# In-situ Dynamic and quasi-in-situ Static Light Scattering on Small Angle Neutron Scattering Instruments

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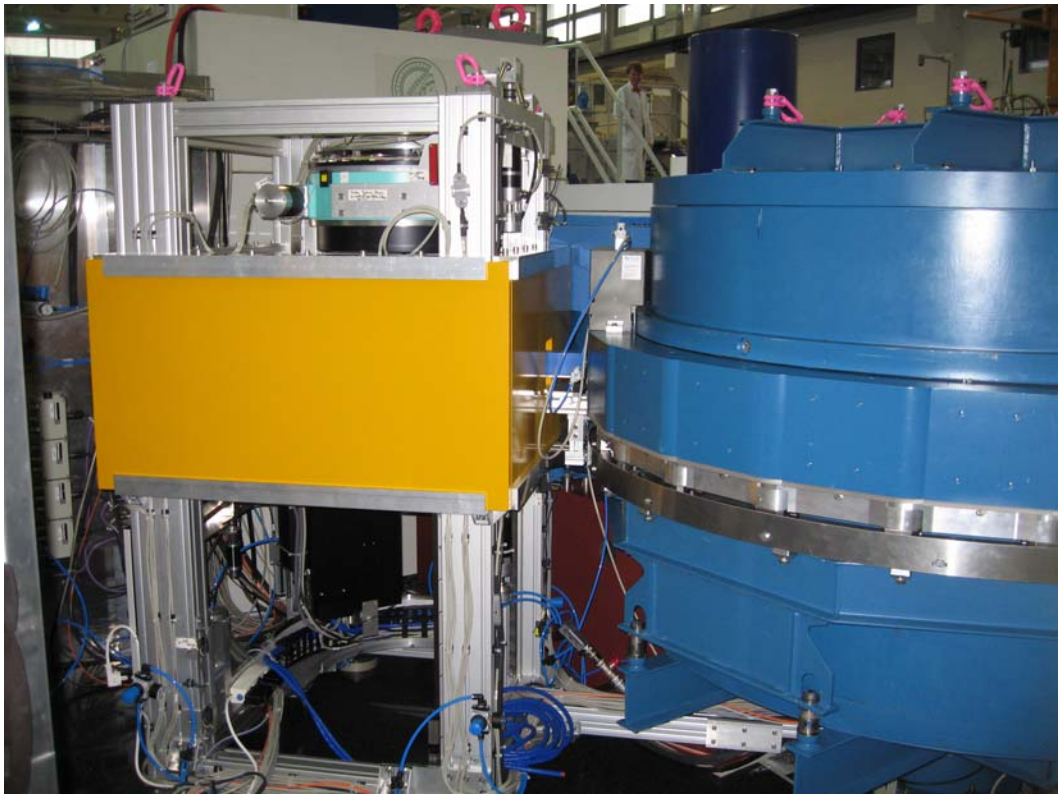
# For the instrument BioDiff large protein crystals are required



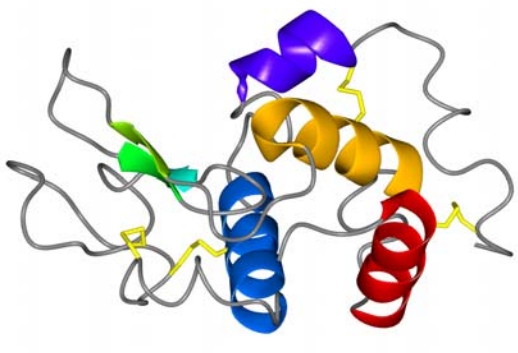
Size of crystals needed:
0.5 mm in all dimensions

- Good understanding of protein crystallisation necessary

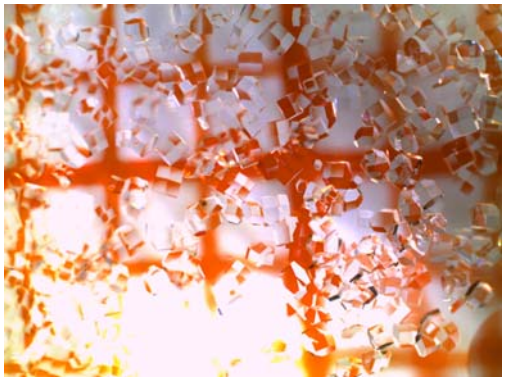
# lysozyme sample as a model for crystalization

- Lysozyme 80 mg/ml in $D_2O$, pH adjusted with 1M NaAc 0,02 µm filtered
- NaCl 6wt% in $D_2O$ buffer 10mM NaAc HAc 0,02 µm filtered

- 1:1 ratio: Lysozyme 40 mg/ml + NaCl 3 wt% in $D_2O$ buffer, @ pH 4.35

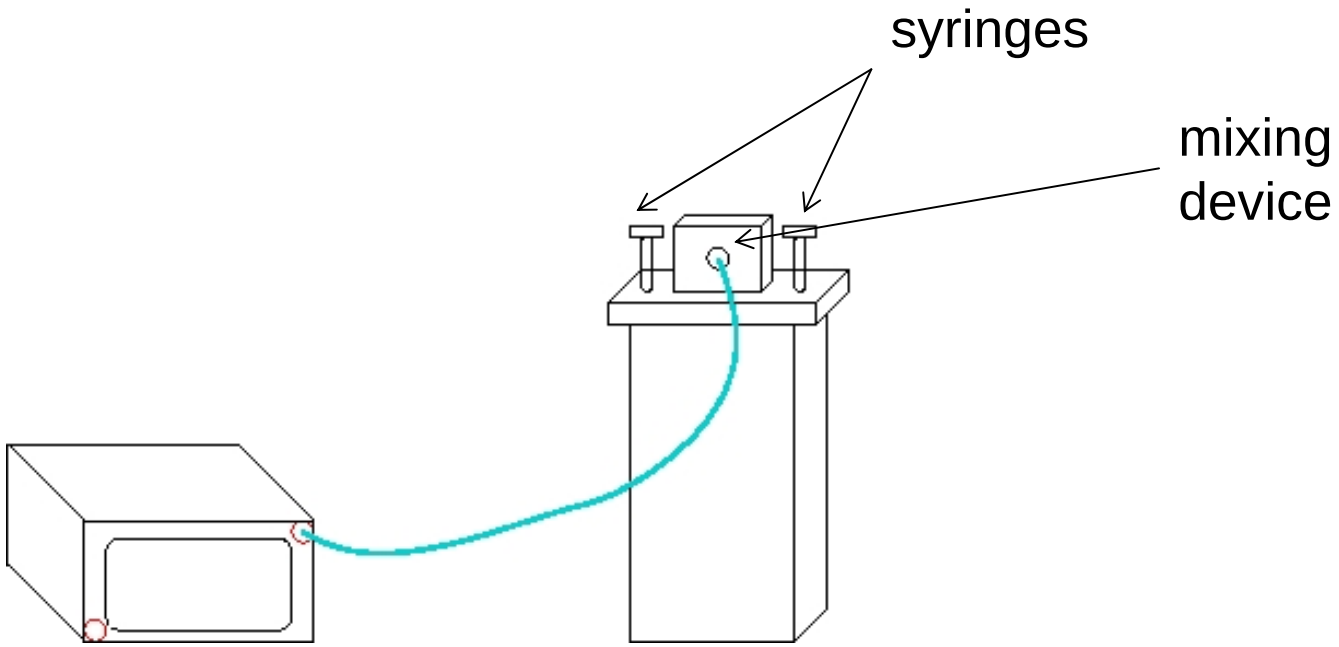


r= 1.9 nm



crystals ca. 0.2 mm

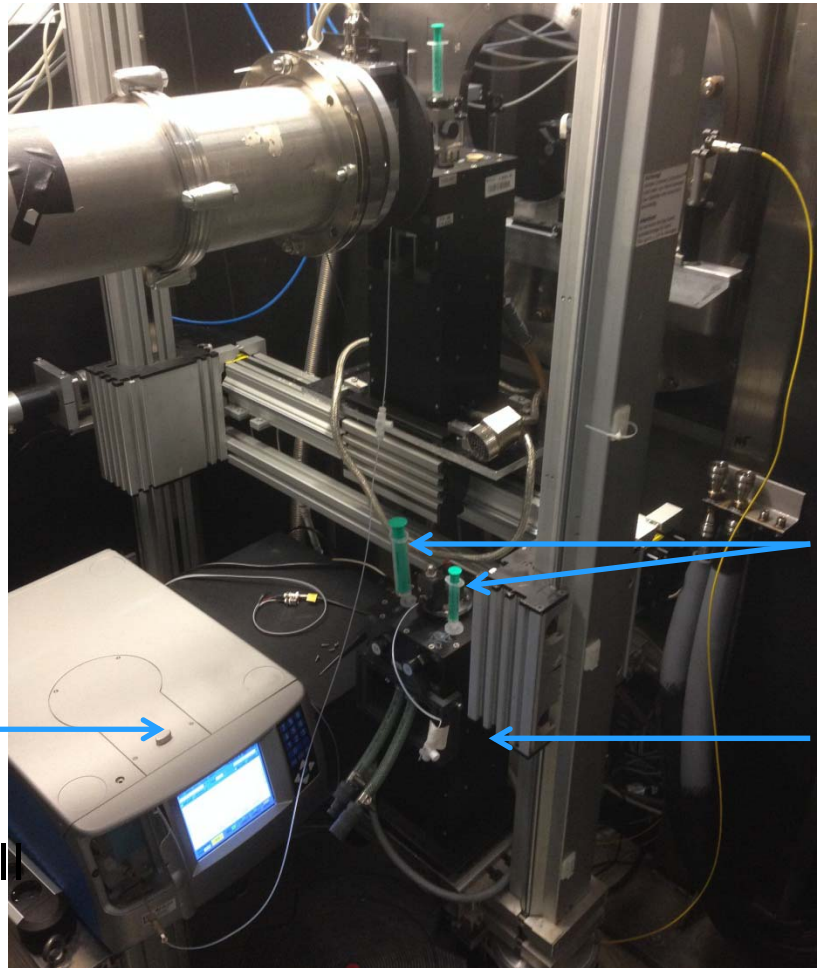
# experimental setup




Static Light Scattering device
Wyatt: Dawn Heleos II

Stopped-Flow device 1

# experimental setup

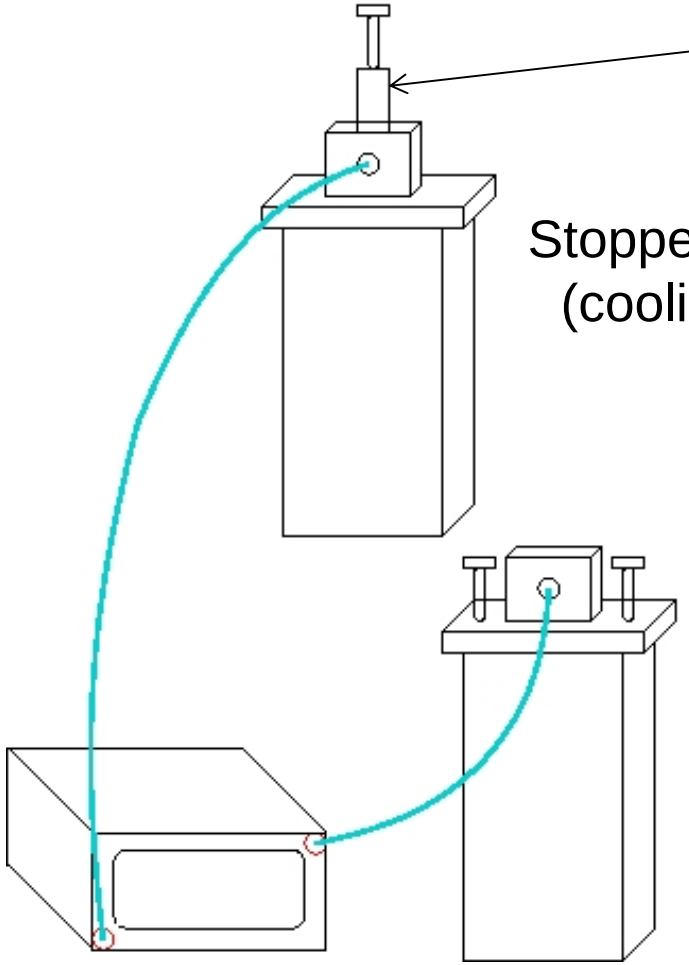


syringes

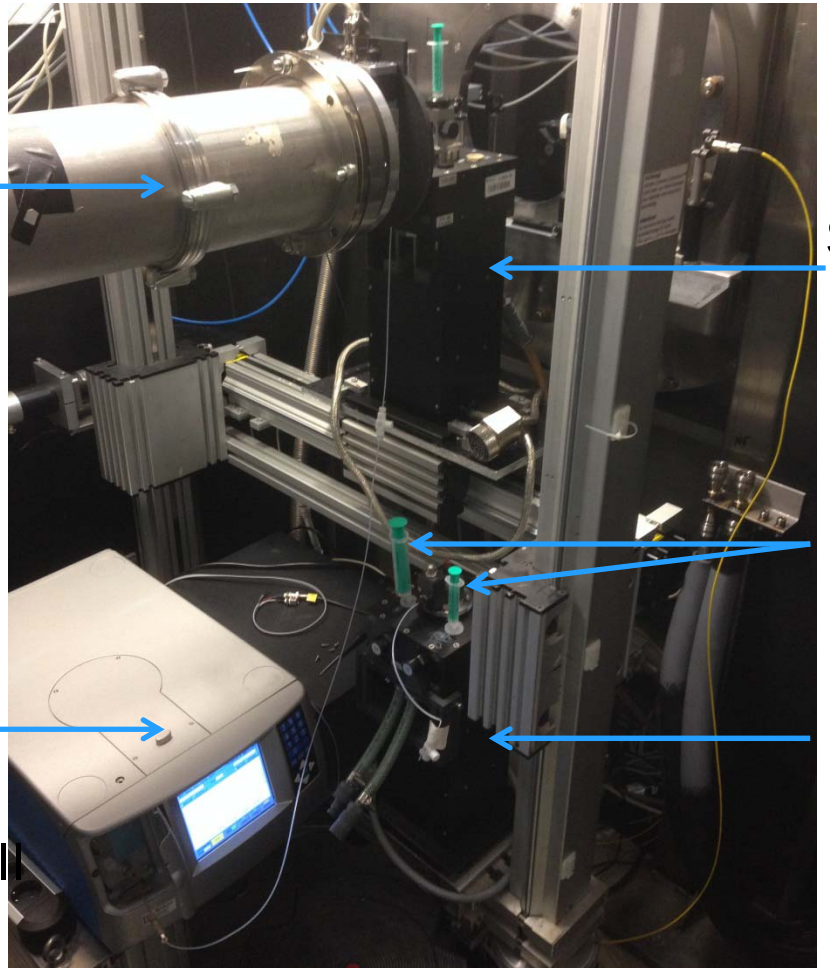
Static Light Scattering device Wyatt: Dawn Heleos II

Stopped -Flow device 1

# experimental setup



# experimental setup



Neutron - guide

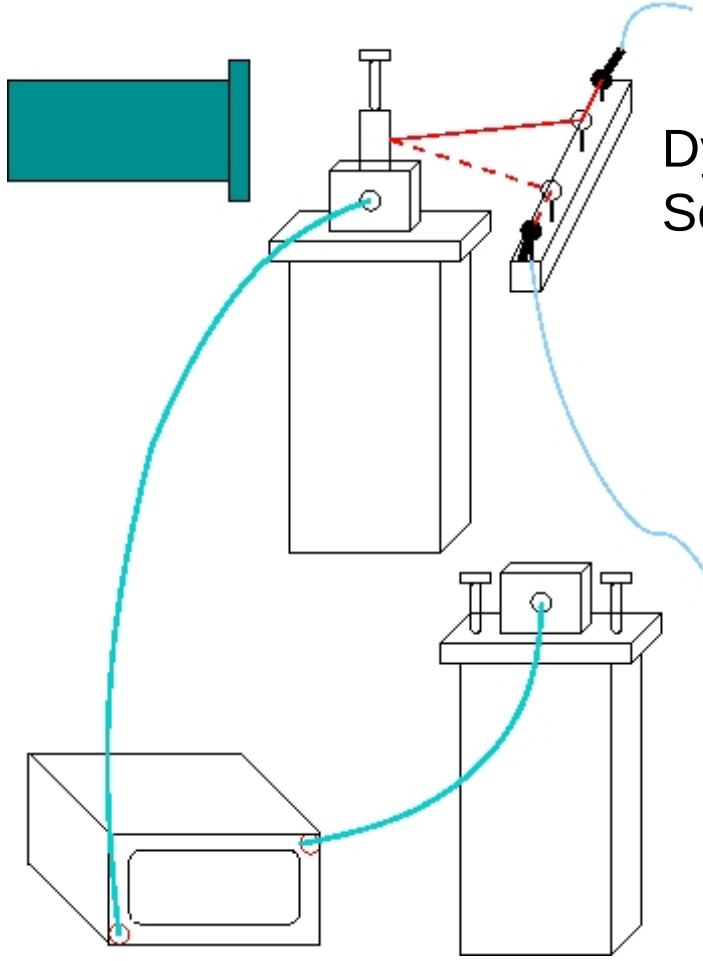
Stopped -Flow device 2
(cooling the system)

syringes

Static Light
Scattering device
Wyatt: Dawn Heleos II

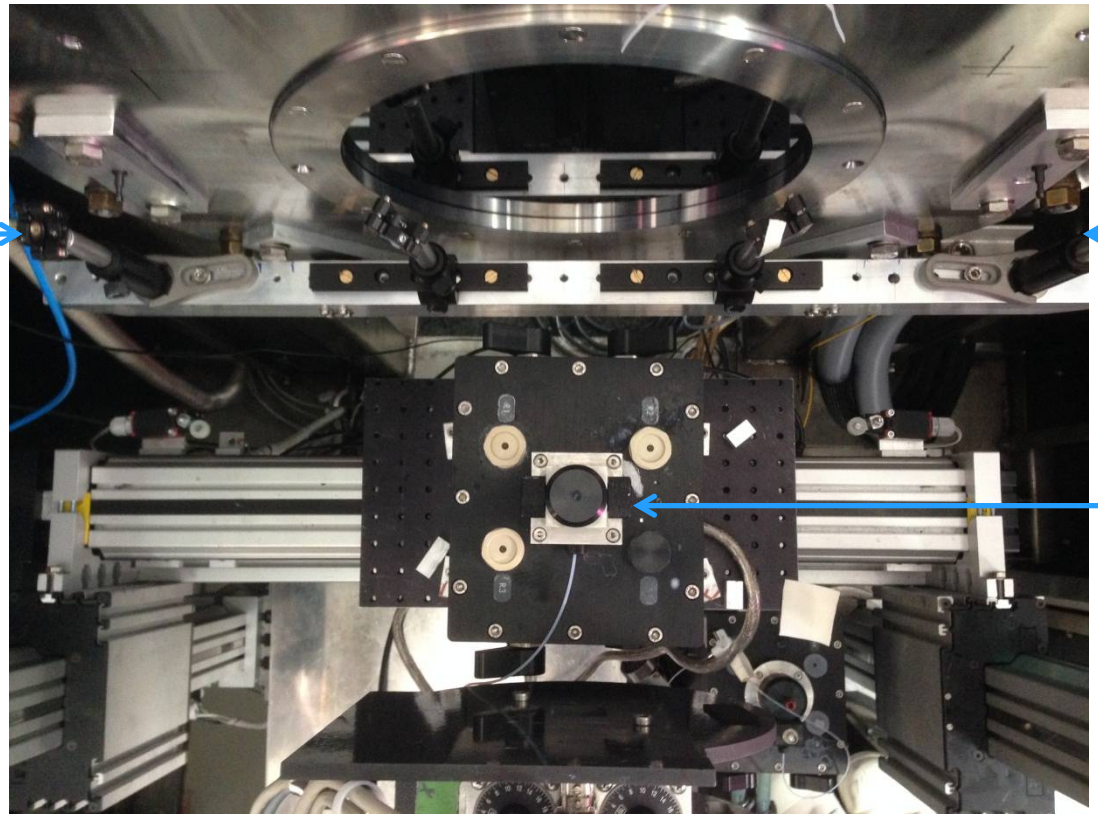
Stopped -Flow
device 1

# experimental setup



# DLS setup

laser fibre
& collimator

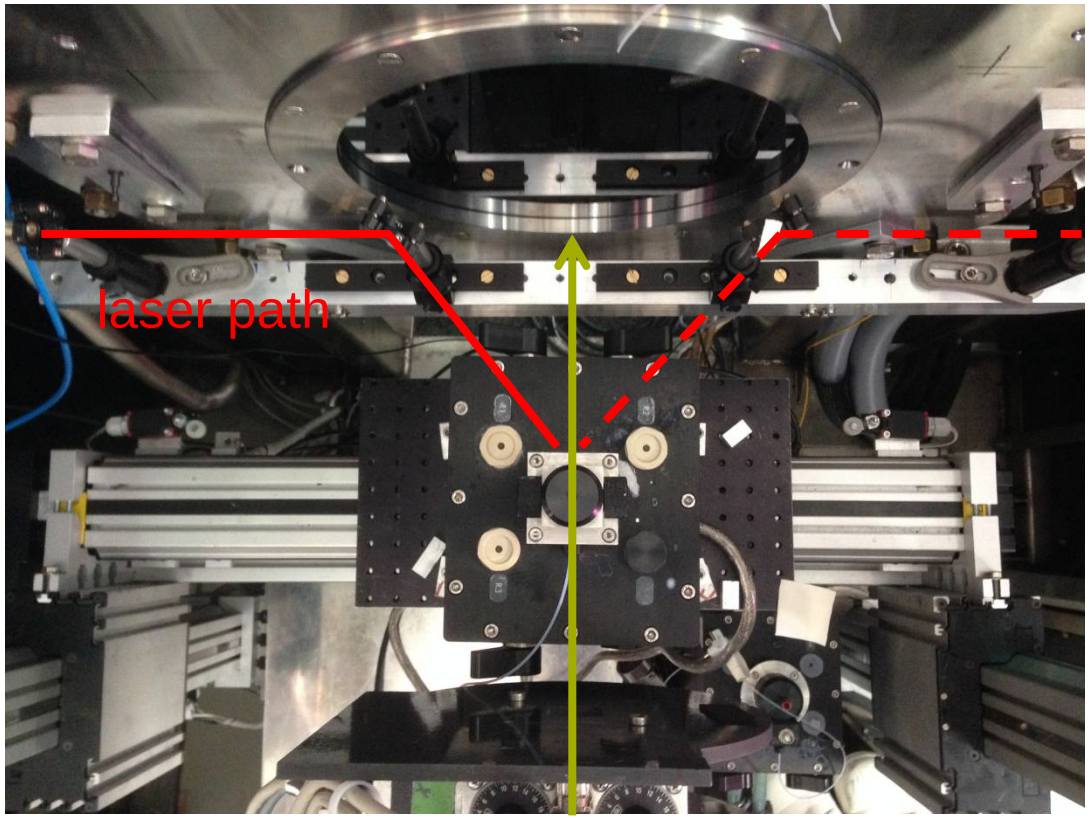


laser fibre
& collimator

cuvette

Final aperture (neutrons)

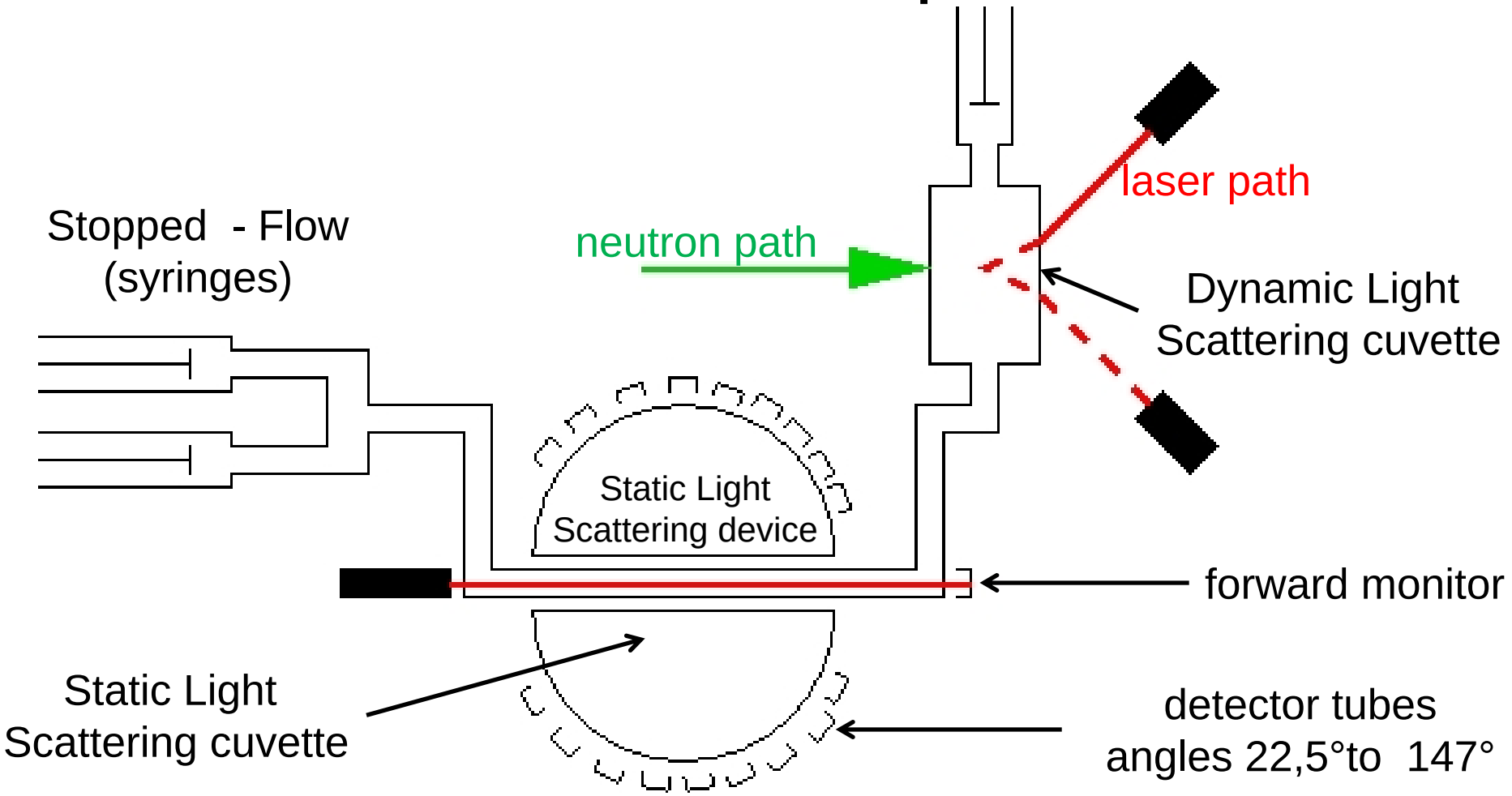
# DLS setup



# Sample cell taken from standard BioLogic Stopped flow apparatus

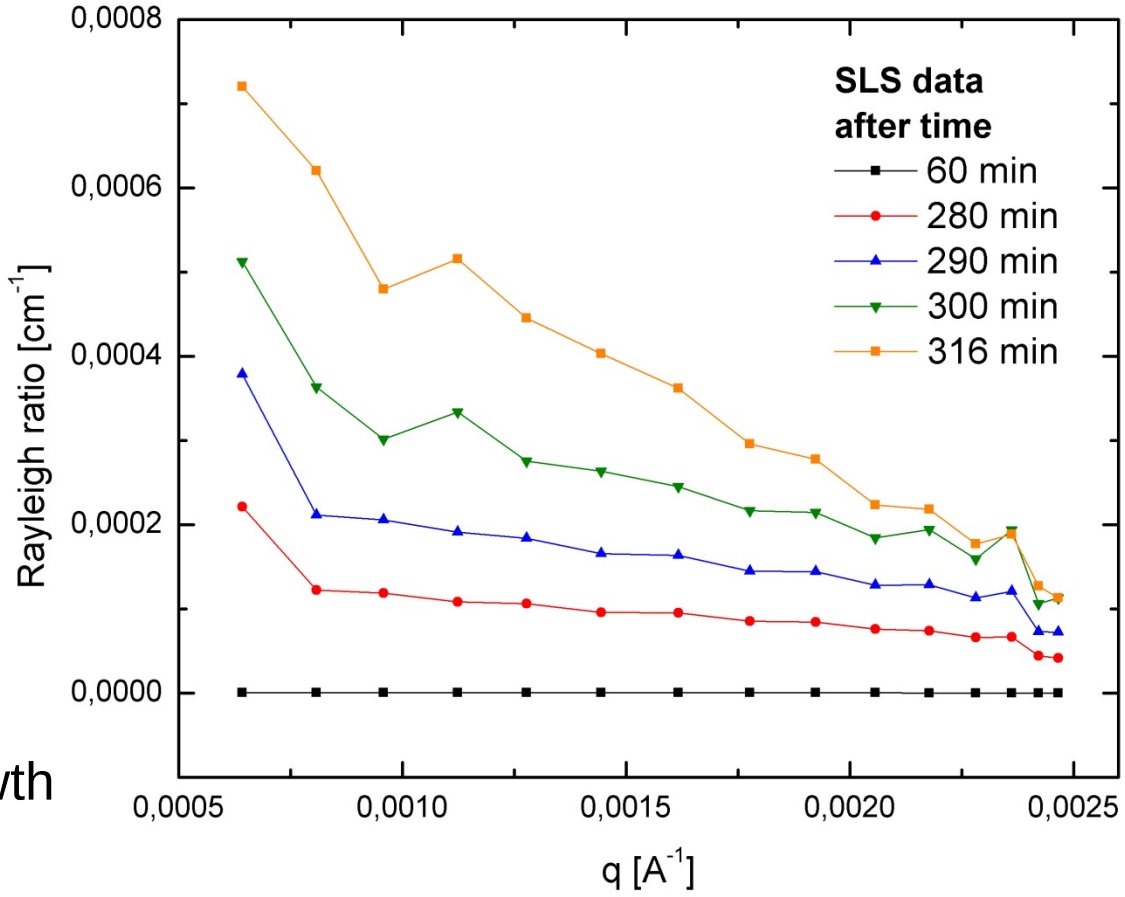
neutrons

# schematic setup



# pre-caracterization: lab measurements with SLS

Lab measurement
with Lysozyme
@ pH 3.35

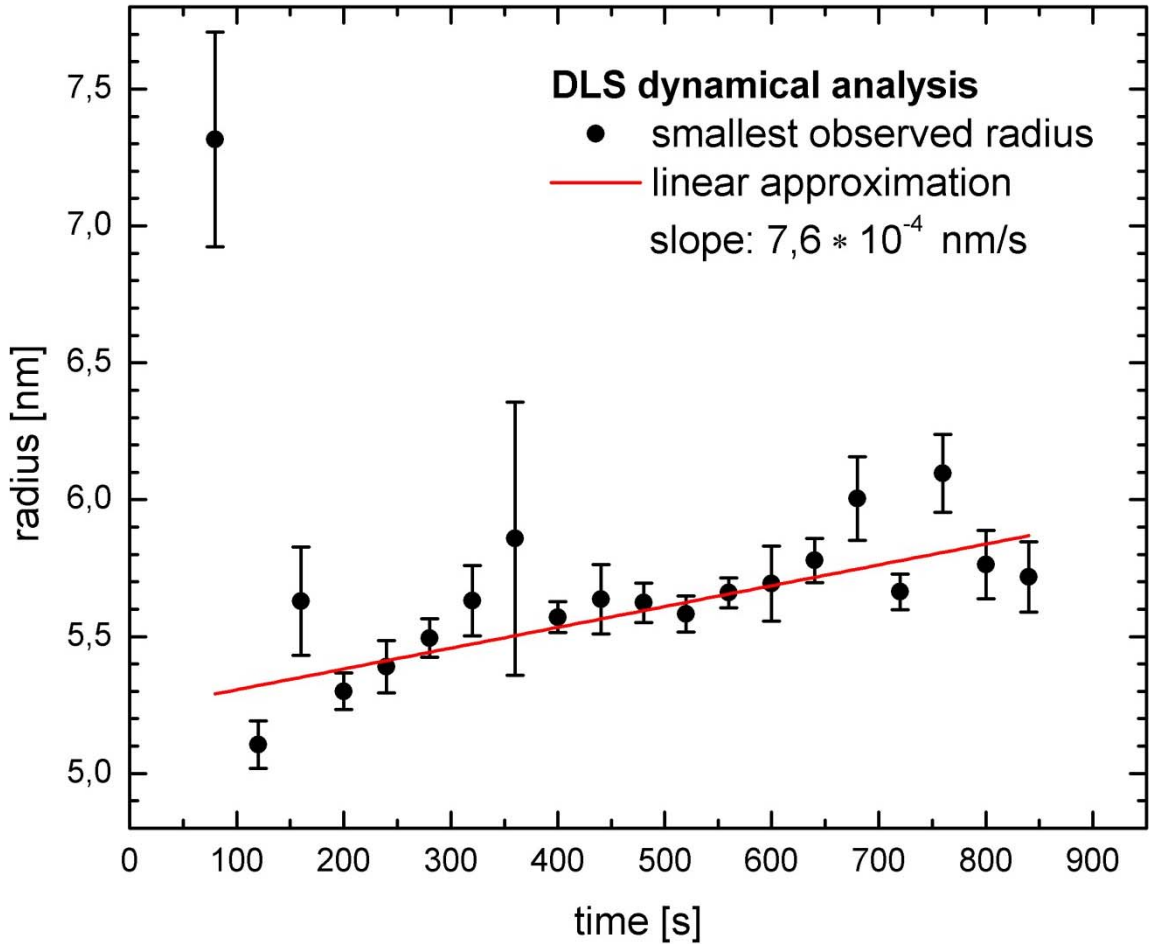



Lysozyme sample
shows cristalization /growth
behaviour over time

# pre-caracterization: lab measurements with DLS

Lab measurement
with Lysozyme
@ pH 4.35

Lysozyme sample shows
growth behaviour over time



# pre-caracterization: lab measurements with DLS

Lab measurement
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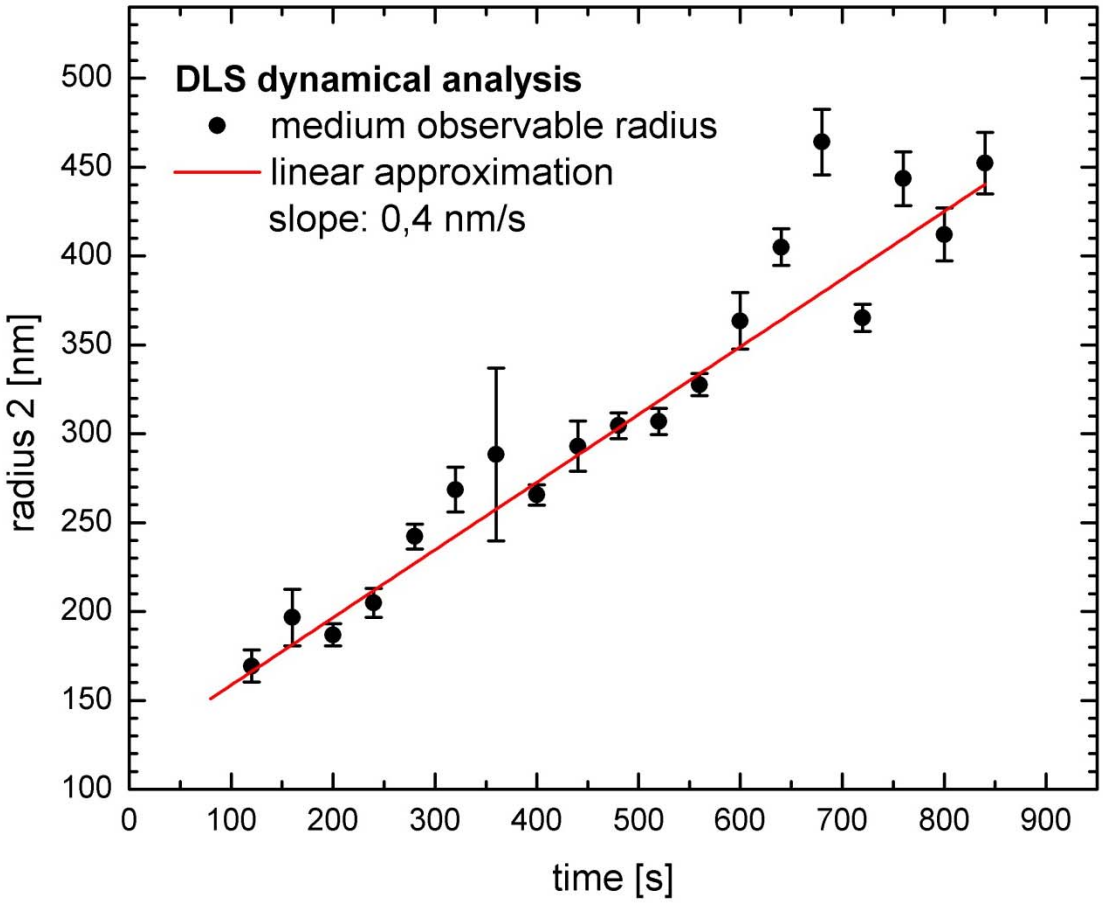
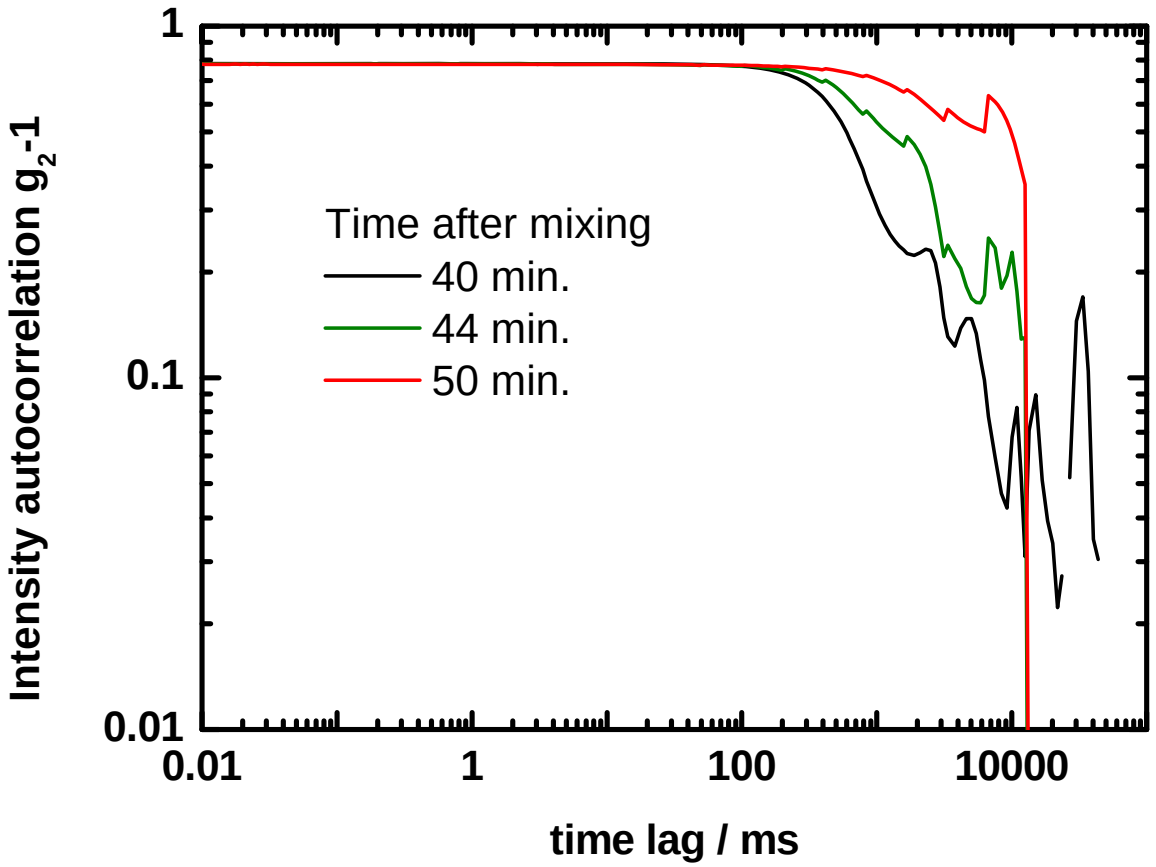
Lysozyme sample shows
growth behaviour over time



# Measurements at KWS-2: in-situ DLS and quasi-in-situ SLS

# in-situ DLS data (first useful curves 40 min. after mixing)

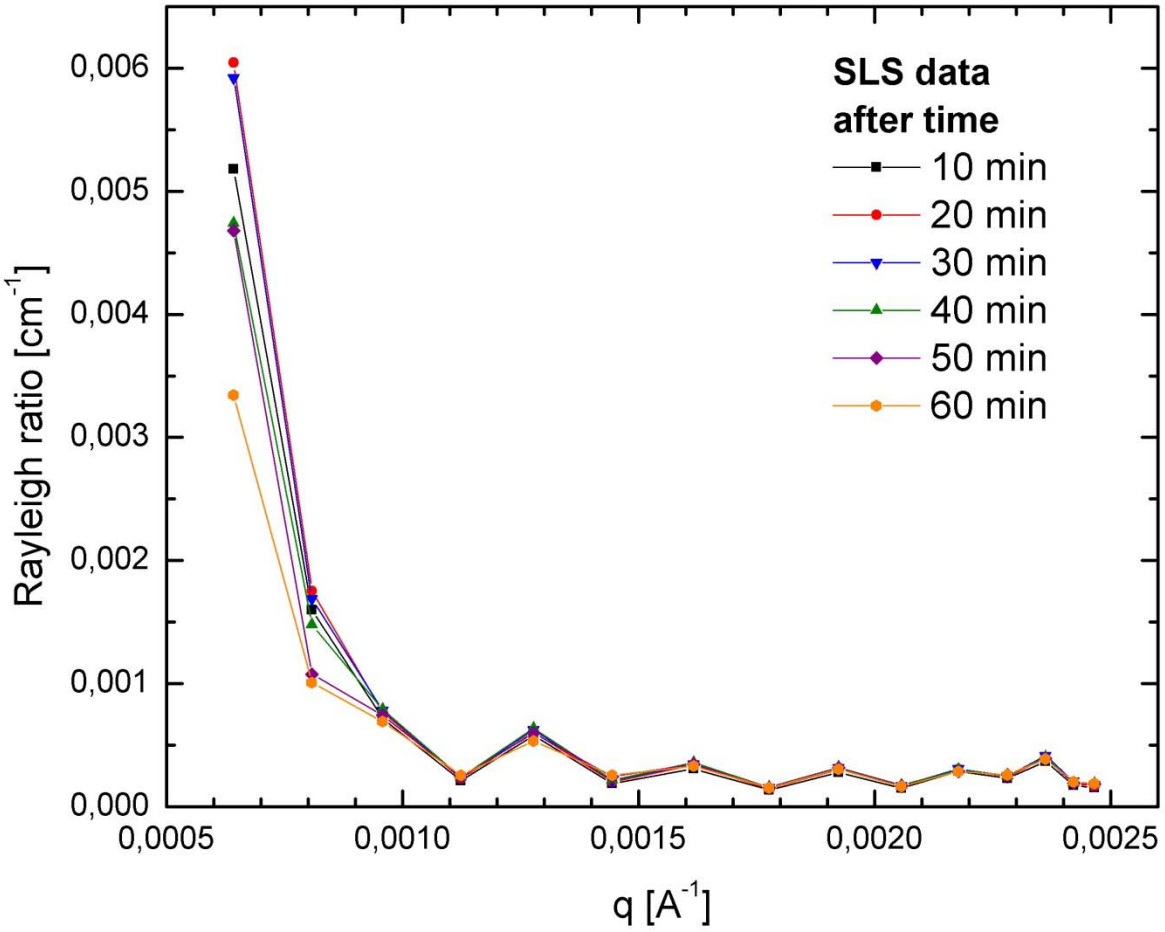



Fit of curve at 40 min. yields a time constant of 10 µs which translates into a hydrodynamic radius of ca. 10 µm.
After 50 min. sample non-ergodic, autocorrelation amplitude < 0.1

# in-situ measurements

measurement @ KWS 2 during the first 60 min after initiation of cristallisation

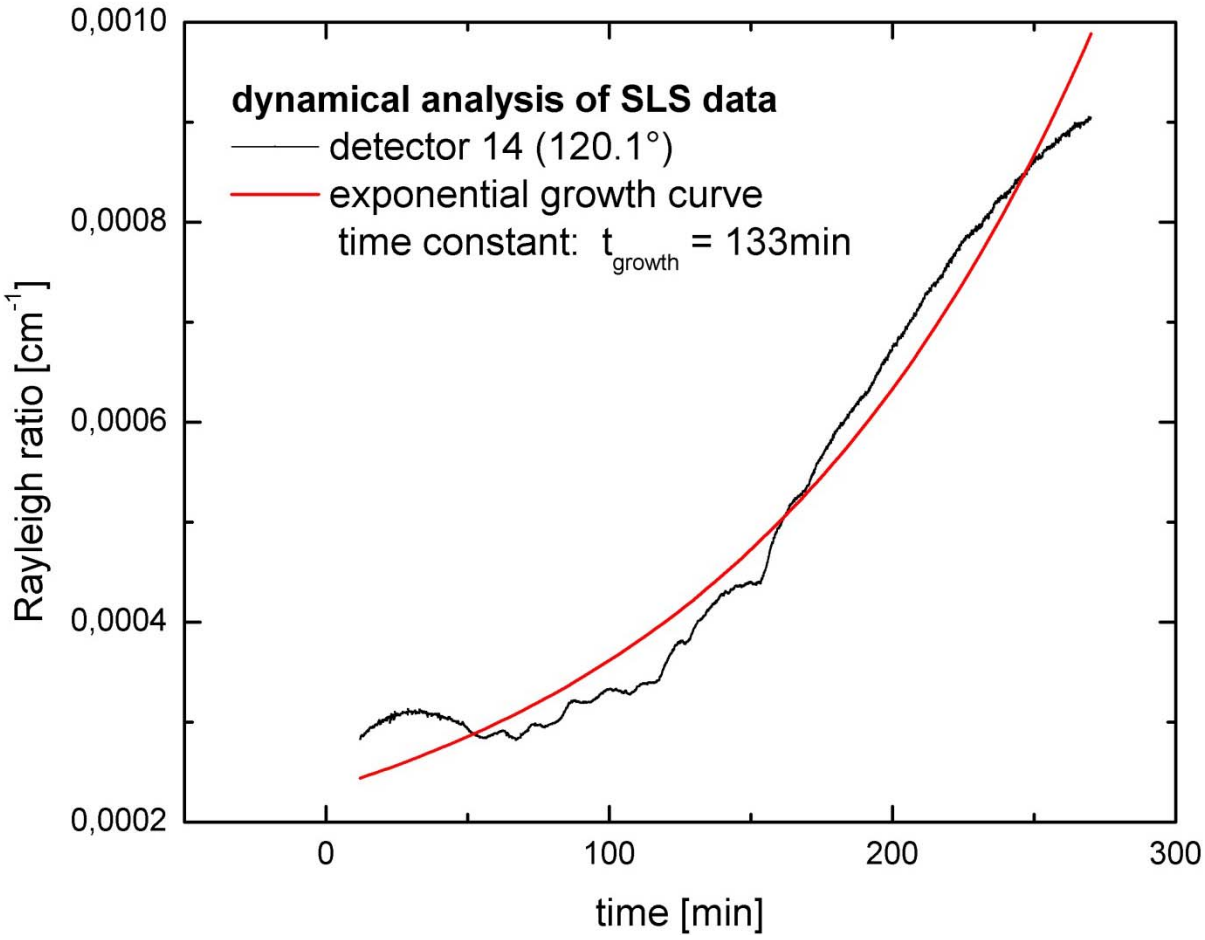
only small changes after 60 min observable



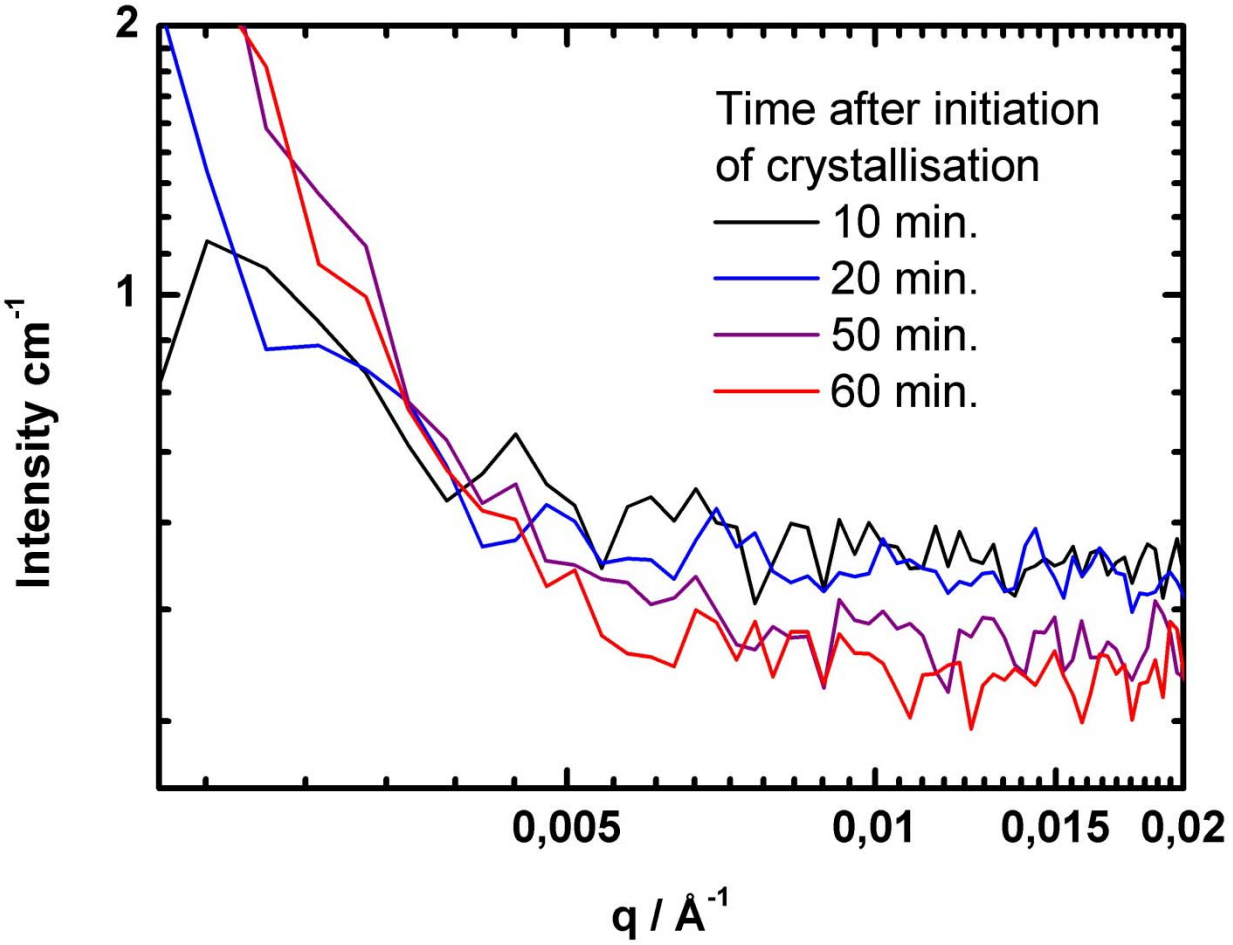
# in-situ measurements

measurement @ KWS 2 during the first 5 h after initiation of cristallisation (after 1h without neutrons)

exponential growth due to formation of lysozyme cristals



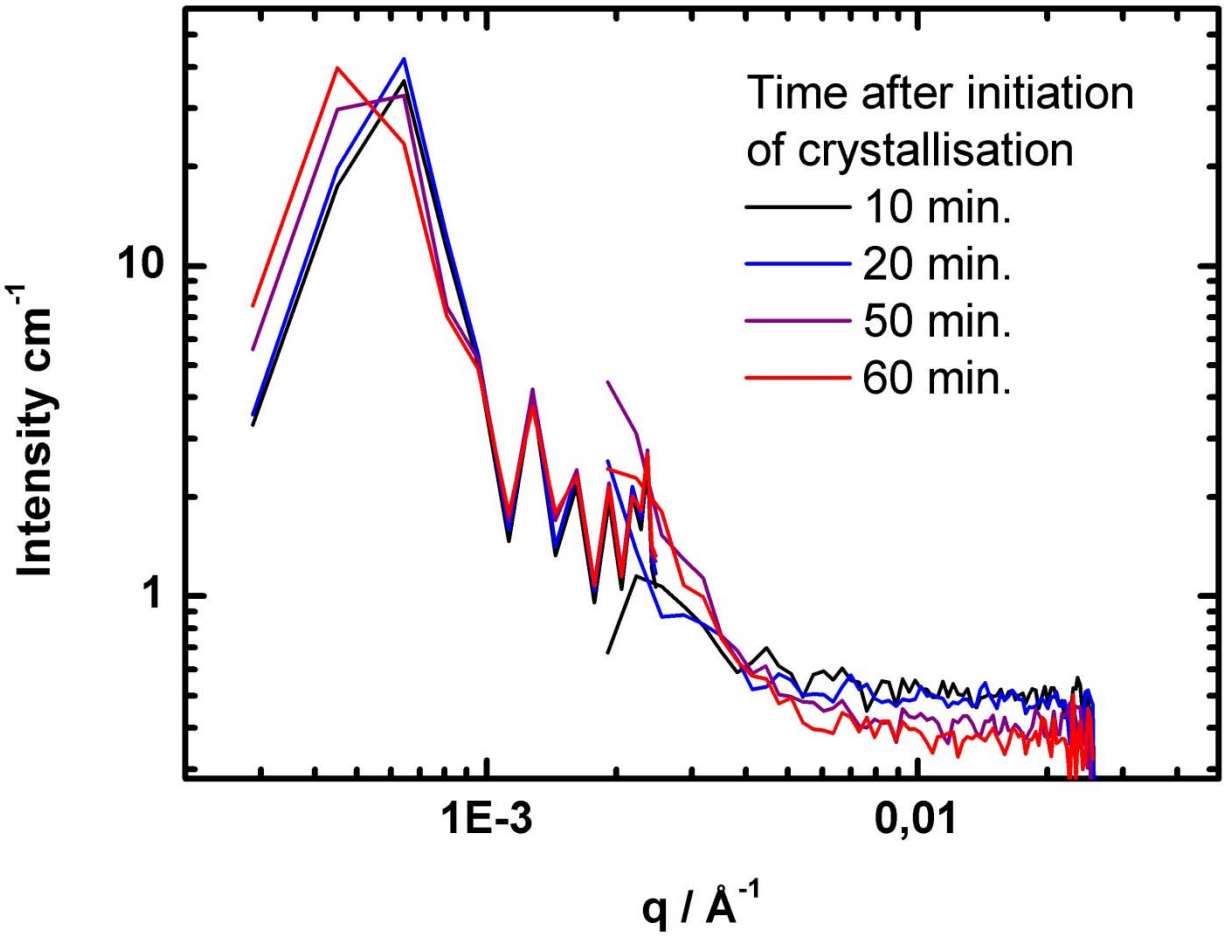
# in-situ measurements




SANS measurement of lysozyme sample during the first 60 min after initiation of cristallisation

time resolution not suffiecient to observe formation of crystallites

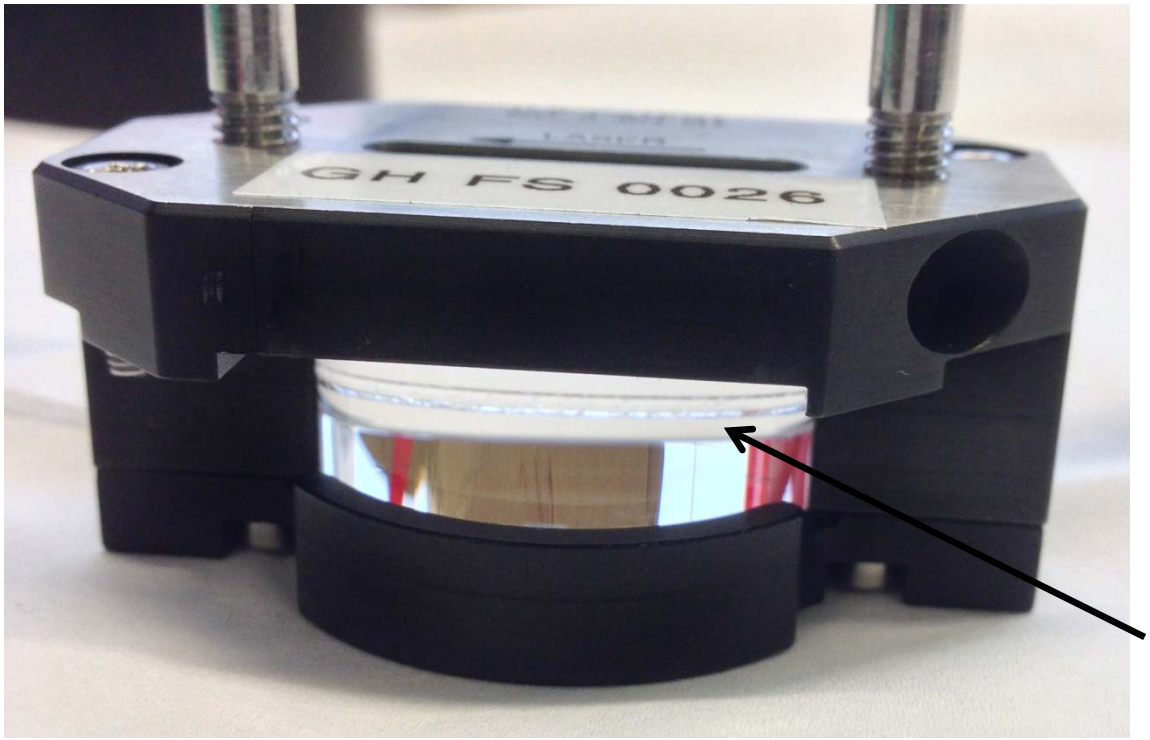
# in-situ measurements




combined SANS and SLS measurement of lysozyme sample during the first 60 min after initiation of cristallisation

# Measurement issues

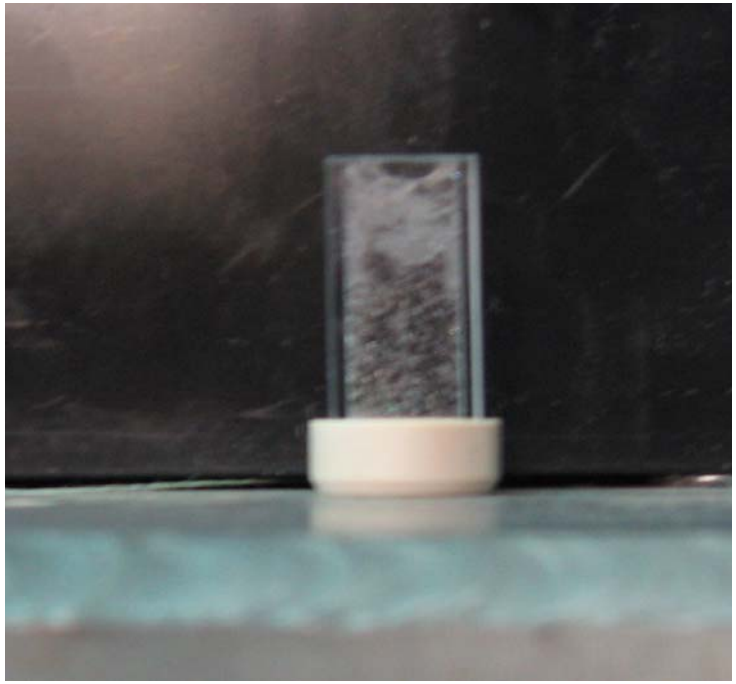
Static Light Scattering cuvette



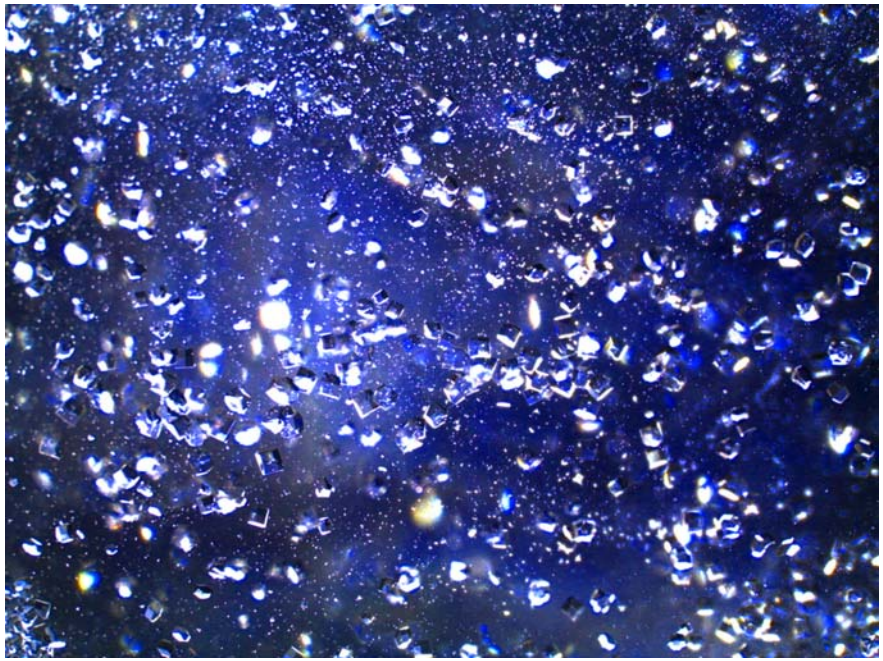

crystal growth on glas surface

sedimentation

# Some lysozyme crystals formed



stopped flow cell

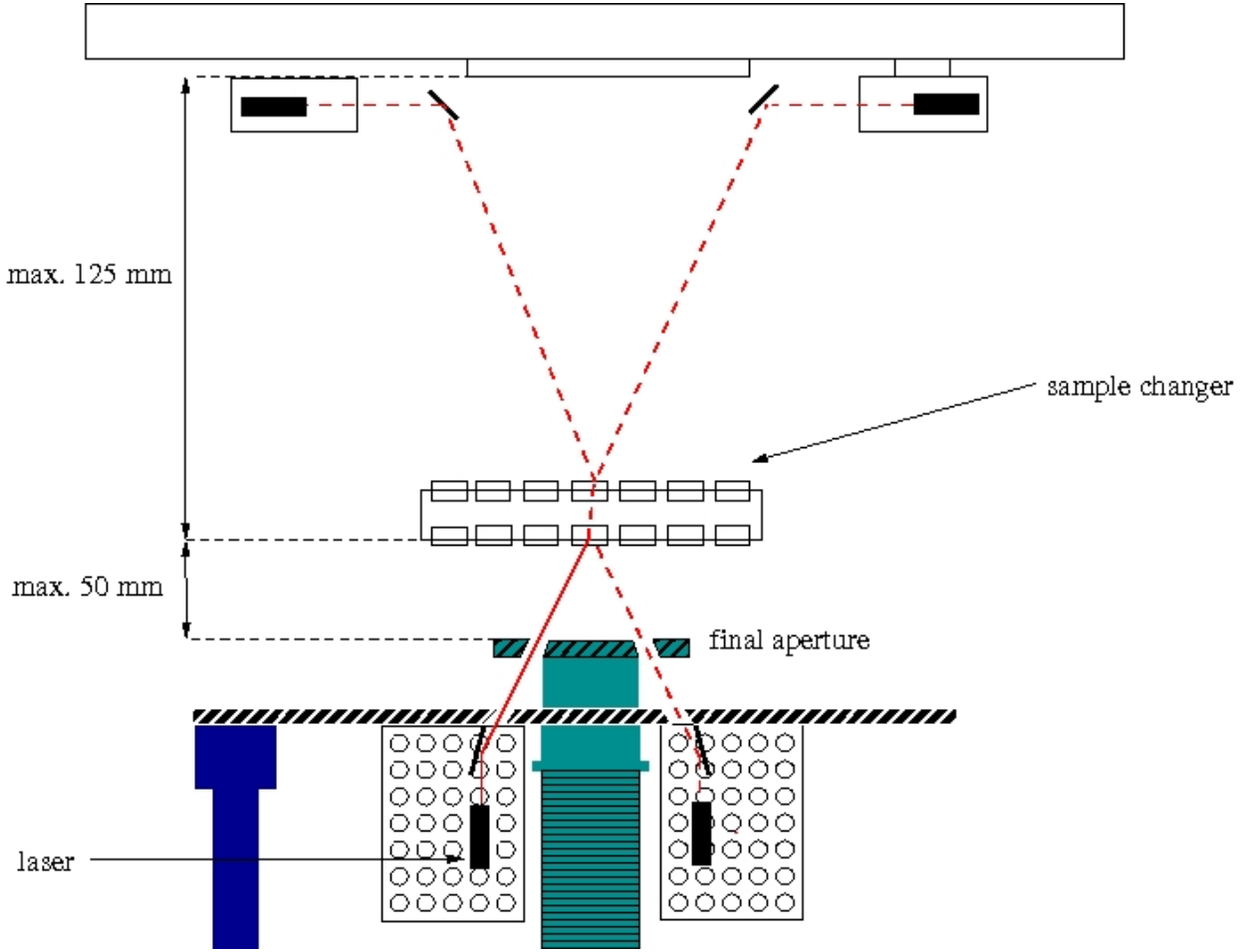


view under a stereo microscope

## Conclusions/Remarks

- more sample characterisation needed (reproducibility)
- different observation volumes (DLS, SANS, SLS)
- different needs for sample concentration (SANS 5%w/v, DLS <0.1w/v%)
- different speeds of data recording (SANS: hours, DLS: minutes, SLS: seconds

# future projects



# Thanks to... ... the KWS2/DLS/SLS-Team:

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- Dieter Richter
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