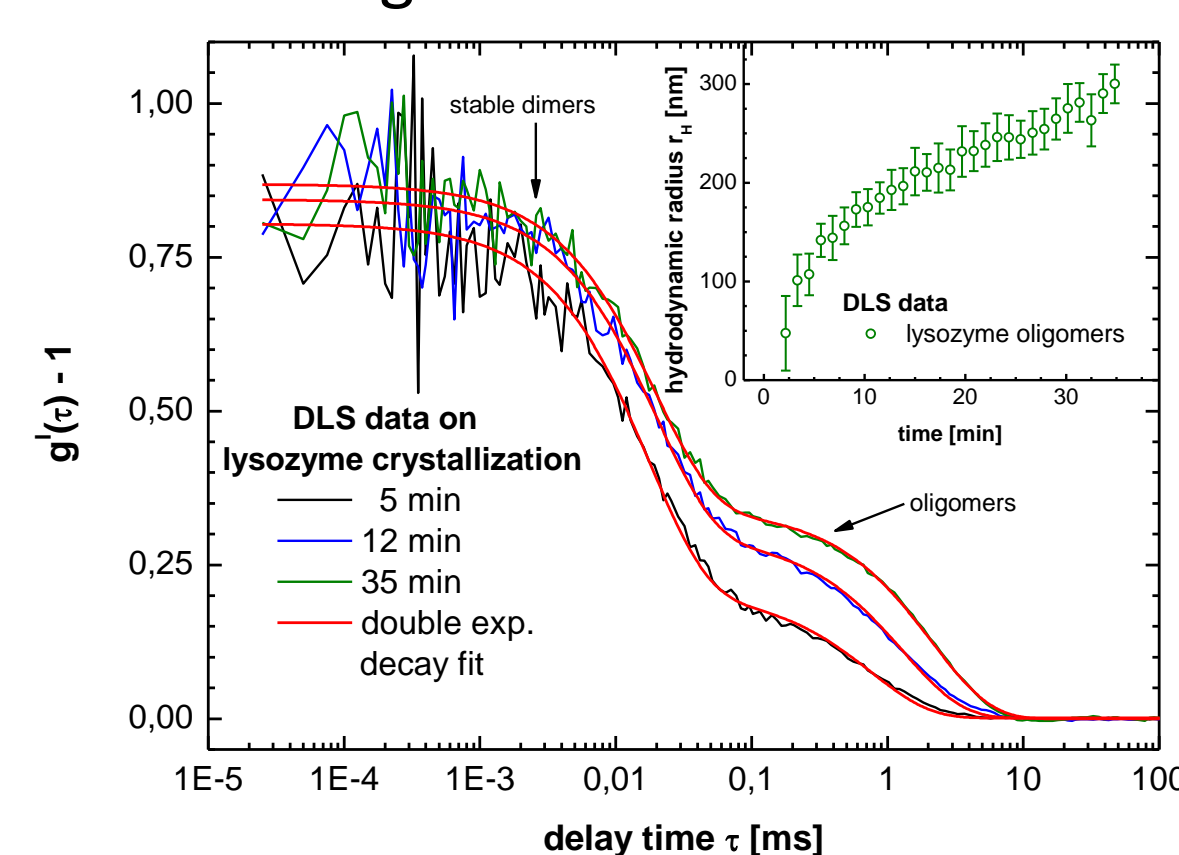
Methods

SANS   +   DLS   +   SLS

Since the crystal growth strongly depends on parameters like Temperature, pH, protein and salt concentration the reproducibility of the crystallization speed between two crystallization runs is sometimes limited. This makes it attractive to obtain as much information from one run as 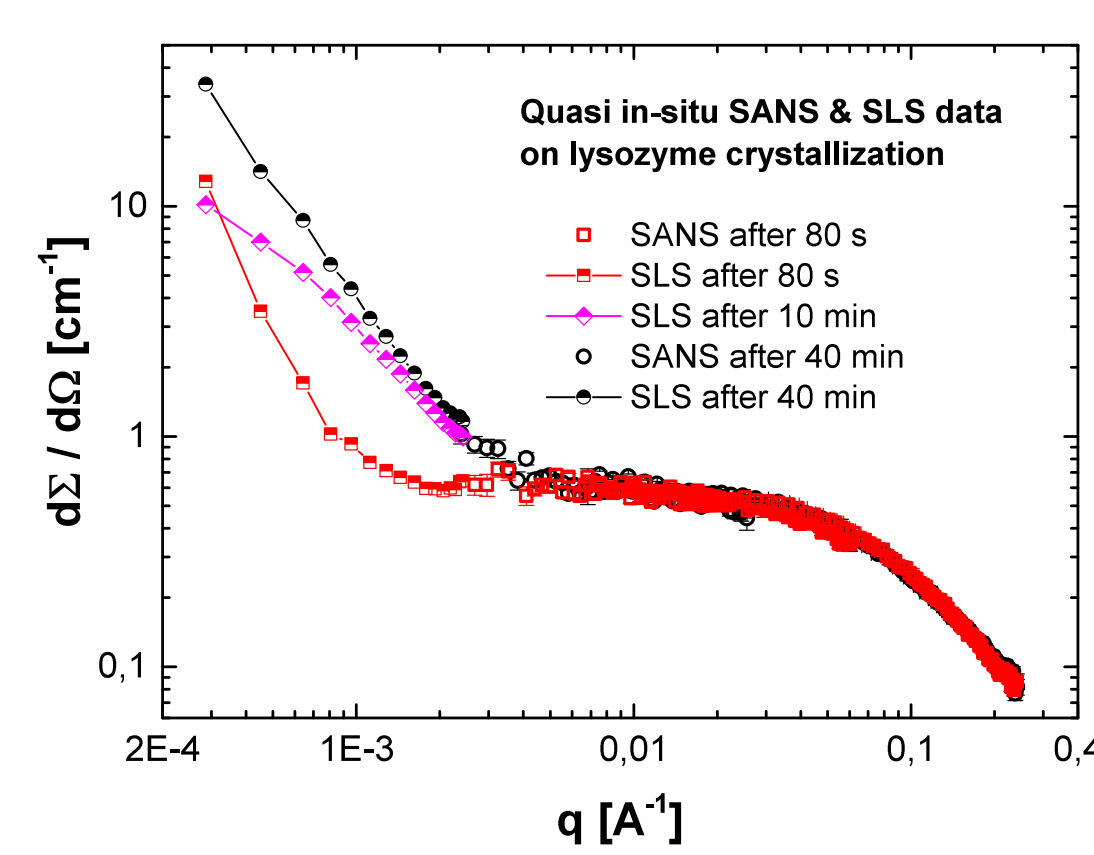possible and use this infromation to align the runs with each other. Here, the DLS data is used to align the results from the SLS and SANS curves.

Results

Results from the dynamic light scattering measurements

DLS data on lysozyme crystallization
— 5 min
— 12 min
— 35 min
— double exp. decay fit

DLS data
○ lysozyme oligomers
— Plots with $d_f(t)$ from SLS
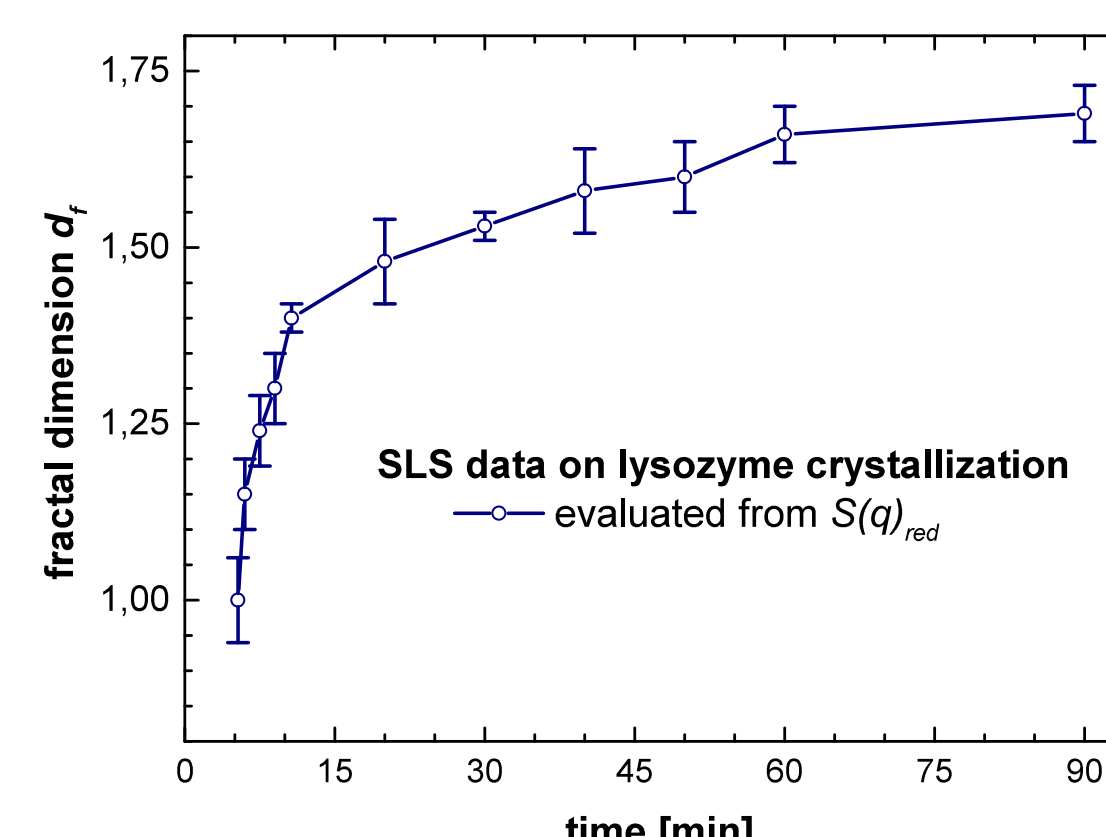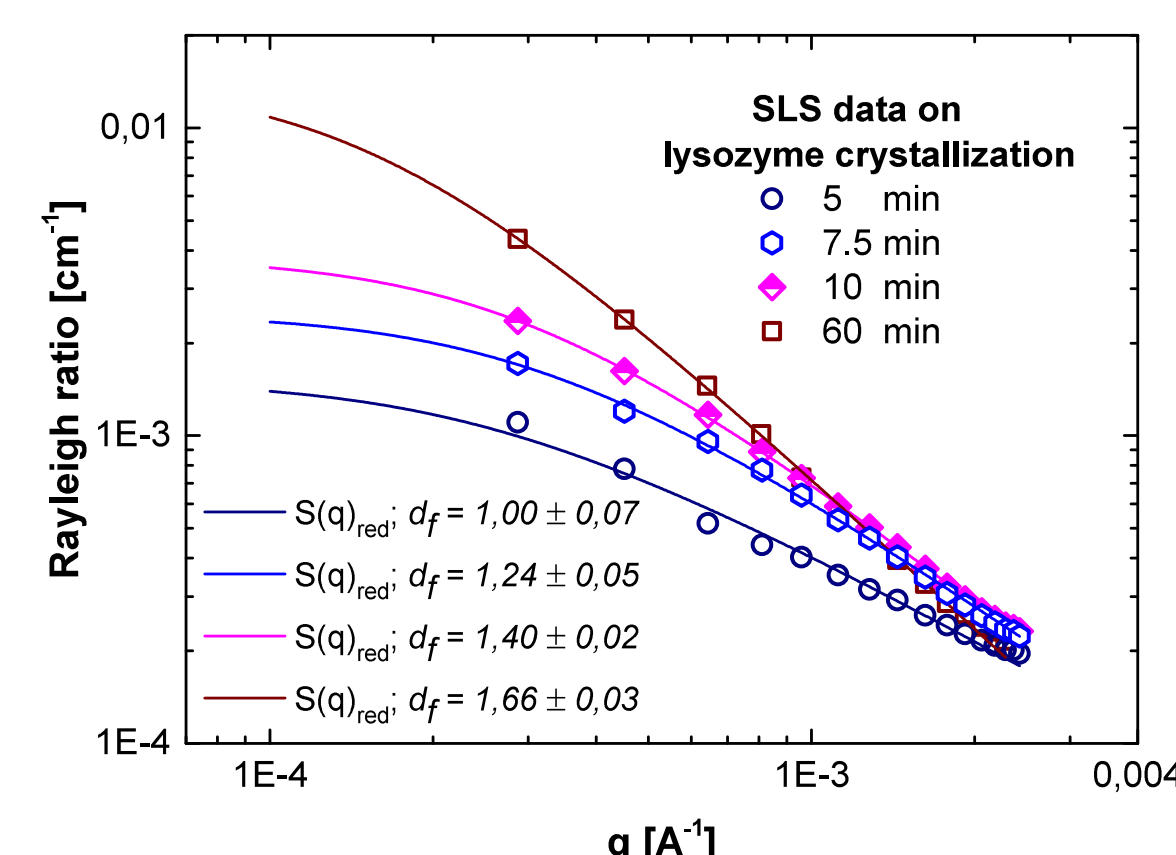$R_h(t) = R_0 (1+ct)^{1/d_f}$

$d_f = 1,6$
$d_f = 1,5$
$d_f = 1,4$
$d_f = 1,0$

Combination of static light scattering with Small angle neutron scattering curves

Quasi in-situ SANS & SLS data on lysozyme crystallization
□ SANS after 80 s
○ SLS after 80 s
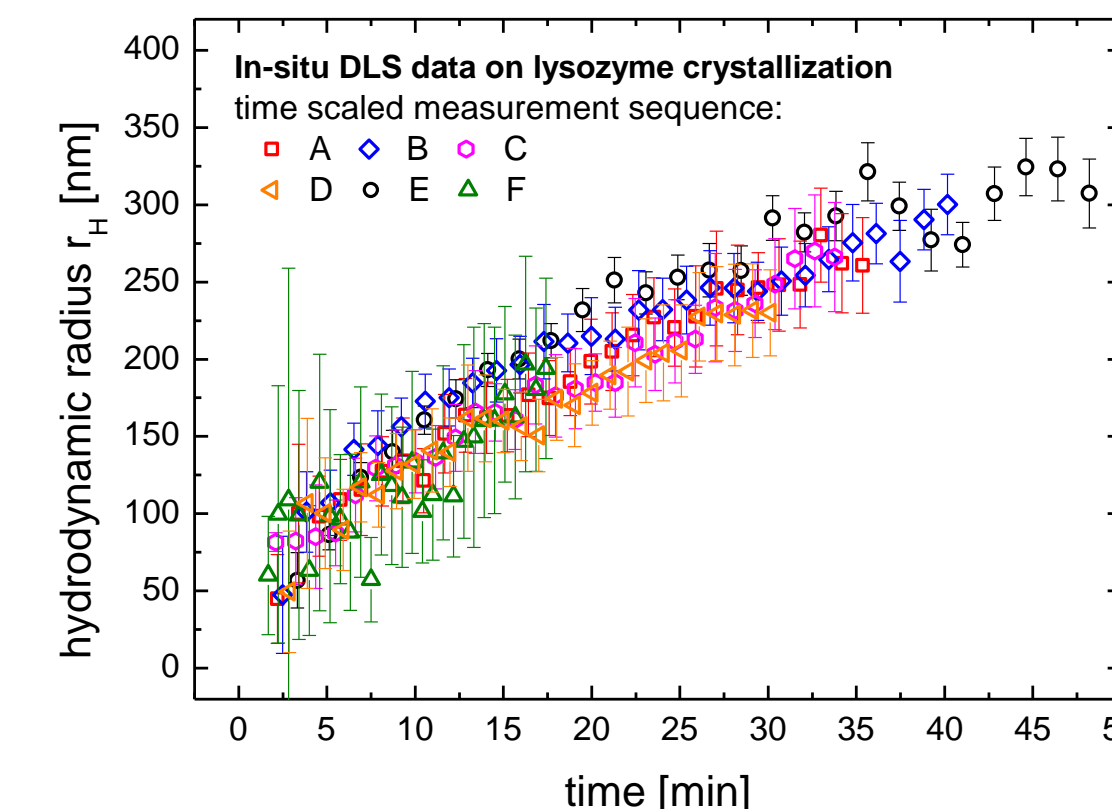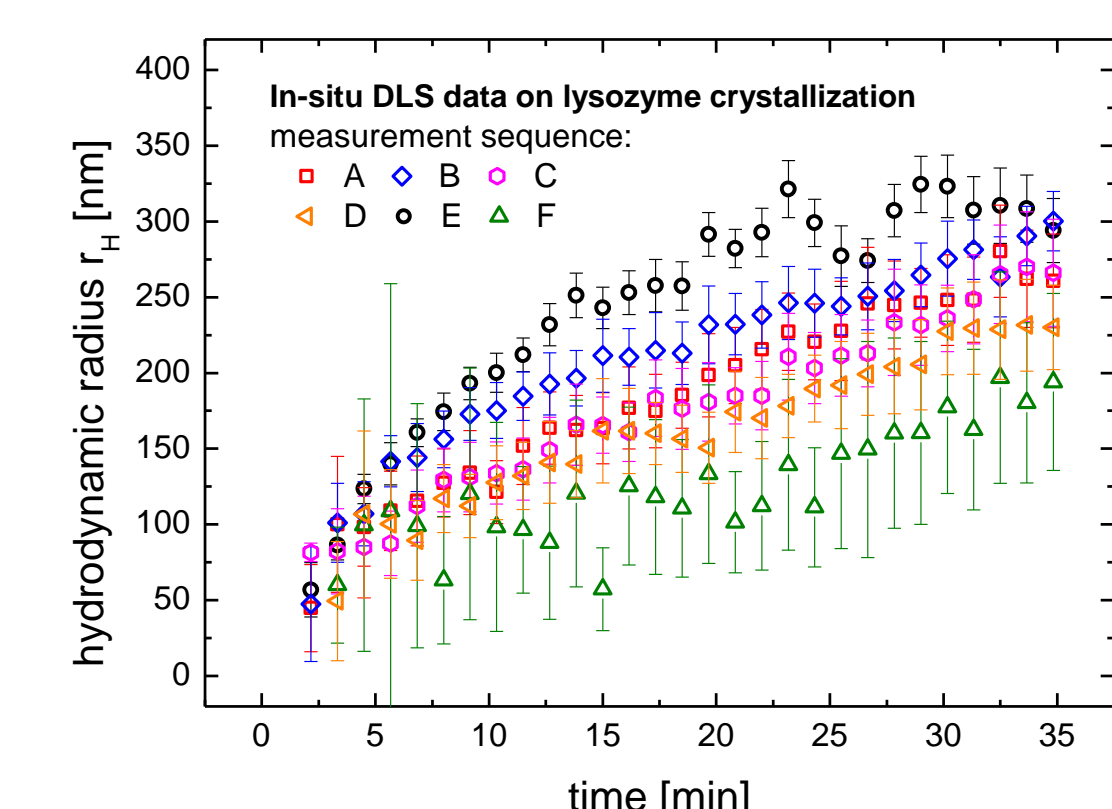□ SLS after 10 min
○ SANS after 40 min
● SLS after 40 min

The new results from this study were an observation of the change in fractal demision in the early phase of the crystallisation process. This can be deduced from fitting the static light scattering data (see figures on the right). This has not been observed previously since structural resolution was missing in earlier studies. A model based approach for interpreting the time dependence of the growth rate of the hydrodynamic radii deduced from the DLS data can only give an average fractal dimension (see figure on the left).

Fitting of the static light scattering curves Reveals a change in fractal dimension in the Early phase of crystal growth

SLS data on lysozyme crystallization
○ 5 min
○ 7.5 min
◆ 10 min
○ 60 min

$S(q)_{red}$: $d_f = 1,00 \pm 0,07$
$S(q)_{red}$: $d_f = 1,24 \pm 0,05$
$S(q)_{red}$: $d_f = 1,40 \pm 0,02$
$S(q)_{red}$: $d_f = 1,66 \pm 0,03$

SLS data on lysozyme crystallization
— evaluated from $S(q)_{red}$

Reproducibility of the measurements

In-situ DLS data on lysozyme crystallization measurement sequence:
□ A ○ B ○ C
◁ D ○ E △ F

In-situ DLS data on lysozyme crystallization time scaled measurement sequence:
□ A ○ B ○ C
◁ D ○ E △ F

The speed of the crystallisation process varies slightly due to small variations in temperature or salt/protein concentration. This can be corrected by a time scaling factor based on the DLS results.

Resulting model

(A) Monomers   (B) Oligomers   (C) Nuclei

Single track

T = 298 K

(E) Fractals

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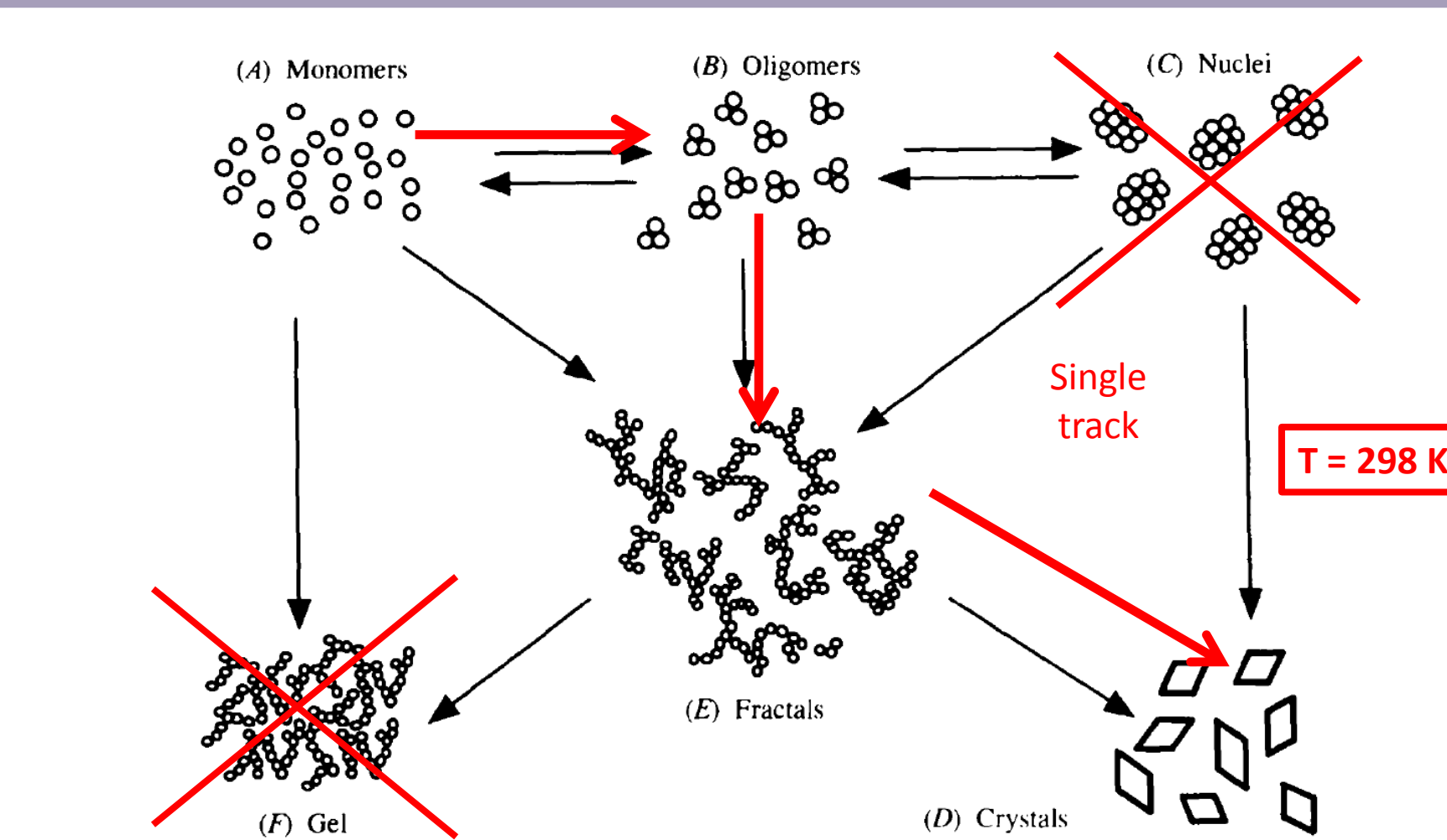
Y. Georgalis, P. Umbach, J. Raptis and Wolfram Saenger, Acta Cryst. 53 (1997) 703-712

Based on the results shown above one can improve the models for the growth of lysozyme crystals. Refering to the model proposed by Georgalis et al. (shown on the left in black) we see more a single track with a co-existence of the fractals and the growing crystals in the final equilibrium state state (see figure on the right).
In the early phase the rising fractal dimension indicates a filling up of the spaces between the branches of the earliest fractals of dimension 1.0. This later results then in a complete space filling and a subsequent crystal growth or in a slow fractal growth presumably when the space filling is not in accordance with the crystal structure.

A)   t < 20 s after mixing   B)   t ~2–5 min   C)

t ~ 10 – 90 min

E)   t > 24 h   D)

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