



The ESRF Phase II Upgrade: Potential impact for Structural Biology

Gordon Leonard
Head, ESRF Structural Biology Group

ESRF – PHASE II SOURCE PROPERTIES										
	Emittance		Beta [m]		λ [\AA]	L [m]	Rms size [μm]		Divergence [μrad]	
	H [nm]	V [pm]	H	V			H	V	H	V
High beta	4	5	37.2	3	6.2	3.2	409	10.8	14.5	10.3
					1	3.2	409	5.6	11.9	6.1
					0.2	4	409	4.7	11.3	4.7
Low beta	4	5	0.37	3	6.2	3.2	50	10.8	104	10.3
					1	3.2	49	5.6	104	6.1
					0.2	4	49	4.7	104	4.7
New lattice	0.13	2	4.7	2.7	6.2	3.2	26.7	10.3	11.4	10.2
					1	3.2	25	4.7	7.4	5.3
					0.2	4	25	3.5	6.8	4.4

POTENTIAL CHARACTERISTICS OF A ESRF PHASE II MX BEAMLINE				
ID29 Beam characteristics with current and Phase-II lattices				
	Current	New Lattice (current optics)	New lattice (perfect optics)	New Lattice (50:1)
Source size (FWHM; H \times V; μm^2)	115 \times 13.2	59 \times 11	59 \times 11	59 \times 11
Divergence (r.m.s. H \times V; μrad^2)	104 \times 6.1	7.4 \times 5.3	7.4 \times 5.3	7.4 \times 5.3
Demagnification ratio	3:1	3:1	3:1	50:1
Beamsize @ sample (μm^2)	\sim 60 \times 30	30 \times 25	20 \times 4	1.2 \times 0.2
Flux @ sample (ph/sec)	\sim 1 \times 10 ¹³	\sim 1 \times 10 ¹⁴	\sim 1 \times 10 ¹⁴	\sim 1 \times 10 ¹⁴
Flux density @ sample (ph/sec/ μm^2)	7.0×10^9	1.7×10^{11}	2.1×10^{12}	2.4×10^{14}
Absorbed dose rate (Gy/sec)	3.2×10^6	7.7×10^7	9.6×10^8	1.2×10^{11}
Time to Henderson Limit (sec) ^a	6.3	0.26	0.021	0.0002

- Smaller beams
 - micro
 - nano
 - μradian divergence
- Increase in flux density
 - 2.5 orders of magnitude
 - 5 orders of magnitude
- Do 'standard' things better
- Faster, better & new experiments
- New scientific opportunities

DOING 'STANDARD' THINGS FASTER & BETTER

1. Microcrystals

- Where? – What? – Optimise? – Trash?
- Smaller beam = finer sampling

2. Large crystals

- Where is 'sweet spot'?
- Smaller beam = finer sampling

3. Crystal structures for fragment-based drug design

<http://www.afmb.univ-mrs.fr/identification-et-optimisation-d>

Particularly pertinent to 'industrial' use of SR: 100s – 1000s of crystal structures required for each target. If we can do this faster can use much larger fragment libraries

Bowler *et al.* & Leonard , Diffraction cartography. *Acta Cryst.* (2010). **D66**, 855–864

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A PERENNIAL PROBLEM

Radiation damage means that the amount of data that can be collected from one crystal is limited.

Complete data set resolution vs. crystal size

Crystal size, μm	Resolution, Å
1.0	5.50
2.0	4.00
3.0	3.50
4.0	2.80
5.0	2.20
6.0	2.00
8.0	1.80
10.0	1.70
15.0	1.60
20.0	1.50
30.0	1.50
50.0	1.50
100.0	1.50

Number of cryocooled crystals of a given size required to achieve dataset resolutions of $d_{\min} = 1.5 \text{ \AA}$ (black) and $d_{\min} = 2.0 \text{ \AA}$ (blue).

Crystal size, μm	Number of crystals ($d_{\min} = 1.5 \text{ \AA}$)	Number of crystals ($d_{\min} = 2.0 \text{ \AA}$)
1.0	100000	10000
2.0	10000	1000
3.0	1000	100
4.0	100	10
5.0	50	5
10.0	10	1
20.0	1	0.1

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NEW EXPERIMENTS: MULTI-CRYSTAL DATA COLLECTION

- Thermolysin, Space Group P6₁22; B-factor=11.5 Å²

Complete data set resolution vs. crystal size

Number of cryocooled crystals of a given size required to achieve dataset resolutions of $d_{\min} = 1.5 \text{ \AA}$ (black) and $d_{\min} = 2.0 \text{ \AA}$ (blue).

- Sample on mesh loop
- Mesh scan of sample
- Detection of protein diffraction
- Series of partial data collection
- Integration of partial sets
- Hierarchical cluster analysis
- Data merging

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NEW EXPERIMENTS: 'SERIAL' MICROCRYSTALLOGRAPHY

- Thermolysin, Space Group P6₁22; B-factor=11.5 Å²

Complete data set resolution vs. crystal size

Number of cryocooled crystals of a given size required to achieve dataset resolutions of $d_{\min} = 1.5 \text{ \AA}$ (black) and $d_{\min} = 2.0 \text{ \AA}$ (blue).

- For a crystal 1x1x1 µm³ in dimensions partial data sets *from about 1000 crystals* would be needed to achieve a final data set resolution of $d_{\min} = 2.0 \text{ \AA}$ (A. Popov, ESRF).
- 'Serial' crystallography the only way to do this efficiently:
 - New experimental set-ups (learn from pioneering work at XFELs)
 - More targets (i.e. biological systems) can be studied – much smaller crystals needed
 - Better electron density maps ('gain in multiplicity')
 - 'Proof of principle' for SR serial crystallography at Petra-III

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RT MILLISECOND SERIAL CRYSTALLOGRAPHY: ESRF ID13

Crystals of bacteriorhodopsin grown in LCP (cubic Lipid Phase). Introduced into the X-ray beam in a 'jet'. Jet exposed to X-rays on ESRF ID13 [13 keV, 8×10^{11} ph/sec; $3 \times 2 \mu\text{m}^2$ spot size]. Diffraction from crystals that pass through X-ray beam measured using fast readout detector.

μcrystal fly-by in 3 subsequent images. Many images contain no diffractions

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BACTERIORHODOPSIN: STRUCTURE REFINEMENT FROM SERIAL DATA

Electron density map in the region of the retinal binding pocket

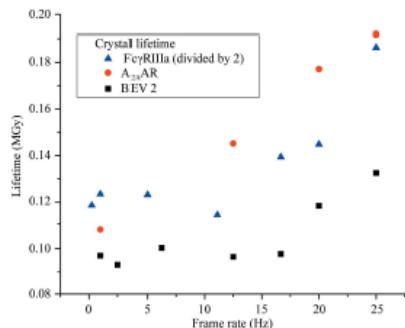
Structure refinement of Bacteriorhodopsin membrane protein crystals from synchrotron LCP-jet serial data at ID13 (refined to $d_{\min} \sim 2.4 \text{ \AA}$; ~1.3 million recorded frames, ~13000 hits, 9655 indexed patterns)

Nogly, P. et al (2015). Lipidic cubic phase serial millisecond crystallography using synchrotron radiation. *IUCrJ* 2. doi:10.1107/S2052252514026487

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'NEW' EXPERIMENTS - ROOM TEMPERATURE PROTEIN CRYSTALLOGRAPHY?



A significant increase in the lifetime at room-temperature resulting from combination of:

- high brilliance X-ray beam
- reduced exposure times [40ms]
- fast readout detector [4ms]

Phase II beam characteristics will mean:

- Even more brilliant X-ray beams
- Much faster (μ sec?) exposure times
- Proper use of Eiger generation detectors
 - readout time $\sim 5\mu$ s
 - 'continuous' data collection.
- An even more significant increase in lifetime?
 - More investigations
 - Higher photon energies?

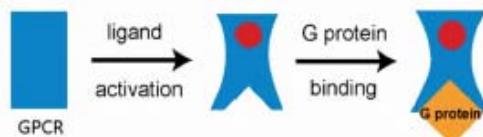
Owen et al. Room-temperature macromolecular crystallography. *Acta Cryst.* (2012), D68, 810–818

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PUMP-PROBE INVESTIGATIONS *IN CRYSTALLO*

- Millisecond or μ second time resolution
- Not just current 'standard' systems (i.e. myoglobin, PYP etc)
- Caged compounds at RT; reactions initiated by laser or X-ray pulse (photo/radio-active substrate or prosthetic group).
- Requires *in crystallo* and *in soluto* spectroscopy
- Acoustic Droplet Ejection, microfluidics to drive diffusion of substrates into nano/microcrystals
- Molecular movies of GPCR activation?



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TIME-RESOLVED STUDIES IN SOLUTION

- Conformational changes upon Calmodulin target binding
- Caged Ca^{2+} , reaction started by photolysis
- Scattering curves recorded with CMOS camera operating @ 2,000 Hz

(a) $R_g^2 (\text{\AA}^2)$ vs Time (msec). The plot shows a rapid initial increase followed by a plateau around 250 \AA^2 .
(b) Normalized $I(\theta)$ vs Time (msec). The plot shows a slight increase from 1.0 to approximately 1.1 over time.

The diagram shows four states of CaM: 1. Two Ca^{2+} ions bind to the C-terminal lobe (<0.5 msec). 2. The protein assumes a compact form (<10 msec). 3. Both lobes bind Ca^{2+} (<30 sec). 4. In the absence of mastoparan, CaM readopts an extended form. 5. In the presence of mastoparan, the compact form is stabilized.

- CaM quickly (<10 ms) assumes a unreported compact form that depends only on Ca^{2+} binding to the C-terminal lobe.
- When both lobes have bound Ca^{2+} , CaM readopts an extended form in the absence of mastoparan while the compact form is stabilised in the presence of mastoparan

Yamada, Matsuo, Iwamoto & Yagi, *SPRING8 Research Frontiers 2012*, 34-35; *Biochemistry*, **51** (2012) 3963.
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MAPPING CONFORMATIONAL LANDSCAPES (IN CRYSTALLO)

Structure

Ensemble of structures of a given protein obtained by X-ray crystallography under various conditions.

Cryo T	Ambient P
Room T	Ambient P
Cryo T	Increasing P
Room T	Increasing P

Additional restraints to give envelope, magnitude of changes, and details of changes obtained by complementary techniques

- BioSAXS – Large-scale movements
- TR-WAXS – Medium-scale movements
- Kinetic Crystallography – Small-scale movements (a few atoms)

Molecular Dynamics Simulations – to validate a scenario involving all available structures, including restraints, and fill up the missing bits

Dynamics

Function

Difference matrix plots for BioSAXS and TR-WAXS, and a 3D ribbon model of the protein structure.

R. Giordano, PhD thesis, 2013

Ensembles of structures not a single structure

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PHASE-II IS NOT JUST FOR MX & SAXS

X-ray fluorescence microscopy of tissues and cells

Coherent Diffraction Imaging at resolution below 5 nm.

Time resolved correlation function
 $c_r(t, \tau) = \frac{\langle I_r(t)I_r(t+\tau) \rangle}{\langle I_r(t) \rangle \langle I_r(t+\tau) \rangle}$

Use increased coherence in *ab initio* determination of macromolecular crystal structures

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PHASE-II SUMMARY

- Smaller beams
 - micro
 - nano
 - μ radian divergence
 - smaller crossfire
 - larger unit cells
- Increase in flux density
 - 5 orders of magnitude
 - smaller crystals
- Do 'standard' things better
 - finer sampling
- Faster, better & new experiments
 - multi-crystal data collection
 - serial microsecond crystallography
 - ultra-fast RT data collection (?)
- 'New' scientific opportunities
 - time-resolved studies

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Thanks for your attention!