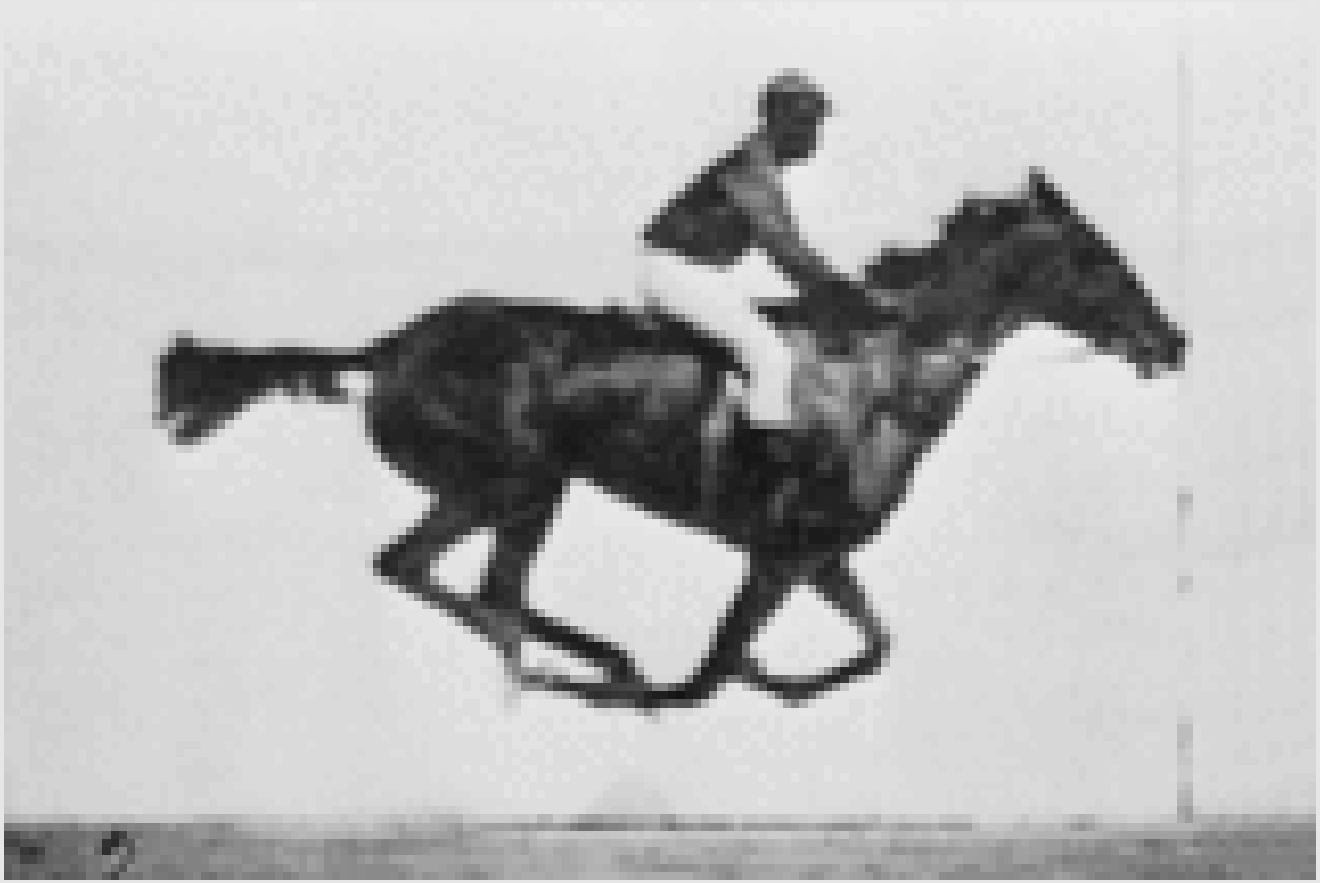
Time-resolved SAXS and SANS

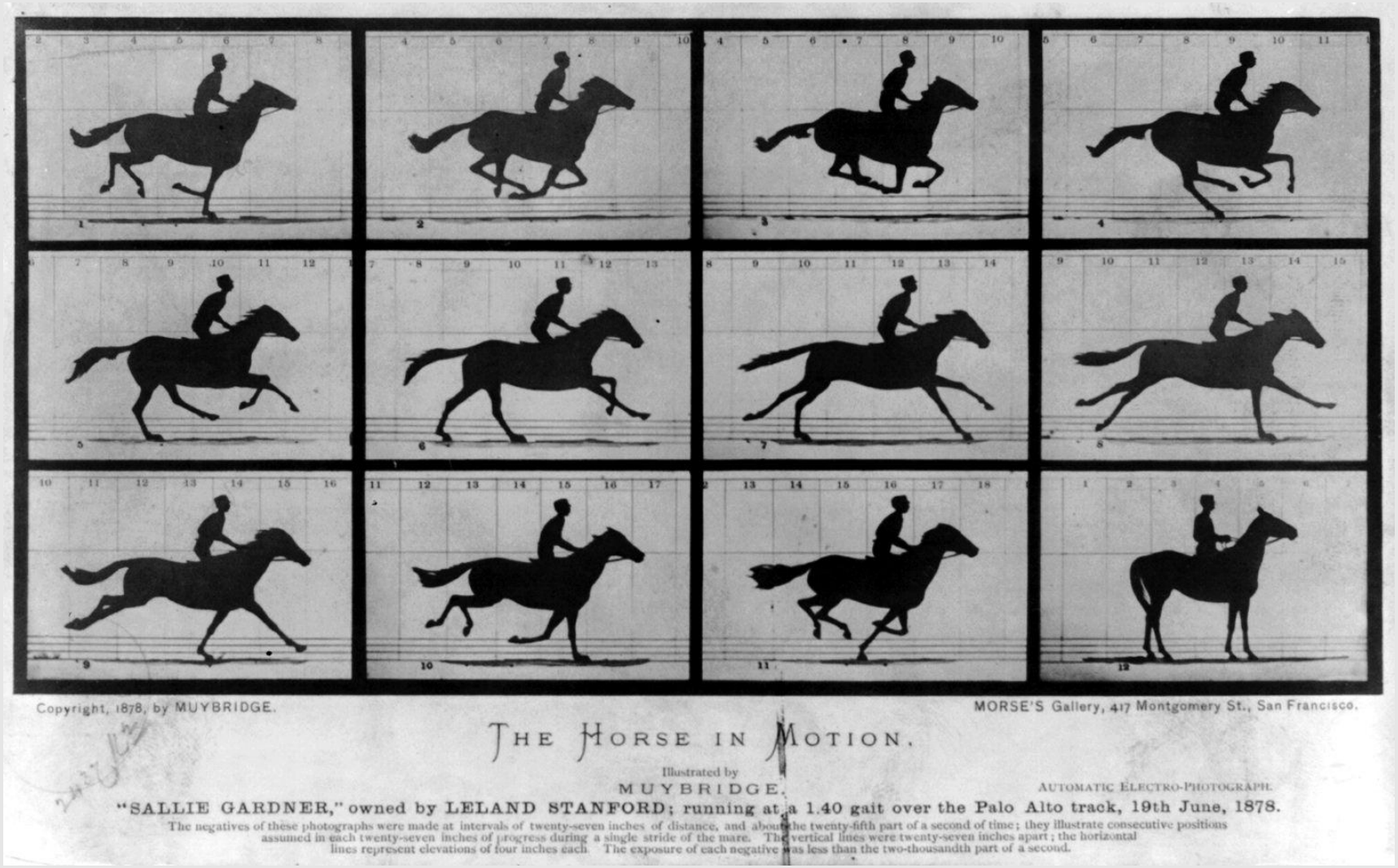
Manfred Roessle, EMBL Hamburg

The horse in motion
Eadweard Muybridge 1877



Sallie Gardner at a gallop

The horse in motion



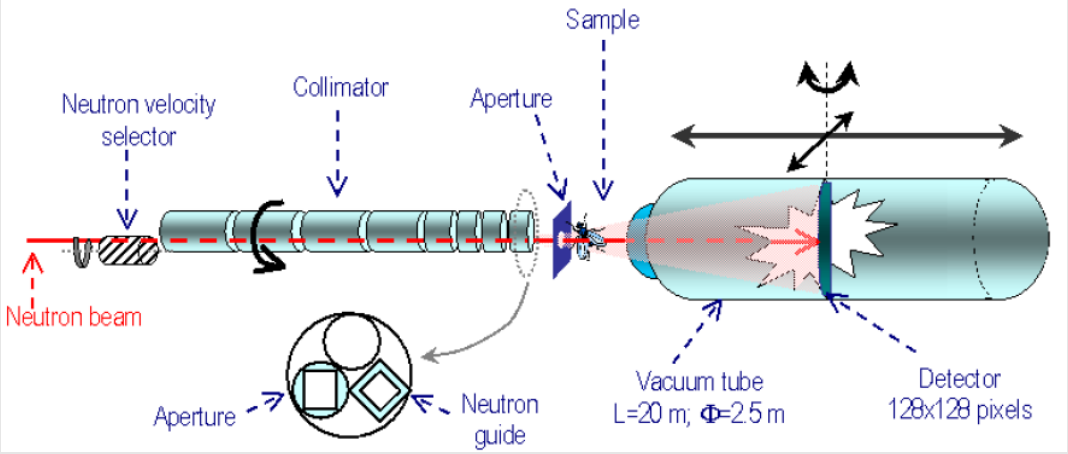
The “BioSANS” instrument D22 at the ILL

- Source-to-sample distances:
  from 1.4m to 17.6 m

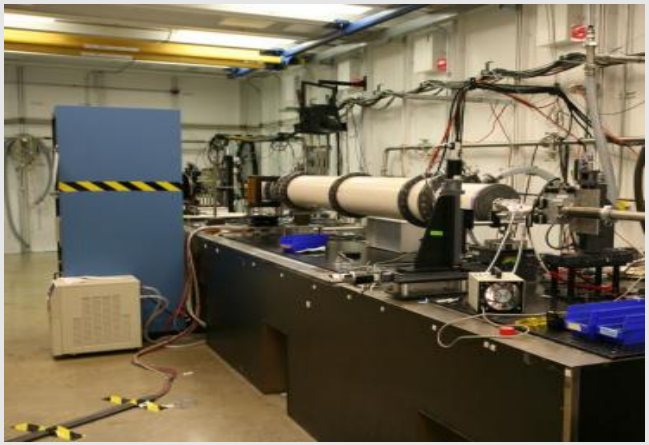
- q-range:
  $1.5 \times 10^{-3}\ nm^{-1} < q < 10\ nm^{-1}$

- Max. flux at specimen:
  1.23×10^{8} neutron/$cm^{-2}\ s^{-1}$

- Spot on sample:
  5 x 5 mm^2



Synchrotron based time-resolved SAXS



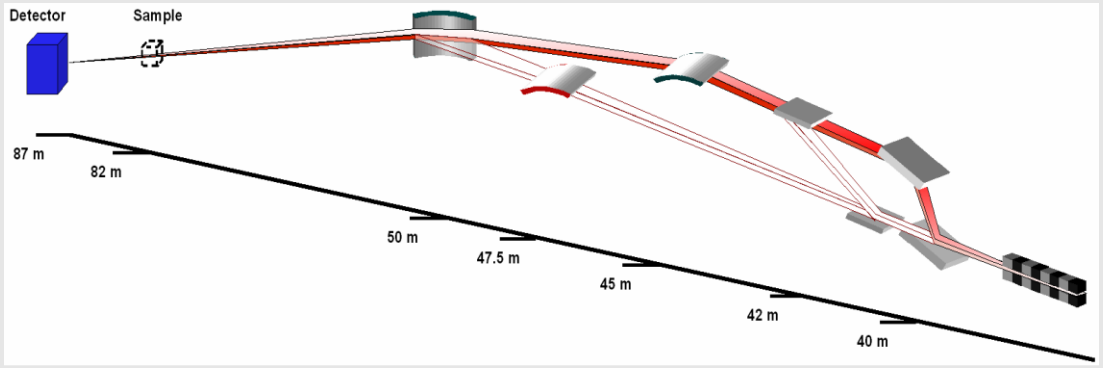
APS, Chicago

- Source-to-sample distances:
  Up to10 m (ID02 ESRF)

- q-range:
  $1x10^{-3}$ nm^{-1} < q < 10 nm^{-1}

- Max. flux at specimen:
  Up to 10^{15} ph/cm^{-2} s^{-1}

- Spot on sample:
  50 x 50 μm^2



Time resolved Small angle scattering

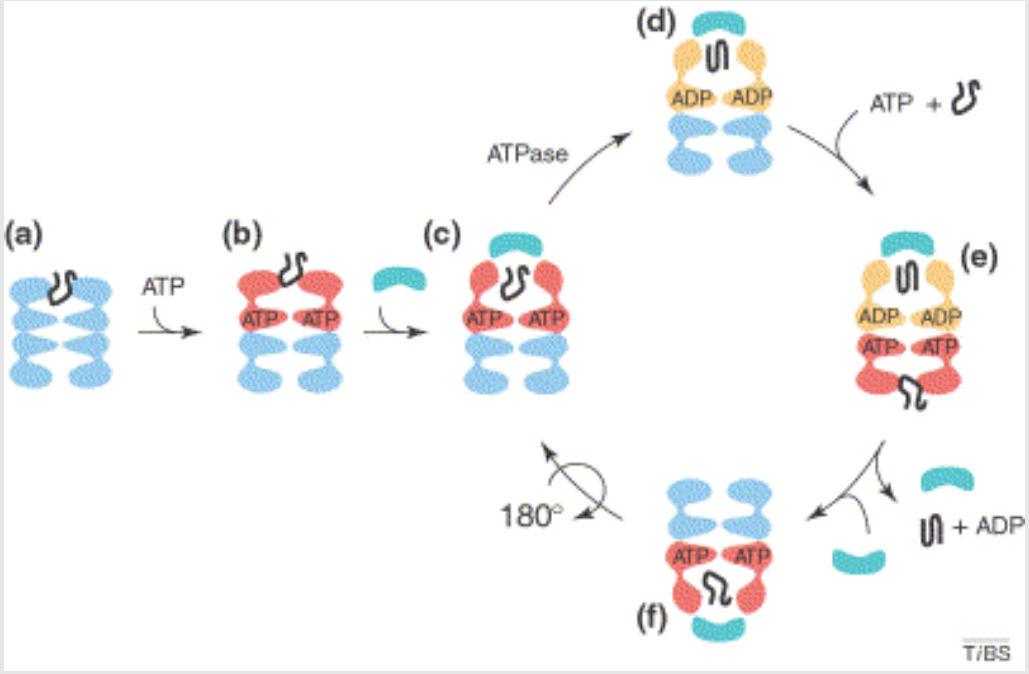
Petra-III inauguration November 2009

SAXS: Using the laser!

Fast kinetics on the chaperonin system GroE

Complex formation kinetics
ATPase activity

The Chaperonin folding machinery



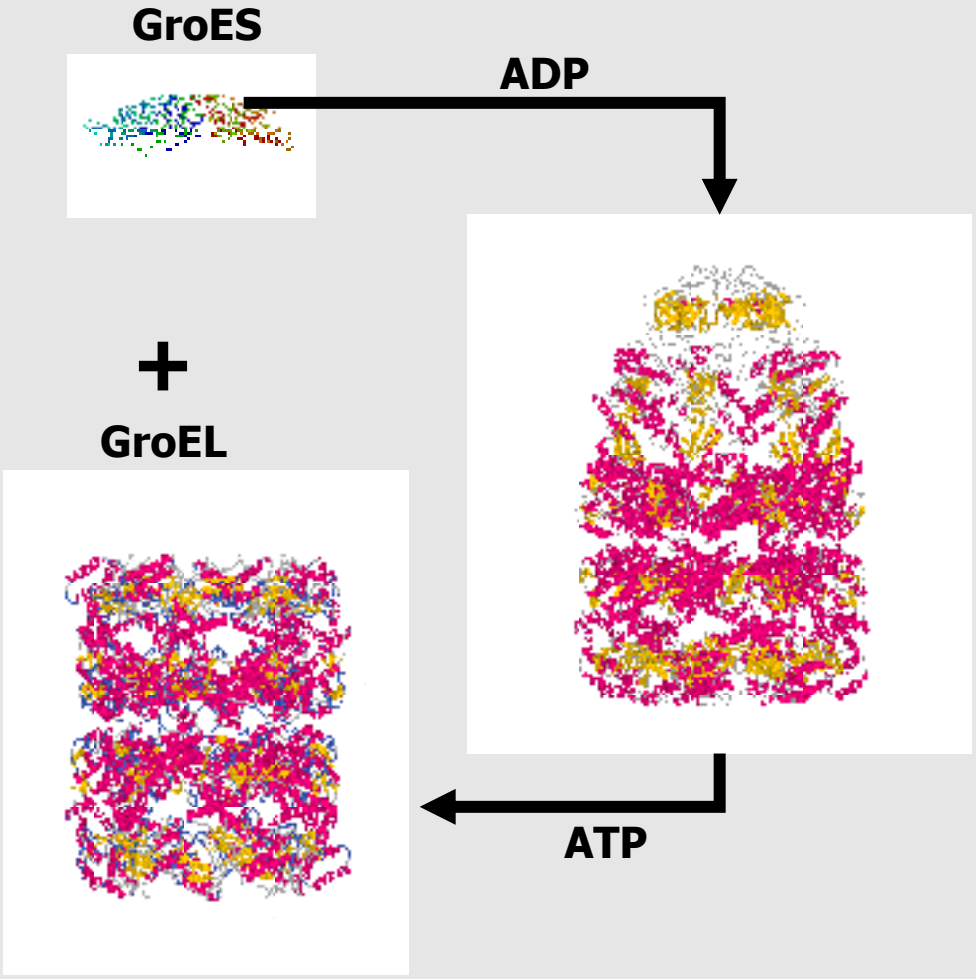
Chaperones of the GroE family are part of the heat shock response of a bacterial cell. It consists of the large GroEL cylindrical protein and a small GroES lid.

The refolding is a multistep ATP driven process and allosteric regulated.

Highly symmetrical particles:
2 x 7 subunits GroEL
1 x 7 subunits GroES

Nice system for small angle scattering!

The Chaperonin folding machinery



main chaperonin GroEL

- two heptameric rings
- 800 kDa MW
- hollow cylinder
- binds denatured protein and facilitate the refolding

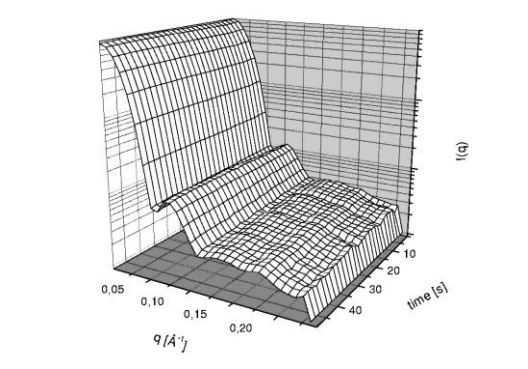
co chaperonin GroES

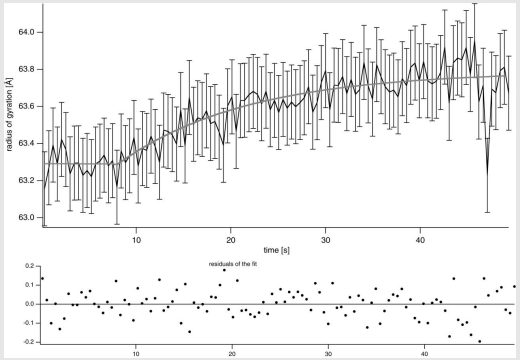
- heptameric dome
- 70 kDa MW
- bind to one end of the GroEL cylinder and close the cavity like a lid

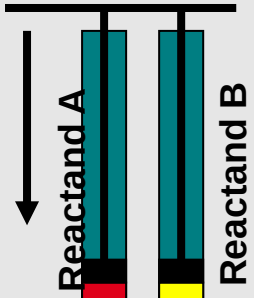
Time resolved SAXS

Investigation of Structural Kinetics

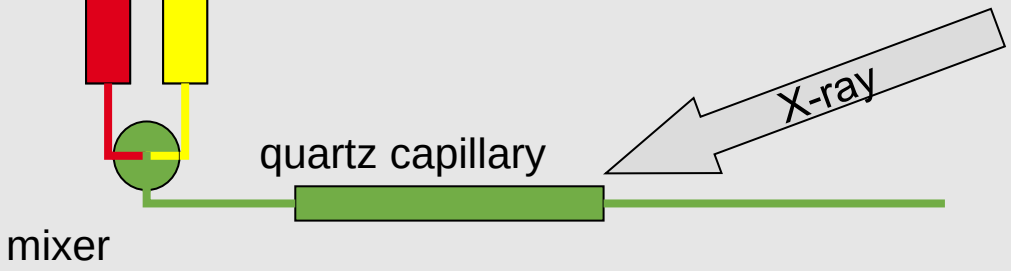
Example:
Reaction kinetics of an ATP driven two component protein system. Classical stopped-flow experiment.

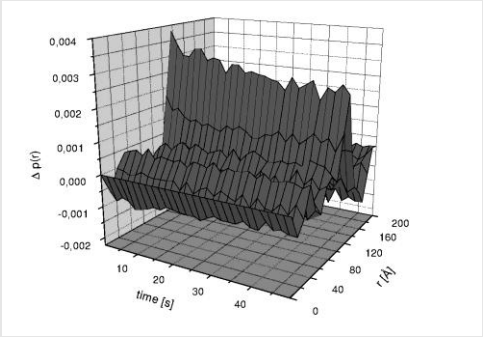






- Typical mixing time in the range of several ms
- Suitable for the sub-second time range
- 50µl to 80µl total volume
- on a third generation synchrotron radiation source such as the ESRF's ID02 about 5 to 10 repetitions necessary



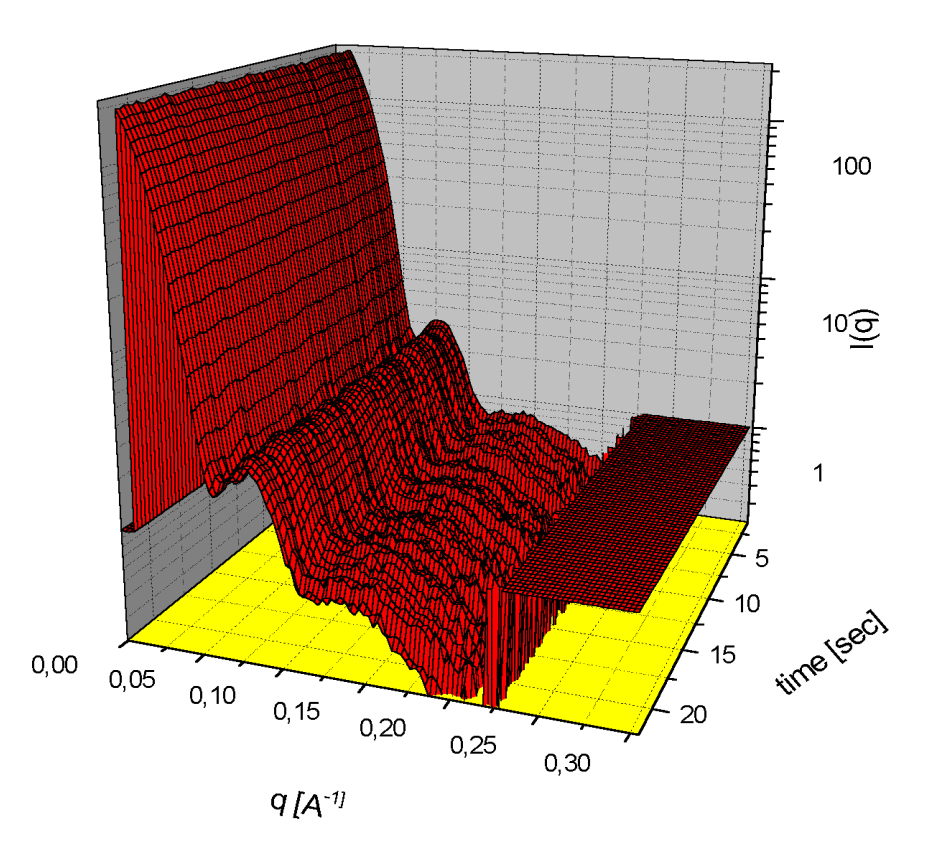


M. Roessle et. al. J.Appl. Cryst.

Repetitive measurements
High sample consumption
Need of a suitable detector system
Time resolution ~ 10ms

The Chaperonin folding machinery

Time-resolved SAXS data recording

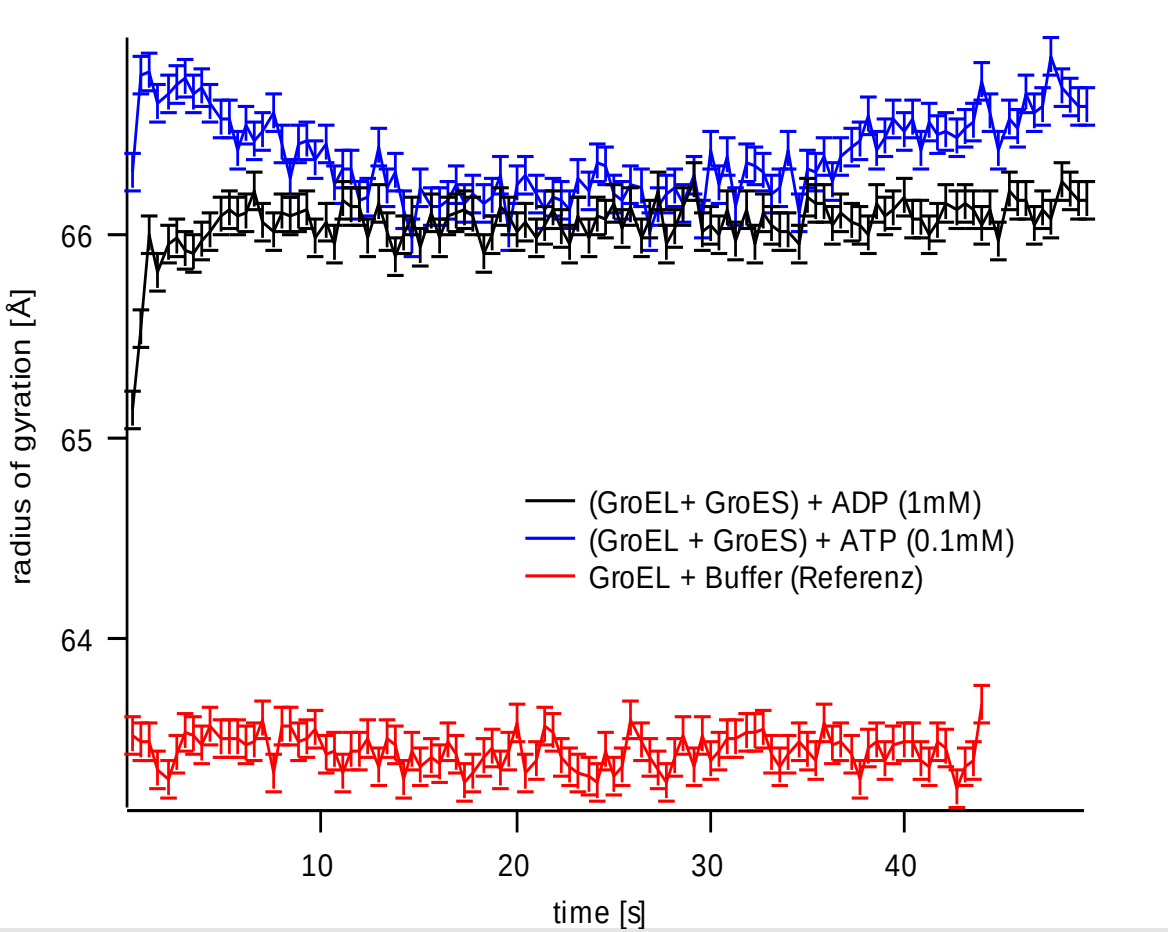


Time resolved SAXS data recording at ID02 ESRF Grenoble

150 ms frame rate
80 µl sample volume
10 repetitions
~ 1 ml total volume

The Chaperonin folding machinery

Formation of the GroEL/GroES complex

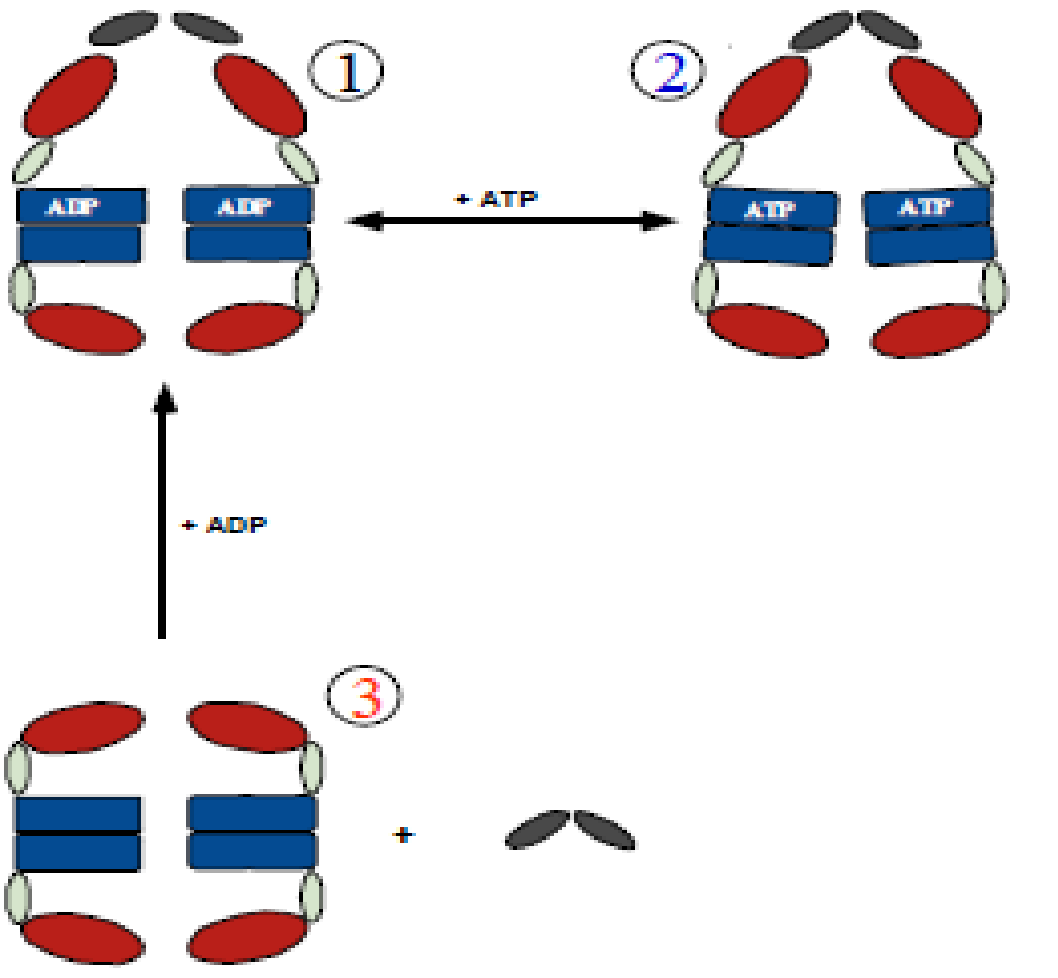


The complex formation is investigated by the time course of the radius of gyration.

The formation of the static GroEL-GroES complex is slower in the presence of ADP, and the ATP introduces a second binding phase in the complex formation kinetics.

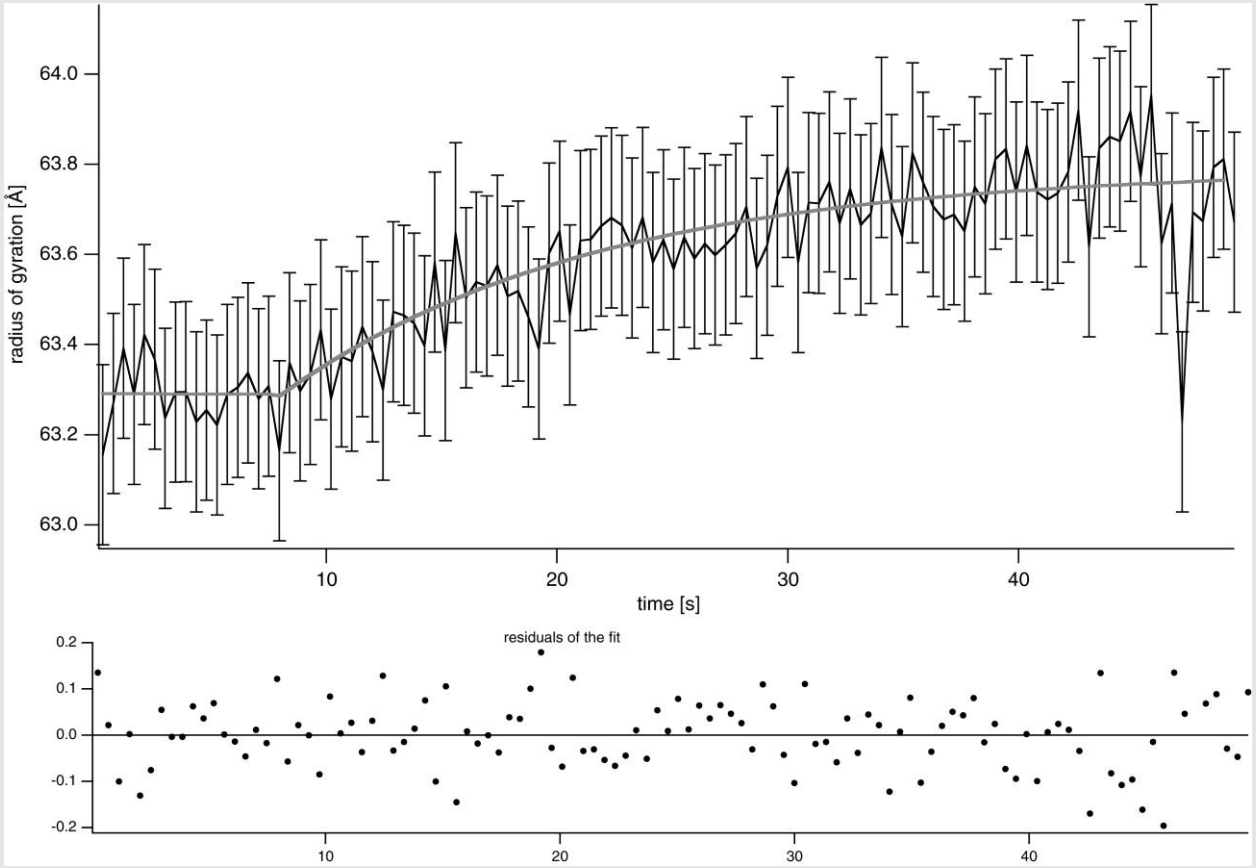
The Chaperonin folding machinery

The GroEL/GroES two stroke motor



The results support the „two stroke motor“ proposed for the chaperion mediated refolding process.
The switching between the ADP and ATP bound state faciltiate the refolding by enlarging the refolding cage under the GroES lid. If ATP bind on the other GroEL ring the GroES is released.

The Chaperonin folding machinery
GroEL ATP ase activity



Cooperative ATP binding mechanism for the ATPase activity.

The early stage of the ATP binding is not visible (< 125 ms), but the lack phase at the beginning indicates a cooperative binding and activity behaviour

SANS: Using the candle….

Slow kinetics on the chaperonin system GroE

Casing experiments
Complex formation with deuterated components

The Thermosome: Open or Closed structure?

Cell, Vol. 93, 125–138, April 3, 1998, Copyright ©1998 by Cell Press

Crystal Structure of the Thermosome, the Archaeal Chaperonin and Homolog of CCT

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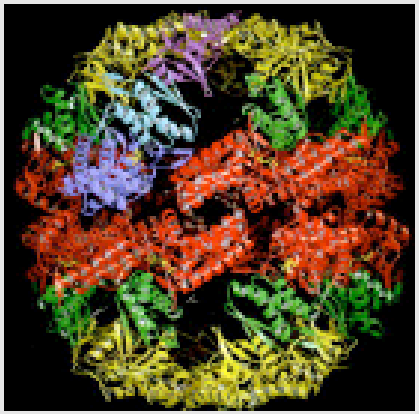
Germany

folded proteins prone to aggregation (Staniforth et al., 1994). This interaction seems to be largely independent of the structure or sequence of the substrate proteins (Viitanen et al., 1992; Horwich et al., 1993), but exposed hydrophobic regions appear to be a common feature for substrate recognition (Fenton et al., 1994). Bound substrate molecules are released in an ATP-dependent manner from the binding regions and are encapsulated in a closed compartment where folding proceeds (Weissman et al., 1995; Hayer-Hartl et al., 1996; Mayhew et al., 1996). Several rounds of binding and release may be required to reach the folded state (Weissman et al., 1994; Mayhew et al., 1996; Rye et al., 1997). A characteristic

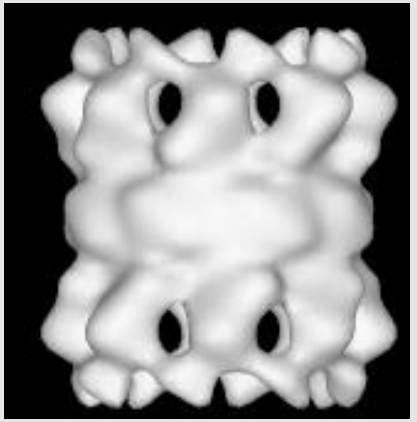


Nature Structural & Molecular Biology **5**, 855–857 (1 October 1998) | doi:10.1038/2296

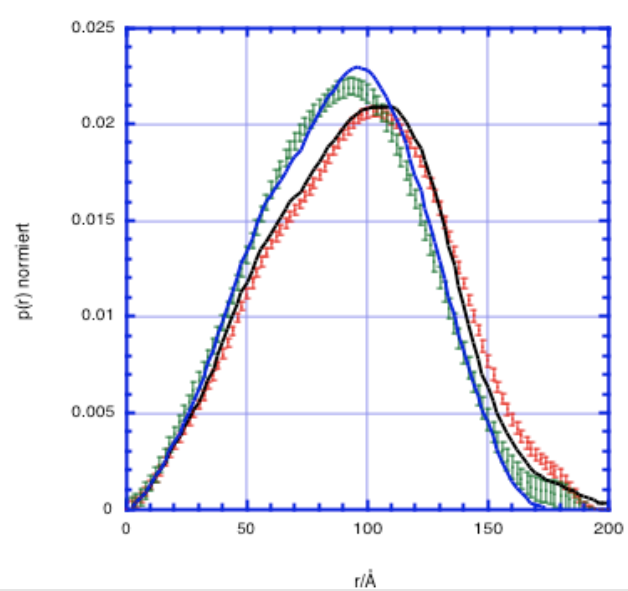
Group II chaperonin in an open conformation examined by electron tomography

Michael Nitsch , Jochen Walz , Dieter Typke , Martin Klumpp , Lars-Oliver Essen & Wolfgang Baumeister

Max-Planck-Institut für Biochemie Martinsried



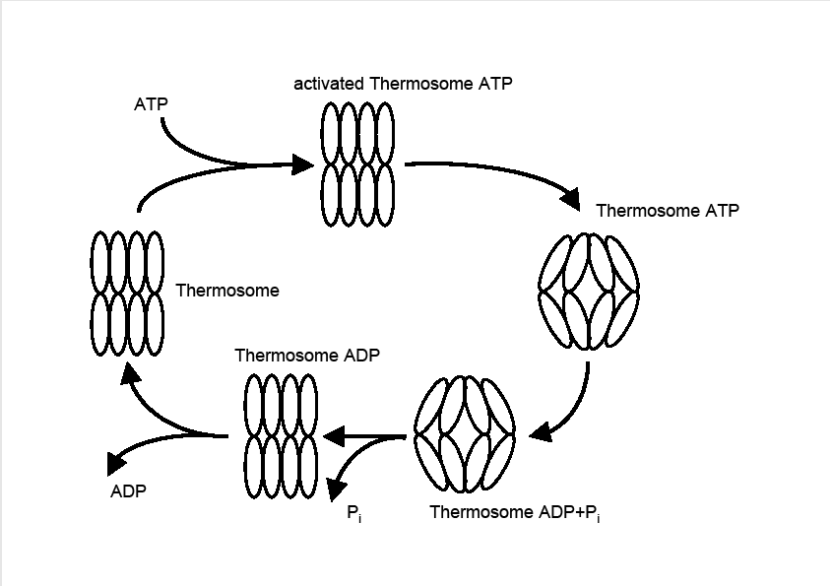
The Thermosome: The complete cycle



Nucleotide conformation

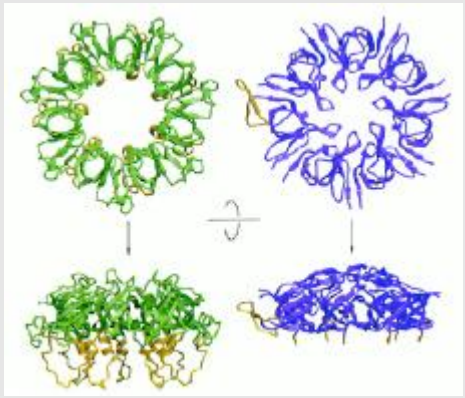
AMP-PNP	open
ADP-AlF	open
ADP-Pi	closed
ADP	open
Pi (control)	open

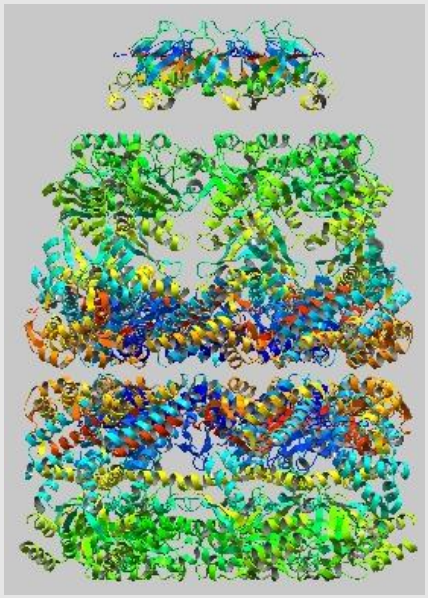
Open and closed conformations exists during the active cycle!

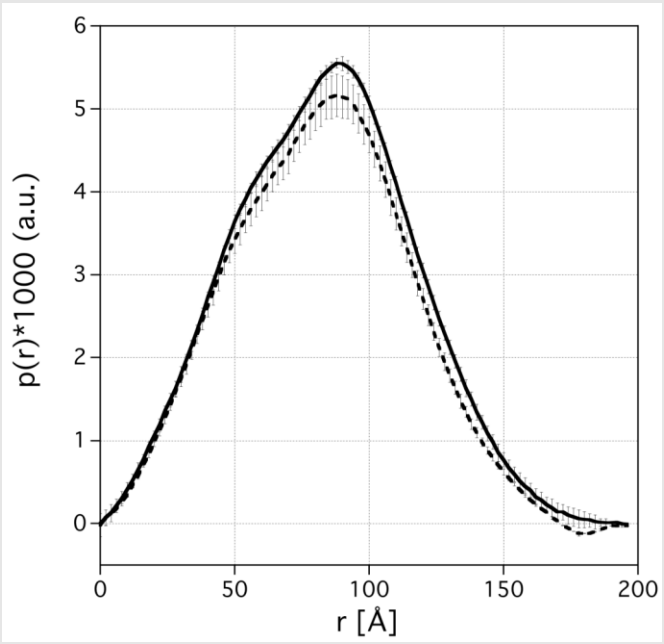


I .Gutsche, et.al CURRENT BIOLOGY, 10:405, 2000.

GP31 the bacteriophage Chaperonin cap

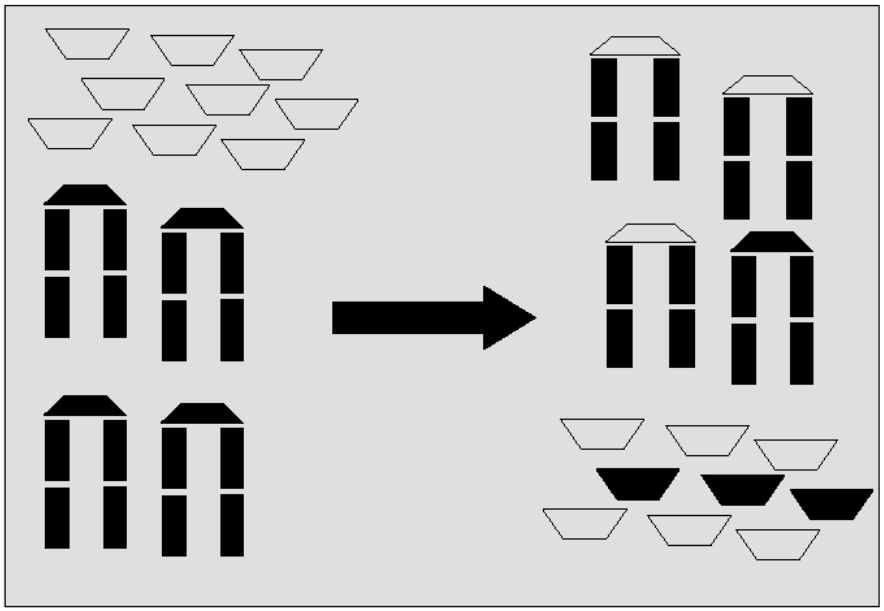






The GroEL-gp31 chaperonin complex, composed of the E. coli GroEL and the bacteriophage T4 encoded gp31, is essential for the folding of the T4 major capsid protein (gp23). Interestingly the E.coli GroEL-GroES complex cannot satisfy the folding requirements of gp23. Although the amino acid sequence of gp31 and GroES is only 14% identical, their structure is quite similar.

Chasing experiments

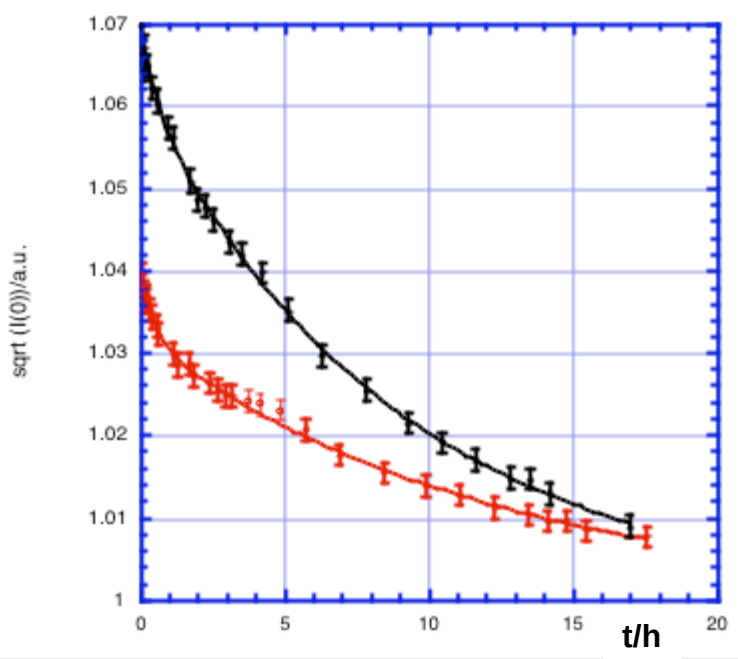


Preloaded GroEL with GP31, both native is mixed with per-deuterated GroES.

The GroES will “chase out” the GP31 from the complex.

This reaction is dependent on the binding constants

Chasing experiments



Chasing of GP31

Chasing of native GroES (control)

Analysis of the I(0) time evolution

- double exponential behavior
- two different reaction mechanisms

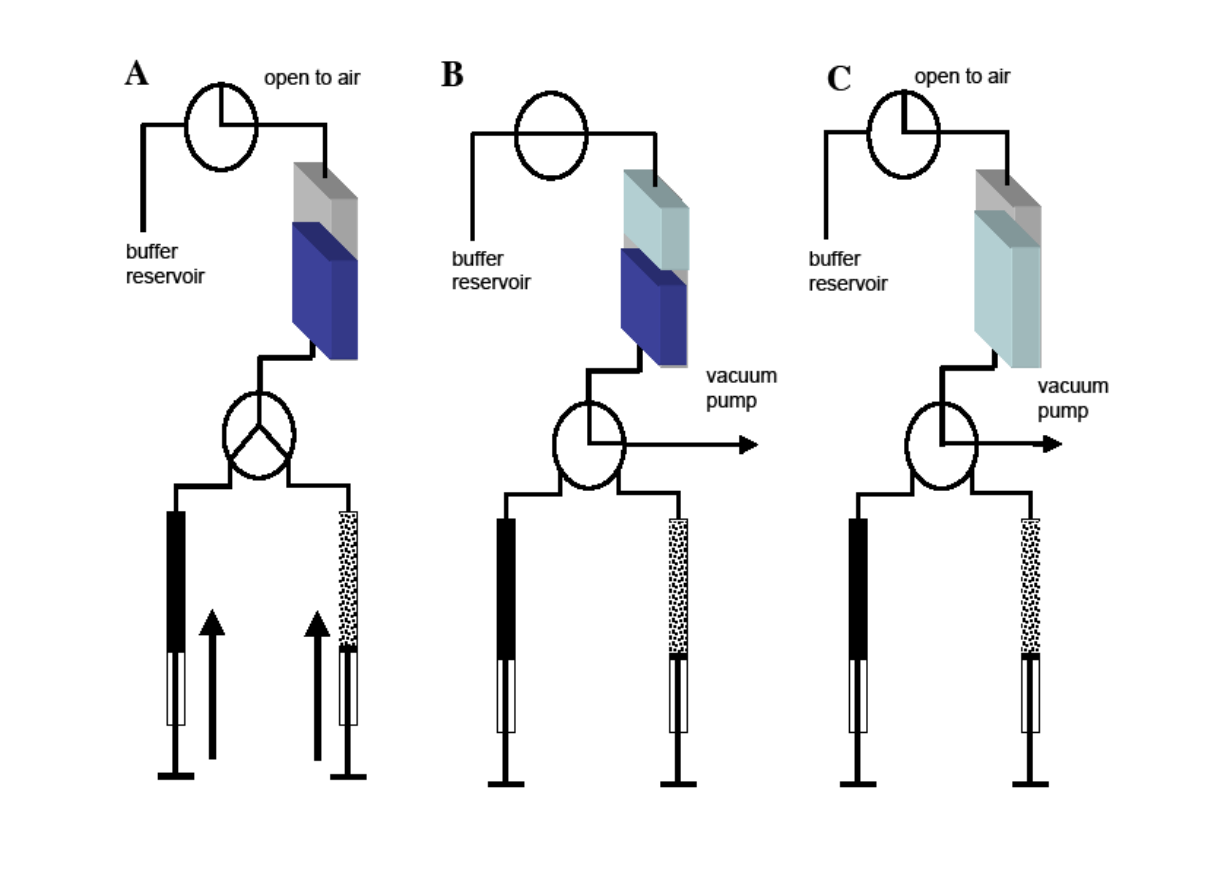
- GroEL and GP31 show different binding constants

Explanation:

Fast reaction the real chasing of bound GroES or GP31 by invisible GroES takes place.

Second slow phase chased GroES or GP31 starts to compete with the invisible GroES.

Time resolved SANS



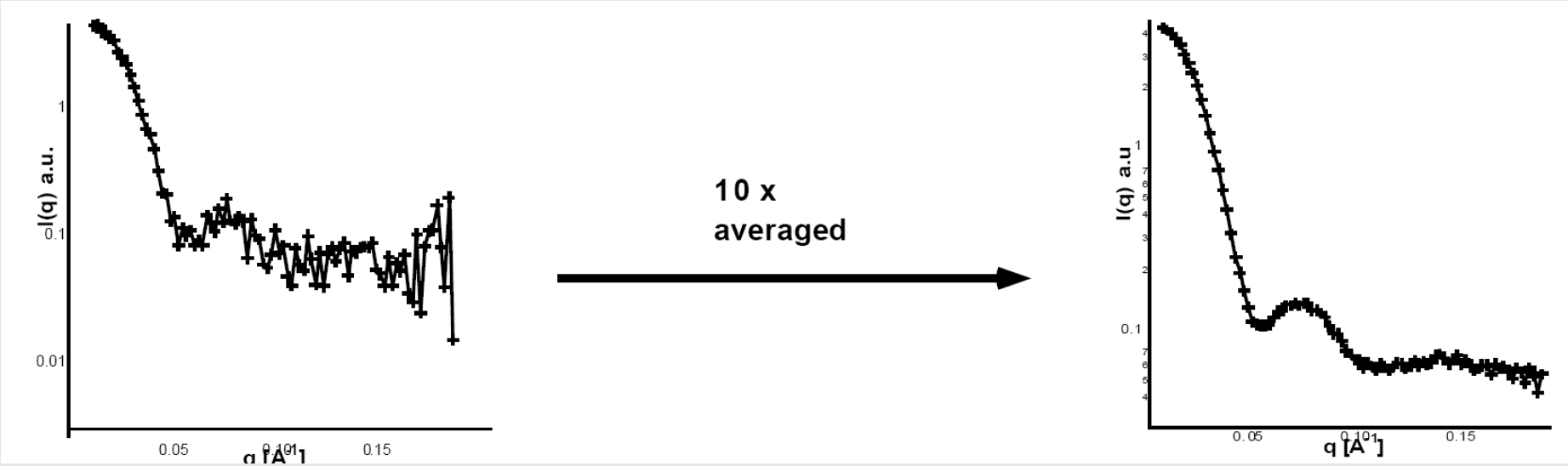
Stopped flow setup for SANS

- 100 µl needed
- large cell
- cleaning is an issue!

- Measurements at high contrast conditions

- D_20 buffer with low incoherent background

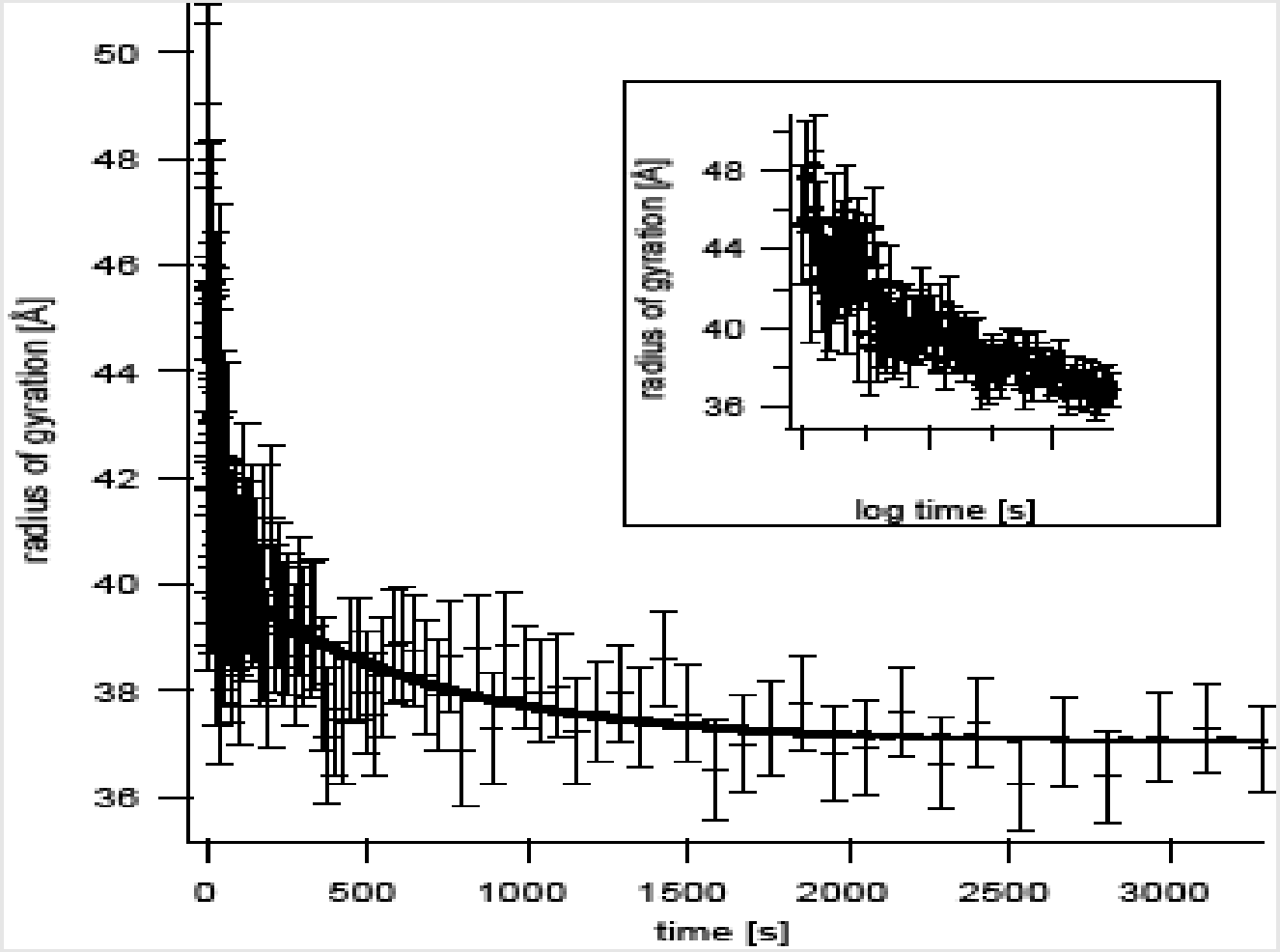
Time-resolved protein solution SANS



1 sec. exposure at D22

The lower flux is partially compensated by the higher scatting contrast of deuterated proteins in D_2O!

Time-resolved protein solution SANS



Formation of the GroEL/GroES$_2$ football complex

Native GroEL with deuterated GroES in 100% D_2O

Rg decreasing indicates the formation of this symmetric complex.

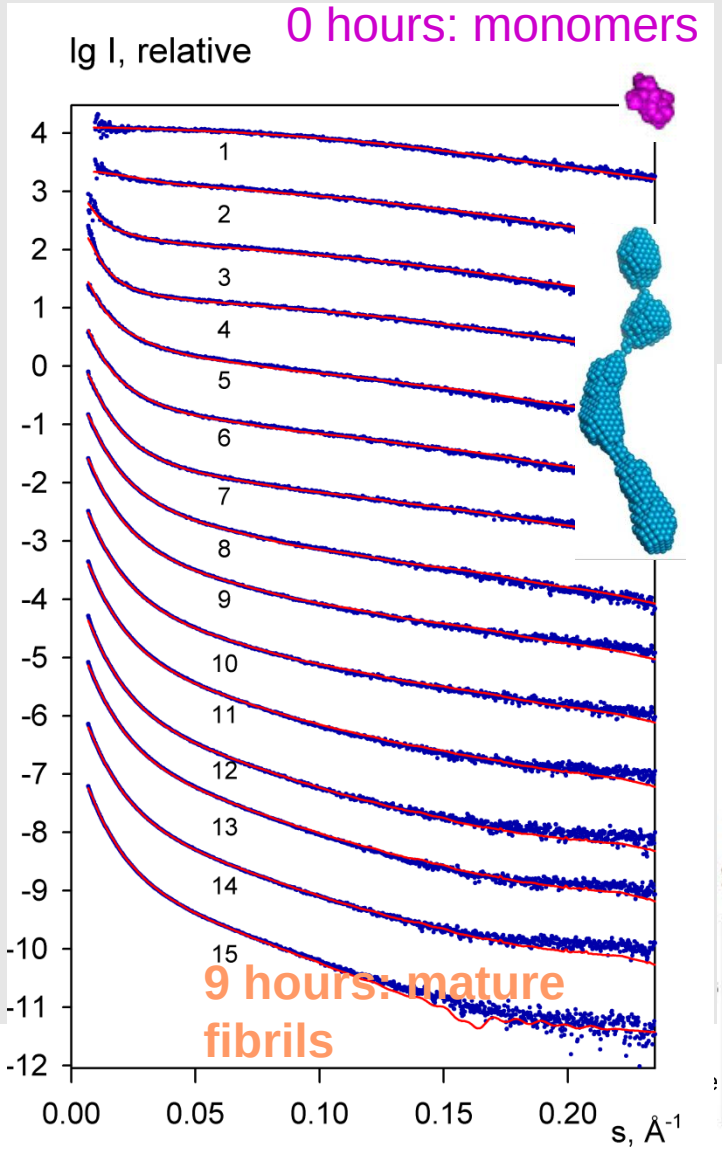
Low flux SAXS: Using a LED lamp…

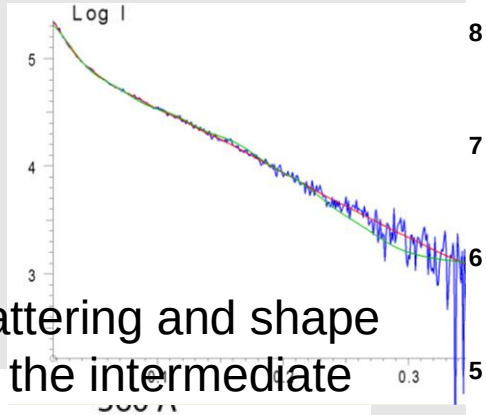
Slow kinetics on Insulin fibrill formation

Formation of large ordered protein complexes investigated by time resolved SAXS
From minutes to hours

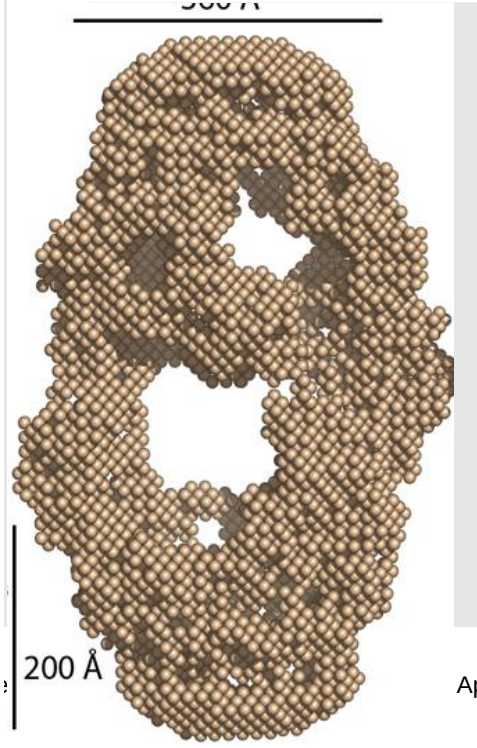
Fibrillation of insulin

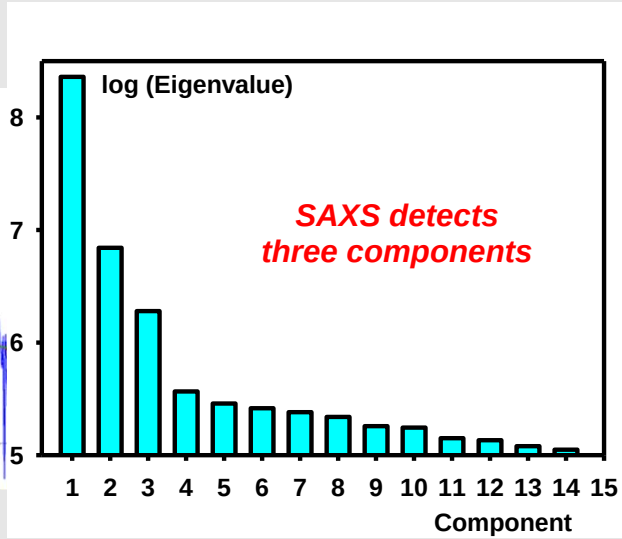
5 g/l 20% acetic acid 0.5M NaCl 45˚C



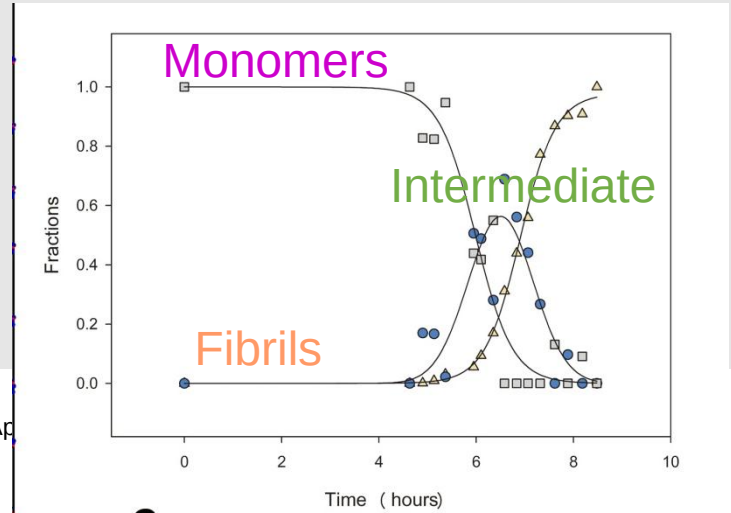


Scattering and shape of the intermediate





Growth rate of fibrils is proportional to volume fraction of intermediates

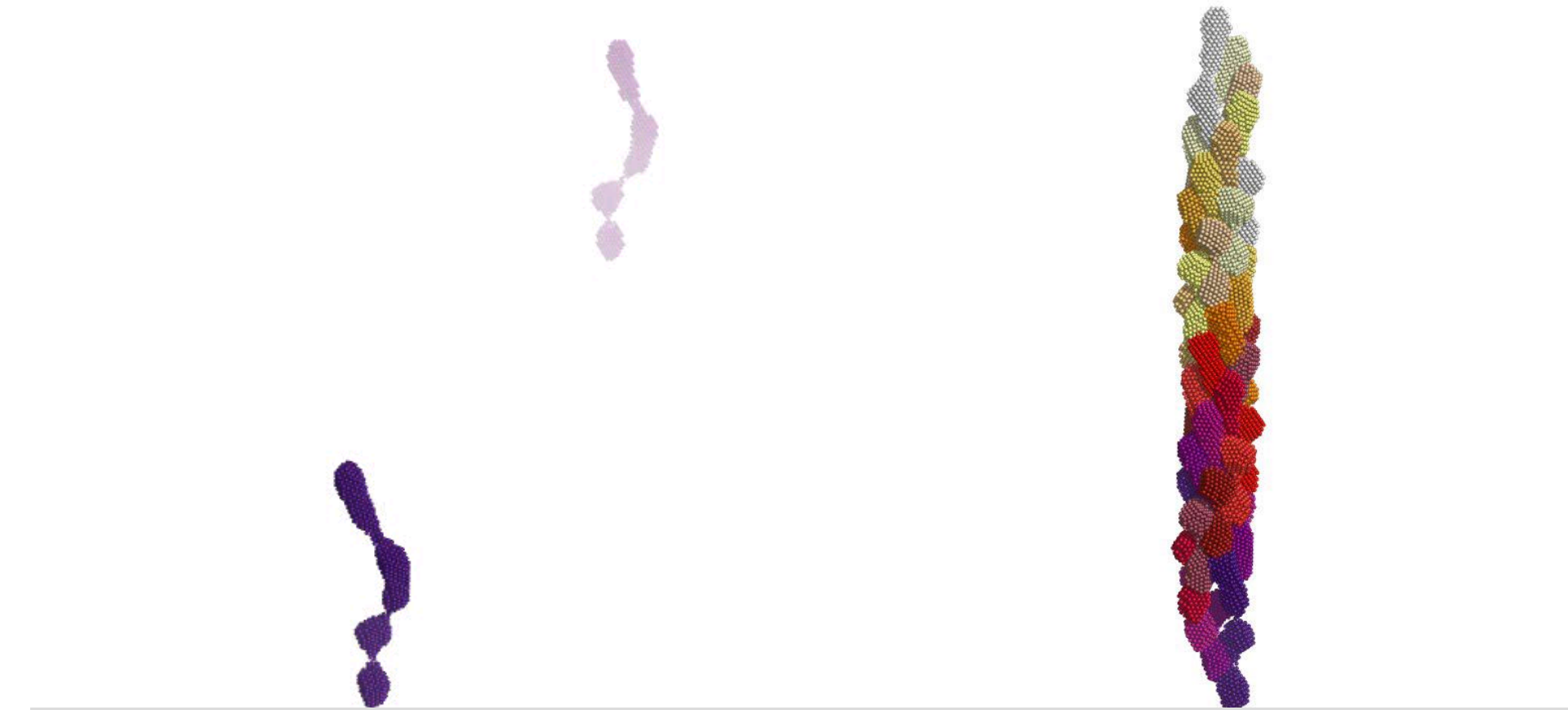


Fibrillation of insulin

Oligomers are fibrillation nuclei and potential targets against amyloidosis

Assembly of protofilaments
from the helical precursors (5-6 units)

Formation of mature fibrils
from intertwinning protofilaments



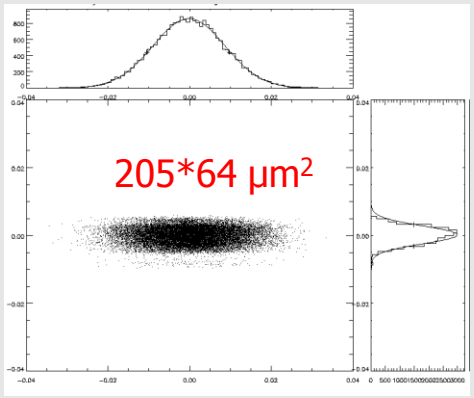
Vestergaard, B., Groenning, M., Roessle, M., Kastrup, J.S., de Weert, M.V., Flink, J.M., Frokjaer, S., Gajhede, M. & Svergun, D.I. (2007) *PLoS Biol.* **5**, e134

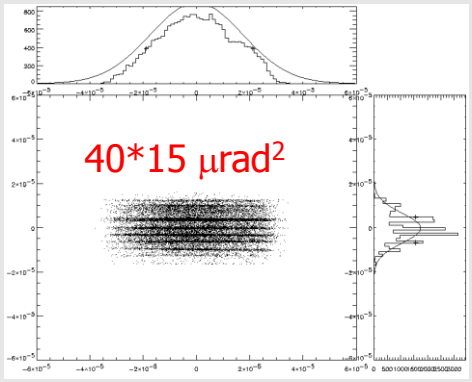
The future is brilliant!

Time resolved SAS on modern high brilliance SAXS beamlines

Parameters of the new BioSAXS beamline at the EMBL Hamburg

- **Standard (DCM) mode 2 x 10^{13} ph/s**
- **High flux (MLM) mode 1 x 10^{15} ph/s**
- **Pink beam mode 9 x 10^{15} ph/s**

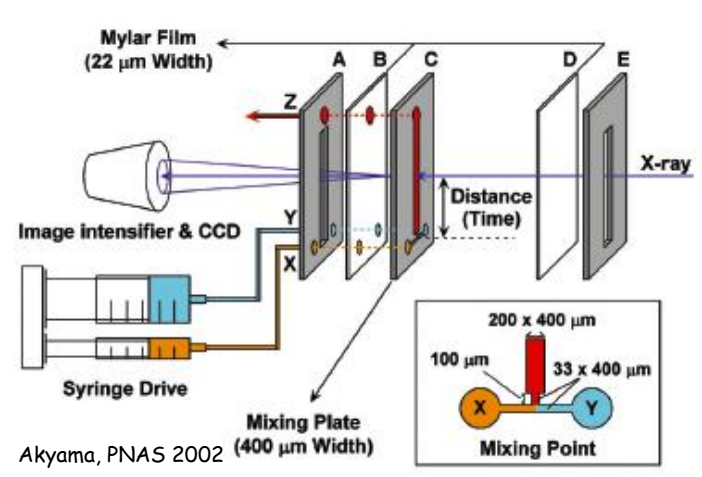




Ray tracing: beam size and divergence @ 8 KeV

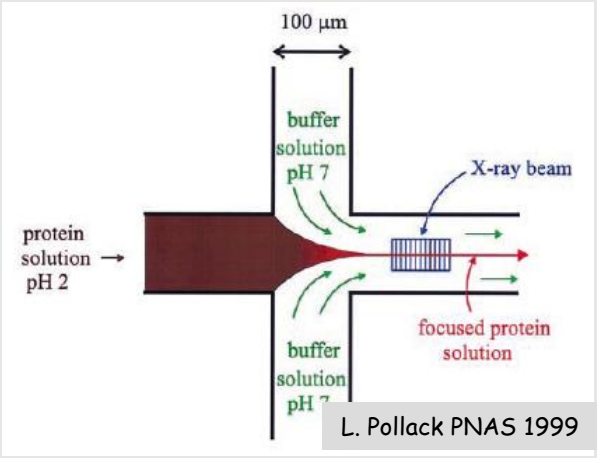
Time resolved small angle scattering:

Fast Mixing in laminar flow geometry by microfluidics

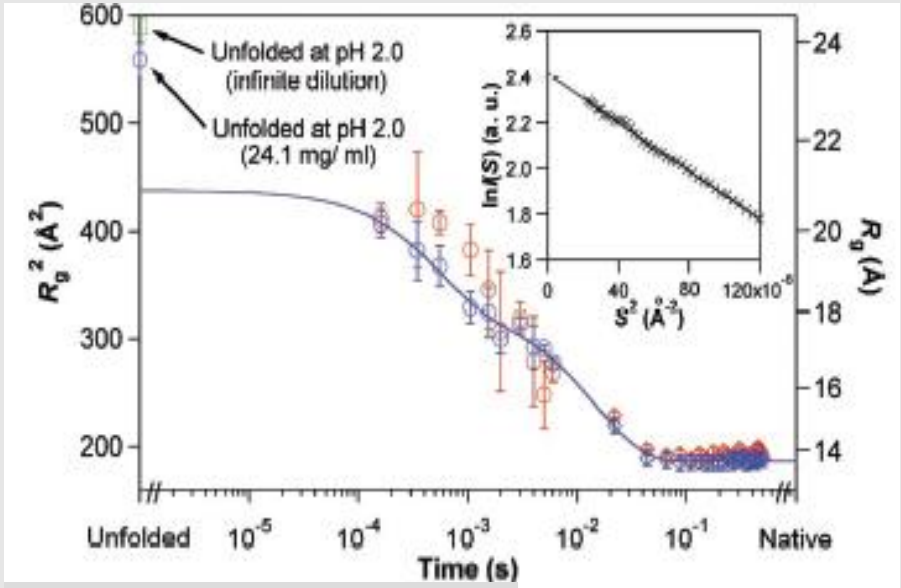


Akyama, PNAS 2002

- fast mixing times ~10μs to ~100μs
- continuous flow method but small sample consumption!
- micromachining or lithographic technology



L. Pollack PNAS 1999

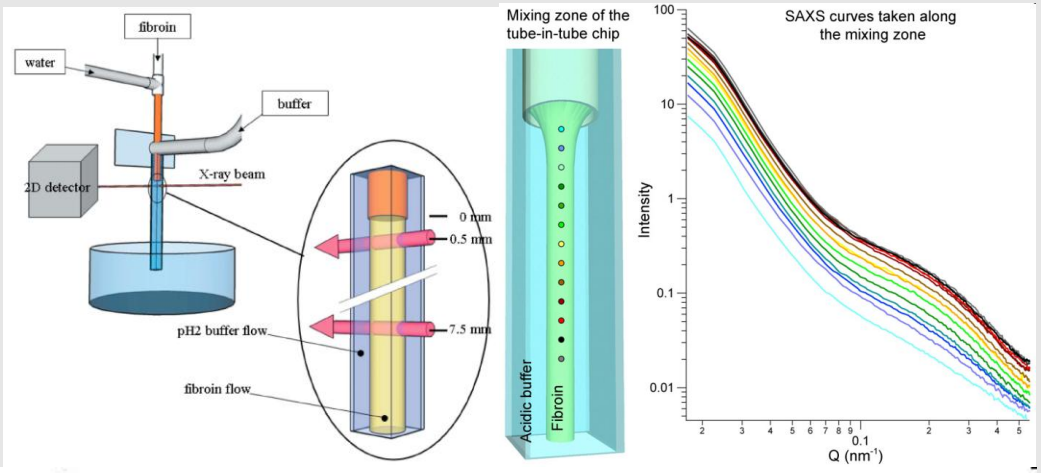


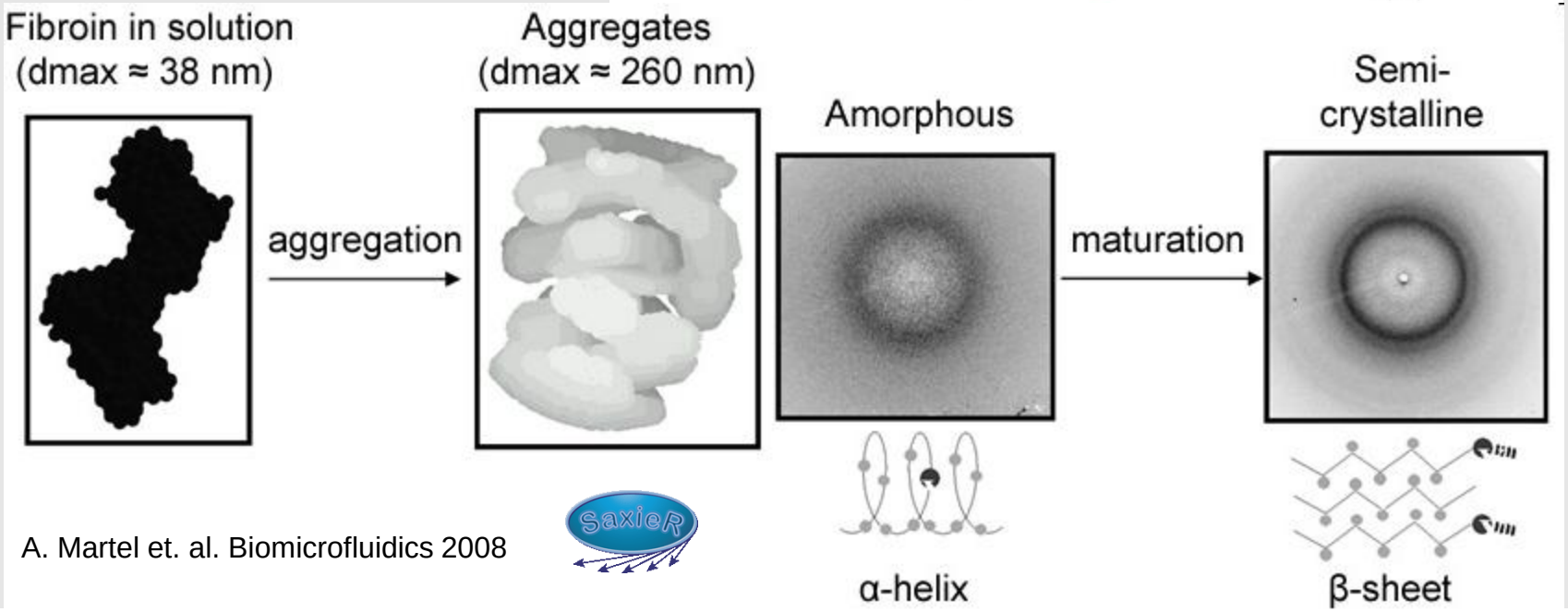
Akiyama et al. PNAS 2002

Online sample preparation
Micro reactors

ESRF microfocus beamline ID 13
Sample environment depends on scientific question

e.g. silk fiber maturation under shear forces





Microfluidics of droplets

⟹ surface energy dominates

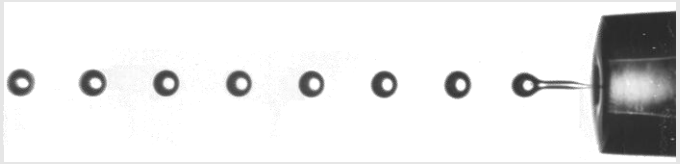
As the surface energy scales with **L^2** the
surface energy dominates over the kinetic energy:

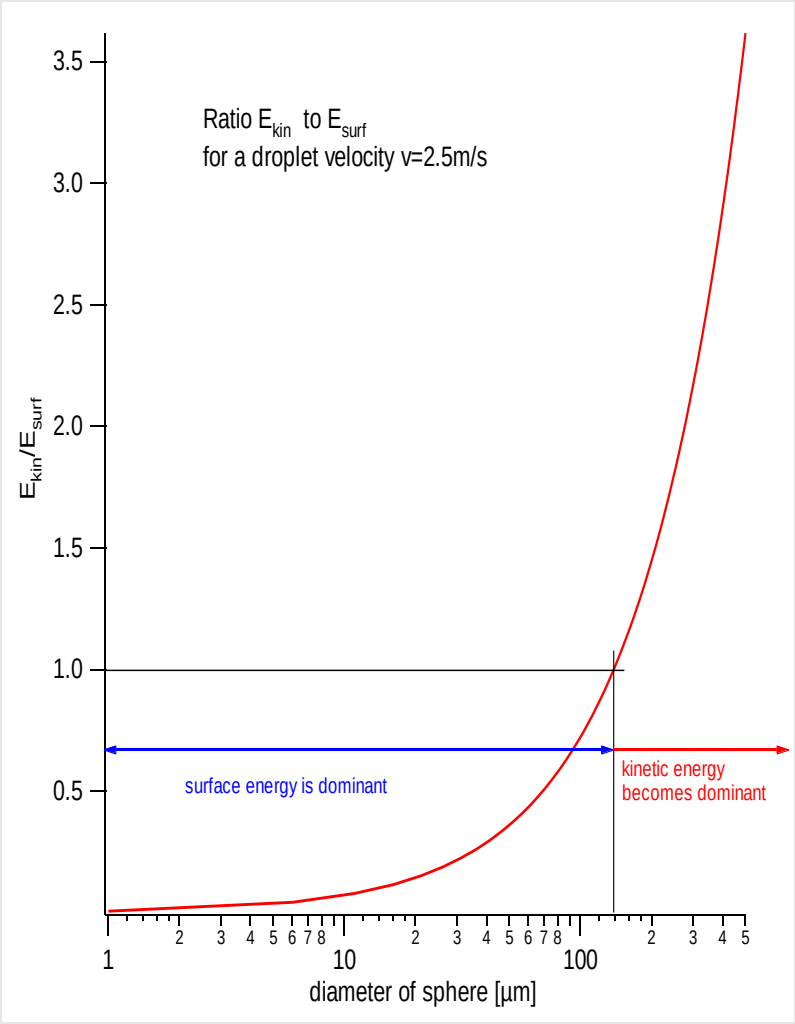
$$E_{surf} = 4\,\sigma\,\pi\,r^2$$

$$E_{kin} = 4/6\,\rho\,\pi\,r^3\,v^2$$

Sphere of radius r

Formation of stable droplets, which can sputtered on a surface without splashing



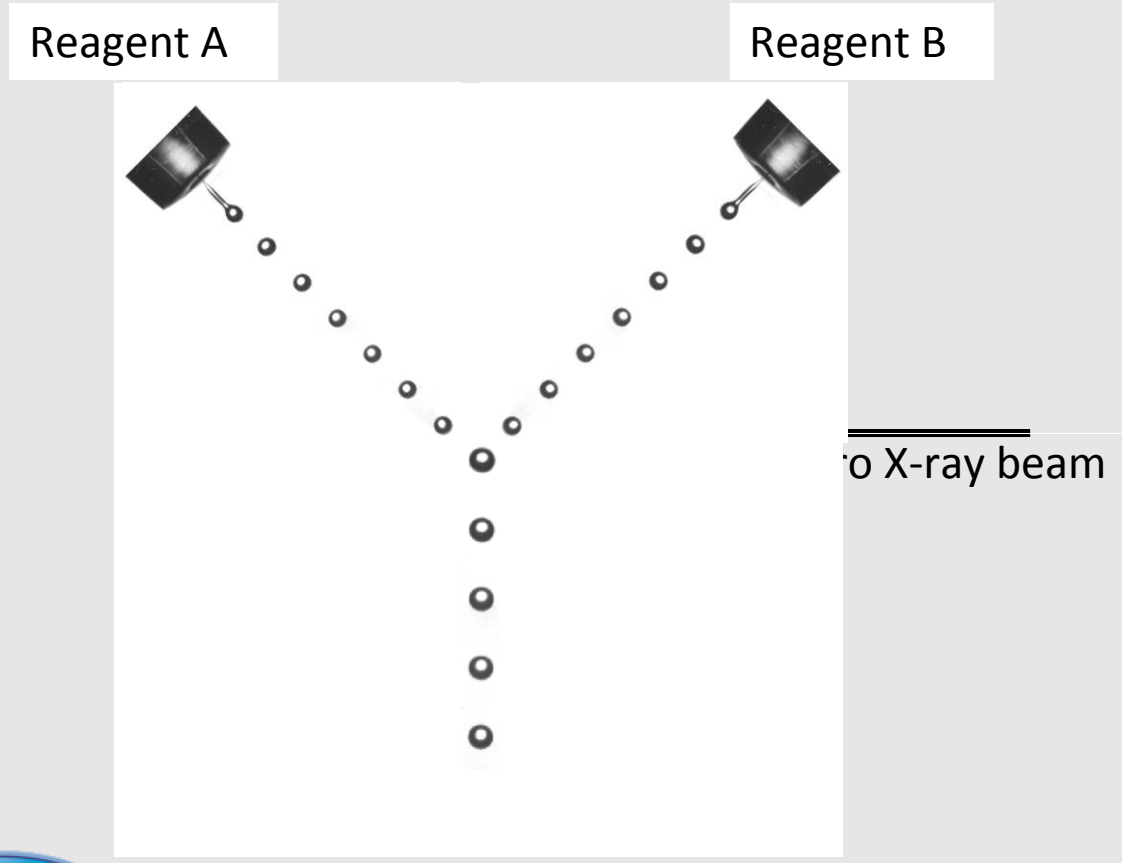


Mixing of droplets on the fly

mixing of the droplets by collision is very fast
t_{mix} ~ 10µs

Following the reaction by scanning the flow after the mixing with the X-ray microbeam.

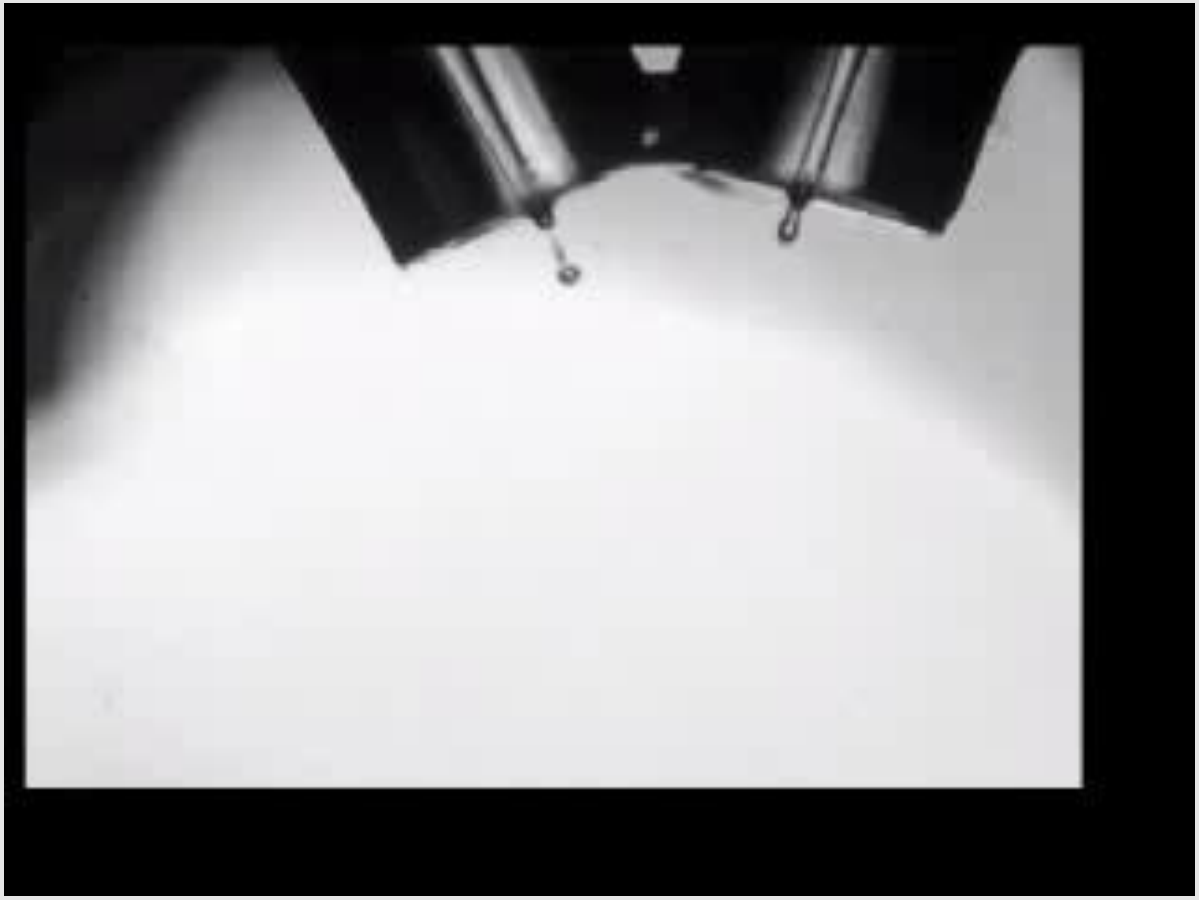
Example:
droplet volume: 65pl
droplet frequency: 1000Hz
exposure time : 10s
time points : 100



→ 65 µl Volume

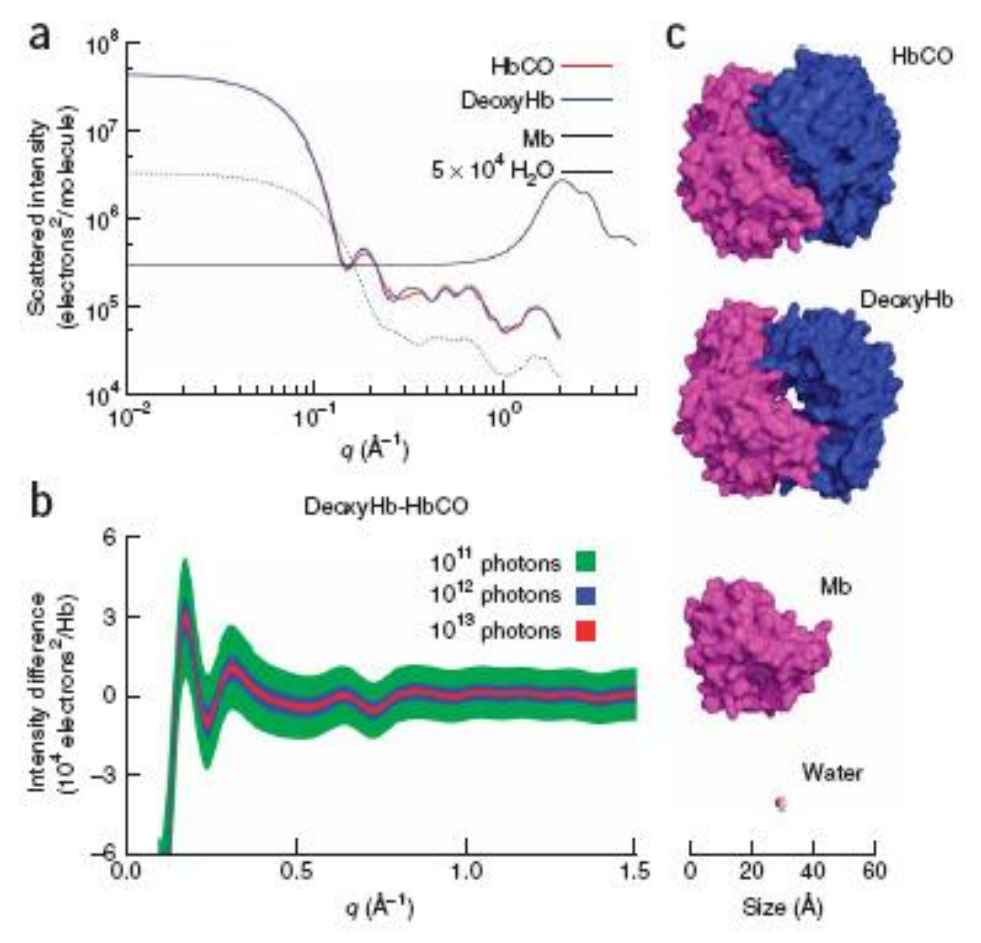
R. Graceffa et.al. 2009
ESRF ID13

Mixing of droplets on the fly

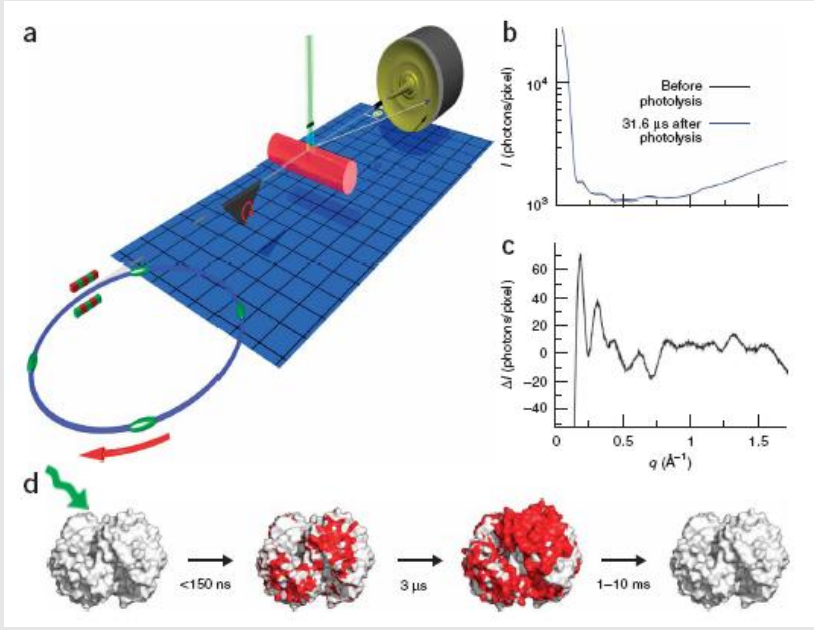


Time resolved SAXS/WAXS

Access to structural dynamics



M. Cammarata et. al. 2008 Nat. Meth



Laser induced conformational change of hemoglobin TR SAXS/WAXS in the sub µs time scale

Petra-III Experimental Hall