

#### The BCA Lonsdale Lecture and Teaching Plenary 2011

### The evolution of synchrotron radiation and the growth of its importance in crystallography

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#### Kathleen Lonsdale 28 January 1903 -- 1 April 1971



Kathleen Lonsdale

President of the IUCr 1966; A key instigator of International Tables; A leading pacifist of her time.





1931 An X-ray analysis of the structure of hexachlorobenzene by the Fourier method

## Synchrotron radiation

- Small focal spots, AND
- Collimated X-ray beams onto the crystal
- Tunable
- And for special applications:-
- White beam for time-resolved fast data
- Defined time structure eg picosecond bunches
- Also plane polarised

## A vision for adoption of synchrotron radiation in crystallography (Daresbury Study Weekend 1979)

- In my talk 'Optimisation of anomalous scattering and structural studies of proteins using synchrotron radiation', Proc. of Daresbury Study Weekend, 26-28 January 1979 DL/SCI/R13. (1979) pages 1–6.
- I conveyed a vision for dealing with:-
- Slow data collection, large unit cells, weak scattering including small crystals, dynamic studies and two-wavelength phase determination (as per Okaya and Pepinsky 1956; '2 lambdas three measurements')
- Parts of this built on the seminal work at Stanford by Keith Hodgson et al (more of whom later in the talk) but my plan was critically supported by Margaret Adams, Guy Dodson, Dorothy Hodgkin and David Phillips:-







Dedicated SR sources and New Rings; key steps for UK

- 1981 Daresbury SRS led by Ian Munro; the first dedicated ('2<sup>nd</sup> generation' SR source)
- 1987 ESRF Foundation Phase Report 'Red Book'; this showed colossal increases in X-ray intensities from a new source, the X-ray undulator. Would the samples stand it?

#### The growth of SR (we could even launch our own Journal)



Figure courtesy of Prof M Hart

# Going relativistic produces the fine collimation emission of SR

 $v \ll C$ γ≈c ٥Ô <u>mc²</u> An SRS bending magnet Wavelength shifter emission (top); Multipole wiggler (middle); Undulator (bottom)





SRS SCW; Shift λ





SRS MPW; Higher intensity

ESRF Undulator; Higher brightness

ESRF greatly improved emittance over SRS



# Undulator emission as a function of magnet gap



Nb much narrower gaps are viable today than the early examples shown here.

# SRS Undulator beamline 5 operated in the visible region (1985/6)





I first encountered undulators when a Soviet Delegation visited Daresbury in 1983.

Shortly after that the early specs for the ESRF emissions from X-ray undulators (then for a 5GeV Machine) became known around that time too.

# So we got our first SRS PX users and results>>>

# Virus structures; a key step involved the methods...



#### J. Appl. Cryst. (1983). 16, 629-636

Oscillation Photography of Radiation-Sensitive Crystals using a Synchrotron Source

BY MICHAEL G. ROSSMANN AND JOHN W. ERICKSON Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907, USA

(Received 18 March 1983; accepted 17 August 1983)

#### SRS Station 7.2 and Hamburg 'DESY':-



We wish to thank Drs Hans Bartunik and Klaus Bartels at DESY in Hamburg, Germany and Dr John Helliwell at the SRS in Daresbury, England for much help and encouragement in the difficult task of X-ray diffraction data collection of the R14 rhinovirus crystals. The difficulties eventually led to the procedure described in this paper. We also wish to

#### Prof M G Rossmann, USA;

In 1985, he became the first scientist to build a model of human rhinovirus-14, HRV-14, one of about 100 known cold virus strains. This was amongst the first group of virus crystal structures solved, and opened up studies of the most technically challenging projects.

Oscillation camera data processing was important especially with virus data; we reexamined the reflection rocking widths and partiality estimates with an SR source



Phil Moore



Helliwell et al J Phys E 1982 SRS 7.2 Triangular monochromator based on the LURE and DESY design: Lemonnier et al and Hendrix, Koch and Bordas



Fig. 15. Reflecting ranges  $\varphi_R$  for zero-level reflections and a vertical rotation axis.



T.J. Greenhough and J.R. Helliwell 'Oscillation camera data processing: reflecting range and prediction of partiality. II Synchrotron sources' (1982) J. Appl. Cryst. <u>15</u>, 493-508.

#### The 'single bounce' monochromator can be curved to minimise the reflected bandpass ie case (b)



J R Helliwell 1984 'Synchrotron X-radiation protein crystallography: instrumentation, methods and applications' Reports on Progress in Physics 47, 1403-1497

An early expt: In pea lectin Howard Einspahr wanted to know 'which was Mn and which was Ca?'; the Mn K edge was accessible on SRS 7.2 at 1.896Å; lets go for it!



 H. Einspahr, K. Suguna, F.L. Suddath, G.Ellis, J.R. Helliwell and M.Z. Papiz 'The location of the Mn:Ca ion cofactors in pea lectin crystals by use of anomalous dispersion and tunable synchrotron X-radiation'. Acta. Cryst. (1985) <u>B41</u>, pp. 336-341. Data collection at softer X-rays is viable; test experiments (Helliwell 1983 unpublished) on lysozyme with the crystal mounted in a mylar 'roll-your-own-capillary', used station 7.2 of the SRS and a wavelength of 2.6Å.





I had explored gradually longer wavelengths:- 1.488Å, 1.739Å, 1.86Å, 2.0Å ... [Helliwell (1984) Reports on Progress in Physics 47, 1403-1497.] Crystallographic approach to solve apocrustacyanin A1:- SIROAS (Xe) and softer X-rays (2 Å), and enhanced sulphur anomalous dispersion.



M. Cianci, P.J. Rizkallah, A. Olczak, J. Raftery, N.E. Chayen, P.F. Zagalsky and J.R. Helliwell "Structure of apocrustacyanin A1 using softer X-rays" (2001) Acta Cryst <u>D57, 1219-1229.</u>

### Beamline instrumentation at SRS 9.6



The SRS Super-conducting wiggler delivered 0.3kWatts into SRS 9.6 sufficient to melt lead!









Miroslav Papiz and the Enraf Nonius FAST TV diffractometer of Uli Arndt

### David Stuart, virus crystallography





BTV was solved by David and his team using ESRF data and FMDV using SRS 9.6 data. Hb of Max Perutz shown for a protein to virus size comparison.

David pictured outside the Diamond Light Source Building

## **Time-resolved**

 Louise Johnson and Janos Hajdu's beautiful time-resolved work on glycogen phosphorylase ('Catalysis in the crystal') was done at SRS 9.6 (Published in EMBO J) and used the flow cell, a quite ingenious device



The flow cell invented by Hal Wycoff, Yale University and championed by David Phillips (pictured)

#### Spin off into chemical crystallography using SRS 9.6



**Marjorie Harding** 

 Table 3. Comparison of crystal scattering powers
 (ignoring the effect of mosaic spread referred to in text)

Sample	Unit-cell scattering efficiency <sup>a</sup> (e <sup>2</sup> Å <sup>-6</sup> )	Sample volume V <sub>x</sub> (µm <sup>3</sup> )	Sample scattering power <sup>b</sup> (e <sup>2</sup> Å <sup>-3</sup> )	Approximate ratio of scattering powers
Piperazine silicate 'normal' size <sup>c</sup>	18 × 10 <sup>-3</sup>	8 × 10 <sup>6</sup>	64000 × 1012	1000
Piperazine silicate microcrystal	18 × 10 <sup>3</sup>	25 × 10 <sup>3</sup>	200 × 1012	3 <
CaF, microcrystal <sup>d</sup>	0-34	200	68 × 1012	1
Protein-lysozyme <sup>e</sup> 'normal' size	7.5 × 10⁻6	8 × 10 <sup>6</sup>	60 × 10 <sup>12</sup>	1 1
Protein-lysozyme microcrystal	7.5 × 10-6	$27 \times 10^{3}$	$0.20 \times 10^{12}$	0.003

Notes: (a)  $\sum f^2/V_{cell}^2$ , where the summation is over the atoms in the *primitive* unit cell with scattering factors f. (b)  $V_x(\sum f^2/V_{cell}^2)$ . (c) Normal size is ca  $0.2 \times 0.2 \times 0.2 \text{ mm.}$  (d) As used by Bachmann, Kohler, Schulz & Weber (1985). (e) Tetragonal lysozyme (Blake, Koening, Mair, North, Phillips & Sarma, 1965). Also allowed for is 33.5wt% of solvent in the crystal.

This effort led by Marjorie on SRS 9.6 FAST was a forerunner of SRS 9.8, 16.2 and now DLS I19.

S.J. Andrews, M.Z. Papiz, R. McMeeking, A.J. Blake, B.M. Lowe, K.R. Franklin, J.R. Helliwell and *M.M. Harding* 'Piperazine Silicate (EU-19) - The structure of a very small crystal determined with synchrotron radiation'. Acta. Cryst. <u>B44</u>, 73-77 (1988).

#### Daresbury Analytical and Research Technical Services (DARTS); an important aspect of the Economic Impact of the SRS



Pierre Rizkallah; Protein Crystallography Supply Manger for DARTS

E.J. Maclean, P.J. Rizkallah and J.R. Helliwell (2006). "Protein Crystallography and Synchrotron radiation; current status and future landscape" European Pharmaceutical Review Issue 2, p71-76

# New Light on Science The Social & Economic Impact of the Daresbury Synchrotron Radiation Source, (1981 - 2008) ence & Technolog

Rapidly tunable optics are also important; the SRS high brightness lattice enhancement of 1985 allowed this optics scheme to be introduced at SRS for MAD work, as well as 'pink' white beam Laue work (when the mono is removed).



Station 9.5 SRS: optical scheme.





This was a common monochromator type for EXAFS: the parallel double crystal monochromator



Figure 23. The contribution of source divergence  $\alpha_v$  and size  $\Delta \theta_{\text{source}}$  to the range of energies reflected by a (+, -) double-crystal monochromator (see figure 22)  $(\theta_1 \neq \theta_2)$ .

The closing of the slit jaws upstream of the monochromator is a simple means of reducing the divergence angle onto the first crystal of the monochromator; this is important when reducing  $\delta\lambda/\lambda$  onto the sample.

## How to solve the phase problem?

We measure a diffraction pattern, take the square roots of the intensities, but then we're stuck:

if we knew the phases we could simply compute a picture of the molecule, but we've lost the information in the experiment!

This is the phase problem, and a large part of crystallography is devoted to solving it.

## **Phasing Techniques**

• Using isomorphous or anomalous differences



http://www-structmed.cimr.cam.ac.uk/course.html

- Or from a substructure
- Or molecular replacement
- Or direct methods

Another way to get three measurements: two wavelengths and an anomalous difference



This is equivalent to two isomorphous heavy atom derivatives. [Helliwell 1979 based on Okaya and Pepinsky 1956] Now introduce selenomethionine in place of methionine

residues in a protein! Se K edge is at 0.98A wavelength



Ce <sup>58</sup>	Pr	Nd	Pm	82 Sm	Eu	Gd <sup>64</sup>	Tb <sup>65</sup>	66 Dy	67 Ho	Er	Tm	Yb	71 Lu
90 Th	91 Pa	92 U	93 Np	94 Pu	Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	103 Lr

This use of multiple wavelengths (at least 2 or more) is called M.A.D.

- Selenomethionine protein production is now a reasonably straightforward technique of protein production.
- One in 57 amino acids, on average, is methionine.
- Intensity changes with wavelength are small but, being all on one crystal, viable to measure.

## Seleno-methionine hydroxymethylbilane synthase fluorescence scan measured at SRS 9.5



#### Five seleniums in 34 kDa



#### Figure 4

Anomalous difference Patterson map (Harker sections  $u = \frac{1}{2}$ , bottom left;  $v = \frac{1}{2}$ , top left; w = 0, top right) for the SRS data (with the anomalous differences averaged across the three wavelength data sets measured). The anomalous difference Patterson map ( $u = \frac{1}{2}$  Harker section) for the ESRF data (again averaged across three wavelengths) is also shown (at bottom right). The labels are SeMet residue numbers. Some peaks which appear close to the Harker sections are cross-vectors between two Se atoms; these are labelled with the numbers of the residues involved.



In Haedener et al 1999 Acta Cryst D we give the SRS 9.5 and ESRF BM14 comparison studies showing viability of the new stations and the ESRF electronic detector



A. Hadener,

Univ. Basle.



MAD electron density map (SRS 9.5 RT data)

#### MAD + DM electron density map

Model fit

Figure 5 Representative MAD electron-density map slab for (a) three wavelengths, (b) three wavelengths plus DM map/phase-improvement. Both maps were calculated from the SRS data measured at ambient temperature. (c) The MAD model for this slab.

Lets now move in more detail towards the 'New rings' and especially ESRF planning and implementation>>> Multi-wavelength anomalous dispersion beam-line at ESRF (BL19 later called BM14) was the first PX station at ESRF and with beamline optics based on SRS 9.5. Andy Thompson left Daresbury to take charge of this development at ESRF. It became the most productive MAD beamline for a long period.





The popularity of MAD methods rested on the relative ease of preparing seleno met proteins or use of metallated versions of a protein as well as sufficient beam intensity to work with small crystals to quickly measure multiple data sets. Cryo cooling was a breakthrough.



Keith Hodgson





Britt Hedman

Stanford's New Rings Workshops in the early to mid 1980s re use of PEP highlighted the need for test assessments of such beams; a NATO Collaborative Research Award with SSRL Stanford led to some pretty seminal work





Focussing of the full SRS wiggler white X-ray beam onto a 20 microns gramicidin test crystal sample to simulate the proposed monochromatic undulator ESRF X-ray intensities to test if microcrystals of biological macromolecules would withstand the X-ray thermal and radiation blast.

## Alignment of the beam onto the crystal is critical; we are taught this from an early age



As mentioned for PEP the ESRF planning required simulation of the effect on protein crystals; beam heating and radiation damage and initiate practical risk assessment experiments:-

J.R. Helliwell and R. Fourme 'The ESRF as a facility for protein crystallography: A report and design study'. ESRP Report IRI-4/83(1983), pp. 1-36.

A beam heating risk management strategy was offered:-

We now of crystal	consider the s at a fixed te	uccess of the co mperature (deter	opper fibre in keeping rmined by the He gas)	g a 10 μm • • •
~	L		grease	
Ø,	[	a < 10 9	\$ ≈ 10,µm	
the la	Copper fibre	is true, of con	crystal	

J.R. Helliwell / Protein crystal perfection and nature of radiation damage



P=positive N=negative electron density

10MRad = 0.1MGy

(Fdamaged – F undamaged) expiα<sub>calc</sub> ie a 20 + 2 Mrad data set at Stanford – a 0+ 7.5Mrad of X-ray dose data set at Daresbury

Splitting of disulphide bridges in proteins due to X-rays; based on a suggestion of Greg Petsko

The European Synchrotron Radiation Facility (ESRF) in Grenoble & the Institut Laue Langevin nuclear reactor is to the right



Sample 40.00m

Pre vertical focusing mirror slits 32.014m

focusing mirror 33,115m

ESRF developed 'Phase I beamline's that were generic ie X-ray undulators for high brilliance applications such as virus crystallography, a microfocus beamline for micro-crystallography or tiny fibres for fibre diffraction or microbeam scanning and a white beam beamline for time-resolved Laue.

EMBL developed with ESRF the microcrystallography diffractometer. Sub-nanosecond time-resolved Laue protein crystallography (eg see Srajer et al *Science1996: Vol. 274 no. 5293 pp. 1726-1729*)

![](_page_40_Picture_1.jpeg)

![](_page_40_Picture_2.jpeg)

Movie kindly provided by Michael Wulff, ESRF; research pioneered with Keith Moffat and later Phil Anfinrud. Interpretation of Laue diffraction patterns involves more steps compared with monochromatic diffraction

- Wavelength normalisation;
- The presence of multiple reflections spots means that their component intensities have to be extracted out;

## Wavelength normalisation curve

![](_page_42_Figure_1.jpeg)

![](_page_42_Figure_2.jpeg)

Prediction of spots colour coded by wavelength:-

Blue = short Xray wavelengths through green, yellow, orange to red (outer edge).

### The Ewald Sphere Construction; reminder

![](_page_43_Figure_1.jpeg)

Shaded = the stimulated region of reciprocal space by the incident wavelength bandpass,  $\lambda_{min}$  to  $\lambda_{max}$ 

D.W.J. Cruickshank, J.R. Helliwell and K. Moffat 'Multiplicity Distribution of Reflections in Laue Diffraction' Acta. Cryst. A43, 656-674 (1987)

![](_page_43_Picture_4.jpeg)

A key result was the predominance (>72.8%) of Bragg reflections in singlet Laue spots and which contradicted the early pioneers' writings such as W L Bragg

> PHYSICS world Synchrotron X-ray diffraction

Colour coding:-Green are singlet reflections Laue spots; Other colours are for doublets, triplets etc We conducted many studies to validate the method and the Daresbury software eg:-

![](_page_45_Figure_1.jpeg)

![](_page_45_Picture_2.jpeg)

Zygmunt Derewenda

FIG. 3. Difference Fourier map calculated with coefficients  $(F_{\text{Later Hg}} - F_{\text{Nymon0}}) \exp \alpha (\text{Pb} + \text{Pt}) \text{mono}$  (a) singles (b) the low-resolution component of energy deconvoluted doubles and in (c) with  $F_{\text{Laue}}$  Hg replaced by monochromatic Hg data. The Laue maps (a) and (b) compare very favorably with (c), the monochromatic map.

Time-resolved structures of hydroxymethylbilane synthase (Lys59Gln mutant) as it is loaded with substrate in the crystal determined by Laue diffraction at ESRF and an electronic area detector (EAD) Helliwell et al J Chem Soc Faraday Trans 1998.

![](_page_46_Figure_1.jpeg)

## The Laue method was enthusiastically taken up at the Grenoble neutron source>>>

### X-rays and neutrons

X-rays; Scattered from electrons proportional to Z (red). Neutrons; scattered from nuclei & evenly across all elements (green)

![](_page_48_Picture_2.jpeg)

Large difference in the neutron cross-section among isotopes
 Neutron diffraction can be used to directly determine the positions of H-isotopes at medium resolutions (~2.5 Å)

#### The EMBL Laue Diffractometer at ILL; LADI I left LADI III right

![](_page_49_Picture_1.jpeg)

![](_page_49_Picture_2.jpeg)

Peter Timmins

- > Gd<sub>2</sub>O<sub>3</sub>-doped neutron sensitive image plate.
- > Typical wavelength range: ~ 2.7 3.6 Å.
- > Narrow (25%0 bandpass 99% spots single.
- > Scanned phonographically.

![](_page_49_Picture_8.jpeg)

Neutron Laue data are processed with the 'Daresbury Laue software'.

## Bound water oxygen and deuterium atoms resolved with neutrons including at liquid helium temp!

![](_page_50_Figure_1.jpeg)

M.P. Blakeley, A.J. Kalb (Gilboa), J.R. Helliwell & D.A.A. Myles (2004) "The 15-K neutron structure of saccharide free concanavalin A" Proceedings of the National Academy of Sciences USA 101, 16405-16410. A few bound waters can be fully resolved with X-rays (as well as bond length analysis) in favorable X-ray cases

![](_page_51_Figure_1.jpeg)

A. Deacon, T. Gleichmann, A.J. Kalb (Gilboa), H. Price, J. Raftery, G. Bradbrook, J. Yariv & J.R. Helliwell

"The structure of concanavalin A and its bound solvent determined with small–molecule accuracy at 0.94Å resolution" (1997) Faraday Transactions, <u>93</u> (24), 4305–4312.

## Research directions for today?

- New SR sources: PETRA III is to be the most brilliant ie the 'ultimate storage ring'
- Our own Diamond Light Source is a 'cutting edge' source with a vibrant research and development programme
- ESRF has a new upgrade programme for nanofocus beams; UK has a 10% share
- Structural biology: continued expansion into large complexes combined with SAXS and EM
- Structural chemistry: continued expansion into eg timeresolved crystallography
- The X-ray laser: towards single molecule diffraction
- New and upgraded neutron sources....

## Acknowledgements especially to:-

- To all my coauthors and collaborators and PhD students for all our joint work together during the decades;
- To the University of Manchester (since 1989) and Daresbury Laboratory (since 1976), but also the ESRF and the Institut Laue Langevin for stimulating environments, as well as my stays at the Universities of York, Oxford and Keele Universities;
- To the various funding agencies a heartfelt thank you!

![](_page_54_Picture_0.jpeg)

## Thankyou

![](_page_54_Picture_2.jpeg)

![](_page_54_Picture_3.jpeg)

![](_page_54_Picture_4.jpeg)

![](_page_54_Figure_5.jpeg)