Time-resolved SAXS and SANS

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The horse in motion
Eadweard Muybridge 1877

Sallie Gardner at a gallop
The horse in motion

Copyright, 1878, by MUYBRIDGE.

"SALLIE GARDNER," owned by LELAND STANFORD; running at a 1.40 gait over the Palo Alto track, 19th June, 1878.

The negatives of these photographs were made at intervals of twenty-seven inches of distance, and about the twenty-fifth part of a second of time; they illustrate consecutive positions assumed in each twenty-seven inches of progress during a single stride of the mare. The vertical lines were twenty-seven inches apart; the horizontal lines represented elevations of four inches each. The exposure of each negative was less than the two-thousandth part of a second.
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: $1.23 \times 10^8$ neutron/cm$^2$ s$^{-1}$
- Spot on sample: $5 \times 5$ mm$^2$
Synchrotron based time-resolved SAXS

- Source-to-sample distances: Up to 10 m (ID02 ESRF)
- q-range: $1 \times 10^{-3}$ nm$^{-1} < q < 10$ nm$^{-1}$
- Max. flux at specimen: Up to $10^{15}$ ph/cm$^{-2}$ s$^{-1}$
- Spot on sample: $50 \times 50$ 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


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 chaperonin mediated refolding process. The switching between the ADP and ATP bound state facilitate 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?

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

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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

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The Thermosome: The complete cycle

Nucleotide conformation
AMP-PNP open
ADP-AIF open
ADP-Pi closed
ADP open
Pi (control) open

Open and closed conformations exists during the active cycle!

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

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.

Chasing of GP31
Chasing of native GroES (control)
Time resolved SANS

Stopped flow setup for SANS

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

- Measurements at high contrast conditions
- D$_2$O buffer with low incoherent background
Time-resolved protein solution SANS

1 sec. exposure at D22

The lower flux is partially compensated by the higher scattering contrast of deuterated proteins in D$_2$O!
Time-resolved protein solution SANS

Formation of the GroEL/GroES$_2$ football complex

Native GroEL with deuterated GroES in 100% D$_2$O

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

SAXS detects three components

Monomers
Intermediate
Fibrils

0 hours: monomers
9 hours: mature fibrils

lg I, relative

log (Eigenvalue)

Component
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 intertwining protofilaments

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 $2 \times 10^{13}$ ph/s
- High flux (MLM) mode $1 \times 10^{15}$ ph/s
- Pink beam mode $9 \times 10^{15}$ ph/s

Ray tracing: beam size and divergence @ 8 KeV
Time resolved small angle scattering:
Fast Mixing in laminar flow geometry by microfluidics

- 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

A. Martel et. al. Biomicrofluidics 2008
Microfluidics of droplets

→ surface energy dominates

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

\[
E_{\text{surf}} = 4 \sigma \pi r^2
\]
\[
E_{\text{kin}} = \frac{4}{6} \rho \pi r^3 v^2
\]

Formation of stable droplets, which can be sputtered on a surface without splashing.
Mixing of droplets on the fly

Mixing of the droplets by collision is very fast
\[ t_{\text{mix}} \approx 10\mu s \]

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

R. Graceffa et.al. 2009
ESRF ID13
Mixing of droplets on the fly
Time resolved SAXS/WAXS
Access to structural dynamics

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

M. Cammarata et. al. 2008 Nat. Meth
Petra-III Experimental Hall