Short Introduction to Time-resolved SAXS

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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

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**Timescales:**

μs to s, hours, days

Muybridge’s horse galloping (1878)
Timescales for (bio)molecular dynamics accessible by mixing

- Microfluidic / continuous flow mixer
- Stopped-flow mixer
- Laser triggering (e.g., flash photolysis)
- Manual/Robotic loading/mixing

Sample Consumption
- little
- large

Time [s]
- nano-sec [$10^{-9}$]
- micro-sec [$10^{-6}$]
- milli-sec [$10^{-3}$]
- sec [$10^0$]
- min [$10^3$]
- hr

Sample Movement
- Folding, Loop motion
- 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 $\lambda$:

$$l = \sqrt{tD} \rightarrow 3 \text{ m}$$

- $D \approx 10^{-5} \text{ cm}^2 / \text{s}$ typ. diffusion constant
- $t \approx 10^{-6} \text{ s}$ diffusion time
- Large Reynolds number $R \approx 2 \times 10^4$
**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.

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Kathuria et al., Biopolymers (2011)
Time-resolved SAXS at BL4-2

- multilayer (Mo/B$_4$C) 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)
**Biologic SFM-400 stopped-flow device**
- 30µl min injection
- Variable flowrate
- >0.25ms deadtime
- Variable mixing ratios

**Opotec Vibrant 355HE tunable laser**
- Wavelength range: 410 nm - 2400 nm
- Peak energy 45mJ, 5ns pulse duration
- Computerized control
- Photoreactions and T-jump experiments

**Photoreactions and T-jump (in preparation)**

**Time-resolved SAXS at BL4-2**
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:

- Shutter
- SF trigger
- Transmission Intensity
- Detector Pulse train
Mixing times

**Mixing 400mM CsCl with water:**

- **Transmission Intensity**
- **Detector Pulse train**
- **Shutter**
- **SF trigger**

**Mixing (~0.8ms)**

**Readout (3ms)**

**Recording (2ms)**

**Water**

**200mM CsCl**

**Mixing times**

**Graph:**
- X-axis: Time (ms)
- Y-axis: Intensity

**Legend:**
- Orange: 200mM CsCl
- Green: Water
**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

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

1st to 10th images (up to 50msec)

- P(r) function of 1st image
Need thorough cleaning of capillary before every shot
Use of radical scavenger is highly recommended (e.g. 5mM DTT)

Radiation damage observed after ~100msec.
Summary

• TR-SAXS Is a very useful technique to identify structural intermediates and characterize kinetics

• it adds “time” to structural biology research

You need:

• 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