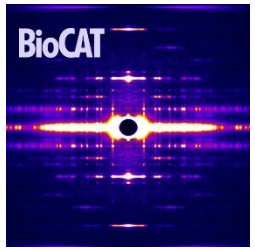


# Upgraded and new capabilities for solution SAXS

Jan. 10, 2025

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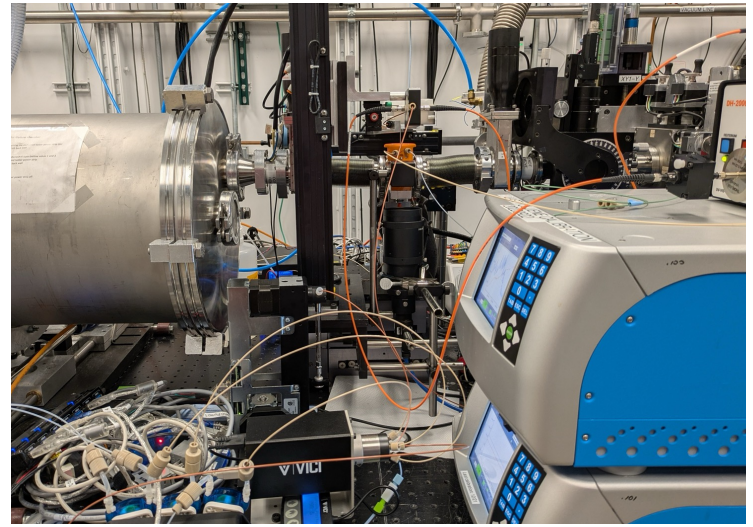
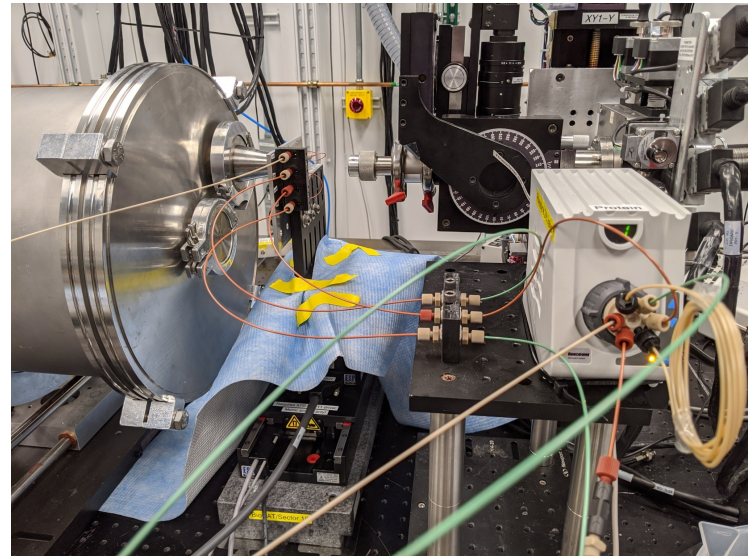
# Overview of solution SAXS capabilities

- BioCAT supports both equilibrium and time-resolved SAXS experiments

- Equilibrium SAXS
  - Chromatography-coupled
    - Size-exclusion chromatography, with a wide range of columns available
    - Ion exchange (IEX) also available (columns available)
  - Asymmetric field flow field fractionation (AF4)-coupled
    - Column-free, tunable separation (range of channels/membranes available)
  - MALS, DLS, dRI and full-spectrum UV-Vis available for all separation-based modes
  - Full temperature control from ~4-40 degrees for chromatography and batch
  - Low-volume batch mode also available (with UV-Vis)
  - Typical $q$-range ~0.0027-0.45 $Å^{-1}$

- Time-resolved SAXS
  - Microfluidic mixtures support time ranges from ~45 $\mu$s to ~1.5 s
  - Typical $q$-range ~0.01-0.65 $Å^{-1}$



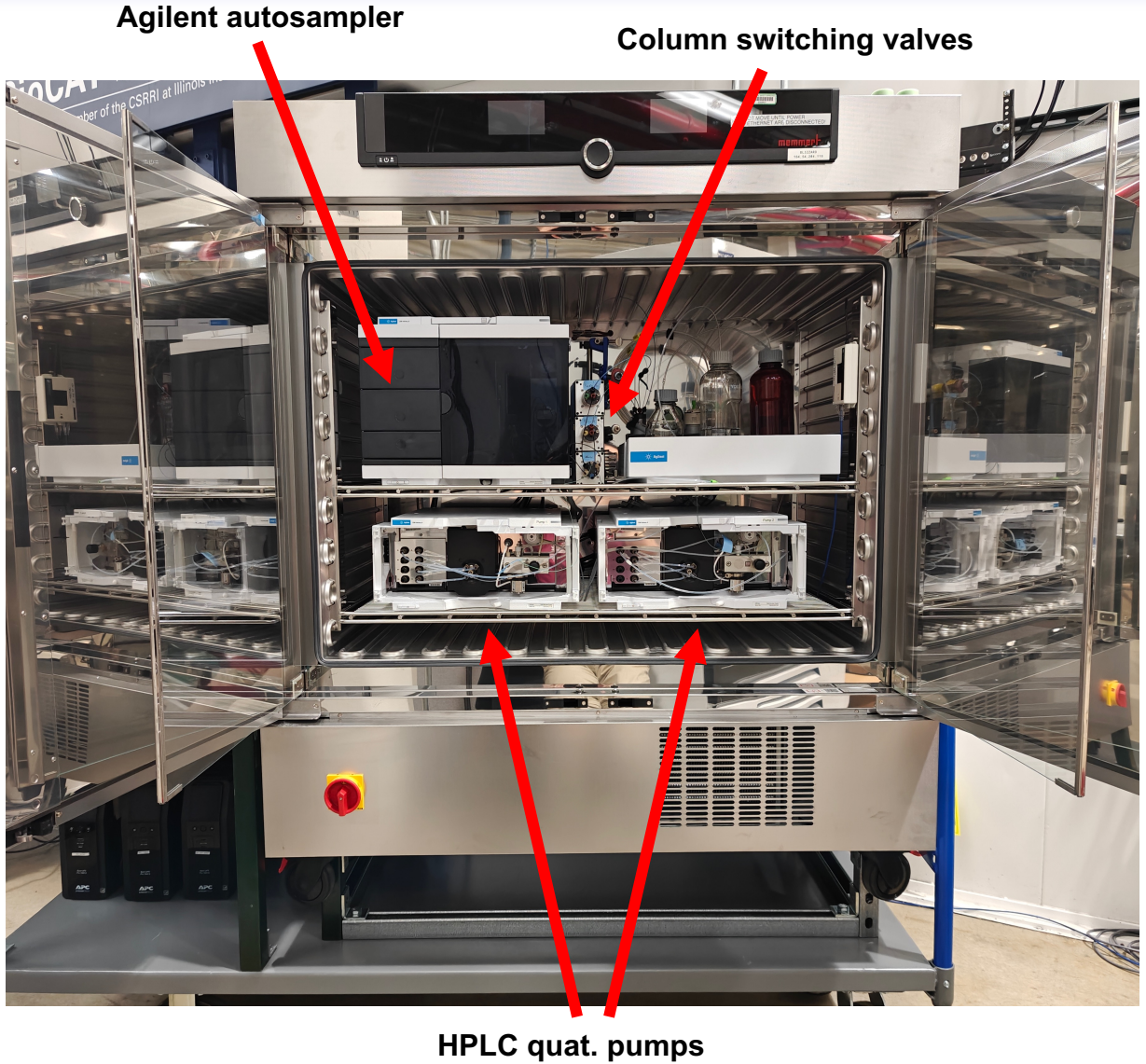


# New capabilities for SEC-MALS-SAXS

- Old systems (AKTA Pure for SEC/IEX-SAXS, 2x Agilent 1260's for SEC-MALS-SAXS) have been retired from routine use

- All chromatography-coupled experiments going forward will use a new, custom, fully temperature-controlled HPLC system
  - Bio-Inert Agilent 1260 Infinity II Pumps and autosampler, custom plumbed with HPLC valves
  - Support for automated buffer and column switching (2 columns, 10 buffers/column)
  - Simultaneous running and equilibration on the same system, with single-button flow path switching
  - Full system temperature control from 4-40 degrees C (entire system housed in Memmert incubator)

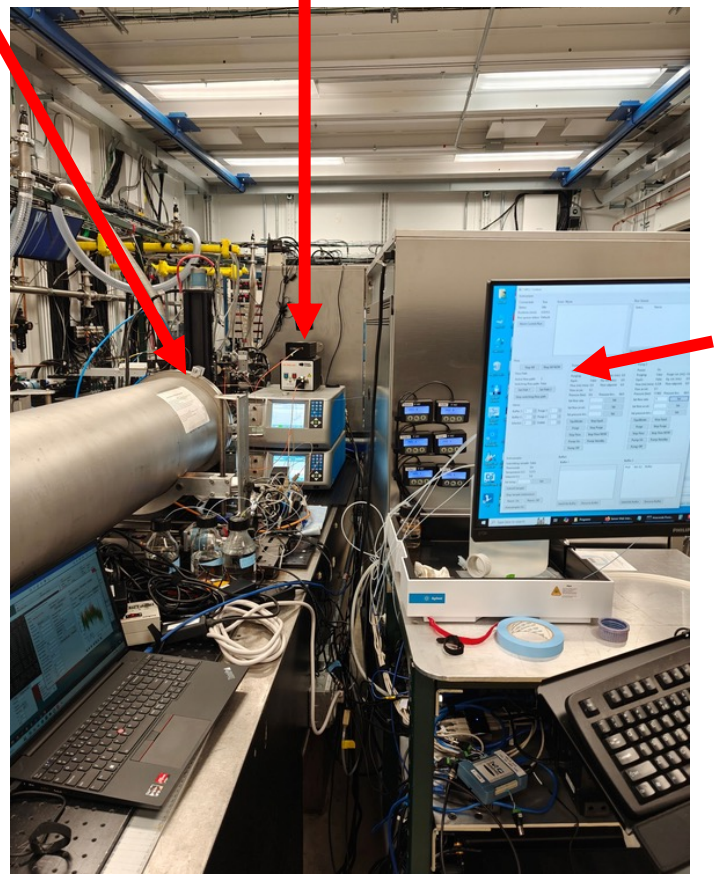
- HPLC control fully integrated into BioCon beamline control software (no need for users to interface with Agilent software)



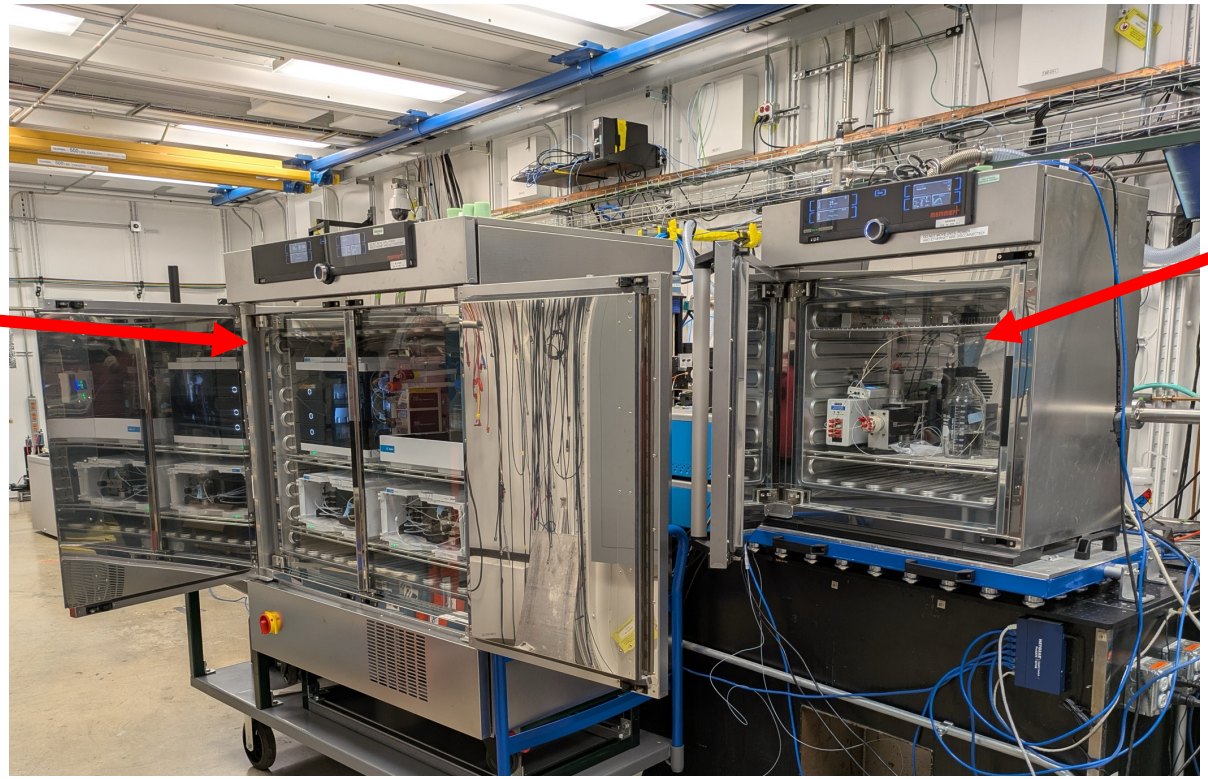
# SEC-MALS-SAXS at the beamline

MALS, RI & UV-Vis detectors

Sample cell

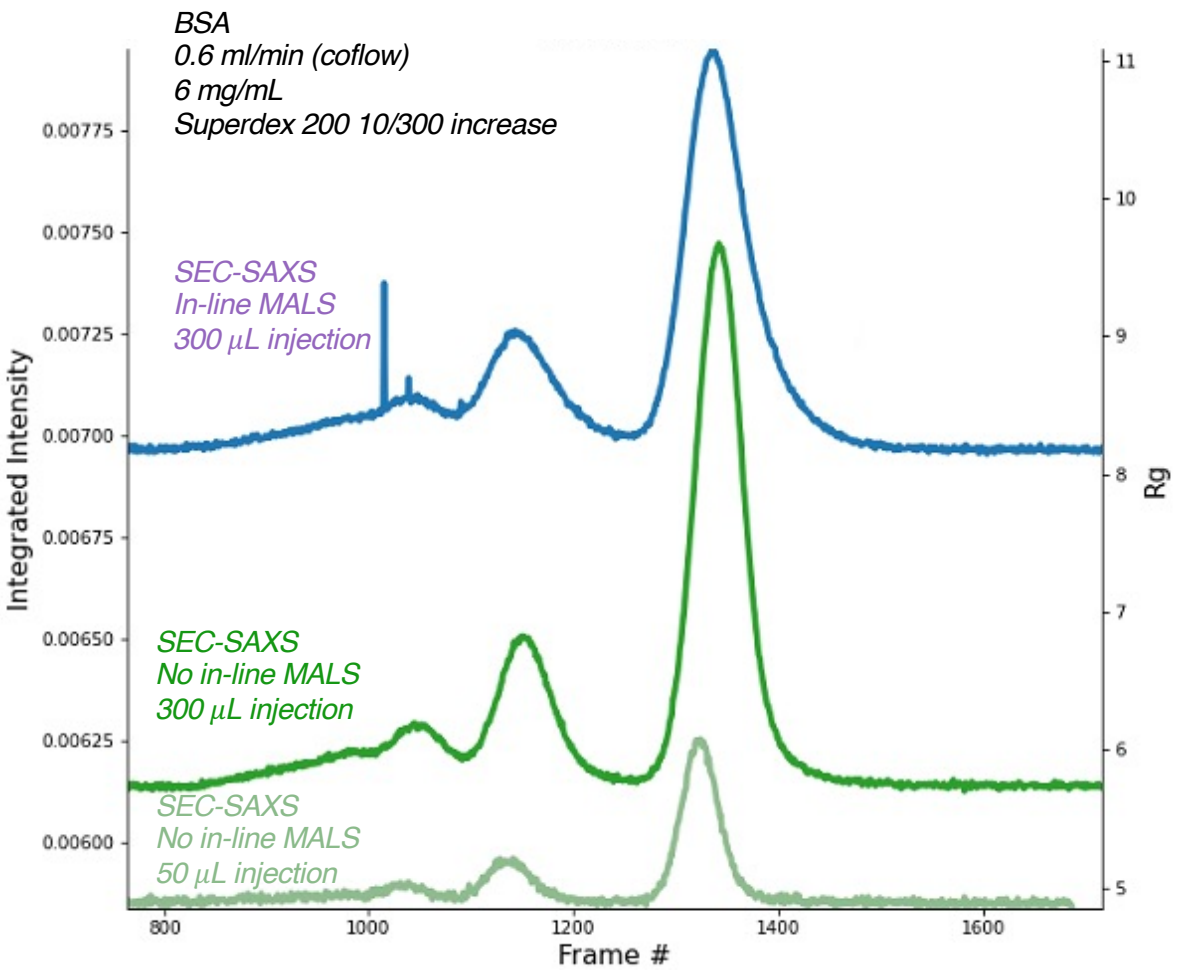


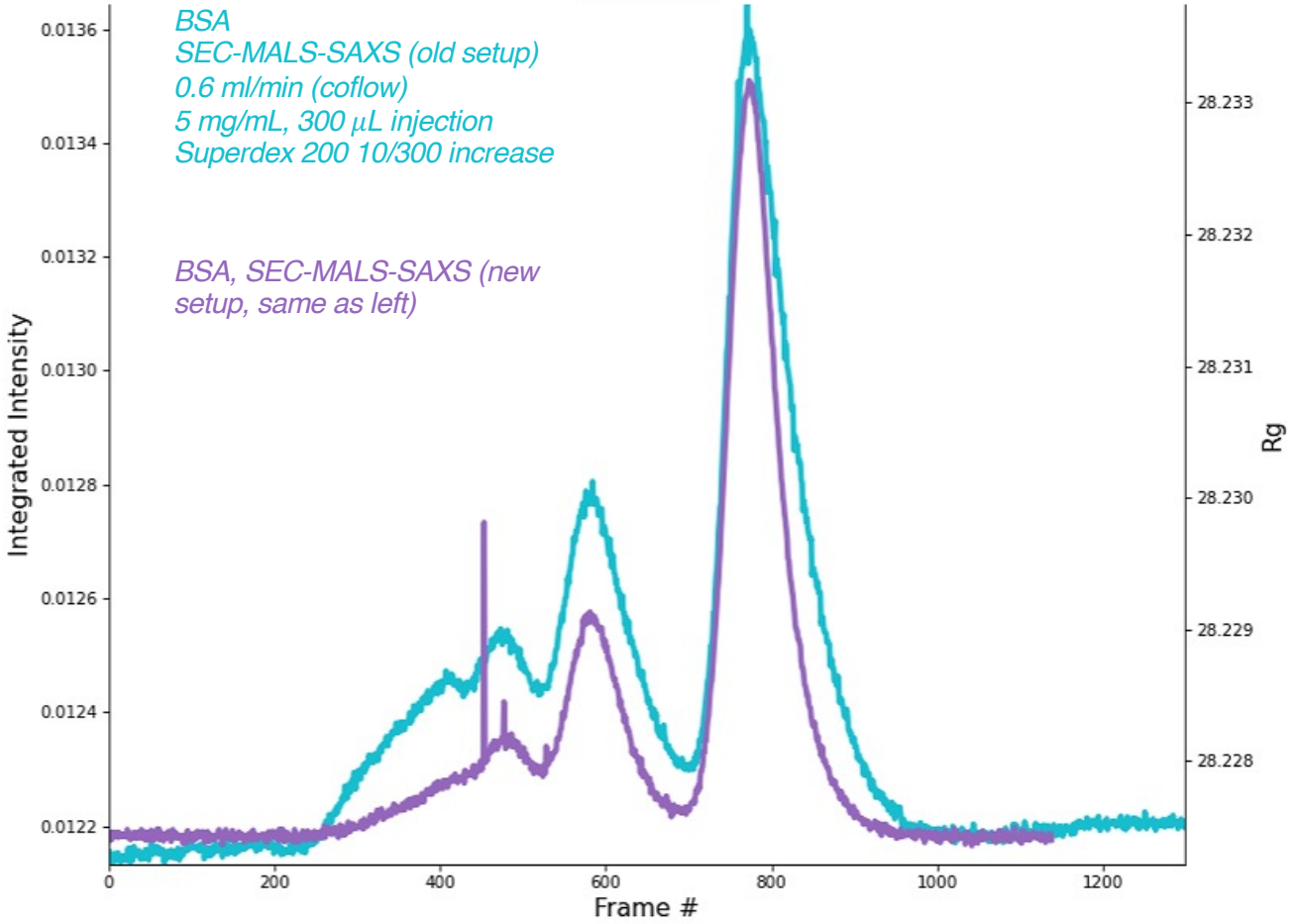
SEC instrument



Coflow fluidics

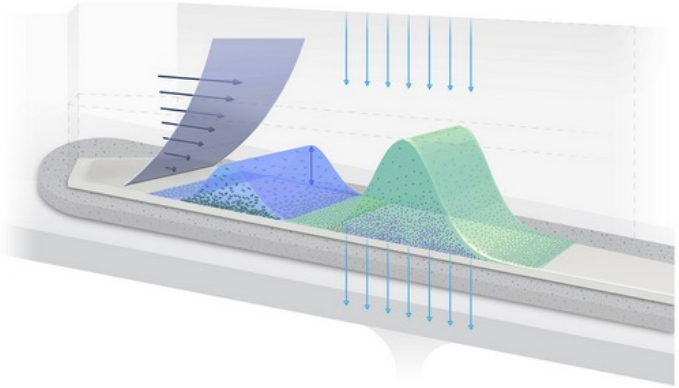
# SEC mode SAXS data



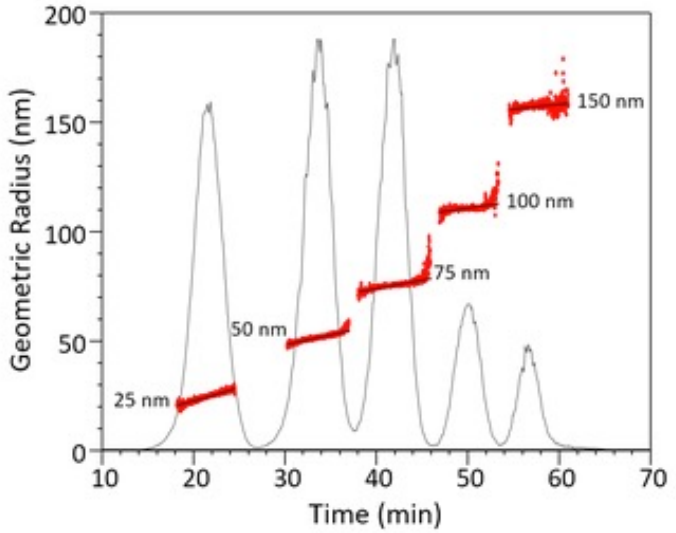



# New capability: AF4-MALS-SAXS

- Asymmetric Field Flow Field Fractionation (AF4) is a column-free separation technique that relies on a parabolic flow profile and diffusion to separate molecules by size
  - No stationary phase, molecules do not experience shear



- Primary expected use cases are systems not amenable to column separation
  - Lipid nanoparticles (LNP's) are of particular interest, as they tend to fall apart on an SEC column.
  - Likely also useful for weak macromolecular complexes or systems with problematic column interactions



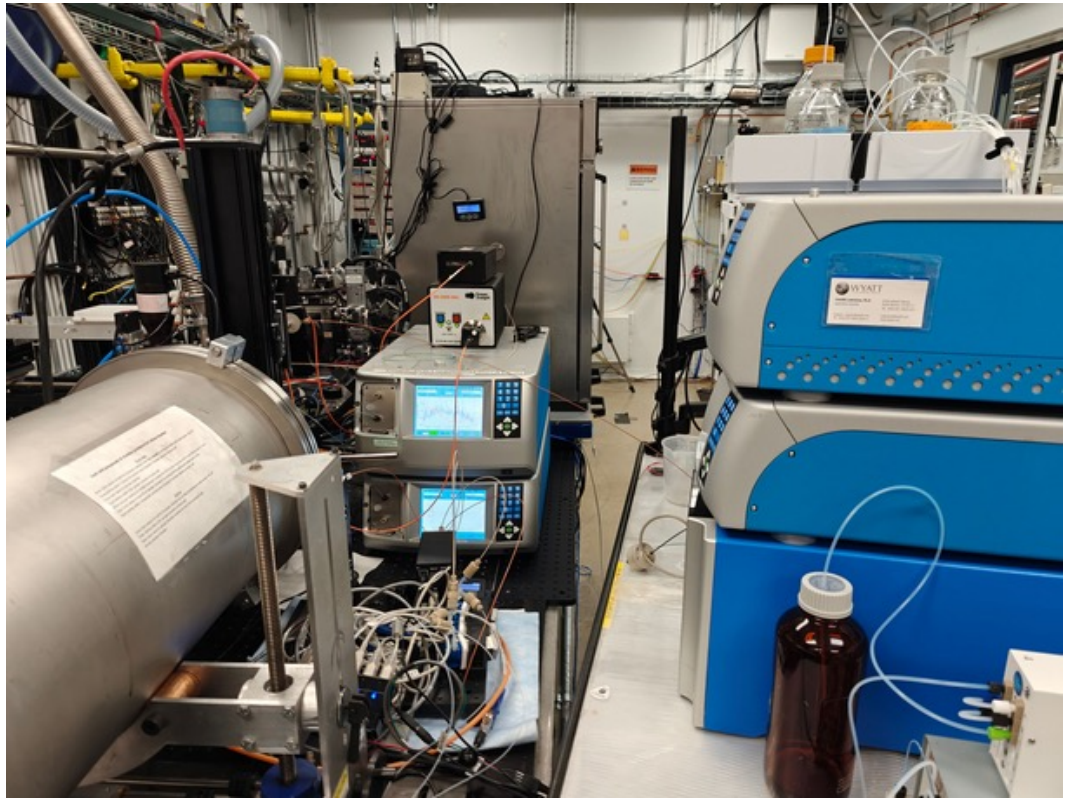

- Routinely available, but separation methods may require advance optimization

https://www.wyatt.com/solutions/techniques/fff-mals-characterization-of-nanoparticles-colloids-macromolecules.html

# New capability: AF4-MALS-SAXS

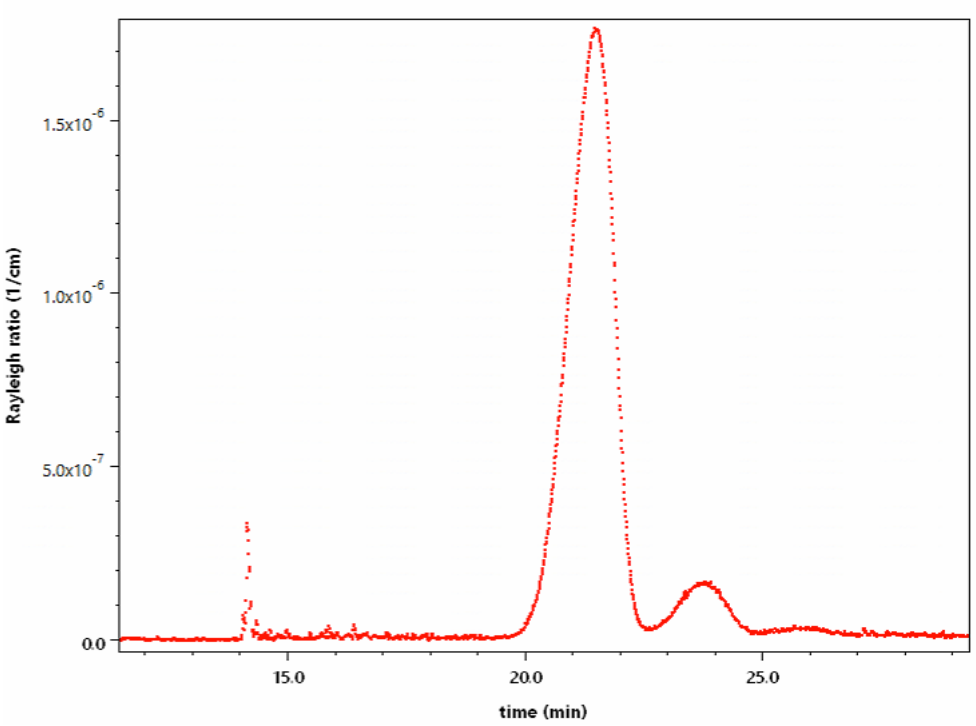
Wyatt Eclipse integrated into HPLC-SEC-MALS stack


Wyatt Eclipse



# New capability: FFF-MALS-SAXS

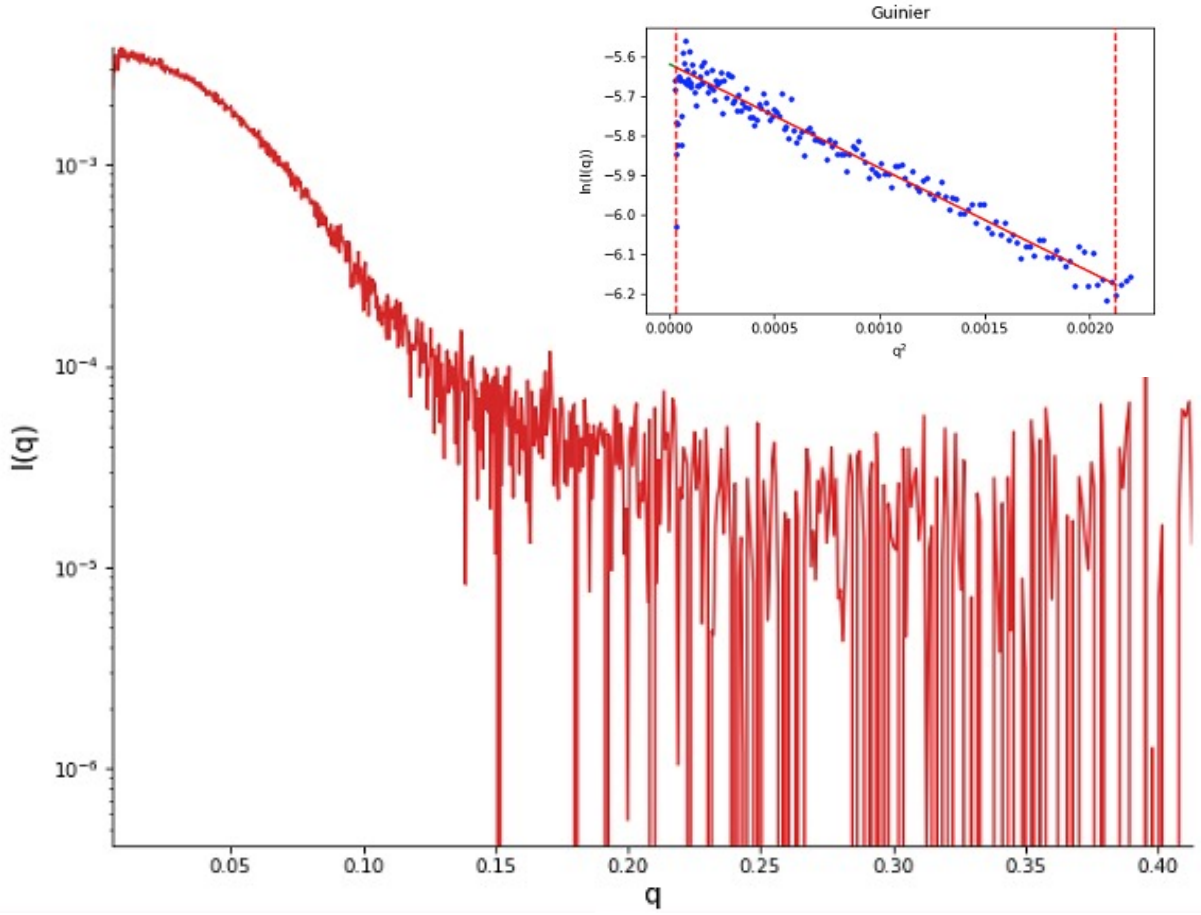
*BSA*
*Custom method, detectors/SAXS cell flow 0.4 ml/min (coflow)*
*6 mg/mL, 300 μL injection*
*Wyatt short channel (variable), 400 μm spacer*
*Regenerated Cellulose, 10 kDa MWCO*
*Dilution control module (DCM) 2.5x*




Note elution is reverse-phase with respect to SEC

Separated BSA monomer

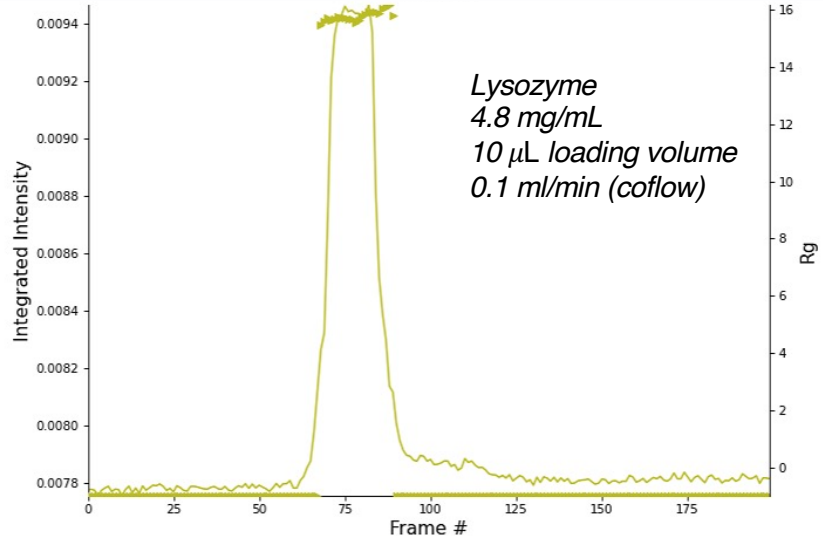
Resolution at SAXS cell still being optimized, but data quality is good

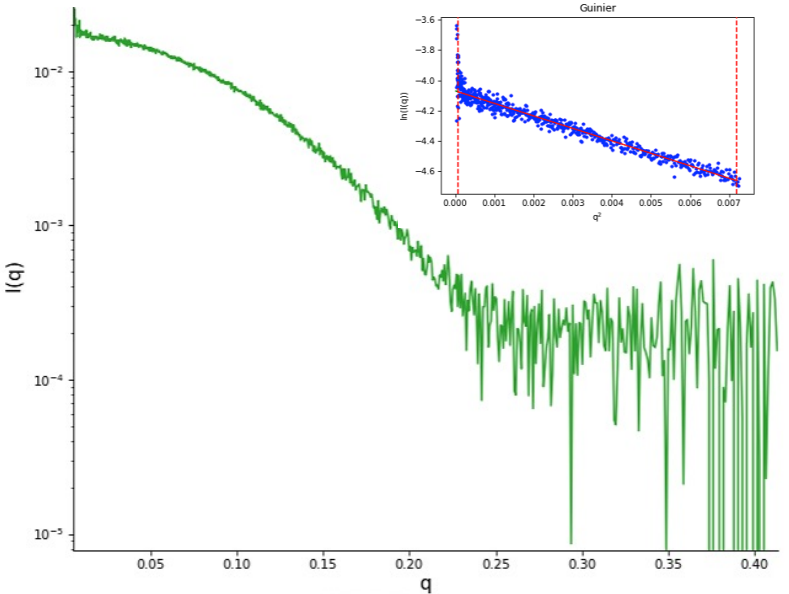


# New capabilities for batch mode SAXS

- Prior to APS-U, batch mode SAXS was manual injection

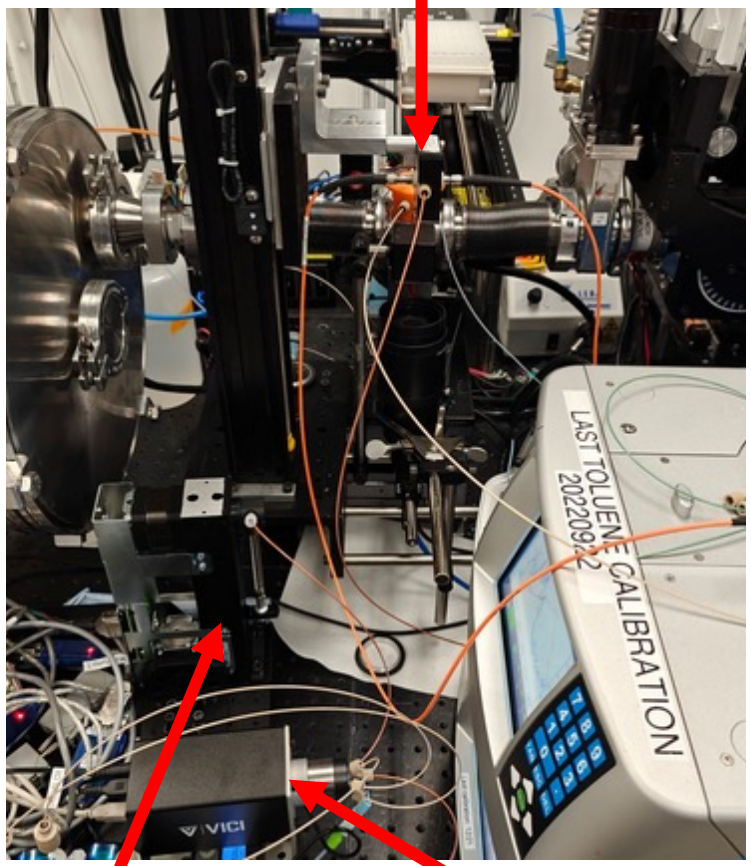
- Batch mode has been overhauled, now automatable via direct needle injection from a 96- or 384-well plate
  - Temperature control for plate and sample cell from 4-40 degrees C (or potentially higher)
  - Direct coflow needle injection allows for good data from volumes as low as 10 $\mu$L
  - A sample can be run in ~3 minutes (including needle cleaning) – full 96-well plate in under 5 hours



# Batch SAXS at the beamline

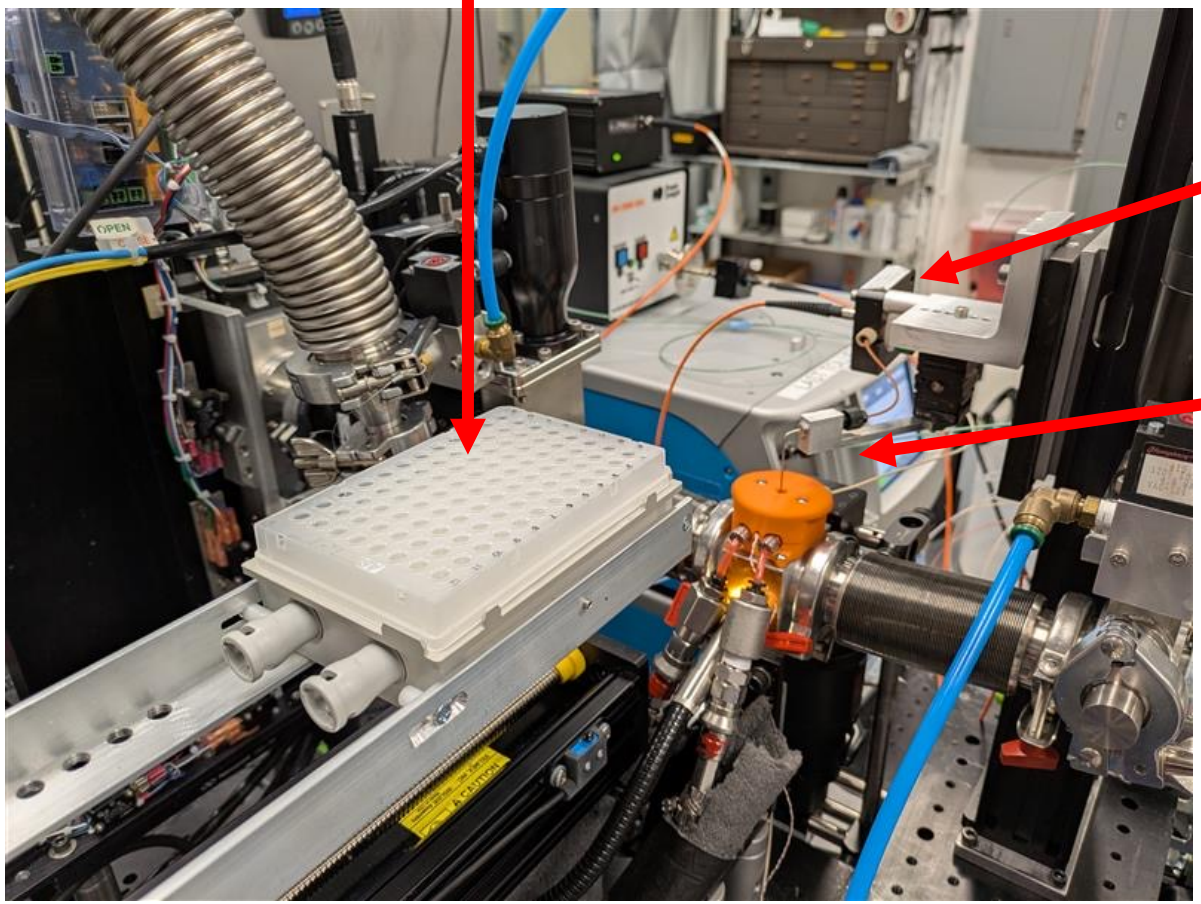
Coflow needle (injection position), on Z-axis linear stage




High precision syringe pump

Valve (for needle cleaning/injection switching)

96-well sample plate (with cooler, on X/Y axis linear stages)

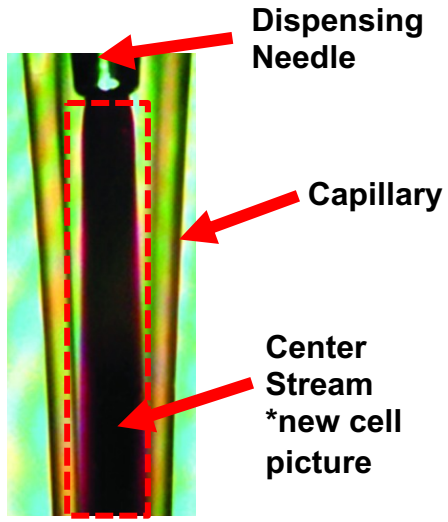


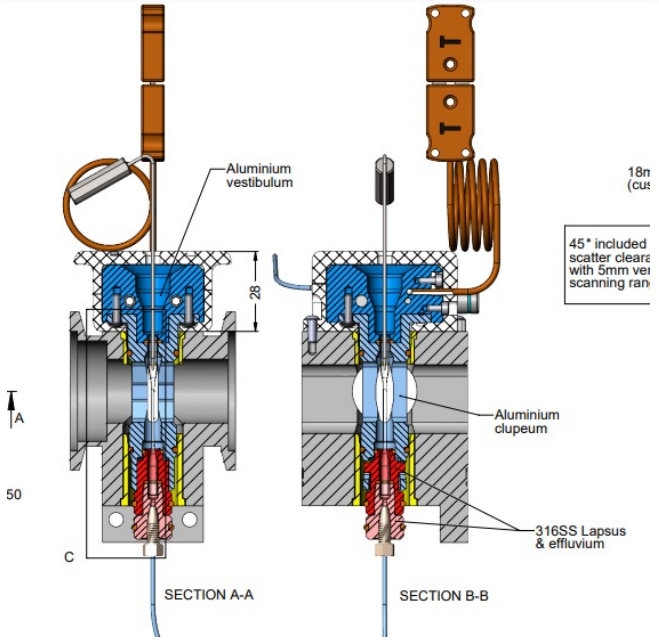
UV cell (measurement available before injection)

Coflow needle (injection position), on Z-axis motor

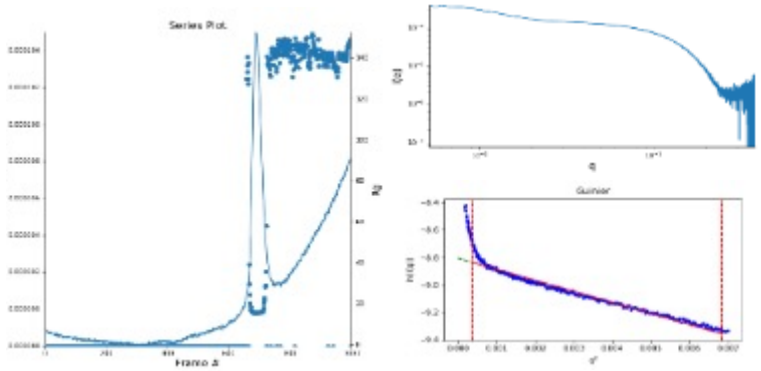
# Updates to other equilibrium systems

- Coflow cell and plumbing have been upgraded for increased flow stability and temperature control
  - New cell design, with improved temperature control (recirculating coolant and active temperature feedback via integrated thermocouple)
  - Inlet plumbing housed in similar incubator to SEC system (improved temperature control)

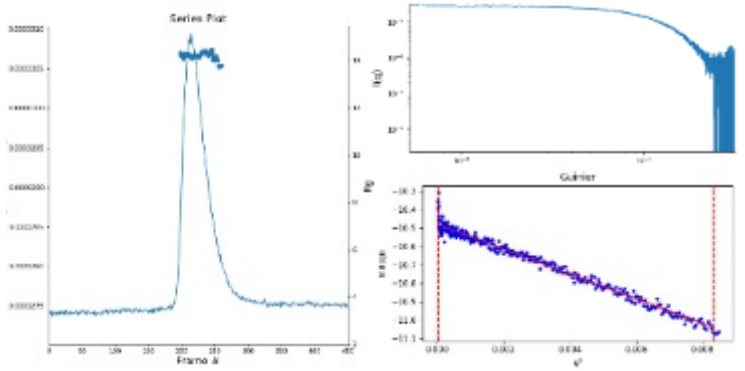



No Co-Flow



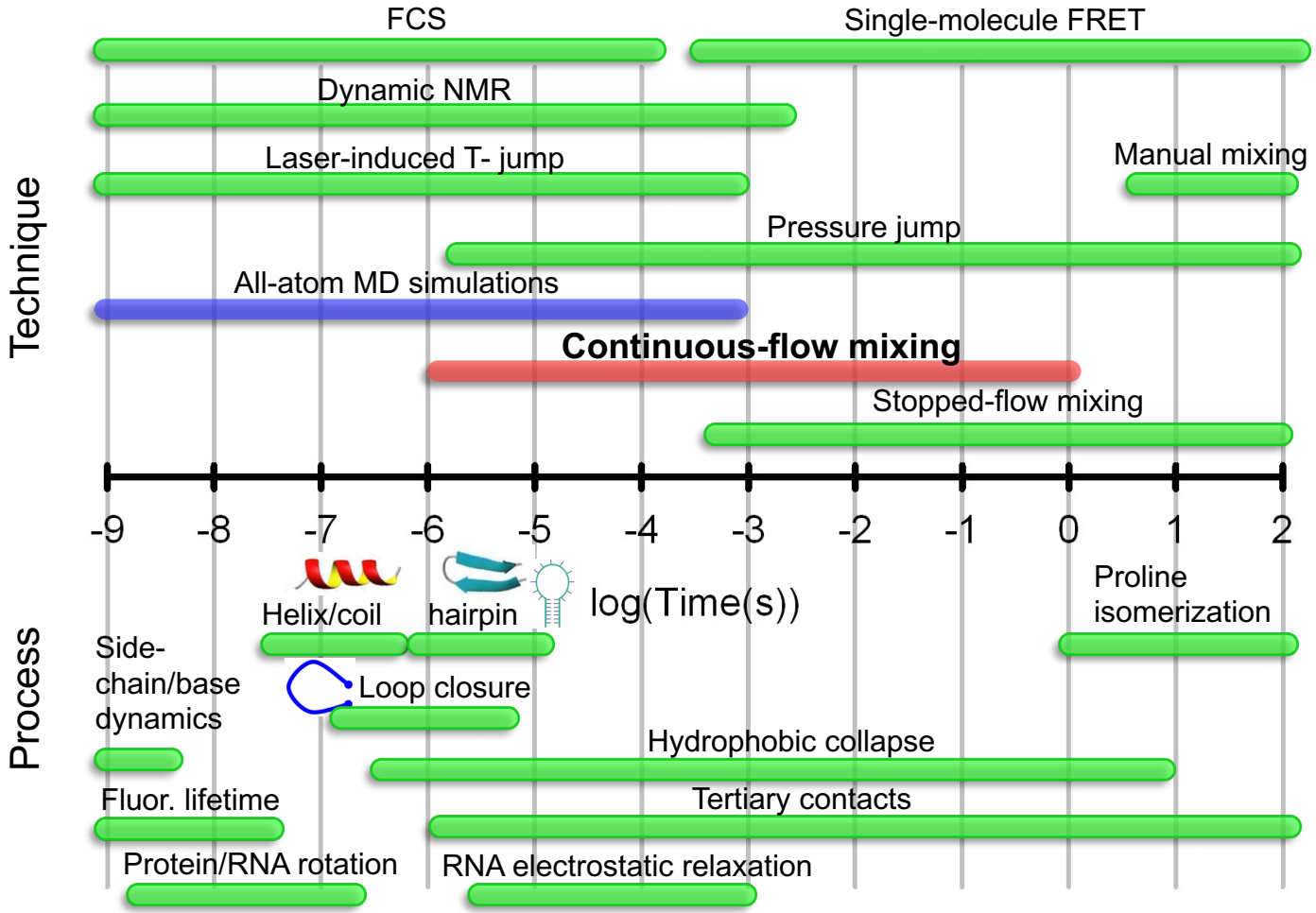
With Co-Flow



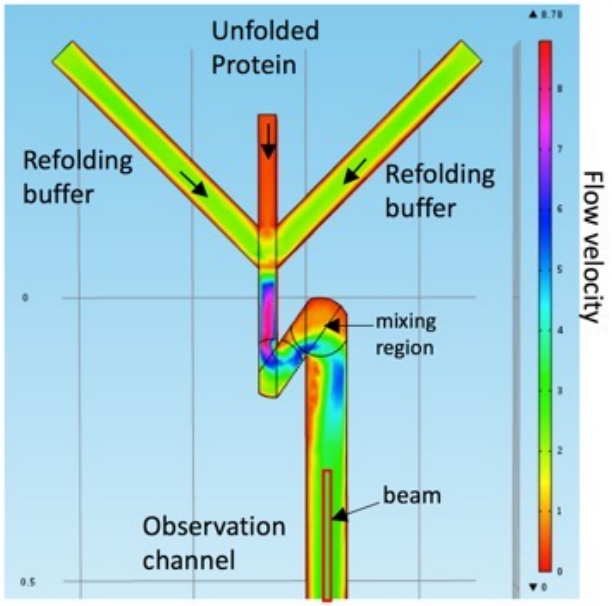
Kirby *et al.*, Acta Cryst. D. **72**:12 (2016)

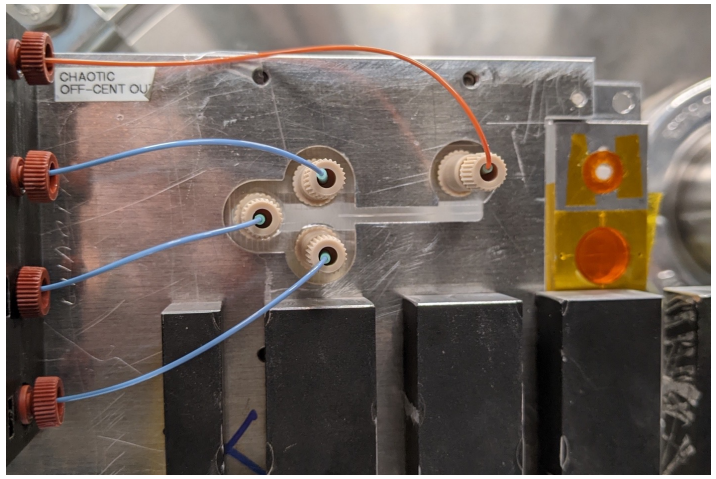
# Time-resolved SAXS

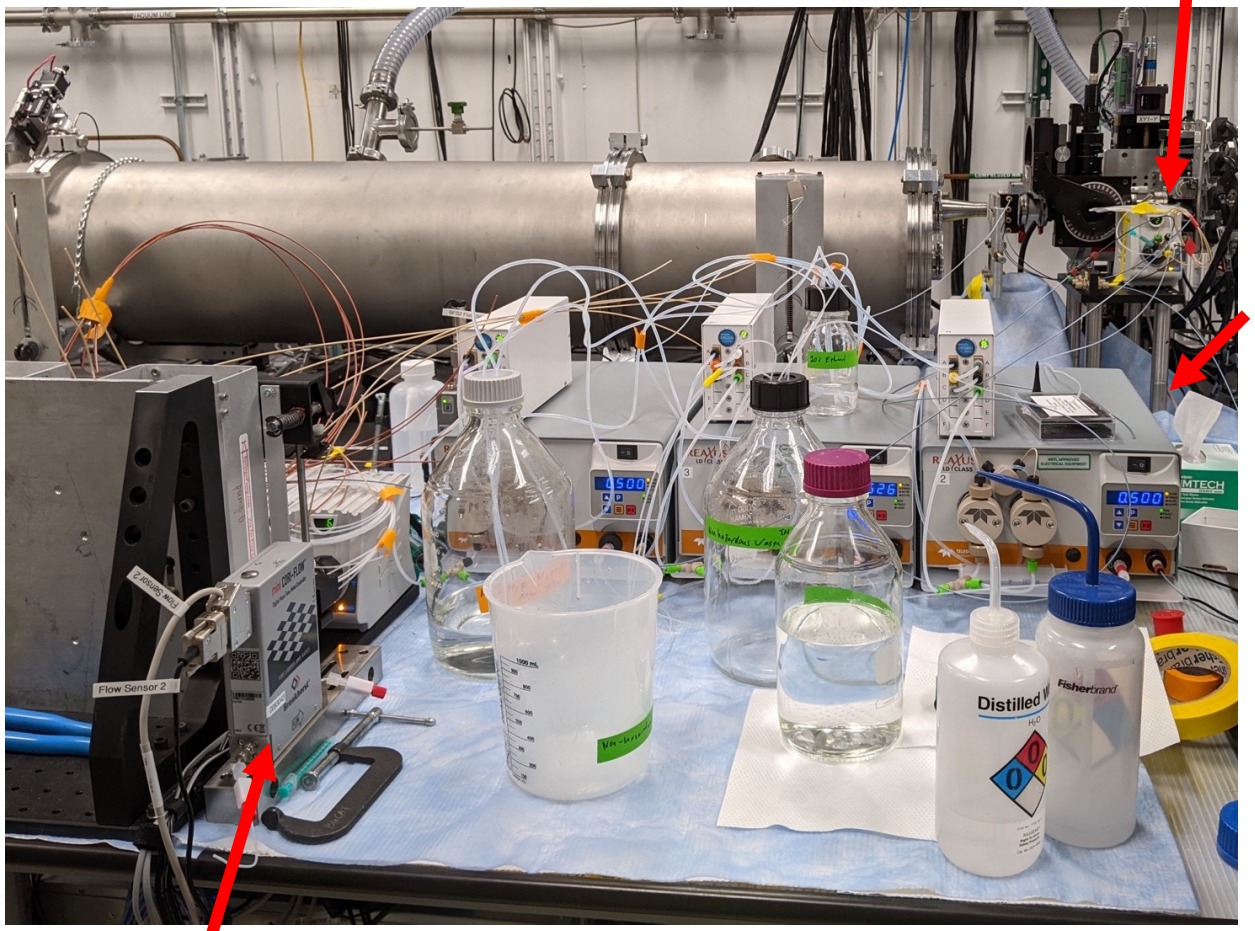
- Capabilities currently similar to pre-APS-U
  - 2 main continuous flow mixers: Chaotic flow mixer for time ranges from ~50-7500 $\mu$s and laminar flow mixers for time ranges ~1 to 1500 ms
  - Development of in-vacuum sample cell (this year), expected to substantially improve S/N
  - New optics (expected early 2026) combined with APS-U expected to significantly expand capabilities (lower $q$, faster minimum times) due to improved microbeam size/shape
- Dedicated RAW GUI currently in development, expected to significantly streamline data processing and analysis
- Time-resolved program is open to general users
  - Experiments often done in close collaboration with BioCAT staff



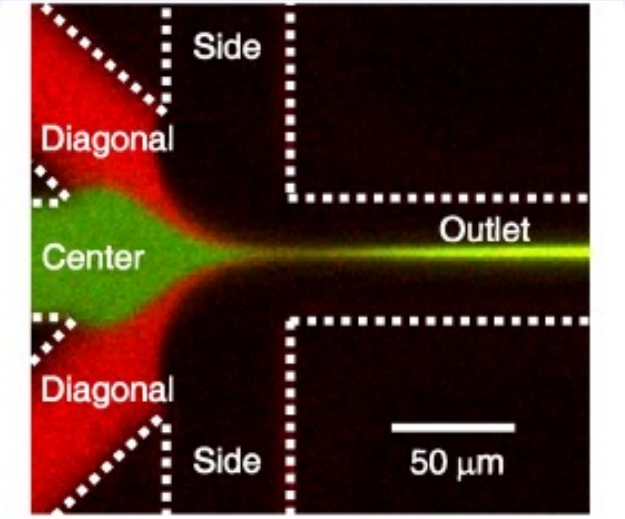
# TR-SAXS: chaotic mixer

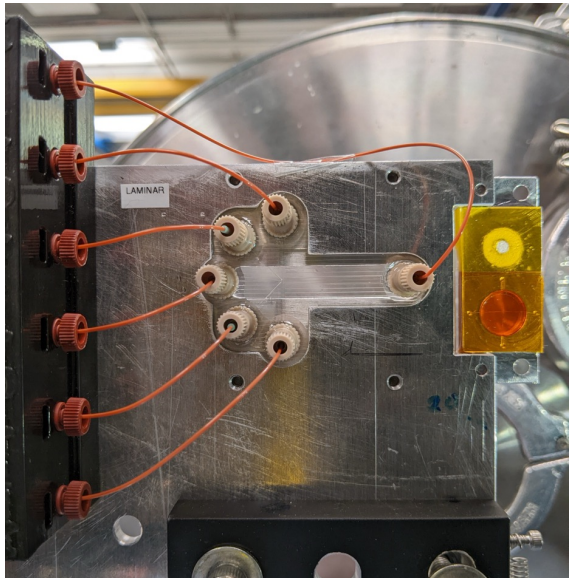


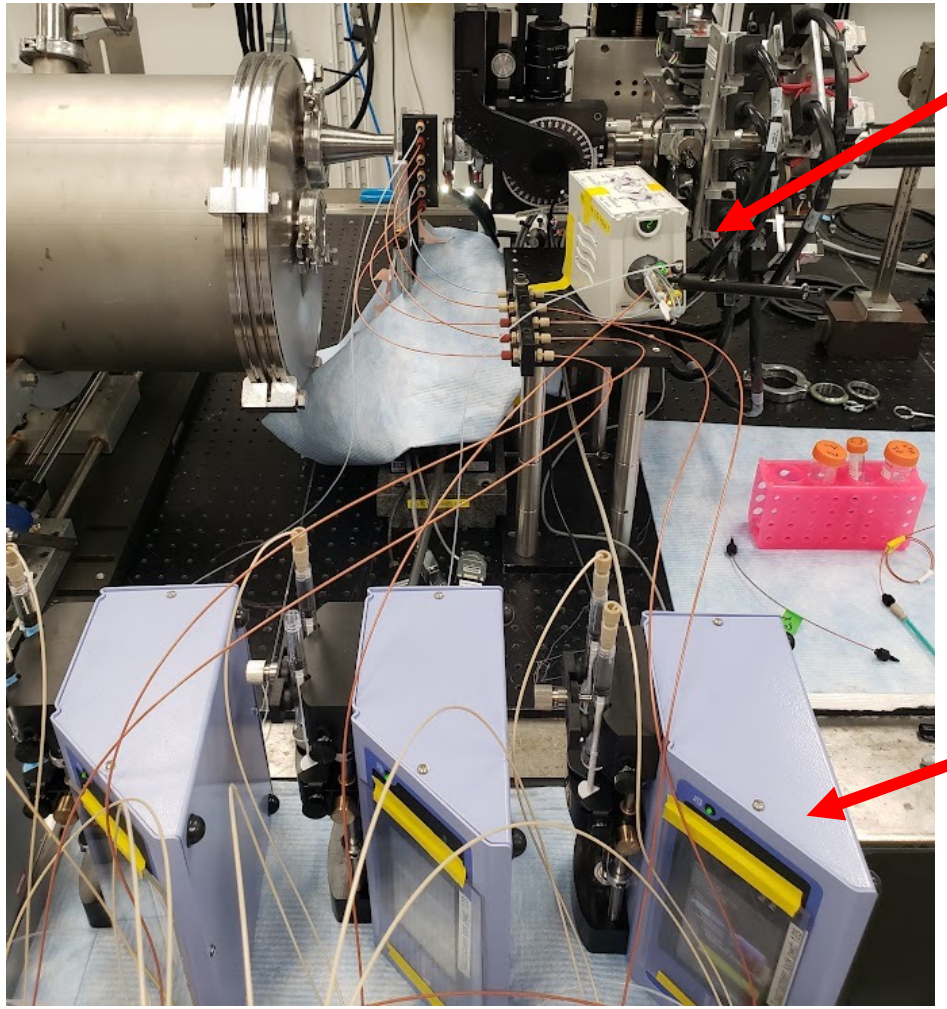



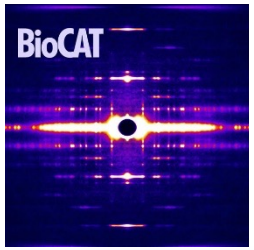
# TR-SAXS: laminar mixer






Park *et al.*, PNAS **105** (2008)




# "Cheat sheet" of equilibrium capabilities

- Also available on BioCAT website (https://www.bio.aps.anl.gov/pages/about-saxs.html)
- Chromatography SAXS
  - Agilent 1260 Infinity II bio-inert pumps and autosampler
  - Various SEC columns
    - Superdex (30, 75 and 200) and Superose 6 10/300 Increase – fractionation range coverage ~0.1 - 5,000 kDa
    - Superdex (75 and 200) and Superose 6 5/150 Increase
    - Superdex 200 3.2/300 Increase
    - Various Silica columns (Wyatt 010S5, 015S5, 030S5, and more)
  - IEX columns: Capto HiRes 5/50, both Q and S resins
- Optional in-line characterization
  - MALS/DLS/dRI
    - Wyatt DAWN HELIOS II (17-angle + DLS)/Optilab tRex, temperature control from 4-40 degrees
  - (In by default) full-spectrum UV-Vis
    - Stellarnet BlackComet spectrometer, microvolume (2 $\mu$L) fiber-coupled cell, OceanInsight D/H light source. Useful range ~210-800 nm
- Full system temperature control from 4-40 degrees C
- AF4-SAXS
  - Agilent 1260 Infinity II autosampler and pump
  - Wyatt Eclipse NEON (with dilution control module)
  - Wyatt short, long and dispersion inlet channels
    - 275, 400 and 525 micron spacers
  - 10 and 30 kDa MWCO membranes, both poletherlsulfone and regenerated cellulose
  - In-line Wyatt DAWN HELIOS II (17-angle + DLS) and Optilab tRex for MALS/DLS/dRI
  - Temperature control ambient or higher (to ~40 degrees C)
- Batch SAXS
  - 96- and 384-well plate loading capabilities
  - Volumes down to 10 $\mu$L
  - Temperature control 4-40+ degrees C
  - ~3 mins/sample, 96-well plate in ~5 hours
  - Full-spectrum UV-Vis measurements available