Time resolved SAXS

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Foreword

• Structural biology: knowing the structure to understand the function

<table>
<thead>
<tr>
<th>Structure</th>
<th>Function</th>
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• Works quite well, we now have a lot of protein structure that shed light on their functions and help to understand how proteins work

• Also had limits:
  • Same functions are performed by protein with different structures
  • IDP: some protein without structure still have functions

What about looking directly at proteins in action?
Time resolved experiment

Study systems whose structures change over time
Time scale of biological processes (protein folding)

- Different time scale
- Necessitates different kind of experiments
Generalities
Time resolved experiments

• Reaction of interest needs to be properly controlled:
  
  • Controlled triggering of the reaction of interest.
  
  • System at equilibrium is perturbed, and one monitor the return to a new equilibrium
Triggering the reaction

Changing the chemical composition of your solvent (Mixing your solution with a reactant)

• Change of pH, salt concentration, introducing ligand or interacting ions...
Pressure jump


Temperature jump

• By mixing

• Using laser pulse

Light triggering

Light acting directly on the protein

Indirectly by releasing caged compounds


Fig. 1. General principle of uncaging.
How fast the reaction should be triggered depends on how fast the system reacts.

- Triggering:
  - Simultaneous, fast and homogeneous triggering at the time scale of the reaction
How fast can you trigger the reaction?

• Depends on the triggering methods
  • Mixing:
    • seconds to ms (with fast mixing devices)
    • Limited by mixing, diffusion time
  • P-Jump:
    • Diffusion of the pressure shockwave: speed of sound ms
    • In practice micros-ms
  • Light triggered reaction:
    • Practically not limited for “direct” triggering (limitation: speed of light)
    • Limited by intermediate reaction in the case of indirect triggering (T-Jump, caged compound)

* Small measurement cell helps.
Monitor the reaction

• Many spectroscopic technics can and have been used

• SAXS is a good technics to study reaction of biological system
  • Samples are in solution, in a quasi-native state. Many reaction takes place in solution and can be triggered in a controlled manner
  • Data can be collected quickly: Possibility to study fast reaction

• SANS: long collection time, limited to very slow reaction

• Different mode of data collection
Continuous vs pump-probe
Continuous vs pump-probe
Continuous vs pump-probe

Perturbation

$\Delta t$

Probe

$\Delta t$

Perturbation

Probe
Limitation – Collection time
Limitation – Collection time
Short collection time: High flux

• Third generation synchrotron

• Multilayer monochromator

• Pink beam
DMM beam of P12

For protein: BSA 2.5 mg/ml
1.35 ms exposure time

100us exposure time
chopper

- Production of short intense beam pulse
- Control of sample exposure (to limit radiation damage)
- Improve time resolution

P12 Chopper
- 2 modes:
  - Stroboscopic
  - pump and probe
Short collection time - Fast detector

• Photon counting detector: Pilatus (300Hz), Eiger (up to 2kHz)

• Detector gating: condition when the detector can count photons on an external signal

• Gas detector (Theoretically, up to 1MHz)
Dead time of the reaction

- Time between the reaction is triggered and the first point is collected (depends on triggering methods and collection time)

Short dead time required to study fast kinetic
Practical tips

• Know your reaction
  • Master your triggering method
  • Know your time scale

• A lot of sample is required
  • Depends on:
    • Radiation damage
    • Number of point in the kinetics
    • Signal to noise ratio desired
  • At least 1 ml of sample, often more, is required
Examples
Slow kinetics – Fibril formation

Amyloid fibrils

• Insoluble protein aggregates
• Implied in different diseases (Alzheimer, Parkinson, Type II diabetes, ...)
• Common structural features (cross beta)
• Nucleation growth

10/28/2019  Time resolved scattering studies - C. Blanchet
SAXS Data

- Singular value decomposition: 3 species
Models
Models
Sub-second kinetics

• Stopped-flow (dead time: 2-10 ms)
MsbA nucleotide binding domain

MsbA is an ATP-binding cassette transporter that transports lipid A and lipopolysaccharide through the inner membrane of Gram-negative bacteria.


Reaction of MsbA NBD with ATP followed by SAXS

Henning Tidow
Inokentijs Josts

Josts et al. Structure (in press)
Stopped flow triggering
MsbA nucleotide binding domain and ATP

- Rapid mixing using stopped-flow
- 35 ms frames collected with different delays after mixing
MsbA nucleotide binding domain and ATP

In the first phase ($t<2.5\text{s}$), rapid increase of the radius of gyration, then ($t>2.5\text{s}$) slow decrease.
ATP-induced transient dimerization of MsbA nucleotide binding domain

Fit with a mixture of monomer and dimers
Calmodulin

Caged compound release by flash photolysis

- DM-nitrophen
Equilibrium measurement
Kinetics
0.5 ms

With mastoparan

Without mastoparan
Model
Ultra-fast time resolved
Ultra short collection time

- Beamline ID09B, ESRF, Grenoble
- Using the pulsed structure of the synchrotron
- About 5000000 bunch/sec
Isolate one bunch

- Isolate one bunch (ms shutter + fast chopper)
Single bunch experiment

• High flux needed

• Repetition of the measurements
Pump and probe experiment

Trigger with Laser pulse

Probe with X-ray

$t$

Bunch length ≈ 100 ps

Resolution: up to 100 ps
T and R states of hemoglobin

Looking at the unbinding of oxygen by hemoglobin
Experimental setup
Structural change in hemoglobin

What is 30fs?

100 fs $\rightarrow$ second
Second $\rightarrow$ 1000000 years

Light travels 9 μm in 30fs
Experimental results
Protein quake
Conclusion

• SAXS is a good tool for time resolved experiments
• Good control on the initiation of the reaction needed
• Use experimental setup adapted to your system
  • Reaction triggering
  • time scale