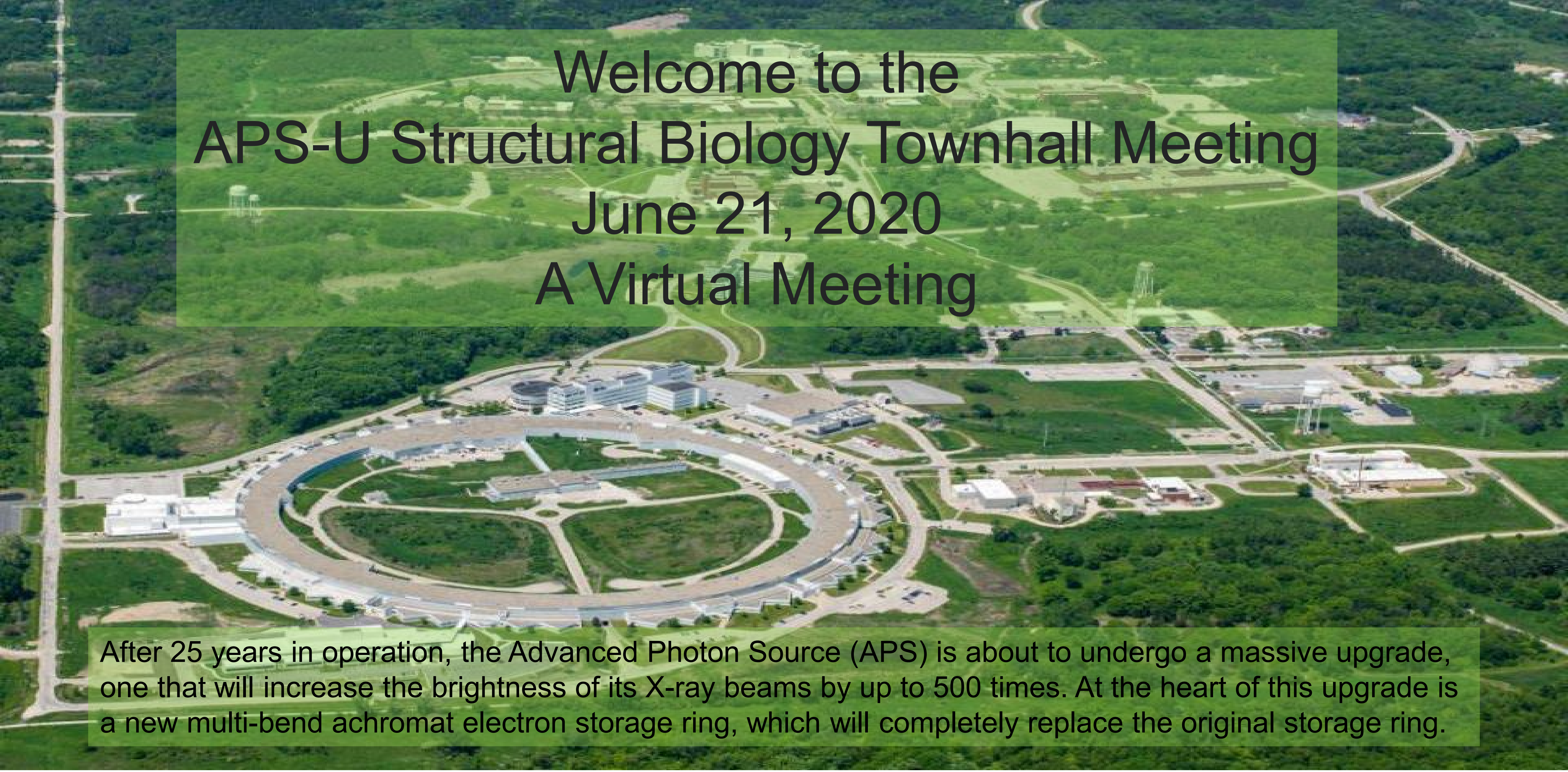


Welcome to the
APS-U Structural Biology Townhall Meeting

June 21, 2020

A Virtual Meeting

After 25 years in operation, the Advanced Photon Source (APS) is about to undergo a massive upgrade, one that will increase the brightness of its X-ray beams by up to 500 times. At the heart of this upgrade is a new multi-bend achromat electron storage ring, which will completely replace the original storage ring.

Argonne
NATIONAL LABORATORY

Welcome to the APS-U Structural Biology Townhall Meeting

Laurent Chapon is Argonne's Associate Laboratory Director for Photon Sciences, and director of the U.S. Department of Energy Office of Science's Advanced Photon Source (APS).

APS-U Structural Biology Townhall Agenda

Start	Duration	End	
1:00 PM	0:05	1:05 PM	Welcome - Laurent Chapon,
1:05 PM	0:25	1:30 PM	APS-U Overview and Life Sciences at the APS, Bob Fischetti
1:30 PM	0:15	1:45 PM	IMCA-CAT, Lisa Keefe
1:45 PM	0:15	2:00 PM	SBC/eBERLight, Andrzej Joachimiak and Karolina Michalska
2:00 PM	0:15	2:15 PM	LS-CAT, Spencer Anderson
2:15 PM	0:15	2:30 PM	SER-CAT, Bi-Chen Wang
2:30 PM	0:15	2:45 PM	GM/CA-CAT, Naga Venugopalan
2:45 PM	0:15	3:00 PM	Break
3:00 PM	0:15	3:15 PM	NE-CAT, Malcolm Capel
3:15 PM	0:15	3:30 PM	LRL-CAT, Jordi Benach
3:30 PM	0:15	3:45 PM	ALS, Marc Allaire
3:45 PM	0:15	4:00 PM	CHESS, Marian Szebenyi
4:00 PM	0:15	4:15 PM	NSLS-II, Sean McSweeney
4:15 PM	0:15	4:30 PM	SSRL, Aina Cohen
4:30 PM	0:30	5:00 PM	Roundtable Q&A
5:00 PM			Adjourn

APS-U Structural Biology Townhall Guidelines

Each APS CAT or Light Source has 15 minutes (10 min. presentation, 5 min. Q&A)

Speakers, please remember to say next slide

A 10-minute notice will be given. After 15 minutes we will move to the next speaker.

Participants can enter questions in the CHAT (your microphone will be disabled)

Speakers can address questions from the CHAT during their Q&A

Argonne staff will monitor the CHAT for unanswered questions which may be addressed during the Roundtable

During the Roundtable participants may use the RAISE HAND function and use their microphone when called on

OVERVIEW OF LIFE SCIENCES AT THE APS AND THE APS-UPGRADE

Bob Fischetti
Life Sciences Advisor to the APS Director
and GM/CA@APS Group Leader
Advanced Photon Source

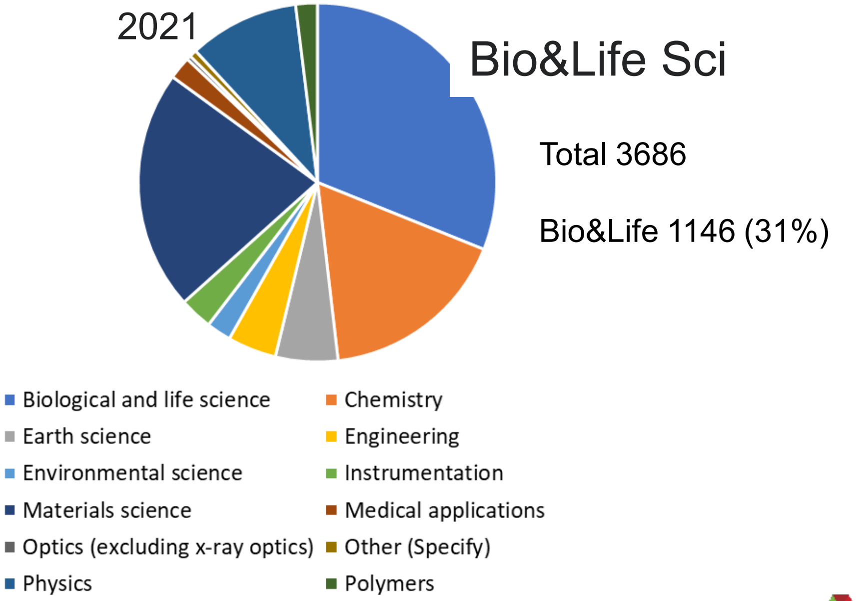
Structural Biology Townhall
June 21, 2019

Outline

- Structural Biology at the Advanced Photon Source
- APS Upgrade (APS-U)
- Radiation damage

Argonne’s Advanced Photon Source (APS) a DOE Office of Science User Facility

- The APS provides high-energy, high-brightness X-ray beams that are ideal for studying the arrangements of molecules and atoms
- Use of the APS (and other Office of Science user facilities) is free for open research intended for publication
- There are 68 beamlines at the APS where over 5300 researchers do their work every year
- Scientists use 15 of these specialized beamlines for macromolecular crystallography, to examine molecular structures and interactions that could yield lifesaving medicines for various diseases
- Biological & Life Sciences accounts for ~$^1/_3$ of APS users
- Users have studied many diseases and drugs – cancer, influenza, Type 2 diabetes, HIV/AIDS, Ebola, Zika, COVID-19

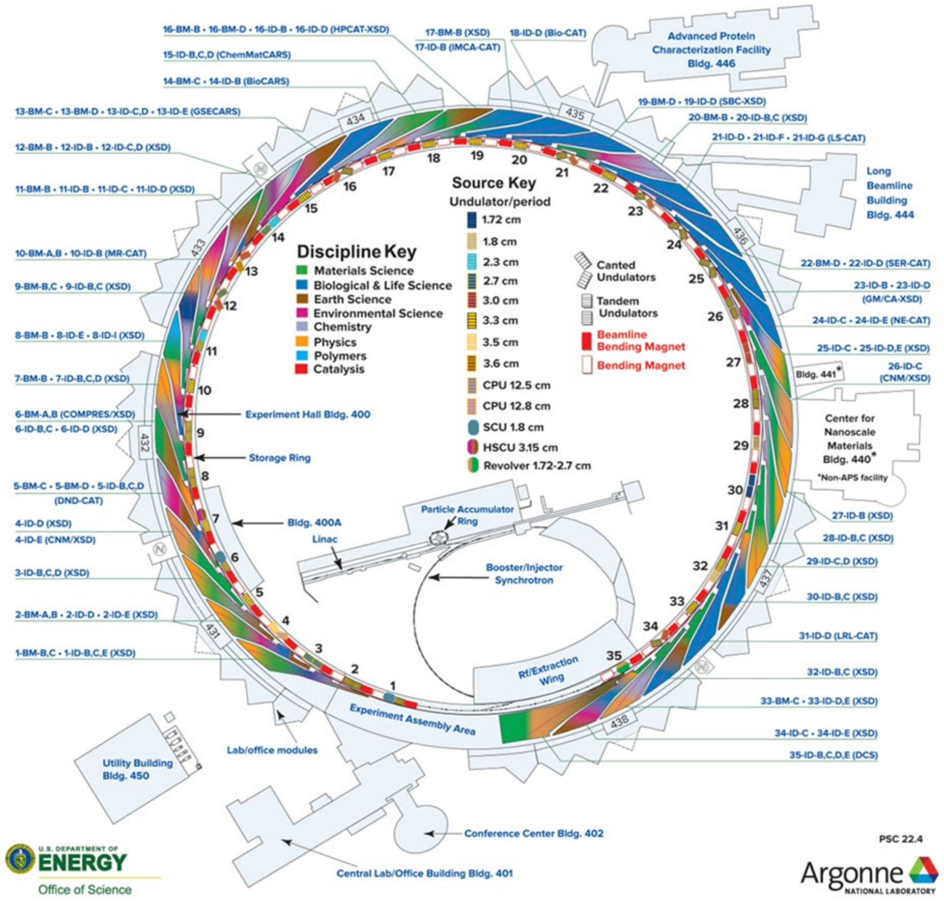


BEAMLINES THAT FULLY OR PARTIALLY SUPPORT LIFE SCIENCES RESEARCH

MX beamlines were built and are operated by CATS

8 Sectors - 15 MX beamlines → 11.8 IDs - 2 BMs

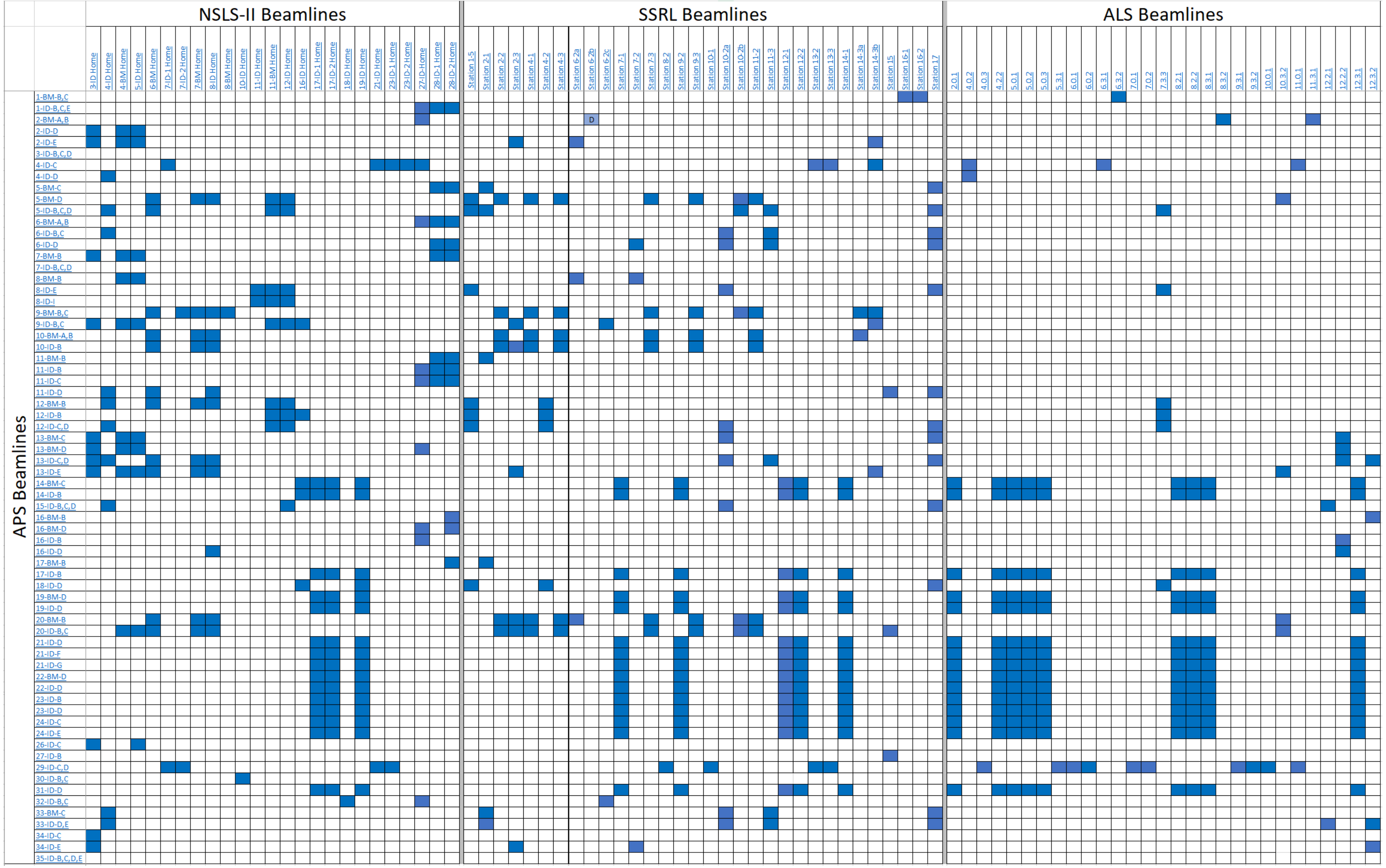
Beamline	Disciplines	Techniques	Operator
14-BM-C	Life Sciences	Macromolecular crystallography, fiber diffraction, BLS2/3	BioCARS
14-ID-B	Life Sciences	Time-resolved cryst., Laue Cryst., WAXS, MX, SMX, BSL2/3	BioCARS
17-ID-B	Life Sciences	Macromolecular crystallography, microbeam, MAD, SAD	IMCA-CAT
19-BM-D	Life Sciences	Macromolecular crystallography, MAD, SAD	SBC-APS-XSD
19-ID-D	Life Sciences	Macromolecular crystallography, MAD, SAD, SMX	SBC-APS-XSD
21-ID-D	Life Sciences	Macromolecular crystallography	LS-CAT
21-ID-F	Life Sciences	Macromolecular crystallography	LS-CAT
21-ID-G	Life Sciences	Macromolecular crystallography	LS-CAT
22-BM-D	Life Sciences	Macromolecular crystallography, MAD, SAD	SER-CAT
22-ID-D	Life Sciences	Macromolecular crystallography, microbeam, MAD, SAD	SER-CAT
23-ID-B	Life Sciences	Macromolecular crystallography, microbeam, MAD, SAD, SMX	GM/CA-APS-XSD
23-ID-D	Life Sciences	Macromolecular crystallography, microbeam, MAD, SAD, SMX	GM/CA-APS-XSD
24-ID-C	Life Sciences	Macromolecular crystallography, microbeam, MAD, SAD	NE-CAT
24-ID-E	Life Sciences	Macromolecular crystallography, microbeam, SAD	NE-CAT
31-ID-D	Life Sciences	Macromolecular crystallography, SAD	LRL-CAT
1-ID-B,C,E	Materials Science	High-energy x-ray diffraction, tomography, phase-contrast imaging	APS-XSD
2-BM-A,B	Physics	Tomography, Phase contrast imaging	APS-XSD
2-ID-D	Life Sciences	Microfluorescence, ptychography	APS-XSD
2-ID-E	Life Sciences	Microfluorescence, tomography	APS-XSD
3-ID-B,C,D	Physics	Nuclear resonant scattering, inelastic x-ray scattering	APS-XSD
5-ID-B,C,D	Materials Science	Powder diffraction, X-ray standing wave, X-ray reflectivity, WAXS	DND-CAT
8-BM-B	Chemistry	Fluorescence spectroscopy	APS-XSD
9-ID-B,C	Chemistry	Nano-imaging, microfluorescence, tomograpahy, U-SAXS, tomography	APS-XSD
12-ID-B	Chemistry	SAXS, WAXS, G-SAXS,	APS-XSD
18-ID-D	Life Sciences	Fiber diffraction, microdiffraction, SXAS, TR-SAXS	BIO-CAT
30-ID-B,C	Physics	Inelastic x-ray scattering (1 meV resolution), nuclear resonant scattering	APS-XSD
32-ID-B,C	Materials Science	Phase contrast imaging, radiography, transmission X-ray microscopy, tomography	APS-XSD



MAPPING OF APS BEAMLINE CAPABILITIES TO DOE FACILITIES

APS > APS-U > Comparable Beamline Options for Users

Denny Mills and Managers from other Light Sources



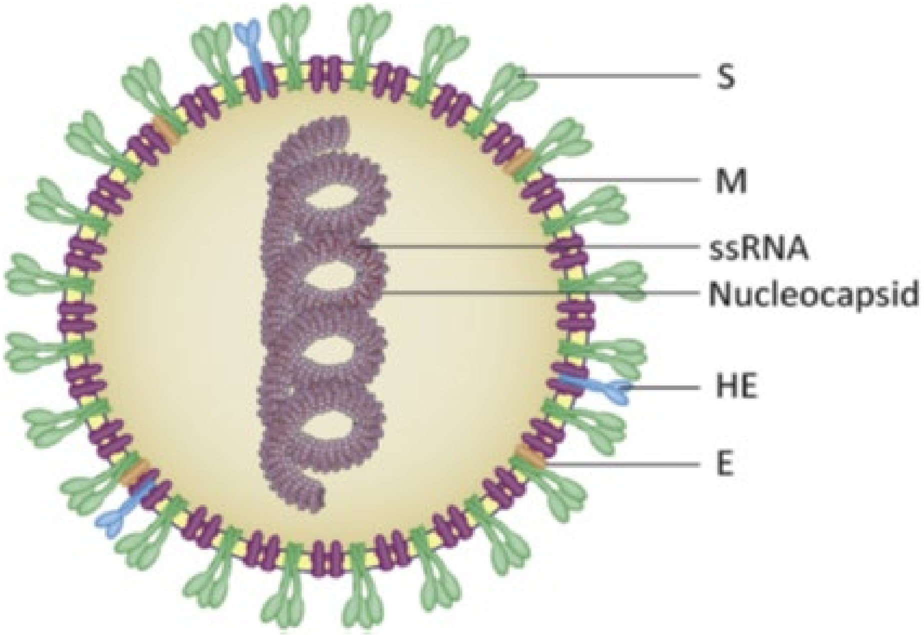
MAPPING OF APS LIFE SCIENCE CAPABILITIES TO OTHER FACILITIES

	NSLS-II															SSRL																	ALS												CHESS			
APS Beamlines	3-ID Home	4-ID Home	4-BM Home	5-ID Home	6-BM Home	10-ID Home	11-BM Home	12-ID Home	16-ID Home	17-ID-1 Home	17-ID-2 Home	19-ID Home	27-ID-Home	28-ID-1 Home	28-ID-2 Home	Station 1-5	Station 2-1	Station 2-3	Station 4-2	Station 6-2a	Station 6-2b	Station 6-2c	Station 7-1	Station 7-2	Station 9-2	Station 10-2b	Station 11-3	Station 12-1	Station 12-2	Station 14-1	Station 14-3b	Station 17	2.0.1	4.2.2	5.0.1	5.0.2	5.0.3	7.3.3	8.2.1	8.2.2	8.3.1	8.3.2	11.3.1	12.3.1	ID7A	ID7B2	ID2A	ID4B
1-ID-B,C,E													■	■	■																																	
2-BM-A,B													■								D																					■	■					
2-ID-D	■		■	■																																												
2-ID-E	■		■	■														■		■											■																	
3-ID-B,C,D																																																■
5-ID-B,C,D		■			■		■	■								■	■									■	■					■						■							■			
8-BM-B			■	■																■				■																							■	
9-ID-B,C	■		■	■			■	■	■									■				■									■																	
12-ID-B							■	■	■							■			■																			■							■			
14-BM-C									■	■	■	■											■		■			■	■	■			■	■	■	■	■		■	■	■			■		■		
14-ID-B									■	■	■	■											■		■			■	■	■			■	■	■	■	■		■	■	■			■	■	■		
17-ID-B										■	■	■											■		■			■	■	■			■	■	■	■	■		■	■	■			■		■		■
18-ID-D									■			■				■			■													■						■							■			
19-BM-D										■	■	■											■		■			■	■	■			■	■	■	■	■		■	■	■			■		■		■
19-ID-D										■	■	■											■		■			■	■	■			■	■	■	■	■		■	■	■			■		■		■
21-ID-D										■	■	■											■		■			■	■	■			■	■	■	■	■		■	■	■			■		■		
21-ID-F										■	■	■											■		■			■	■	■			■	■	■	■	■		■	■	■			■		■		
21-ID-G										■	■	■											■		■			■	■	■			■	■	■	■	■		■	■	■			■		■		
22-BM-D										■	■	■											■		■			■	■	■			■	■	■	■	■		■	■	■			■		■		■
22-ID-D										■	■	■											■		■			■	■	■			■	■	■	■	■		■	■	■			■		■		■
23-ID-B										■	■	■											■		■			■	■	■			■	■	■	■	■		■	■	■			■		■		■
23-ID-D										■	■	■											■		■			■	■	■			■	■	■	■	■		■	■	■			■		■		■
24-ID-C										■	■	■											■		■			■	■	■			■	■	■	■	■		■	■	■			■		■		■
24-ID-E										■	■	■											■		■			■	■	■			■	■	■	■	■		■	■	■			■		■		
30-ID-B,C						■																																										
31-ID-D										■	■	■											■		■			■	■	■			■	■	■	■	■		■	■	■			■		■		
32-ID-B,C													■									■																										

SARS CoV-2 Viral Protein Composition

Viral structure

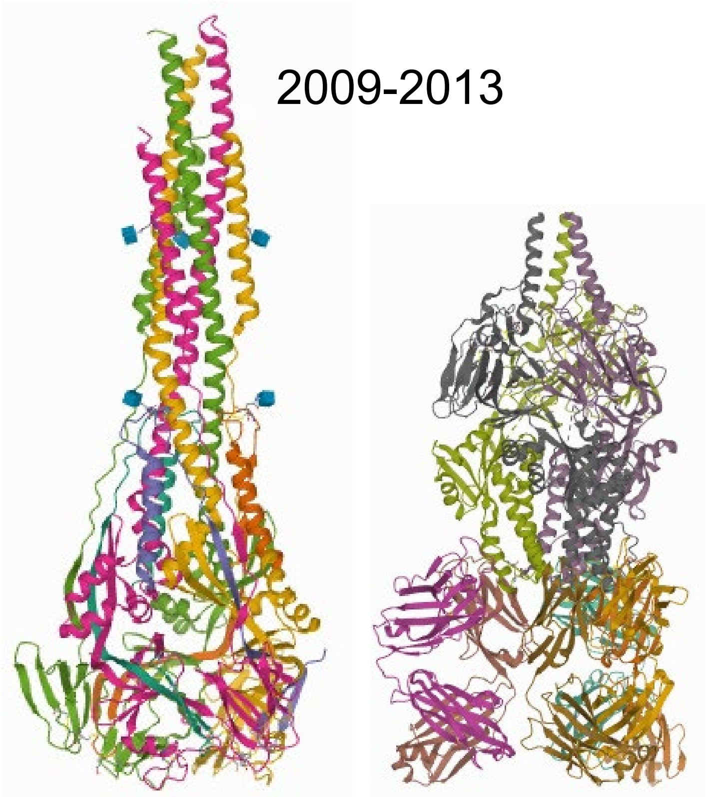
- The SARS CoV-2 virus is ~120 nm in diameter (1/1000 of a human hair)
- It is composed of 28 unique proteins
- Potentially all of the proteins are antiviral or vaccine targets
- Important protein targets include:
  - NSP3 (papain-like protease): involved in replication and may interfere with immune response
  - NSP5 (main protease, 3CLpro): involved in replication
  - Spike: initiates infection by binding to the cell receptor
- Prior to the COVID-19 pandemic, researchers utilizing the APS determined 70 coronavirus protein structures
- APS beamlines rank 3rd in the number of SARS CoV-2 structures (>280)
- APS beamlines provided the most diverse coverage of the 28 unique proteins
- PAXLOVID™ was developed using IMCA-CAT beamline



Viruses. Family Coronaviridae.
DOI: http://dx.doi.org/10.1016/B978-0-12-803109-4.00017-9

COVID-19 Vaccine & Antibody Development

2009-2013



2013: Respiratory Syncytial Virus (RSV) Fusion Glycoprotein (F). Early vaccines generated ineffective antibodies against F in the post-fusion state (left). Human antibody D25 stabilizing metastable F in the pre-fusion state (right). APS 22-ID

McLellan, Graham, et al.

2013-2017

2015: Middle East respiratory syndrome coronavirus (MERS-CoV) receptor binding domain with spike protein trimers (one up) stabilized in the prefusion state with the 2-proline mutation.

2020: This has been incorporated in SARS-CoV-2 mRNA vaccines.

Cryo-EM, APS 19-ID, SSRL

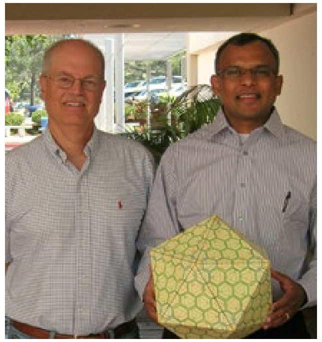
2020

2020: SARS CoV-2 RBD (green) with multivalent neutralizing nanobody NB20 (brown) which may be more effective and resistant to mutations. APS 23-ID

Adenovirus deliver vehicle for COVID-19 vaccines

“Crystal Structure of Human Adenovirus at 3.5 Å Resolution,” Science 329, 1071 (2010).

- Largest biomolecule structure determined to atomic resolution with X-rays
- Modified adenovirus delivers DNA for cells to produce stabilized SARS CoV-2 spike protein used in Janssen’s (Johnson & Johnson) and AstraZeneca’s vaccines

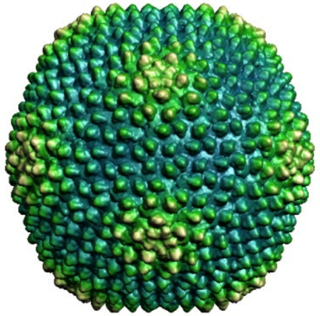


Vijay Reddy
Glen Nemerow

Myoglobin (2W6X)

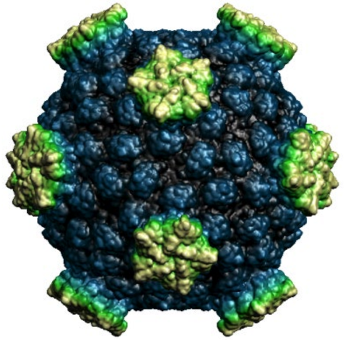
Ribosome (70S; 2X9S)

Bacteriophage PRD1; 1W8X



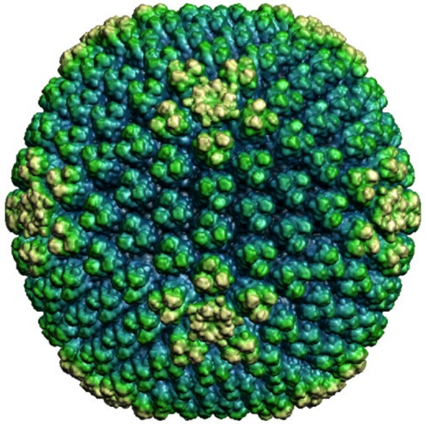
pT=25

Reovirus core (1EJ6)



T=1

Adenovirus (1VSZ/2BLD)



pT=25 ←T number

Dia. (Å):	42	280	702	754	960
# a.a. in IAU:	153	3,394	4,915	4,370	15,155
DNA/RNA (Kbp):	N/A	3.3	14.9	23.5	35.9

Protein Data Bank Deposits

Number of Protein Data Bank Deposits																		
Source	**Feb 2020**	**Mar 2020**	**Apr 2020**	**May 2020**	**Jun 2020**	**Jul 2020**	**Aug 2020**	**Sep 2020**	**Oct 2020**	**Nov 2020**	**Dec 2020**	**Jan 2021**	**2/3/2021**	**2/10/2021**	**2/17/2021**	**2/24/2021**	**3/3/21**	**Facility total**
ALS	0	0	0	1	0	0	2	1	4	1	103	0	0	0	1	0	0	113
APS	0	13	13	18	8	12	20	7	4	4	9	6	8	2	6	5	4	139
Australian Synchrotron	0	0	1	1	2	0	0	0	1	0	0	6	1	0	0	2	0	14
BESSY	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
CHESS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
CLS	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0	0	3
CryoEM	1	5	5	8	8	38	16	25	22	33	35	6	6	5	1	3	13	230
DLS	0	77	19	18	10	2	0	52	0	6	10	106	0	0	0	0	11	311
ELETTRA	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	5	8
Home source	2	1	1	2	4	4	1	3	1	0	2	2	0	1	0	0	1	25
Korea	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
LCLS	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0	0	4
MAX-IV	0	0	0	0	1	0	0	0	1	0	2	0	0	0	0	0	0	4
NMR	0	0	1	0	0	0	0	2	1	1	0	0	0	0	0	0	0	5
NSLS-II	0	0	0	0	1	0	0	5	0	0	4	1	0	0	1	0	3	15
ORNL-neutron	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	2
PETRA3	0	1	1	3	1	0	2	0	2		27	1	0	0	0	1	4	43
Shanghai	1	3	6	7	7	2	1	17	1	5	0	1	0	0	3	0	1	55
SIRUS	0	0	0	0	0	0	2	0	2	1	6	1	2	0	0	0	0	14
SLS	0	0	0	5	0	1	0	0	1	0	0	4	0	0	0	0	0	11
Spring-8	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	2
SSRL	0	0	0	3	0	5	2	0	12	0	35	2	0	1	3	0	0	63
Taiwan	0	0	0	0	2	0	0	0	1	2	0	0	0	0	0	0		5
Weekly/Monthly total	**4**	**103**	**47**	**66**	**45**	**64**	**46**	**117**	**54**	**53**	**236**	**139**	**17**	**11**	**15**	**12**	**42**	1071
Accumulative total	**4**	**107**	**154**	**220**	**265**	**329**	**375**	**492**	**546**	**599**	**835**	**974**	**991**	**1002**	**1017**	**1029**	**1071**	

Users of US light sources have contributed over 25% of the structures

Nobel-Prize winning RESEARCH AT THE APS

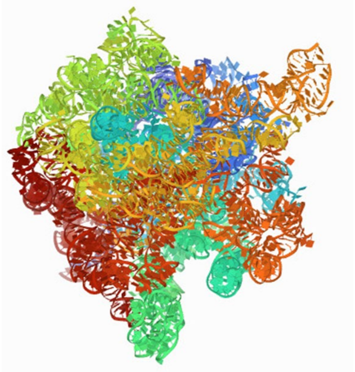
Important discoveries about human physiology and clues to new pharmaceuticals to combat disease

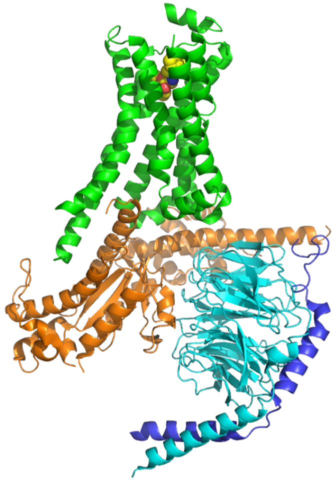
Ada Yonath
Weizmann Institute
of Science, Israel

Thomas Steitz
Yale University, U.S.

Venkatraman Ramakrishnan
MRC Laboratory of
Molecular Biology, U.K.

2009 Nobel Prize in Chemistry
Structure and
function of
the ribosome





2012 Nobel Prize in Chemistry
Studies of
G-protein-coupled
receptors (GPCRs)

Brian K. Kobilka
Stanford University, U.S.

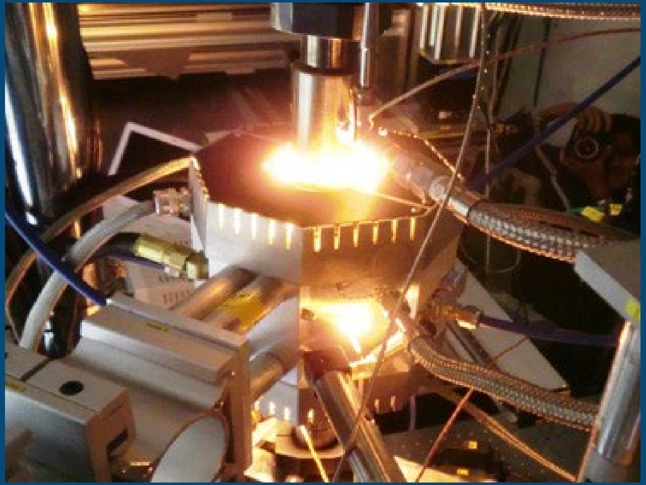
Robert J. Lefkowitz
Duke University, U.S.

APS-U: THE ULTIMATE 3D MICROSCOPE

High Energy

Penetrating bulk materials and operating systems

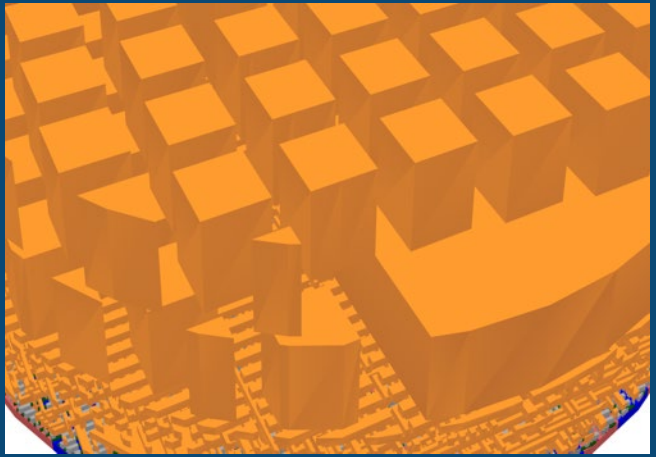
- World's brightest source of hard x-rays
- 3D mapping deep inside samples
- X-ray cinematography in previously inaccessible regimes



Brightness

Providing macroscopic fields of view with nm-scale resolution

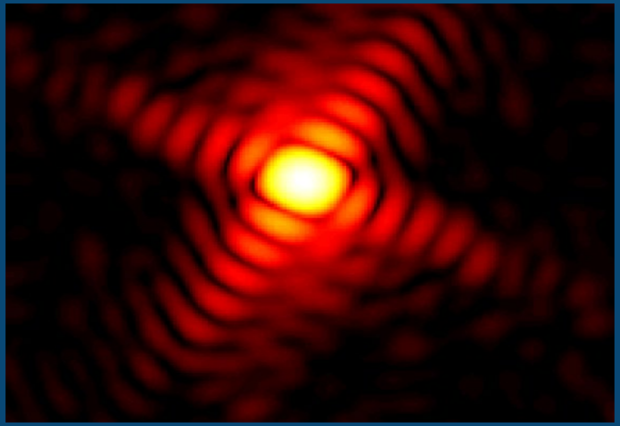
- Multi-scale imaging connecting nanometer features across macroscopic dimensions
- Fast sampling with chemical, magnetic, electronic sensitivity



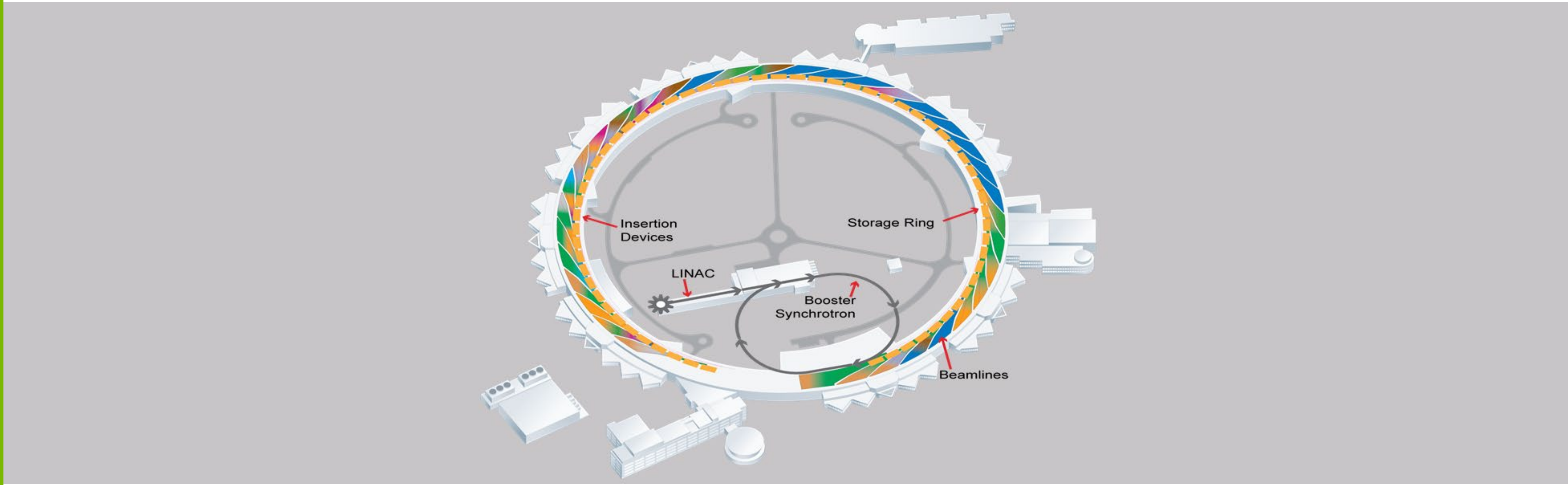
Coherence

Enabling highest spatial resolution even in non-periodic materials

- Extends lens-less imaging to hard x-ray domain, with resolution down to <1 nm, localizing atoms
- Increases phase contrast for fast full-field imaging
- Correlation methods improve by 10,000x-1,000,000x



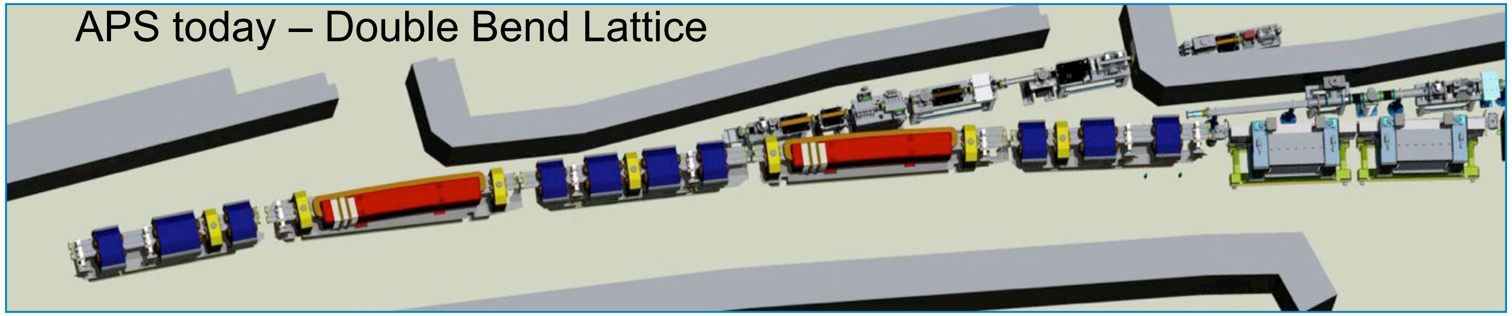
The APS accelerator complex and beamlines



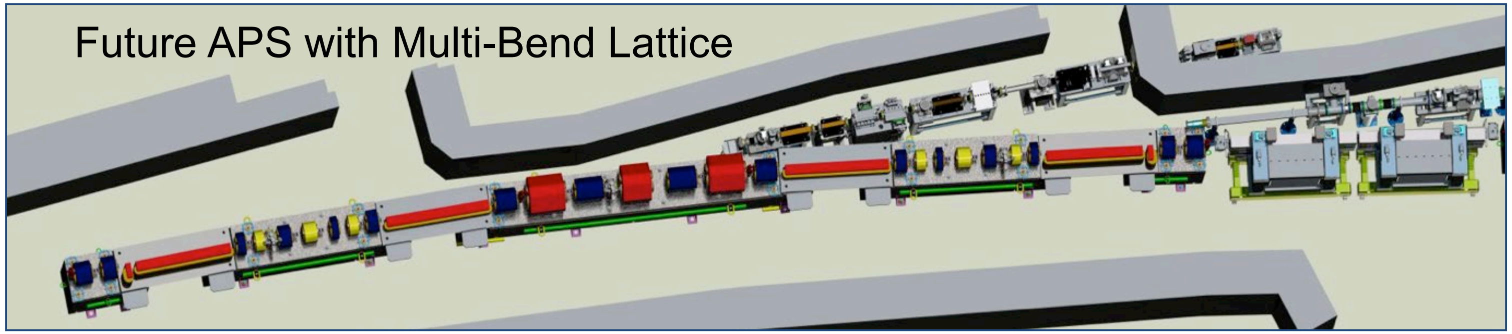
- LINAC - electric field accelerates the electrons to 450 million electronvolts (MeV).
- Booster Synchrotron – radio frequency cavities accelerate electrons from 450 MeV to 7 billion electron volts (7 GeV). (Speed >99.999999% of the speed of light.)
- Storage Ring - The 7-GeV electrons are injected into the 1104-m-circumference storage ring, a circle of more than 1,000 electromagnets and associated equipment, located in a radiation-proof concrete enclosure inside the experiment hall, which is large enough to encircle Chicago's Wrigley Field.
- Insertion Devices – APS is a "third-generation" light sources optimized to produce insertion device radiation in the hard (highly penetrating)
- Beamlines - comprise crystal and/or mirror optics designed to tailor the X-ray beam for specific types of experiments. The beam is delivered to a radiation-proof experiment station that contains the sample under investigation; additional optics that may be needed to analyze and characterize the scattering, absorption, or imaging process; and detectors to collect data from the interaction of x-ray beam and sample.

APS Upgrade multi-bend achromat lattice concept

~50-fold reduction in horizontal emittance



APS today – Double Bend Lattice



Future APS with Multi-Bend Lattice

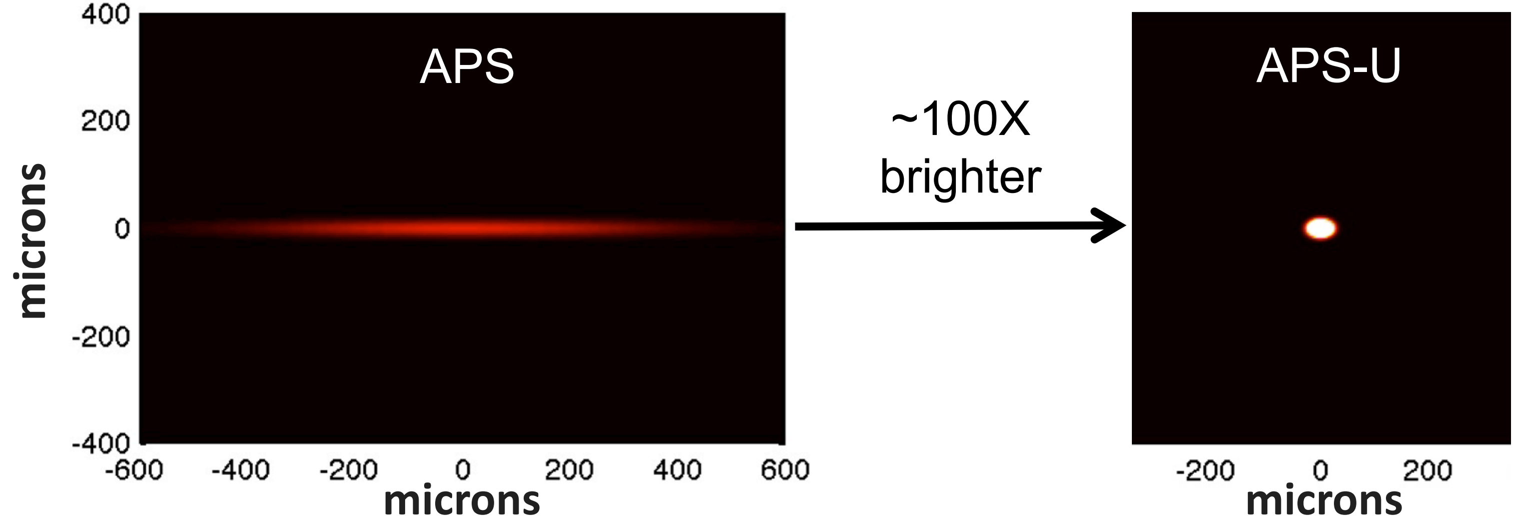
Emittance is a measure of the average spread of particle coordinates in position-and-momentum phase space.

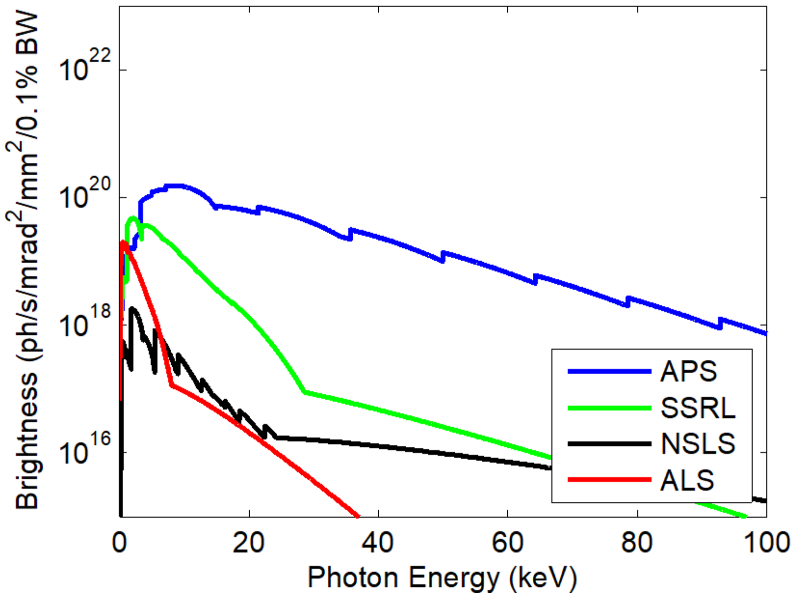
$$\varepsilon_x = C_L \frac{E^2}{N_D^3}$$

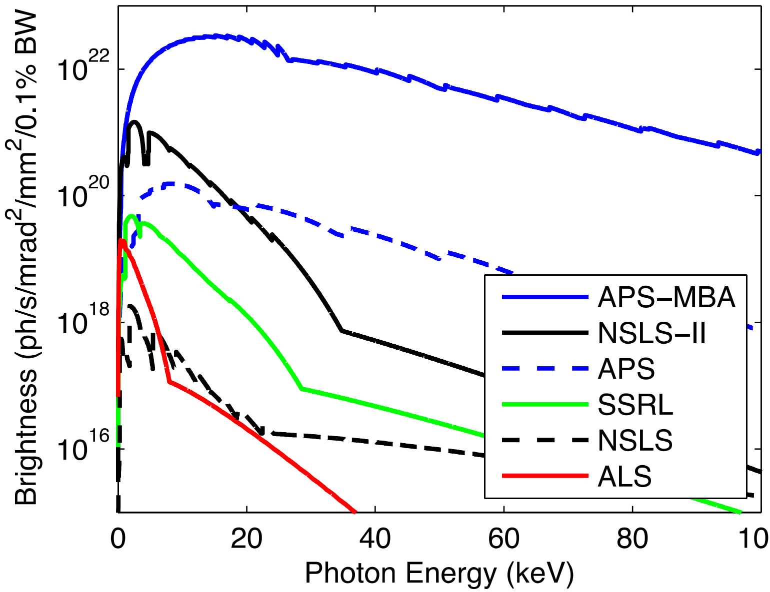
C_L = Ring circumference
E = Beam energy (E = 6 GeV for APS MBA)
N_D = Number of dipoles per sector (N_d = 7 for APS MBA)

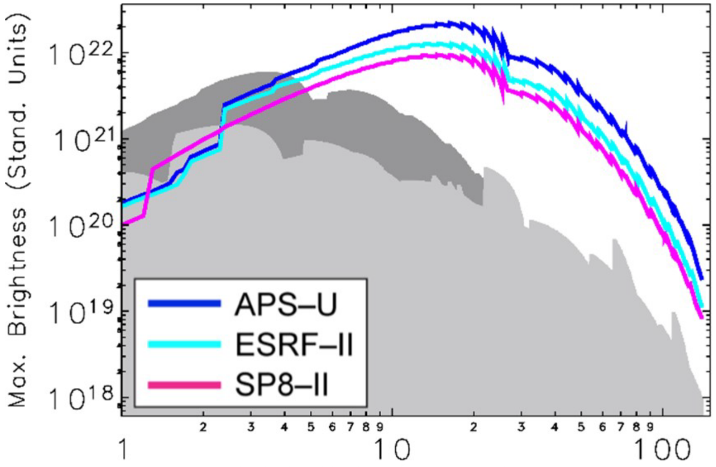
APS-U / APS emittance ~ 40 - 50 : 1

APS Upgrade (APS-U) Multi Bend Achromat (MBA) Lattice







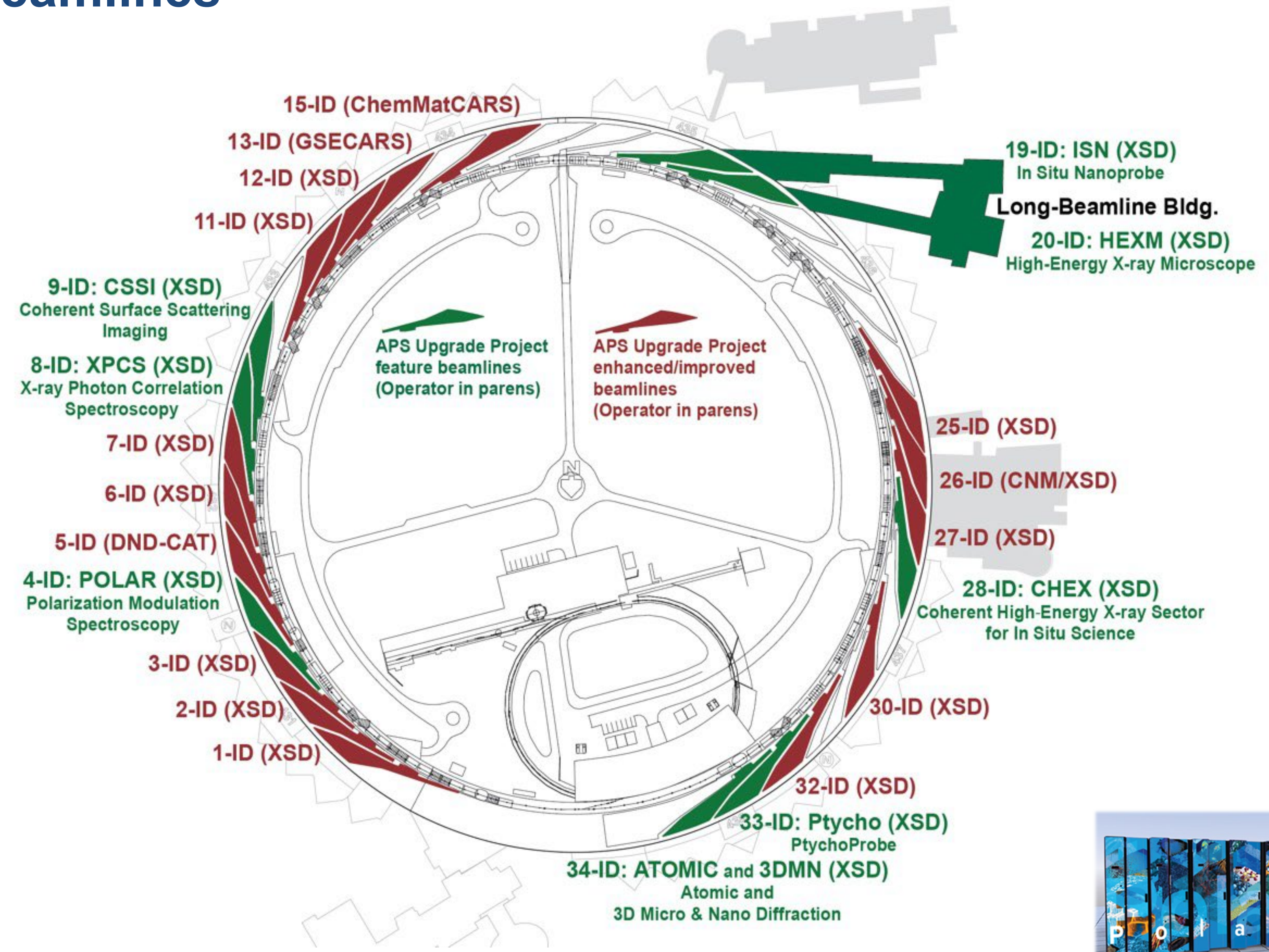


Curves for APS, ESRF, and SP8 upgrades based on present designs, assuming identical undulators

Brightness vs. x-ray energy at top beamlines among U.S. DOE synchrotron facilities

APS Upgrade Project

Beamlines



- New storage ring, **42 pm** emittance @ 6 GeV, 200 mA
- New and updated insertion devices, including SCUs
- Combined result in brightness increases of up to 500x

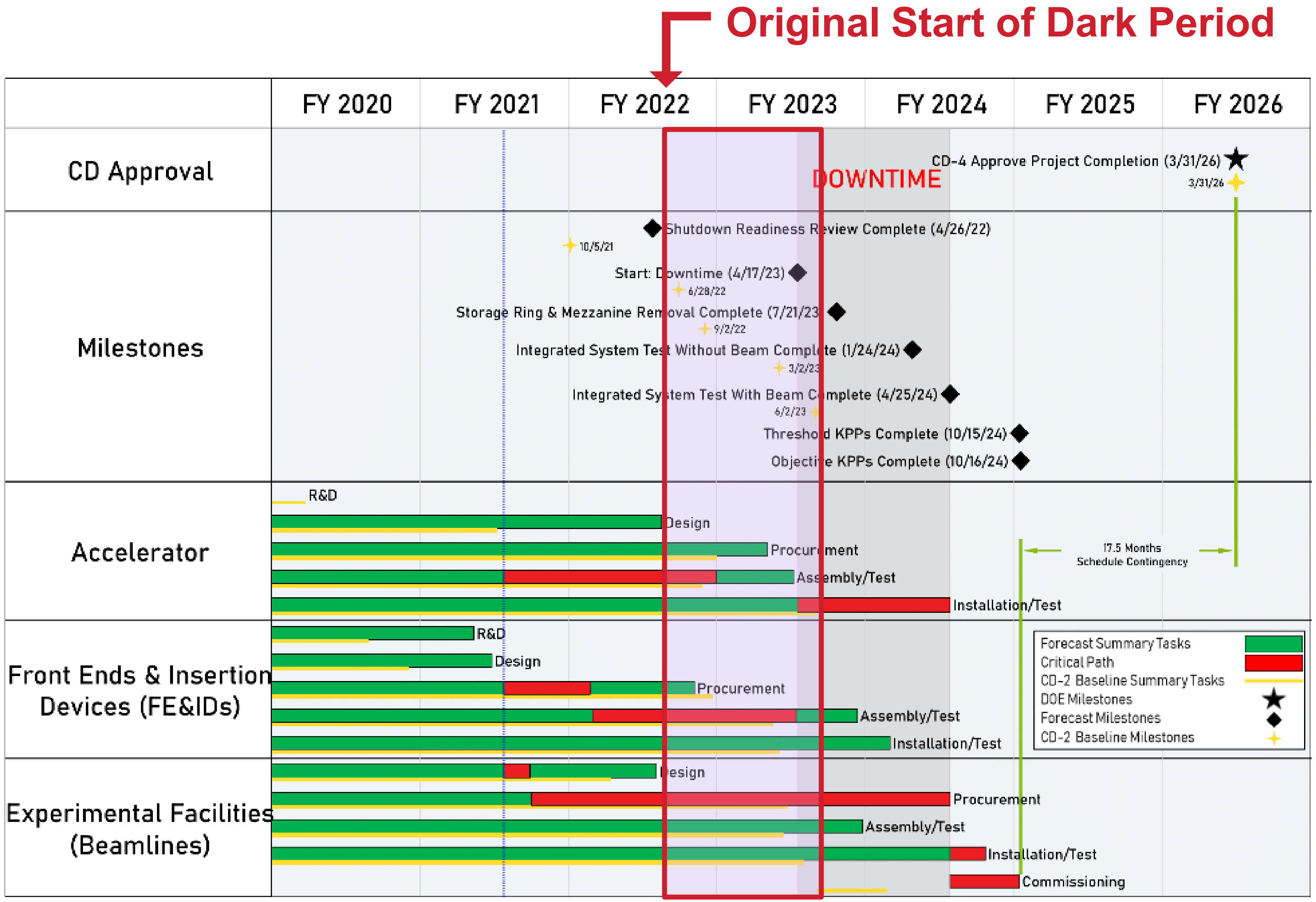
- 9 new feature beamlines + Long Beamline Building
- 15 enhanced and improved beamlines
- Realign 17 bending magnet beamlines
- Exploit high performance computing, AI

Coming Fall 21'

Coming Late 22'

New Schedule – Announced May 10, 2021

- Original June 2022 shutdown schedule impacted by pandemic
- Start of the storage ring installation period shifted by 10 months to **April 17, 2023**
- No change in duration of 1-year installation period (key deliverable)
- Resume x-ray operations in April 2024



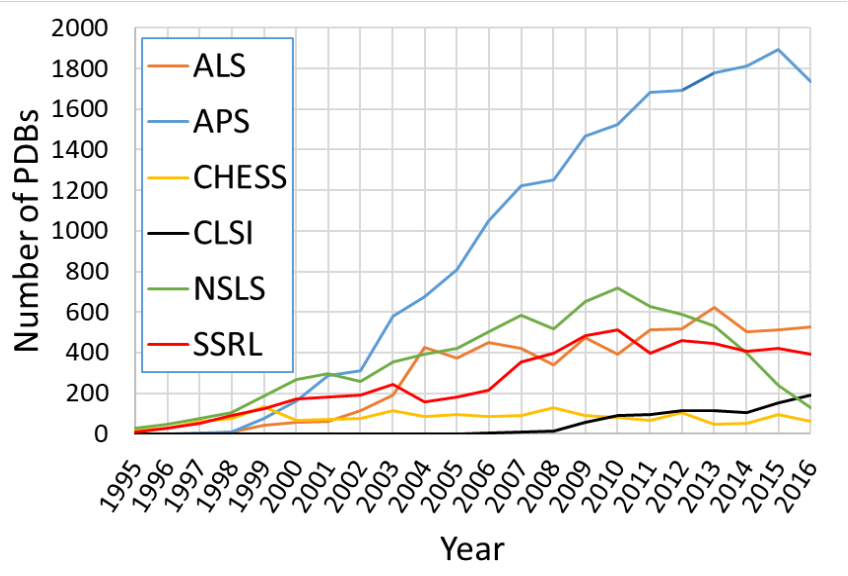
The Impact of the APS dark period on structural biology

Assessment began in 2017

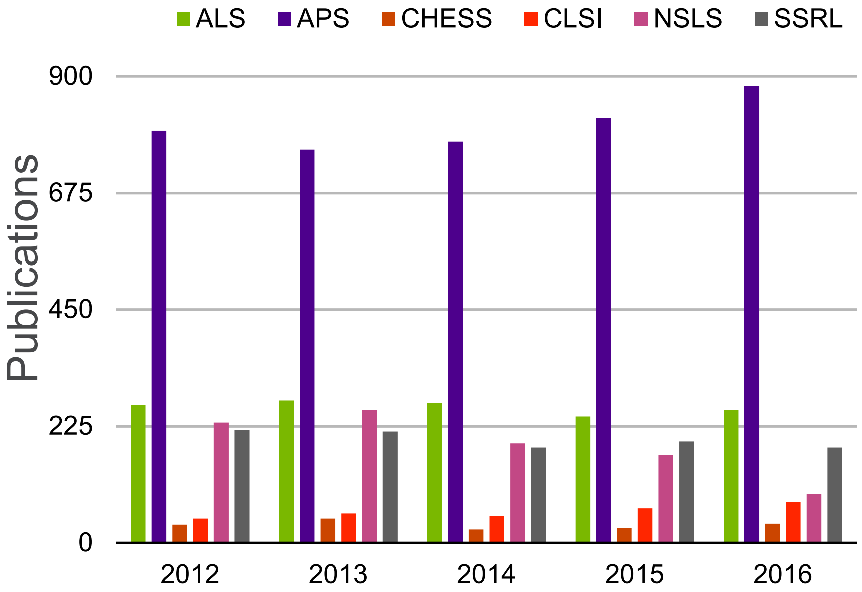
Facility	**Life Sciences Representatives**
ALS	Paul Adams and Corie Ralston
APS	Bob Fischetti
NSLS-II	Sean McSweeney
SSRL	Britt Hedman, Aina Cohen, and Keith Hodgson
LCLS	Soichi Wakatsuki
CHESS	Rick Cerione, Marian Szebenyi and Richard Gillilan

STRUCTURAL BIOLOGY PRODUCTIVITY METRICS: PDBS AND PUBLICATIONS

APS beamlines account for about 50% of the US productivity



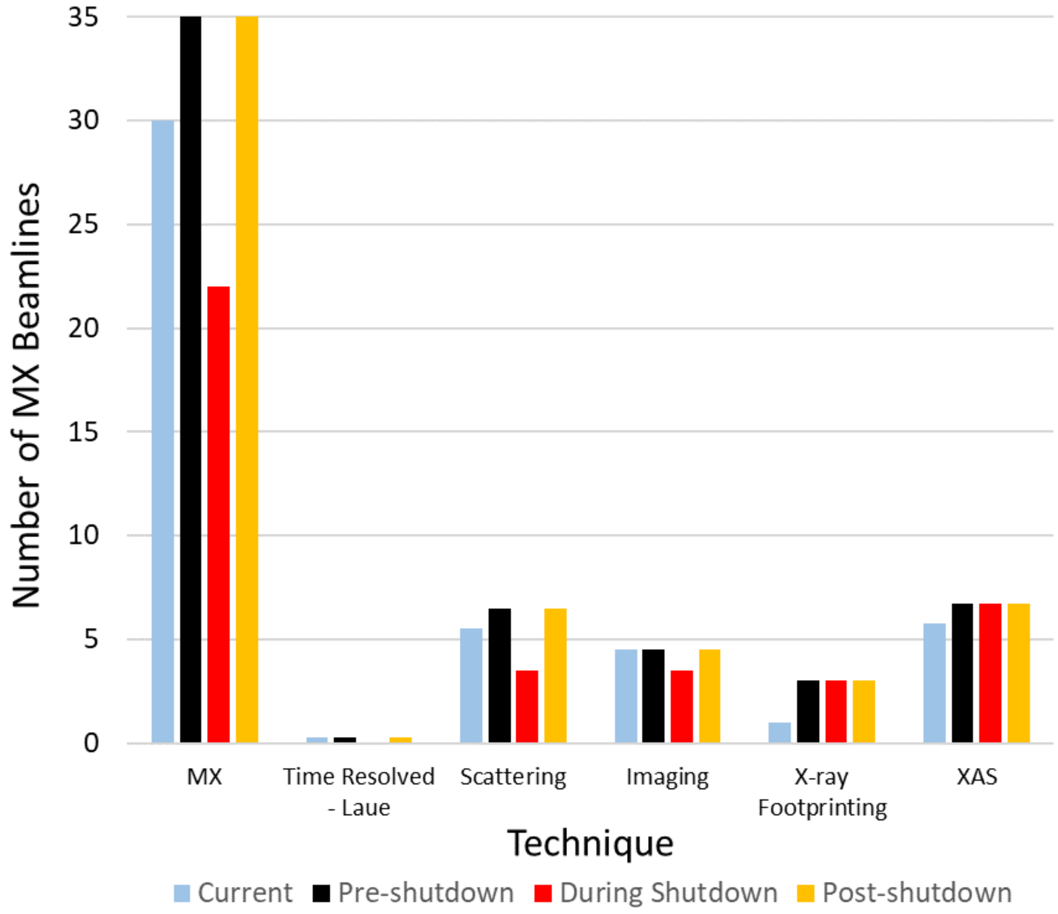
PDB depositions by lightsource



Annual publications by lightsource

APS structural biology beamlines examine about 300,000 samples annually resulting in more than 1800 PDB depositions and in over 850 peer reviewed publications.

Number of US beamlines by technique

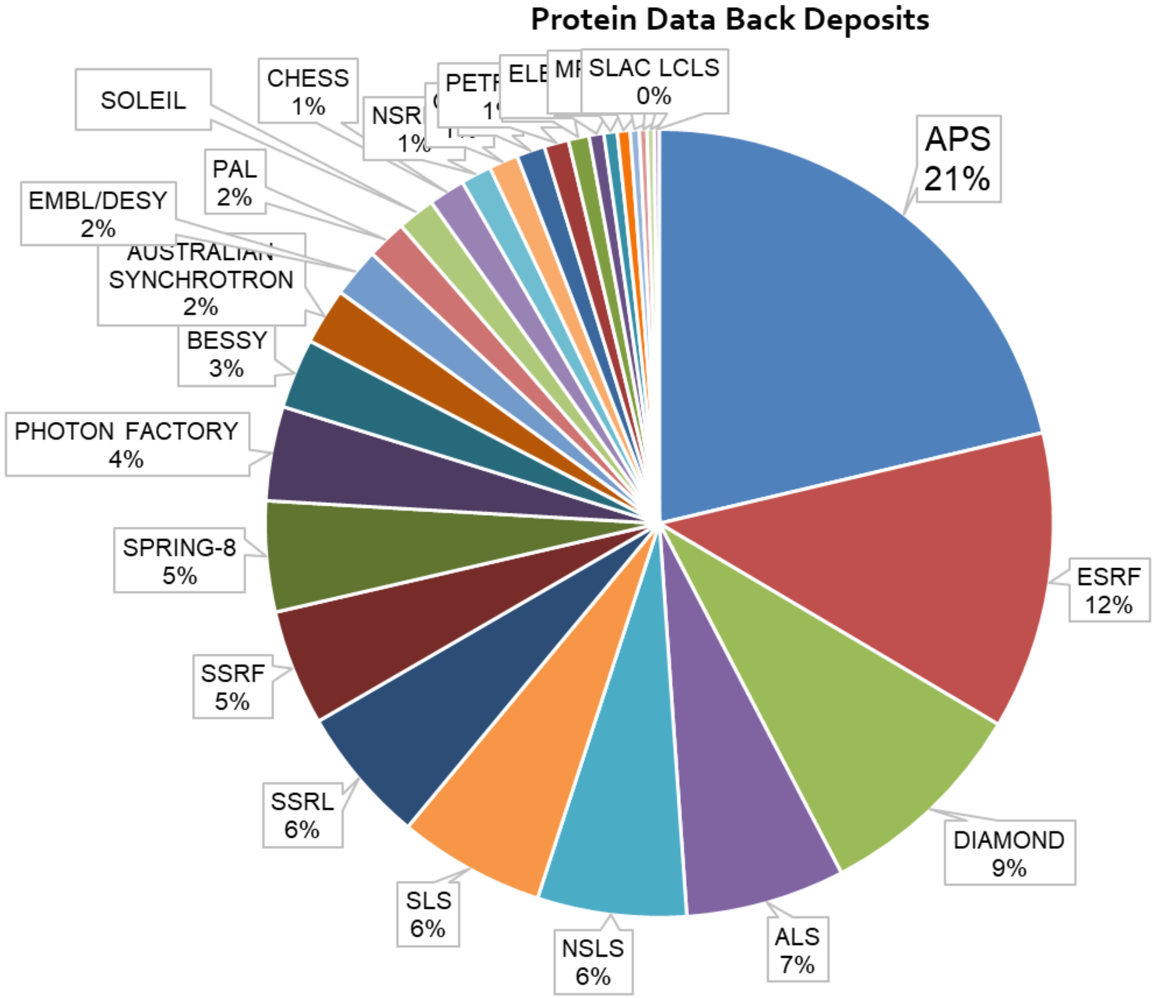


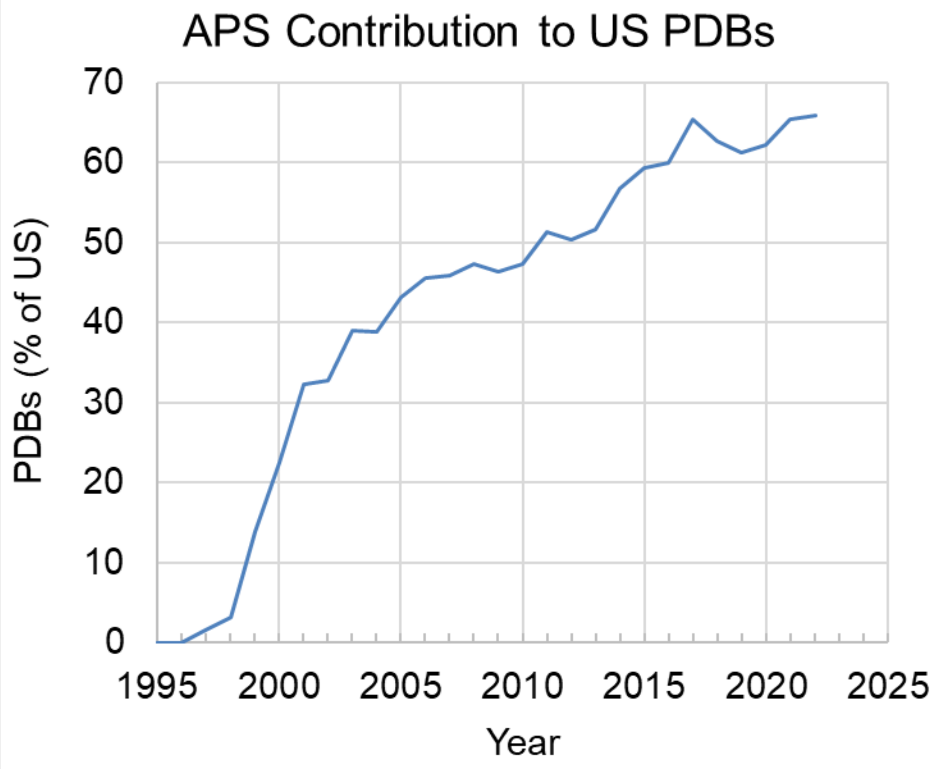
APS-U storage ring replacement is scheduled to take 12 months.
MX beamline capacity will be reduced by 37% during that period.

Protein data bank deposits based on X-ray data

Total from all X-ray sources: 163,819
Total from all synchrotron sources: 135,481
APS beamlines have contributed: 28,603 deposits (21.1%)

APS beamlines are contributing
over 60% of US output





These numbers do include pharma research!

Data as of February 15, 2022

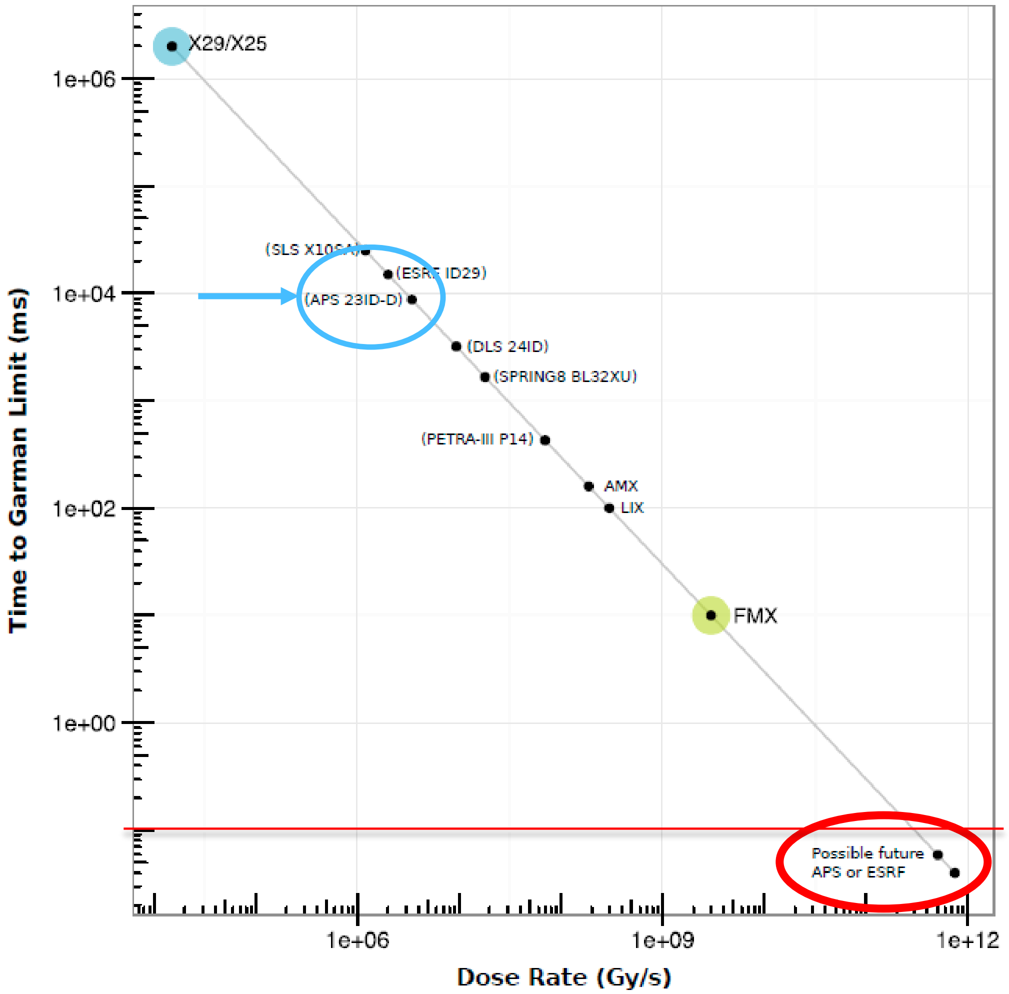
Time to Garman Limit with 4th generation synchrotron sources – <100 µsec!

Garman limit[1] ~ 3.0 x 10^7 Gray (35% intensity loss)

1 sec

1 msec

100 µsec



Data complied by Bob Fischetti, updated and plotted by Sean McSweeney

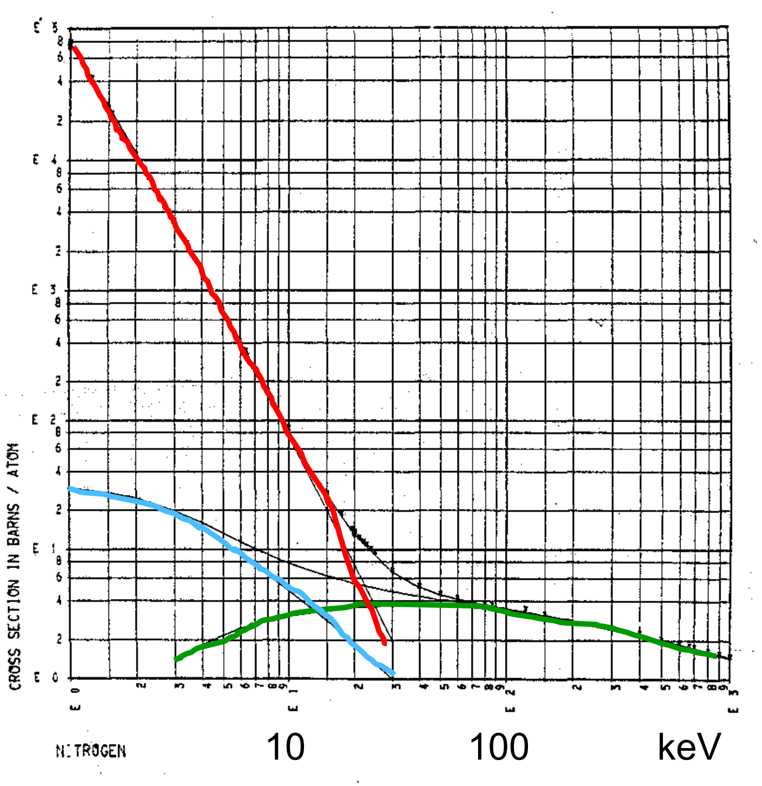
[1] Owen, R.L., Rudino-Pinera, E. & Garman, E.F. *Proc Natl Acad Sci U S A* **103**, 4912-7 (2006)

Interaction of X-rays and mater - briefly

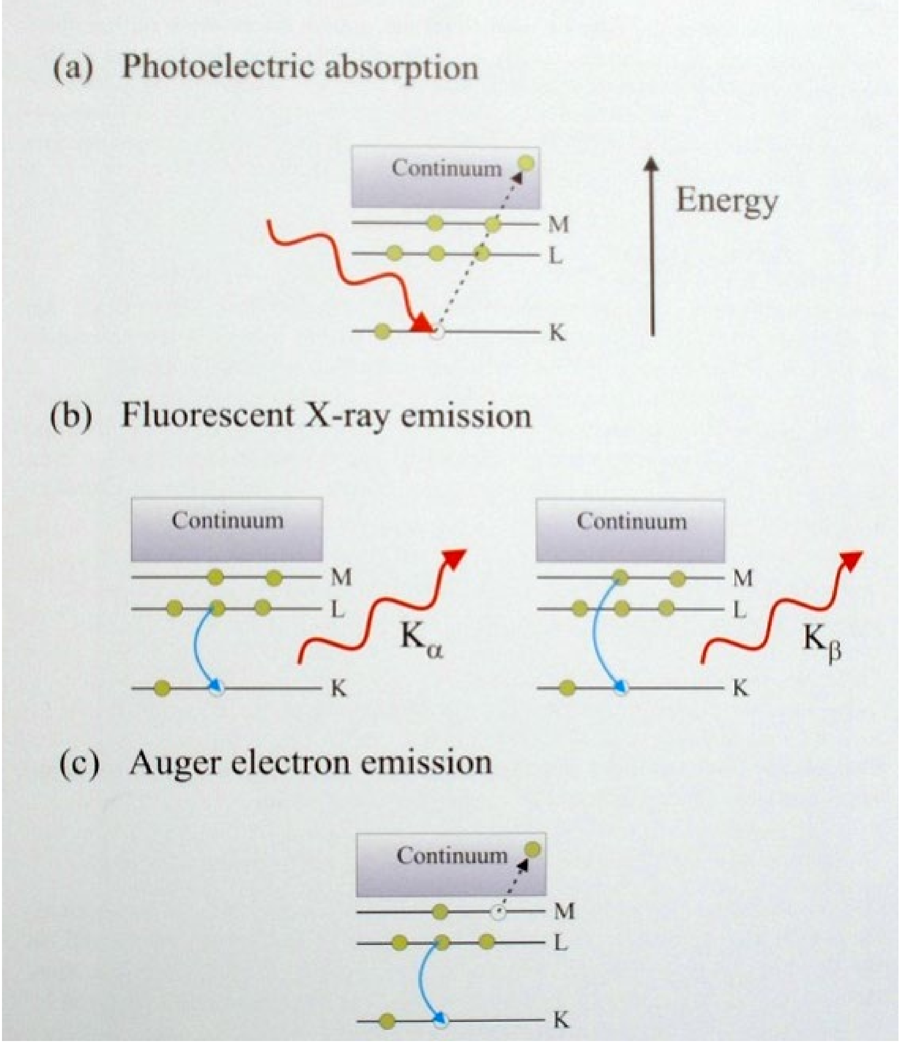
🟥 Photoelectric effect

🟦 Thompson scattering - elastic

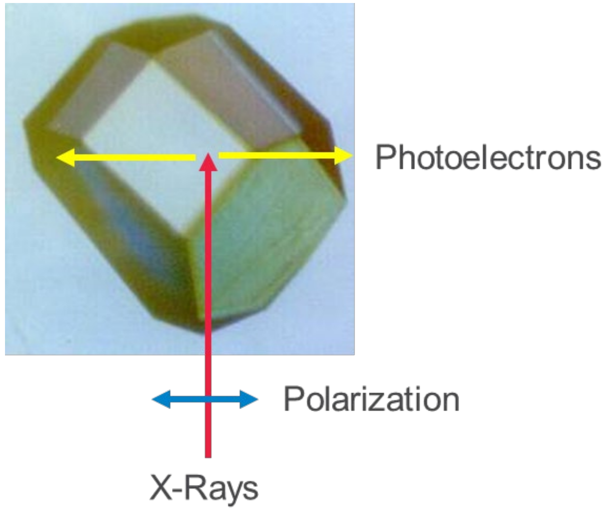
🟩 Compton scattering - inelastic

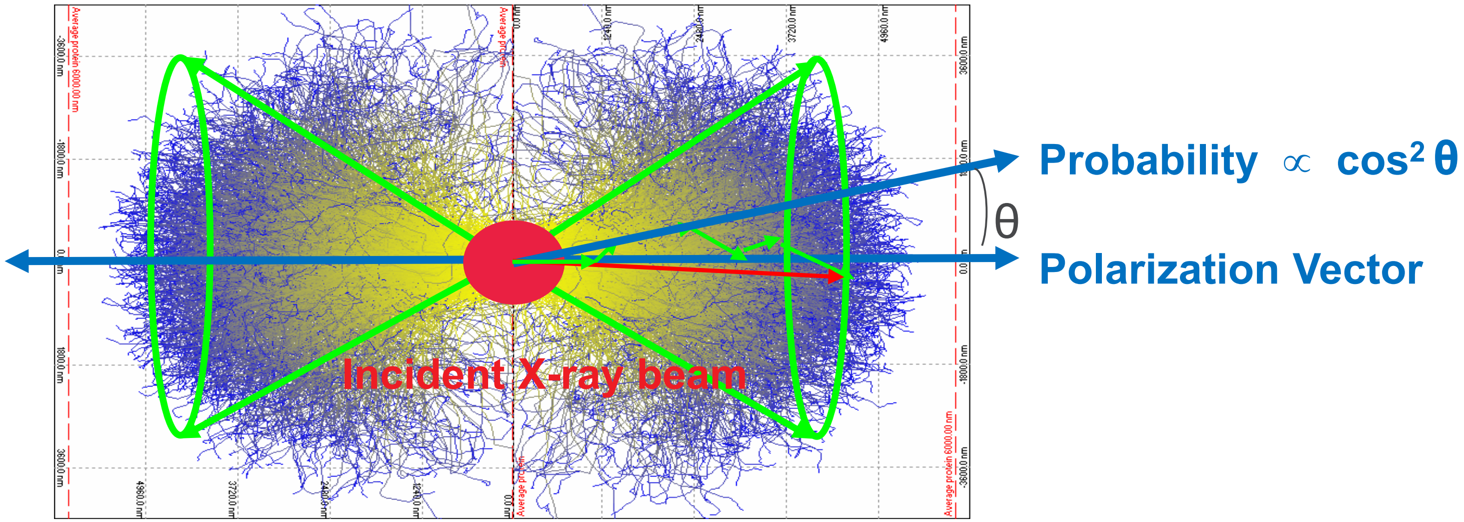


“Typical protein”
C: 1818; H: 7286; N: 420; O: 2673; S: 25.
This corresponds to a 50% solvent content.

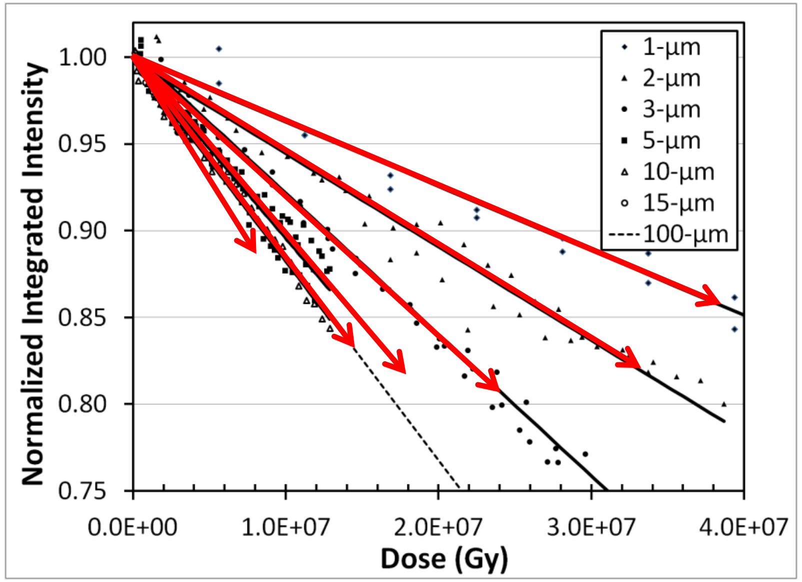


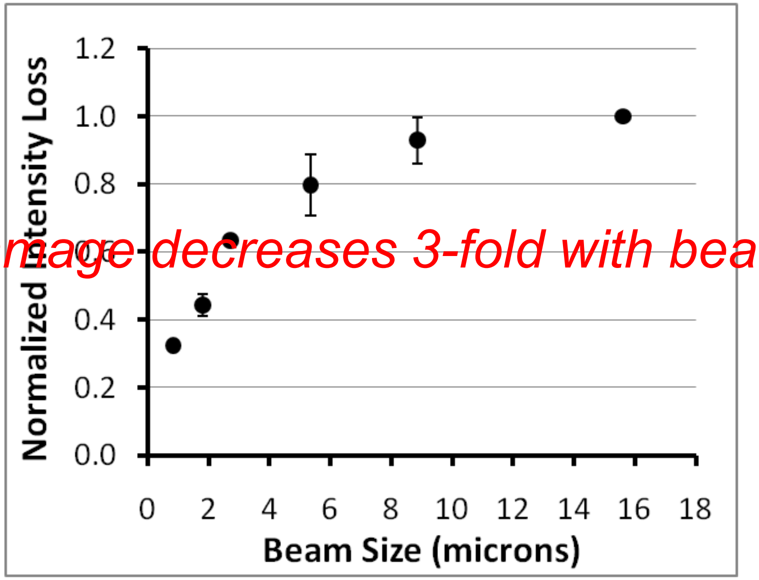
Crystal decay (intensity loss) vs. beam size and dose





Casino Monte Carlo Simulation (by Gerd Rosenbaum)



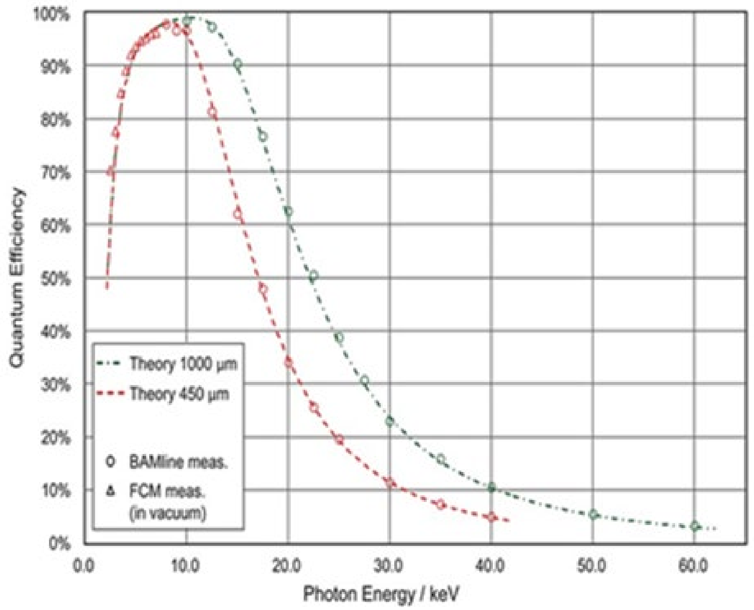


Damage decreases 3-fold with beam size

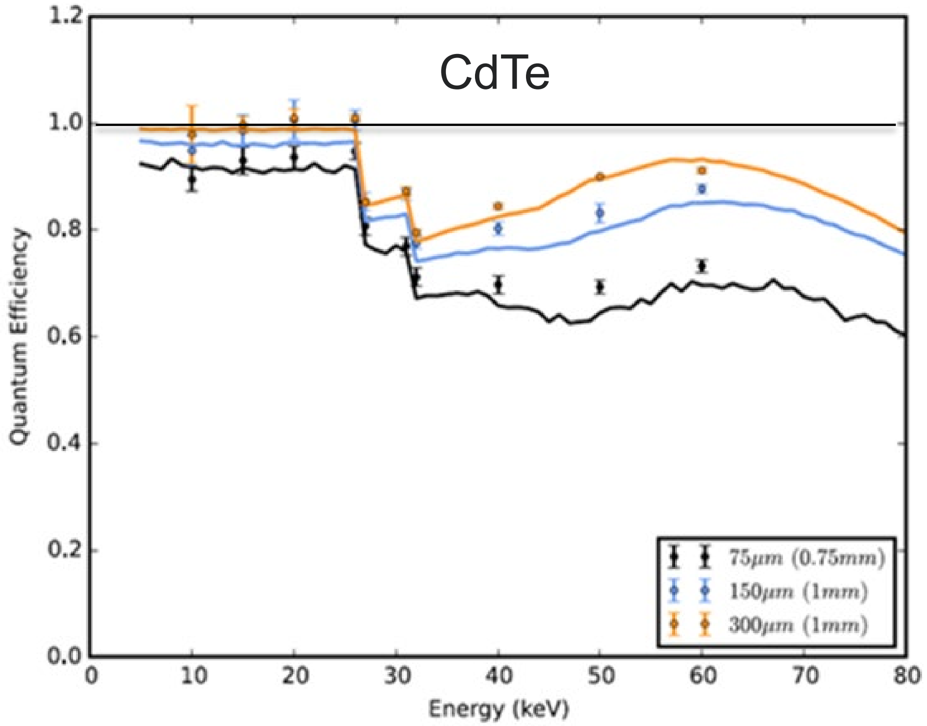
R. Sanishvili, et. al. & S. Vogt & R. Fischetti (2011) Radiation Damage in Protein Crystals is Reduced with a Micron-Sized X-Ray Beam, *PNAS* **108**, 6127 - 6132

Detector Quantum (QE) efficiency vs energy

Si



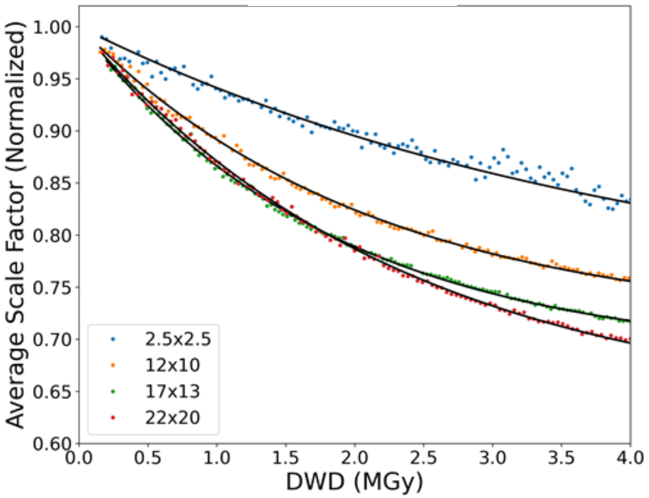
The QE of Si-sensors of 450- and 1000-micron thickness (Data courtesy of Dectris).



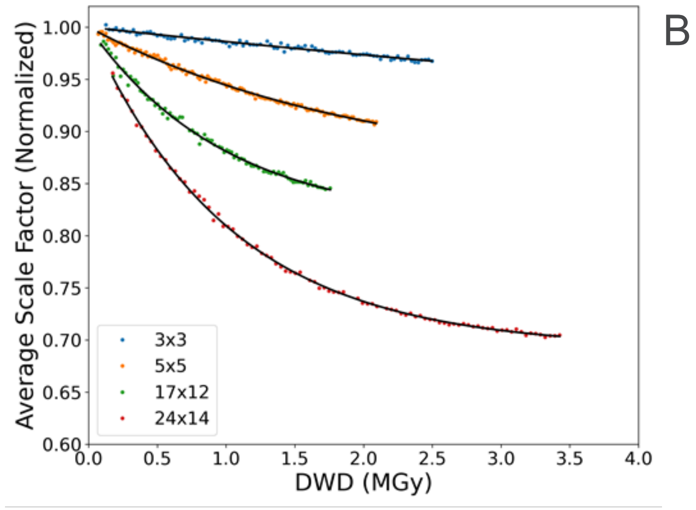
The QE of a CdTe sensor for three pixel sizes and sensor thicknesses on an Eiger2 ASIC as measured (dots) and simulated (continuous lines). The CdTe sensor thickness is indicated in parentheses. (Zambon et. al. 2017).

Intensity loss vs. Diffraction Weighted Dose

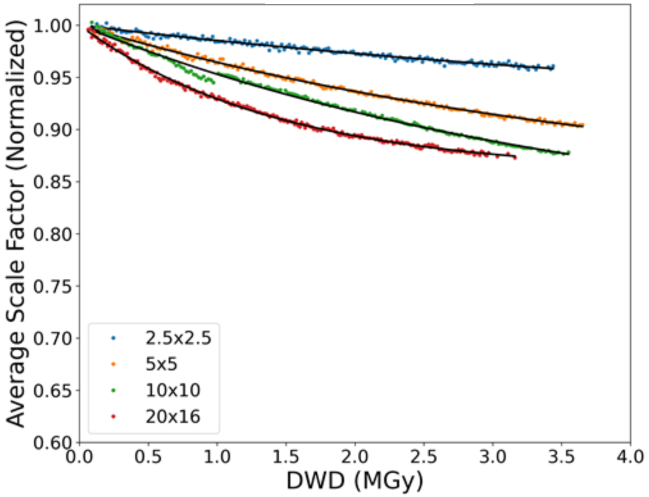
12.0 keV



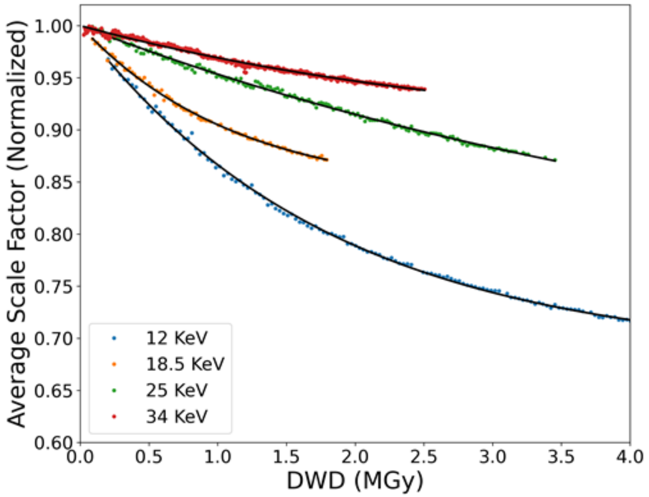
18.5 keV



25.0 keV



~16×8 microns



Quantitating radiation damage vs X-ray energy and beam size

- Repetitive data collection over the same 0-1 degree
- For a given energy and Dose radiation damage is reduced with decreasing beam size
- For the same beam size radiation damage is reduced with increasing X-ray energy

David Kissick, Naga Venugopalan, Qingping Xu, GM/CA team

Beamlines Townhalls & Workshops

Targeted communications with specific user communities

Structural Science Workshop	*Jan. 11-15*
CHEX (28-ID) Workshop Series	*Apr. 30 – Oct. 22*
ASL & Timing (25-ID)	*Sept. 1*
XPCS (8-ID)	*Sept. 20*
CSSI (9-ID)	*Oct. 6*
HEXM (20-ID)	*Oct. 25*
Polar (4-ID)	Nov. 12
Spectromicro/Bionanoprobe (2-ID)	Nov. 30
ISN(19-ID)/Ptycho (33-ID)	Dec. 2
Atomic/3DMN (34-ID)	Dec. 6
Structural Biology	Jun. 21

Other resources

https://www.aps.anl.gov/APS-Upgrade/Meetings-Workshops

Science Workshops for the APS-U: 2018/2019/2020

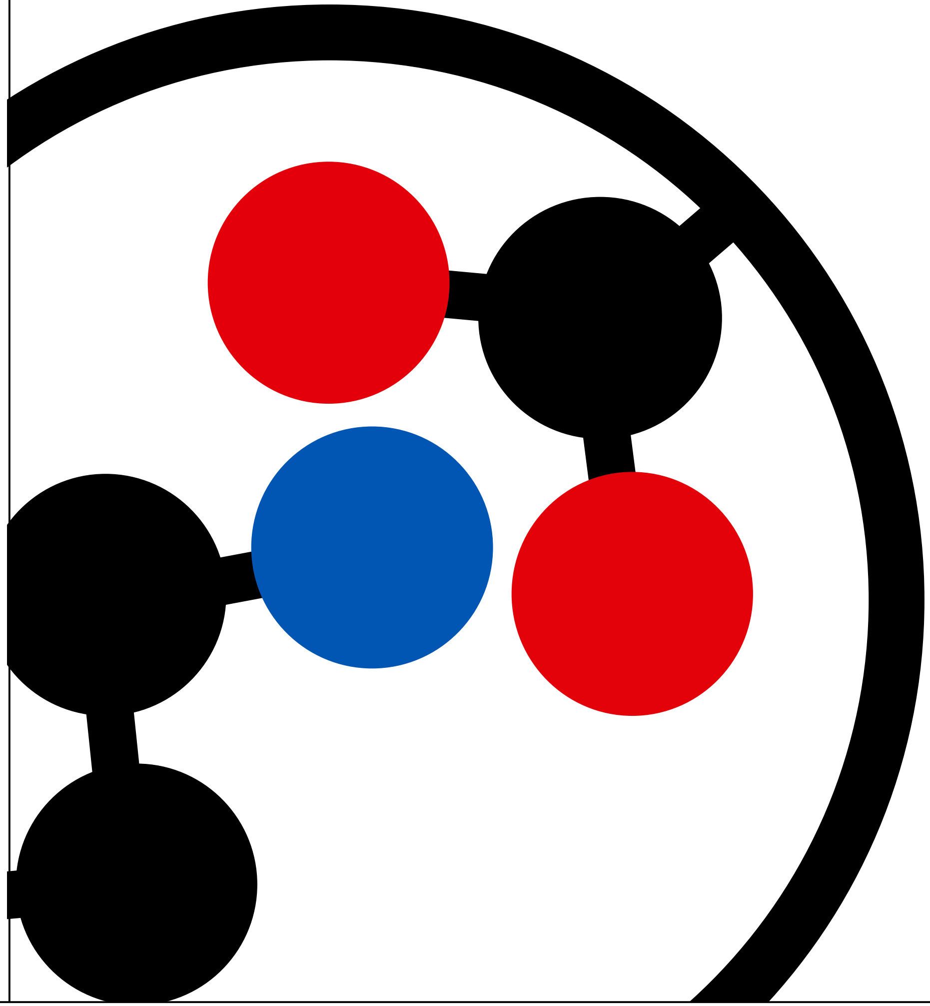
1. Biological Science Opportunities Provided by the APS Upgrade, August 20-21, 2018
2. Opportunities in Biological and Environmental Research Uniquely Enabled by the APS Upgrade, August 28-29, 2018 (tentative)
3. Condensed matter physics, x-ray scattering and x-ray spectroscopy at the APS-U, September 25-26, 2018
4. Discovery, synthesis and development of emerging materials and the role of the APS-U, September 27-28, 2018
5. Time resolved chemistry at the APS-U, October 1-2, 2019
6. Catalysis research, x-rays and the APS-U, October 3-4, 2019
7. Industrial processes and the role of APS-U, September 21-22, 2020
8. Materials under extreme conditions at the APS-U, September 23-24, 2020
9. Structure, Kinetics, and Dynamics in Soft Materials, 2020 (exact date TBD)

Thank you for your attention!

APS-U Structural Biology Townhall Agenda

Start	Duration	End	
1:00 PM	0:05	1:05 PM	Welcome - Laurent Chapon,
1:05 PM	0:25	1:30 PM	APS-U Overview and Life Sciences at the APS, Bob Fischetti
1:30 PM	0:15	1:45 PM	IMCA-CAT, Lisa Keefe
1:45 PM	0:15	2:00 PM	SBC/eBERLight, Andrzej Joachimiak and Karolina Michalska
2:00 PM	0:15	2:15 PM	LS-CAT, Spencer Anderson
2:15 PM	0:15	2:30 PM	SER-CAT, Bi-Chen Wang
2:30 PM	0:15	2:45 PM	GM/CA-CAT, Naga Venugopalan
2:45 PM	0:15	3:00 PM	Break
3:00 PM	0:15	3:15 PM	NE-CAT, Malcolm Capel
3:15 PM	0:15	3:30 PM	LRL-CAT, Jordi Benach
3:30 PM	0:15	3:45 PM	ALS, Marc Allaire
3:45 PM	0:15	4:00 PM	CHESS, Marian Szebenyi
4:00 PM	0:15	4:15 PM	NSLS-II, Sean McSweeney
4:15 PM	0:15	4:30 PM	SSRL, Aina Cohen
4:30 PM	0:30	5:00 PM	Roundtable Q&A
5:00 PM			Adjourn

Pharma Data & Upgrades @ IMCA-CAT



June 21, 2022

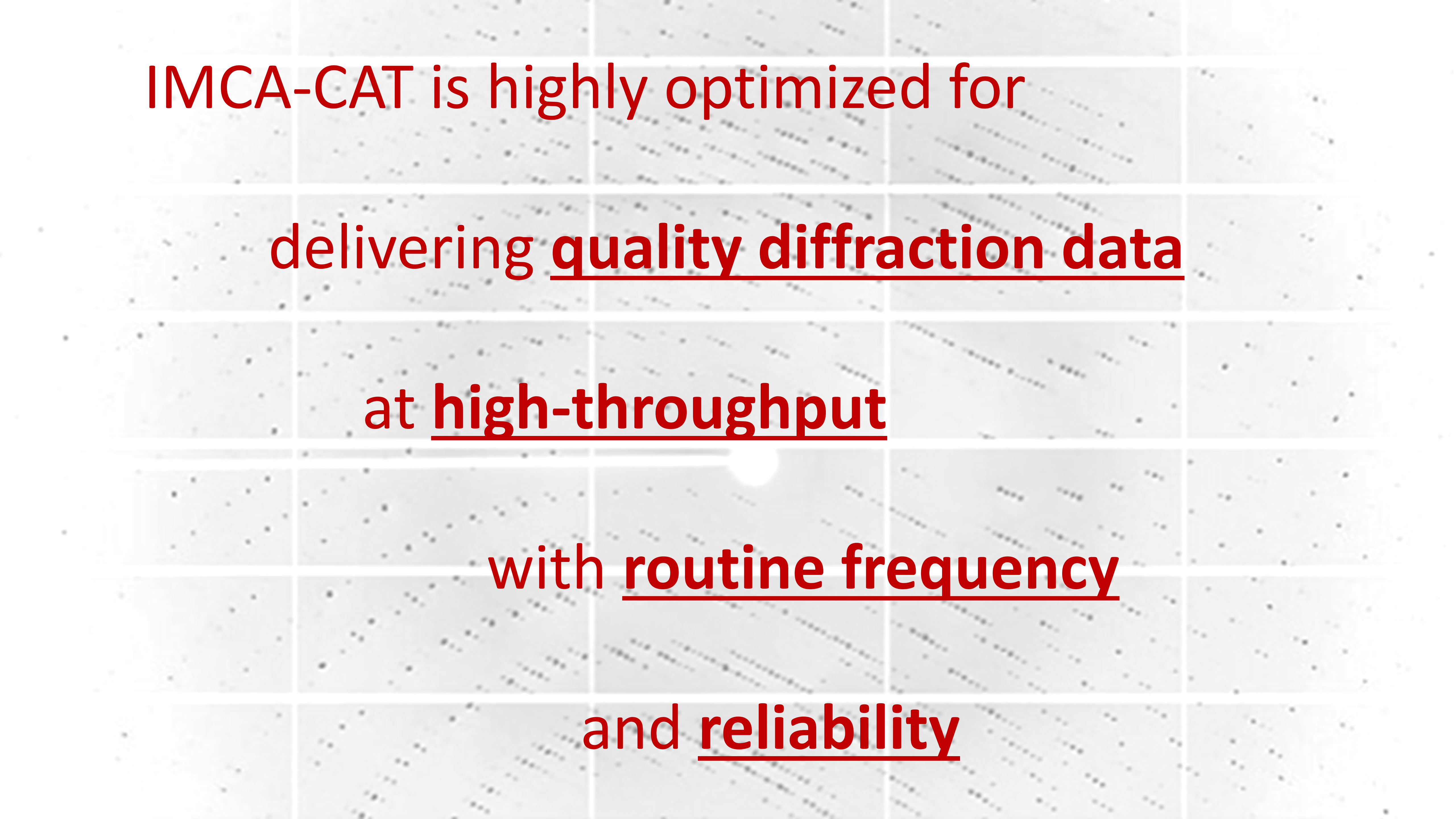
Lisa J Keefe, PhD
Executive Director of IMCA-CAT
Vice President for Advancing Therapeutics
Hauptman-Woodward Medical Research Institute

IMCA-CAT
Industrial Macromolecular Crystallography Association
Collaborative Access Team

is a structural biology resource for IMCA
and the broader community of pharma companies

abbvie
evotec
Bristol Myers Squibb™
janssen
PHARMACEUTICAL COMPANIES OF Johnson&Johnson
MERCK
NOVARTIS
Pfizer
RELAY
THERAPEUTICS

IMCA-CAT’s mission is to accelerate pharma drug discovery



IMCA-CAT is highly optimized for

delivering **quality diffraction data**

at **high-throughput**

with **routine frequency**

and **reliability**

IMCA-CAT operations are optimized for industry

High-Throughput

Auto Mode

Reliable and Fast

- quality data
- >400 data sets daily
- robot reliability: 99.99%
- auto processing by Global Phasing

Access

Mail-In

Routine and Efficient

- expert data collection
- weekly flow of data
- trusted data security

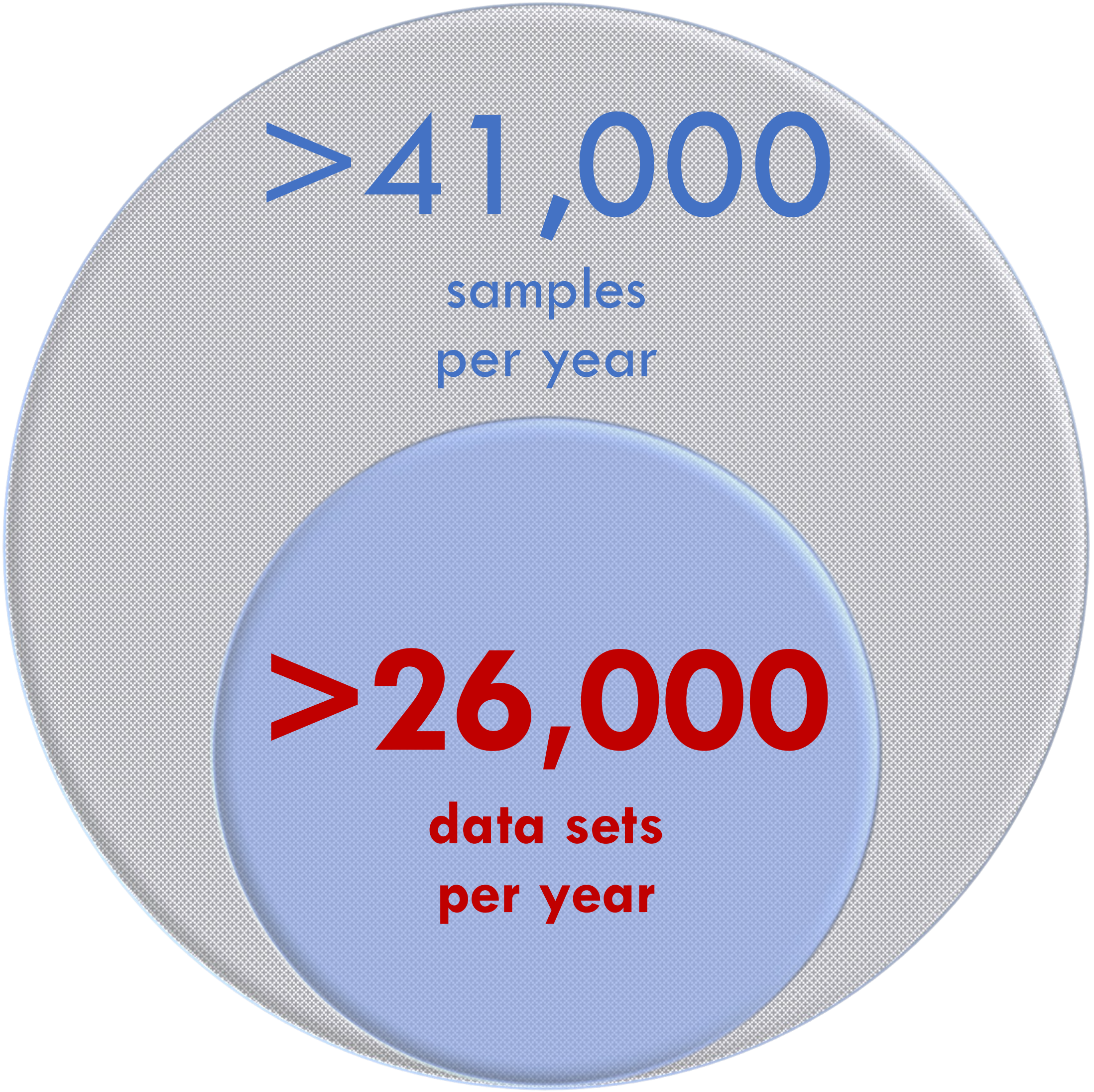
Scheduling

Flexible

Predictable for Continuous Flow of Data

- data when needed
- prompt queueing
- fast turnaround (within a few days)

During APS-U, data will be collected for industry for:



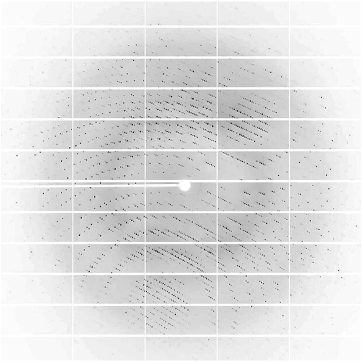
>41,000

samples
per year

>26,000

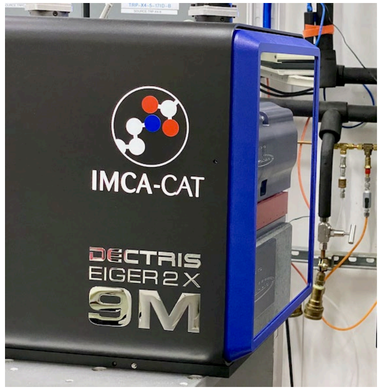
**data sets
per year**

During APS-U, IMCA-CAT will collect data for industry

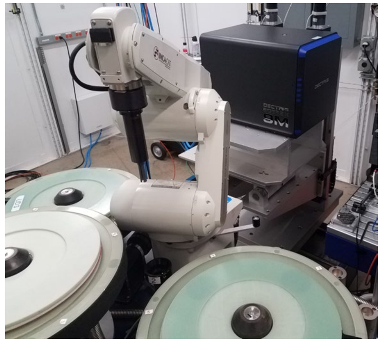


IMCA-CAT beamline will be upgraded to deliver a small and highly intense beam during the APS-U

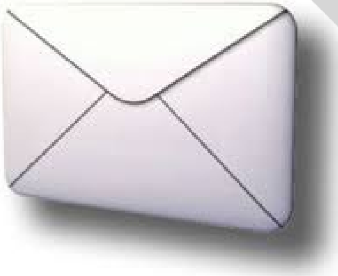
Detector



Robot

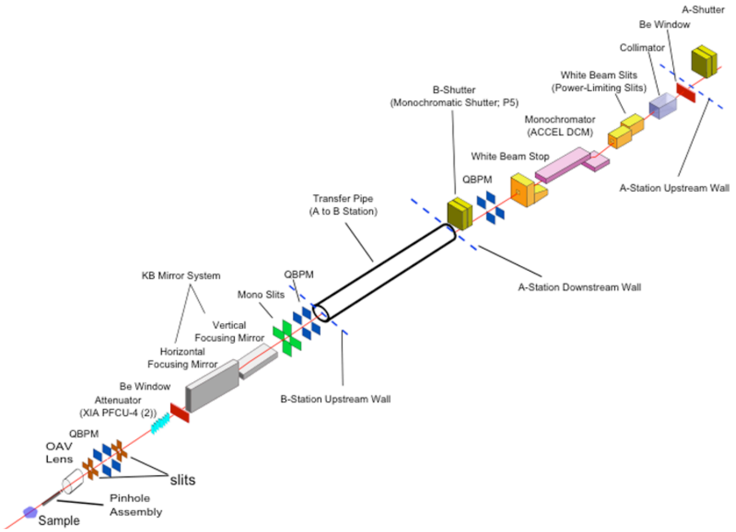


Software



micro
focus

focused to 5 μm x 5 μm



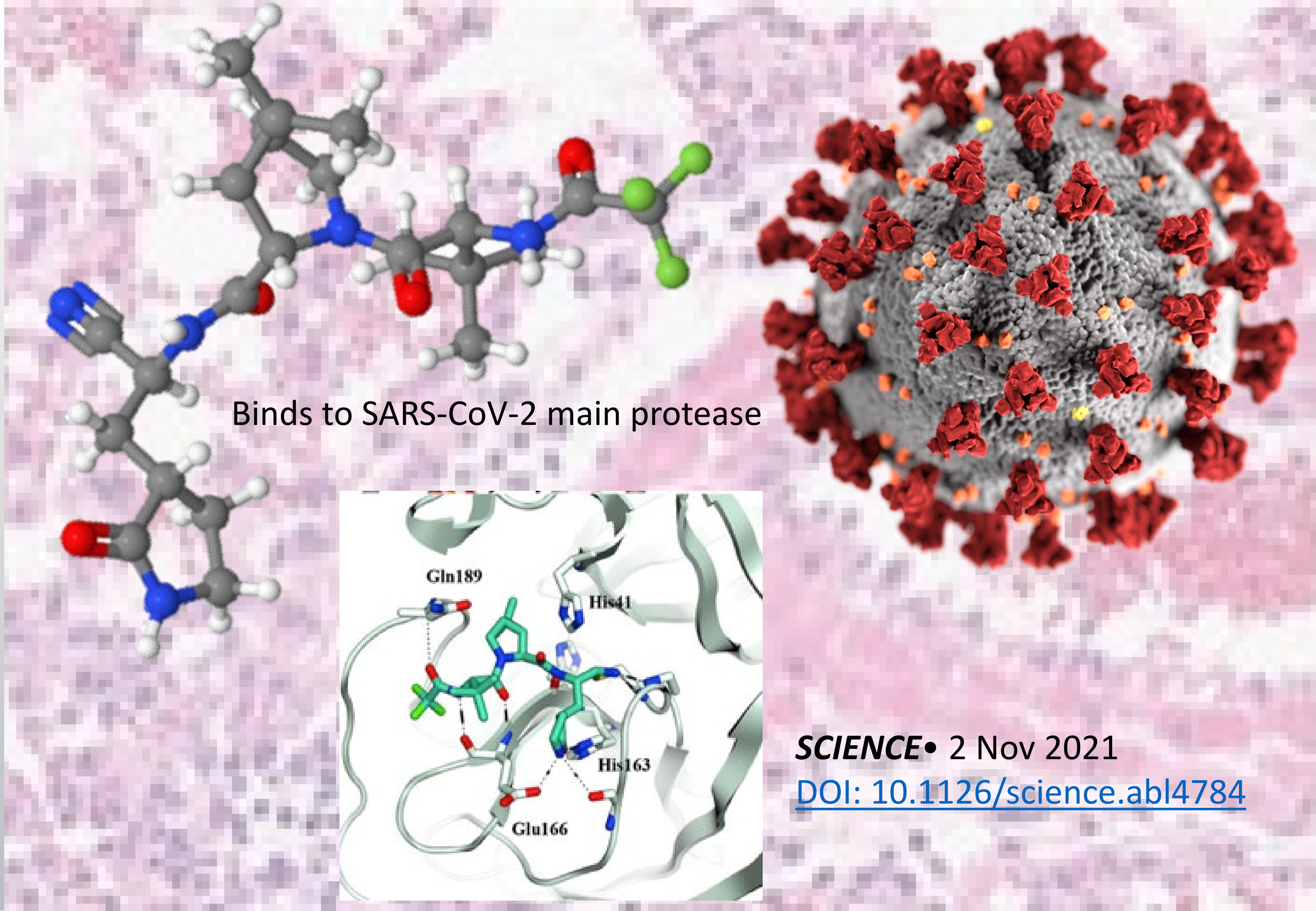
Beamline Optics Upgrade:

- **state-of-art monochromator**
- **highest quality focusing mirrors**
- **high precision goniometer**

IMCA-CAT is critical for shortening pharma drug discovery pipelines

A Structure-Based Drug Design Success

PAXLOVID™



Binds to SARS-CoV-2 main protease

SCIENCE • 2 Nov 2021

DOI: 10.1126/science.abl4784

IMCA-CAT

Industrial Macromolecular Crystallography Association
Collaborative Access Team

Industry

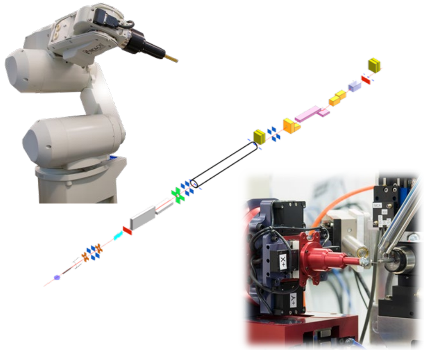
IMCA Members

abbvie
Bristol Myers Squibb™
evotec
janssen
PHARMACEUTICAL COMPANIES OF Johnson&Johnson
MERCK
NOVARTIS
Pfizer
RELAY THERAPEUTICS

IMCA-CAT Subscribers

Experiment

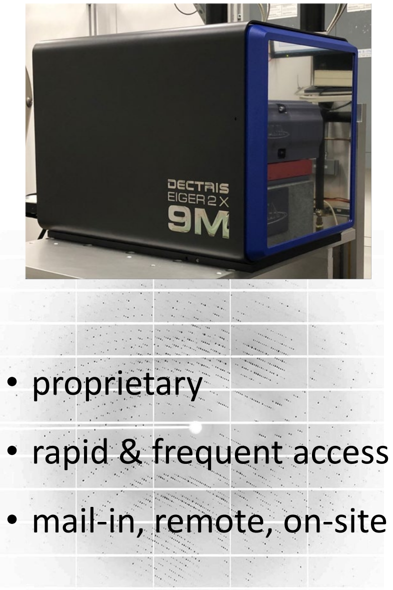
Beamline 17-ID @ APS



- focused, intense beam
- mini beam 5-50 µm
- pucks: Unipuck, ACTOR, ALS

Capabilities

- diffraction rastering
- collect-along-vector
- auto collect & process

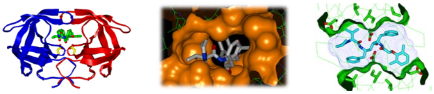


- proprietary
- rapid & frequent access
- mail-in, remote, on-site

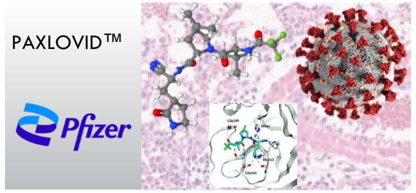
Productivity

41,000+
samples annually

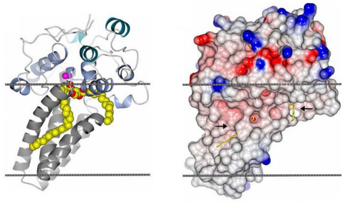
- high-throughput
- fast, encrypted data transfer
- real-time integration to company pipelines

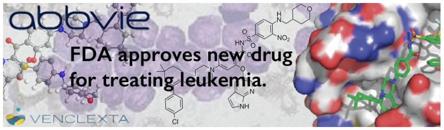


Discovery



- micro crystals
- membrane proteins
- MAD / SAD
- *in situ*





FDA approves new drug for treating leukemia.

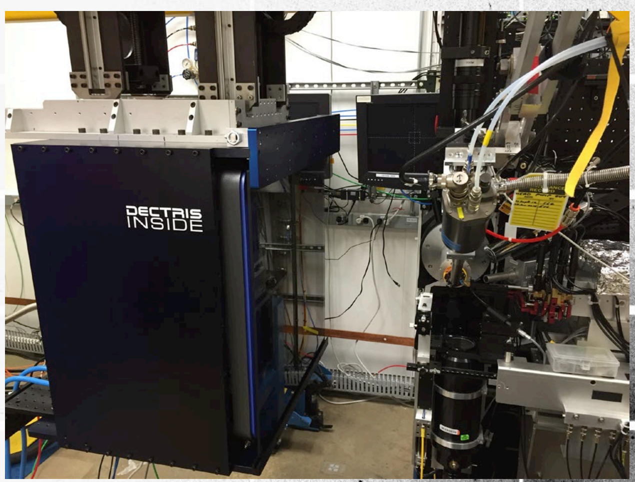
www.imca-cat.org

APS-U Structural Biology Townhall Agenda

Start	Duration	End	
1:00 PM	0:05	1:05 PM	Welcome - Laurent Chapon,
1:05 PM	0:25	1:30 PM	APS-U Overview and Life Sciences at the APS, Bob Fischetti
1:30 PM	0:15	1:45 PM	IMCA-CAT, Lisa Keefe
1:45 PM	0:15	2:00 PM	SBC/eBERLight, Andrzej Joachimiak and Karolina Michalska
2:00 PM	0:15	2:15 PM	LS-CAT, Spencer Anderson
2:15 PM	0:15	2:30 PM	SER-CAT, Bi-Chen Wang
2:30 PM	0:15	2:45 PM	GM/CA-CAT, Naga Venugopalan
2:45 PM	0:15	3:00 PM	Break
3:00 PM	0:15	3:15 PM	NE-CAT, Malcolm Capel
3:15 PM	0:15	3:30 PM	LRL-CAT, Jordi Benach
3:30 PM	0:15	3:45 PM	ALS, Marc Allaire
3:45 PM	0:15	4:00 PM	CHESS, Marian Szebenyi
4:00 PM	0:15	4:15 PM	NSLS-II, Sean McSweeney
4:15 PM	0:15	4:30 PM	SSRL, Aina Cohen
4:30 PM	0:30	5:00 PM	Roundtable Q&A
5:00 PM			Adjourn

Structural Biology Center

19-ID/19-BM Beamlines, Sector 84 Facilities



6,260 PDB deposits
2,616 publications
219,355 total citations
214 H-index

SBCcollect loop auto-centering
Crystal "point-and-click" centering
Remote access

Protein purification and crystallization

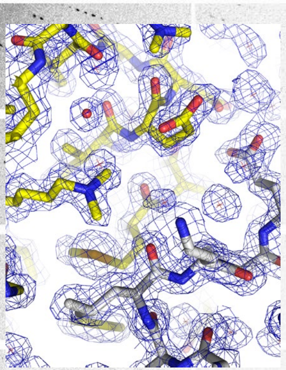
Highly flexible beam size from 5 x 5 µm to 200 × 200 µm

PILATUS3 6M Detector - 30 images/sec
ACTOR robot
nano xyz-head
SSX capabilities
19-BM fast sample exchange robot

Diffraction data

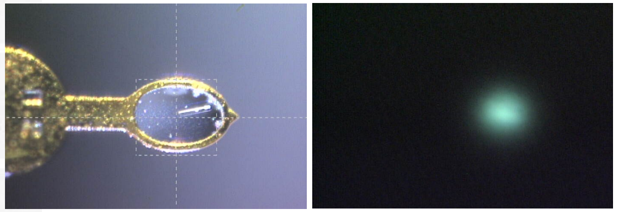
Electron density

Structure

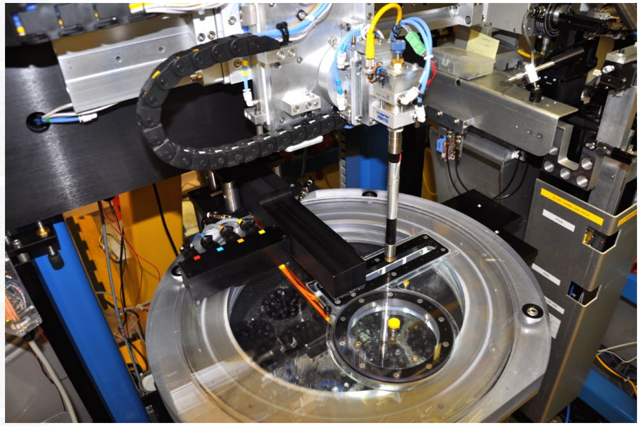


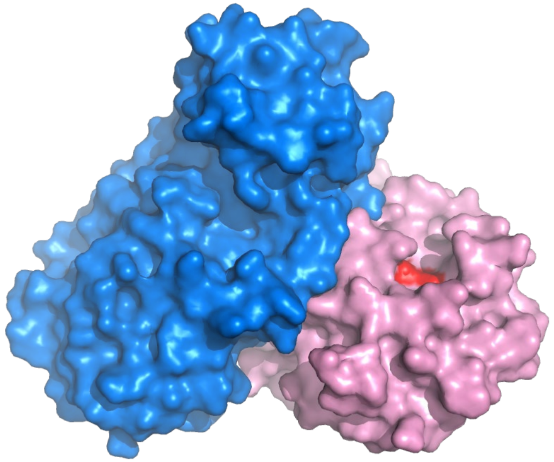
HKL3000: semi-automated structure determination

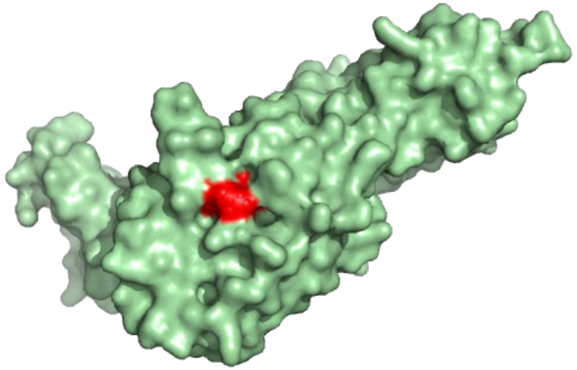
Sample centering

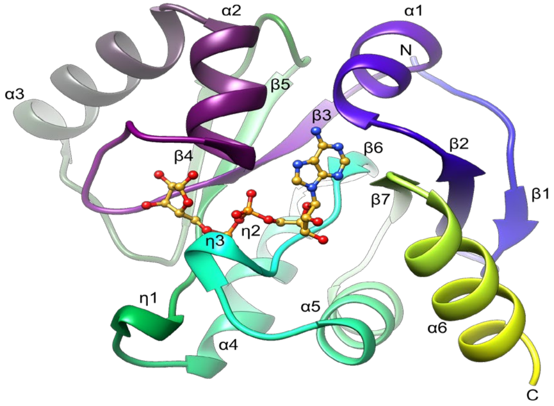


Microcrystals and mini-beams





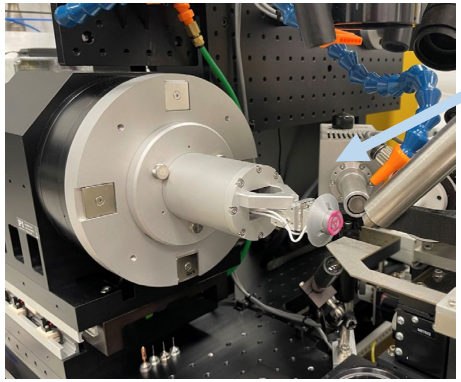


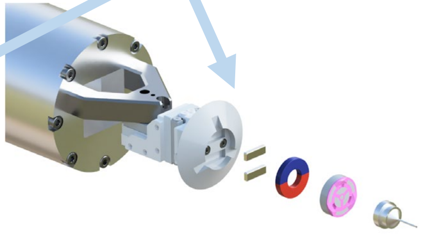


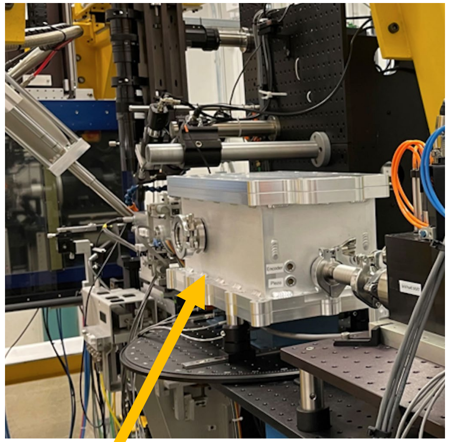
New 19-ID Capabilities

- Modular goniostat was installed at SBC 19-ID with new magnetic mount.
- This goniostat can accommodate variety sample holders designed and 3D printed at the SBC:
  - Compound Refractive Lens increased the x-ray flux at the sample 8 times, enabling serial crystallography with smaller crystals experiments.
  - ALEX holder for SSX
  - Plate scanner
  - Capillary holder for microfluidics experiments (ice formation, chemical reactions etc.)
- These sample holders and their controllers can be ported on other APS beamlines.

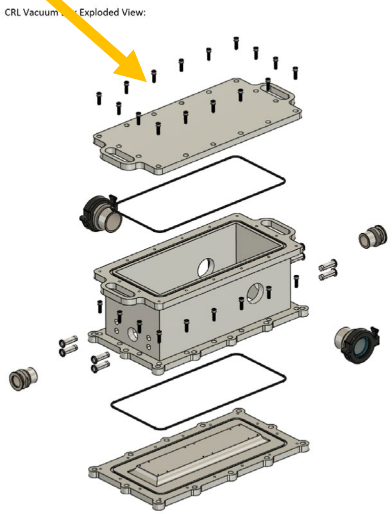
Magnetic Mount



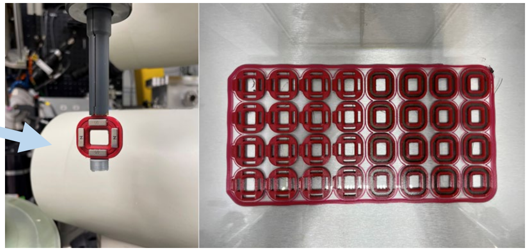




CRL Box



ALEX Holder



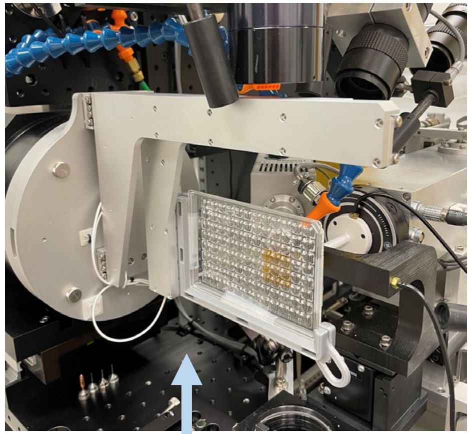
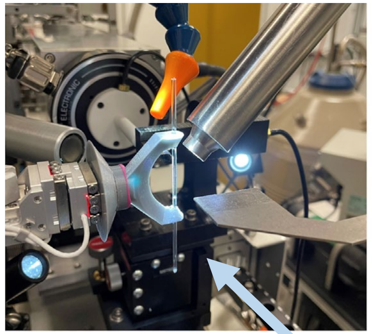


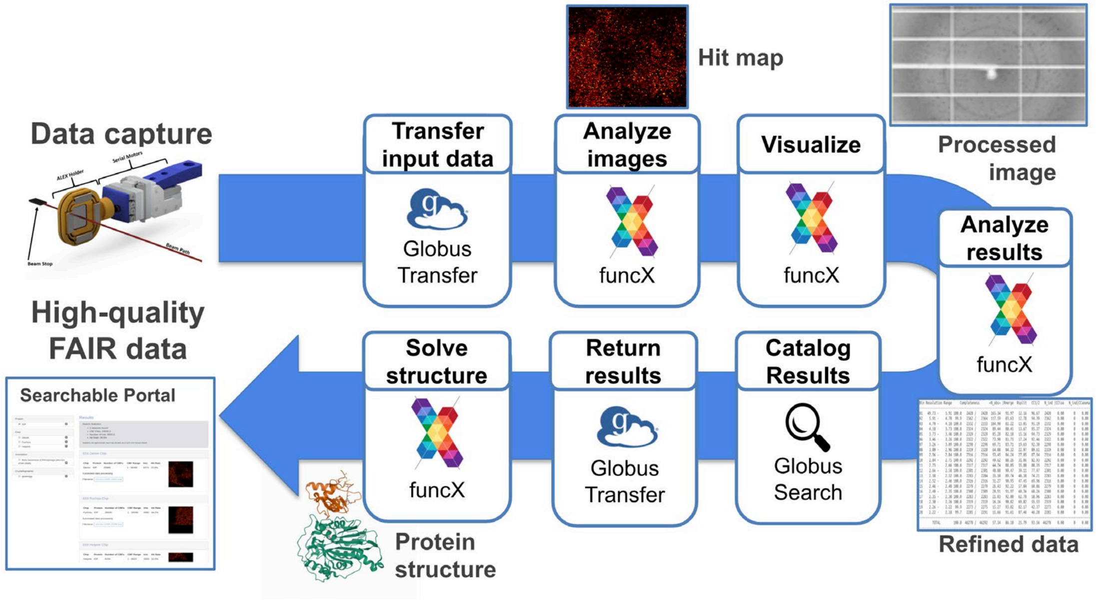
Plate Scanner

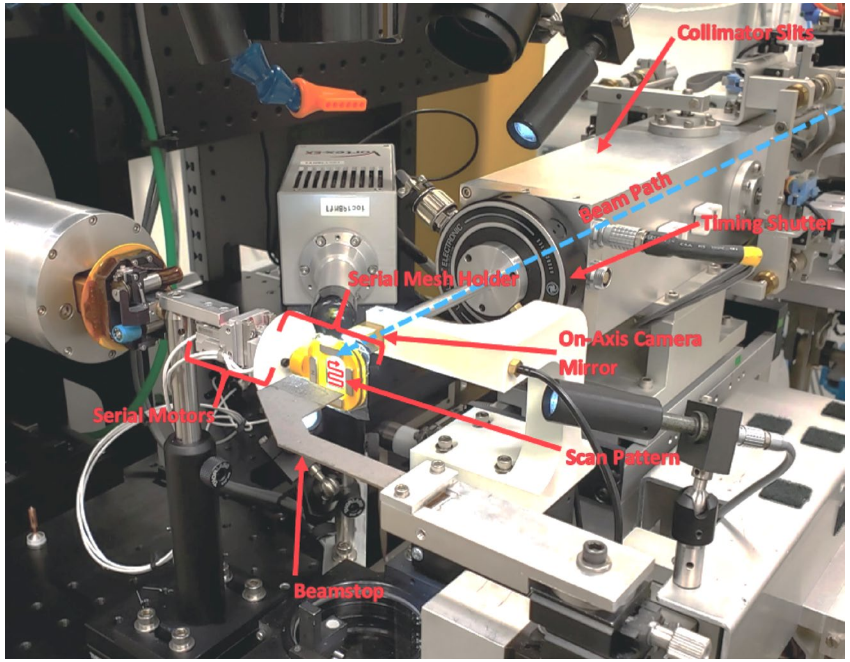


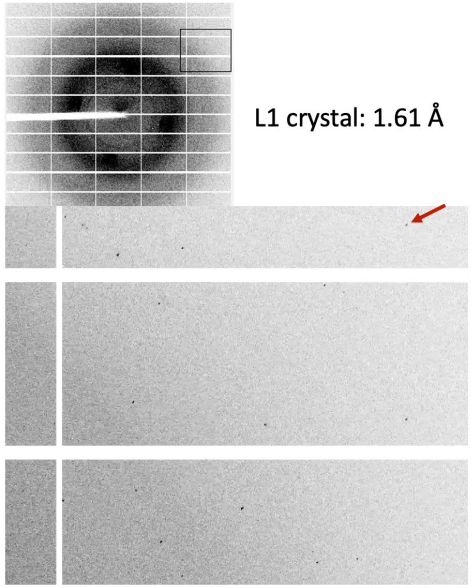
Capillary Holder

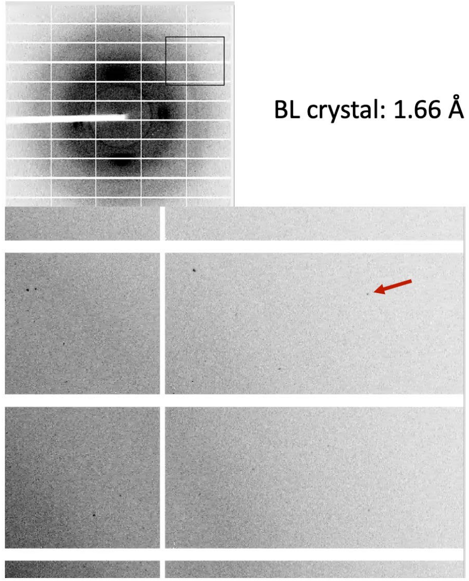
19-ID Set up for Serial Crystallography

- Dynamic crystallography implemented at 19-ID and available to users.
- Rapid data collection for serial crystallography.
- Visualizing chemical reaction in the crystal of enzyme.
- Sector 19 is linked to Argonne Leadership Computing Facility to rapidly process diffraction images and provide near real time feedback to diffraction experiments.









SBC – User Support During “Dark” Period

- SBC will continue support of MX user operations till APS “dark” period, starting ~ April 17, 2023
- SBC conducted survey how we can help users to go through the APS “dark” period?
- Users expressed interest and SBC will apply for beamtime at several light sources:
  - BNL/NSLS-II, SSRL/SLACMX and LBNL/ALS MX Beamlines
- SBC will help to coordinate user success and SBC staff will provide limited user support.
- Sector 84 (APCF) molecular biology capabilities will stay open

Structural Biology Center Transitioning to eBERlight

Current status

- SBC is funded by DOE Biological and Environmental Research
- Operates 2 MX beamlines (19-ID, 19-BM) for the general user community

Need for change

- BER community needs access to other techniques
- APS-U brings new opportunities
- 19-ID will host In Situ Nanoprobe

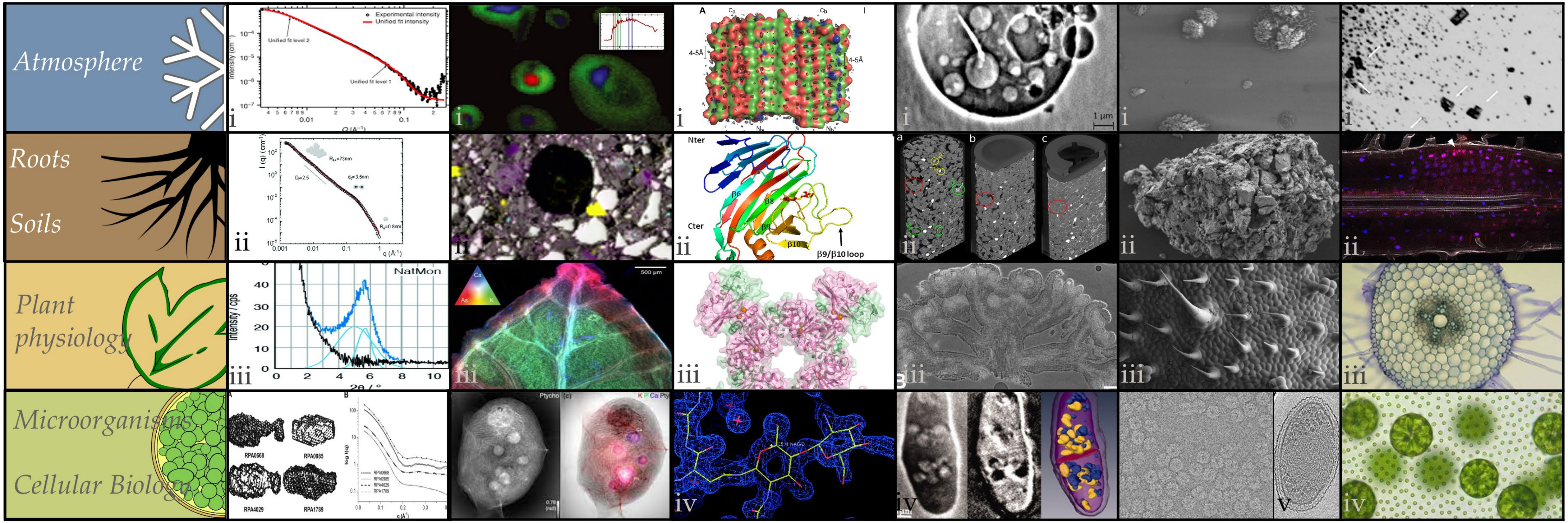
Future program

- eBERlight will serve as a liaison between BER researchers and the APS
- Leverage additional ANL resources
- Integrate with other DOE/BER facilities

Multimodal Approach to BER Science at APS-U

APS-U Techniques | Complementary

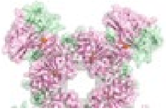
Solution SAXS | *Point Probe XFM / XAS* | *Crystallographic MX* | *Full-field Imaging XMIC* | *Electron Microscopy EM* | *Non-Destructive Optical MIC*



nm - μm | *nm - mm* | *atomic* | *nm - mm* | *<nm - mm* | *μm - mm*

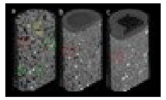
eBERlight Capabilities for Biological and Environmental Science

Crystallography
21-ID (LS-CAT)

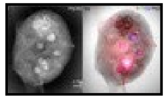


Full-field imaging

2-BM
32-ID



X-ray microscopy
2-ID-D
2-ID-E
(ISN,
25-ID-D,E)



Molecular biology

Advanced Protein
Characterization
Facility

Sample preparation

Cryolab at APS
Lab infrastructure
in bld. 203

Microfluidics

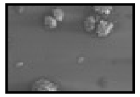
Advanced Protein
Characterization
Facility

Computing

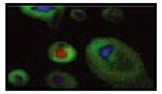
Argonne Leadership
Computing
Facility

Electron microscopy

Center for Nanoscale
Materials
Picoprobe

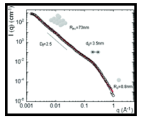


X-ray absorption spectroscopy
9-BM
20-BM
25-ID-D,E
10-ID, BM



Small-angle X-ray scattering

9-ID
12-ID
12-BM



eBERlight Organization

Comprehensive program for BER community starting FY24

Virtual Collaborative Access Team (CAT) managed by X-ray Science Division

Guaranteed beamtime across several beamlines (max. 2 beamlines equivalent)

Effort distribution:

- 25% crystallography (LS-CAT)
- 65% microscopy & imaging (XSD)
- 10% X-ray absorption spectroscopy & SAXS (XSD)

50% of the eBERlight guaranteed beamtime to support CAT members (BER researchers), 50% to support General User Program

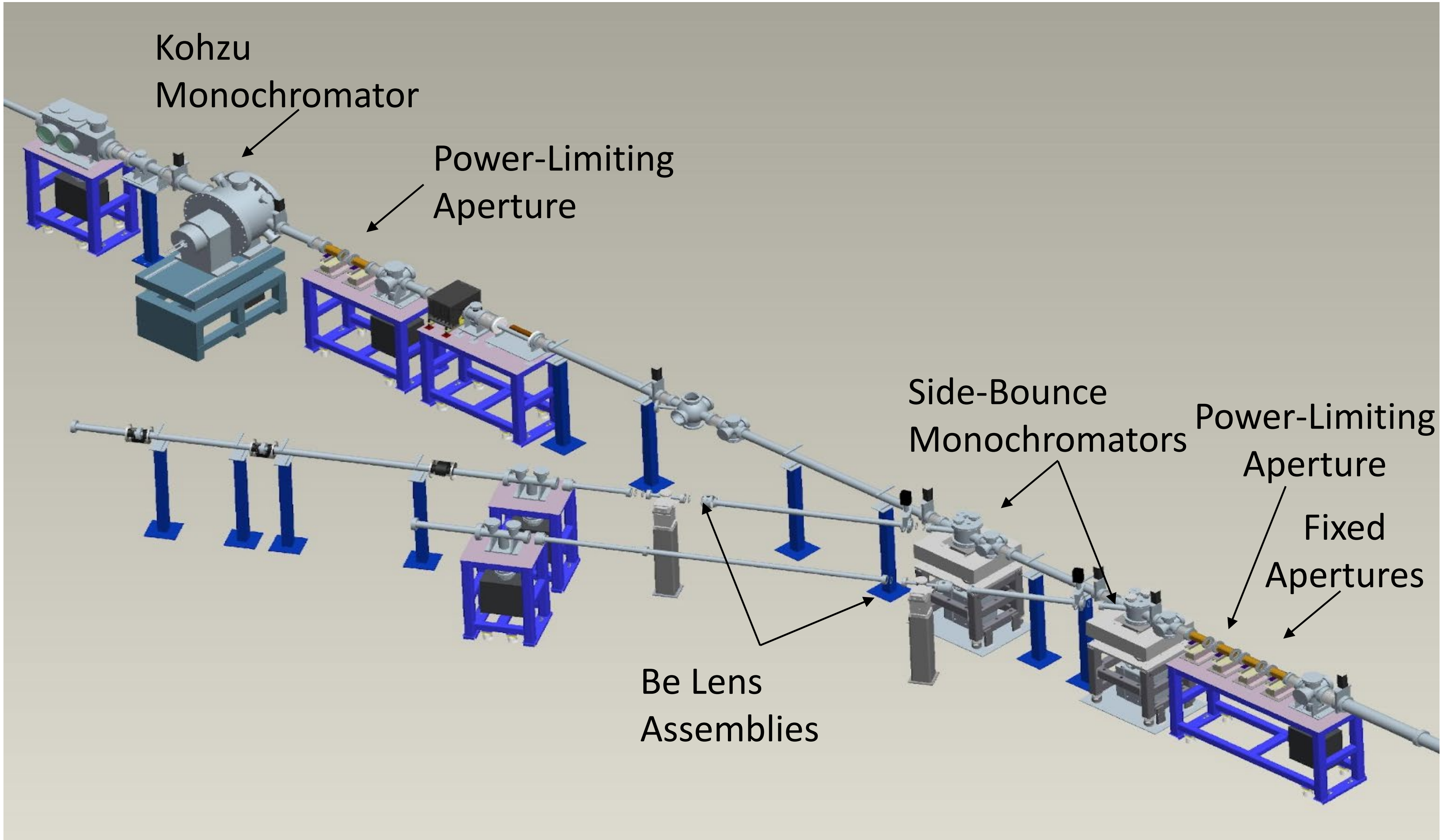
APS-U Structural Biology Townhall Agenda

Start	Duration	End	
1:00 PM	0:05	1:05 PM	Welcome - Laurent Chapon,
1:05 PM	0:25	1:30 PM	APS-U Overview and Life Sciences at the APS, Bob Fischetti
1:30 PM	0:15	1:45 PM	IMCA-CAT, Lisa Keefe
1:45 PM	0:15	2:00 PM	SBC/eBERLight, Andrzej Joachimiak and Karolina Michalska
2:00 PM	0:15	2:15 PM	LS-CAT, Spencer Anderson
2:15 PM	0:15	2:30 PM	SER-CAT, Bi-Chen Wang
2:30 PM	0:15	2:45 PM	GM/CA-CAT, Naga Venugopalan
2:45 PM	0:15	3:00 PM	Break
3:00 PM	0:15	3:15 PM	NE-CAT, Malcolm Capel
3:15 PM	0:15	3:30 PM	LRL-CAT, Jordi Benach
3:30 PM	0:15	3:45 PM	ALS, Marc Allaire
3:45 PM	0:15	4:00 PM	CHESS, Marian Szebenyi
4:00 PM	0:15	4:15 PM	NSLS-II, Sean McSweeney
4:15 PM	0:15	4:30 PM	SSRL, Aina Cohen
4:30 PM	0:30	5:00 PM	Roundtable Q&A
5:00 PM			Adjourn

LS-CAT Upgrade

Spencer Anderson
Operations Manager, LS-CAT
6/21/2022

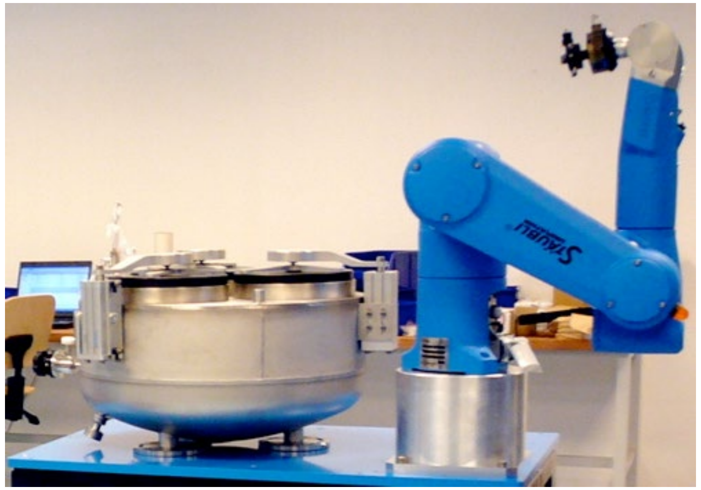
Sector 21 Component Layout



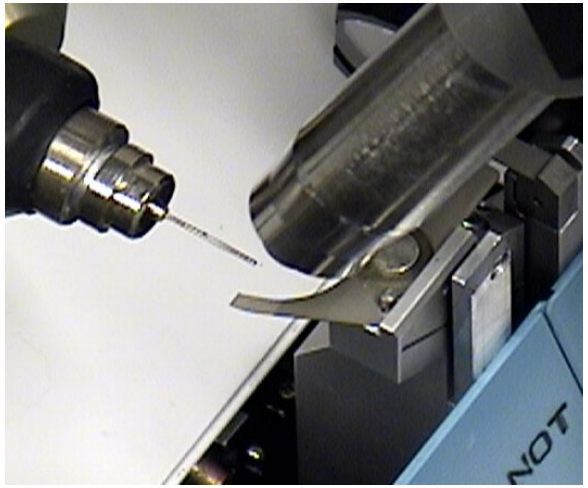
ID-F/G Side Stations

- fixed energy above Se peak
    - ID-F 12.668 keV
    - ID-G 12.670 keV

- current beam 50µm
    - **poorly focused/relatively low flux**
    - 1-3s per image exposure
    - CCD limited to ~30 frames per minute

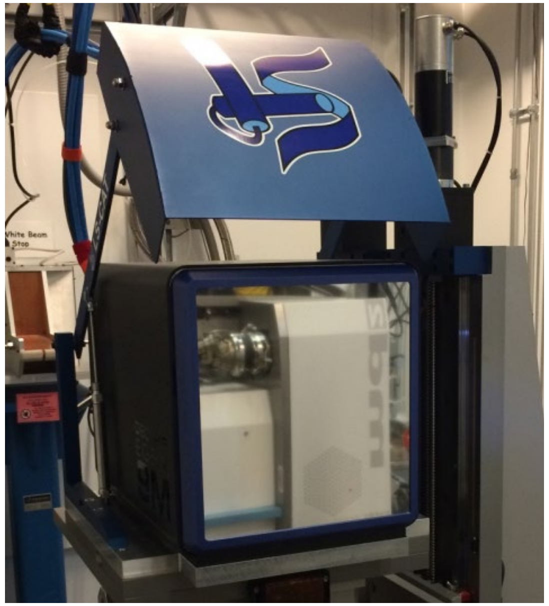


Cryogenic Automated Transfer System (CATS)



MD2 sample environment

MX300 CCD detector



Dectris Eiger 9M

21-ID-D

Dectris Eiger2 X 16M

- tunable from 6-20 keV
- bimorph mirrors focus to ~25μm
- ~~MD2 microdiffractometer~~
- MD3-UP microdiffractometer
- ~~Dectris Eiger 9M PAD~~
- Eiger2 X 16M PAD
- *? Compound Refractive Lens Transfocator*
- *new sample changer*

MD2-S microdiffractometer

MD3-UP microdiffractometer

21-ID-E

NEVER FINISHED

- needs monochromator
- original designed to be tunable:

12.5 – 12.8 keV
13.4 – 13.6 keV

- bimorph mirror
- CCD detector
- CATS sample changer

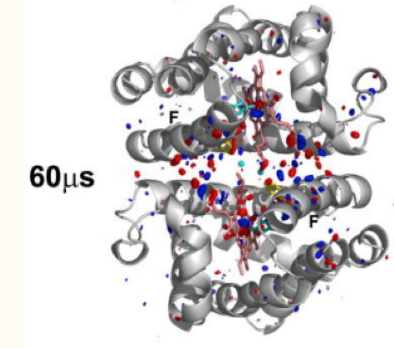
Post Upgrade Crystallography

- Microcrystallography
  - MD3-UP designed for small samples
  - detector readout 130Hz or faster

- Serial crystallography
  - plates
  - chips
  - capillaries

- Dynamic crystallography
  - redox sensitive proteins
  - caged compounds
  - ID-D designed for white beam

microfluidic chip designed for crystal growth and collection



$Fo^{light} - Fo^{dark}$ difference map of *dimeric Scapharca hemoglobin*

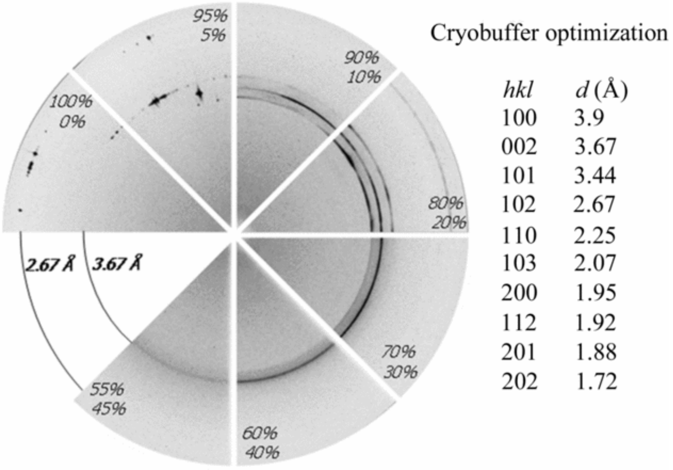
Sui et al. A capillary-based microfluidic device enables primary high-throughput room-temperature crystallographic screening. J. AppL Cryst. (2021). 54, 1034-1046.

Srajer and Royer. Time-resolved x-ray crystallography of heme proteins. Methods Enzymol. 2008; 437: 379–395.

On Site Clinics

Start up again if pandemic ever ends…

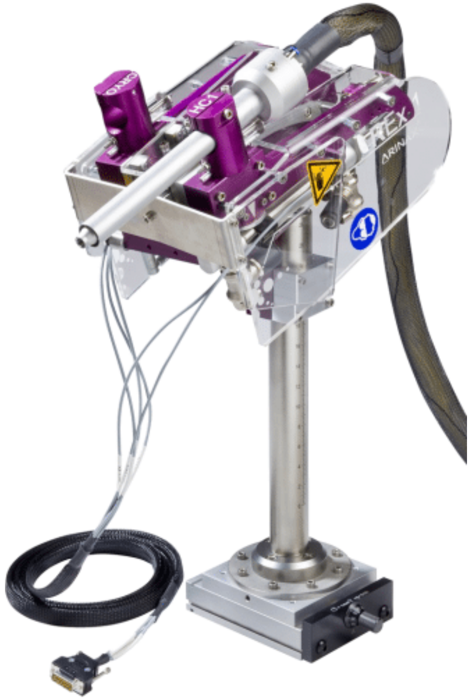
- *crystallographic theory*
- *data collection and analysis*
- ***increase focus on sample preparation***
- *room temperature vs frozen*
- *cryo protection*
- *freezing method*
- *humidity manipulation*



Cryobuffer optimization

hkl	*d* (Å)
100	3.9
002	3.67
101	3.44
102	2.67
110	2.25
103	2.07
200	1.95
112	1.92
201	1.88
202	1.72

change in ice rings with cryoprotectant concentration



Arinax REX Rapid Nozzle Exchanger

Mitegen Watershed Workstation

Summary

- LS-CAT designed for high flux and already compatible with post APS-U beam

- continue routine single crystal experiments on side stations, but with a huge improvement in flux

- ID-D (and ID-E) designed for more challenging serial and dynamic crystallography experiments

- ID-D in great shape with new microdiffractometer and PAD

- prioritize finishing ID-E since challenging experiments take lots of beamtime

APS-U Structural Biology Townhall Agenda

Start	Duration	End	
1:00 PM	0:05	1:05 PM	Welcome - Laurent Chapon,
1:05 PM	0:25	1:30 PM	APS-U Overview and Life Sciences at the APS, Bob Fischetti
1:30 PM	0:15	1:45 PM	IMCA-CAT, Lisa Keefe
1:45 PM	0:15	2:00 PM	SBC/eBERLight, Andrzej Joachimiak and Karolina Michalska
2:00 PM	0:15	2:15 PM	LS-CAT, Spencer Anderson
2:15 PM	0:15	2:30 PM	SER-CAT, Bi-Chen Wang
2:30 PM	0:15	2:45 PM	GM/CA-CAT, Naga Venugopalan
2:45 PM	0:15	3:00 PM	Break
3:00 PM	0:15	3:15 PM	NE-CAT, Malcolm Capel
3:15 PM	0:15	3:30 PM	LRL-CAT, Jordi Benach
3:30 PM	0:15	3:45 PM	ALS, Marc Allaire
3:45 PM	0:15	4:00 PM	CHESS, Marian Szebenyi
4:00 PM	0:15	4:15 PM	NSLS-II, Sean McSweeney
4:15 PM	0:15	4:30 PM	SSRL, Aina Cohen
4:30 PM	0:30	5:00 PM	Roundtable Q&A
5:00 PM			Adjourn

APS Structural Biology Virtual Town Hall Meeting

SER-CAT

SouthEast Regional Collaborative Access Team

B.C. Wang, John Rose, John Chrzas and

SER-CAT Team

June 21, 2022

SER-CAT's Vision/Goals and Future Challenges

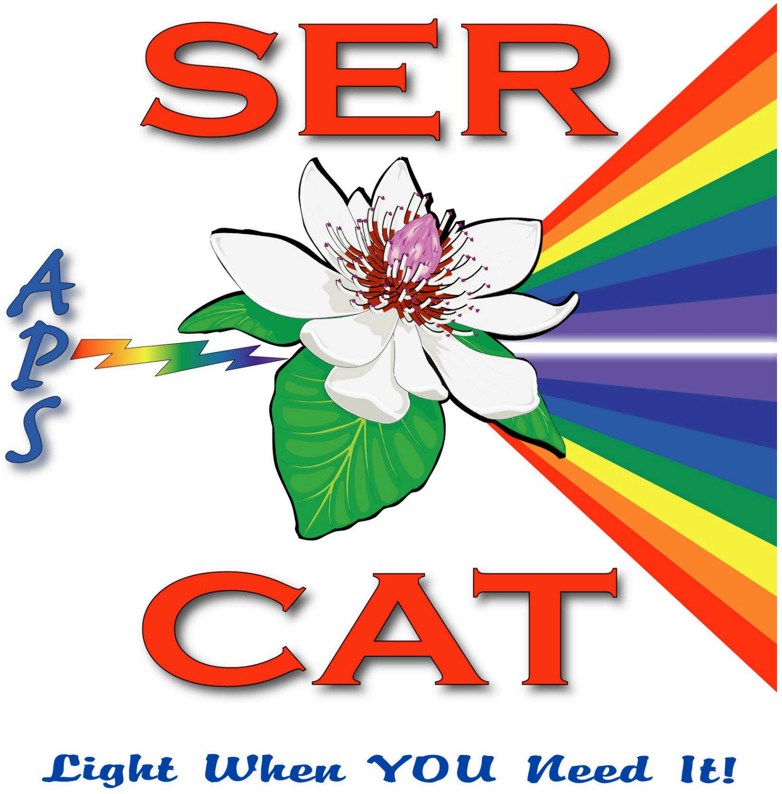
Vision/Goals

- The construction of SER-CAT's two synchrotron beamlines at the APS was initiated in 1997 with **a grant from the Georgia Research Alliance (GRA) as an enabling technology for research and education** for the institutions **in the State of Georgia.**

- The first (**22ID**) of the two beamlines was commissioned in 2002 when it started providing "***Light When You Need it***" as a virtual home synchrotron source.

- SER-CAT has since become a **shared Research Resource** for institutions, mostly in the Southeast, in **promoting structural biology programs and faculty recruiting. A SER-CAT Trust Estate fund** was also established at UGA in 2001 to assist SER-CAT's future **operations and upgrade**.

- SER-CAT has now grown into a consortium of **23 institutions.**

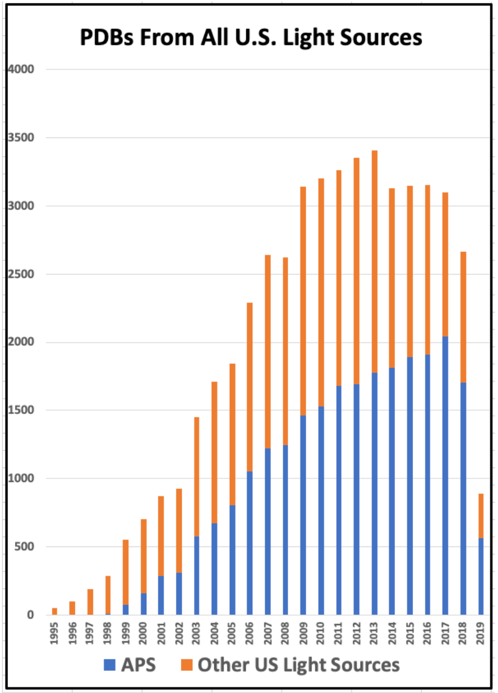


SER-CAT Program's Response to the APS-U

1. SER-CAT VALUES ITS USERS – ***Working with several facilities in the U.S. to find beam time for SER-CAT users during APS dark period.***

2. SER-CAT VIEWS THE APS DARK PERIOD AN OPORTUNITY – To optimize and upgrade SER-CAT Operation and Equipment to support future structure-based new science enabled by the 4th generation storage ring at the APS, first of its kind in the U.S.

Challenges with # 1 about



- **APS generates over half of all PDB entries coming from U.S. facilities (2008 – 2018)**
- **Other US facilities do not have the extra capacity (or undulator beamlines) required to address this need**
- **APS Users will have to make GU applications at other facilities – the lines will be long**

Challenges with # 2 about

Will discuss the plans in later slides

SER
APS
CAT
Light When YOU Need It!

SER-CAT Response to the APS Dark Period

SER-CAT VALUES ITS USERS

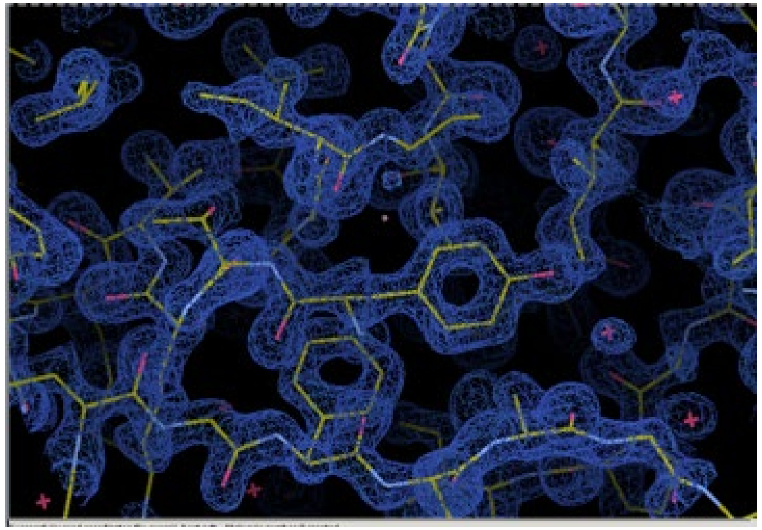
- **SER-CAT will serve as the central hub for the scheduling and management of SER-CAT's beamtime at these collaborative facilities.**
- **SER-CAT will provide a second layer of user support during data collection - answering questions and providing suggestions as they currently do.**
- **SER-CAT will also assist users in data processing and analysis - including tackling "Problem data sets"**
- **SER-CAT will also help users apply for GU time at other U.S. facilities - providing links and other materials**

Goal

The user ships their crystals to the beamline and uses familiar software for mounting, centering & data collection to collect the best possible data from the crystal.

The SER-CAT - BCSB/ALS Collaboration

- **In preparation for the APS "dark" period, we established a Partnership program with ALS-BCSB in 2020**
- **We held a virtual training workshop using BCSB beamlines on March 18, 2021, the day before SER-CAT's 2021 Annual Symposium**
- **In addition, we provided hands-on remote data collection training runs to individual SER-CAT users during May – June 2021 & 2022 on ALS Beamlines 501 and 502**
- **SER-CAT Users are now ready to use ALS 501 & 502 during APS-U**



A standard Test Run on ALS 502

Extreme S-SAD phasing test using bovine a trypsin crystal and data collected using 1.0 Å X-rays ($\Delta F'' = 0.24$)

New York Structural Biology Center

SER-CAT is in negotiations with the New York Structural Biology Center to come on as an additional member using their NYX beamline (19ID) at NSLS-II.

SER-CAT Upgrade

- ***Sector 22 will have a canted front-end installed to enable a second undulator resource 22ID-E to replace 22BM.***

- ***The first priority is to ensure 22ID-D comes out of the upgrade fully operational.***

- ***The user program on 22ID-D must be established as soon as possible once the APS provides X-rays.***

- ***The new beamline (22ID-E) will come online 6-12 months after 22ID-D is operational.***

22ID-D Upgrade

- *New FMB-Oxford Monochromator to replace the current one.*
    - *2 sets of crystals pairs, Si(111) and Si(220), in vacuum tank*
    - *Provide large energy range 5-24 keV*
    - *Provide improved energy resolution option with Si(220) crystals*
- *IDT K-B mirror system*
    - *Currently installed with the horizontal mirror in service*
- *New MD3 diffractometer with new sample robot*
- *Full automation of routine sample screening and data collection*
- *Upgrade all motion control and vacuum systems*
- *Network upgraded in 2020*
- *Equipment Protection System (EPS) upgraded in 2020*
- *Upgrade to Generation 4 Personnel Safety System (PSS)*

22ID-E Upgrade

Bringing 2nd ID X-ray Beam from Station 22ID-A to the Future Station 22ID-E (currently 22BM-C)

- *Side Bounce single crystal monochromator*
  - *Si (220)*
- *MD2 diffractometer with sample robot*
- *CRL focusing*
  - *Multiple lens stacks for range of focus options*
- *Full automation of routine sample screening and data collection*

Light When YOU Need It!

Acknowledgements

UGA & GRA

Lars Ljungdahl
Mike Cassidy
Susan Shows
Joe Key
Karen Holbrook

BCSB/ALS

Marc Allaire
Corie Ralston
Stacey Ortega

SER-CAT

John P. Rose
Zheng-Qing (Albert) Fu
Gerold Rosenbaum
Jim Fait
Unmesh Chinte
Norma Duke
Rod Salazar
Lily Li
Larry DeLucas (UAB)

John Chrzas
Zhongmin Ji
John Unik
John Gonczy
Palani Kandavelu
Michael Molitsky
Kathy Morris
Jackie Taylor
Craig Smith (UAB)

APS

Gopal Shenoy
Denny Mills
Susan Barr Strasser
J. Murray Gibson
Stephen Streiffer
Robert Fischetti

NIGMS/NIH
SER-CAT Institutions, APS/ANL
Ramsay - Georgia Research Alliance Endowment Fund
University of Georgia Research Foundation

Light When YOU Need It!

APS-U Structural Biology Townhall Agenda

Start	Duration	End	
1:00 PM	0:05	1:05 PM	Welcome - Laurent Chapon,
1:05 PM	0:25	1:30 PM	APS-U Overview and Life Sciences at the APS, Bob Fischetti
1:30 PM	0:15	1:45 PM	IMCA-CAT, Lisa Keefe
1:45 PM	0:15	2:00 PM	SBC/eBERLight, Andrzej Joachimiak and Karolina Michalska
2:00 PM	0:15	2:15 PM	LS-CAT, Spencer Anderson
2:15 PM	0:15	2:30 PM	SER-CAT, Bi-Chen Wang
2:30 PM	0:15	2:45 PM	GM/CA-CAT, Naga Venugopalan
2:45 PM	0:15	3:00 PM	Break
3:00 PM	0:15	3:15 PM	NE-CAT, Malcolm Capel
3:15 PM	0:15	3:30 PM	LRL-CAT, Jordi Benach
3:30 PM	0:15	3:45 PM	ALS, Marc Allaire
3:45 PM	0:15	4:00 PM	CHESS, Marian Szebenyi
4:00 PM	0:15	4:15 PM	NSLS-II, Sean McSweeney
4:15 PM	0:15	4:30 PM	SSRL, Aina Cohen
4:30 PM	0:30	5:00 PM	Roundtable Q&A
5:00 PM			Adjourn

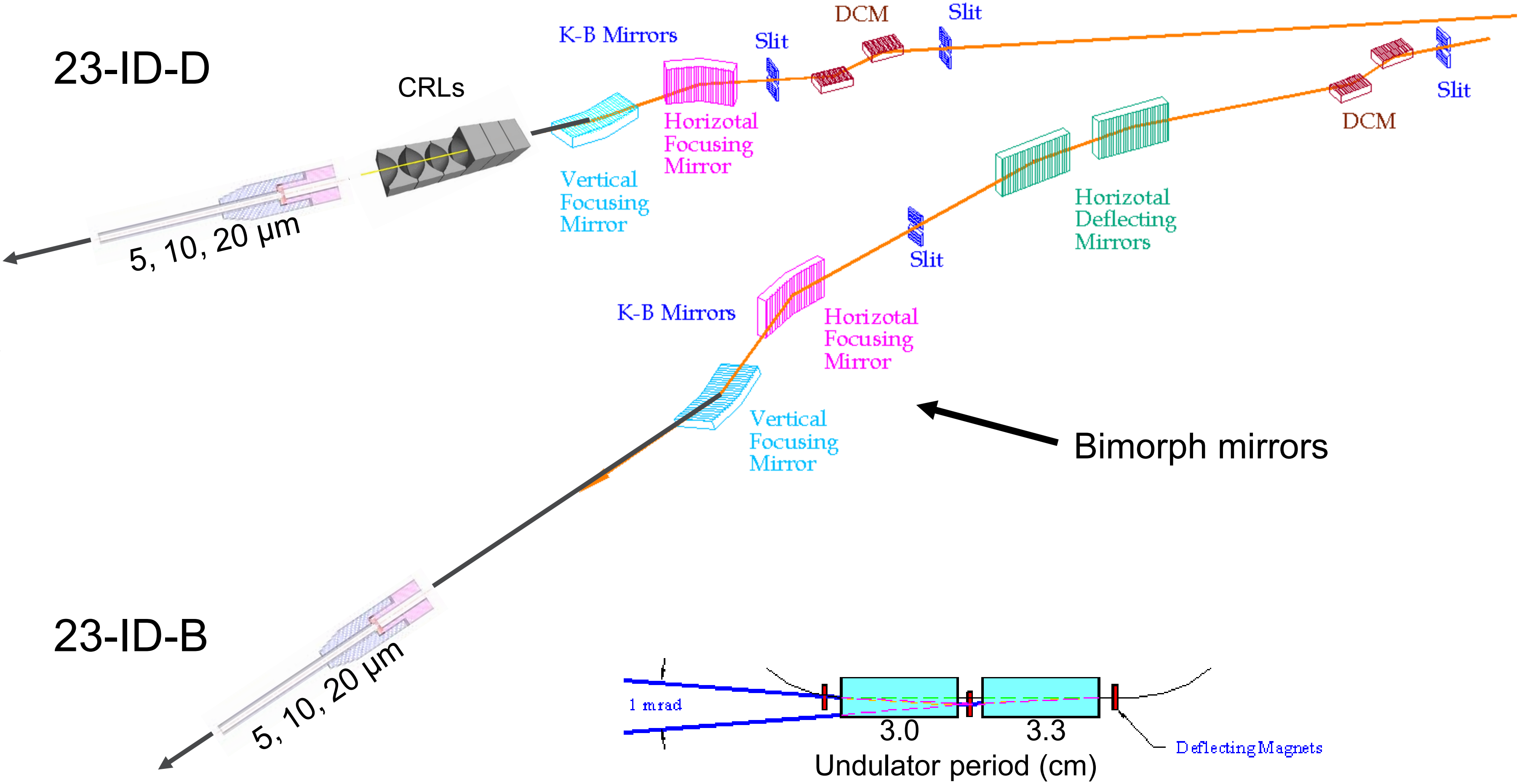
GM/CA @APS

Nagarajan Venugopalan (Naga)

APS Upgrade Structural Biology Town Hall
21 June 2022

GM/CA Dual-canted Undulator beamlines layout

Rapid energy tunability – High intensity and positional stability – Low convergence optics



23-ID-D

Pilatus3 6M
5-20 keV

21 x 20 µm^2
1 µm with CRLs

Bimorph mirrors

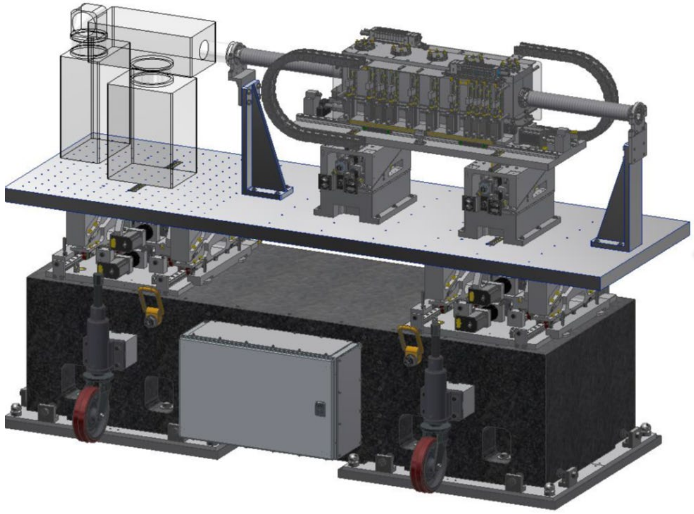
23-ID-B

Eiger 16M
3.5-20 keV
20 x 80 µm^2

3.0 cm device optimized for Se SAD/MAD phasing

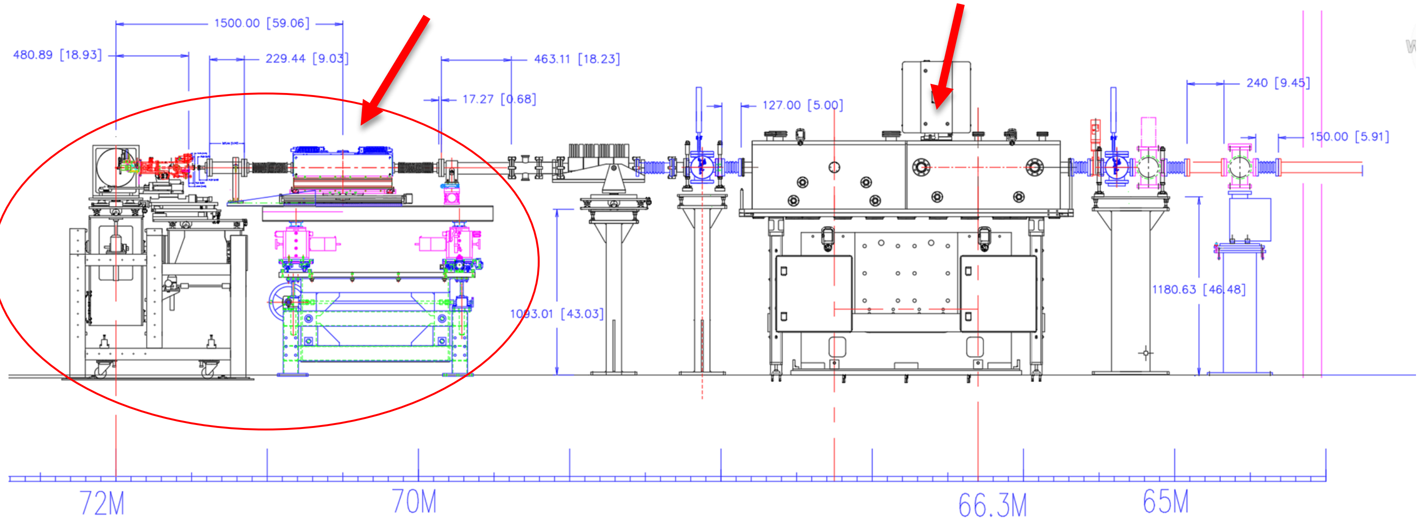
GM/CA-Hardware Upgrades: 23-IDD

New end station table and sample environment



CRL Transfocator

New focusing mirrors

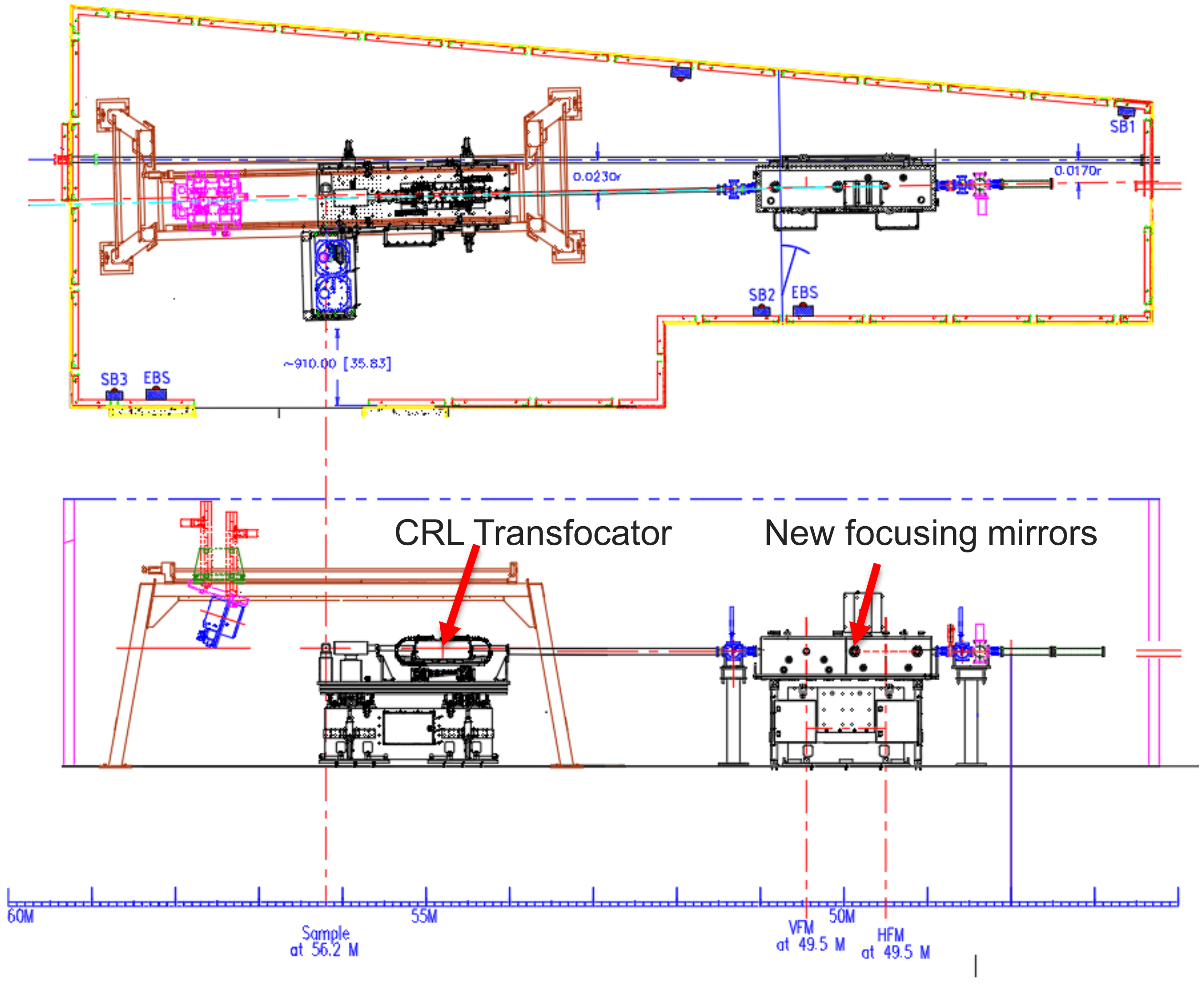


	Optic	Intensity (photon/sec)	Beam size (μm×μm, FWHM)	Flux density (photon/sec/μm²)
APS	**Mirrors**	3.5×10^{13}	62.2 × 3.0	2.4×10^{11}
APS-U*		7.1×10^{13}	4.4 × 3.2	6.5×10^{12}
APS	**CRLs**	4.3×10^{12}	15.0 × 0.5	7.0×10^{11}
APS-U*		1.6×10^{13}	0.7 × 0.5	5.7×10^{13}

Exciting new frontier!

A crystal will survive <1 msec → need rapid beam delivery and detector framing

GM/CA-Hardware Upgrades: 23-IDB

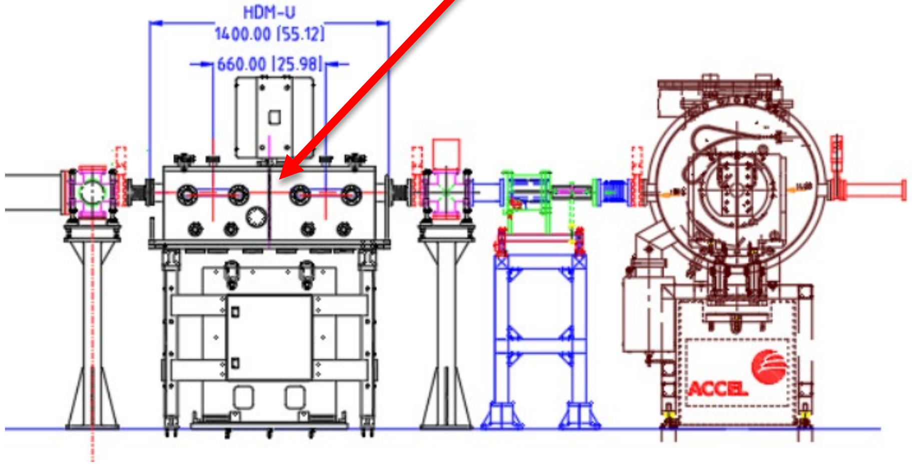


CRL Transfocator

New focusing mirrors

23-IDA

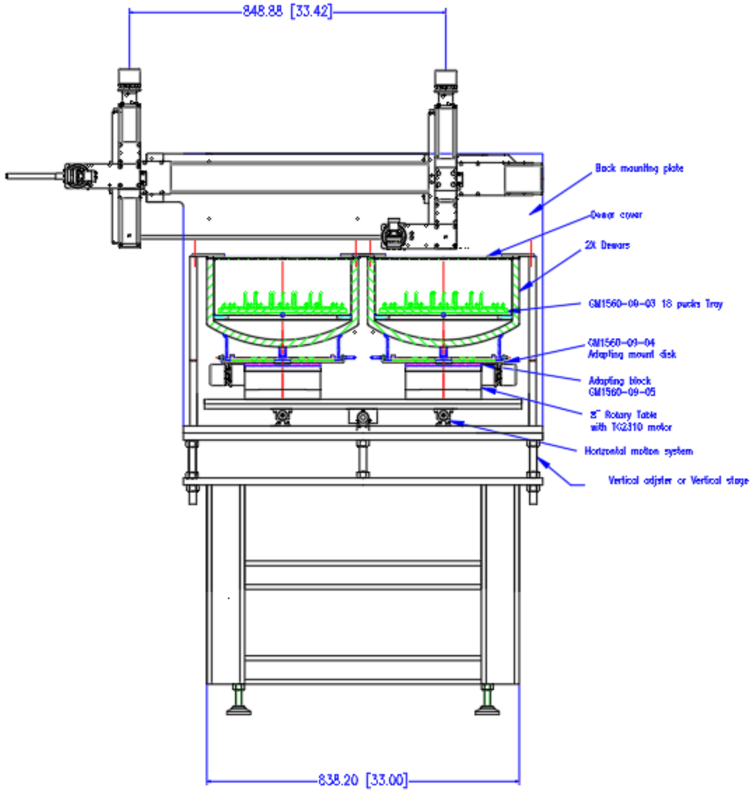
New Horizontal Deflecting mirrors



Installation during the dark period

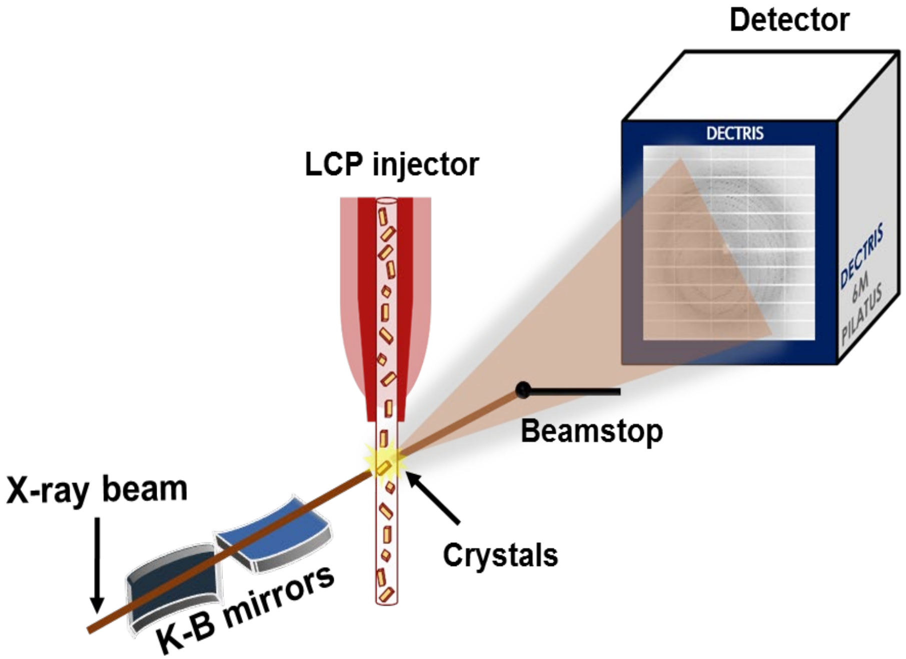
Increased Dewar capacity

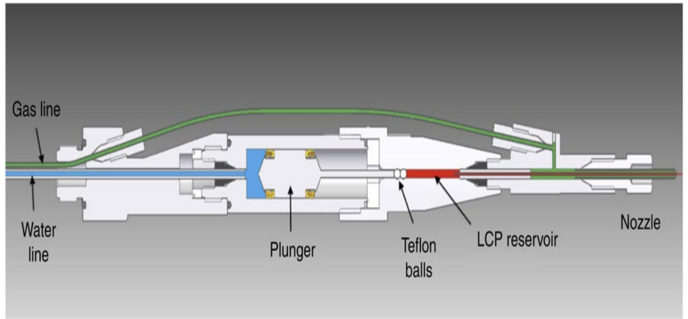
Current Robot Dewar
18 pucks, 288 Samples



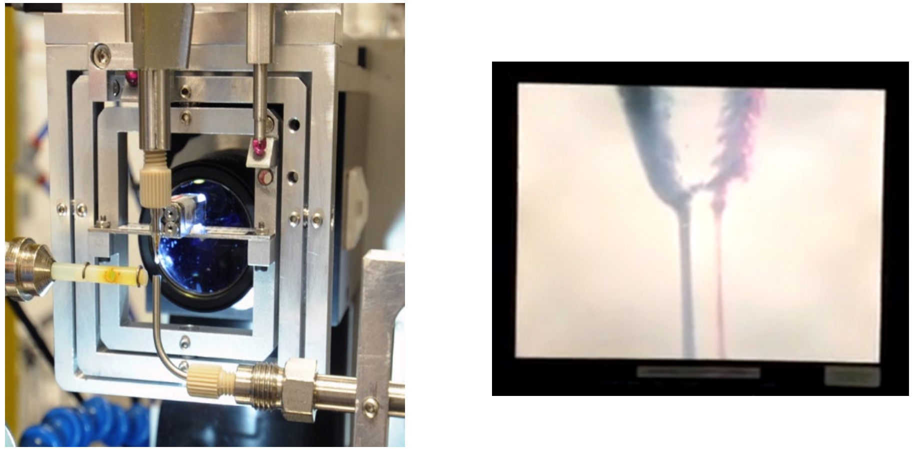
Double the capacity, 2 dewars side-by-side
36 pucks, 576 Samples

Serial Synchrotron Crystallography: LCP Injector





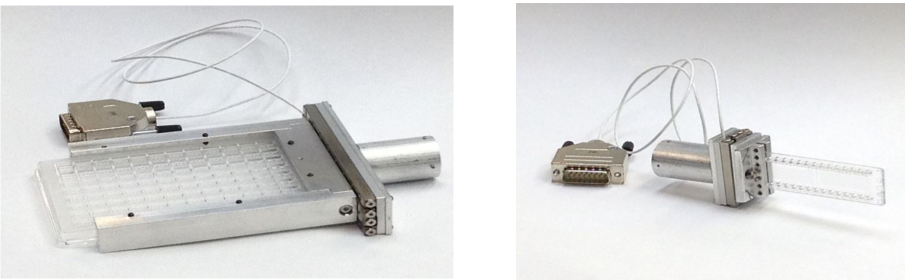
LCP Injector
Curtesy of Uwe Weierstall

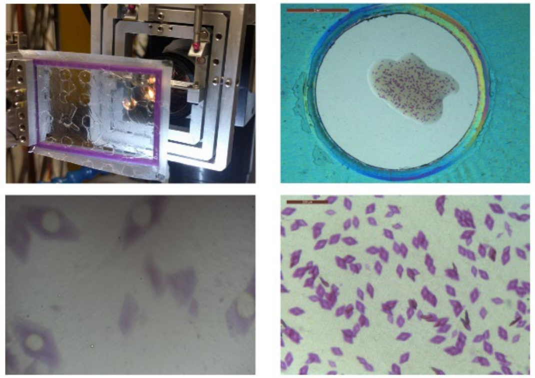


LCP Injector on 23ID-D

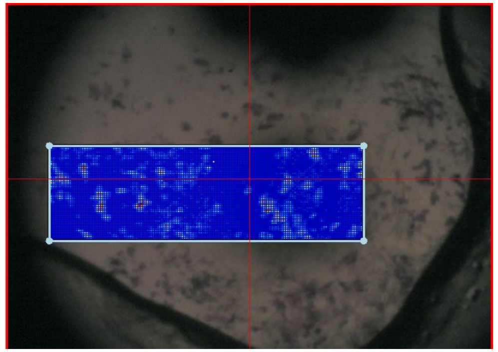
J.M. Martin-Garcia, C.E. Conrad, G. Nelson, N. Stander, N.A. Zatsepin, J. Zook, L. Zhu, J. Geiger, E. Chun, D. Kissick, M. Hilgart, C. Ogata, A. Ishchenko, N. Nagaratnam, S. Roy-Chowdhury, J. Coe, G. Subramanian, A. Schaffer, D. James, G. Ketwala, N. Venugopalan, S. Xu, S. Corcoran, D. Ferguson, U. Weierstall, U., J.C.H. Spence, V. Cherezov, P. Fromme, R.F. Fischetti, W. Liu, IUCrJ, 4, 439-454 (2017).

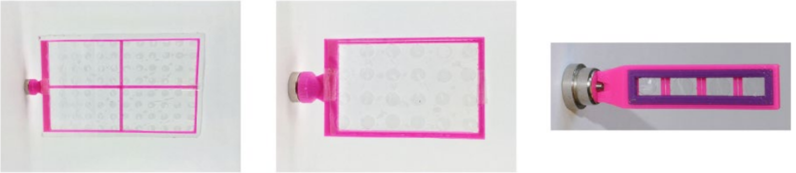
Fixed target Serial Crystallography





T.D. Murray, A.Y. Lyubimov, C.M. Ogata, H. Vo, M. Uervirojnangkoorn, A.T. Brunger, J.M. Berger, Acta Cryst. D71, 1097 – 1997 (2015).
J. Broecker, V. Klingel, W.-L. Ou, A.R. Balo, D.J. Kissick, C.M. Ogata, A.L. Kuo, O.P. Ernst, Cryst. Growth Des. 16(11), 6318-6326 (2016)
J. Broecker, T. Morizumi, W.-L. Ou, V. Klingel, A. Kuo, D.J. Kissick, A. Ishchenko, M.-Y. Lee, S. Xu, O. Makarov, V. Cherezov, C.M. Ogata, O.P. Ernst, Nature Protocols 13(2), 260-292 (2018).





PyBluIce: Data collection software upgrades

Serial Crystallography

- Injector collect
- Mesh collect
- Large cell count raster

Sustained HDR

(High data rate)

- Full detector speed
- Faster spot finding

Automation

- Auto-raster
- Multiple modes of data collection

Scriptability

- Modular approach

Beamline Computing upgrades:

- 1 Petabyte size storage array network for ID lines, additional 0.5 Petabyte size storage will be merged from BM
- 8 computers with 100 Gig connectivity
- Network switch with dual 100 Gig connectivity to ALCF (Argonne Leadership Computing Facility)
- Working with Nicholas Schwarz group at APS, arranging for real time data transfer and data processing at ALCF
- Merged 2 Globus servers, increasing Globus online data transfer speeds
- Procure faster computers

Computing Access During Dark Period:

Requirements:

  - Registered APS Users
  - ESAF submission
  - Completed Training

The future is bright!

- Upgraded X-ray focusing optics will deliver an intense beam as small as 1-µm
- Routine Serial Synchrotron Crystallography
    - LCP Injector
    - Fixed Target
- New software tools for routine serial crystallography, High Data rate and Automation
- Computing upgrades: large storage and real time data transfer and processing

- Exciting times are ahead!

Thank You

APS-U Structural Biology Townhall Agenda

Start	Duration	End	
1:00 PM	0:05	1:05 PM	Welcome - Laurent Chapon,
1:05 PM	0:25	1:30 PM	APS-U Overview and Life Sciences at the APS, Bob Fischetti
1:30 PM	0:15	1:45 PM	IMCA-CAT, Lisa Keefe
1:45 PM	0:15	2:00 PM	SBC/eBERLight, Andrzej Joachimiak and Karolina Michalska
2:00 PM	0:15	2:15 PM	LS-CAT, Spencer Anderson
2:15 PM	0:15	2:30 PM	SER-CAT, Bi-Chen Wang
2:30 PM	0:15	2:45 PM	GM/CA-CAT, Naga Venugopalan
2:45 PM	0:15	3:00 PM	Break
3:00 PM	0:15	3:15 PM	NE-CAT, Malcolm Capel
3:15 PM	0:15	3:30 PM	LRL-CAT, Jordi Benach
3:30 PM	0:15	3:45 PM	ALS, Marc Allaire
3:45 PM	0:15	4:00 PM	CHESS, Marian Szebenyi
4:00 PM	0:15	4:15 PM	NSLS-II, Sean McSweeney
4:15 PM	0:15	4:30 PM	SSRL, Aina Cohen
4:30 PM	0:30	5:00 PM	Roundtable Q&A
5:00 PM			Adjourn

APS-U Structural Biology Townhall Agenda

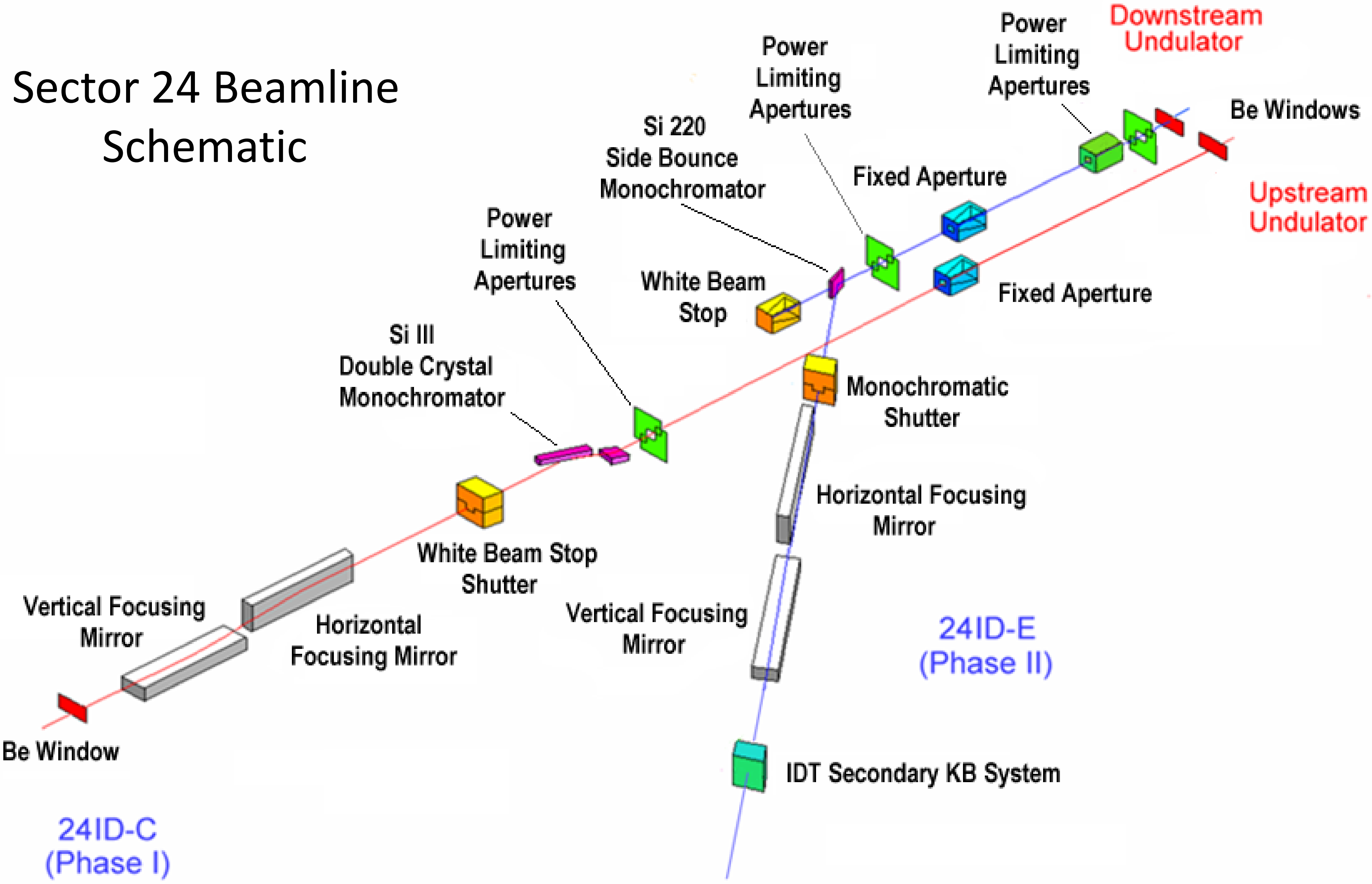
Start	Duration	End	
1:00 PM	0:05	1:05 PM	Welcome - Laurent Chapon,
1:05 PM	0:25	1:30 PM	APS-U Overview and Life Sciences at the APS, Bob Fischetti
1:30 PM	0:15	1:45 PM	IMCA-CAT, Lisa Keefe
1:45 PM	0:15	2:00 PM	SBC/eBERLight, Andrzej Joachimiak and Karolina Michalska
2:00 PM	0:15	2:15 PM	LS-CAT, Spencer Anderson
2:15 PM	0:15	2:30 PM	SER-CAT, Bi-Chen Wang
2:30 PM	0:15	2:45 PM	GM/CA-CAT, Naga Venugopalan
2:45 PM	0:15	3:00 PM	Break
3:00 PM	0:15	3:15 PM	NE-CAT, Malcolm Capel
3:15 PM	0:15	3:30 PM	LRL-CAT, Jordi Benach
3:30 PM	0:15	3:45 PM	ALS, Marc Allaire
3:45 PM	0:15	4:00 PM	CHESS, Marian Szebenyi
4:00 PM	0:15	4:15 PM	NSLS-II, Sean McSweeney
4:15 PM	0:15	4:30 PM	SSRL, Aina Cohen
4:30 PM	0:30	5:00 PM	Roundtable Q&A
5:00 PM			Adjourn

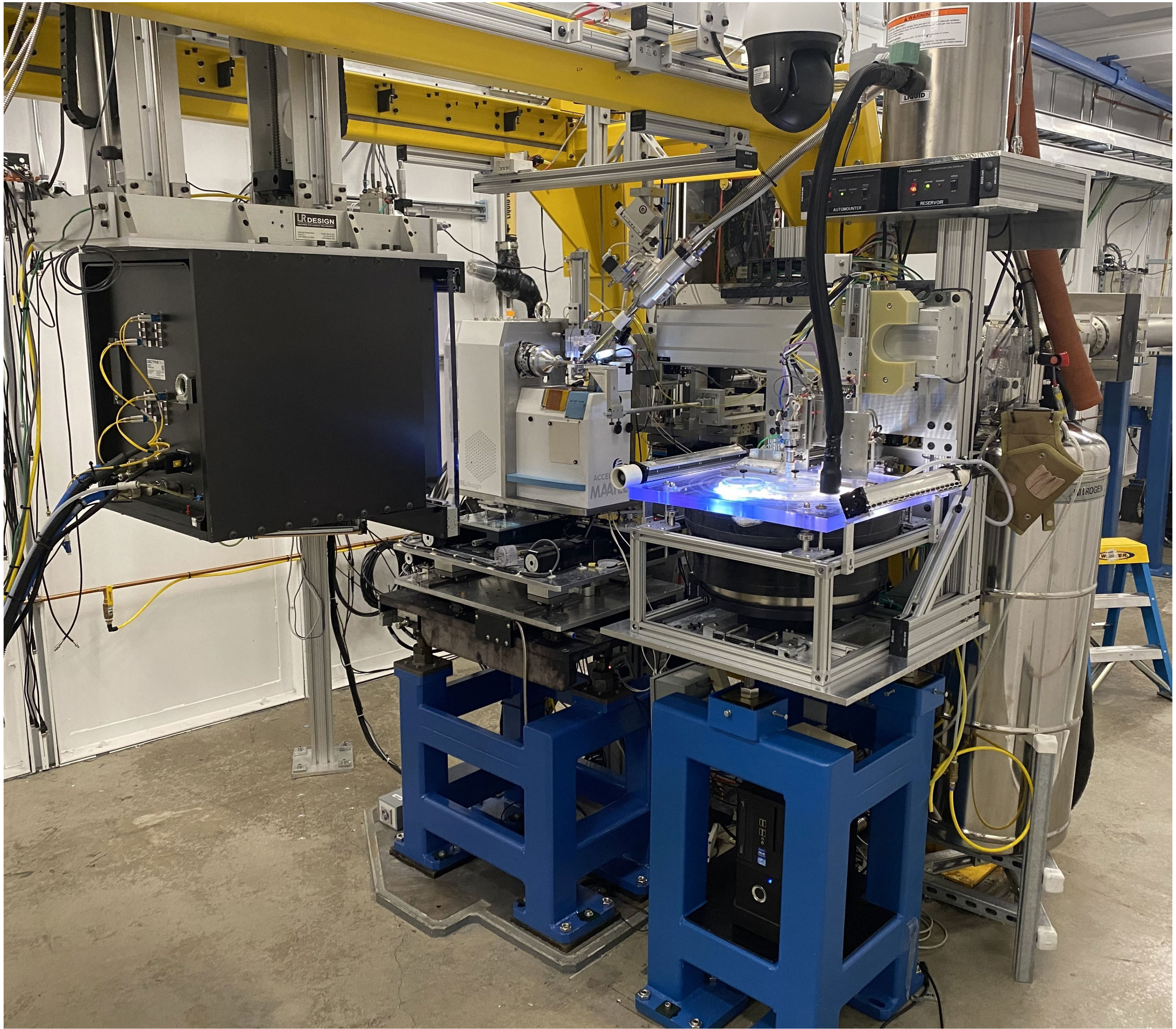
Improvements at NE-CAT Driven by APSU

Malcolm Capel
NE-CAT Deputy Director
Dept. Chemistry and Chemical Biology,
Cornell University

V5

Sector 24 Beamline Schematic



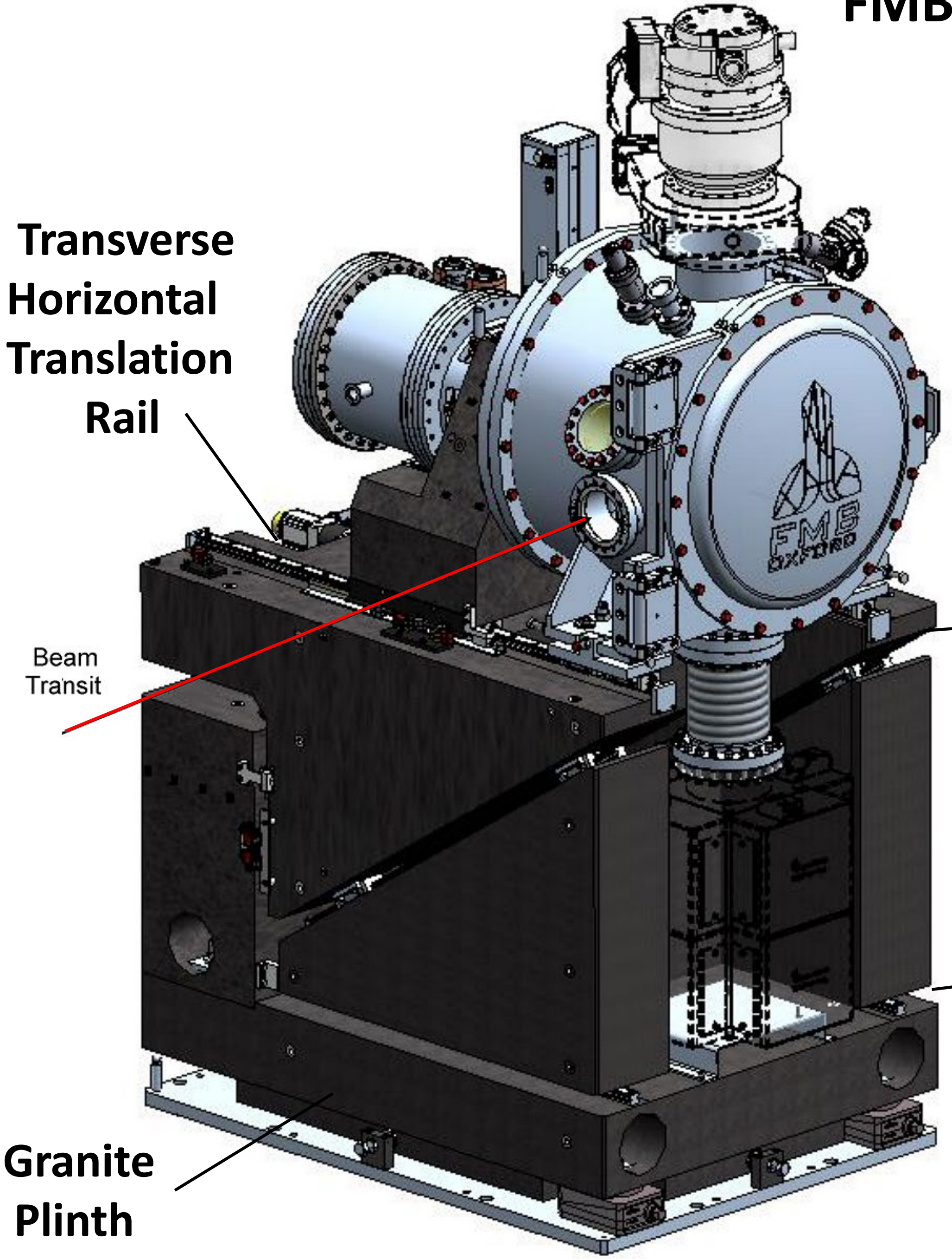


Common Endstation

1) Eiger 1/2 16M
2) A-Frame Detector Positioner
3) Arinax MD2 Microdif-factometer
4) Locally Designed 14 Puck ALS robot

APSU-Related Upgrades

FMB-Oxford Dual Channel Cut Monochromator



Transverse Horizontal Translation Rail

Vertical Lift Wedge Drive.

Horizontal Compensator For Vertical Wedge

Granite Plinth

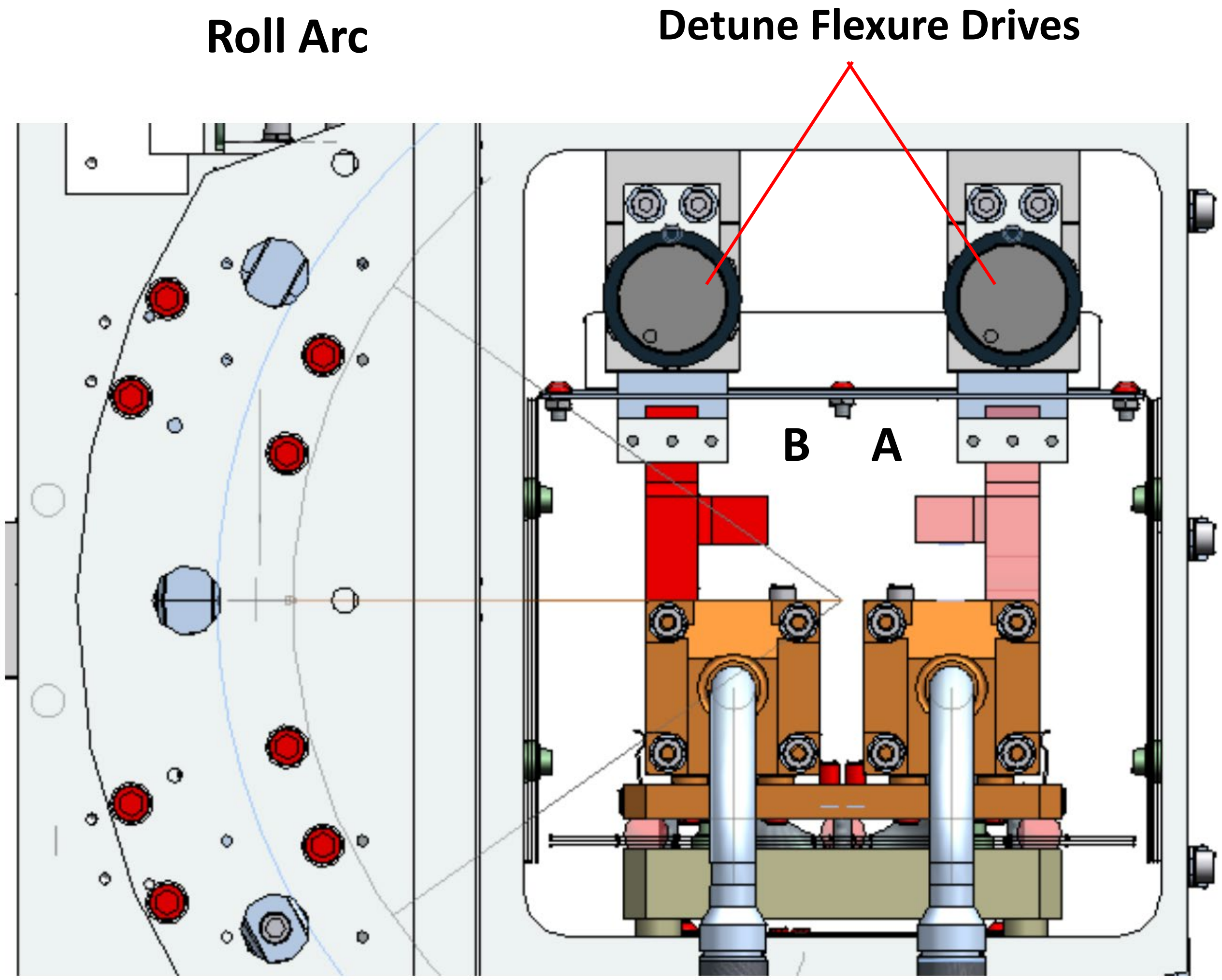
Dual Channel cut of differing channel widths optimized to reduce overall vertical shift of beam to less than ±0.35 mm over the spectral range of 6 to 20 KeV.

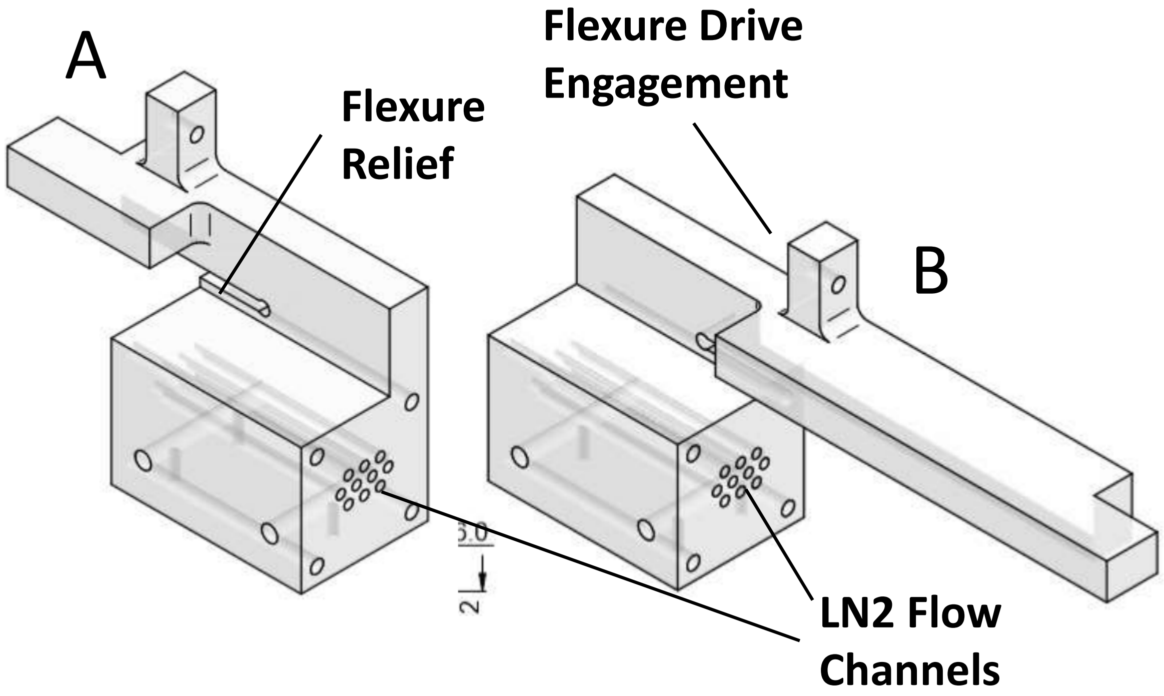
Channel cut selection via horizontal translation of entire vacuum chamber.

Minimize LN2 flow-related monochromatic beam position instabilities …mechanically more simple than existing system.

2nd leaflet detune for 3rd harmonic rejection or flux compensation for thermal drift.

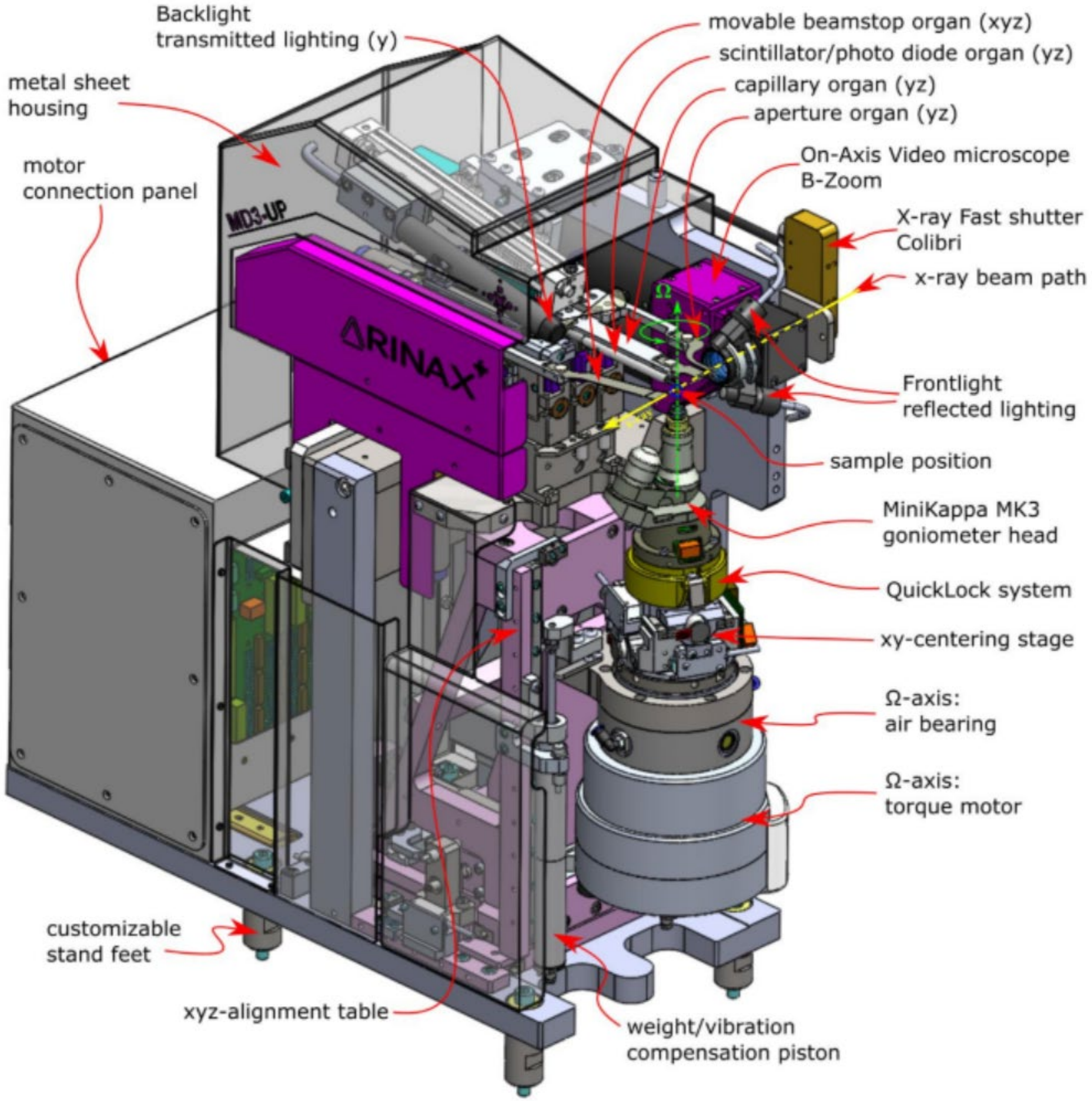
Channel Cut Design and Roll Configuration





Crystal	A	B
Cut	Si<111>	Si<111>
Energy Range	6-8 keV	8-20 keV
Channel width	13.1mm	12.7mm
Beam Offset	24.7 – 25.4mm	24.6 – 25.3mm

Replacement of Aging Arinax MD2 microdiffactometers: MD3UP



Vertical omega axis eliminates gravitational effects on sphere of confusion (< 200 nm).

New high precision mini-kappa head.

Greatly improved sample / beam visualization & illumination (high resolution /speed digital cameras).

x,y,z beam stop.

fast magnetic levitation data shutter

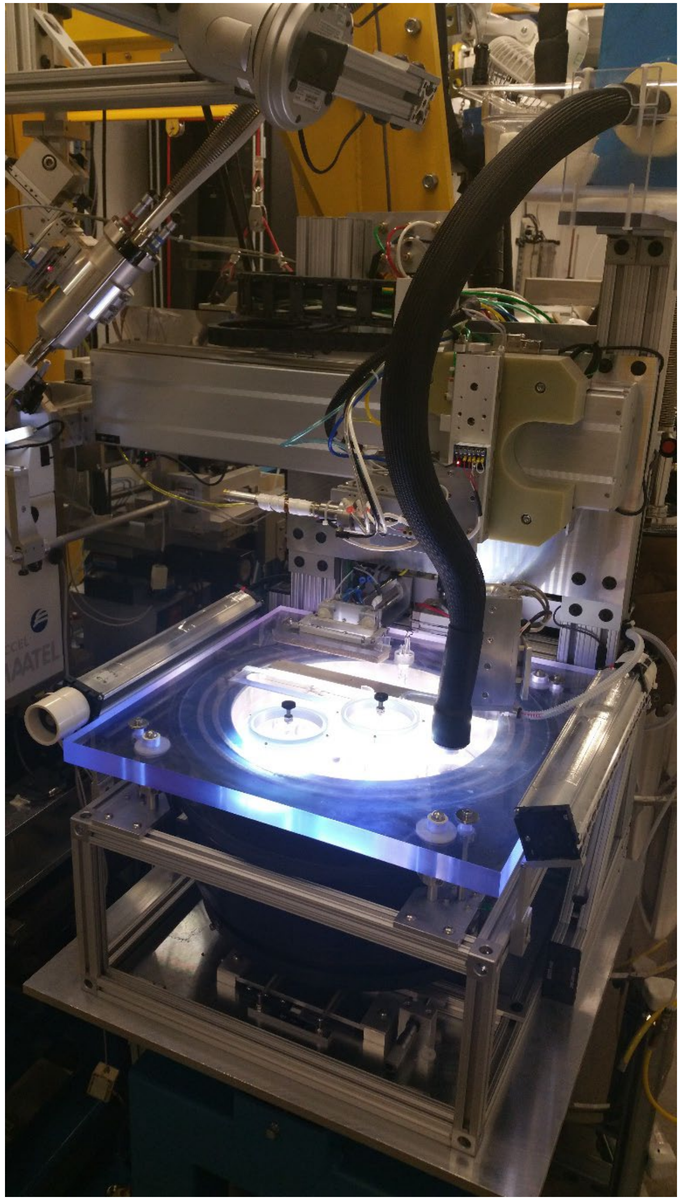
Extensive control API for beam line integration
    Motion control
    Visualization

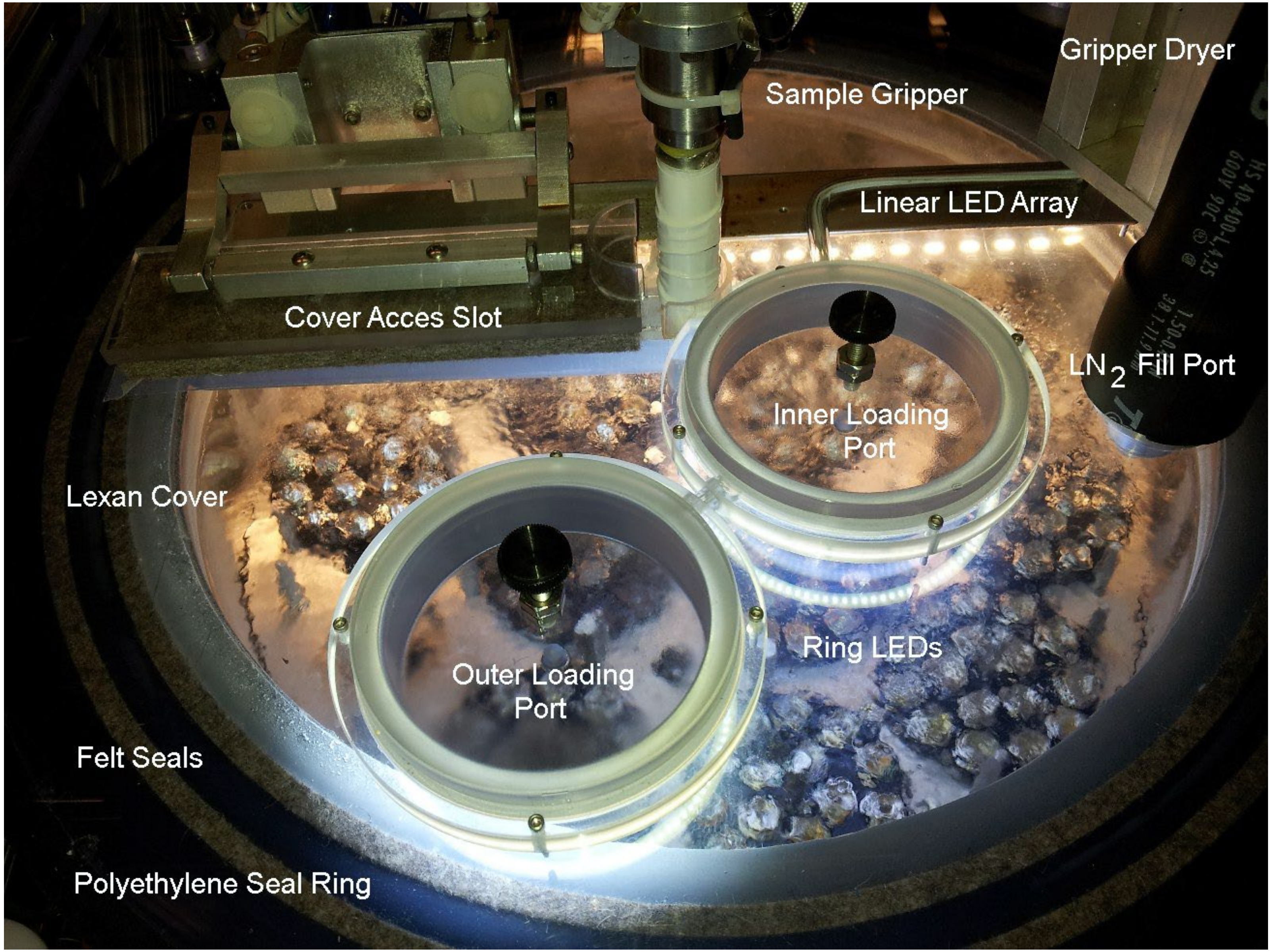
Both units delivered and cold-commissioned
    Nov 2021 – Jan 2022
    Software integration in process

C-Line & new robot MD3 installed during dark period,
E-Line MD3, robot installed after C-Line recommissioning

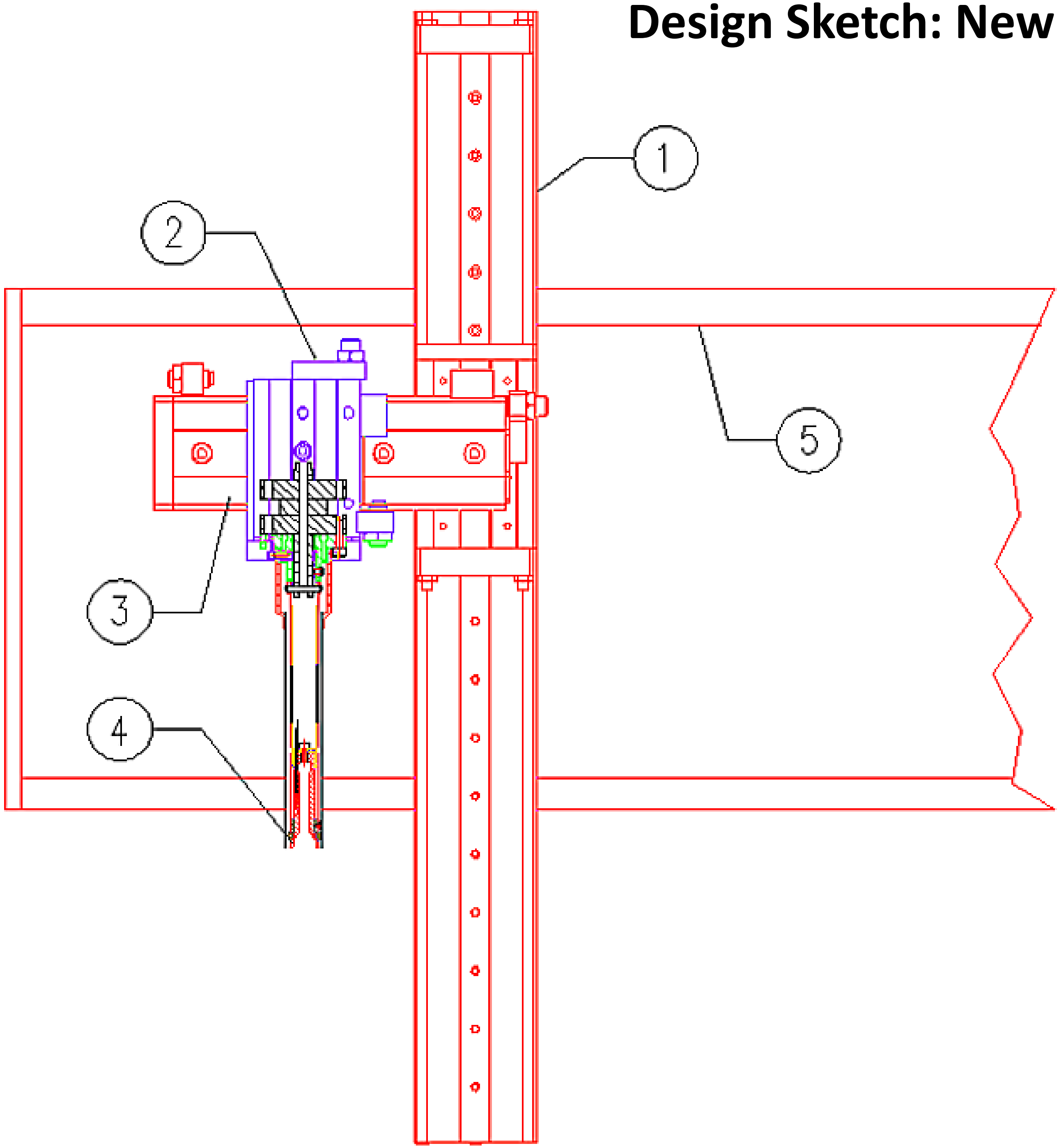
New 30 Puck (480 sample)
MD3-Compatible, ALS-Style Autoloader

Extant Sample Mounter: 14 (ALS/Uni) Pucks, 224 Samples





Design Sketch: New Gripper Manipulator



1) Large vertical pneumatic stage

2) Voice-coil linear drive stage

3) Horizontal pneumatic stage

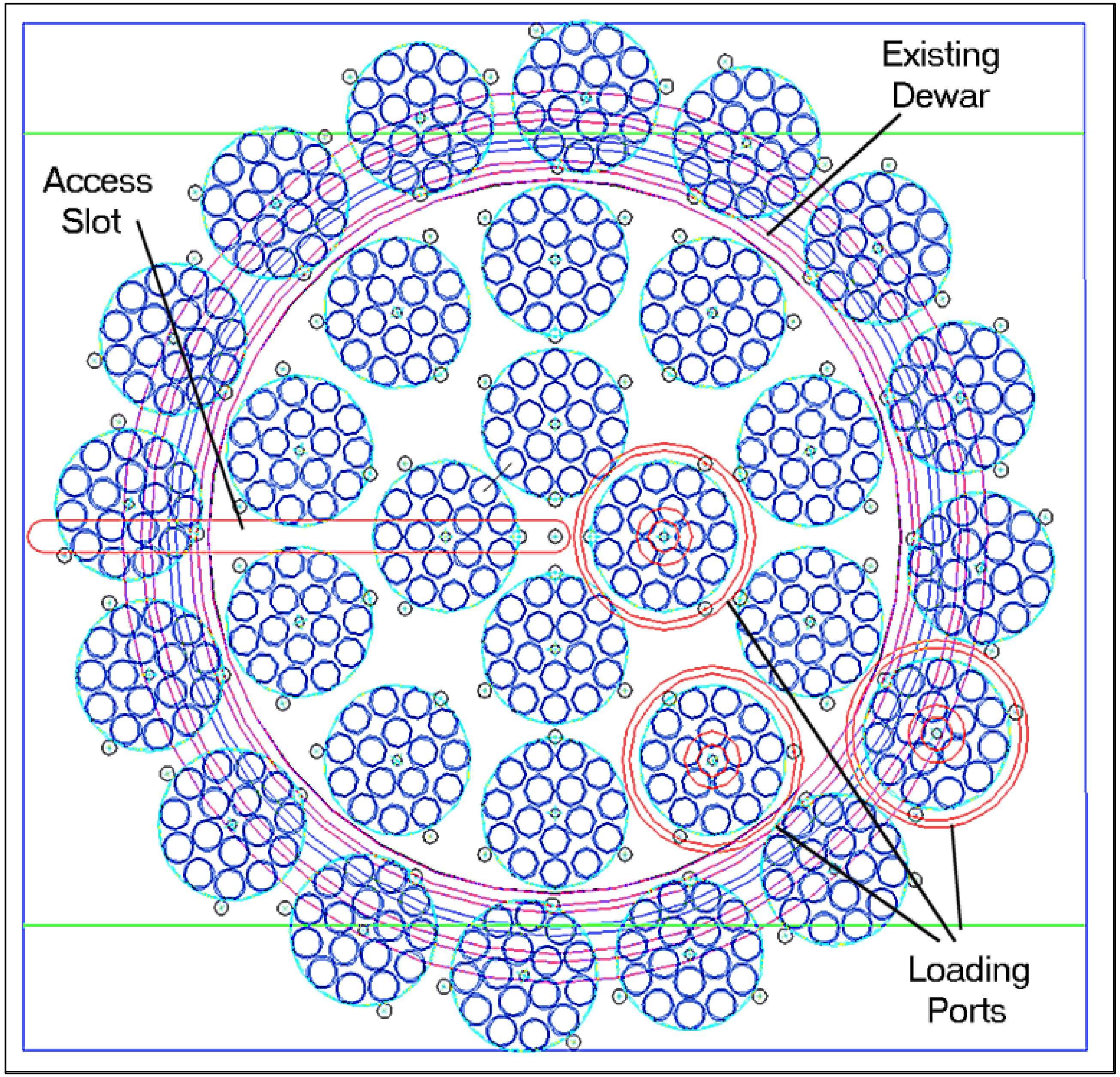
4) Sample gripper

5) Horizontal linear magnetic pulse motor stage.

The sample dewar is located 35 cm below the midline of the linear magnetic pulse stage. MD3 situated to the left of the field of this figure.

The figure shows the configuration immediately before installation on the MD3 omega stage.

Puck Plate Plan View



Remaining Effort on Project

1) Design & fabricate dewar components:
    - Dewar seal assemblies
    - Puck support plates
    - Dewar rotary stage
    - Lexan dewar cover.
    - Support structure

2) Software revision
    - optimization of duty cycle
    - (30 second dismount/mount cycle time).

3) Cryofab Dewars on hand.

Additional Upgrades

1) Replacement of both monochromator cryopumps (FMB-Oxford Cryopump-XV).

2) Replacement of both sample cryocoolers (Oxford Cryosystems Cryostream-800)

3) Sydor photoresistive diamond BPMS: post monochromator , endstation.

4) Replacement of entire motion control system: mcu, drivers.

5) Replacement of all UHV components: gate valves, ion pumps, pump controllers.

6) Software:
    - Developement of fully-automated unattended crystal survey system (snaps & runs)
    - Improved grid and vector (helix) scan speed

Computing Cluster and Storage

- Computer cluster built in three phases Total: 312 HT Cores 2.1TB RAM
  - Phase 1: 32 x Intel Xeon X5520 4C/8T 2.3 GHz processors 576GB RAM
  - Phase 2: 8 x Intel Xeon E5-2687W 8C/16T 3.1 GHz processors 256GB RAM
  - Phase 3: 10 x Intel Xeon E5-2687W4 12C/24T 3.1 GHz processors 1.28TB RAM

100 GBS Infiniband interconnect: cpu interconnect & shared memory backplane

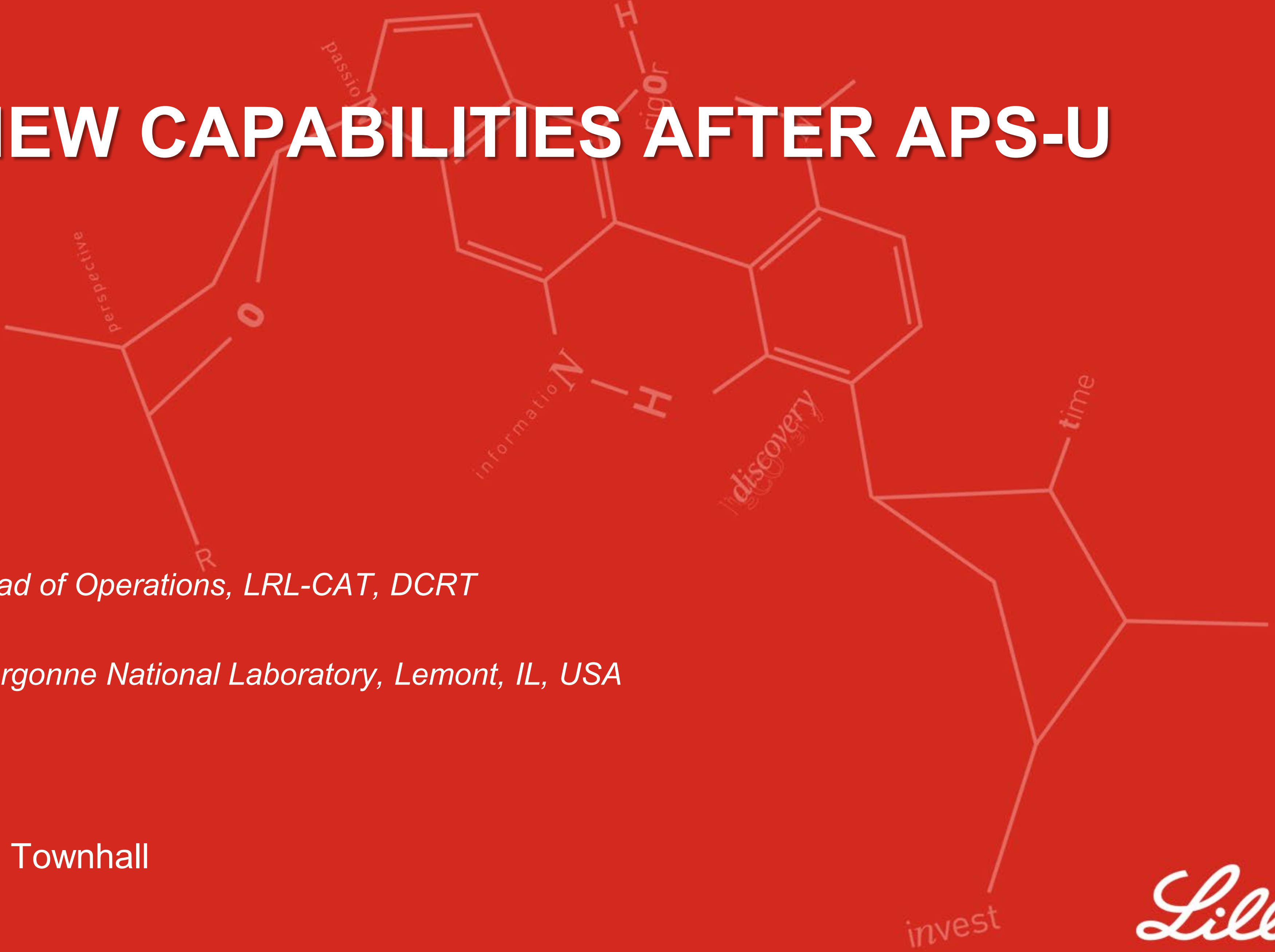
- Total formatted disk storage space is 0.92 PB
  - 1 x Nexsan SATABeast storage arrays (total 30TB)
  - 2 x Nexsan SATABeast2 storage arrays (total 120TB)
  - 1 x Nexsan E48 plus 2 expansion units (total 340TB)
  - 1 x Nexsan Beast P (total 430TB)

Cluster and Storage Facilities will remain available to users during APSU dark period.

APS-U Structural Biology Townhall Agenda

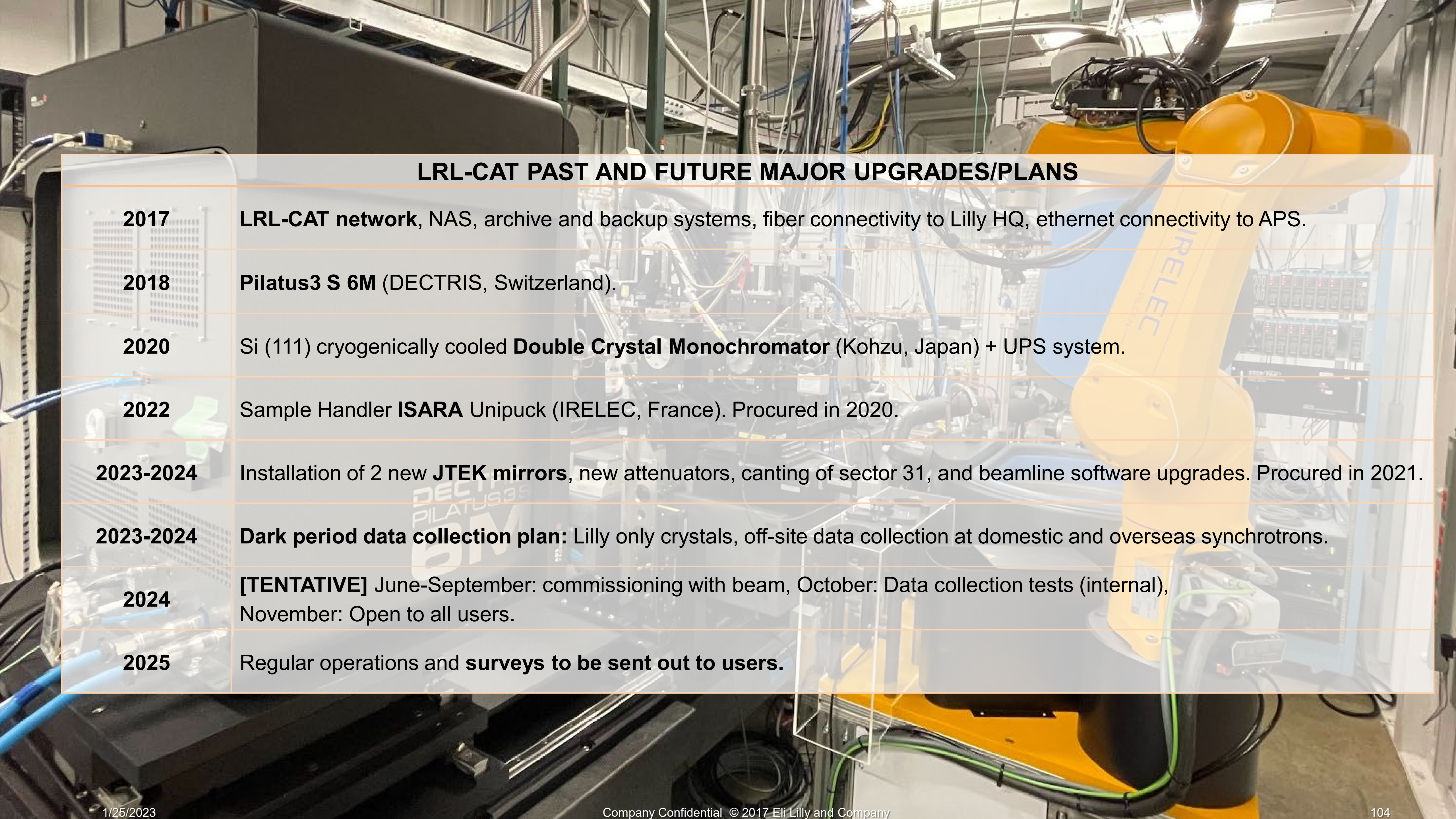
Start	Duration	End	
1:00 PM	0:05	1:05 PM	Welcome - Laurent Chapon,
1:05 PM	0:25	1:30 PM	APS-U Overview and Life Sciences at the APS, Bob Fischetti
1:30 PM	0:15	1:45 PM	IMCA-CAT, Lisa Keefe
1:45 PM	0:15	2:00 PM	SBC/eBERLight, Andrzej Joachimiak and Karolina Michalska
2:00 PM	0:15	2:15 PM	LS-CAT, Spencer Anderson
2:15 PM	0:15	2:30 PM	SER-CAT, Bi-Chen Wang
2:30 PM	0:15	2:45 PM	GM/CA-CAT, Naga Venugopalan
2:45 PM	0:15	3:00 PM	Break
3:00 PM	0:15	3:15 PM	NE-CAT, Malcolm Capel
3:15 PM	0:15	3:30 PM	LRL-CAT, Jordi Benach
3:30 PM	0:15	3:45 PM	ALS, Marc Allaire
3:45 PM	0:15	4:00 PM	CHESS, Marian Szebenyi
4:00 PM	0:15	4:15 PM	NSLS-II, Sean McSweeney
4:15 PM	0:15	4:30 PM	SSRL, Aina Cohen
4:30 PM	0:30	5:00 PM	Roundtable Q&A
5:00 PM			Adjourn

LRL-CAT: NEW CAPABILITIES AFTER APS-U



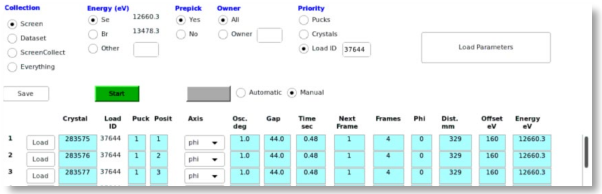
Jordi Benach, Ph.D.
Sr. Director-Chemistry & Head of Operations, LRL-CAT, DCRT
Eli Lilly and Company
Advanced Photon Source, Argonne National Laboratory, Lemont, IL, USA

APS-U Structural Biology Townhall
June 7, 2022



LRL-CAT PAST AND FUTURE MAJOR UPGRADES/PLANS	
2017	**LRL-CAT network**, NAS, archive and backup systems, fiber connectivity to Lilly HQ, ethernet connectivity to APS.
2018	**Pilatus3 S 6M** (DECTRIS, Switzerland).
2020	Si (111) cryogenically cooled **Double Crystal Monochromator** (Kohzu, Japan) + UPS system.
2022	Sample Handler **ISARA** Unipuck (IRELEC, France). Procured in 2020.
2023-2024	Installation of 2 new **JTEK mirrors**, new attenuators, canting of sector 31, and beamline software upgrades. Procured in 2021.
2023-2024	**Dark period data collection plan:** Lilly only crystals, off-site data collection at domestic and overseas synchrotrons.
2024	**[TENTATIVE]** June-September: commissioning with beam, October: Data collection tests (internal), November: Open to all users.
2025	Regular operations and **surveys to be sent out to users.**

Mail-in - High-throughput - Automation - Unscheduled - Fast data turnaround



Collection
● Screen
○ Dataset
○ ScreenCollect
○ Everything

Energy (eV)
● Se 12660.3
○ Br 13478.3
○ Other

Prepick
● Yes
○ No

Owner
● All
○ Owner

Priority
○ Pucks
○ Crystals
● Load ID 37644

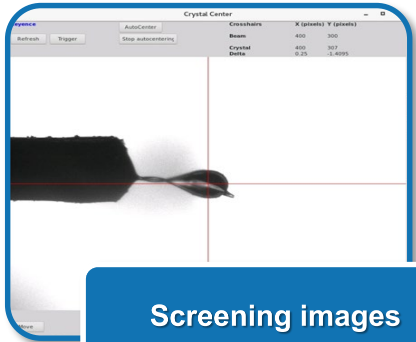
○ Automatic ● Manual

		Crystal	Load ID	Puck	Posit	Axis	Osc. deg	Gap	Time sec	Next Frame	Frames	Phi	Dist. mm	Offset eV	Energy eV
1	Load	283575	37644	1	1	phi	1.0	44.0	0.48	1	4	0	329	160	12660.3
2	Load	283576	37644	1	2	phi	1.0	44.0	0.48	1	4	0	329	160	12660.3
3	Load	283577	37644	1	3	phi	1.0	44.0	0.48	1	4	0	329	160	12660.3

LRL-CAT ~ in-house X-ray diffractometer

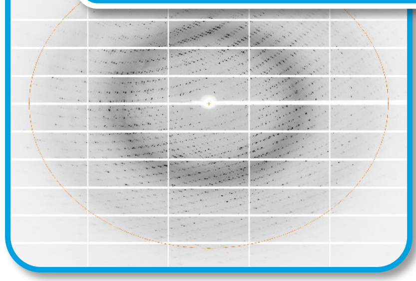
Crystal harvested
Shipment created

Dewar received
samples loaded



Crosshairs	X (pixels)	Y (pixels)
Beam	400	300
Crystal	400	307
Delta	0.25	-1.4095

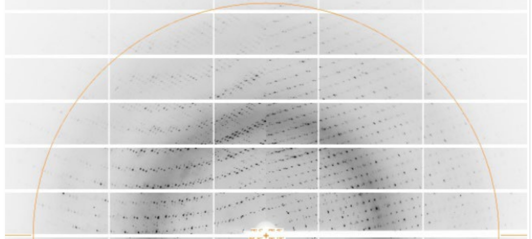
Screening images
18-24 h after harvest



Datasets available
18-32 h after harvest

900-1800 frames x 0.2 °

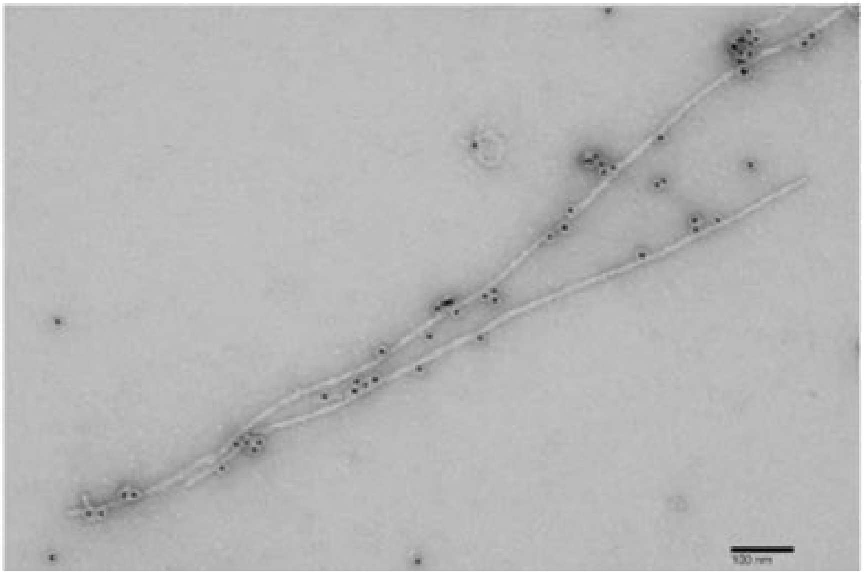
scored, center-verified, resolution estimated

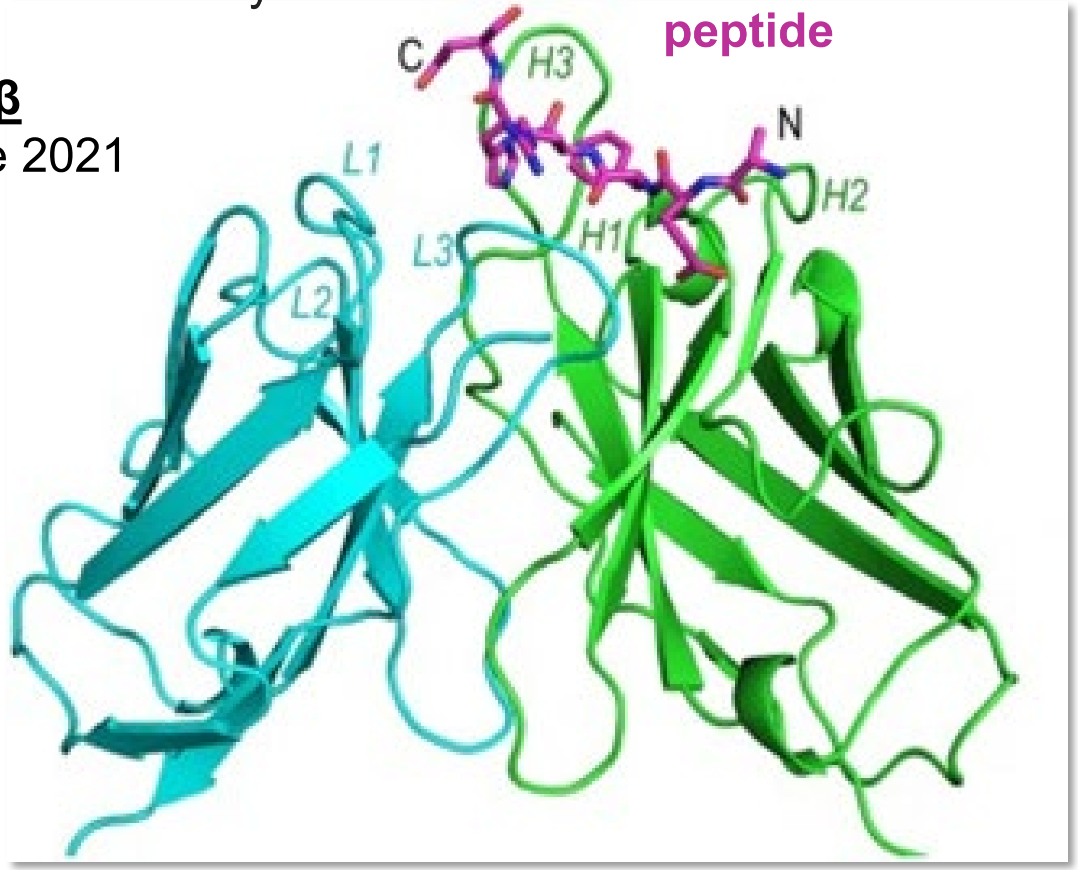


4 x frames

LRL-CAT SCIENCE: Alzheimer's disease (Biogen Inc.)

- A human-derived antibody targeting amyloid-β (Aβ)
- Crystal structures of Aducanumab complexed with a small piece of this amyloid were determined from data collected at LRL-CAT
- Aducanumab selectively targets the **aggregated species of Aβ**
- Aducanumab was approved for medical use in the FDA in June 2021



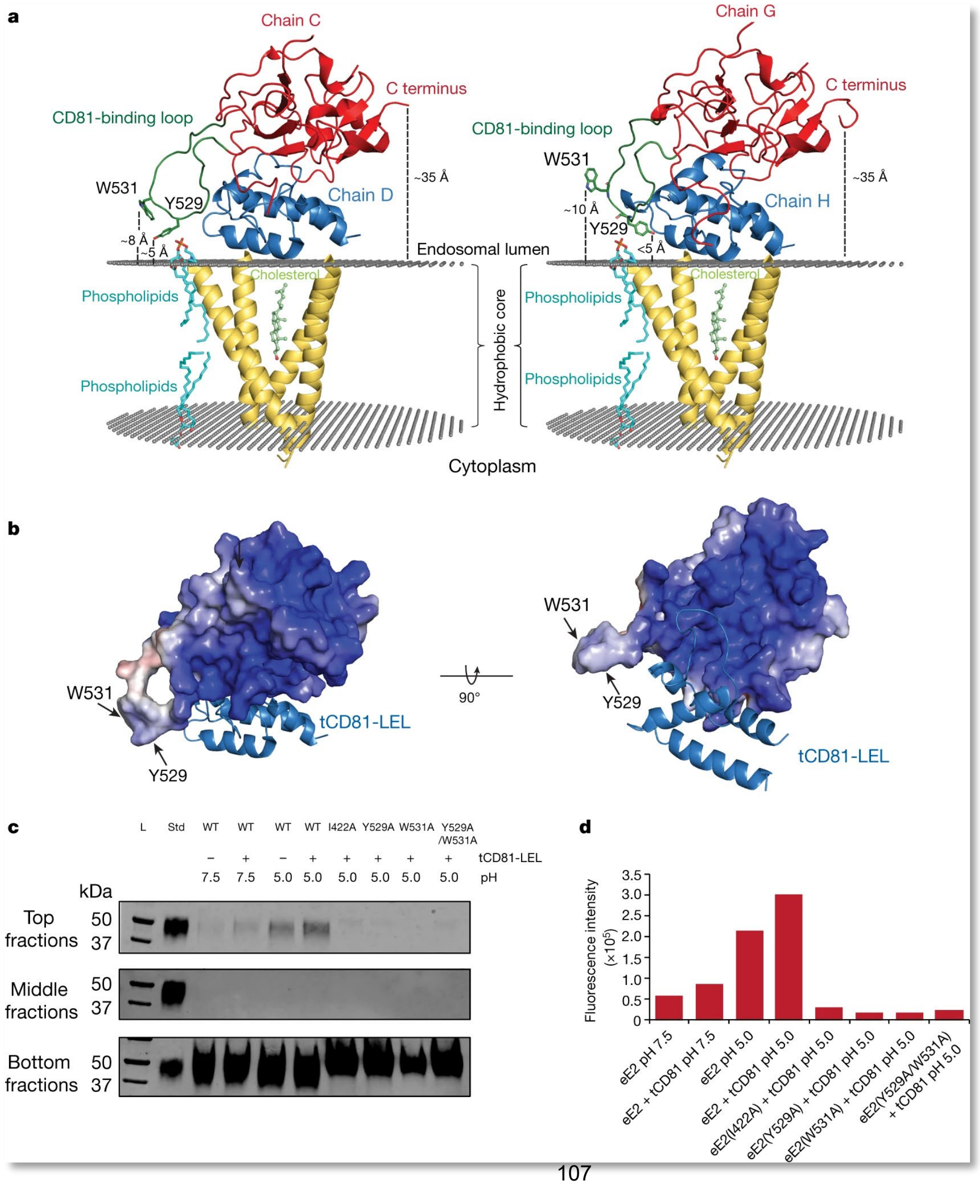


Structural and kinetic basis for the selectivity of aducanumab for aggregated forms of amyloid-[beta]
Arndt, J.W. *et al.* Sci. Rep. 8 (Nature), 6412-1-6412-16 (2018)

LRL-CAT SCIENCE : Hepatitis C Virus (NIH/NIAID)

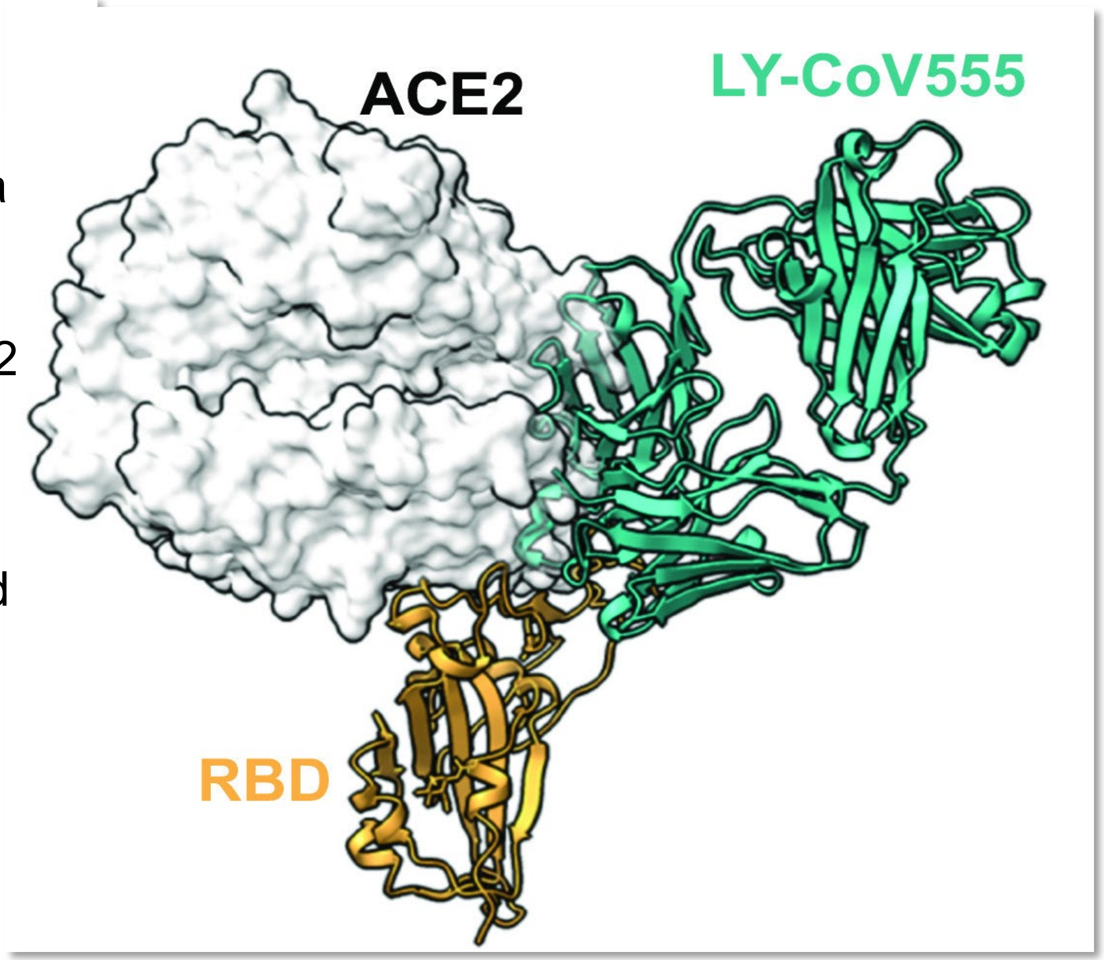
- Hep C virus: chronic liver disease, cirrhosis and hepatocellular carcinoma in humans, and afflicts more than 70 million people worldwide
- Better understanding the binding of hepatitis C virus to the host cells
- Acidification and receptor binding result in a conformational change in E2 in preparation for membrane fusion

Structural insights into hepatitis C virus receptor binding and entry
Kumar, A. *et al.* Nature 598, 521-525 (2021)



LRL-CAT SCIENCE : COVID-19 (AbCellera Biologics and Eli Lilly)

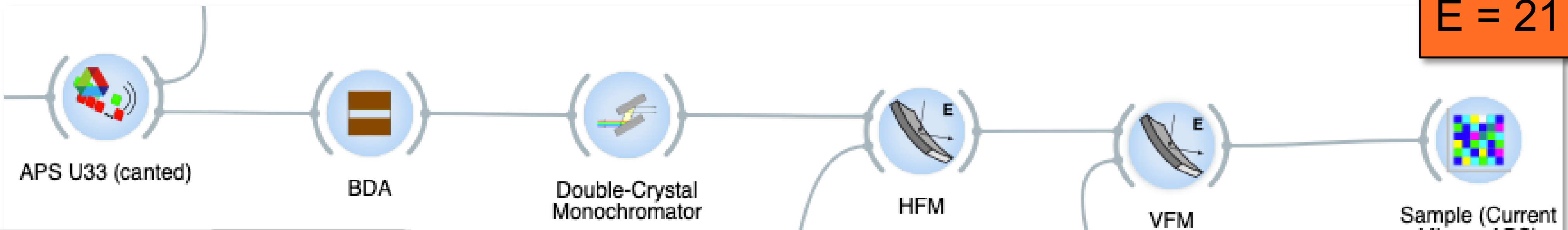
- Bamlanivimab is a monoclonal antibody developed by AbCellera Biologics and Eli Lilly as a treatment for COVID-19
- LY-CoV555 antibody blocks the interaction between SARS-CoV2 and human ACE2 receptor
- The medication was granted an emergency use authorization (EUA) by the FDA in November 2020, and the EUA was revoked in April 2021

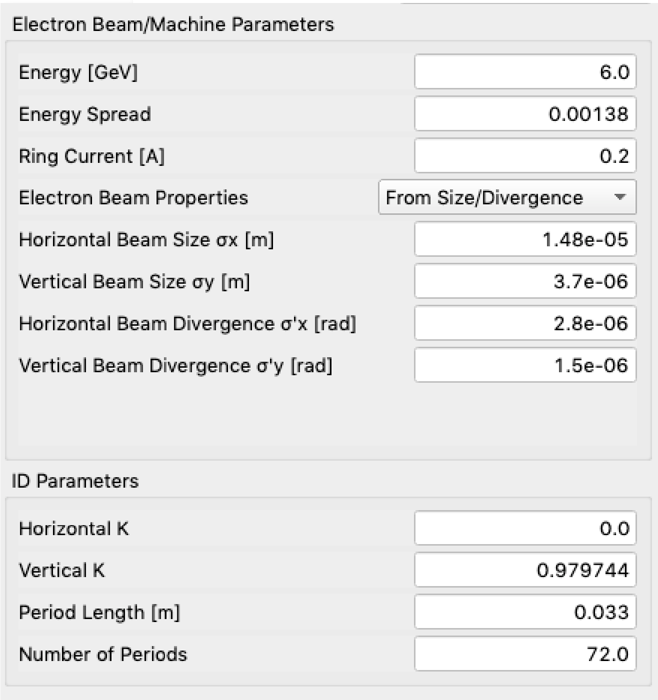


The neutralizing antibody, LY-CoV555, protects against SARS-CoV-2 infection in nonhuman primates
Jones, B.E. *et al.* Sci. Transl. Med. 13 (593), (2021)

APS WILL DELIVER A MUCH SMALLER SOURCE

E = 21 KeV



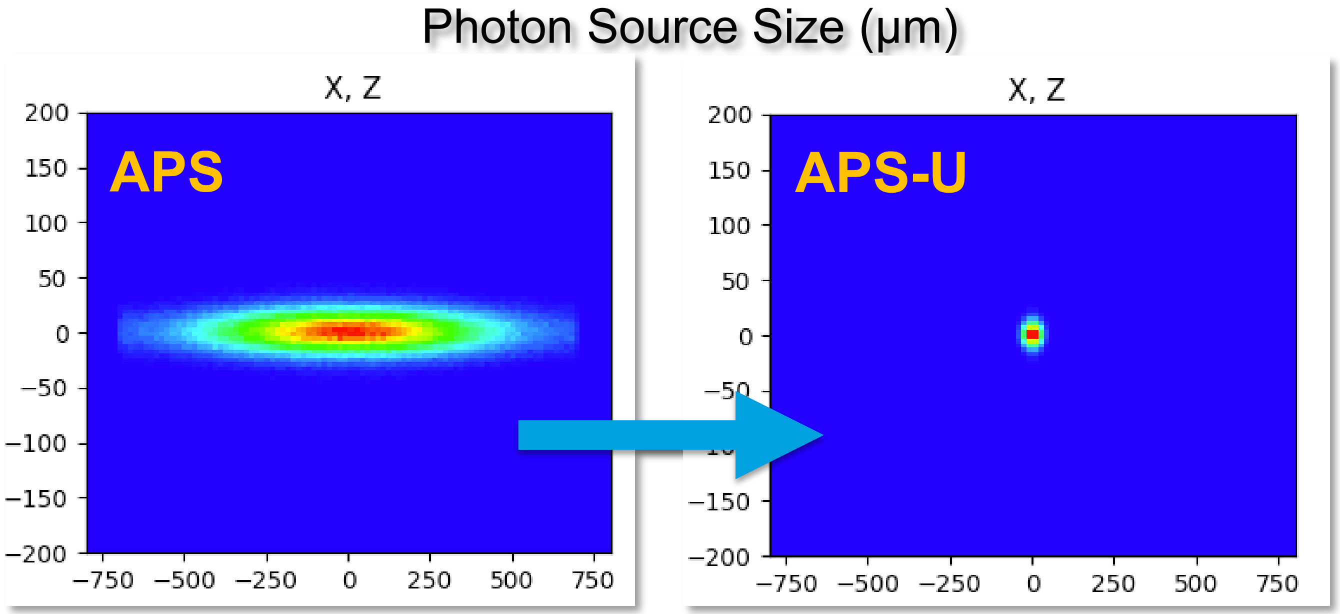


Electron Beam/Machine Parameters

Energy [GeV]	6.0
Energy Spread	0.00138
Ring Current [A]	0.2
Electron Beam Properties	From Size/Divergence
Horizontal Beam Size σx [m]	1.48e-05
Vertical Beam Size σy [m]	3.7e-06
Horizontal Beam Divergence σ'x [rad]	2.8e-06
Vertical Beam Divergence σ'y [rad]	1.5e-06

ID Parameters

Horizontal K	0.0
Vertical K	0.979744
Period Length [m]	0.033
Number of Periods	72.0



Simulations and slide provided by Luca Rebuffi (APS)

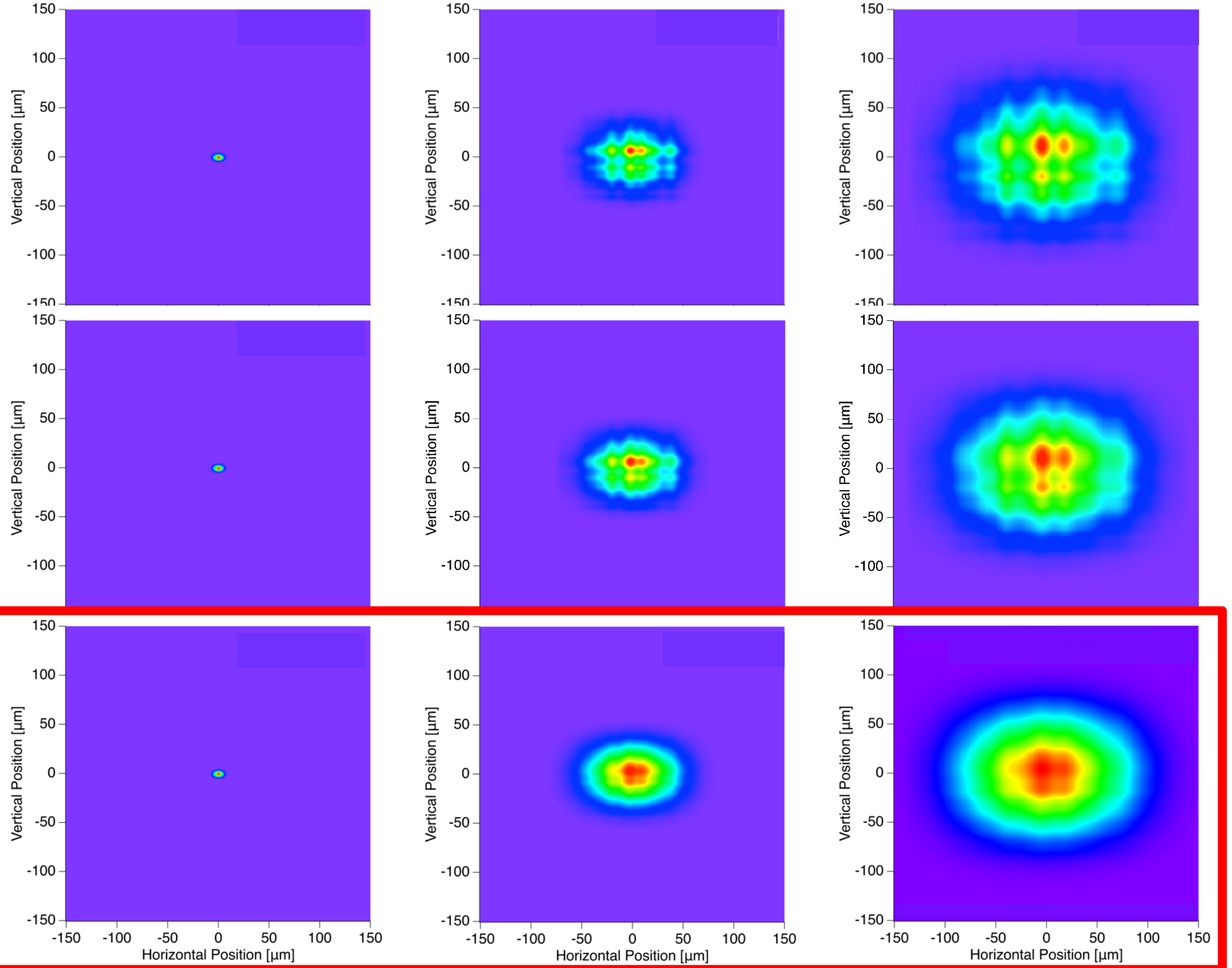
LRL-CAT CHOSE THE BEST FOCUSING MIRRORS

7.5 x 2.0 μm^2 FWHM | **50 x 50 μm^2 FWHM** | **100 x 100 μm^2 FWHM**

Figure error rms: ~5-10 nm
Slope error rms: ~0.15 μrad

Figure error rms: ~4 nm
Slope error rms: ~0.1 μrad

Figure error rms: < 0.5 nm
Slope error rms: < 0.5 μrad
(IRELEC/JTEC)



Pictures provided by Luca Rebuffi (APS)

LRL-CAT POST APS-U: AUTOMATED & BI-MODAL

Highly automated – Unscheduled – Mail-in – Auto-collection & processing – On-the-fly data transfer – Fast data turnaround

HIGH THROUGHPUT MODE

beam size range ~25x25 to 100x100 µm^2 (HxV)

SBDD, fragment screening, established projects

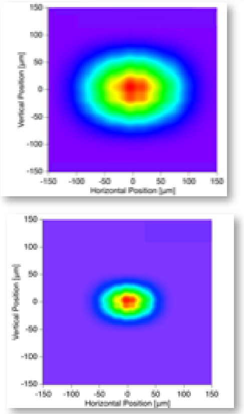
- Fast
- Improved loop and X-ray centering routines
- Improved strategy data collection routines
- Automated data processing

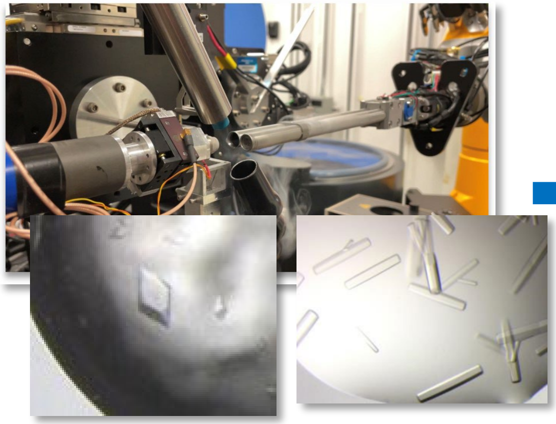
MICRO-FOCUS MODE

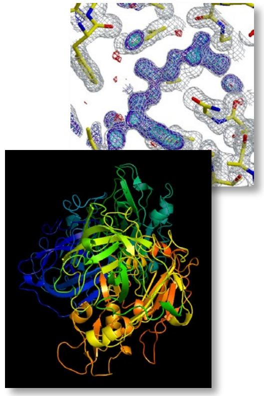
beam size ~7x2 µm^2 (HxV)

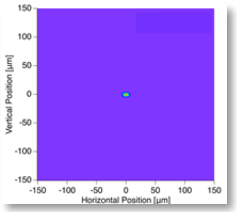
Mem. prot, macromolecular interactions, micro-crystals

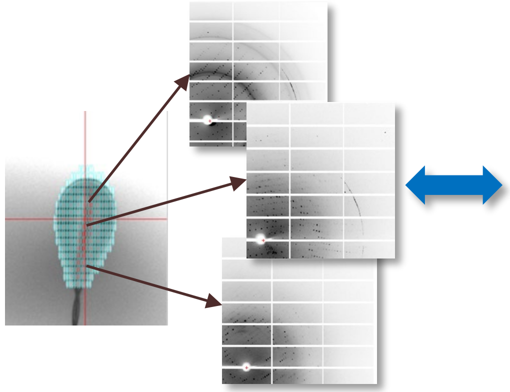
- Very fine X-ray crystal centering & cartography
- Iterative automated data collection to:
  - ✓ Improve resolution, completeness, multiplicity, anomalous signal
  - ✓ Achieve or improve *de novo* phasing
  - ✓ Optimize macromolecule/ligand e-density maps

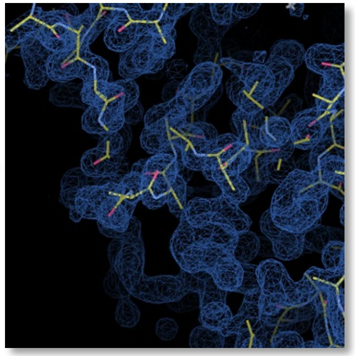












Thank you!

APS-U Structural Biology Townhall Agenda

Start	Duration	End	
1:00 PM	0:05	1:05 PM	Welcome - Laurent Chapon,
1:05 PM	0:25	1:30 PM	APS-U Overview and Life Sciences at the APS, Bob Fischetti
1:30 PM	0:15	1:45 PM	IMCA-CAT, Lisa Keefe
1:45 PM	0:15	2:00 PM	SBC/eBERLight, Andrzej Joachimiak and Karolina Michalska
2:00 PM	0:15	2:15 PM	LS-CAT, Spencer Anderson
2:15 PM	0:15	2:30 PM	SER-CAT, Bi-Chen Wang
2:30 PM	0:15	2:45 PM	GM/CA-CAT, Naga Venugopalan
2:45 PM	0:15	3:00 PM	Break
3:00 PM	0:15	3:15 PM	NE-CAT, Malcolm Capel
3:15 PM	0:15	3:30 PM	LRL-CAT, Jordi Benach
3:30 PM	0:15	3:45 PM	ALS, Marc Allaire
3:45 PM	0:15	4:00 PM	CHESS, Marian Szebenyi
4:00 PM	0:15	4:15 PM	NSLS-II, Sean McSweeney
4:15 PM	0:15	4:30 PM	SSRL, Aina Cohen
4:30 PM	0:30	5:00 PM	Roundtable Q&A
5:00 PM			Adjourn

BioSciences

MX at the Advanced Light Source

Marc Allaire

Head of BCSB

Overview

BERKELEY LAB

U.S. DEPARTMENT OF ENERGY

MBIB Molecular Biophysics & Integrated Bioimaging

The Advanced Light Source

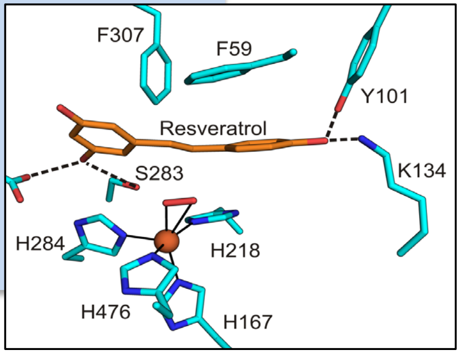
- ALS at Lawrence Berkeley National Laboratory
- (DOE/BES supported synchrotron facility
- Specialized in soft X-ray science, but spans UV to hard X-rays
- 40+ experimental stations (beamlines)
- More than 2000 users, 1000 publications per year

Biosciences at the ALS

BioSciences

Macromolecular X-ray Crystallography

- Provides atomic resolution insight into biomolecules
- 7 Beamlines
- Funded by HHMI, Industry, Universities, NIH
- 15 Staff

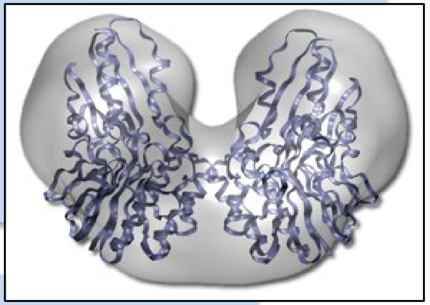


Soft X-ray Tomography

- Unique to ALS
- Rapid 3-dimensional reconstructions of biological samples (cell biology)
- Funded by NIH & DOE/BER
- Lead - Carolyn Larabell
- 7 Staff

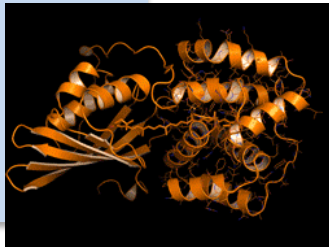
Small Angle X-ray Scattering

- Provides information about biomolecules in solution
- Combined of with crystallography and soft X-ray tomography
- Funded by NIH, DOE/BER
- Lead - Greg Hura
- 6 Staff



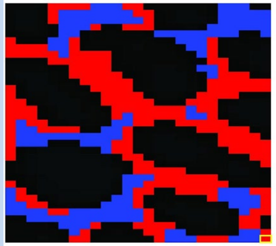
X-ray Footprinting

- Probes interactions between proteins
- Funded by NIH
- Lead - Corie Ralston
- 1 Staff



Synchrotron Infrared Spectroscopy

- Probes chemistry of live samples
- Funded by DOE/BER
- Lead – Hoi-Ying Holman
- 2 Staff (also ALS)

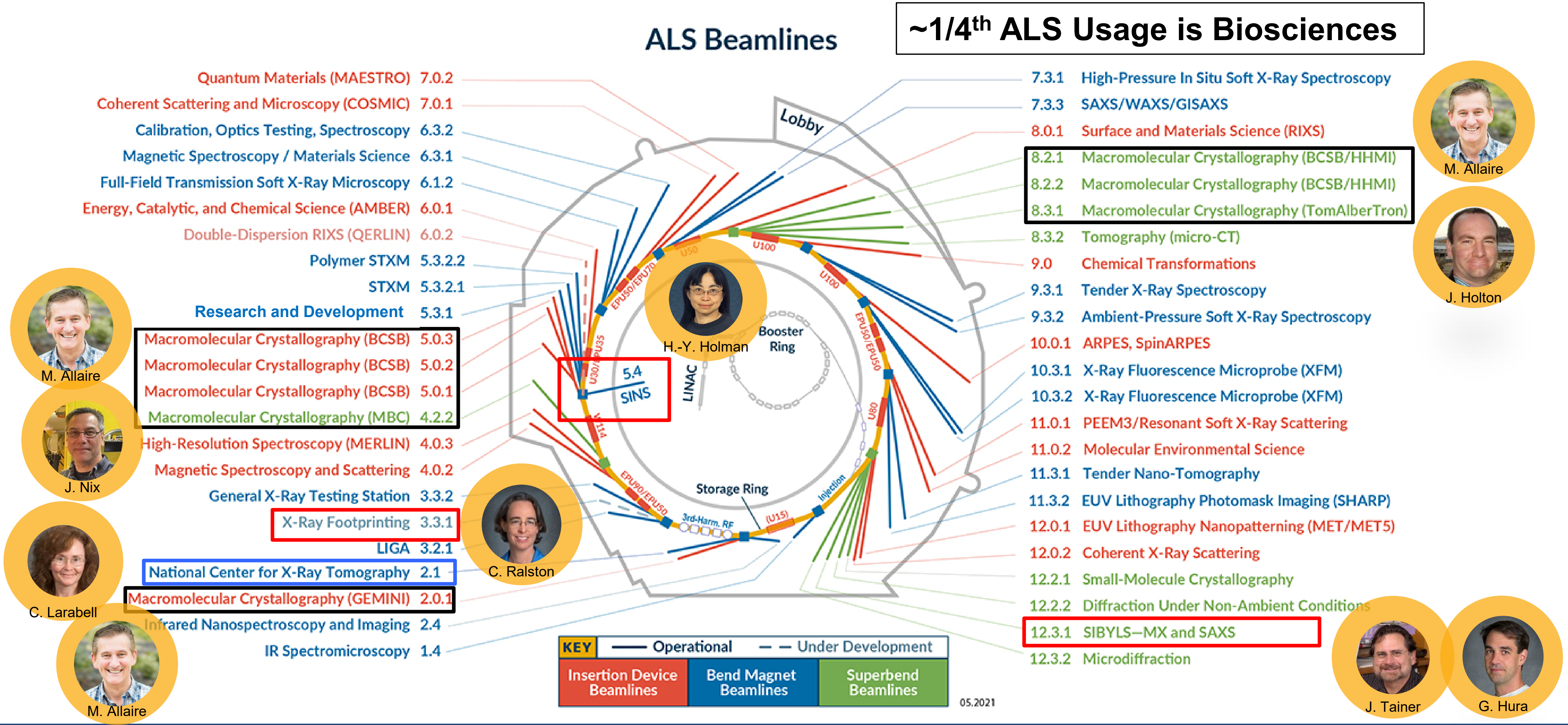


BERKELEY LAB U.S. DEPARTMENT OF ENERGY MBIB Molecular Biophysics & Integrated Bioimaging

MX at the ALS

BioSciences

~1/4th ALS Usage is Biosciences



ALS Beamlines

Quantum Materials (MAESTRO)	7.0.2
Coherent Scattering and Microscopy (COSMIC)	7.0.1
Calibration, Optics Testing, Spectroscopy	6.3.2
Magnetic Spectroscopy / Materials Science	6.3.1
Full-Field Transmission Soft X-Ray Microscopy	6.1.2
Energy, Catalytic, and Chemical Science (AMBER)	6.0.1
Double-Dispersion RIXS (QERLIN)	6.0.2
Polymer STXM	5.3.2.2
STXM	5.3.2.1
Research and Development	5.3.1
Macromolecular Crystallography (BCSB)	5.0.3
Macromolecular Crystallography (BCSB)	5.0.2
Macromolecular Crystallography (BCSB)	5.0.1
Macromolecular Crystallography (MBC)	4.2.2
High-Resolution Spectroscopy (MERLIN)	4.0.3
Magnetic Spectroscopy and Scattering	4.0.2
General X-Ray Testing Station	3.3.2
X-Ray Footprinting	3.3.1
LIGA	3.2.1
National Center for X-Ray Tomography	2.1
Macromolecular Crystallography (GEMINI)	2.0.1
Infrared Nanospectroscopy and Imaging	2.4
IR Spectromicroscopy	1.4

7.3.1	High-Pressure In Situ Soft X-Ray Spectroscopy
7.3.3	SAXS/WAXS/GISAXS
8.0.1	Surface and Materials Science (RIXS)
8.2.1	Macromolecular Crystallography (BCSB/HHMI)
8.2.2	Macromolecular Crystallography (BCSB/HHMI)
8.3.1	Macromolecular Crystallography (TomAlberTron)
8.3.2	Tomography (micro-CT)
9.0	Chemical Transformations
9.3.1	Tender X-Ray Spectroscopy
9.3.2	Ambient-Pressure Soft X-Ray Spectroscopy
10.0.1	ARPES, SpinARPES
10.3.1	X-Ray Fluorescence Microprobe (XFM)
10.3.2	X-Ray Fluorescence Microprobe (XFM)
11.0.1	PEEM3/Resonant Soft X-Ray Scattering
11.0.2	Molecular Environmental Science
11.3.1	Tender Nano-Tomography
11.3.2	EUV Lithography Photomask Imaging (SHARP)
12.0.1	EUV Lithography Nanopatterning (MET/MET5)
12.0.2	Coherent X-Ray Scattering
12.2.1	Small-Molecule Crystallography
12.2.2	Diffraction Under Non-Ambient Conditions
12.3.1	SIBYLS—MX and SAXS
12.3.2	Microdiffraction

KEY	— Operational	– – Under Development
🟥 Insertion Device Beamlines	🟦 Bend Magnet Beamlines	🟩 Superbend Beamlines

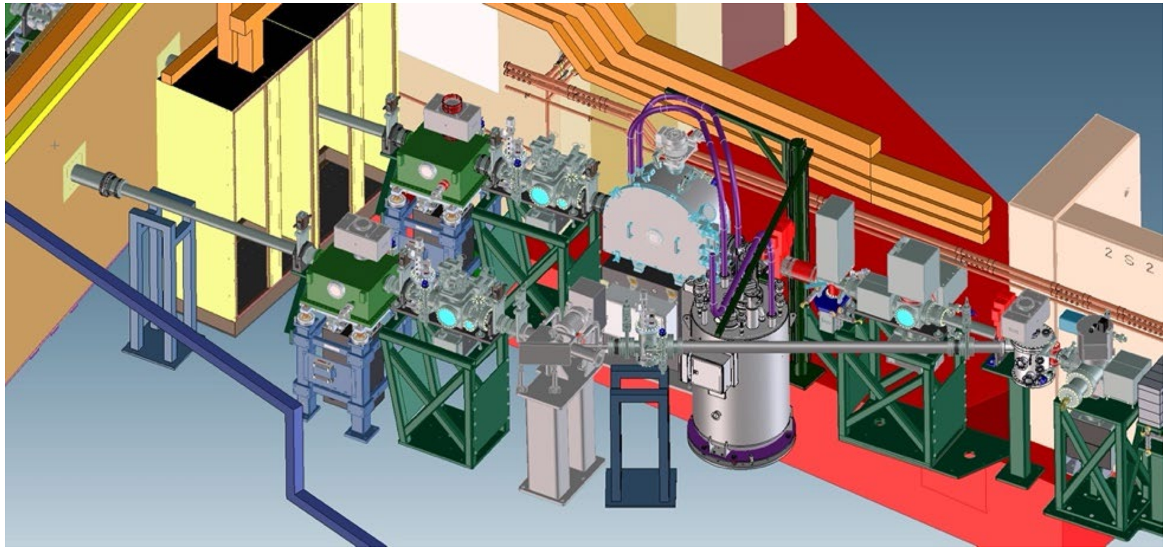
05.2021

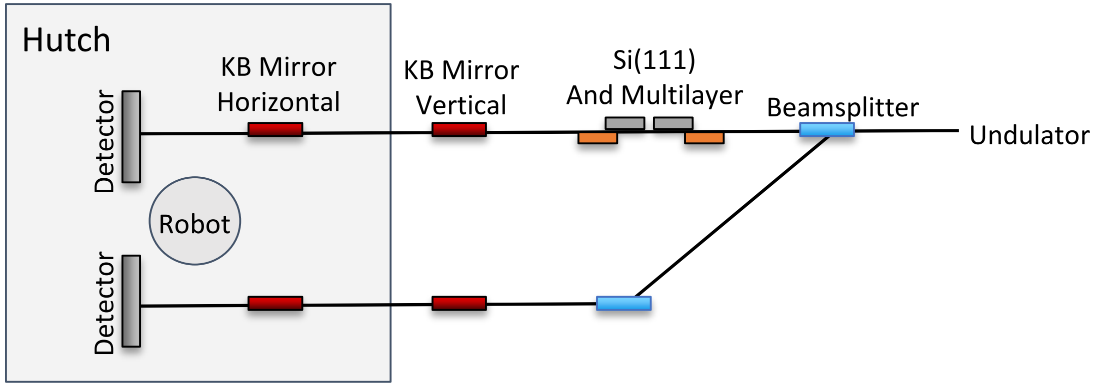
MX beamlines at the ALS

	2.0.1	4.2.2	5.0.1	5.0.2	5.0.3	8.2.1	8.2.2	8.3.1
Contact	Allaire	Nix	Allaire					Holton
Detector	Pilatus 6M	RDI CMOS 8M	Pilatus 2M	Pilatus 6M	Pilatus 2M	ADSC Q315r	ADSC Q315r	Pilatus 6M
Frame rate (Hz)	100	25	25	25	25	0.3	0.3	100
Fluorescence detector	Vortex EX90	Vortex VX100	none	Vortex EM100	none	Oxford NaI:Tl	Oxford NaI:Tl	Vortex EX100
Source	Undulator (IVID)	Super-Bend (5T)	56-pole 11.4 cm wiggler (1.9T)			SUPERBEND (5T)		
Primary mirror RMS slope error (μRad)	None	Silicon 0.2	Silicon 1.0	Silicon 1.0	Silicon 1.0	Silicon 0.2	Silicon 0.2	Silicon 0.2
Energy range (keV)	5 - 15	5.5 - 16	12.7	5 - 16	12.7	5 – 16 ML: 10 - 13	5 - 16	5 - 17
Mono-chromator	Cryo Si(111) 0.4% ML	Sagittal Si(111)	Si(220)	Cryo Si(111)	Si(220)	Cryo Si(111) 0.4% ML	Si(111)	Si(111)
Flux (ph/s) 100 μm dia. hole	2x10^{12} ML 1x10^{13}	1x10^{12}	3x10^{11}	1.5x10^{12}	5x10^{11}	6x10^{11} ML 2x10^{12}	6x10^{11}	1x10^{12}
focus size (v x h; μm)	15 x 15	85 x 55	350 x 150	350 x 150	350 x 150	100 x 50	75 x 40	80 x 60
Collimation/ defocus (μm)	15 - 100	20 - 400	20 - 150	10 - 150	20 - 150	20 - 150	20 - 150	15 - 100
Robot	NATX-ray	Rigaku Actor	Berkeley Auto Mounter	Berkeley Auto Mounter	Berkeley Auto Mounter	Rigaku Actor	Rigaku Actor	Cool Hand Luke
Pin / puck compatibility	SPINE ALS Unipuck	ALS Rigaku Unipuck	SPINE ALS Unipuck	SPINE ALS Unipuck	SPINE ALS Unipuck	SPINE ALS Rigaku Unipuck	SPINE ALS Rigaku Unipuck	All pins Staff unpacking
Sample capacity	24 x 16	5 x 16	12 x 16	12 x 16	6 x 16	5 x 16	5 x 16	52 pins + “infinite”
GU fraction	(25%)	25%	25%	25%	25%	25%	25%	25%

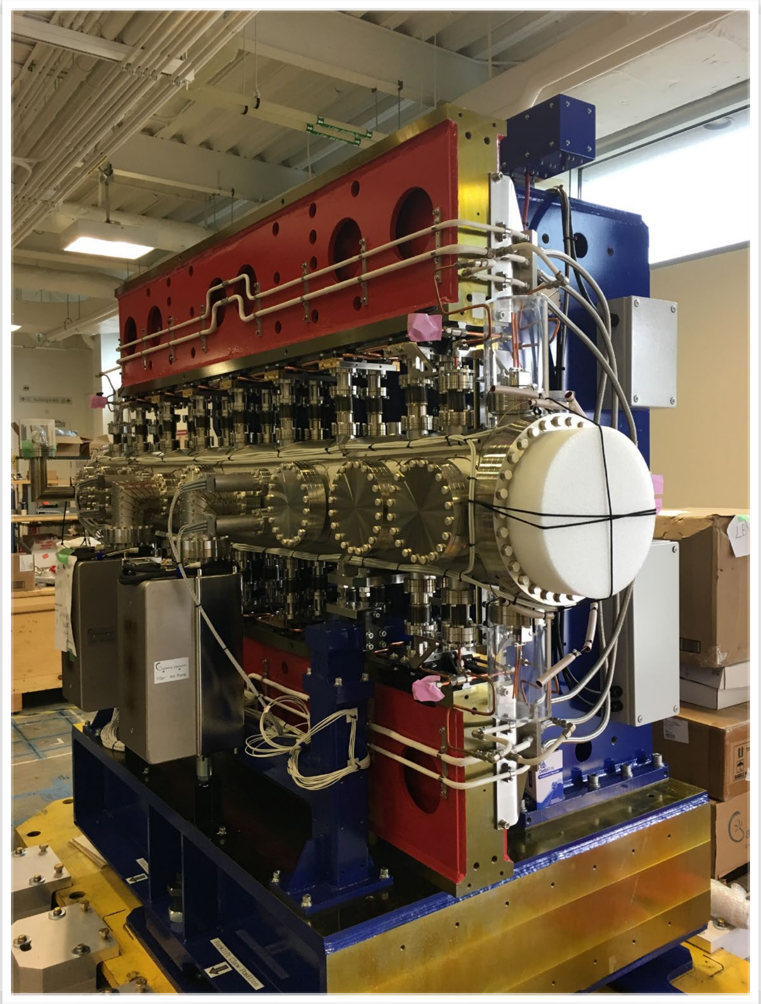
Gemini Microfocus Beamline

- The need to study smaller crystals requires smaller, brighter beams
- The new Gemini beamline will provide:
  - Brightness: 100-fold over current beamlines and additional 10-fold boost with multilayer when running at Se edge
  - Focus: 15 x 15 µm focused beam size
  - Spot separation: less than 1 mrad divergence allows large unit cells
  - Efficiency: 8 sec sample exchange with robotic sample mounting; large puck storage capacity; shutterless data collection; fully remote and automated options
- Successful collaboration between MBIB, ALS and LBNL Engineering



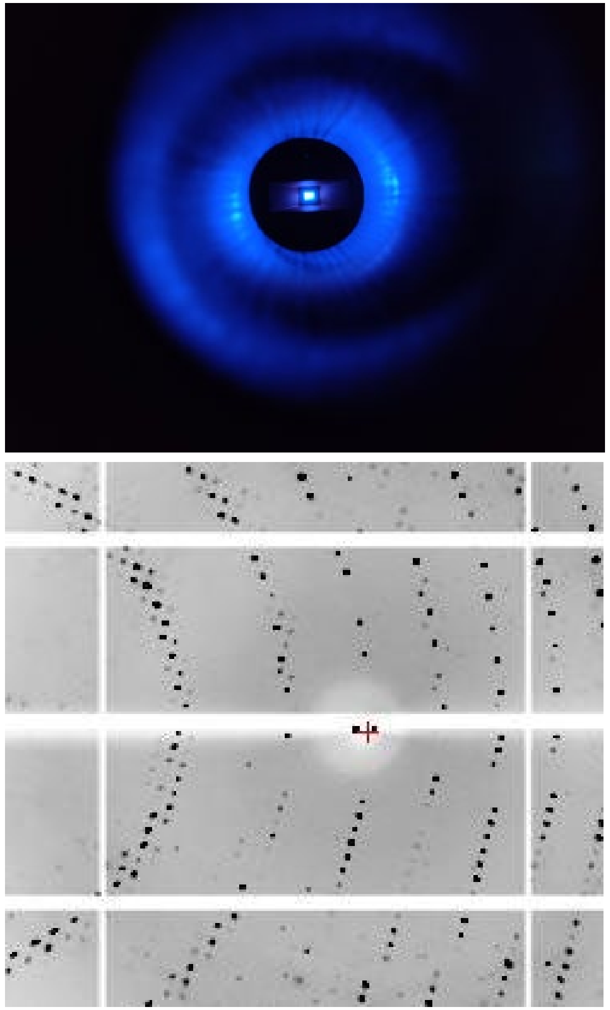


Gemini Microfocus Beamline



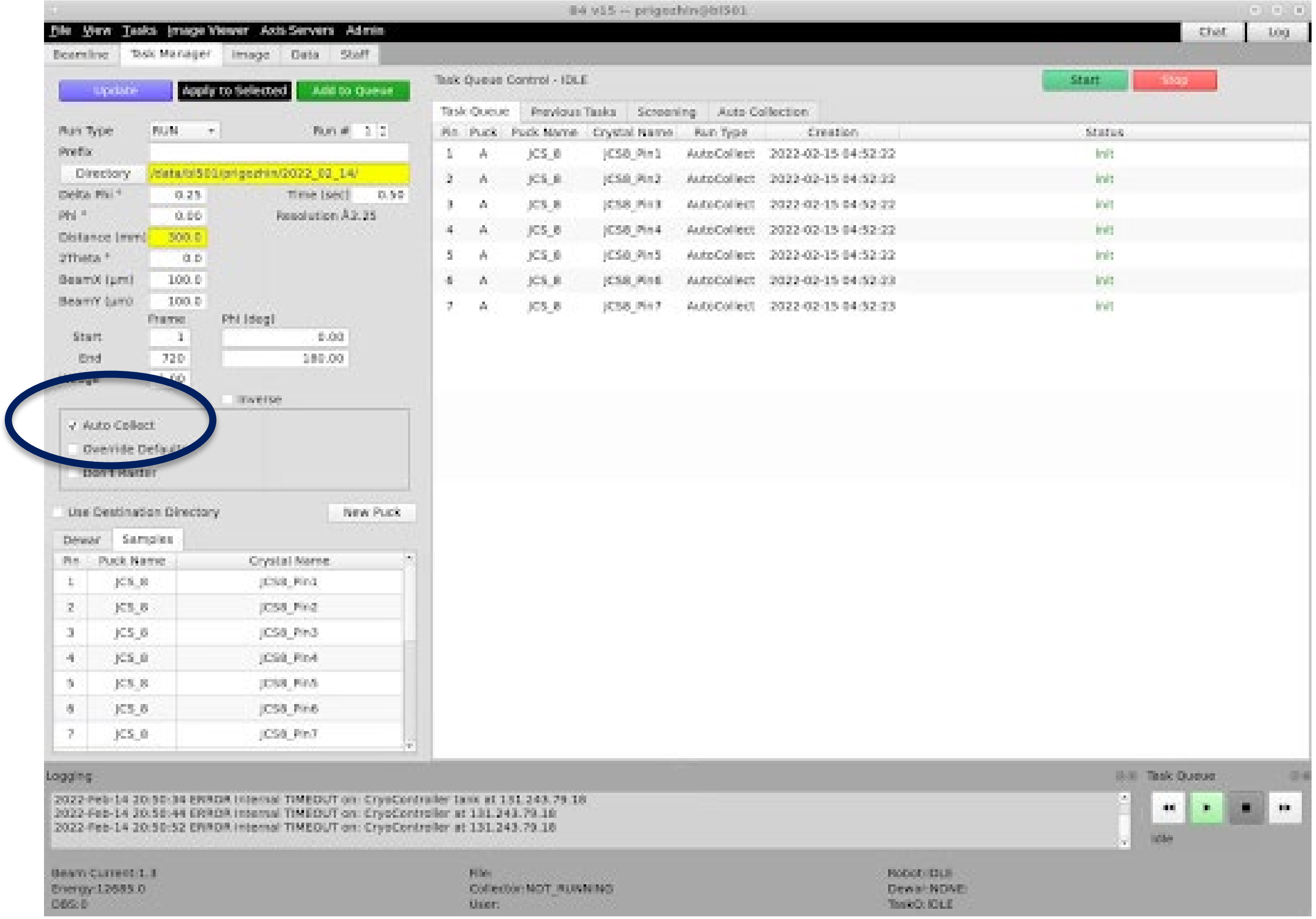
Leda Insertion Device

X-ray Optics



Light and Data

Recent development



Run Type: RUN
Run #: 1
Prefix:
Directory: /data/bl501/prigozhin/2022_02_14/
Delta Phi °: 0.25
Time (sec): 0.50
Phi °: 0.00
Resolution Å: 2.25
Distance (mm): 300.0
2Theta °: 0.0
BeamX (µm): 100.0
BeamY (µm): 100.0

	Frame	Phi (deg)
Start	1	0.00
End	720	180.00

☐ Inverse

✓ Auto Collect
☐ Override Default
☐ Don't Raster

☐ Use Destination Directory

Pin	Puck Name	Crystal Name
1	JCS_8	JCS8_Pin1
2	JCS_8	JCS8_Pin2
3	JCS_8	JCS8_Pin3
4	JCS_8	JCS8_Pin4
5	JCS_8	JCS8_Pin5
6	JCS_8	JCS8_Pin6
7	JCS_8	JCS8_Pin7

Pin	Puck	Puck Name	Crystal Name	Run Type	Creation	Status
1	A	JCS_8	JCS8_Pin1	AutoCollect	2022-02-15 04:52:22	Init
2	A	JCS_8	JCS8_Pin2	AutoCollect	2022-02-15 04:52:22	Init
3	A	JCS_8	JCS8_Pin3	AutoCollect	2022-02-15 04:52:22	Init
4	A	JCS_8	JCS8_Pin4	AutoCollect	2022-02-15 04:52:22	Init
5	A	JCS_8	JCS8_Pin5	AutoCollect	2022-02-15 04:52:22	Init
6	A	JCS_8	JCS8_Pin6	AutoCollect	2022-02-15 04:52:23	Init
7	A	JCS_8	JCS8_Pin7	AutoCollect	2022-02-15 04:52:23	Init

Logging

```
2022-Feb-14 20:50:34 ERROR Internal TIMEOUT on: CryoController task at 131.243.79.18
2022-Feb-14 20:50:44 ERROR Internal TIMEOUT on: CryoController at 131.243.79.18
2022-Feb-14 20:50:52 ERROR Internal TIMEOUT on: CryoController at 131.243.79.18
```

Task Queue
Idle

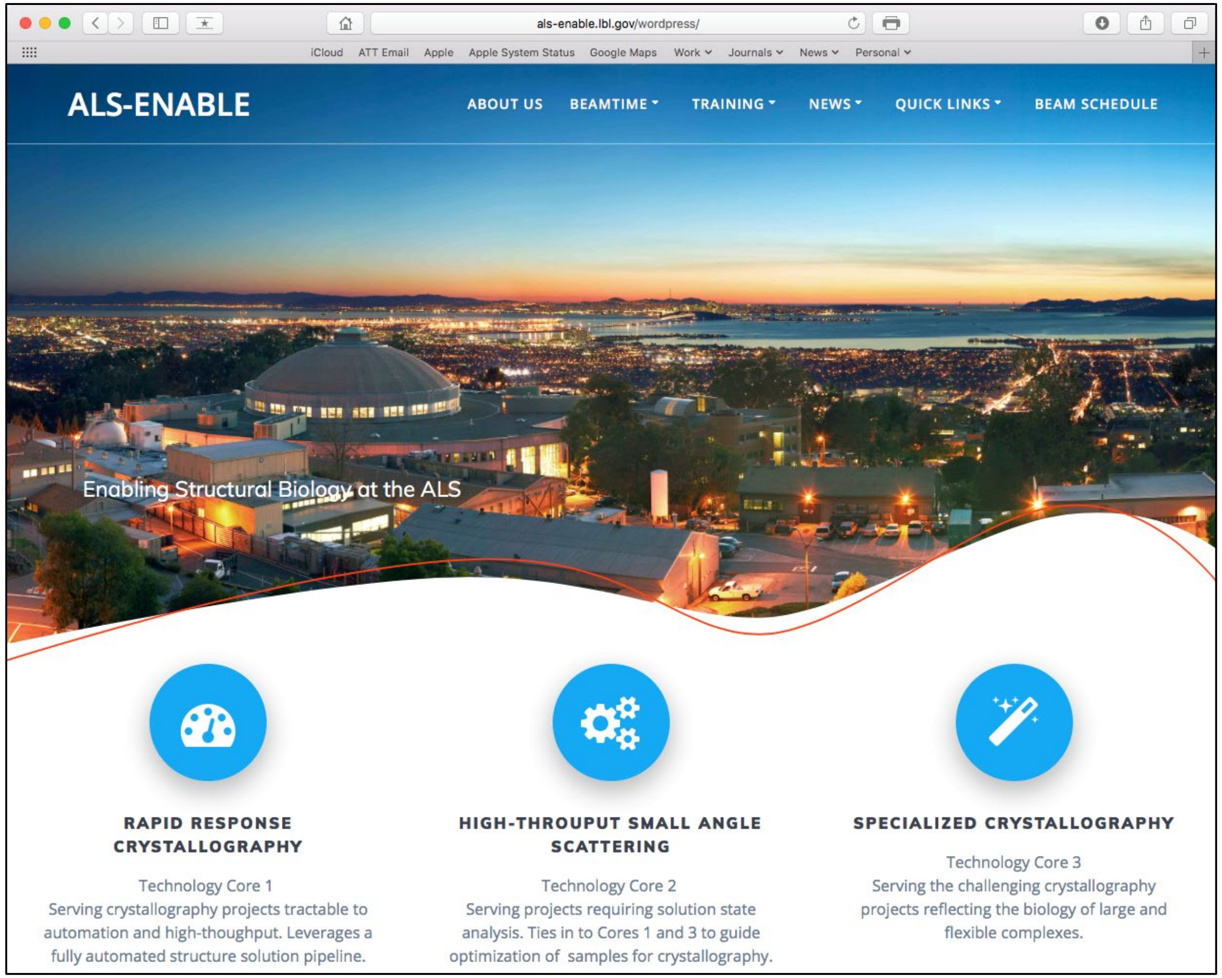
Beam Current: 1.3
Energy: 12685.0
OBS: 0

File:
Collector: NOT_RUNNING
User:

Robot: IDLE
Dewar: NONE
TaskQ: IDLE

ALS-ENABLE

- NIH P30 grant to support user access to ALS structural biology beamlines (als-enable.lbl.gov)



ALS-ENABLE

Enabling Structural Biology at the ALS

RAPID RESPONSE CRYSTALLOGRAPHY

Technology Core 1
Serving crystallography projects tractable to automation and high-thoughput. Leverages a fully automated structure solution pipeline.

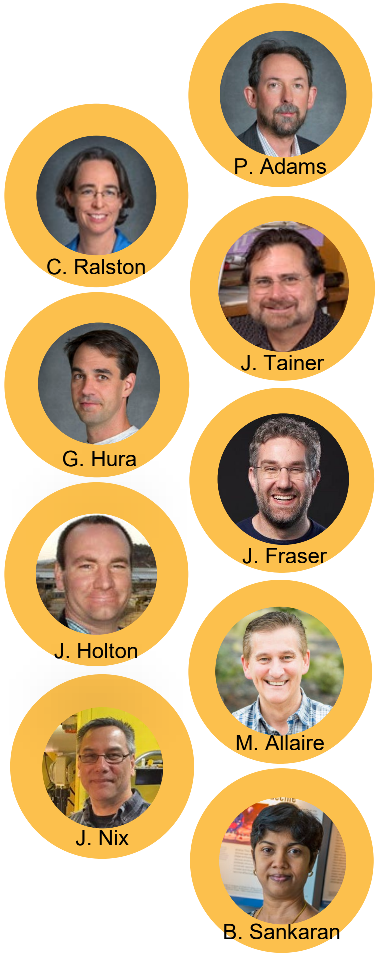
HIGH-THROUPUT SMALL ANGLE SCATTERING

Technology Core 2
Serving projects requiring solution state analysis. Ties in to Cores 1 and 3 to guide optimization of samples for crystallography.

SPECIALIZED CRYSTALLOGRAPHY

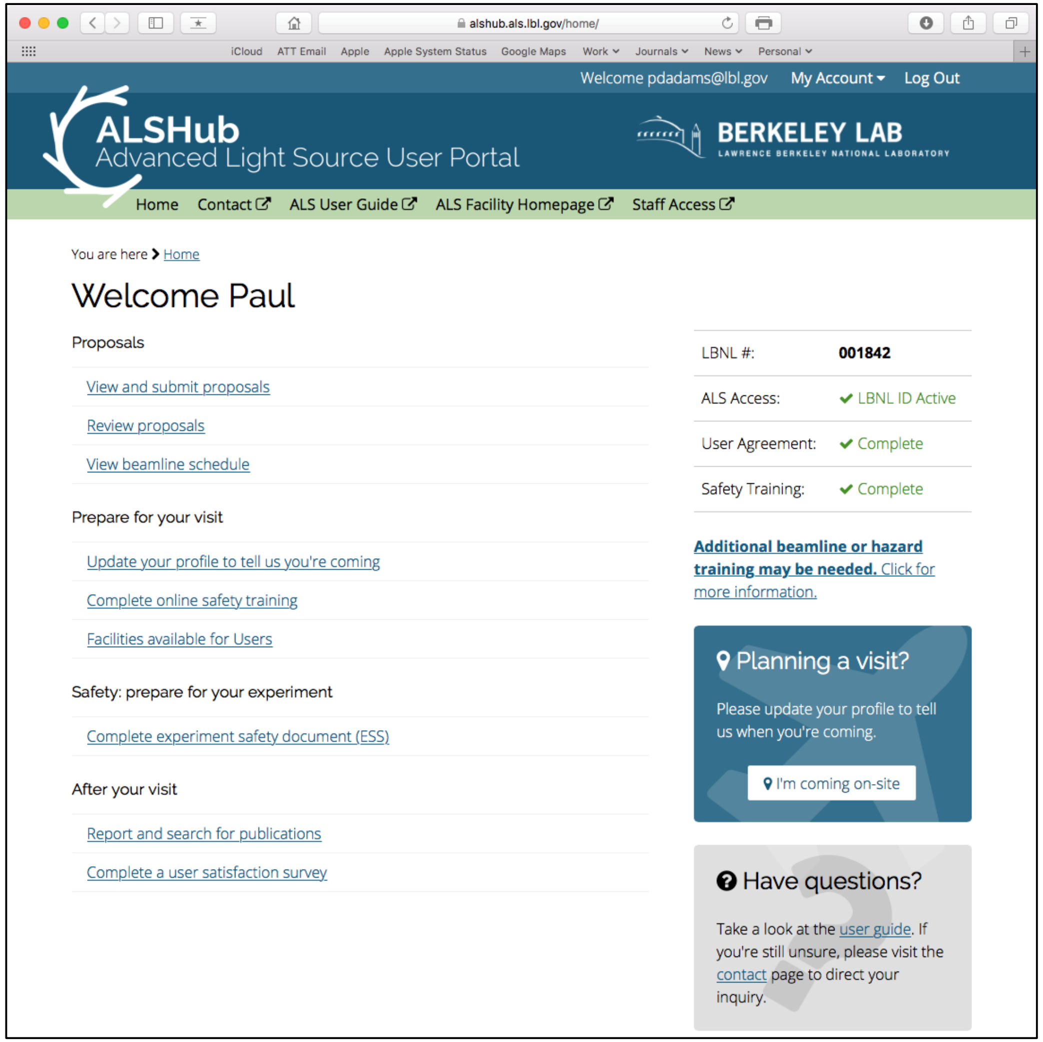
Technology Core 3
Serving the challenging crystallography projects reflecting the biology of large and flexible complexes.

- Mature Synchrotron Resource
- Supports general user access to MX, SAXS and X-Ray Footprinting beamlines
- Automated data collection
- Collaborative projects
- High throughput SAXS
- Room temperature data collection
- In plate data collection



User Access

- Non-peer reviewed access to Participating Research Teams (PRT), who support operations and beamline development
  - TomAlberTron (James Holton – jmholton@lbl.gov)
  - MBC (Jay Nix – jcnix@lbl.gov)
  - BCSB (Marc Allaire – mallaire@lbl.gov)
- Peer reviewed access through General User proposals (als.lbl.gov/users/user-guide)
  - Merit-based review
  - ALS administered (alsuser@lbl.gov)
  - 6-month and RAPIDD
    - Register as an ALS user
    - Apply for beamtime
    - Establish a user agreement
    - Proprietary research requires reimbursement for the cost of the photons



You are here > Home

Welcome Paul

Proposals

- View and submit proposals
- Review proposals
- View beamline schedule

Prepare for your visit

- Update your profile to tell us you're coming
- Complete online safety training
- Facilities available for Users

Safety: prepare for your experiment

- Complete experiment safety document (ESS)

After your visit

- Report and search for publications
- Complete a user satisfaction survey

LBNL #:	**001842**
ALS Access:	✓ LBNL ID Active
User Agreement:	✓ Complete
Safety Training:	✓ Complete

Additional beamline or hazard training may be needed. Click for more information.

Planning a visit?

Please update your profile to tell us when you're coming.

Have questions?

Take a look at the user guide. If you're still unsure, please visit the contact page to direct your inquiry.

Preparing in view of the APS upgrade

- Mature Synchrotron Resource at the ALS
- GUI upgrade for ease of operations
- Down to 4-hour shift for rapid response
- Pixel-Array Detectors on 8.2.1/8.2.2
- Increased GU fraction (200% to 400%)
- Remote access on all beamlines
- Automated data collection
- Increase resource for collaborative projects
- Room-temperature data collection
- In-situ data collection from plates
- Remote training on beamline use
- Outreach at conferences

Dewars waiting to be shipped

APS-U Structural Biology Townhall Agenda

Start	Duration	End	
1:00 PM	0:05	1:05 PM	Welcome - Laurent Chapon,
1:05 PM	0:25	1:30 PM	APS-U Overview and Life Sciences at the APS, Bob Fischetti
1:30 PM	0:15	1:45 PM	IMCA-CAT, Lisa Keefe
1:45 PM	0:15	2:00 PM	SBC/eBERLight, Andrzej Joachimiak and Karolina Michalska
2:00 PM	0:15	2:15 PM	LS-CAT, Spencer Anderson
2:15 PM	0:15	2:30 PM	SER-CAT, Bi-Chen Wang
2:30 PM	0:15	2:45 PM	GM/CA-CAT, Naga Venugopalan
2:45 PM	0:15	3:00 PM	Break
3:00 PM	0:15	3:15 PM	NE-CAT, Malcolm Capel
3:15 PM	0:15	3:30 PM	LRL-CAT, Jordi Benach
3:30 PM	0:15	3:45 PM	ALS, Marc Allaire
3:45 PM	0:15	4:00 PM	CHESS, Marian Szebenyi
4:00 PM	0:15	4:15 PM	NSLS-II, Sean McSweeney
4:15 PM	0:15	4:30 PM	SSRL, Aina Cohen
4:30 PM	0:30	5:00 PM	Roundtable Q&A
5:00 PM			Adjourn

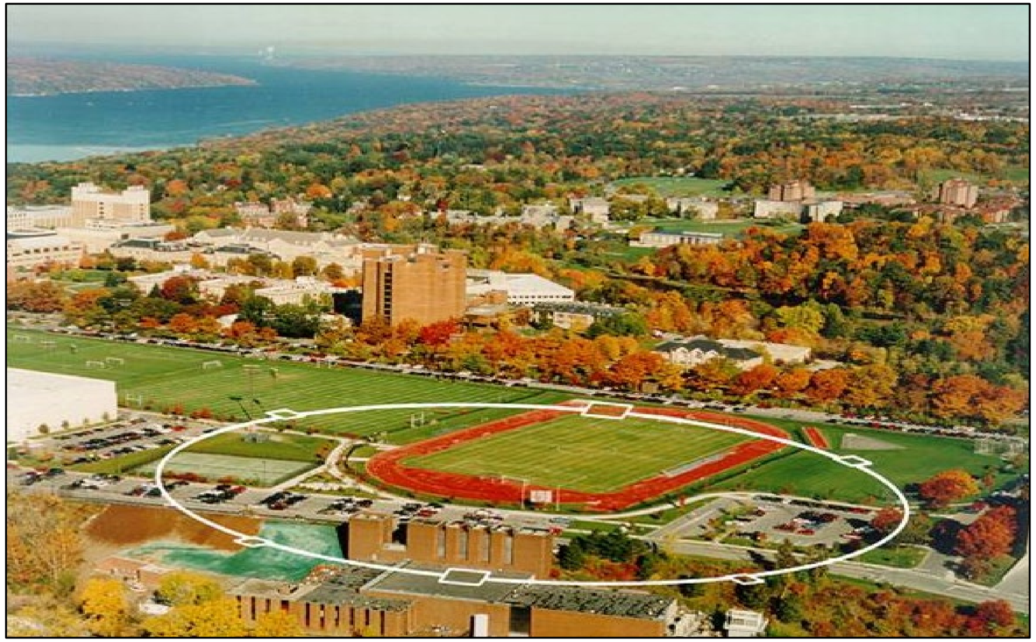
Cornell High Energy Synchrotron Source

Marian Szebenyi for the CHESS team

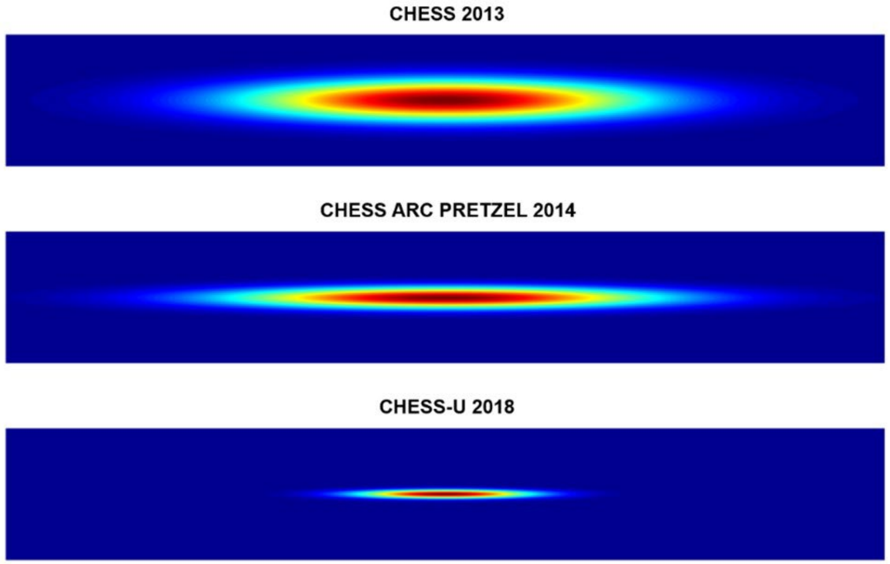
Cornell University
Cornell Laboratory of Accelerator-based ScienceS and Education

CHESS basics

Located on Cornell's central campus, CHESS hosts a wide range of X-ray experiments, with a focus on innovation.



2019 upgrade to storage ring and beamlines.



Electron beam size at X-ray source position

CESR now runs at 6 GeV, 100-200 mA.
All beamlines are fed by insertion devices.

Beamlines

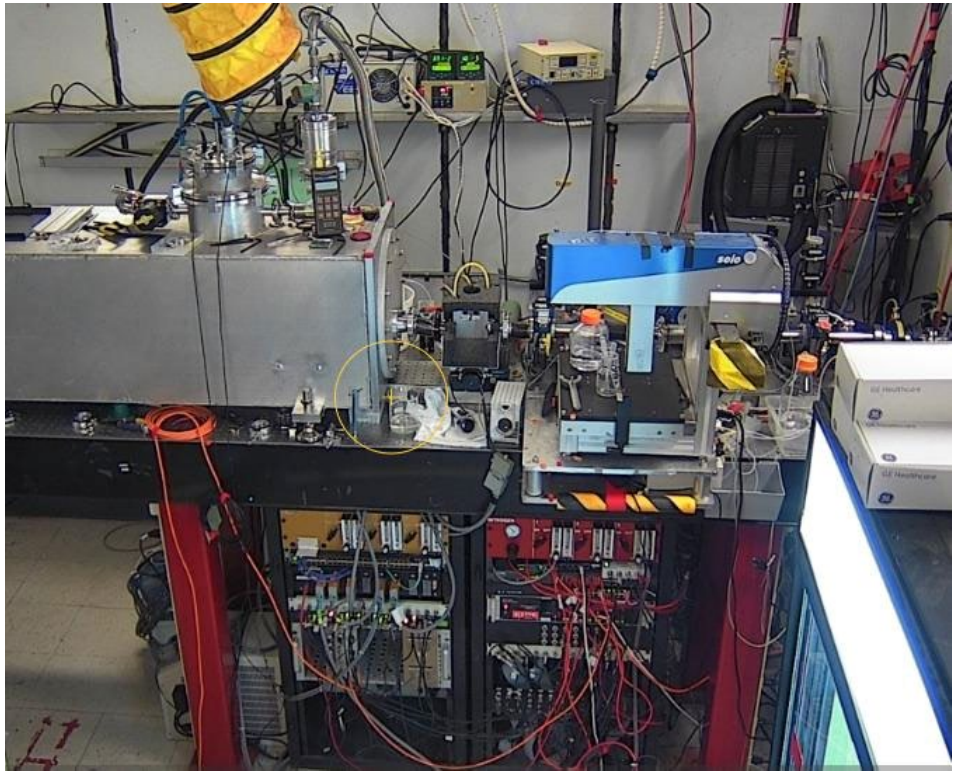
- **1A3:** Structural Materials (SMB)
- **2A:** Photon In-Photon Out spectroscopy (PIPOXS)
- **3A:** Forming & Shaping Technology (FAST)
- **3B:** Functional Materials (FMB)
- **4B:** Q-Mapping for Quantum Materials (<QM>^{2})
- **7A:** Biological Small-Angle X-ray Scattering (BioSAXS)
- **7B2:** Flexible Macromolecular Crystallography (FlexX)

Beamlines are supported by a mix of funding agencies, for different purposes.

CHEXS **MacCHESS** **MSN-C**

BioSAXS standard features

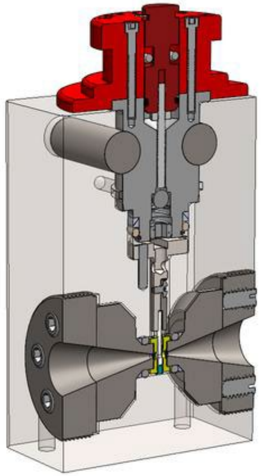
- Tunable multilayer monochromator, 1.5% bandwidth, 7-14 KeV.
- Beam size 250 x 200 or 25 x 30 µm, q range 0.005-0.7 Å^{-1}.
- EIGER 4M detector, in vacuum tank. For most experiments, no air gap between sample and detector.
- Options for sample loading:
  - From trays using robot
  - Directly from size-exclusion (SEC-SAXS) or ion-exchange column
  - Manually (e.g. for loading high-pressure cell)
- In-line DLS/MALS



BioSAXS operation modes

- Most users come in person and make use of the wet lab facilities for final sample preparation.
- For samples needing no special preparation, a mail-in option is available.
  - Support for this option is dependent on the availability of staff to run the samples; if funding is obtained for additional automation this will be less of a problem.
- For a complex experiment where the user cannot come in person, a joint mode with communication over NoMachine and Zoom is possible.

BioSAXS for developing techniques



High pressure:

- Static cell for up to 4 kbar pressure.
- Chromatography system, pressurized throughout, to 1 kbar.

Non-standard samples:

- Anaerobic systems.
- Membranes mounted on a support; required installation of special equipment.
- Other examples: cell nuclei, nanoparticles (potential drug carriers).

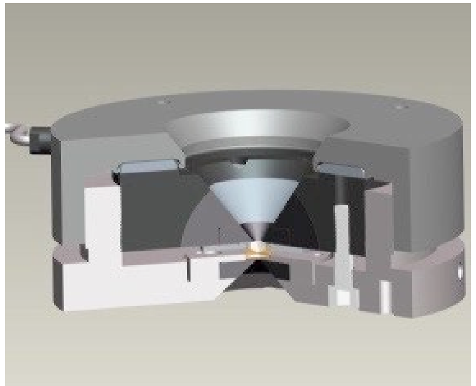
Test for BioCAT:

- Good data collected from muscle fibers.

FlexX for standard MX

- Tunable multilayer monochomator: 9-14 KeV, 0.6% bandwidth.
- Beam diameter 100 µm using collimator or 10 µm using CRL, ~$2\text{x}10^{11}$ ph/sec on sample.
- EIGER2 16M detector, BAM-2 automounter (ALS, Unipuck, SPINE pins), Oxford cryosystem, annealer.
- **ADX** data collection GUI, automatic data reduction using **fast_dp**.
- Common crystallographic software installed.
- Remote experiment control using NoMachine, rapid data retrieval using Globus.
- High pressure cryocooling available.

FlexX for developing techniques



High pressure:

- VivoDAC cell allows precise control of pressure in 0-15 kbar range. Can mount 2-3 crystals per cell.
- Usable data sets can be obtained from <10 crystals.

Serial MX (fixed-target):

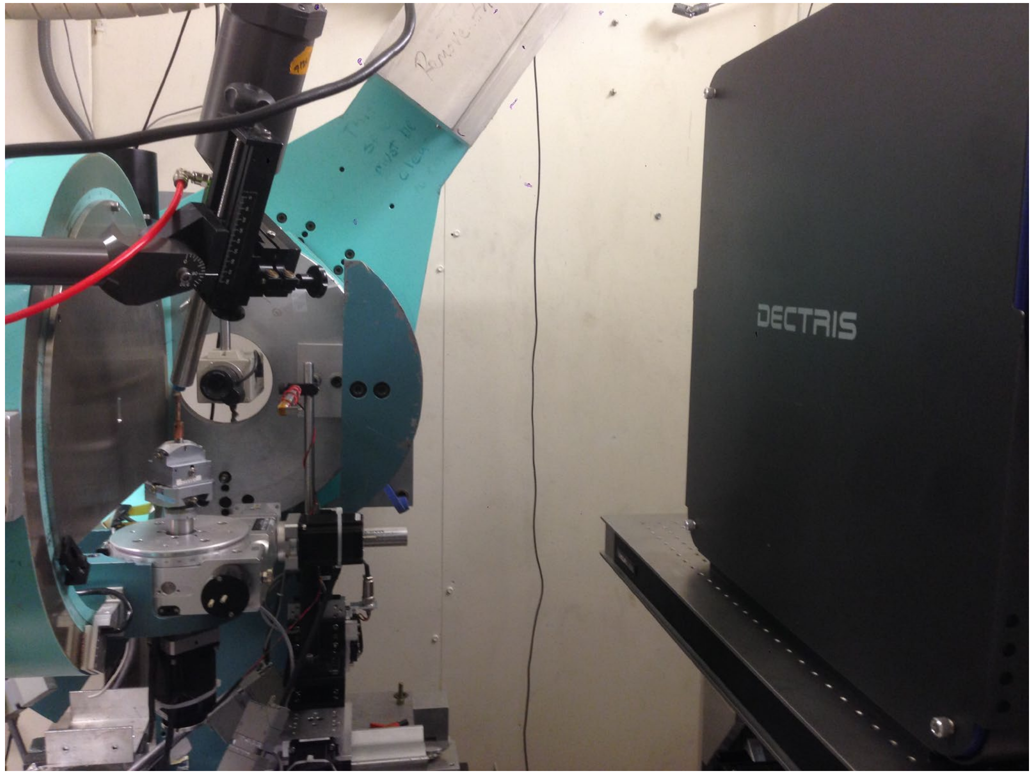
- Chips, tools, and humidity controlled enclosure for mounting crystals are available. **ADX** includes option for rastering across chip, collecting small oscillation at each point.
- Structures have been determined using serial data from CHESS.

Other:

- Unusual samples including frozen embryos have been examined.

<QM>^{2}

- Tunable from 6-70 KeV, with 0.01% bandwidth.
- Has 4-circle diffractometer, Pilatus2 6M detector.
- Useful for crystallography (MAD, diffuse scattering experiments) requiring a narrow X-ray bandwidth.
- Caveat: MX not a priority, could be difficult to get time.



PIPOXS

- Provides absorption and emission X-ray spectroscopy, including EXAFS, HERFD-XANES, resonant and non-resonant XES.
- Helps determine geometric and electronic structure of catalysts, and relate structure to activity.
- Can be used to study active sites in metalloenzymes.
- Photon energy range 4 – 30 KeV with Si(111),
- 7 – 58 KeV with Si(311).
- Microfluidic system enables time-resolved observation
- of ongoing reactions.

CHESS
CORNELL HIGH ENERGY
SYNCHROTRON SOURCE

Getting beamtime

- Typical running schedule is mid-Jan to mid-Mar, mid-Apr to late-Jun, late-Sep to Thanksgiving; 6 days per week, 24 hours per day.
- Submit a proposal application on-line; proposals are good for 2 years. For additional visits after the first, submit a beamtime request (BTR).
- For BioSAXS and MX, submit any time; response is usually rapid.

Summary

- CHESS BioSAXS and MX beamlines provide excellent routine service; in-person, mail-in and remote modes are supported.
- Non-standard experiments are welcome; we have special expertise in high pressure BioSAXS and MX, as well as fixed-target serial MX.

For more information:

- CHESS web site www.chess.cornell.edu/.

APS-U Structural Biology Townhall Agenda

Start	Duration	End	
1:00 PM	0:05	1:05 PM	Welcome - Laurent Chapon,
1:05 PM	0:25	1:30 PM	APS-U Overview and Life Sciences at the APS, Bob Fischetti
1:30 PM	0:15	1:45 PM	IMCA-CAT, Lisa Keefe
1:45 PM	0:15	2:00 PM	SBC/eBERLight, Andrzej Joachimiak and Karolina Michalska
2:00 PM	0:15	2:15 PM	LS-CAT, Spencer Anderson
2:15 PM	0:15	2:30 PM	SER-CAT, Bi-Chen Wang
2:30 PM	0:15	2:45 PM	GM/CA-CAT, Naga Venugopalan
2:45 PM	0:15	3:00 PM	Break
3:00 PM	0:15	3:15 PM	NE-CAT, Malcolm Capel
3:15 PM	0:15	3:30 PM	LRL-CAT, Jordi Benach
3:30 PM	0:15	3:45 PM	ALS, Marc Allaire
3:45 PM	0:15	4:00 PM	CHESS, Marian Szebenyi
4:00 PM	0:15	4:15 PM	NSLS-II, Sean McSweeney
4:15 PM	0:15	4:30 PM	SSRL, Aina Cohen
4:30 PM	0:30	5:00 PM	Roundtable Q&A
5:00 PM			Adjourn

Brookhaven™ National Laboratory

U.S. DEPARTMENT OF ENERGY

Structural Biology at NSLS-II

Seán McSweeney

smcsweeney@bnl.gov

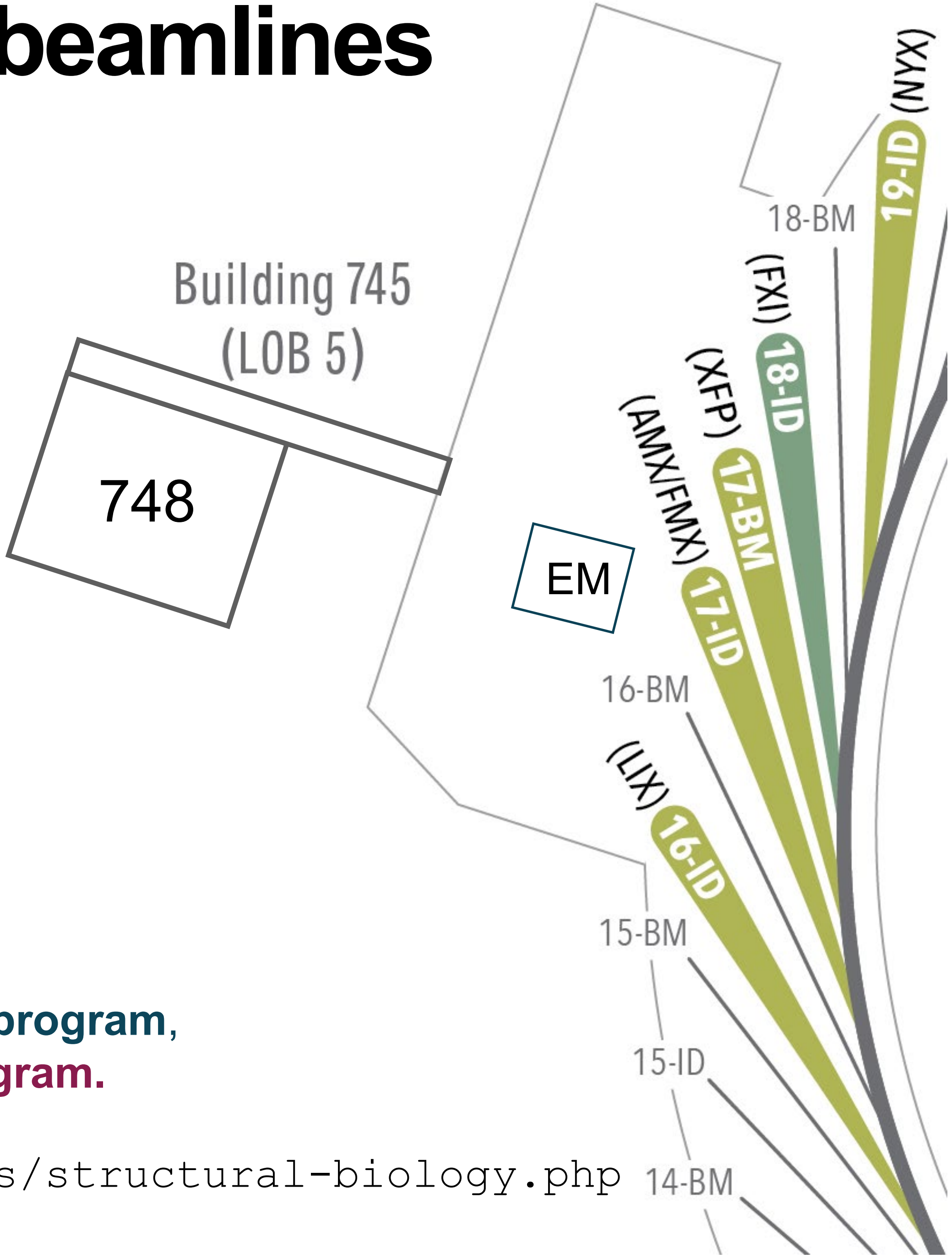
June 21st 2022

@BrookhavenLab

Structural Biology Program beamlines

Are clustered near NSLS-II building 745..

- X-ray scattering: **LIX**
- Macromolecular Crystallography: **AMX**, **FMX**, **NYX**
- X-ray Footprinting: **XFP**
- Electron Microscopes: 745 & 748



Facility Beamlines: 100% of access through General User program,
Partner Beamlines: 50% access through General User Program.

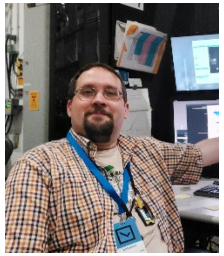
Brookhaven National Laboratory

https://www.bnl.gov/nsls2/programs/structural-biology.php

17-BM: X-Ray Footprinting of Biological Materials (XFP)

Partner Beamline built (NSF $$$) and operated by Case Western Reserve University (spokesperson: Prof. Mark Chance).

3PW source, 4.5-16 keV “pink beam” X-rays to sample in focused and defocused modes



Erik Farquhar

Mike Sullivan

Vijaya Lakshmi (“Vijji”) Kanchustambham

CASE WESTERN RESERVE UNIVERSITY EST. 1826

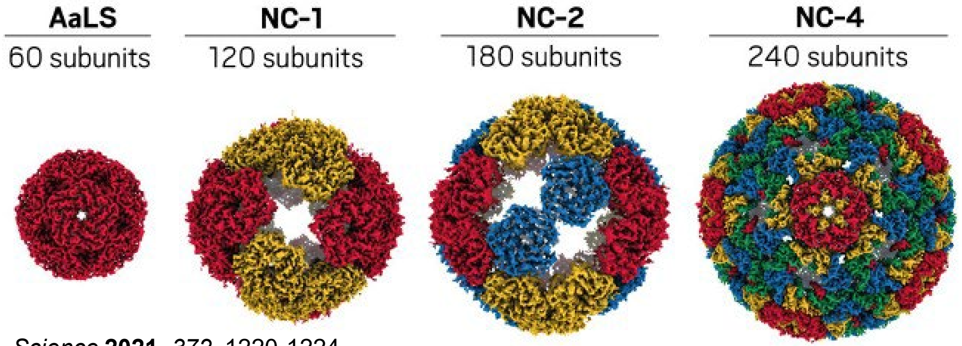
SCHOOL OF MEDICINE

Premise:

X-ray radiolysis of water gives OH radicals that react with *solvent accessible* regions of proteins and nucleic acids (“controlled radiation damage”).

Map damage onto structure, observe effects from binding of a drug, etc. Complements MX, SAXS, CryoEM.

Science example: Evolution of virus structure

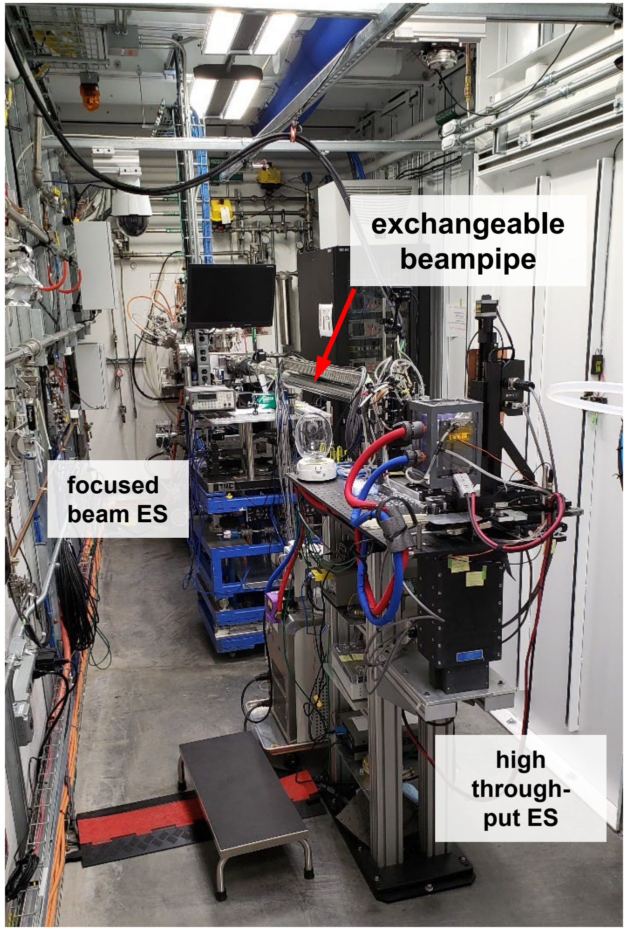


Science **2021**, *372*, 1220-1224

RNA-binding protein can assemble into a virus-like particle (capsid).

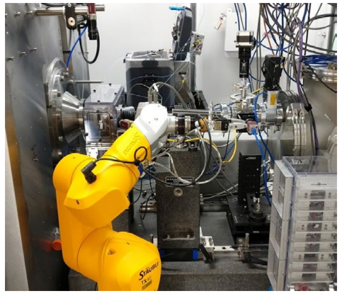
XFP used to show increasing protection of the nucleic acids

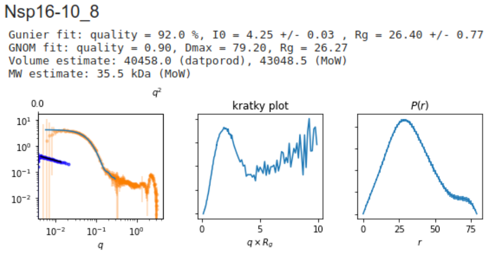
Model for how viruses evolved to protect their genetic code.



LIX   Solution scattering

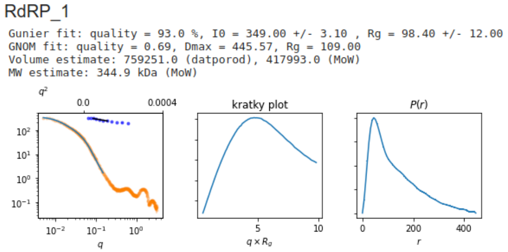
The protein is in solution, with integrated chromatography units. Kinetics in different ways are possible – allowing study reactions or formation of complexes.





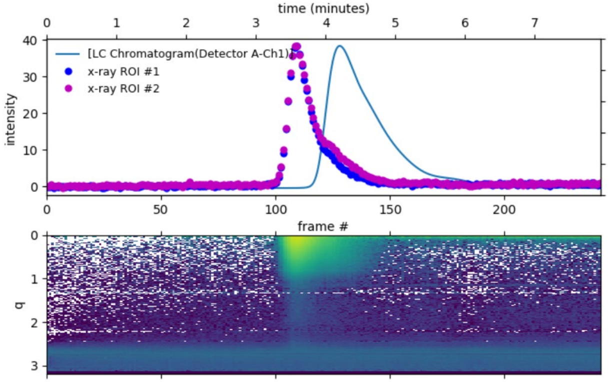
Nsp16-10_8

```
Gunier fit: quality = 92.0 %, I0 = 4.25 +/- 0.03 , Rg = 26.40 +/- 0.77
GNOM fit: quality = 0.90, Dmax = 79.20, Rg = 26.27
Volume estimate: 40458.0 (datporod), 43048.5 (MoW)
MW estimate: 35.5 kDa (MoW)
```



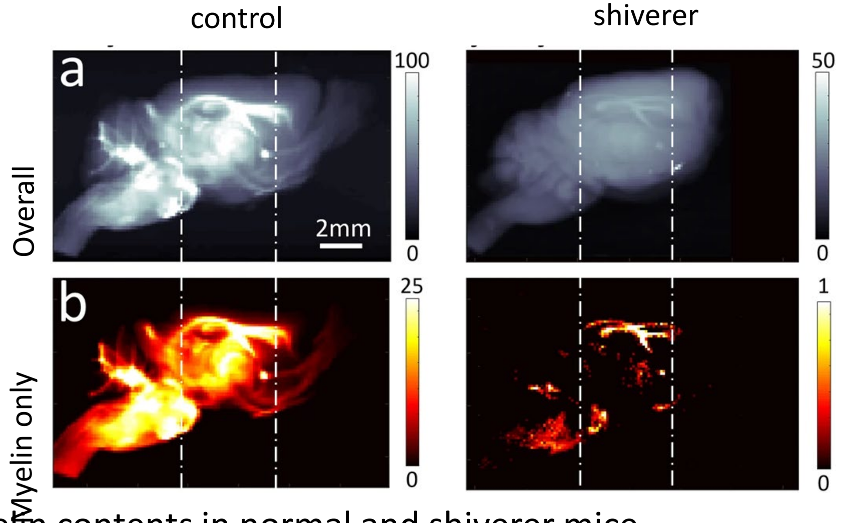
RdRP_1

```
Gunier fit: quality = 93.0 %, I0 = 349.00 +/- 3.10 , Rg = 98.40 +/- 12.00
GNOM fit: quality = 0.69, Dmax = 445.57, Rg = 109.00
Volume estimate: 759251.0 (datporod), 417993.0 (MoW)
MW estimate: 344.9 kDa (MoW)
```

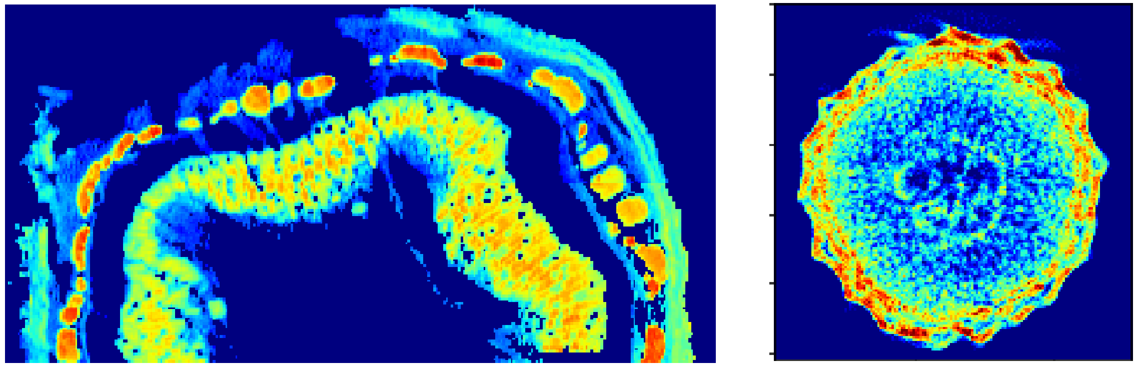


Scanning structural mapping

Visualize the spatial distribution of structural components in biological tissues based on their scattering signatures.



Brain myelin contents in normal and shiverer mice



Cellulose in physical (left) and virtual (right) sections of plants

NYX Protein Crystallography Beamline

- NYX is a partner user beamline funded and operated by the New York Structural Biology Center (NYSBC).
- Our objective is to provide high energy resolution x-rays to optimize the signal-to-noise ratio for anomalous scattering experiments.
- Spokesperson: Wayne Hendrickson.
- Staff
  - Kevin Battaile; LBS
  - Dieter Schneider; co-LBS
  - Randy Abramowitz; Mechanical Technician
  - Michael Skinner; Software Engineer

The Rockefeller University
Science for the benefit of humanity

Columbia University
in the City of New York

Memorial Sloan Kettering Cancer Center

The City University of New York

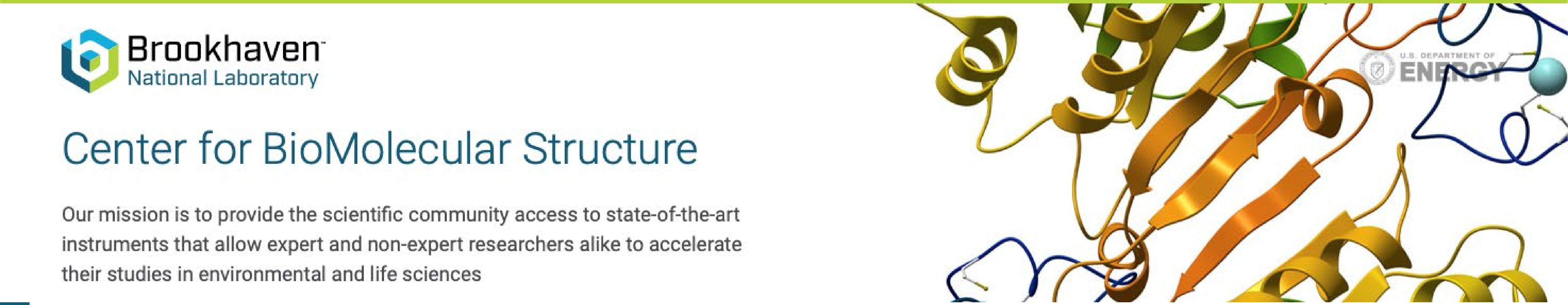
Icahn School of Medicine at Mount Sinai

New York University

Einstein
Albert Einstein College of Medicine

Weill Cornell Medical College

Wadsworth Center
New York State Department of Health



Brookhaven National Laboratory

Center for BioMolecular Structure

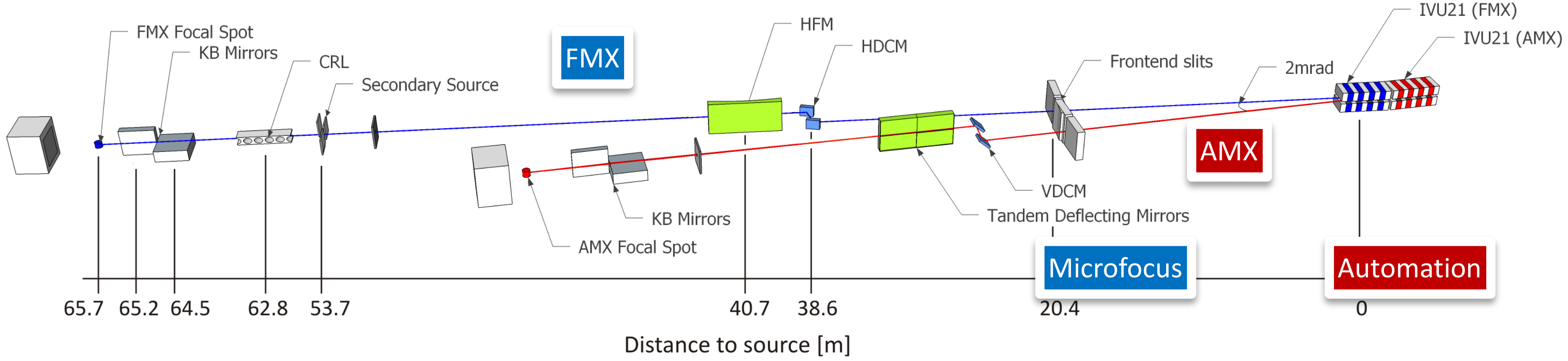
Our mission is to provide the scientific community access to state-of-the-art instruments that allow expert and non-expert researchers alike to accelerate their studies in environmental and life sciences

To enable the resources at CBMS:

- NIH/NIGMS P30 supports Structural biology at LiX, FMX and AMX
- DOE-BER supports structural biology and also bioimaging.

`https://www.bnl.gov/nsls2/lifesciences`

17ID-1 & 2: AMX and FMX – Automation and microfocus x-ray beams.



FMX and AMX: Two beamlines with overlapping and complementary capabilities

Energy range	5 – 30 keV	5 – 18 keV
Wavelength range	0.4 – 2.5 Å	0.7 – 2.5 Å
Flux at focus at 12.7 keV	3.5×10^{12} ph/s	5×10^{12} ph/s
Focal spot min (H×V)	1 × 1.5 μm^2	7 × 5 μm^2
Focal spot range	1 – 10 µm	5 (– 20) µm
Detector	Eiger 16M	Eiger 9M

Sample changers using **unipuck bases** and **SPINE pins**. We find these are **essential for reliable high throughput automation**. >95% of experiments are remote access.

Brookhaven National Laboratory

Access to NSLS-II

Three main forms:

- **General User** proposal: single PI, single beamline
- **Rapid Access** proposals: rolling call, beamtime usually within two to three weeks, if space in schedule
- **Block Allocation Groups**: multi-PI, multi project applications gaining access to all beamlines in the program from one application. Good for two years

Our suggestion to you if interested in access to NSLS-II

Block Allocation Group (BAG)

- **The BAG** may be science themed, or geographical, could be a APS-CAT
- **Lowers administrative overhead**: one application, essentially rapid access once active. Makes life easier for you and us.
- Enables you to establish “mini-shifts” for each PI/group in the BAG.
- **Will need an active BNL user account:** changes in our cyber security policy make this a requirement for access to the IT infrastructure.
- **Next deadlines:** Sept 31 2022, for experiments to begin Jan 2023, Jan 31 2023, for experiments to begin May 2023.

Scheduling

- We try to run a hybrid system of user self-scheduling and us setting the schedule.
- Flexible and adapted through each NSLS-II operation cycle.
- Late availability shifts advertised.
- We are looking to roll out fully automated overnight data collection as our preferred way of using all the nighttime shifts.
- The aim is to maximize time available for experiments without staff burnout.

Macromolecular crystallography short term aims

- Further optimize use of micro-focus beamlines.
- Fully Automated data collection for most experiments.
- Guided automation for xtals < 20micron.
- Common user interface across all three MX beamlines. (**Done**)
- On the fly data analysis and merging of all data collected. (**Available**)
- Easy access to data, and experiment summaries through ispyb database. (**In process**)
- Integrated analysis workflows with SAXS, x-ray footprinting and cryo-em.
- Collaboration and optimization of instruments for room temperature and time-resolved measurements
- Very high throughput automated fragment/ligand screening :
    - goal ~ 1000 samples / day (data collection to map)
- Dynamics through collection of many datasets from “similar”crystals.

Unfortunately
not in
the photo


Grace


John & Tom

Jun Aishima

Jun


Matt


Jake


Liguo


Vijji


Tom

APS-U Structural Biology Townhall Agenda

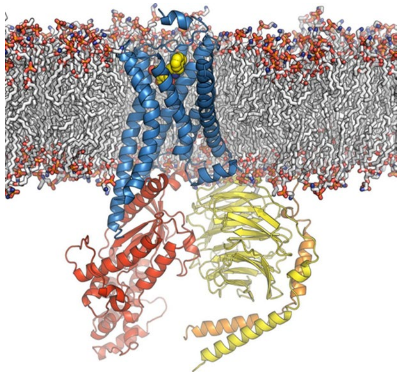
Start	Duration	End	
1:00 PM	0:05	1:05 PM	Welcome - Laurent Chapon,
1:05 PM	0:25	1:30 PM	APS-U Overview and Life Sciences at the APS, Bob Fischetti
1:30 PM	0:15	1:45 PM	IMCA-CAT, Lisa Keefe
1:45 PM	0:15	2:00 PM	SBC/eBERLight, Andrzej Joachimiak and Karolina Michalska
2:00 PM	0:15	2:15 PM	LS-CAT, Spencer Anderson
2:15 PM	0:15	2:30 PM	SER-CAT, Bi-Chen Wang
2:30 PM	0:15	2:45 PM	GM/CA-CAT, Naga Venugopalan
2:45 PM	0:15	3:00 PM	Break
3:00 PM	0:15	3:15 PM	NE-CAT, Malcolm Capel
3:15 PM	0:15	3:30 PM	LRL-CAT, Jordi Benach
3:30 PM	0:15	3:45 PM	ALS, Marc Allaire
3:45 PM	0:15	4:00 PM	CHESS, Marian Szebenyi
4:00 PM	0:15	4:15 PM	NSLS-II, Sean McSweeney
4:15 PM	0:15	4:30 PM	SSRL, Aina Cohen
4:30 PM	0:30	5:00 PM	Roundtable Q&A
5:00 PM			Adjourn

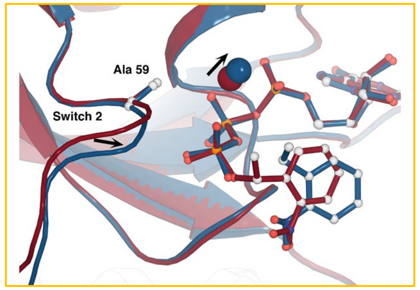
Macromolecular Crystallography

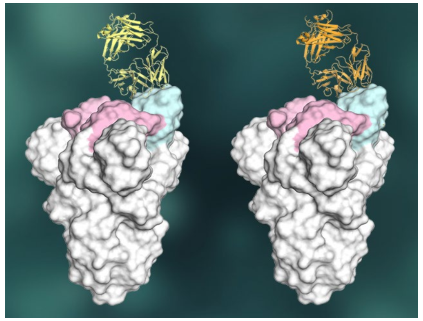
at the Stanford Synchrotron Radiation Lightsource

APS Upgrade Structural Biology Virtual Townhall Meeting

Aina Cohen – ACohen@SLAC.Stanford.edu
June 21, 2022







SMB SSRL

U.S. DEPARTMENT OF ENERGY NIH

SLAC NATIONAL ACCELERATOR LABORATORY

SLAC National Accelerator Laboratory

SLAC

NATIONAL ACCELERATOR LABORATORY

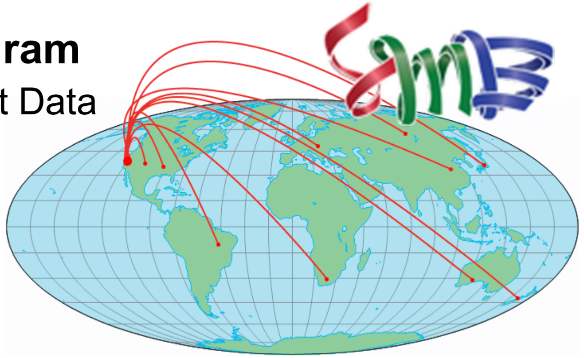
Stanford Synchrotron Radiation Lightsource (SSRL)

SLAC NATIONAL ACCELERATOR LABORATORY

Macromolecular Crystallography Group

Remote Access Program

- >98% of Users Collect Data Remotely
- You Control the Experiment
- Saves Time and Money
- Access to Cutting-edge Equipment
- Fosters Collaboration



SSRL:

- **BL12-1** Undulator, Microcrystal
- **BL 12-2** Undulator, Microcrystal
- **BL9-2** Wiggler Multi-wavelength
- **BL14-1** Bend Multi-wavelength

Beamtime Inquiries Welcome

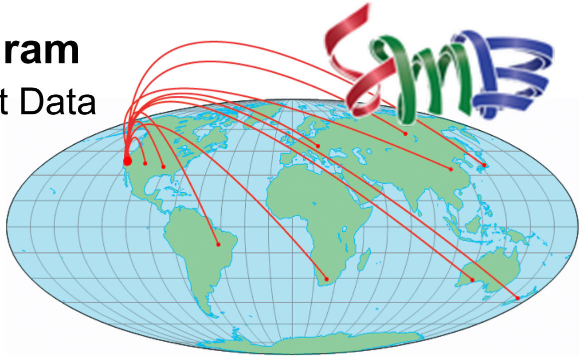
Aina Cohen: ACohen@SLAC.Stanford.edu
Lisa Dunn: Lisa@SLAC.Stanford.edu

Stanford Synchrotron Radiation Lightsource (SSRL)


2004 – First Remote Control Experiments

Remote Access Program

- >98% of Users Collect Data Remotely
- You Control the Experiment
- Saves Time and Money
- Access to Cutting-edge Equipment
- Fosters Collaboration



SSRL:

- **BL12-1** Undulator, Microcrystal
- **BL 12-2** Undulator, Microcrystal
- **BL9-2** Wiggler Multi-wavelength
- **BL14-1** Bend Multi-wavelength

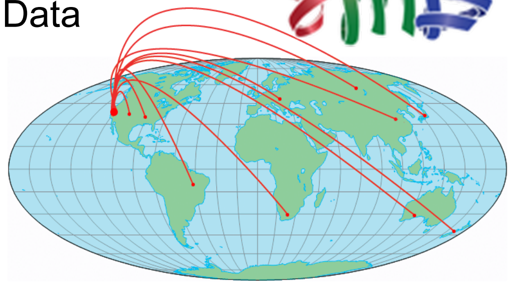
Beamtime Inquiries Welcome

Aina Cohen: ACohen@SLAC.Stanford.edu
Lisa Dunn: Lisa@SLAC.Stanford.edu

Improved Remote Access Facilities

Remote Access Program

- >97% of Users Collect Data Remotely
- You Control the Experiment
- Saves Time and Money
- Access to Cutting-edge Equipment
- Fosters Collaboration



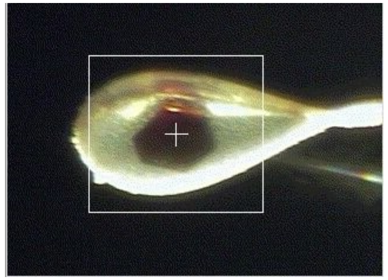
Beamtime Inquiries Welcome

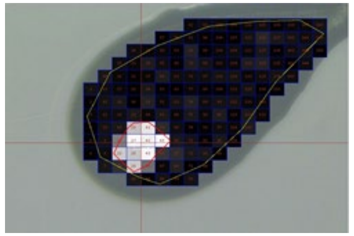
Aina: ACohen@SLAC.Stanford.edu
Lisa: Lisa@SLAC.Stanford.edu

SSRL:

- **BL12-1** Undulator Microcrystal
- **BL12-2** Undulator, Microcrystal
- **BL 9-2** Wiggler Multi-wavelength
- **BL14-1** Bend Multi-wavelength

15 seconds loop centering





73 second down to **18 seconds** in shutterless mode (150 pts)

- **Faster robotic sample exchange** (~25 s)
- **Faster automated loop centering** (~15 s)
- **Shutterless data collection** (EIGER or PILATUS)
- **Shutterless grid searches to locate crystals**

Fully Autonomous Data Collection and Processing

User:

1. Ship Samples to SSRL in Uni-pucks or Cassettes
2. Use Blu-ice Screening Tab to choose parameters for automation

Automated Steps:

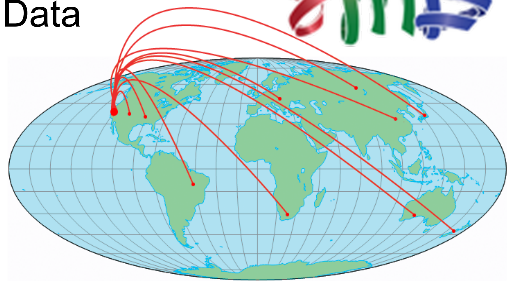
1. Robot mounts sample pin
2. Sample loop or mesh centering (video)
3. Low dose X-ray grid search to center the best crystal into the beam and to set the best beamsize.
4. Video snapshot of crystal
5. Shutterless X-ray diffraction data collection (or screening)
   – strategy coming soon
1. All data collected is automatically processed! (XDS, results in /data directory with diffraction images)

Repeat for next crystal…

Improved Remote Access Facilities

Remote Access Program

- >97% of Users Collect Data Remotely
- You Control the Experiment
- Saves Time and Money
- Access to Cutting-edge Equipment
- Fosters Collaboration



SSRL:

- **BL12-1** Undulator Microcrystal
- **BL12-2** Undulator, Microcrystal
- **BL 9-2** Wiggler Multi-wavelength
- **BL14-1** Bend Multi-wavelength

Beamtime Inquiries Welcome

Aina: ACohen@SLAC.Stanford.edu
Lisa: Lisa@SLAC.Stanford.edu

Fully Autonomous Data Collection and Processing

User:

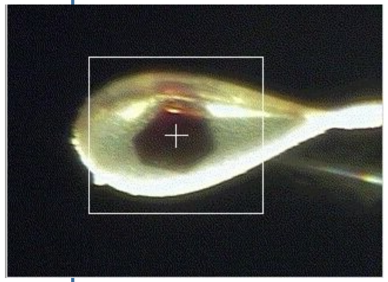
1. Ship Samples to SSRL in Uni-pucks or Cassettes
2. Use Blu-ice Screening Tab to choose parameters for automation

Automated Steps:

1. Robot mounts sample pin
2. Sample loop or mesh centering (video)
3. Low dose X-ray grid search to center the best crystal into the beam and to set the best beamsize.
4. Video snapshot of crystal
5. Shutterless X-ray diffraction data collection (or screening)
   – strategy coming soon

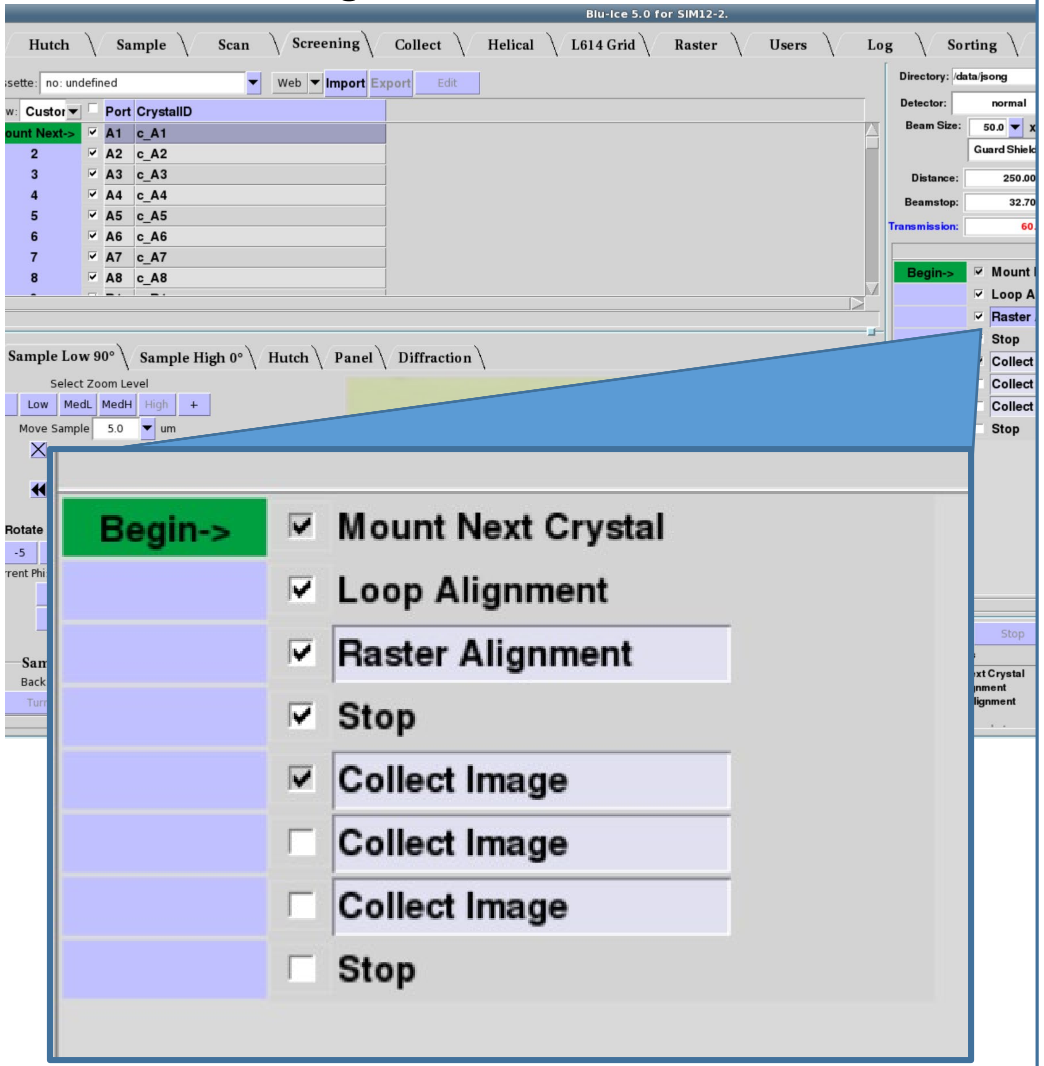
1\. All data collected is automatically processed! (XDS, results in /data directory with diffraction images)

Repeat for next crystal…



Improved Remote Access Facilities

Blu-ice Screening Tab



sette: no: undefined

Directory: /data/jsong

Detector: normal

Beam Size: 50.0

Distance: 250.00

Beamstop: 32.70

Transmission: 60.

Move Sample 5.0 um

w: Custor	☐	Port	CrystalID
ount Next->	☑	A1	c_A1
2	☑	A2	c_A2
3	☑	A3	c_A3
4	☑	A4	c_A4
5	☑	A5	c_A5
6	☑	A6	c_A6
7	☑	A7	c_A7
8	☑	A8	c_A8

Begin->

☑ Mount Next Crystal

☑ Loop Alignment

☑ Raster Alignment

☑ Stop

☑ Collect Image

☐ Collect Image

☐ Collect Image

☐ Stop

Fully Autonomous Data Collection and Processing

User:

1. Ship Samples to SSRL in Uni-pucks or Cassettes
2. Use Blu-ice Screening Tab to choose parameters for automation

Automated Steps:

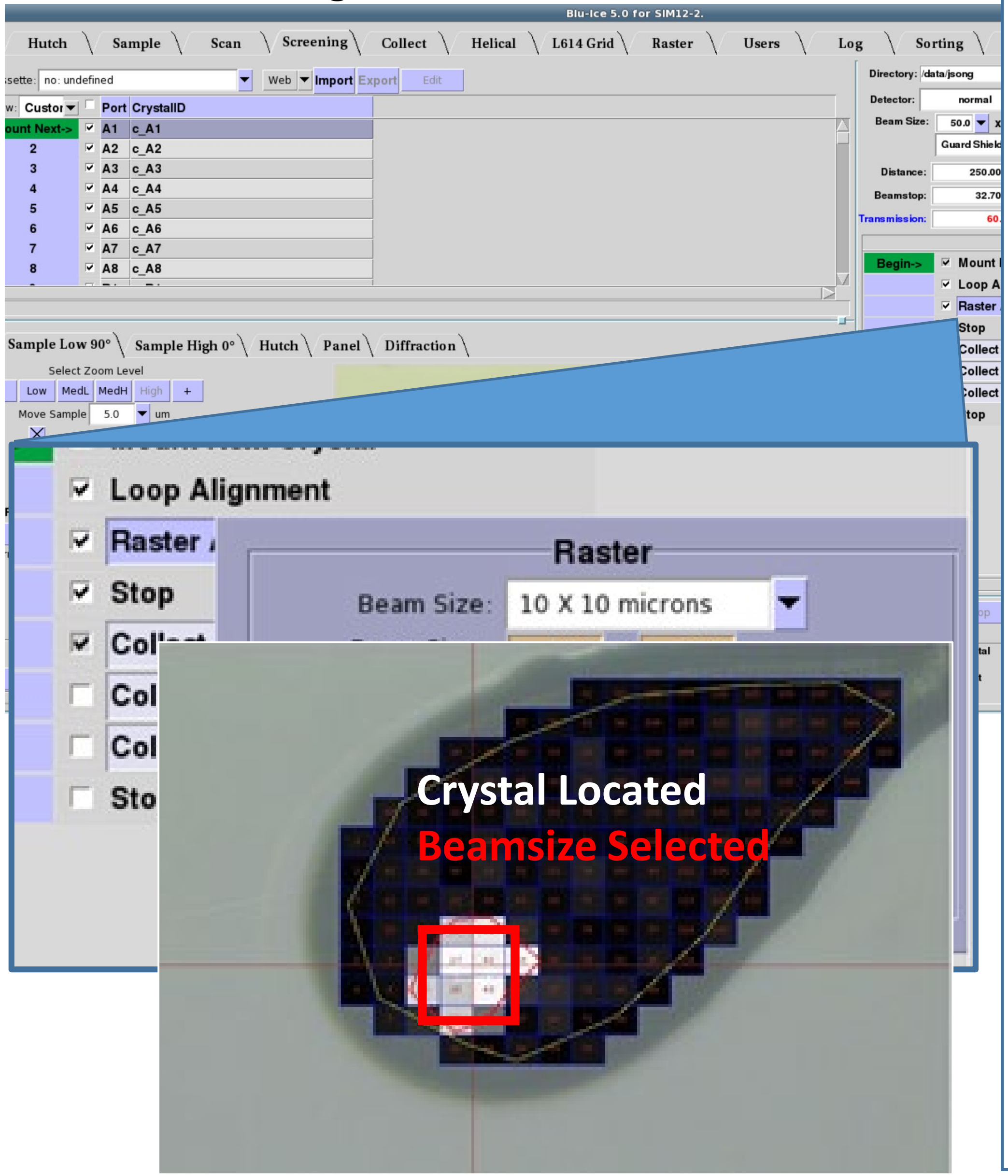
1. Robot mounts sample pin
2. Sample loop or mesh centering (video)
3. Low dose X-ray grid search to center the best crystal into the beam and to set the best beamsize.
4. Video snapshot of crystal
5. Shutterless X-ray diffraction data collection (or screening)
   – strategy coming soon

1. All data collected is automatically processed! (XDS, results in /data directory with diffraction images)

Repeat for next crystal…

Improved Remote Access Facilities

Blu-ice Screening Tab



Fully Autonomous Data Collection and Processing

User:

1. Ship Samples to SSRL in Uni-pucks or Cassettes
2. Use Blu-ice Screening Tab to choose parameters for automation

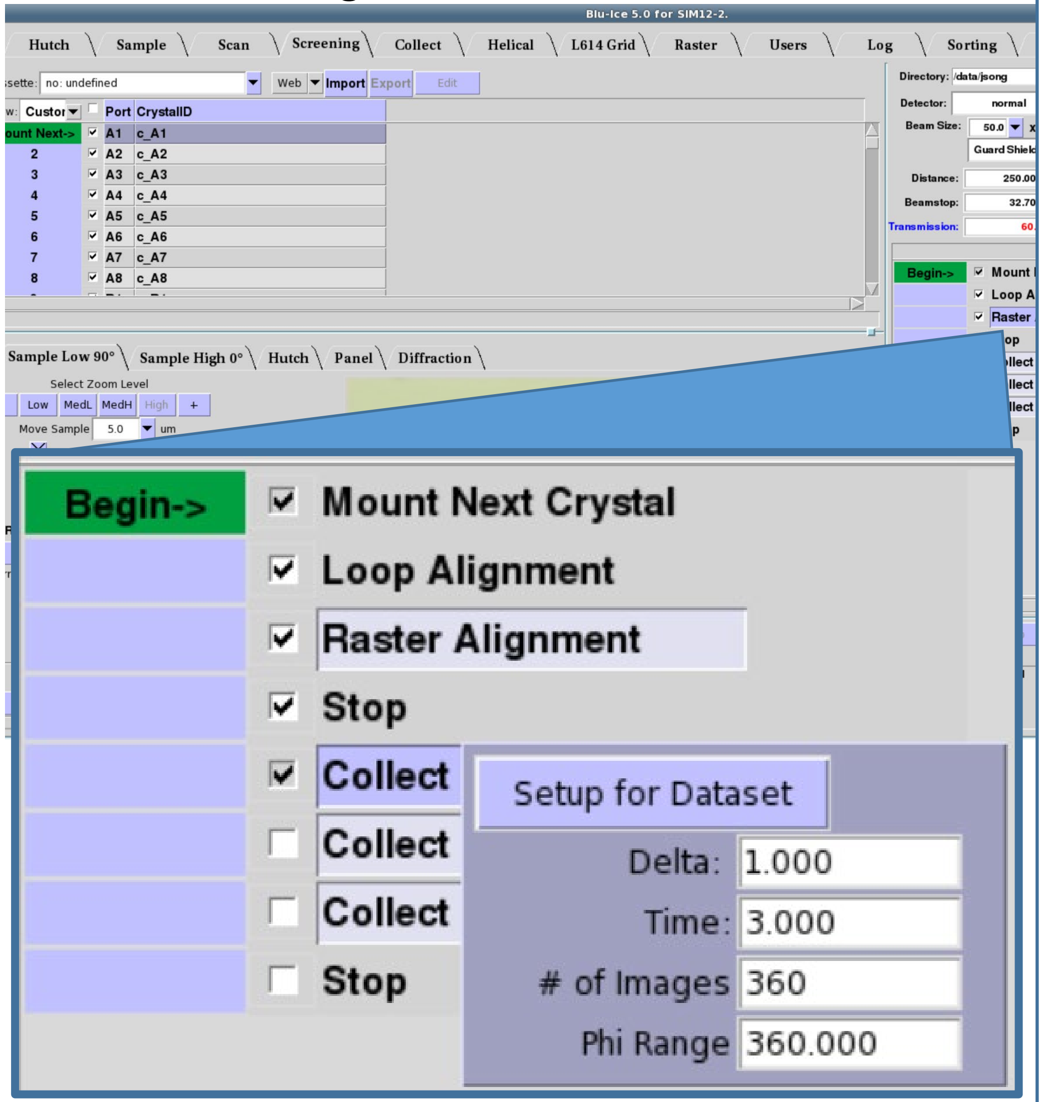
Automated Steps:

1. Robot mounts sample pin
2. Sample loop or mesh centering (video)
3. Low dose X-ray grid search to center the best crystal into the beam and to set the best beamsize.
4. Video snapshot of crystal
5. Shutterless X-ray diffraction data collection (or screening)
   – strategy coming soon
1. All data collected is automatically processed! (XDS, results in /data directory with diffraction images)

Repeat for next crystal…

Improved Remote Access Facilities

Blu-ice Screening Tab



☑ Mount Next Crystal
☑ Loop Alignment
☑ Raster Alignment
☑ Stop
☑ Collect
☐ Collect
☐ Collect
☐ Stop

Setup for Dataset

Delta: 1.000
Time: 3.000
# of Images 360
Phi Range 360.000

/data/username/auto-processing_imagename

Fully Autonomous Data Collection and Processing

User:

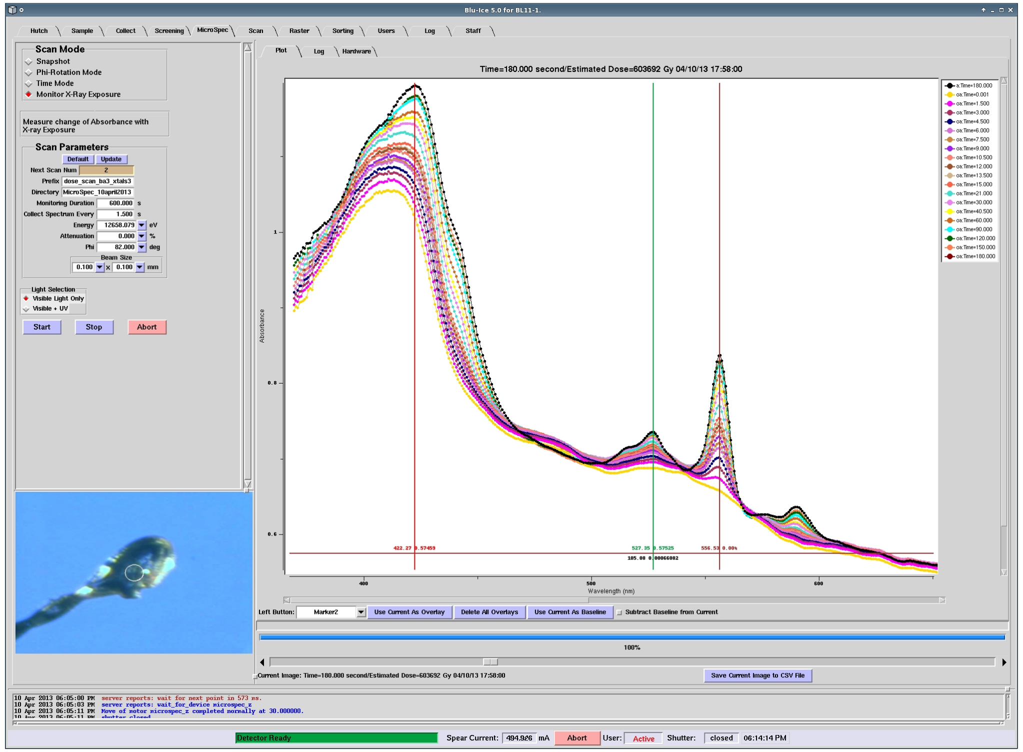
1. Ship Samples to SSRL in Uni-pucks or Cassettes
2. Use Blu-ice Screening Tab to choose parameters for automation

Automated Steps:

1. Robot mounts sample pin
2. Sample loop or mesh centering (video)
3. Low dose X-ray grid search to center the best crystal into the beam and to set the best beamsize.
4. Video snapshot of crystal
5. Shutterless X-ray diffraction data collection (or screening)
   – strategy coming soon
1. All data collected is automatically processed! (XDS, results in /data directory with diffraction images)

Repeat for next crystal…

Fully Remote Access
UV-Visible Microspectrometry at BL9-2



Scan Mode
◇ Snapshot
◇ Phi-Rotation Mode
◇ Time Mode
◆ Monitor X-Ray Exposure

Measure change of Absorbance with X-ray Exposure

Scan Parameters
Next Scan Num: 2
Prefix: dose_scan_ba3_xtals3
Directory: MicroSpec_10april2013
Monitoring Duration: 600.000 s
Collect Spectrum Every: 1.500 s
Energy: 12658.079 eV
Attenuation: 0.000 %
Phi: 82.000 deg
Beam Size: 0.100 x 0.100 mm

Light Selection
◆ Visible Light Only
◇ Visible + UV

Time=180.000 second/Estimated Dose=603692 Gy 04/10/13 17:58:00

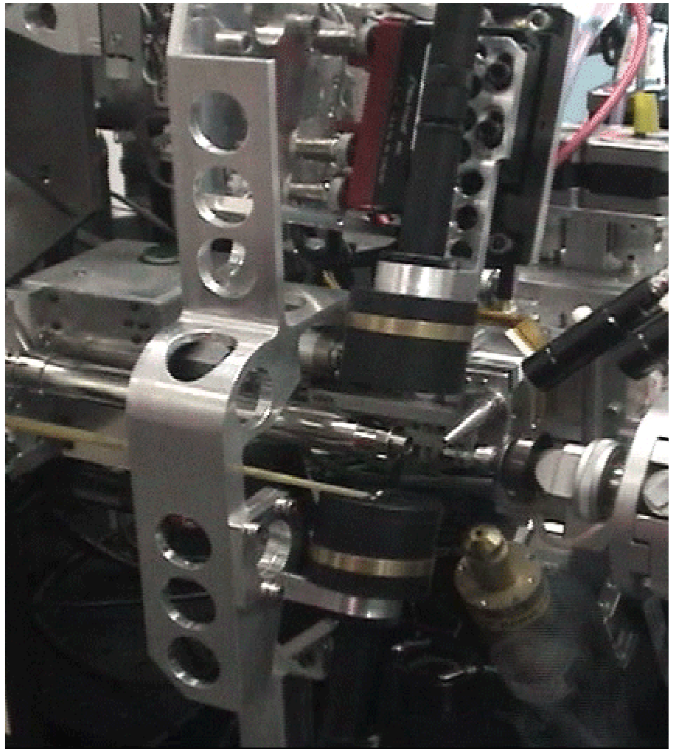
Left Button: Marker2
☐ Subtract Baseline from Current

Current Image: Time=180.000 second/Estimated Dose=603692 Gy 04/10/13 17:58:00

```
10 Apr 2013 06:05:00 PM  server reports: wait for next point in 573 ms.
10 Apr 2013 06:05:03 PM  server reports: wait_for_device microspec_z
10 Apr 2013 06:05:11 PM  Move of motor microspec_z completed normally at 30.000000.
```

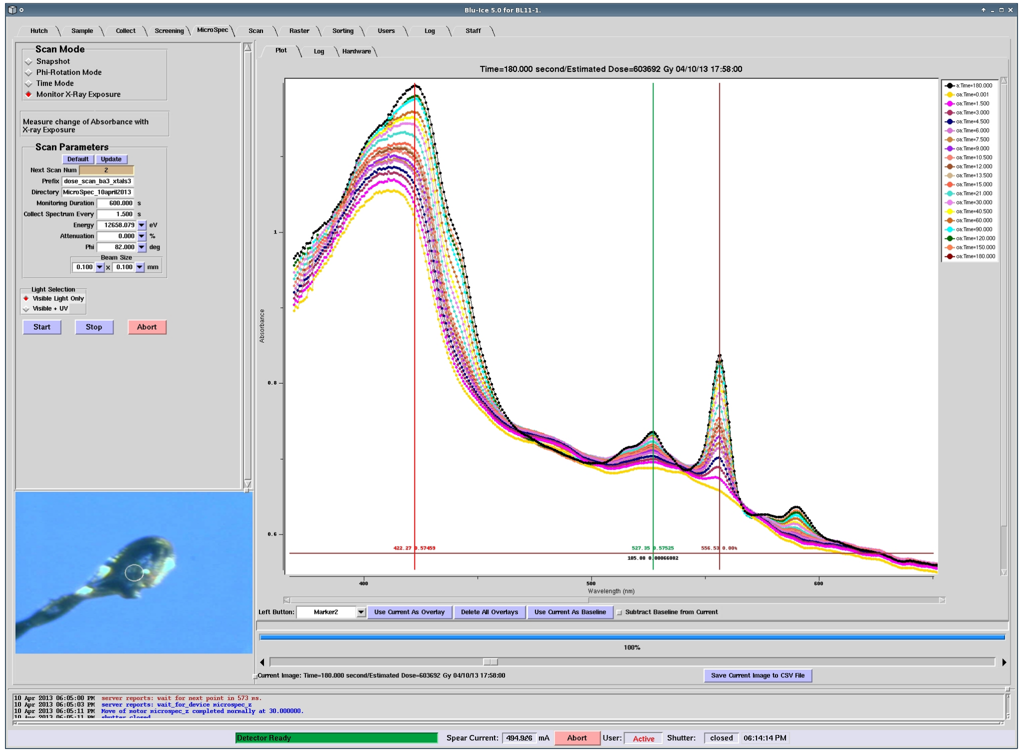
Detector Ready | Spear Current: 494.926 mA | User: Active | Shutter: closed | 06:14:14 PM

Monitor the spectra as a function of X-ray dose (Gy)



Illumination and Collection Objectives

Fully Remote Access
UV-Visible Microspectrometry at BL9-2



Scan Mode

- ○ Snapshot
- ○ Phi-Rotation Mode
- ○ Time Mode
- ● Monitor X-Ray Exposure

Measure change of Absorbance with X-ray Exposure

Scan Parameters

Next Scan Num: 2
Prefix: dose_scan_ba3_xtals3
Directory: MicroSpec_10april2013
Monitoring Duration: 600.000 s
Collect Spectrum Every: 1.500 s
Energy: 12658.079 eV
Attenuation: 0.000 %
Phi: 82.000 deg
Beam Size: 0.100 x 0.100 mm

Light Selection
- ● Visible Light Only
- ○ Visible + UV

Time=180.000 second/Estimated Dose=603692 Gy 04/10/13 17:58:00

Left Button: Marker2
☐ Subtract Baseline from Current

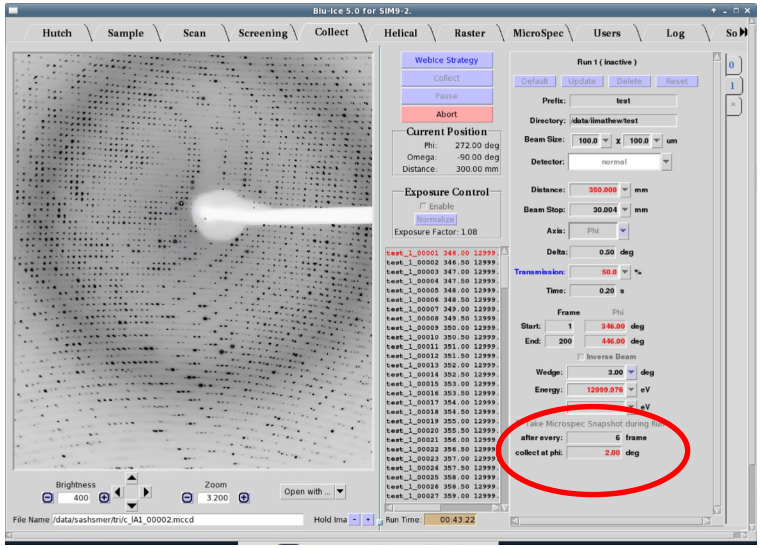
100%

Current Image: Time=180.000 second/Estimated Dose=603692 Gy 04/10/13 17:58:00

```
10 Apr 2013 06:05:00 PM  server reports: wait for next point in 573 ms.
10 Apr 2013 06:05:03 PM  server reports: wait_for_device microspec_z
10 Apr 2013 06:05:11 PM  Move of motor microspec_z completed normally at 30.000000.
```

Detector Ready | Spear Current: 494.926 mA | Abort | User: Active | Shutter: closed | 06:14:14 PM

Monitor the spectra as a function of X-ray dose (Gy)



Run 1 (inactive)

Current Position
Phi: 272.00 deg
Omega: -90.00 deg
Distance: 300.00 mm

Exposure Control
☐ Enable
Exposure Factor: 1.08

```
test_1_00001 346.00 12999.
test_1_00002 346.50 12999.
test_1_00003 347.00 12999.
test_1_00004 347.50 12999.
test_1_00005 348.00 12999.
test_1_00006 348.50 12999.
test_1_00007 349.00 12999.
test_1_00008 349.50 12999.
test_1_00009 350.00 12999.
test_1_00010 350.50 12999.
test_1_00011 351.00 12999.
test_1_00012 351.50 12999.
test_1_00013 352.00 12999.
test_1_00014 352.50 12999.
test_1_00015 353.00 12999.
test_1_00016 353.50 12999.
test_1_00017 354.00 12999.
test_1_00018 354.50 12999.
test_1_00019 355.00 12999.
test_1_00020 355.50 12999.
test_1_00021 356.00 12999.
test_1_00022 356.50 12999
test_1_00023 357.00 12999
test_1_00024 357.50 12999.
test_1_00025 358.00 12999.
test_1_00026 358.50 12999.
test_1_00027 359.00 12999.
```

Run Time: 00:43:22

Prefix: test
Directory: /data/iimathew/test
Beam Size: 100.0 x 100.0 um
Detector: normal
Distance: 350.000 mm
Beam Stop: 30.004 mm
Axis: Phi
Delta: 0.50 deg
Transmission: 50.0 %
Time: 0.20 s

	Frame	Phi	
Start:	1	346.00	deg
End:	200	446.00	deg

☐ Inverse Beam
Wedge: 3.00 deg
Energy: 12999.976 eV

☐ Take Microspec Snapshot during Run
after every: 6 frame
collect at phi: 2.00 deg

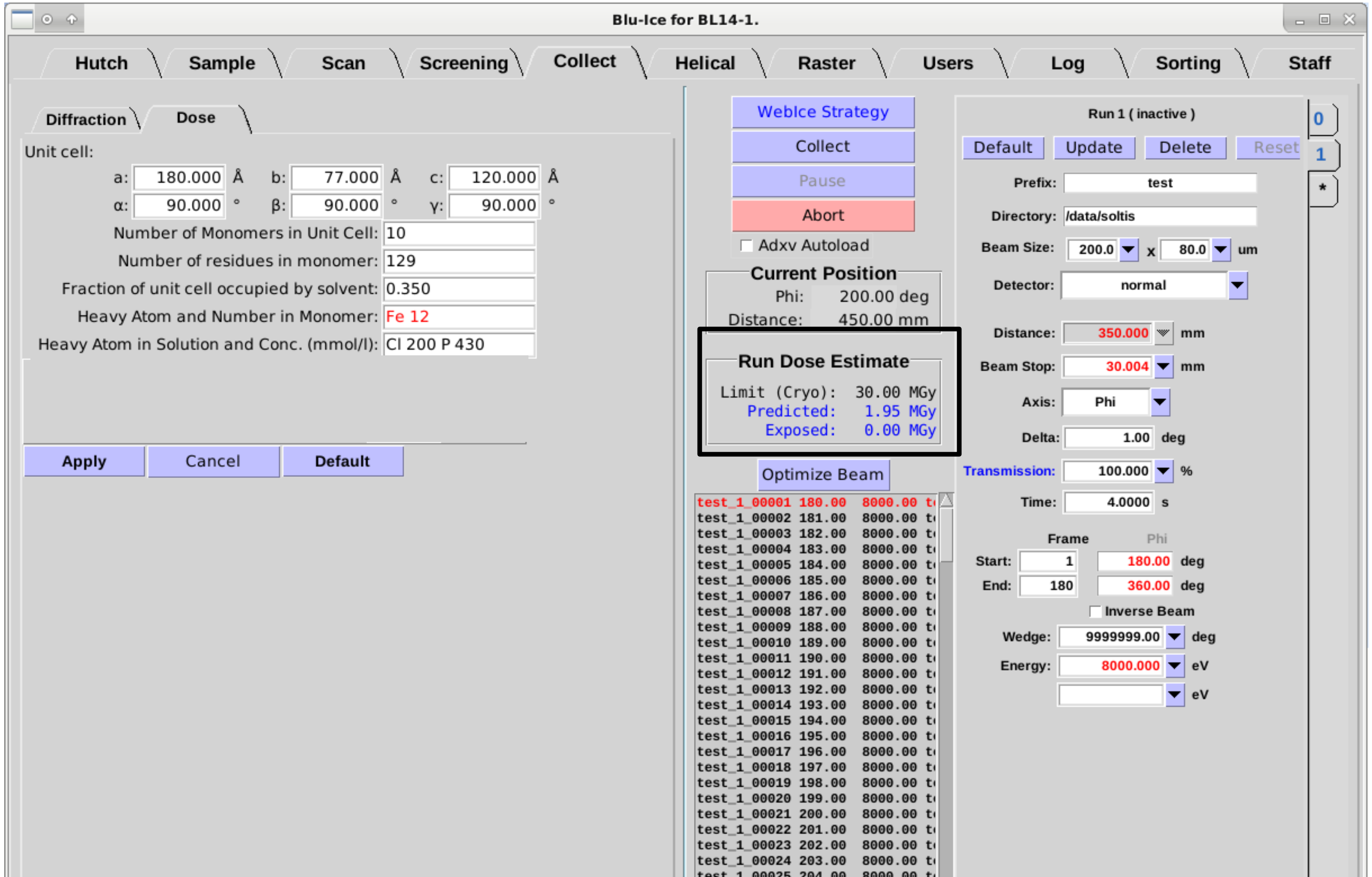
Brightness: 400
Zoom: 3.200
File Name /data/sashsmer/tri/c_lA1_00002.mccd

Interleave microspec-snapshots with diffraction data collection

Real-time X-ray Dose Calculator Aids Experimental Strategy

- Feedback on the predicted total x-ray exposure based on the experimental Run definition
- Raddose3D is used to calculate the Average Weighted Dose

O.B. Zeldin, S. Brockhauser, J. Bremridge, J.M. Holton, and E.F. Garman, "Predicting the X-ray lifetime of protein crystals" *PNAS* **110**, 51 (2013)



Unit cell:
a: 180.000 Å b: 77.000 Å c: 120.000 Å
α: 90.000 ° β: 90.000 ° γ: 90.000 °
Number of Monomers in Unit Cell: 10
Number of residues in monomer: 129
Fraction of unit cell occupied by solvent: 0.350
Heavy Atom and Number in Monomer: Fe 12
Heavy Atom in Solution and Conc. (mmol/l): Cl 200 P 430

☐ Adxv Autoload

Current Position
Phi: 200.00 deg
Distance: 450.00 mm

Run Dose Estimate

```
Limit (Cryo):  30.00 MGy
   Predicted:   1.95 MGy
     Exposed:   0.00 MGy
```

```
test_1_00001 180.00  8000.00 t
test_1_00002 181.00  8000.00 t
test_1_00003 182.00  8000.00 t
test_1_00004 183.00  8000.00 t
test_1_00005 184.00  8000.00 t
test_1_00006 185.00  8000.00 t
test_1_00007 186.00  8000.00 t
test_1_00008 187.00  8000.00 t
test_1_00009 188.00  8000.00 t
test_1_00010 189.00  8000.00 t
test_1_00011 190.00  8000.00 t
test_1_00012 191.00  8000.00 t
test_1_00013 192.00  8000.00 t
test_1_00014 193.00  8000.00 t
test_1_00015 194.00  8000.00 t
test_1_00016 195.00  8000.00 t
test_1_00017 196.00  8000.00 t
test_1_00018 197.00  8000.00 t
test_1_00019 198.00  8000.00 t
test_1_00020 199.00  8000.00 t
test_1_00021 200.00  8000.00 t
test_1_00022 201.00  8000.00 t
test_1_00023 202.00  8000.00 t
test_1_00024 203.00  8000.00 t
test_1_00025 204.00  8000.00 t
```

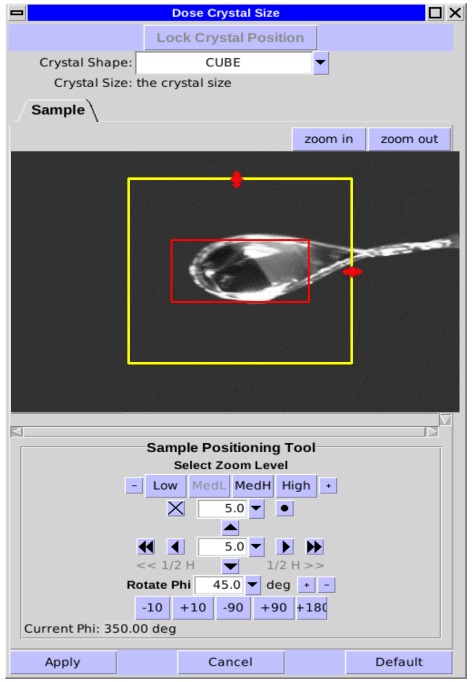
Run 1 (inactive)
Prefix: test
Directory: /data/soltis
Beam Size: 200.0 x 80.0 um
Detector: normal
Distance: 350.000 mm
Beam Stop: 30.004 mm
Axis: Phi
Delta: 1.00 deg
Transmission: 100.000 %
Time: 4.0000 s

	Frame	Phi	
Start:	1	180.00	deg
End:	180	360.00	deg

☐ Inverse Beam
Wedge: 9999999.00 deg
Energy: 8000.000 eV
eV

Crystal Size Widget

- Live video feed of crystal
- Controls to align crystal
- Crystal shape selection for the graphic overlay
- Crystal size adjusted with Push/Pull handles on the overlay

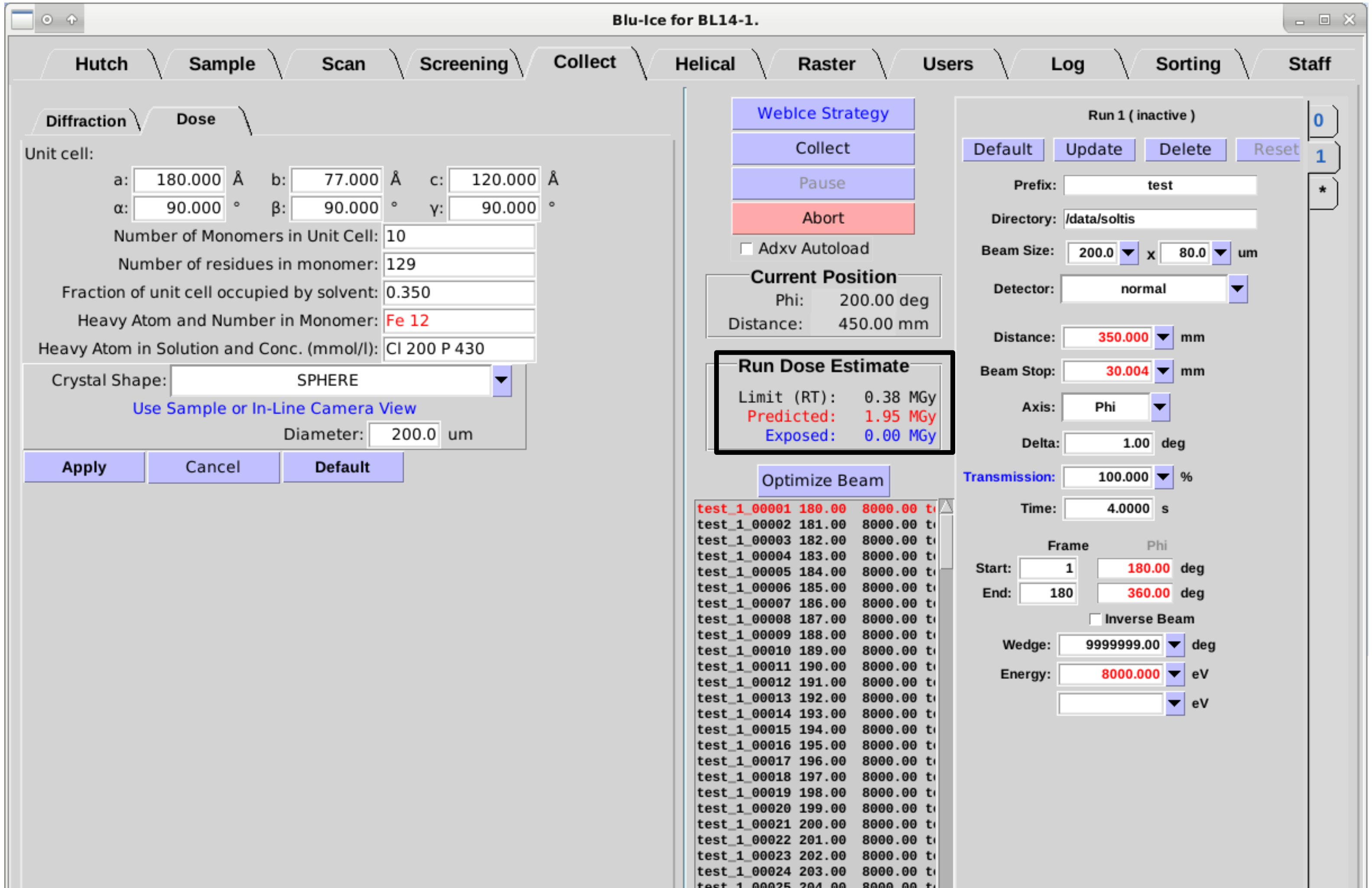


Crystal Shape: CUBE
Crystal Size: the crystal size
5.0
5.0
Rotate Phi 45.0 deg
Current Phi: 350.00 deg

Real-time X-ray Dose Calculator Aids Experimental Strategy

- Feedback on the predicted total x-ray exposure based on the experimental Run definition
- Raddose3D is used to calculate the Average Weighted Dose

O.B. Zeldin, S. Brockhauser, J. Bremridge, J.M. Holton, and E.F. Garman, "Predicting the X-ray lifetime of protein crystals" *PNAS* **110**, 51 (2013)



Unit cell:

a: 180.000 Å b: 77.000 Å c: 120.000 Å

α: 90.000 ° β: 90.000 ° γ: 90.000 °

Number of Monomers in Unit Cell: 10

Number of residues in monomer: 129

Fraction of unit cell occupied by solvent: 0.350

Heavy Atom and Number in Monomer: Fe 12

Heavy Atom in Solution and Conc. (mmol/l): Cl 200 P 430

Crystal Shape: SPHERE

Use Sample or In-Line Camera View

Diameter: 200.0 um

☐ Adxv Autoload

Current Position

Phi: 200.00 deg

Distance: 450.00 mm

Run Dose Estimate

```
Limit (RT):   0.38 MGy
Predicted:    1.95 MGy
Exposed:      0.00 MGy
```

```
test_1_00001 180.00  8000.00 t
test_1_00002 181.00  8000.00 t
test_1_00003 182.00  8000.00 t
test_1_00004 183.00  8000.00 t
test_1_00005 184.00  8000.00 t
test_1_00006 185.00  8000.00 t
test_1_00007 186.00  8000.00 t
test_1_00008 187.00  8000.00 t
test_1_00009 188.00  8000.00 t
test_1_00010 189.00  8000.00 t
test_1_00011 190.00  8000.00 t
test_1_00012 191.00  8000.00 t
test_1_00013 192.00  8000.00 t
test_1_00014 193.00  8000.00 t
test_1_00015 194.00  8000.00 t
test_1_00016 195.00  8000.00 t
test_1_00017 196.00  8000.00 t
test_1_00018 197.00  8000.00 t
test_1_00019 198.00  8000.00 t
test_1_00020 199.00  8000.00 t
test_1_00021 200.00  8000.00 t
test_1_00022 201.00  8000.00 t
test_1_00023 202.00  8000.00 t
test_1_00024 203.00  8000.00 t
test_1_00025 204.00  8000.00 t
```

Run 1 (inactive)

Prefix: test

Directory: /data/soltis

Beam Size: 200.0 x 80.0 um

Detector: normal

Distance: 350.000 mm

Beam Stop: 30.004 mm

Axis: Phi

Delta: 1.00 deg

Transmission: 100.000 %

Time: 4.0000 s

	Frame	Phi	
Start:	1	180.00	deg
End:	180	360.00	deg

☐ Inverse Beam

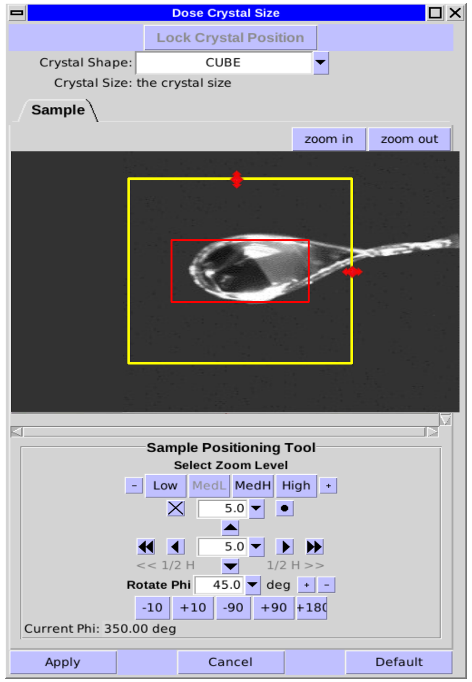
Wedge: 9999999.00 deg

Energy: 8000.000 eV

eV

Crystal Size Widget

- Live video feed of crystal
- Controls to align crystal
- Crystal shape selection for the graphic overlay
- Crystal size adjusted with Push/Pull handles on the overlay



Crystal Shape: CUBE

Crystal Size: the crystal size

5.0

5.0

Rotate Phi 45.0 deg

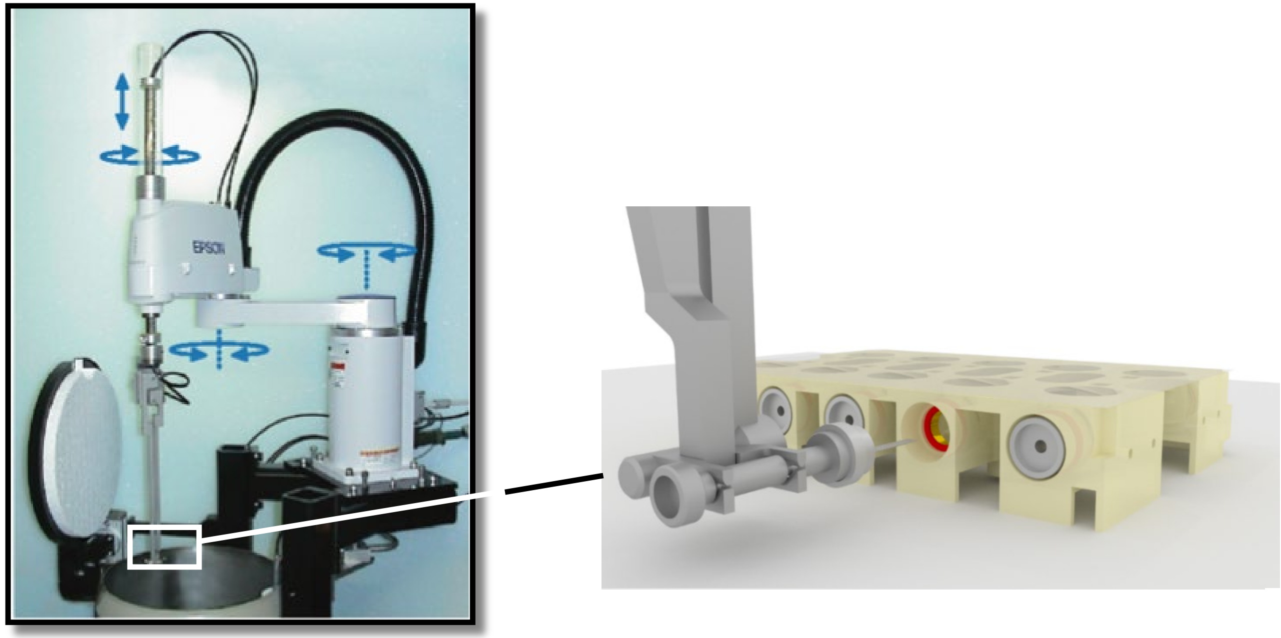
Current Phi: 350.00 deg

Microfocus Beam Line 12-1

Remote Access at Controlled Humidity and Physiological Temperatures

- Robotic Sample Mounting of Samples at Controlled Humidity
- First offered used at LCLS-MFX
- Based on a new in-situ crystallization plate
- 18 different user groups across the US so far

Samples inside at controlled humidity



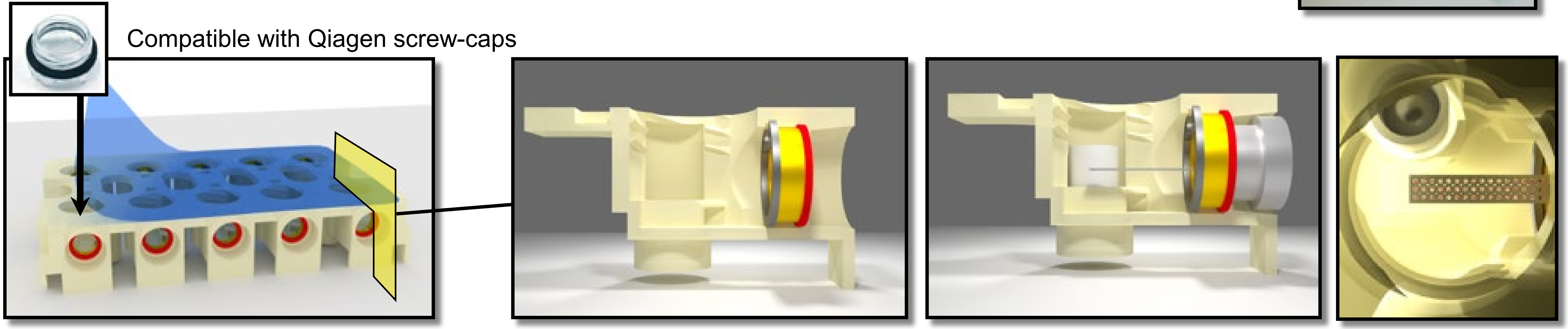
https://www-ssrl.slac.stanford.edu/smb-mc/content/users/manuals/remote-access-at-elevated-temperatures-and-controlled-humidity

- System will be duplicated on other SSRL MC beamlines

Microfocus Beam Line 12-1

Remote Access at Controlled Humidity and Physiological Temperatures

- Robotic Sample Mounting of Samples at Controlled Humidity
- First offered used at LCLS-MFX
- Based on a new in-situ crystallization plate
- 18 different user groups across the US so far - first August 2020



Compatible with Qiagen screw-caps

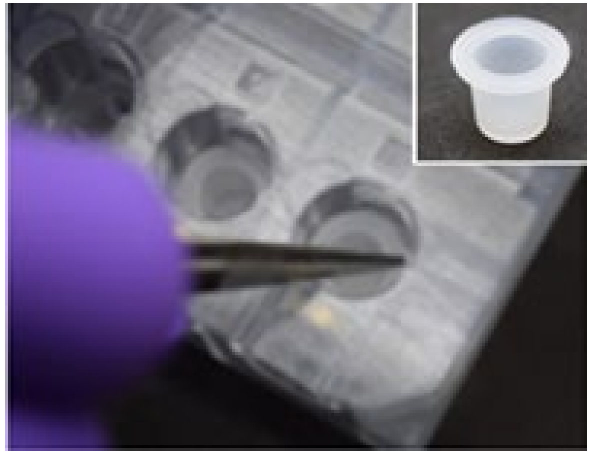
Ring magnet holds sample base inside

- System will be duplicated on other SSRL MC beamlines

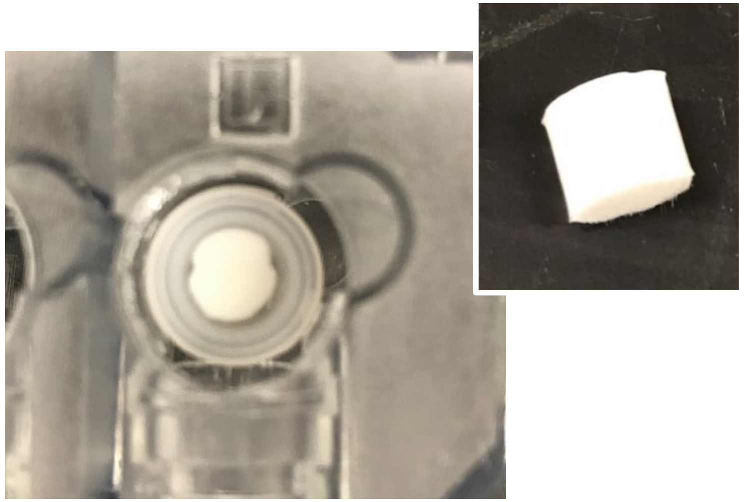
Microfocus Beam Line 12-1

Remote Access at Controlled Humidity and Physiological Temperatures

- Robotic Sample Mounting of Samples at Controlled Humidity
- First offered used at LCLS-MFX
- Based on a new in-situ crystallization plate
- 18 different user groups across the US so far



Disposable silicone liner cups hold crystallization solutions and make cleaning and reuse of plates easy.



Soft foam insert prevents splashing or spilling of the well solutions during transport.

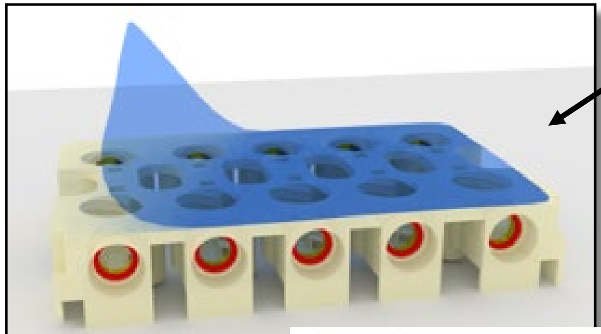
SSRL Crystallization Plate Liner Cups

SSRL Crystallization Plate Liner Cups are disposable silicone cups used to hold cry
package contains 100 cups.

Commercially Available:
www.crystalpositioningsystems.com

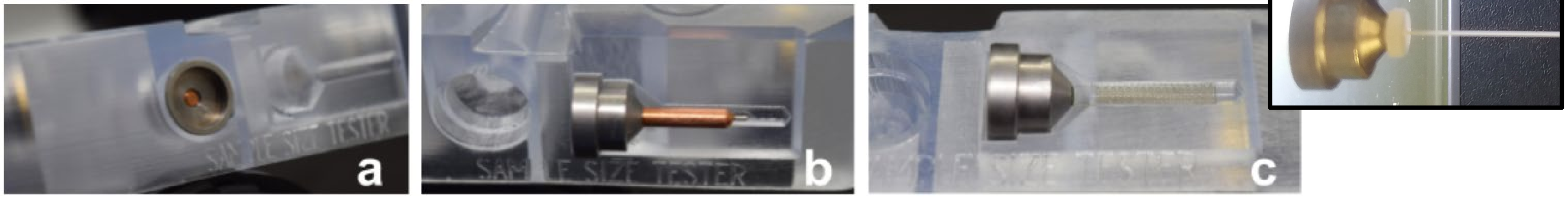
Remote Access at Controlled Humidity and Physiological Temperatures

- Robotic Sample Mounting of Samples at Controlled Humidity
- First offered used at LCLS-MFX
- Based on a new in-situ crystallization plate
- 18 different user groups across the US so far



Tester for sample pins compatibility

- Uses a magnetic sample base
- If sample on base fits inside the tester it is compatible with the SSRL robot



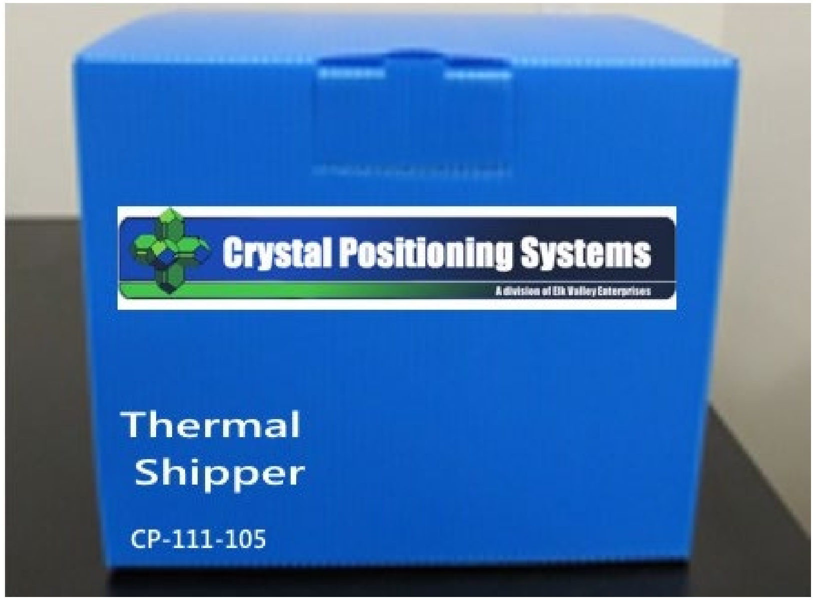
Remote Access at Controlled Humidity and Physiological Temperatures

- Robotic Sample Mounting of Samples at Controlled Humidity
- First offered used at LCLS-MFX
- Based on a new in-situ crystallization plate
- 14 different user groups across the US so far

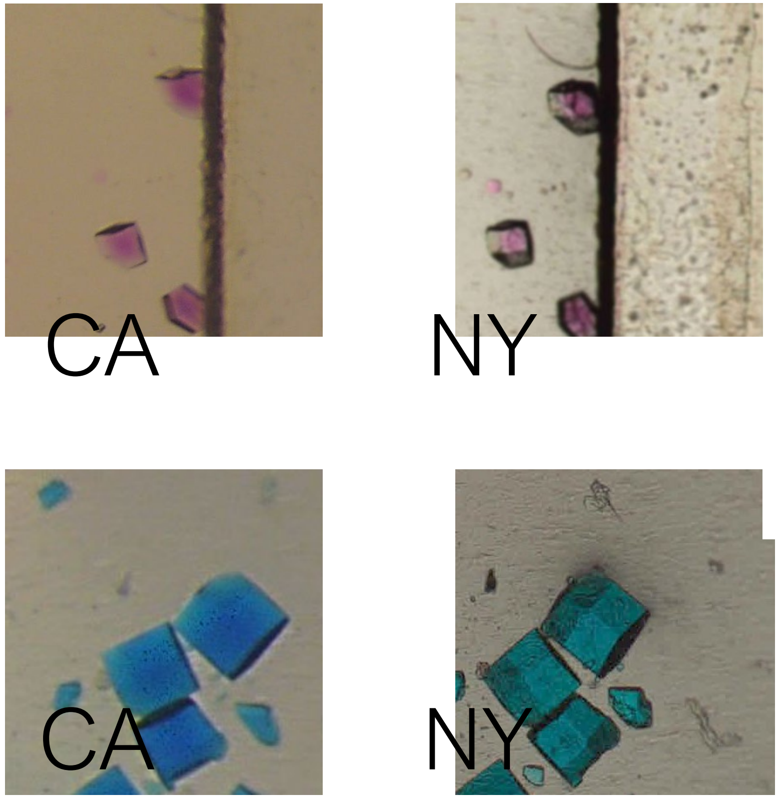
Microfocus Beam Line 12-1

Remote Access at Controlled Humidity and Physiological Temperatures

- Robotic Sample Mounting of Samples at Controlled Humidity
- First offered used at LCLS-MFX
- Based on a new in-situ crystallization plate
- 14 different user groups across the US so far



- **Maintains a 20 ^{o}C (+/- 5) environment for 7 days**
- **Gel packs further stabilize the temperature**
- **Foam inserts securely hold crystallization trays**



Prototype plates and shipper tested between Buffalo, NY and Stanford, CA

Microfocus Beam Line 12-1

Remote Access at Controlled Humidity and Physiological Temperatures

- Robotic Sample Mounting of Samples at Controlled Humidity
- First offered used at LCLS-MFX
- Based on a new in-situ crystallization plate
- 14 different user groups across the US so far

Remote Control

Sample

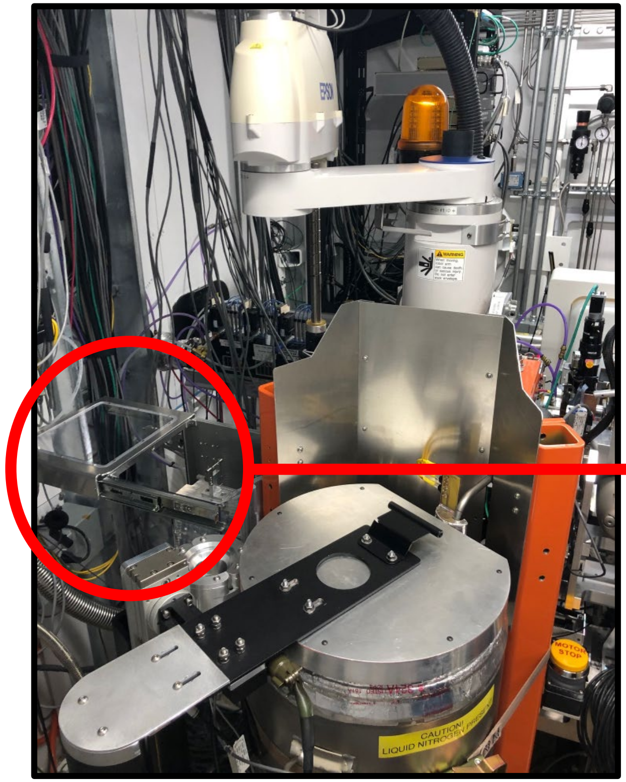
Sample Annealing

Sample_Temperature

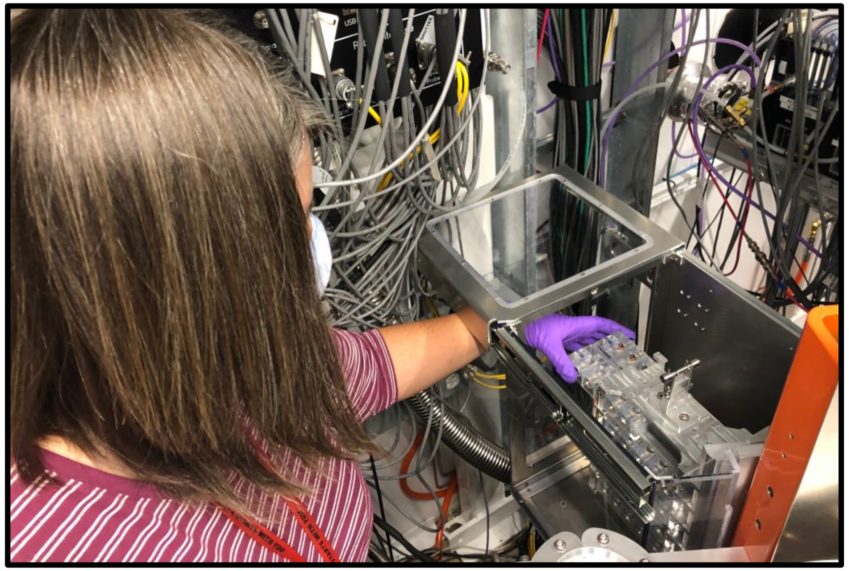
Humidity_Control

99.99 K

Set Point: 270.00 K

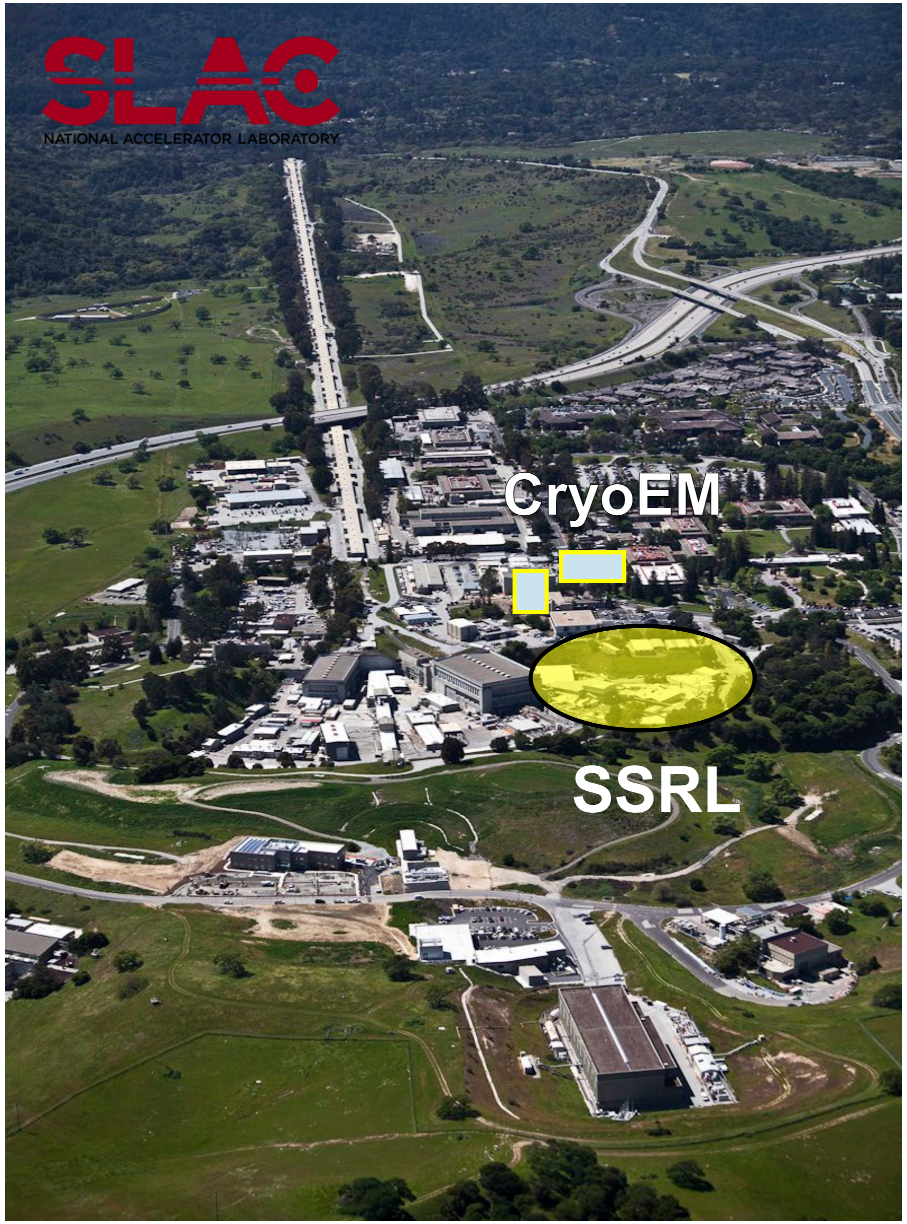


Humidity controlled enclosure holds 5 plates / 50 samples

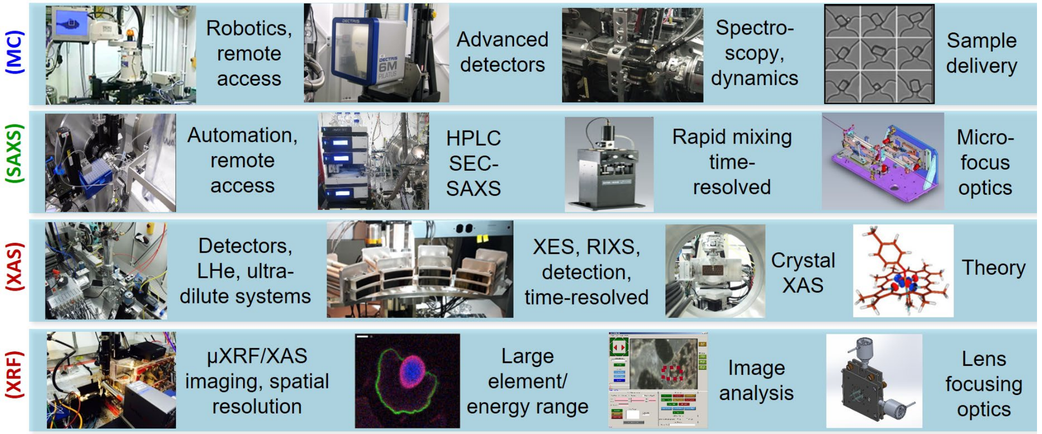


User-support inserts plates prior to beamtime

SSRL Structural Molecular Biology Facilities



Structural Molecular Biology Capabilities

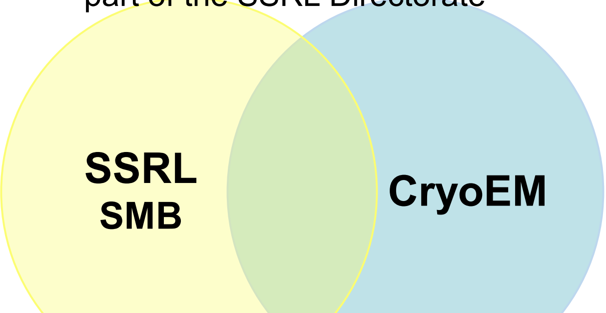


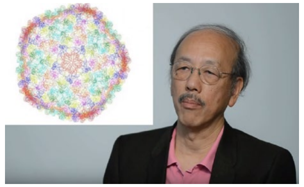
(MC)	Robotics, remote access	Advanced detectors	Spectroscopy, dynamics	Sample delivery
(SAXS)	Automation, remote access	HPLC SEC-SAXS	Rapid mixing time-resolved	Micro-focus optics
(XAS)	Detectors, LHe, ultra-dilute systems	XES, RIXS, detection, time-resolved	Crystal XAS	Theory
(XRF)	µXRF/XAS imaging, spatial resolution	Large element/ energy range	Image analysis	Lens focusing optics

2018: SMB served ~1,100 unique proposal users on 396 proposals, from 38 U.S. states, 18 countries; >220 pubs, ~40 theses; 8 beam line stations

CryoEM Facilities at SLAC

part of the SSRL Directorate



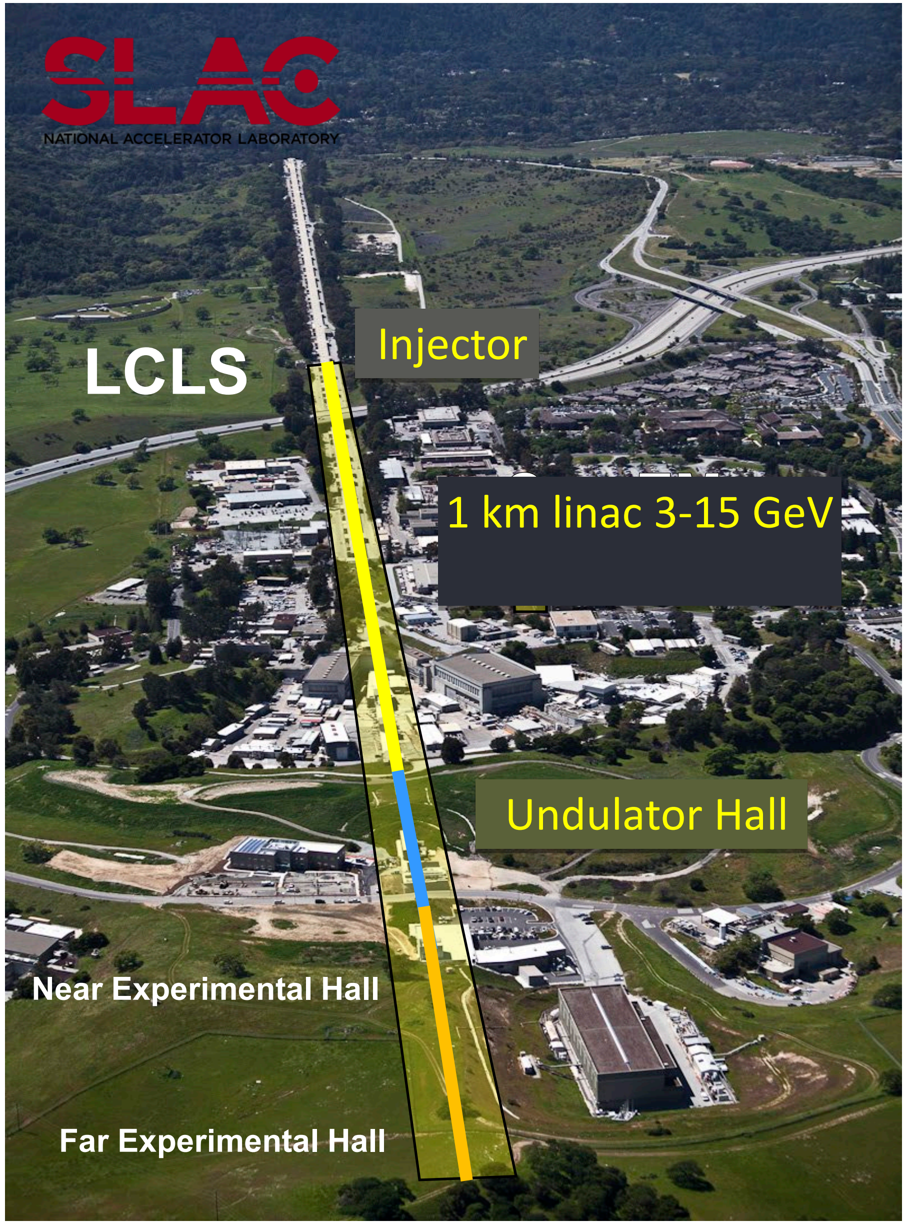


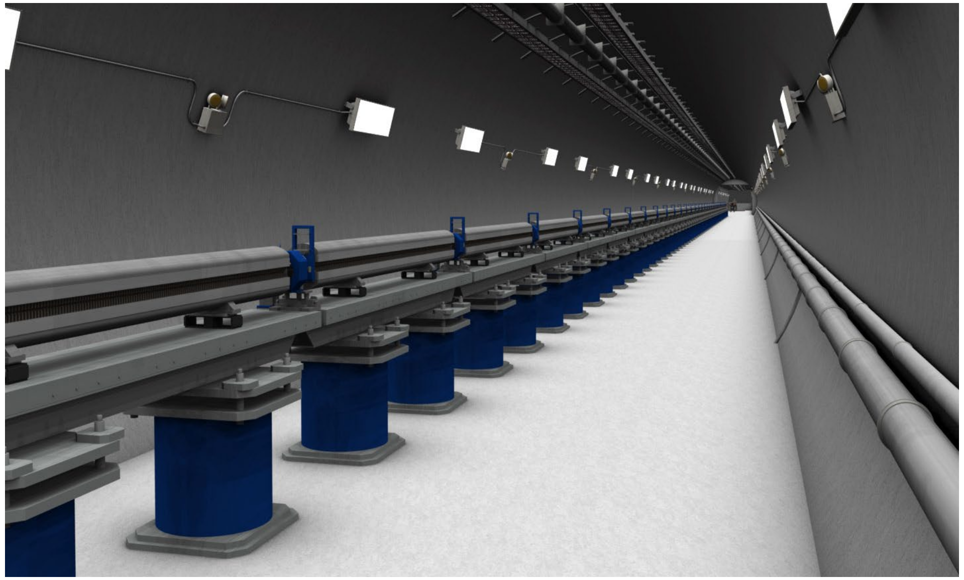
Professor Wah Chiu

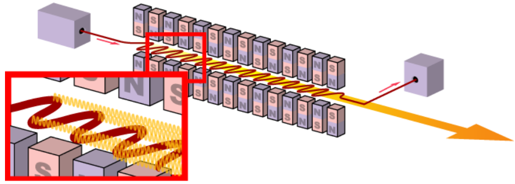
- **NIH National Service Center for Cryo-EM**

Combined User Admin Software and Scheduling
Experimental Methods and Software Developments

LCLS X-ray Free Electron Laser (XFEL)







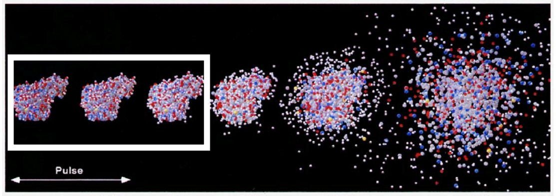
Undulator Hall

electron motion in phase
with field of emitted photons

Hard X-rays at LCLS

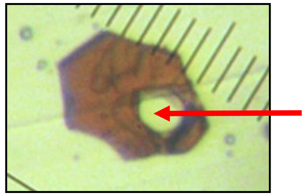
Photons/pulse: 10^{12} (9500 eV)
Pulse duration: 40 fs
Pulses/second: 120

Diffraction before Destruction

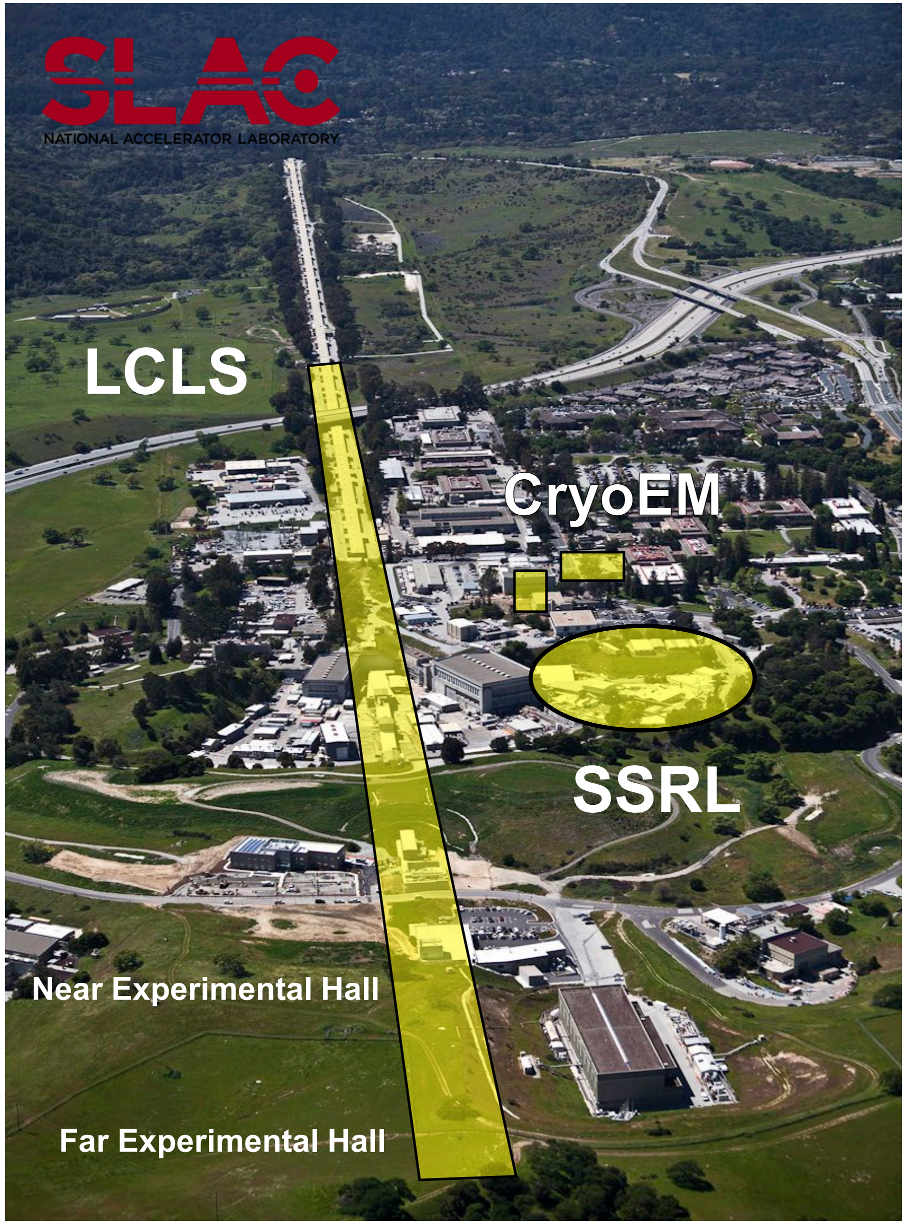


Neutze et al., Nature, vol 406, 752 (2000)

- Very small crystals
- Room temperature
- Metalloenzymes
- Time resolved studies



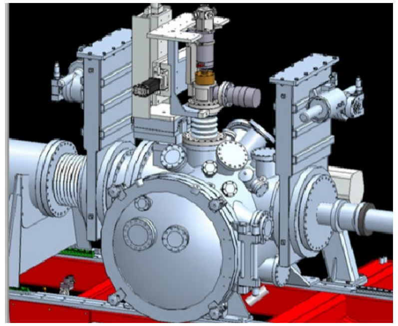
LCLS X-ray Free Electron Laser (XFEL)



Crystallography Experiments at LCLS

🟥 CXI

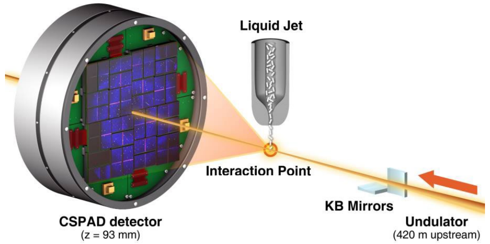
Coherent X-ray Imaging



- In Vacuum environment
- Beam size 100 nm to 4 µm

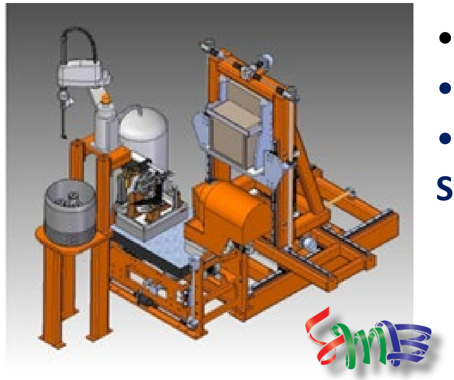
Crystal injector experiments

- Crystal slurries (100 nm to 5 µm)
- Difficult to crystallize
- Time resolved studies



🟥 MFX

Macromolecular Femtosecond Crystallography



Operation, Support & Development
Collaboration of LCLS & SSRL-SMB

- In atmosphere (air or helium)
- Beam size 3 µm (up to 50 µm)
- Flexible sample environment

Standard Goniometer Setup

- Target individual crystals (>5 µm)
- Controlled temperature (RT to cryo)
- Delicate samples or limited supply
- High resolution (large complexes, metalloenzymes, RT - hydration)
- Time-resolved studies

Thank you for your attention

Talk to us about your ideas for new experiments.

Becoming an SSRL User: https://smb.slac.stanford.edu/forms/becominguser/

Lisa Dunn – user scheduling administrator – Lisa@SLAC.Stanford.edu

Aina Cohen – Acohen@SLAC.Stanford.edu

Learn more at a remote access training workshop!

APS-U Structural Biology Townhall Agenda

Start	Duration	End	
1:00 PM	0:05	1:05 PM	Welcome - Laurent Chapon,
1:05 PM	0:25	1:30 PM	APS-U Overview and Life Sciences at the APS, Bob Fischetti
1:30 PM	0:15	1:45 PM	IMCA-CAT, Lisa Keefe
1:45 PM	0:15	2:00 PM	SBC/eBERLight, Andrzej Joachimiak and Karolina Michalska
2:00 PM	0:15	2:15 PM	LS-CAT, Spencer Anderson
2:15 PM	0:15	2:30 PM	SER-CAT, Bi-Chen Wang
2:30 PM	0:15	2:45 PM	GM/CA-CAT, Naga Venugopalan
2:45 PM	0:15	3:00 PM	Break
3:00 PM	0:15	3:15 PM	NE-CAT, Malcolm Capel
3:15 PM	0:15	3:30 PM	LRL-CAT, Jordi Benach
3:30 PM	0:15	3:45 PM	ALS, Marc Allaire
3:45 PM	0:15	4:00 PM	CHESS, Marian Szebenyi
4:00 PM	0:15	4:15 PM	NSLS-II, Sean McSweeney
4:15 PM	0:15	4:30 PM	SSRL, Aina Cohen
4:30 PM	0:30	5:00 PM	Roundtable Q&A
5:00 PM			Adjourn

APS-U Structural Biology Townhall Roundtable Guidelines

Participants may use the RAISE HAND function and use their microphone when called on. You can address your question to a specific speaker or to any of the speakers.

Questions not addressed during the individual Q&A can be raised here or Argonne staff may bring them to the table

I would like to acknowledge
All the speakers
Andre Salles, Beth Schlesinger and Jade Thomas

APS-U Structural Biology Townhall Agenda

Start	Duration	End	
1:00 PM	0:05	1:05 PM	Welcome - Laurent Chapon,
1:05 PM	0:25	1:30 PM	APS-U Overview and Life Sciences at the APS, Bob Fischetti
1:30 PM	0:15	1:45 PM	IMCA-CAT, Lisa Keefe
1:45 PM	0:15	2:00 PM	SBC/eBERLight, Andrzej Joachimiak and Karolina Michalska
2:00 PM	0:15	2:15 PM	LS-CAT, Spencer Anderson
2:15 PM	0:15	2:30 PM	SER-CAT, Bi-Chen Wang
2:30 PM	0:15	2:45 PM	GM/CA-CAT, Naga Venugopalan
2:45 PM	0:15	3:00 PM	Break
3:00 PM	0:15	3:15 PM	NE-CAT, Malcolm Capel
3:15 PM	0:15	3:30 PM	LRL-CAT, Jordi Benach
3:30 PM	0:15	3:45 PM	ALS, Marc Allaire
3:45 PM	0:15	4:00 PM	CHESS, Marian Szebenyi
4:00 PM	0:15	4:15 PM	NSLS-II, Sean McSweeney
4:15 PM	0:15	4:30 PM	SSRL, Aina Cohen
4:30 PM	0:30	5:00 PM	Roundtable Q&A
5:00 PM			Adjourn