Synchrotron Radiation and Structural Biology

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Outline:

- Why study Structural Biology?
- Why use Synchrotron Radiation for Structural Biology?
- How to use Synchrotron Radiation for Structural Biology
- Examples
- The Future of synchrotron-based MX

Why Study Structural Biology?

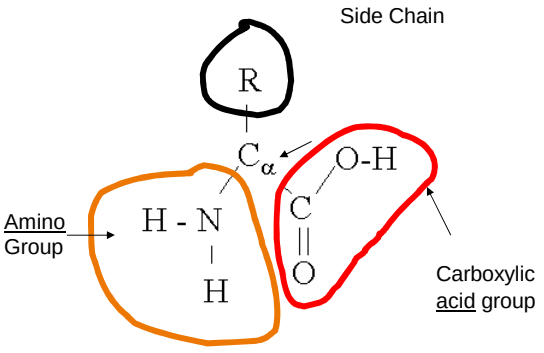
The ultimate goal of molecular biology is to understand biological processes in terms of the chemistry and physics of the macromolecules that participate in them. One of the essential differences between the chemistry of living systems and that of the nonliving is the great structural complexity of biological 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.

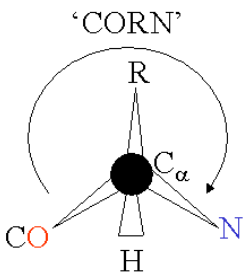
Introduction to Protein Structure, C-I. Branden & John Tooze, Garland Publishing Inc., 1991

'Form' = 'Function'

WHAT IS A PROTEIN?

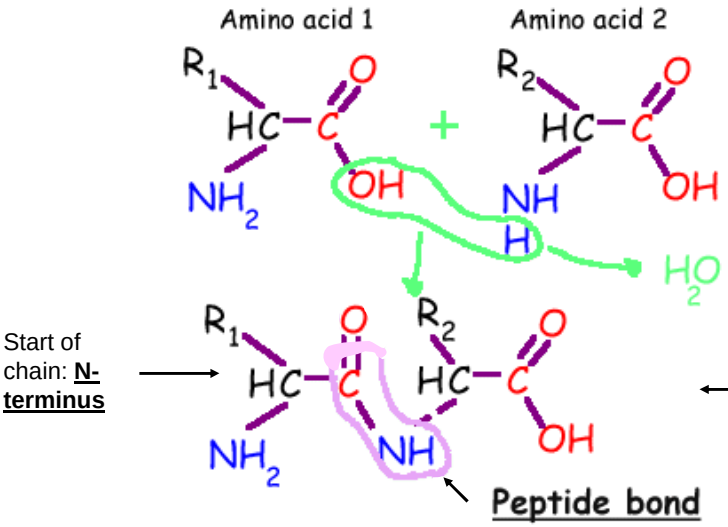
A sequence of amino acids joined end-to-end to form a linear polypeptide chain.



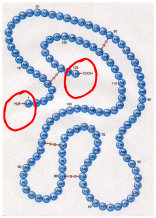


Proteins contain L-amino acids

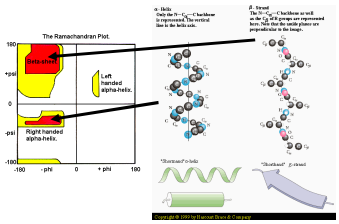
THE PEPTIDE BOND



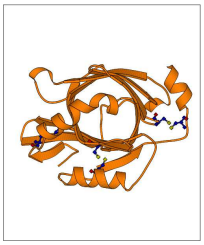
The Structure of Proteins



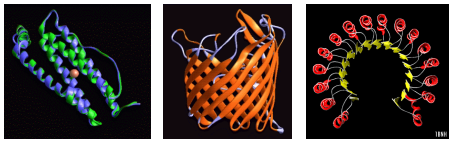
The amino acid sequence (N→C) of a protein is it's **primary structure**



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

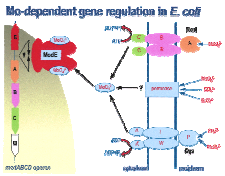


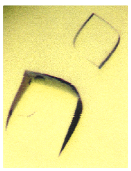
Examples of the α-, β- and α/β **tertiary** structures of different proteins

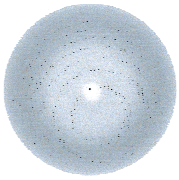
How to Study Protein Structure?

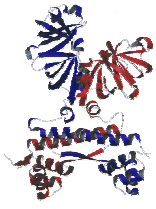
- Nuclear Magnetic Resonance
- Electron Microscopy and Diffraction
- Circular Dichroism/ Light Scattering
- Solution Scattering (SAXS/SANS)
- **X-ray (Neutron) Crystallography = Macromolecular Crystallography (MX)**

MX in five pictures



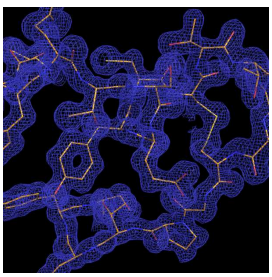




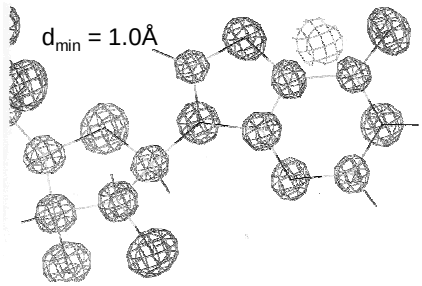


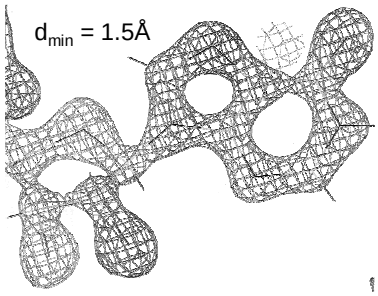
Atomic model of macromolecule of interest = atomic coordinates (x,y,z) + displacement factor (B, U_{ij}).

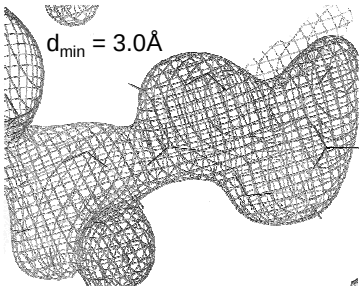
'Form = Function'

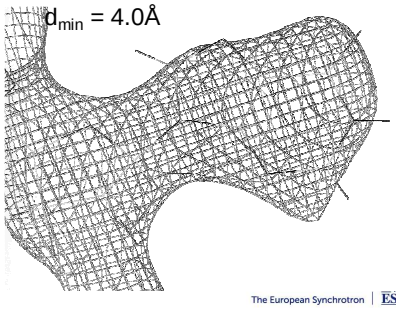


NOT ALL ELECTRON DENSITY MAPS ARE EQUAL





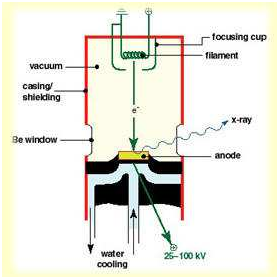




The European Synchrotron | ESRF

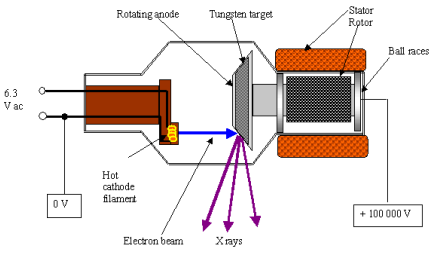
The generation of X-rays for MX

Sealed tube source



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

Rotating anode source



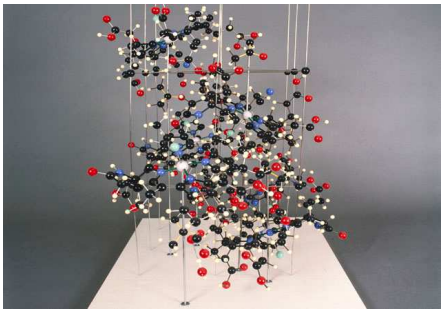
Spinning the target helps dissipate head load → higher power and higher intensity of X-rays.

Commonly used targets: Mo, Cu, Cr

The European Synchrotron | ESRF

The first crystal structures of biological macromolecules

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.

[Max Perutz]

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

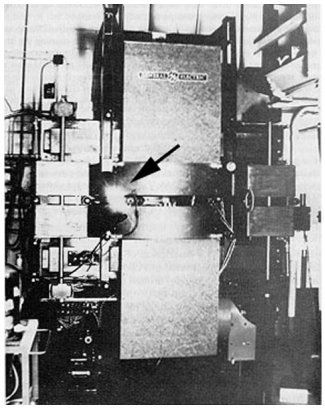
Crystals of macromolecules have large unit cells. Biological macromolecules are also conformationally variable. Growing large crystals is not easy

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

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

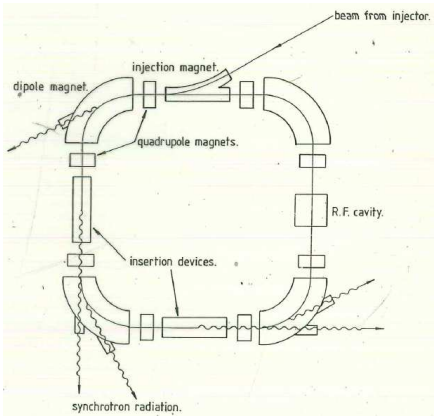
Synchrotron Radiation

Synchrotron Radiation



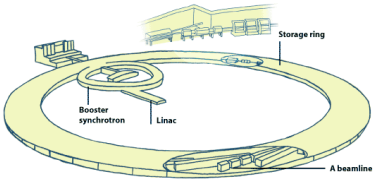
Elder et al., (1947) "Radiation from Electrons in a Synchrotron" *Phys. Rev.*, **71**, 829-830.

A Storage Ring



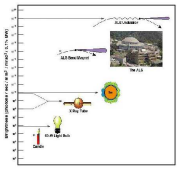
1st Generation – parasitic use
2nd Generation - dedicated
3rd Generation – dedicated, higher energy

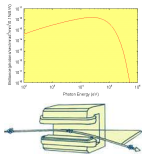
The European Synchrotron Radiation Facility (ESRF)



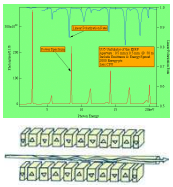
ESRF: 3rd generation; Energy 6GeV; circumference 844 m; >600 staff; >4000 visitors/year

Synchrotron Radiation is ideal for macromolecular crystallography





Bending magnet

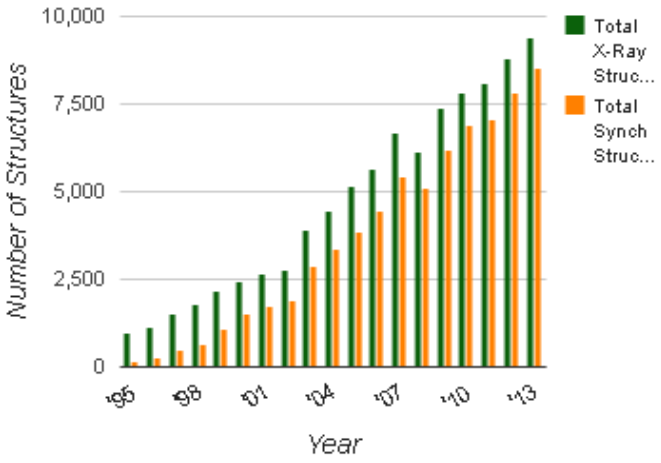


Undulator

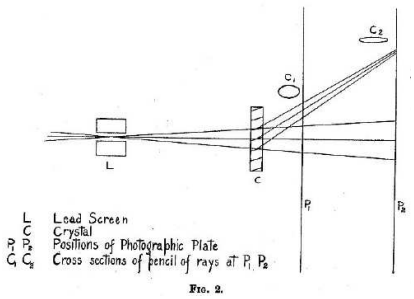
- Brilliance at 3rd generation synchrotron sources
  - better resolution, smaller samples, time resolution
- Wavelengths from IR to hard X-rays
  - Anomalous dispersion, fluorescence techniques
- Time structure (ps)
  - Time resolved studies
- Coherence
  - Imaging

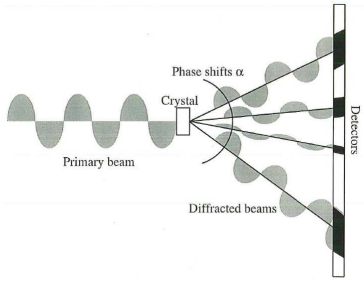
There are now many synchrotrons worldwide

Synchrotron facilities are very productive



Doing MX at synchrotron sources

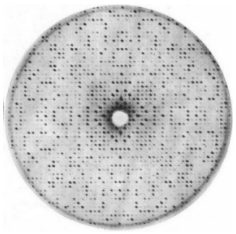




$$n\lambda = 2d\sin\theta$$

- (usually) monochromatic beam
- (usually) rotate crystal
- not photographic plates as detectors!
- much more technology

The evolution of detectors for structural biology experiments

Photographic Film	Image Plate ~1990	CCD ~1996	Pixel ~2004
			
Read-out ~60min Atomic resolution	Ø 345 mm Read-out ~80sec 17 bit dynamic range	225 x 225 mm^2 (3072 x 3072 pixels) Read-out ~1sec 16 bit dynamic range	424 x 435 mm^2 (2463 x 2527 pixels) Read-out ~2msec 20 bit dynamic range

Facilities for Structural Biology at the ESRF

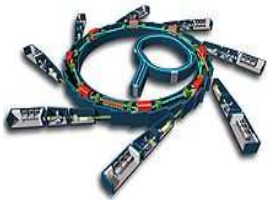
Currently available SB beam lines

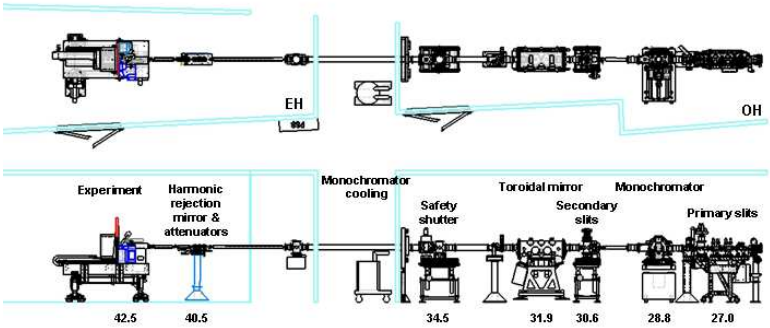
	Energy [keV]	Beam size [mm^2]	Flux [ph/s]	detector	Frame rate [Hz]
ID23-1	6-20	10-40	3x10^{12}	Pilatus 6M	25
ID23-2	14.2	5x7	4x10^{11}	Pilatus3 2M	250
ID29	6-20	10-50	5x10^{12}	Pilatus 6M	25
ID29S	optical spectroscopy (CRYOBENCH; UV/vis absorption, fluorescence, Raman)				
BM29	7-15	500 (100)	2x10^{13}	Pilatus 1M	100

Future SB beam lines

MASSIF-1	12.8	20-150	10^{13}	Pilatus3 2M	250
MASSIF-2	12.8	20-100	10^{13}	tbd	
MASSIF-3	12.8	>10	5x10^{13}	Eiger 4M	750
ID30B	6-20	20-200	10^{13}	Pilatus3 6M	100

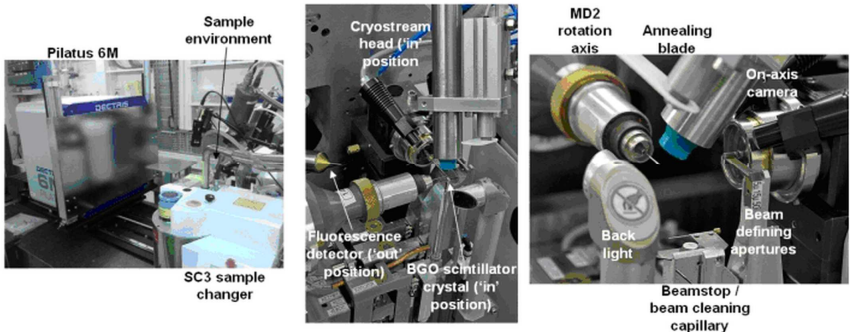
ID29: A beamline for Structural Biology



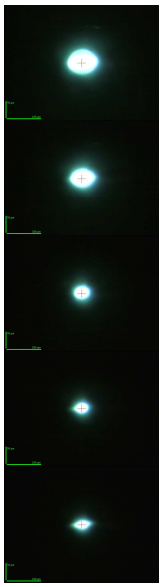


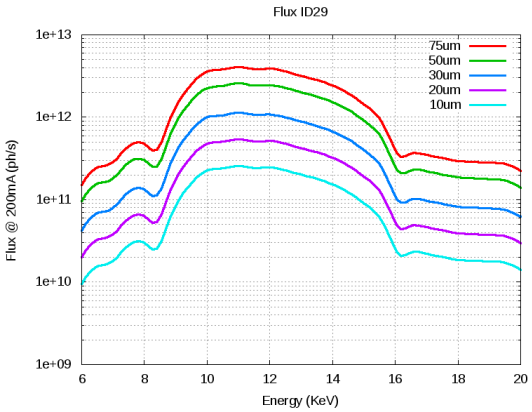
The layout of ID29 at ESRF: Radiation source: One of two 1.6m long undulators (U_{21}; U_{35}); beam conditioning in Optics Hutch (OH), data collection in Experimental Hutch (EH).

The experimental set-up at ID29 at ESRF

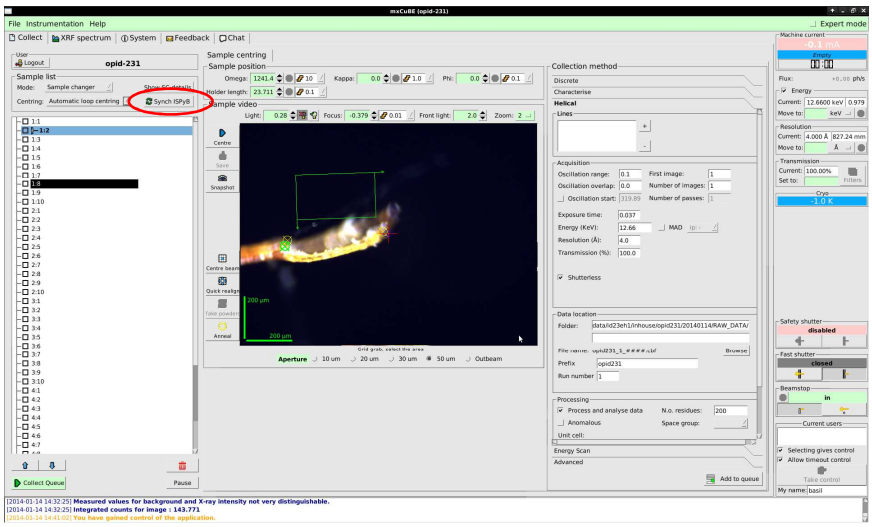


The X-ray beam at ID29 at ESRF



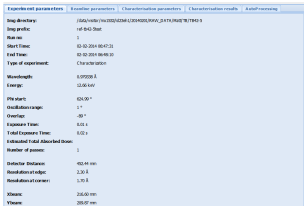


MX Experiments are (highly) automated



MX experiments (and results) are recorded

Parameters & Results



Crystal Snapshots

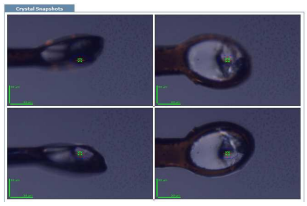
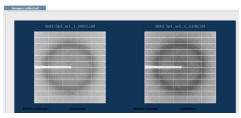
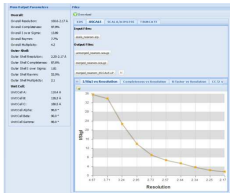


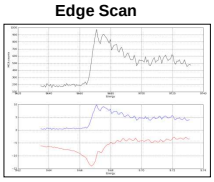
Image thumbnails



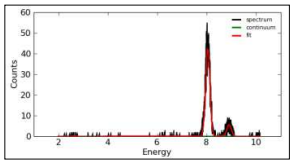
Autoprocessing results



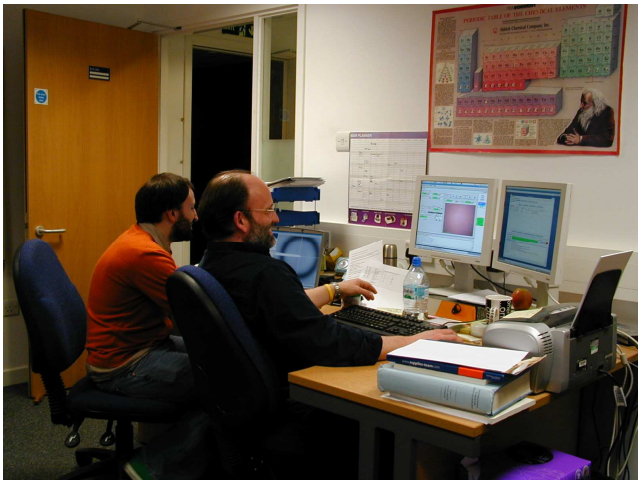
Edge Scan



XRF Spectrum



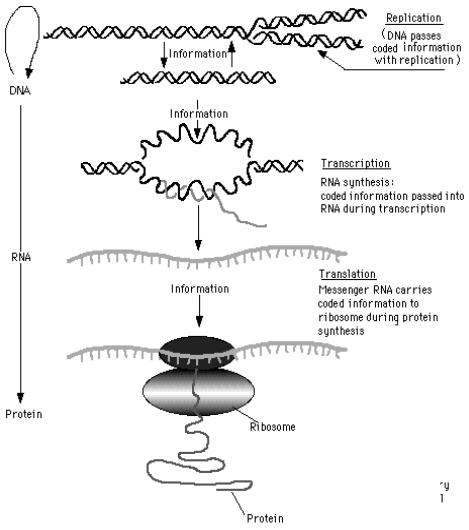
Many synchrotron beamlines can be accesses from anywhere



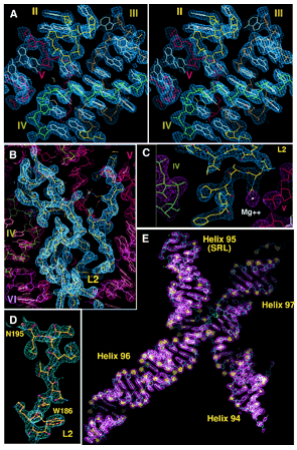
Examples of the power of MX

- The structure of the ribosome
- G-Protein Coupled Receptors
- Potassium Channels
- Photosynthesis
- How anaesthetics work
- Mitochondrial Complex 1

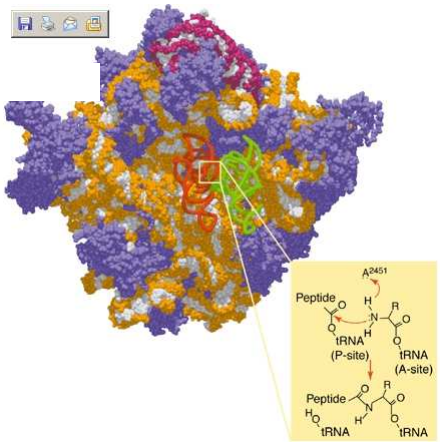
Structural information on the ribosome before MX



The crystal structure of the ribosome



High resolution electron density

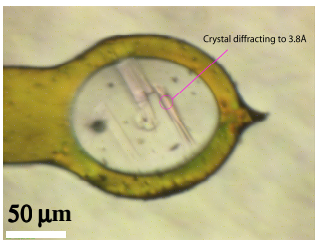


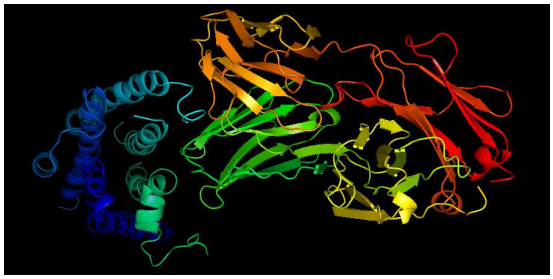
Culmination of decades of work!

Ramakrishnan, Steitz, Yonath: Nobel Prize in Chemistry, 2009

G-Protein Coupled Receptors (GPCRs) – MX with microfocus beams

human b2 adrenergic receptor



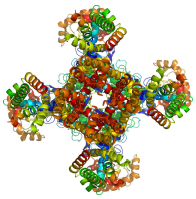


Need microfocus beams: ESRF ID13, ID23-2 and APS GM/CA CAT; 23ID-B

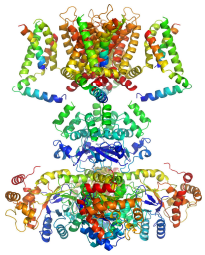
Rasmussen et al., *Nature* (2007) **450**,383

Kobilka, : Nobel Prize in Chemistry, 2012

Landmarks in MX - neurotransmitters



Landmarks in Macromolecula Crystallography - neurotrans

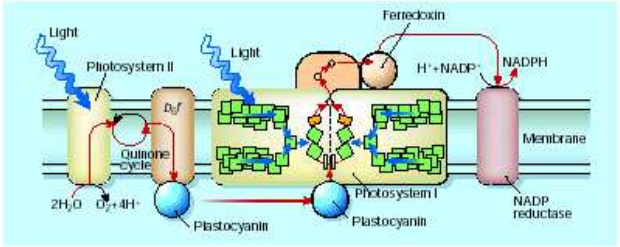


Neurotransmitters play an essential role in signal transduction. The resolution of the structure and the biophysical properties of the **Voltage dependant K+ channel** led to the Nobel Prize for Chemistry for ESRF user Rod McKinnon (Rockefeller University N.Y.) in 2003.

Photosynthesis - photosystems I and II

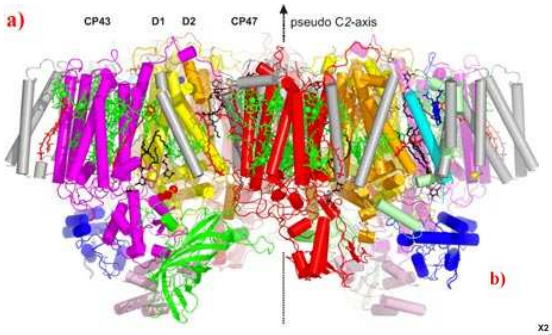
Photosynthesis

$$6\,CO_2 + 12\,H_2O + \text{light } (h\upsilon) \longrightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$$

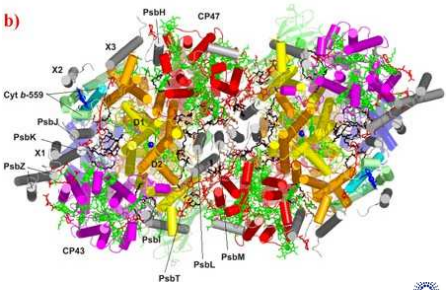


W. Kühlbrandt (2001) *Nature* 411, 896

Photosystem II (*T. elongatus*)

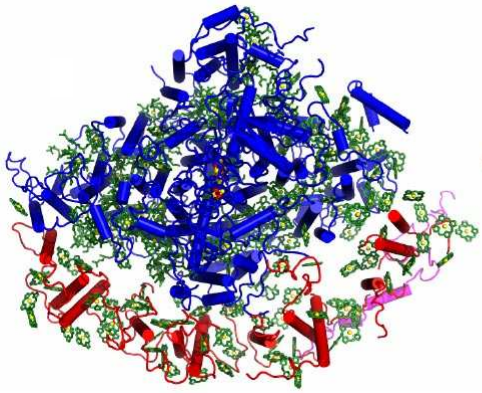


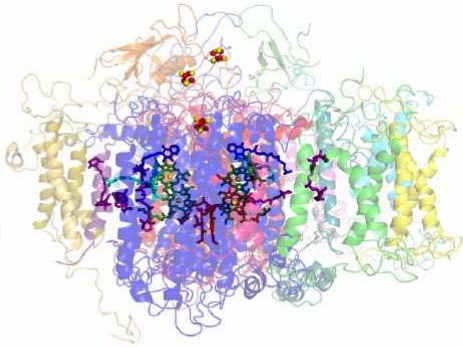
Dimer
20 protein subunits
77 co-factors
35 chlorophylls,
11 β-carotens,
2 pheophytins, 2 haem,
Mn_4Ca, 14 lipids
3.0 Å resolution



Loll, Kern. Saenger, Zouni, Biesiadka (2005), *Nature* 438, 1040

Photosystem I from Plant (*Pisum sativum*)

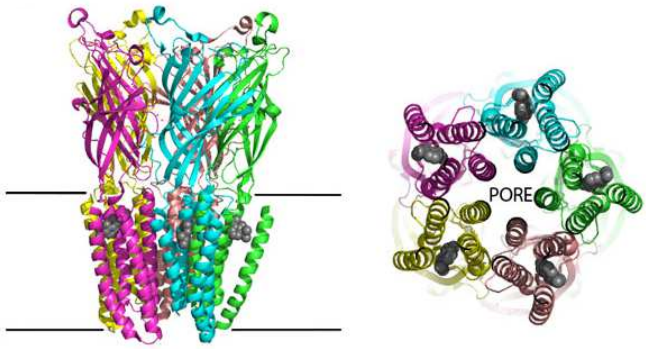




12 core subunits, 4 light harvesting membrane proteins 45 transmembrane helices, 167 chlorophylls, 3 Fe-S clusters 2 phylloquinones, Resolution 3.4 Å

Amunts, Dory & Nelson, (2007) *Nature* 447, 58-63

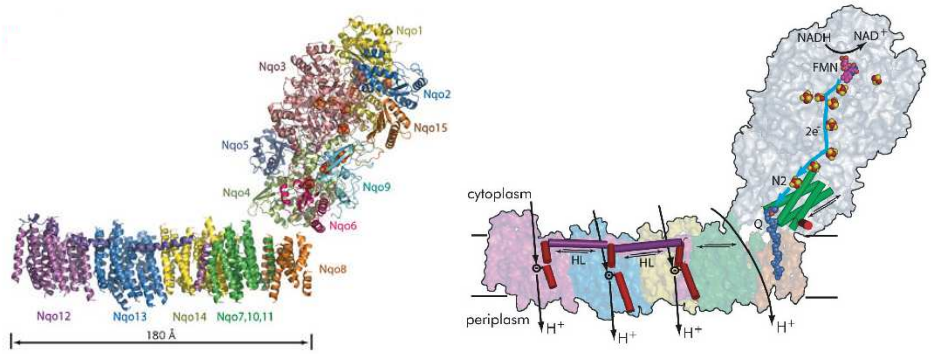
The molecular mechanism of pain relief



Pentameric ligand-gated ion channels (pLIGICs): Neurotransmitters bind in the extracellular domain and cause the opening or closing of the channel located in the membrane domain. Propofol (an anaesthetic, grey spheres) binds in a hydrophobic pocket between helices and interferes with the opening and closing of the channel

Nury, H. et al., (2011). X-ray Structures of general anaesthetics bound to a pentameric ligand-gated ion channel, *Nature* **469**, 428-431.

Architecture of respiratory complex I



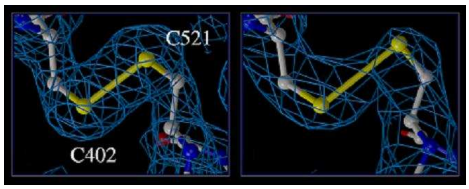
Complex I plays a central role in cellular energy production, providing about 40% of the proton-motive force required for ATP synthesis. Complex I dysfunction has been implicated in many human neurodegenerative diseases. Complex I appears to resemble a steam engine, where the energy of electron transfer is used to move a piston, which then drives, instead of wheels, a set of discontinuous helices.

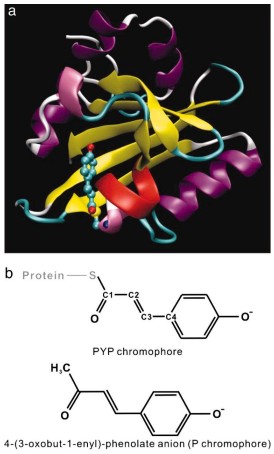
R.G. Efremov, R. Baradaran and L.A. Sazanov, Nature 465, 441-445 (2010).

MX on its on does not give all the answers: sometimes need to combine with other techniques to give improved interpretation of X-ray data

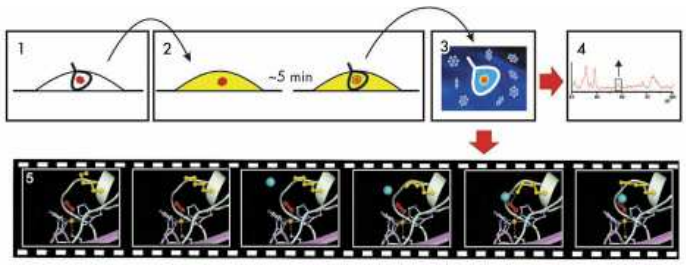
Combining MX and Spectroscopy

- Oxidation state of redox proteins
- Photoactive groups
- Intermediate states
- Active site changes
- Ligands (Raman)
- Monitoring of radiation damage





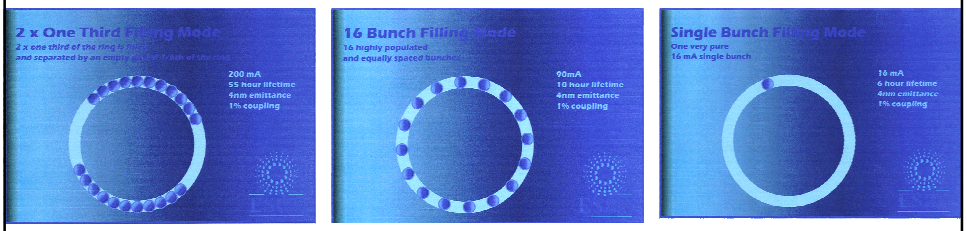
Off-line characterisation of crystals using spectroscopy - Improved interpretation of X-ray data



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.

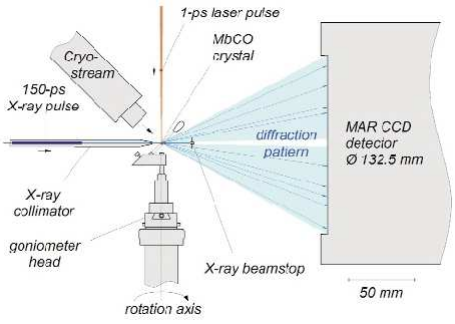
Katona *et al.,* (2007), *Science* 316, 449-53

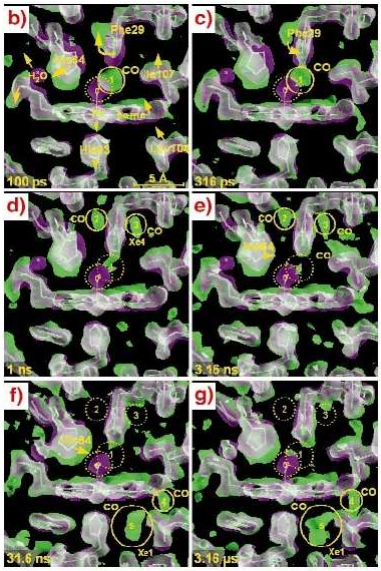
Storage Ring Filling Modes at ESRF



Synchrotron Radiation is 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





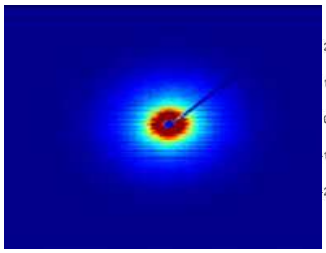
Structure of MbCO at different time delays after photolysis. The bound CO dissociates, eventually becoming trapped in sites 4 and 5, where it remains out to the microsecond time scale.

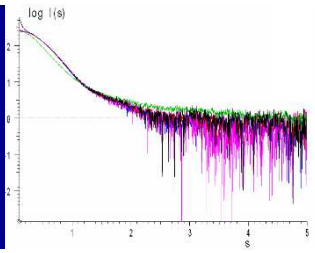
F. Schotte *et al.,* (2003), *Science*, 300, 1944-1947.

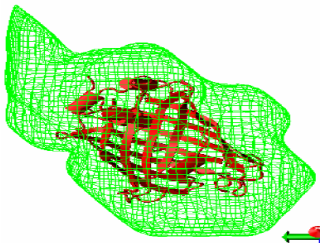
Structural Biology at synchrotrons without crystals - SAXS

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





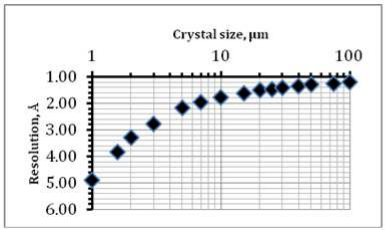


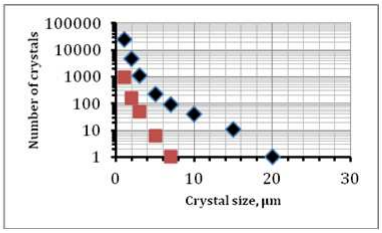
*molecular shape, molecular interactions, kinetics, etc…

Smaller crystals & larger unit cells

Crystals of macromolecules have large unit cells. Biological macromolecules are also conformationally variable. Growing large crystals is not easy. 'Microcrystals' - crystals comprising inly a few unit cells - to become the norm (?)

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

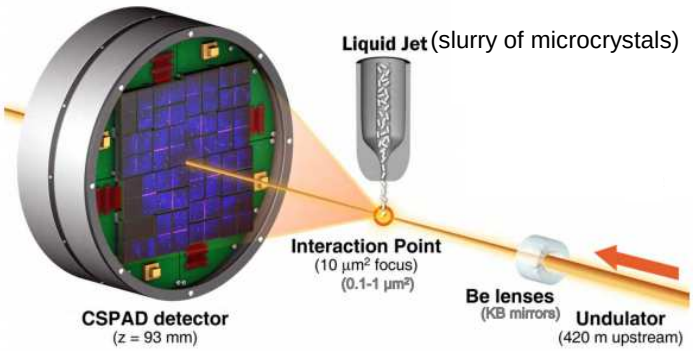
→ New Paradigm for macromolecular crystallography: Multi-crystal data collection

Or

→ Avoid radiation damage

Macromolecular crystallography at X-FELs

High resolution serial femtosecond crystallography

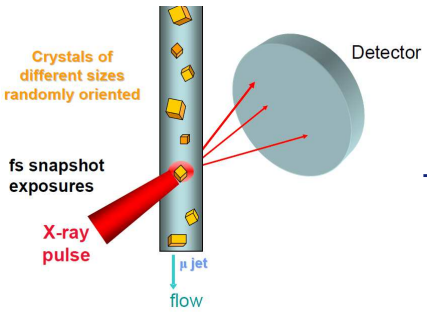


Cornell-SLAC Pixel Array Detector

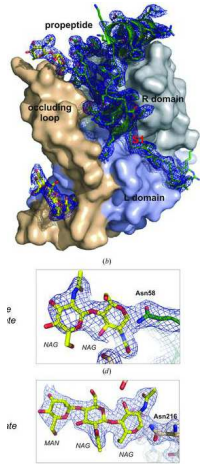
Boutet et al Science **337**:362 (2012)

Paradigm shift in the way that diffraction data are collected

Macromolecular crystallography at X-FELs

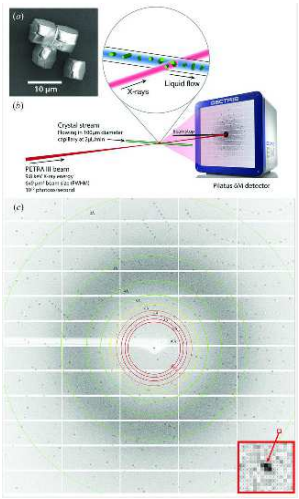


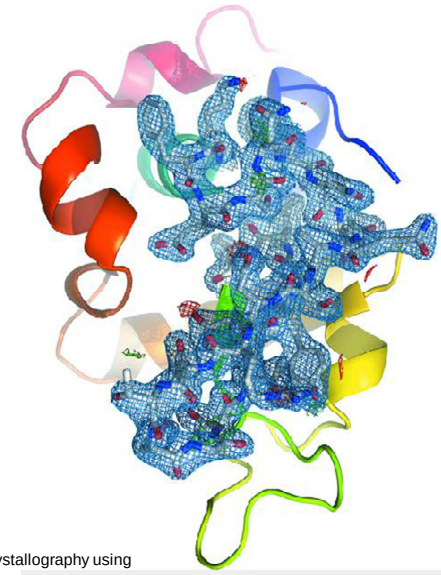
Chapman, *et al.* (2011). Femtosecond X-ray protein nanocrystallography *Nature* **470**, 73-77.



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

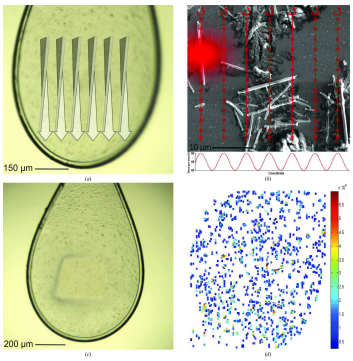
Serial Crystallography at synchrotron sources

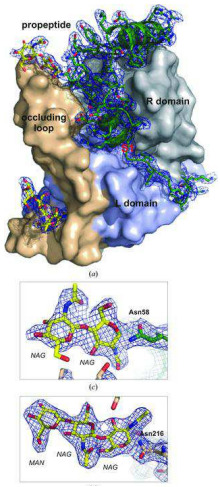




Stellato, F., et al. (2014). Room-temperature macromolecular serial crystallography using synchrotron radiation. *IUCrJ*, **1**, 204-212

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

Serial crystallography: solid supports or jets?

Jets

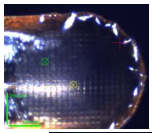
- Fast
- Room temperature data collection
- Better set-up for pump probe?
- Sample intensive/wasteful
- (poor) data quality for SAD/MAD?

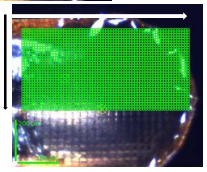
Solid supports (i.e. grids)

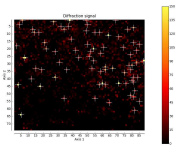
- Automatable
- Can pre-scan grid, collect data from all crystals
- Properly defined rotation range
  - much more data /crystal
- Measure some fully recorded reflections
  - Easier to process/merge partial data sets
  - Better data quality
  - Easier structure solution (MAD/SAD)
- Slower than with jet
- Room temperature data collection more difficult?
- Not 'proper' serial crystallography

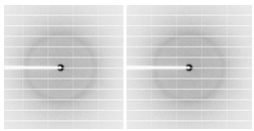
Serial crystallography using solid supports has been automated at ESRF

sample holder alignment
(using κ- and Ω angles)
↓
overlaying mesh
(several 1000 data points)
↓
fast helical oscillations
(> 1 sec per line)
↓
Fast identification of Diffraction spots (= crystal)
↓
ranking of hits
&
data collection, merging







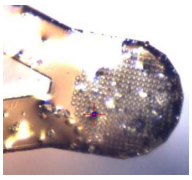


Prototype protocols implemented on ESRF ID29
C. Mueller-Dieckmann
Sasha Popov
Ulrich Zander
Olof Svensson
G. Leonard
D. deSanctis

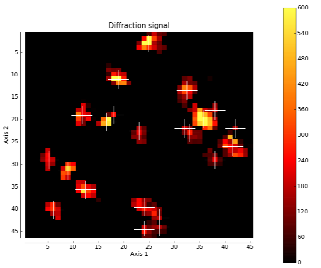
collaboration with
Gleb Bourenkov (EMBL Hamburg)
Gordely Group (IBS Grenoble)

Automatic solid-support SSX: proof of principle

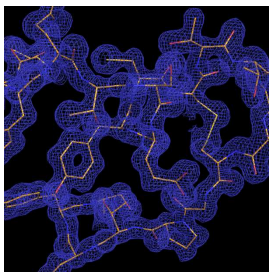
Thaumatin



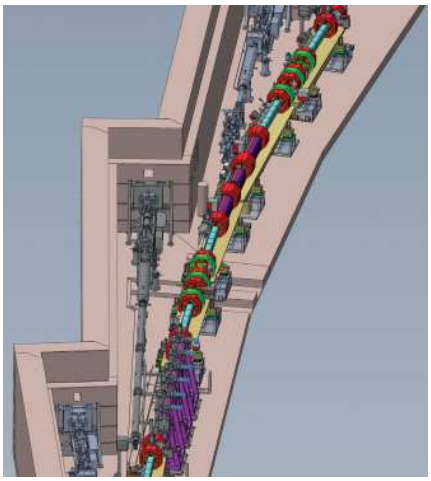
crystal size: ~ 20 µm in each dimension
22 hits
data collection: ±5º rotation on each hit



all 22 sub-data sets processable
all sub-data sets were merged
- resolution: 1.3 Å
- completeness: 99.8%
- <I/σI>: 13.13
- R_{meas}: 14.8%



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**

- Much smaller crystals
- Serial crystallography

Summary:

- Synchrotron radiation and Storage Rings
  - Dominated the era of macromolecular crystallography (~ 90,000 entries in PDB)
  - Radiation damage can limit information obtained
- Serial Crysallography
  - X-FELs & synchrotrons
    - 4th Generation Synchrotron sources
      - Will dominate the future of Macromolecular Crystallography

Thanks for your attention!