Synchrotron Radiation and Structural Biology

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Outline:

Proteins and nucleic acids

Why study Structural Biology?

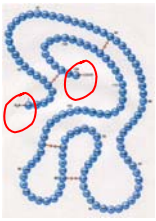
Why use Synchrotron Radiation for Structural Biology?

How to use Synchrotron Radiation for Structural Biology

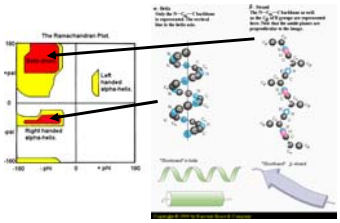
Examples

Future developments

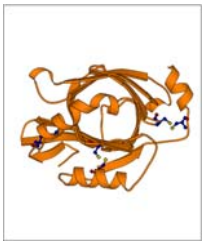
The Structure of Proteins



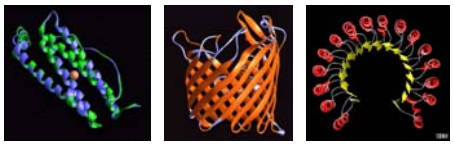
The amino acid sequence (N→C) of a protein is it's **primary structure**



Secondary structure:
Regions of the amino acid chain adopt certain conformations (α-helix; β-strand)

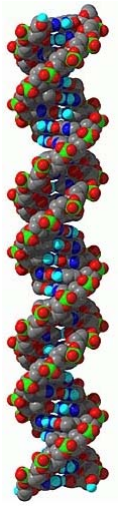


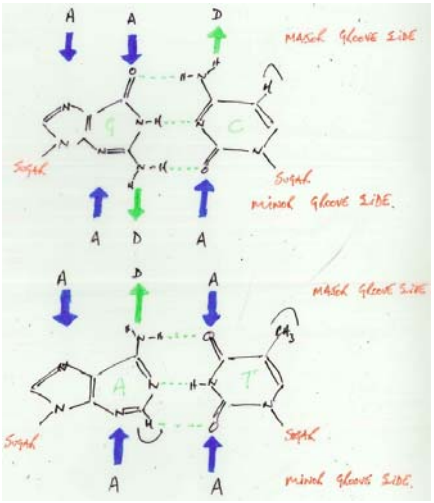
The three-dimensional arrangement of secondary structure elements results in the **tertiary structure** of a protein



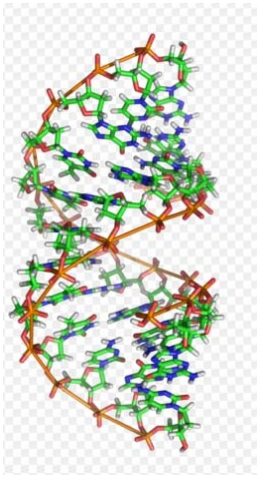
Nucleic acids are also biological macromolecules

B-DNA





A-DNA /RNA



Why Study Structural Biology?

The ultimate goal of molecular biology is to understand biological processes in terms of the chemistry and physics of the macromolecules that participate in them. One of the essential differences between the chemistry of living systems and that of the nonliving is the great structural complexity of biological macromolecules. We shall not unravel the chemistry of life in molecular detail without knowing at atomic or close to atomic resolution the structure of biological macromolecules, especially the proteins.

Introduction to Protein Structure, C.-I. Branden & John Tooze, Garland Publishing Inc., 1991

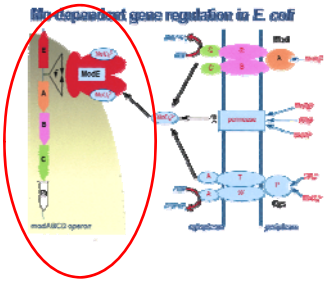
Form = Function

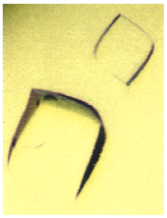
The functionality of proteins and nucleic acids, including their interactions with each other are vital in all living organisms: malfunction causes disease

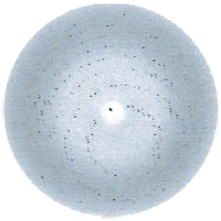
How to Study the structure of biological macromolecules?

- Circular Dichroism/ Light Scattering (2ndy; 4ry structure only?)
- Nuclear Magnetic Resonance
- Electron Microscopy and Diffraction
- **Solution Scattering (SAXS/SANS)**
- **X-ray (Neutron) Crystallography = Macromolecular Crystallography**

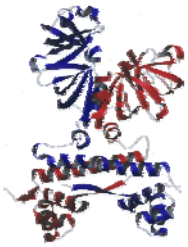
FORM = FUNCTION: Macromolecular Crystallography in 5 Pictures

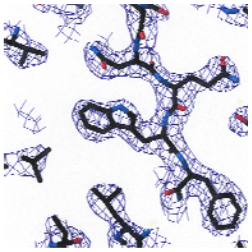






Atomic model of macromolecule of interest = atomic coordinates (x,y,z) + displacement factors (B, U_{ij}).

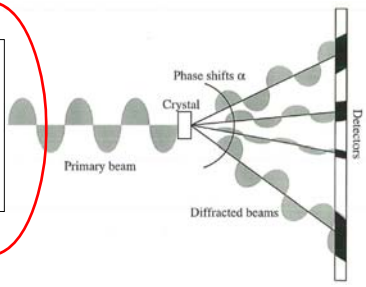




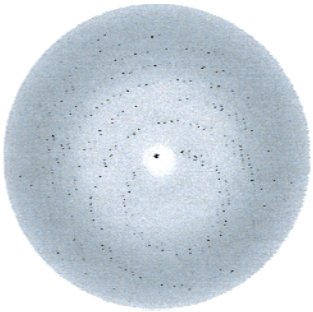
$$\rho_{(x,y,z)} = \frac{1}{V_c}\sum_h\sum_k\sum_l F_{hkl}\ \exp(-2\pi i(hx+ky+lz))$$

Doing (macromolecular) crystallography

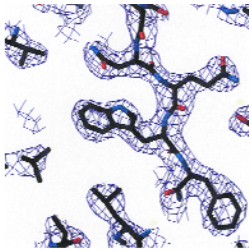
X-ray source



$$n\lambda = 2d\sin\theta$$



- (usually) monochromatic beam
- (usually) rotate crystal



Solve phase problem

$$\rho_{(x,y,z)} = \frac{1}{V_c}\sum_h\sum_k\sum_l F_{hkl}\ \exp(-2\pi i(hx+ky+lz))$$

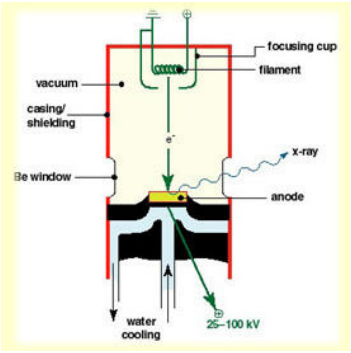
$$F_{hkl} = \sum_j |F_{hkl}|\exp -2\pi i(\phi)$$

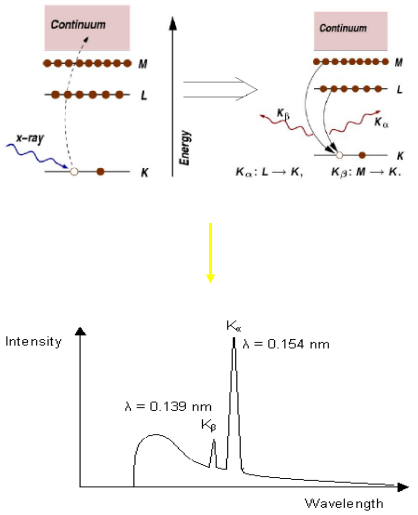
Amplitude Phase

$$|\boldsymbol{F_{hkl}}| \propto \sqrt{\boldsymbol{I_{hkl}}}$$

Generating X-rays

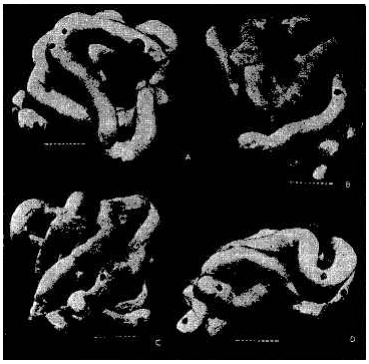
Sealed tube sources





The first crystal structures of biological macromolecules

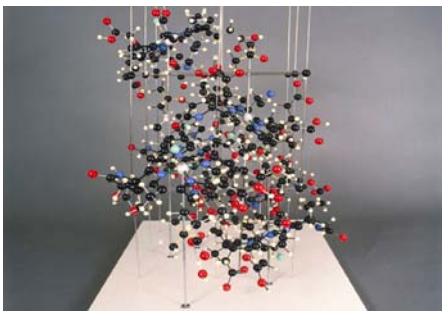
Myoglobin



Kendrew, J.C. et al., (1958). A three-dimensional model of the myoglobin molecule obtained by x-ray analysis. *Nature* **181,** 662–666.

[Max Perutz]

Vitamin B_{12}



Crowfoot Hodgkin, D. et al., (1955). Structure of Vitamin B_{12} : The Crystal Structure of the Hexacarboxylic Acid derived from B_{12} and the Molecular Structure of the Vitamin. *Nature* **176**, 325 – 328.

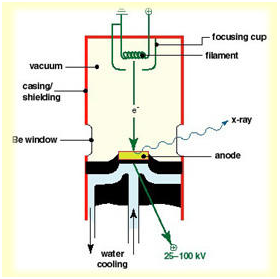
Smaller crystals/large unit cells need higher intensity X-rays

$$E(hkl) = \frac{e^4}{m^2 c^4 \omega} I_0 \lambda^3 LPA \frac{V_x}{V_o^2} |F(h)|^2$$

Energy of a diffracted beam is inversely proportional to square of unit cell volume → for crystals of macromolecules need more intense sources of X-rays to get maximum information (i.e. data resolution). Small crystals diffract less well than big ones.

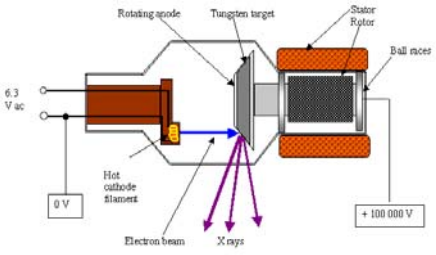
The generation of more intense X-rays

Sealed tube source



Heating problems mean can't make these too powerful (anode would melt)

Rotating anode sources

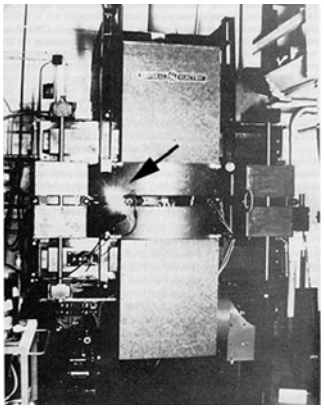


Spinning the target helps dissipate head load → higher power and higher intensity of X-rays.

Commonly used targets: Mo, Cu, Cr

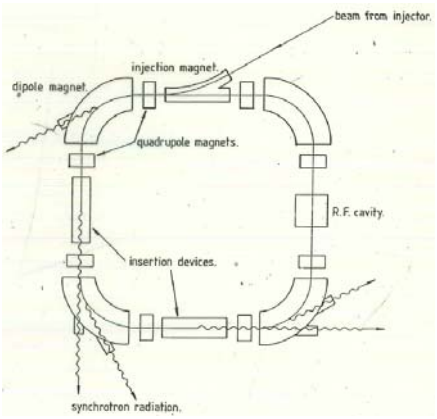
Synchrotron Radiation

Synchrotron Radiation



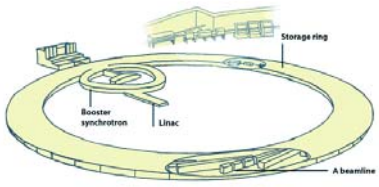
Elder et al., (1947) "Radiation from Electrons in a Synchrotron" *Phys. Rev.*, **71**, 829-830.

A Storage Ring



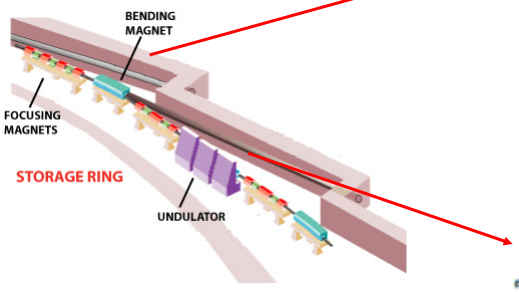
1st Generation – parasitic use
2nd Generation - dedicated
3rd Generation – dedicated, higher energy

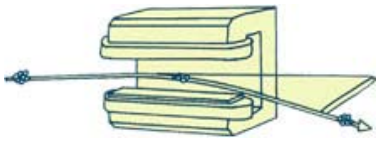
Modern Synchrotron sources



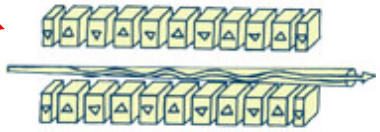
ESRF: Energy 6GeV; circumference 844 m

Inside a Storage Ring



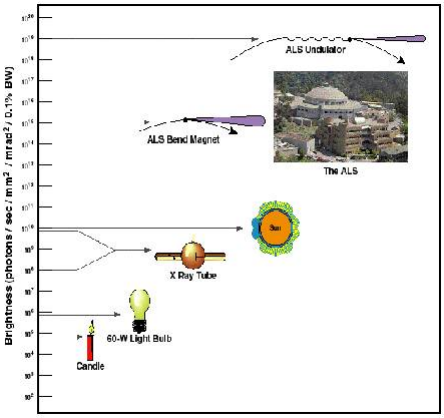


BM: Wide divergent fan

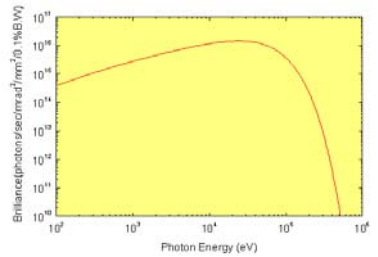


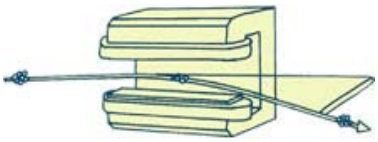
Undulator: Highly collimated, intense beam

Synchrotron Radiation is very bright

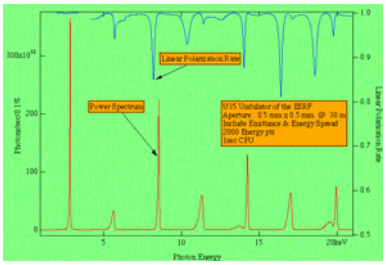


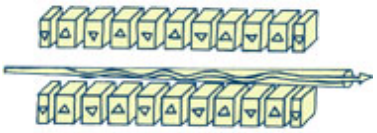
Synchrotron radiation has broad spectral range





Bending magnet spectrum



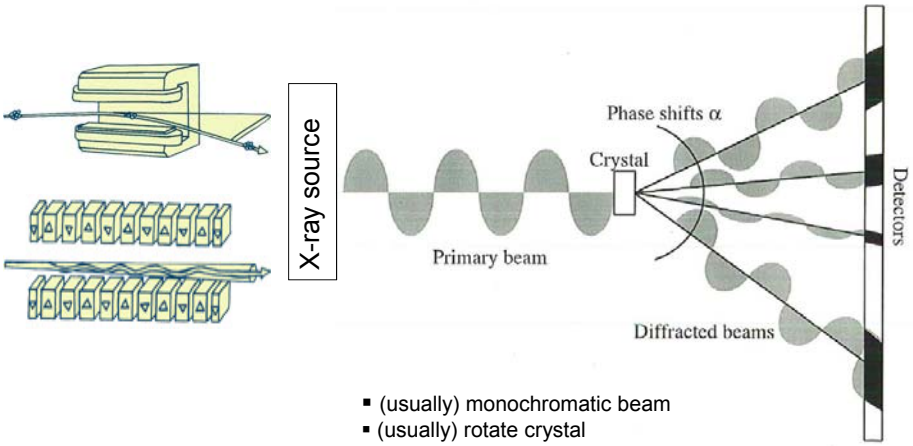


Undulator spectrum

Synchrotron Radiation is ideal for macromolecular crystallography

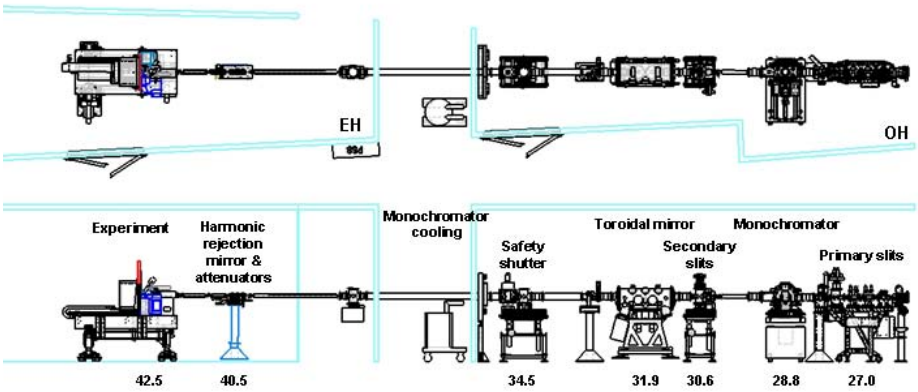
- Brilliance at 3rd generation synchrotron sources
  - better resolution, smaller samples
- Wavelengths from IR to hard X-rays
  - Anomalous dispersion, fluorescence techniques
- Time structure (ps)
  - Time resolved studies

Doing (macromolecular) crystallography at synchrotron sources



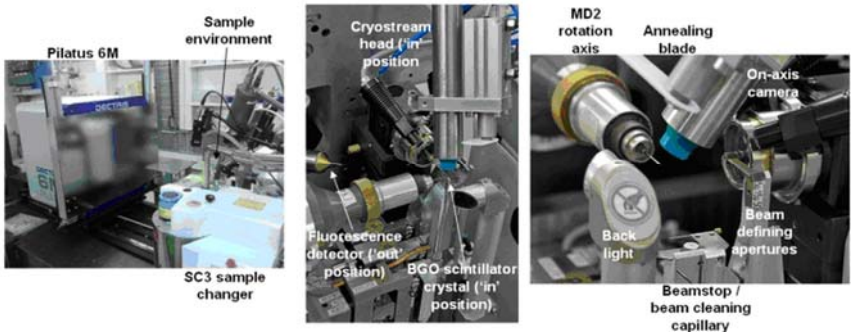
- (usually) monochromatic beam
- (usually) rotate crystal

A synchrotron beamline for MX (ESRF ID29)

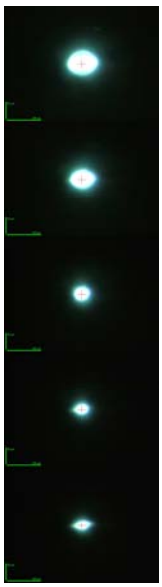


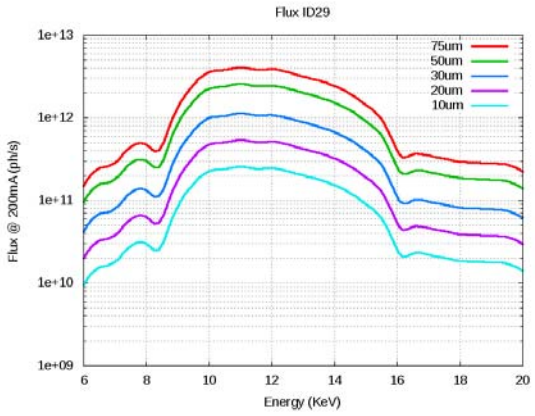
The layout of ID29 at ESRF: Beam conditioning in Optics Hutch (OH), data collection in Experimental Hutch (EH).

The experimental set-up at ID29 at ESRF

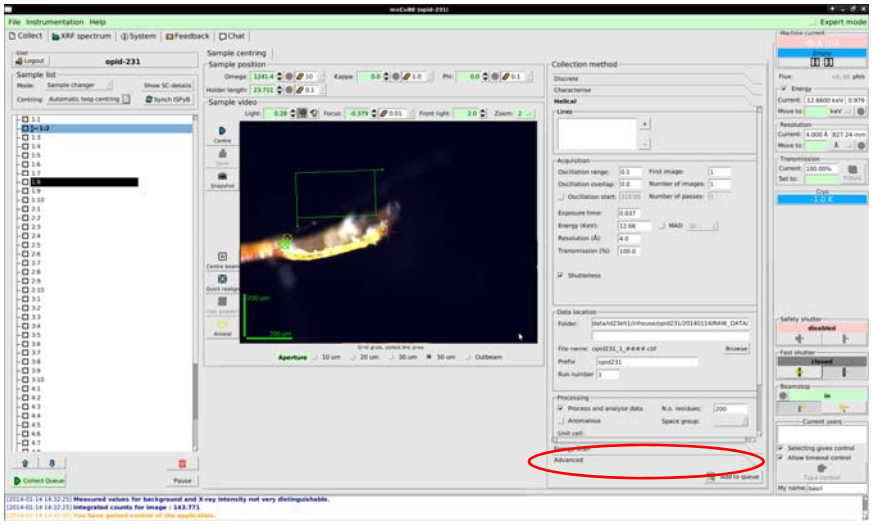


The X-ray beam at ID29 at ESRF



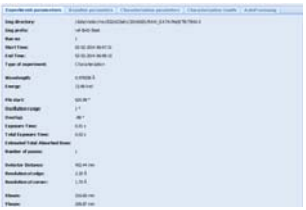


GUIs can make complicated experiments straightforward



Experiments (and results) are recorded

Parameters & Results



Crystal Snapshots

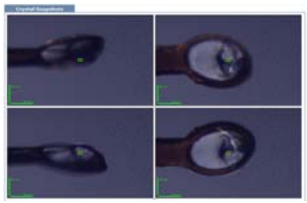
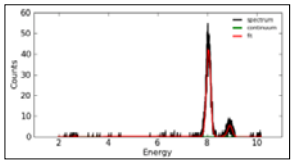
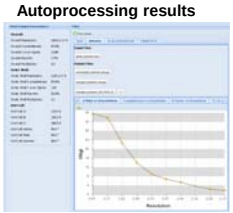


Image thumbnails

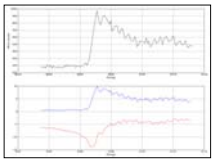
XRF Spectrum



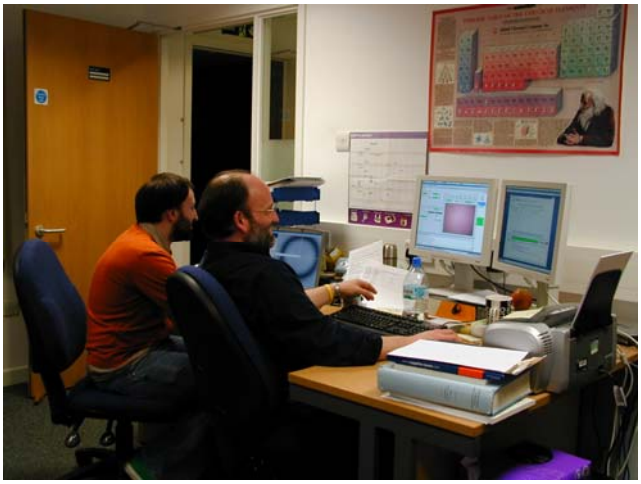
Autoprocessing results



Edge Scan

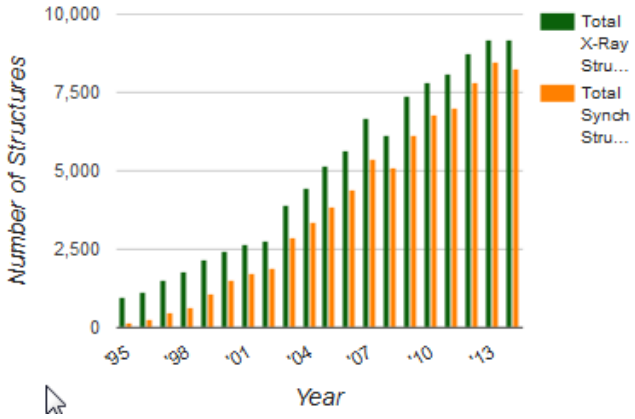


Many synchrotron beamlines can be accesses from anywhere

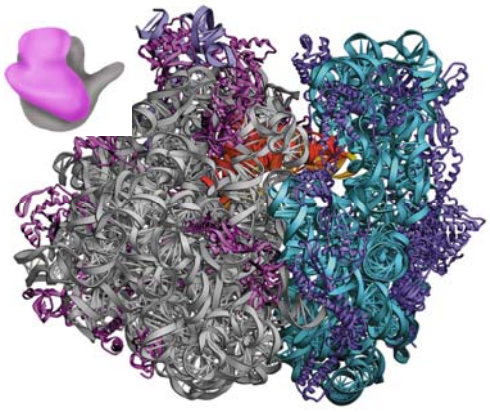


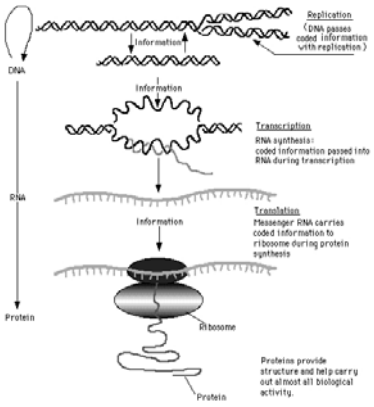
There are now many synchrotrons worldwide

Synchrotron facilities are very productive

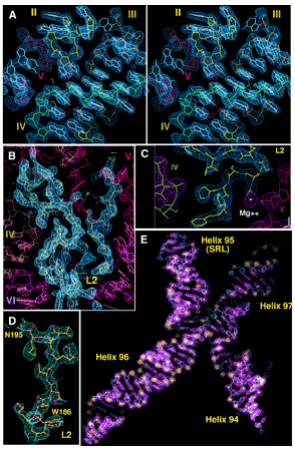


LANDMARKS IN MX - THE CRYSTAL STRUCTURE OF THE RIBOSOME

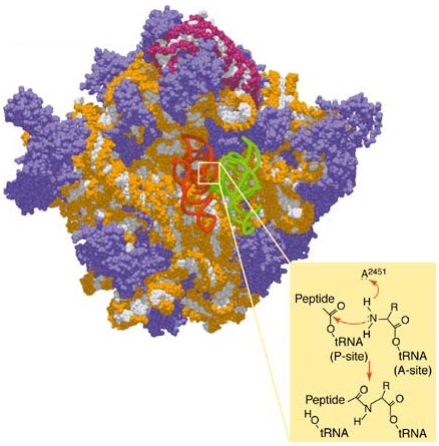




LANDMARKS IN MX - RIBOSOME MECHANISM?

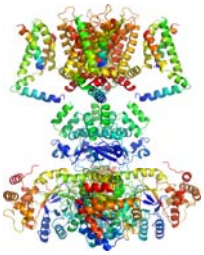


High resolution electron density



Culmination of decades of work!

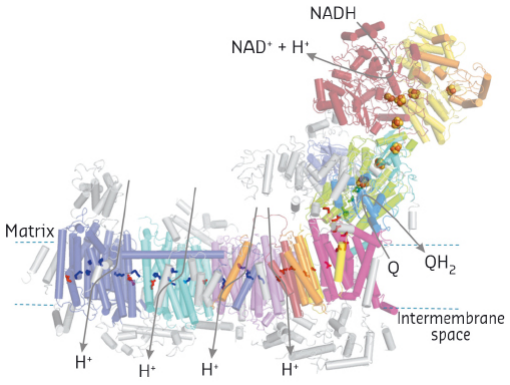
LANDMARKS IN MX - NEUROTRANSMITTERS



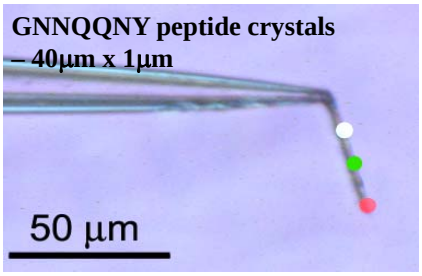
Neurotransmitters play an essential role in signal transduction. The resolution of the structure and the biophysical properties of the **Voltage dependant K+ channel** led to the Nobel Prize for Chemistry for ESRF user Rod McKinnon (Rockefeller University N.Y.) in 2003.

MECHANISTIC CLUES FROM THE STRUCTURE OF MITOCHONDRIAL COMPLEX I

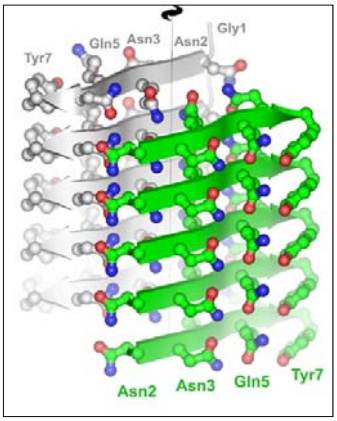
Mitochondria gain energy by oxidising hydrogen extracted from nutrients, converting it into a proton gradient that is then used to synthesise adenosine tri-phosphate (ATP), the universal energy currency of the cell. Complex I couples the transfer of two electrons from NADH (reduced nicotinamide adenine dinucleotide) to ubiquinone with the pumping of four protons across the inner mitochondrial membrane.
Defects in human complex I are the most frequent cause of inherited mitochondrial disorders and are implicated in numerous (neuro)degenerative diseases and ageing.



Small molecules are also important in Structural Biology



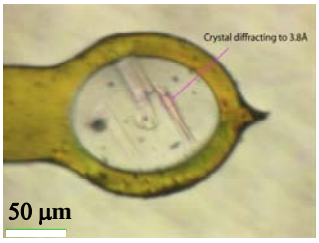
Nelson *et al., Nature* (2005) **435**, 773



Neurodegenerative diseases are associated with the formation of amyloid fibrils. Spines of these have a common structure. Interface between the two β-sheets is 'dry' (no water molecules). Also no hydrogen bonds from one sheet to its mate. Structure suggests routes towards developing therapies for Alzheimer's and related amyloidoses.

MORE SUCCESS WITH MICRO-FOCUS X-RAY BEAMS

human b2 adrenergic receptor

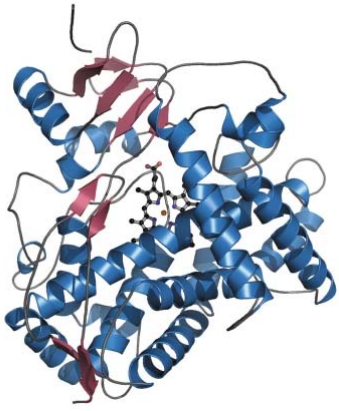


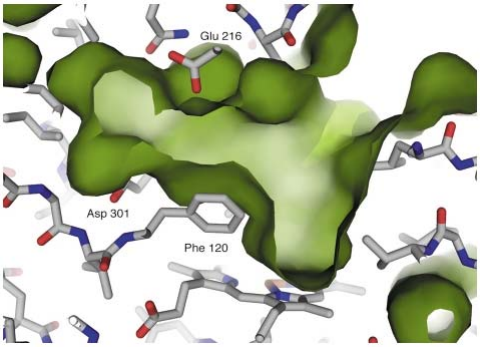
ESRF ID13, ID23-2 and APS GM/CA CAT; 23ID-B

Rasmussen et al., *Nature* (2007) **450**,383

Macromolecular Crystallography and drug design

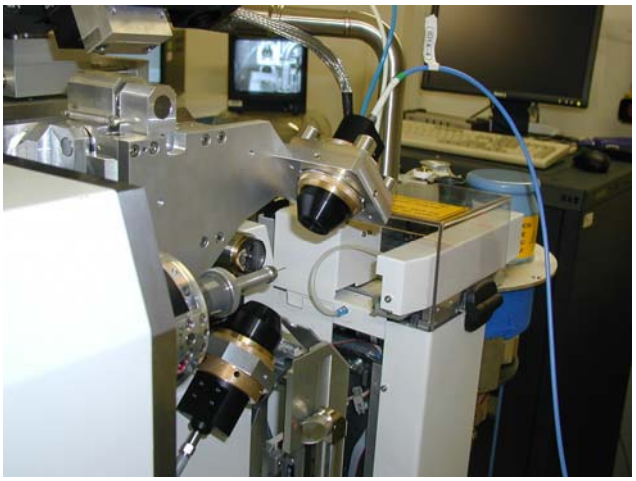
Crystal structure of human cytochrome P450 2D6





Structural information helps to explain how this molecule catalyses the metabolism of at least 20% of known drugs.

COMBINING CRYSTALLOGRAPHY AND SPECTROSCOPY



Bourgeois *et al.,* (2002) *J. Appl. Crystallogr*, 35, 319–326.

On-line characterisation of crystals using spectroscopy - Improved interpretation of X-ray data

- Oxidation state of redox proteins

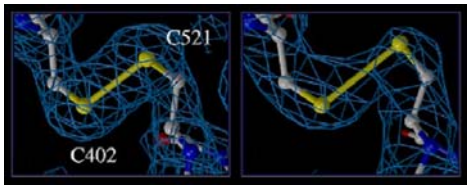
- Photoactive groups

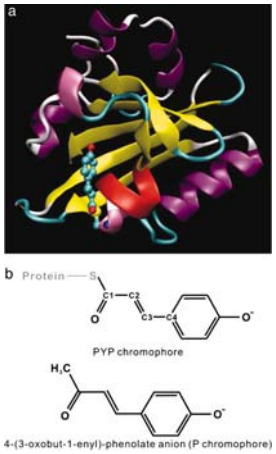
Intermediate states

Active site changes

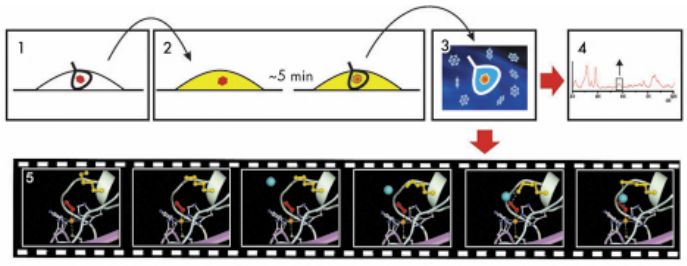
Ligands (Raman)

Monitoring of radiation damage





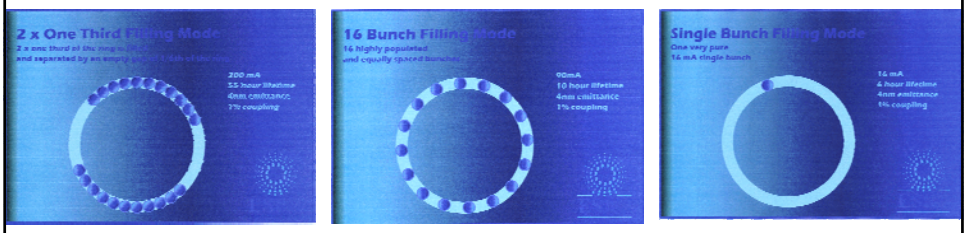
Off-line characterisation of crystals using spectroscopy - Improved interpretation of X-ray data



Single crystal analysis of a superoxide reductase point mutant showed three intermediate states trapped in crystal. The resulting 'film'of the reaction of the reaction pathway was validated using raman spectroscopy.

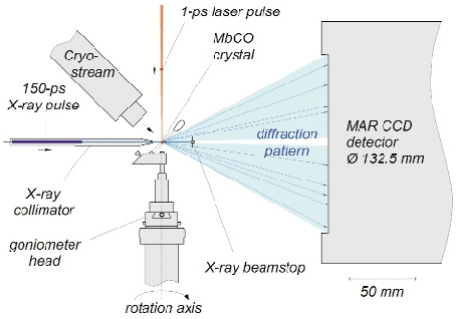
Katona *et al.,* (2007), *Science* 316, 449-53

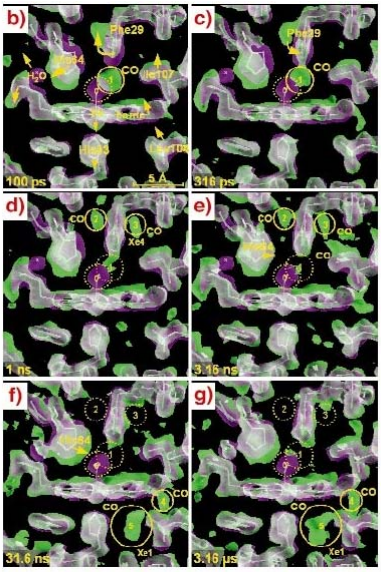
Storage Ring Filling Modes at ESRF



Synchrotron Radiation has a 'pulsed' time structure. In 16- or single-bunch modes we can take advantage of this to probe fast structural changes in macromolecules using Laue diffraction techniques

Time-resolved MX using the Laue technique (White/Pink beam)





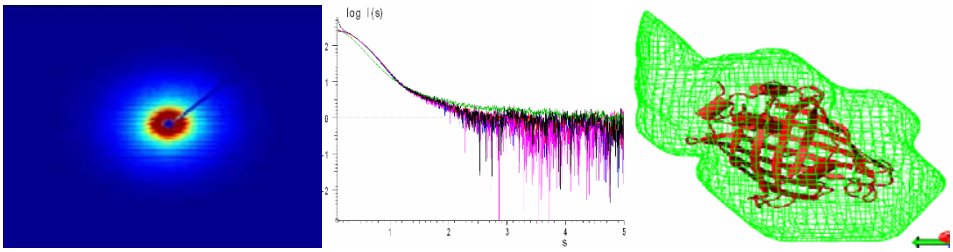
Structure of MbCO at different time delays after photolysis. The bound CO dissociates, eventually becoming trapped in sites 4 and 5, where it remains out to the microsecond time scale.

F. Schotte *et al.,* (2003), *Science*, 300, 1944-1947.

Structural Biology at synchrotrons without crystals - SAXS

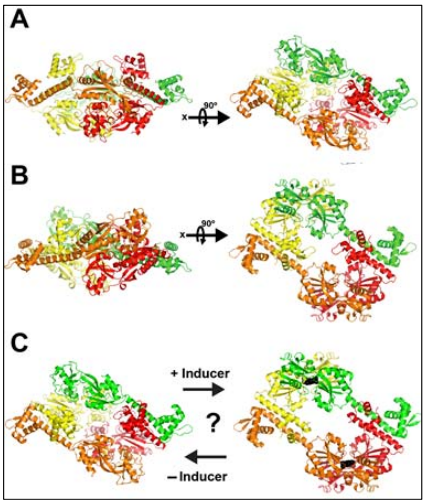
Small Angle X-ray Scattering (SAXS) is a technique for studying structure (and other things*) at low resolution in solution & under normal biophysical/biochemical conditions

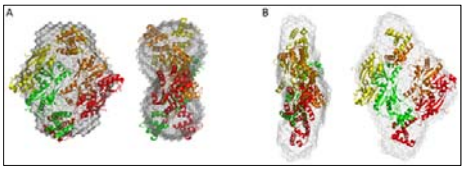
Information from SAXS: - model independent parameters (Rg, I(0))
- *ab initio* shape determination
- rigid body modelling

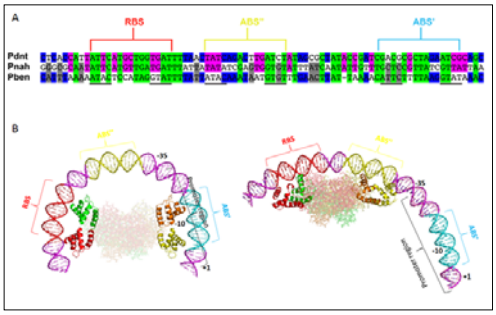


***molecular shape, molecular interactions, kinetics, etc…**

STUDIES IN SOLUTION CAN SOMETIMES REVEAL THINGS CRYSTALS CAN'T

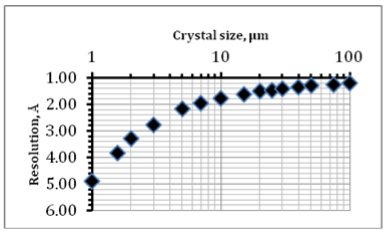


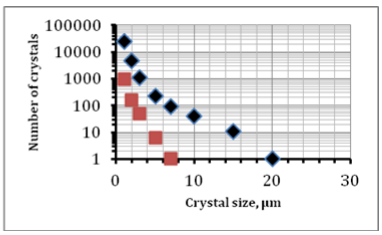




Lerche et al., submitted

RADIATION DAMAGE IS A PROBLEM





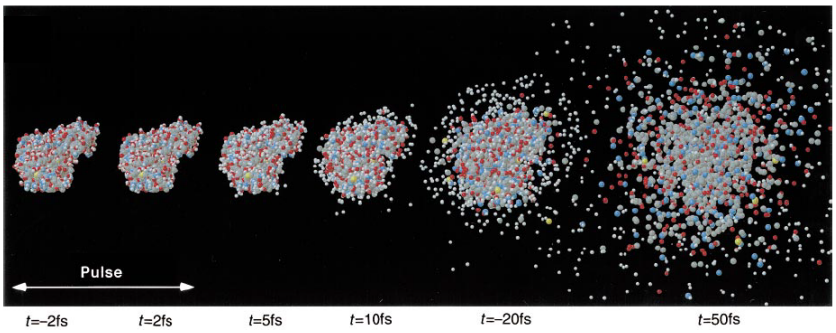
The resolution of a *complete* diffraction dataset that will be yielded from a *single* microcrystal of a biological macromolecule will remain limited by radiation damage. Many such crystals will be required for the collection of even moderate resolution diffraction data

→ New Paradigm for macromolecular crystallography: Multi-crystal data collection

Or

→ Avoid radiation damage

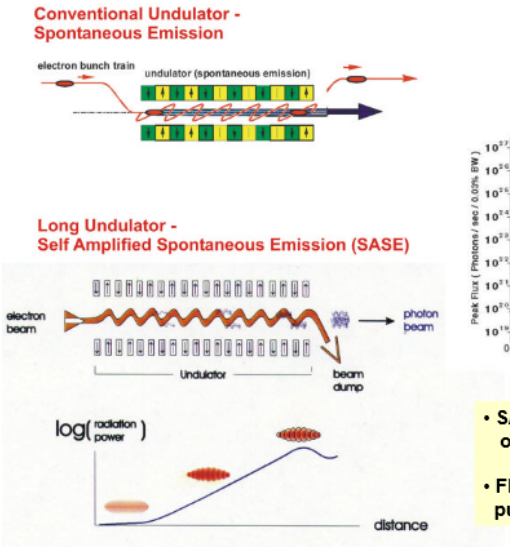
AVOIDING RADIATION DAMAGE

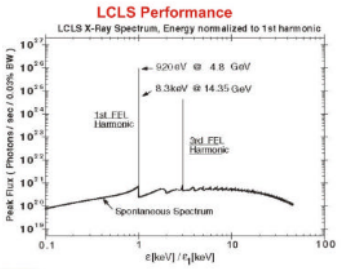


Radiation damage after exposure to extremely high X-ray dose occurs after a time lag of a few 10s of femtoseconds. Can we produce X-ray sources that might allow collection of diffraction data crystal on these timescales?

Neutze, R. et al. (2000). Potential for biomolecular imaging with femtosecond X-ray pulses. *Nature* **406**, 752-757.

FREE ELECTRON LASERS

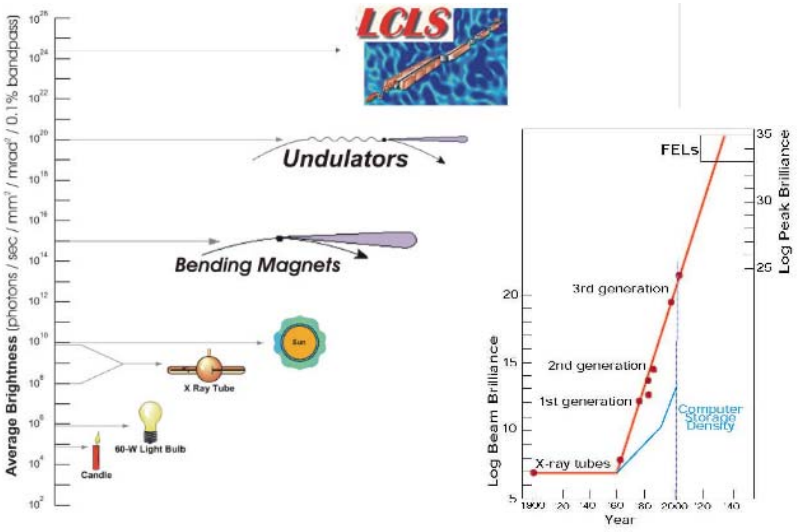




- SASE gives 10^6 intensity gain over spontaneous emission
- FELs can produce ultrafast pulses (of order 100 fs)

http://www-ssrl.slac.stanford.edu/stohr/xfel-pic.pdf

X-FELs X-ray beams are much brighter than synchrotron X-ray beams



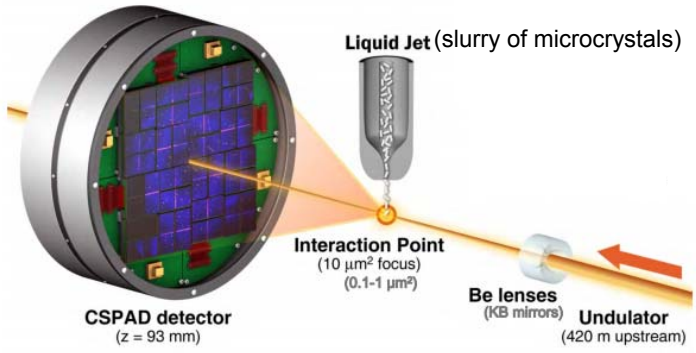
http://www-ssrl.slac.stanford.edu/stohr/xfel-pic.pdf

A PROBLEM WITH USING X-FEL X-RAY BEAMS?

When exposed to X-FEL X-ray beams crystals will vaporise. How can one collect diffraction data? Will one see any diffraction?

MACROMOLECULAR CRYSTALLOGRAPHY AT X-FELS

High resolution serial femtosecond crystallography



Cornell-SLAC Pixel Array Detector

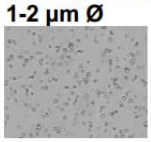
Boutet et al Science **337**:362 (2012)

Paradigm shift in the way that diffraction data are collected

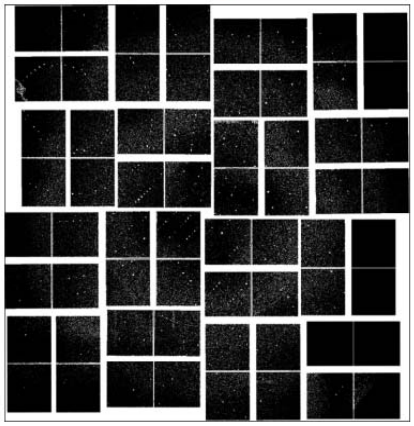
MACROMOLECULAR CRYSTALLOGRAPHY AT X-FELS

High resolution femtosecond diffraction of micron-sized lysozyme crystals

Lysozyme crystals
1-2 µm Ø



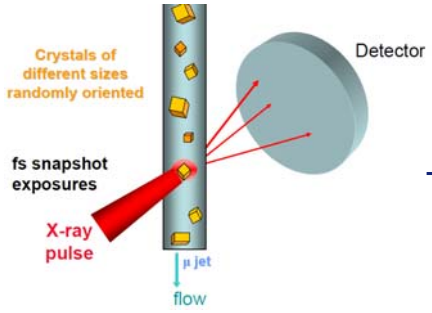
40 fs pulse
10 µm² focus
Transmission 15%
0.6 mJ/sample
33 MGy/pulse
9.4 keV , λ =1.32 A
Resolution 1.9 Å



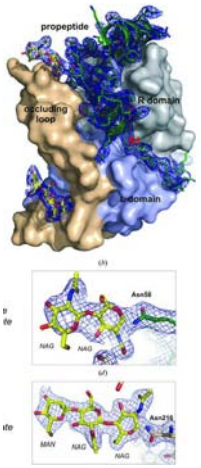
Crystals pass through beam in random orientations. Single 'still' image from each crystal. 1000s of images combined to produce a complete data set

I. Schlichting, ESRF Users' Meeting, February 2014

MACROMOLECULAR CRYSTALLOGRAPHY AT X-FELS

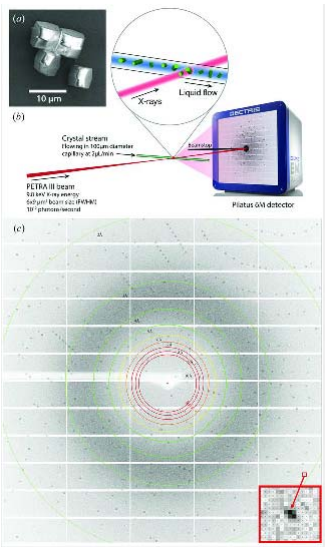


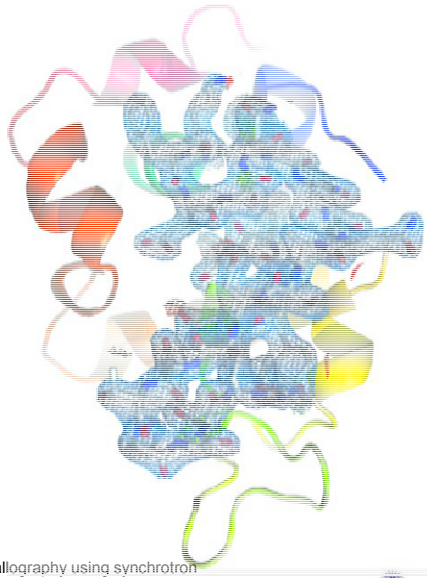
Chapman, *et al.* (2011). Femtosecond X-ray protein nanocrystallography *Nature* **470**, 73-77.



Redecke et al. (2013) Natively Inhibited Trypanosoma brucei Cathepsin B Structure Determined by Using an X-ray Laser. *Science* **339**, 227-30.

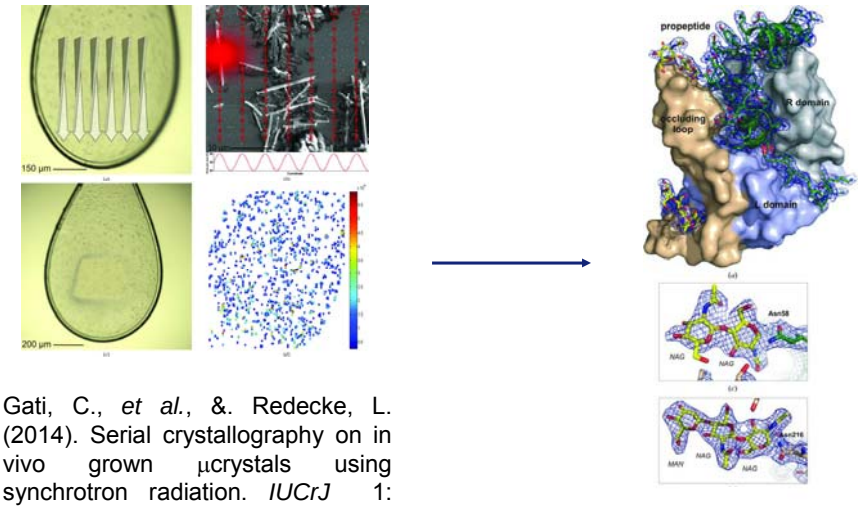
Serial Crystallography at synchrotron sources





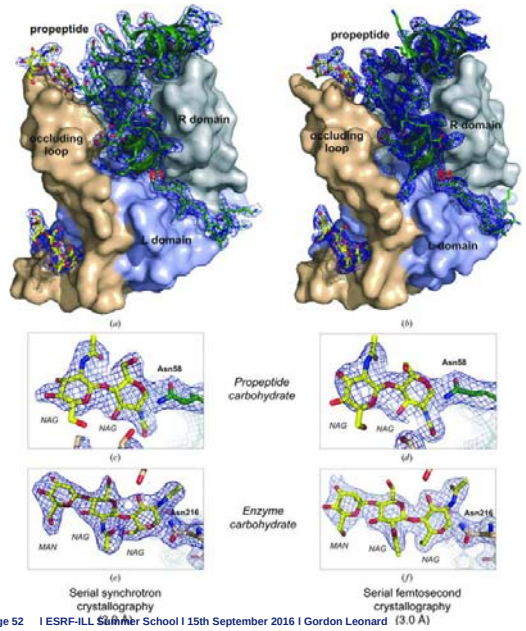
Stellato, F., et al. (2014). Room-temperature macromolecular serial crystallography using synchrotron radiation. *IUCrJ*, **1**, 204-212

Serial Crystallography at synchrotron sources



Gati, C., *et al.*, &. Redecke, L. (2014). Serial crystallography on in vivo grown µcrystals using synchrotron radiation. *IUCrJ* 1: doi:10.1107/S2052252513033939

Jets (X-FEL) vs Solid supports (SR Source)

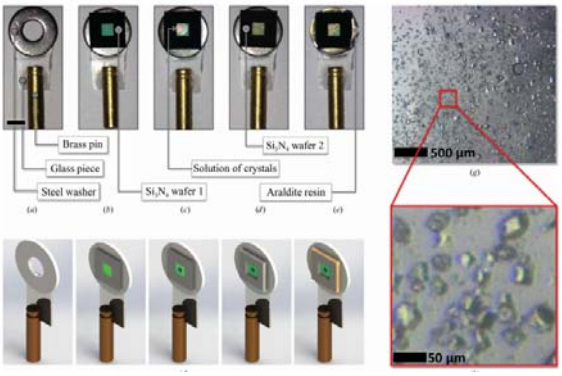


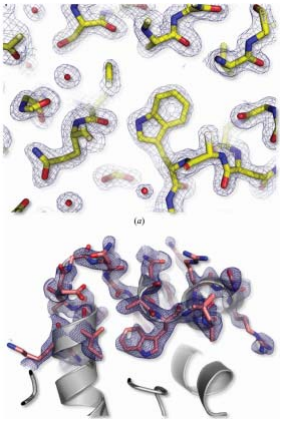
Similar/same results?

X-FEL: Redecke et al. (2013) Natively Inhibited *Trypanosoma brucei* Cathepsin B Structure Determined by Using an X-ray Laser. *Science* **339**, 227-30.

SSX: Gati, C., *et al.*, &. Redecke, L. (2014). Serial crystallography on in vivo grown mcrystals using synchrotron radiation. *IUCrJ* 1: doi:10.1107/S2052252513033939

‘Rastering’ Serial Crystallography at Synchrotron Sources (Grids)

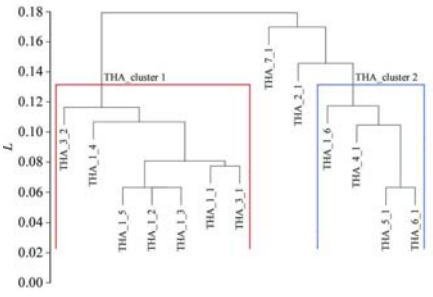


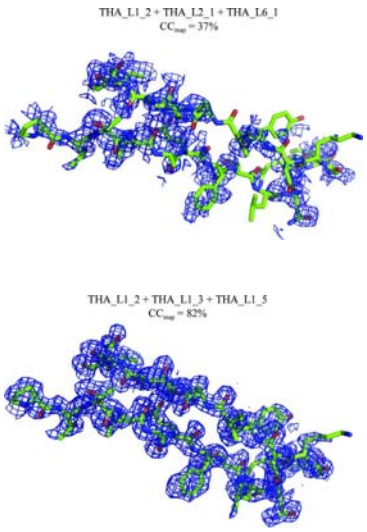


Coquelle, N. et al., (2015). Raster-scanning serial protein crystallography using micro- and nano-focused synchrotron beams. *Acta Cryst*. **D71**, 1184–1196; DOI: 10.1107/S1399004715004514

The effect merging partial data sets from non-isomorphous crystals

$$dist(i,j) = \sqrt{1 - cc_i^2(i,j)}$$

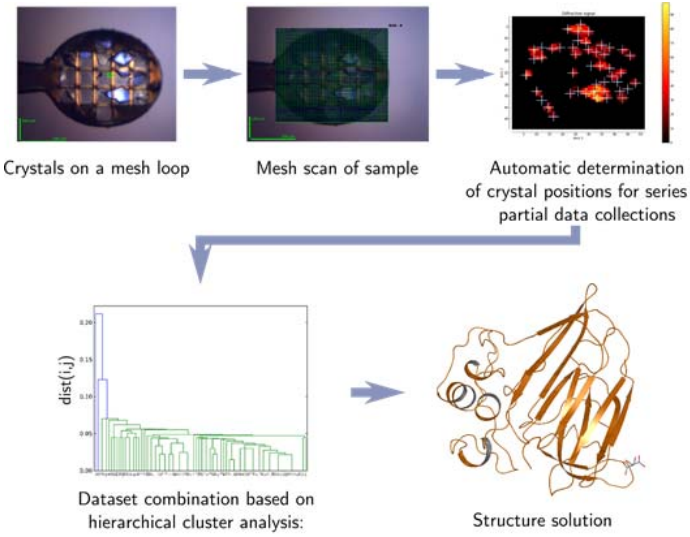




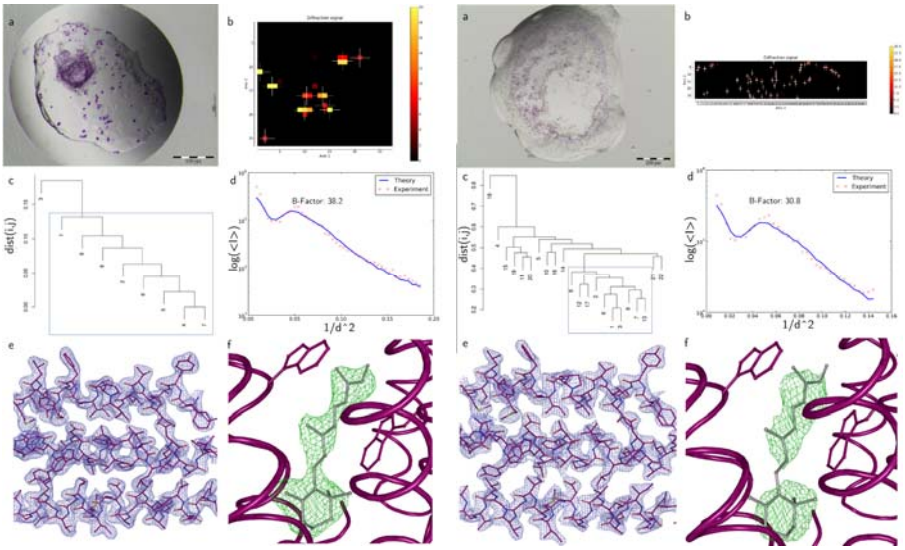
Also, identification of different conformations
→ mapping of conformational landscapes

Giordano, R., Leal, R. M. F., Bourenkov, G. P., McSweeney, S. & Popov, A. N. (2012). *Acta Cryst.* D68, 649-658.

Optimising SSX at cryo-temperatures

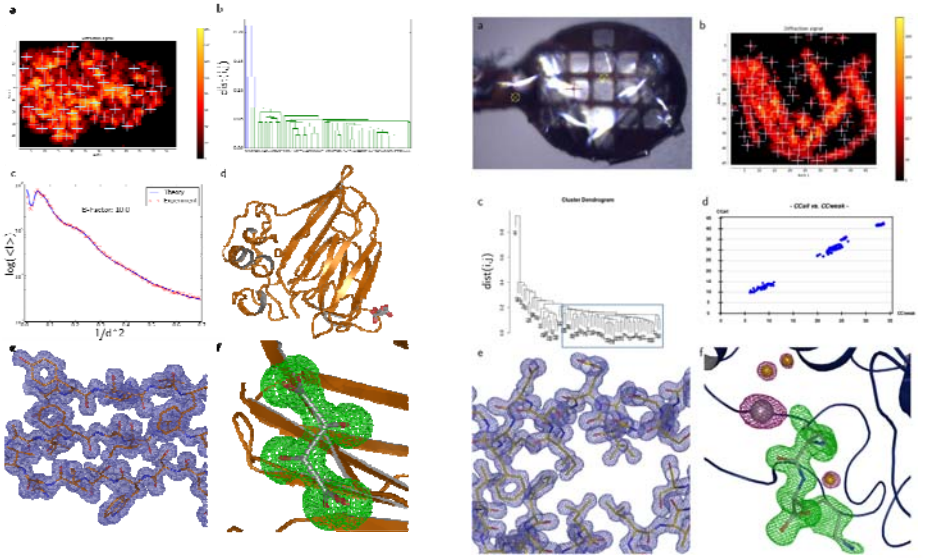


Automatic solid-support SSX/MDX: proof of principle



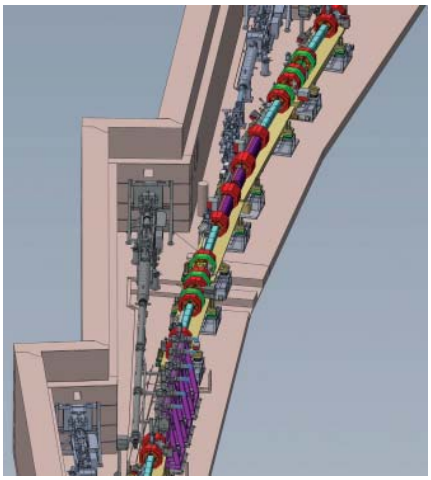
Zander, U. et al., (2015). *Acta Cryst.* Under review

Automatic solid-support SSX: proof of principle



Zander, U. et al., (2015). *Acta Cryst.* Under review

ESRF as a 4th generation synchrotron source



- Hybrid seven-bend achromat design
- 30-fold reduction in horizontal emittance
- Much smaller X-ray beams
- Much brighter X-ray beams

See ESRFnews, Number 67, July 2014 for more details

ESRF ID29 at 4th generation ESRF

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**
Low res. data collection	**?**	Yes	Yes	**Yes**
μbeam MAD[e]	**Yes**	Yes	n/a	**n/a**
μfocus MAD	**No**	No	Yes	**Yes**
Serial μcrystallography	**?**	?	Yes	**Yes**

Thanks for your attention!