

■ National Synchrotron Light Source II

Biosciences at NSLS-II: status and plan

Sean McSweeney

March 2019

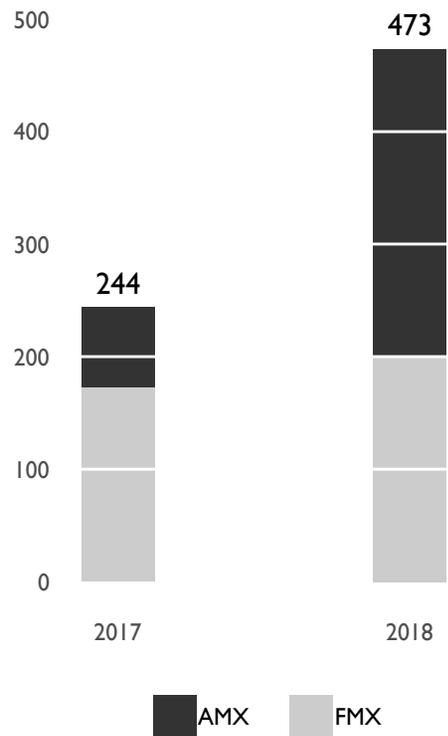


Outline.

- FMX and AMX: development of the macromolecular crystallography program.
- LiX: small angle scattering developments.
- XFP: developments and first science examples.
- NYX: status
- CryoEM : progress and next steps.
- Medium term vision for a coordinated bioscience program

Macromolecular Crystallography User programs.

Cumulative number of User groups on the MX beamlines.

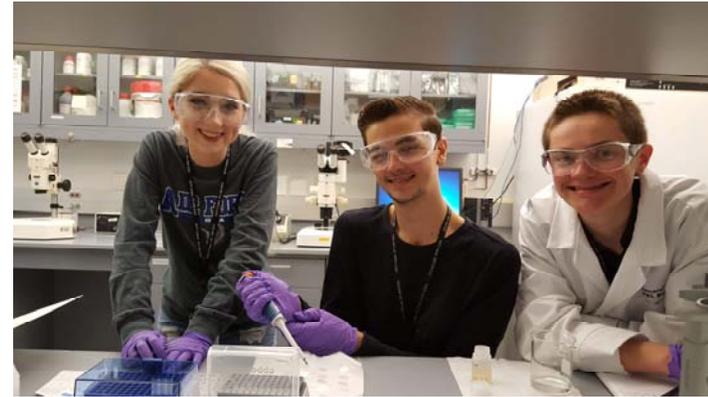
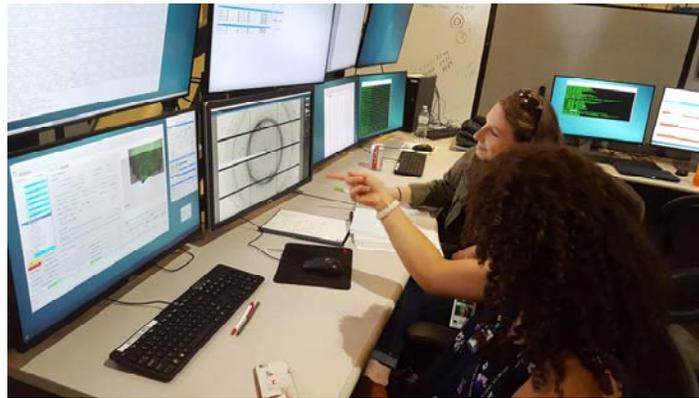
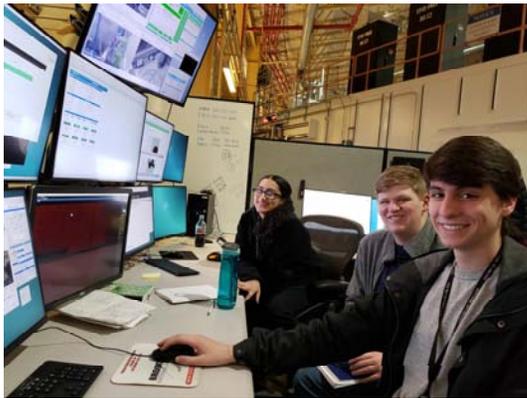
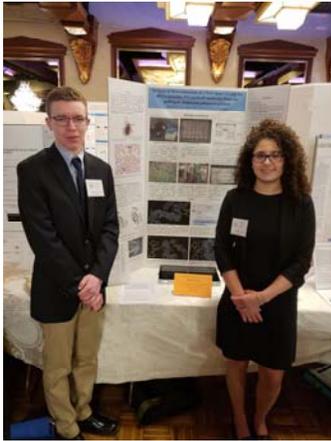


We have 22 Block allocation groups, supporting the work of about 120 PIs.

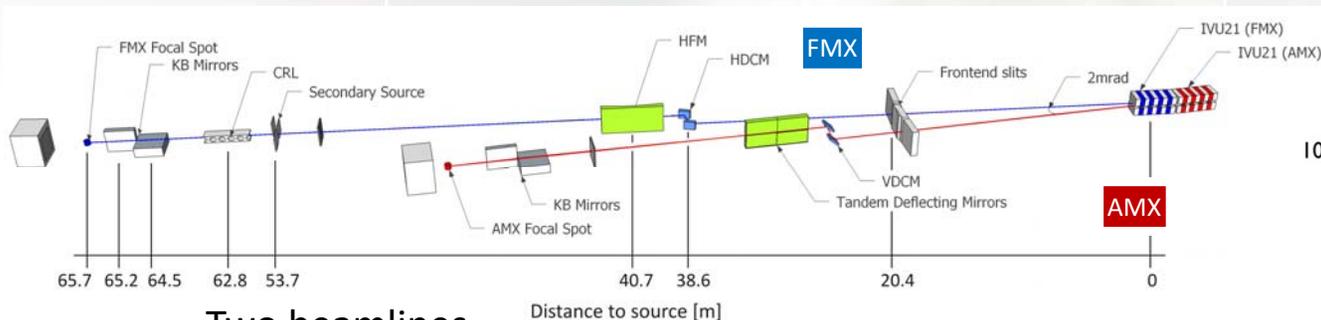
Four more BAG applications were received at the latest allocation deadline ~25 PIs

More indicators of throughput in 2018:
Unique users is 165 on AMX and 139 on FMX,
and have seen 18 publications and 32 PDB deposits.

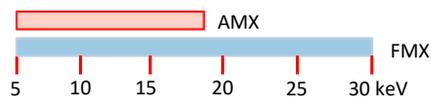
Our most enthusiastic BAG in action. Long Island High School students in action...



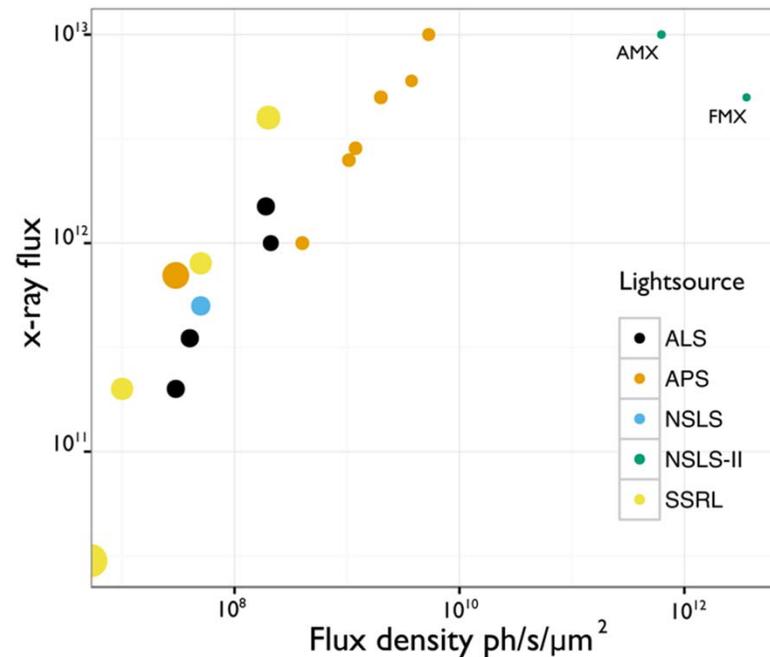
Twin themes – Automation & microfocus FMX & AMX



Two beamlines
with overlapping and complementary capabilities



US structural biology beamlines.

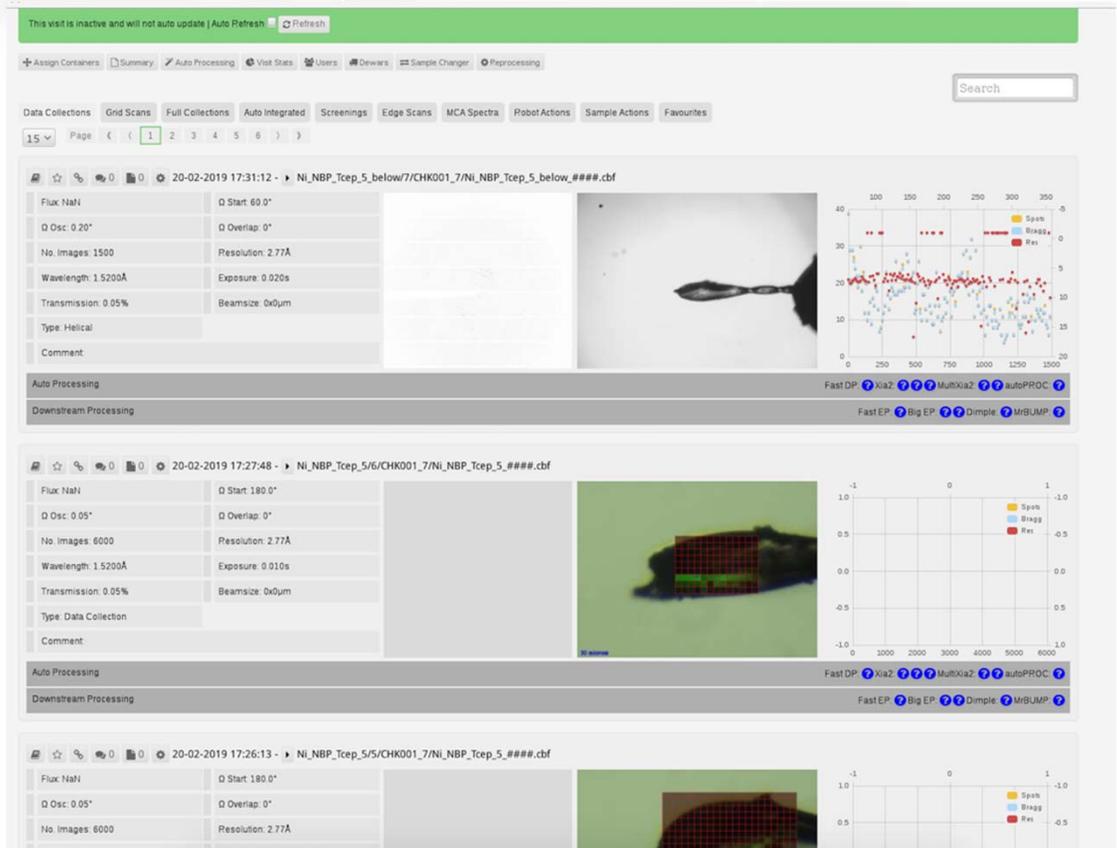


Data from biosync web site October 2018

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We are taking in good ideas from elsewhere, I would like us to be doing more of this.

- To deal with the data management questions we installed the database ISPyB with the interface SynchWeb.
- Collaborated with Diamond Lightsource to roll this out.
- Continuation allows us to build out for SAXS and cryoEM and for serial crystallography.
- Diamond are investigating roll out to other beamlines and techniques. We are staying informed.
- Development coordinated by Structural Biology & DAMA group.

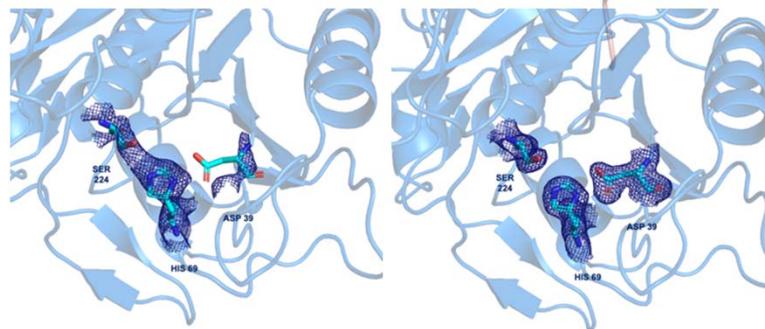
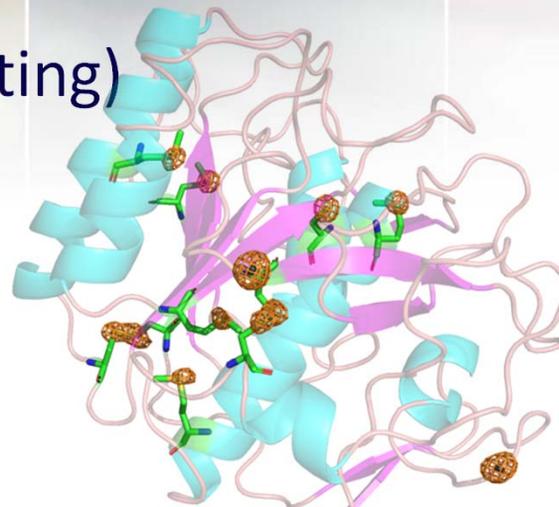


Jet Serial MX at FMX - Native Phasing (also workshop at the 2019 User Meeting)

Wavelength	7.11 keV
Exposure Time	10 ms
Detector	Eiger 16 M
# images collected	920 000
# indexed patterns	310 057
% indexed	33.7 %
Resolution range [Å]	25.2 – 2.0 (2.1 – 2.0)
Unique Reflections	33 100 (3 304)
Average multiplicity	1 837.1 (749.9)
I/sigI	23.1 (3.1)
Rsplit	2.8 (36.0)
CC*	99.98 (95.92)
CCano	0.37 (-0.06)
R _{work} /R _{free}	14.3/17.8

- 10 Sulfurs, 2 di-sulfide bridges
- 2 Ca (Ca-SAD also successful!)
- Data processed with CrystFEL 0.8.0
- CCall/CCweak 27.5/14.2 and CFOM 41.7 (SHELXCDE via CRANK2) Final FOM 0.3
- Auto-building with Buccaneer and Parrot
- Minimum indexed patterns required for successful S-SAD 130 000 (Multiplicity 840)

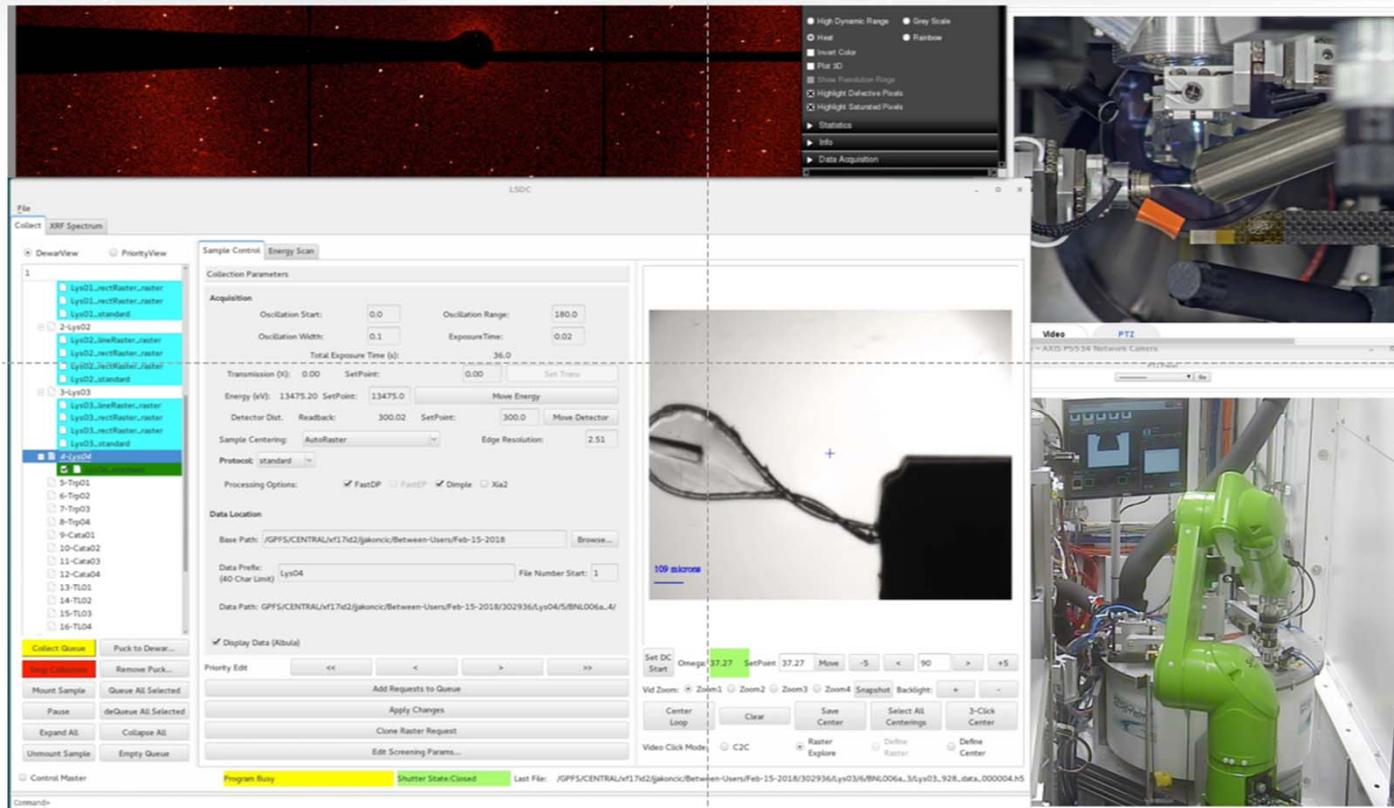
Anomalous difference density map contoured at 5.0 σ calculated from the ~300 000 indexed images and the final, refined phases.



$2F_o - 2F_c$ electron density map after SAD phase retrieval (left) and after refinement (right), contoured at 1.0 σ . In sticks are the Proteinase K catalytic triad.

Sabine Botha, Nadia Zatsepin

Automation of MX data collection with a 5 μm beam

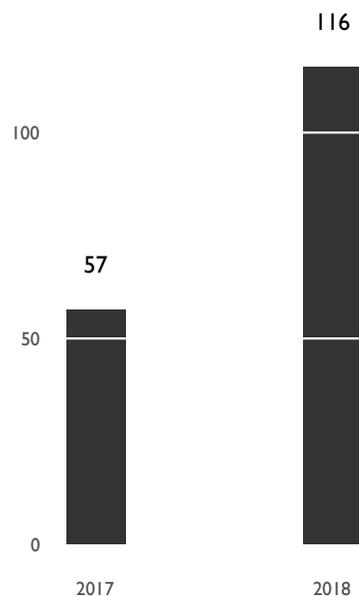




LiX Lin Yang

LiX user program development.

Cumulative number of User groups on the LiX beamline.



LiX is a multifunction Life Sciences Scattering beamline, currently supporting two major modes of operation: microbeam scanning and protein solution scattering.

Roughly 49% of the User time on LiX is used for solution scattering. Three minor modes of operation are used to support this work:

- “Static” Solution Scattering
- On-line HPLC scattering.
- Automated high capacity mode (recently commissioned)

Protein Solution Scattering at LiX: some lessons learned

Its be rough getting here:

- We started with an beamline control system that was intimidating to Users. We have fixed (are fixing) this issue.
- We increased automation and reliability of the instruments means less wasted sample.
- The online HPLC system in use is close to instruments in the lab and folks are comfortable with this tool.
- We integrated data analysis so that a model can be produced quickly after a measurement.

Solution scattering workbench

- Hands-on training with beam time

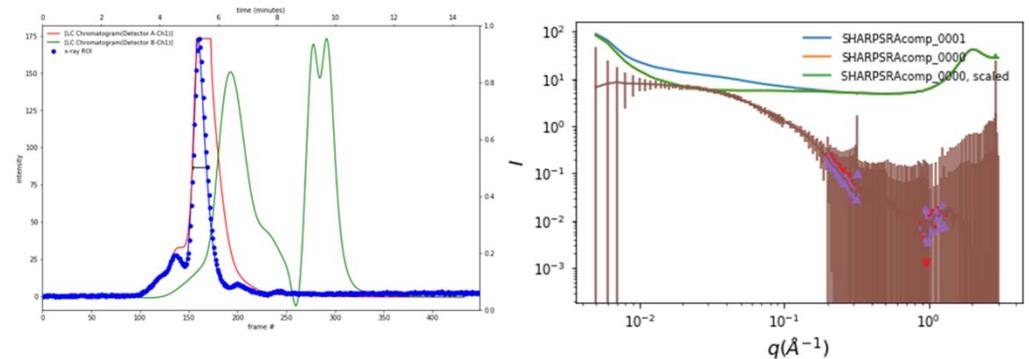
- 2 sessions so far (2018-2 and 3)
- Planning 2019-1
- Different focus every time (e.g. software installation and data processing in 2018-3)
- We aim for 10 participants per session.



2018-2 solution scattering workbench

- Intended to help improve beamline productivity

- Help to attract more users.
- More efficient user training and consultation in groups.
- Supplement other outreach activities



Data collected by Leeper group (Kennesaw State), who attended the workbench and has subsequently submitted a beam time proposal



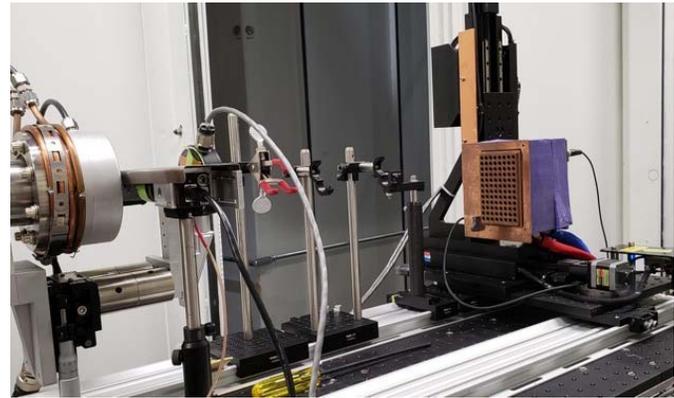
XFP

Erik Farquhar & Mark Chance

Current Status of XFP (17-BM)

CWRU School of Medicine operated Partner BL

- 3 FTEs (1 Ph.D. scientist, 2 support staff) based at NSLS-II.
- NIH/NIBIB P30 funding through August 2019
- partner user agreement with NSLS-II through April 2020



A Brief History:

- constructed with NSF MRI + CWRU funding 2014-2016.
- first light summer 2016, science commissioning 2016-2017: JSR BL capabilities paper just accepted, novel time-resolved FP of GPCRs (Brian Kobilka) in *Cell*.
- GU program active since June 2017

Capabilities & Integration with NSLS-II Structural Biology

- accommodate high-throughput and difficult high-dose experiments
- community expanding beyond origins at CWRU/JHU: Stockley (Leeds), Montfort (Arizona), Drummond/Sosnick (Chicago)
- unique BSL2 footprinting capability (specialty areas: virus assembly, prions)
- now accepting BAGs & proprietary users
- building connections with neighbors – controls, coordination with LiX

Future of XFP Program

NIGMS programmatic interests dictate a pivot away from NIH for support of XFP by the Partner, as well as re-emphasis on X-ray footprinting science.

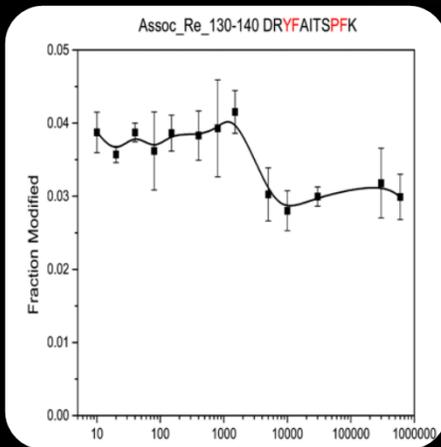
Future: CWRU XFP program will be funded via continued financial commitment from institution and an initial 3 year new technology development partnership with a new sponsor (pending).

Three elements:

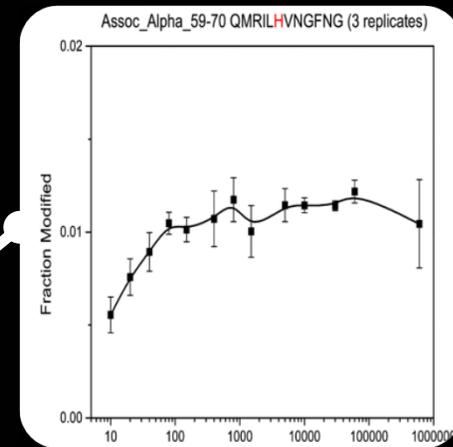
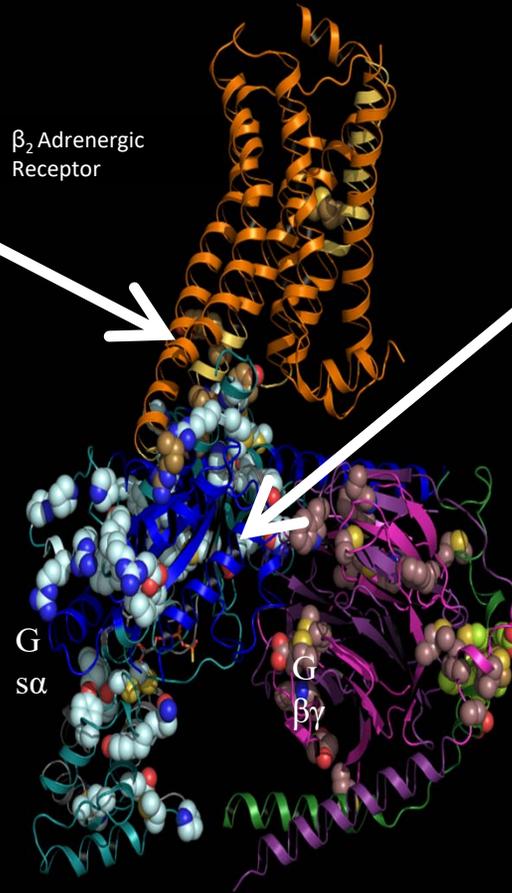
1. continue to support X-ray footprinting by CWRU investigators and centers funded by NIH/NSF/DOD in multiple departments
2. develop new XFP technologies via new CWRU center on bio-nano sensors (funding pending).
3. engage community problems and validate technology developments with vigorous GU program

CWRU will commit additional staff to specifically support CWRU + bio-sensor efforts. Current beamline staff focus on technology development, GU program support, and tighter integration with NSLS-II Structural Biology program

Time Resolved FP probes kinetic events during GPCR–G protein association



Changes in the ionic lock motif indicate a ~1000 ms kinetic transition during the association process. Changes in this region are consistent with structures of the activated state of the receptor where G α terminus is bound and a remodeling of ordered solvent in this region.



Early association events are considerably less well understood --representative graph from N terminal region in G α suggests that immediately upon GPCR association, allosteric changes are communicated to the nucleotide binding site within 100 ms



NYX

Dieter Schneider & Wayne Hendrickson

NYX Partner Beamline: Status

NYX
NYSBC

Technical commissioning results:

Design energy resolution essentially achieved
Native focus currently twice the design value
Beam stabilization achieved
Energy change in 6.5 – 10 keV range exercised
Sample changing robot in operation

Scientific commissioning ongoing

First results published

Remaining challenges

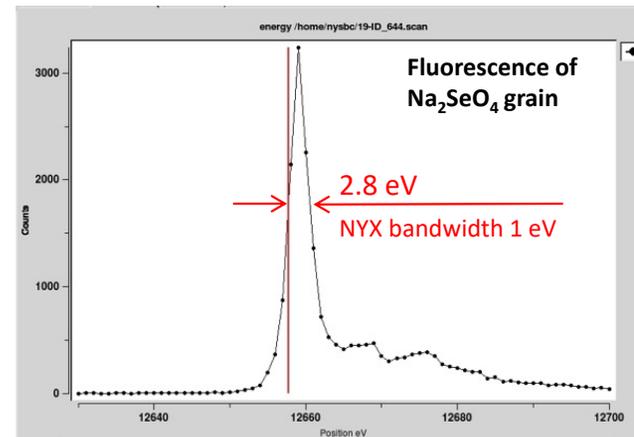
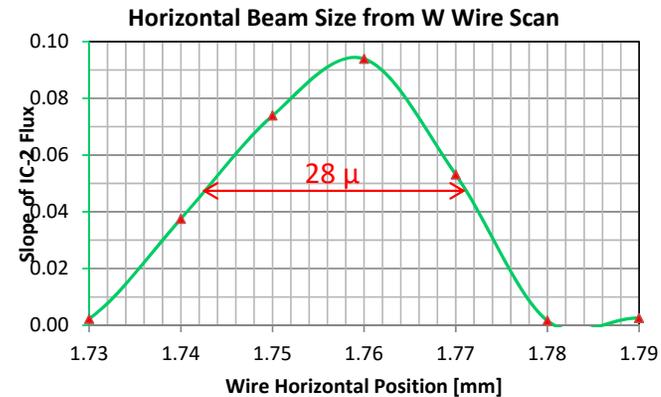
Experimental station ongoing upgrades
Experiment control software

Measured NYX Parameters:

E-range	6.3 – 18 keV
$\Delta E/E$	8×10^{-5}
Focal spot size	32 μm (H) x 22 μm (V)
Flux at 12 keV	2×10^{12} ph/s

NYX Optics:

DCM with asymmetrically cut (6°) Si[111] crystals to provide high energy-resolution
1st crystal tangentially bent to collect full white beam cone
2nd crystal sagittally bent providing horizontal focusing
Vertically focusing with elliptically bent mirror



Primary aims:

- Begin General User program in May
- Serve Partner and General Users
- Continue beamline upgrades

Scientifically:

- Collect data on specimen requiring high energy resolution: *i.e.* Thioredoxin
- Solve S-SAD structure at NYX E_{\min}

Technically:

- Perfect sample changing robot
- Develop remote use capability
- Pursue controls and software upgrades



Mahrugh Usmani, Mark McRae, Xiangpeng Kong (NYU), Samantha Nyovanie, and Jingyun Dong, at NYX Feb 15, 2019

The SAC asked to consider:

Response:

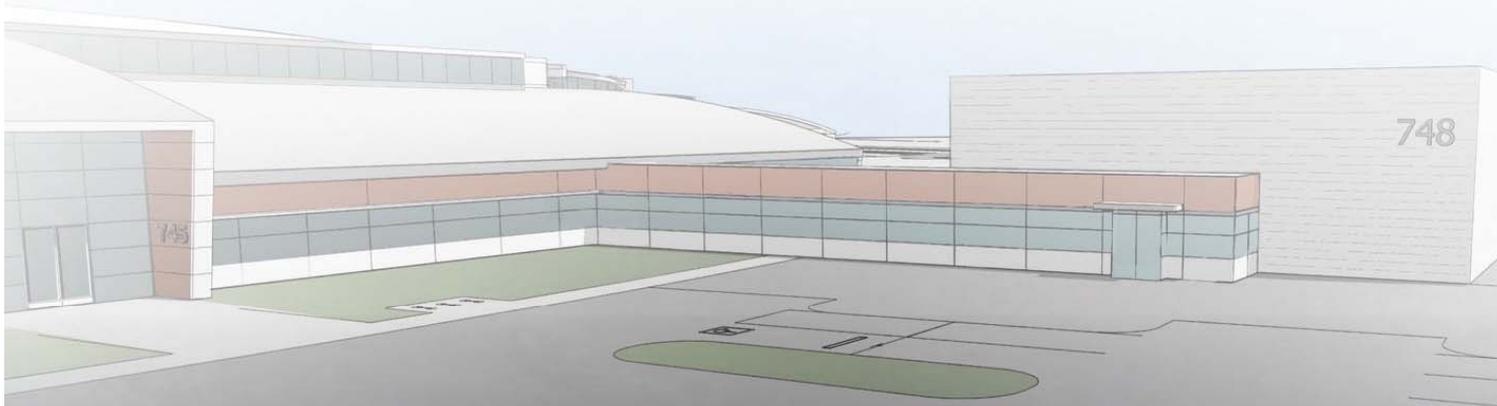
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- | | |
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| <ul style="list-style-type: none">▪ Options for continued funding | <p>Funding of NYX remains through NYSBC at the same level as in the past two years</p> |
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- | | |
|---|--|
| <ul style="list-style-type: none">▪ Options to update outdated aspects | <ul style="list-style-type: none">• Detector: apply in 2020 for NIH HEI grant for an Eiger 9M• Controls: take advantage of NSLS-II-provided 1/3 FTE for controls engineering• Optics: acquire h-white beam slits, augment beam diagnostics (BPMs, screens) |
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| <ul style="list-style-type: none">▪ Options to integrate NYX with NSLS-II structural biology (SB) program | <ul style="list-style-type: none">• NYX not part of the SB NIH grant application• NYX is integrated with SB through<ul style="list-style-type: none">○ General User program (40% of beam time)○ common beam time calendar○ productive contact with its scientific staff |
|--|--|
-



CryoEM

Laboratory for BioMolecular Structure

CryoEM Building 748 Design Development Team



Concept Design

Detailed Design

Lab Design

Groundbreaking Event, December 13, 2018



Marty Faller



Ana Stojanovic Ranjbar



Mike Bromfield



Steve Cannella



Peggy Caradonna



Chris Channing



Julian Adams



Jeff Keister



Qun Liu



Architecture (HDR)

Detailed Design (continued)

Procurement



Gabriels Kleiman



Kevin Lemans



Tom Joos



Mike Kretschmann



Tom Nehring



Jim Wright



Phil Gardner



Dave Pareglio



Jose Velez



U.S. DEPARTMENT OF
ENERGY

Office of
Science

BROOKHAVEN
NATIONAL LABORATORY



NEW YORK
STATE OF
OPPORTUNITY.
Empire State
Development

CryoEM – Screening Microscope



Nearly Complete
February 7th

Screening Microscope

- Order placed September 27th, 2018
- Received November 29th, 2018
- Microscope lab completed and released for use December 2018
- Installation started January 28th, 2019
- Installation and acceptance test likely to run to end of March 2019
- Start operation with some training

Biochemistry and sample preparation labs to be handed over in the next weeks.



Ongoing work and next actions

- Recruitment continues, active search and out reach happening.
- Established Joint Appointments with Microscopists at Stony Brook and Yale.
- Developing the plan for a long term sustainable facility.
- Starting to explore partnerships. Based upon mechanism of the Partner User Agreements in place at NSLS-II.
- Expect to start training in sample preparation and characterization by the summer.
- Building delivery Dec 2019, Krios delivery Dec 2019.

Medium Term planning for Biosciences

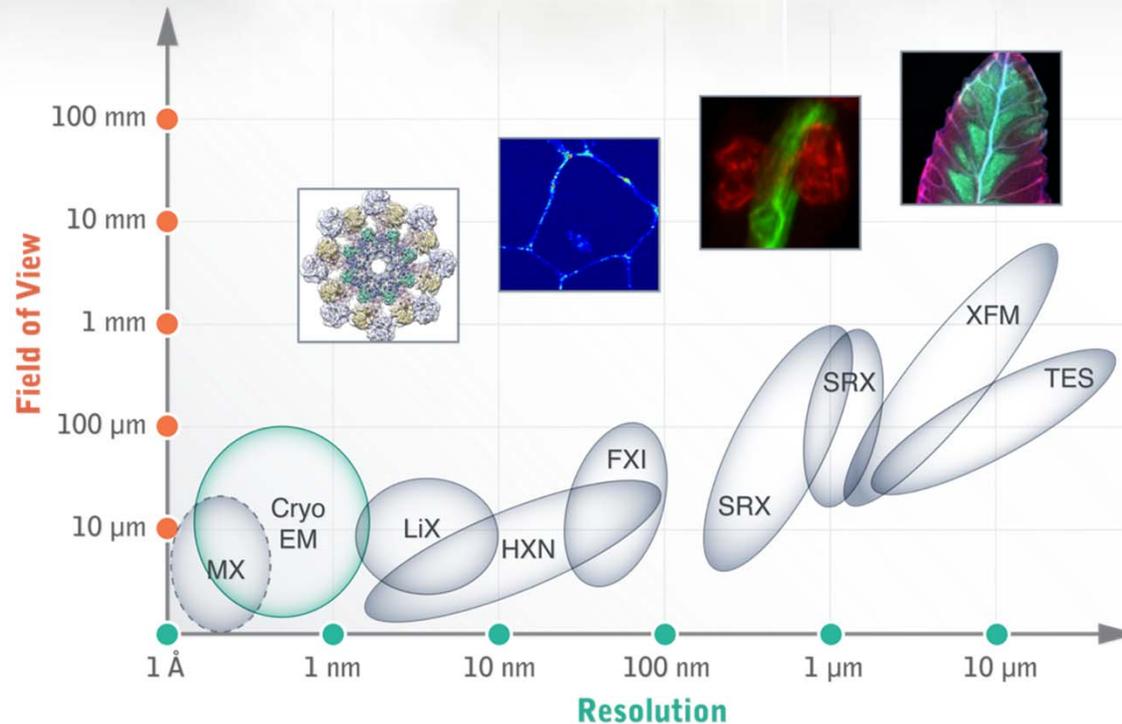
Pillars of our strategy building upon NSLS-II

- Components of the work
 - *Establish* the links from Structural Biology with Imaging Prog. & cryoEM facility. Encapsulated in our renewal proposal.
 - *Reinforce* the collaboration within Structural Biology Prog.
 - Develop the opportunity presented through electron microscopy and tomography.
 - Coordinated out-reach to research community.
 - Establish on-site training in all aspects of the use of the facility.
 - Integrate with other capabilities at BNL.
 - Recruit and retain the team capable to support these actions.

Integrate with other capabilities at BNL

- Strengthened synchrotron Imaging and establish cryo-electron tomography, start investigating “bio-cryo-tomography” more generally. (Imaging Group)
- Computational Science to improve the use of data analysis, innovation with Machine Learning and high throughput computation. (Shinjae Yoo)
- Collaborate with CFN staff for innovative sample preparation, and support for instruments. (Oleg Gang)
- Work closely with the Biology department for research activities and for support of visitors. (John Shanklin, Biology Dept Chair)

Biomolecular Characterization and Imaging available at NSLS-II



Through our programs we are blurring the overlap between structural biology and imaging. Our challenge is to make these instruments accessible and attractive to the broad community of researchers interested in Biology questions.

Organizing for the future.

Recognizing the need to adapt our management to the needs of the future funding and the demands they pose for us, we have chosen to redevelop our management strategy to allow for development a cohesive strategy, agility in dealing with issues and effective delegation. We created three scientific Cores, and two cross cutting activities to support our efforts.

- **Macromolecular Crystallography:** Lead - Jean Jakonicic, responsible for overview of MX activities.
- **Scattering Core:** Lead - Lin Yang, responsible for scattering science, and outreach with XFP and the Neutron sources at ORNL.
- **Bioimaging Core:** Lead - Ryan Tappero, with responsibility for developing our Bioimaging capabilities.
- **Training & Outreach:** Lead - Vivian Stojanoff, with responsibility for the thematic training.
- **Integrated Technology Core:** Lead - Martin Fuchs, provides solutions to the maintenance and development needs through the portfolio.

Concluding Comments

- We have increased the use of three structural biology beamlines, while maintaining reliability, developing new instruments and collaborating more broadly.
- Two Partner User Beamlines are maturing and adapting to changing circumstance. We continue to work together to provide “joined up structural biology support”
- The challenge provided by cryoEM is being embraced and a plan is being established to provide a sustainable facility.
- Biosciences within BNL is becoming more integrated and is leveraging the resources available. Next step will be to become even more outward looking as we establish strong foundations