What can we do with serial crystallography at a synchrotron (?)

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Serial crystallography on *in vivo* grown microcrystals using synchrotron radiation

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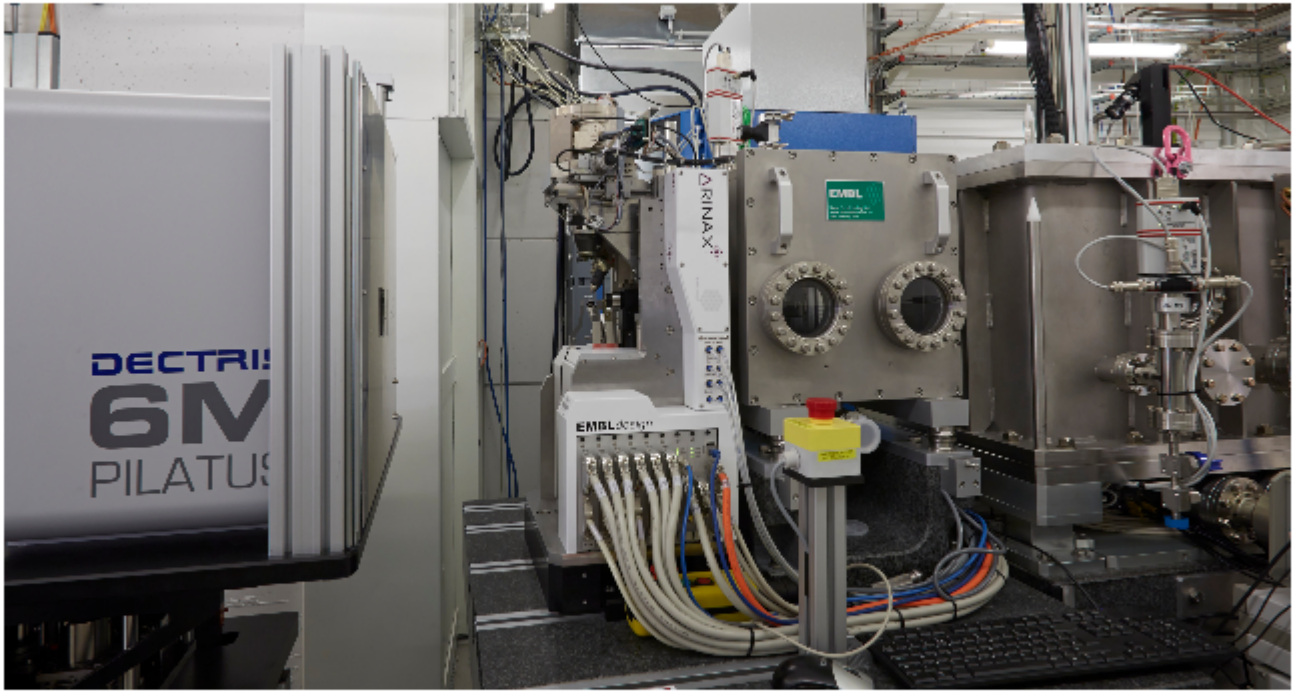
PETRAIII, Beamline P14



user operation 02/2013-02/2014

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40

Beam parameters (micro-focusing mode)

@ 10 keV

- Beam size in microfocus mode

  5-6(h) x 4-5 (v)
- Flux

  6 10^{12} ph/sec
- Peak dose rate

  100 MGy/sec

Data Collection @P14

- The only way to exploit the full microfocus flux is to continuously move sample through the beam
- Vertical spindle MD3 provides the necessary dynamic mechanical precision, and sophisticated motion control
- Shutterless helical ("the 4D") scan use the full beam – helical data collection runs at the same rate as regular d.c.
- Convenient micro-crystallography tools - diffraction-based sample localization etc. - via MxCuBEv2, in progress

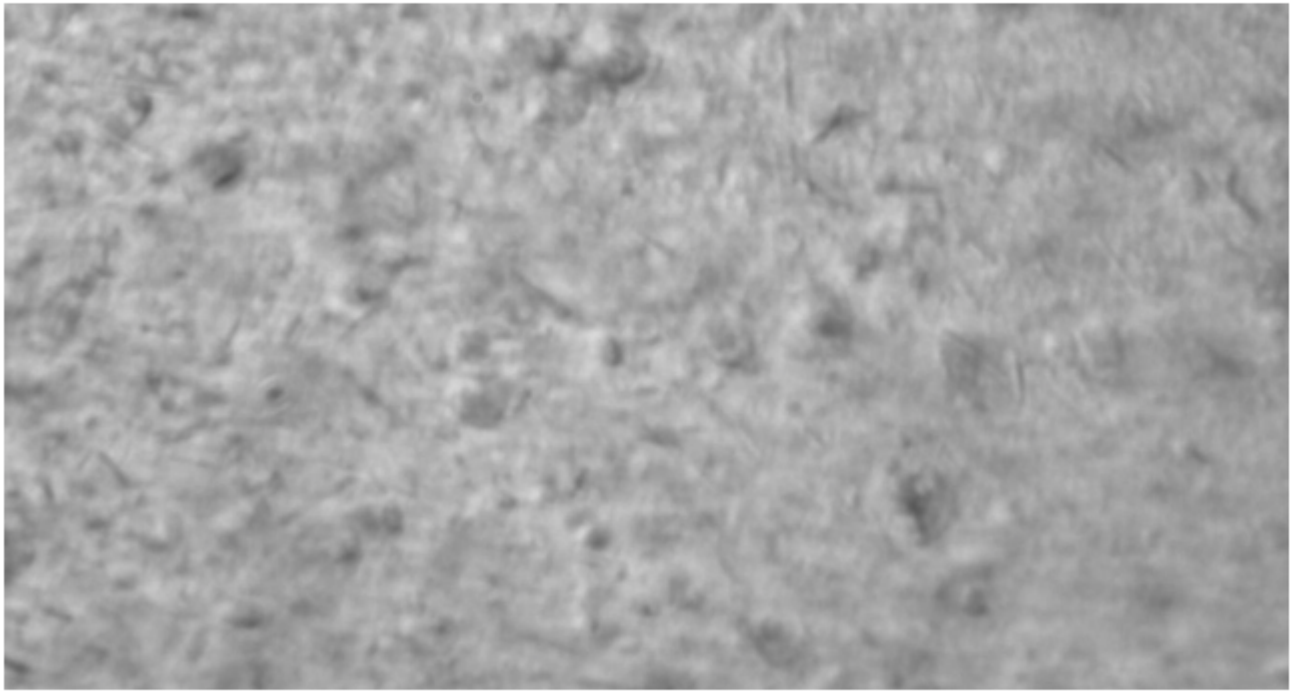
  Using line focus is very efficient
- For regular data collection the flux traded off for flexibility in beam definition, and stability. It matches the detector framing rate (PILATUS 6MF 25Hz)

The case for serialization

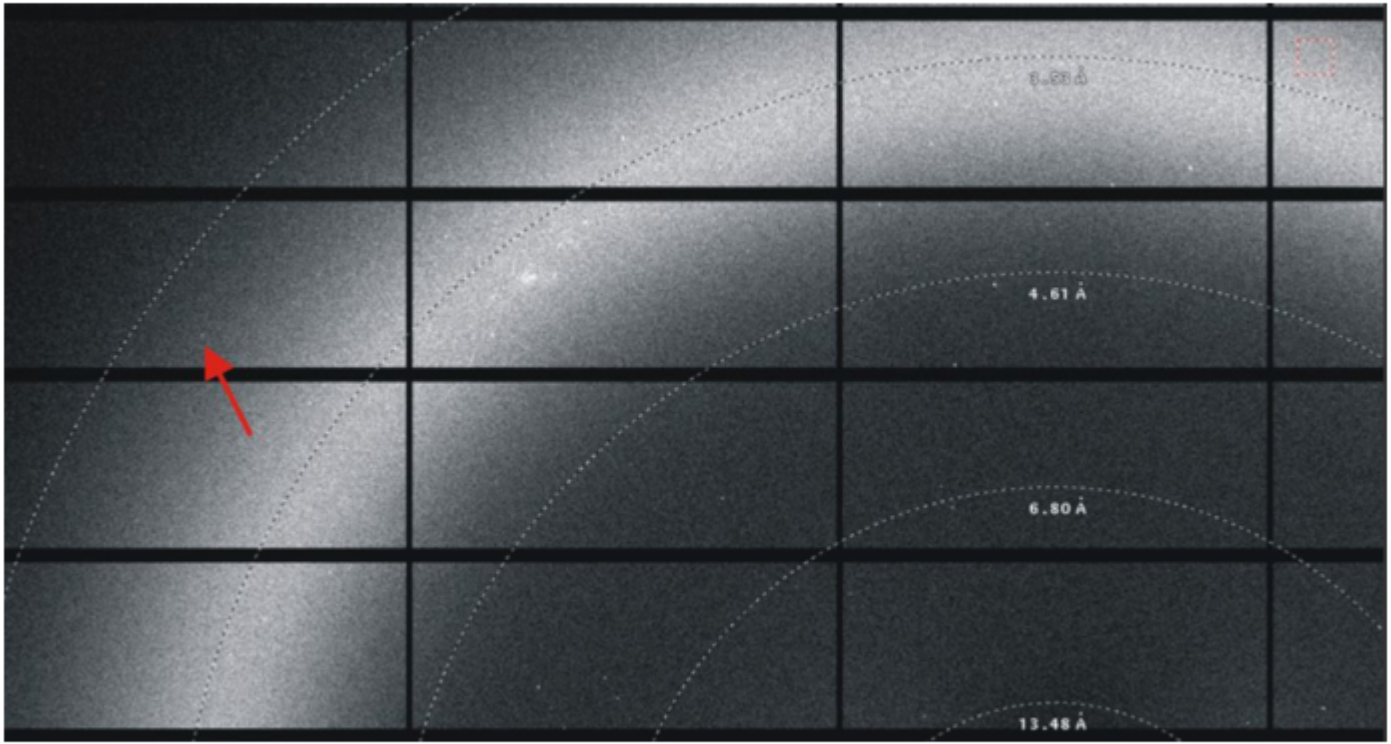
- Approximately a live-dose per rocking width of rotation is required to obtain some interpretable diffraction data
- Optical optical localization is difficult or impossible

- small crystals (<≈ 3 µm in two dimensions) represent typical example
- not determined by the crystal size alone, but by a complete set of diffraction properties, radiation sensitivity and by the sample environment

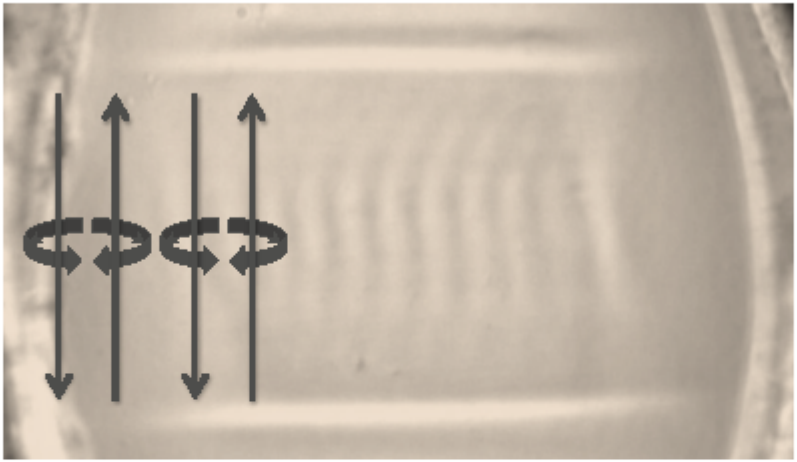
Microcystaline suspension in a standard cryo mount, MD3 OAV image



Diffraction image, exposure dose 34 MGy



Fast rastering by rotation exposures



- series of helical scans along vertical spindle axis, ± 10° to ± 60°
- horizontal translations of sample mount between line scans, pitch = beam FWHM
- flat mount aligned normal to the beam with Kappa
  - approximately equal dose at each point
- synchronized shutterless acquisition @ detector

Small but <u>scalable</u> wedge of rotation data on every "random" hit

in-vivo grown CatB crystals

T.Brucei procatepsin B (CatB)

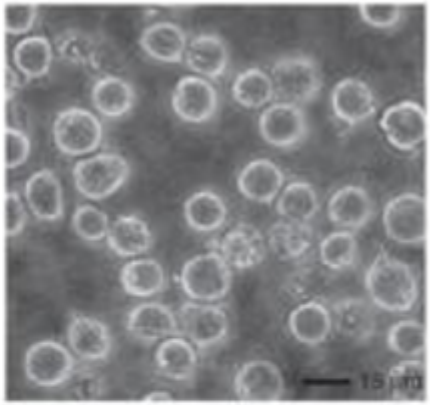
Spontaneously crystallizes in baculovirus-infected insect cells

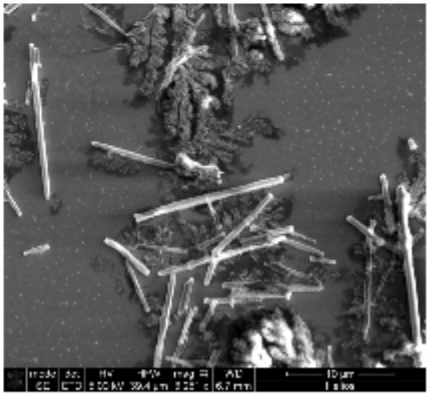
Average dimensions 0.9*0.9*11 μm^3
10^7 unit cells

Concentration $5x10^8$ crystals/millilitre^{-1}

Structure determined previosly using Serial Femtosecond Crystallography (SFX)

Redecke, L. *et al.* (2013) *Science*, **339**, 227-230.





CatB data collection

Sample

- Crystalline suspension + 40% w/w glycerol
- Mounted on standard 20 µm nylon loop, ⦸ 700 µm
- Sample volume 13 nl
- In total ~5000 crystals

Beam

- 4 x 5 μm^2 (FWHM)
- flux of 1.2 $x10^{12}$ photons
- Energy 10.00 keV.

Data collection

- Scanned region 600x600 µm
- 120 helical scans x 240 exposure
- 1 sec, 0.375° rotation, 2.5 µm translation
- 5 µm translaton beween lines

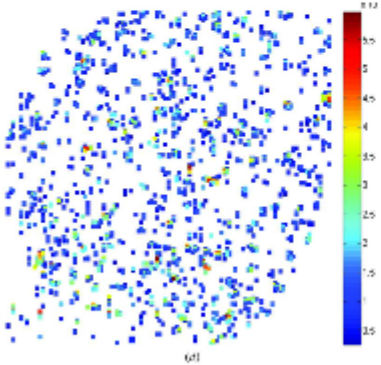
  Dose 50 to 60 MGy
- 28800 frames in 8 hours

CatB data processing

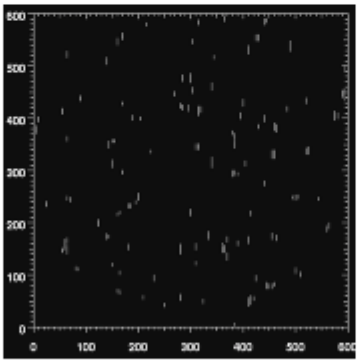
2233 frames indexed by CRYSTFEL (White et al. 2012)

1734 frames grouped into 595 rotation wedges
130 wedges (557 frames) successfully integrated by XDS
120 wedges retained in scaling by SCALA (C.C. to merged data >70%)
80 crystals contributed in total

Spatial distributions

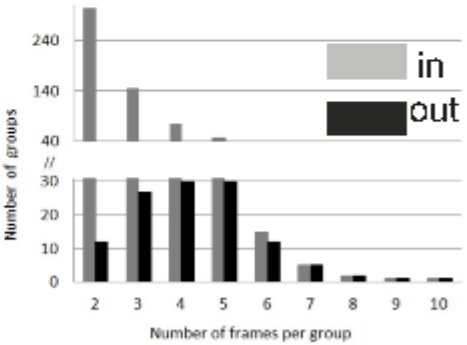


CRYSTFEL hits



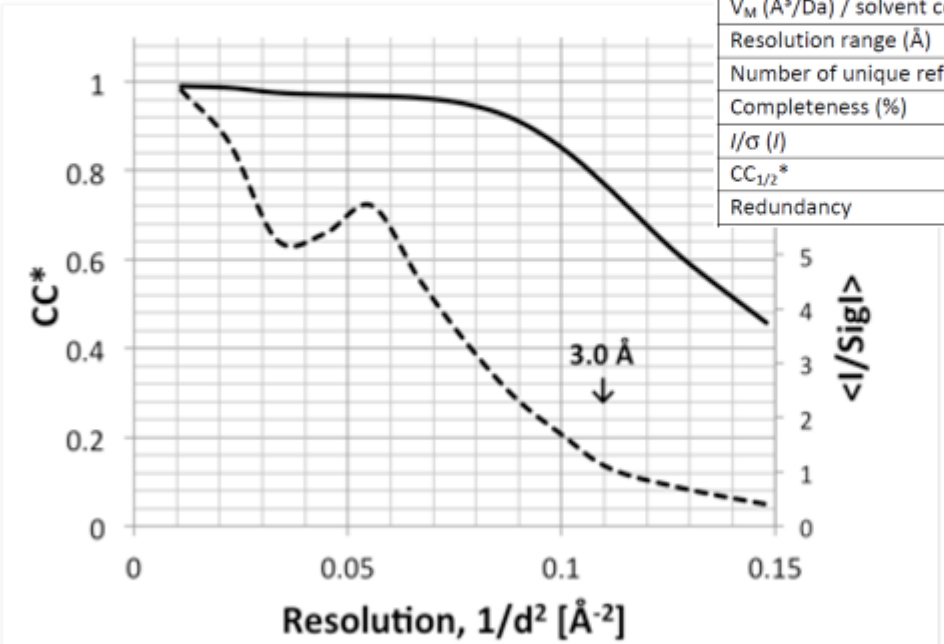
Final data

Wedge size distribution



CatB data statistics

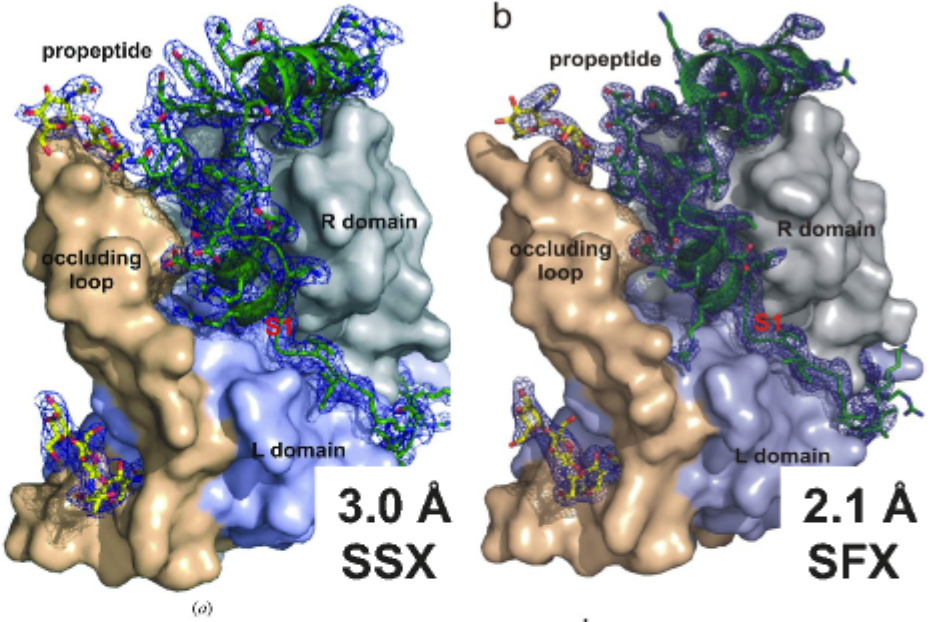
Data collection	
Light source / Beamline	PETRA III / P14
Maximum dose (MGy)	50-60
Space group	$P4_22_12$
Cell dimensions *a*, *b*, *c* (Å)	123.5, 123.5, 54.3
V_M (Å³/Da) / solvent content (%)	2.99 / 58.6
Resolution range (Å)	88.1 - 3.0 (3.16 - 3.00)[a]
Number of unique reflections	8,881
Completeness (%)	99.8 (99.9)
$I/\sigma(I)$	3.7 (1.0)
$CC_{1/2}$*	0.79 (0.97)
Redundancy	12.3 (12.6)

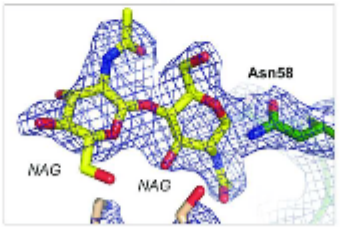


Structure solution

Molecular replacement

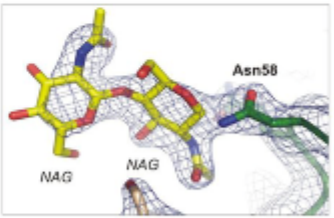
62 residues propeptide
and two carbohydrate chains
newly built into density

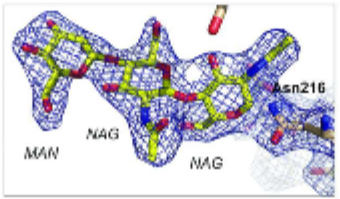




d

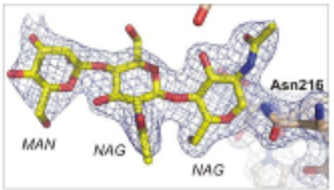
propeptide carbohydrate





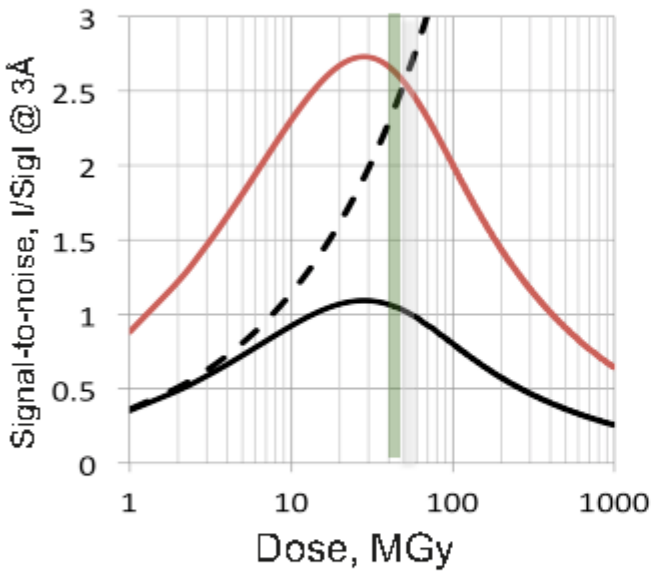
f

enzyme carbohydrate



Rw = 22.3%
Rf = 26.4%

Why the resolution is low?



Modeling with BEST

- (Log)decay rate 0.8 Å^2/MGy as in MX "on average"
- Background scattering as in CatB experiment
- Average diffraction intensity –"-
- the same 80 crystals

Simulated I/SigI vs Dose

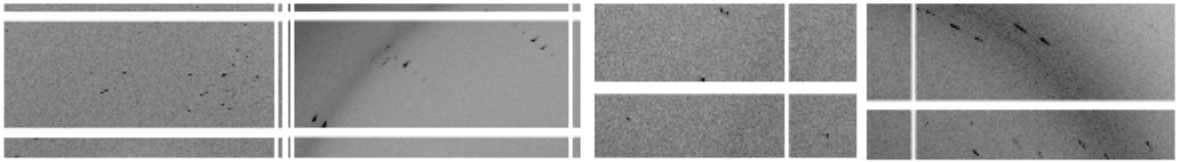
—— our experiment

—— if beam size was 1x1 μm^2 FWHM

– – FEL case: hypothetical experiment with no radiation damage

More experiments

- Substantial set of test microcrystal systems exercised, data analysis in progress (Cornelius Gati, CFEL)
- Some “real systems” tried
- Diffraction quality of microcrystals is the bottleneck
- Sample preparation is difficult and requires many trials
- *On-line data analysis is missing severely!*

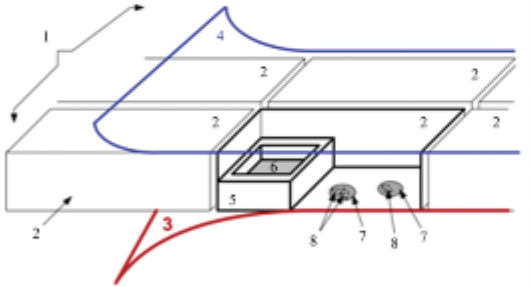


Extrapolations - Rapid screening @100K ?

Tag	Flux ph/sec	Beam size μm^2	Frame rate	Time to raster 0.5x0.5 mm^2 (min)
P14 as of last Monday	$5\ 10^{12}$	5x6	6 flux limited	**70**
P14 collecting all flux	$2\ 10^{13}$	5x6	25	**15**
P14 + better optics (?)	$2\ 10^{13}$	3x3	250	**4**
Ideal	$2\ 10^{13}$	1x1	3000	**3**

Room temperature, in-situ ?

Crystal Direct™ plate scanner mounted on MD3



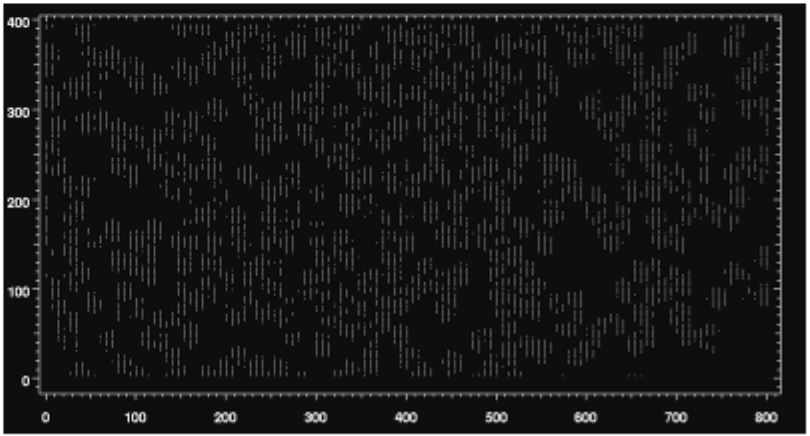
Cipriani, Marquez et al. (2012) 'CrystalDirect: a new method for automated crystal harvesting based on laser-induced photoablation of thin films.' Acta Cryst D68:1393.

Data collection

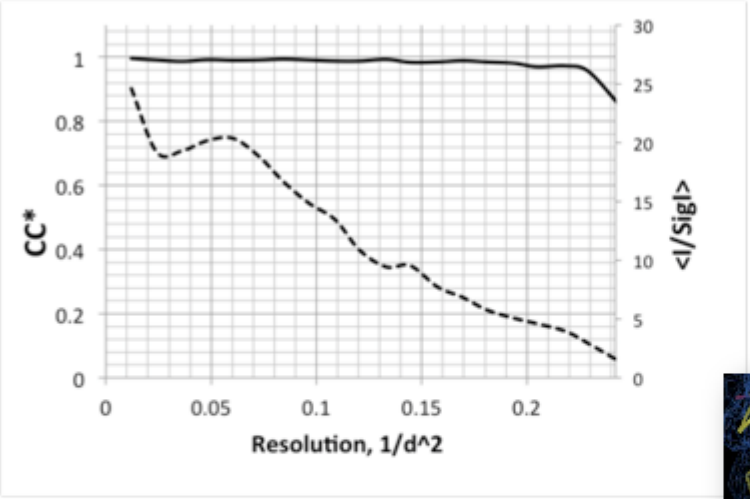
Insulin in CrystalDirect™ plate – data collection

- Insulin in $I2_13$, a = 78 Å
- Typical crystal size: 10 x 10 x 10 μm^3
- 5 µm beam at 5% transmission
- 120 x 400 µm 4D scans (20° rotation, Δ = 6.7 µm
- 50 ms per frame of 0.09° + 2 µm translation
- 24000 frames, 800 x 400 μm^2

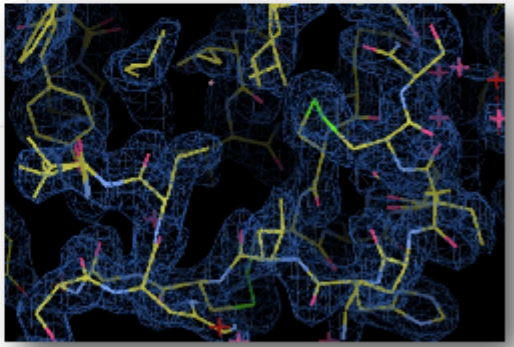
900 partial data sets
400 crystals
Resolution 2.10 Å
Completeness 100.0%
Average multiplicity 180



Cubic insulin - data



Indexing ambiguity!!!



- d_{min} = 2.1 Å
- $R_{w,f}$ = 0.15, 0.18

Dense microcrystalline precipitates: abnormal outgassing ?

Conclusions

- Serial synchrotron cryo-crystallography works principally
- Potentially addresses new range of weak diffractors in an old, “Massif-like” manner
- Data resolution is inferior to SFX, but less sample is used – complementarity is a future?
- The technique is conceptually simple and built on standard components
- Scalable – we need higher flux, smaller beam, faster detectors
- Need better, faster, on-line data analysis software
- *In-situ* variant is promising