

Other Activities

Vivian Stojanoff

User Program

- *Transition User program - LSBR <>SMB closeout
- *LSBR User Program

User Access

- *Proposal types

User Ancillary Facilities

- *Laboratories, Sample Preparation

Dissemination

- *Web development

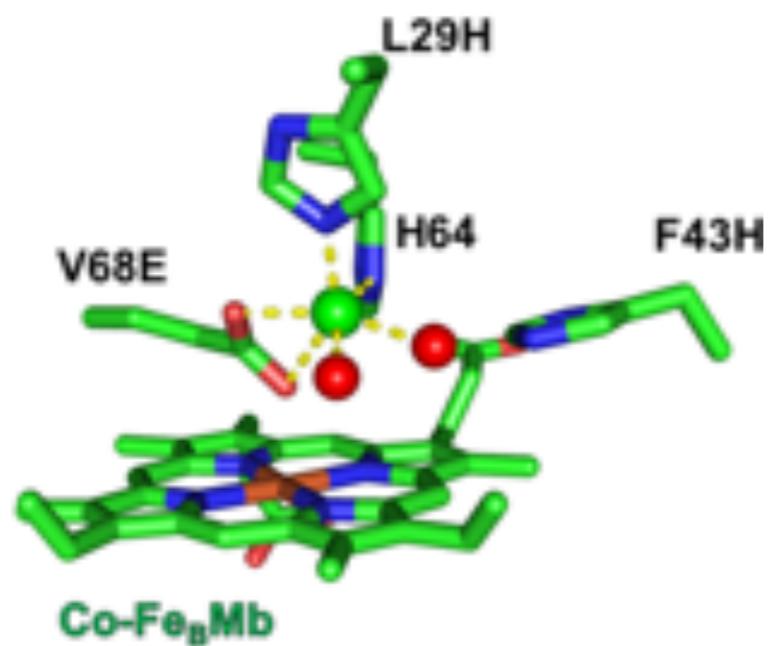
Outreach activities

What is being planned

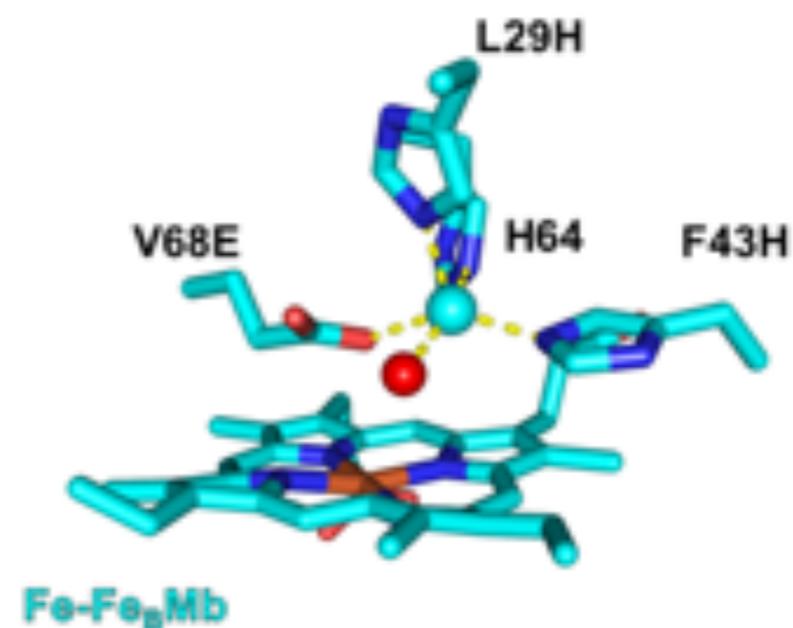
Transition Program - Productivity*

	Publications
Published	24
Submitted	5
In preparation	9

	Structures
PDB released	53
PDB On Hold (HPUB)	9
Structures being Refined	62



Yi Lu Group University of Illinois



*according to Survey processed September, 2016

Transition Program - Productivity*

24 Publications in peer review journals; 8 in premier journals

I J Domochowski *Angew Chem* (2016) 55:1733-1736 DOI: 10.1002/anie.201508990

B W Roose et al *Human Carbonic Anhydrase II complexed with two-faced guest*
(5EKH, 5EKJ, 5EKM)

G. Cingolani *Nat Commun* (2016) 8: 14310

R. K. Lokareddy et al *Portal protein functions akin to a DNA-sensor that couples genome-packing to icosahedral capsid maturation*
(5JJ1, 5JJ3)

M Garcia-Diaz *EMBO J* (2016) 35:1957-2060 DOI:10.15252/emboj.201694332

M J Burak et al *A fidelity mechanism in DNA polymerase lambda promotes error-free bypass of 8-oxo-dG*
(5III, 5IIJ, 5IIK, 5IIM, 5IIN, 5IIO)

J McLellan *Nat.Chem.Biol.* (2015) DOI: 10.1038/nchembio.1982

M B Battles et al *Molecular Mechanism of Respiratory Syncytial Virus Fusion Inhibitors*
(HPUB 5)

D W Christianson *Nat.Chem.Biol.* (2016) 12 741-747 DOI: 10.1038/nchembio.2134

Y Hai and *Histone deacetylase 6 structure and molecular basis of catalysis and inhibition*
(5EEF,5EEI, 5EEK)

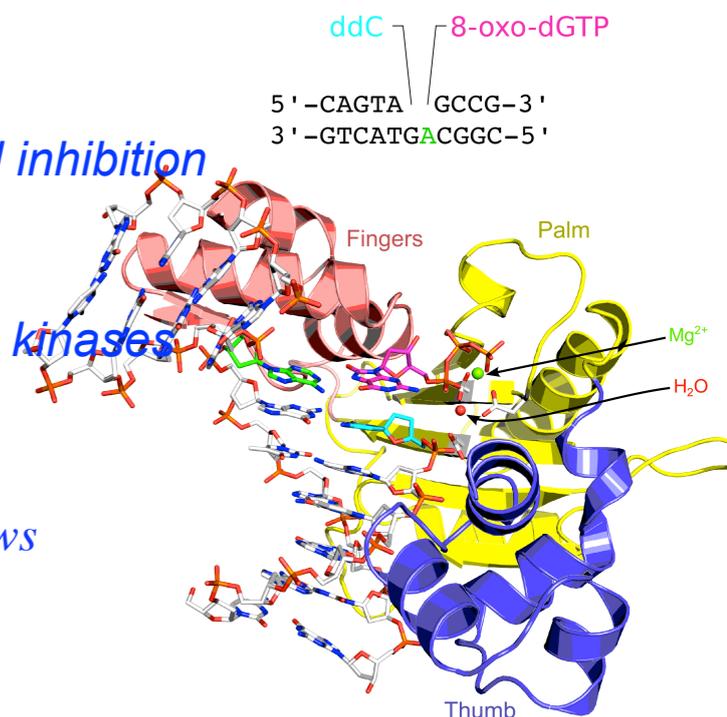
Y. Ha *PNAS* (2016) 113:8711-8716 DOI::10.1073/pnas.1522112113

Y. Muftuoglu et al *Mechanism of substrate specificity of phosphatidylinositol phosphate kinases*
(5E3S, 5E3T, 5E3T)

D. Jeruzalmi *Nucleic Acids Res.* (2016) DOI: 10.1093/nar/gkw1288

N. Orlova et al *The replication initiator of the cholera pathogen's second chromosome shows structural similarity to plasmid initiators*
(5UBD, 5UBE, 5UBF)

<Average Impact Factor>** = 6.09



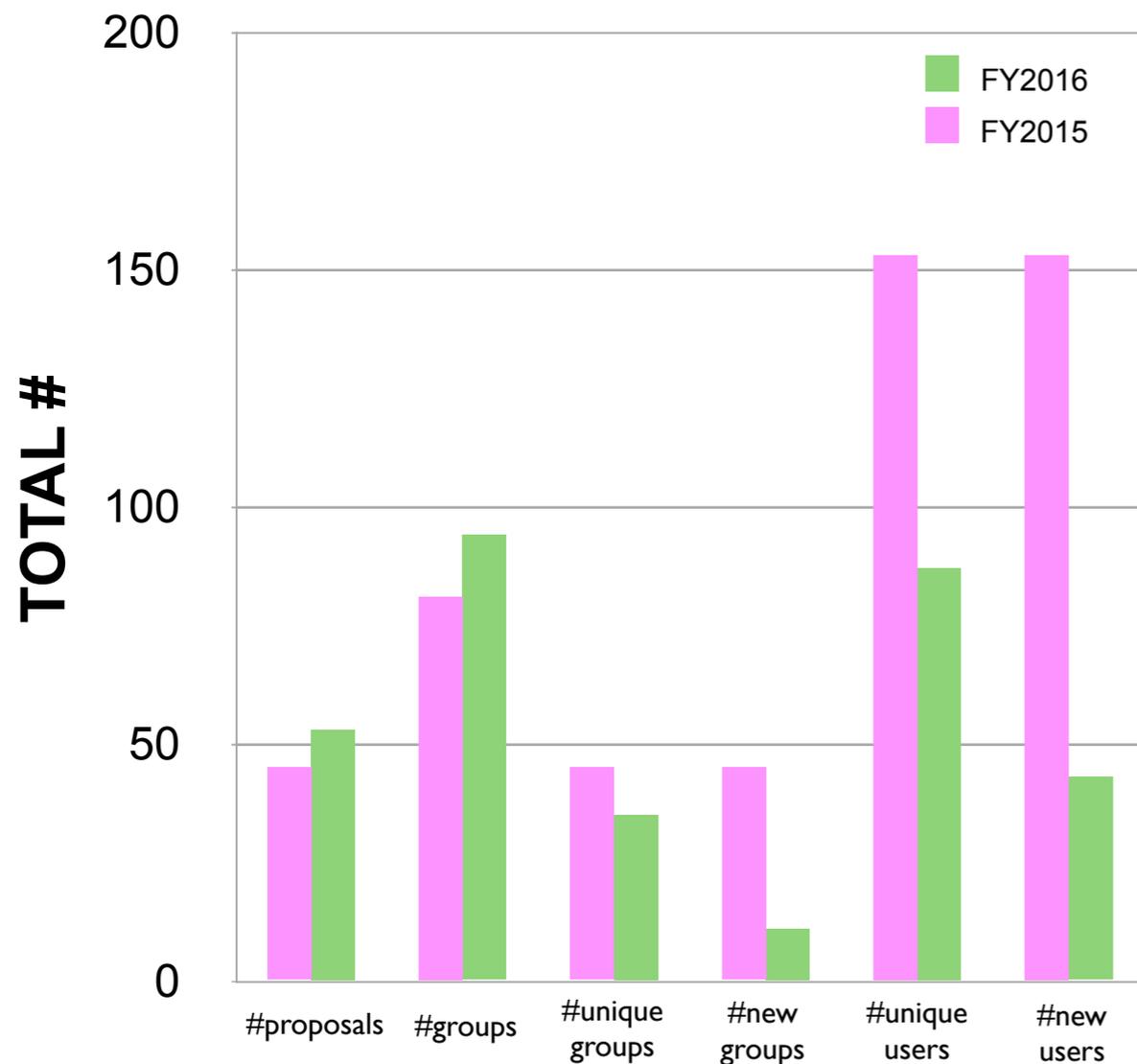
Kip Guja - Garcia-Diaz Lab (SUNY); EMBO J

*according to Survey processed September, 2016; Updated March 2017

**<http://www.citefactor.org/journal-impact-factor-list-2015.html>

Transition Program - Impact

A total of 53 proposals were submitted to the program over its duration.



#proposals = total #proposals submitted in a given year

#groups = total #groups which visited the BL in a given year

#unique groups = total #groups which visited the BL at least once in a given year

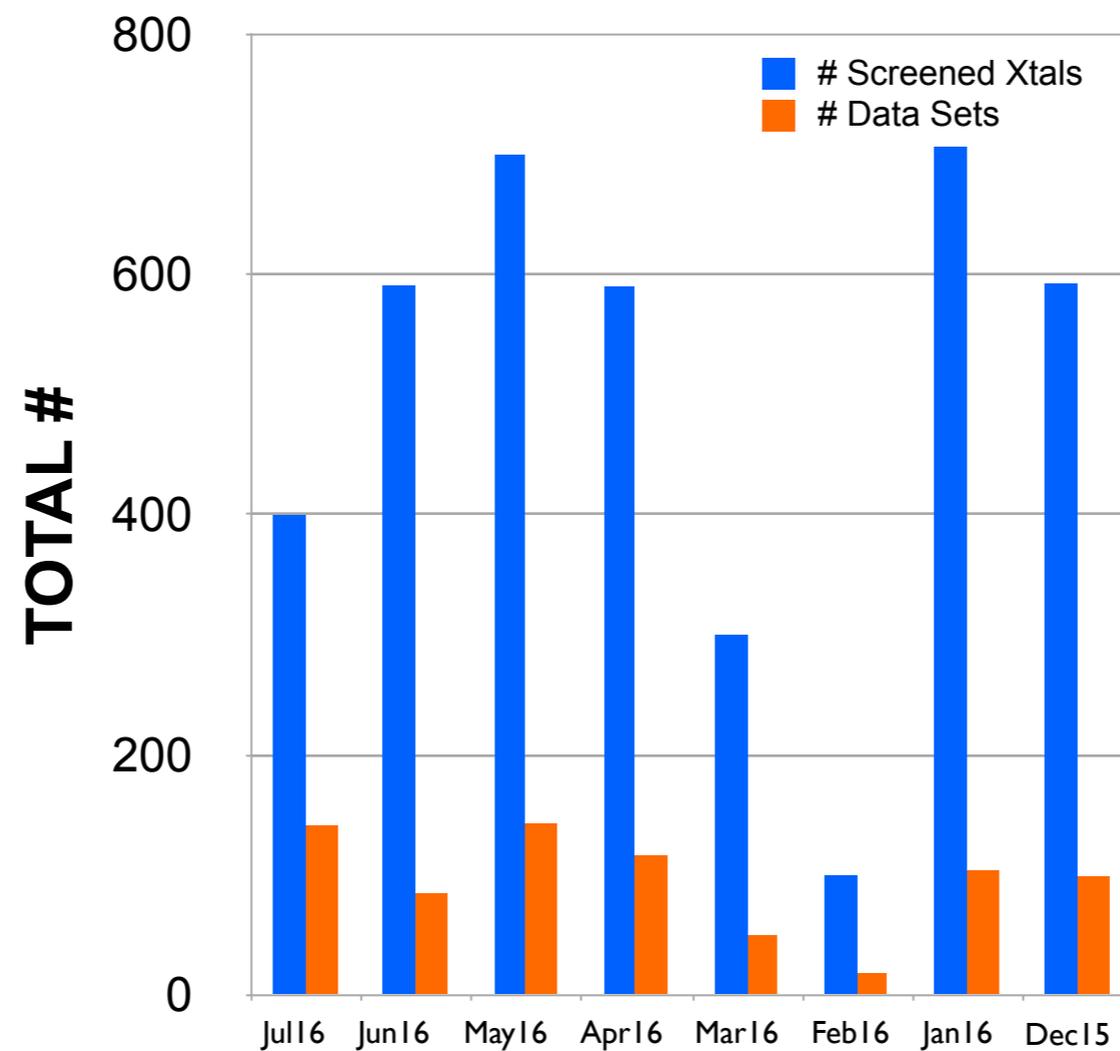
#new groups = #groups that visited the BL at least once in a given year and did not visit the previous year

#unique users = #users that visited the BL at least once in a given year

#new users = #users which visited the BL at least once in a given year and did not visit the previous year

Transition Program - Impact

Data sets collected and estimate of crystals screened in FY16.



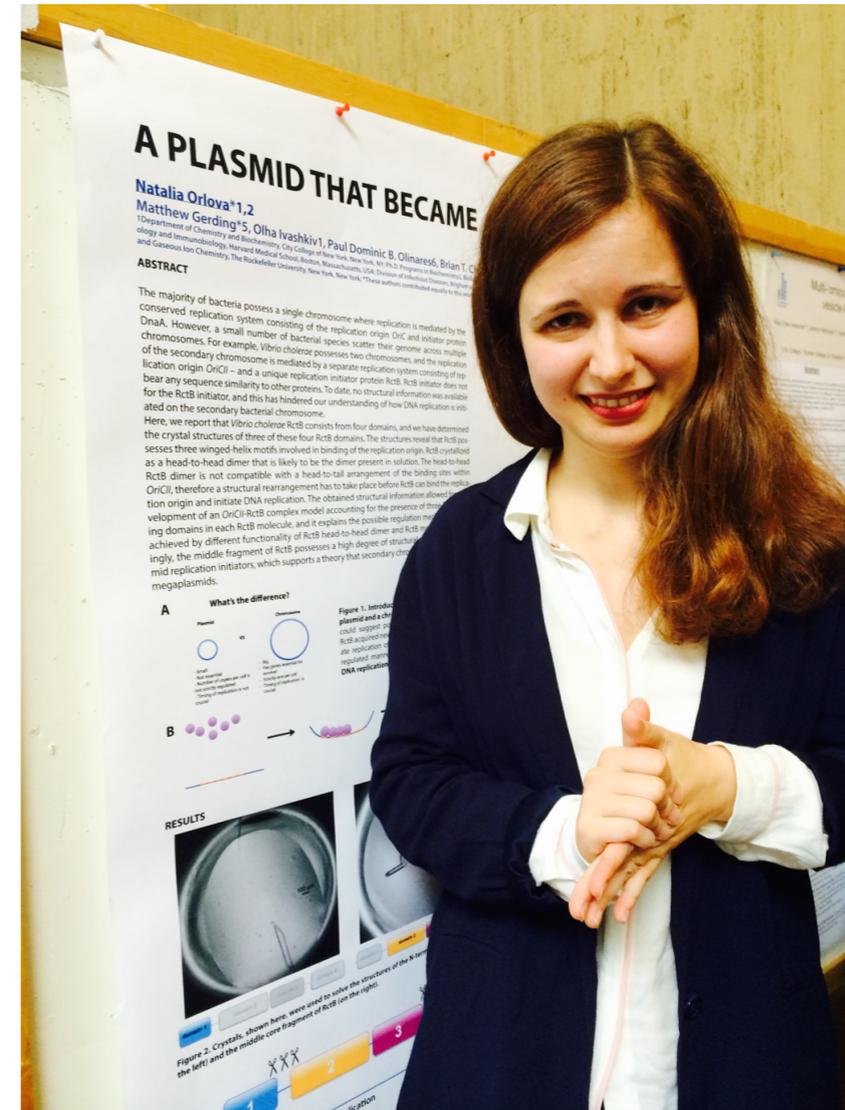
The program received a total of 79 days of beam time in FY16 on BL14-I at the SSRL or the equivalent of 237 eight hour shifts.

Transition Program - Workforce Impact

Effectively PhD students and post doctoral fellows benefited from these program

FY16	Users
Faculty	16
Post Doctoral fellows	~25
PhD students	~46

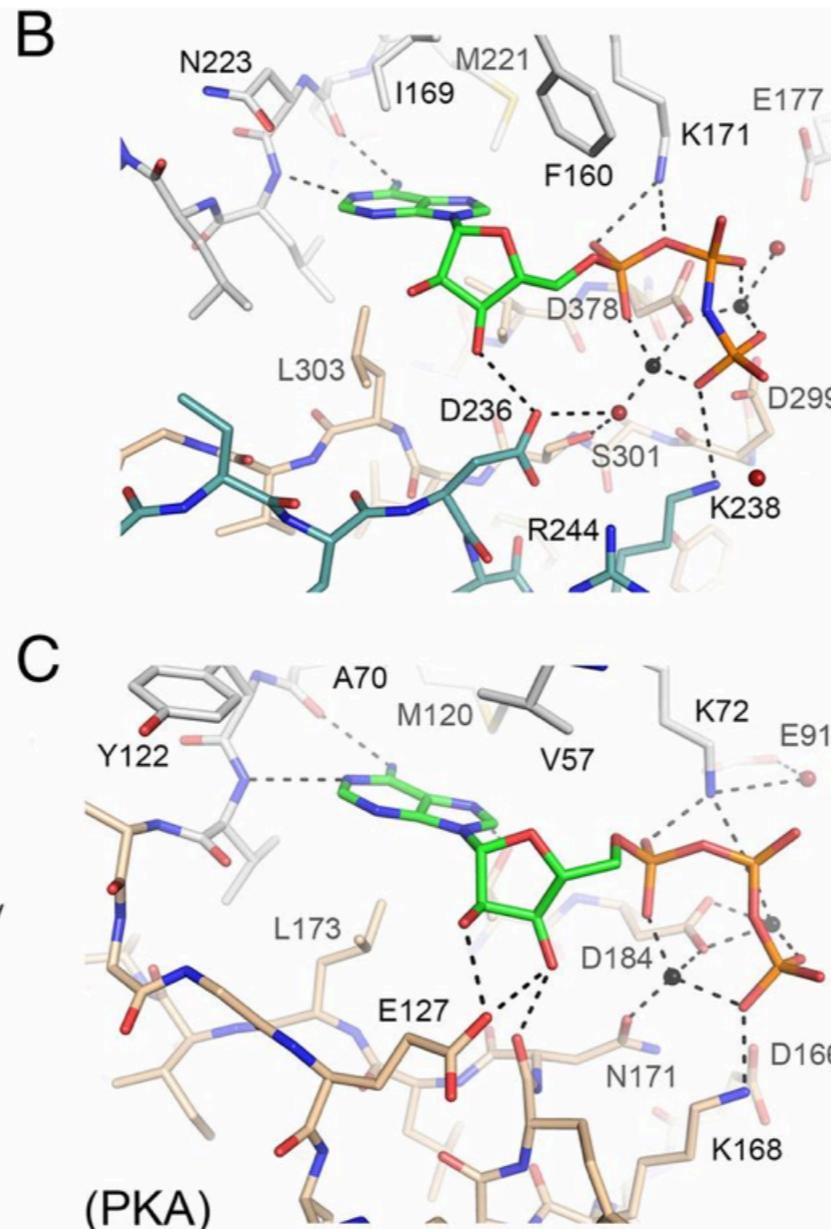
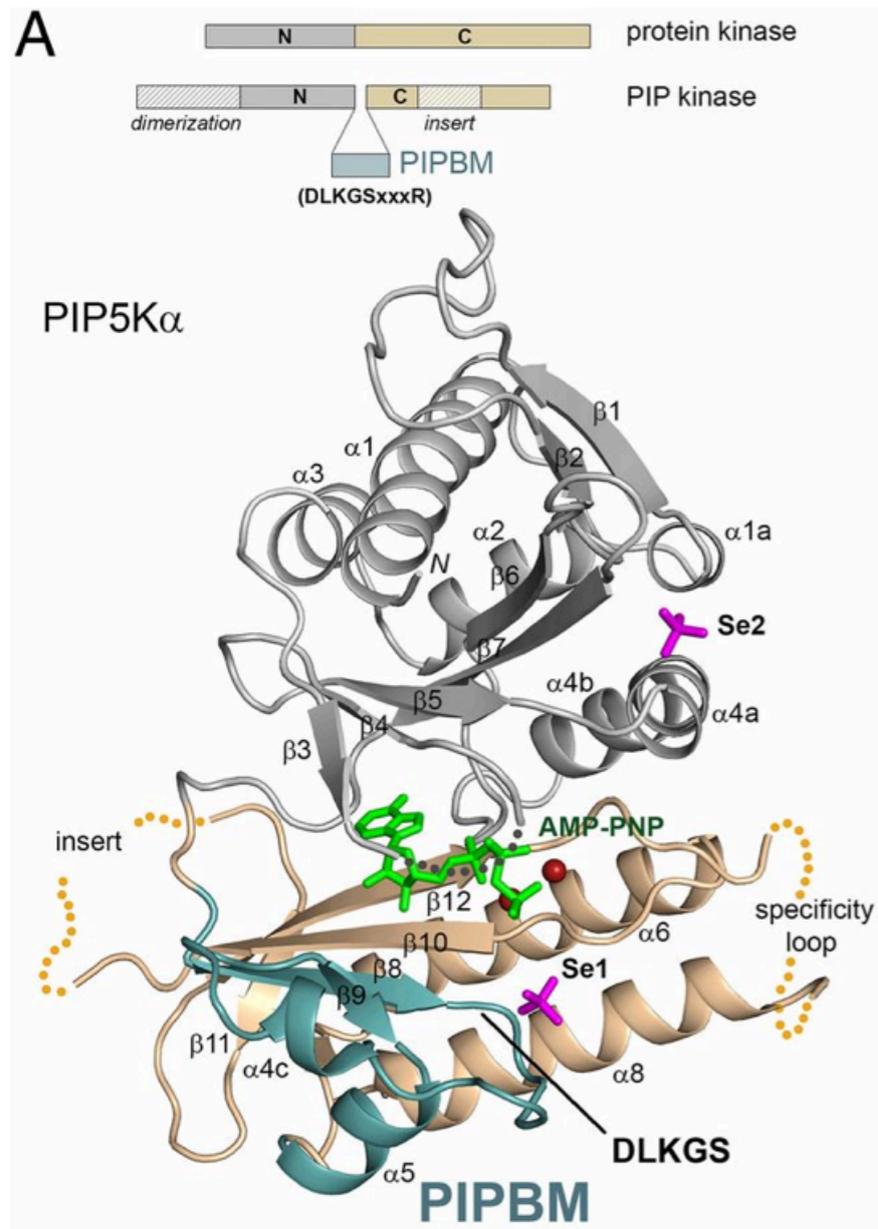
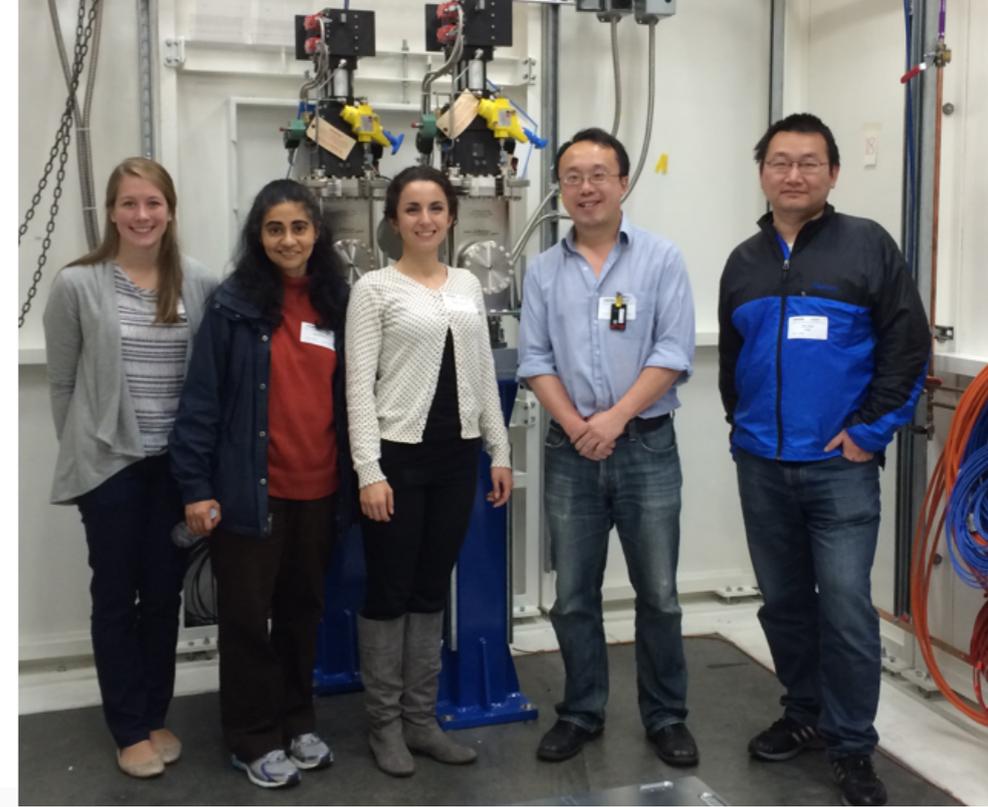
In FY16 only 16 PI's participated in the experiments. About 53% of the user base were PhD's. Several thesis work were finished during this period and new students who never had used a synchrotron facility came on board.



Natalia Orlova - Jeruzalmi Lab; The Graduate Center, City University of New York (CUNY) presenting her PhD research at the New York Structural Biology Discussion Group Meeting, August 2016. Submitted: The replication initiator of the cholera pathogen's second chromosome shows structural similarity to plasmid initiators

The Ya Lab – YALE

The Ya group have been long time users of the Brookhaven National Lab Facilities for their molecular structure determination. In a visit to the LSBR beamlines last Fall Yagmur Muftuoglu (center left) presented her results on the *Mechanism of substrate specificity of phosphatidylinositol phosphate kinases* part of her PhD thesis she defended last May 2016



Overall structure and ATP binding site. (A) Domain organization. The schematic compares PIPKs with protein kinases. The overall structure of the zebrafish PIP5K α in complex with AMP-PNP is shown below the schematic with the N-lobe (gray), the C-lobe (light tan), and the PIPB domain (blue). Disordered regions of the protein are indicated by dotted curves. Bound AMP-PNP and SeO₄²⁻ are shown as stick models. (B) Structural details of the ATP-binding site of PIP5K α . Hydrogen bonds are indicated by dotted lines. Red spheres represent water, and black spheres represent metal ions. (C) ATP-binding site of protein kinase A (PDB ID code 1ATP). Structural illustrations were all generated on PyMOL.

PNAS (2016) 113:8711-8716 DOI::10.1073/pnas.1522112113

LSBR User ACCESS

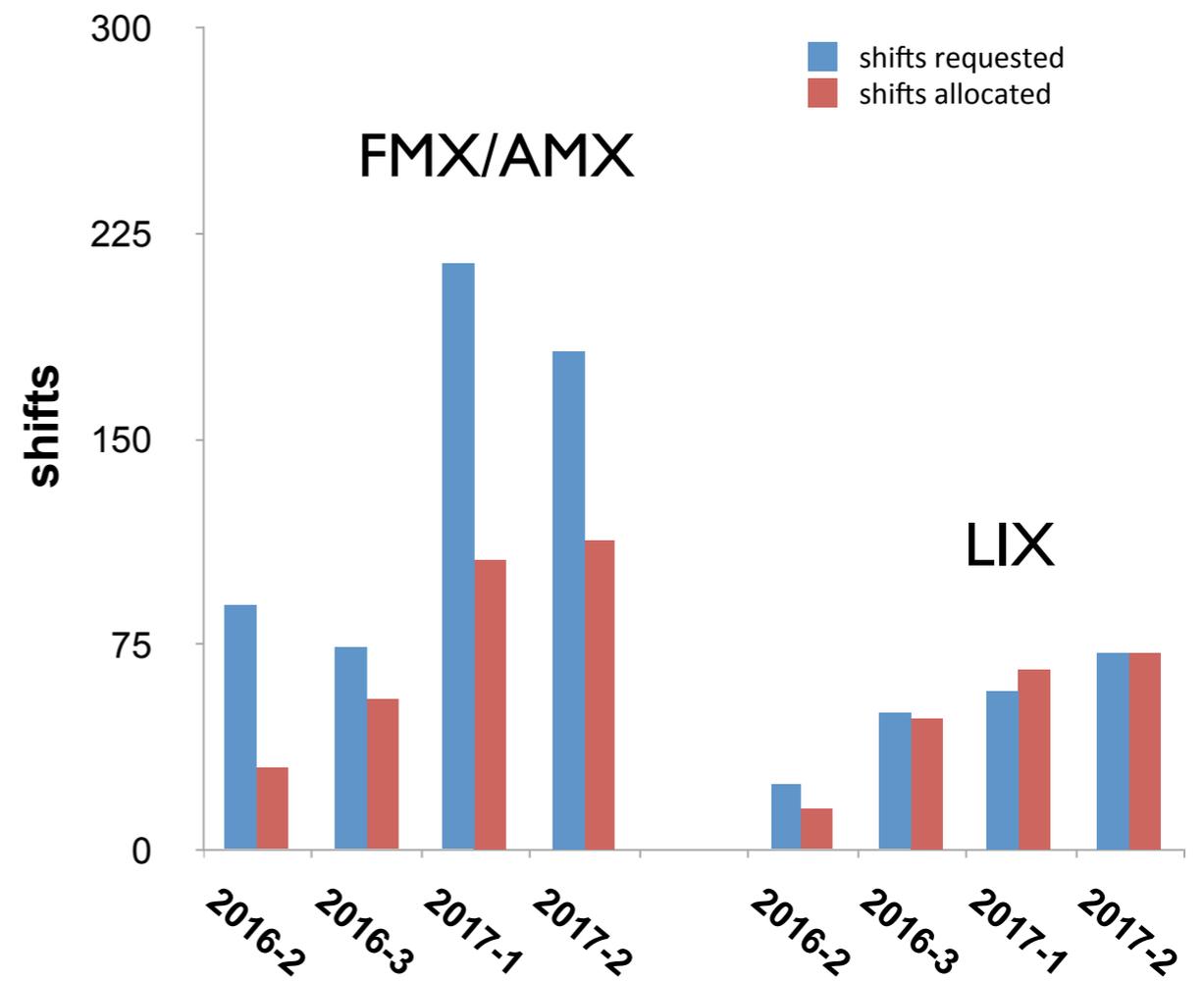
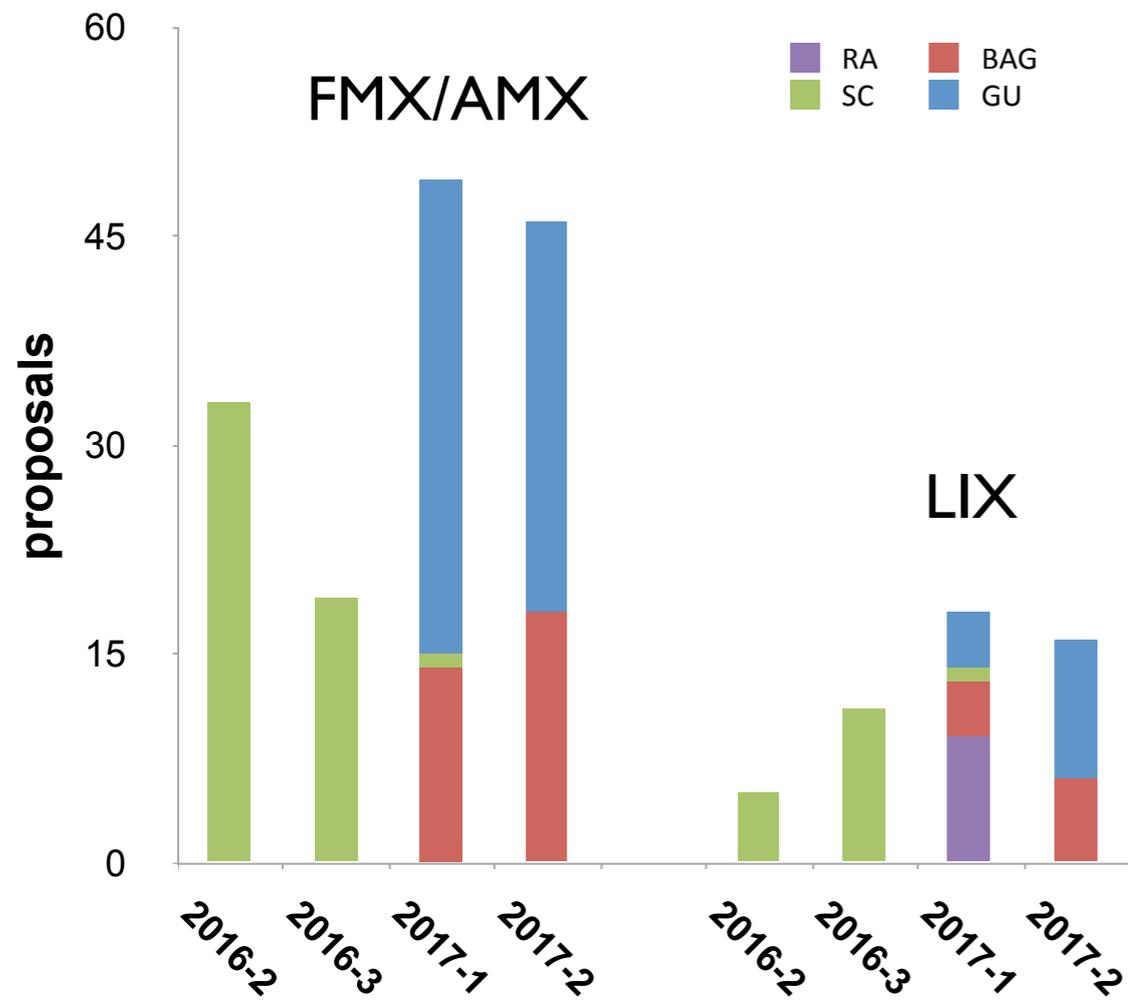
Proposals are submitted through the web-based Proposal Allocation, Safety, and Scheduling System (PASS) system



Proposal type	Block Allocation Groups (BAG)	Rapid Access (RA)	General Users (GU)
Proposal Length	2 years (6 cycles)	1 cycle	1 year (3 cycles)
Research Groups	Multiple PI's	Single Project	Single PI
Beam Time	flexible; MX and LIX	single request; single beam line	single beam line
Beam Time request future cycles	BRT	-	BRT

LSBR User Program

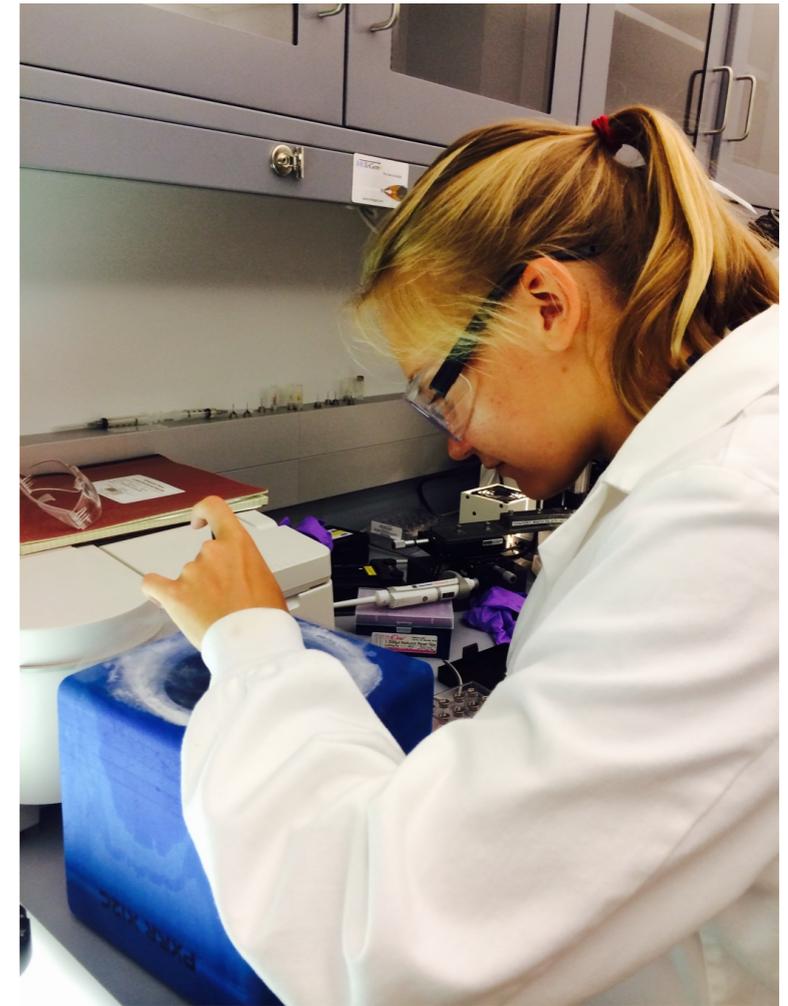
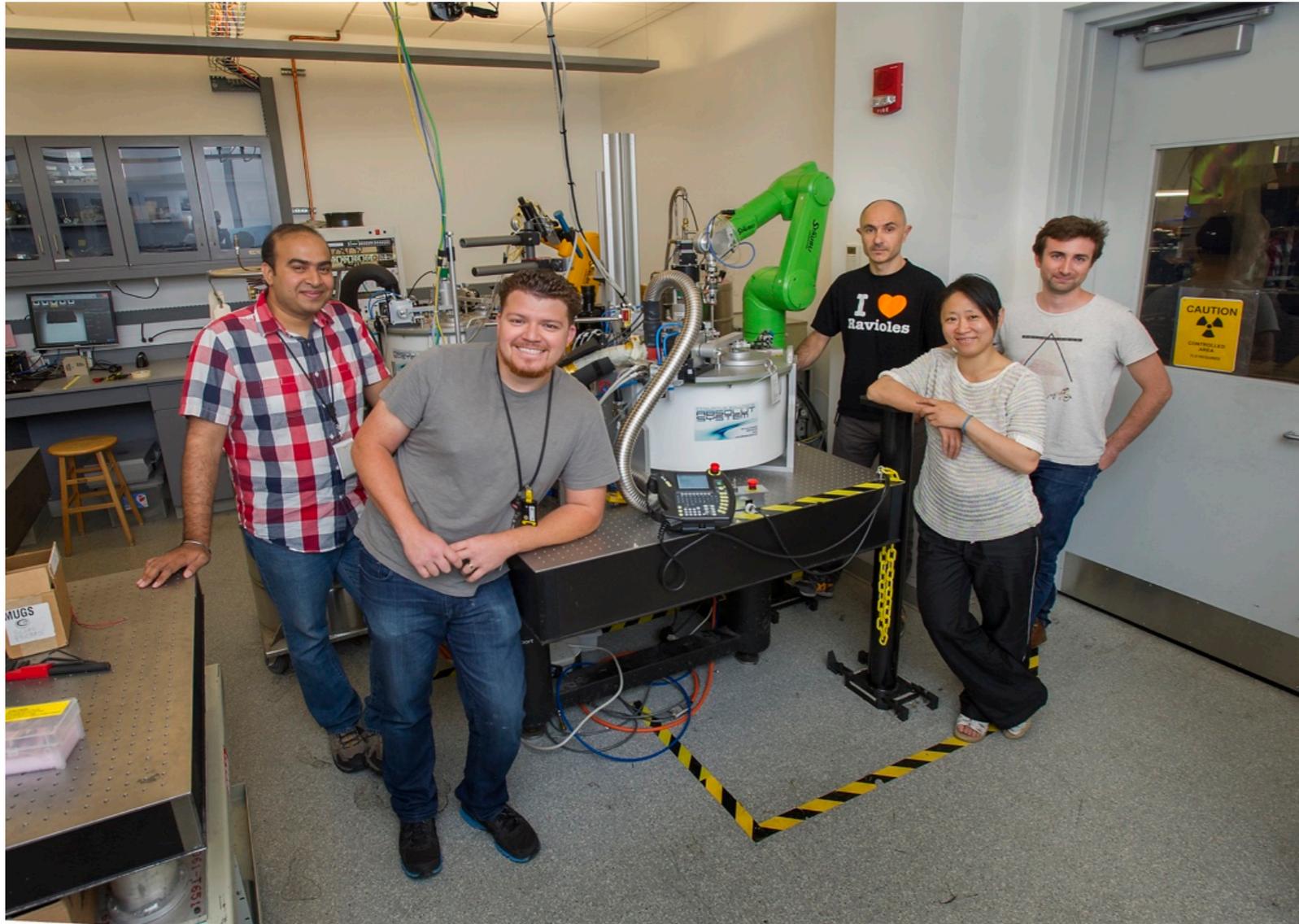
Scheduling currently being pursued in collaboration with the NSLS-II User Office



LSBR User Ancillary Facilities

Labs in LOB1 - Wet Labs

- Structural Biology - Crystallization
- Biochemistry Lab - Purification and sample characterization



Labs in LOB1 - Dry Labs Staging Labs for beam line instrumentation

- automation, robotics and sample visualization
- microfluidics and sample environment for LIX

Dissemination

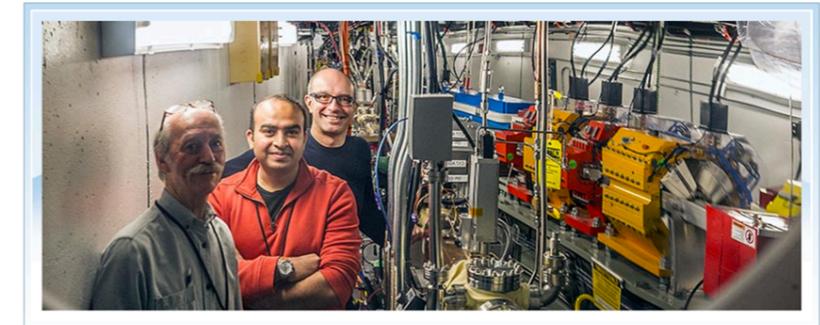
The LSBR webpages are being updated

The screenshot shows the top navigation bar of the Brookhaven National Laboratory website, featuring the logo and the text "Life Science Biomedical Technology Research" and "U.S. DEPARTMENT OF ENERGY". Below the navigation bar, there are several menu items: Home, Facilities, Users, Staff, Contact, Science Highlights, and NSLS-II. The main content area features a large image of a protein structure with dimensions 110 Å and 75 Å, and a label "gp4". Below the image, there is a paragraph describing the mission of the Life Science and Biomedical Technology Research (LSBR) resource at the National Synchrotron Light Source II. A yellow button labeled "Apply for Beam Time" is positioned to the right of the text. At the bottom of the page, there are three buttons: "Science Highlights See all", "News & Events See all", and "Announcements".

Include:

- *science highlights, news& events, announcements
- *specific beam line characteristics and manuals
- *user instructions

Macromolecular Crystallography



X-ray Crystallography allows for the three-dimensional structure determination of macromolecules proteins, DNA, RNA or assemblies such as viruses and ribosomes. The Life Science MX beam lines will enable research on molecular interactions, on enzyme catalysis reactions, on the action of drugs and disease mechanisms.

Core Beamlines

AMX and FMX are two beamlines with overlapping capabilities. While the AMX 'mini' beam supports efficient structure determination and high-density throughput for chemical library or mutation activity screening, the FMX 'micro beam' enables the study of macromolecular complexes, weakly diffracting samples and radiation-sensitive crystals.

Partner User Beamlines

The New York Structural Biology Center beamline, NYX, will provide the highest energy resolution of the macromolecular crystallography beam lines enabling multiwavelength and single wavelength anomalous diffraction.

Ancillary Facilities

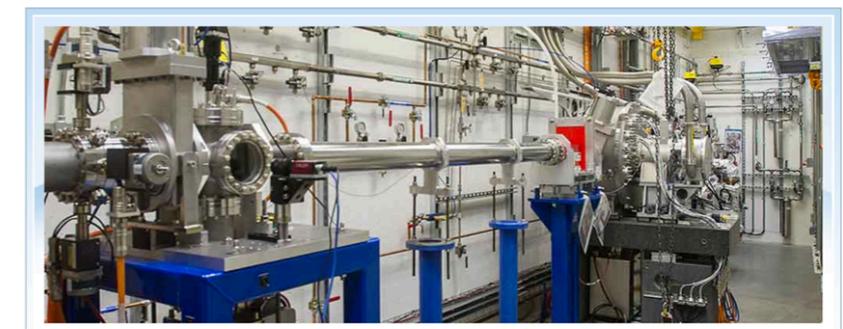
The core facilities are supported by a structural biology Laboratory that enables high throughput crystallization and screening. A UV-Vis and Raman spectroscopy Laboratory enables the study of metalloprotein in solution or crystals. [More...](#)

AMX Beamline

FMX Beamline

Highly Automated Macromolecular Crystallography (AMX)

Solution Scattering



The increased interest in the functional mechanisms of hierarchical complex systems of the biological molecules saw a growing interest in solution scattering methods. These methods are ideally positioned to monitor a broad range of interactions simultaneously elucidating structural applications at different levels.

Core Beamlines

The LIX end station is unique in that it is easily reconfigurable. This beamline will host four solution scattering setups incorporated in a single module: (1) windowless 8-cell sample holders, (2) flow through cell, (3) In line High Pressure Liquid Chromatography (HPLC) flow cell and (4) Time resolved scattering based on microfluidic mixer. The high-throughput 8-cell windowless cell will hold ~12µL of solution sample and will decouple the time-consuming process of cleaning up the sample holder from the actual scattering measurements. Sharing a kinematical mounting interface LIX further allows grazing incidence studies and imaging measurements.

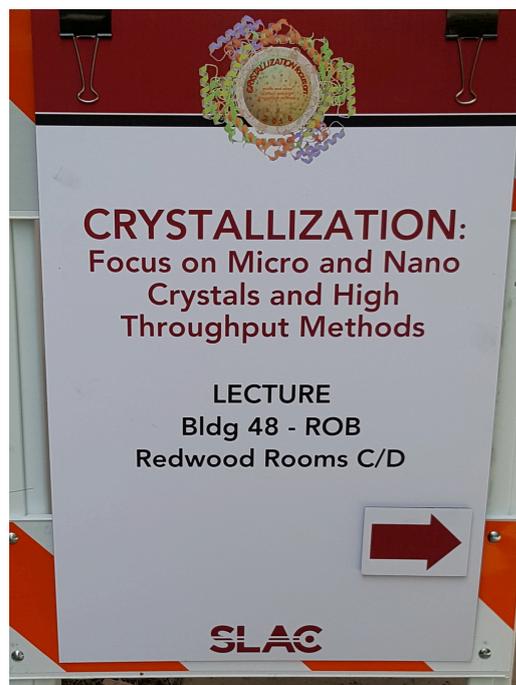
Laboratory Facilities

The core beam line is supported by a biology and chemistry Laboratory that enables sample purification. Equipped with an FPLC chromatography system and several centrifuges it allows researchers to prepare their samples on site. [See user laboratory details.](#)

Education and Outreach Activities

Workshops	Graduate Courses	Outreach
Crystallization focus on:	Yale University	Tours
Exploring Life Science with a New Light	NYU	NSLS-II Open House
WISE Stony Brook	Stony Brook	
<i>Future Users</i>	<i>Future Users</i>	<i>Community Communication</i>

Crystallization focus on ... micro and nano crystals and high throughput methods 2016 at SLAC



- 43 registered participants
- 16 participants brought proteins
- 15 participants screened for conditions
- 06 got crystals and tested them for diffraction
- 13 academic and government scientists Speakers and Tutors
- 07 non-academic Speakers and Tutors
- 09 Lectures
- 11 Tutorials
- 08 Practical parallel sessions
- 02 Practical Demonstration sessions
- 09 Crystallization robotic systems
- 04 Company Talks
- 05 Companies participating with Crystallization robotics
- 06 Companies providing supplies - consumables

SPONSORS

Crystallization Supplies

Molecular Dimension
Anatrace
Hampton Research
Mitegen
Qiagen
Millipore

Protein Crystallization Robots

Art Robbins Instruments
Formulatrix
Douglas Instruments
TTPLabtech
Labcyte

Visualization

JANSI
DeepCrystal

Travel Grants

The Pittsburgh Diffraction Society
IUCr International Union of Crystallography

Exploring Life Science with a New Light



In collaboration with NSLS-II User Administration and the Office of Educational Programs

Participants of the course are expected to submit proposals to NSLS-II. As a result of this workshop 4 proposals were submitted and a core group of four teachers started a protein crystallization program with their research students.

Participation in Graduate Courses



Graduate Students enrolled in the Advanced Structural Biology (HBH 585-SBU) learned about Structural

What is being planned

User Program

- *LSBR User Program ramp up

User Access

- *BAG's
- *Rapid Access

User Ancillary Facilities

- *Wet Laboratories LOB5
- *Dry Labs LOB5

Dissemination

- *Web development continuous development (manuals, instructions, etc ...)

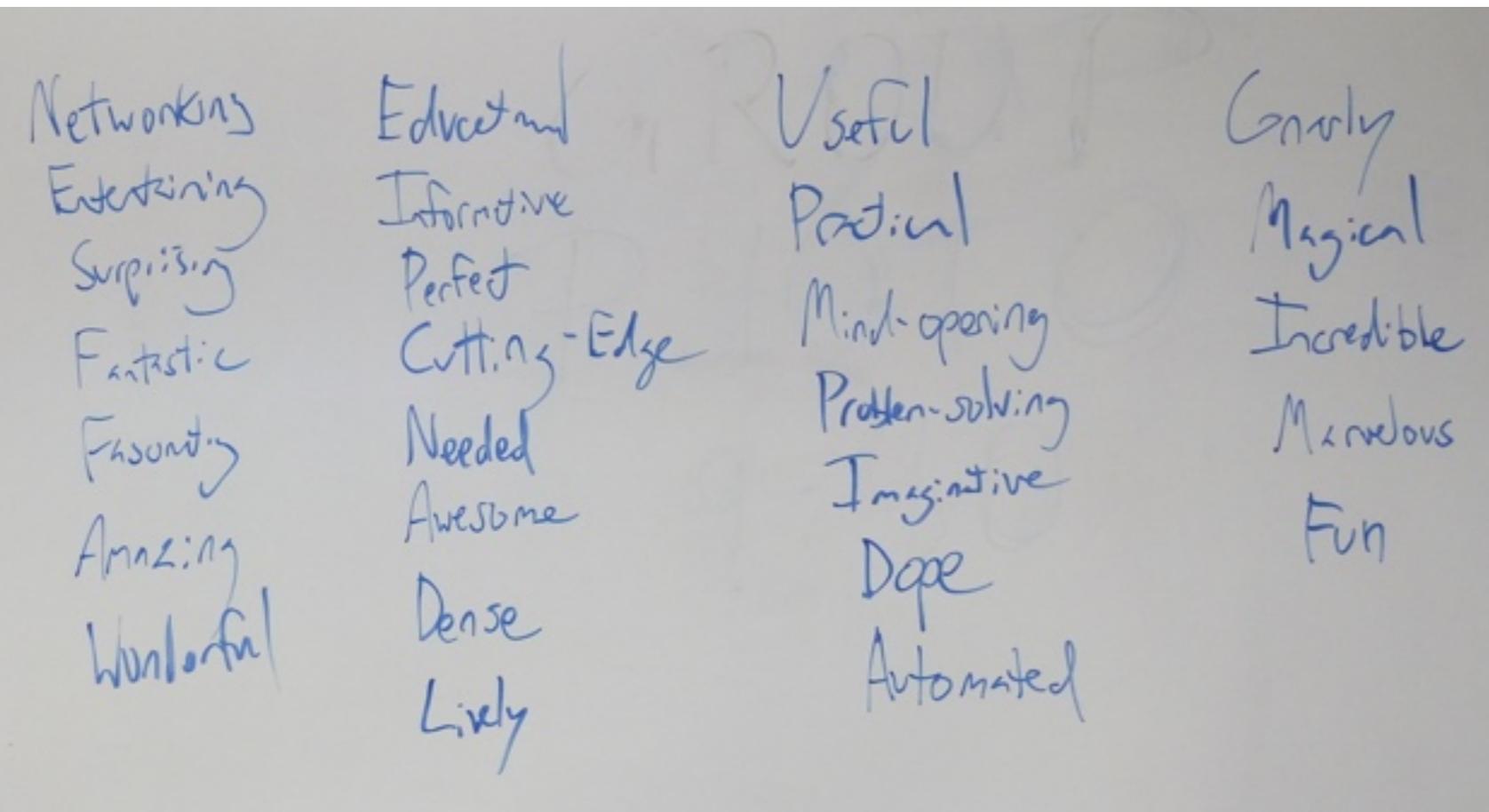
Outreach activities

- *Industry Open House (April)
- *BAG training session (June)
- *Workshop - "*Exploring Proteins with a New Light*" (OEP) - (June)
- *BAG training session (September)

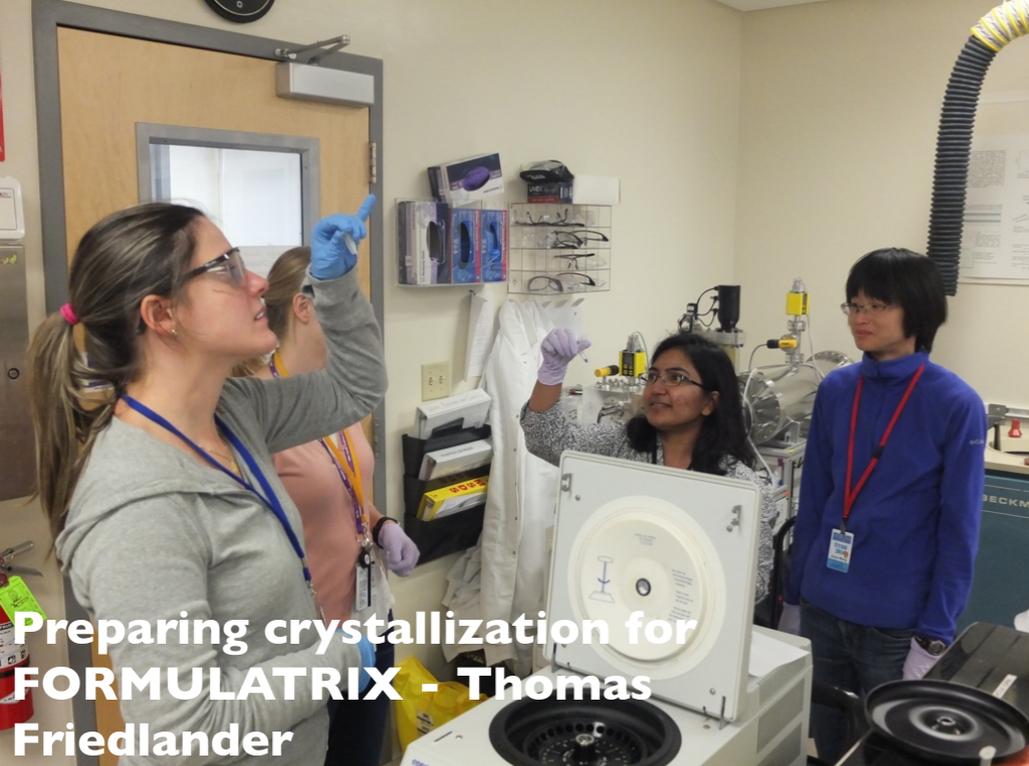
Crystallization focus on micro and nano crystals and high throughput methods 2016 at SLAC Group Photo



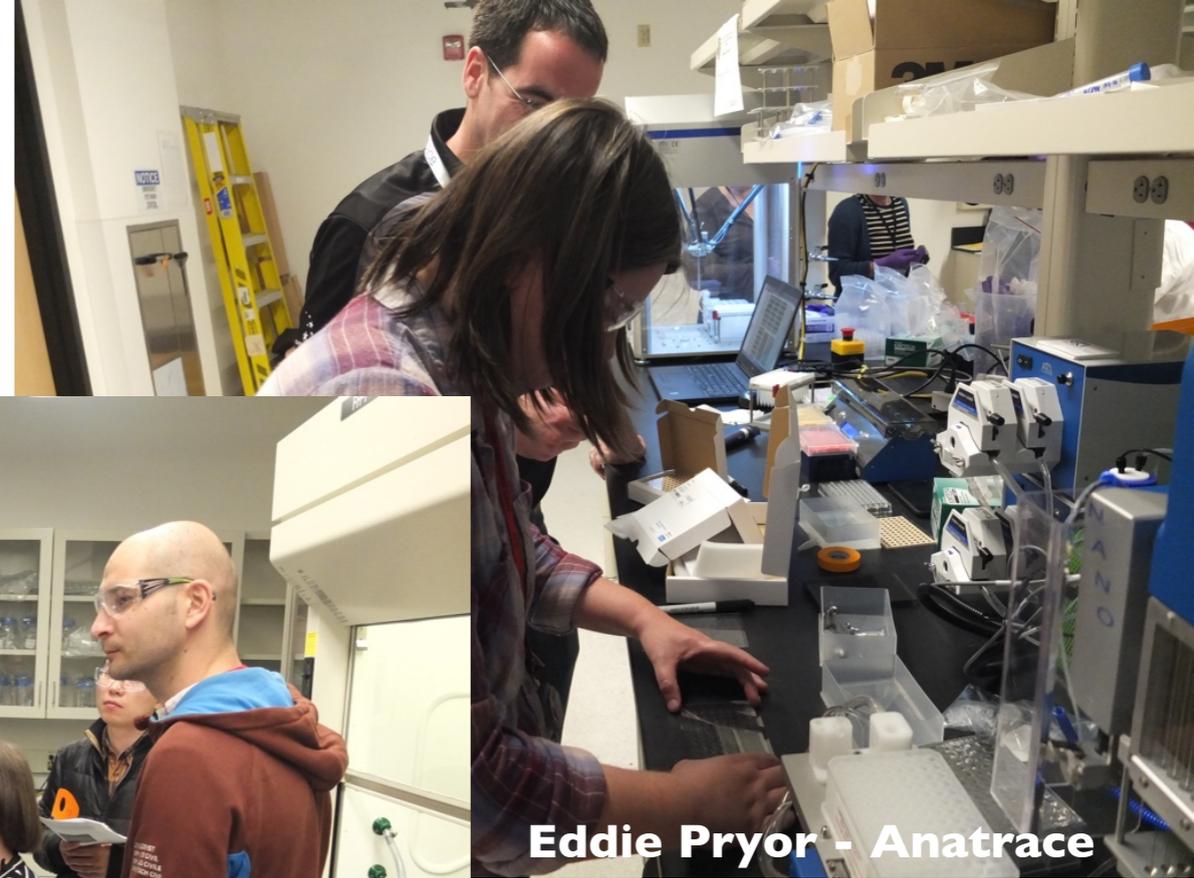
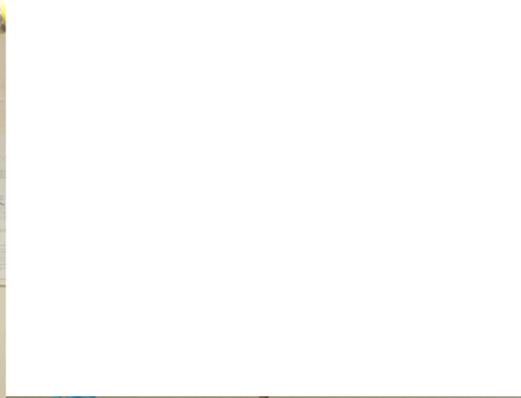
Travel Grant recipients



Participant feedback during close out session - each of the participant was allowed only one word to characterize their experience during the course. Not all were present soem participants were still working with tutors on their projects.



Preparing crystallization for FORMULATRIX - Thomas Friedlander

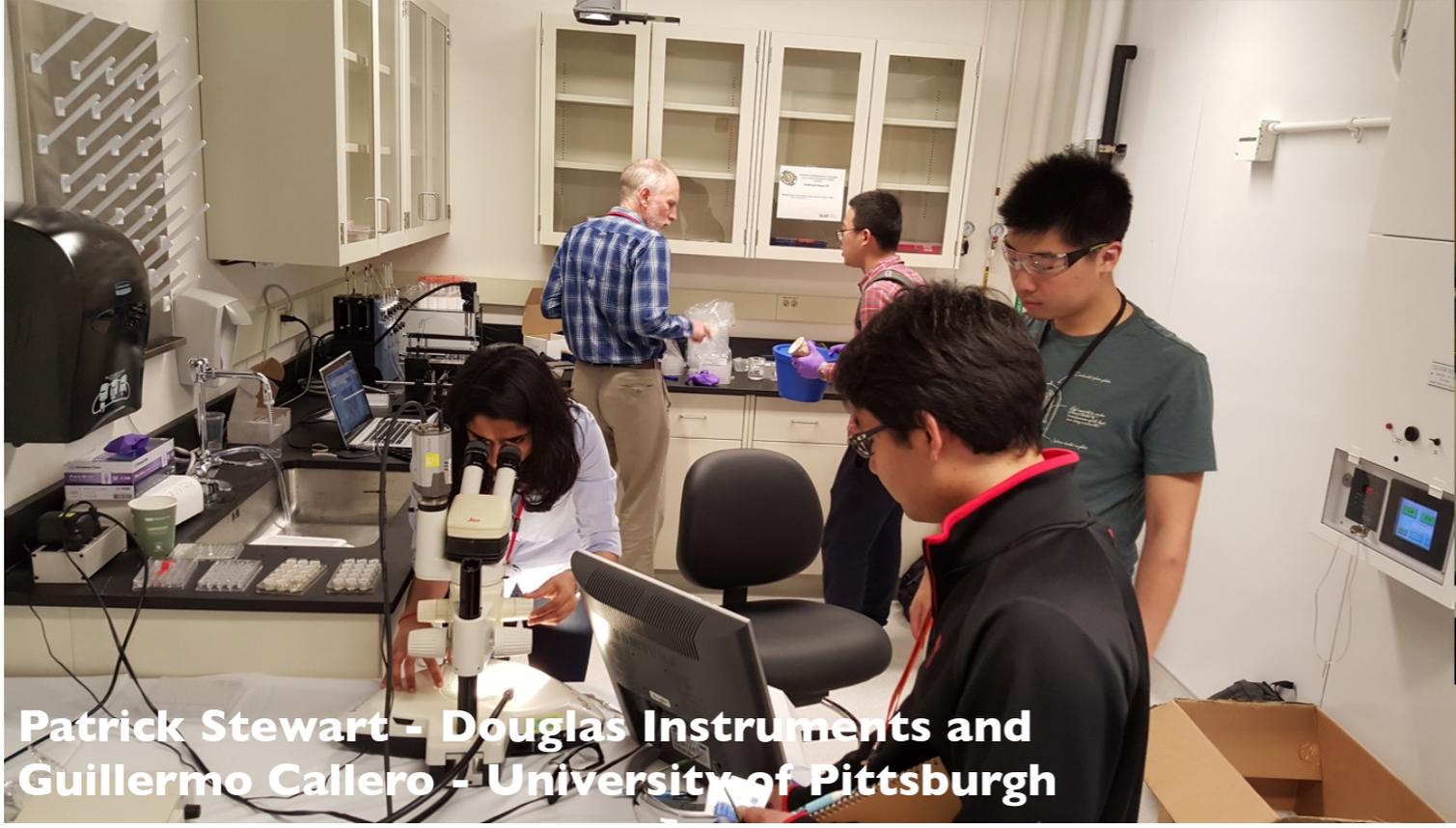


Eddie Pryor - Anatrace



Patrick Loll - Drexel University

Practical Sessions
Tutorials



Patrick Stewart - Douglas Instruments and Guillermo Callero - University of Pittsburgh



Raymond Sierra - LCLS

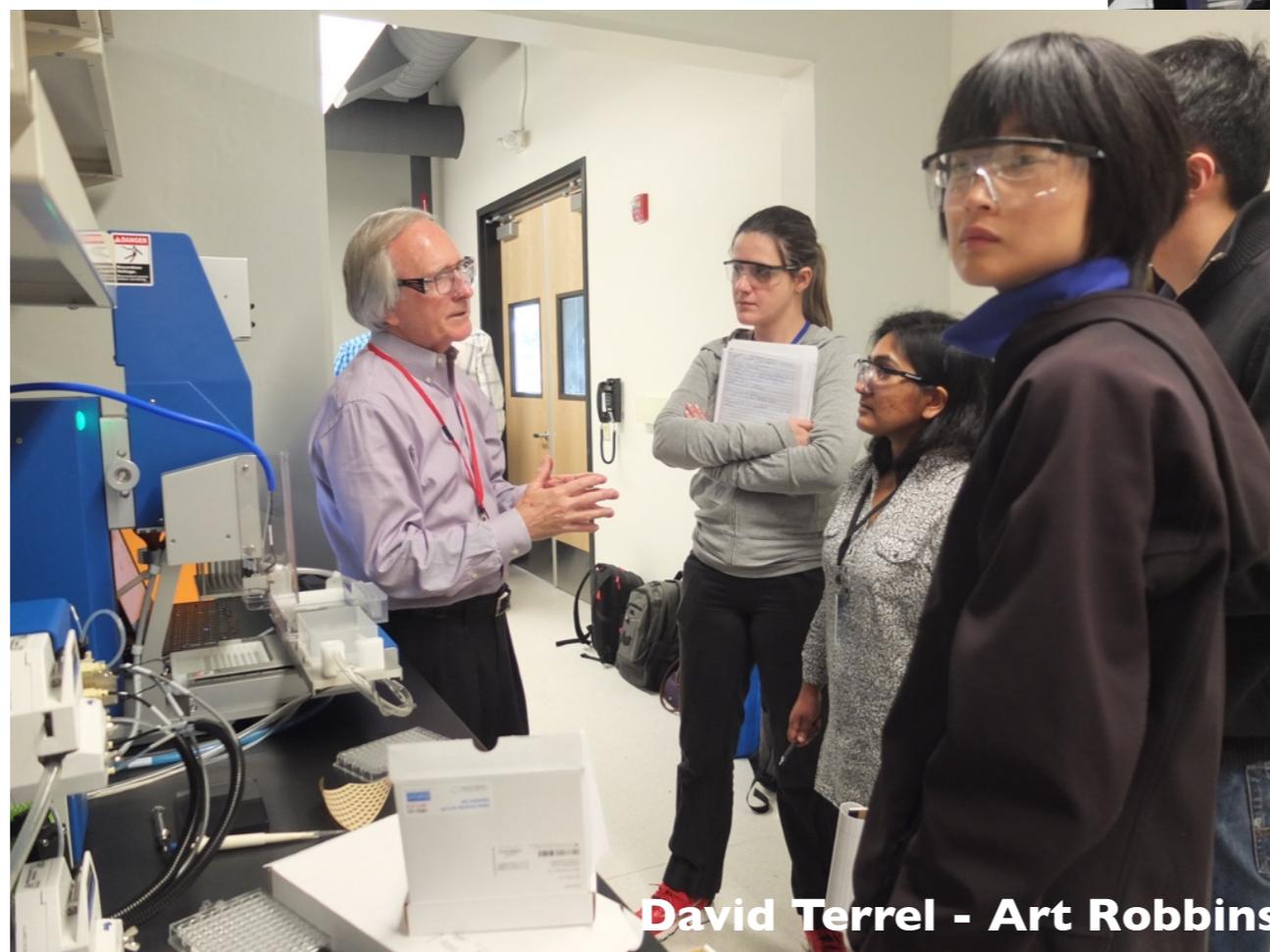
Practical Sessions *Tutorials*



Martin Caffrey - Trinity College



**Howard Lee and Tim Allison Labcyte
Liz Baxter SSRL**



David Terrel - Art Robbins



Estimated Contributions received as donations

Consumables and Supplies

U\$21,025.00

Crystallization Robots Companies

Consumables

Scientific and Professional

9.5 FTE full time 4 days

Academic Contributions

Membrane Proteins

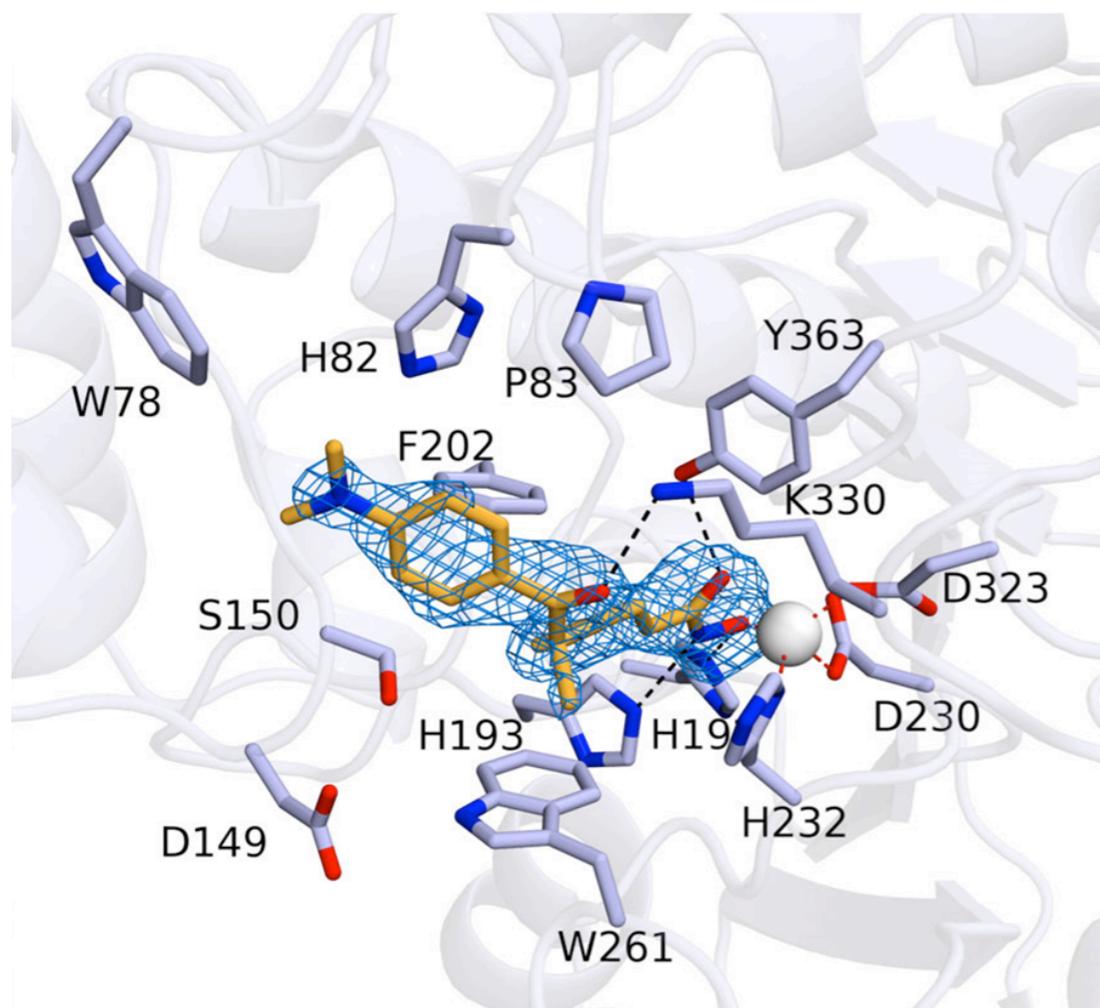
Specific Consumables used
in tutorials



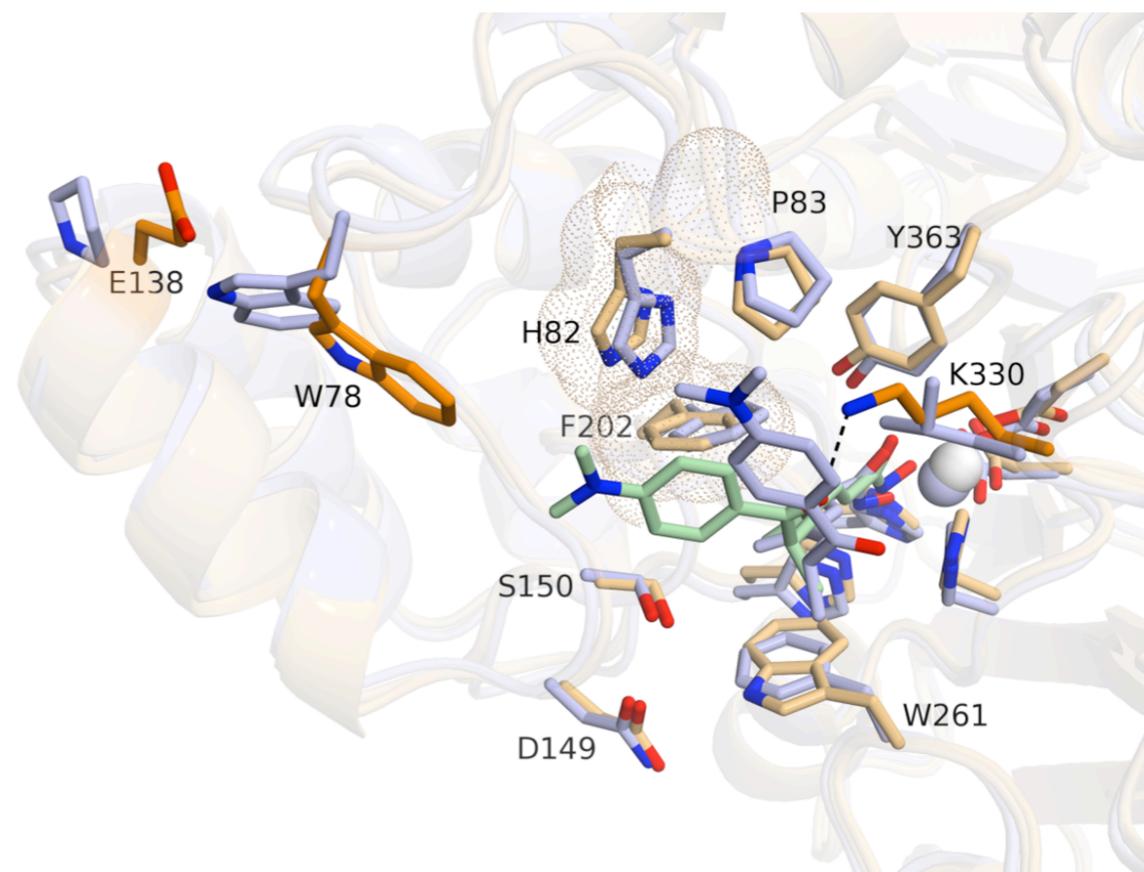
*... as a vendor it was very useful setting
REAL FEEDBACK*

The Christianson Lab – UPENN

Metal-dependant histone deacetylases is one of the subjects being investigated by the Christianson group. Graduate student Yang Hai determined the structures of human and zebrafish HDAC6, a sizzling hot target for cancer chemotherapy. Histone deacetylases promote high binding affinity between histones and DNA and are involved in a series of pathways, of importance for environmental processing and human diseases

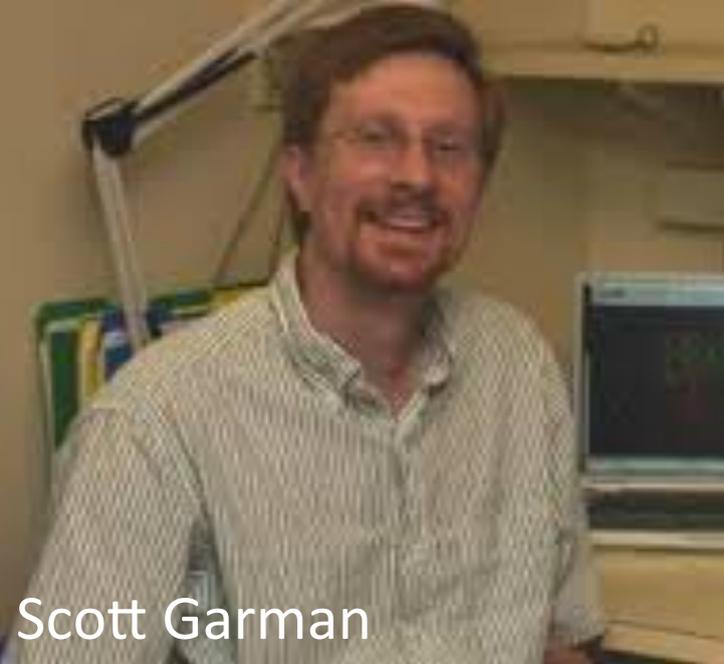


Simulated annealing omit map of zebrafish histone deacetylase 6 catalytic domain 1 in complex with trichostatin A



Structural comparison of CD1 and CD2 domain active sites of zebrafish histone deacetylase.

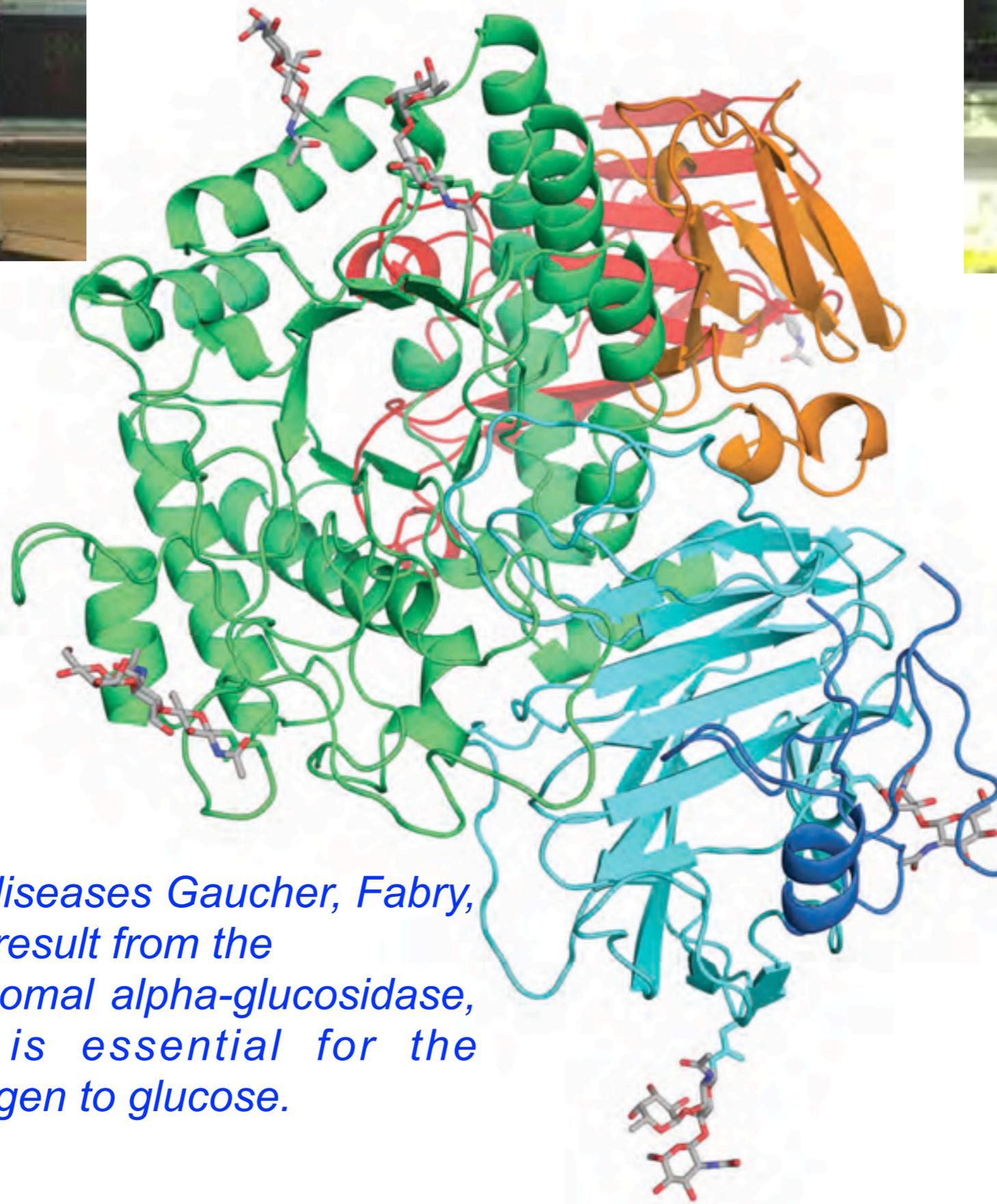
The Garman Lab UMASS



Scott Garman



Derrick Demming



Lysosomal storage diseases Gaucher, Fabry, Pompe, Tay-Sachs, result from the deficiency of Lysosomal alpha-glucosidase, an enzyme that is essential for the degradation of glycogen to glucose.

Ribbon diagram of Acid Alpha Glucosidase colored by domain. Trefoil (blue), N-terminal β -sandwich (cyan), catalytic TIM barrel (green), proximal C-terminal β -sandwich (orange), distal C-terminal β -sandwich (red), N-linked glycans (grey).
Courtesy D. Demming, PhD student in the Garman Lab.



The Ragusa Lab Dartmouth College

Michael Ragusa, Assistant Professor Dartmouth College. A former member of the Page Lab (Brown University), Ragusa is establishing his group at the Chemistry Department.

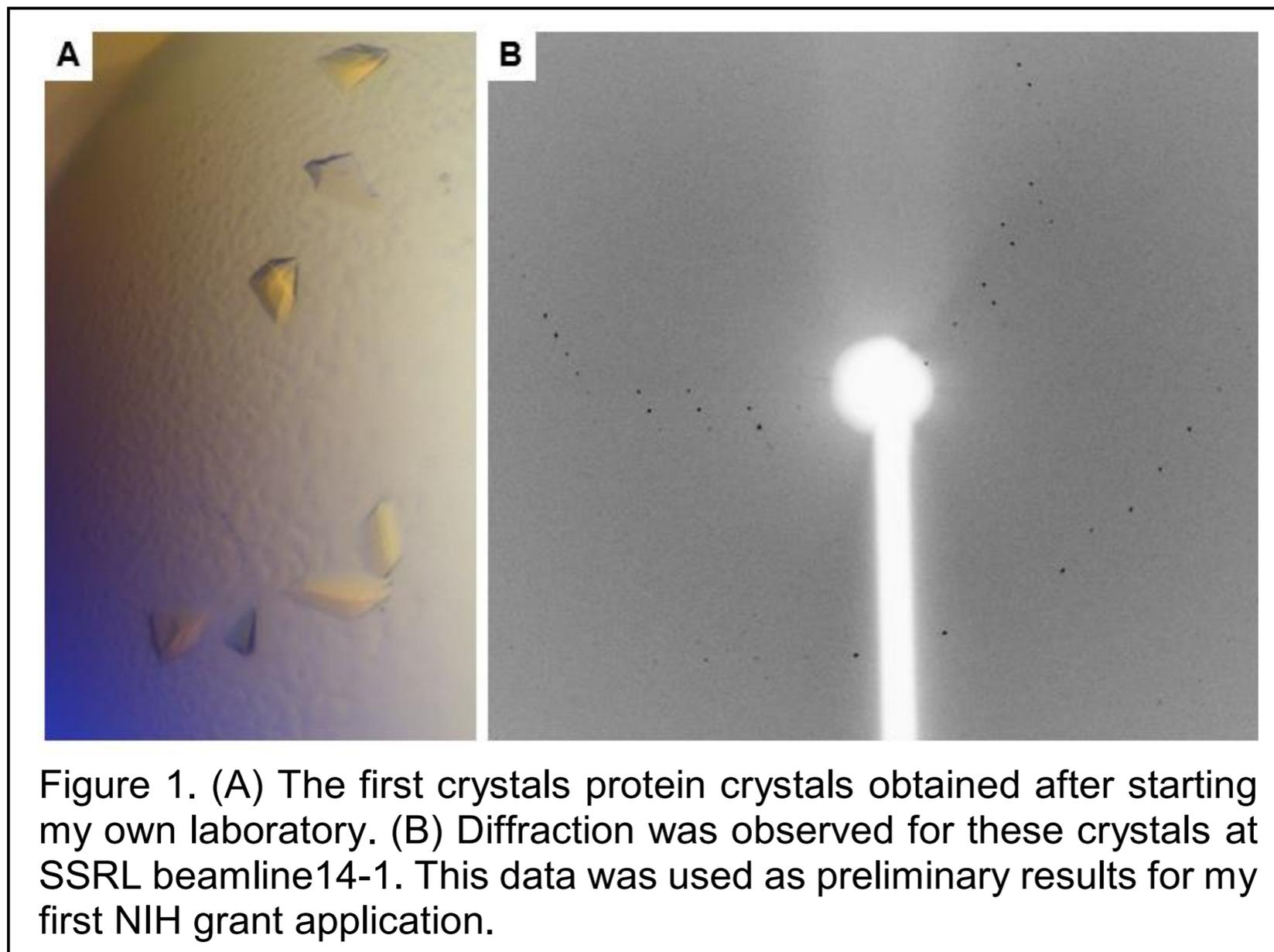
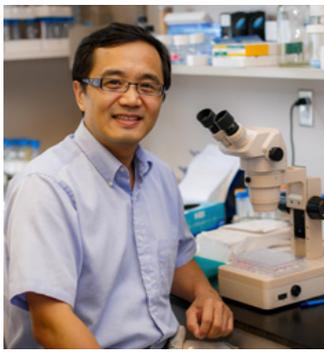
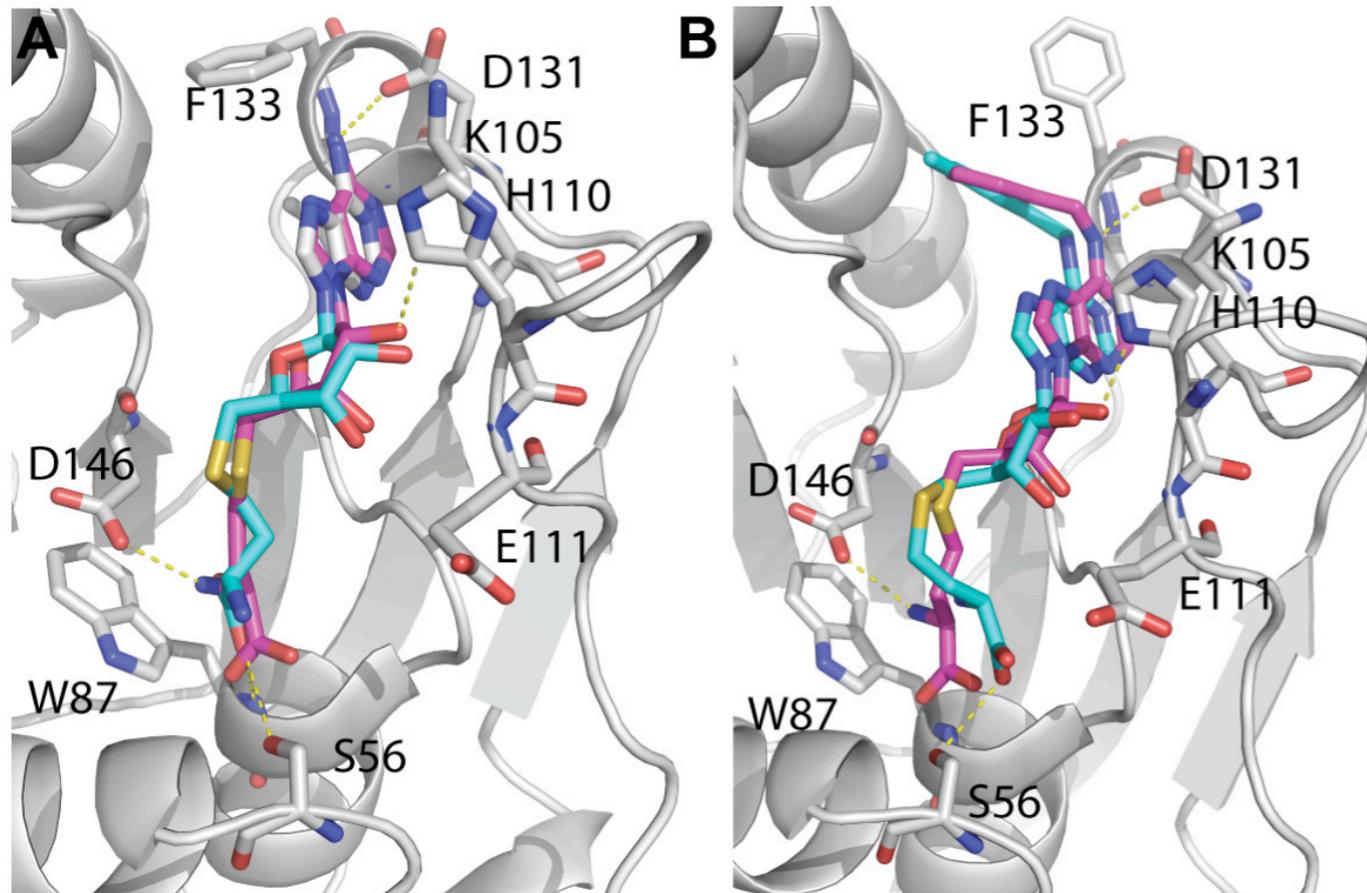


Figure 1. (A) The first crystals protein crystals obtained after starting my own laboratory. (B) Diffraction was observed for these crystals at SSRL beamline14-1. This data was used as preliminary results for my first NIH grant application.



Novel Broad Spectrum Inhibitors Targeting the Flavivirus Methyltransferase – Li Group

The flavivirus methyltransferase (MTase) is an essential enzyme that sequentially methylates the N7 and 2'-O positions of the viral RNA cap, using S-adenosyl-L-methionine (SAM) as a methyl donor. We report here that small molecule compounds, which putatively bind to the SAM-binding site of flavivirus MTase and inhibit its function, were identified by using virtual screening. In vitro methylation experiments demonstrated significant MTase inhibition by 13 of these compounds, with the most potent compound displaying sub-micromolar inhibitory activity. The most active compounds showed broad spectrum activity against the MTase proteins of multiple flaviviruses. Two of these compounds also exhibited low cytotoxicity and effectively inhibited viral replication in cell-based assays, providing further structural insight into flavivirus MTase inhibition.



Comparison of experimentally determined and docked conformations of SAH (A) and the SAH-based inhibitor 36A (B) in the SAM-binding pocket of the DENV3 MTase.

The MTase was in cartoon representation in grey color with representative contact residues in stick representation. Ligands (SAH or 36A) were in stick representation. Colors for atoms unless specified: oxygen, red; nitrogen, blue; carbon for MTase residues, grey; carbon for ligands (crystallography-determined), magenta; carbon for ligands (docked), cyan.

The following Slides are a summary of the Crystallization
Course we organized at SLAC