Time-resolved Scattering of Proteins in Solution: New Opportunities for an ERL

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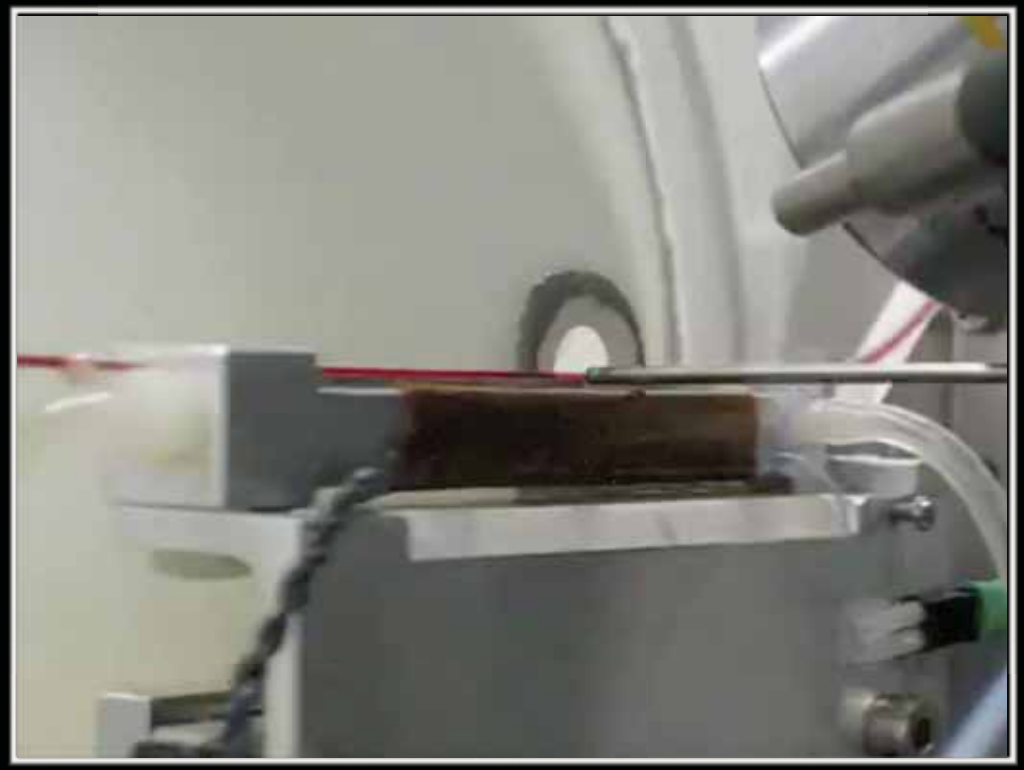
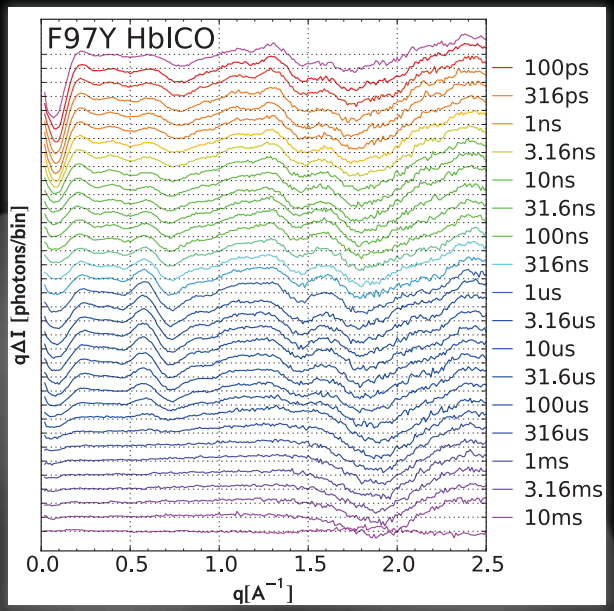
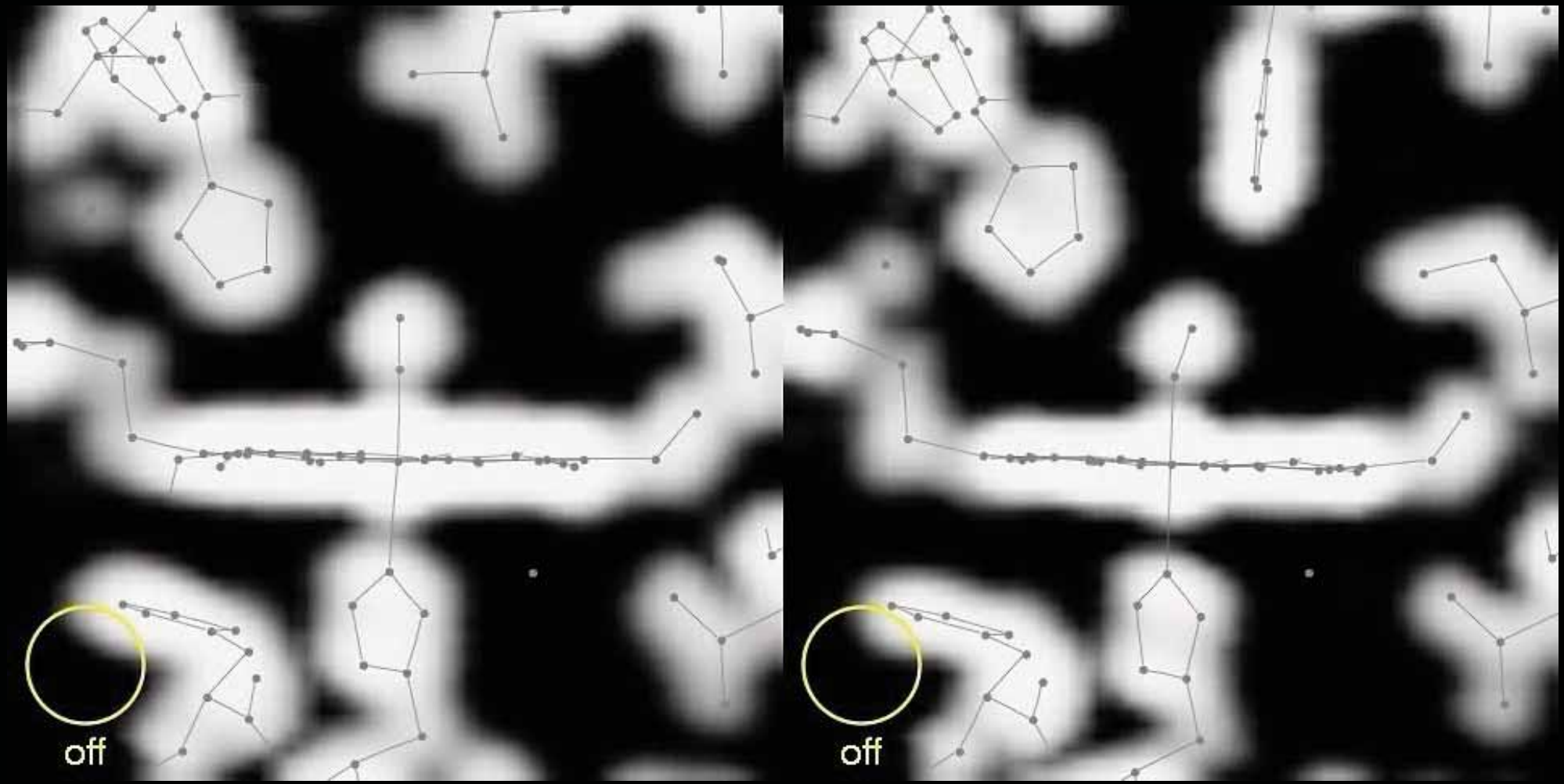




Wild-type MbCO *L29F MbCO*

*(MbCO + Mb*CO)*



Movie posted as supplementary online material for:
Schotte et al., J. Struct. Biol., **147(3)**, 235-246 (2004)

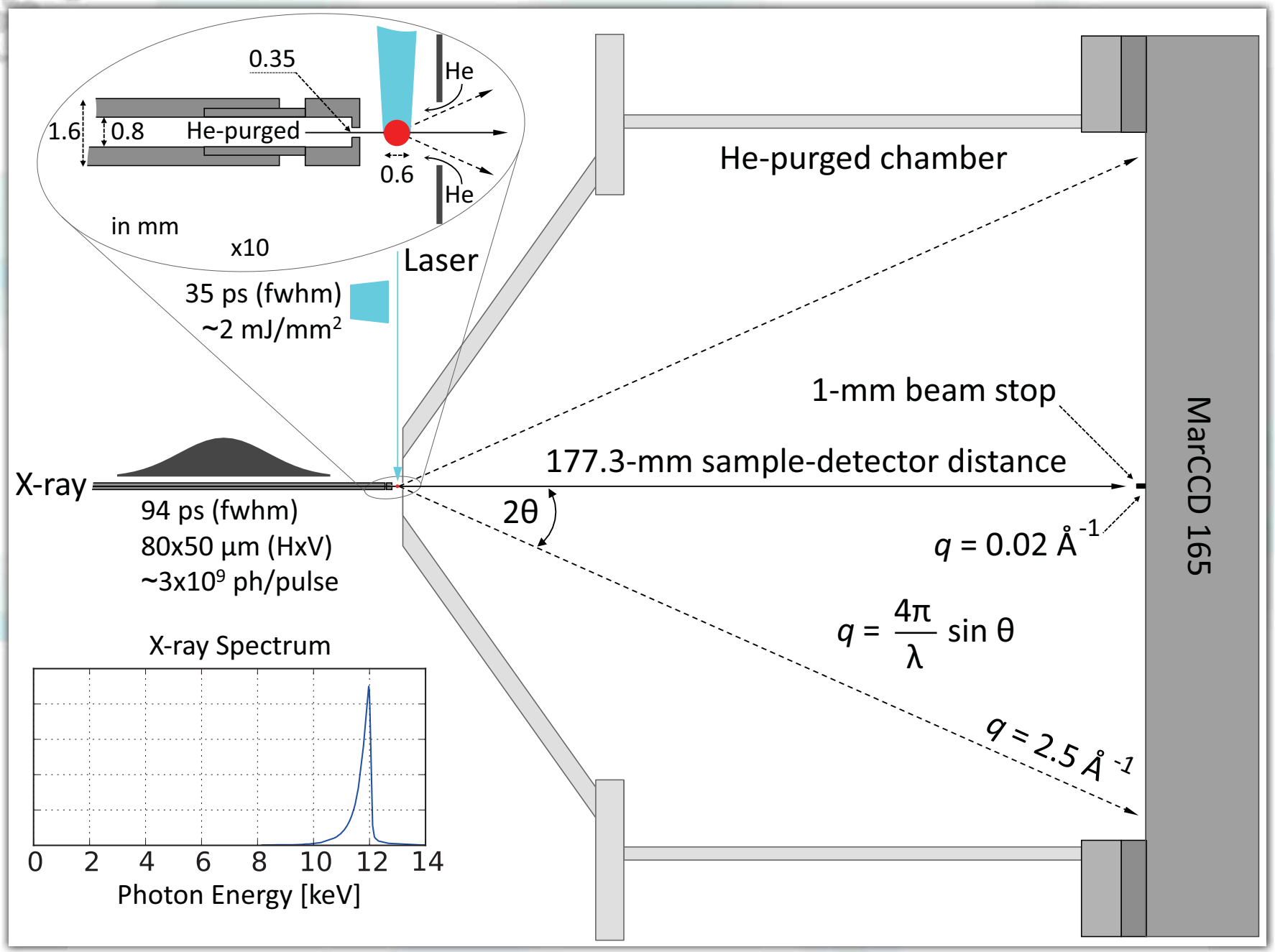
See also Schotte et al., Science, 300, 1944-1947 (2003)

Time-resolved Small- and Wide-angle X-ray Scattering of proteins in solution (SAXS/WAXS)

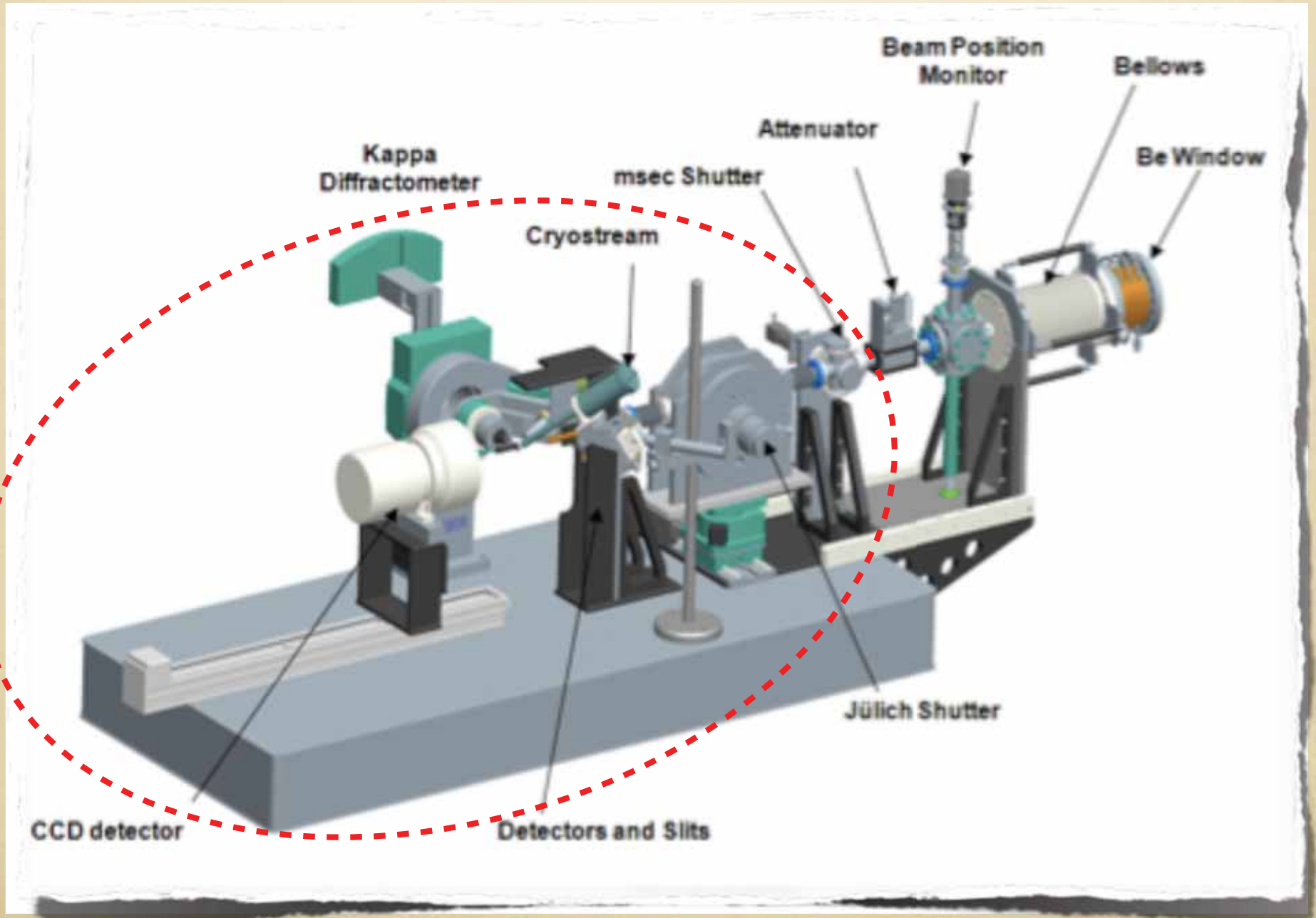
What do we measure?

- 1-D scattering fingerprint
- **SAXS** region informs about size and shape, and is sensitive to volume changes as well as mass transport into and out of the protein.
- **WAXS** region is sensitive to structure at higher resolution.

Time-resolved SAXS/WAXS diffractometer

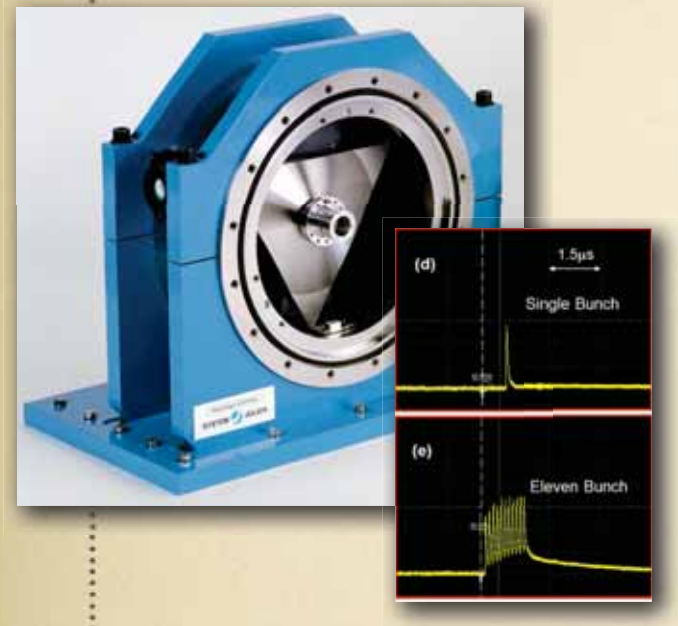


APS:BioCARS Experimental Hutch

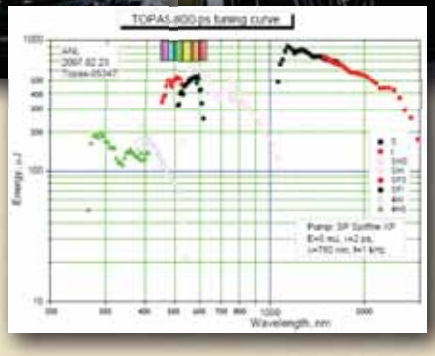


NIDDK Contributions to BioCARS Upgrade

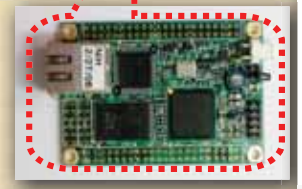
High-speed Chopper Upgrade $72 K

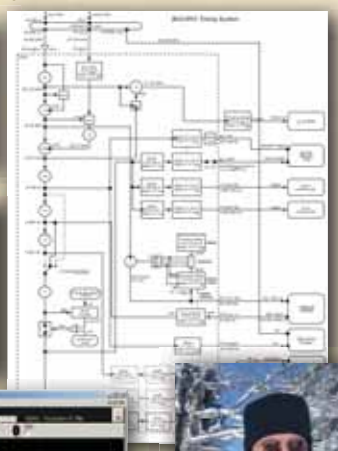


High-power Laser System: ~$500 K

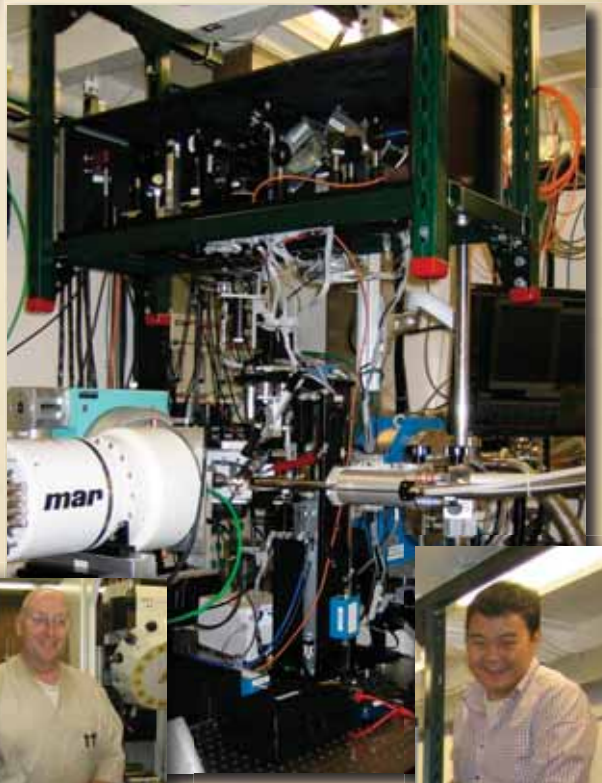


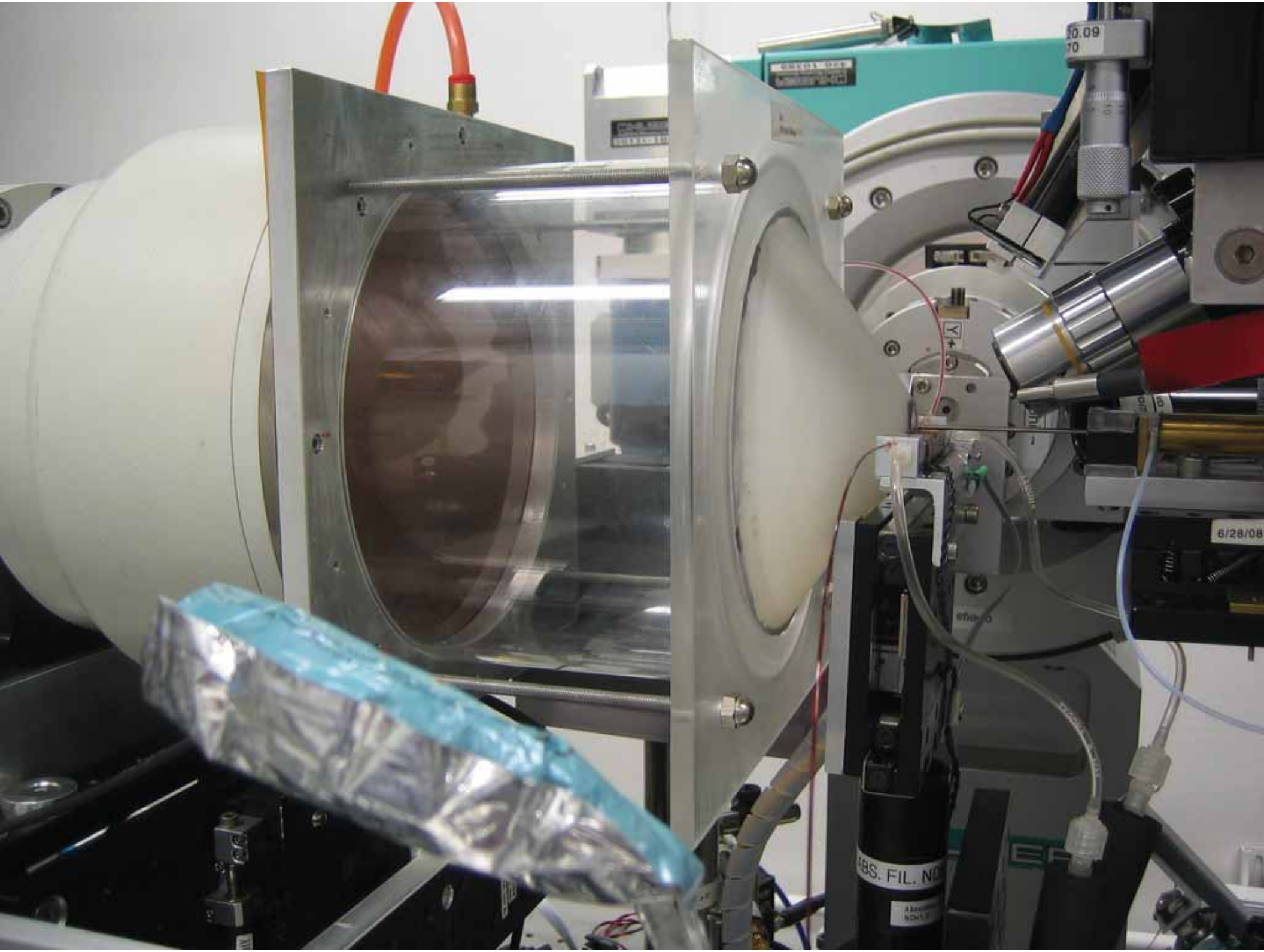
FPGA-based Timing System / LaueCollect

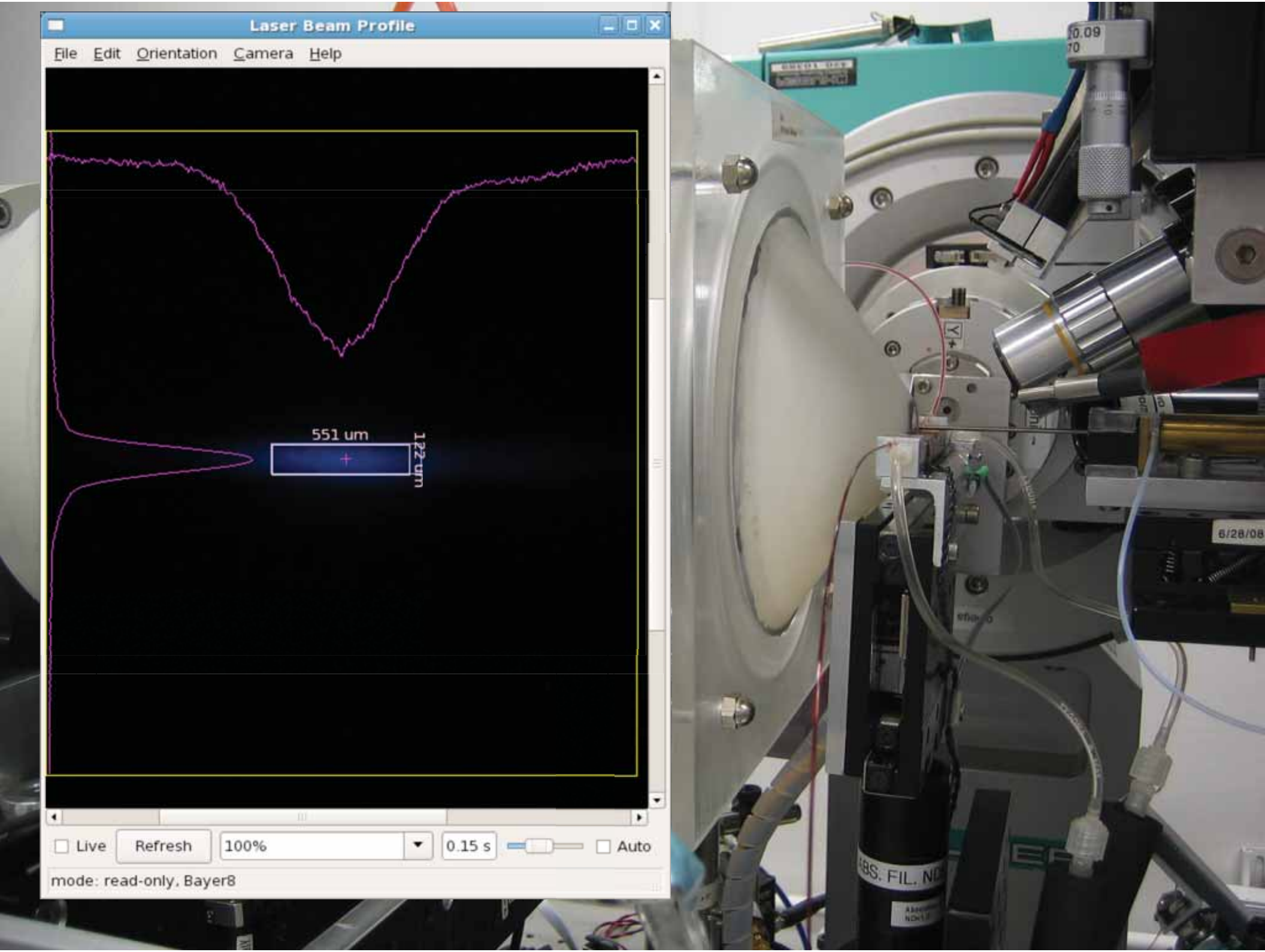


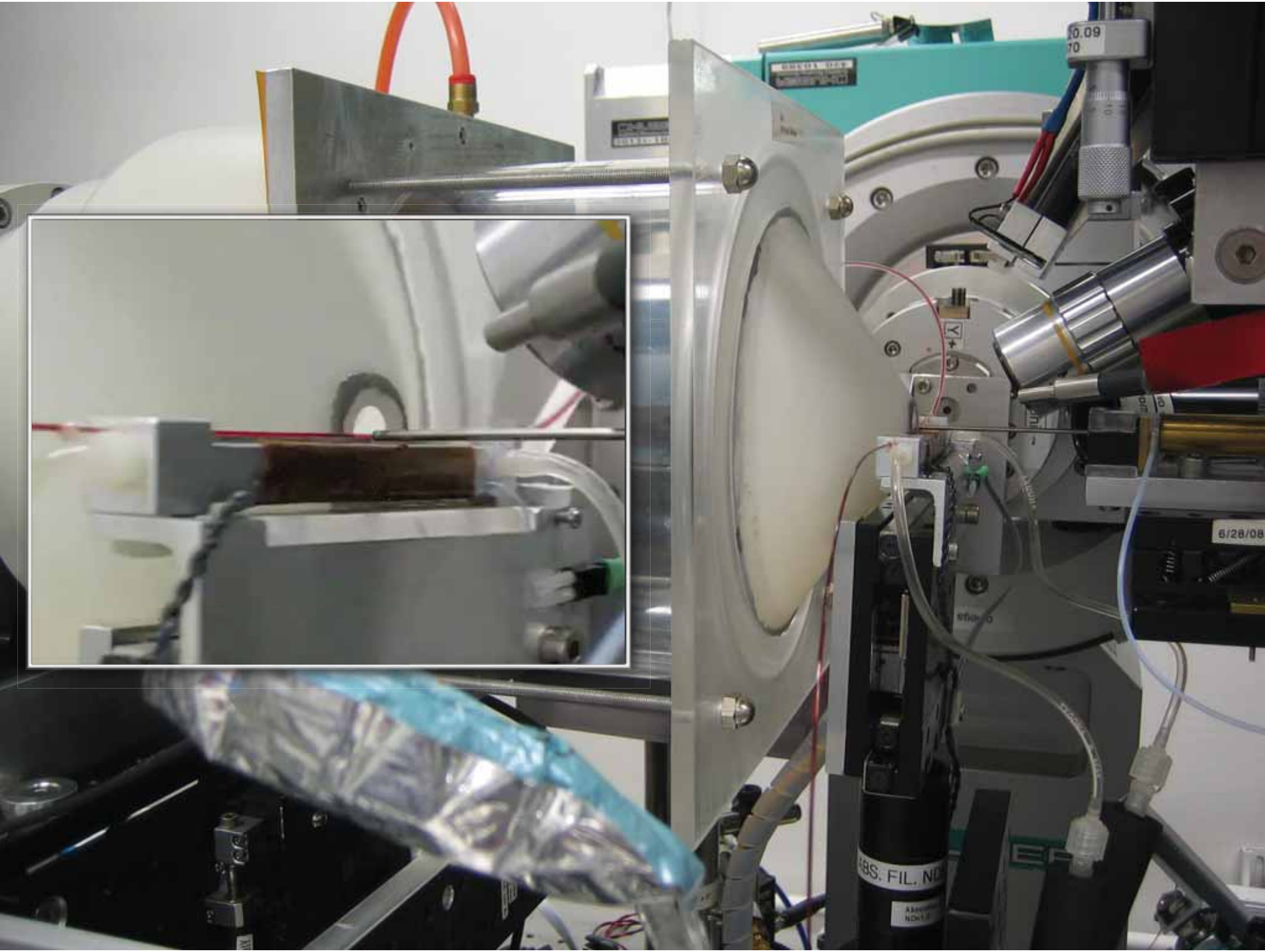


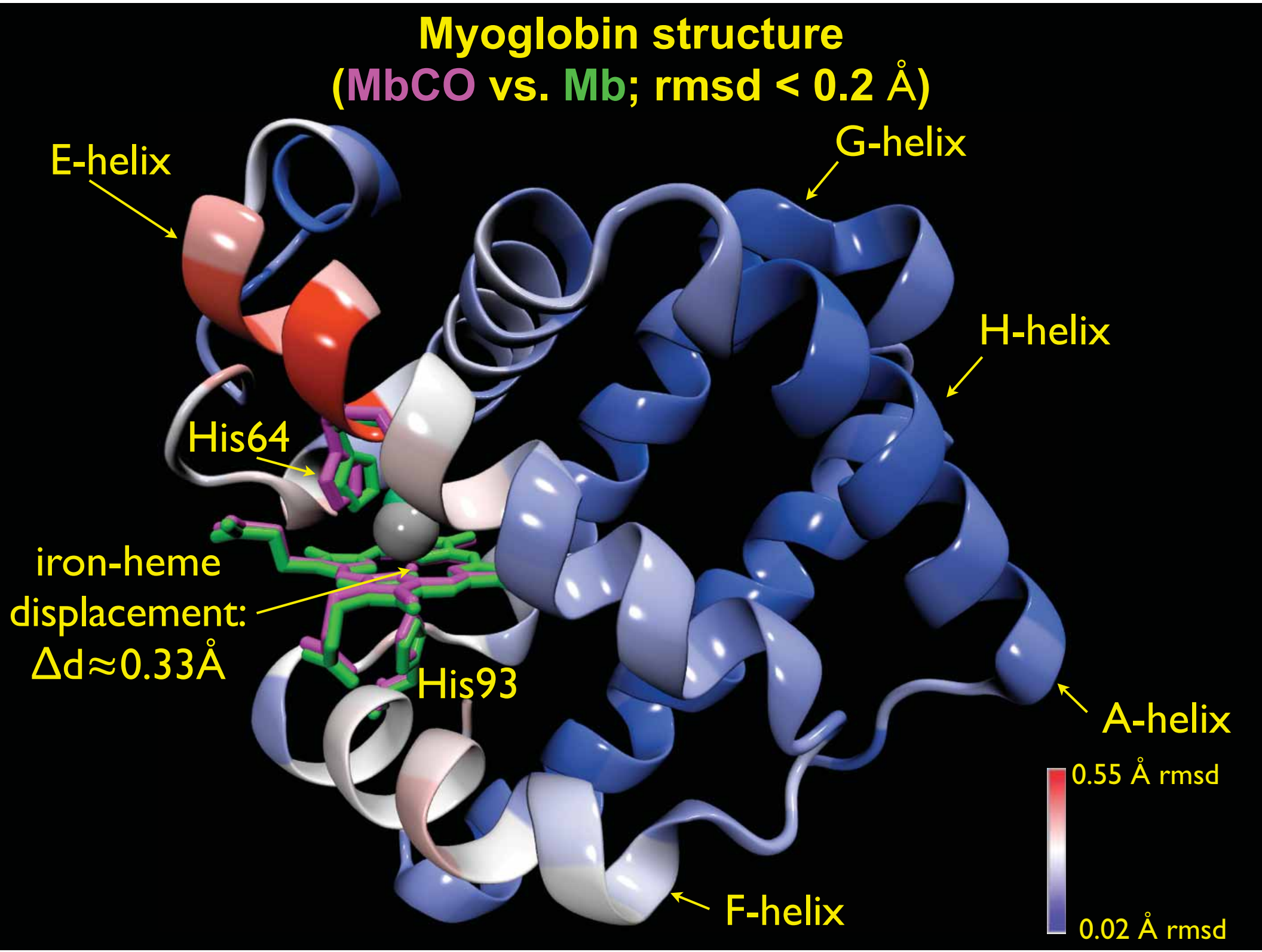
Beam-conditioning optics and Diffractometer: ~$120 K




6/28/08

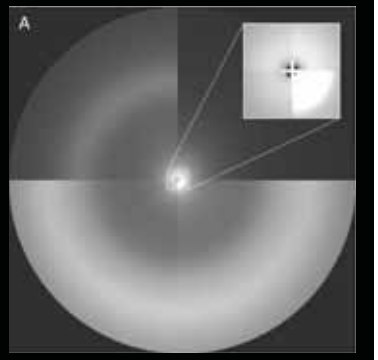

Laser Beam Profile
File Edit Orientation Camera Help
551 um
122 um
Live
Refresh
100%
0.15 s
Auto
mode: read-only, Bayer8


6/28/08

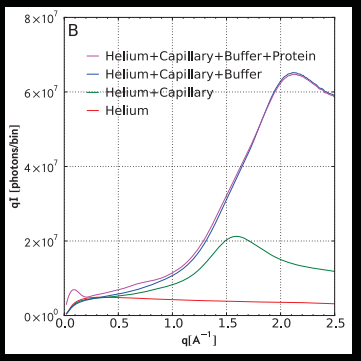

Myoglobin structure
(MbCO vs. Mb; rmsd < 0.2 Å)
E-helix
G-helix
H-helix
His64
iron-heme
displacement:
Δd≈0.33Å
His93
A-helix
F-helix
0.55 Å rmsd
0.02 Å rmsd

Static SAXS/WAXS of MbCO

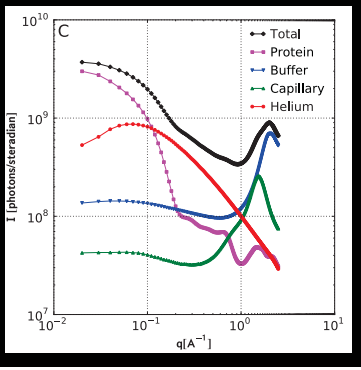
Composite MarCCD Image

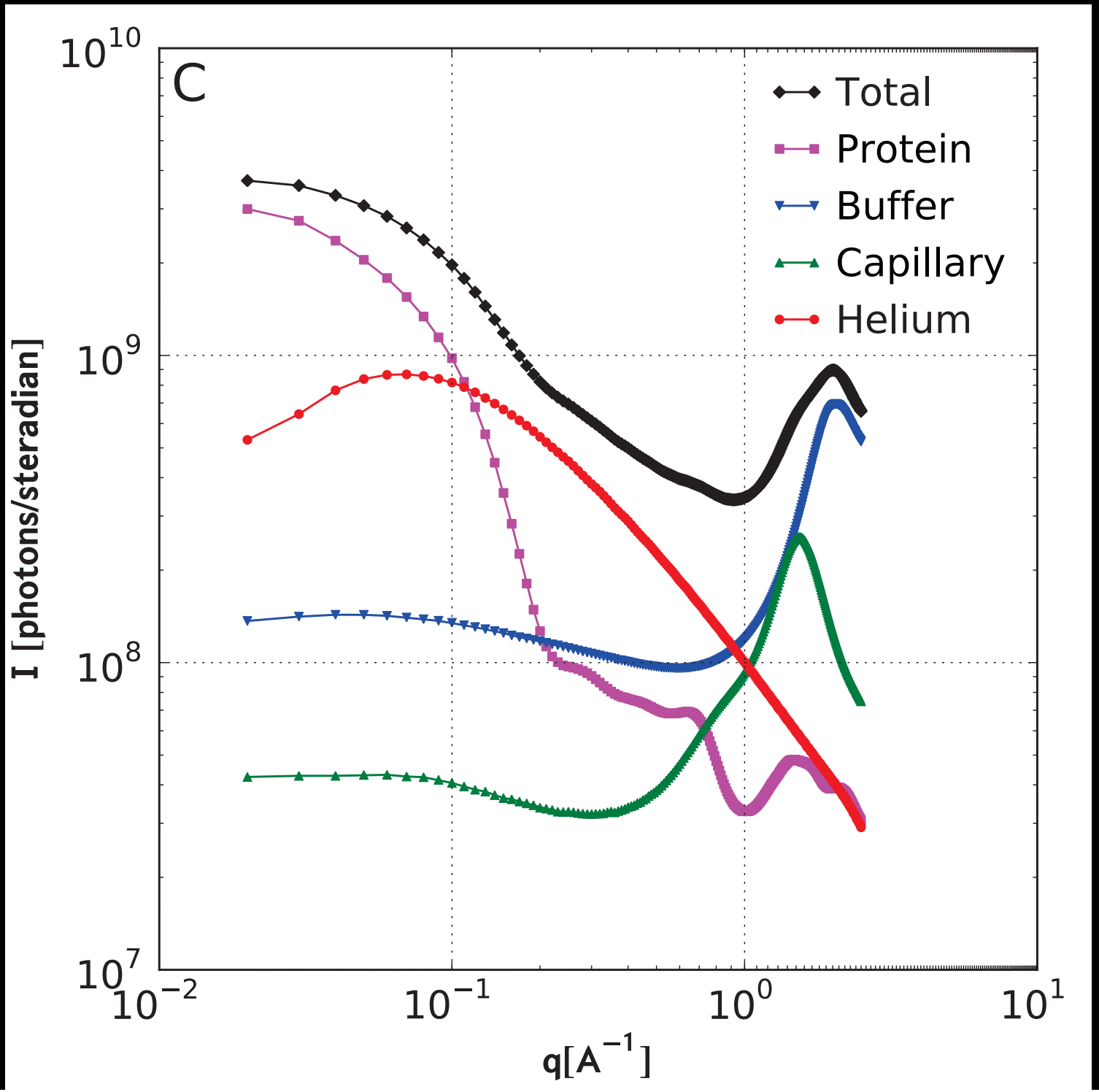


Angular Integration



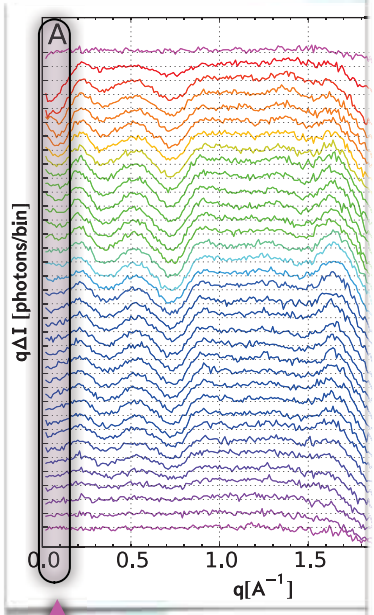
Decomposition



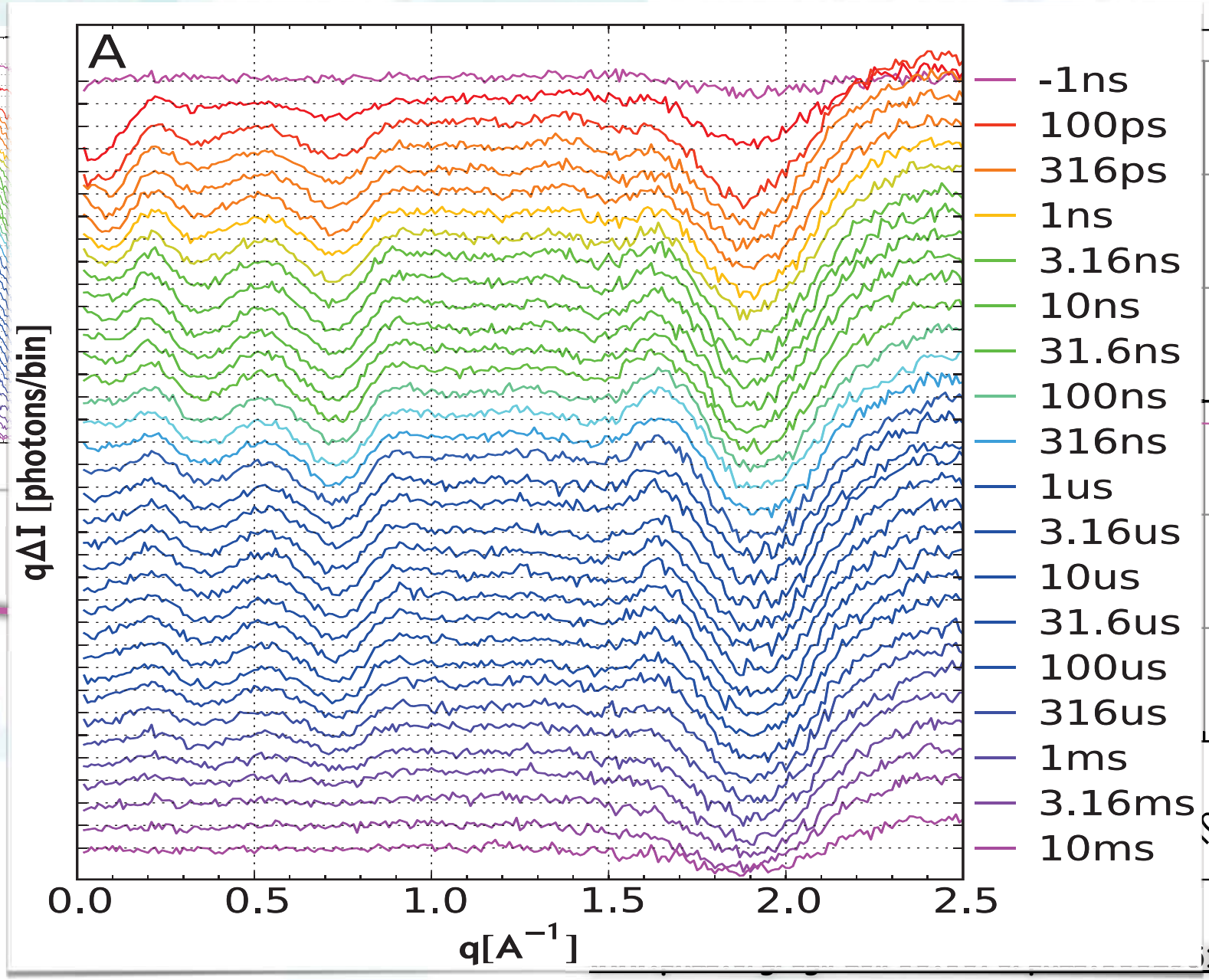


Time-resolved SAXS/WAXS

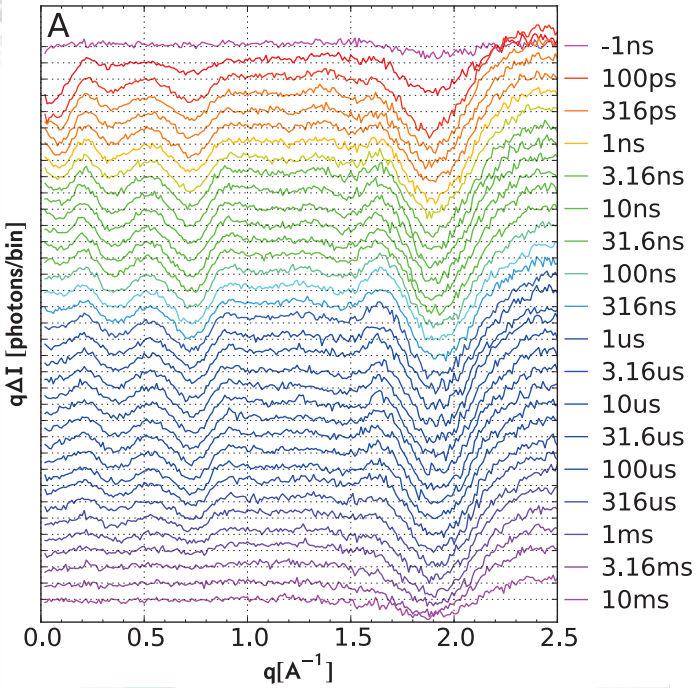
SAXS region is sensitive to volume changes and mass transport:
$I(q=0) \propto (n_p - n_b)^2$, where n_p and n_b are the number of electrons in the protein and in an equivalent volume of buffer (21,580 Å^3), respectively.

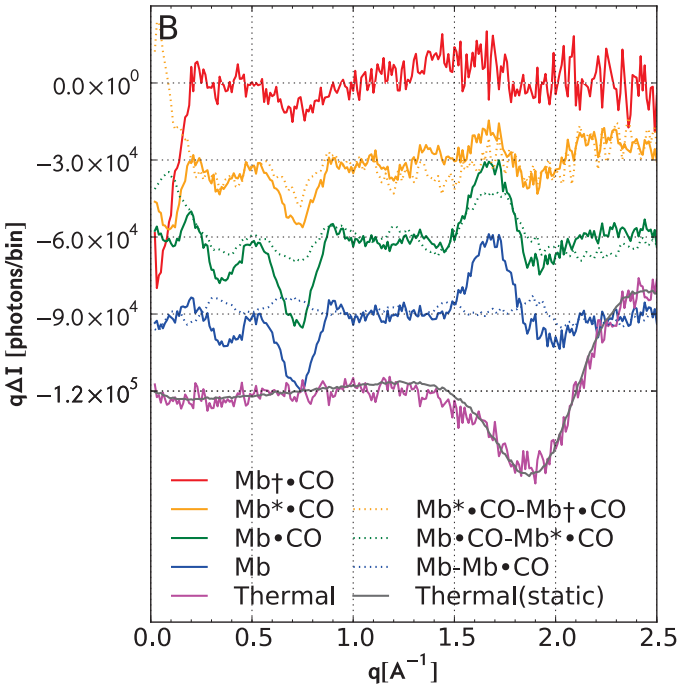


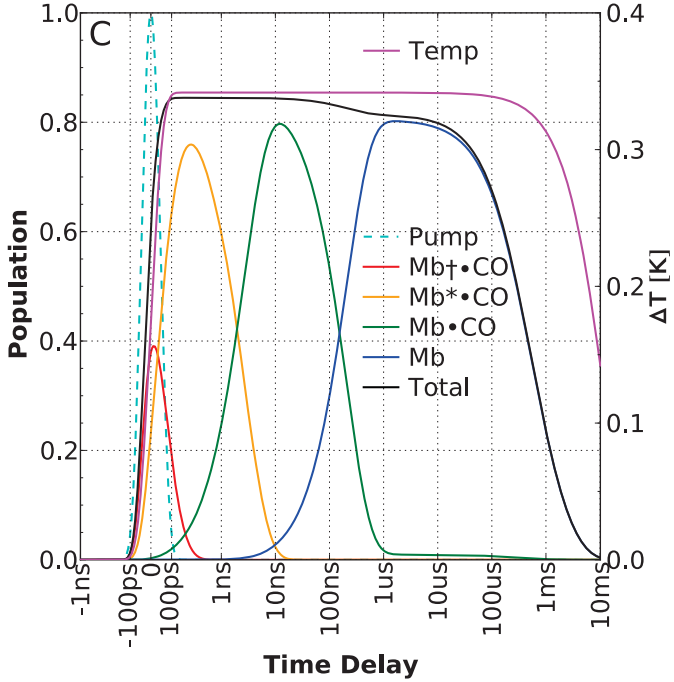
$\int q\Delta I dq$



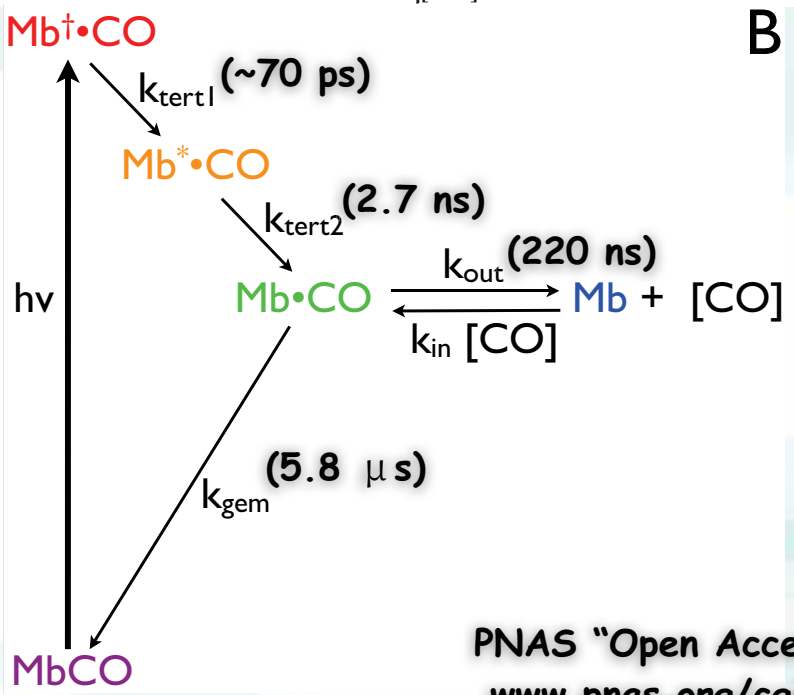
Time-resolved SAXS/WAXS of photolyzed MbCO







B



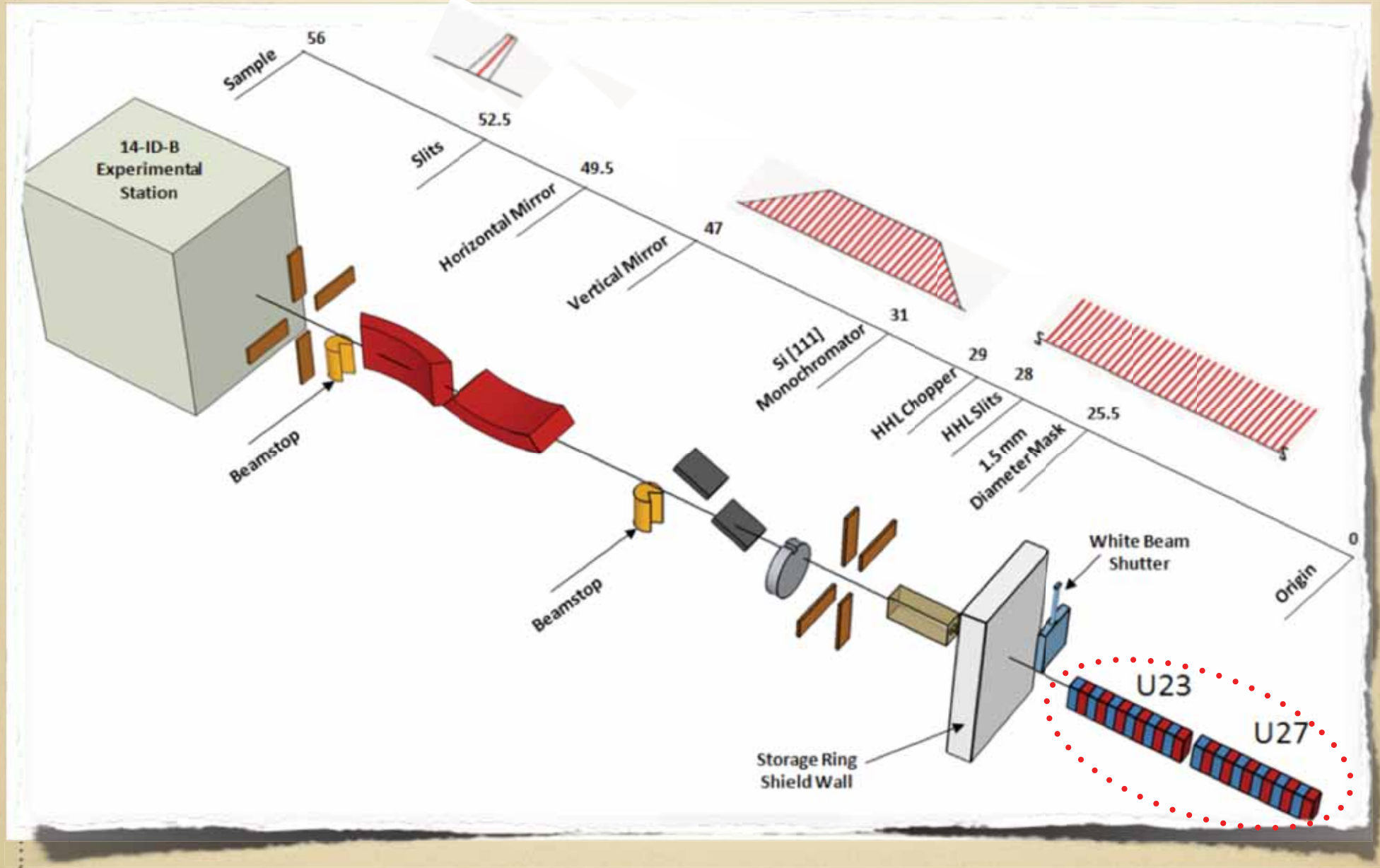
PNAS "Open Access":
www.pnas.org/cgi/doi/10.1073/pnas.1002951107

Its all about photons…

BioCARS

- 6×10^{9} photons on the detector at time of readout (integrate 1100 pump-probe pairs)
- few percent of total scattering comes from protein (50 mg/ml)
- few percent change in scattering due to pump-induced structural change
- Signal of interest: ~10^{-4}

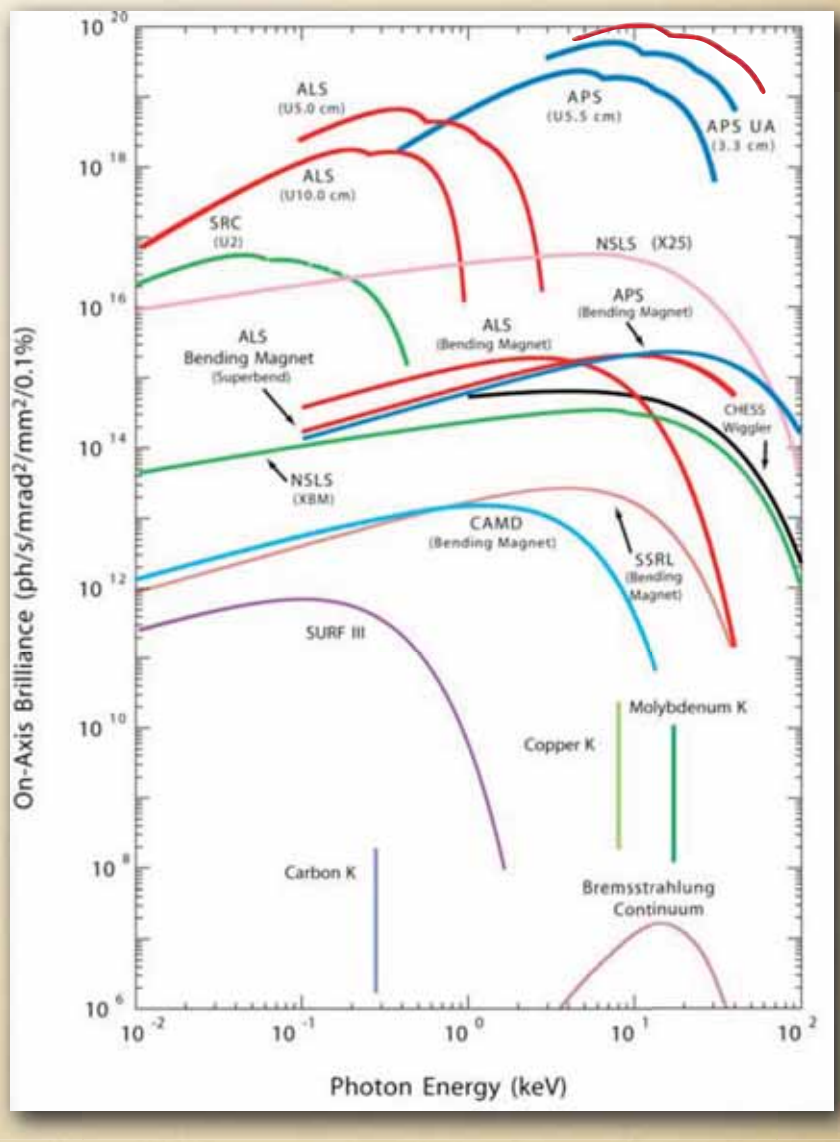
BioCARS ID14B Upgrade



Short-period undulators

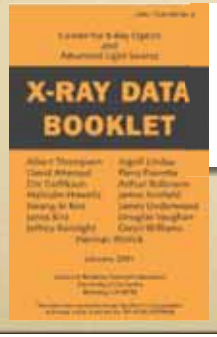
U23+U27 Undulators

(NIH/NIDDK paid $327K for magnets)



Source Characteristics

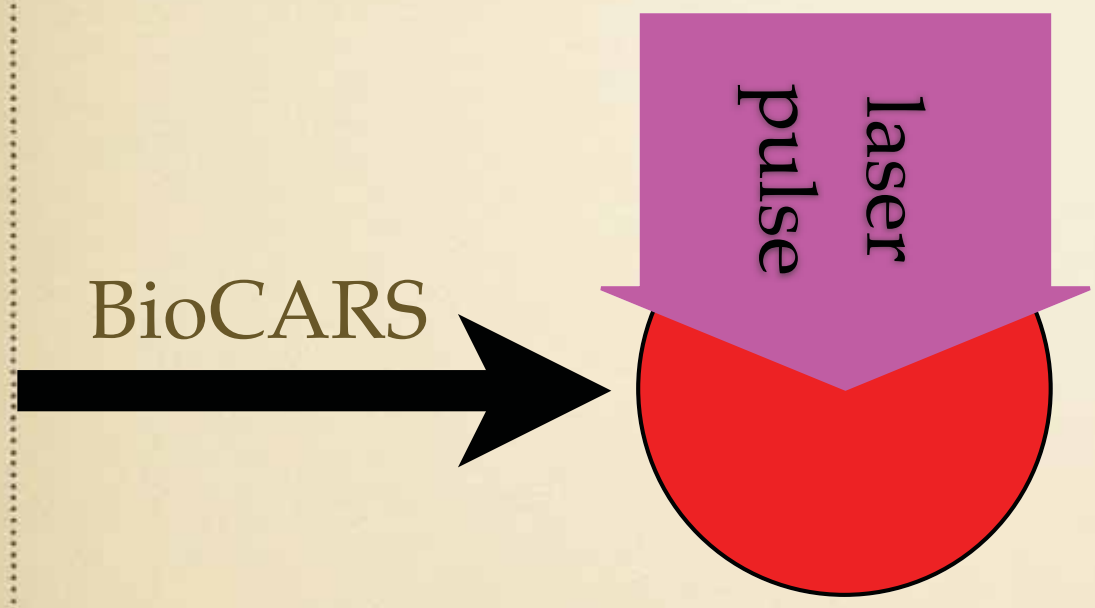
	APS (BioCARS)	LCLS	Cornell ERL (high flux)
E: electron energy (GeV)	7	14.35	5
bunch charge (nC)	**15**	**1**	**1**
pulse duration (ps)	**94**	**0.1**	**0.1**
L: undulator length (m)	2.4+2.4	130	25
undulator period (mm)	23+27	30	18
minimum gap (mm)	10.5	6	5
x-ray energy (keV)	12	8	8
repetition rate (Hz)	**41**	**120**	**up to 100,000**
x-ray flux (ph/pulse)	**3×10^{9}**	**1.5×10^{12}**	**$\sim5\times10^{8}$**



$$P_T[\mathrm{kW}] = 0.633\ E^2[\mathrm{GeV}]\,B_0^2[\mathrm{T}]\,L[\mathrm{m}]\,I[\mathrm{A}]$$

~ 20% of BioCARS

Sample geometry



BioCARS

600 μm

Pump-probe geometry limits time resolution:
Δt=2 ps

Cornell ERL

100 μm

Group velocity mismatch limits time resolution:
Δt=0.11 ps
n_{water} (589 nm) = 1.33

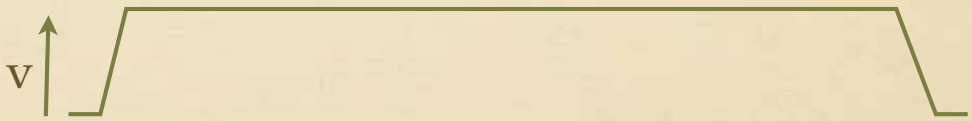
Spot Size/Repetition Frequency

BioCARS focus

- 25-mm translation range
- Translate sample 240 μm/shot to expose "fresh" volume
- move-stop-acquire sequence
- 5g acceleration allows 41 Hz operation

Cornell ERL focus (target)

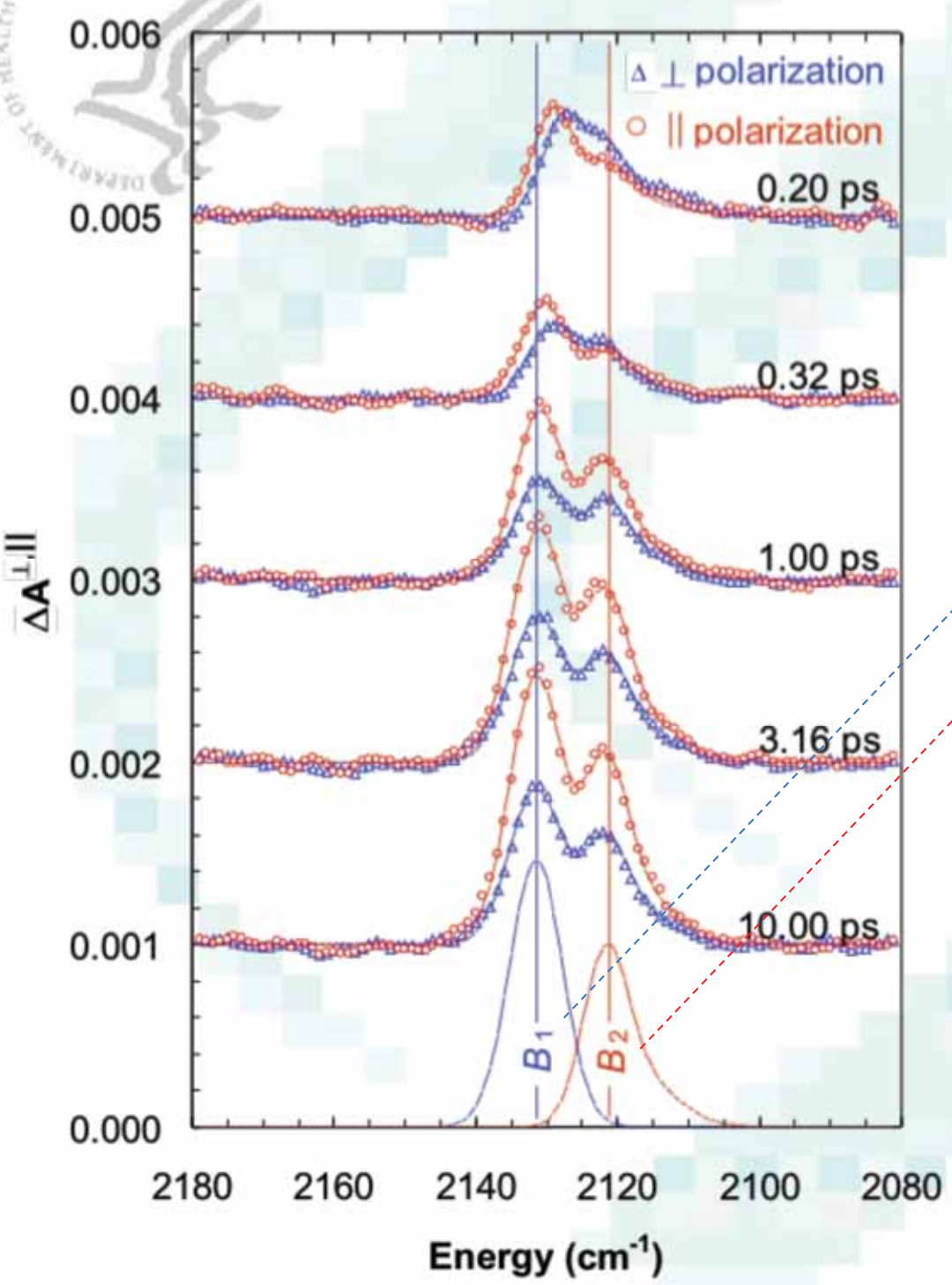
- 25-mm translation range
- 5g acceleration

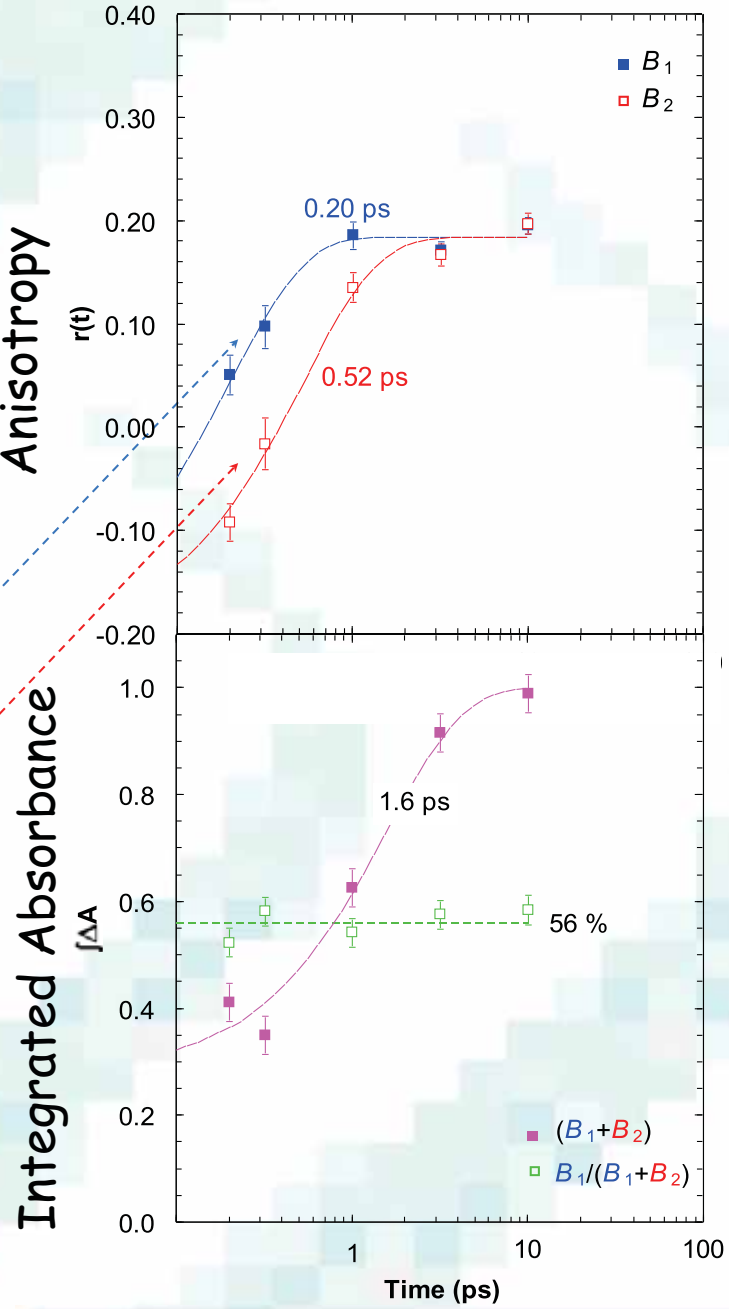


- 156 mm/s sample translation (restrict acceleration to 10% of stroke; $\Delta\tau$<30μs)
- 20-μm spot size (50-μm separation) → 450 pulses in 144 ms
- ~ 2800 Hz data acquisition (back and forth scanning)

~2 times faster data collection than BioCARS

Time-Resolved Polarized *B*-state Spectra

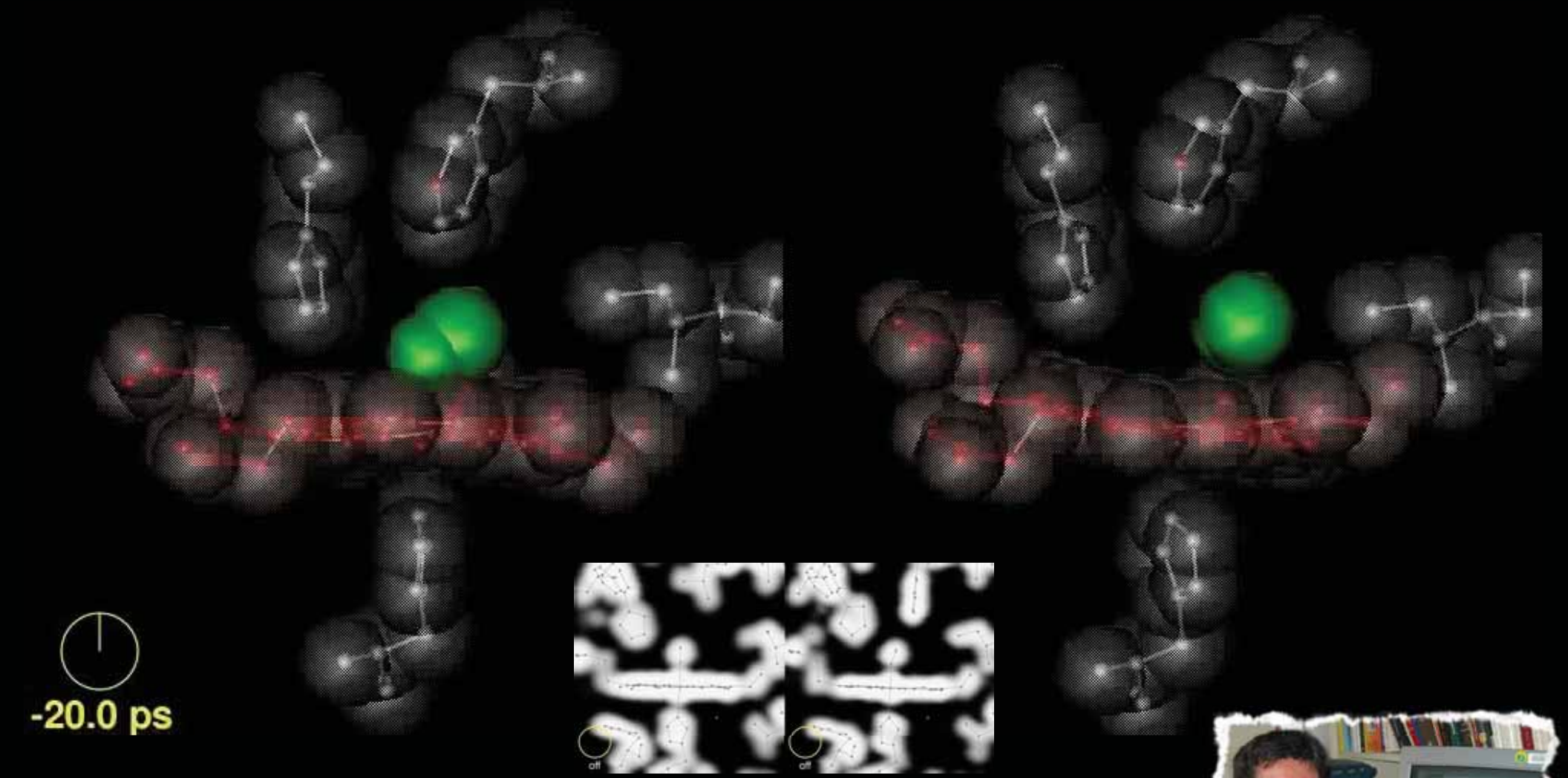




Lim, Jackson, and Anfinrud,
Nature Struc. Biol. **4**, 209 (1997)

Ligand migration trajectories in L29F MbCO

Phe site *Xe4 site*



Movie posted as supplementary online material for:

Hummer, Schotte, and Anfinrud,

PNAS 101, 15330-15334 (2004)

Summary and Outlook

- Time-resolved SAXS patterns afford exquisite sensitivity to protein volume changes and mass transport into and out of the protein.

- Time-resolved WAXS fingerprints contain a wealth of structural information down to 2.5 Å, and provide stringent constraints for putative models of conformational states and structural transitions between them.

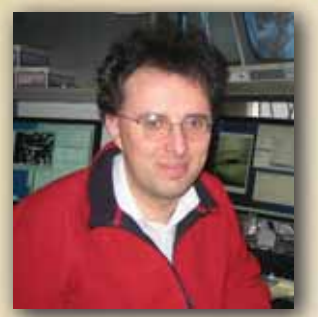
- Cornell ERL could improve the time resolution of SAXS/WAXS to ~100 fs.

- Time-resolved SAXS/WAXS is a valuable complement to time-resolved Laue crystallography, time-resolved laser spectroscopy, and computational modeling, and is proving increasingly useful in studies of protein structure, function, and dynamics.

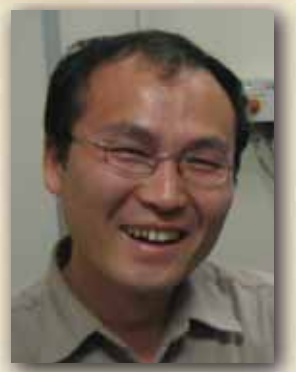
(Snowmaggedon 2010)

Acknowledgements

LCP



Dr. Friedrich Schotte



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Bernie Howder

Outside Collaborators

Prof. John Olson
Rice U.

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U Mass

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- Zhong Ren, Vukica Šrajer

Rice University

- Jayashree Soman (Mb, Hb crystals)

LCLS

- Marco Cammarata
- David Fritz

Germany

- Dwayne Miller
- Ilme Schlichting