Goniometer-based Diffraction of Single Crystals

Paul Scherrer Institut
Jan 26, 2015

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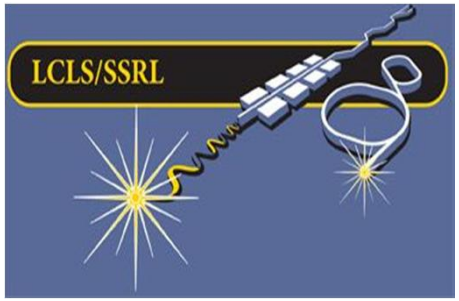
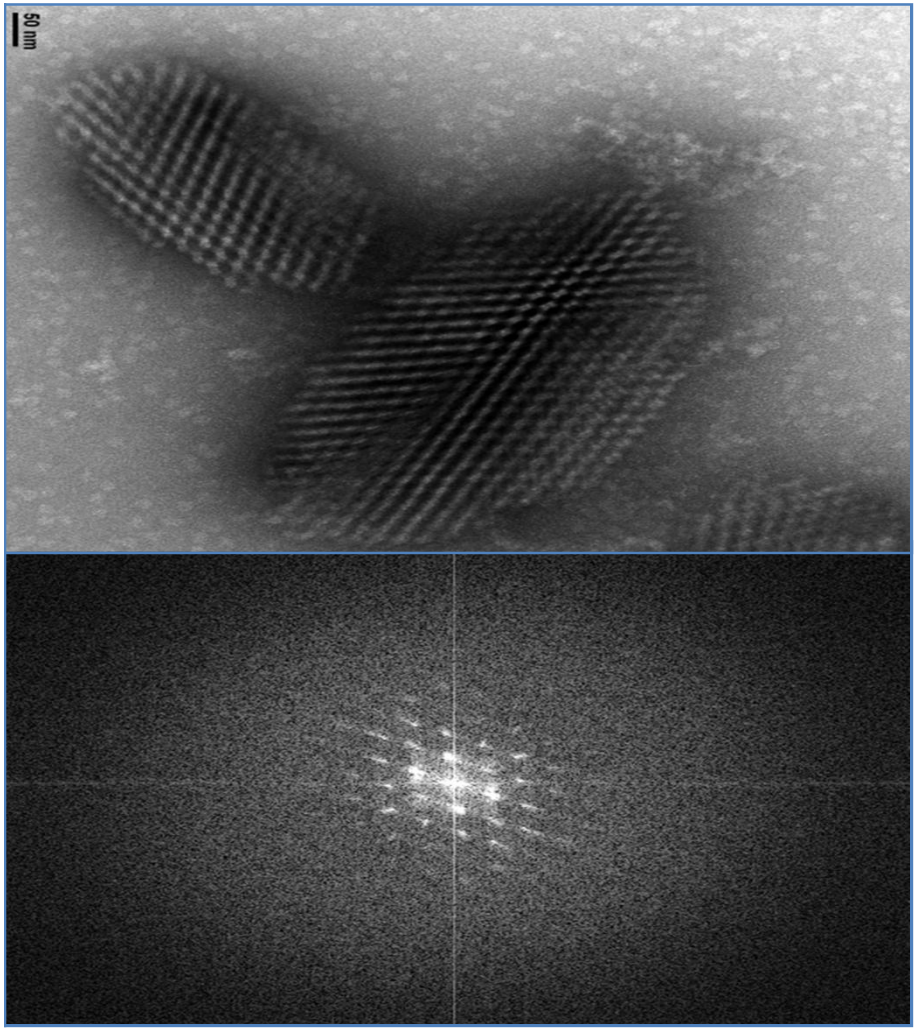
SLAC
NATIONAL ACCELERATOR LABORATORY



TEM Imaging of Nano-Crystals

Calero Laboratory
Structural Biology Department

FEI Tecnai T12

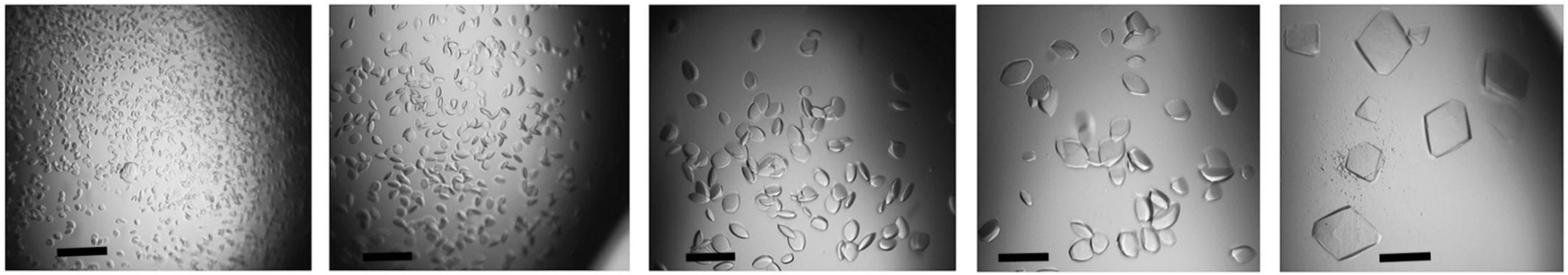


RNA Pol II with TFIIB + 50-merDNA / 5-merRNA hybrid

Stevenson H. P *et al.* (2014) *Proc Natl Acad Sci USA* 111, 8470-8475.

Controlled Seeding

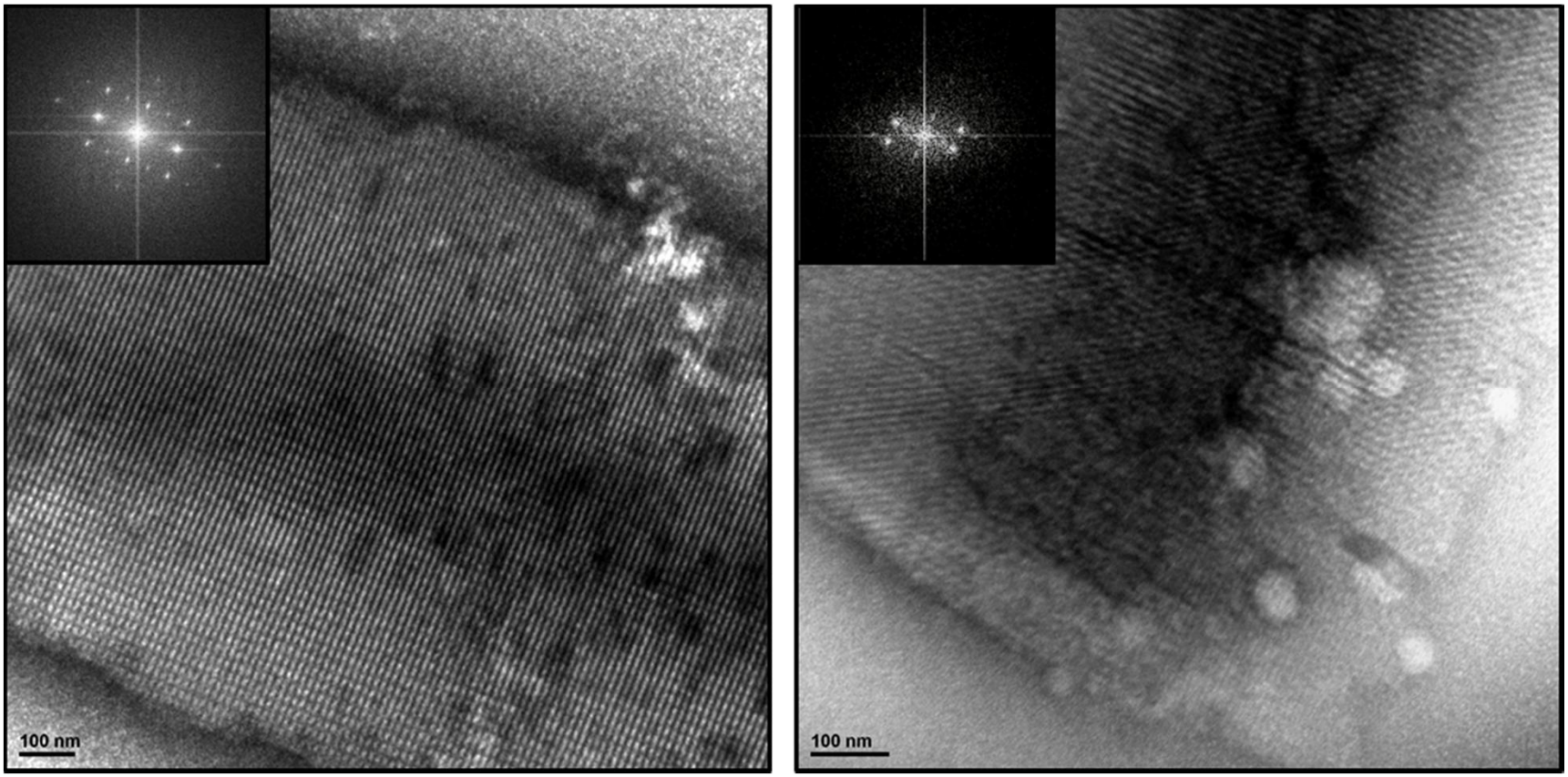
- Crystallization condition optimization
- Nano-seeding experiments
- Controlled crystal size



RNA Pol II with TFIIB + 50-merDNA / 5-merRNA hybrid

Calero G. *et al.* (2015) *in preparation*

GDVN Injector Tests



Pre-Injector

Post-Injector

TEM images of Pol II-GFP crystals pre- and post-injector

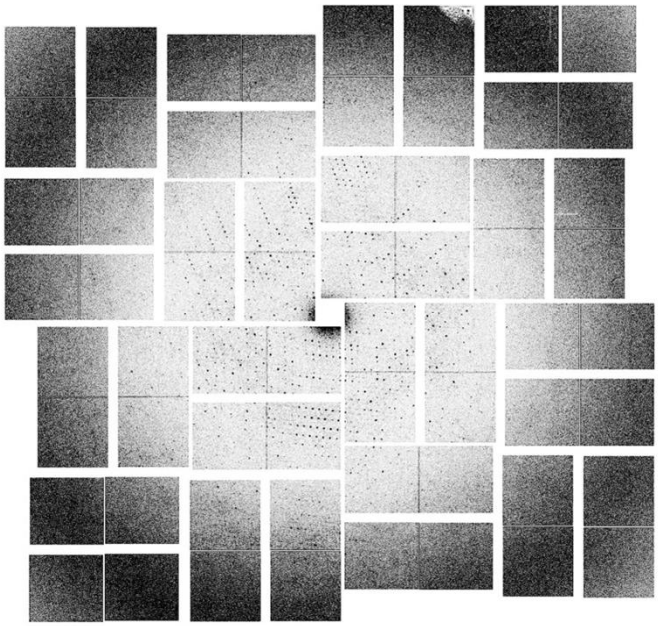
Stevenson H. P., *et al.* (2014) *Philosophical transactions of the Royal Society of London. B, Biological sciences* 369(1647).

Comparison of Methods using Crystals of a Large Multi-Protein Complex

RNA Pol II with TFIIB + 50-merDNA / 5-merRNA hybrid

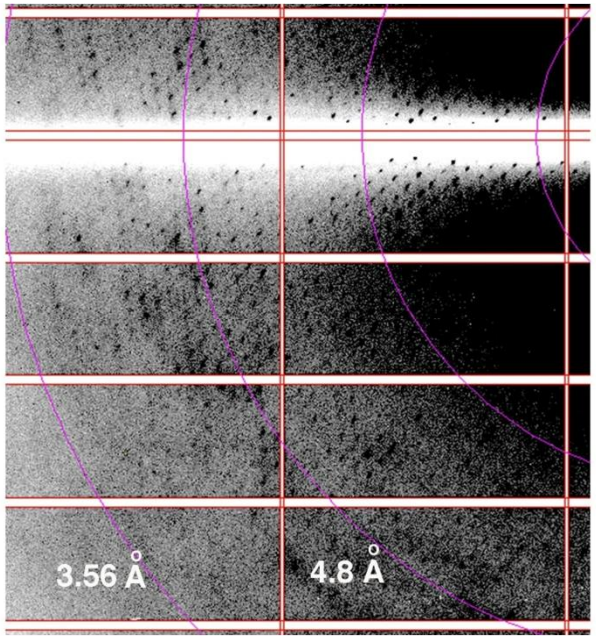
- Unit Cell: $P2_12_12_1$ a = 220 Å, b= 252 Å, c = 410 Å
- TEM used to identify nano-crystals in crystallization screen
- 5 µm and smaller crystals screened at LCLS-CXI (November 2013)
- Larger crystals (20 µm to 50 µm) obtained through controlled seeding
- Screened at micro-focus beamline SSRL 12-2 and LCLS-XPP (December 2013)

LCLS CXI



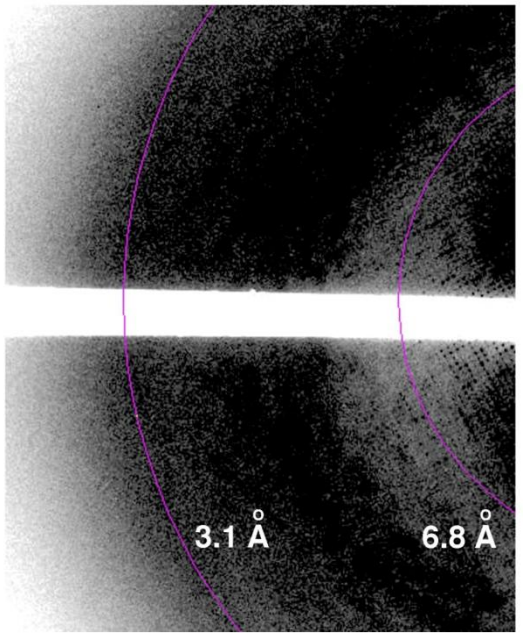
4.0 Å

SSRL 12-2



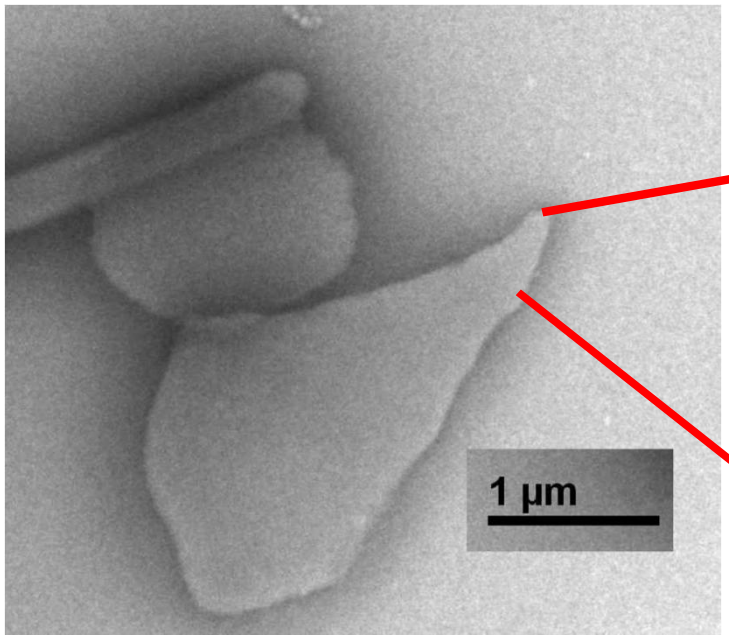
3.7 Å

LCLS XPP

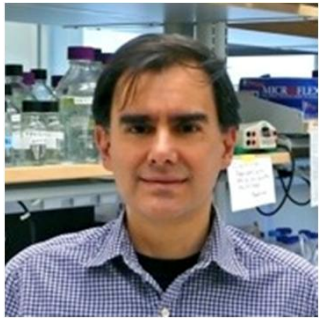


3.3 Å

GDVN Injector Screening at LCLS-CXI

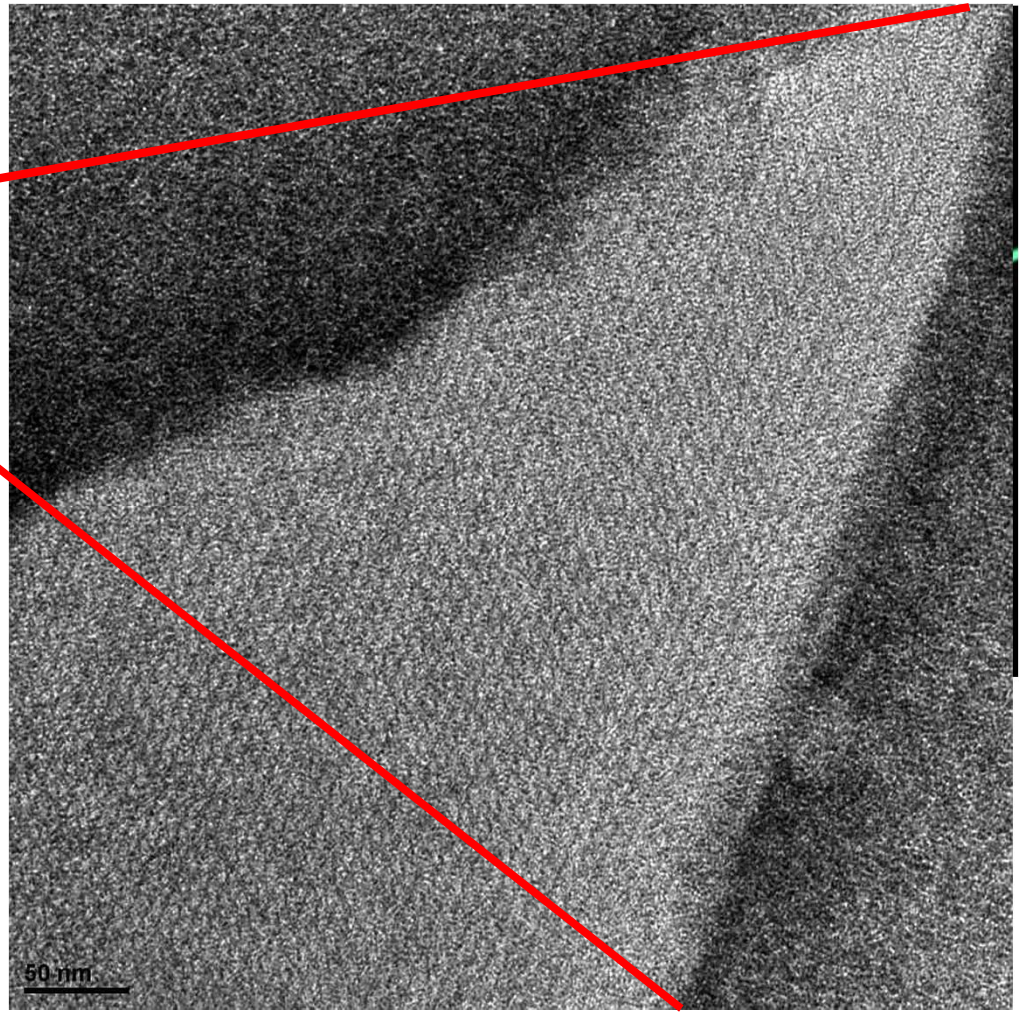


TEM analysis of HPCD crystals used for GDVN screening



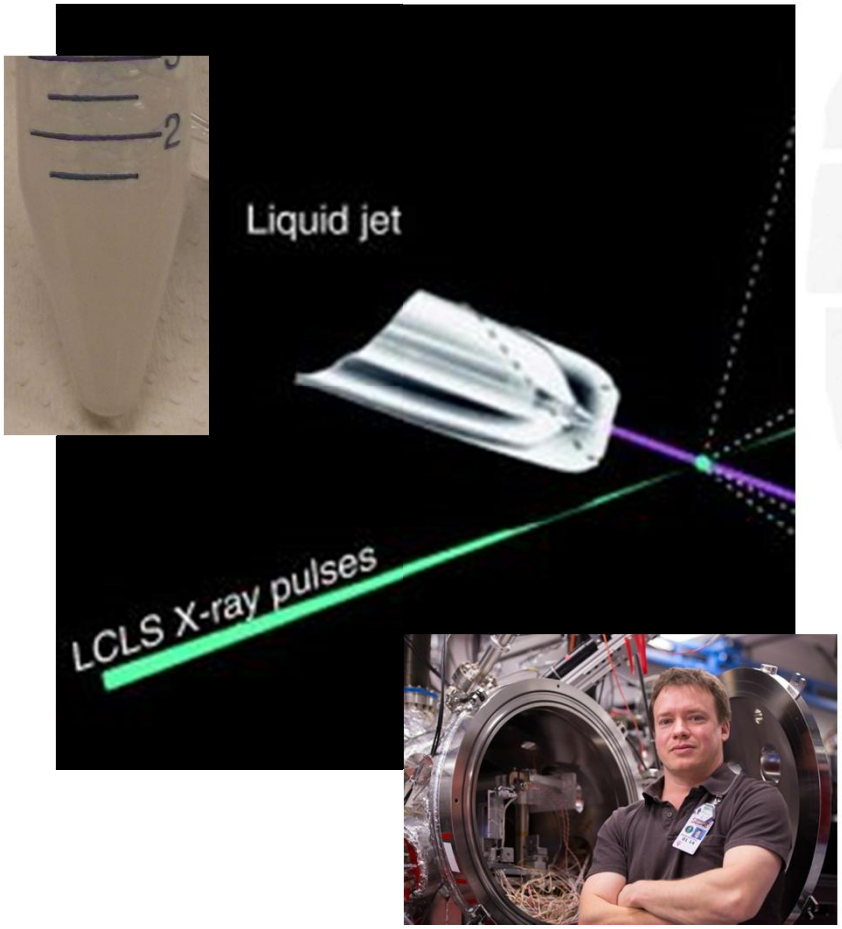
Guillermo Calero (Pitt)

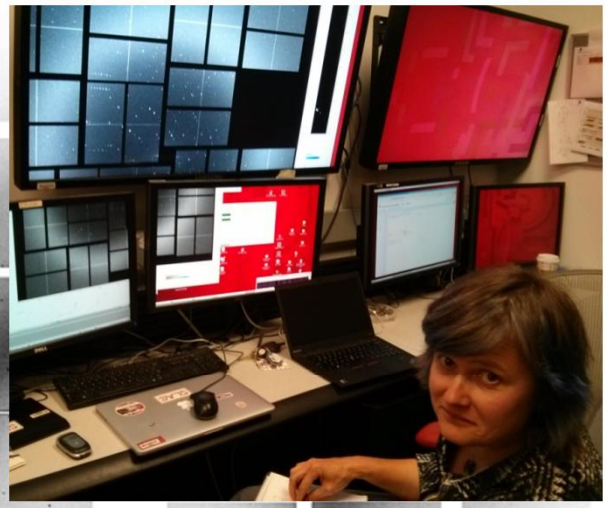
Elena Kovaleva (SSRL)



GDVN Injector Screening at LCLS-CXI

CXI July 14th 2014
HPCD in the GDVN injector
10 Minutes of Beamtime

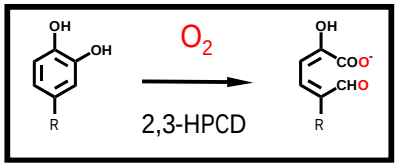




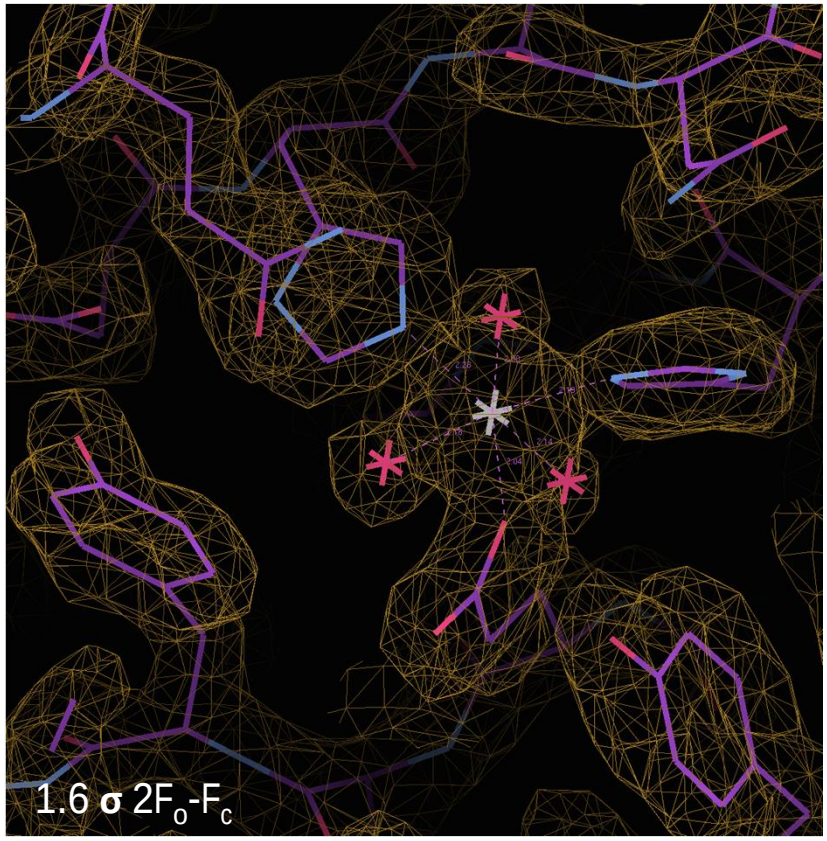
Diffraction Pattern of HPCD crystals
using the GDVN injector at CXI

10 minute structure of a Dioxygenase collected at LCLS-CXI

HPCD – Aromatic ring-cleaving dioxygenase from *B. fuscum.*
An extradiol dioxygenase that activates molecular O_2 to catalyze oxidative ring-opening of aromatic substrates.



John D. Lipscomb Group
University of Minnesota



Data collection, processing and refinement statistics

Collection time	10 min
Program	cctbx.fel
# images merged	5431
Spacegroup	$P2_12_12$
Cell dimensions	a = 112 Å, b = 155 Å, c = 102 Å
Resolution range	21.3 – 2.0 Å
Total reflections	1,328,339
Redundancy	12.9 (1.6)
Mean I/σI	12.8 (2.5)
$CC_{1/2}$	77.3% (20.5%)
Completeness	91.2% (54.7%)

Aaron Brewster

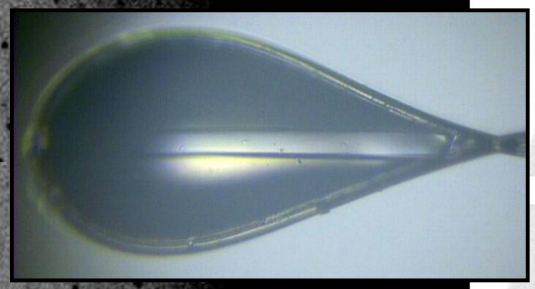
Refinement (REFMAC)

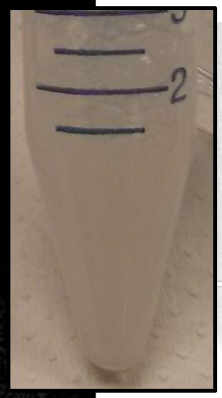
R/R_{free} = 20.9/27.5, FOM = 0.81

Macro-Crystals / Nano-Crystals

XPP May 2014
Long Crystals
Typical pattern

1.6 Å



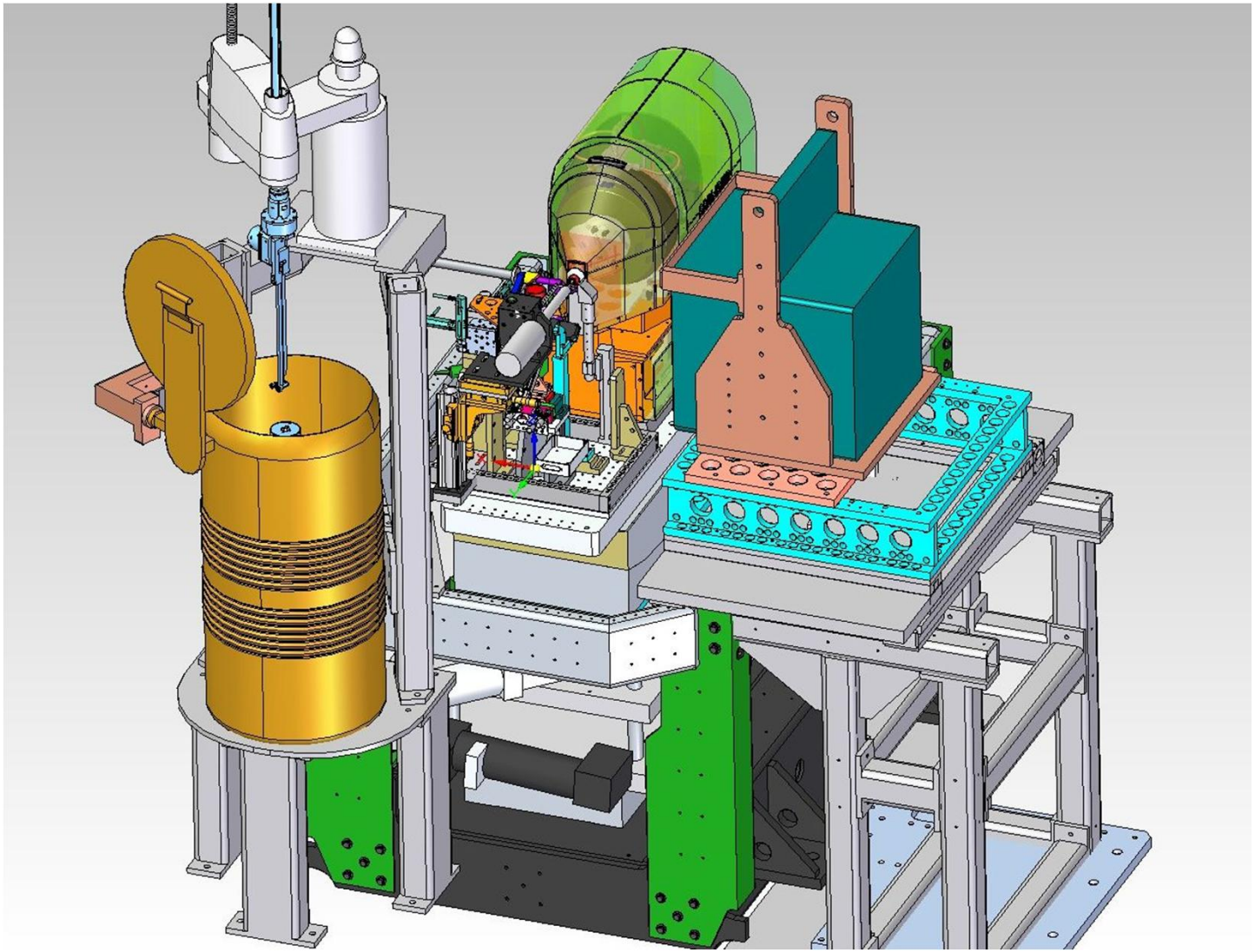


CXI July 2014
Injector with nano/micro-crystals
Best pattern

1.7 Å

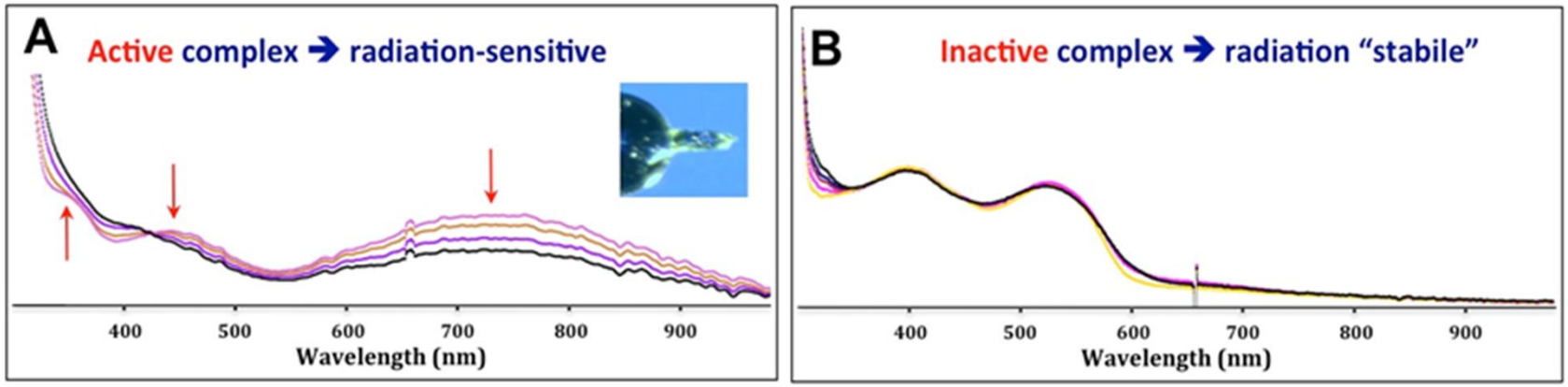
Goniometer Setup at LCLS-XPP

Collaboration between LCLS-XPP and SSRL-SMB



Experimental Goals

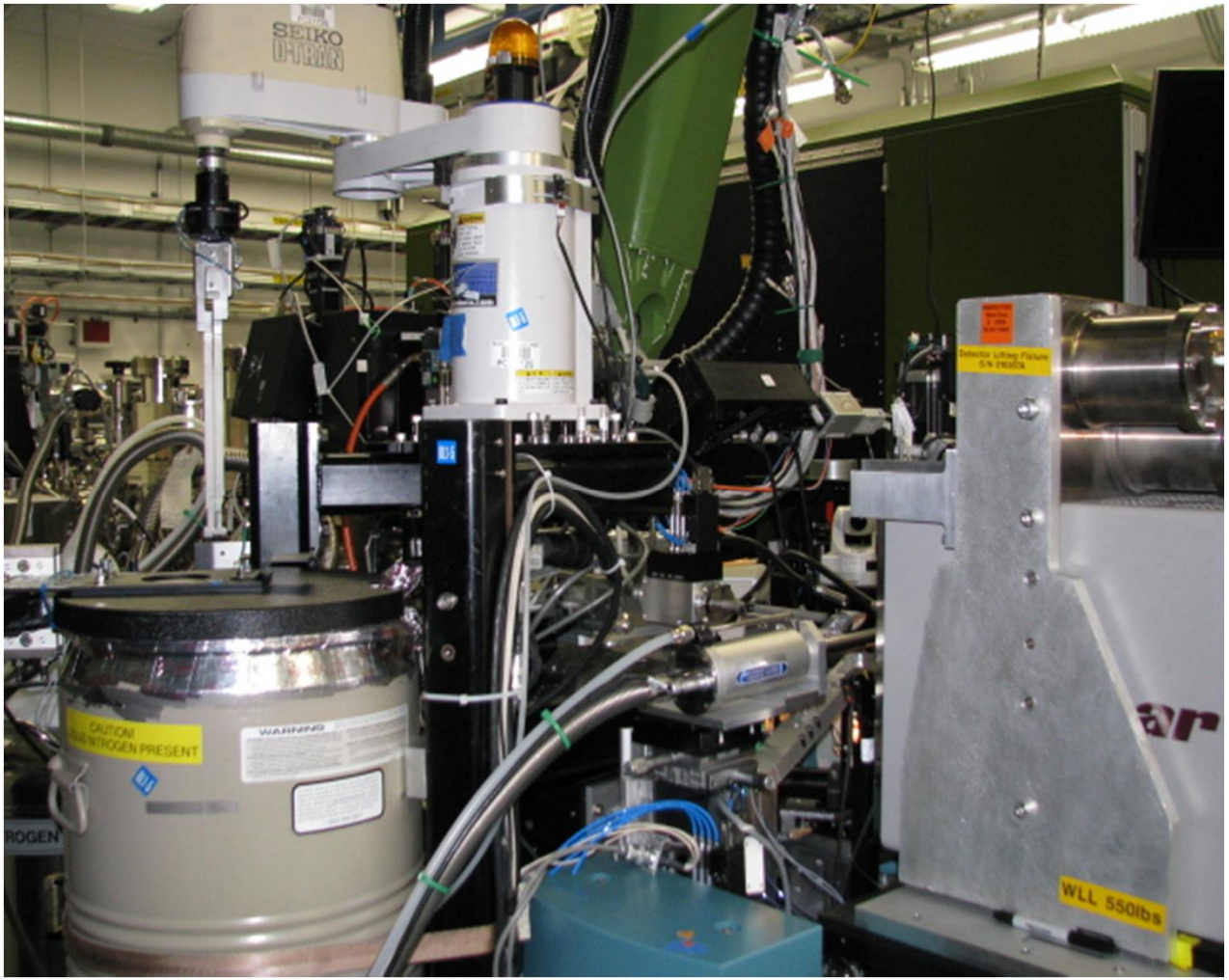
- Extend the diffraction resolution obtained from weakly diffracting crystals
- Room temperature studies
  - Study protein conformational dynamics
  - Monitor reactions within crystals – time resolved studies
  - Problems with cryo-protection
- Chemically accurate structures of metalloenzymes



Stability of Fe-coordinated intermediates of HPCD in the X-ray beam monitored by UV-Vis spectroscopy at SSRL BL11-1

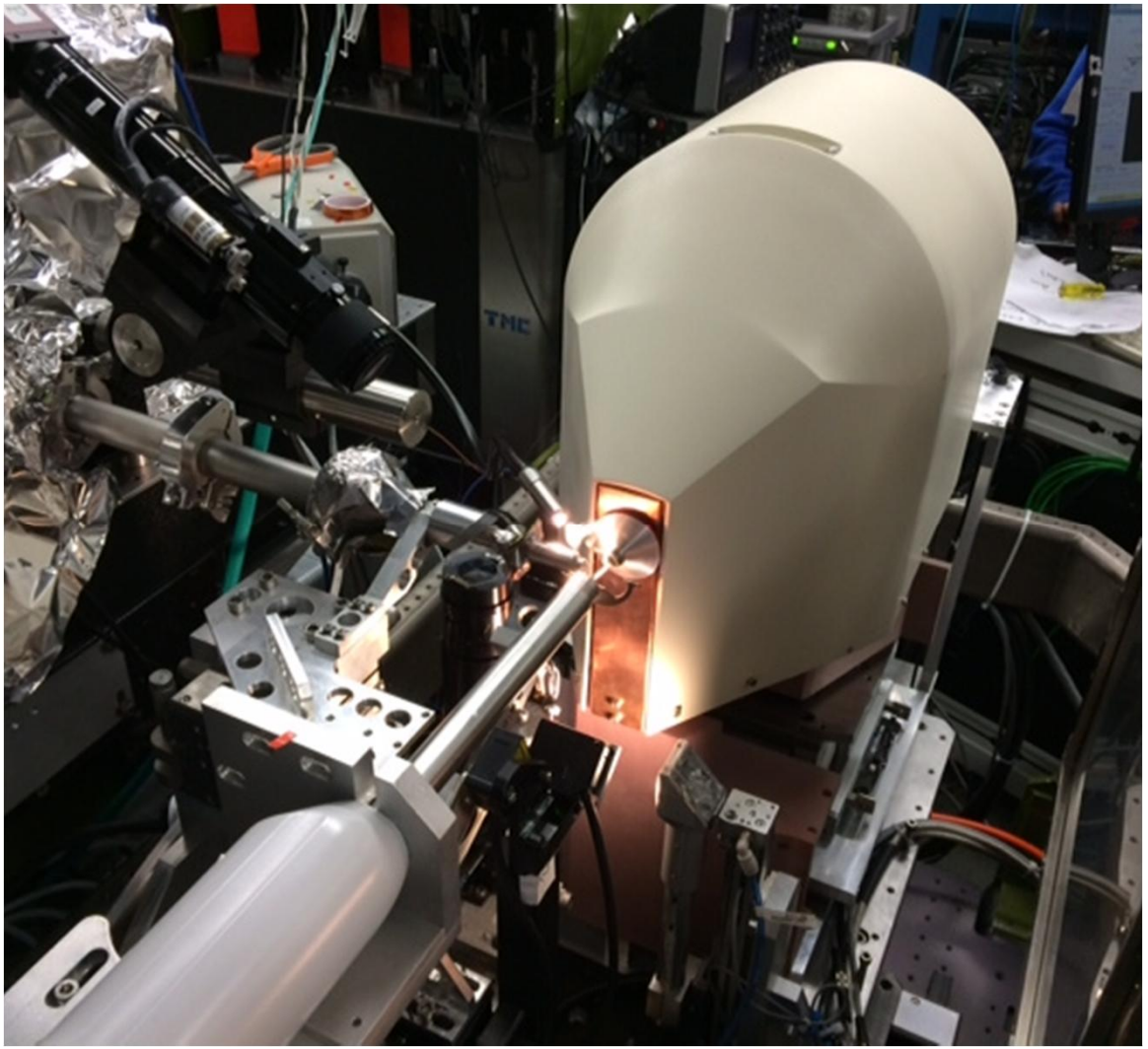
Goniometer Setup at LCLS-XPP

Collaboration between LCLS-XPP and SSRL-SMB

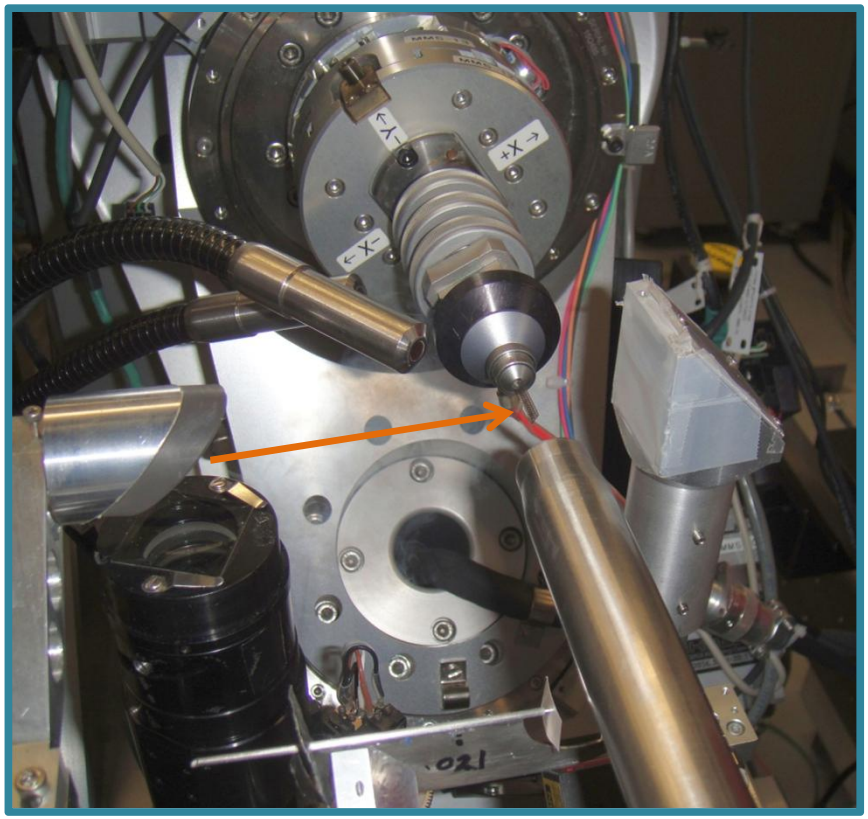


Goniometer Setup at LCLS-XPP

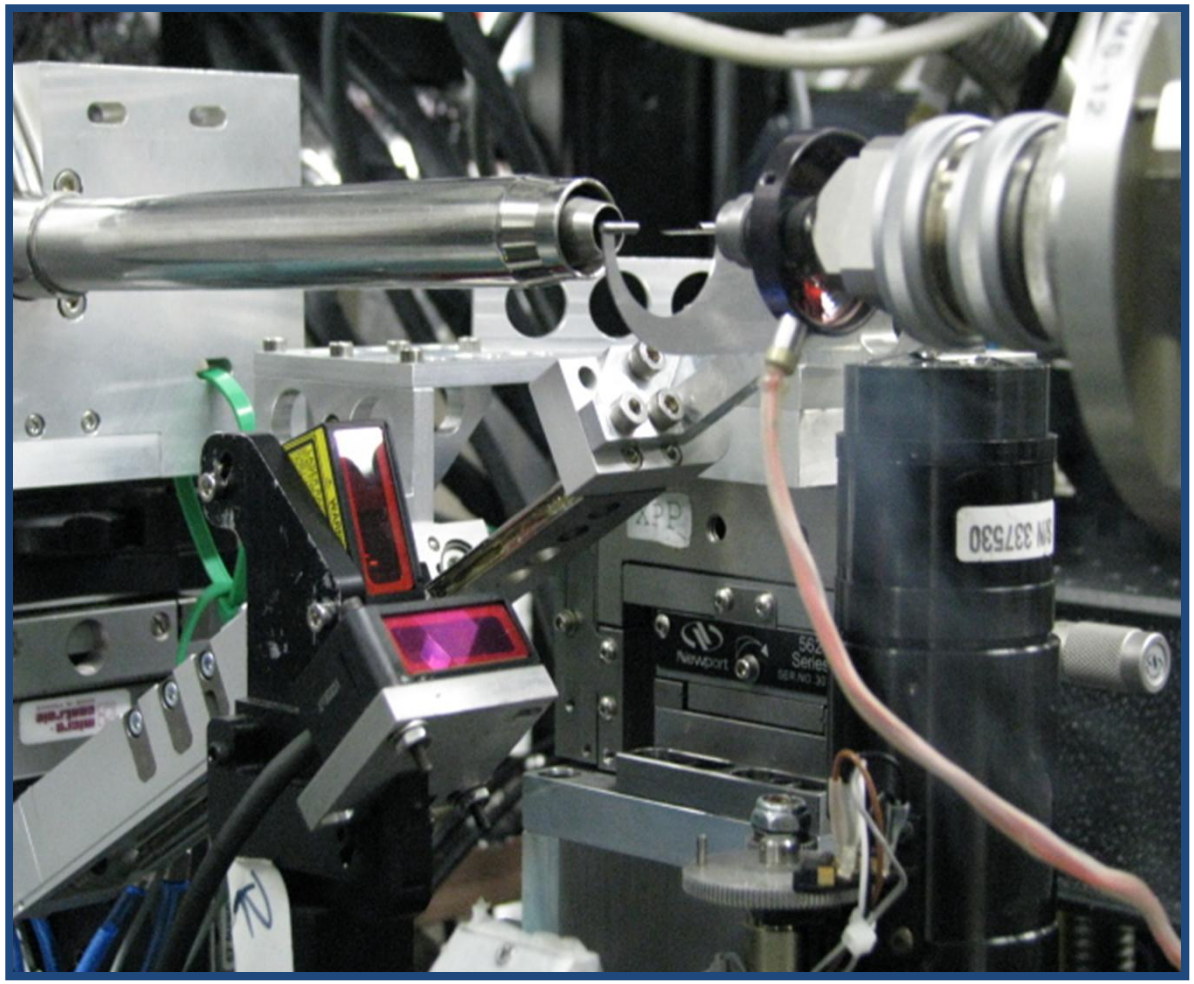
Collaboration between LCLS-XPP and SSRL-SMB



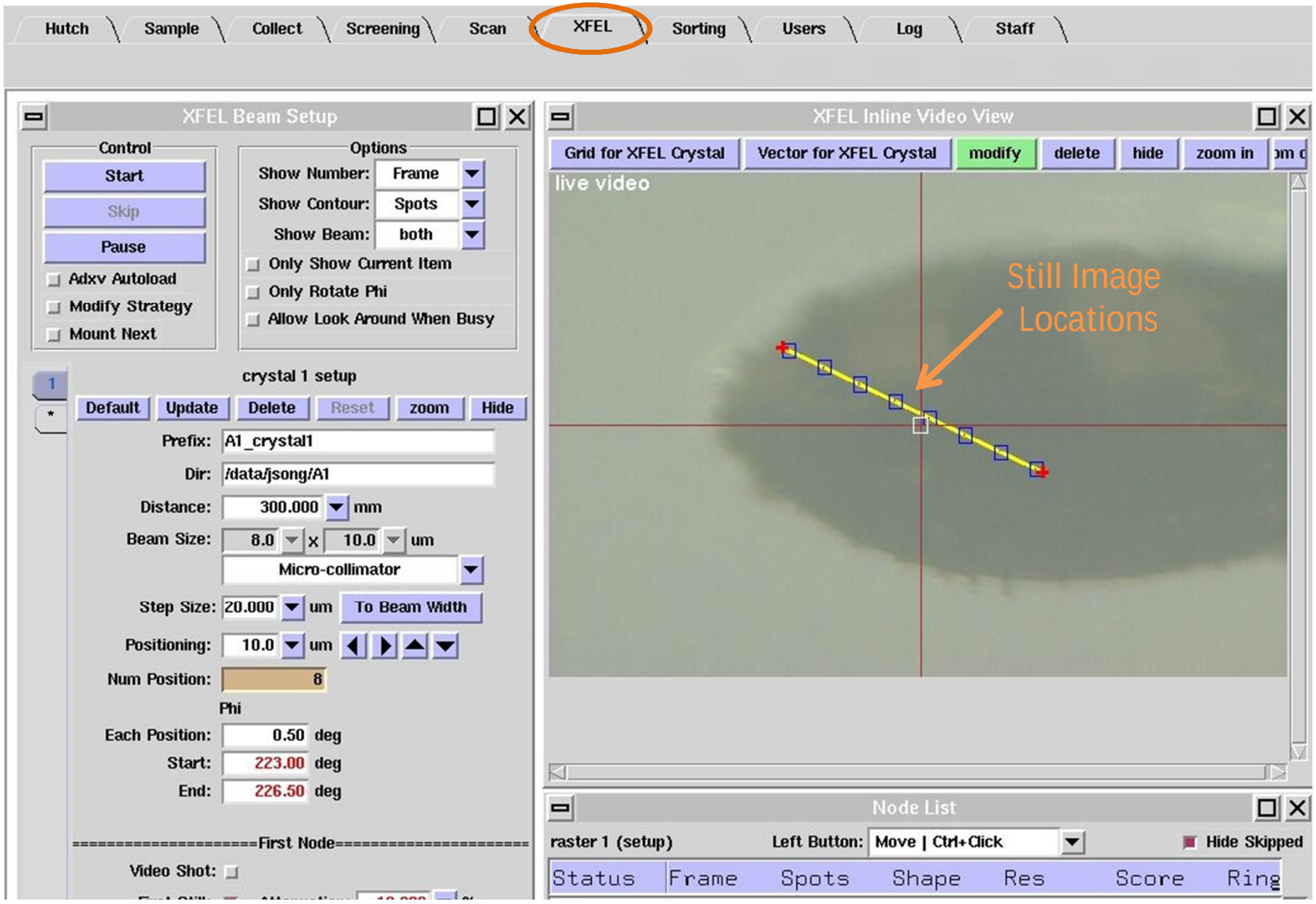
Goniometer Setup at LCLS-XPP



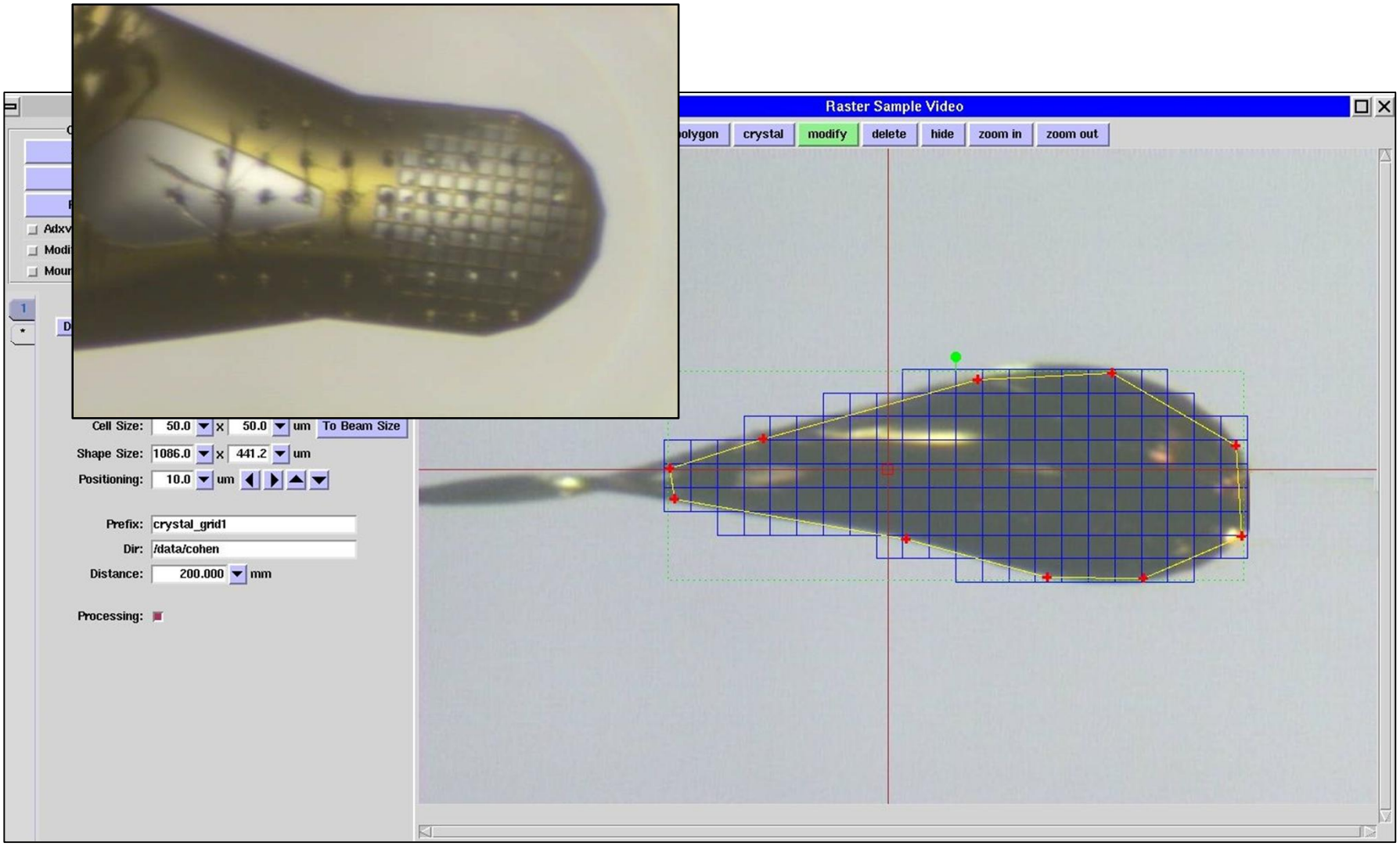
Goniometer Setup at LCLS-XPP



Blu-Ice Control System



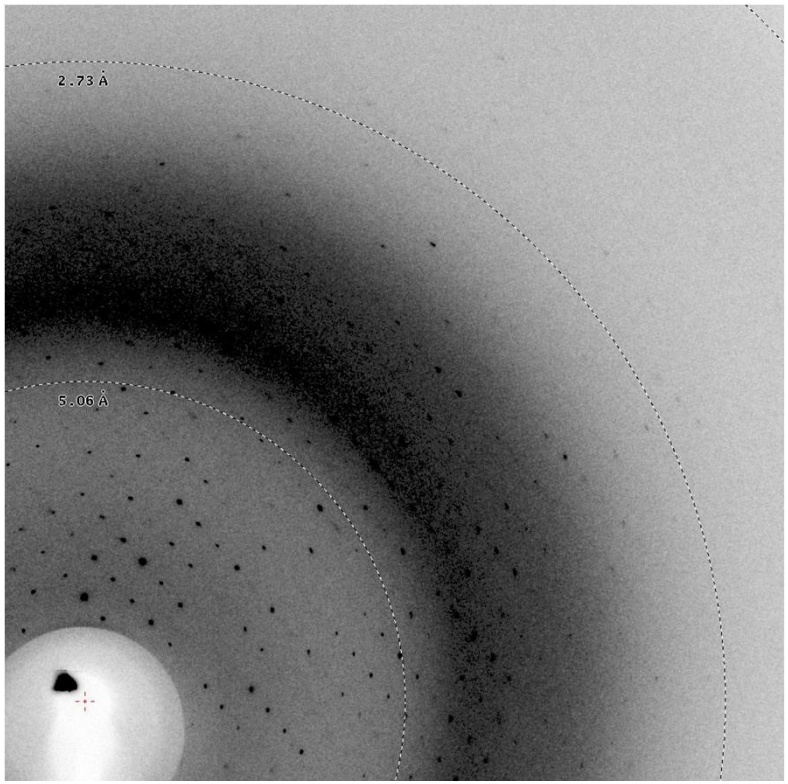
Raster Data Collection Mode



Raster Data Collection

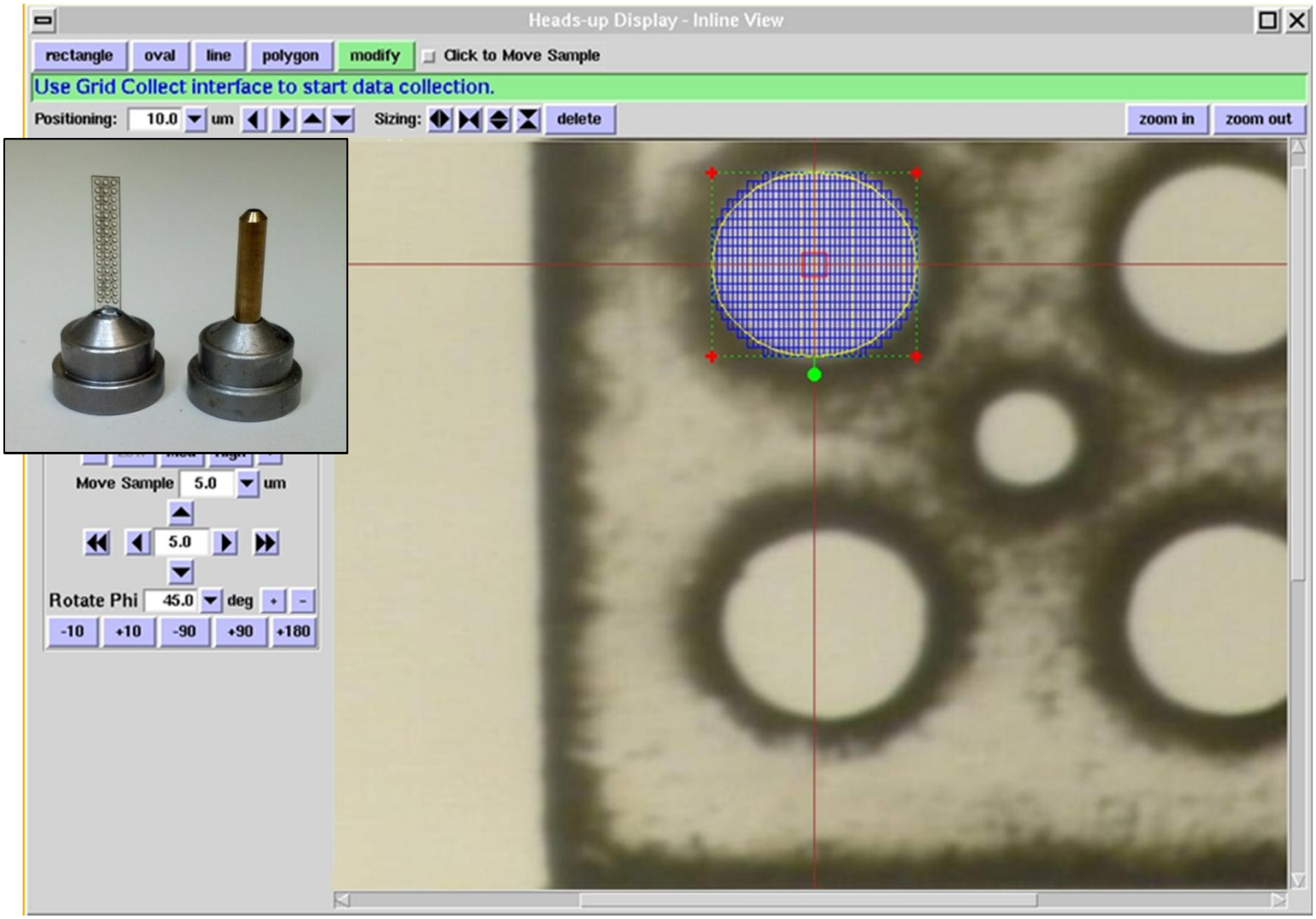
X-ray Diffraction of a GPCR Crystal at LCLS-XPP

Best diffraction to 2.3 Å (2.8 Å at achieved at APS)
Typical diffraction to 2.9 Å in most frames (typical from APS 3.3 Å)
Anecdotally, diffraction spots show less “streaky” shape.



Bill Weis, Brian Kobilka et al., June 22, 2013

Rastering within
High Density Sample Containers

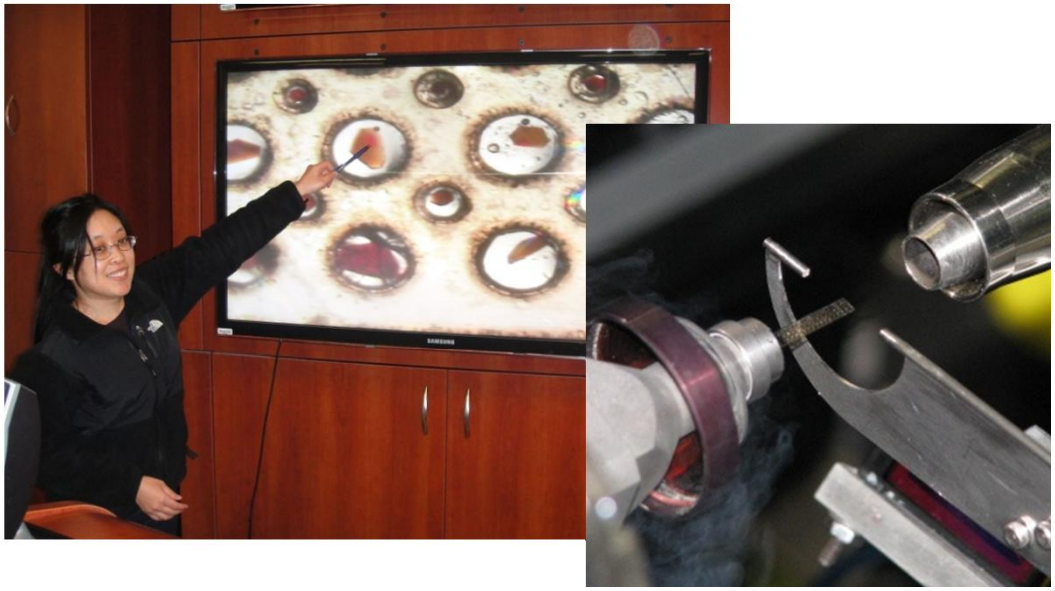


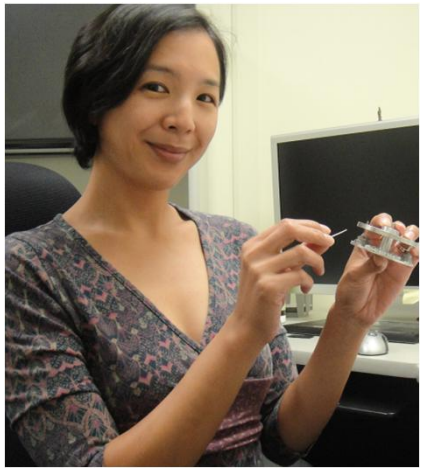
Sample Mounting Grids

Problem: metal sites suffer radiation damage: reduction and structural changes

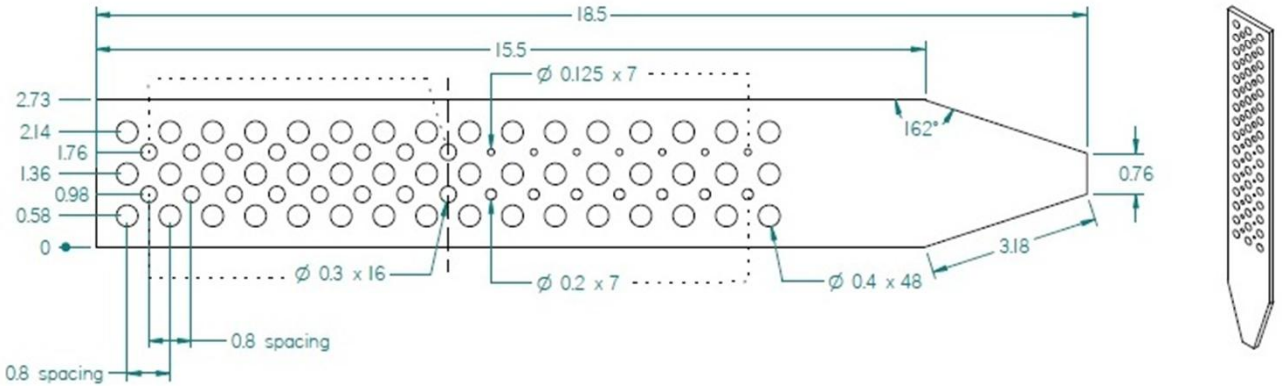
- Holds up to 70 crystals
- 125 µm, 200 µm and 400 µm holes
- Compatible with the SAM robot

Comparison of grid to copper-magnetic pin

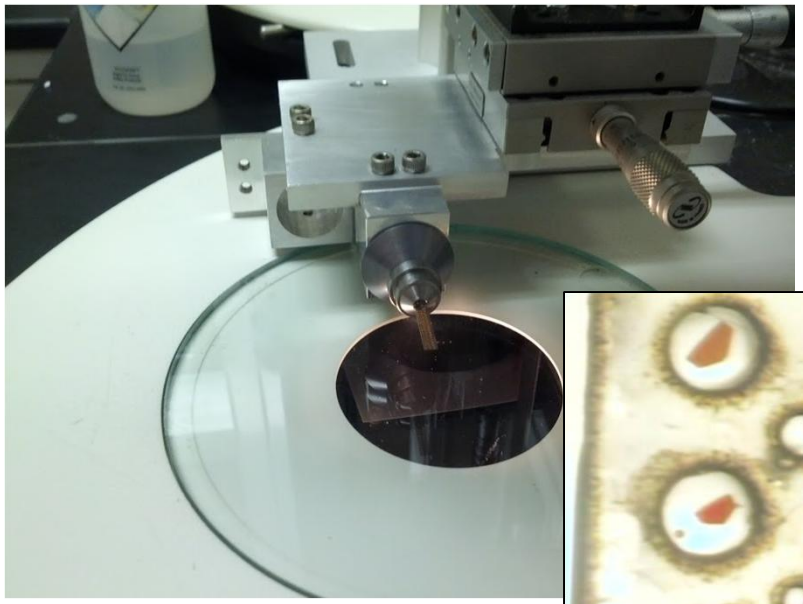


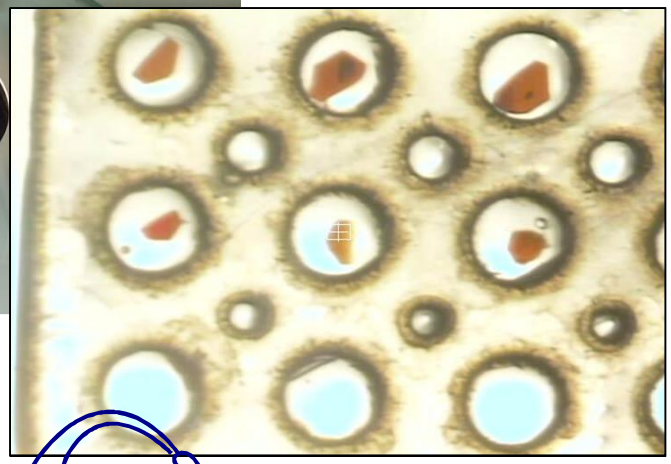


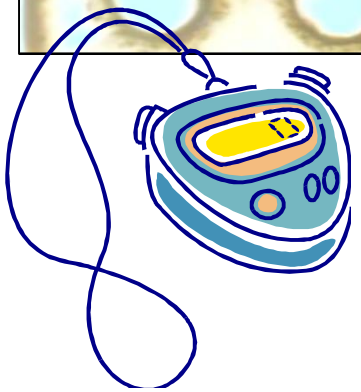
Fixture holds grids in place as epoxy cures



Populating Grids with Crystals





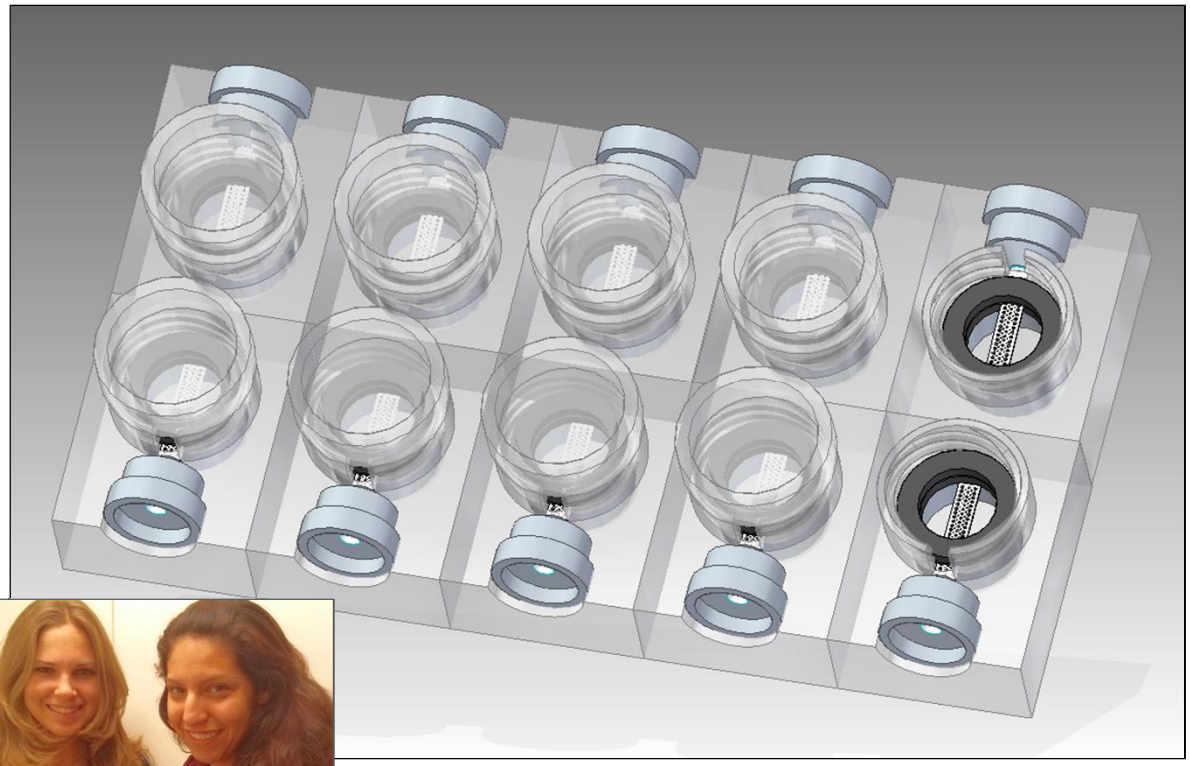


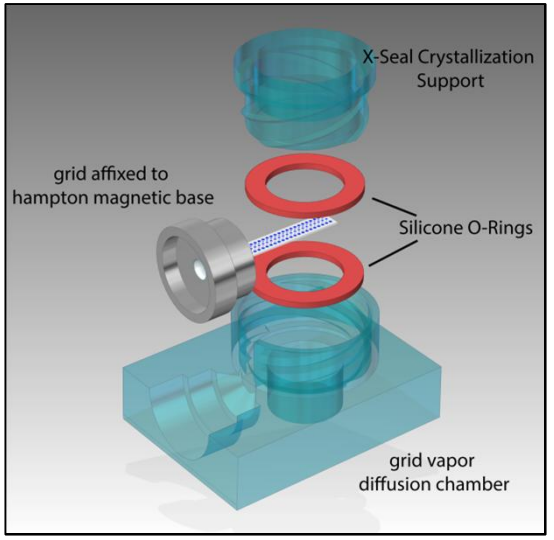
- Holds up to 70 crystals in 125, 200 or 400 µm holes
- Compatible with the SAM robot
- Samples loaded into grid using nylon loop tool
- Paratone-N is helpful to prevent dehydration
- Samples flash-frozen in liquid nitrogen

http://smb.slac.stanford.edu/hardware/sample_mounting_grids/

Crystallization in Grids

- Grid adhered to magnetic pin base before crystallization
- Thin sheet of polycarbonate adhered to grid
- Hanging drop crystallization tray with double o-ring seal

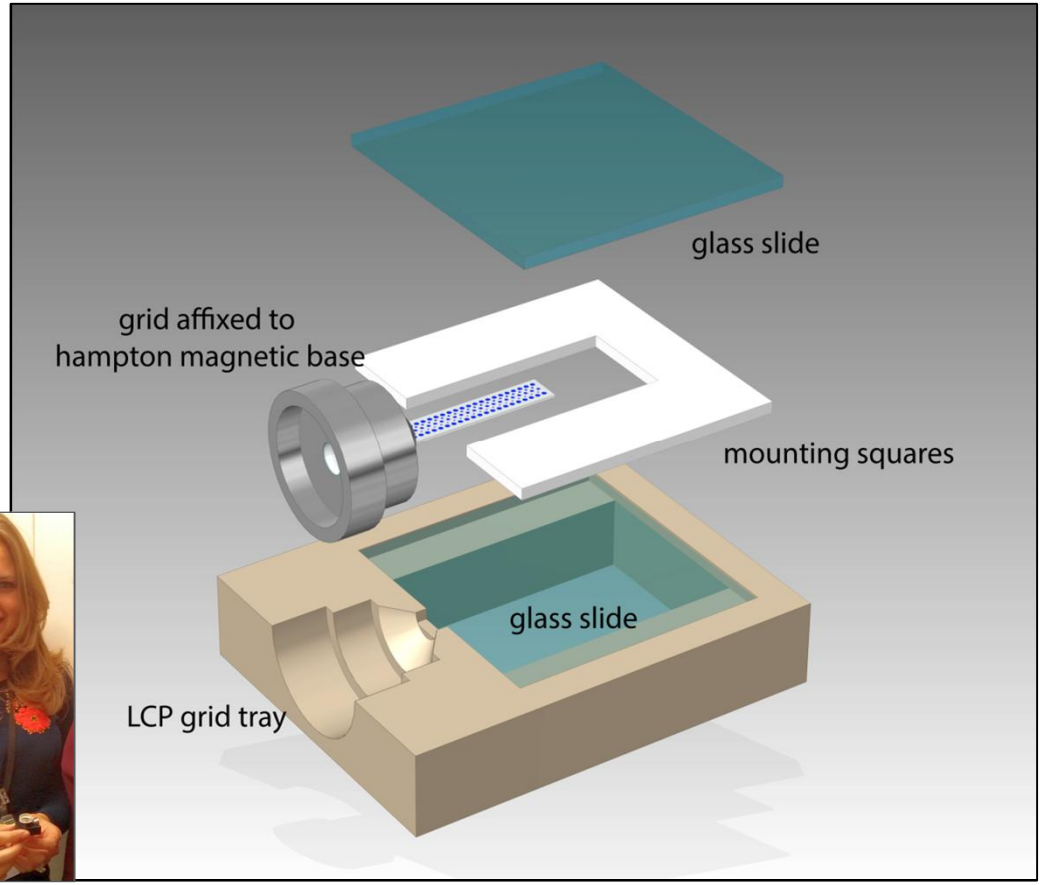


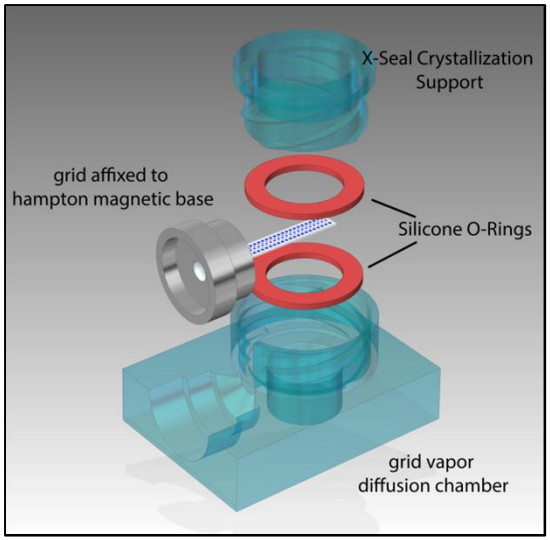


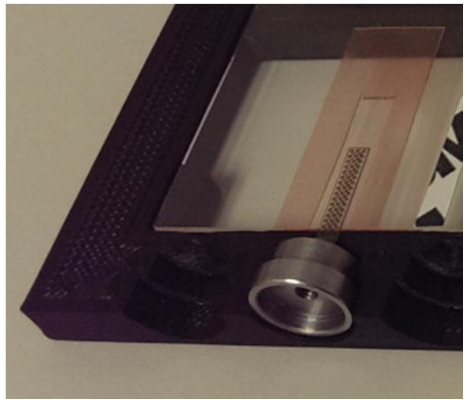
ACohen@slac.stanford.edu

LCP Crystallization in Grids

- Grid adhered to magnetic pin base before crystallization
- Thin sheet of polycarbonate adhered to grid
- Hanging drop crystallization tray with double o-ring seal
- LCP crytallization between glass slides

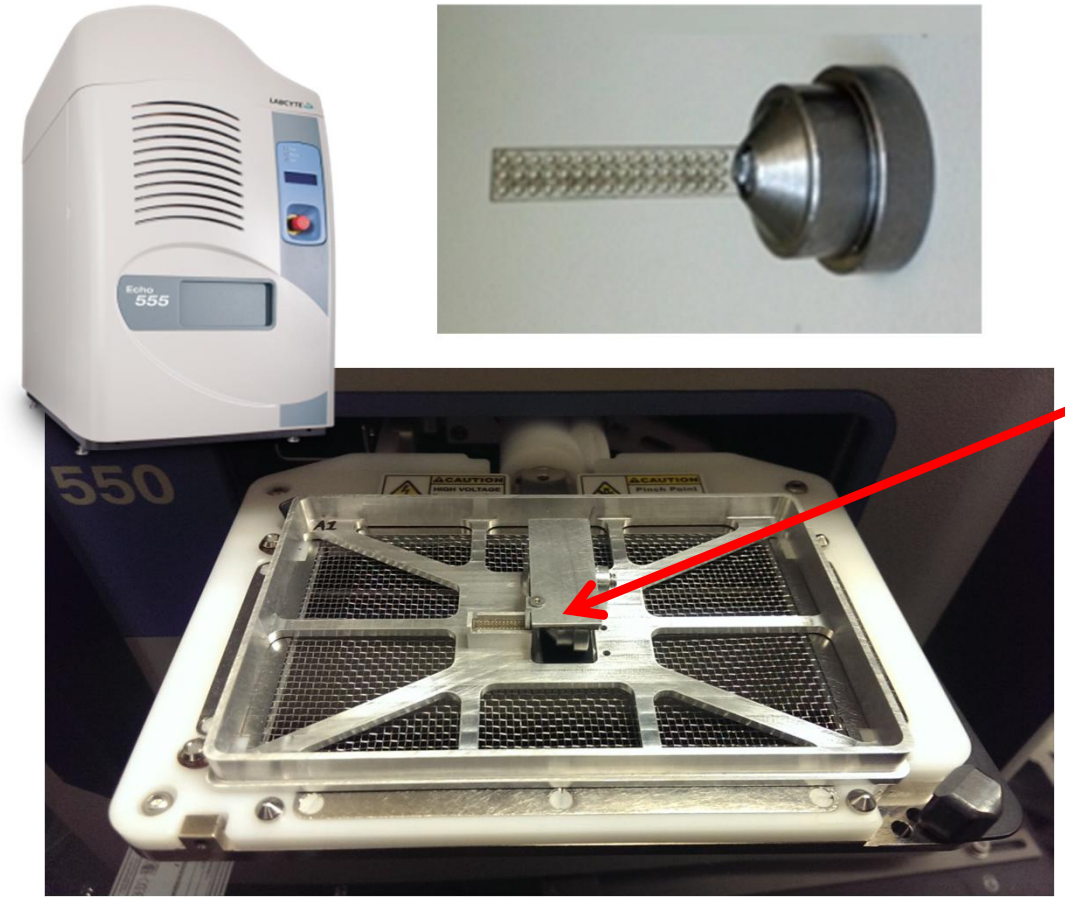


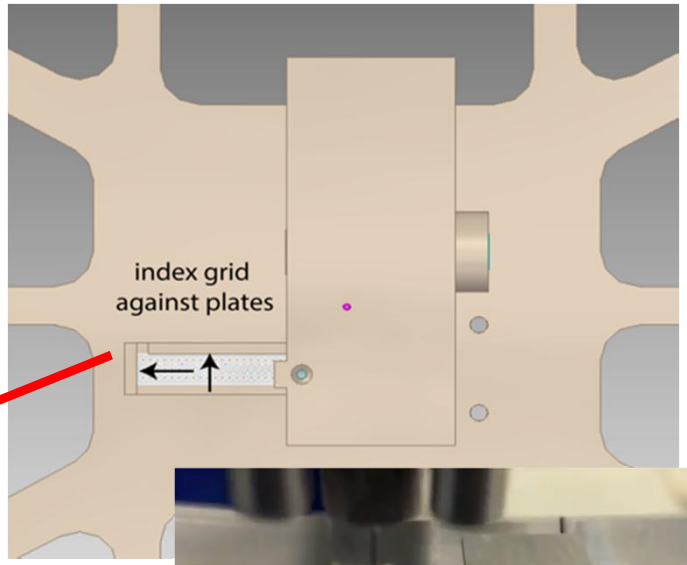


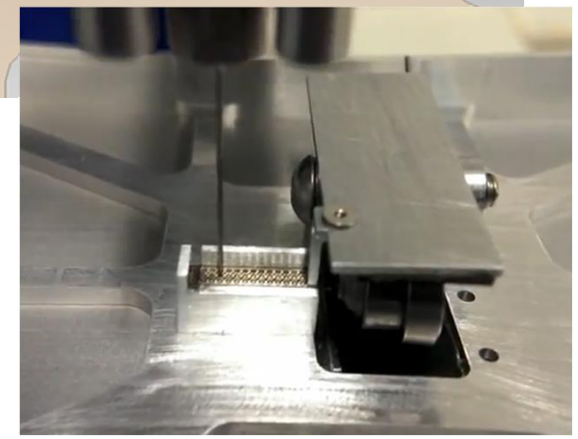


Automation for Filling Grids

- Adaptor in standard microplate form factor
- Compatible with a variety of fluid handing robots
- Labcyte Echo (hanging drop) and Art Robbins Gryphon (LCP)





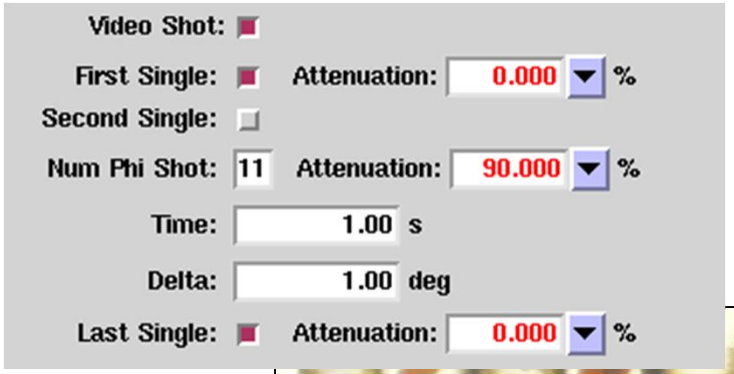


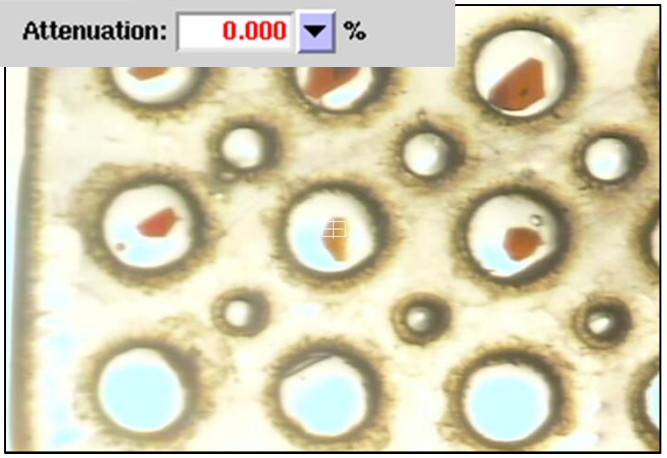
Gryphon

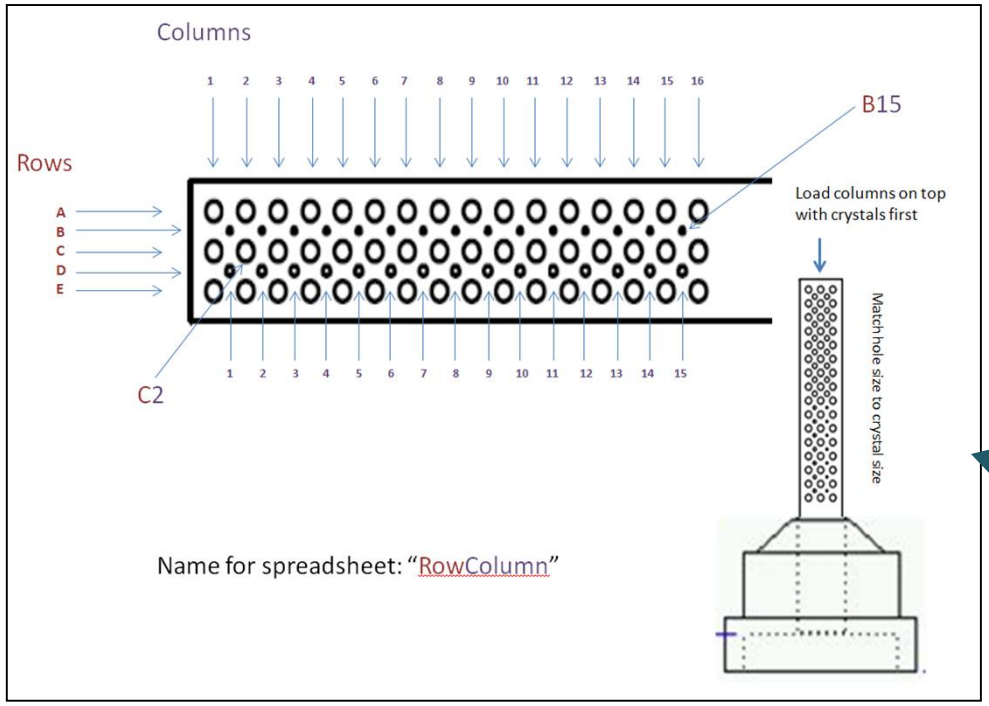
Grid Data Collection Mode

Grid Collection Sequence

- Move to the center of the hole
- Optional – Pause to click to center
- Still Image
- Optional – 2nd Still Image
- Optional – Oscillation Data
- Optional – End Still Image
- Move to next Crystal in Grid …

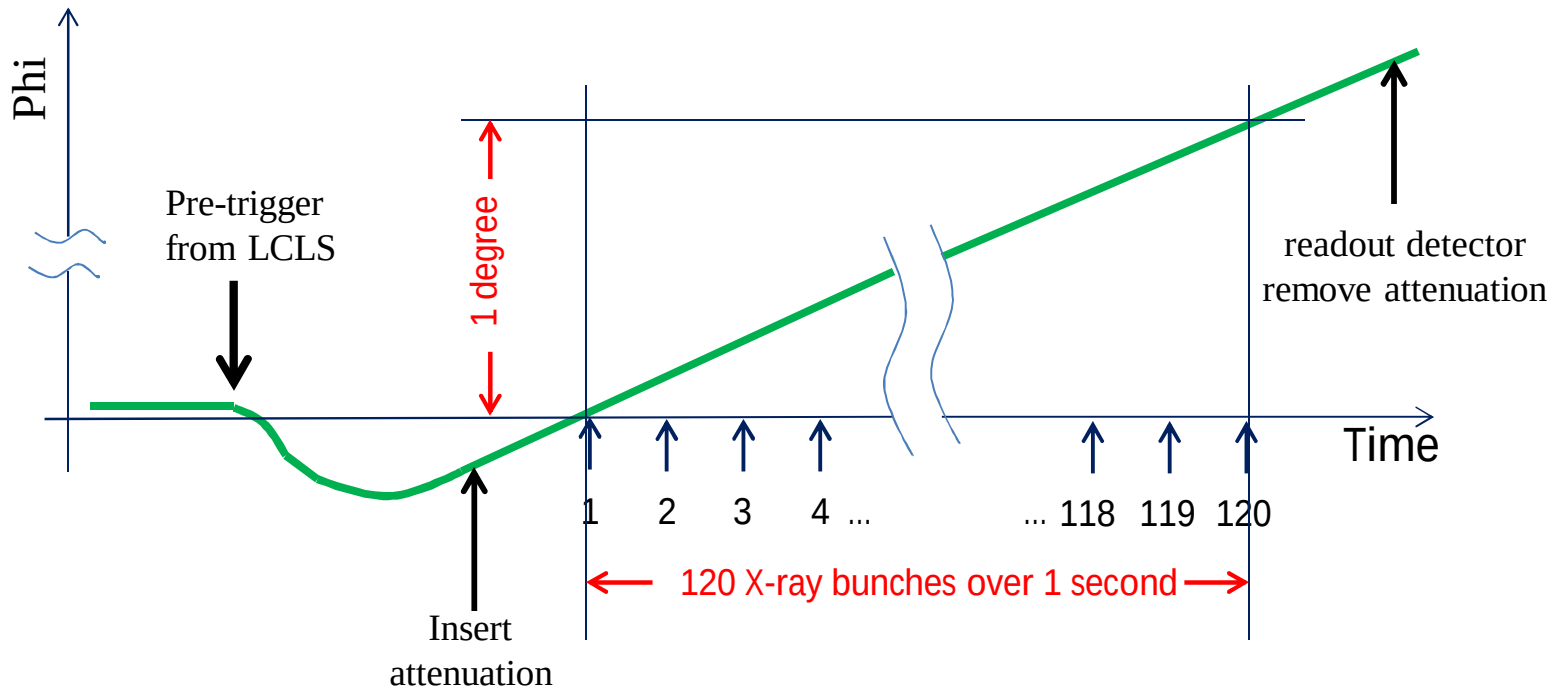






Holes containing crystals are listed in the Blu-Ice Excel Spreadsheet for automated screening

Oscillation Data Acquisition at LCLS

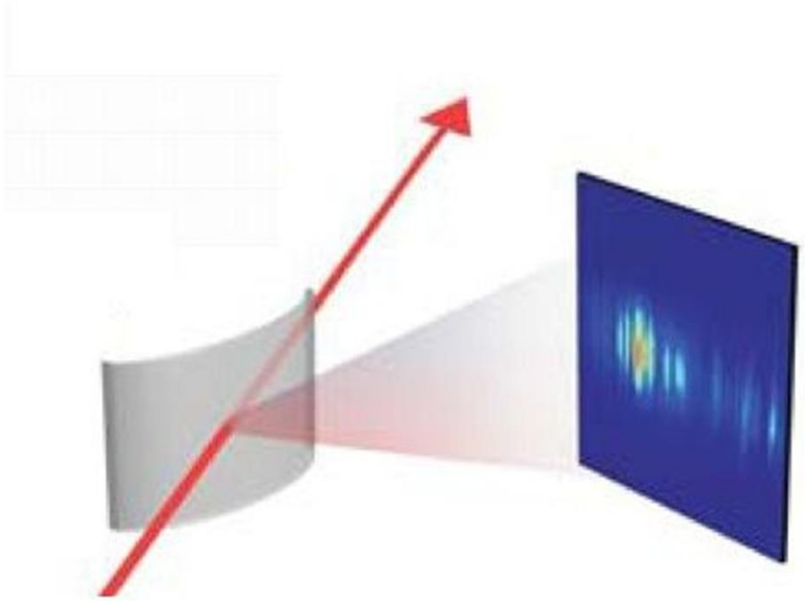


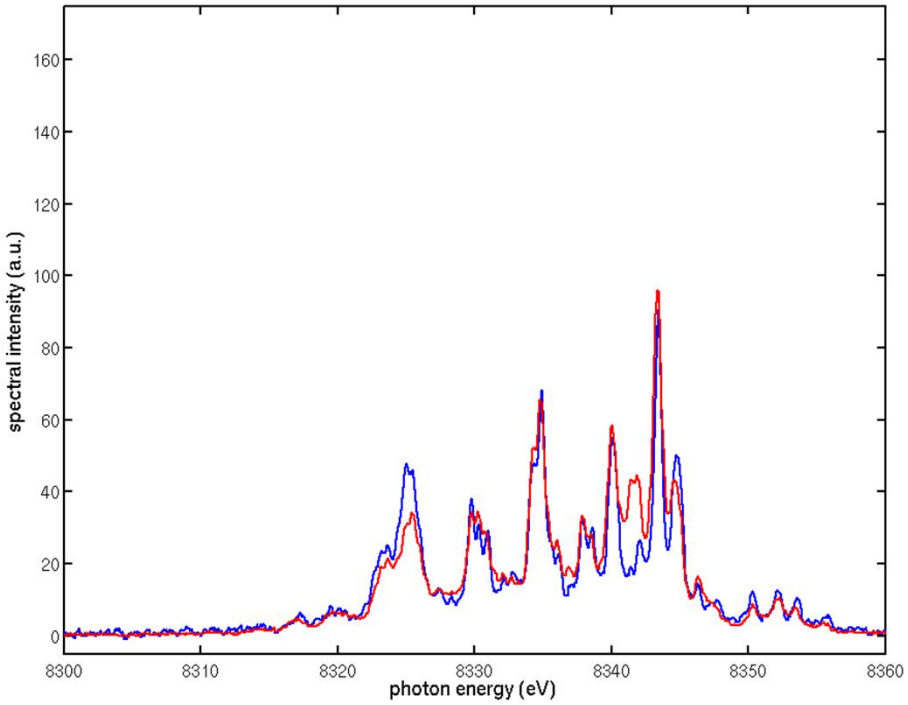
- The arrival angle of the first X-ray bunch is recorded for verification by latching the encoder value when it arrives.

Angle vs. bunch coincidence better than 0.01 degrees.

Energy Spectrometer

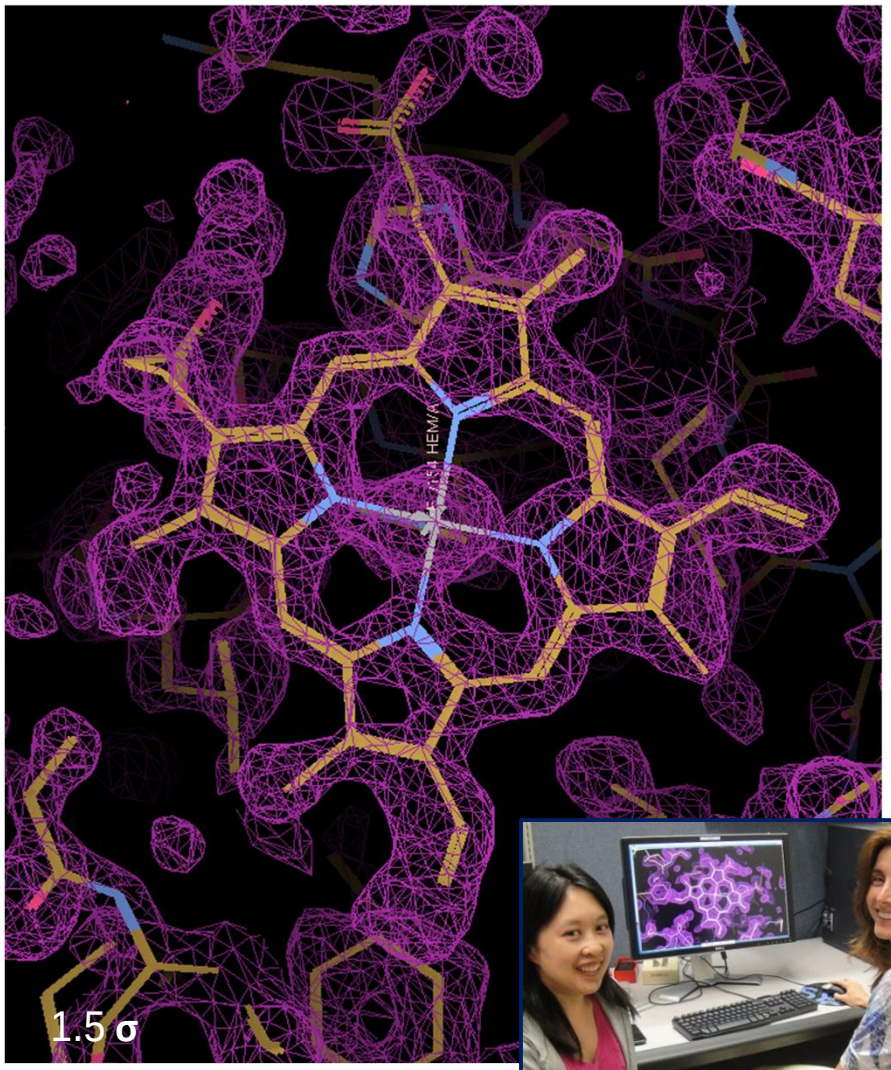
- Measures energy spectrum for every XFEL pulse
- In-line bent Si membrane deflects ~13% beam to CCD
- Developed by LCLS-XPP scientists
- A spectrum from every pulse used for data collection is saved





Zhu et al, *Applied Physics Letters* 101, 034103 (2012)

Myoglobin Grid Results



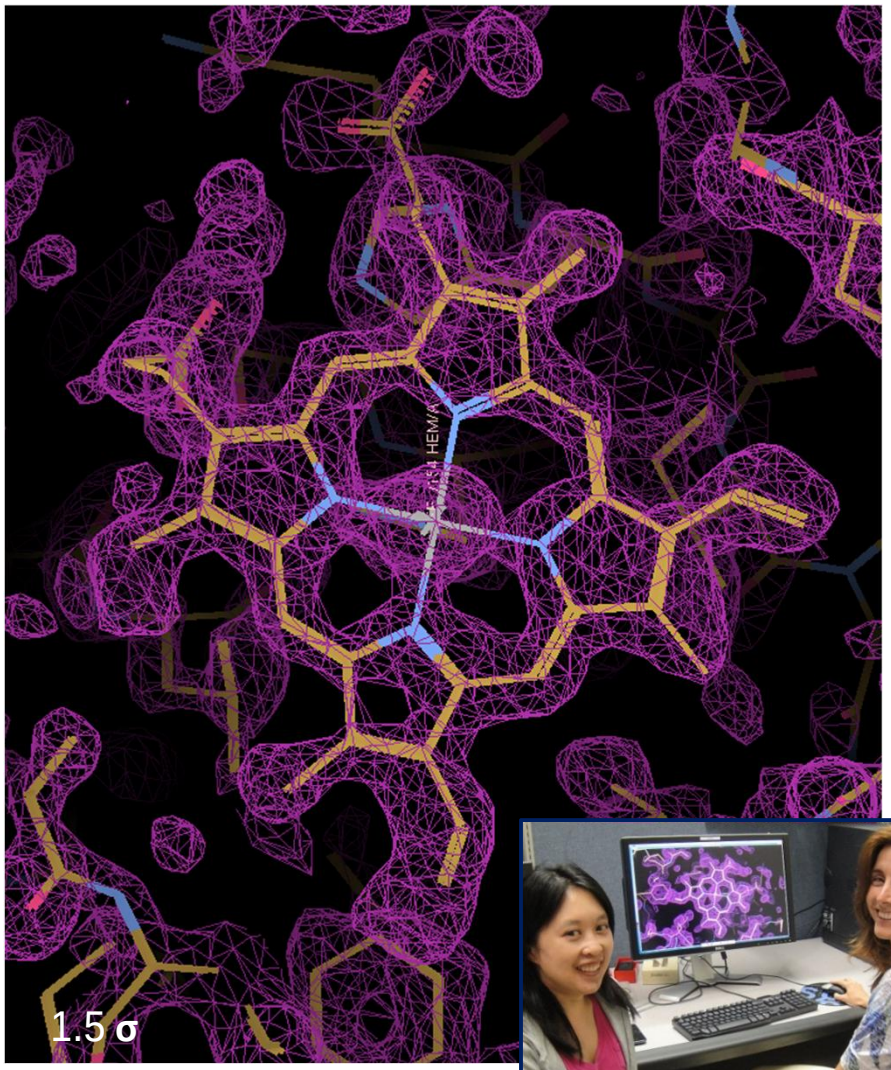
$2F_o$-F_c (first shot)

- 932 crystals exposed (32 grids):
  - 1 'damage free' still image / crystal
  - 11° oscillation (attenuated at 120Hz)
  - 1 end shot / still image
- 253 crystals in final dataset
- Oscillations:
  - indexing and unit cell refinement XDS
  - pointless for c-axis orientation
- 253 still images:
  - wavelength from spectrometer
  - integrated individually with mosflm
  - scaled with scala and merged

92% complete to 1.4 Å

CC1/2=0.77

Myoglobin Grid Results



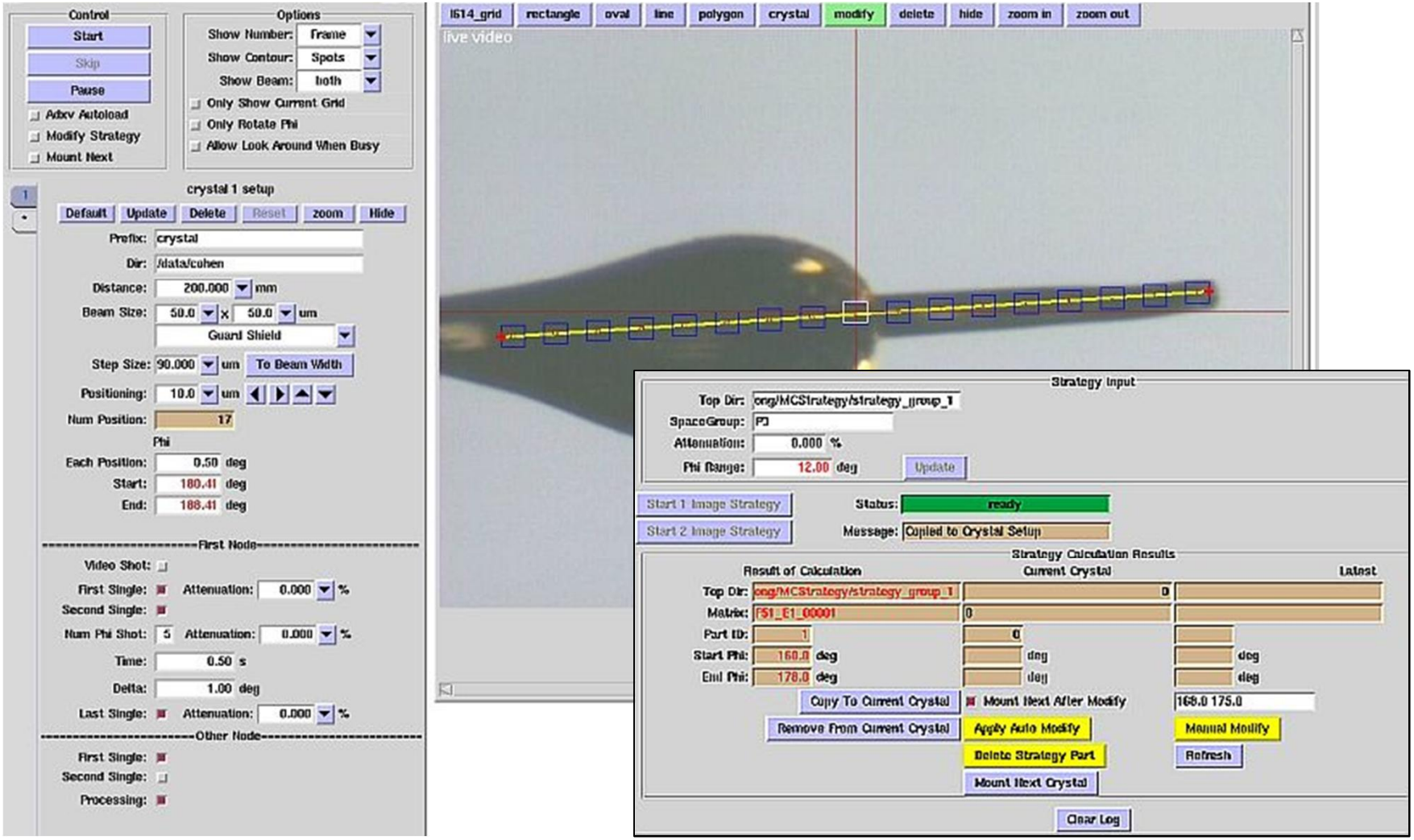
$2F_o$-F_c (first shot)

- 932 crystals exposed (32 grids):
  - 1 ‘damage free’ still image / crystal
  - 11° oscillation (attenuated at 120Hz)
  - 1 end shot / still image
- 739 crystals in final dataset
- Oscillations:
  - indexing and unit cell refinement XDS
  - pointless for c-axis orientation
- 739 still images:
  - wavelength from spectrometer
  - processed with cctbx.xfel

99.9% complete to 1.36 Å

CC1/2=0.96

Long Crystal Data Collection Mode

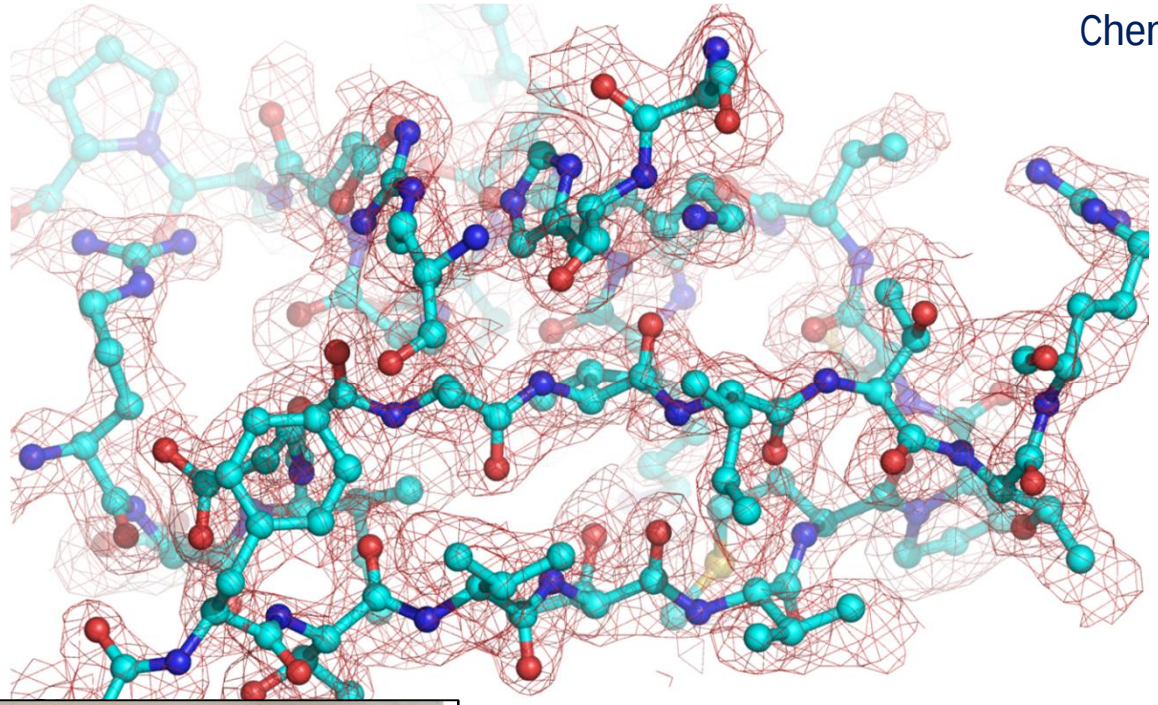


Multi-Crystal Strategy

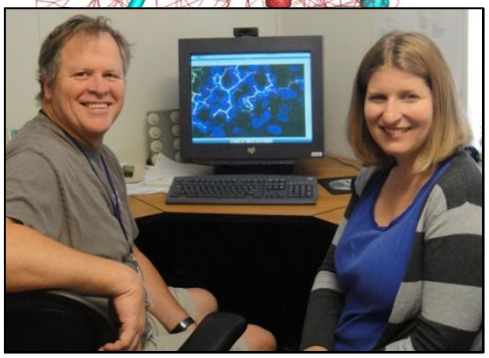
Radiation Damage Free Structure Hydrogenase

Hydrogenase efficiently catalyzes hydrogen production

John Peters Group
Chemistry and Biochemistry
MONTANA STATE UNIVERSITY



Oleg Zadvornyy and Steve Keable in the LCLS XPP control room, June 2013



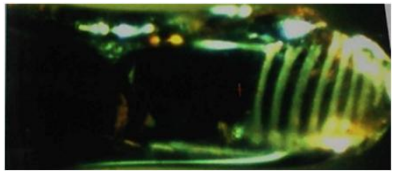
5 crystals (2 long + 3 medium length)
$P4_22_12$; a = b = 111.18 Å, c = 103.54 Å
110 still images
Processing using mosflm/scala
94 % complete to 1.6Å
multiplicity 4.5

Molecular replacement
(using 3c8y from PDB)
- rotation peak = 9.9 (bkgd = 3.8)
- translation, R = 0.43, score = 0.5
- contrast = 26.3

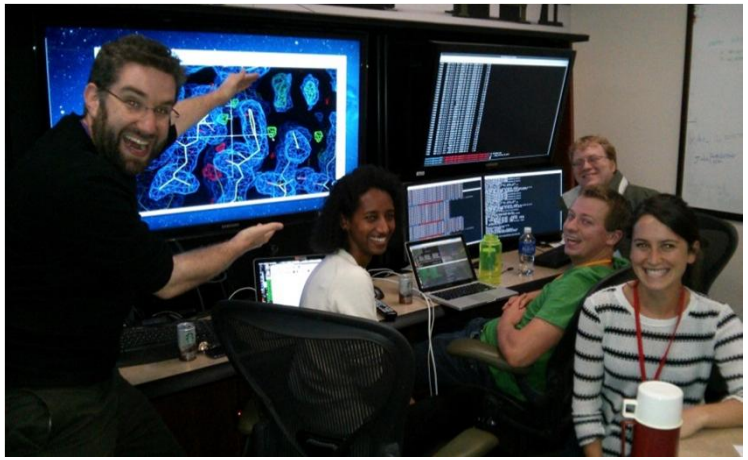
Molecular Refinement (REFMAC)
R = 0.346, R_{free} = 0.389, FOM = 0.63

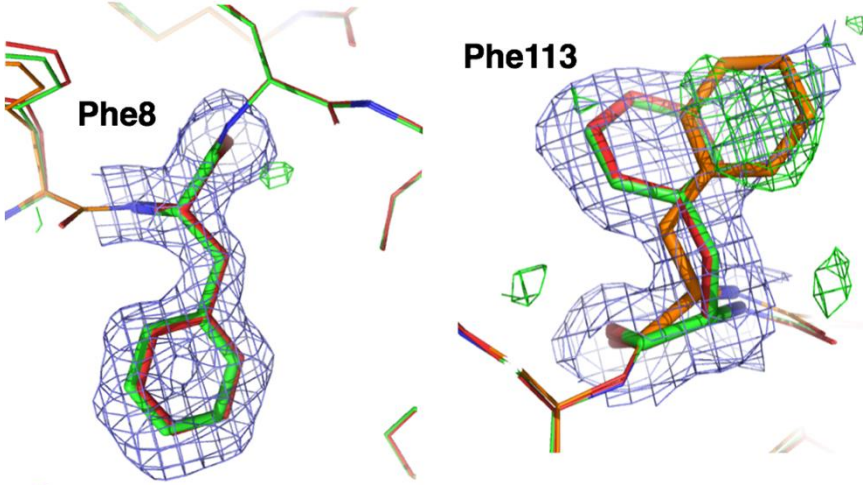
Room temperature structure of Cyclophilin A: functional conformational dynamics

University of California San Francisco



The Fraser lab and Aaron Brewster at 3:30AM after an LCLS shift with data processed in real time and maps displayed on the monitor





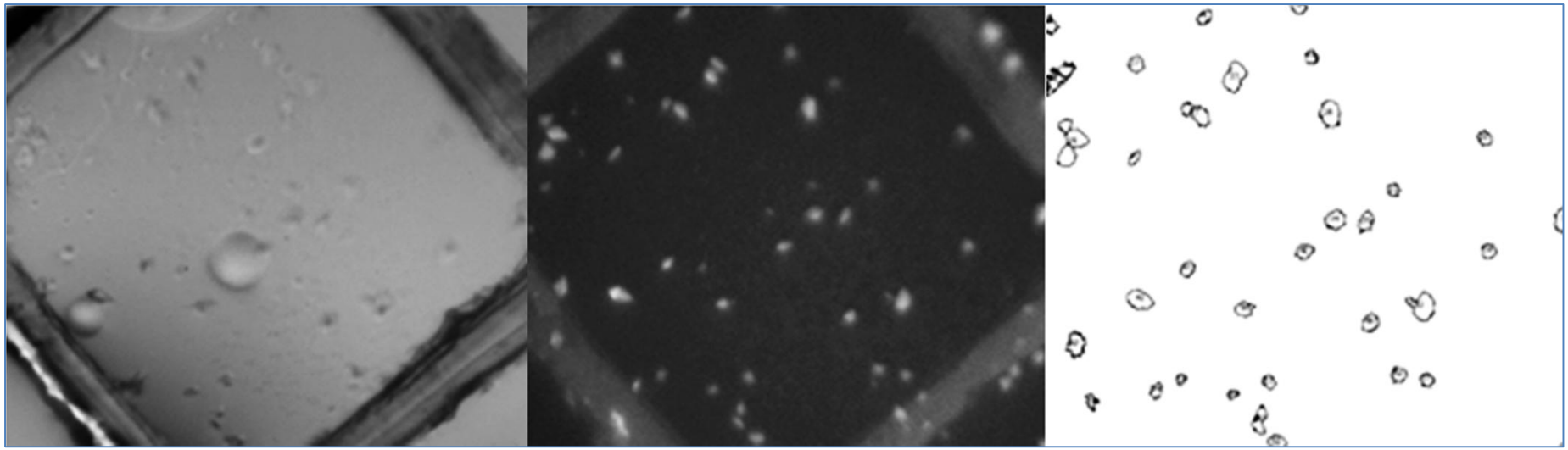
Electron density map for CypA confirms multiple conformations for Phe113, which is part of the dynamic allosteric network validated in Fraser *et al*, Nature, 2009. In contrast,Phe8 has electron density consistent only with a unique conformation.

611 frames across 43 crystals. Data integration and post-refinement using cctbx.xfel.

Resolution Range		Completeness		<N_obs>	|Qmeas	Qw	CC1/2	N_ind	|CCiso	N_ind|	<I/sigI>
45.10 -	3.23	99.0	3438 /	3473	18.71	28.37	27.23	87.90	3429	89.44	3424	116.76
3.23 -	2.56	98.8	3279 /	3318	15.51	39.24	38.60	81.80	3266	88.03	3277	49.42
2.56 -	2.24	98.7	3234 /	3276	12.57	47.92	47.34	71.99	3197	81.43	3234	34.45
2.24 -	2.04	98.0	3197 /	3262	9.85	53.98	53.04	69.88	3155	73.94	3196	29.85
2.04 -	1.89	95.2	3096 /	3251	7.43	56.15	55.32	64.19	3006	69.55	3095	24.92
1.89 -	1.78	90.5	2926 /	3232	5.19	50.35	47.40	51.76	2735	61.36	2925	21.71

Crystal Mapping using UV imaging

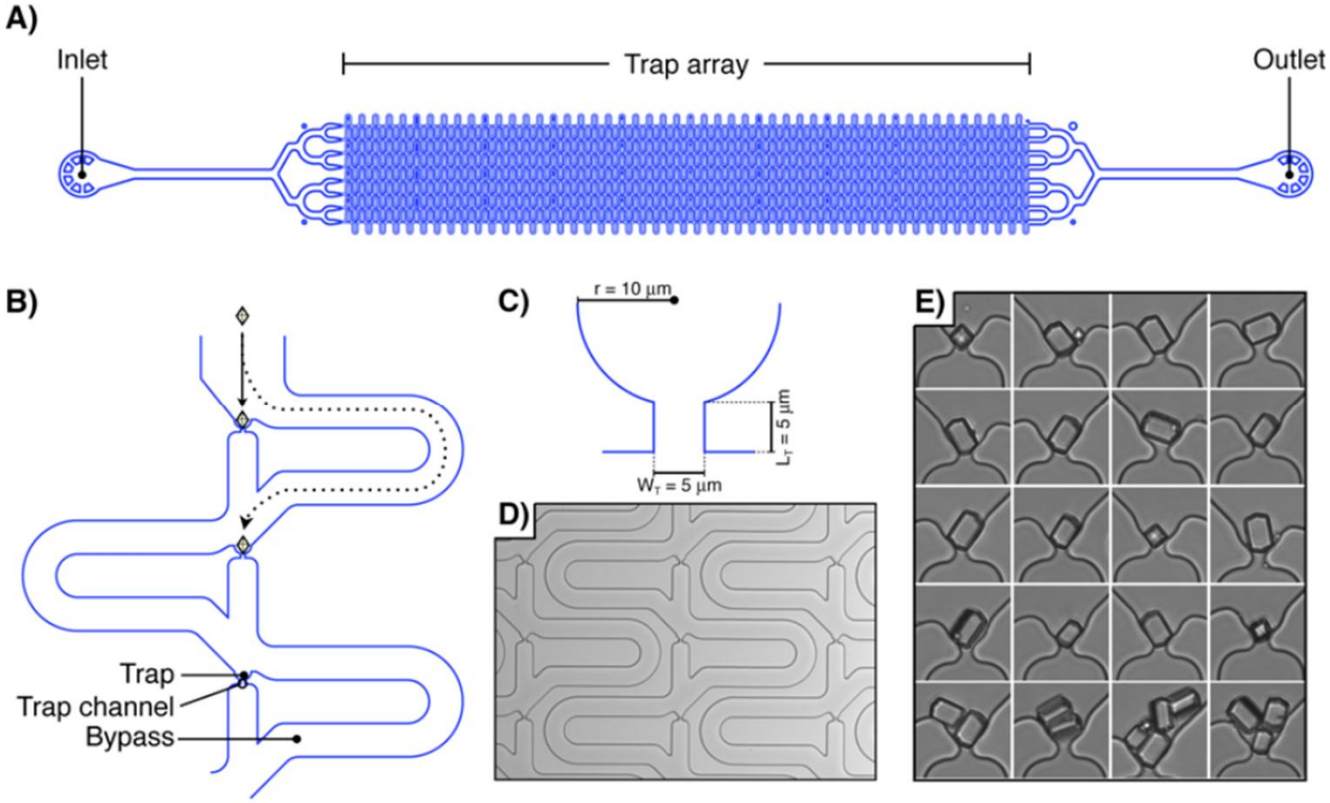
Low Background Carbon Supports



A video image (left) and UV image (middle) of a carbon support populated with 1-2 µm RNA Polymerase II crystals. The calculated crystal positions from automated image analysis are shown on the right.

High Density Sample Containers

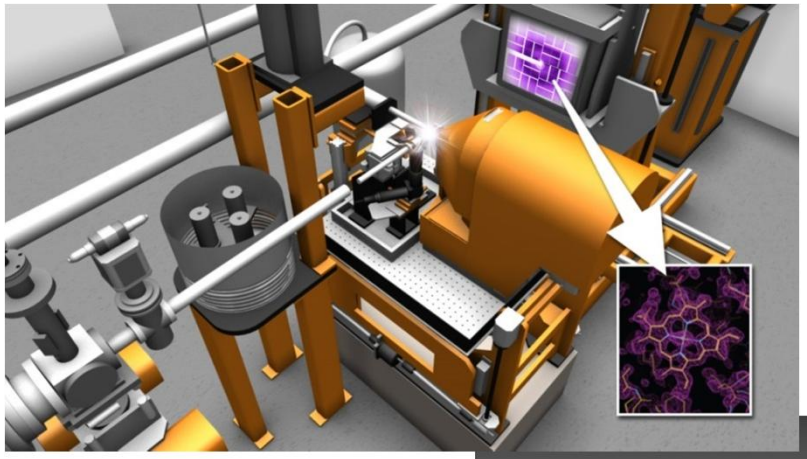
- Sample are held in defined locations

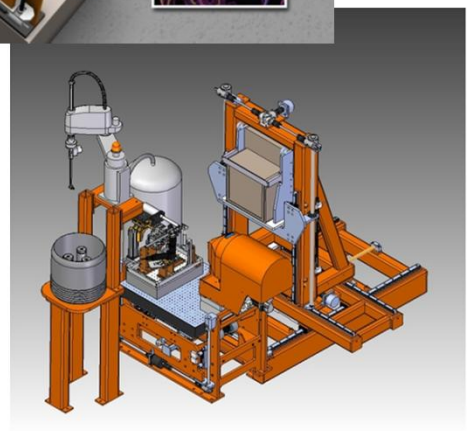


Microfluidic Crystal Traps Developed by
Artem Y. Lyubimov, James Berger, Axel Brunger and co-workers

Two New Facilities for Structural Molecular Biology Research

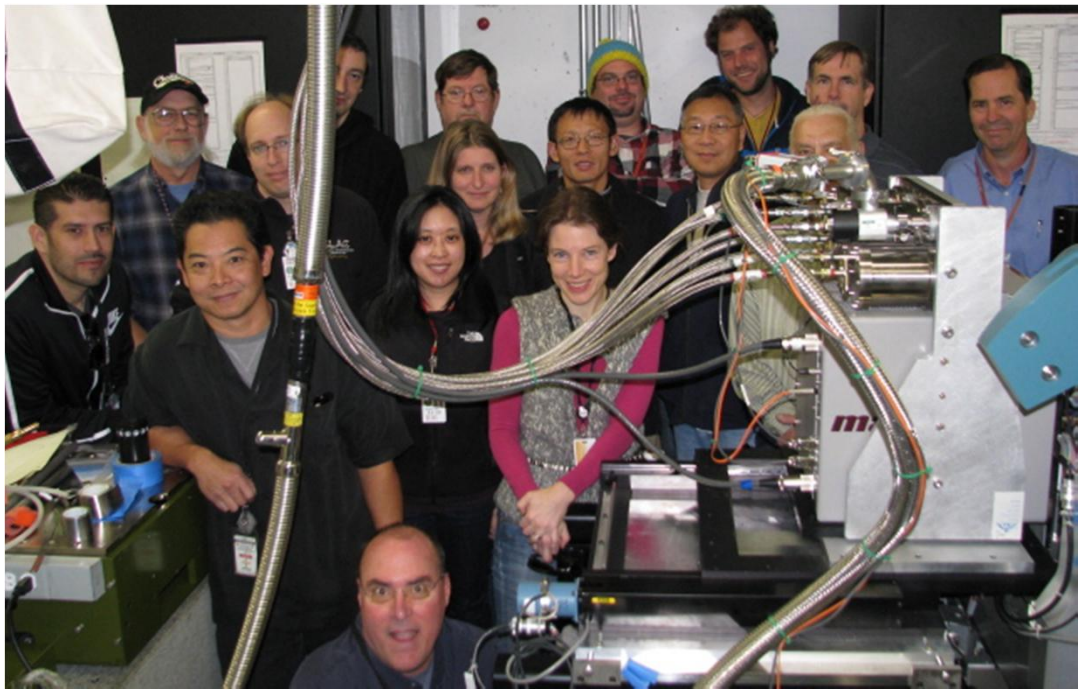
SLAC
NATIONAL ACCELERATOR LABORATORY





- MFX Hutch at LCLS
- BL12-1 at SSRL
  - synergistic operation
  - in atmosphere operation
  - goniometer based crystallography
  - injector / drop on demand diffraction

Thank you for your attention



LCLS-XPP and SSRL-SMB groups