MX Breakout Session

Bob Fischetti

Workshop on Science Opportunities with an MBA Lattice
Advanced Photon Source

October 21-22, 2013

MX Working Group for APS MBA-lattice Science Case

Internal
Robert Fischetti, APS and GM/CA (Team coleader)
Keith Brister, LS-CAT
Malcolm Capel, NE-CAT
Andrzej Joachimiak, Argonne, SBC-CAT
Kanagalaghatta Rajashankar (Raj), NE-CAT
Stephen Wasserman, LRL-CAT

External
Bill Weis, Stanford University (Team coleader)
Steve Harrison, Harvard University
Janet Smith, University of Michigan
Bi-Cheng Wang, University of Georgia

MX Breakout Session Agenda

1:00 Overview of committee recommendations, and beamline performance - Bob Fischetti
1:20 Mitigating radiation damage - Gerd Rosenbaum
1:40 Sample handling and alignment techniques - Malcolm Capel
2:00 Ultra-high multiplicity to detect a weak phasing signal - Janet Smith
2:30 How to think about diffraction data from tiny crystals - Steve Harrison
3:00 Town Hall Discussion
5:00 Adjourn

New Scientific Opportunities

Structure determination of

- Membrane proteins grown in lipidic cubic phase (LCP)
- Large macromolecular complexes and molecular machines

Examples

- Kinetochore: component of mitotic spindle – control of chromosome segregation in cell division.
- Nuclear pore: controls export and import of large molecules to nuclear space.

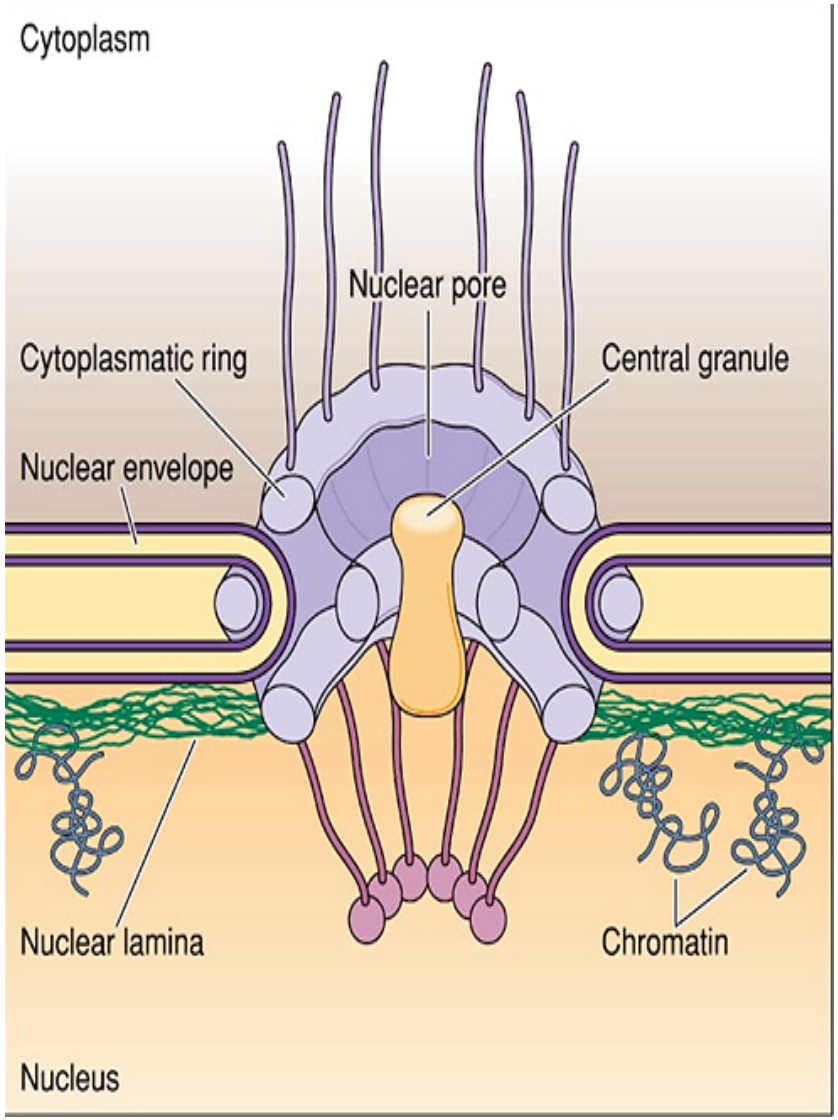
Large Scale Efforts

- Many components and subcomponents, all available only in low yield
- Generally produce small, heterogeneous crystals -> extensive survey efforts

Significance of MBA Lattice Upgrade

- Small, brilliant beams for optimal data acquisition from micro-crystals

Nuclear Pore



Controls import and export of macromolecules to/from nuclear space.

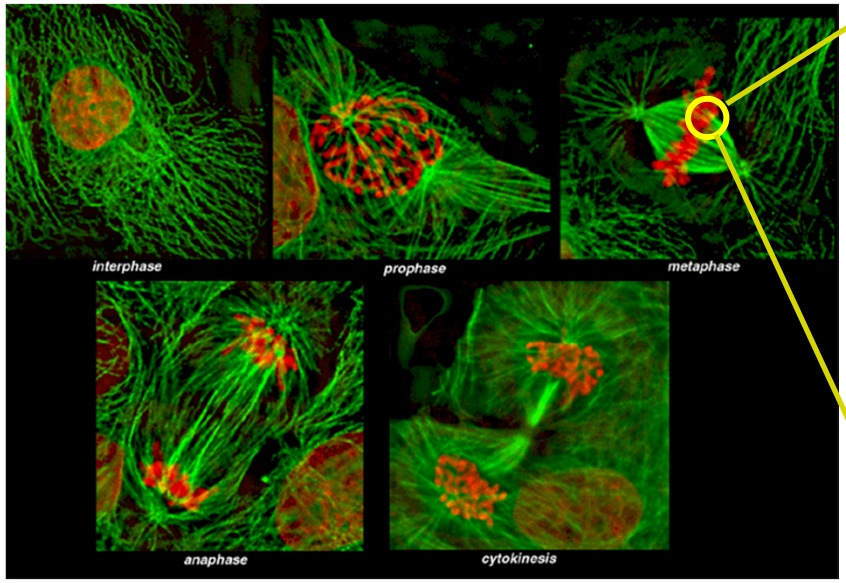
30 distinct proteins, in multiple Copies.

Thomas Schwartz, MIT (NE-CAT)

Andre Hoelz, Cal. Tech (GM/CA)

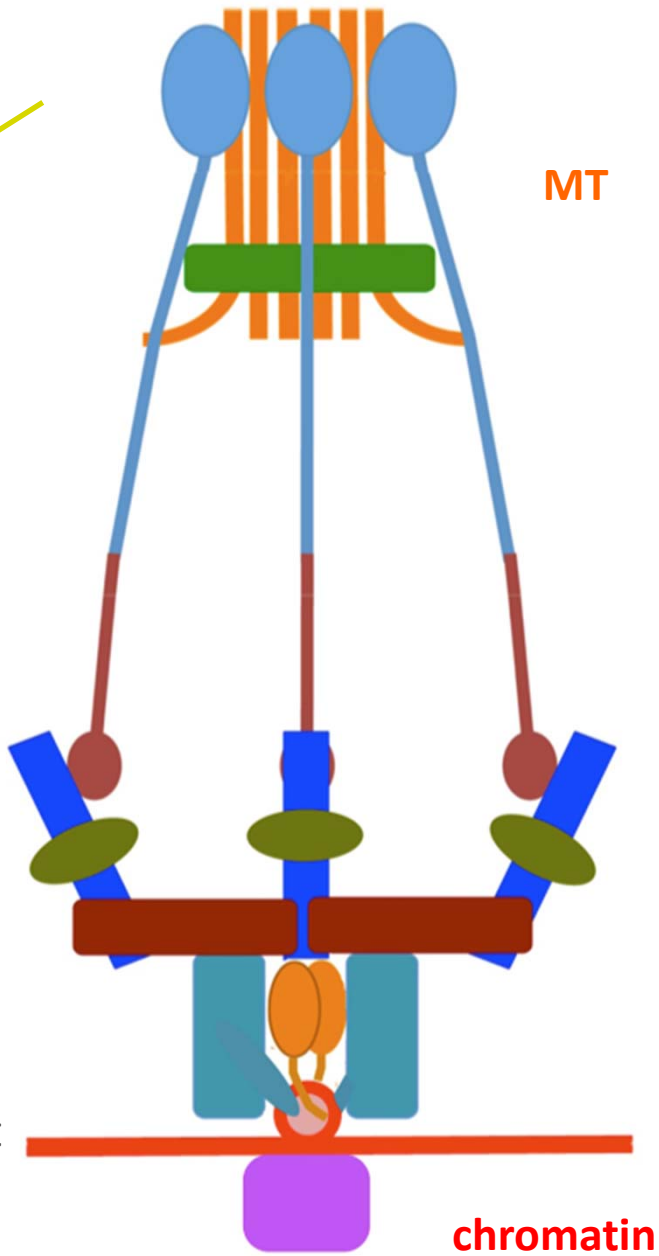
Kinetochores

assemblies that attach chromosomes to microtubules of mitotic spindle



Mitosis:
spindle microtubules geen; chromosomes, red
from W. Earnshaw

~20 Distinct Proteins
Many present in multiple copies

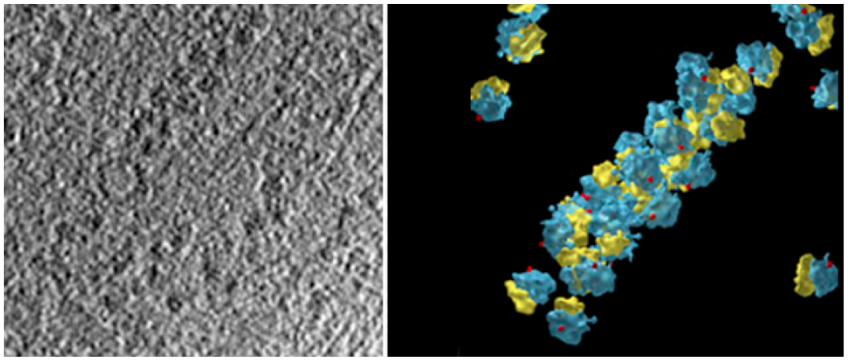


schematic yeast kinetochore

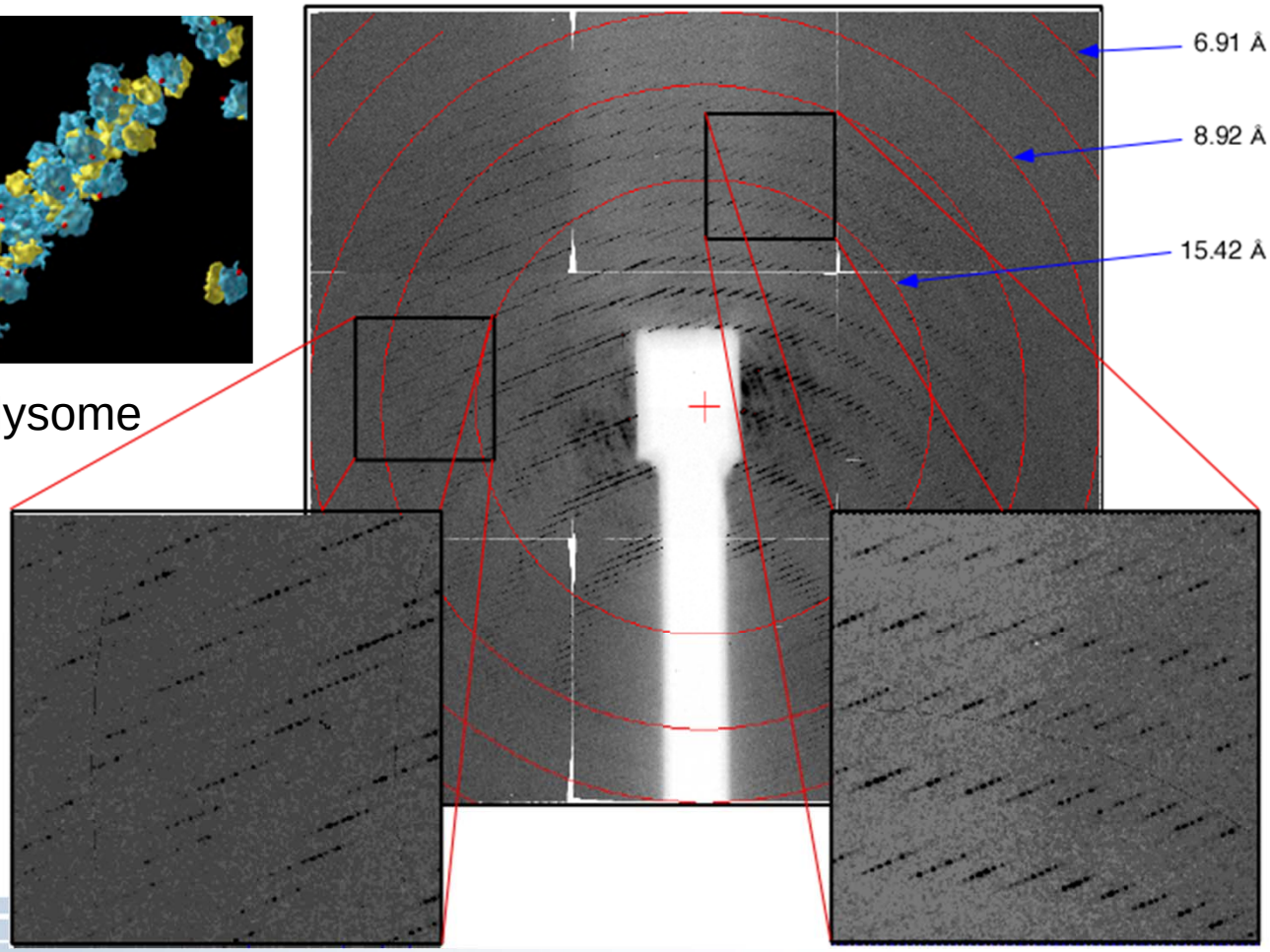
500 Å

Structure determination of very large macromolecular assemblies

- Diffraction pattern obtained at the SBC 19ID beamline from complete ribosomal particles (10 – 12 MDa per asymmetric unit,(five ribosomes)). The crystallographic unit cell is centered, with 2700 Å as the longest axial length. (Courtesy of Dr. Jaime Cate, University of California Berkeley).



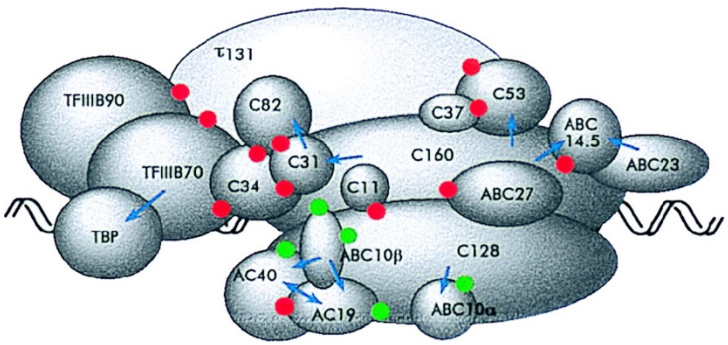
- EM reconstruction of polysome

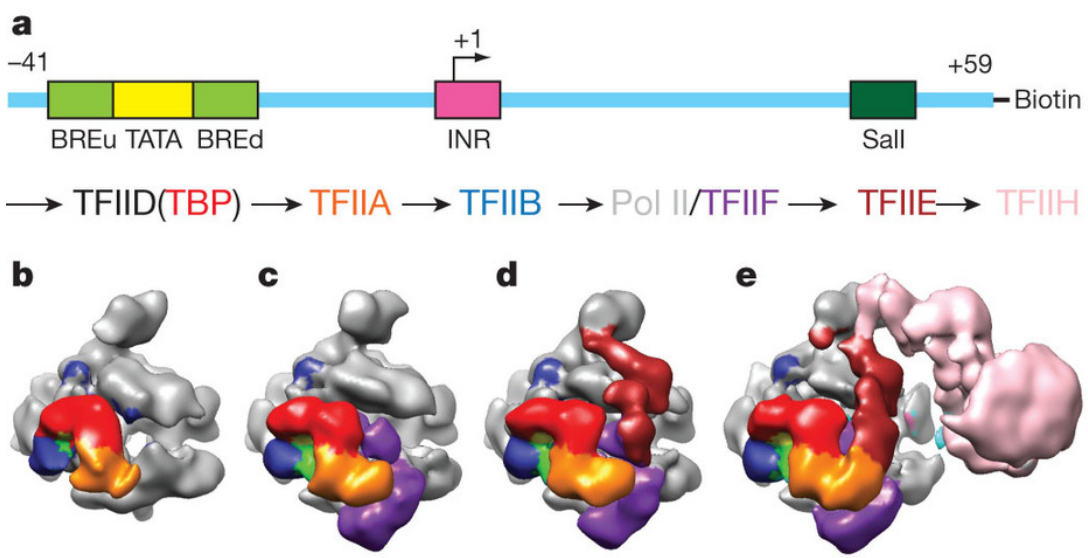


K. Grünewald & W. Baumeister

Structure determination of very large transcription initiation machines

- The preinitiation complex is a large complex of proteins that is necessary for the transcription of protein-coding genes in eukaryotes and archaea. The preinitiation complex helps position RNA polymerase II over gene transcription start sites, denatures the DNA, and positions the DNA in the RNA polymerase II active site for transcription.
- Only EM reconstructions are available for these large and complex assemblies.





Time to the Garman Limit (cryo-cooled)

Garman limit[1] ~ 3.0 x 10^7 Gray (35% intensity loss)
Deposited energy in sample – not incident energy!

E ~ 12.68 keV

Beamline	Divergence (µrad, FWHM)	Smallest beam width (µm)	Smallest beam height (µm)	Flux (ph/sec)	Flux density (ph/s/µm2)	Dose rate (Gy/s)	Time to Garman limit (msec)
APS-U MBA 23-ID-D	3200 x 1200	0.40	0.50	5.3E+13	3.4E+14	1.0E+11	0.29
NSLS-II FMX‡	1700 x 700	1.00	0.50	5.0E+12	1.3E+13	3.9E+09	7.60
DLS VMX¥		0.50	0.50	1.0E+12	5.1E+12	1.0E+09	29.90
APS-U MBA 23-ID-D	270 x 180	6.10	5.20	6.1E+13	2.4E+12	7.6E+08	39.52
Petra3 MX2	500 x 300	4.00	1.00	5.0E+12	1.6E+12	4.9E+08	60.81
SPring8 BL32XU§	1520 x 980	0.90	0.90	6.2E+10	9.7E+10	3.0E+07	992.99
Petra3 MX1	200 x 150	28.00	13.00	1.0E+13	3.5E+10	1.1E+07	2766.63
APS 23-ID-D*	400 x 100	5.00	5.00	5.4E+11	2.8E+10	8.5E+06	3518.81
DLS I24	2000 x 50	8.00	8.00	1.0E+12	2.0E+10	6.2E+06	4864.40
ESRF ID23-2†	550 x 360	7.50	7.50	4.0E+11	9.1E+09	2.8E+06	10688.38
APS 23-ID-D*	400 x 100	70.00	25.00	2.00E+13	1.46E+10	4.50E+06	6650.55

*APS 23-ID intensities are for 12.0 keV except where noted
§SPring8 BL32XU intensities area at 12.398 keV
†ESRF Upgrade may have changed these numbers
‡NSLS-II AMX/FMX intensities are at 12.7 keV

[1] Owen, R.L., Rudino-Pinera, E. & Garman, E.F. *Proc Natl Acad Sci U S A* **103**, 4912-7 (2006)
[2] RADDOSE http://biop.ox.ac.uk/www/garman/lab_tools.html

Microcrystallography of Biological Macromolecules

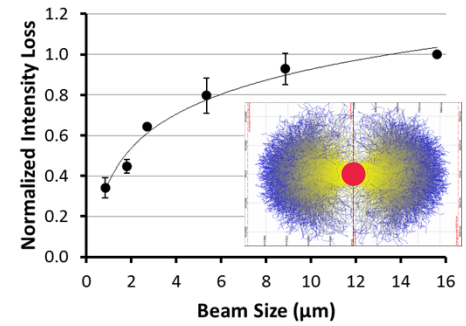
Microcrystallography has enabled the determination of high impact, 3D structures of proteins & protein complexes

APS-U & MBA-lattice will significantly expand the MX horizon

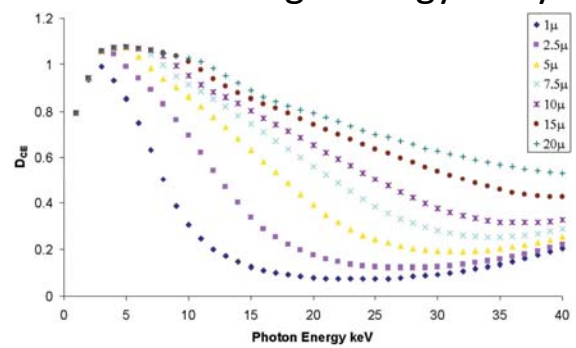
- Exploit the high Brightness
  - Nano/Microcrystals – currently inaccessible
  - Improved S/N and resolution for small (0.5 – 5 µm), inhomogenous and/or weakly scattering crystals
- Exploit APS high energy source
  - Reduced radiation damage – photoelectron escape
- Enhanced modes of data collection
  - *In situ*, Rastering, Helical and Shutterless data collection

Reduced Radiation Damage

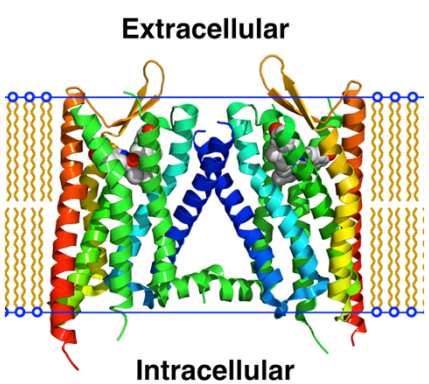
With microbeams

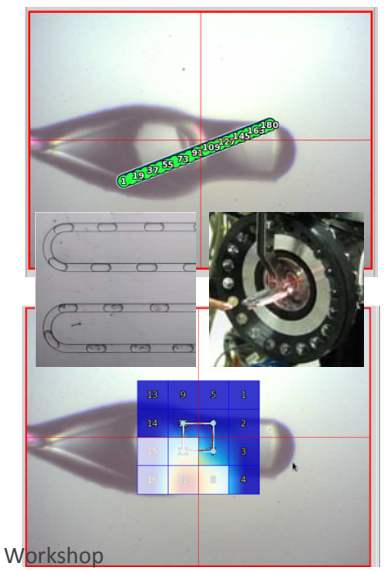


Or high energy X-rays

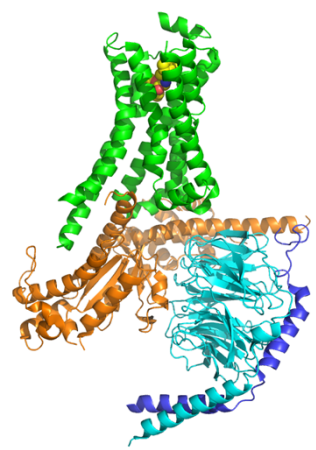


k-opioid GPCR
Ray Steven's lab



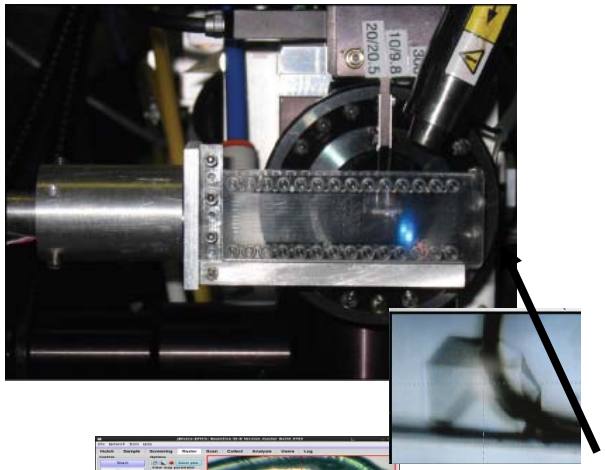


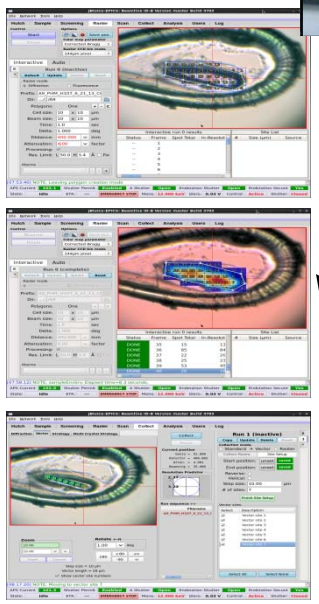
β_2 adrenergic receptor-Gs protein complex
Kobilka & Weis labs



2012 Nobel Prize in Chemistry

In Situ Room Temperature Data Collection





Sample

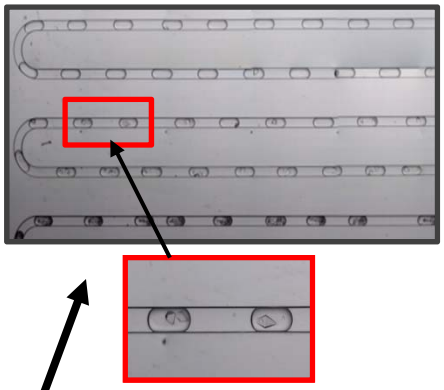
Raster

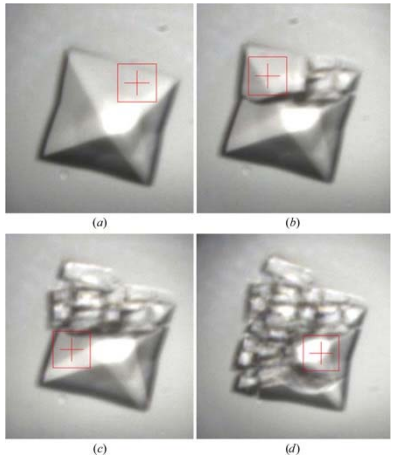
Vector

Mini- to Micro-beam tools

Blurring the line between synchrotron data collection and serial femtosecond crystallography. Higher brightness and faster detectors employed in the search for every shrinking crystals of increasing complexity and biological importance.

- In Situ screening will provide important diffraction feedback on limited quantities of biological material at an early stage in crystallization trials.
- Samples introduced by novel delivery systems (e.g. acoustic drop)
- Data collection on samples with large amounts of small crystals complexed to a variety of compounds.
- Data collection on high symmetry space groups (e.g. viruses)
- Development of data collection tools extending to the µm level

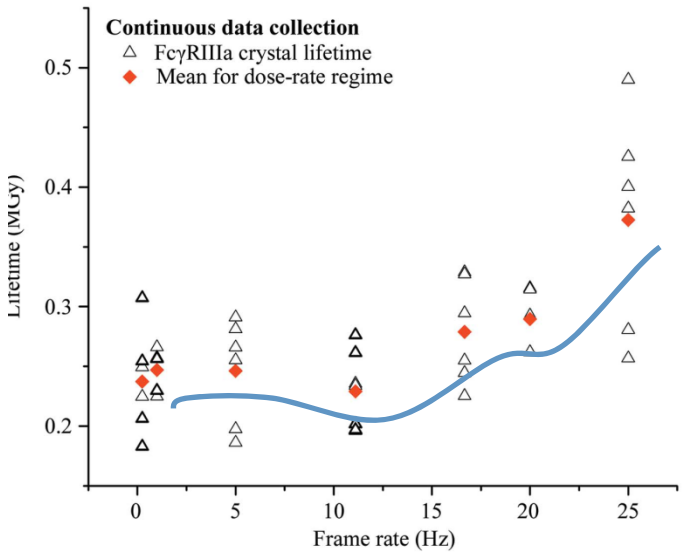


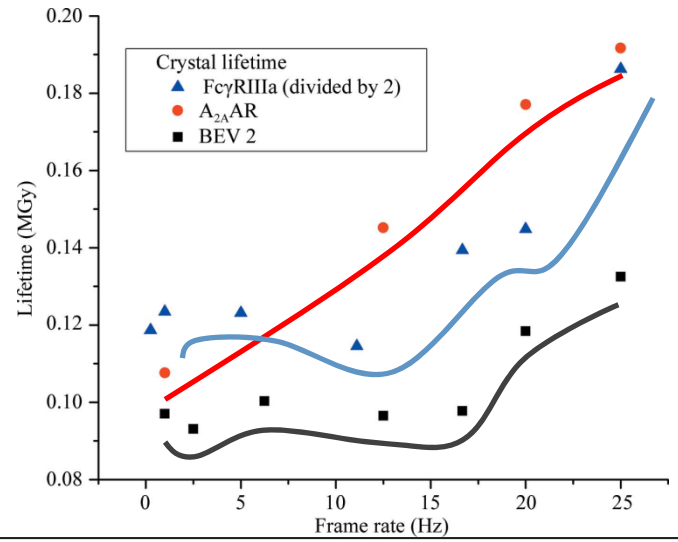


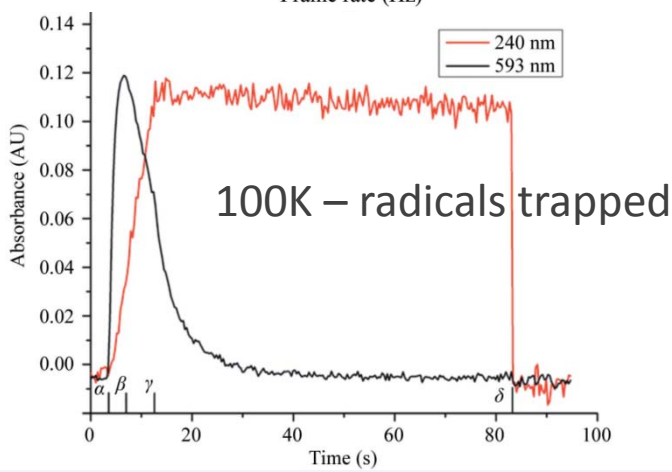
In situ data collection from a virus crystal at 3 different Positions.
D. Axford et. al., Acta Cryst. (2012) D68, 592-600

Craig Ogata

Outrunning 2nd Rad Dam at Room Temp – Beware!







Crystal lifetime
Start-stop (dark progression)
40 msec exp; 4 sec dead time → 0.186 MGy
Shutterless
>90 msec exp → 0.257 MGy
40 msec exp → 0.373 MGy

Compare cryo-cooled → 30 MGy

Black – generation of aqueous or solvated e-, followed by 1st order decay
Red – postulated to be hydroxyl radical

Owen *et. al.* Acta Cryst. (2012) D**68**, 810-818

Near-ambient data collection? Reduced free radical migration

Viruses: for SV40, polymavirus, reovirus cores, and rotavirus DLPs, none of which could be frozen, "supercooling" (setting nominal temperature of cold-air flow just above level that would lead to ice-crystal formation in the capillary) allowed "undamaged" exposures from additional positions on a large crystal.

Example: SV40 crystals, in a high-salt mother liquor, were cooled to -15°C, measured on a thermocouple at the exit of the nozzle; we could then record equally good patterns from non-overlapping parts of the same crystal (100μ collimator; crystals >250μ across) if we set the temperature to -5°C or 0°C, radiation damage from one position on the crystal degraded data recorded subsequently from non-overlapping positions elsewhere on the crystals.

Guess: increased viscosity of the mother liquor near its freezing point retards free-radical diffusion. Would a this approach, with a very intense beam and hence very short exposures, reduce damage during a single exposure from a small crystal to levels comparable to those seen for comparable exposure at 80°K? In other words, can we make diffusion of damaging species slow compared with exposure time?

High Multiplicity and S-SAD

28 crystals – 18 isomorphous

Three steps of diminishing difficulty

Difficulty

Solving the S sub-structure

Low-resolution data only (d_{min} 5.2 Å OK; 4.5 Å failed)

Ultra-high multiplicity (100-fold OK; 50-fold failed)

Phase calculation from the S sub-structure

Moderate-resolution data (d_{min} 4.5 Å OK; 3.0 Å failed)

High multiplicity (33-fold OK; 17-fold failed)

Phase refinement (2-fold NCS, 75% solvent)

Extension in small steps (100 steps OK; 20 steps marginal)

Low multiplicity (6-fold)

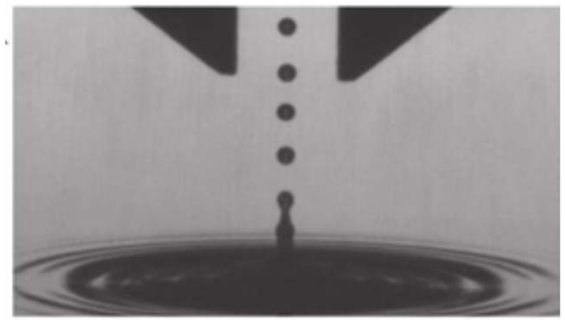
MX Workshop Report

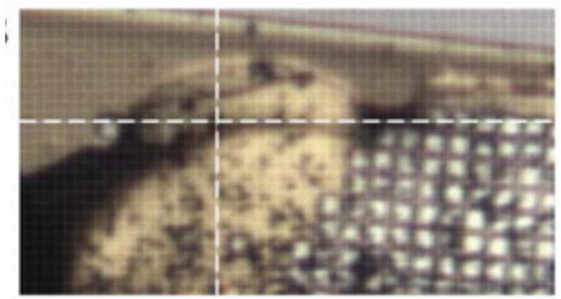
Enabling Technologies and R&D

- Micro-beam micro-quantity & Micro-crystal Crystallography
- *In situ* diffraction
- Pink-beam or large convergence angle crystallography
- Out-running secondary radiation damage
- Weak phasing signal detection with high multiplicity data sets
- Multiple Crystal data collection
- Large Homogenous Crystal
- X-ray Diffraction Near Edge Spectroscopy
- New sample delivery/Handling Visualization methods
- Better integration of crystal growth and preserving isomorphism
- Improved stability of sample and endstation components
- Detectors optimized for high energy
- Complementarity of XFEL and 4th Gen Synchrotron

Acoustic Droplet Ejection

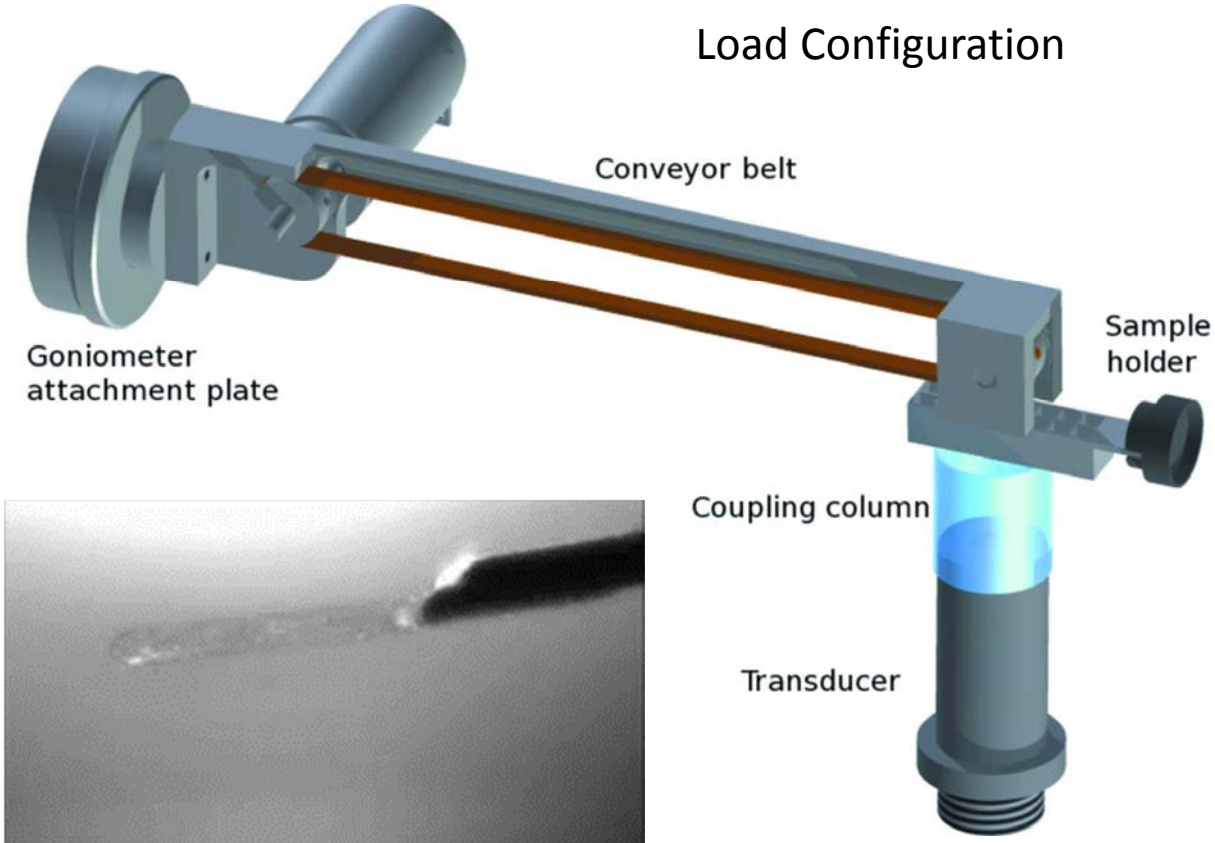
2.5 nl ejected droplet
175 µ diameter

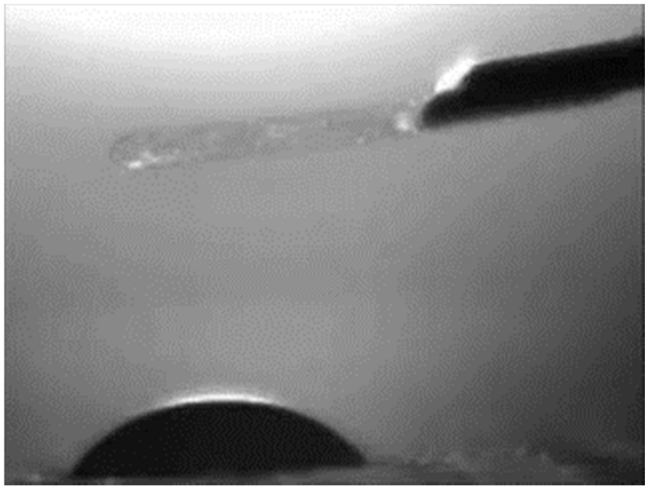




Slurry of insulin microcrystals
Supported by 25x25µ grid

Load Configuration

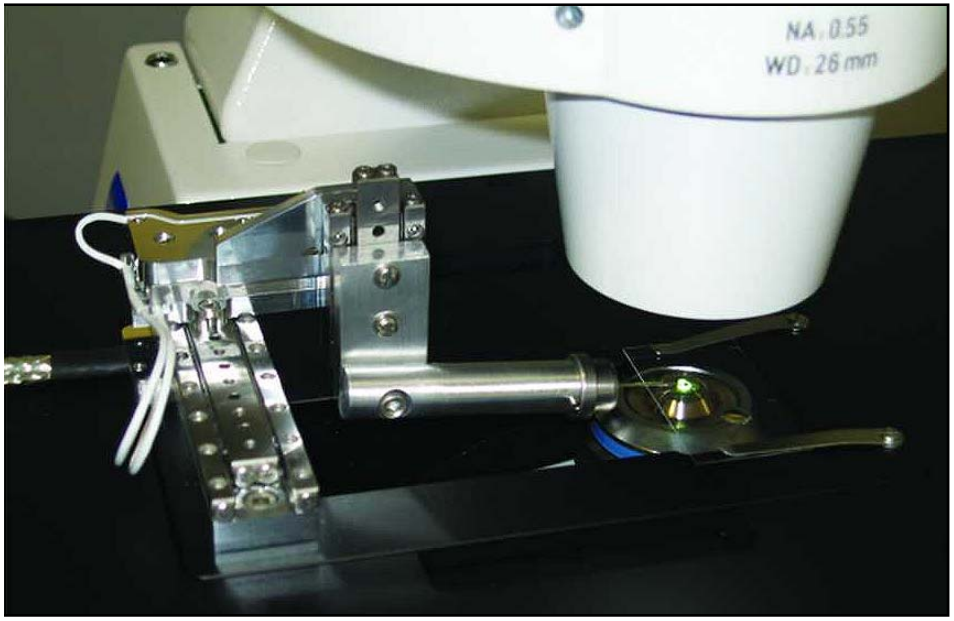


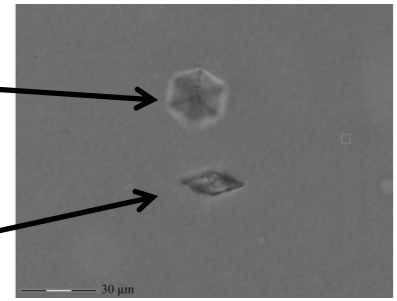


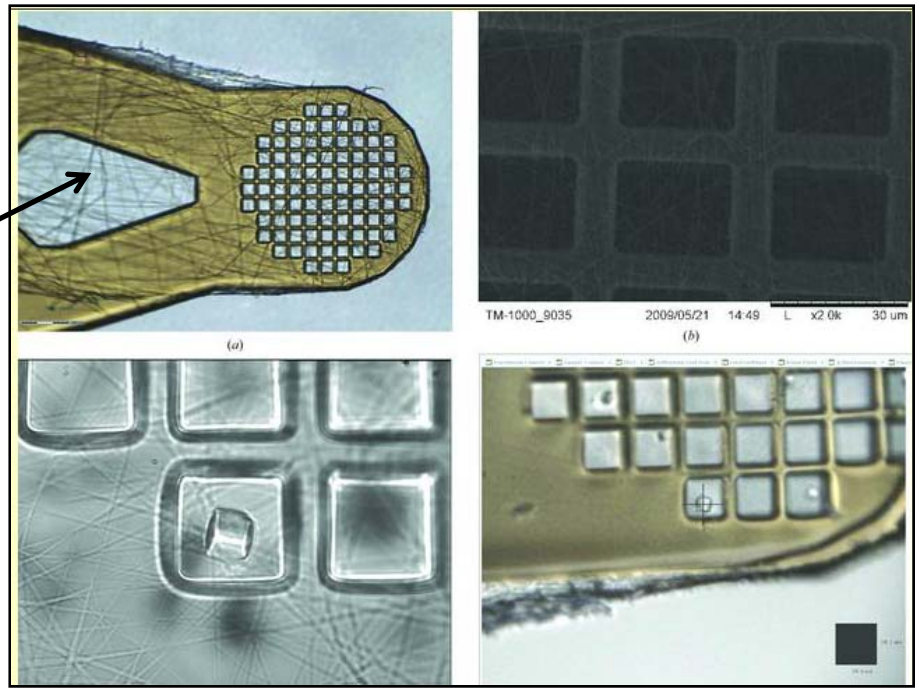
Alexei Soares, BNL/NSLS

Optical Traps

Strong E field gradient at waist of focused laser beam -> repulsive or attractive forces on dielectric particles with refractive index mismatch to solvent. Potential minimum at focus of beam.







Electro-spun PMMA fibers provide Floor to micromesh wells

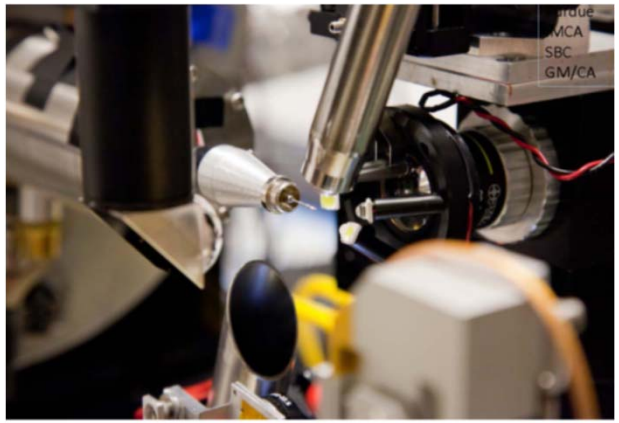
DLS

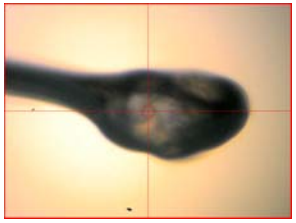
A. Wagner, et al Acta. Cryst. D Vol 69, pp 1297-1302, Jul 2013

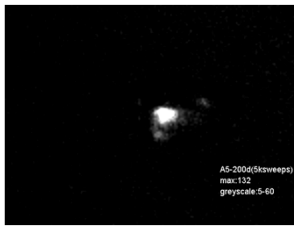
Second Order Non-linear Imaging of Chiral Crystals (SONICC) / Two-Photon Excitation UV Fluorescence

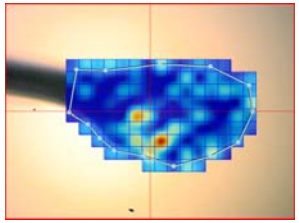
- High-sensitivity technique to detect sub-micron sized crystals, even in turbid media such as lipidic cubic phase (LCP).
- Measures Second Harmonic Generation (SHG) signal that arises from interaction of high-field laser with anharmonic polarizability tensor of chemical bonds.
- Multi-CAT collaboration

  GM/CA, IMCA, SBC
- Pioneered by Garth Simpson's group at Purdue University.





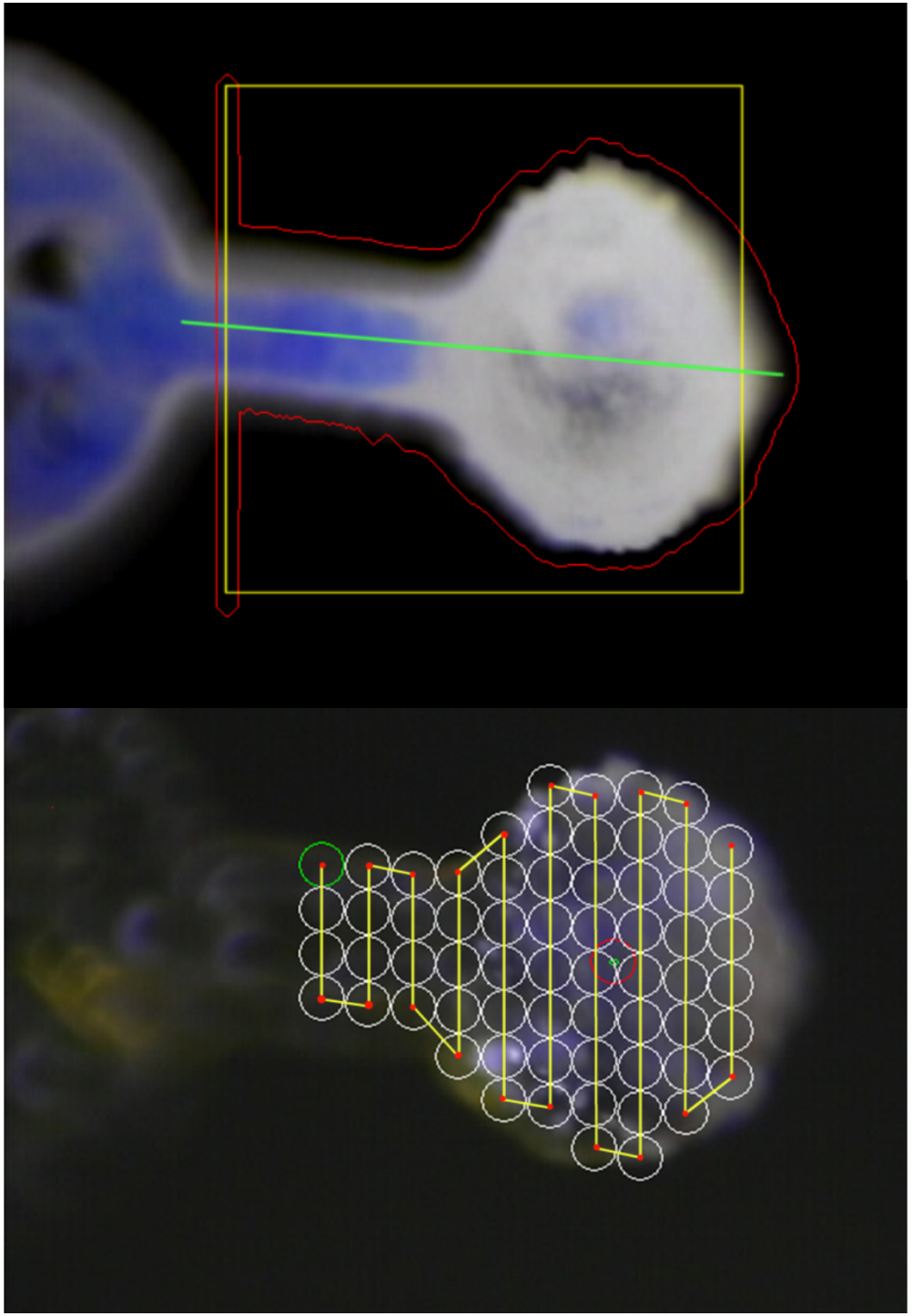


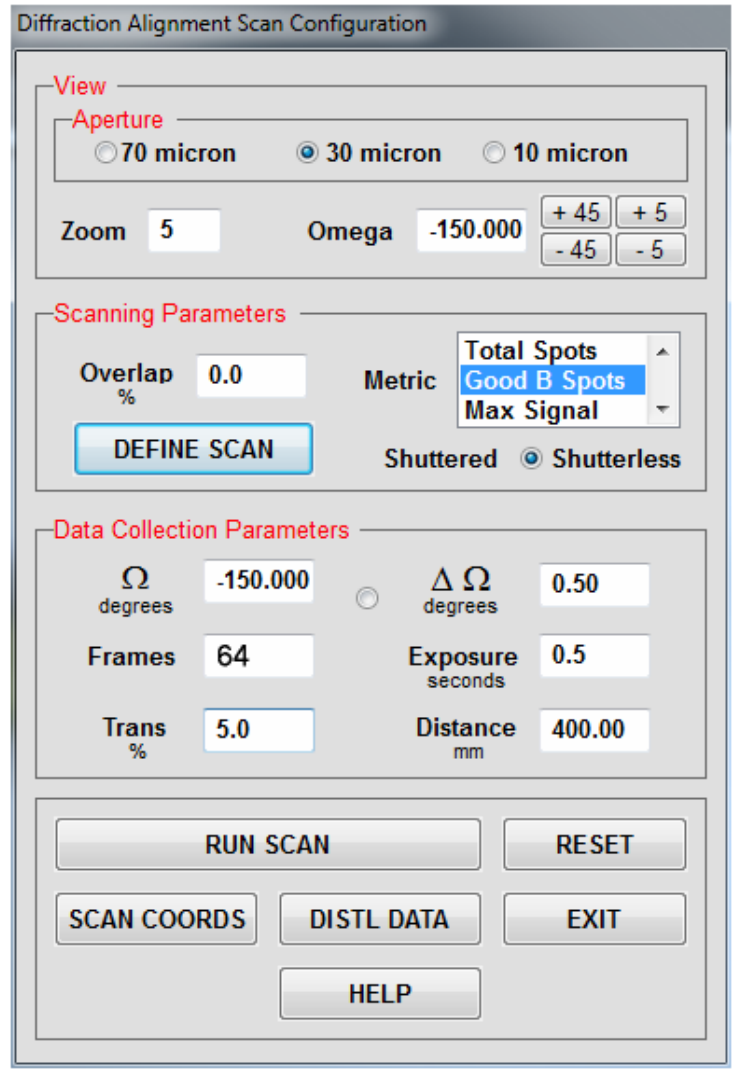


Sample containing human opioid receptor in lipidic cubic phase from Vadim Cherezov (TSRI).
Top: sample in bright field
Middle: SONICC image in laser focal plane
Bottom: Diffraction in JBluice .

MICHAEL BECKER, ANL

Setup




Diffraction Alignment Scan Configuration
View
Aperture
70 micron
30 micron
10 micron
Zoom
5
Omega
-150.000
+ 45
+ 5
- 45
- 5
Scanning Parameters
Overlap
%
0.0
Metric
Total Spots
Good B Spots
Max Signal
DEFINE SCAN
Shuttered
Shutterless
Data Collection Parameters
Ω
degrees
-150.000
Δ Ω
degrees
0.50
Frames
64
Exposure
seconds
0.5
Trans
%
5.0
Distance
mm
400.00
RUN SCAN
RESET
SCAN COORDS
DISTL DATA
EXIT
HELP