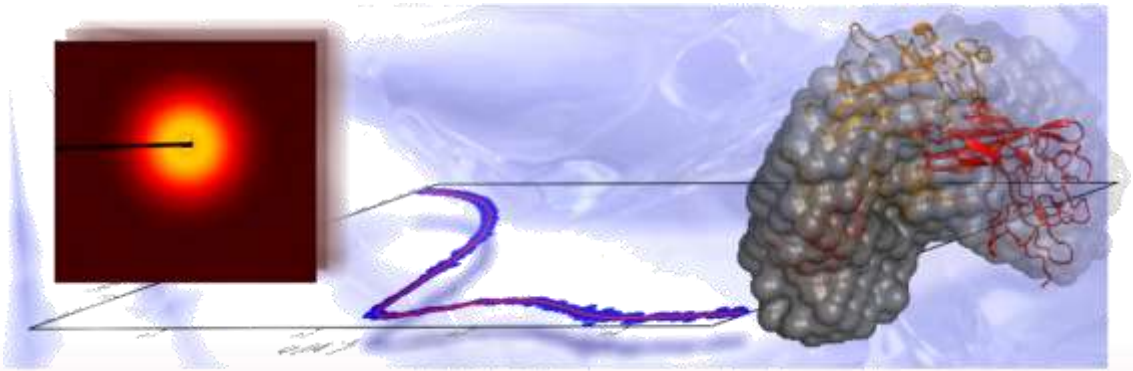
Short Introduction to Time-resolved SAXS



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BioSAXS beamline BL 4-2

BioSAXS Workshop, March 28-30, 2016

Why do TR-SAXS?

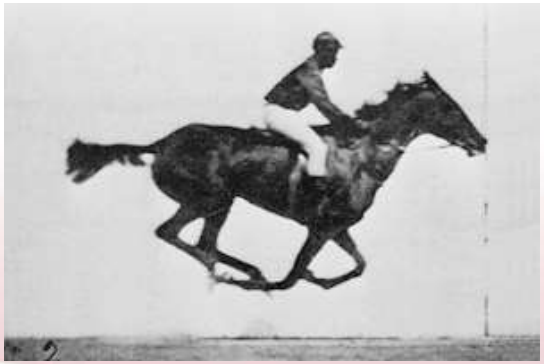
Cons:

- many pitfalls and technical difficulties
- needs lots of material (compared to static SAXS)
- rad. Damage issues

Pros:

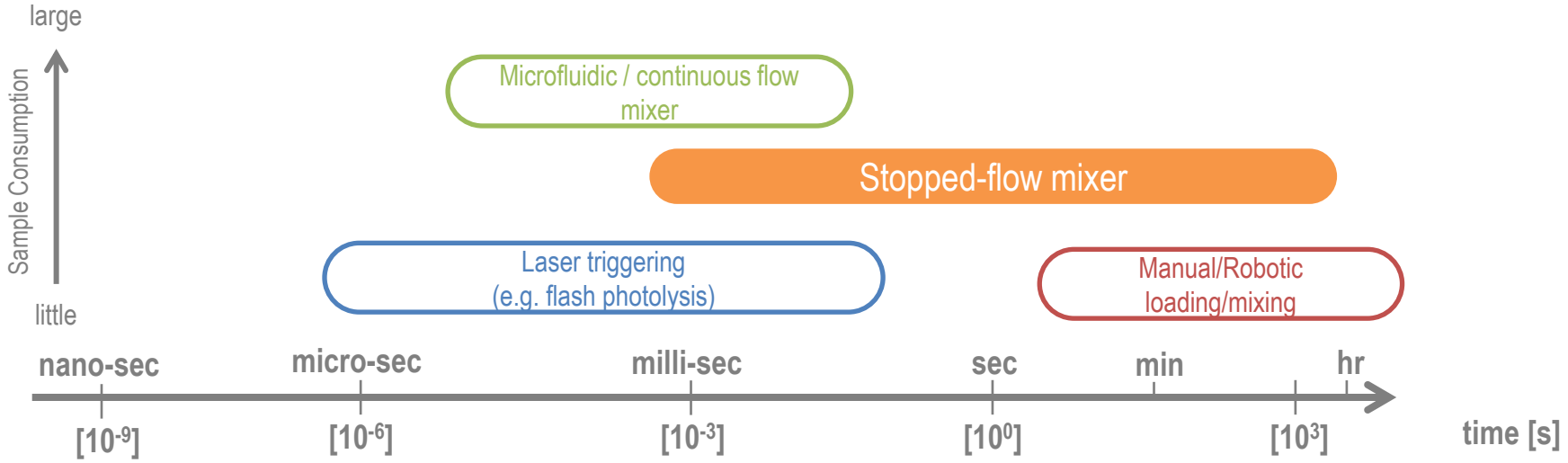
- It's cool
- watch reaction in real time
- sensitive to intermediate states in reaction
- details in the kinetic might be functional relevant
- connect to MD simulation/theory

Timescales: µs to s, hours, days



Muybridge's horse galloping (1878)

Timescales for (bio)molecular dynamics accessible by mixing



Folding, Loop motion

Local ← Domain motion, Oligomerization, Assembly, Dissociation → Large scale

What do you need to do TR-SAXS

sizable change between initial and final state (several A in Rg and or Dmax)

initiate the reaction (mixer, flash, heater, pressure …)

ability to collect data at specific time points

Systems that can be studied using TR-SAXS

- conformational changes in response to environmental changes (pH-jump, T-jump, p-jump …)
- Changes due to ligand binding
- Complex formation
- …

How to initiate the reaction

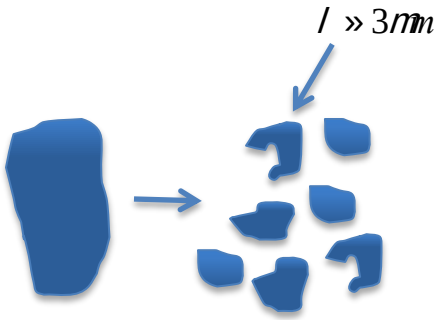
- TR-SAXS in solutions most often use mixing as reaction trigger (although flash, heat-jump or pressure jump also used)
- For mixing there are two types: turbulent or laminar flow mixing
- two basic concepts of mixing devices
  - continuous flow (CF)
  - stopped-flow (SF)

Final step of mixing governed by diffusion over a length λ:

$$l = \sqrt{tD} \Rightarrow l \approx 3mm$$

$D \approx 10^{-5} cm^2/s$ typ. diffusion constant

$t \approx 10^{-6} s$ Diffusion time



Large Reynolds number $R \approx 2x10^4$

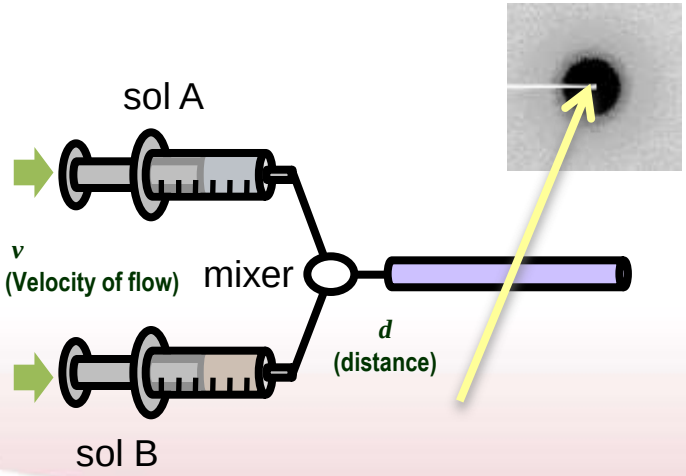
Types of mixer

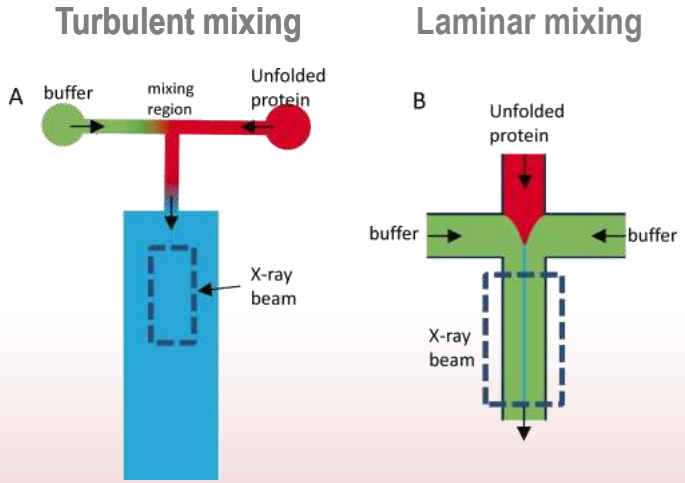
Stopped-Flow (SF) mixer

- turbulent mixer
- mix and then stop the flow
- time resolution: ~1ms
- time resolution limited by frame rate and photon flux
- sample consumption (several mg).

Continuous-Flow (CF) mixer

- turbulent or laminar
- continuously flow and mix
- achievable time resolution: ~20us
- time resolution limited by beam size
- high sample consumption (> hundreds of mg) laminar mixers.better

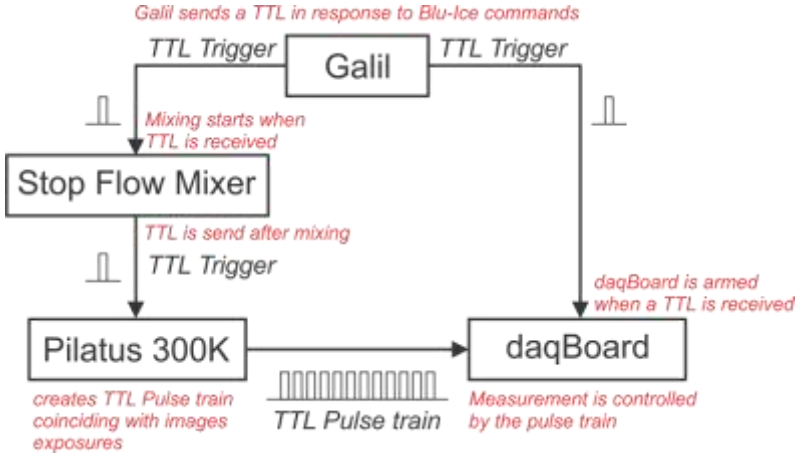


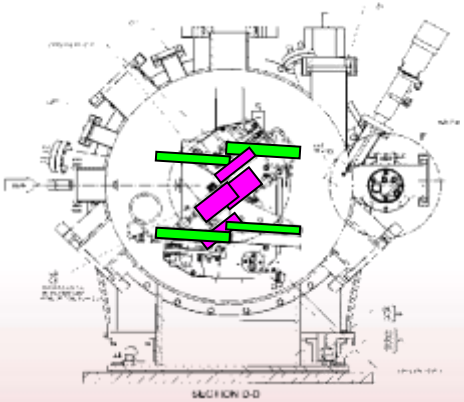


Kathuria et al., Biopolymers (2011)

Time-resolved SAXS at BL4-2

- multilayer (Mo/B_4C) monochromator with 2% energy bandpass for increased photon flux (~10^{14} ph/s)
- using PCI data acquisition boards gated by pulse train from detector for intensity monitoring
- all hardware under Blu-ICE control
- dedicated BluIce interface for TR-SAXS experiments
- fast PAD detector Pilatus 300k
- currently: time resolution 5ms (detector limited)





Integrated Si(111) and Mo/B_4C Mono

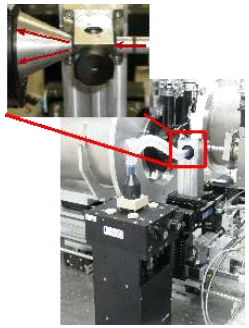
Blu-Ice unser interface

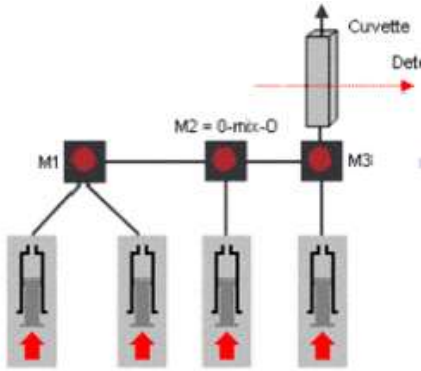
Pilatus 300K
- 200Hz frame rate
- photon counting

Time-resolved SAXS at BL4-2

Stopped-flow Mixer

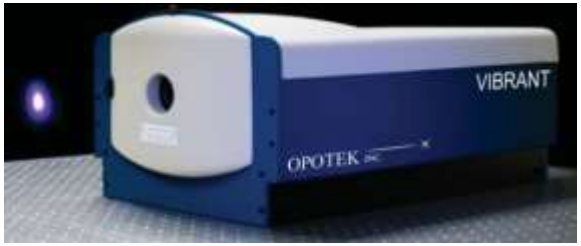
- Biologic SFM-400 stopped-flow device
- 30µl min injection
- Variable flowrate
- >0.25ms deadtime
- Variable mixing ratios





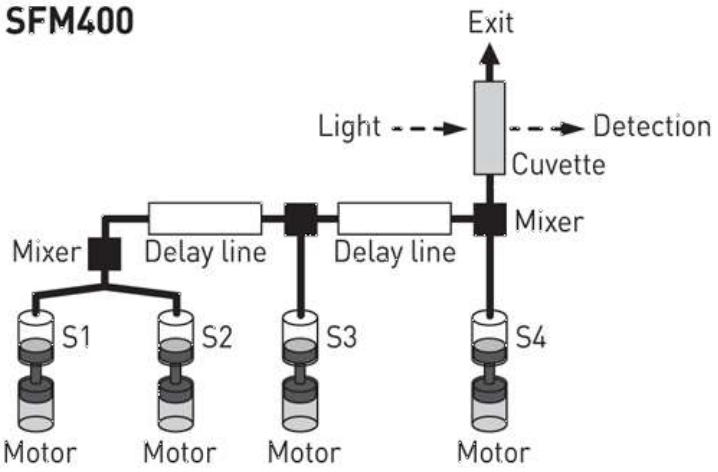
Photoreactions and T-jump (in preparation)

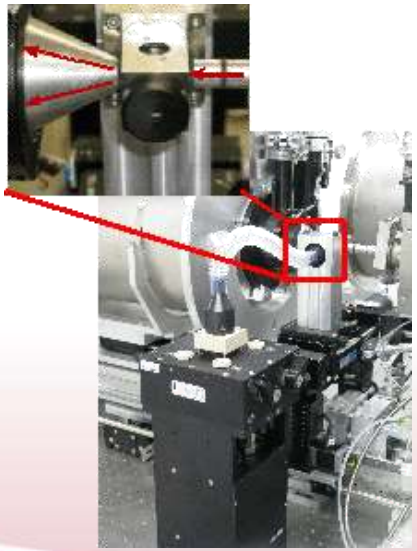
- Opotec Vibrant 355HE tunable laser
- wavelength range: 410 nm- 2400 nm
- peak energy 45mJ, 5ns pulse duration
- computerized control
- photoreactions and T-jump experiments



Stopped-flow Mixer

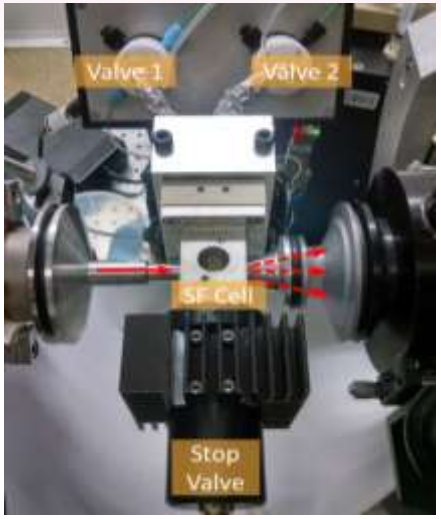
- Biologic SFM-400 stopped-flow device
- four motorized syringes
- Variable flowrate and mixing ratios
- >0.25ms deadtime
- 30µl min injection
- using ‘extension cord’ due to space constraints

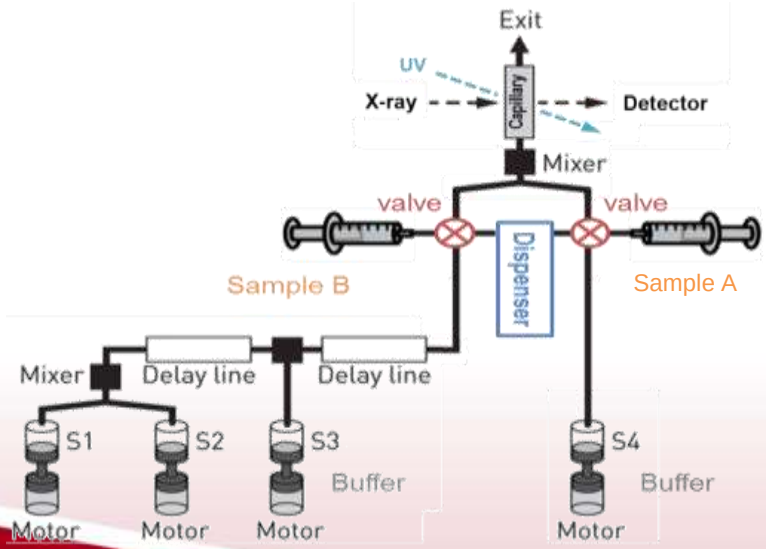


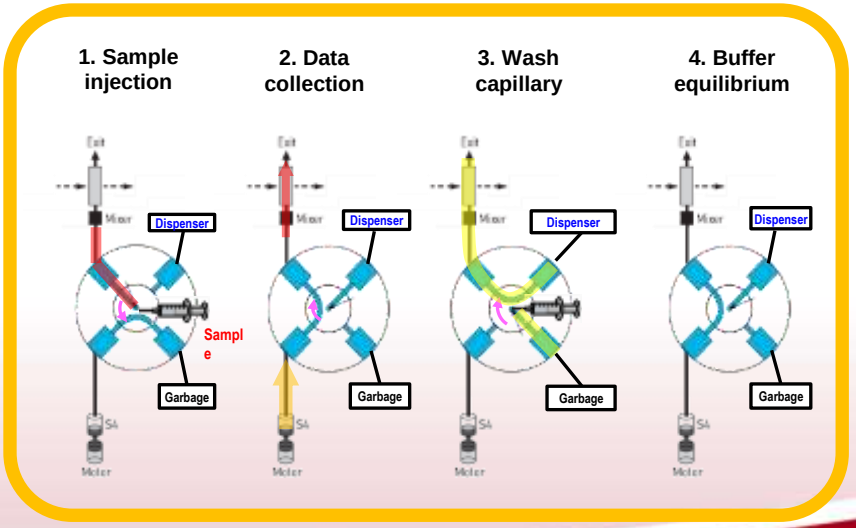


Customized Stopped-flow Mixer

- adding sample injection ports with motorized valves
- valve control integrated into Blu-Ice interface
- eliminates sample consuming priming of fluid paths
- simultaneous UV spectrum recording (in preparation)
- allows automated and thorough cleaning of capillary without compromising sample (!!!)







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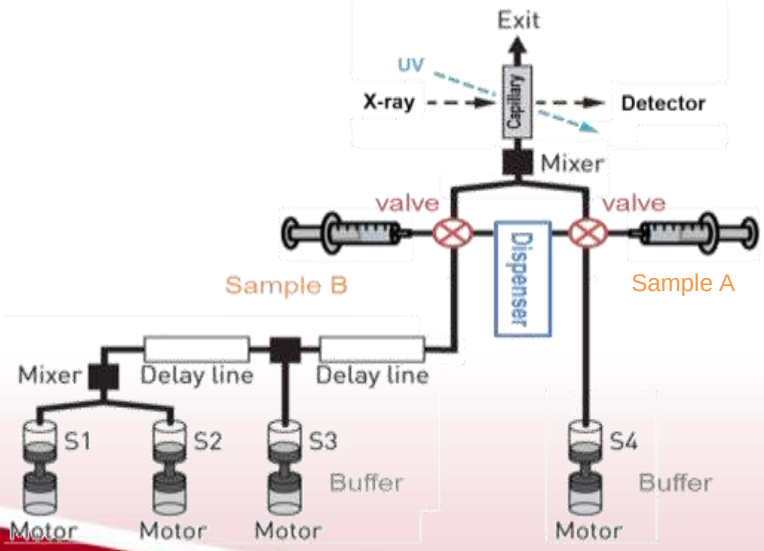
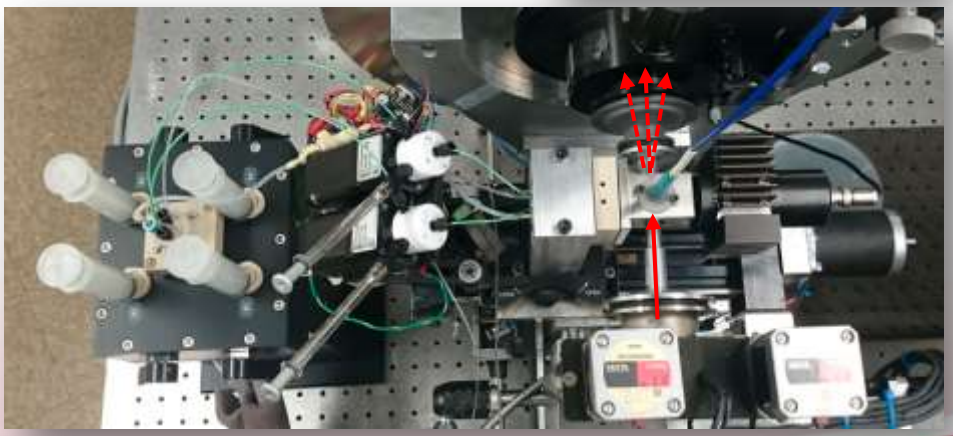
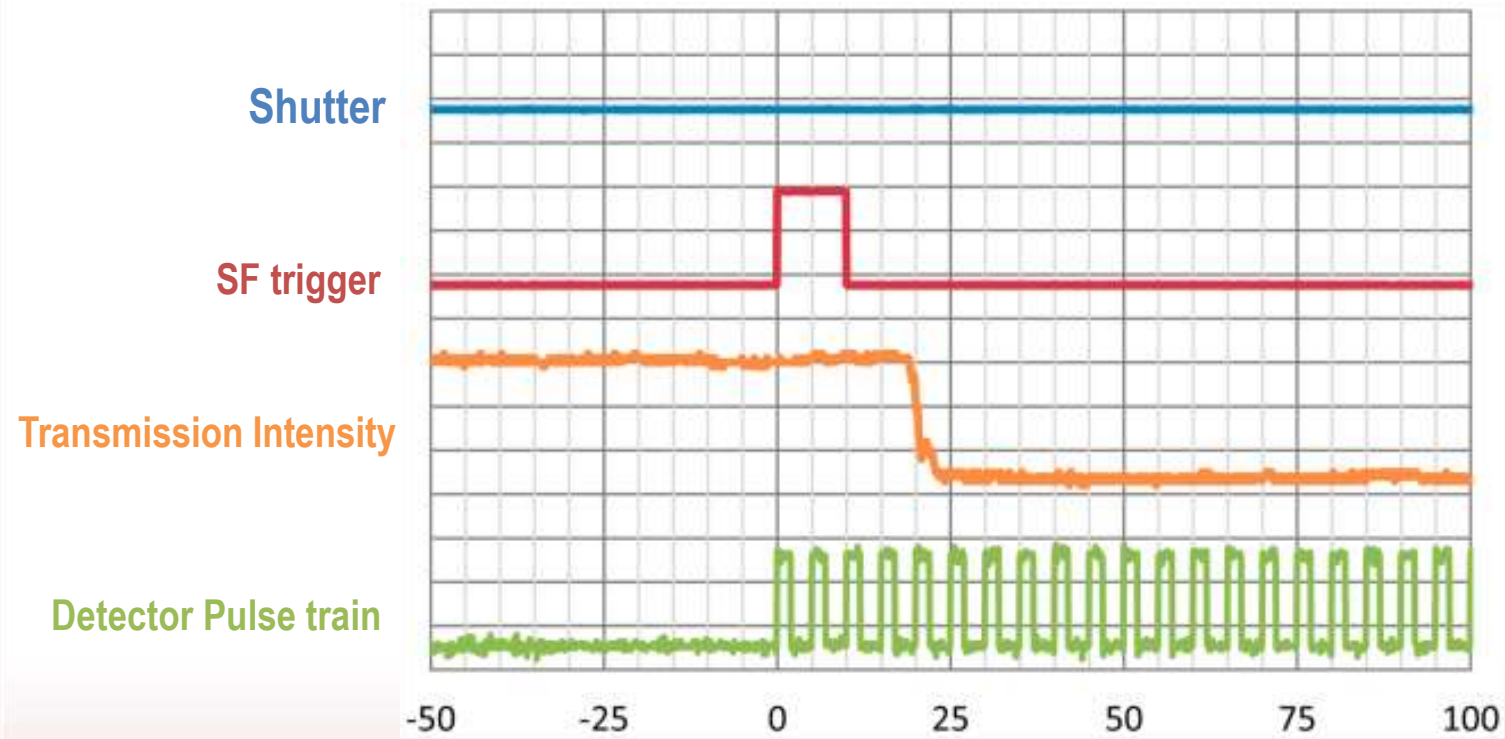




Mixing times

Mixing 400mM CsCl with water:



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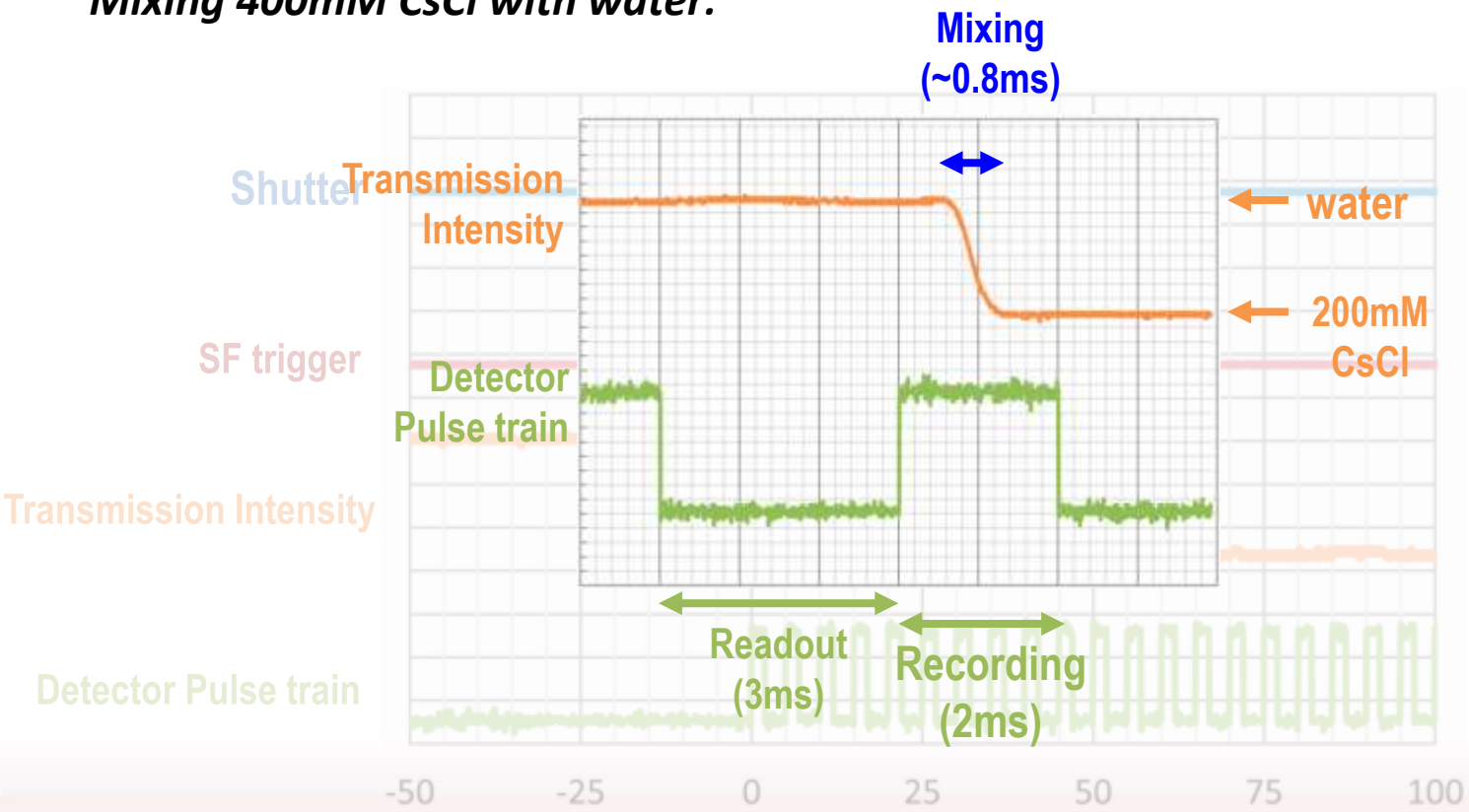


Si(111) vs multilayer

10mg/ml BSA (66.5kDa) with different exposure time

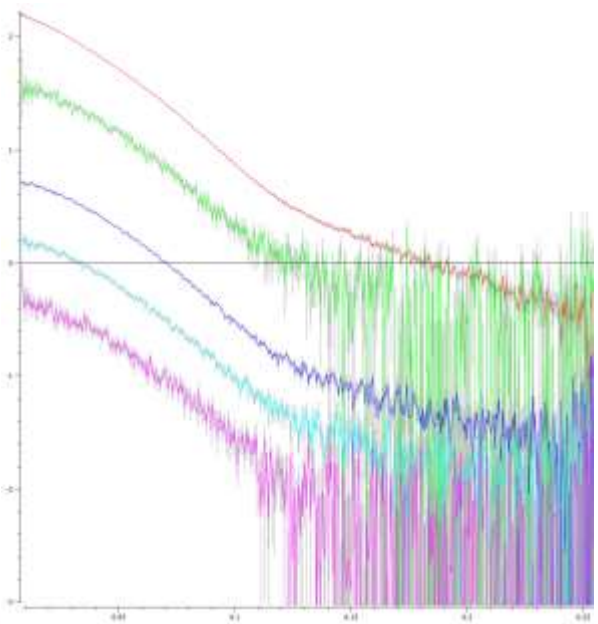
Multilayer: 15msec

Multilayer: 2msec

Si(111): 95msec

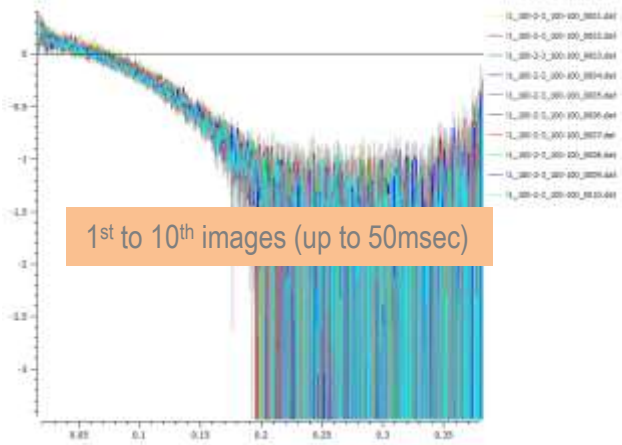
Si(111): 15msec

Si(111): 2msec



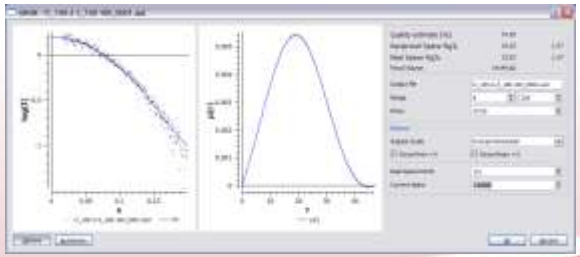
q (Å^{-1})

2msec exposure of 10mg/ml Lysozyme (5msec repetition, multilayer beam)



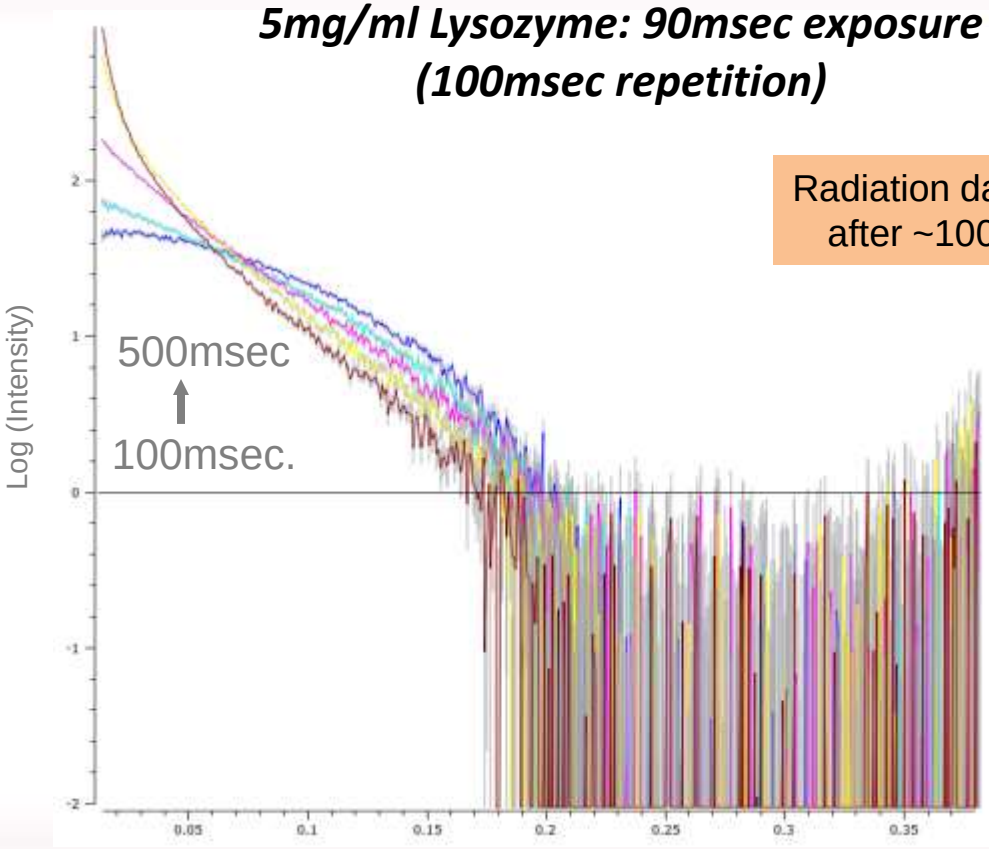
q (Å^{-1})

- P(r) function of 1st image



Radiation Damage

5mg/ml Lysozyme: 90msec exposure (100msec repetition)



Radiation damage observed after ~100msec.

- Need thorough cleaning of capillary before every shot
- Use of radical scavenger is highly recommended (e.g. 5mM DTT)

Summary

- TR-SAXS Is a very useful technique to identify structural intermediates and characterize kinetics
- it adds “time” to structural biology research

You need:s

- a system with large change between initial and final state (well pre-characterized by static SAXS)
- lots of sample (at least 10mg, better more):
    - depends on time scale: typically the faster the reaction the more sample;
- a way to disturb the system out of equilibrium
- ability to collect data at specific time points (enough photons/s and fast detectors

Thank you