U[ers] Can’t Touch This
Remote Access with SSRL at SLAC

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SSRL-SMB-PX talks at MCE 2021

In case you missed it…

Tuesday March 16th
Aina Cohen
Next Generation Remote Experiments

Wednesday March 17th
Art Lyubimov
Crystallography Live: Processing and Analysis of Xray Diffraction Data in Real Time
Thank you for your attention!
Fast Tracked Research Leads to Drug and Vaccines in Clinical Trials

Synchrotron & CryoEM research started one year ago and LCLS in August 2020

SSRL BL12-1: Structure-guided inactivation of the SARS-CoV-2 spike protein using nanobodies (Koenig, Science 2021)

LCLS-MFX: SARS-CoV-2 main protease structures at near-physiological temp to guide drug repurposing (Durdagi, bioRxiv/2020)

Cryo-EM: Structure of spike proteins and glycans of human coronavirus NL63 directly from virus particles (Zhang, bioRxiv/2020/245696)

Groups from across the US and abroad used SSRL, LCLS and CryoEM facilities at SLAC for COVID-19 related research

Proposals awarded time: 51
MC Fragments/inhibitors screened: > 1088
MC PDB deposits: 38
MC Publications: 11
Remote Access with SMB at SLAC

- Standard @ SSRL/SMB
  - Remote Data Collection from anywhere in the world (Soltis et al., Acta Cryst. D64, 210, 2008)

- Advanced features @ Beamline 12-1
  - Serial and Dynamics
  - Elevated Temperatures
  - Humidity Control

- Look to the future
**NoMachine NX**
- Unix-based
- Access to internal network
  - Beamline control
  - SSRL data storage
  - Data processing

**Crystal server**
- Sample database
- Spreadsheets

**Blu-Ice Control Software**
- Experiment control, tcl/tk, C/C++
- Support enables user
- Simple and intuitive graphical interface
  - (McPhillips et al., J. Synch. Rad. 9, 401, 2002)

**User support**
- Zoom, phone, email
### Shipping Samples to SSRL

#### General Information
- **Name:**
- **Institution:**
- **Phone:**

#### Access Information
- **Type of Access:**
  - beam time
  - beamline staff
  - collaboration
- **Access Additional Information:**
  - Access Description:
  - User at SSRL: Yes
  - Removing Yes/No
- **Proposal Number:**
- **Note of Special Request:**
- **Are you the Principal Investigator on the Proposal?**
  - Yes
  - No

#### Sample Information
- **Number of Liquid Nitrogen Barrels:**
- **Total Number of Cavities:**
- **Total Number of Ice Pucks:**
- **Number of Blue Shipping Containers for Elevated Temperature:**
Spreadsheet based
- Online accessible
  - Editable
  - Upload/download
- Loads into Blu-Ice
  - Through beamline assignment (database or Blu-Ice)
Beamline control

Sample control
- Anneal, wash
- Queue next sample

Scans
- Excitation scans
- MAD scans
  - Runs AutoCHOOCH
  - Displays energies for $f'$, $f''$, plus a calculated remote energy
Beamline control

Sample control

- Anneal, wash
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UV-Vis Microspectroscopy @ BL9-2

- Non-invasive
- Confirm identity of reaction intermediates trapped within a crystal
- Monitor reactions occurring in the crystal
  - triggering by lasers or within flow cells
- Monitor X-ray induced structural changes
  - e.g. metalloproteins
  - Radiation damage
- Fully automated for spectroscopy between diffraction data (interweave)
- Both the X-ray exposure time and X-ray dose are recorded for each spectrum measured
- in-situ UV-vis microspectrophotometer
  - (Cohen et al., Protein Pept. Lett. 23, 283 2016)
Standard @ SSRL/SMB – Experimental Setup

Screening
- Automatic
  - Exchange samples in 25 seconds
- Loop centering (15 seconds)
- Raster alignment
- WebIce strategy
  - Requires 2 images

Rastering
- Microbeam, matching crystal size
- Polygon, oval, line
- 90° line raster for centering
- Scoring
- Diffraction-based crystal alignment (Song et al., J. Synch. Rad. 14, 191, 2007)

Art Lyubimov
(talked yesterday)

Diffraction images scored automatically
Results are displayed in Blu-Ice and WebIce and saved in spreadsheet
WebIce

- Mosaicity
- Oscillation
- Resolution
- Exposure time
- Number of images
- Detector, beamstop distances

>* Import collection strategy into Blu-Ice*

### Coming Soon

- Integrate into Blu-Ice
Standard data collection

- Rotation, wedge
- Helical

Beamline 12-1

- Microbeam: 5 μm² to 100 μm²
- High Intensity: 5 x10¹² photons/sec
- 5-15 keV range, S-SAD phasing optimized
- EIGER 16M (133 Hz or 750 ROI)
- Crystal rotation speeds <90°/s
- Instant feedback
- Automated hit finding + data processing
  - Any dataset more than 5 frames is fully autoprocessed in users’ directory
  - XDS, POINTLESS, XTRIAGE, AIMLESS, TRUNCATE
  - MOLEREP, REFMAC5
The Interceptor

- Live processing and scoring to track basic diffraction properties of a single crystals in real time
  - Resolution score
  - +max diffraction intensity score
  - Ice ring penalty
  - Elongated spot penalty
- Fully automated, persists in background
- Can handle high-speed bursts (~300 images) up to 100Hz
Serial Crystallography at SLAC
SSRL/SMB: 12-1 and MFX

Five Beamlines at SSRL dedicated to Macromolecular Crystallography Research

X-ray Free Electron Laser – LCLS-MFX Instrument

Synergistic User Operations
Similar Equipment & Controls Software

New Rapid Access Proposal Mechanism
BL12-1 / Gateway to LCLS

Aina Cohen (talked Tuesday)
Standard setups for goniometer and injector

Collaboration between SSRL-SMB & LCLS-HXR staff

Blu-Ice Control Software (developed at SSRL)
- User friendly - familiar to the user community
- Highly automated

High Speed Microcrystal Goniometer
- Rapid sample motions with +/- 0.5 µm SOC

Automated Sample Exchange with Stanford Robot
- Highly reliable (>1M samples mounted at SSRL)
- Cryogenic studies enable safe transport & storage
- Support room temperature samples at controlled humidity

UV-fluorescence microscopy and visual mapping of crystal samples (Barnes et al., PNAS. 116(19), 9333-9339, 2019)
Advanced Features – Serial Crystallography

Gas Dynamic Virtual Nozzle

Mobile Electro-spinning Sample Holder (MESH)

LCP/Viscous Media Injector

Mixing Injectors

Lois Pollack (Cornell) collaboration

A LCP injector has been acquired for BL12-1

Weierstall, Uwe et al. Nature communications 5 (2014)


Lois Pollack (Cornell) collaboration

Sierra, Acta Cryst D (2012)

Thin polymer crystallization chip

Megan Shelby, Deepshika Shamraj Gil bile, Matthew Coleman, Matthias Frank, Tonya Kuhl
LLNL, UC Davis
Advanced Features – Serial Crystallography

Fixed target

Full automation is possible if it fits within the robot gripper

If it has known dimensions and fiducials, we can automate it!

http://www.mitegen.com/
Cohen, et al., PNAS (2014)
Gati et al. IUCrJ (2014)
Gati et al., IUCrJ (2014)
Heymann et al. IUCrJ (2014)
Sui et al. Lab Chip, 16 (2016)
Reactions triggered within crystals during (or prior to) data collection (μs to ms time scale)

- **Microfluidics to trap/mix solutions around crystals**
  - Sarah Perry and Shuo Sui (UMass Amherst)
  - SMB-MX Group (SLAC/SSRL)

- **Liquid/Crystal Mixing Injectors**
  - Lois Pollack (Cornell) collaboration

- **Light activation/Caged Compounds**
  - Jonathan Clinger (Cornell U)
  - SMB-MX Group (SLAC/SSRL)

- **“Kinetic crystallography”**

- **Cryo-trap intermediates**
  - In assembly for BL12-1
Validating intermediate states
Freeze-trap reactions in cryostream at fixed time-delays after adding substrate to crystal

Monitor/Follow Radiation Damage
Radiation damage is accrued site-specifically around metal centers

Support for photo-triggered reactions
UV light for uncaging compounds, activating reactions
All automated!

Fully remote pump probe (timing control)
On-axis system to be installed at BL12-1 and in planning for MFX
**Time-resolved studies of transcription**

Remote collection @ 12-1 crystals exposed to UV light (breaking cage) temperature increase from 100K to 170K rapid helical data collection (2sec/dataset)

Illumination with the UV source breaks the nitrobenzyl group (NPE) *in crystallo* allowing ATP release from RNA-Pol II, metal coordination (blue) and phosphodiester bond formation (red).

The Calero Lab (University of Pittsburgh)
Advanced Features – Elevated Temperatures

- Measurements at physiological temperatures
- Understanding temperature effects in protein structure and interactions
- Potentially no additional disorder from cryoprotectants
- Side chain conformation insights
- Time scale resolution, transient steps

Aina Cohen
(talked Tuesday)
Advanced Features – Elevated Temperatures

First used at LCLS-MFX, but now standard at 12-1

Aina Cohen (talked Tuesday)

Samples inside at controlled humidity
**Fraser Group** (UCSF - QBI Corona Virus Research Group)

- Studies of conserved “macro domain,” enzyme shown to promote virulence in coronavirus
- Screening of fragments done at BL9-2 and BL12-2
- Measurements at physiological temperatures associated with infection in humans were done at BL12-1
- Understanding how temperature affects the enzyme structure and interactions may provide insight to develop antiviral therapeutics
Advanced Features – Hydration/Dehydration

Bovine Liver Catalase
- starting RH=96%, final RH=90%, 1% RH steps,
- 300 seconds equilibration time between steps

Humidity range of 30.0% to 99.8% ±0.05% RMS
Advanced Features– Hydration/Dehydration
Arinax Humidity Control Device
Automated switch between humid RT or cryo-conditions
- Flash cooling after controlled dehydration experiments
- Samples both cryo and RT supported for same beamtime
- Flash-cooling experiments to trap reaction intermediates

Flash-cooling 60 ms to switch

In assembly for BL12-1
Advanced Features – Nozzle Switcher

Cryo-trapping Dynamics
Use in conjunction with light, spray, drop

Cryo-trapping Experiment to Observe Phytochrome Photoconversion Intermediates

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- Look to the future
To the future!

- Multi-crystal database
- Blu-Ice Strategy
- Dynamics automation
  - Timing
  - Automate EIGER with gonio
    - 90°/second, fast framing, all remote
- Optical sources, Raman installment
  - More sources! More fibers!
- Nozzle switcher installment
- New optics: CRLs, multilayer monochromator
- EIGER2 XE 16M
  - move EIGER 16 to BL12-2
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Irimpan Mathews   Henry Meier

LBNL/ALS
James Holton

If you have additional questions, please email me at jwierman@slac.stanford.edu
Questions we ask ourselves

- How much processing do we automate?
- How much data collection automation/autonomous?
  - The thought: the further the user gets from the nuts and bolts of data collection, the less they appreciate intricacies in the data.
  - “Automation is more work for staff, less for users” - N.P.
- When do we intervene with data collection?
  - e.g. “Yikes, they are totally missing the crystal!”
- Where does data come from for strategy?
  - Can users override acquired data?
  - Does every collection generate a strategy?
- How do we handle storing data?
  - Cloud?
  - Massive physical storage?
  - Junk it!
- How to teach new users?
Front End Glamour Shot