Introduction to Macromolecular Crystallography and Round Beams

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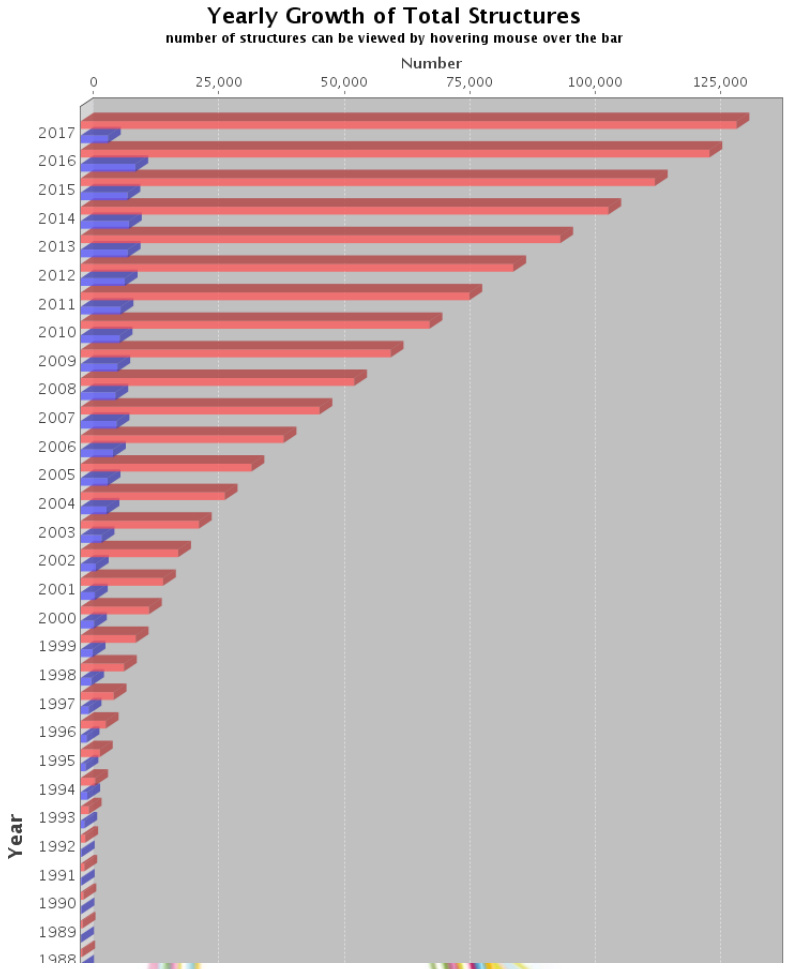
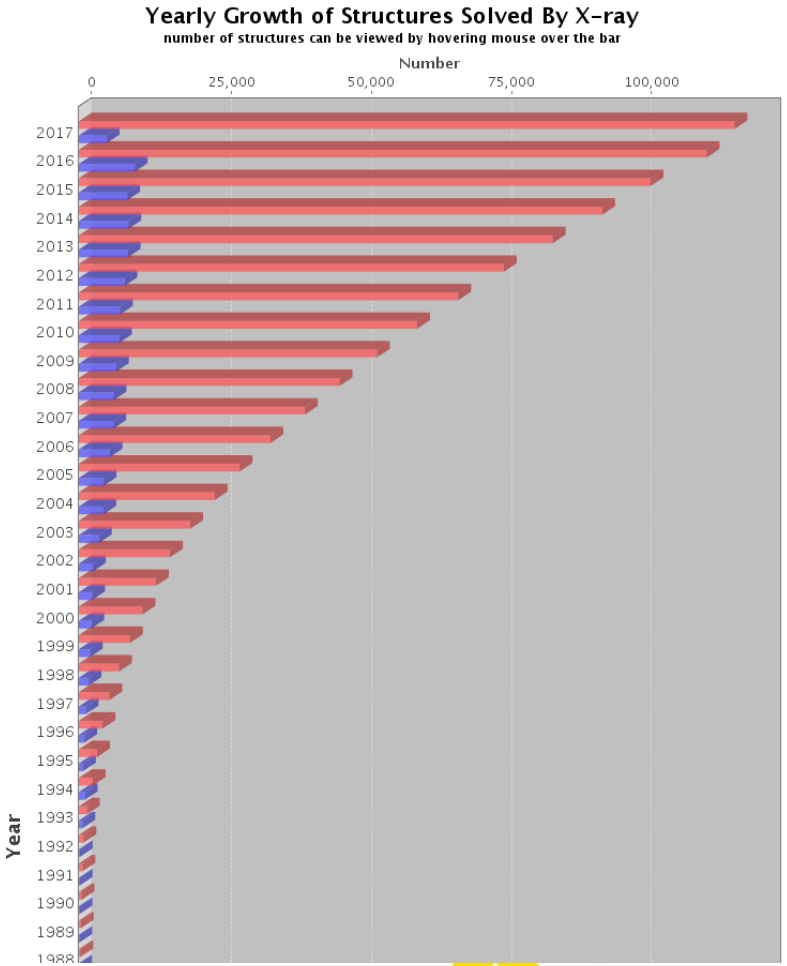
Introduction

- Macromolecular crystallography has played a key role in our understanding of biological systems over the last 40-50 years.
- In the last 20-30 years synchrotron radiation, first parasitic, and now purpose generated has played the largest role in the success of structural biology

PDB depositions



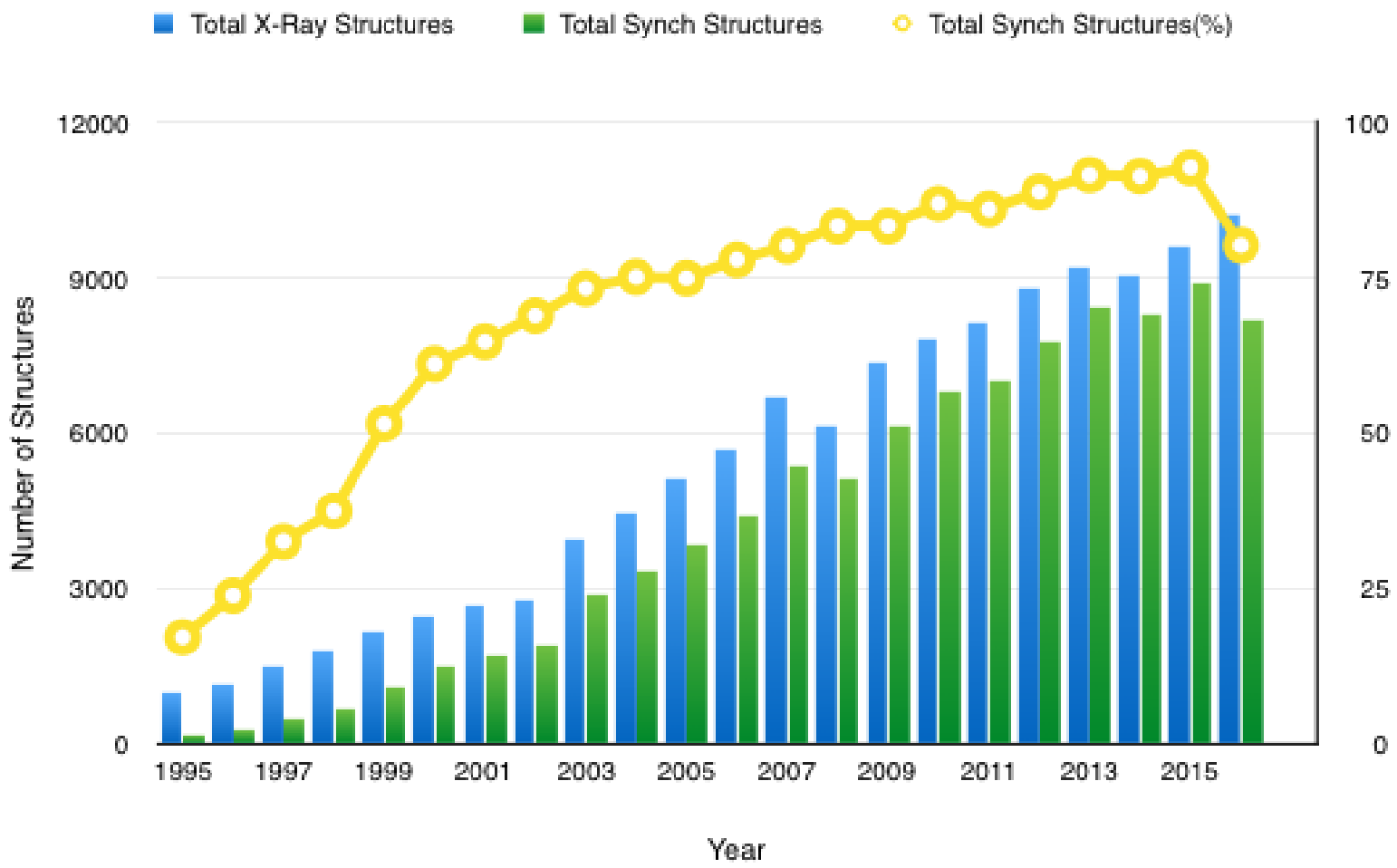


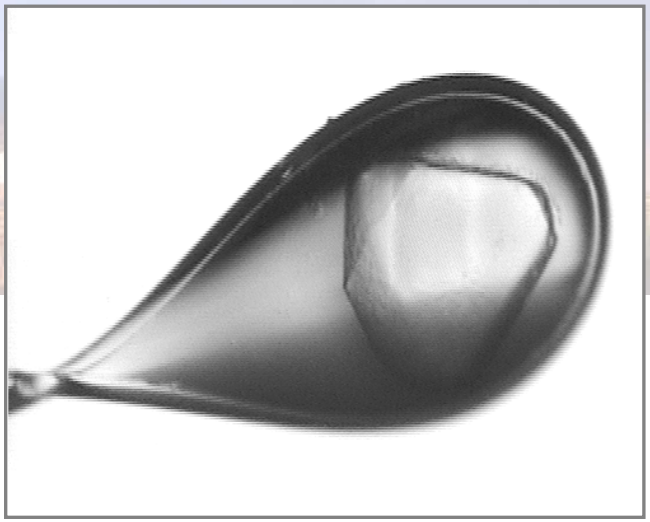
Protein Data Bank structures by method

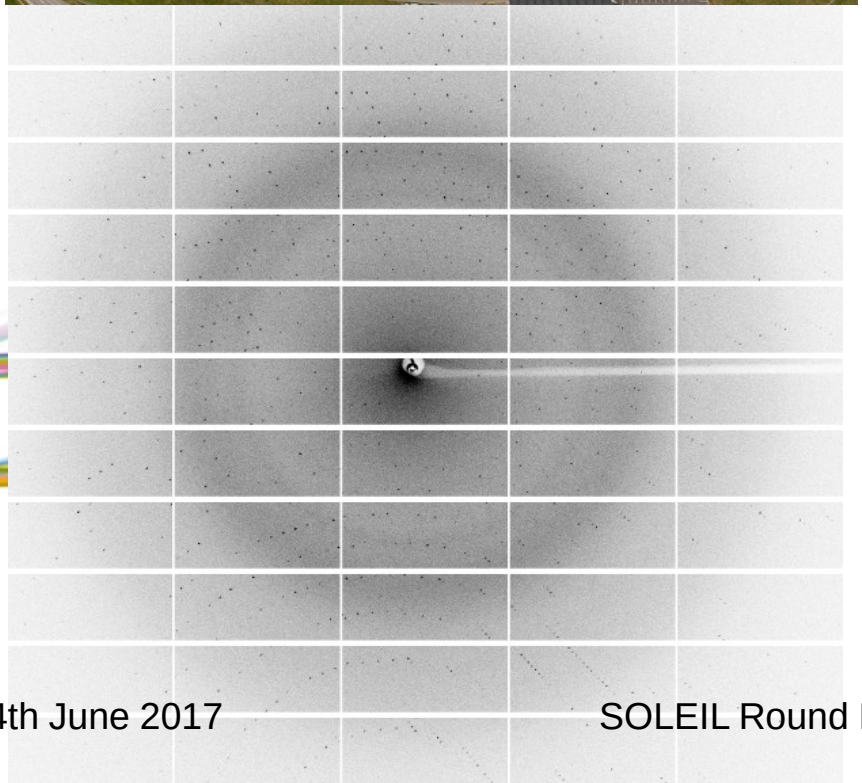
- Macromolecular crystallography is by far the most successful method for structure determination

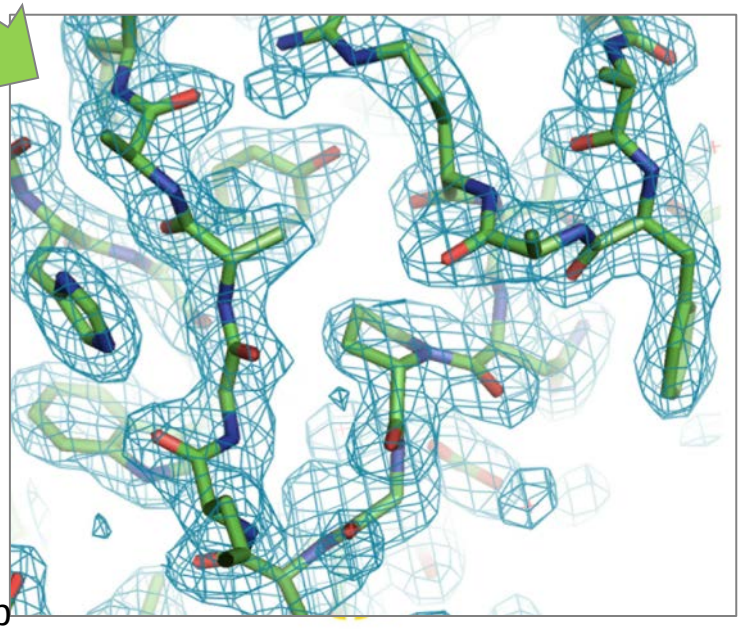
		Molecule Type				
		Proteins	Nucleic Acids	Protein/NA Complexes	Other	**Total**
Exp. Method	X-ray	109653	1846	5580	4a	**117083**
	NMR	10398	1209	241	8	**11856**
	Electron Microscopy	1115	30	400	0	**1545**
	Hybrid	102	3	2	1	**108**
	Other	192	4	6	13	**215**
	Total	**121460**	**3092**	**6229**	**26**	**130807**

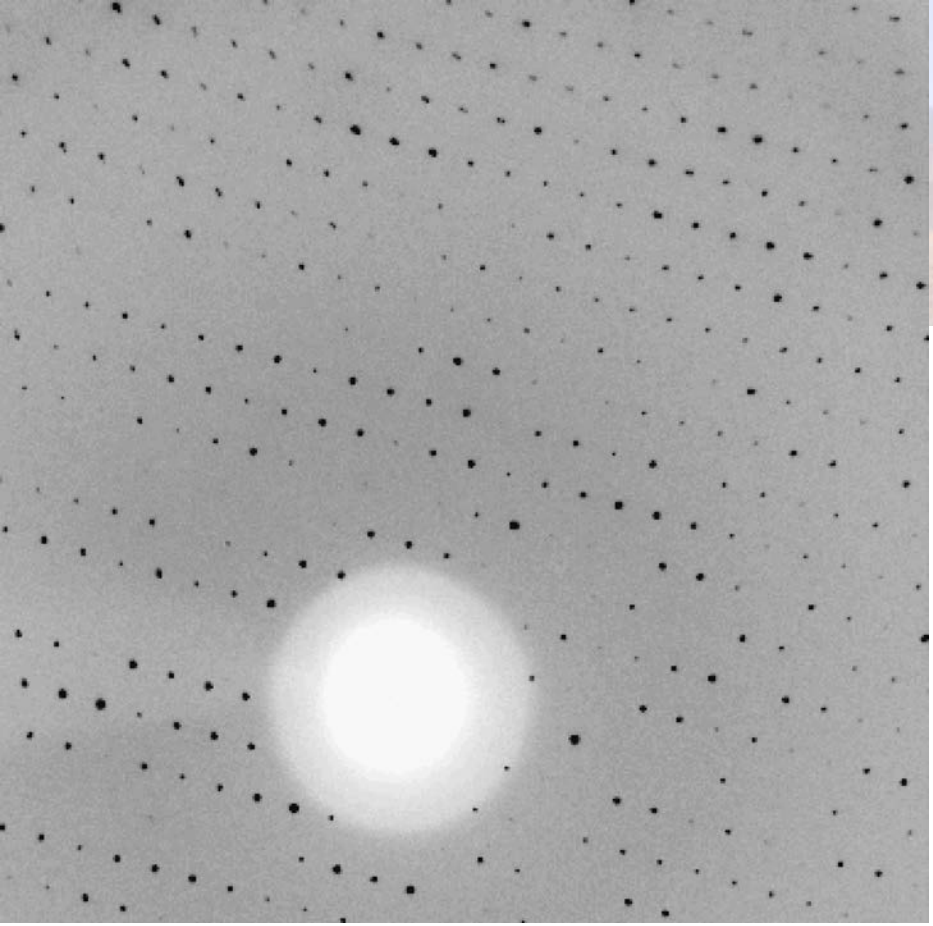
Impact of synchrotron radiation on MX







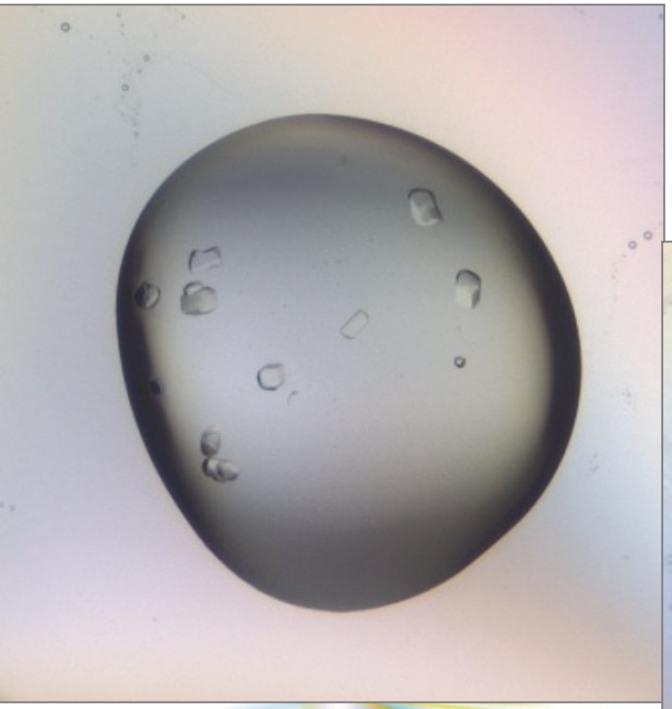


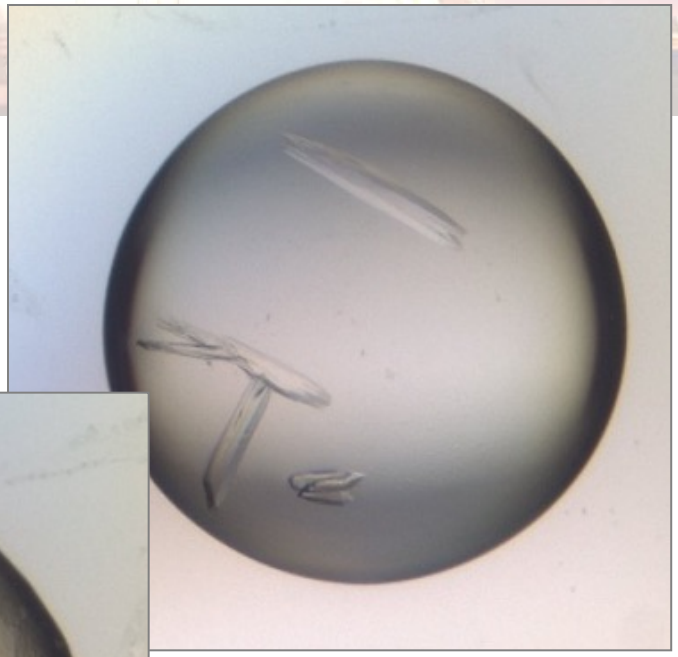


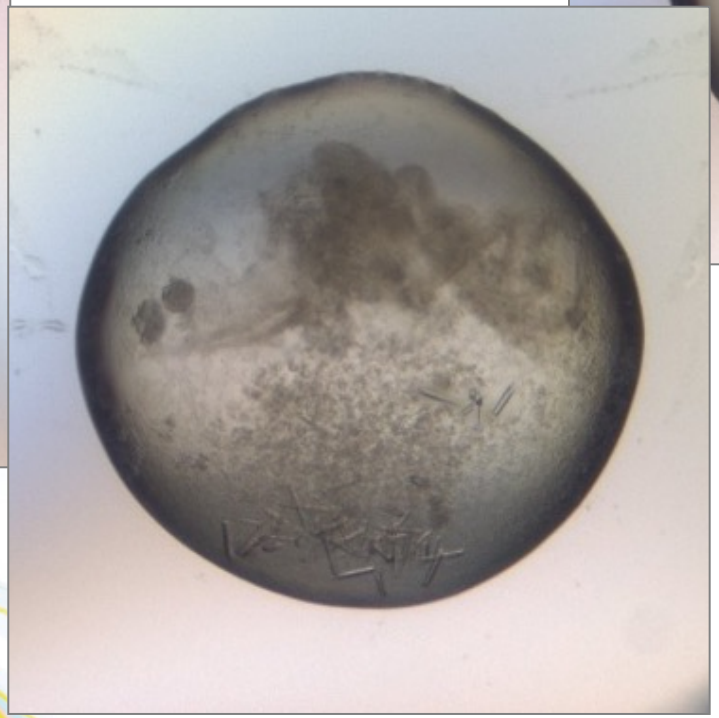
Phi = 0°

Phi = 90°

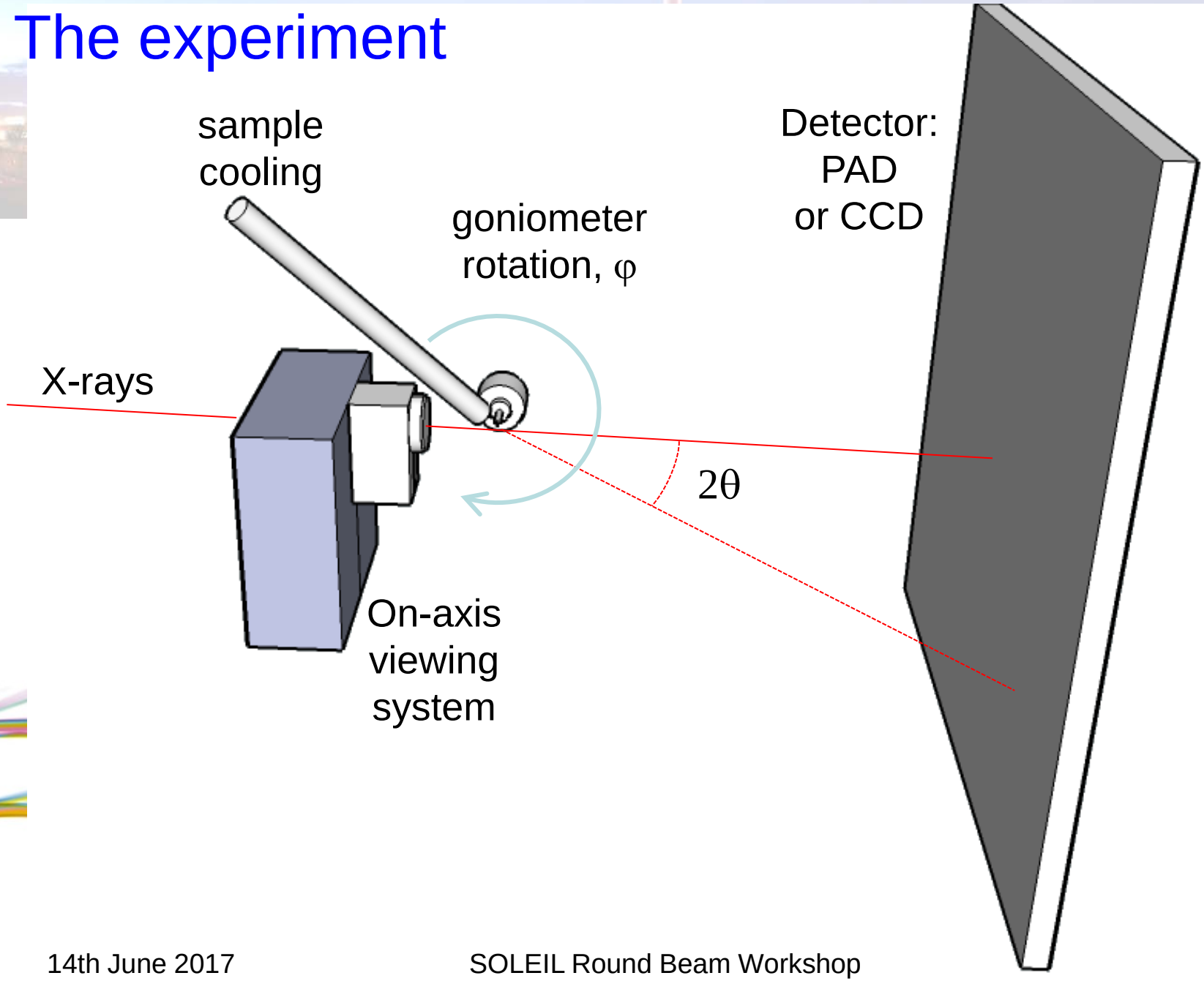
Crystals come in all shapes and sizes







The experiment



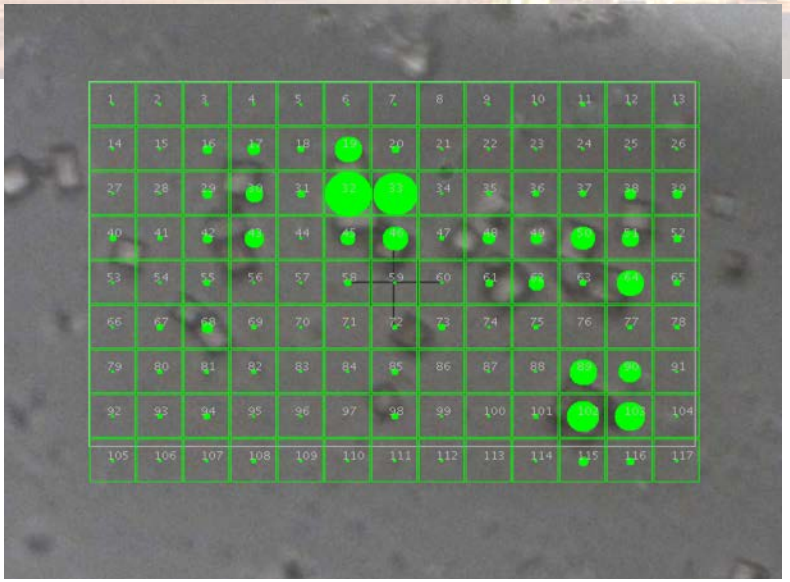
MX Trends in last decade

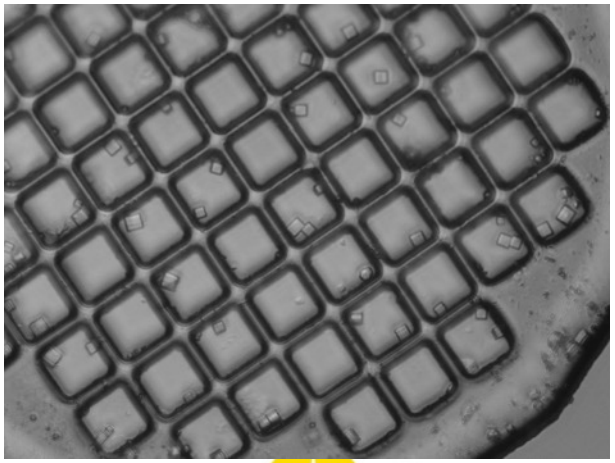
- Micro/nano-crystallography
  - use of multiple crystals per data set
- Room temperature and in situ crystallography
- Serial crystallography at both XFELs and Synchrotrons (see next talk by Bourenkov)
- Ultra-High throughput (seconds per complete data set)
  - data collection at up to 1 kHz frame rates

Microcrystallography

- Crystals dispersed on a micromesh
  - density of crystals on mesh regulated by slurry concentration
- Residual liquid wicked or blotted away
- Micromesh cryocooled and mounted on goniometer with mesh plane perpendicular to beam
- Mesh area divided into ‘manageable’ sized regions of ~100x100um
- raster scanning to locate best hits
  - very fast due to shutterless scanning
  - 200 grid points in about a minute?
- data collection from best hits
- move to next region

- Typical parameter
  - 0.05 or 0.1 deg images with between 1 – 10 degs per crystal depending on size
  - 1 sec/deg is typical with between ~10^{11} – 10^{12} ph/s (4 – 10 um size)

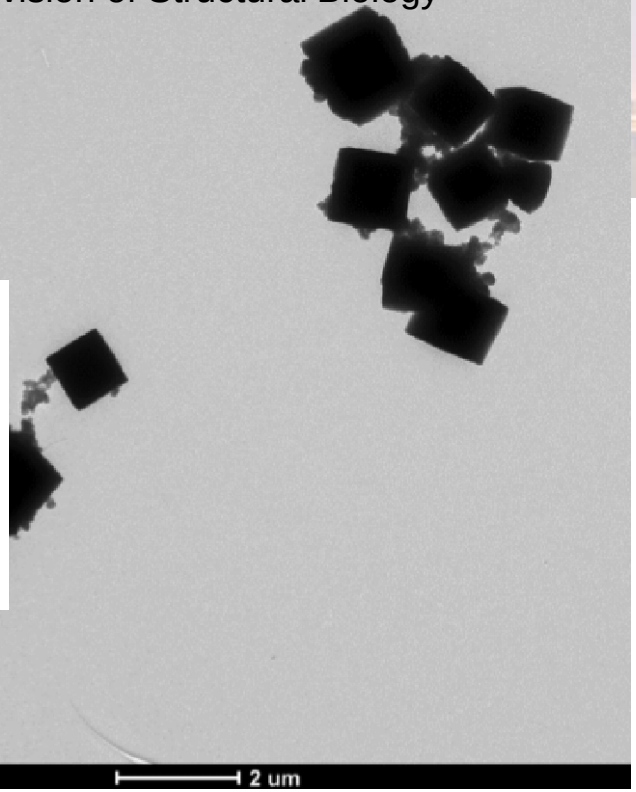




CPV17 ~ 1 μm^3 crystals

- 238 residues

TEM image courtesy of Geoff Sutton, University of Oxford Division of Structural Biology



Weak data with very low background!

8	1	4	9	4	3	1	5	3
3	0	5	5	5	5	4	2	1
2	2	2	4	7	2	5	3	1
2	2	2	13	17	15	11	4	3
5	2	1	8	24	21	15	6	3
2	3	3	4	6	12	10	2	4
5	1	5	1	6	5	5	1	8
4	5	2	4	3	2	1	3	3
6	4	2	3	4	2	3	5	1

1	2	5	0	1	3	0	3	3
2	3	1	1	2	1	4	2	3
2	0	2	3	2	1	1	1	2
2	3	3	6	8	4	0	2	2
2	0	3	9	13	2	1	2	0
3	5	0	3	3	3	1	3	2
4	0	2	4	1	0	1	1	1
1	3	4	1	3	1	1	3	2
2	0	1	4	4	3	4	2	1

diamond

ARTICLE

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Structure of CPV17 polyhedrin determined by the improved analysis of serial femtosecond crystallographic data

Helen M. Ginn[1], Marc Messerschmidt[2,†], Xiaoyun Ji[1,3], Hanwen Zhang[1], Danny Axford[4], Richard J. Gildea[4], Graeme Winter[4], Aaron S. Brewster[5], Johan Hattne[5], Armin Wagner[4], Jonathan M. Grimes[1,4], Gwyndaf Evans[4], Nicholas K. Sauter[5], Geoff Sutton[1] & David I. Stuart[1,4]

The X-ray free-electron laser (XFEL) allows the analysis of small weakly diffracting protein crystals, but has required very many crystals to obtain good data. Here we use an XFEL to determine the room temperature atomic structure for the smallest cytoplasmic polyhedrosis virus polyhedra yet characterized, which we failed to solve at a synchrotron. These protein microcrystals, roughly a micron across, accrue within infected cells. We use a new physical model for XFEL diffraction, which better estimates the experimental signal, delivering a high-resolution XFEL structure (1.75 Å), using fewer crystals than previously required for this resolution. The crystal lattice and protein core are conserved compared with a polyhedrin

Table 1 | XFEL data collection and refinement statistics.

	CPV17
Data collection	
Space group	I23
Cell dimensions	
a, *b*, *c* (Å)	106.1, 106.1, 106.1
α, β, γ (°)	90, 90, 90
Resolution (Å)	28.30–1.75(1.79–1.75)*
R_{pim} (%)	7.4 (33.8)
R_{split} (%)	11.8 (58.4)
$CC_{½}$	98.0 (38.6)
Completeness (%)	100 (100)
Redundancy	52.4 (11.1)
Refinement	
Resolution (Å)	28.36–1.75
No. of reflections	20,122
R_{work}/R_{free}	12.2/15.4
No. of atoms	
Protein	1,914
Ligand/ion	32
Water	174
B-factors	
Protein	23.7
Ligand/ion	34.4
Water	32.7
R.m.s. deviations	
Bond lengths (Å)	0.010
Bond angles (°)	1.39

CPV17, CPV type 17; XFEL, X-ray free-electron laser.
Number of crystals used: 5,554.
*Highest-resolution shell is shown in parenthesis.

Table 2 | Synchrotron data collection and refinement statistics.

	CPV17
Data collection	
Space group	I23
Cell dimensions	
a, *b*, *c* (Å)	104.9, 104.9, 104.9
α, β, γ (°)	90.0, 90.0, 90.0
Resolution (Å)	74.16–2.20 (2.26–2.20)*
R_{merge} (%)	66.5 (325.9)
$I/\sigma I$	6.4 (1.4)
Completeness (%)	99.9 (100.0)
Multiplicity	47.8 (25.4)
Refinement	
Resolution (Å)	74.16–2.20
No. of reflections	9,376
R_{work}/R_{free}	14.7%/19.9%
No. of atoms	
Protein	1,907
Ligand/ion	32
Water	146
B-factors	
Protein	22.5
Ligand/ion	50.0
Water	32.2
R.m.s deviations	
Bond lengths (Å)	0.013
Bond angles (°)	1.675

CPV17, CPV type 17.
Number of crystals used: 768.
*Highest-resolution shell is shown in parenthesis.

GPCRs on I24 at Diamond

Structure of class B GPCR corticotropin - releasing factor receptor 1

K. Hollenstein, J. Kean, A. Bortolato, R. K. Y. Cheng, A. S. Doré, A. Jazayeri, R. M. Cooke,

ARTICLE

doi:10.1038/nature12357

Structure of class B GPCR corticotropin-releasing factor receptor 1

Kaspar Hollenstein[1], James Kean[1], Andrea Bortolato[1], Robert K. Y. Cheng[1], Andrew S. Doré[1], Ali Jazayeri[1], Robert M. Cooke[1], Malcolm Weir[1] & Fiona H. Marshall[1]

Structural analysis of class B G-protein-coupled receptors (GPCRs), cell-surface proteins that respond to peptide hormones, has been restricted to the amino-terminal extracellular domain, thus providing little understanding of the membrane-spanning signal transduction domain. The corticotropin-releasing factor receptor type 1 is a class B receptor which mediates the response to stress and has been considered a drug target for depression and anxiety. Here we report the crystal structure of the transmembrane domain of the human corticotropin-releasing factor receptor type 1 in complex with the small-molecule antagonist CP-376395. The structure provides detailed insight into the architecture of class B receptors. Atomic details of the interactions of the receptor with the non-peptide ligand that binds deep within the receptor are described. This structure provides a model for all class B GPCRs and may aid in the design of new small-molecule drugs for diseases of brain and metabolism.

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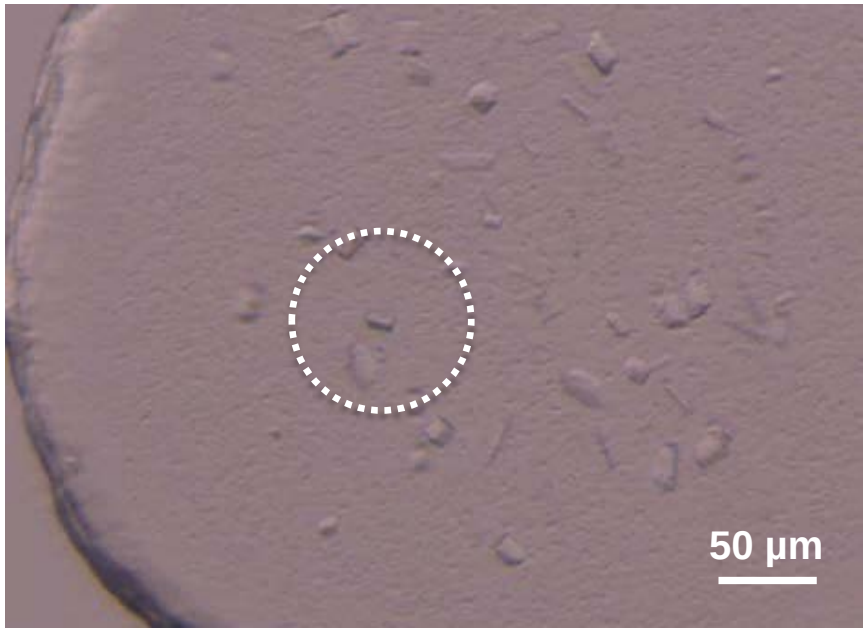
Nature (2013) **499** 438-443.

Crystals between 5 - 15 µm in dimension



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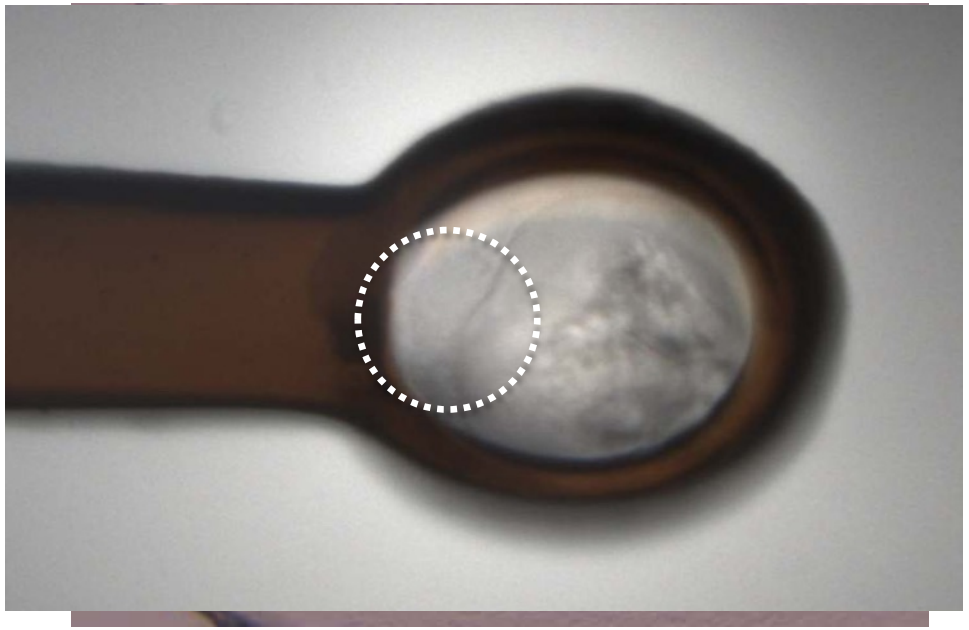

Crystals between 5 - 15 µm in dimension

Grown in LCP - invisible once harvested and cryo-cooled.

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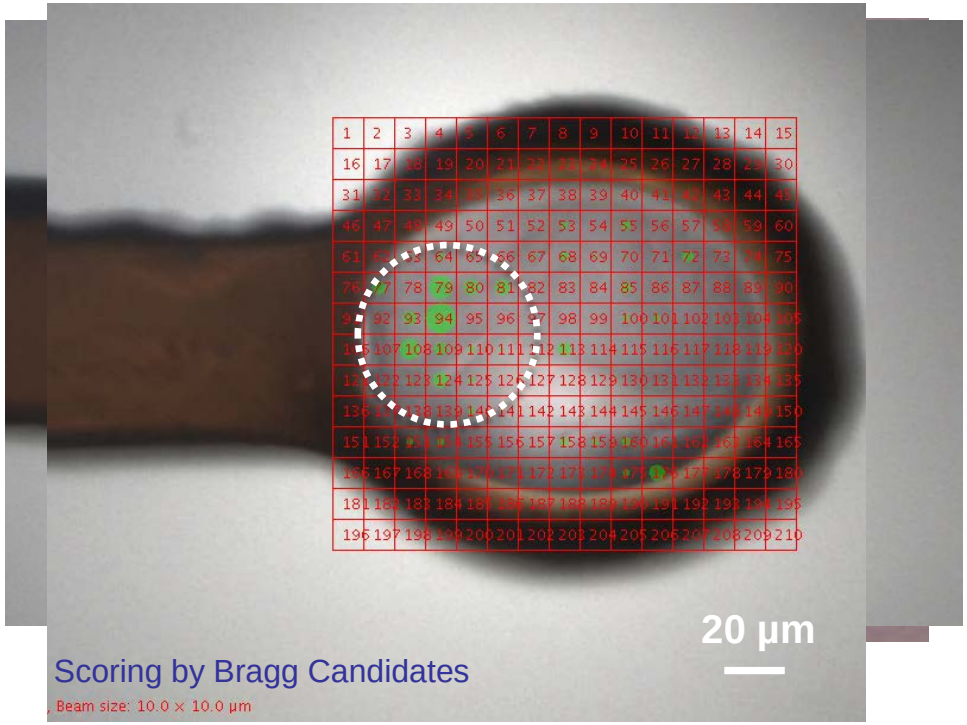
Crystals between 5 - 15 µm in dimension

Grown in LCP - invisible once harvested and cryo-cooled.

<u>Experimental strategy</u>

Grid scan over entire loop with attenuated beam to locate crystal.



For small crystals (~5 µm) reduce beamsize, and then repeat grid scan around sweet spot from gridscan #1 before collecting data

Structure determined to 3 Å resolution using 35 crystals

Early in situ setup on Diamond I24

Plate mounted at 0°

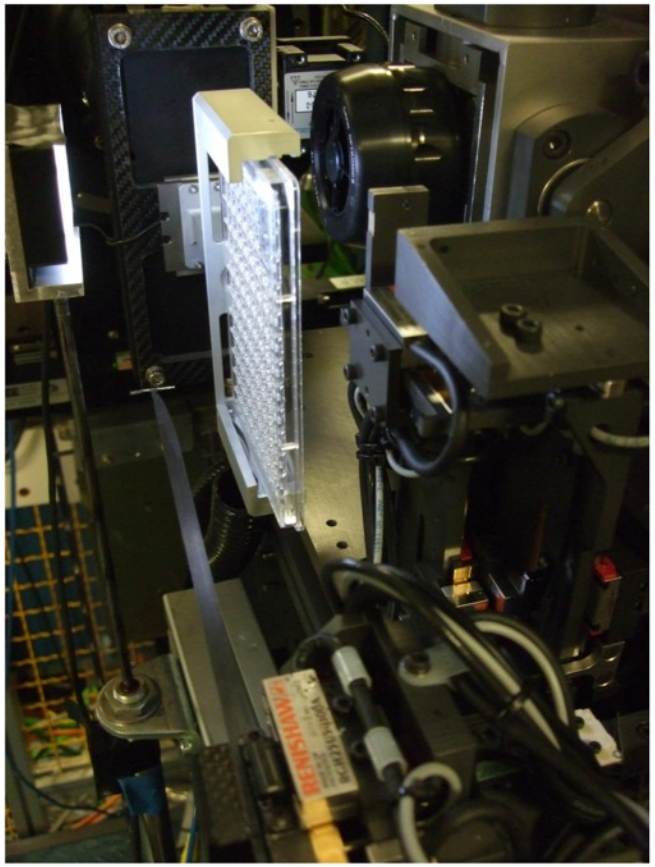
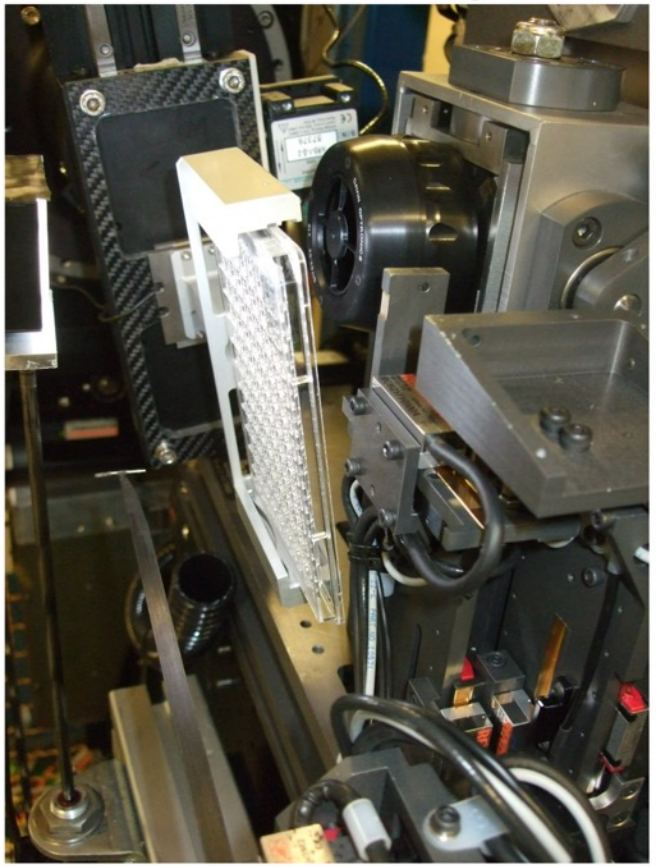


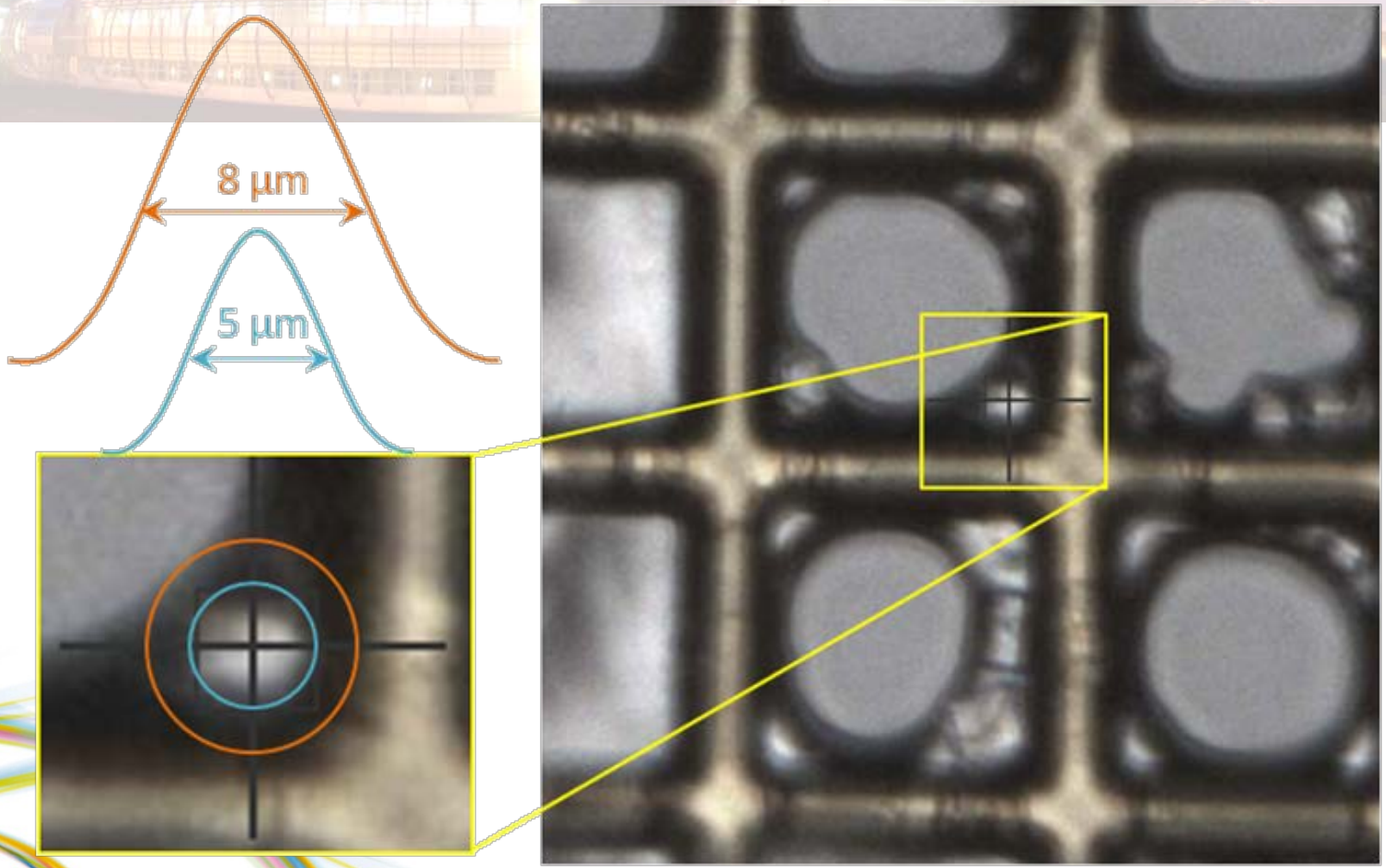
Plate mounted at tilted angle



Which beam parameters matter?

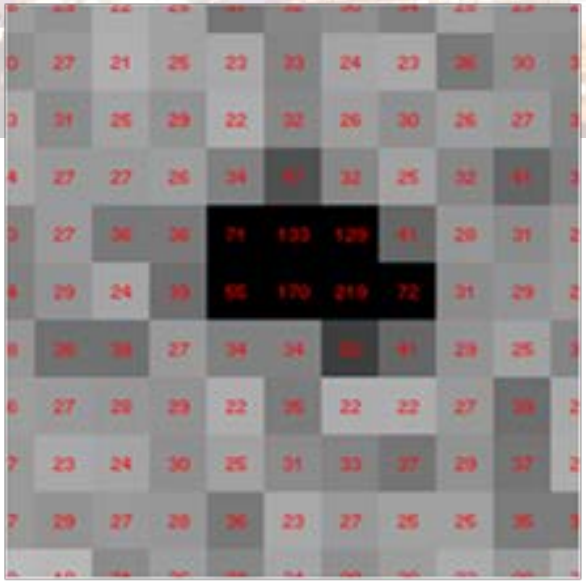
- Beam spot size and shape at the sample position
  - matching beam to crystal size and shape to reduce background scatter from surrounding material
  - need optics that can allow beam size to be varied independently vertically and horizontally over 1-2 orders
    - e.g. I24 Diamond 5 – 50 um; VMXm Diamond 0.5 – 10 um
- Beam divergence
  - confining diffracted beam spots to match detector pixel size thereby reducing impact of measured background on measurement signal to noise
- X-ray energy tuneability
  - tune X-ray energy to important heavy atom absorption edges (5 – 20 keV)
  - benefit from effects such as photoelectron escape to reduce radiation damage > 20 keV)
- Band-width of ID harmonics (for pink beam experiments using multilayer monochromators)
  - to gain an order of magnitude flux increase for time-resolved serial crystallography experiments
- Bunch timing (less so unless very, very high brilliances are available)

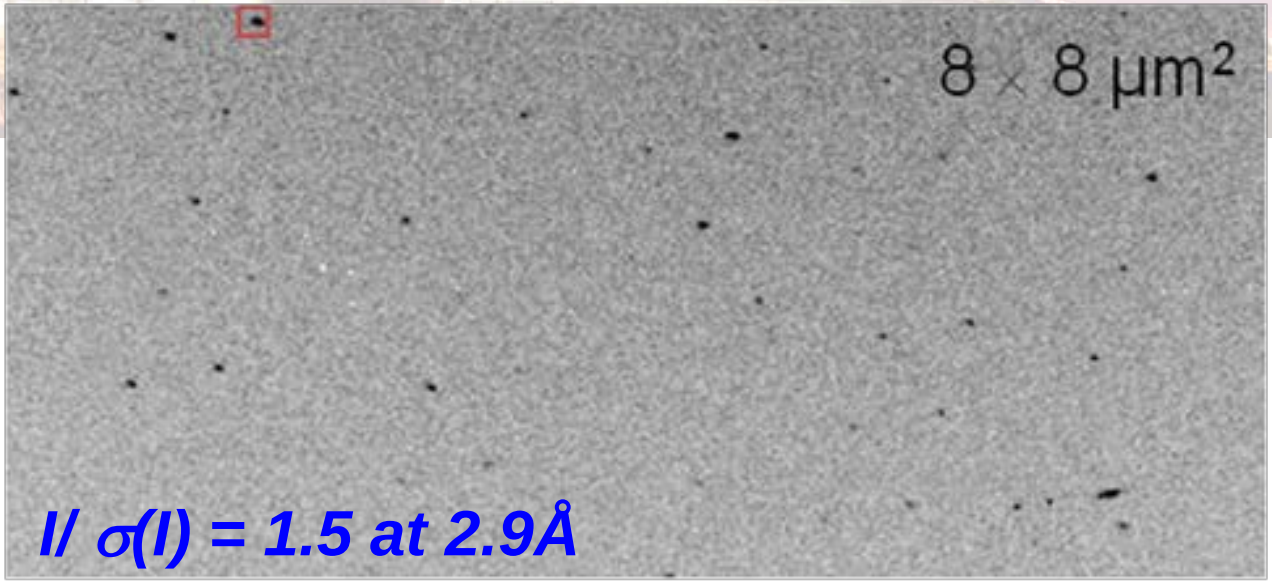
Matching your beam to your sample



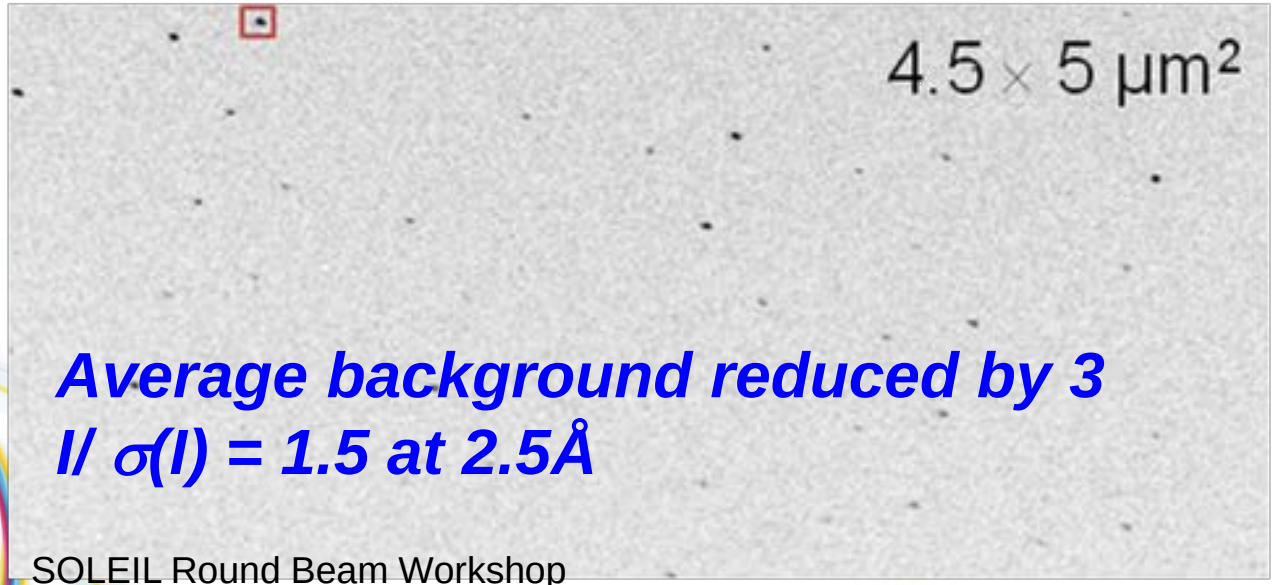
AcMNPV polyhedra crystals approx 5×5×5 µm^3 in size were mounted on a micromesh grid
Data were collected with two beamsizes: 8×8 µm^2 and 4.5×5 µm^2

Matching your beam to your sample





I/ σ(I) = 1.5 at 2.9Å

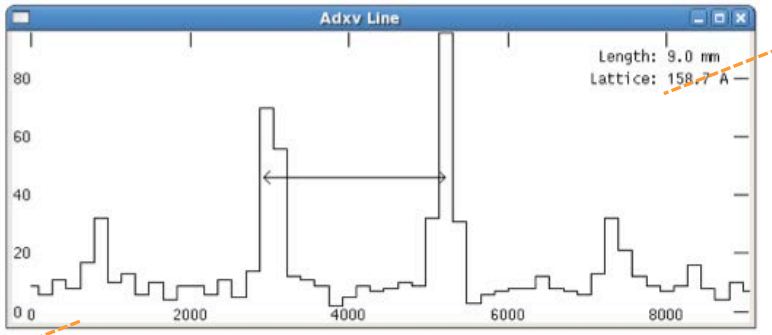


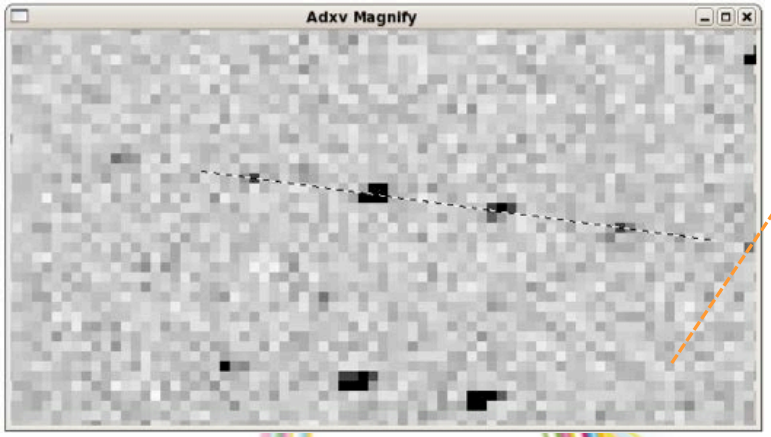
Average background reduced by 3
I/ σ(I) = 1.5 at 2.5Å

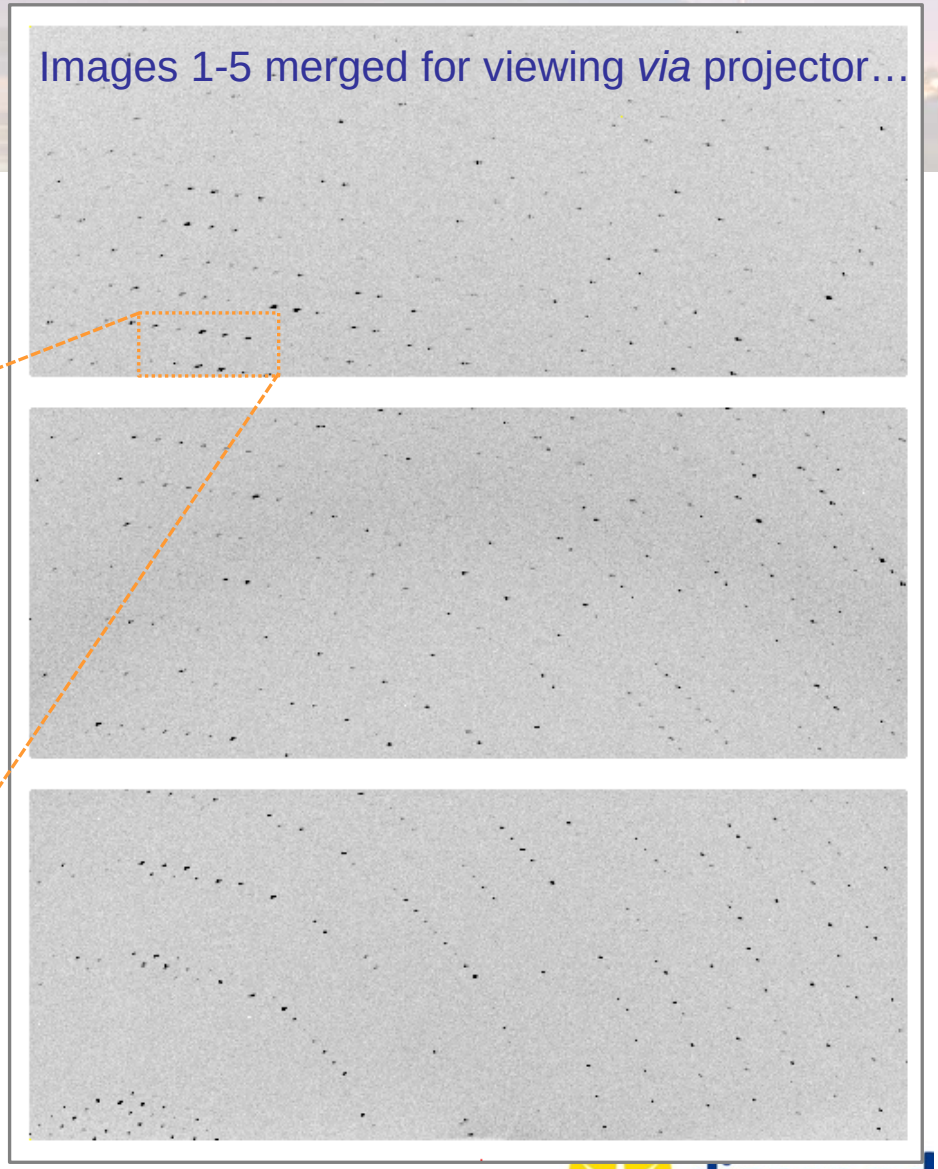
Low divergence

A22 Foot and mouth disease virus measured on I24 at Diamond

Beam defocused to 30×30 μm^2
exposure time 3.05 ms
(exposure period 4ms)







Matching beam divergence to detector properties

- Current state-of-the-art 2D pixel-array detectors for crystallography have pixel sizes of ~ 50 – 75 microns
- To record data extending to high resolution we position detectors at between 200 and 500 mm from sample
- This means that divergences of beams at sample should be ~< 100 urads
- (Future advances in detector technology may drive this number down)

Current issues

- Currently for many MX beamlines the vertical (electron beam) X-ray source size is much smaller that required
  - X-ray optics are limiting factor in achieving small vertical beams at sample (not vert. source)
  - creating larger beams to match sample size is not straightforward (in fact it is still a challenge for many beamlines)
  - CRLs provide good options
- Large horizontal source size means very large demagnification ratios 50-100:1 resulting in high divergences > 500 urad
  - also reduced space for end-station components
- Large mirrors (~1m) are required to capture all available flux

Could round beams help (or more generally, versatility in e-beam parameters)?

- Trading off vertical beam size against horizontal to create a more isotropic source shape would potentially simplify optical configurations
- A reduced overall emittance would hopefully keep horizontally divergence to acceptable levels (< 0.1mrad)
  - mirror optics would be smaller
  - required acceptance for in-line optics (CRLs etc) are smaller
  - working distance at sample larger
- Can machine optics and X-ray optics work together to create clean (structureless) and variable beam shapes and sizes, low divergence X-ray beams at the sample
  - fast changes of e-beam parameters could provide fast changes of X-ray beam parameters at sample?

Acknowledgments

- Thanks to colleagues in the MX group at Diamond Light Source for contributing slides

THANK YOU FOR YOUR ATTENTION