Time resolved scattering studies

Clement Blanchet

Time resolved studies?

- Studies of systems that changes over time
- Collect data at different time point of the reaction

Time resolved experiment

- Perturb a system

- Monitor the return to equilibrium

Time resolved studies: ingredients

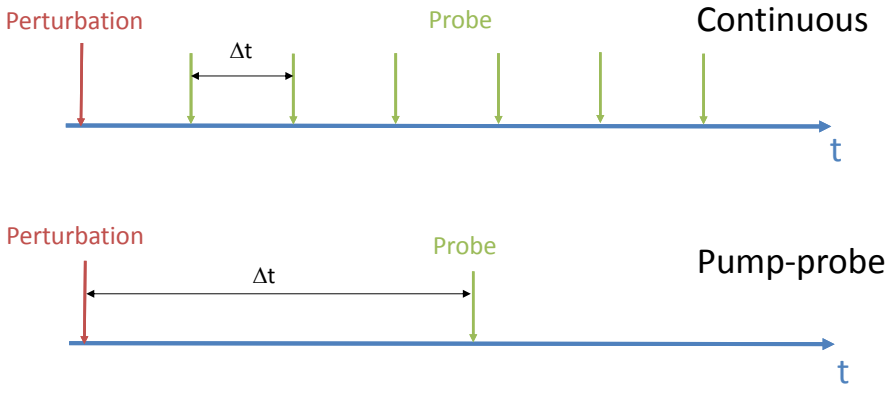
Perturbation

- Modification of physical conditions (T, P)
- Modification of chemical conditions (fast mixing, caged compound)
- Modification of the system (photolysis,…)

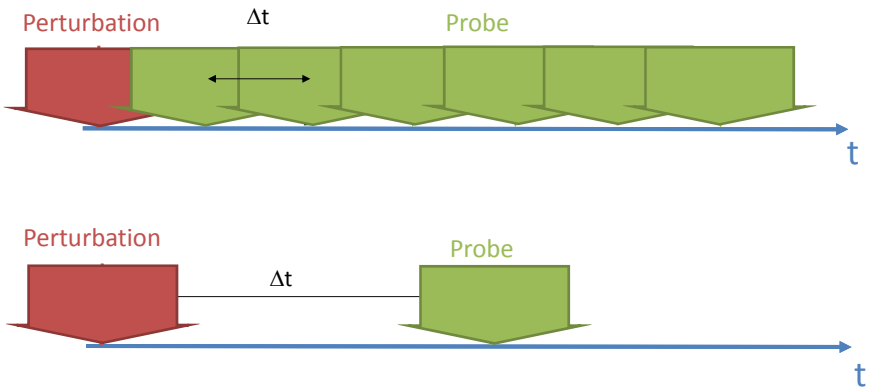
Probe

- Anything that can collect information on your system
- In our case : SAXS (SANS has generally too long collection time for time resolved studies).

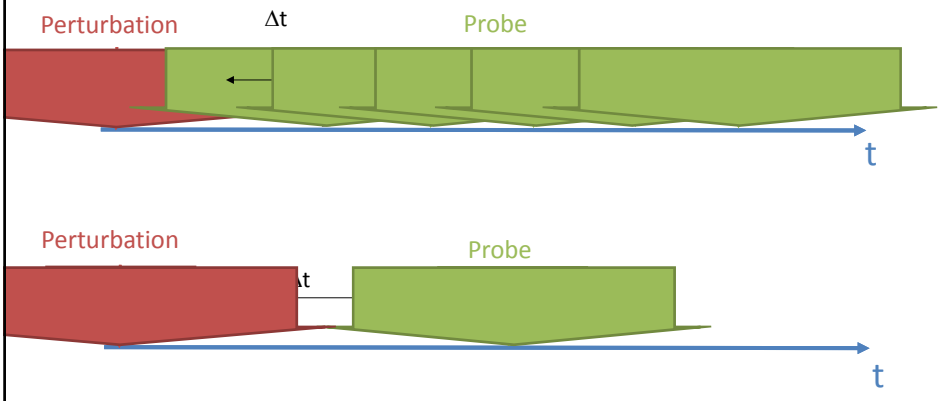
Continuous vs pump-probe



Continuous vs pump-probe



Continuous vs pump-probe



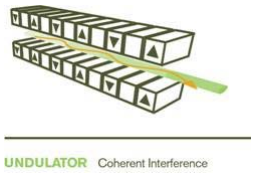
Monitor the reaction

- Fast reaction: short perturbation
- Fast reaction: short collection time

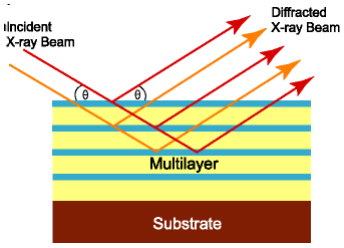
- Many photons needed to collect a proper SAXS data → High flux
- Fast detector

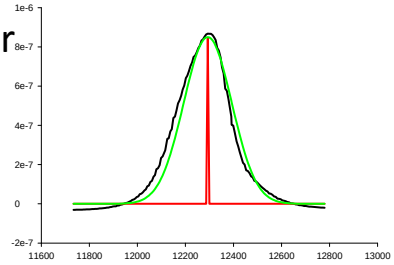
Short collection time: High flux

- Third generation synchrotron

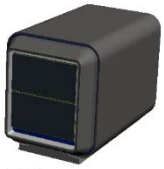


- Multilayer monochromator

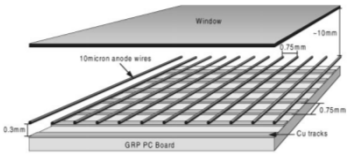




Fast detector



- Photon counting detector: Pilatus (300Hz), Eiger (3kHz)
- CCD(up to 5kHz)
- Gas detector (1MHz)

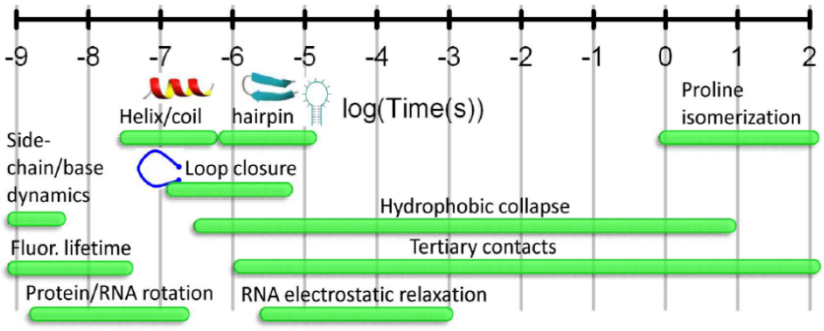


- Overcome detector limitation: use short beam pulse (using shutter, chopper,…)

Dead time

- Time between the beginning of the reaction and the first data point
- Depends on:
  - How fast the reaction is triggered
  - How fast the first point can be collected
- Short dead time needed to study fast kinetic

Time scale of biological processes
(protein folding)



Examples

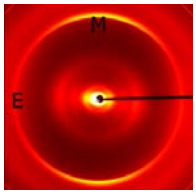
- "Slow Kinetics"
  - Fibril formation
- Sub-Second kinetics
  - Stopped-flow
- Millisecond kinetics
  - Continuous flow
  - Caged compound
- Ultrafast kinetics

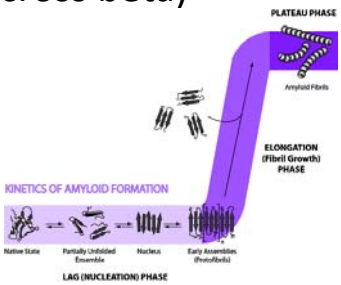
Slow kinetics – Fibril formation

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**

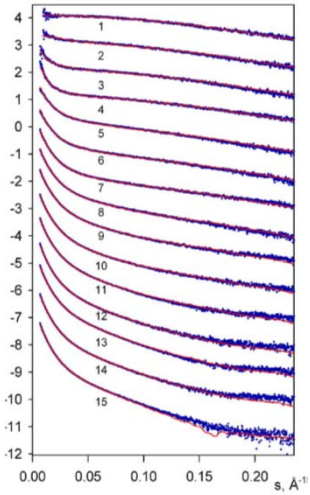
Amyloid fibrils

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

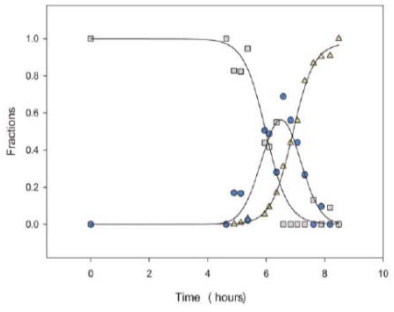




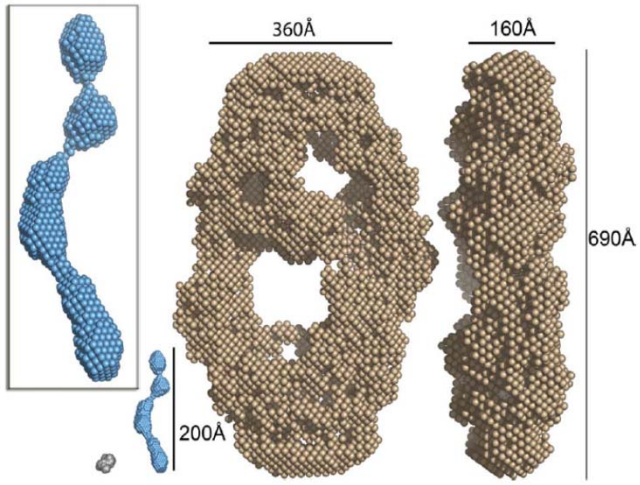
SAXS Data



- Singular value decomposition: 3 species



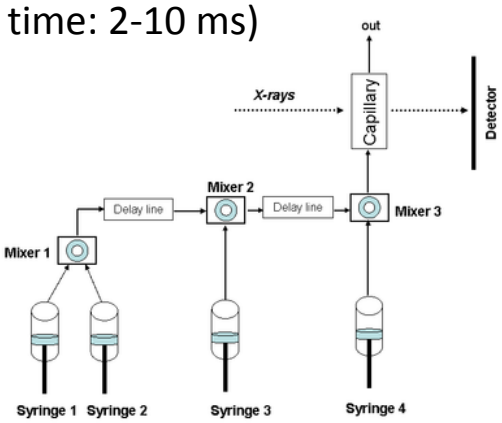
Models



Models

Sub-second kinetics

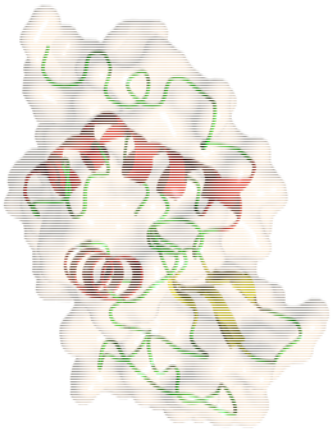
- Stopped-flow (dead time: 2-10 ms)



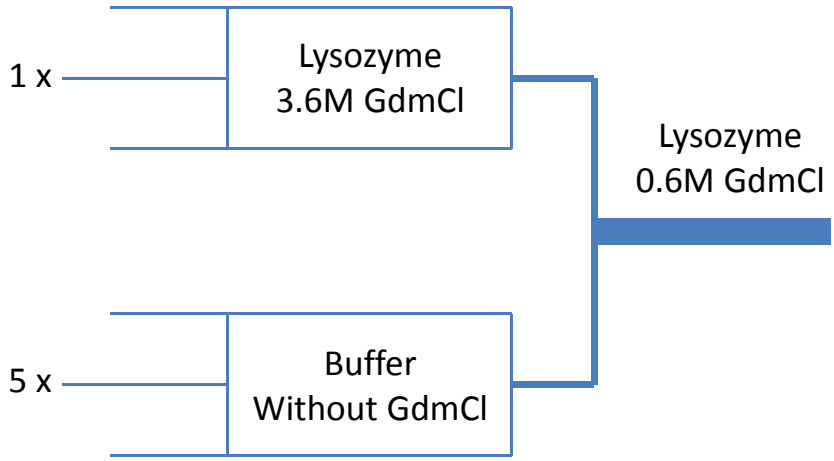
Stopped flow - Example

Characterization of Transient Intermediates in Lysozyme Folding with Time-resolved Small-angle X-ray Scattering

Segel et al.
JMB, 1999, Volume 288 (3), 489-499

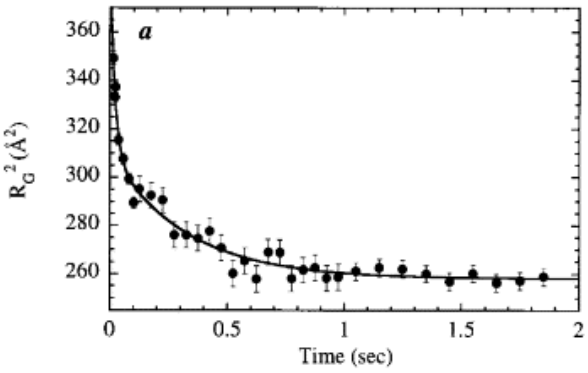


Lysozyme Folding



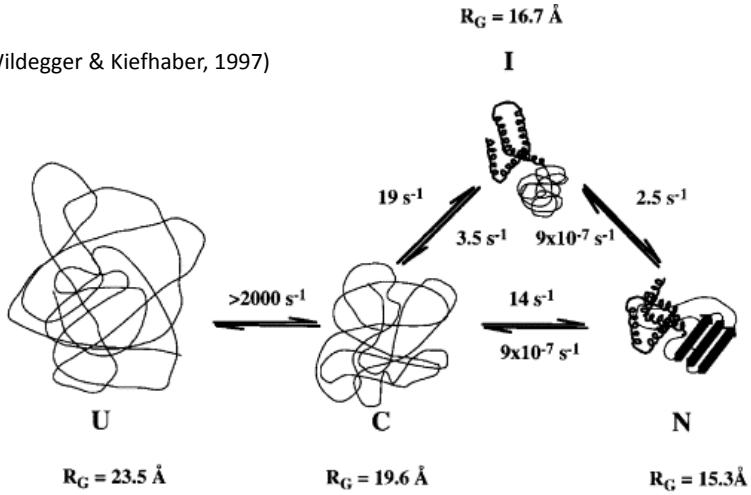
Lysozyme Folding

- Evolution of Rg in time



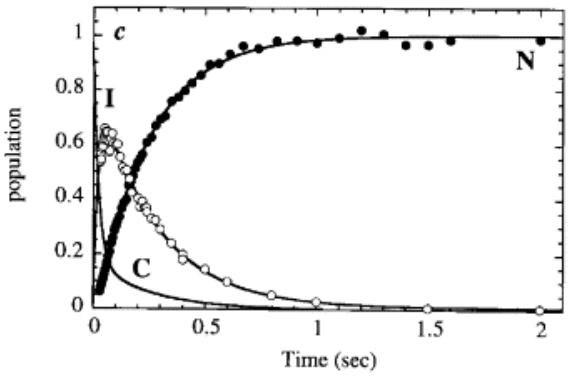
Singular value decomposition

(Wildegger & Kiefhaber, 1997)



Interrupted refolding experiment

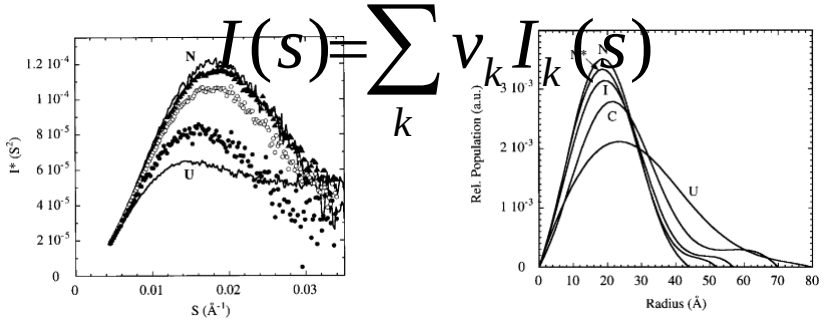
- Double mixing step monitored by fluorescence



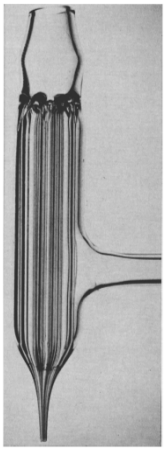
Reconstruction of the scattering profile

$$I(s,t) = \nu_C(t) I_C(s) + \nu_I(t) I_I(s) + \nu_N(t) I_N(s)$$

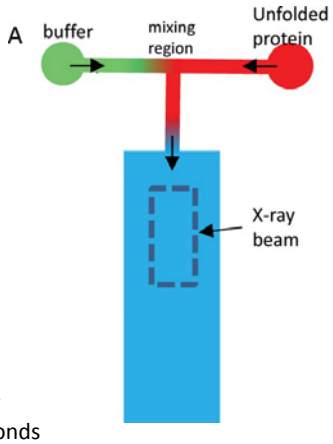
$$I(s) = \sum_k \nu_k I_k(s)$$



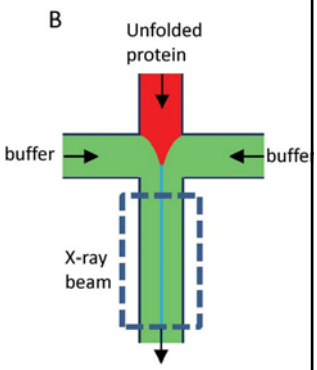
Continuous flow



Concentric capillary mixer
Mixing time: 30 microseconds
Moskowitz & Bowman, *Science*, 1966



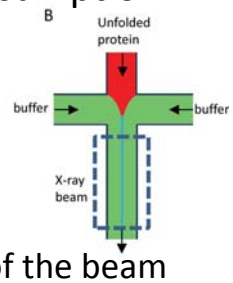
Turbulent mixing



Laminar mixing

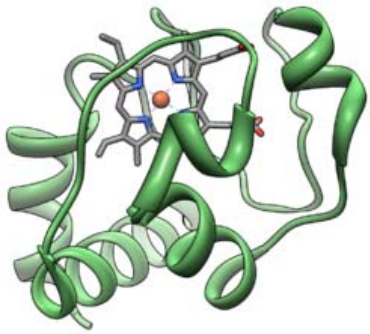
Continuous flow

- Continuous flow → high sample consumption
  - Microfluidic continuous flow system



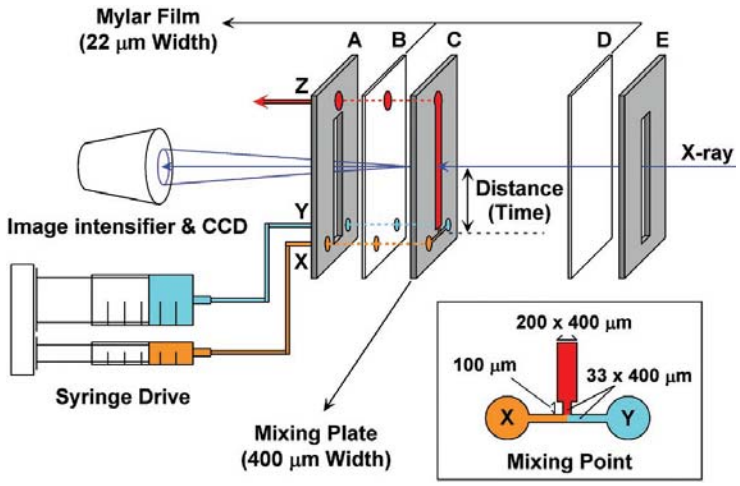
- Space <-> time
  - low flux OK
  - time resolution <-> flow rate and size of the beam
- Dead time (SAXS) ≈150 microseconds

Example continuous flow

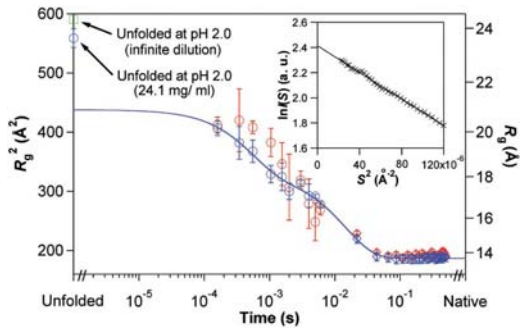


Conformational landscape of cytochrome c folding studied by microsecond-resolved small-angle x-ray scattering. Akiyama *et al.* PNAS 2002

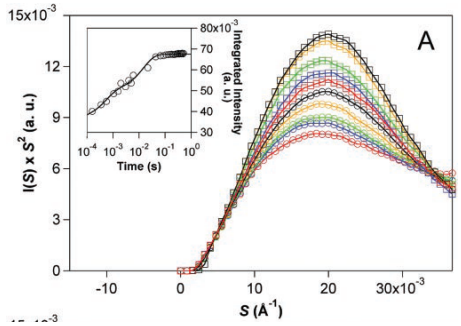
Continuous flow



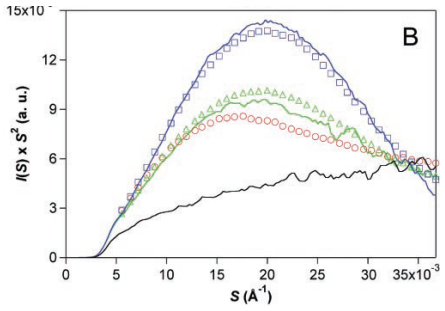
Radius of gyration



Kratky plots

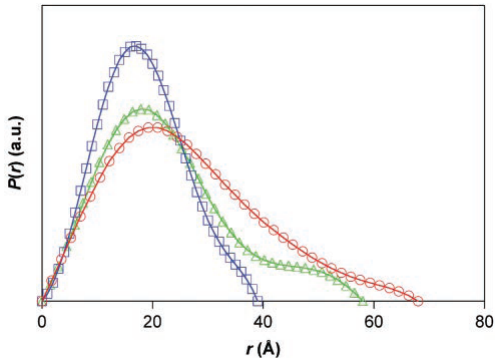


Singular value decomposition



$$U \leftrightarrow I \rightarrow II \rightarrow N^*$$

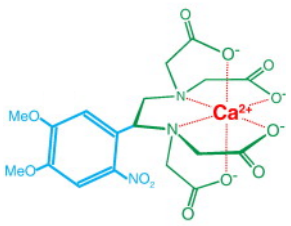
Conformational landscape of Cyto C



Scheme	Component I		Component II		Component N	
	R_g, Å	D_{max}, Å	R_g, Å	D_{max}, Å	R_g, Å	D_{max}, Å
U ↔ I → II → N*	20.5	66	17.7	58	13.9	39

Caged compound release by flash photolysis

- DM-nitrophen

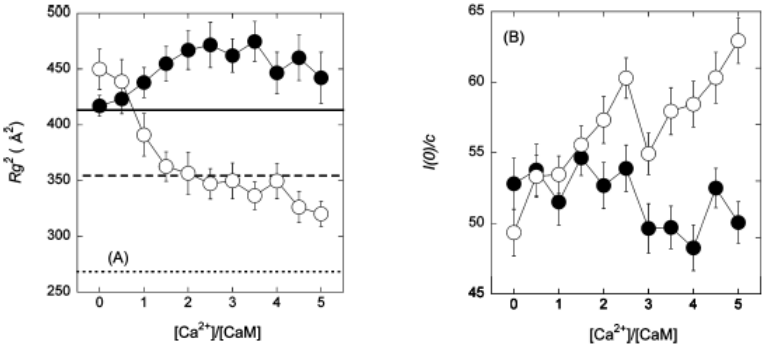


Calmodulin

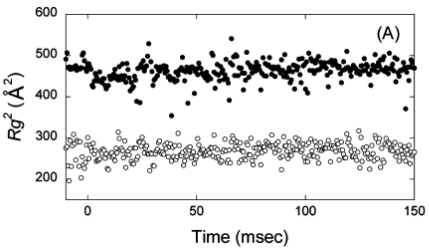
A Compact Intermediate State of Calmodulin in the Process of Target Binding. Yamada *et al.* Biochemistry 2012

Mastoparan

Equilibrium measurement



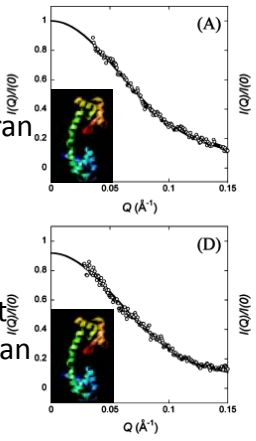
Kinetics



0.5 ms

With mastoparan

Without mastoparan

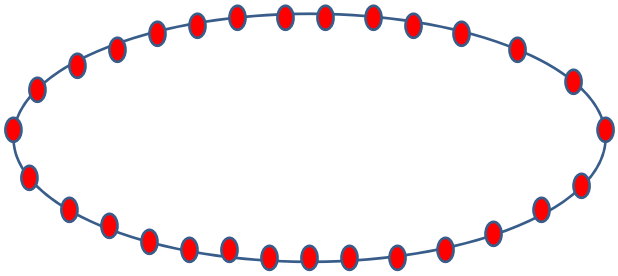


Model

Ultra-fast time resolved

Ultra short collection time

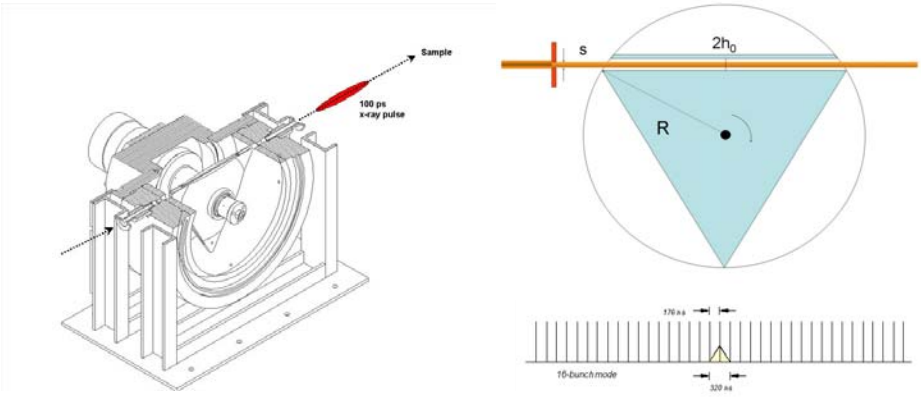
- Beamline ID09B, ESRF, Grenoble
- Using the pulsed structure of the synchrotron



- About 5000000 bunch/sec

Isolate one bunch

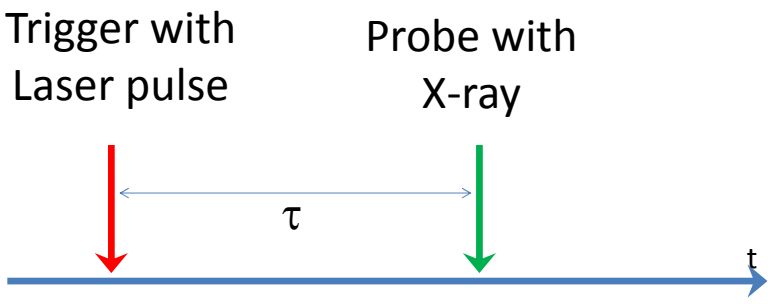
- Isolate one bunch (ms shutter + fast chopper)



Single bunch experiment

- High flux needed
- Repetition of the measurements

Pump and probe experiment



Bunch length ≈ 100 ps
→Resolution: up to 100 ps

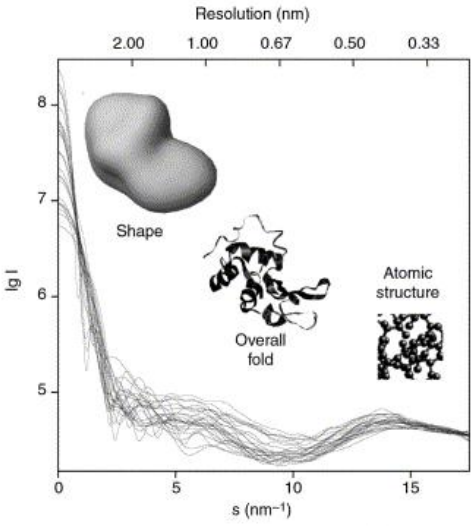
What is 100ps?

100 psec → second

Second → 315 years

Light travels 3 cm in 100ps

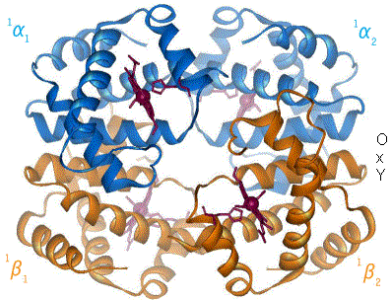
Too fast for SAXS



TR WAXS

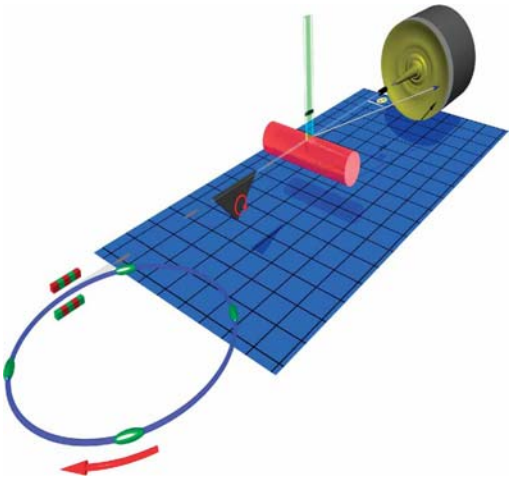
Tracking the structural dynamics of proteins in solution using time-resolved wide-angle X-ray scattering. Cammarata et al. Nature 2008.

T and R states of hemoglobin

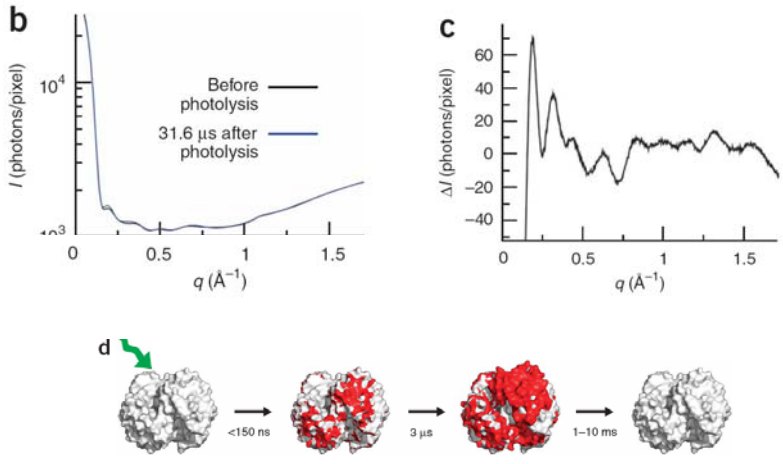


Looking at the unbinding of oxygen by hemoglobin

Experimental setup



Structural change in hemoglobin



Conclusion

- SAS can be used to study kinetic
- For fast reaction:
  - Special setup required to triggered the reaction
  - High flux is needed: third generation source (impossible with lab source and neutrons)