The Care and Nurture of Undulator Datasets.

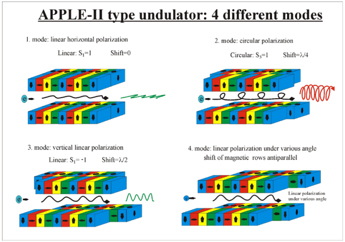
Seán McSweeney

European Synchrotron Radiation Facility
Grenoble, France

02/02/2009

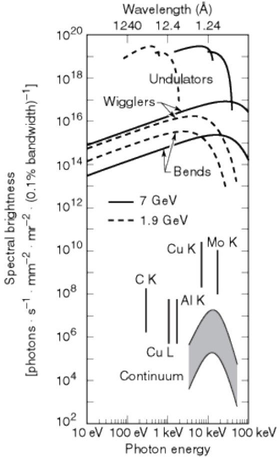
Outline

The production of Undulator Radiation.



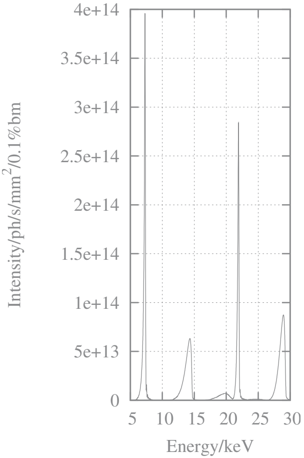
- The magnetic-field induces small changes in electron trajectory.
- At each peak light is emitted.
- With appropriate construction **Constructive interference** occurs.
- The properties of the light emitted is determined by the magnetic field, magnetic period and the electron beam energy.

X–ray Radiation Sources.



- Undulator sources give many orders of magnitude more flux than any other X-ray source.
- achieving the potential flux requires **extremely** homogenious magnetic fields and gaps *and good beamlines.*

Undulator Spectrum



- spectrum is high peaks (and low troughs).
- the peaks vary according to the magnetic field - gap.
- in some cases undulator will produce one peak
  - ESRF - ID14: U23.5, U24.
- Very high intensity.
- Very low divergence.
- Small focal spot sizes.

Consequences

Consequences

- Even cryo-cooled crystals have a finite lifetime.
- Data collection rates can be very fast.
- Every flaw in your crystal will be revealed.
- multiple xtals may be necessary for a complete data-set.

but. . .

- data collection will be possible from all sorts of samples.
- structure determination from very large cells, weak diffraction . . .
- “impossible” samples will be tractable.
- much more rapid screening and characterisation . . .

Not so simple

Optimal Usage can be Learned.

- The power of these devices can make this sort of beamline difficult to use.
- How to use these beamlines?
- Follow The Rules !
- There is an Economic and Scientific advantage to knowing how to uses these sources.

The "Rules"

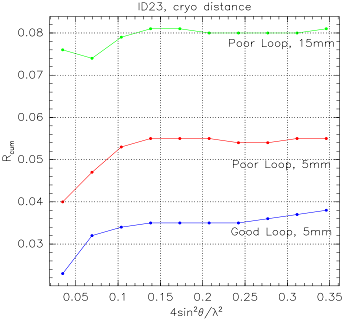
1. Do the Simple Things Properly.
2. Speed Kills.
3. Everything Dies.
4. Have a Plan.
5. Use Good Crystals.

Simple things

are easy to get right

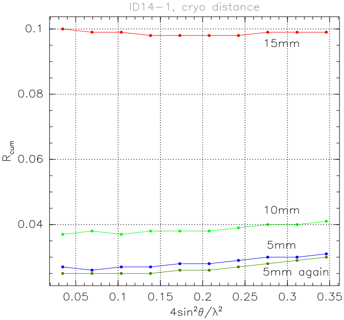
- Make (and use) good sample pins.
- Put your crystal in the beam !
- Get the cryo-stream set up properly.
- Put the backstop in place

Sample Pins



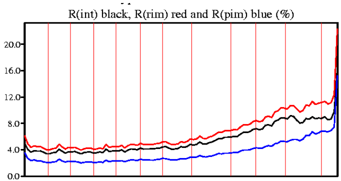
- pins should be well glued into the base
- loops should be reasonably close to the pin.
- bases should fit the goniometer head.
- SPINE standard add more.

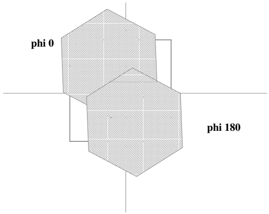
Cryostream - Xtal Effects.



- The alignment of the cryo-stream is important!
- cryo -xtal distance can effects data quality.
  - move the cryo back-in.
- This has the effect of magnifying many other problems.

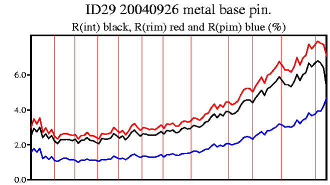
Center Xtals Properly !

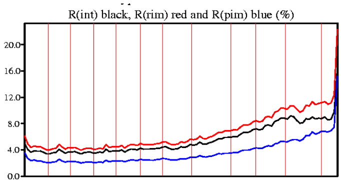




- The data quality is on the edge of being OK.
- The problem is with the beamline!
- However close inspection of the crystal, revealed that whilst the crystal stayed in the beam, it was not stationary.

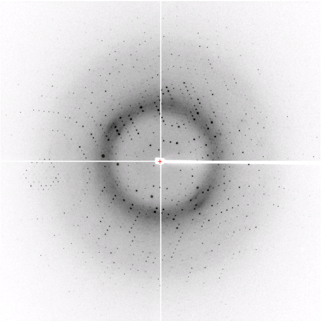
After re-centering.

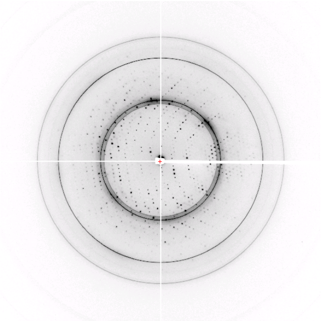




- carefully re-centering the xtal, produced a small but significant improvement in data quality.
- Note also that the low resolution data are much improved.

Beamstops are Easy !





Speed Kills

Speed will damage your data in a number of ways.

- At high velocity spindles may not move smoothly.
- Employing an inappropriate strategy may result in incomplete data.
- poorly made pins will wave the crystal about in the X–ray beam.

Velocity

Mechanical spindles have problems/

- Angular velocity oscillates, and is not stable during the exposure.
- Spindle may be accelerating during exposure.

To combat these issues most modern rotation axis use "air-bearing" spindles. These provide a high speed, rigid rotation axis suitable for MX.

Data Collection Strategy

Data collection rates of **more than 40** images/min are possible. The temptation to “just collect” is severe, this temptation should NOT be given into.

There is no shame in leaning how to ***think****.*

Data Collection Strategy 2

RABID data collection

- "Prog. X will sort out the overlaps" - is slower and less accurate than getting the job done properly.
- "Oops I have no Anomalous completeness" - doesn't help in phasing.
- "Damn, your only xtal has died " - doesn't make you friends back in your lab.

Take 5 mins to develop a plan to collect the data. You will save that time later.

programs exist to do (much) of this job for you - `BEST` **really is**

All Crystals Die

Some questions before starting

- Do you want phases or high resolution ? - you are unlikely to get both from one xtal.
- Do you have enough signal for SAD rather than MAD - is the solvent content on your side ? or NCS...
- Do you have more than one crystal ? - will you use then all ?

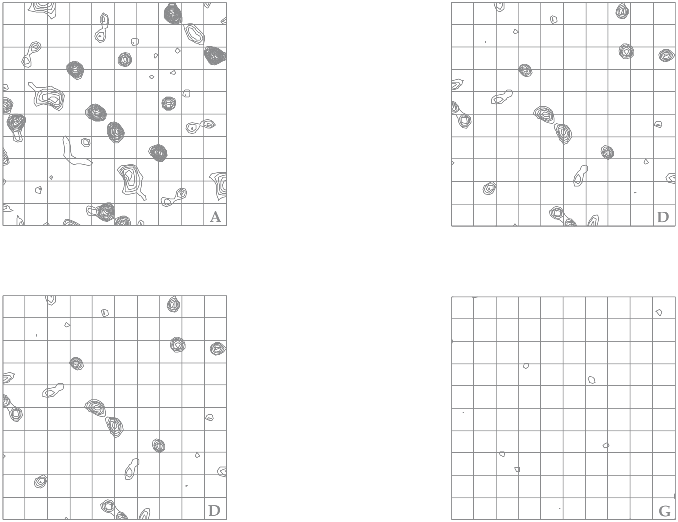
The good thing (from my point of view) is that you will make an experiment and not just collect data.

Radiation Damage

Rules of thumb

- The Henderson Limit $2x10^{7}Gy$, by which time half the diffraction power is lost. Now refined as the Gaman limit
  - Stay well away!
- At ESRF we estimate you have between 100 and 500 seconds of exposure time available but is beamline dependent.
- A program exists to estimate absorbed dose - `raddose`.

An example



RD as a phasing source.

A number of investigators (initially Ravelli *et-al*) demonstrated that the changes induced by irradiation held useful phasing information.

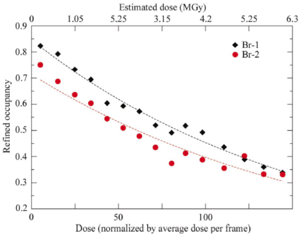


Figure 3
Refined values of the occupancies for each of the two Br atoms in scenario III. The dashed lines represent exponential fits with fitted parameters $0.846\exp(-0.00625d)$ for Br-1 and $0.714\exp(-0.00588d)$ for Br-2, in good agreement with the q_0 and β_d values refined in scenario IV (see Table 3). The absolute values for dose (upper horizontal axis) were obtained from data reported by Ennifar *et al.* (2002) and should only be considered as rough estimates. With these estimates, the exponential decay parameters for Br-1 and Br-2 are β = (6.7 MGy)$^{-1}$ and β = (7.1 MGy)$^{-1}$, respectively, in good agreement with the D_1 value of 7.40 (0.8) MGy reported by Ennifar *et al.* (2002).

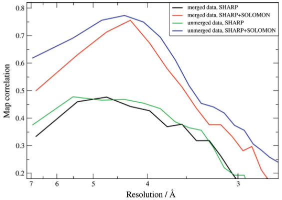


Figure 3
Ypa2: quality of the phases. The plots represent the correlation coefficients (as a function of resolution) of maps computed from experimental phases with respect to a map computed from the final refined structure. Black: SAD phases computed from merged data (images 1–180). Red: SAD phases computed from merged data (images 1–180), after density modification. Green: RIP-aided SAD phases computed from unmerged data (images 1–180). Blue: RIP-aided SAD phases computed from unmerged data (images 1–180), after density modification.

Rule 3 summary

Use the minimum flux consistent with your aims.

- For phasing be conservative
- For high resolution data collection
  - Collect a low resolution pass first.
  - Be prepared to use several crystals.
- Process the data as you go and check data quality often.

Have a Plan.

As well as following the previous rules it is important **to have a plan** for how to use the beamtime, and how to address the demands of each data collection.
Remember it is possible to collect a lot of data if you are prepared. 40 data-sets per day is easily possible 100's of datasets if organised . . .

What should the Plan contain?

Plan should contain

- Priority ordering - vital thru' to "if time"
- for each sample
  - basic crystallographic information.
  - type of experiment to be performed.
  - expected and desired diffraction limits.
- contact information (incase things go wrong).

General Comments

- Fill the detector - they are expensive, why leave most of it with no diffraction on it !
- Process as you go.
- Think about the background scatter.
- Mosaic crystals often give better data. . .
- *Know what and why you are collecting these data*.

Phasing Experiments

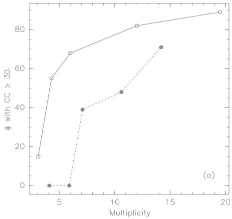
be conservative

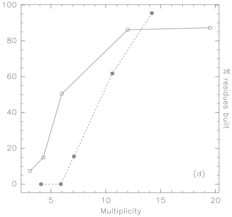
- Collect to lower resolution - I collect to the point where R_{symm} would be 10%.
- Collect a complete SAD data set before moving wavelength - remote or peak ?
- Aim for multiplicity and completeness.
- Collect a complete, weak, dataset and then add more data.
- More signal is probably better than more data.
- Start sub-structure search ASAP. Run `SHELX` for enough trials

MR and high resolution

- Collect the low resolution data **first**
- Refine cell and mosaicity well in order to get a good strategy.
- Data quality if not such an issue - not all “bad” data is useless.

Fit the experiment to your aims





- To find sites, very high multiplicity is not so important
- Accuracy of phases improve with multiplicity.

- To auto build lots of sites multiplicity can be vital!

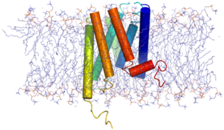
Use Good Crystals!

Don’t save good crystals.

It is amazing how often an experimenter leaves a good crystal “for best”.
Finding the crystal can be a challenge!

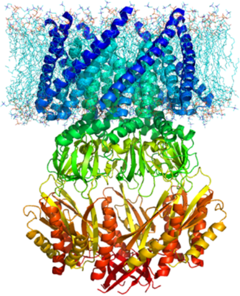
Screening

GPCR



1043 Crystals

Mechanosensitive Channel



"many 100's of crystals"

1 in 30

Data collections 2008

Tue 11 Nov, 18:18

- **id14eh1** Sample Evaluations: 13364, Data Sets: 1853
- **id14eh2** Sample Evaluations: 13294, Data Sets: 1444
- **id14eh4** Sample Evaluations: 14696, Data Sets: 2748
- **id29** Sample Evaluations: 18089, Data Sets: 3240
- **id23eh1** Sample Evaluations: 19232, Data Sets: 3782
- **id23eh2** Sample Evaluations: 17796, Data Sets: 1873
- **Total Sample Evaluations**: 96471, **Total Data Sets**: 14940

ISPyB

3% success in 2008

- 120,000 crystals tests.
- 17,000 data sets.
- 542 PDB deposits (-30% wrt 2007. . .)
- **So one in 30 datasets lead to PDB entry.**

Conclusion

- Be informed.
- Plan
- Exploit the advantage of doing the simple things well
- There is a competitive advantage in being able to use these sources efficiently.