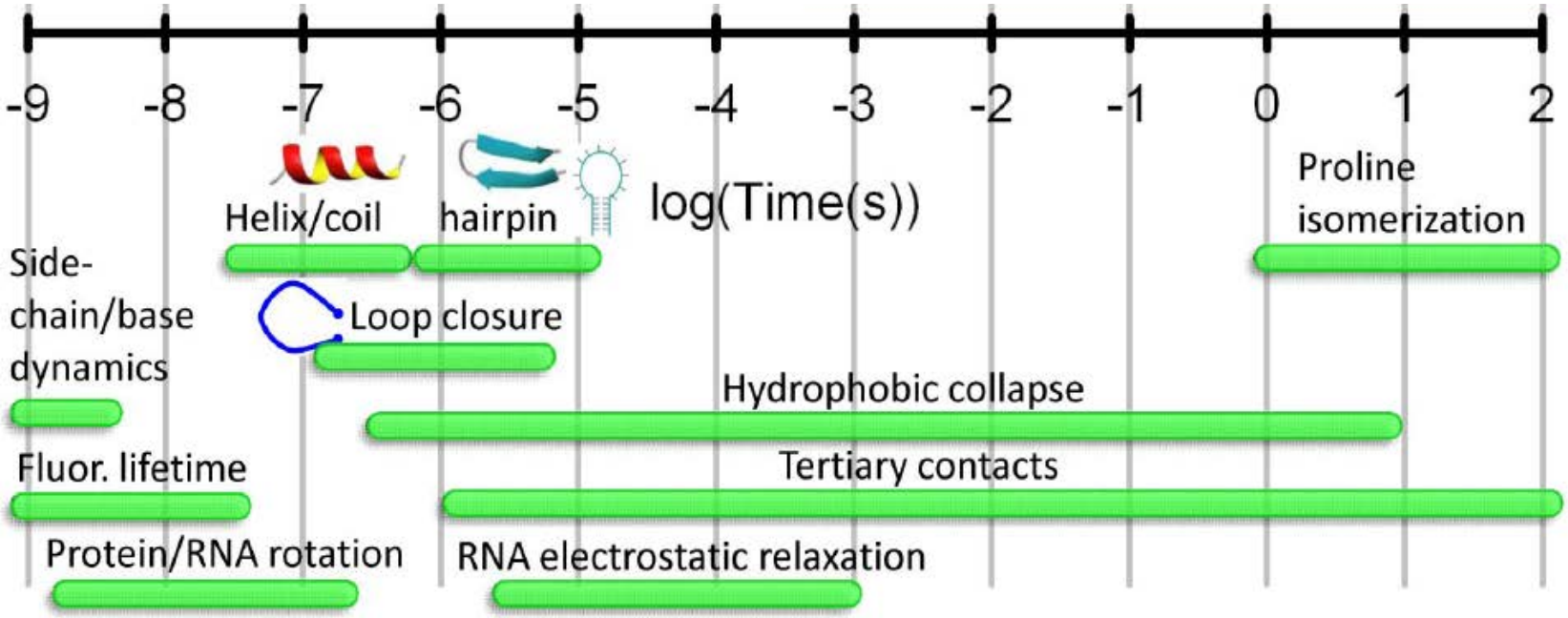
Time resolved scattering experiments

Clement Blanchet

Time resolved experiments?

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

Time scale of biological processes (protein folding)



Different time scales

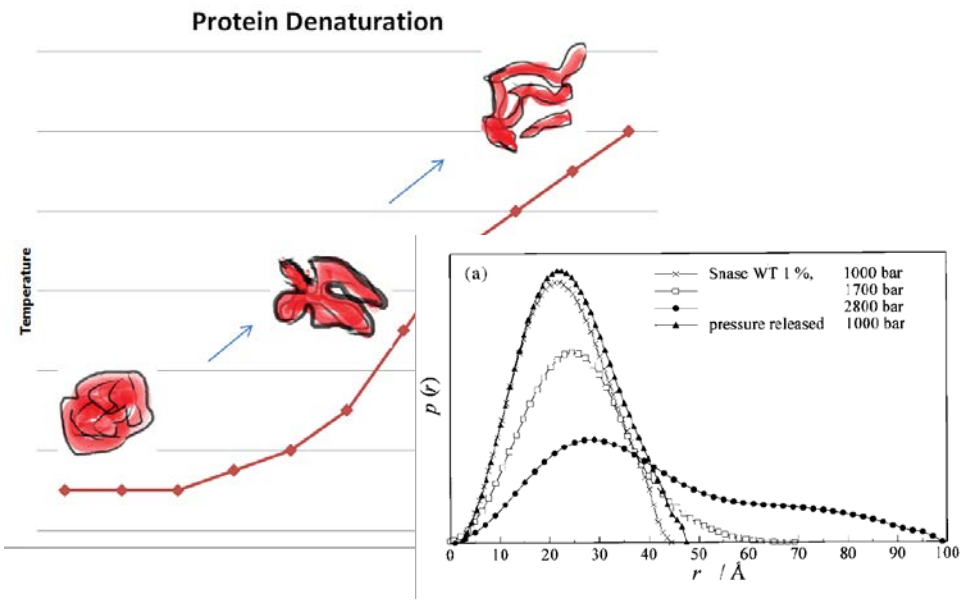
Different experimental setups

Ingredients of time resolved experiments

- Controlled triggering of the reaction of interest
- Way of monitoring the reaction

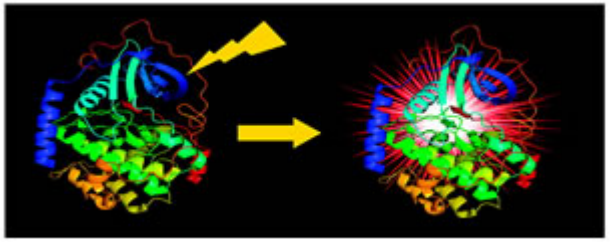
Triggering

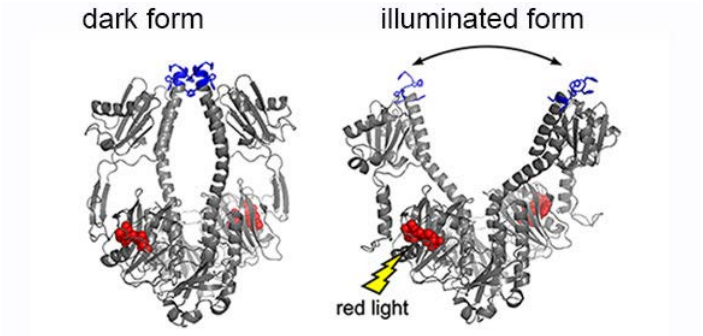
Modification of physical condition (T, P)



Modification of chemical conditions

- pH jump
- Denaturing agent
- Addition of salts or other additives

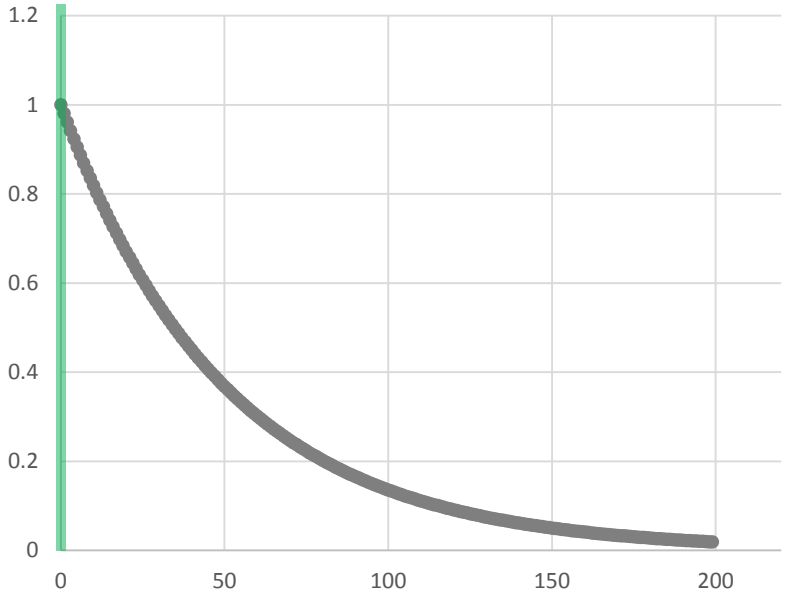


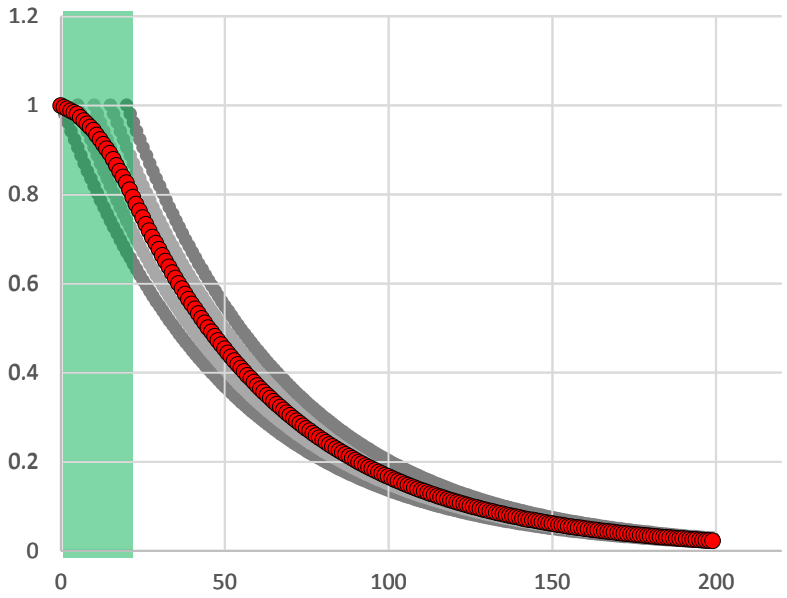


Modification of the system itself
Flash photolysis, photosensitive protein

Limitation – triggering

- Triggering:
  - Simultaneous, fast and homogeneous triggering at the time scale of the reaction





How fast can you trigger the reaction?

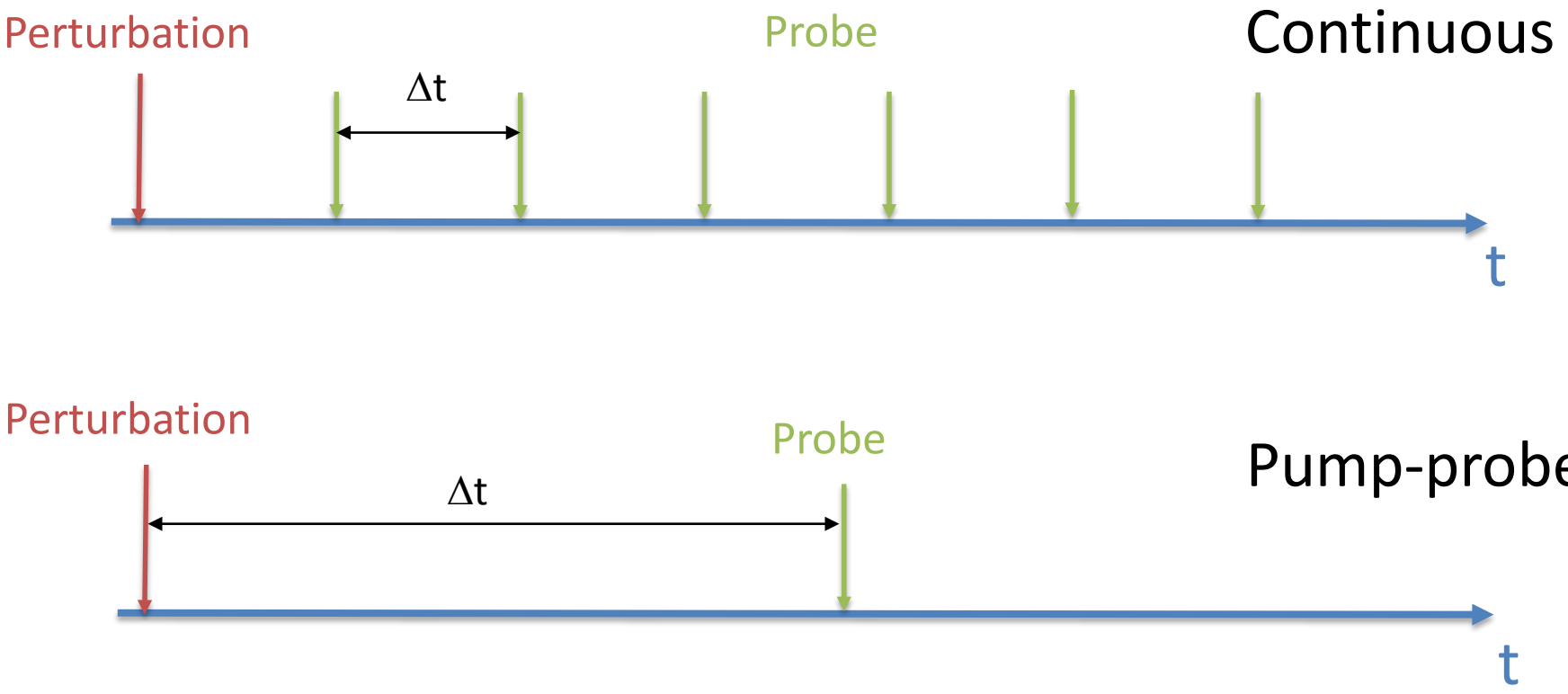
- Depends on the triggering methods
  - Mixing:
    - seconds to ms (with fast mixing devices)
    - Limited by mixing, diffusion time
  - P-Jump:
    - Diffusion of the pressure shockwave: speed of sound ms
    - In practice micros-ms
  - Light triggered reaction:
    - Practically not limited for “direct”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

* Small measurement cell helps.

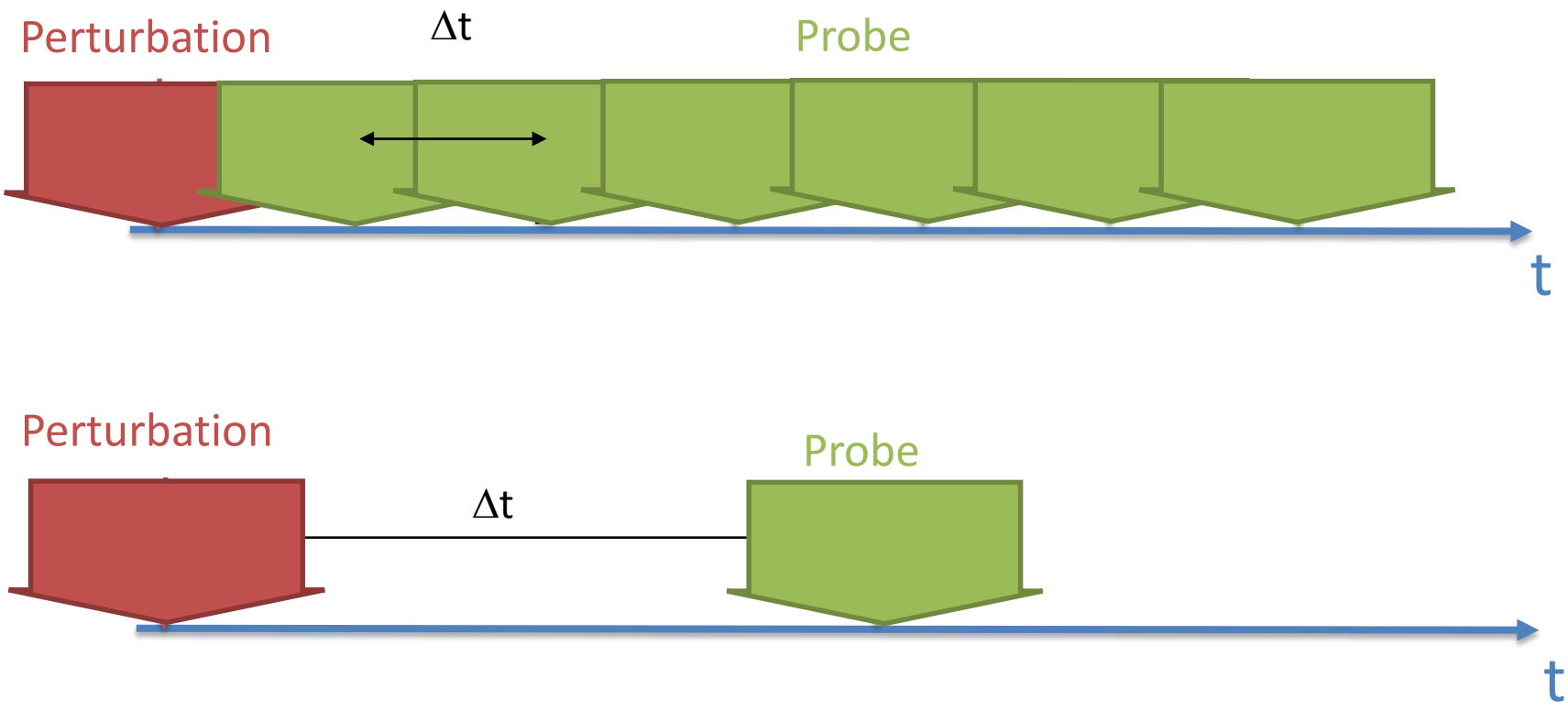
Monitoring the reaction

- 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

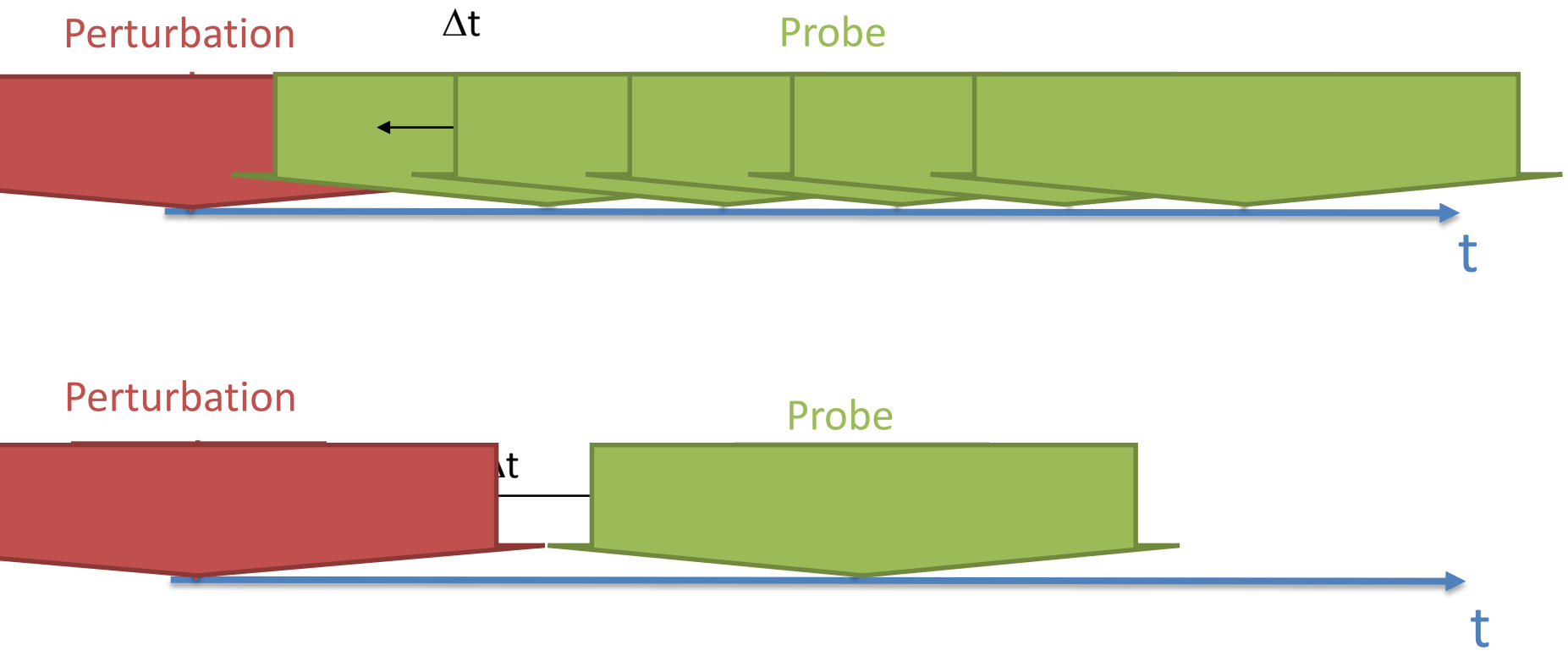
Continuous vs pump-probe



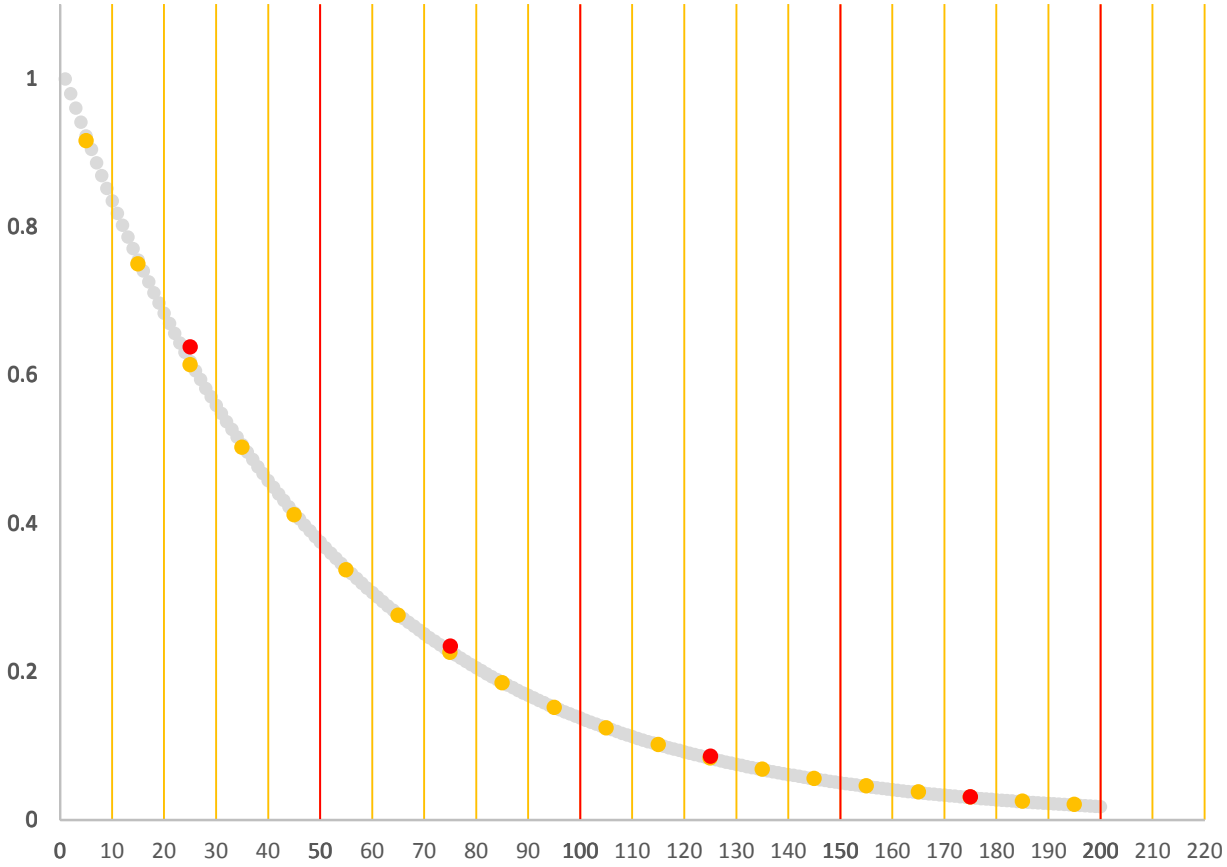
Continuous vs pump-probe



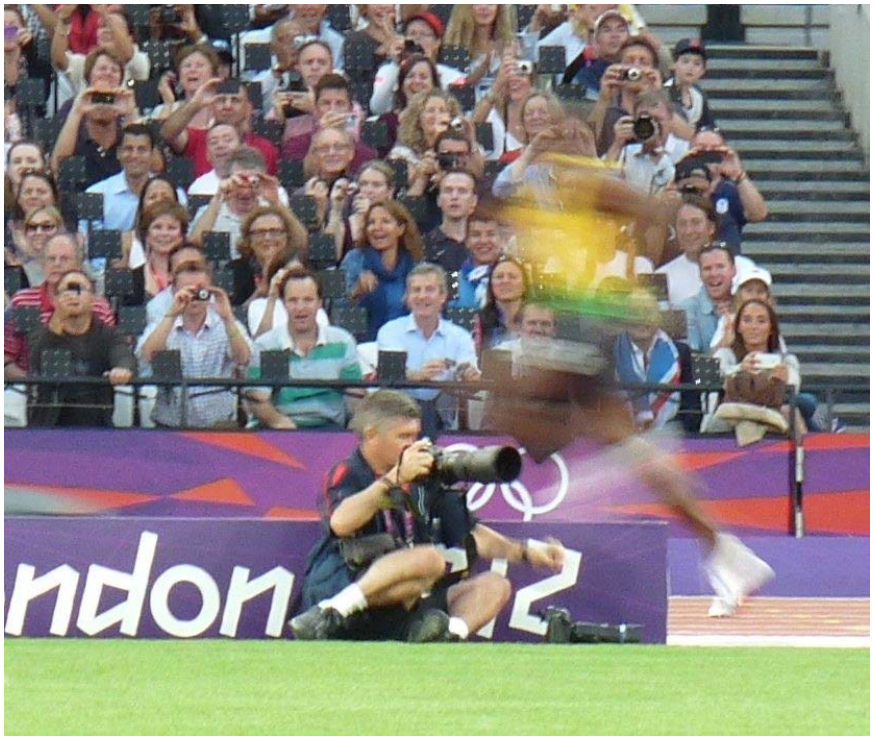
Continuous vs pump-probe



Limitation – Collection time



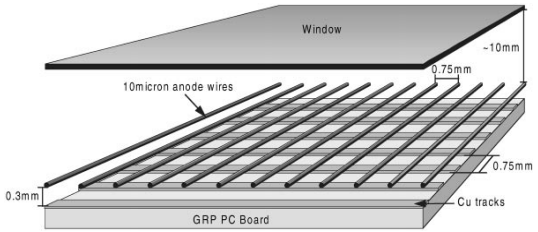
Limitation – Collection time



Short collection time - Fast detector

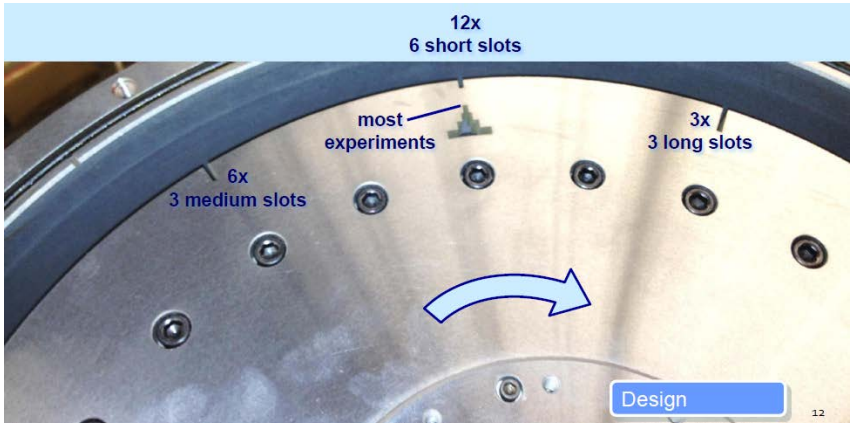
- Photon counting detector: Pilatus (300Hz), Eiger (up to 3kHz), Xfel detectors,…

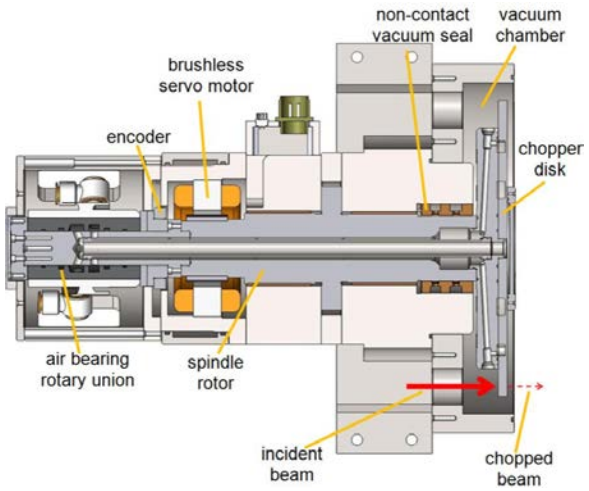
- Gas detector (Theoretically, up to 1MHz)



Short collection time – Short X-ray pulse

- Use short beam pulse to overcome the detector limitation (using fast shutter, chopper,…)

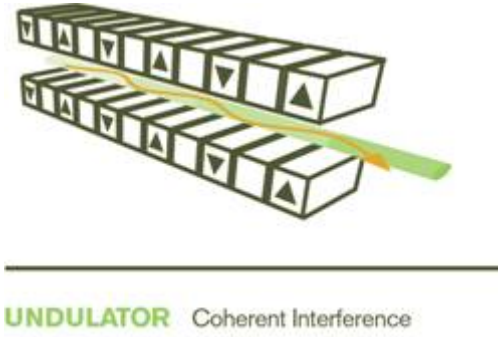




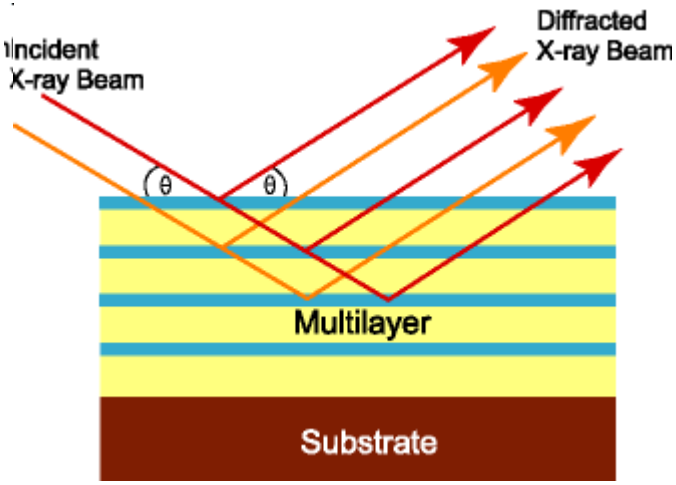
Chopper

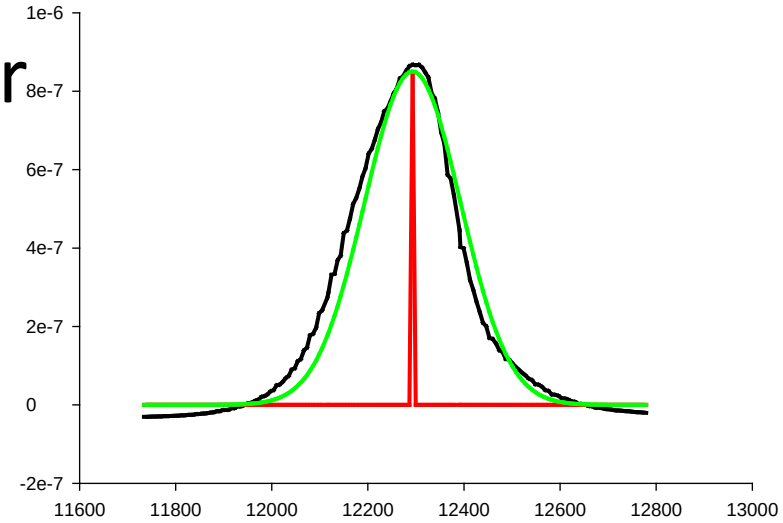
Short collection time: High flux

- Third generation synchrotron

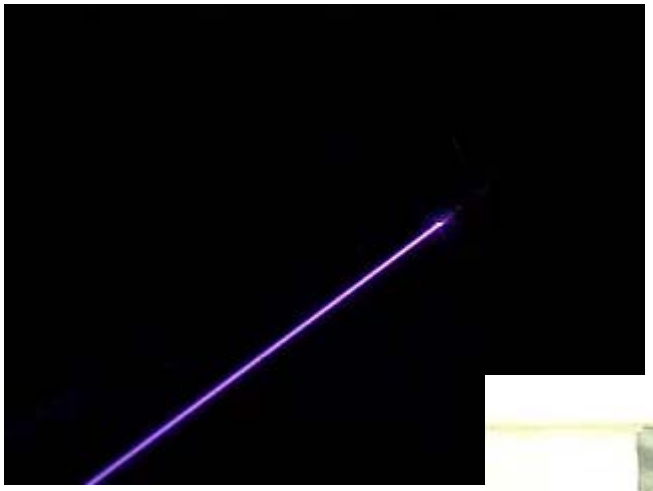


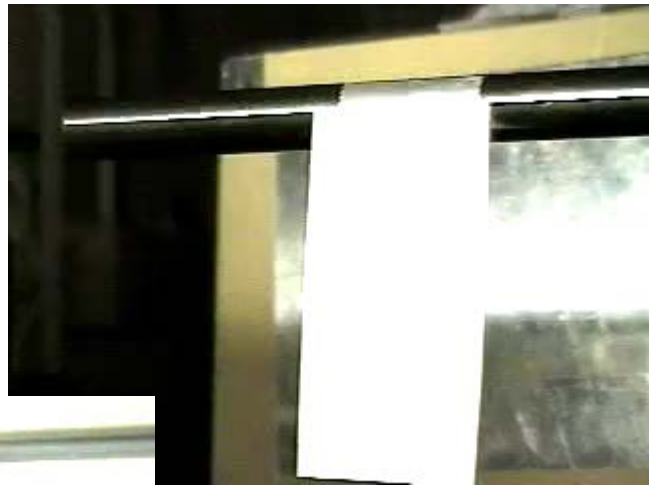
- Multilayer monochromator



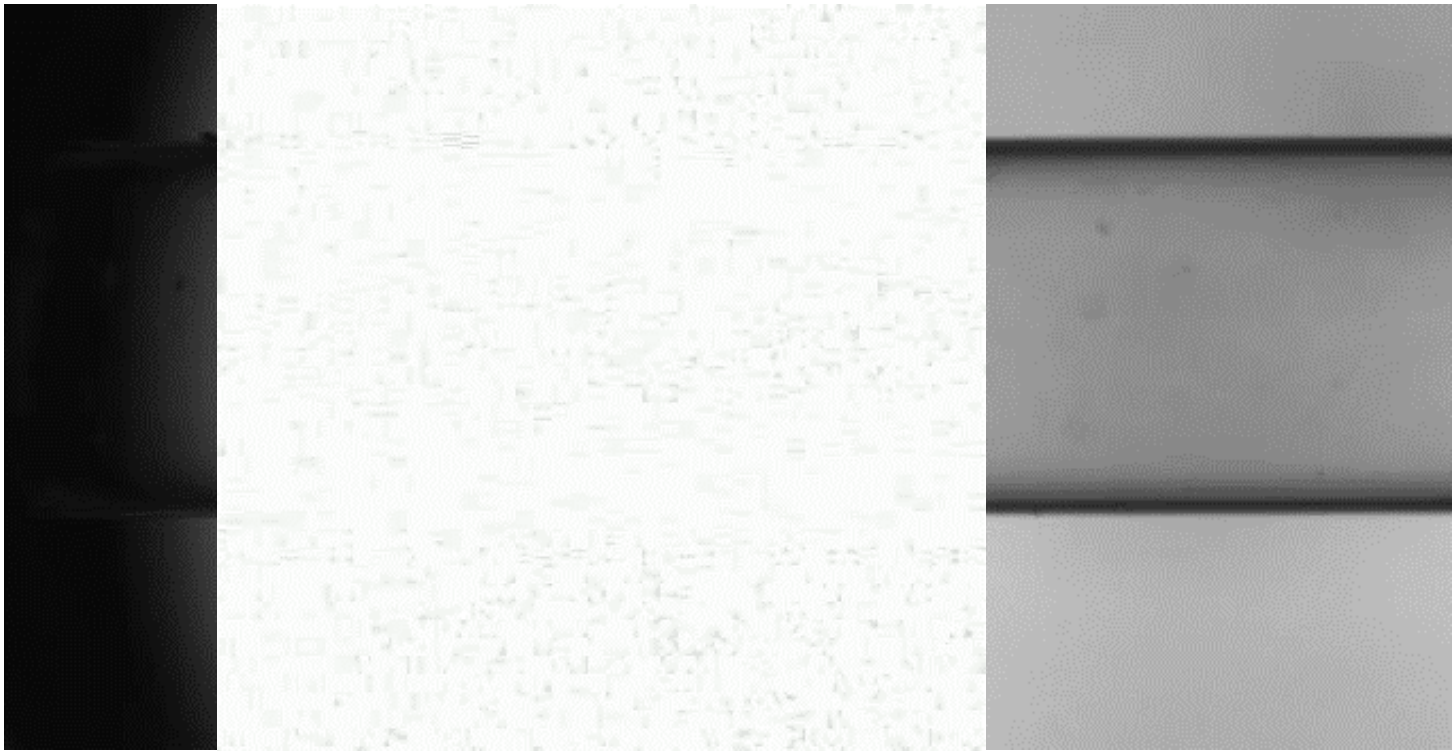


Example high flux beam (BL40XU, Spring8)

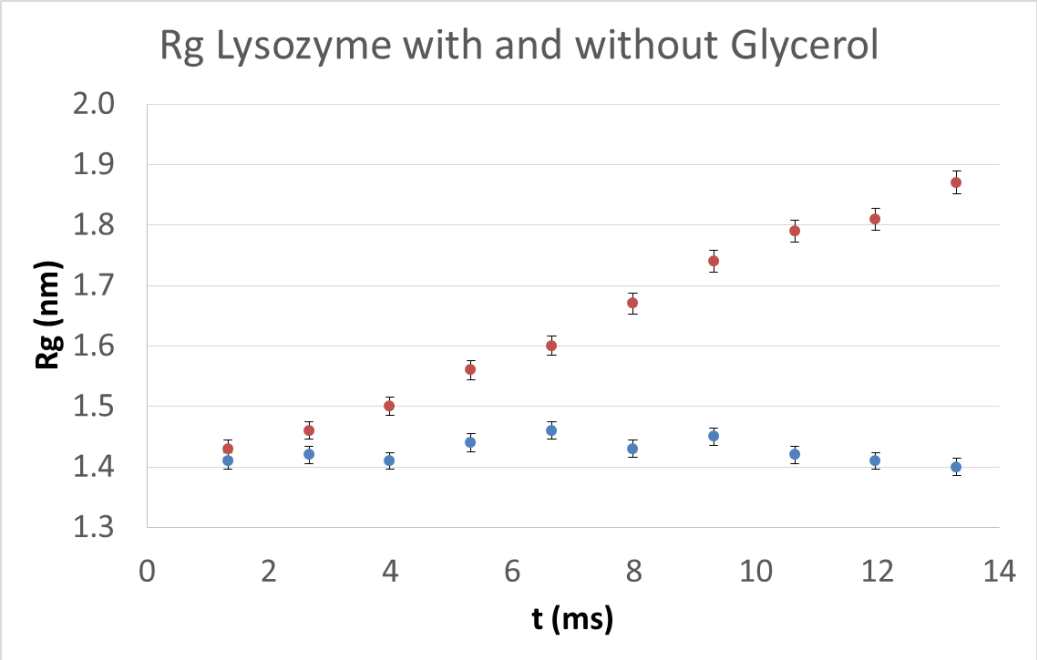




But careful radiation damage

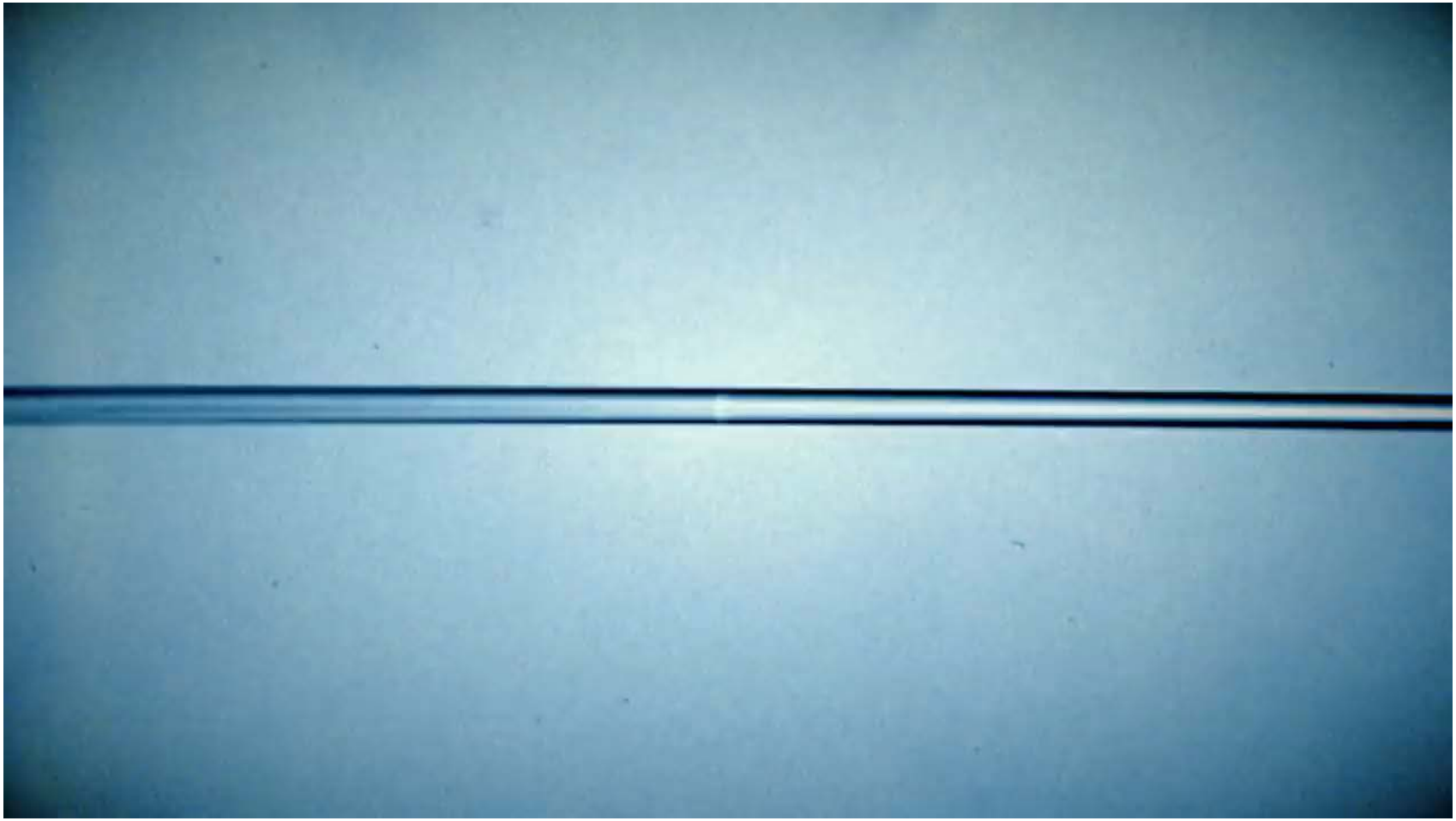


But careful radiation damage



- Adapt collection strategy (pump and probe)
- Use short pulses

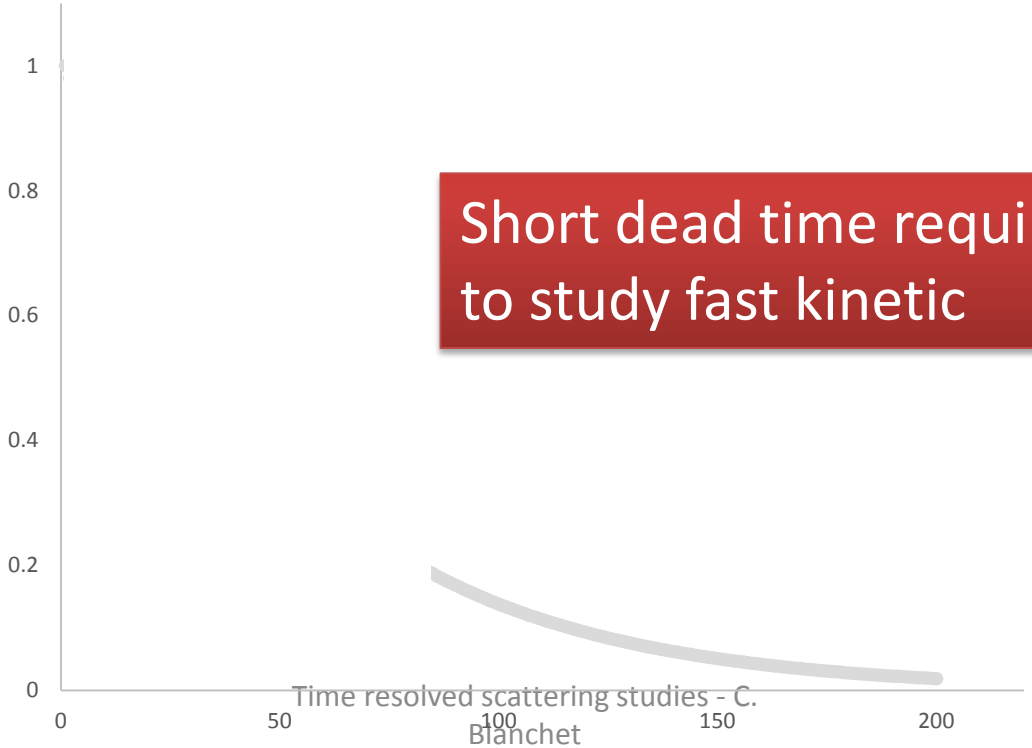
FEL Beam (SLAC Stanford)



Stan, C. A., Milathianaki, D., Laksmono, H., Sierra, R. G., McQueen, T. A., Messerschmidt, M., ... & Guillet, S. A. (2016). Liquid explosions induced by X-ray laser pulses. *Nature Physics*.

Dead time

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



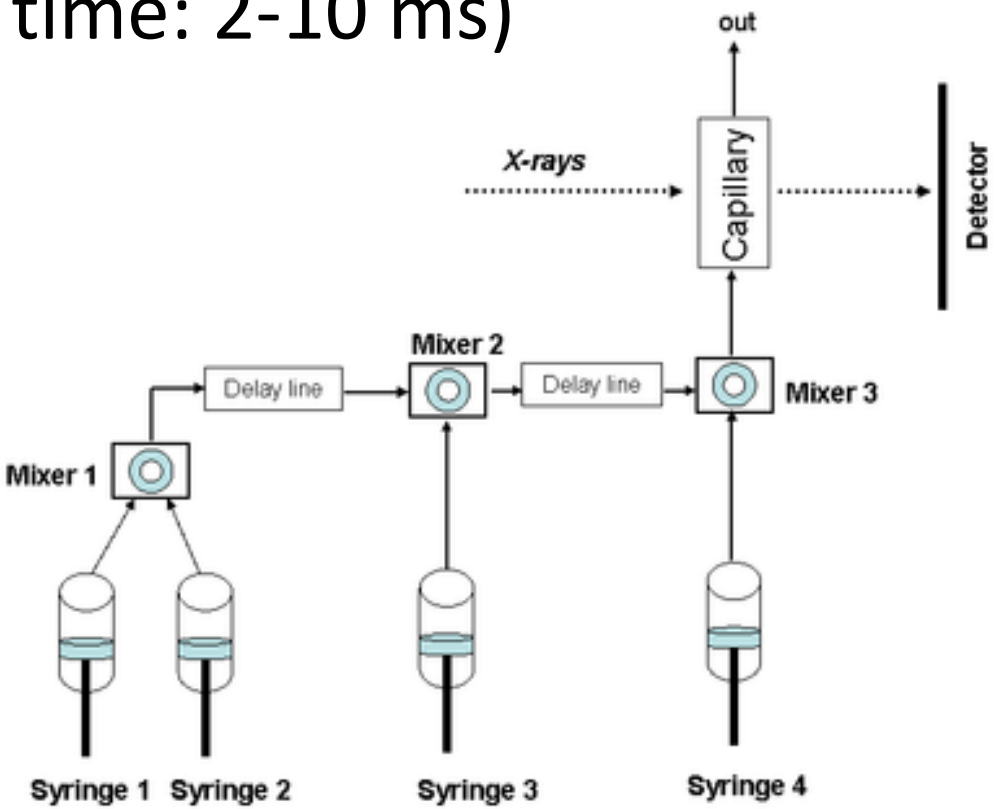
Short dead time required to study fast kinetic

Examples

- Sub-Second TR experiments
  - Stopped-flow
- Millisecond TR experiments
  - Continuous flow
  - Caged compound
- Ultrafast TR experiments
  - Synchrotron
  - FEL

Sub-second kinetics

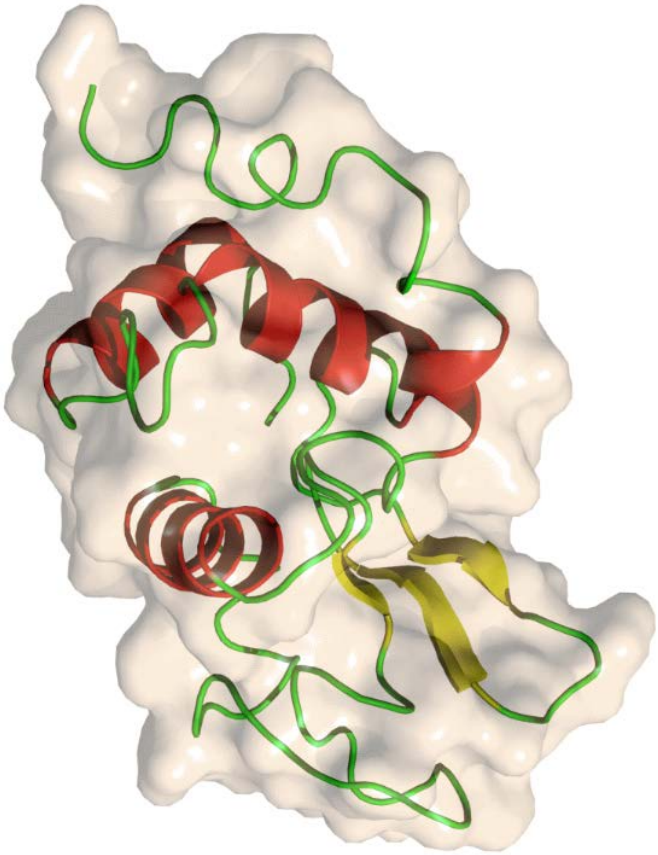
- Stopped-flow (dead time: 2-10 ms)



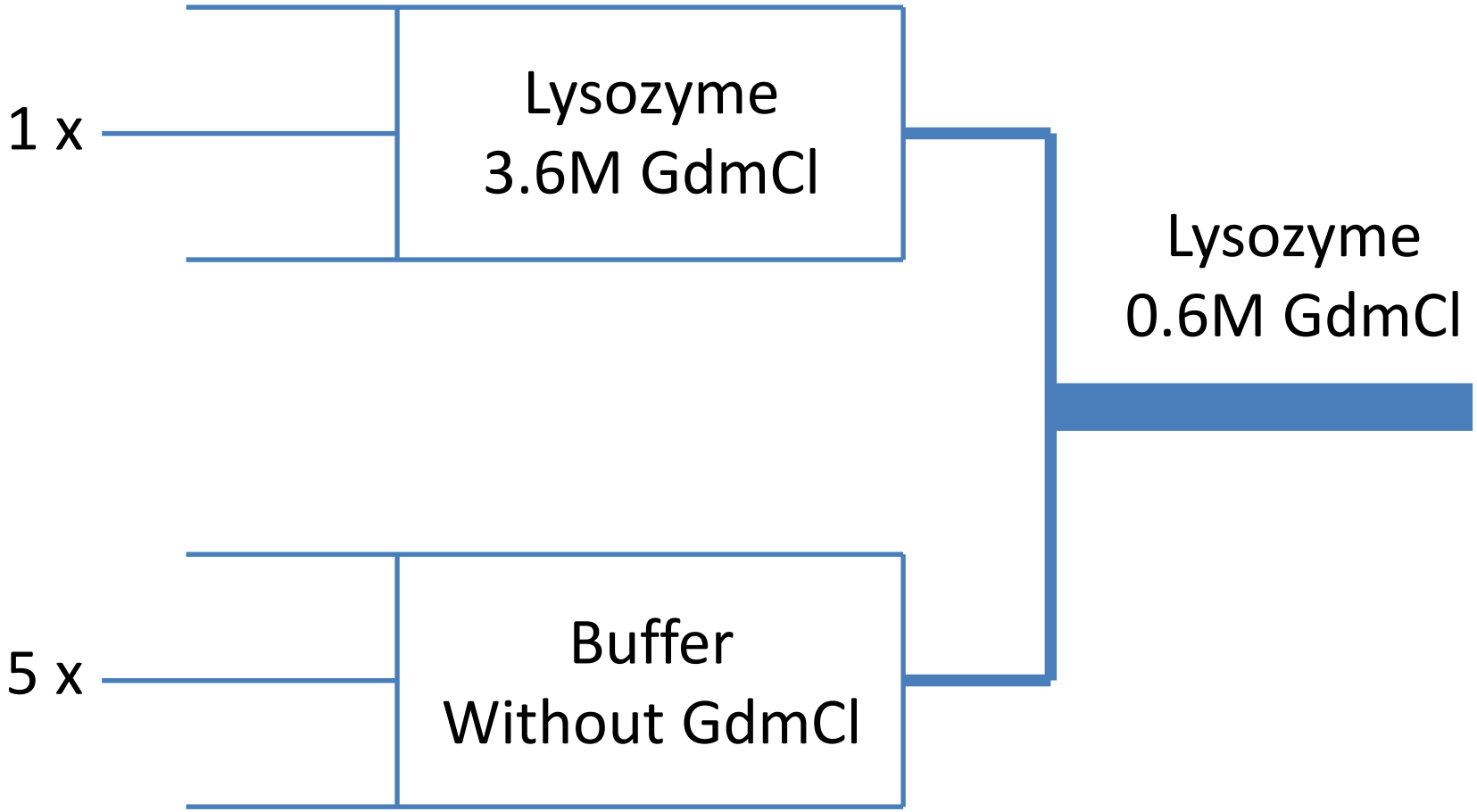
Stopped flow - Example

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

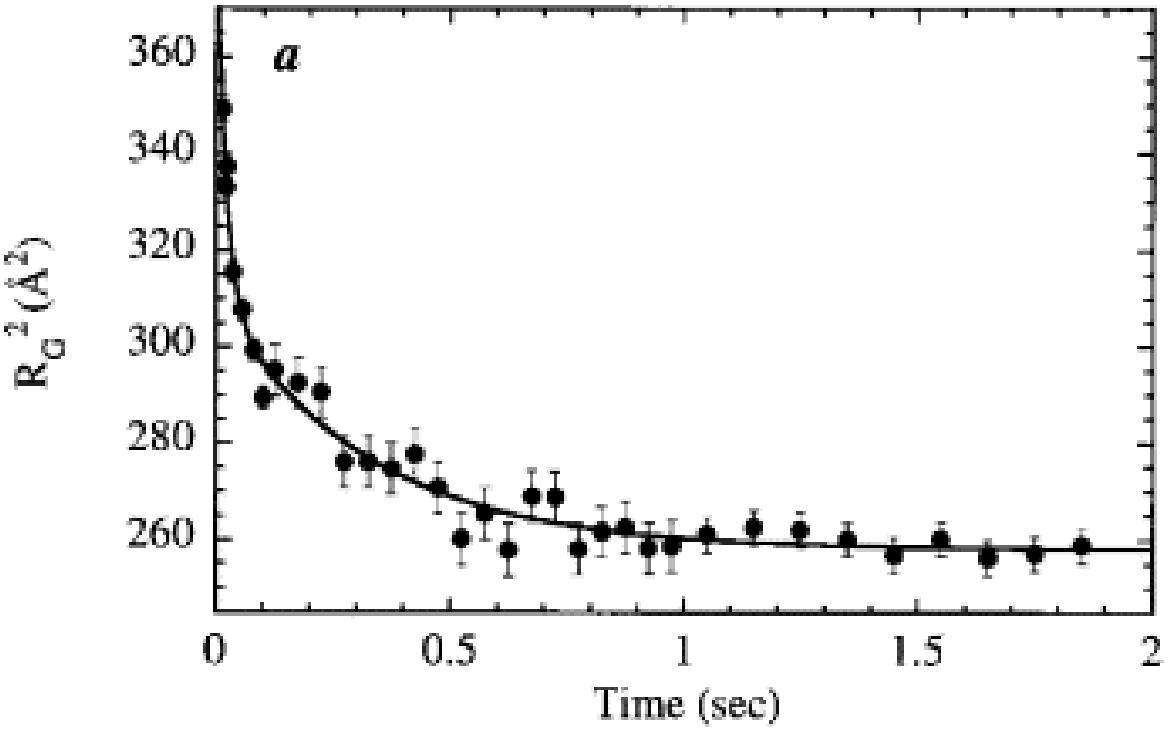


Lysozyme Folding



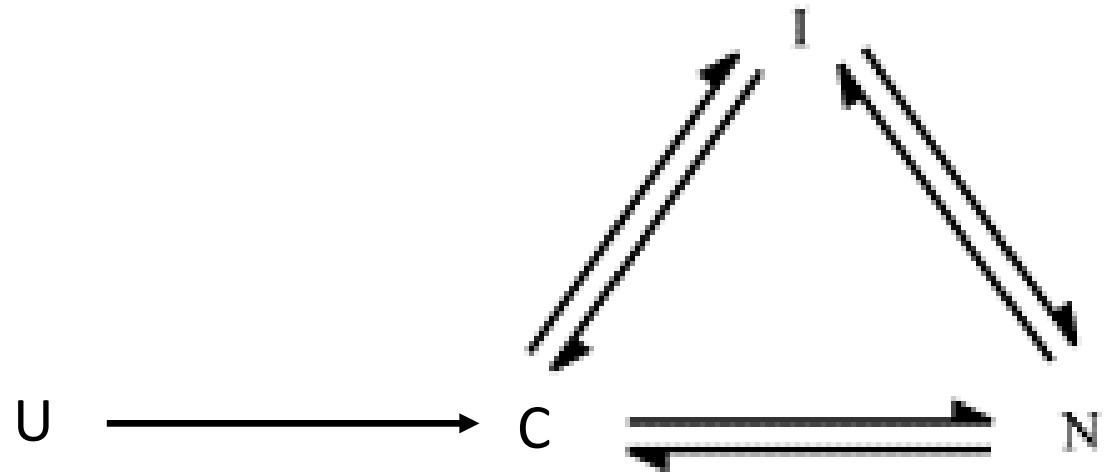
Lysozyme Folding

- Evolution of Rg in time



Refolding model

(Wildegger & Kiefhaber, 1997)

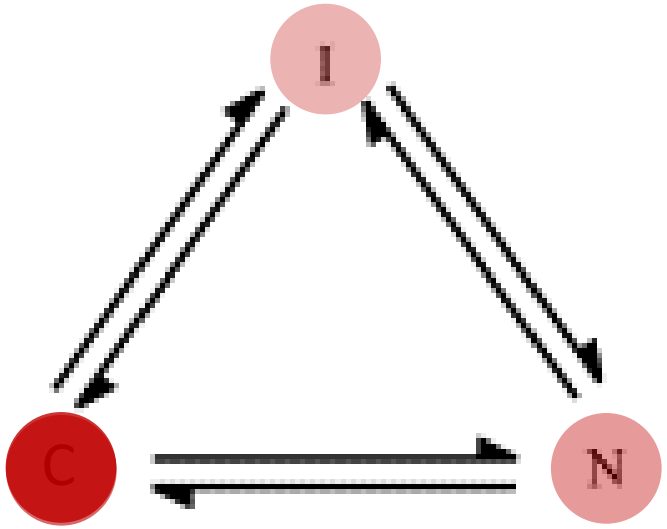


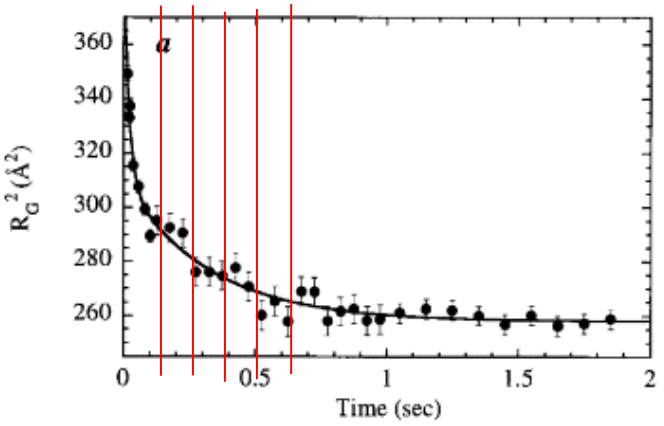
Rg = 23.5 A

Rg = 19.6 A

Interrupted refolding experiment

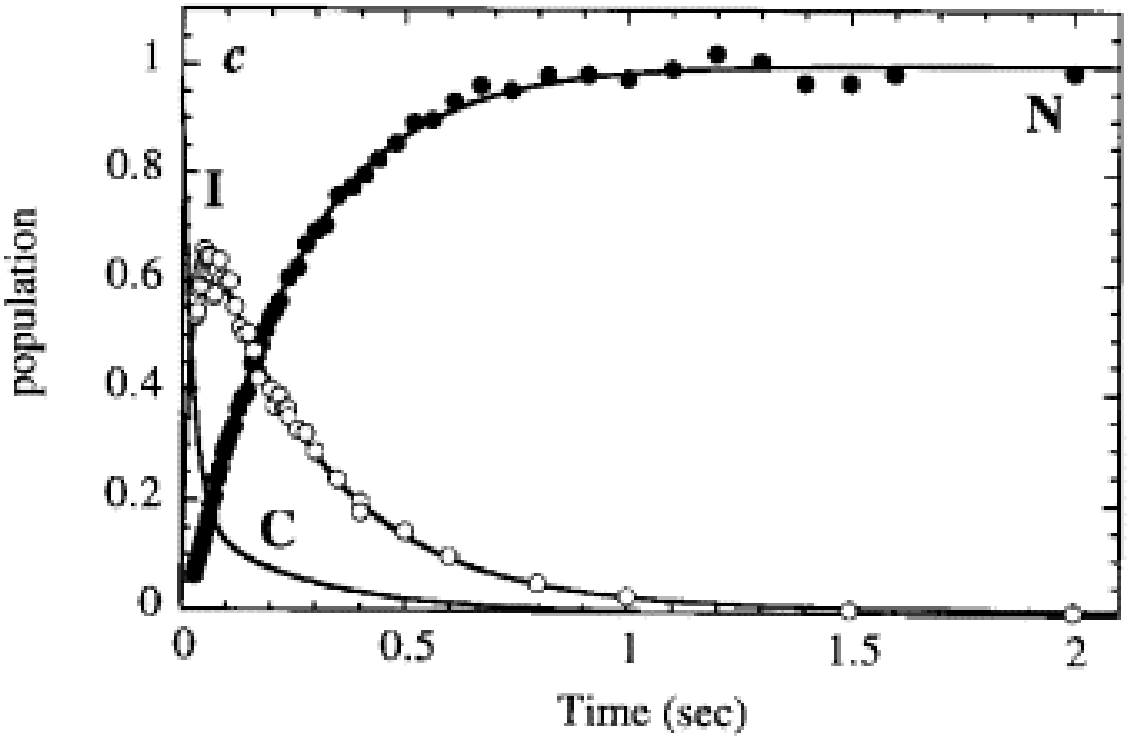
- Double mixing step monitored by fluorescence





Interrupted refolding experiment

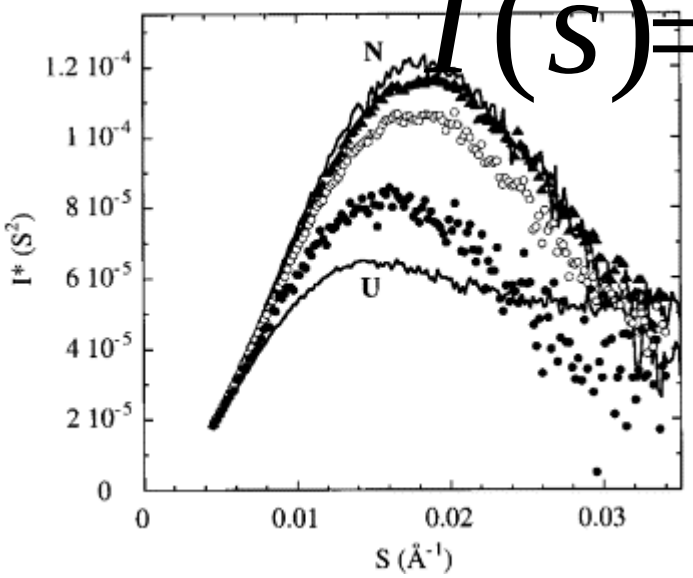
- Double mixing step monitored by fluorescence

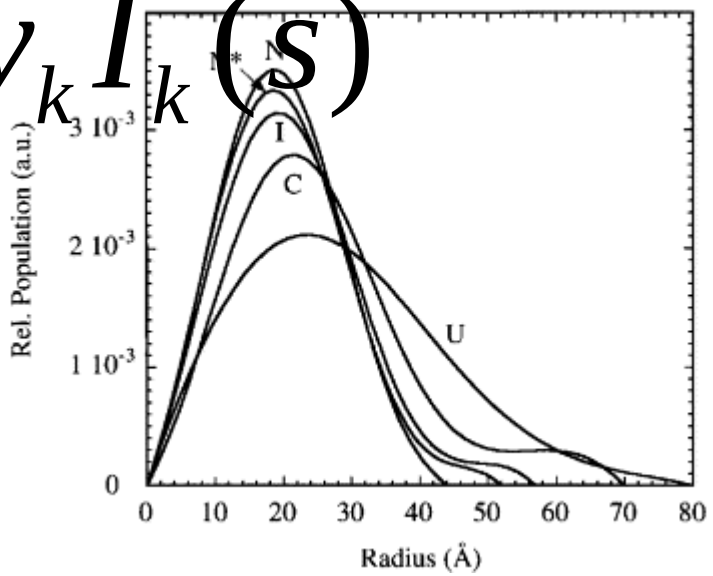


Reconstruction of the scattering profile

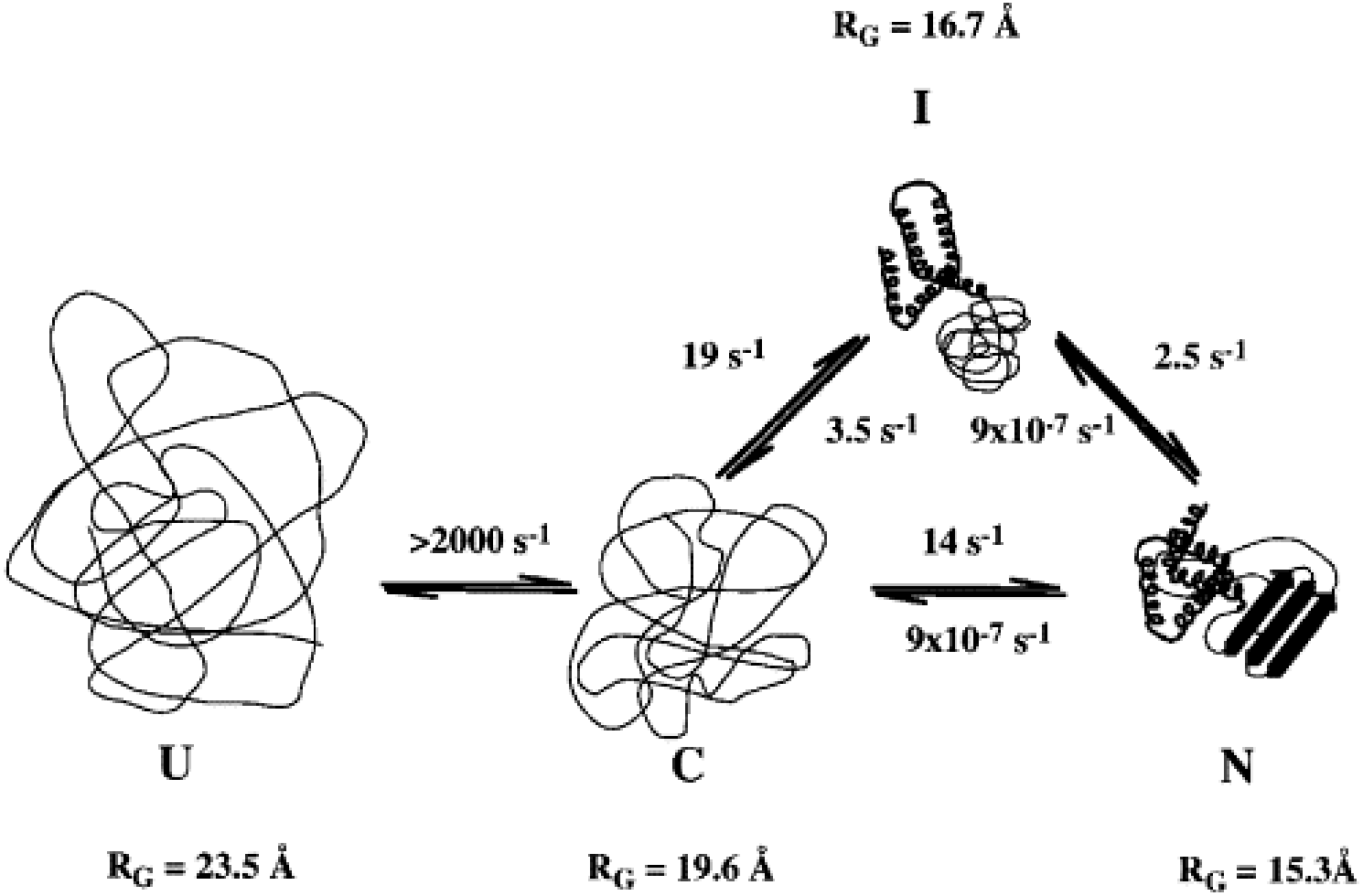
$$I(s,t) = \nu_C(t) I_C(s) + \nu_I(t) I_I(s) + \nu_N(t) I_N(s)$$

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

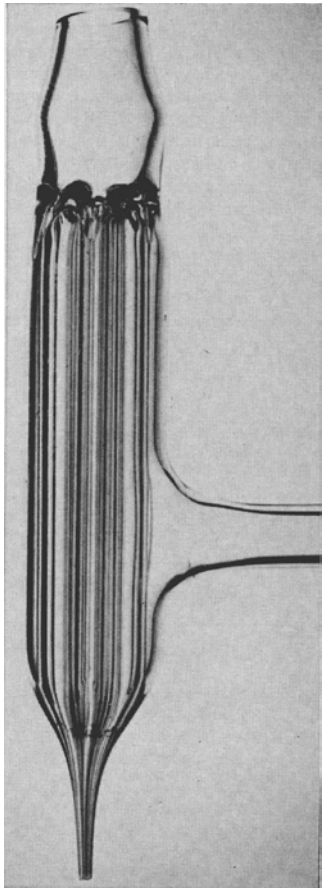




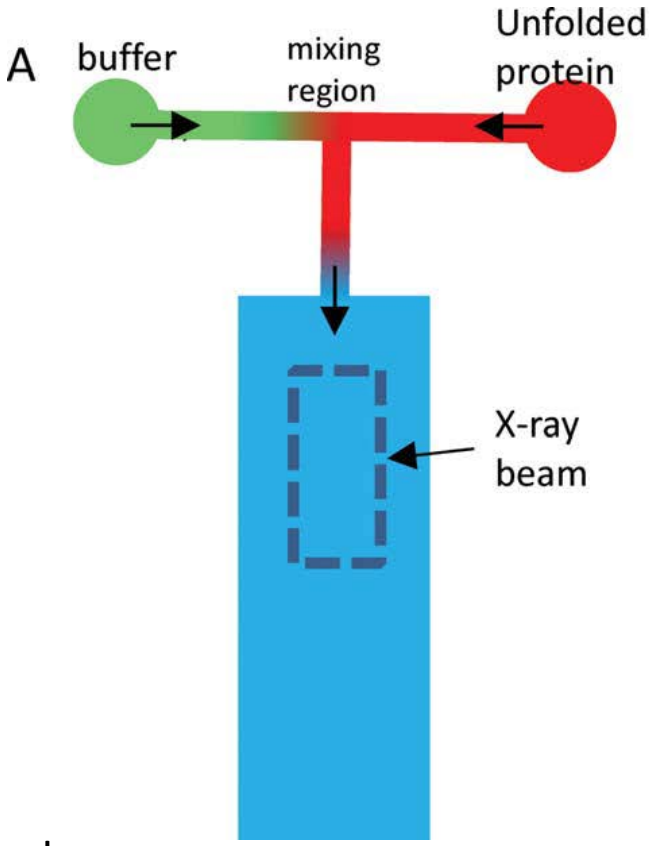
Refolding model



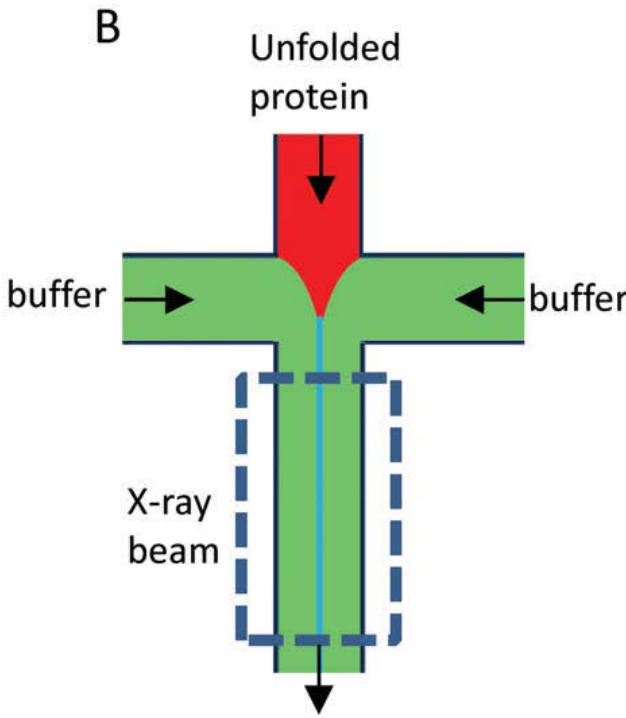
Continuous flow



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



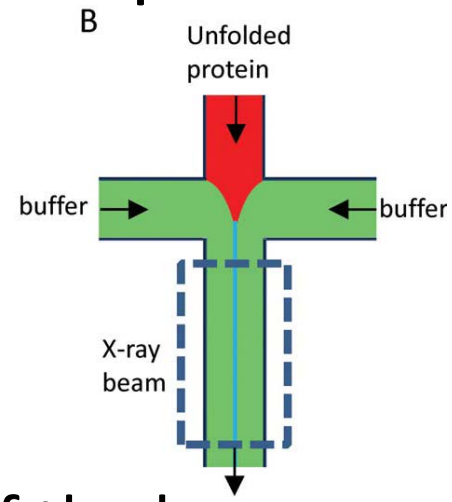
Turbulent mixing



Laminar mixing

Continuous flow

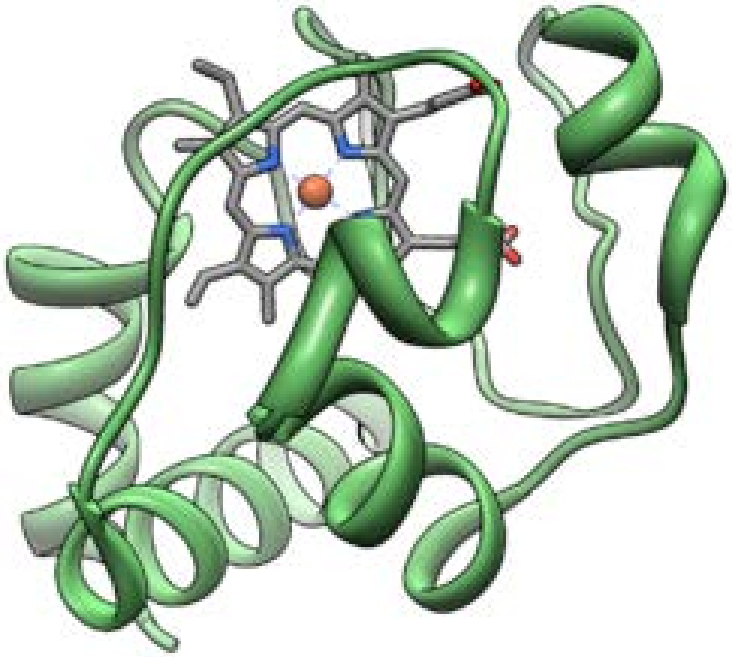
- Continuous flow → high sample consumption
  - Microfluidic continuous flow system



- Space <-> time
  - low flux OK
  - time resolution <-> flow rate and size of the beam

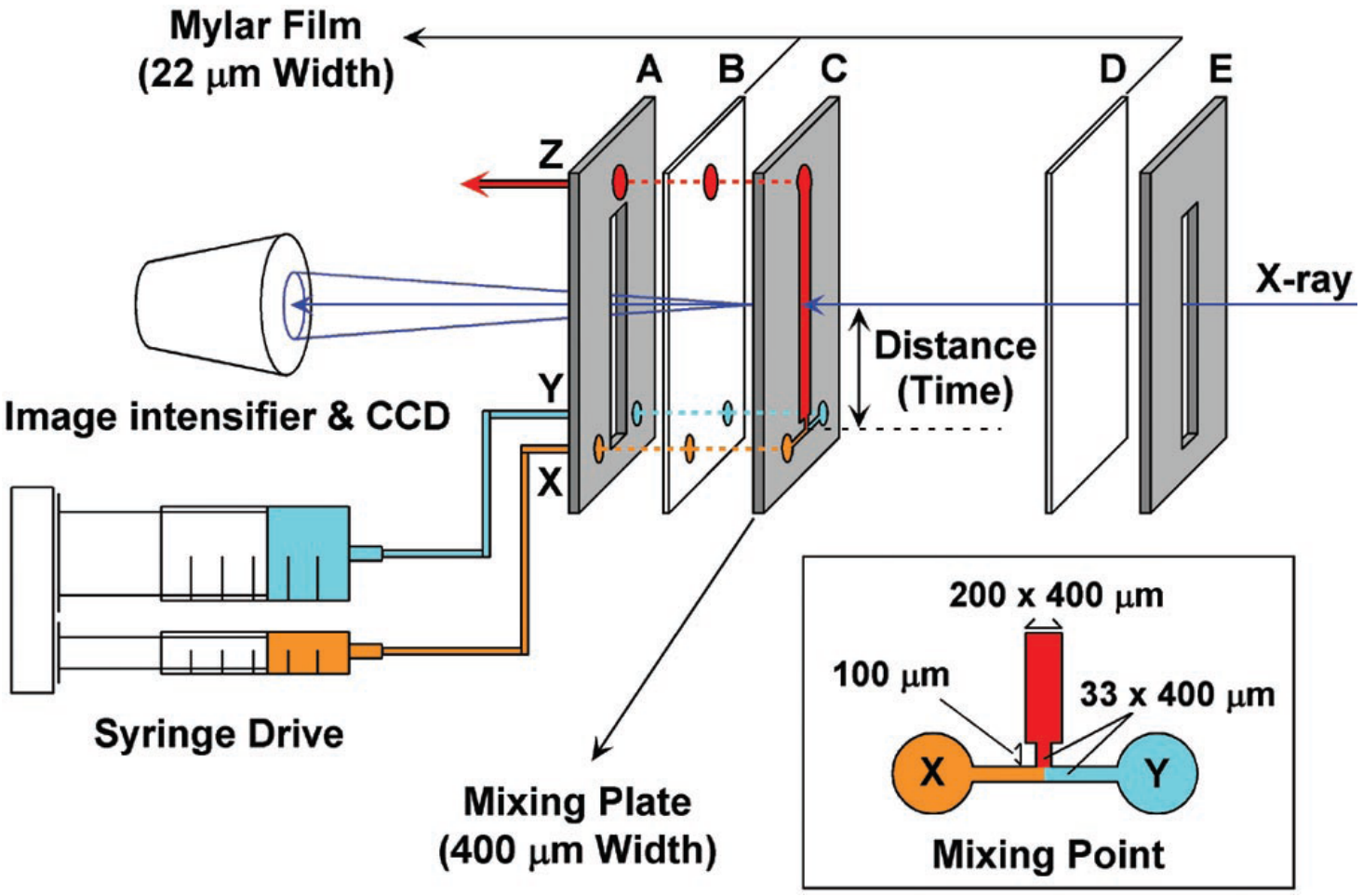
- Dead time (SAXS) ≈150 microseconds

Example continuous flow

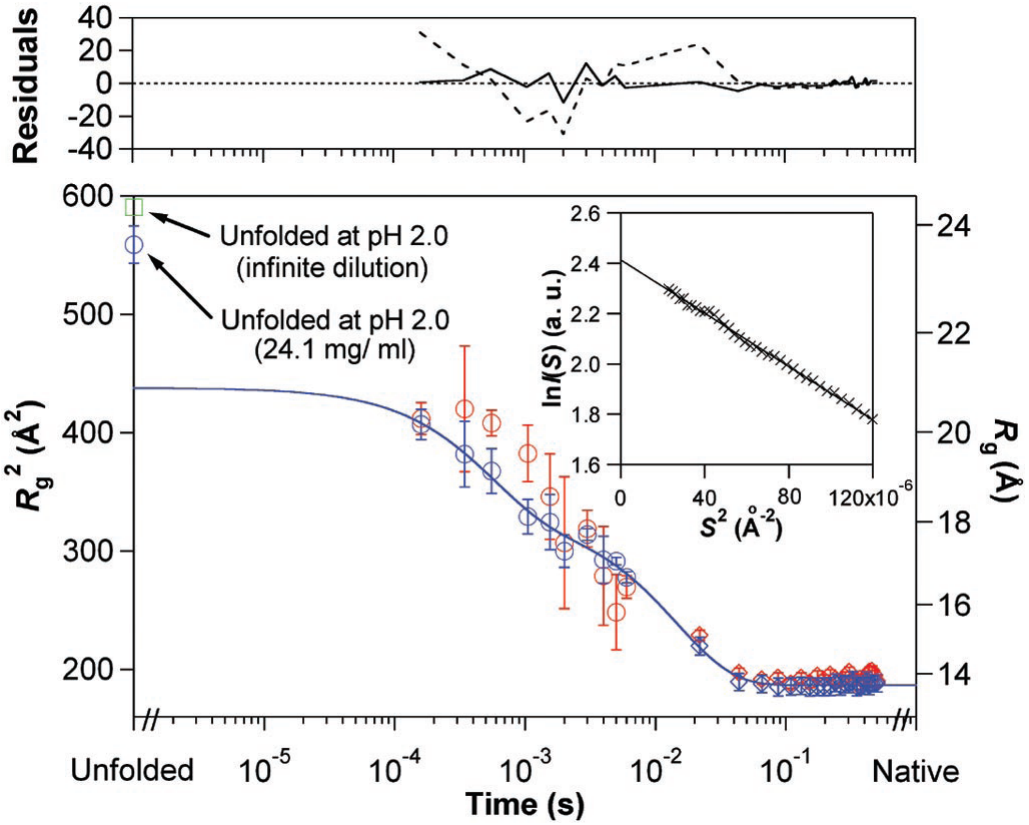


Conformational landscape of cytochrome c folding studied by microsecond-resolved small-angle x-ray scattering. Akiyama *et al.* PNAS 2002

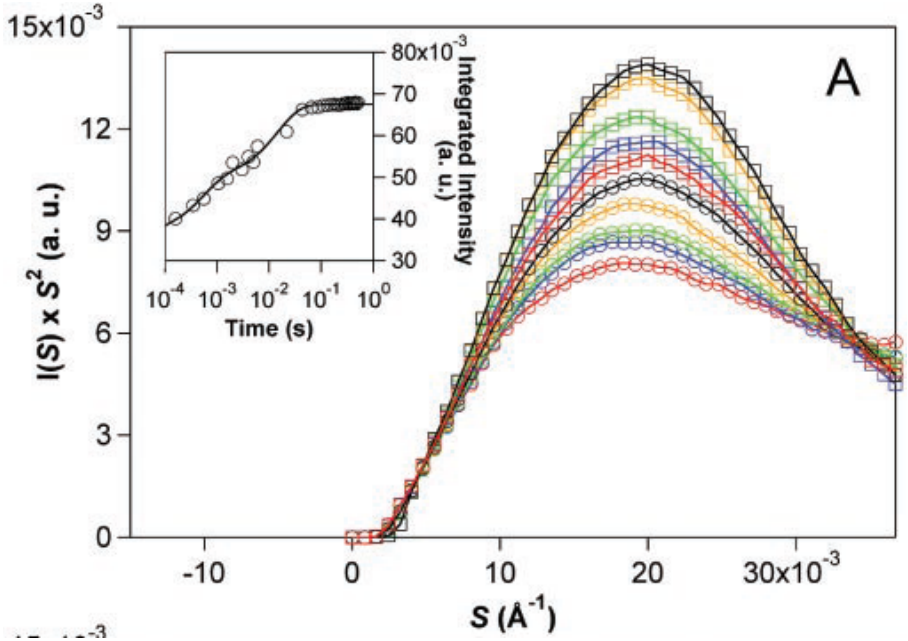
Continuous flow



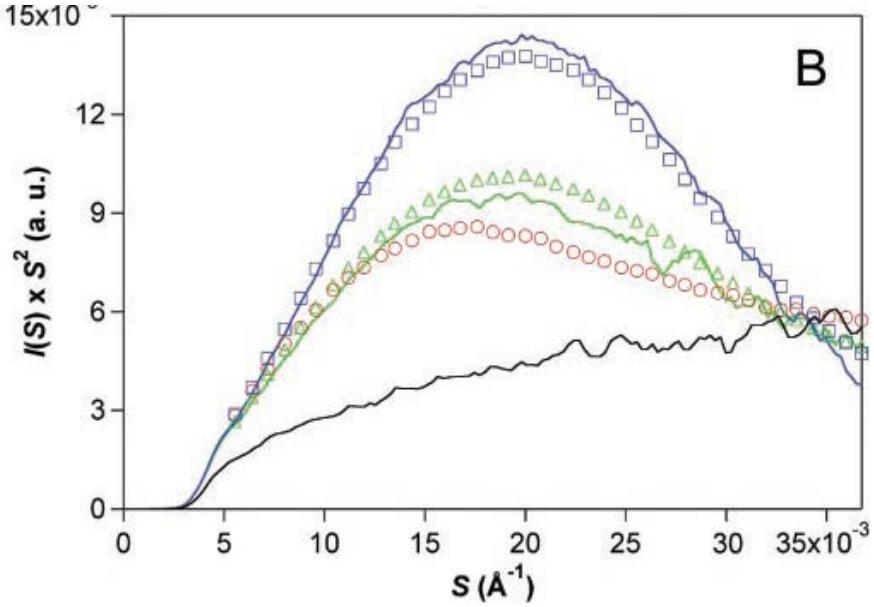
Radius of gyration



Kratky plots

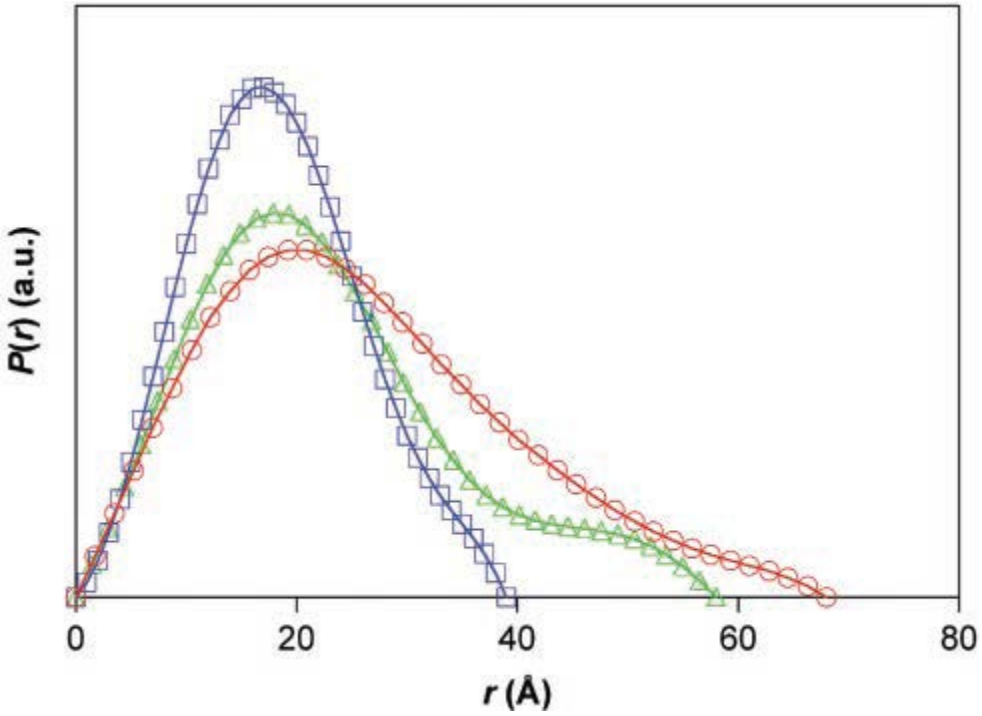


SAXS Curves



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

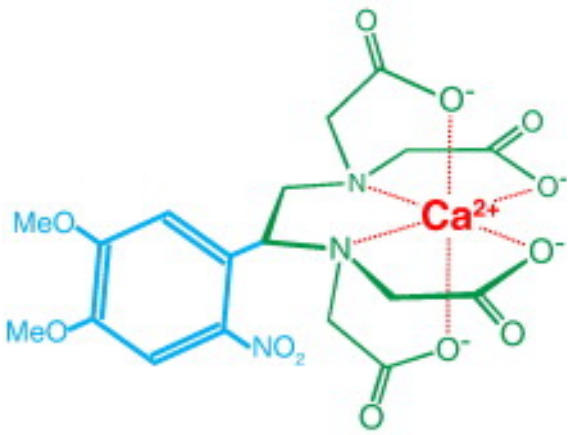
Conformational landscape of Cyto C



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

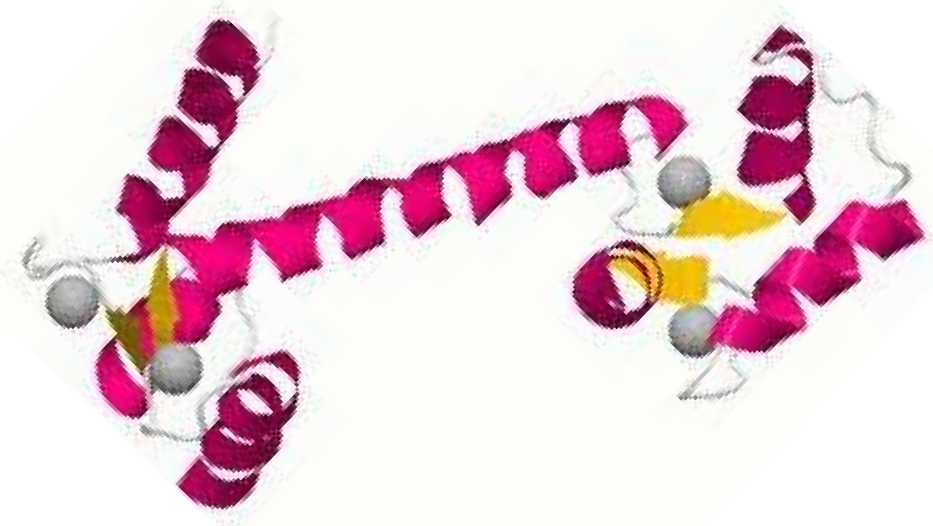
Caged compound release by flash photolysis

- DM-nitrophen



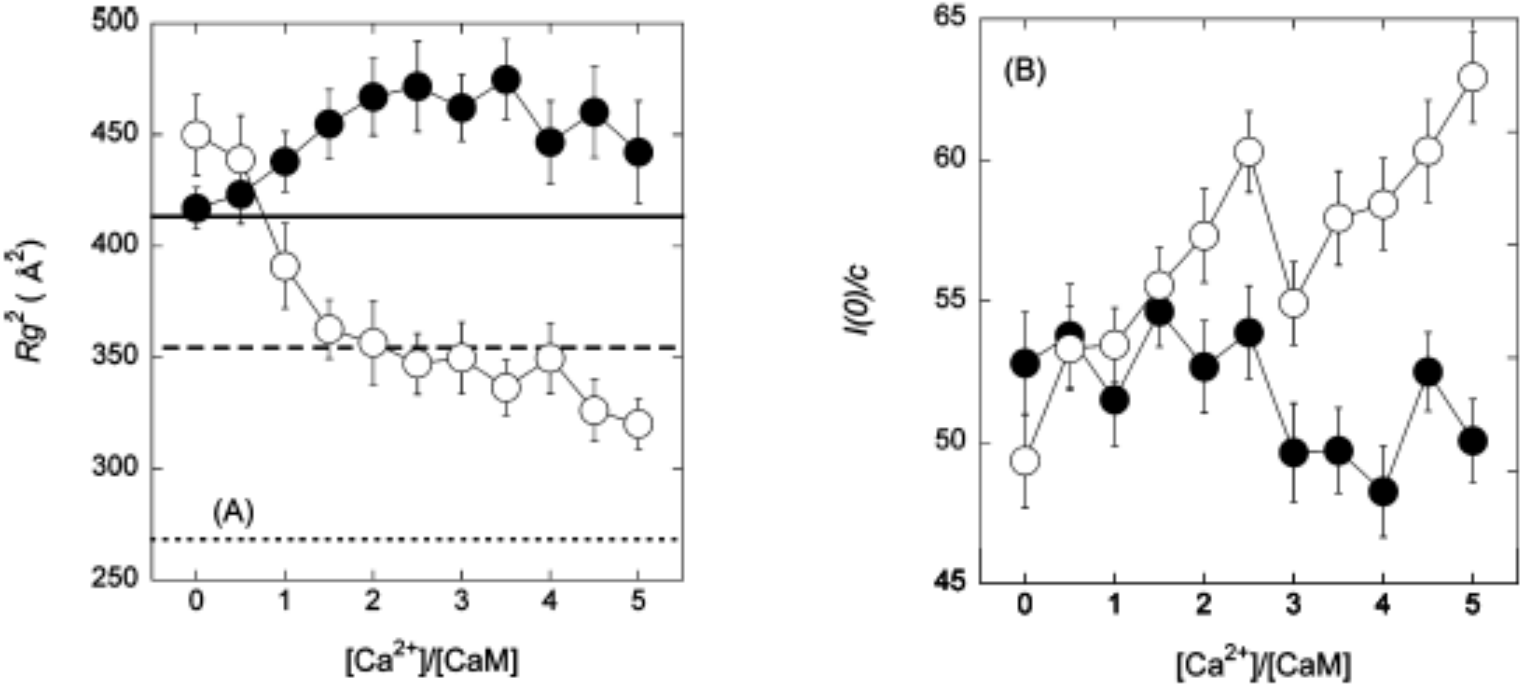
Calmodulin

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



Mastoparan

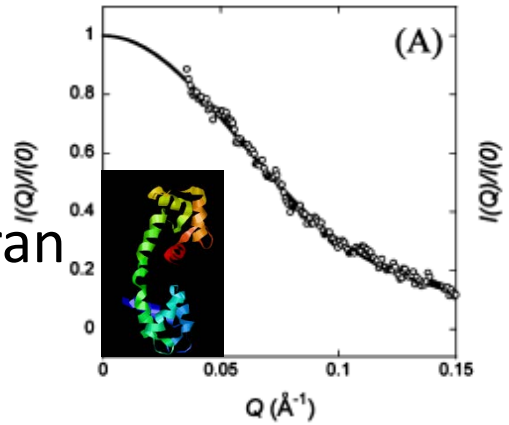
Equilibrium measurement



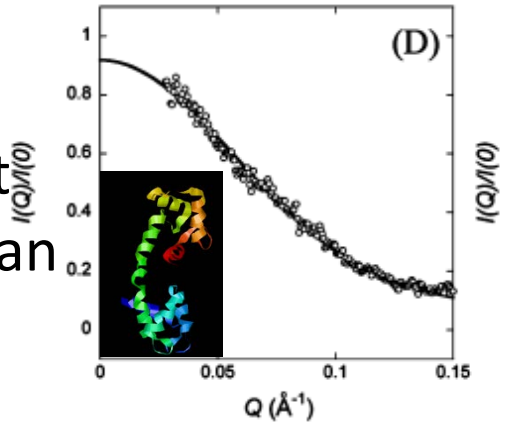
Kinetics

0.5 ms

With mastoparan



Without mastoparan

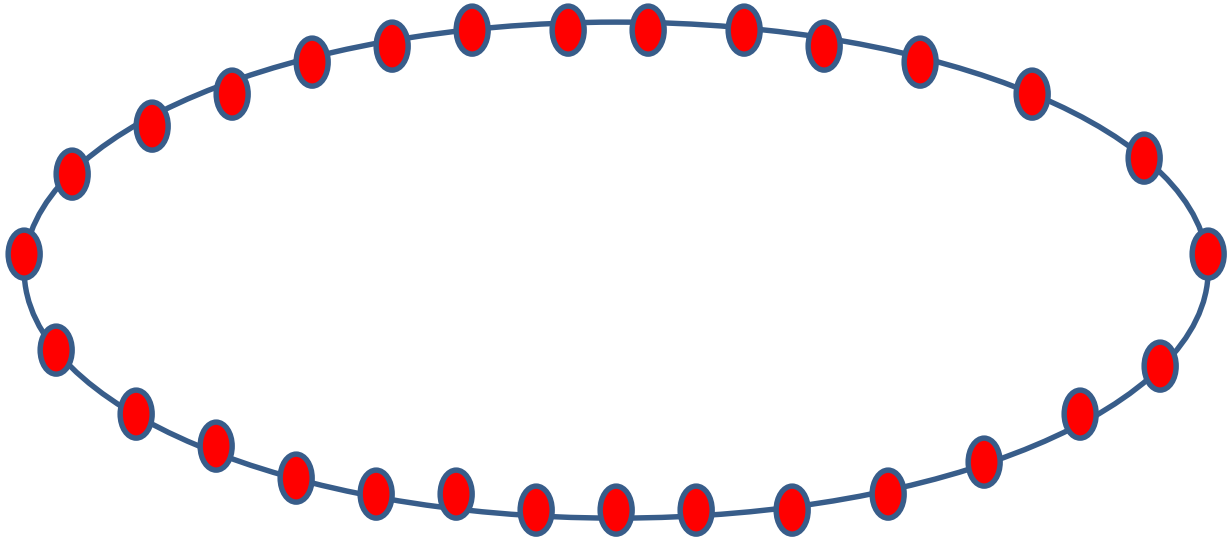


Model

ULTRA-FAST TIME RESOLVED

Ultra short collection time

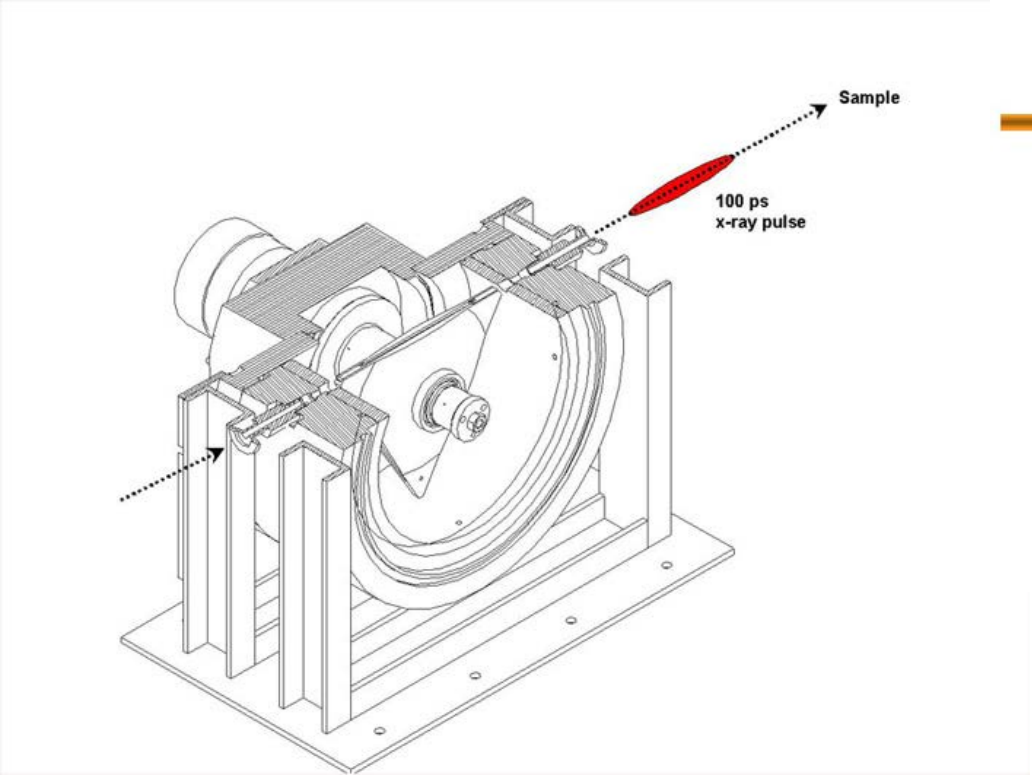
- Beamline ID09B, ESRF, Grenoble
- Using the pulsed structure of the synchrotron

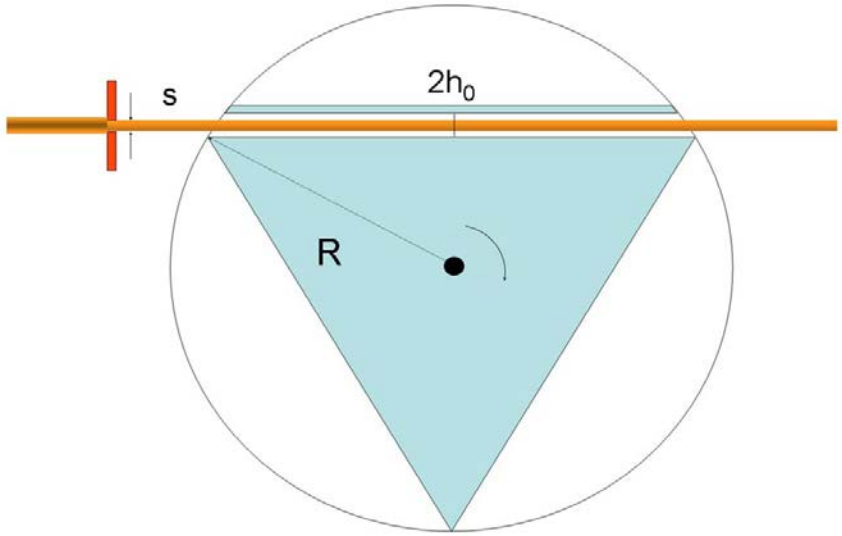


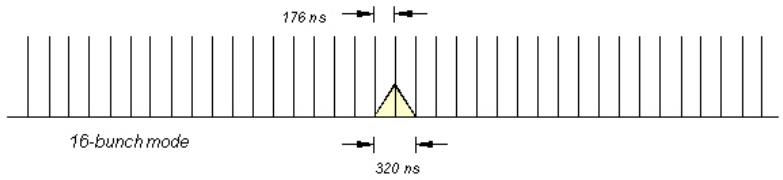
- About 5000000 bunch/sec

Isolate one bunch

- Isolate one bunch (ms shutter + fast chopper)







Single bunch experiment

- High flux needed

- Repetition of the measurements

Pump and probe experiment

Trigger with Laser pulse

Probe with X-ray

Bunch length ≈ 100 ps

→Resolution: up to 100 ps

What is 100ps?

100 ps → second

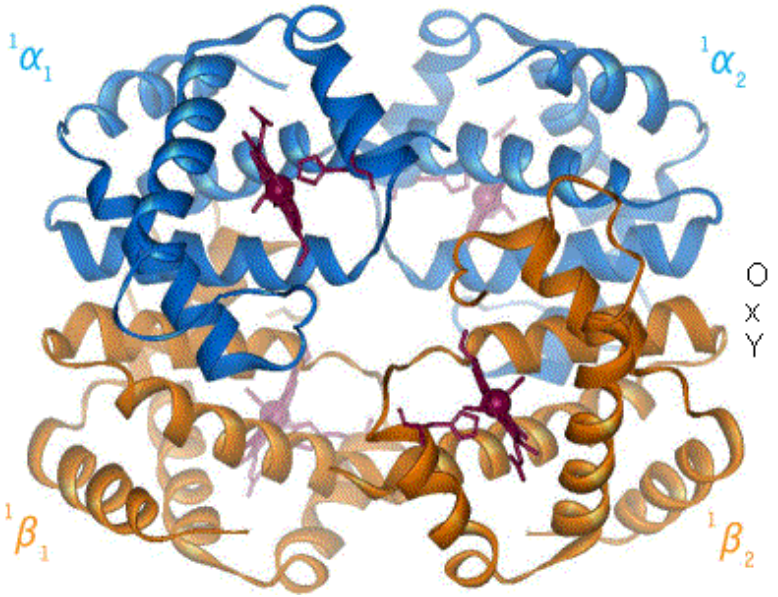
Second → ?

Light travels ? in 100ps

TR WAXS

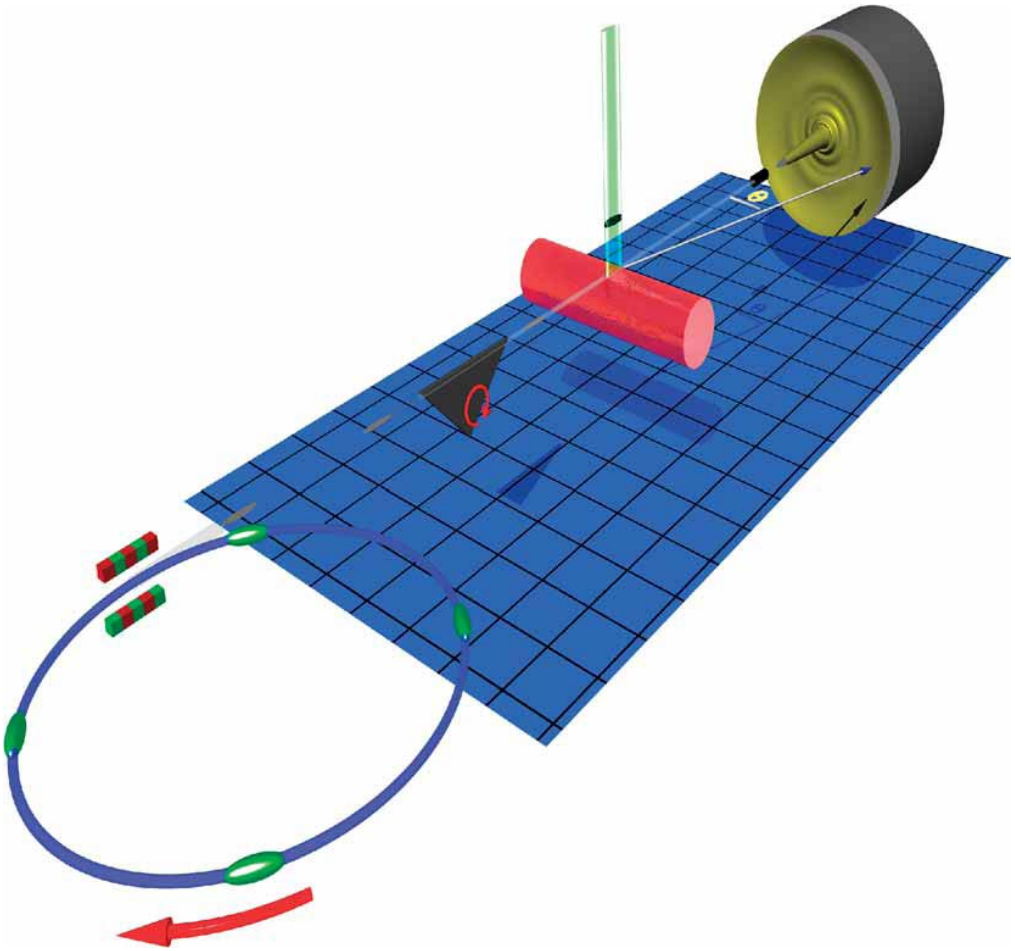
Tracking the structural dynamics of proteins in solution using time-resolved wide-angle X-ray scattering. Cammarata et al. Nature 2008.

T and R states of hemoglobin

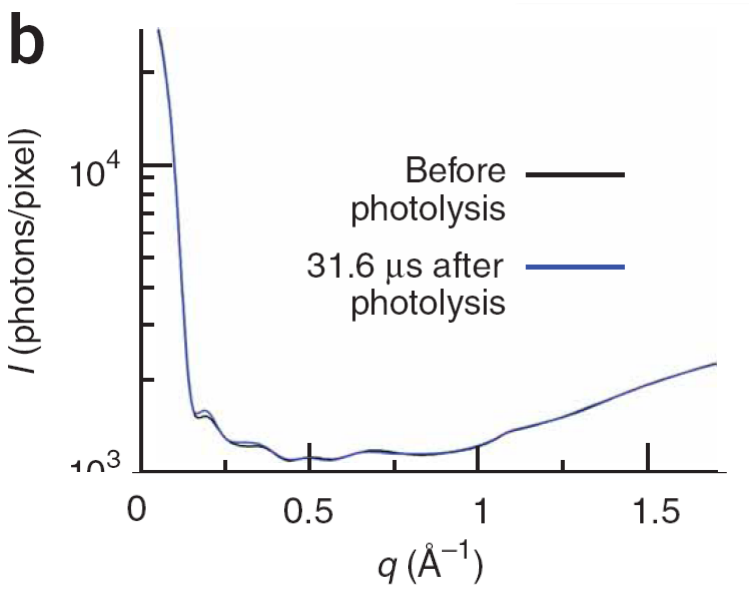


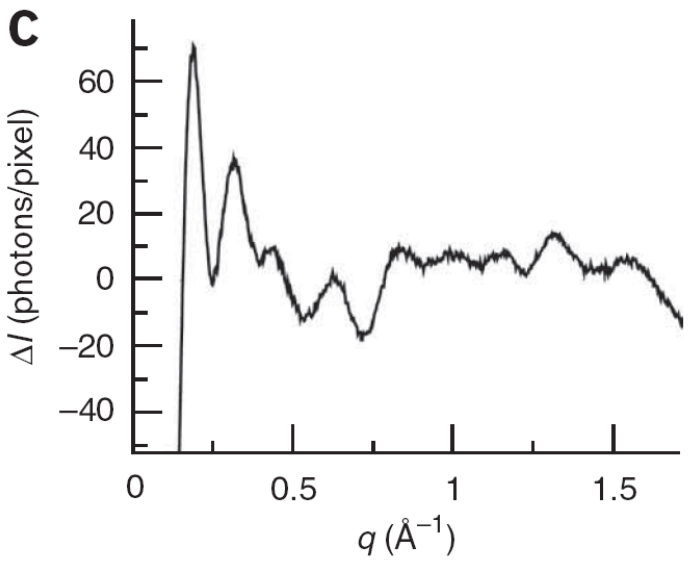
Looking at the unbinding of oxygen by hemoglobin

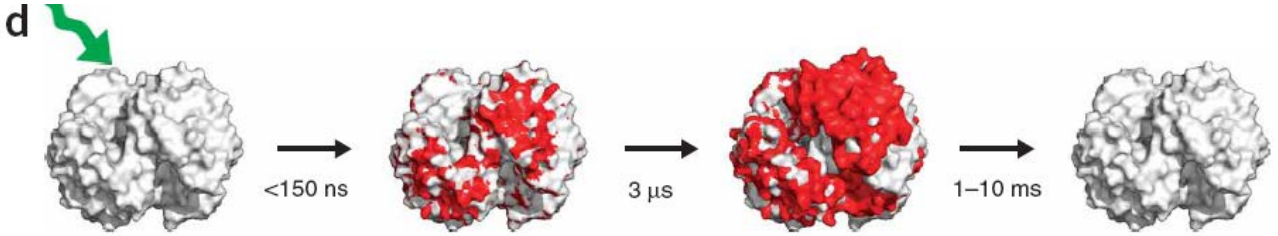
Experimental setup



Structural change in hemoglobin

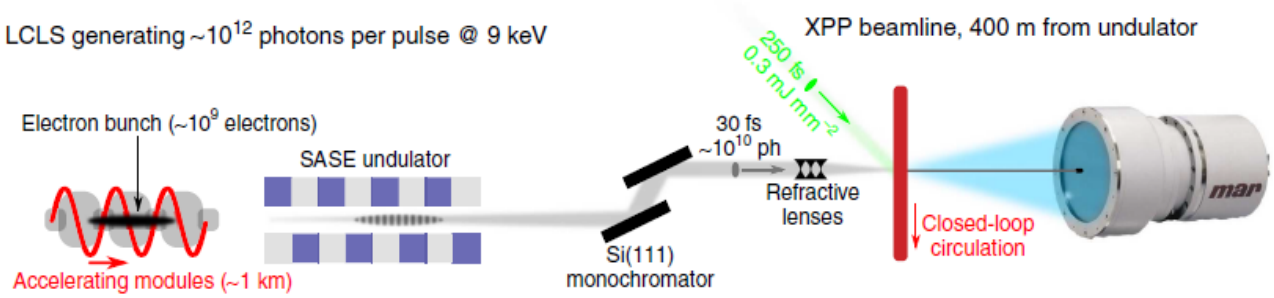






FEL

Levantino, M., Schirò, G., Lemke, H. T., Cottone, G., Glownia, J. M., Zhu, D., ... & Cammarata, M. (2015). Ultrafast myoglobin structural dynamics observed with an X-ray free-electron laser. *Nature communications*, *6*.



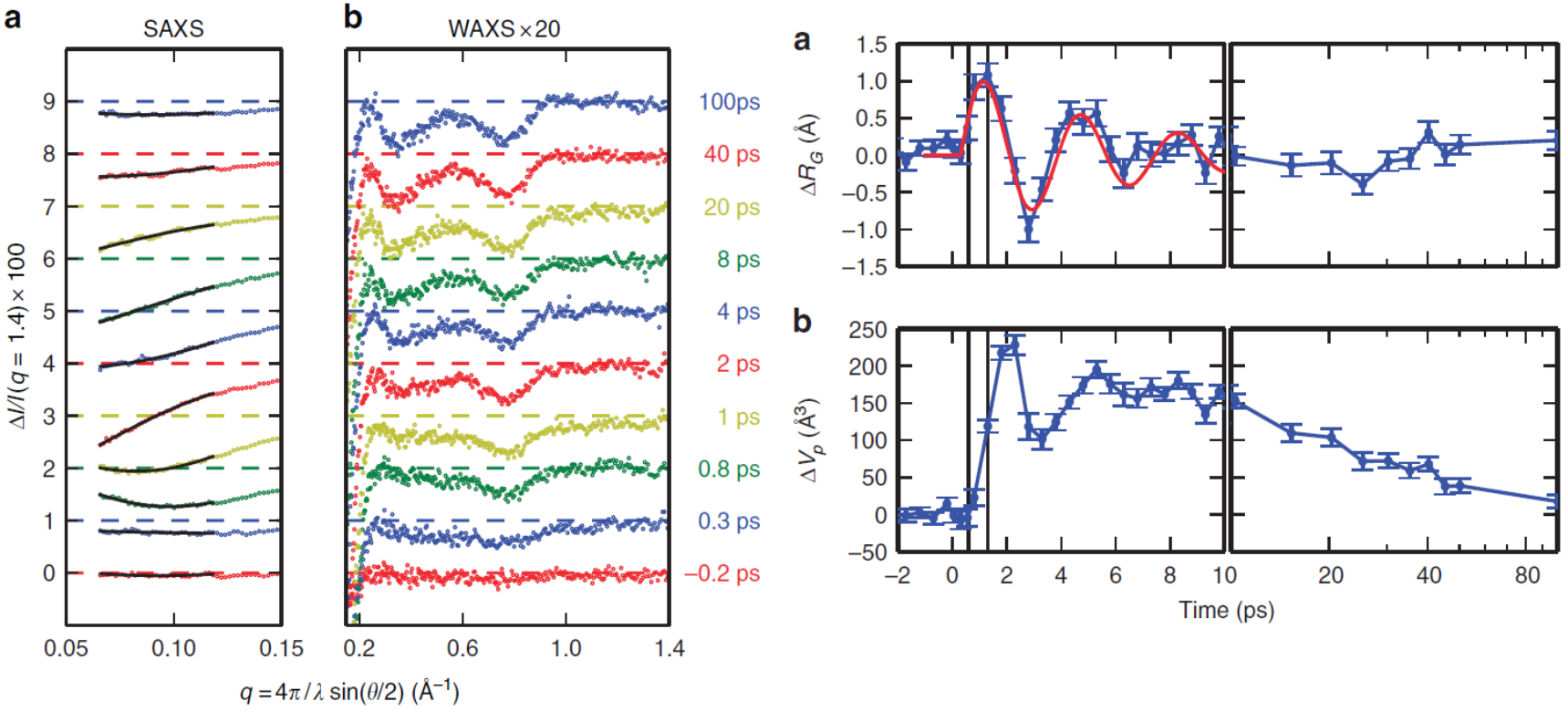
What is 30fs?

100 fs → second

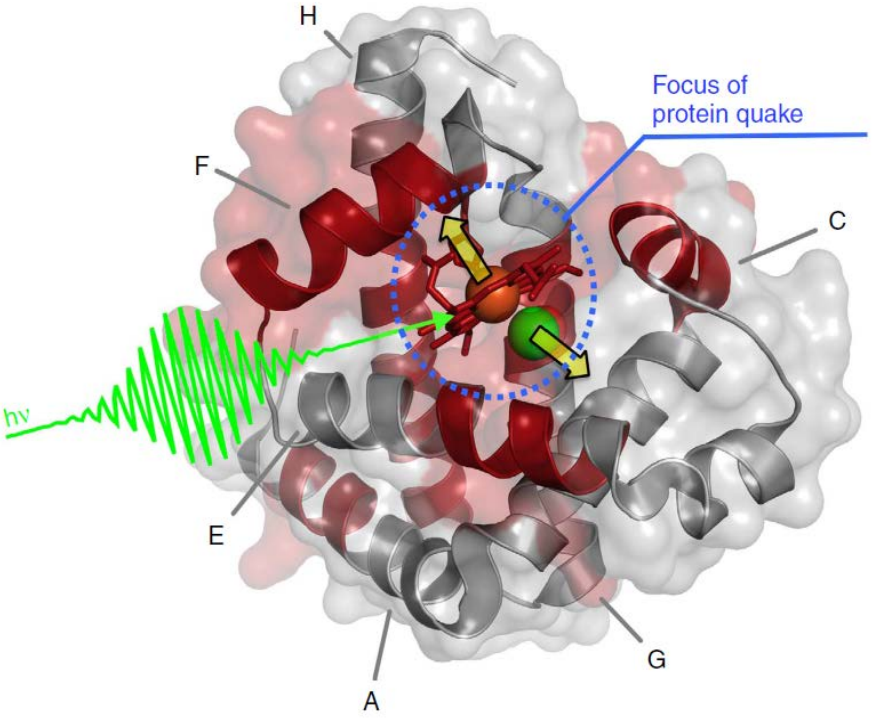
Second → 1000000 years

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