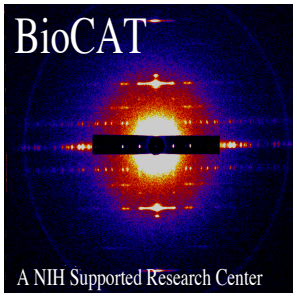

Solution small angle x-ray scattering: A versatile biophysical tool to study biological macromolecules

Jesse Hopkins
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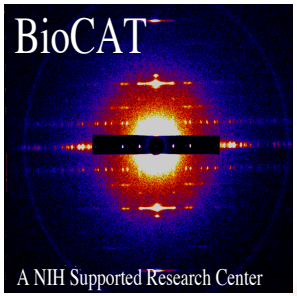

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Tom Irving
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Srinivas Chakravarthy
Beamline scientist

Weikang Ma
Beamline scientist

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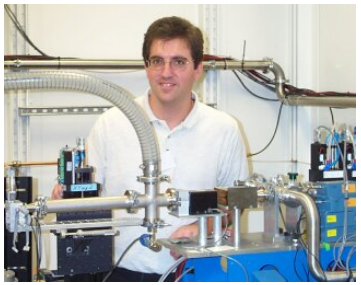

Rick Heurich
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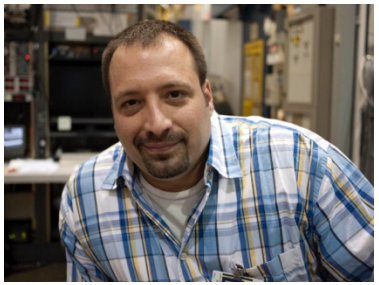

Mark Vukonich
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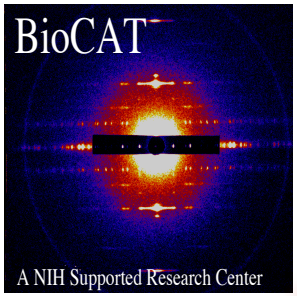
U.S. DEPARTMENT OF ENERGY | Office of Science

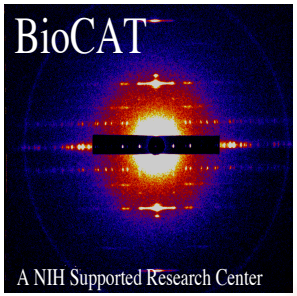
NATIONAL INSTITUTES OF HEALTH



What is solution SAXS?

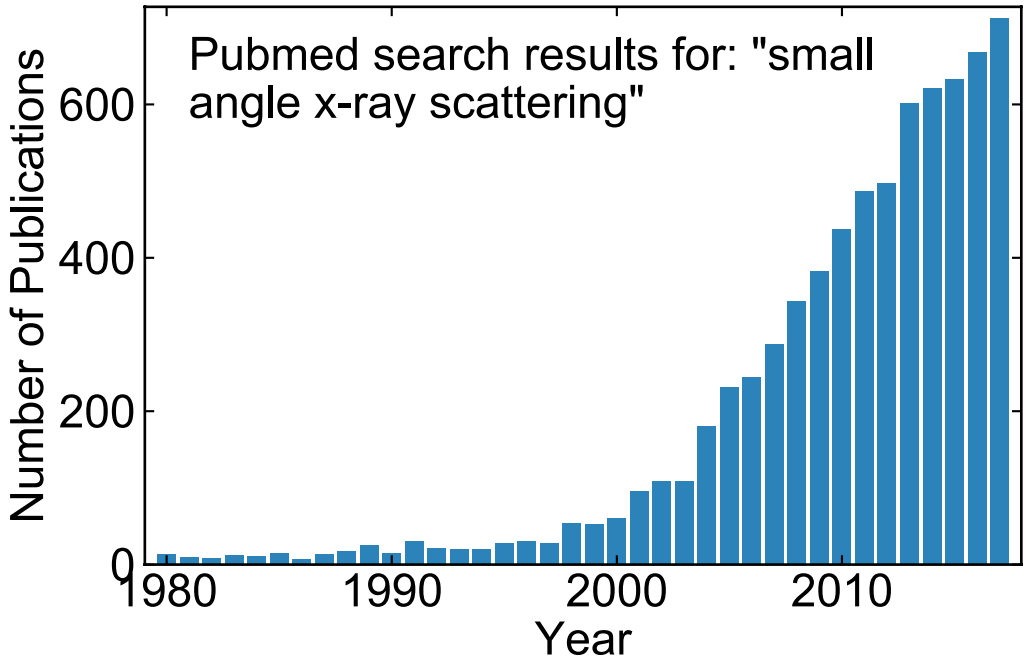
- Biophysical technique for studying isolated macromolecules in solution

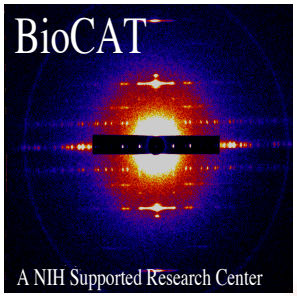
- Complimentary to high-resolution structural techniques
  - Diffraction
  - NMR
  - Electron Microscopy



Why do solution SAXS?

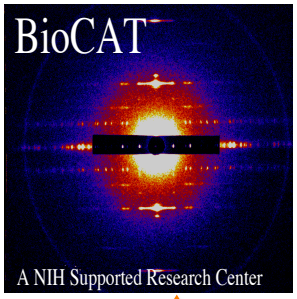
- Studies molecules in solution
  - No crystals or labels needed
- Wide range of buffer conditions accessible
  - Salt, pH, ligand concentration . . .
- Study mixtures, complexes, flexibility, disorder
- Beamlines and software analysis accessible to non-experts



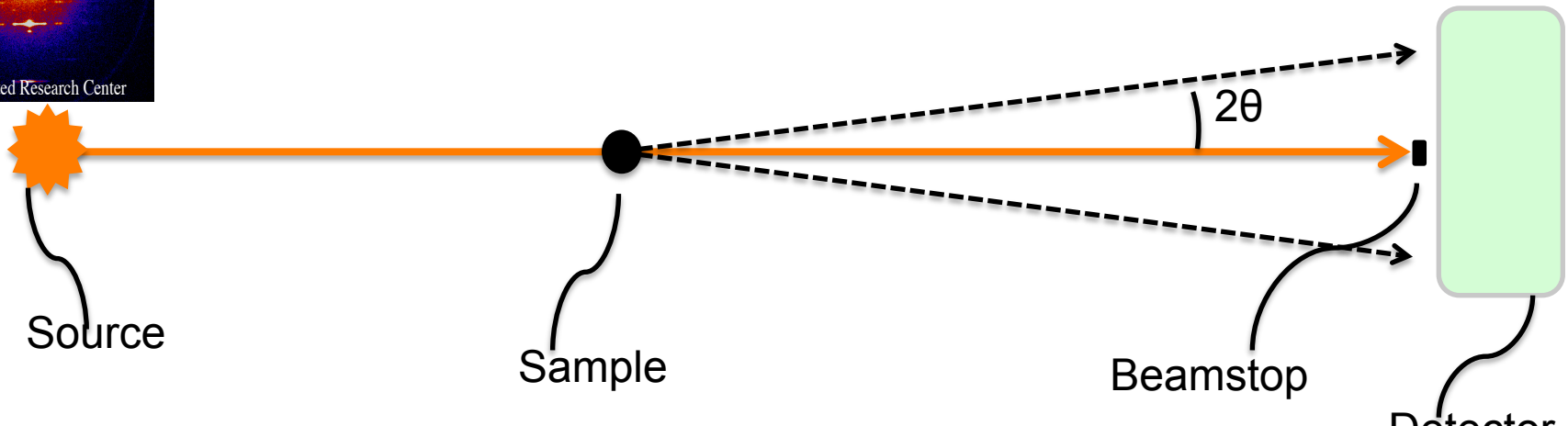


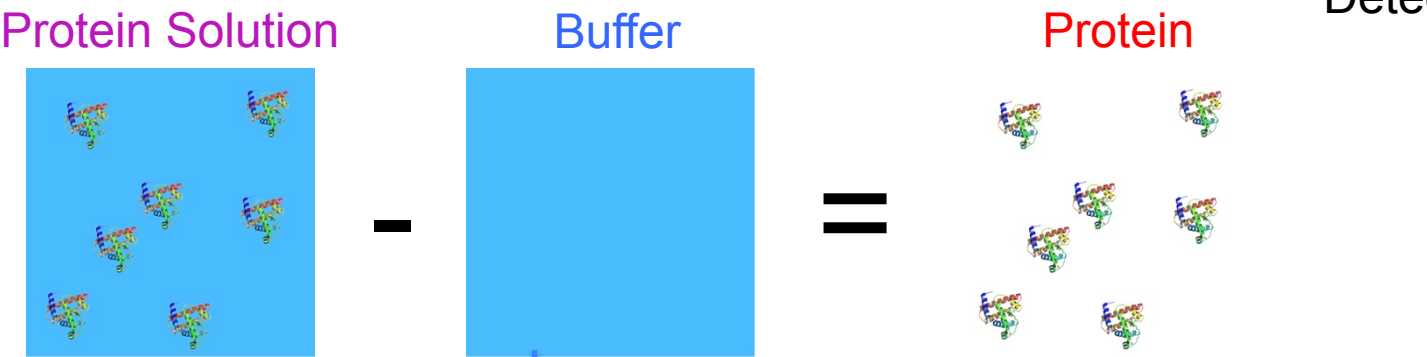
What can you study with SAXS?

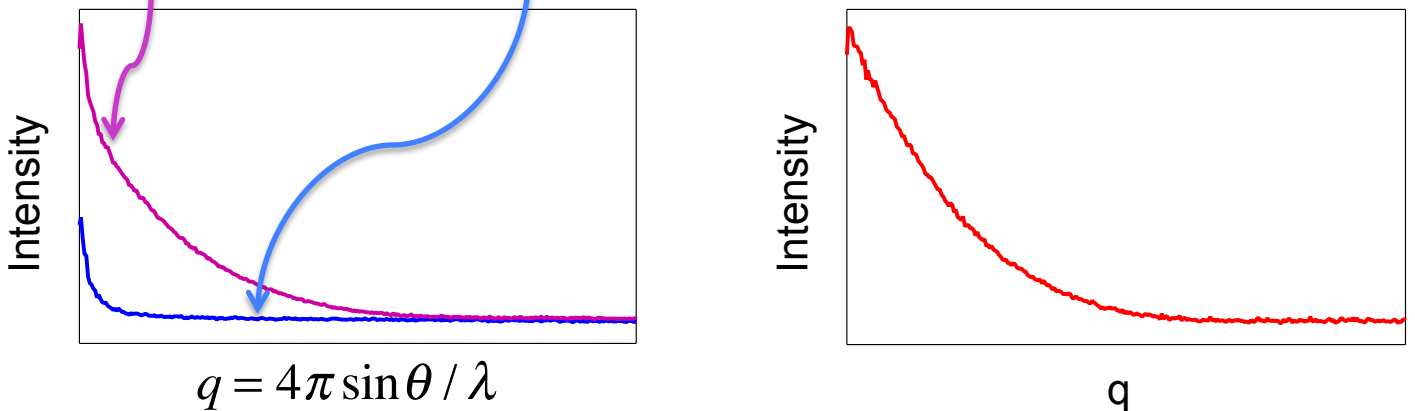
- Purity (monodispersity)
- Oligomeric State
- Shape/size/anisometry
- Flexibility and disorder
- Foldedness
- Conformation
- Complexes
- Interparticle interactions
- Time resolved SAXS can provide this information for dynamic changes in the macromolecule, with ~100 μs resolution

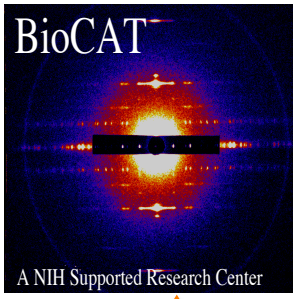


A typical SAXS experiment

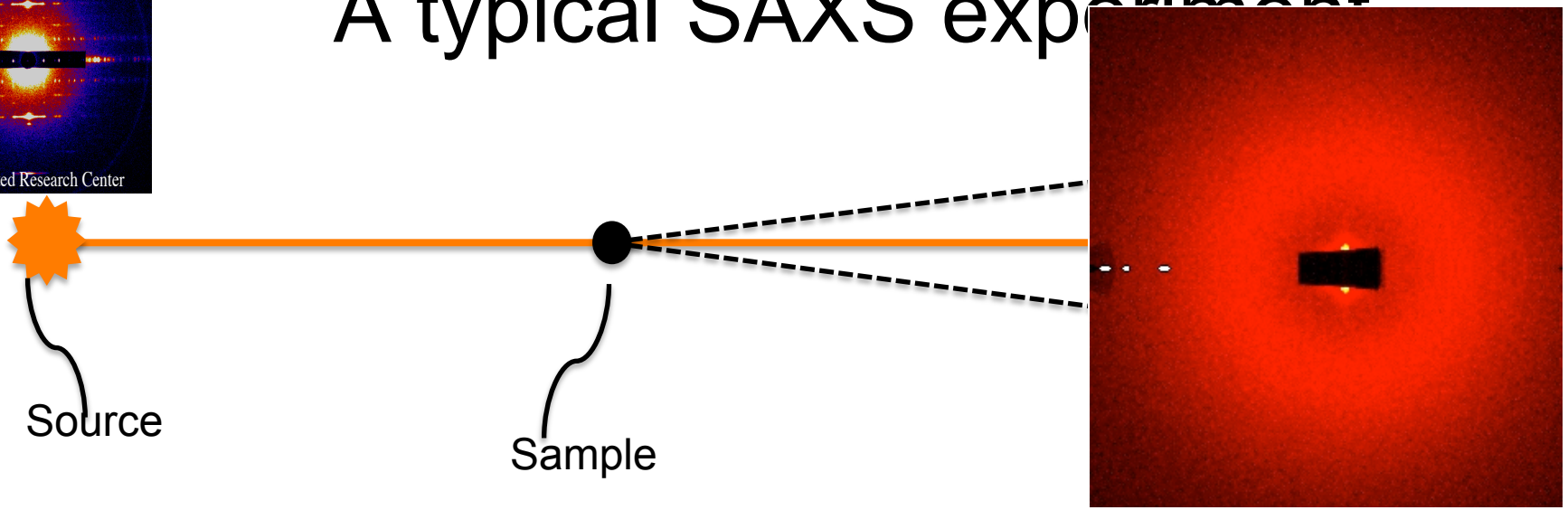


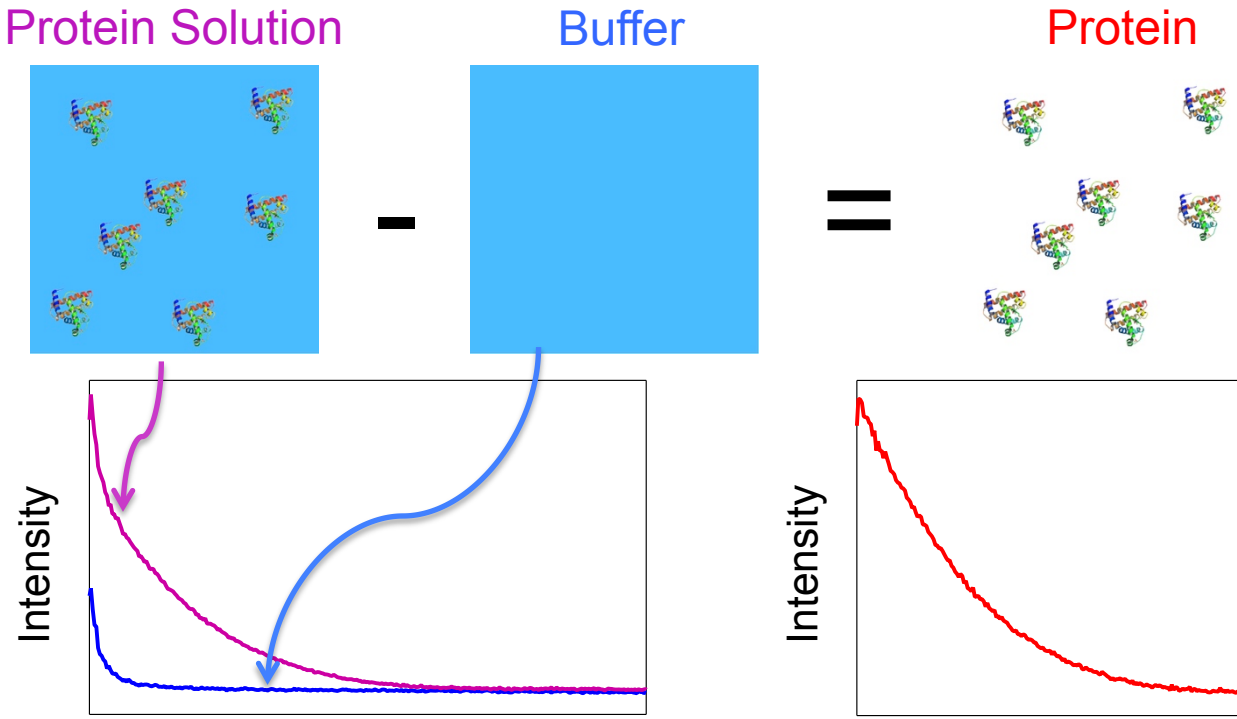


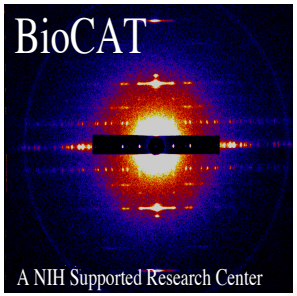




A typical SAXS experiment

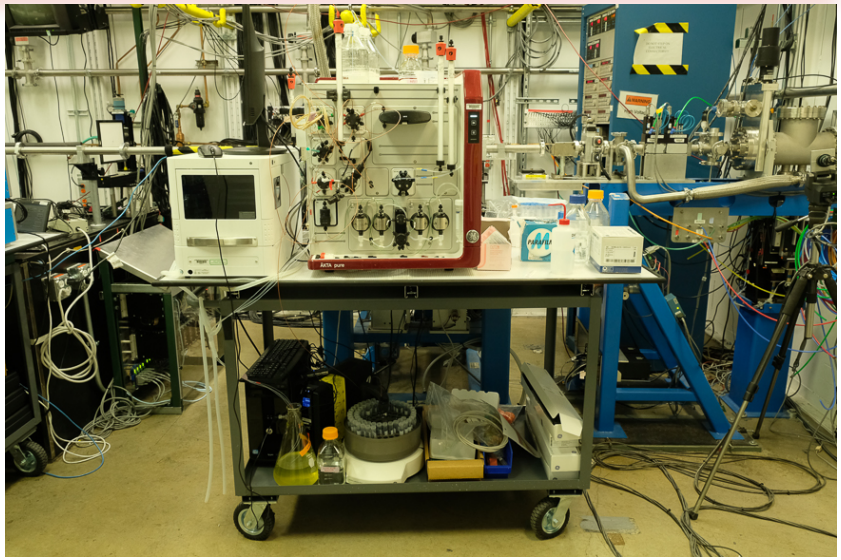


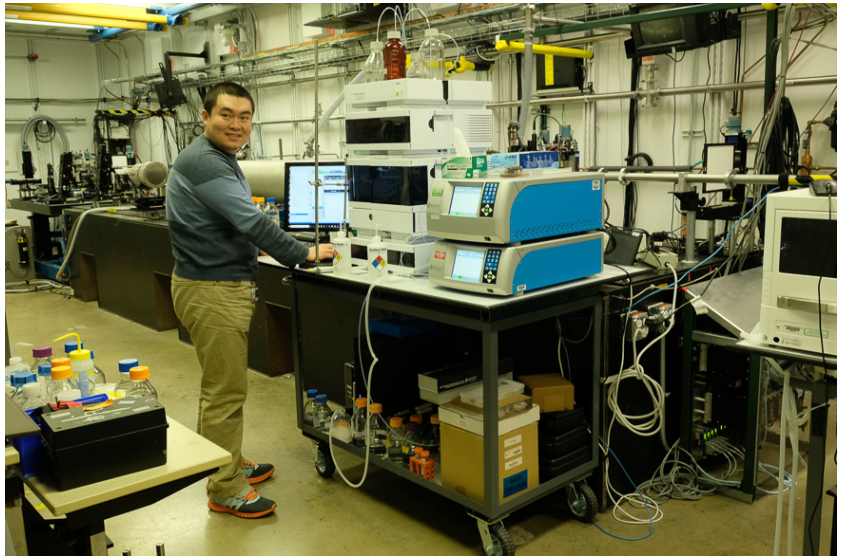




SAXS at BioCAT

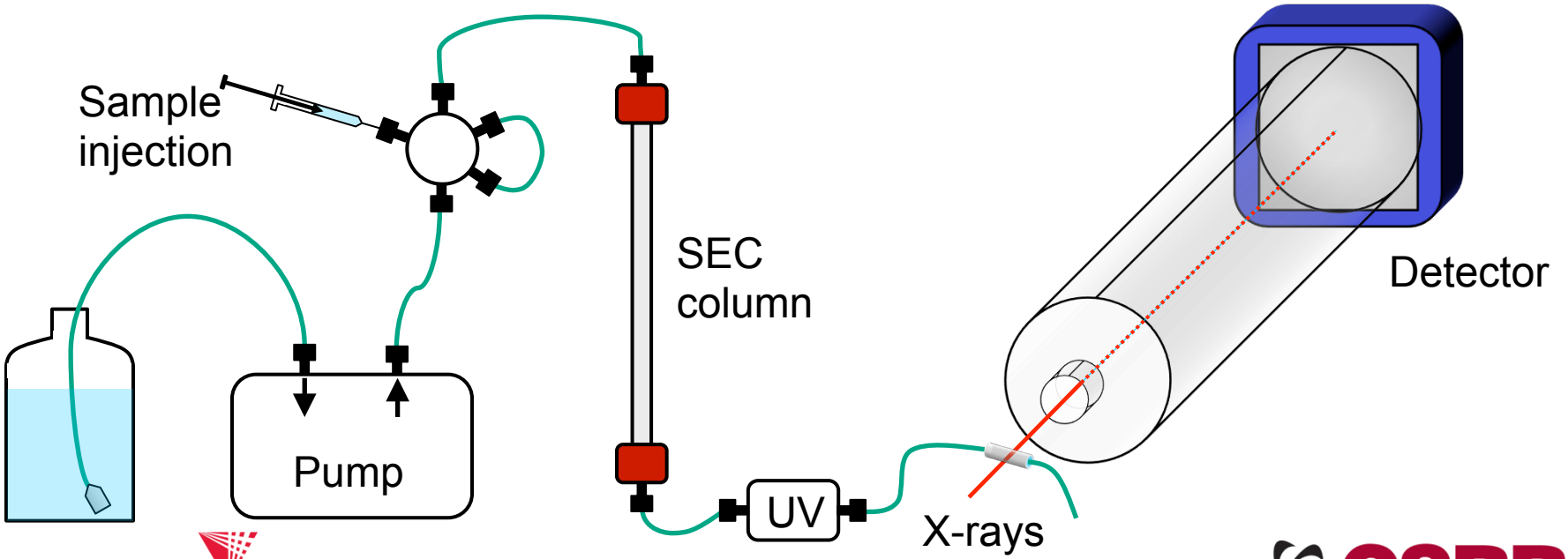
- Equilibrium SAXS
  - Size exclusion chromatography coupled SAXS (SEC-SAXS)
  - Size exclusion chromatography, multi-angle light scattering, dynamic light scattering, refractive index coupled SAXS (SEC-MALS-SAXS)
  - Batch mode



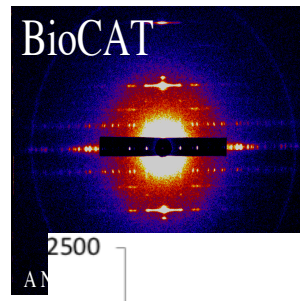


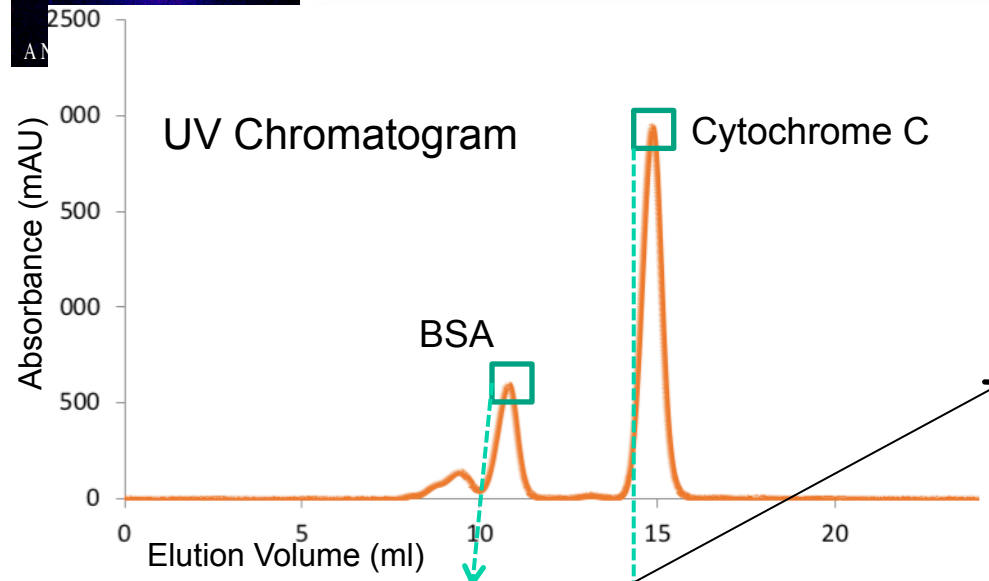
What is SEC-SAXS and why do it?

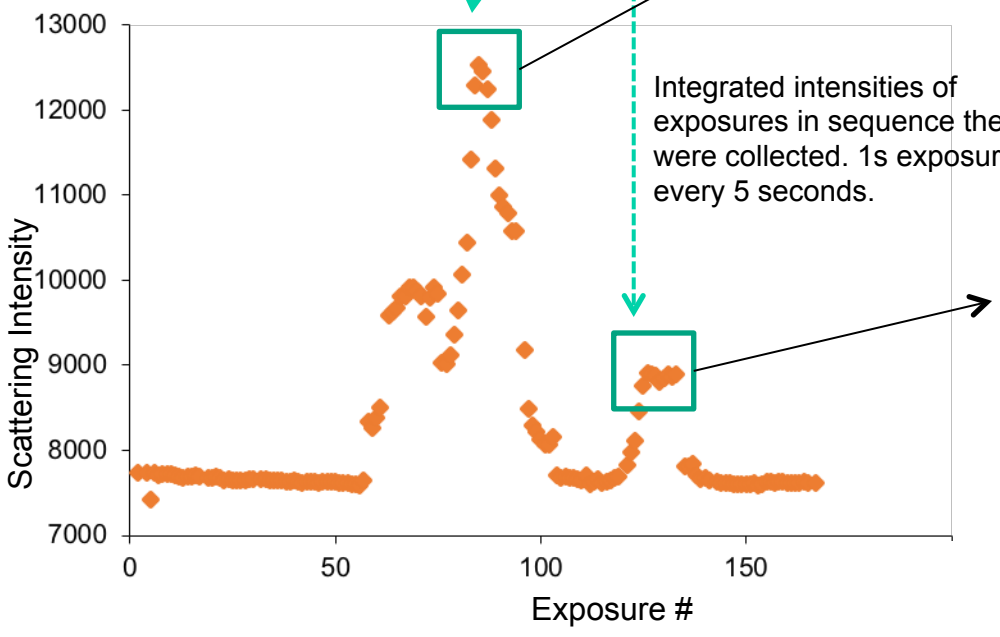
- Sample run through sizing column
  - Separates macromolecules by size
- Column output flows directly through SAXS cell
- Data collected continuously during elution

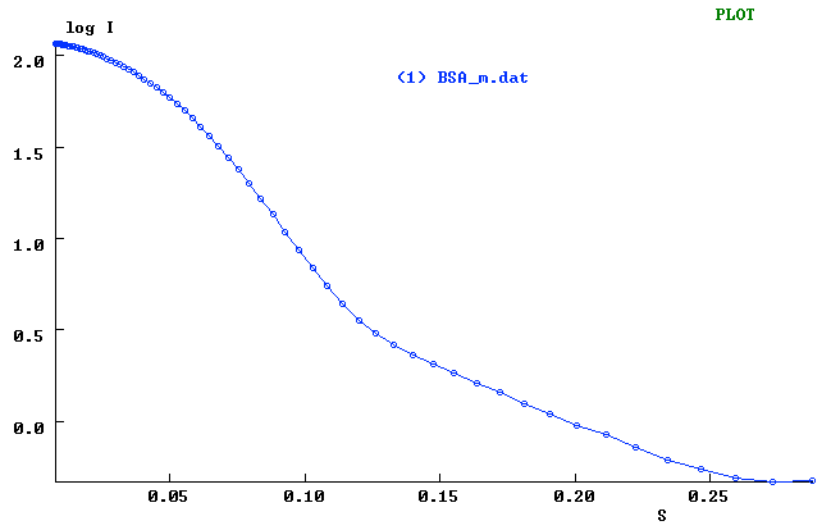


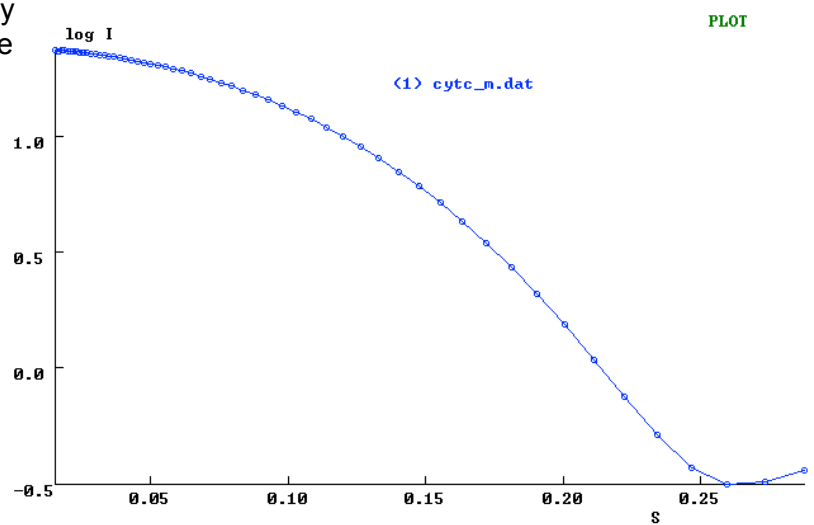
Power of SEC-SAXS: Simultaneous Data Collection for BSA and Cytochrome C

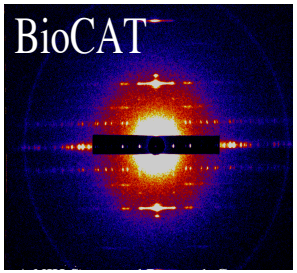




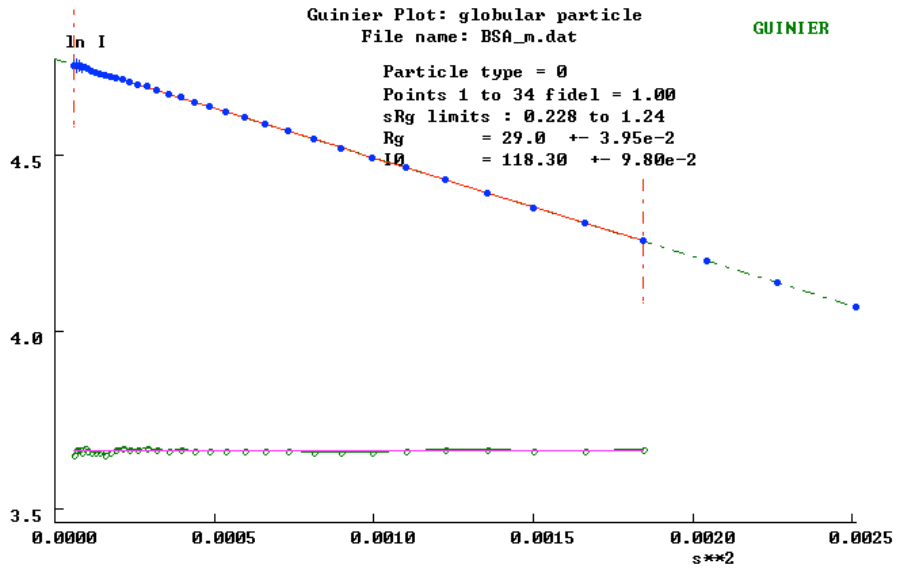


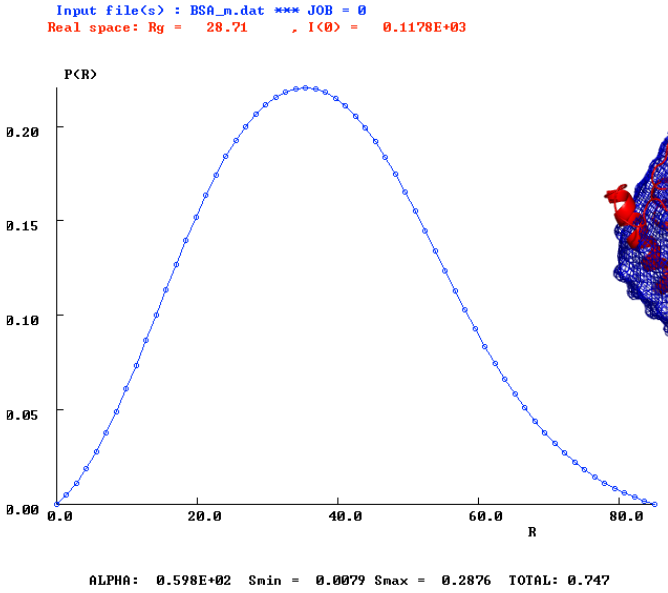


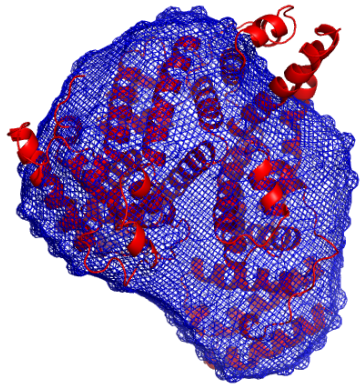




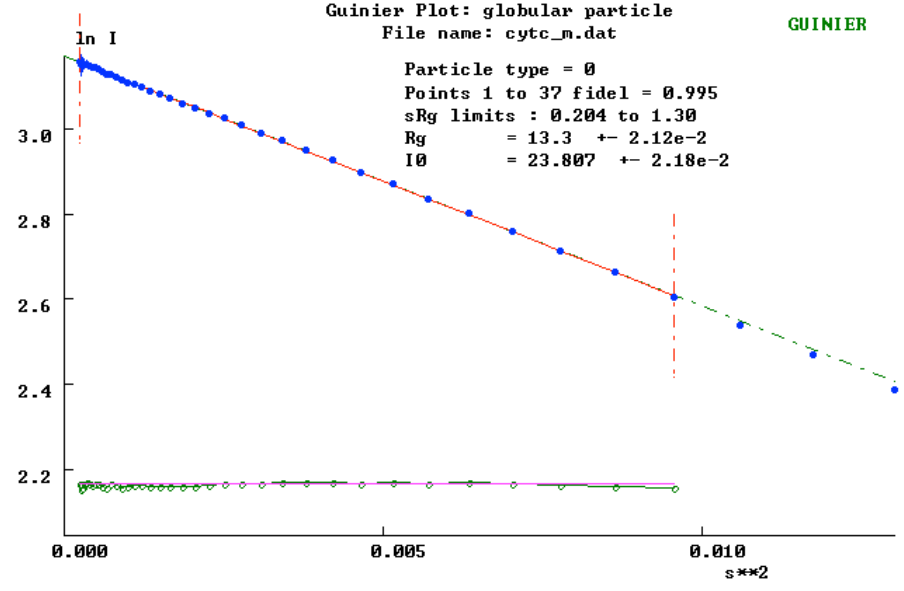
Power of SEC-SAXS: Simultaneous Data Collection for BSA and Cytochrome C

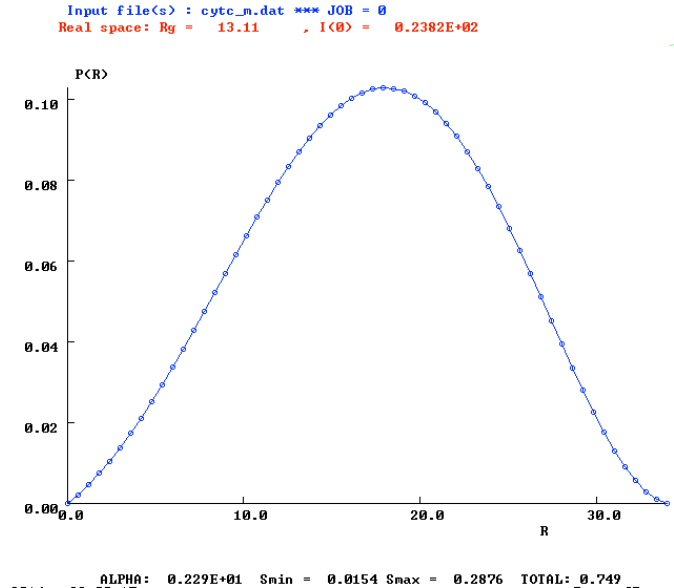






BSA





CytC

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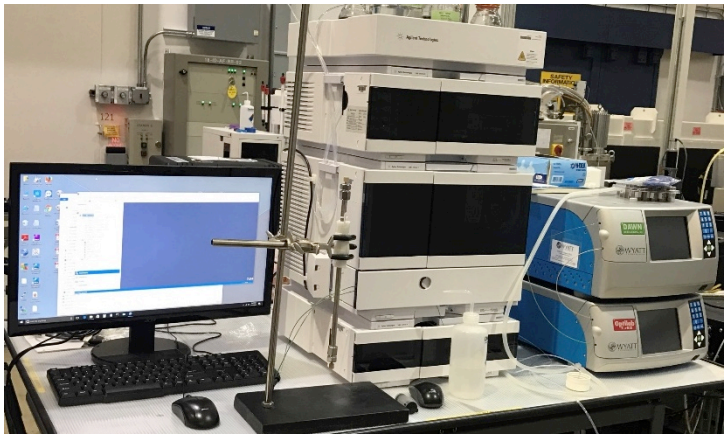
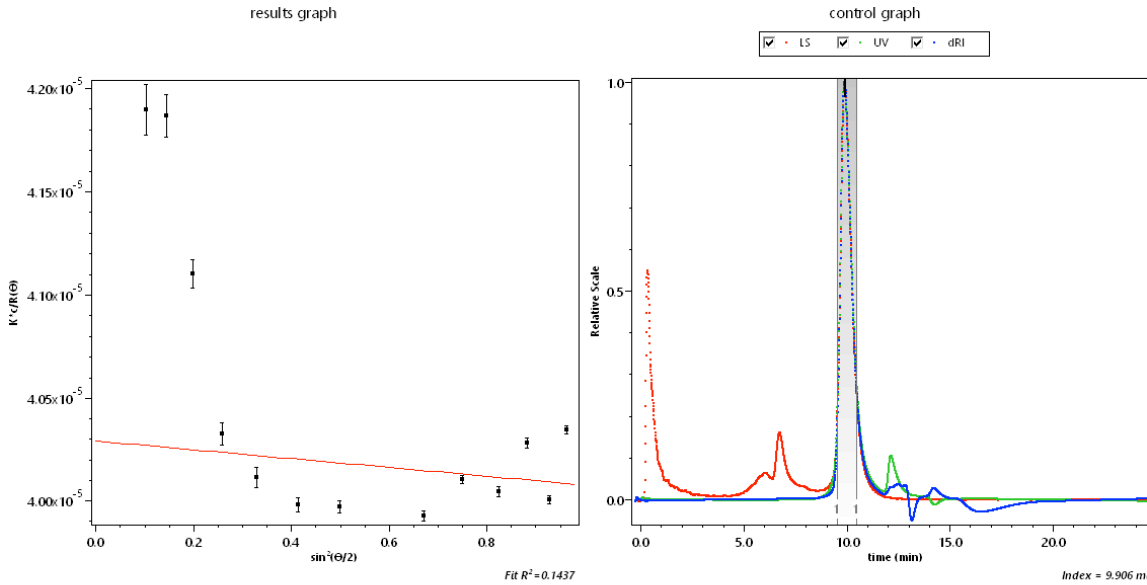
SEC-MALS-DLS-SAXS – A More Complete Biophysical Characterization of Macromolecules





	Value
Molar Mass	(2.482 ± 0.017) e+4 g/mol
rms radius	0.0 ± 0.0 nm
Peak Number	1
Slice Index	1220
Model	Zimm
Fit Degree	1
Abscissa Position	9.906 min
Concentration	(6.344 ± 0.000) e-1 mg/mL
dn/dc (mL/g)	0.1850

→ Molar Mass = 2.482+/-0.017 e^{+4} g/mol

→ Concentration = 6.344 e^{-1} mg/ml

Infrastructure needed for SEC-MALS-DLS-dRI-SAXS. Demonstration of the utility of SEC-MALS-DLS-dRI. Shown here are the light scattering, UV, QELS and dRI signals and their use to determine the concentration, molecular weight and hydrodynamic radius of the protein (next slide).

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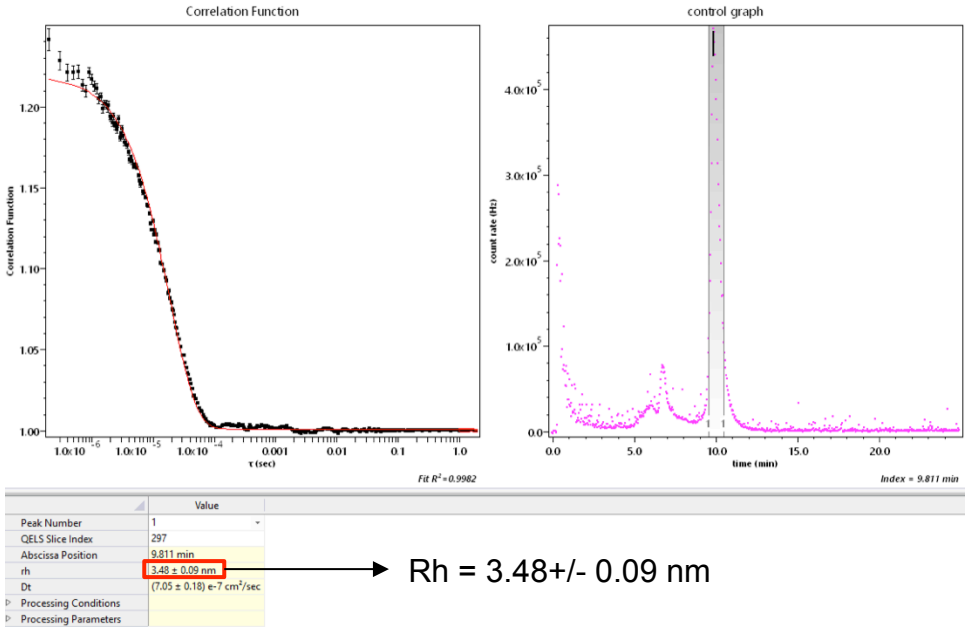
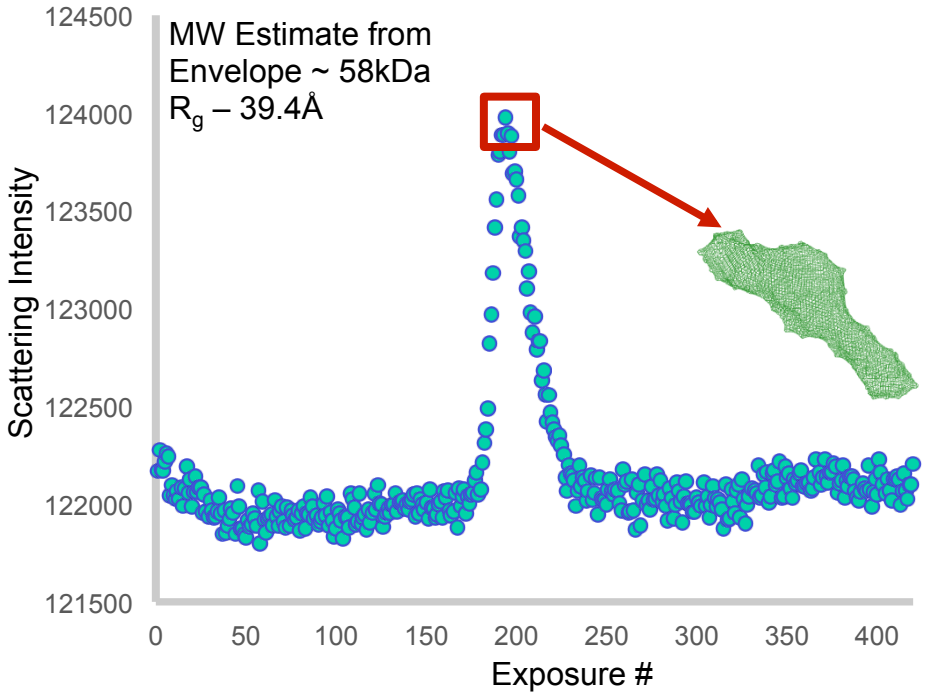
SEC-MALS-DLS-SAXS – A More Complete Biophysical Characterization of Macromolecules



Rh = 3.48+/- 0.09 nm



Shown here is the QELS signal and its use to determine the hydrodynamic radius of the protein. On the right panel is shown the scattering chromatogram while collecting 1 second X-ray exposures every 3 seconds. Each point is the summed up intensity of each exposure plotted in the order they were acquired. Also shown is a low resolution envelope calculated ab initio using DAMMIF using 5 exposures at the peak averaged after buffer subtraction. Molecular weight of the protein which is wrongly estimated to be ~58kDa because of the extended conformation. The Mw (weight averaged molar mass) from MALS is ~25 kDa which is very close to the 23.3kDa theoretical molecular mass of the protein in monomeric form.

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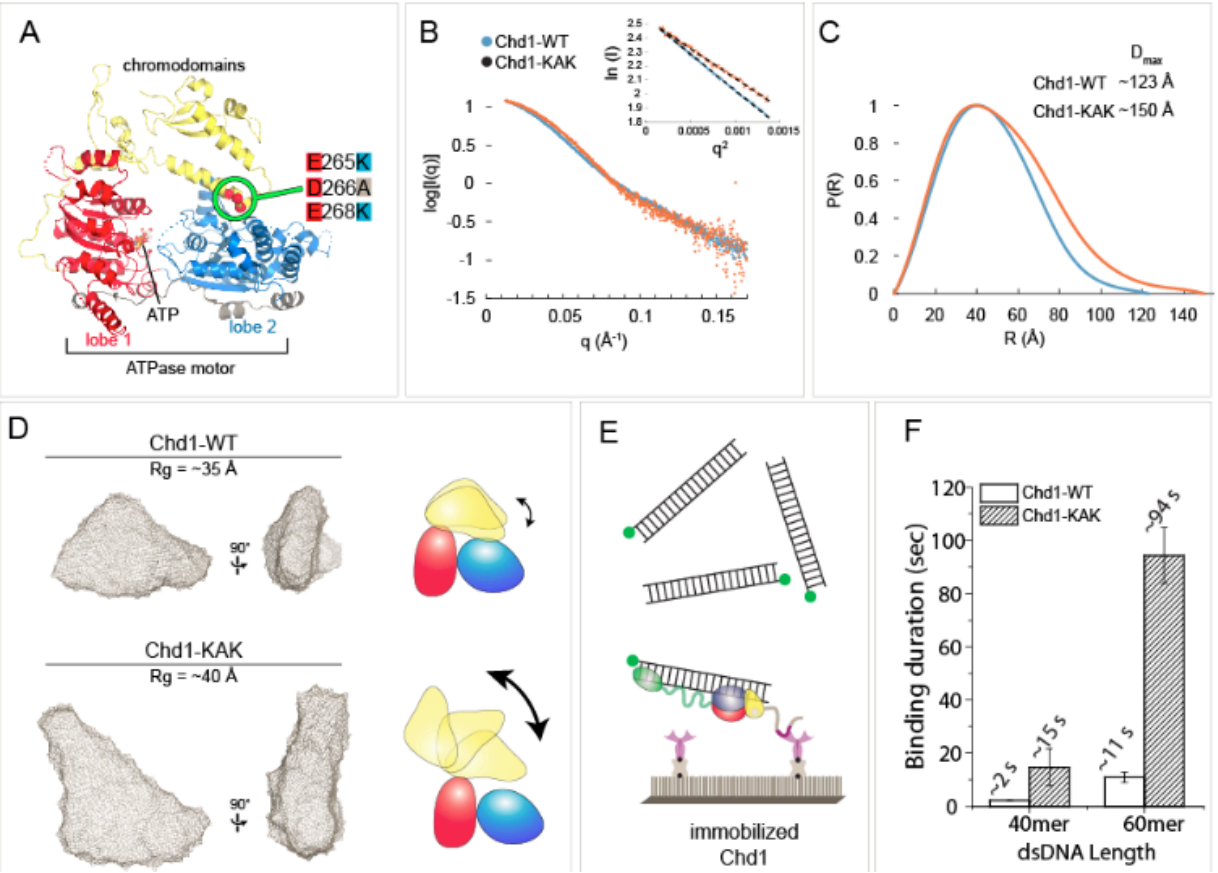
Highlights – Equilibrium SAXS with Single Molecule FRET

SAXS helped in clarifying the auto-regulatory function of the chromodomains in substrate specificity & in the generation of intermediates during nucleosome sliding by the chromatin remodeler CHD1. ***Qiu et al., The Chd1 Chromatin Remodeler Shifts Nucleosomal DNA Bidirectionally as a Monomer.*** *Mol Cell.* ***2017 Oct 5;68(1):76-88.e6.***



The KAK mutant displays an extended structure and a more stable interaction with double stranded DNA (A) Crystal structure of the chromodomain-ATPase portion of Chd1, highlighting the location of KAK mutation at the chromo-ATPase interface. (B) Small angle X-ray scattering (SAXS) profiles for Chd1-WT and Chd1-KAK proteins consisting of just the chromodomain and ATPase motor. Guinier plot analysis (inset) shows that samples were free from aggregation. (C) P(R) distributions for SAXS data shown in (B). (D) Ab initio bead models generated by DAMMIN. Cartoons on the right illustrate possible structural changes associated with the KAK mutation. (E) Schematic of experiment in which Cy3 labeled dsDNA (40 or 60 bp) was added to surface immobilized Chd1-WT or Chd1-KAK proteins. Note that these constructs contain the DBD. (F) Binding duration for dsDNA to both proteins (n=500 binding events).

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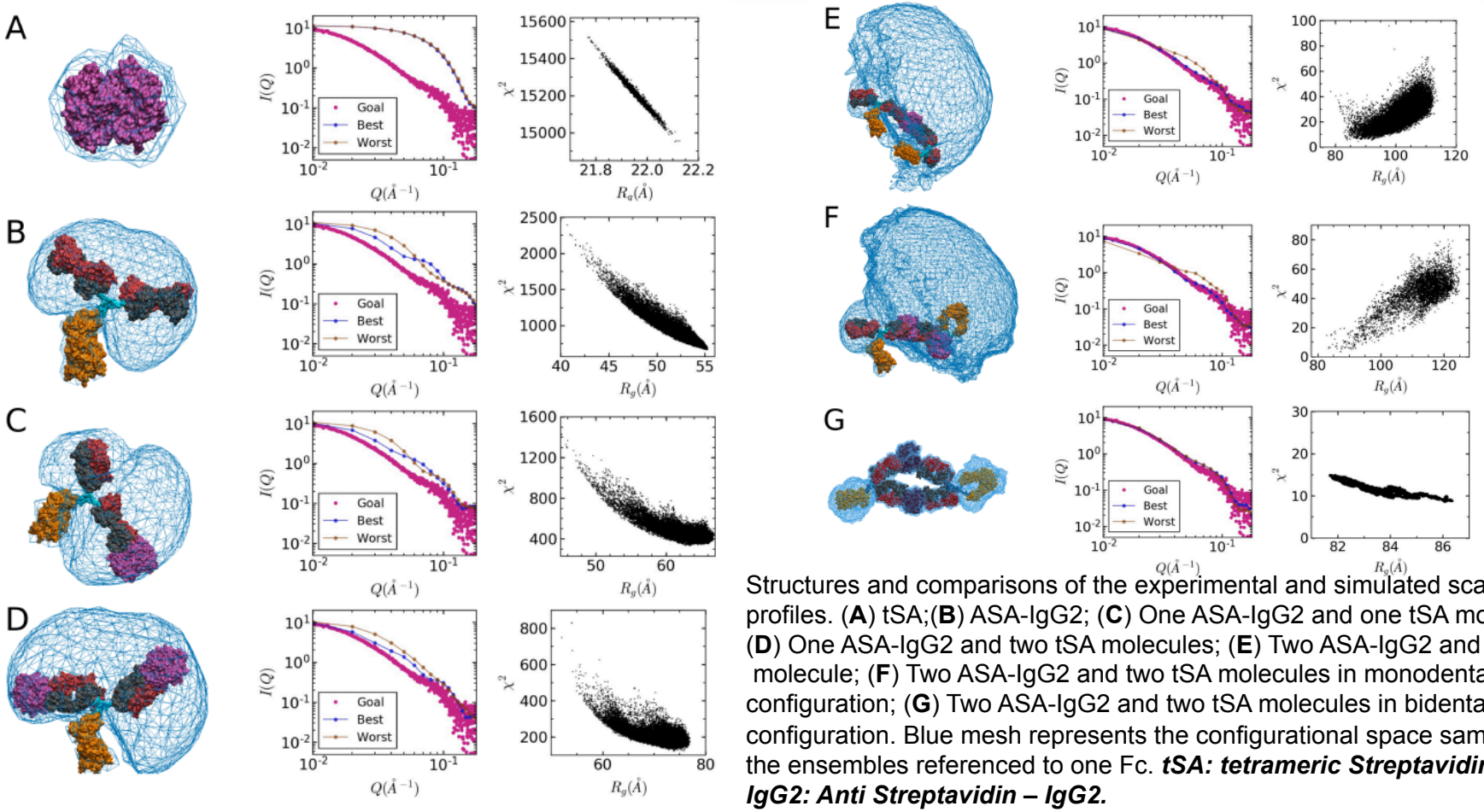
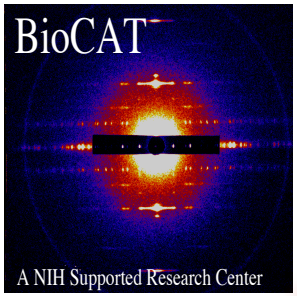
Highlights – SAXS with MD



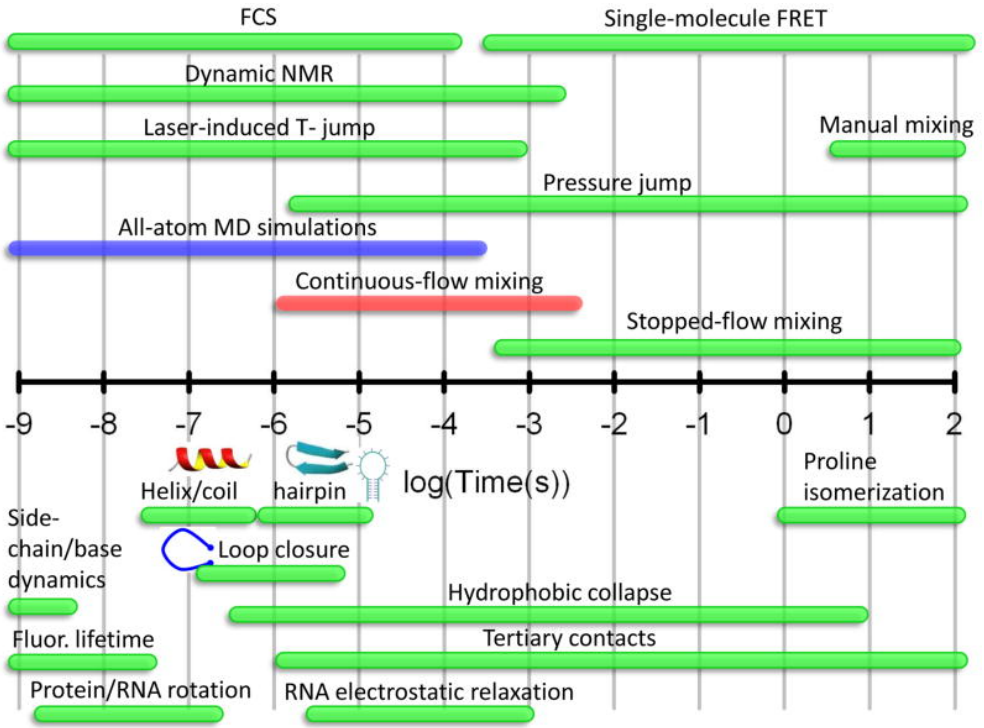
Structures and comparisons of the experimental and simulated scattering profiles. (**A**) tSA;(**B**) ASA-IgG2; (**C**) One ASA-IgG2 and one tSA molecule; (**D**) One ASA-IgG2 and two tSA molecules; (**E**) Two ASA-IgG2 and one tSA molecule; (**F**) Two ASA-IgG2 and two tSA molecules in monodentate configuration; (**G**) Two ASA-IgG2 and two tSA molecules in bidentate configuration. Blue mesh represents the configurational space samples by the ensembles referenced to one Fc. ***tSA: tetrameric Streptavidin, ASA-IgG2: Anti Streptavidin – IgG2.***

The use of SEC-SAXS and ensemble modeling is the only way to characterize antibody–antigen complexes using SAXS. Castellanos et al., Antibodies 2017 6(4), 25.



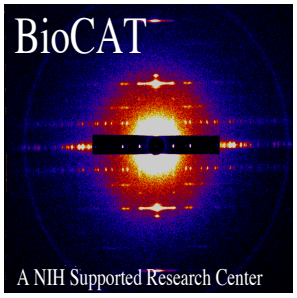
SAXS at BioCAT

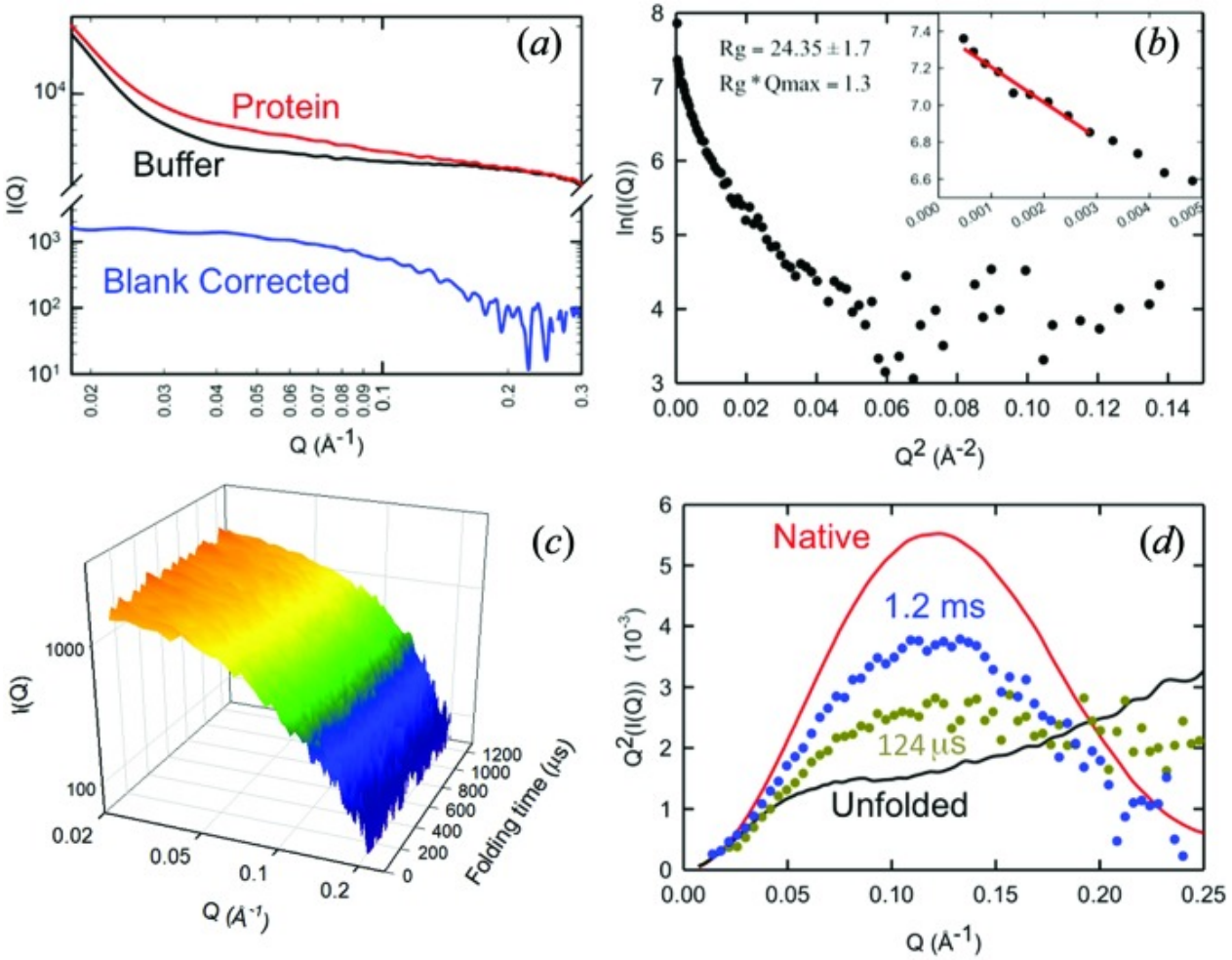
- Non-equilibrium time resolved SAXS
  - Continuous flow mixing
    - ~100 μs minimum time point
    - Flow helps prevent radiation damage
  - Stopped flow mixing
    - ~1 ms minimum time point
    - Established technology



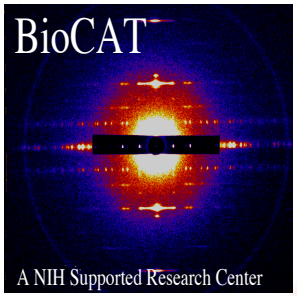
Kathuria et al., 2011, Biopolymers, 95(8)

CF-SAXS developed in close collaboration with Osman Bilsel at U. Mass. Amherst





a. Raw data for cytochrome c and buffer at a representative time point (100 µs) along the channel. b. Guinier fit of the data (100-148 µs points averaged). c. The blank-corrected scattering curves for 3.5 mg/ml Cyt c over the 0.1 – 1.2 ms time range after initiation of folding. Each scattering curve is the average of ~10 frames of 200ms exposure. d. Kratky plots at representative time points.



Summary

- BioCAT supports state-of-the-art equilibrium and non-equilibrium solution SAXS
- SAXS can provide information on:
  - Size and shape
  - Flexibility and disorder
  - Conformation
  - Complexes
  - And more…
- Powerful complementary technique