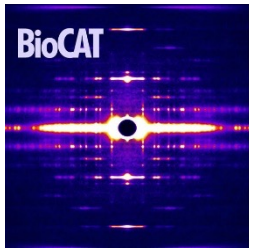

Time Resolved SAXS

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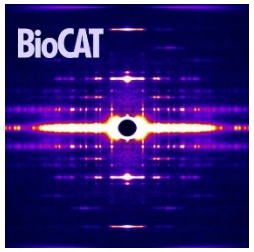
ILLINOIS INSTITUTE OF TECHNOLOGY

June 23, 2022

CSRRI

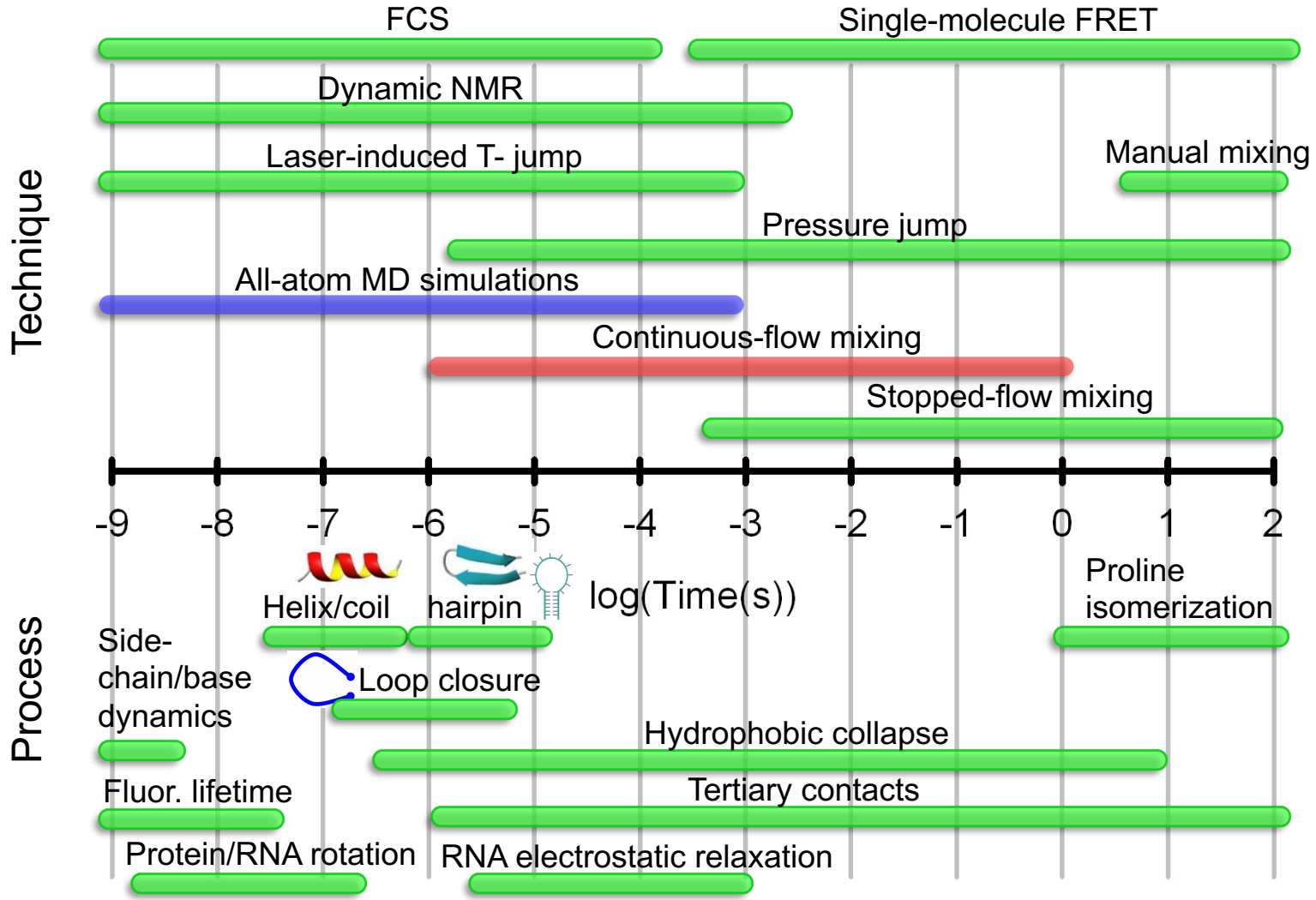


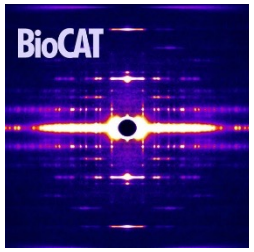
Time resolved SAXS

- Different methods for different time resolutions
  - XFEL pump probe
    - Minimum timescales < 100 fs
    - Direct laser excitation required
  - Synchrotron pump probe
    - Minimum timescales ~100 ps
    - Direct laser excitation or T jump
  - Fast mixing
    - Minimum timescales ~100 μs
    - At synchrotrons or XFELs
  - Other
    - Pressure jump, T jump, manual mixing, etc.
    - Minimum timescales determined by reaction initiation process, details of measurement
- Important to pick a method that matches your timescales
  - XFEL and pump probe are more costly in sample, often harder to get time to do
- Consider initiation method

Time resolved SAXS

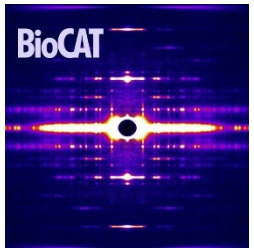
S.V. Kathuria et al *Biopolymers* **2011**, 95, 550-558.





Time resolved SAXS

- SAXS is a global, solution based technique
- Any changes you can study with equilibrium SAXS can also be studied with TR-SAXS
- Complementary to site-specific probes like FRET
- At BioCAT:
  - Stopped flow mixing (≳1 ms)
  - Continuous flow mixing
    - Chaotic flow mixing (~80 μs to 75 ms)
    - Laminar flow mixing (~1 ms to 1.5 s)



Stopped flow mixing

- Overview:
  - Mix together two liquids
    - Typically protein solution, and a solution inducing a reaction
  - Immediately after mixing, solution enters sample cell
  - Close off sample cell
  - Watch reaction evolve with time in sample cell

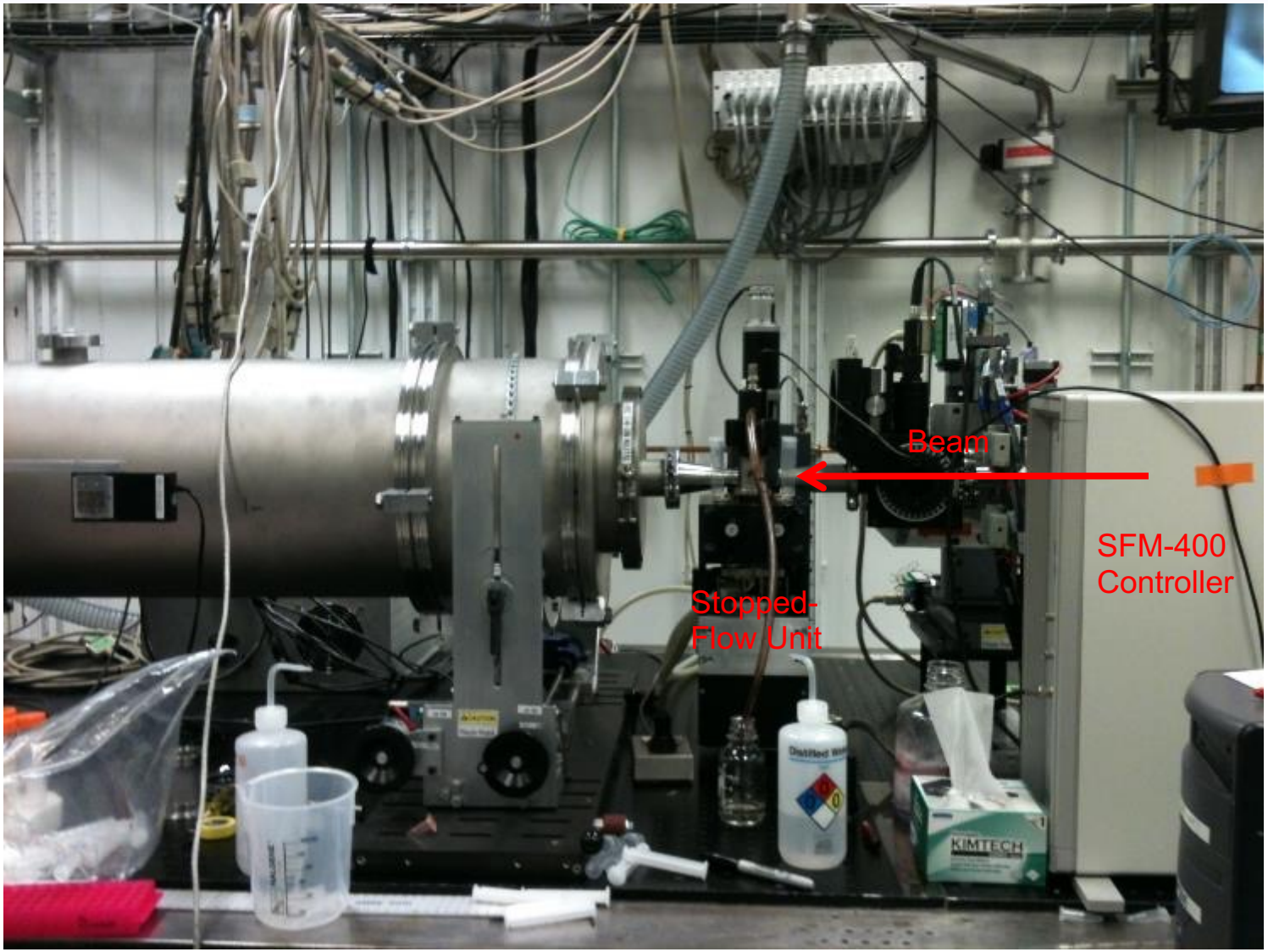
- Equipment commercially available
  - Straightforward, long history

- Earliest time point ~1 ms, latest however long you're willing to wait

- Measuring same sample over and over again can create radiation damage
  - Use limited total exposure, multiple injections to measure full time range

- Time resolution only as good as the fastest exposure
  - Lowers signal to noise, multiple injections to build up signal

Stopped flow mixing



Stopped flow mixing

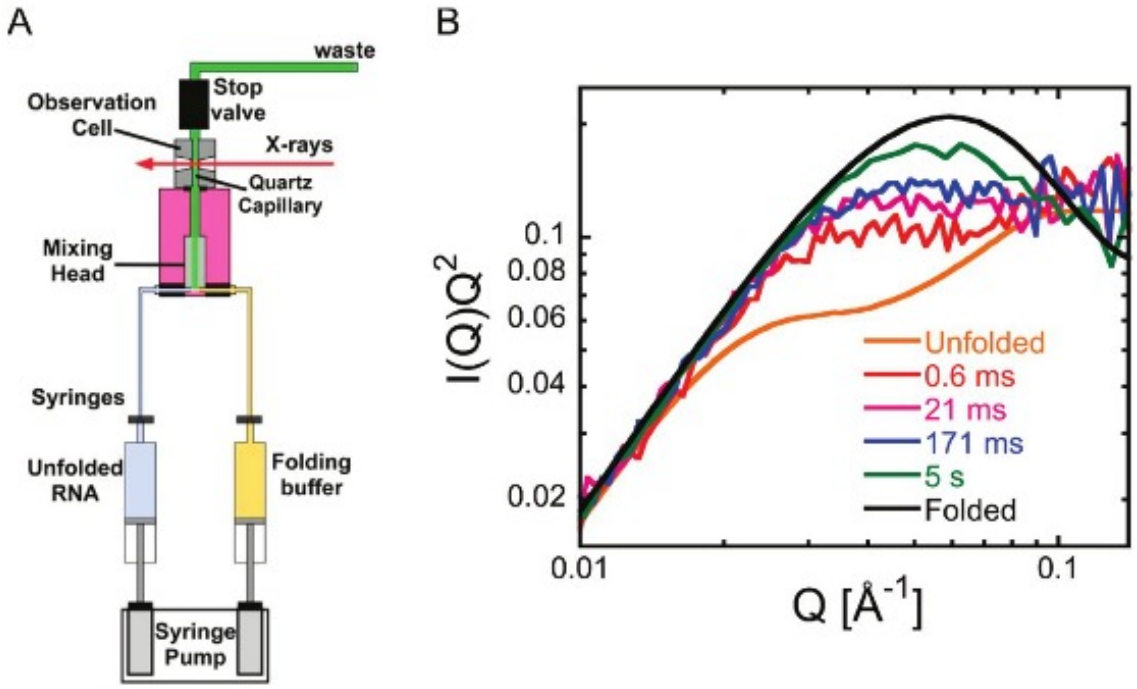
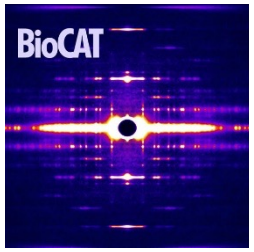


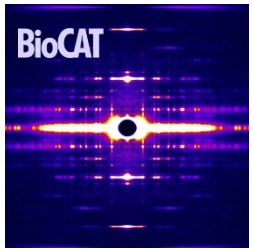
Figure 1. Time-resolved SAXS of *Azoarcus* ribozyme folding. (A) Schematic view of the stopped-flow mixer (SFM400). Syringes were loaded with unfolded RNA (1 mg/mL after mixing) in 20 mM Tris-HCl and folding buffer containing $MgCl_2$. The dead time (~0.6 ms) was minimized by a high flow rate and short distance from the small-volume mixer to the observation point. (B) Kratky plots of real-time folding data in 1.5 mM $MgCl_2$. Curve at 5 s (green) were in 5 mM $MgCl_2$. For time-resolved measurements (≤200 ms), 15−20 identical 1 ms data sets were averaged. Scattering data up to 5 s were acquired for 50 ms and averaged over 4 shots. For unfolded RNA in 20 mM Tris-HCl (orange) and folded RNA in 5 mM $MgCl_2$ (black) at equilibrium, data were collected for 1.6 s (4 times).

Roh et al., J Am Chem Soc. 2010 Jul 28;132(29):10148-54



Stopped flow mixing

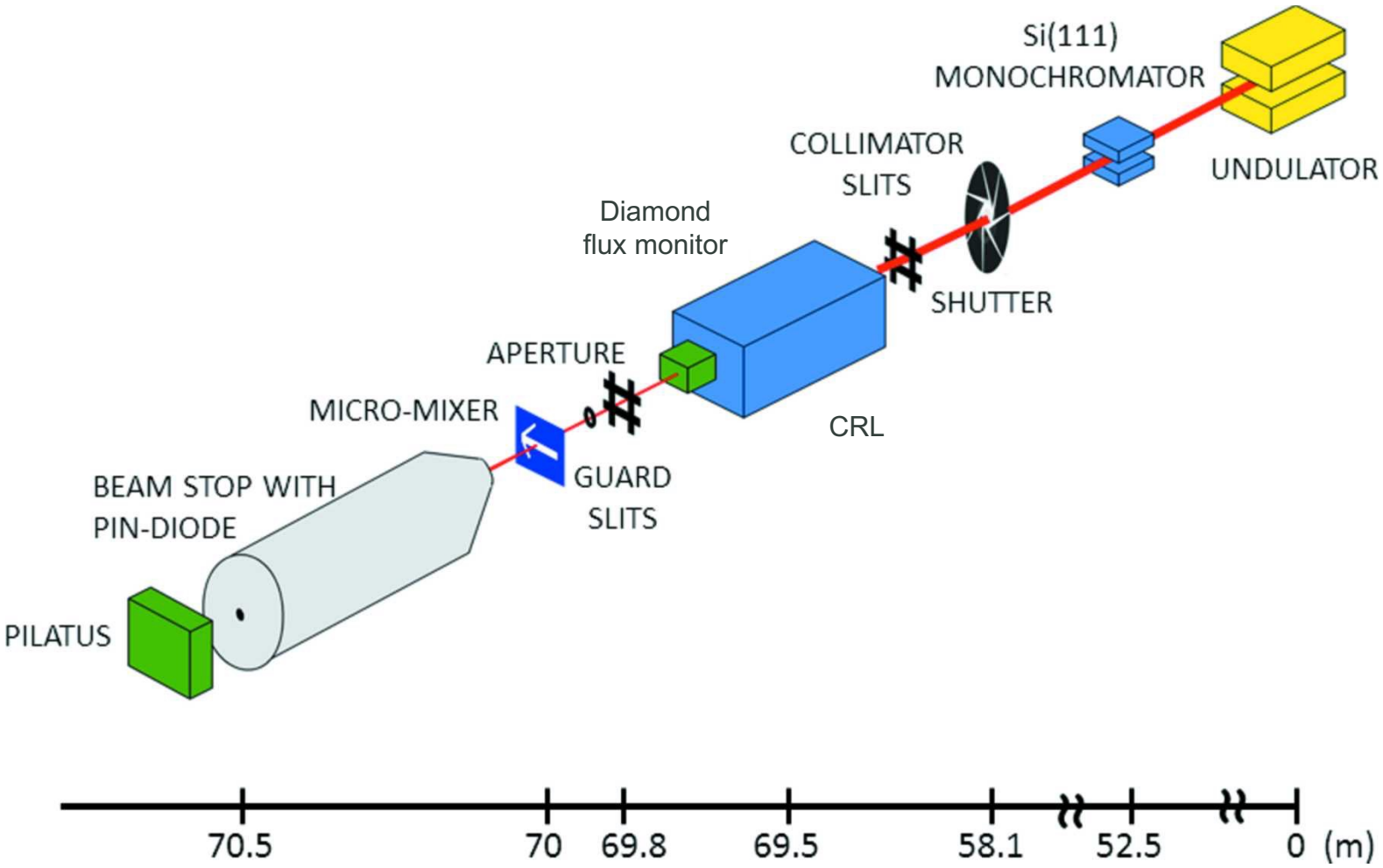
- Due to radiation damage concerns, sample consumption, we don't recommend this approach
  - Exceptions for long time points, certain types of experiments
- Has been used very successfully at several beamlines
  - BL4-2 at SSRL
  - ID07 at ESRF



Continuous flow mixing

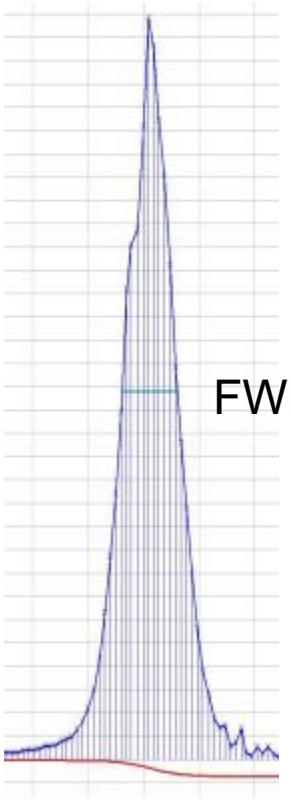
- Overview
  - Fast mixing done in microfluidic mixers
  - Mix together two liquids
    - Typically protein solution, and a solution inducing a reaction
  - Immediately after mixing, solution enters long observation region
  - Continuously flow sample through mixer
    - Constantly refreshing mixed solution
  - Observe reaction at different points in the observation region after mixing for different time points
- Equipment generally not commercially available
  - Only 1 beamline routinely provides this approach (BioCAT)
  - Some groups bring their own equipment to beamlines
- Time ranges depend on mixers used
  - For SAXS, earliest time point ~100 μs
  - Latest time point ~10 s
  - May need to use multiple mixers to achieve desired time range
- Minimizes radiation damage due to continuous flow
- Measured time point no longer depends on exposure time, can optimize signal to noise with more exposure

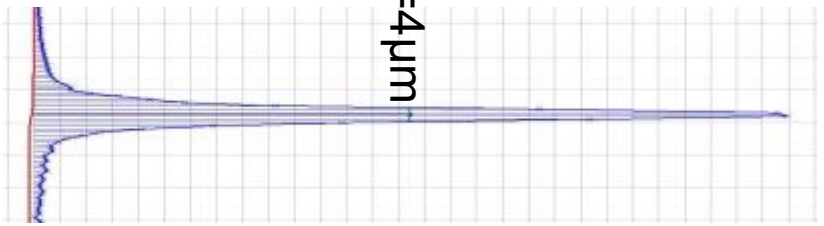
Continuous flow mixing



Continuous flow mixing

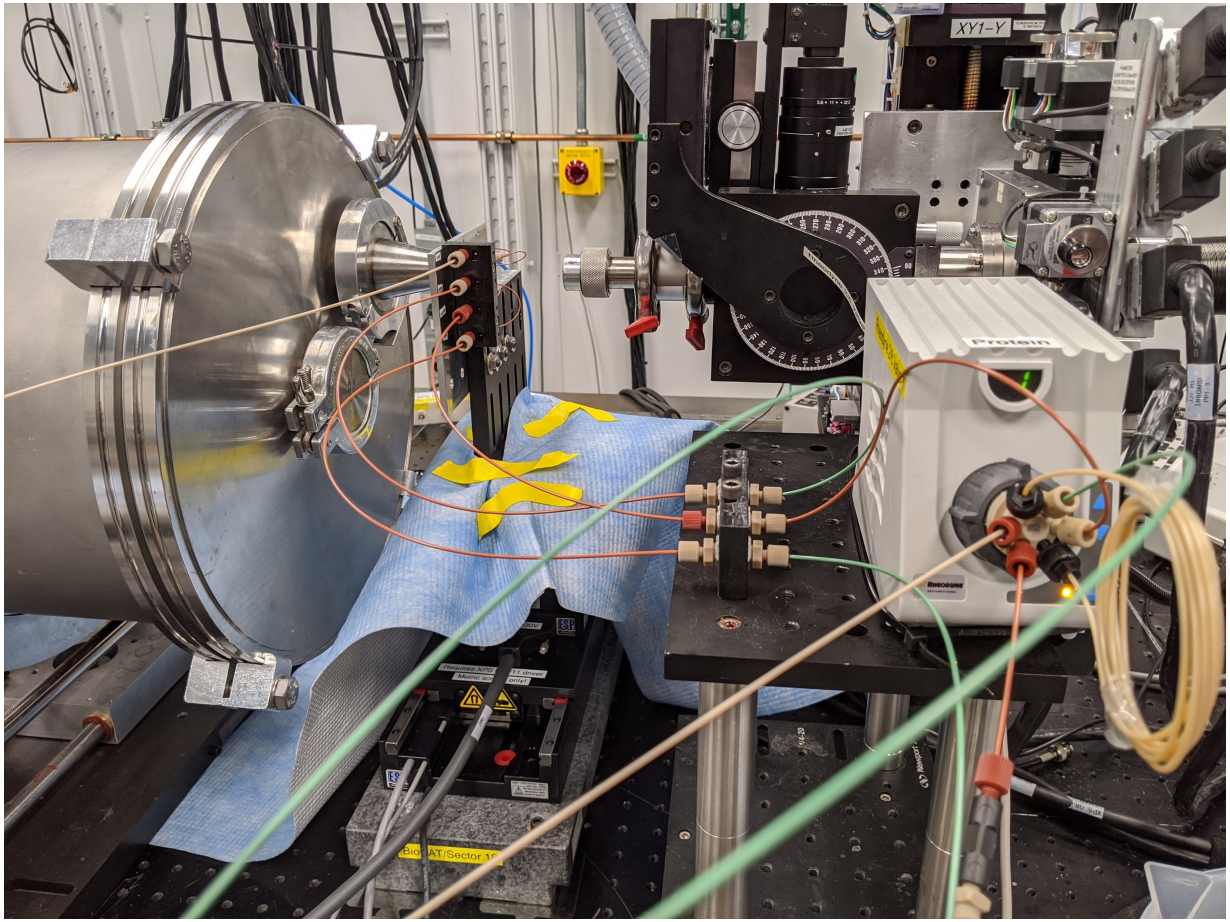
- Small observation channel requires microfocused beam
- We use compound refractive lens
  - Some use KB mirrors
- Focal Length = 1.88m
- Low Divergence
- High flux density
  - ~10^{12} ph/s @ 12 keV





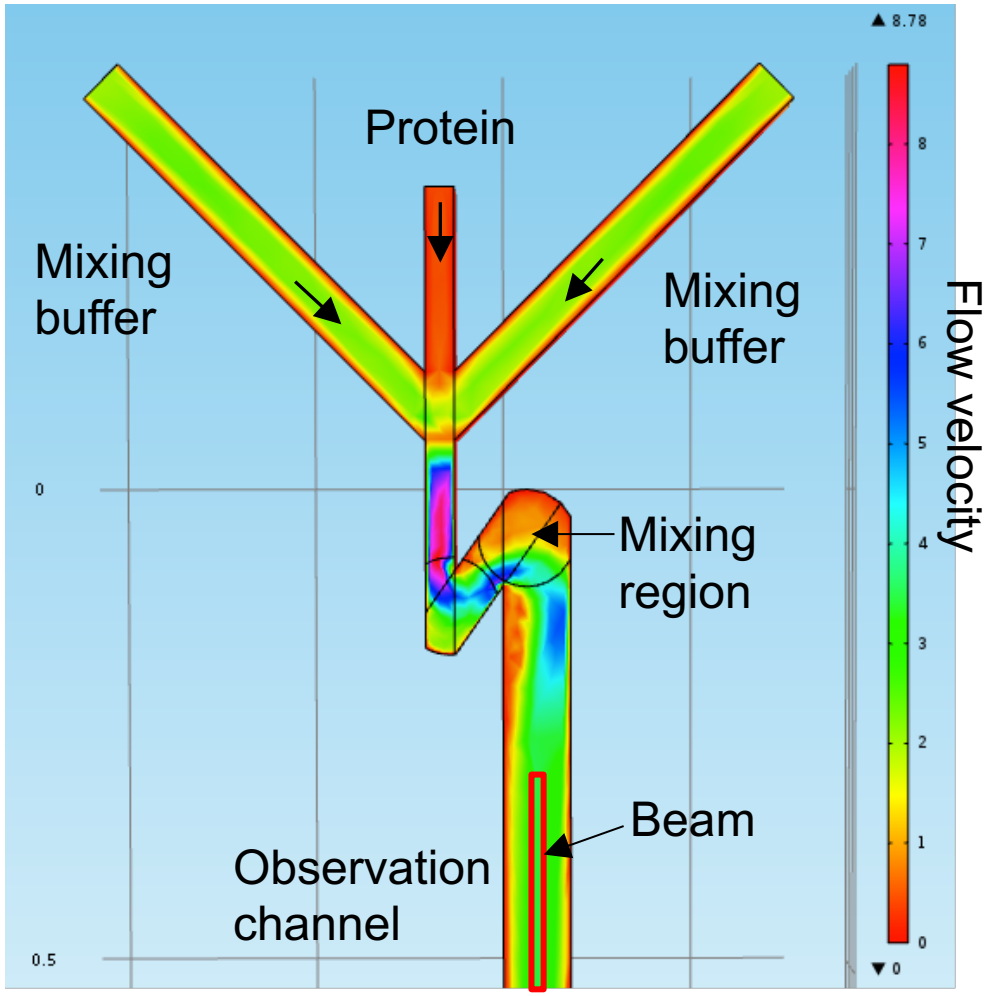
Continuous flow mixing

- Mixer observation region is not completely uniform to x-rays
- Different time points require scanning along the observation region
  - Parasitic scattering variations require point-by-point buffer subtraction
- Scanning has to be perfectly synchronized with exposure and extremely precise for good buffer subtraction
  - To minimize sample consumption, synchronization with start of mixing also required
  - Lots of time and effort required to get this right

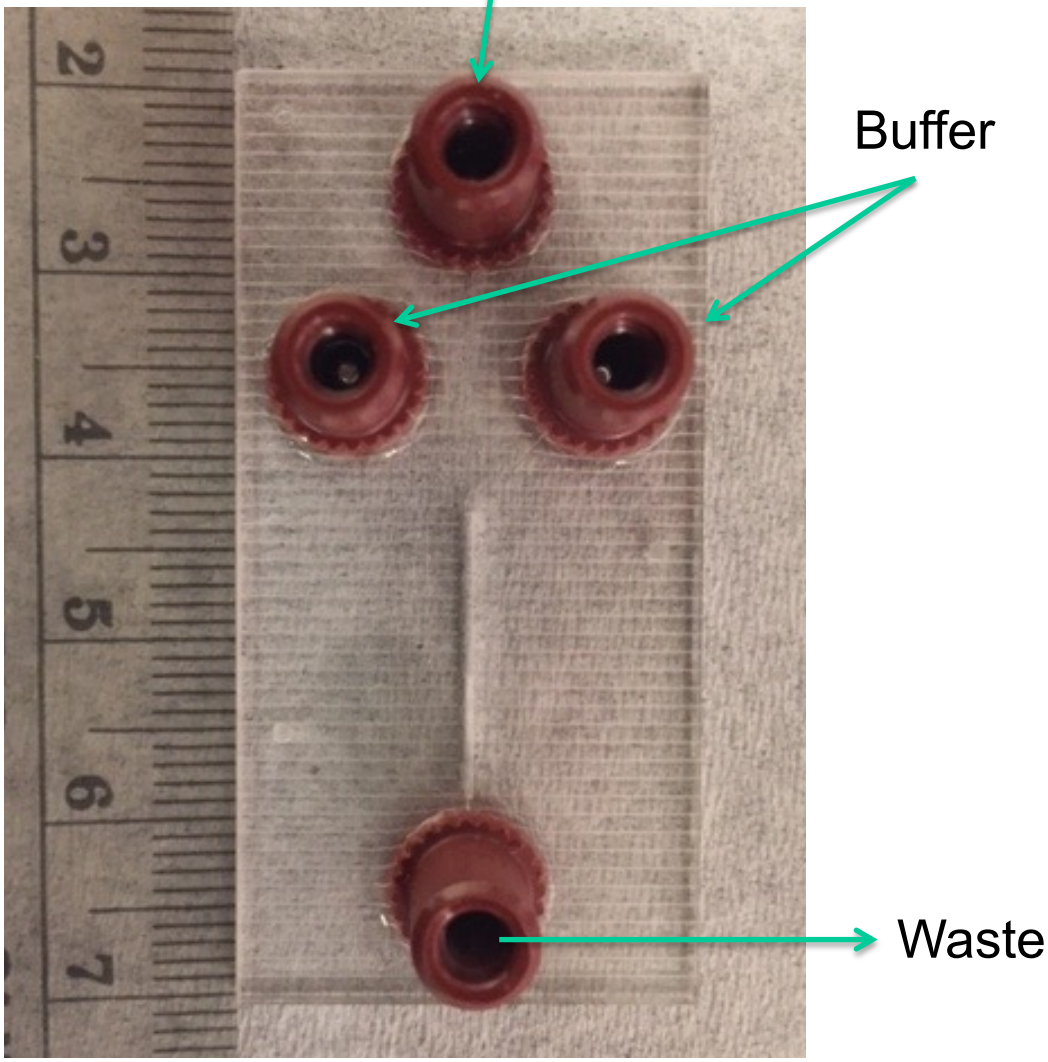


Chaotic flow mixing

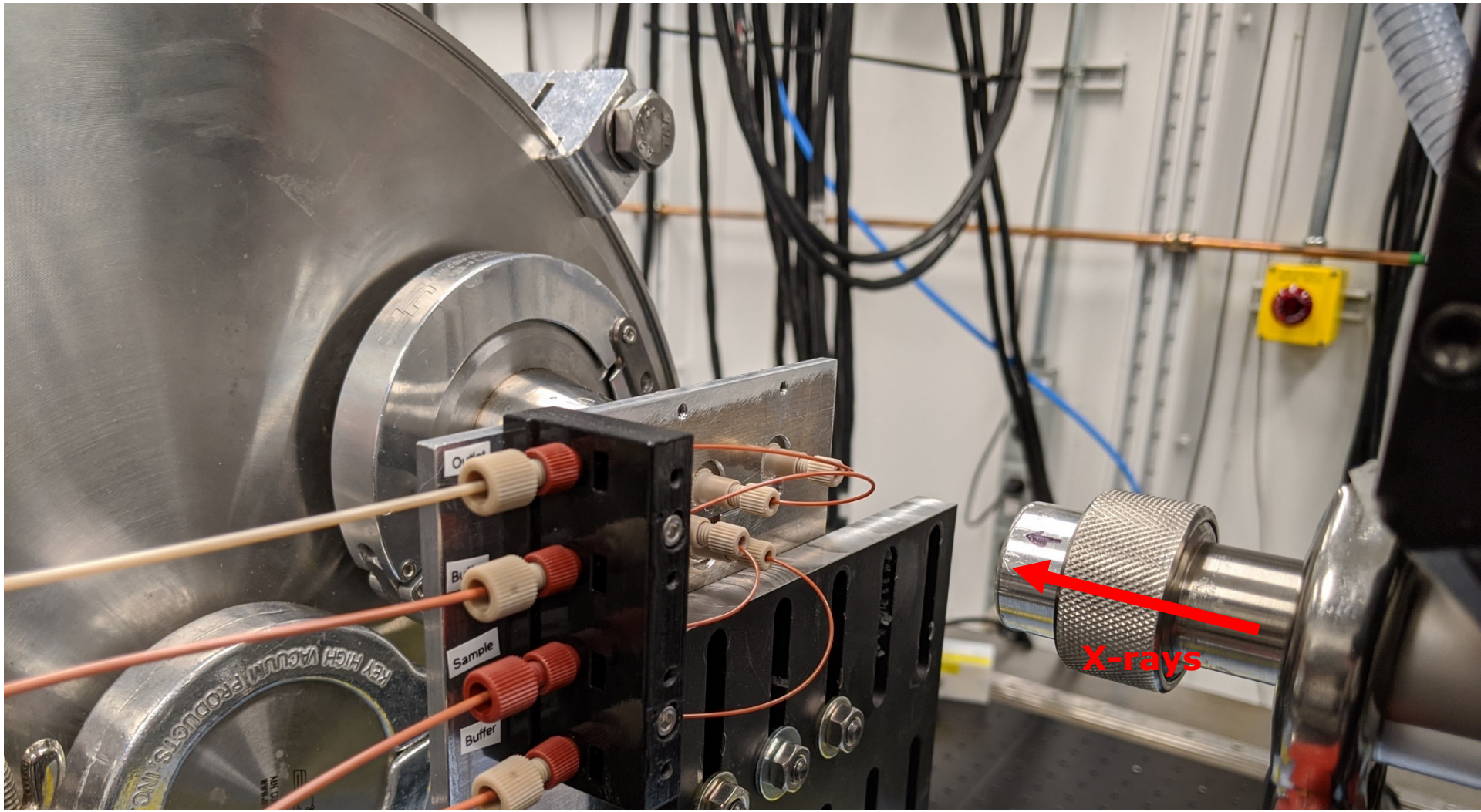
- Mixer design and development in collaboration with Matthews group at U. Mass (Osman Bilsel)
  - Ongoing project for ~10 years
  - Current designs achieve lowest time points, use least sample
- Design guided by CFD
- Fabricated in quartz to withstand pressures and intense microfocus x-ray beam (Translume)
- ~80 µs to 75 ms



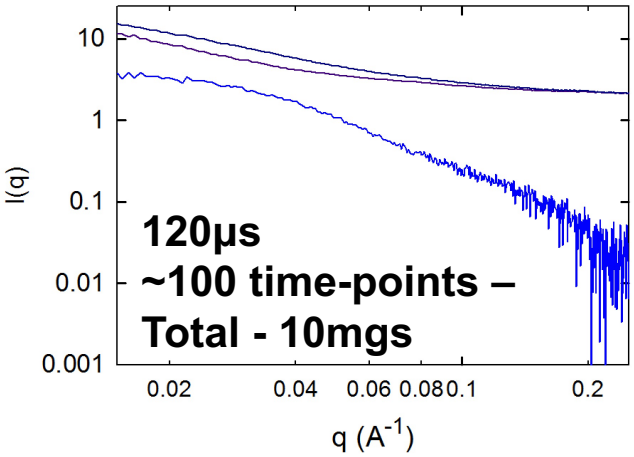
Chaotic flow mixing

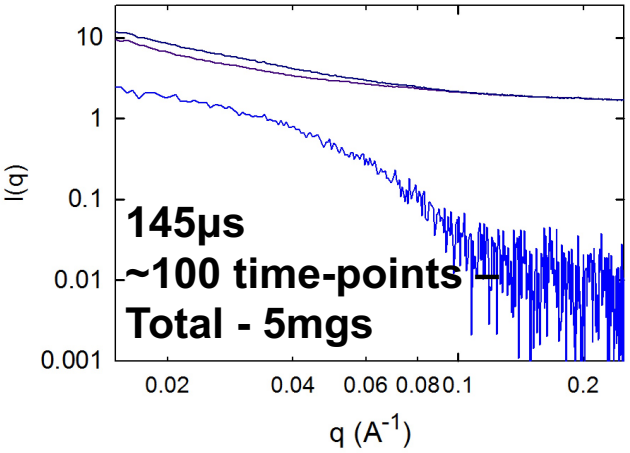


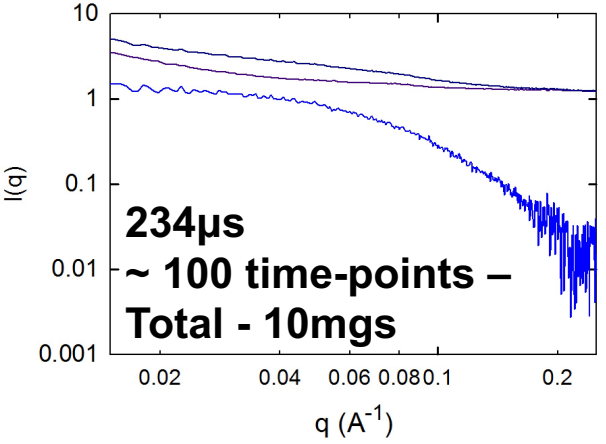
Chaotic flow mixing



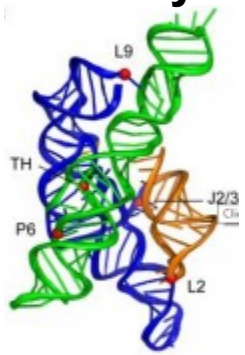
Chaotic flow mixing



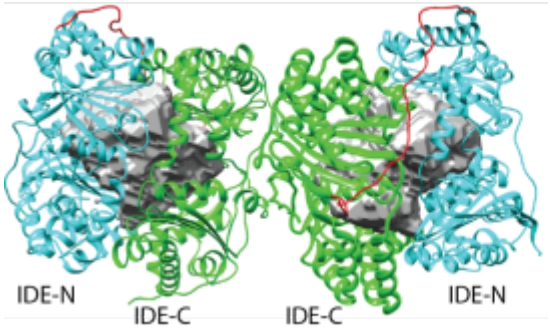




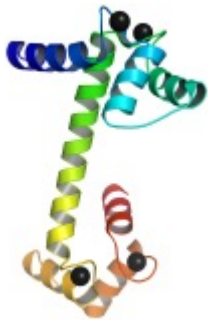
Ribozyme



IDE dimer

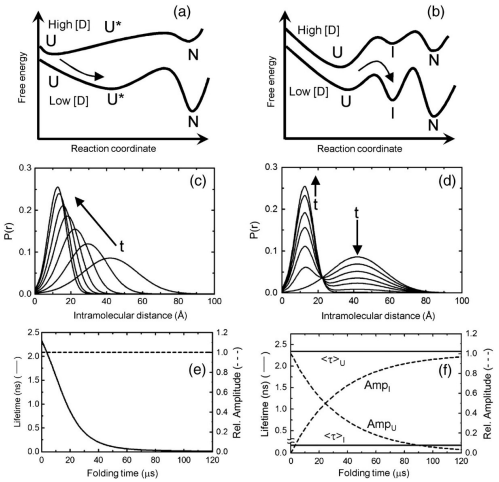


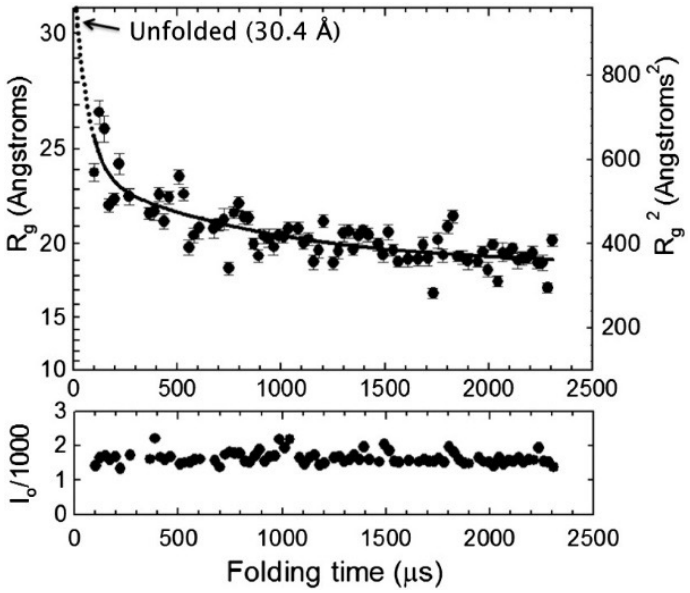
Calmodulin



TR-SAXS – Example 1

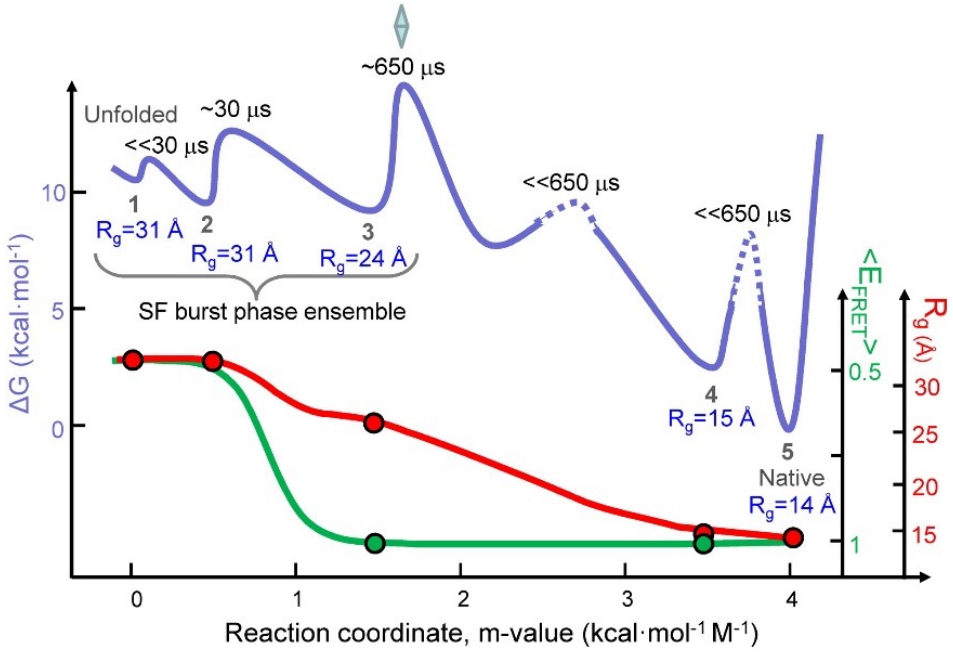
- Refolding experiment using Cytochrome C
- Testing whether refolding collapse is continuous or barrier limited
- Combined SAXS and FRET to test both global and local dynamics during refolding
- Developed a model for barrier-limited collapse based on changes in Rg in both equilibrium unfolding and time resolved SAXS data



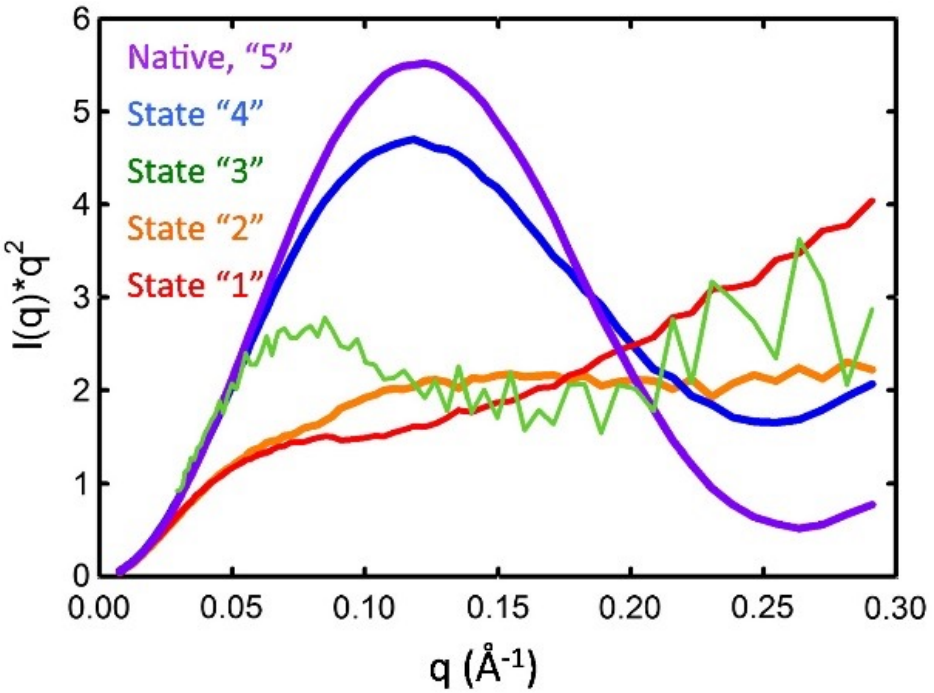


Microsecond Barrier-Limited Chain Collapse Observed by Time-Resolved FRET and SAXS. Kathuria et al., JMB. 2014 May 1;426(9):1980-94.

TR-SAXS – Example 1

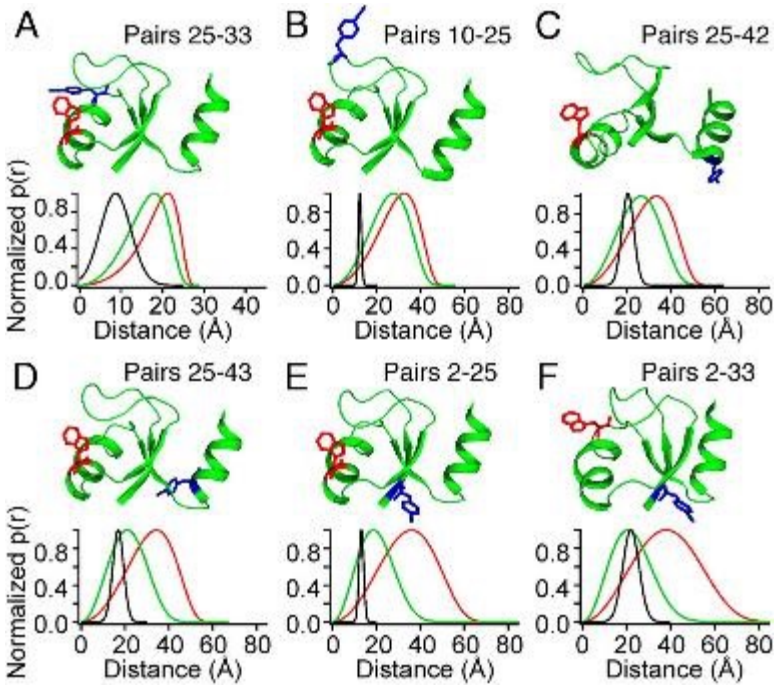


Kinetic model for cytochrome c folding.



Kratky plots of kinetic and equilibrium species. Shows intermediate state.

TR-SAXS – Example 2

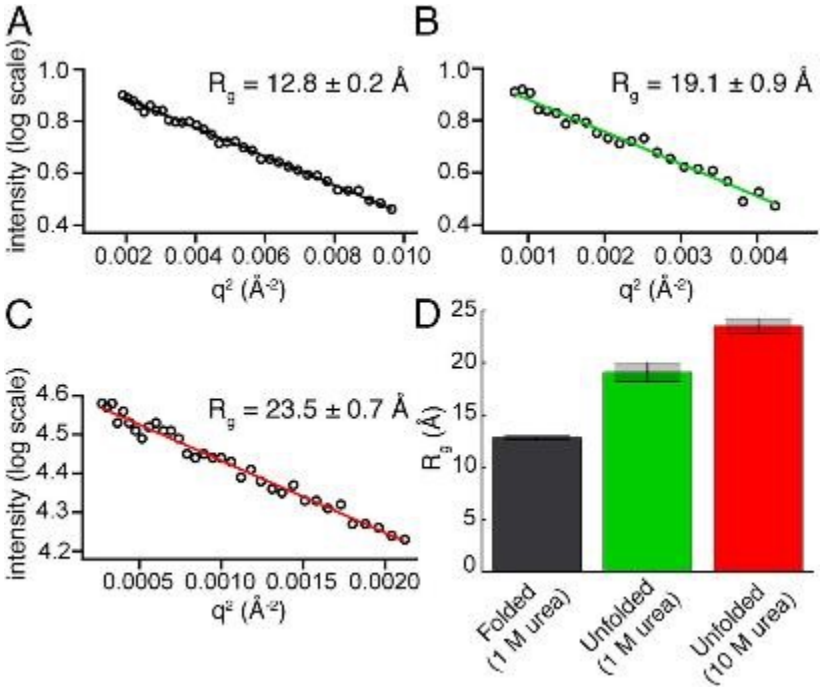


(*A–F*) FRET provides evidence for compaction. Ribbon diagrams illustrating the location of the FRET pairs are shown together with the distance distributions. Red, unfolded state in 10 M urea; green, unfolded state in 1 M urea; black, folded state in 1 M urea.

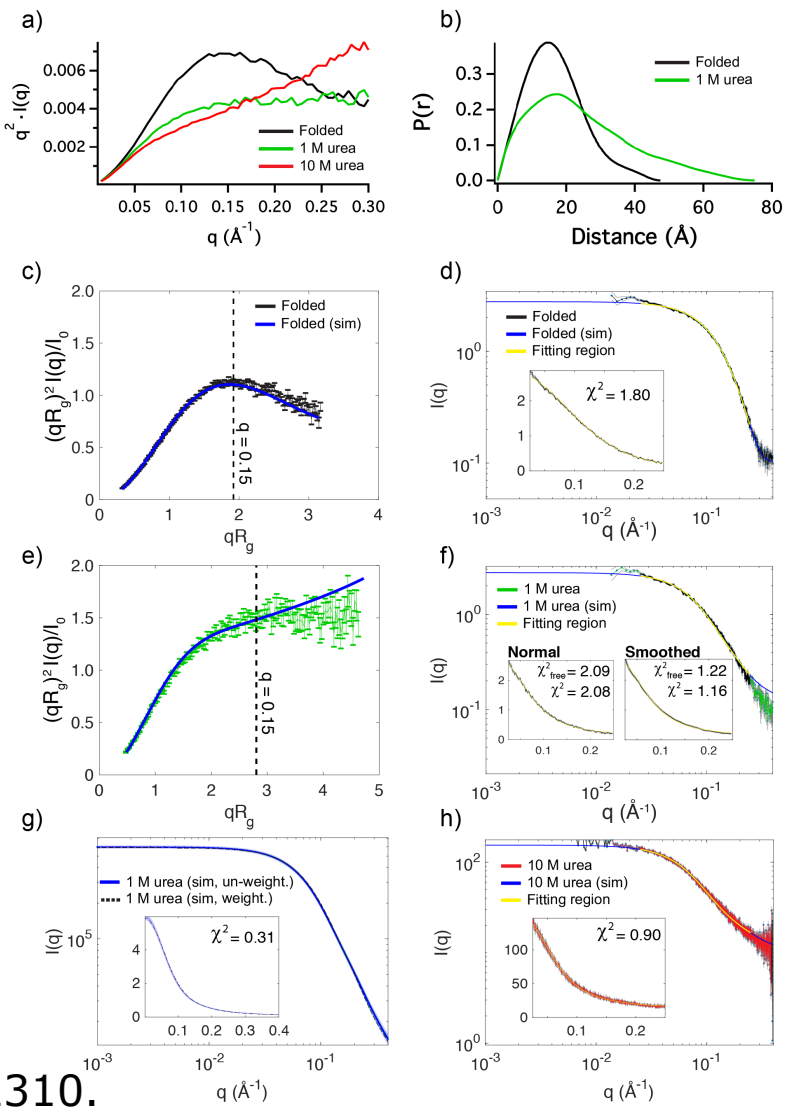
- Studying refolding in NTL9
- Using FRET and SAXS show that there is an initial fast contraction, followed by slower folding
- FRET combined with MD shows initial contracted state has specific structural preferences
- SAXS shows that the overall dimensions are consistent with a random coil, due to ensemble averaged conformational fluctuations

Peran et al. *PNAS*, 2019 Jun 18;116(25):12301-12310.

TR-SAXS – Example 2



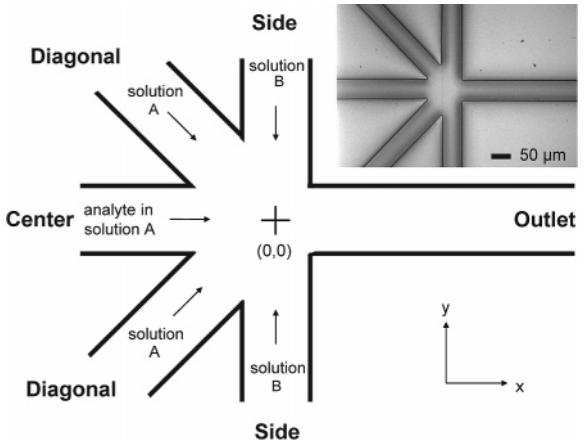
Guinier analysis of SAXS data. (*A*) Continuous-flow data for the native state in 1 M urea. (*B*) the unfolded state in 1 M urea. (*C*) Equilibrium data for the unfolded state in 10 M urea. (*D*) Comparison of average radii of gyration across the folded and unfolded states. Error bars are those calculated from the Guinier fits.



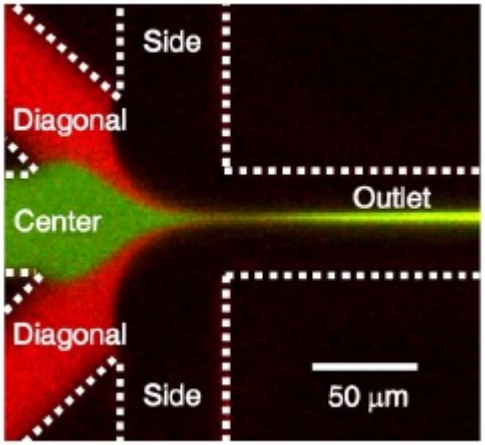
Peran et al. *PNAS*, 2019 Jun 18;116(25):12301-12310.

Laminar flow mixing

- Mixer development with Arleth group (U. Copenhagen), based on design from Pollack group (Cornell)
- Simulated using CFD
- Fabricated in quartz (Translume)
- ~1 ms to 1.5 s

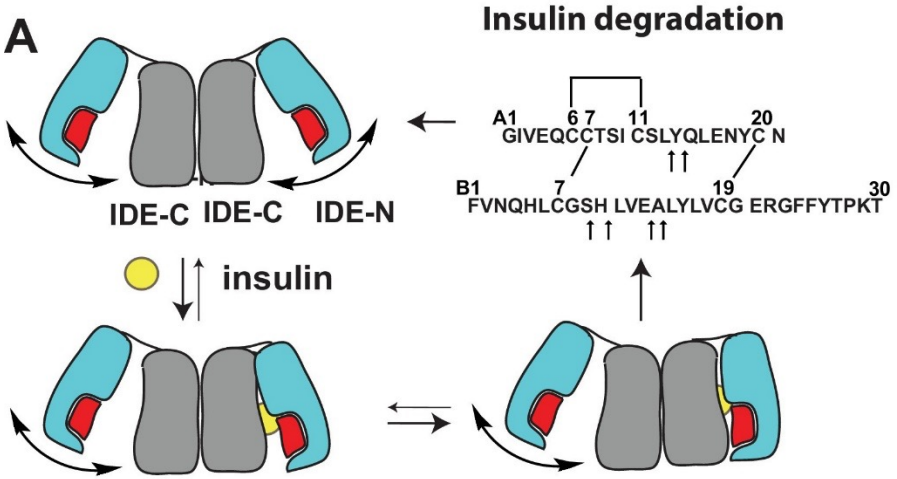


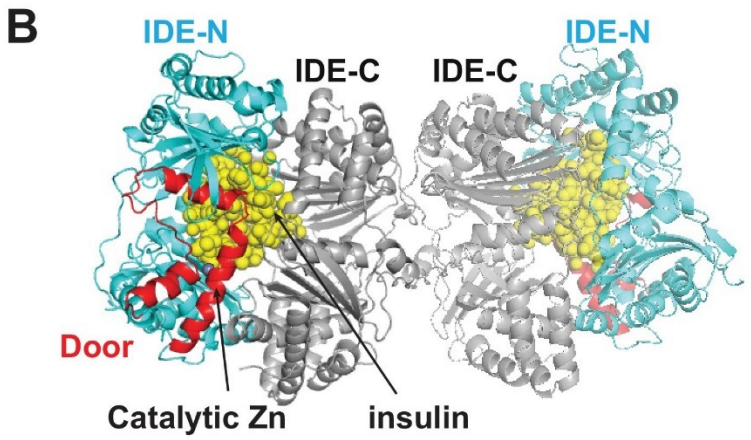
Park et al. Anal. Chem. 2006



TR-SAXS – Example 3

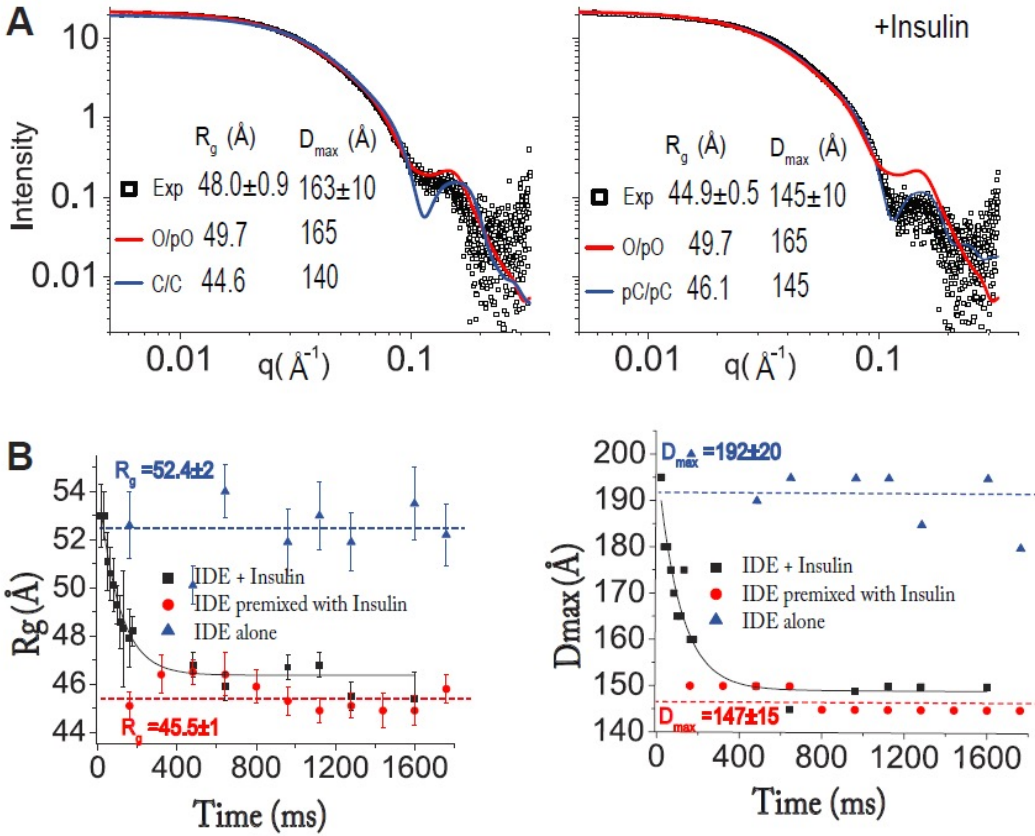
- Studying mechanism of insulin capture and degradation in insulin degrading enzyme (IDE)
- Important process for type two diabetes and Alzheimer's disease
- Used cryoEM and x-ray crystallography to solve high resolution structures of insulin bound (closed) and unbound (open) states
- Used equilibrium SAXS to determine which states were present in solution
- Used time resolved SAXS to determine that open-close transition is rate limiting for insulin degredation

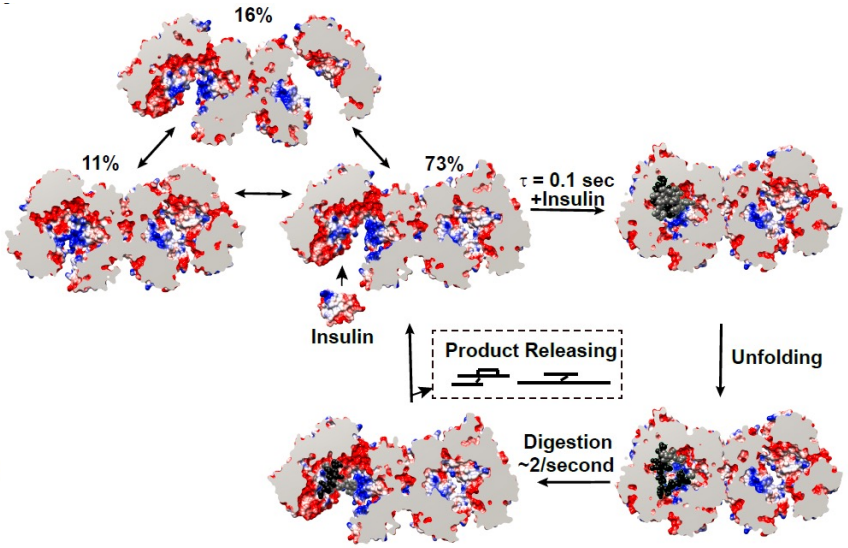




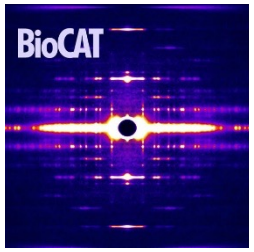
Zhang et al. *Elife* 2018 Mar 29;7. pii: e33572.

TR-SAXS – Example 3





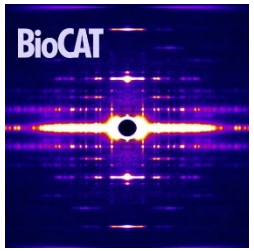
Zhang et al. *Elife* 2018 Mar 29;7. pii: e33572. doi: 10.7554/eLife.33572.



Time resolved SAXS at BioCAT

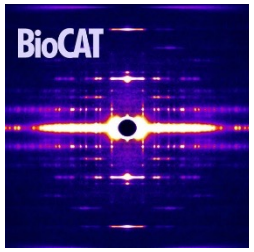
- Chaotic flow mixing
  - Provides fastest timescales (~80 μs to 75 ms)
  - Uniform mixing
  - Larger sample consumption (~10-100 mg per time series)

- Laminar flow mixing
  - Slower timescales (~1 ms to 1.5 s)
  - Non-uniform mixing (concentration of protein and reactant varies with time)
  - Low sample consumption (~1-10 mg per time series)



Doing TR-SAXS at BioCAT

- Talk to beamline scientists before arriving to plan experiments
  - Jesse Hopkins: jhopkins1@iit.edu
  - Max Watkins: mwatkins2@iit.edu
- TR-SAXS users first must do equilibrium SAXS measurements at BioCAT
  - Check sample quality (must be good enough for batch mode!)
  - Determine end points
  - Is expected change happening, visible with SAXS?
- Ideally other preliminary experiments to determine relevant timescales
  - Binding/off rates
  - FRET
  - Time resolved CD
  - Etc . . .
- Plan for at least 10 mg (laminar flow) or 100 mg (chaotic flow) of sample for first experiment
  - Less maybe possible depending on size of macromolecule, and time range of interest



Summary

- TR-SAXS (or WAXS) possible on timescales from <100 fs to days
- Pick appropriate initiation technique for timescale, desired reaction
- Most common (at synchrotrons) is mixing reactions
  - Stopped flow
  - Continuous flow
- BioCAT provides time ranges from ~80 μs to 1.5 s via continuous flow mixing, > 1 ms via stopped flow mixing