ESRF | The European Synchrotron

The ESRF Phase II Upgrade: Potential impact for Structural Biology

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ESRF – PHASE II SOURCE PROPERTIES

	Emittance		Beta [m]		λ [Å]	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

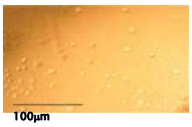
POTENTIAL CHARACTERISTICS OF A ESRF PHASE II MX BEAMLINE

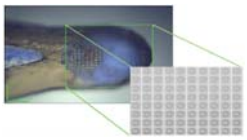
ID29 Beam characteristics with current and Phase-II lattices				
	Current	New Lattice (current optics)	New lattice (perfect optics)	**New Lattice (50:1)**
Source size (FWHM; H × V; μm²)	115 × 13.2	59 x 11	59 x 11	**59 x 11**
Divergence (r.m.s. H × V; μm²)	104 × 6.1	7.4 x 5.3	7.4 x 5.3	**7.4 x 5.3**
Demagnification ratio	3:1	3:1	3:1	**50:1**
Beamsize @ sample (μm²)	~60 x 30	30 x 25	20 x 4	**1.2 x 0.2**
Flux @ sample (ph/sec)	~1 x 10^{13}	~1 x 10^{14}	~1 x 10^{14}	**~1 x 10^{14}**
Flux density @ sample (ph/sec/μm²)	7.0 x 10^{9}	1.7 x 10^{11}	2.1 x 10^{12}	**2.4 x 10^{14}**
Absorbed dose rate (Gy/sec)	3.2 x 10^{6}	7.7 x 10^{7}	9.6 x 10^{8}	**1.2 x 10^{11}**
Time to Henderson Limit (sec)[c]	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

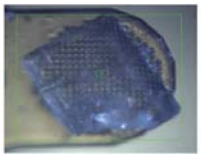
1. Microcrystals

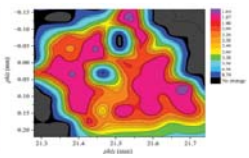




- Where? – What? – Optimise? – Trash?
- Smaller beam = finer sampling

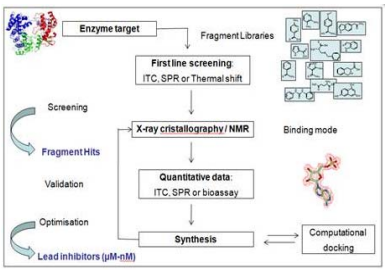
2. Large crystals





- 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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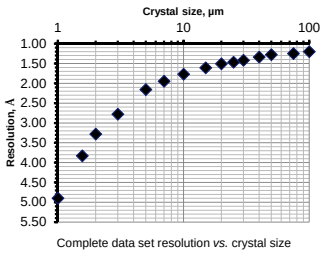
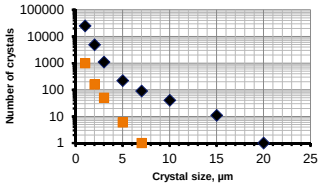
A PERENNIAL PROBLEM

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



Complete data set resolution *vs.* crystal size



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

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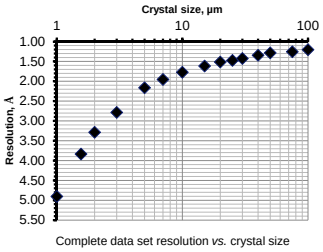
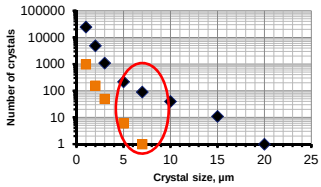
NEW EXPERIMENTS: MULTI-CRYSTAL DATA COLLECTION

- Thermolysin, Space Group $P6_122$; B-factor=11.5 Å^2

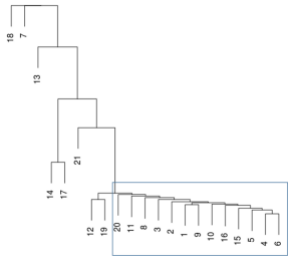


Complete data set resolution *vs.* crystal size



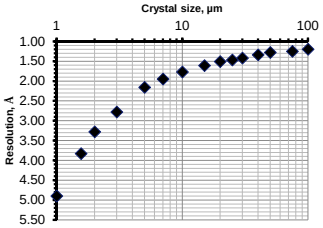
Number of cryocooled crystals of a given size required to achieve dataset resolutions of d_{min} = 1.5 Å (black) and d_{min}= 2.0 Å (blue).

- 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

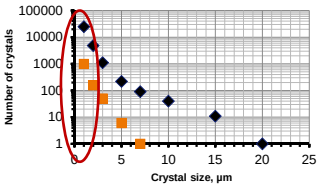


NEW EXPERIMENTS: 'SERIAL' MICROCRYSTALLOGRAPHY

- Thermolysin, Space Group $P6_122$; B-factor=11.5 Å^2



Complete data set resolution *vs.* crystal size

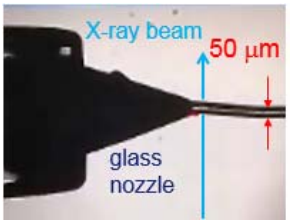


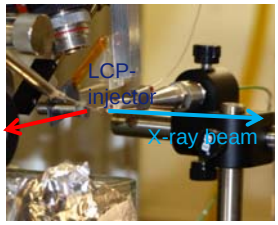
Number of cryocooled crystals of a given size required to achieve dataset resolutions of d_{min} = 1.5 Å (black) and d_{min}= 2.0 Å (blue).

- For a crystal 1x1x1 µm^3 in dimensions partial data sets *from about 1000 crystals* would be needed to achieve a final data set resolution of d_{min} = 2.0 Å (A. Popov, ESRF).

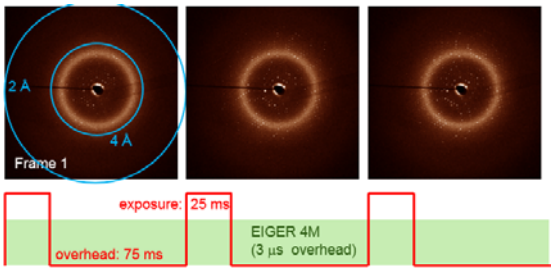
- 'Serial' crystallography the only way to do this efficiently:
  - New experimental set-ups (learn from pioneering work at XFELs)
  - More targets (i.e. biological systems) can be studied – much smaller crystals needed
  - Better electron density maps ('gain in multiplicity')
  - 'Proof of principle' for SR serial crystallography at Petra-III

RT MILLISECOND SERIAL CRYSTALLOGRAPHY: ESRF ID13



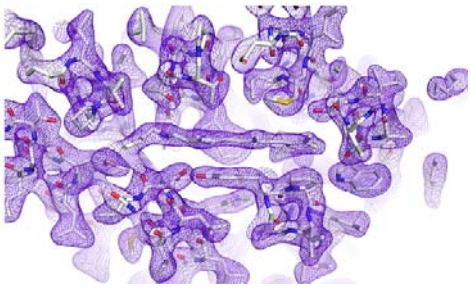


Crystals of bacteriodhopsin grown in LCP (cubic Lipid Phase). Introduced into the X-ray beam in a 'jet'. Jet exposed to X-rays on ESRF ID13 [13 keV, $8x10^{11}$ ph/sec; 3x2 μm^2 spot size]. Diffraction from crystals that pass through X-ray beam measured using fast readout detector.

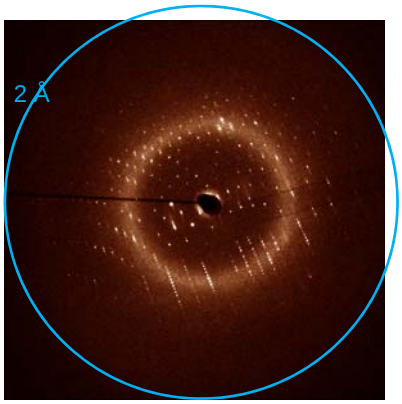


µcrystal fly-by in 3 subsequent images. Many images contain no diffractions

BACTERIORHODOPSIN: STRUCTURE REFINEMENT FROM SERIAL DATA



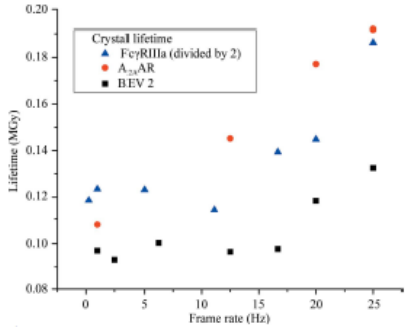
Electron density map in the region of the retinal binding pocket



Structure refinement of Bacteriorhodopsin membrane protein crystals from synchrotron LCP-jet serial data at ID13 (refined to d_{min} ~ 2.4 Å; ~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

'NEW' EXPERIMENTS - ROOM TEMPERATURE PROTEIN CRYSTALLOGRAPHY?



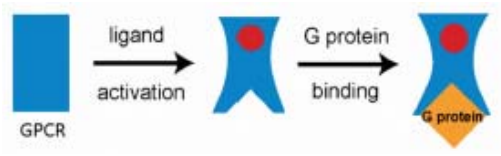
A significant increase in the lifetime at room-temperature resulting from combination of:
- high brilliance X-ray beam
- reduced exposure times [40ms]
- fast readout detector [4ms]

Phase II beam characteristics will mean:
- Even more brilliant X-ray beams
- Much faster (μsec?) exposure times
- Proper use of Eiger generation detectors
  - readout time ~5μs
  - '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

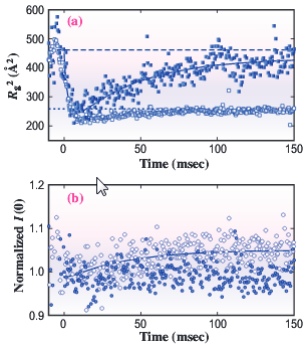
PUMP-PROBE INVESTIGATIONS *IN CRYSTALLO*

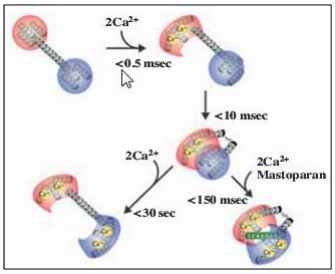
- Millisecond or μsecond time resolution
- Not just current 'standard' systems (i.e. myoglobin, PYP etc)
- Caged compounds at RT; reactions initiated by laser or X-ray pulse (photo/radio-active substrate or prosthetic group.
- Requires *in crystallo* and *in soluto* spectroscopy
- Acoustic Droplet Ejection,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



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

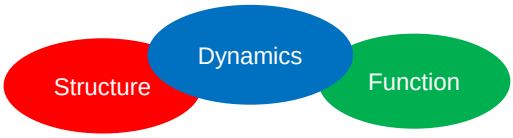


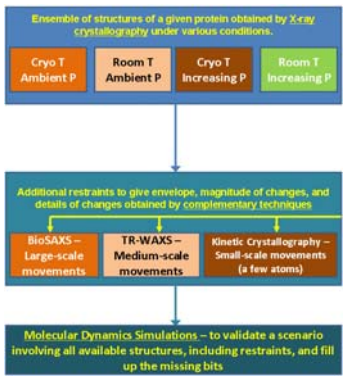


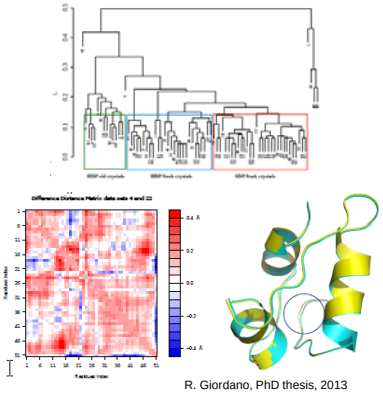
- CaM quickly (<10 ms) assumes a unreported compact form that depends only on Ca^{2+} binding to the C-terminal lobe.
- When both lobes have bound Ca^{2+}, CaM readopts an extended form in in the absence of mastoparan while the compact form is stabilised in the presence of mastoparan

Yamada, Matsuo, Iwamoto & Yagi, *SPring8 Research Frontiers* 2012, 34-35; *Biochemistry,* **51** (2012) 3963.

MAPPING CONFORMATIONAL LANDSCAPES (*IN CRYSTALLO*)



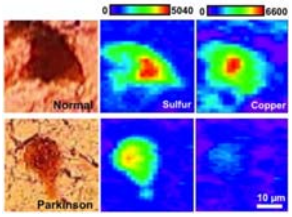




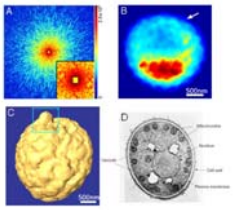
R. Giordano, PhD thesis, 2013

Ensembles of structures not a single structure

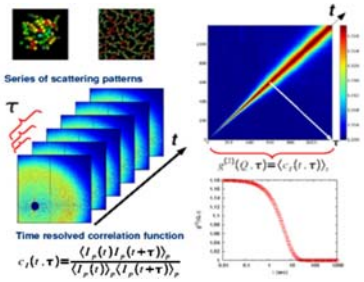
PHASE-II IS NOT JUST FOR MX & SAXS



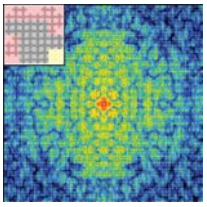
X-ray fluorescence microscopy of tissues and cells



Coherent Diffraction Imaging at resolution below 5 nm.



X-ray photon correlation spectroscopy (XPCS): dynamics of proteins on sub-µsecond time scales.



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

PHASE-II SUMMARY

- **Smaller beams**
  - micro
  - nano
  - µradian 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

Thanks for your attention!