ESRF | The European Synchrotron

Synchrotron Radiation and Structural Biology

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Outline:

Proteins and nucleic acids

Why study Structural Biology?

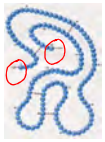
Why use Synchrotron Radiation for Structural Biology?

How to use Synchrotron Radiation for Structural Biology

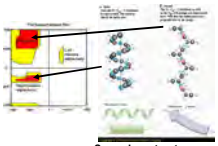
Examples

Current & future developments

The Structure of Proteins



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



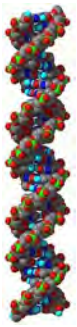
Secondary structure: Regions of the amino acid chain adopt certain conformations (α-helix; β-strand)

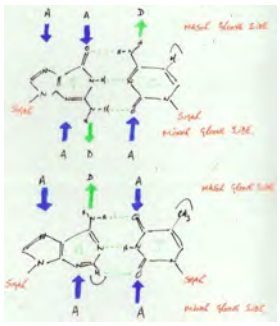
The three-dimensional arrangement of secondary structure elements results in the **tertiary structure** of a protein

Quaternary Structure: How protein associate with themselves (multimers, etc) and with other proteins

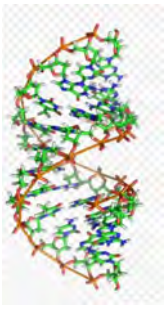
Nucleic acids are also biological macromolecules

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. Branden & John Tooze, Garland Publishing Inc., 1991

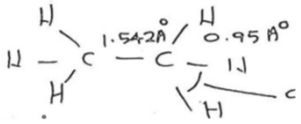
Form = Function

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

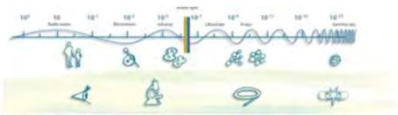
MICROSCOPY AND RESOLUTION

In all microscopy techniques what we can see is limited by the wavelength of light used.

$$res_{\max} = \lambda / 2$$

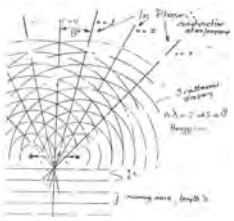


To see individual atoms in molecules need to use light with 0.5 Å > λ > 4.0 Å. Hence the need to use X-rays.

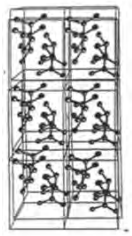


DIFFRACTION

Diffraction occurs when radiation/light passes through an aperture of similar dimensions to the wavelength of the radiation. Edges of aperture act as secondary sources of radiation and get constructive/destructive interference from 'sources'.

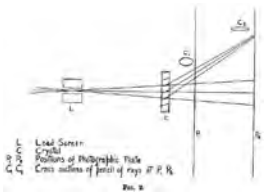


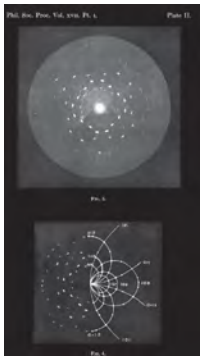
$$n\lambda = 2d \sin\theta$$



Crystals are made up of many identical units – **unit cells** – stacked together in three dimensions. The contents of each unit cell are identical. Crystals act as diffraction gratings for X-rays.

X-RAY CRYSTALLOGRAPHY





[W.L, Bragg] X-rays are waves 'reflected' by planes in a crystal

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

W.L. Bragg (1912) Proc. Camb. Phil. Soc., XVII (I), pp. 43-57.

THE FIRST CRYSTAL STRUCTURE

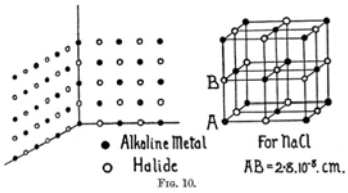
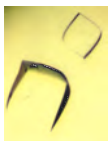


FIG. 10.

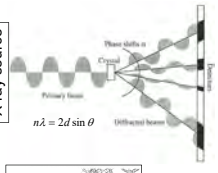
'….results would seem to indicate clearly the association of each diffracting centre with a single atom.'

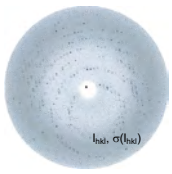
W. L. Bragg (1913) The Structure of Some Crystals as Indicated by Their Diffraction of X-rays. *Proc. R. Soc. Lond. A* **89**, 248 – 277; doi: 10.1098/rspa.1913.0083

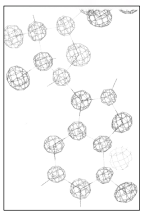
X-RAY CRYSTALLOGRAPHY



X-ray source







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

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

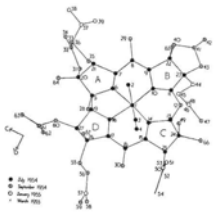
$$F_{hkl} = \sum_j |F_{hkl}|\exp -2\pi i(\phi)$$

Amplitude — Phase

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

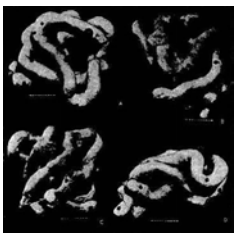
RESOLUTION

Vitamin B_{12}



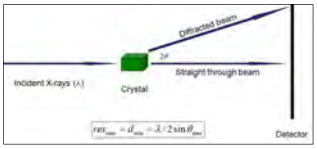
Crowfoot Hodgkin, D. et al., (1955). Structure of Vitamin B_{12} : The Crystal Structure of the Hexacarboxylic Acid derived from B_{12} and the Molecular Structure of the Vitamin. *Nature* **176**, 325 – 328.

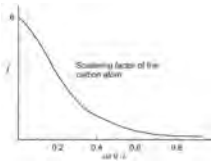
Myoglobin



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

DIFFRACTION DATA RESOLUTION

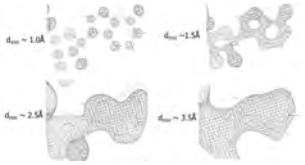




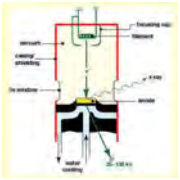
$$E(hkl) = \frac{e^4}{m^2c^4\omega} I_o \lambda^3 LPA \frac{V_c}{V_z^2} |F(h)|^2$$

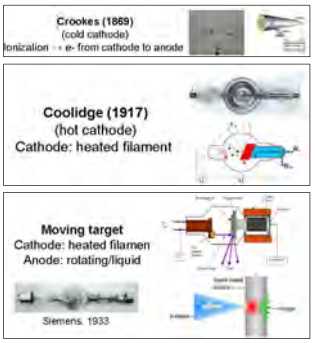
- For a crystal of a given size, energy of a diffracted beam is:
  - proportional to incident X-ray beam
  - proportional to volume of crystal
  - inversely proportional to square of unit cell volume

  → for crystals of macromolecules (large unit cells, small crystals) we need intense sources of X-rays to get maximum information (i.e. data resolution).



GENERATING X-RAYS





SYNCHROTRON RADIATION INTENSE AND HAS A BROAD SPECTRAL RANGE

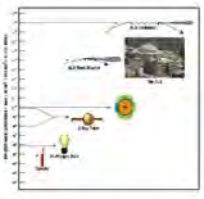
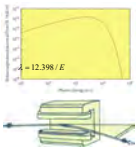
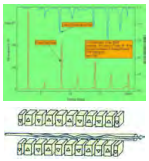


Fig. 3 Comparison of the brightness of the bending magnet and undulator sources at the Advanced Light Source (ALS), to that of other common light sources.



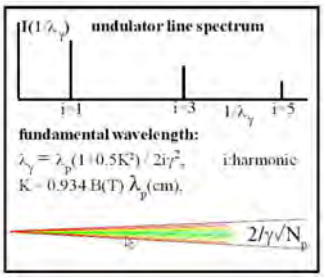
Bending magnet spectrum



Undulator spectrum

UNDULATOR SYNCHROTRON RADIATION IS HIGHLY COLLIMATED

$$1/\gamma = mc^2 / E$$



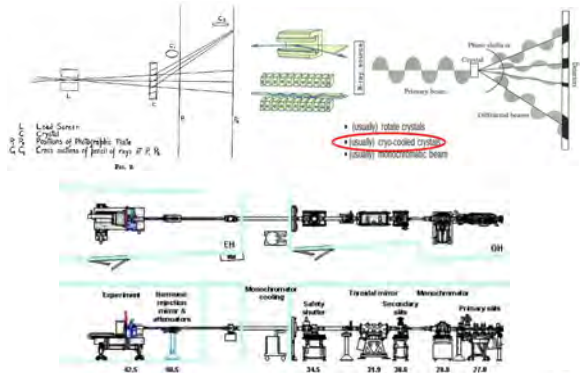
http://www-ssrl.slac.stanford.edu/primer.pdf

Intensity + collimation = 'brilliance'

SYNCHROTRON RADIATION IS IDEAL FOR MACROMOLECULAR CRYSTALLOGRAPHY

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

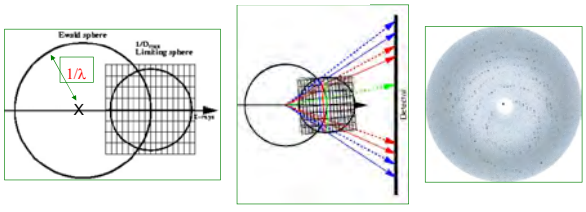
MX AT SYNCHROTRON SOURCES



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

MONOCHROMATIC MX WITH 2D DETECTORS

$$n\lambda = 2d_{hkl}\sin\theta$$

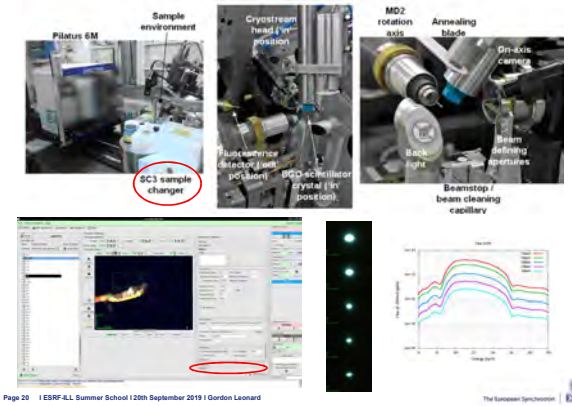


Rotating the crystal (X) means that the reciprocal lattice also rotates, reciprocal lattice points pass through the surface of the Ewald sphere resulting in diffracted beams.

Due to 'denseness' of reciprocal lattice in macromolecules – large unit cells - we can't collect the unique part of the sphere in one large rotation.

Have to break this down into a series of (much) smaller parts (oscillations) - typically (much) smaller than 0.5° (larger for crystals of small molecules) which are added together form the data set

Experimental set-up at ID29 at ESRF



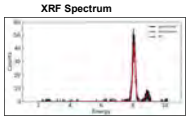
EXPERIMENTS (AND RESULTS) ARE RECORDED (ISPYB)

Parameters & Results

Crystal Snapshots

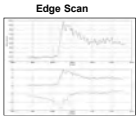
Image thumbnails

XRF Spectrum



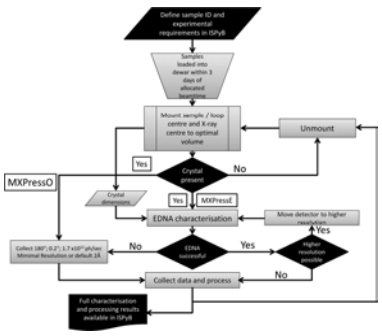
Autoprocessing results

Edge Scan

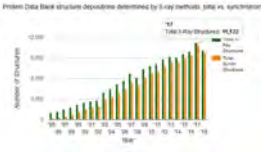


EXPERIMENT DATABASE + AUTOMATION = REMOTE ACCESS

AUTOMATIC WORKFLOWS

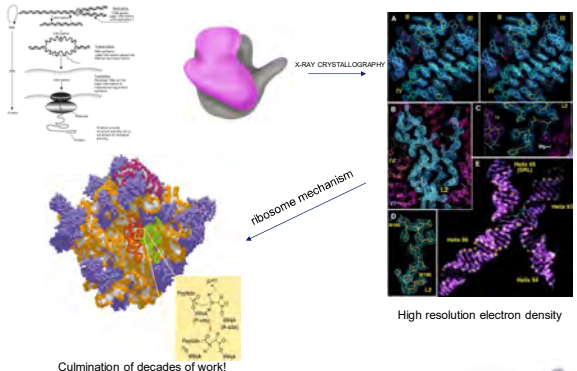


NOW MANY VERY PRODUCTIVE SYNCHROTRONS WORLDWIDE



http://biosync.sbkb.org/
http://www.rcsb.org/

LANDMARKS IN MX - THE CRYSTAL STRUCTURE OF THE RIBOSOME

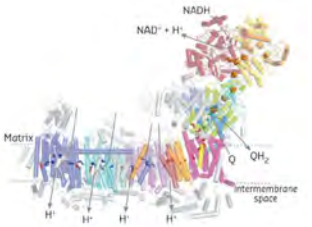


High resolution electron density

Culmination of decades of work!

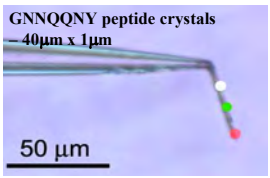
MECHANISTIC CLUES FROM THE STRUCTURE OF MITOCHONDRIAL COMPLEX I

- Mitochondria gain energy by oxidising hydrogen extracted from nutrients, converting it into a proton gradient that is then used to synthesise (ATP), the universal energy currency of the cell. **Complex I** couples the transfer of two electrons from NADH to ubiquinone with the pumping of four protons across the inner mitochondrial membrane.

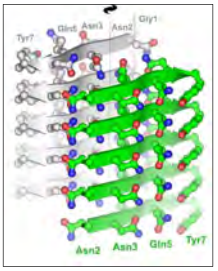


- Defects in human complex I are the most frequent cause of inherited mitochondrial disorders and are implicated in numerous neurodegenerative diseases and ageing.

SMALL MOLECULES ARE ALSO IMPORTANT IN STRUCTURAL BIOLOGY



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

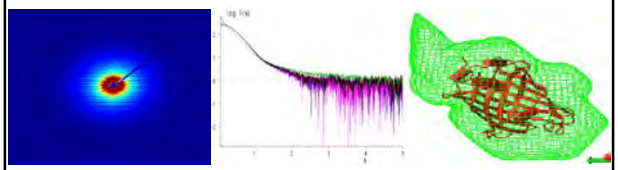


Neurodegenerative diseases are associated with the formation of amyloid fibrils. Spines of these have a common structure. Interface between the two β-sheets is 'dry' (no water molecules). Also no hydrogen bonds from one sheet to its mate. Structure suggests routes towards developing therapies for Alzheimer's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

STRUCTURAL BIOLOGY AT SYNCHROTRONS WITHOUT CRYSTALS - SAXS

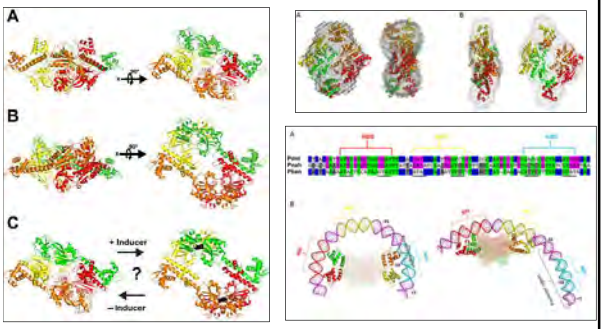
Small Angle X-ray Scattering (SAXS) is a technique for studying structure (and other things*) at low resolution in solution & under normal biophysical/biochemical conditions

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



Michael Lerche, Cyril Dian, Adam Round, Rosa Lönneborg, Peter Brzezinski &Gordon A. Leonard (2015). The solution configurations of inactive and activated DntR have implications for the sliding dimer mechanism of LysR transcription factors. Scientific Reports, 6:19988 ; DOI: 10.1038/srep19988

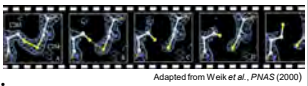
Radiation damage in X-ray crystallography

Global radiation damage

- **Reciprocal space** – degradation of diffraction properties of a crystal
  - ↗ Wilson *B* factor
  - ↗ Mosaicity
  - ↗ Relative unit cell volume
  - ↘ Resolution (Å)
- Before 1990: **room temperature** crystallography requires data from several crystals to obtain a complete dataset
- 1990s: **cryo-crystallography** extends the lifetime of crystals to be able to record a full dataset (or more) on one single crystal (Garman & Schneider, *J. Appl. Cryst.* (1997))
- **Even at cryo-temperatures maximum absorbed dose/crystal ~20 - 30MGy**

Specific radiation damage

- 2000s: 3rd generation synchrotron sources revealed damage in the **real space**, on specific chemical groups

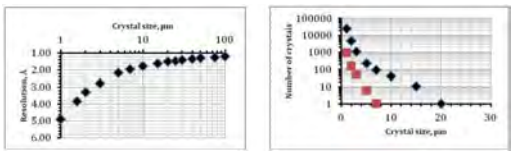


Adapted from Weik *et al.*, *PNAS* (2000)

- **Specific** radiation damage is a concern in crystallography at **cryogenic temperature**
- The onset of **specific** damage is faster than **global** damage at **cryogenic temperature**

→ **Decoupling of the two types of radiation damage**

GLOBAL RADIATION DAMAGE, CRYSTAL SIZE AND DATA SET RESOLUTION



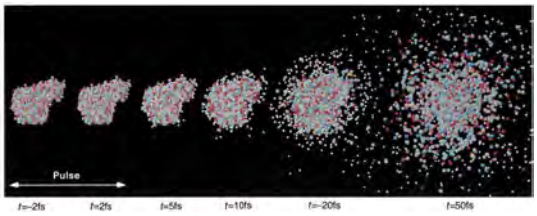
The resolution of a *complete* diffraction dataset that will be yielded from a *single* microcrystal of a biological macromolecule will remain limited by radiation damage. Many such crystals will be required for the collection of even moderate resolution diffraction data

→ New (old) paradigm for macromolecular crystallography: Multi-crystal data collection

Or

→ Avoid radiation damage

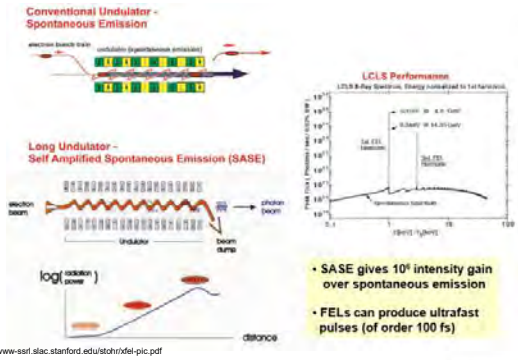
AVOIDING RADIATION DAMAGE



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?

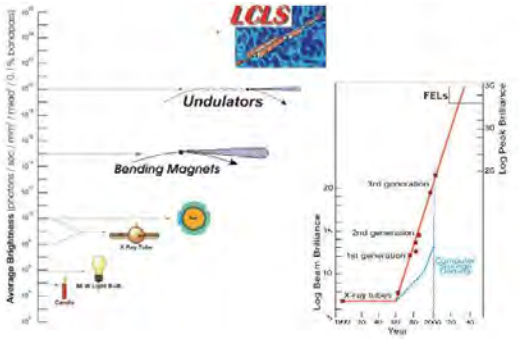
Neutze, R. et al. (2000). Potential for biomolecular imaging with femtosecond X-ray pulses. *Nature* **406**, 752-757.

FREE ELECTRON LASERS



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

X-FELS X-RAY BEAMS ARE MUCH BRIGHTER THAN SYNCHROTRON X-RAY BEAMS

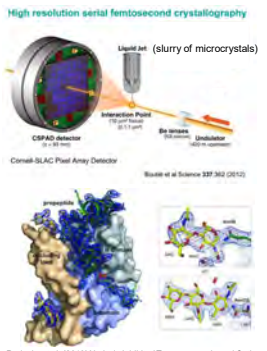


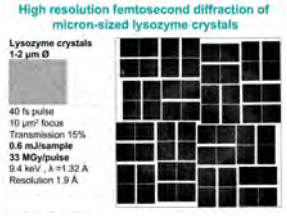
http://www-ssrl.slac.stanford.edu/stohr/xfel-pic.pdf

BUT.....

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

SERIAL FEMTOSECOND CRYSTALLOGRAPHY (X-FELS)

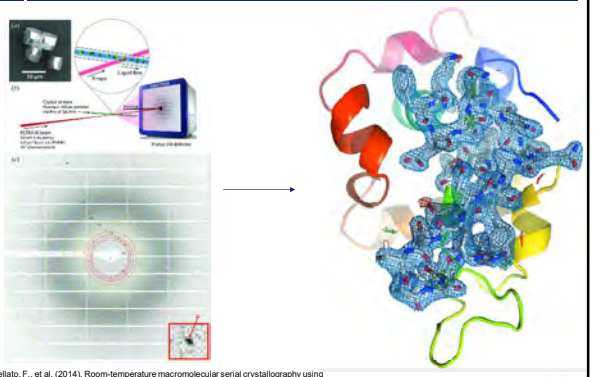




Crystals pass through beam in random orientations. Single 'still' image from each crystal. 1000s of images combined to produce a complete data set. I. Schlichting, ESRF Users' Meeting, February 2014

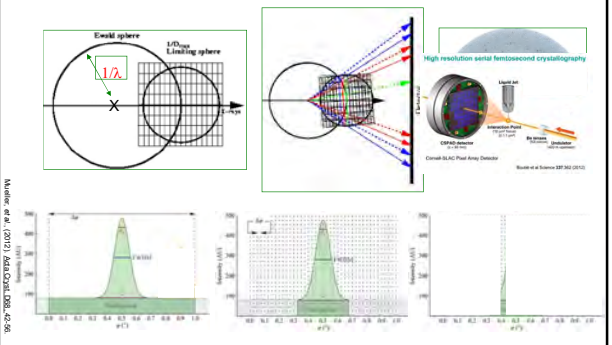
Redecke et al. (2013) Natively Inhibited Trypanosoma brucei Cathepsin B Structure Determined by Using an X-ray Laser. *Science* **339**, 227-30.

SERIAL CRYSTALLOGRAPHY AT SYNCHROTRON SOURCES



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

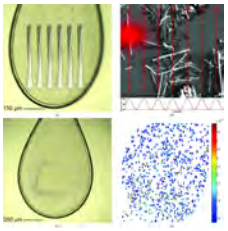
COLLECTING DIFFRACTION DATA WITH 2D DETECTORS

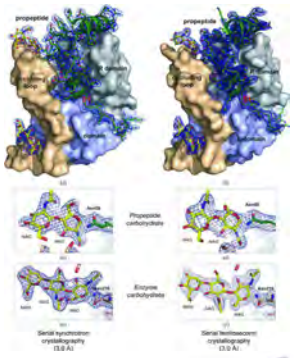


Mueller, et al. (2012). Acta Cryst. D68, 42-56.

$$I = \sum (P_i - B_i) \qquad I / \sigma(I) = \frac{I}{\left[\sum (P_i + B_i)\right]^{1/2}}$$

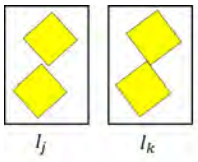
Serial Crystallography at synchrotron sources (II)



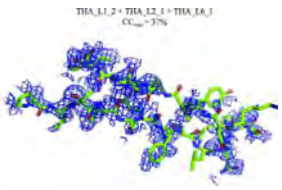


Gati, C., *et al.*, &. Redecke, L. (2014). Serial crystallography on in vivo grown μcrystals using synchrotron radiation. *IUCrJ* 1: doi:10.1107/S2052252513033939

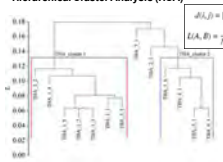
NON-ISOMPORPHISM

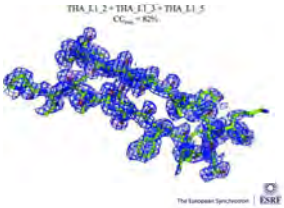


$I_j \neq I_k$



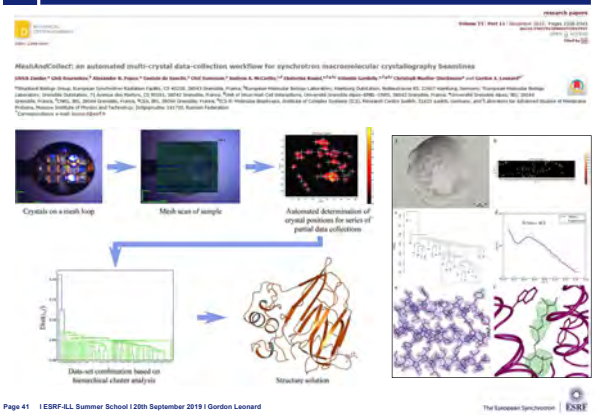
Hierarchical Cluster Analysis (HCA)



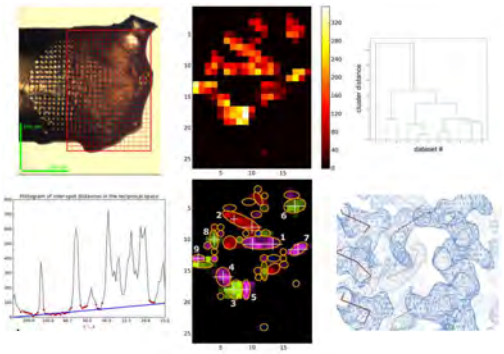


Giordano, et al., (2012). Acta Cryst. D68, 649-658.

'FIXED-TARGET' SSX : TAKING ADVANTAGE OF OSCILLATION DATA COLLECTION



WORKFLOWS FOR MULTI-CRYSTAL (SERIAL) CRYSTALLOGRAPHY : MESHBEST



Igor Melnikov et al., (2018). The complex analysis of X-ray mesh scans for macromolecular crystallography. *Acta Cryst.* **D74**, https://doi.org/10.1107/S2059798318002735

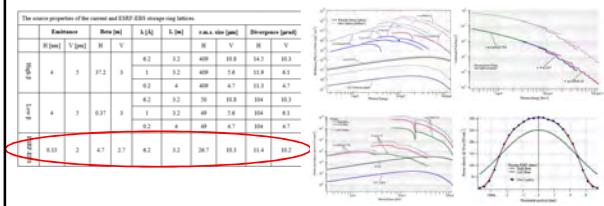
ESRF-EBS: AN AMBITIOUS UPGRADE PROGRAMME TO PREPARE THE FUTURE

ESRF Extremely Brilliant Source (ESRF-EBS)

- FIRST of a new generation of synchrotron storage rings
- ~100 times more brilliant and coherent X-rays
- Programme to exploit the qualities of this new and so far unique extremely brilliant X-ray so
  - Creation of new beamli
  - Innovative detecto
  - « Data as a Sc

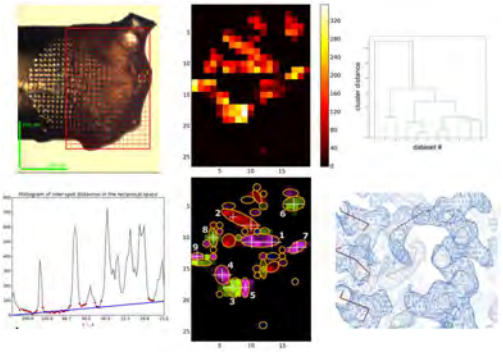
ESRF-EBS: 10th December 2018 – 25th August 2020

WHAT DOES ESRF-EBS REALLY MEAN?



- Smaller source size
- Lower divergence
- **Smaller X-ray beams**
- **Much brighter beams**
- All straight sections equal
- Higher coherence fraction

WORKFLOWS FOR MULTI-CRYSTAL (SERIAL) CRYSTALLOGRAPHY : MESHBEST

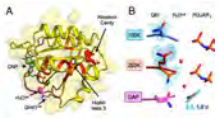


Igor Melnikov et al., (2018). The complex analysis of X-ray mesh scans for macromolecular crystallography. *Acta Cryst.* **D74**, https://doi.org/10.1107/S2059798318002735

ROOM TEMPERATURE DATA COLLECTION AND PUMP-PROBE INVESTIGATIONS

Room-temperature Data Collection

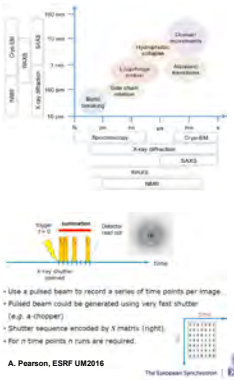
Crystal structures obtained at cryogenic temperatures can obscure functionally important conformations: i.e. for small GTPase H-ras only the RT structure showed the catalytically essential glutamine residue in the conformation that is required for GTP hydrolysis.



Fraser et al., PNAS 108, 16247-16252, 2011

Because of radiation damage, serial crystallography is (probably) the only route to obtaining RT structures.

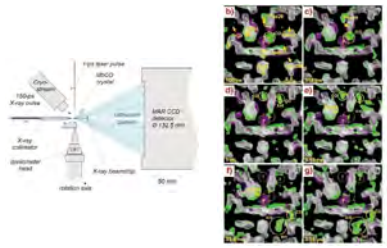
Pump-probe *in crystallo*



A. Pearson, ESRF UM2016

TIME-RESOLVED MX USING THE LAUE TECHNIQUE (WHITE/PINK BEAM)

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

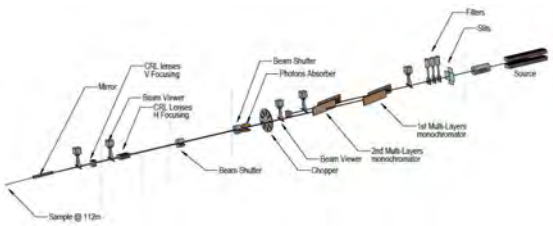


Structure of MbCO at different time delays after photolysis. The bound CO dissociates, eventually becoming trapped in sites 4 and 5, where it remains out to the microsecond time scale.

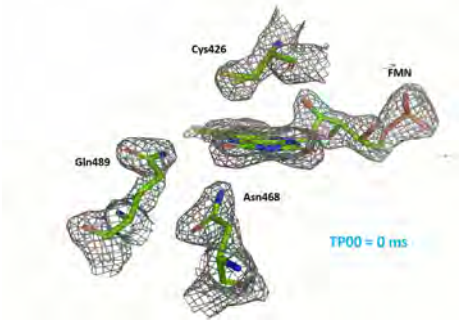
F. Schotte *et al.*, (2003), *Science*, 300, 1944-1947.

EBSL8 - A BEAMLINE FOR (TIME-RESOLVED)SERIAL CRYSTALLOGRAPHY

- High flux (10^{16} ph/s), large bandwidth (~1%)
- Beamsize: 0.5 μm – 10 μm
- Tuneable: 10-30 keV



MOVIE OF PHOTOADDUCT FORMATION (MONOCHROMATIC X-RAYS, 60 MSEC TIME RESOLUTION)



Thanks for your attention!