UPGRADE PROGRAMME – PHASE I

CONCEPTUAL DESIGN REPORT

Macromolecular crystallography	
Current designated sector:	Facility goes to:
ID29	ID29

1.1 ID CARD

ID29 is a fully independent MX MAD beamline with a high level of automation and a high photon flux over an energy range from 6 keV to 20 keV. The proposed refurbishment of the beamline comprises an optimised setup for the use of lower energies (5 keV to 8 keV) as well as the option of a microfocused beam over the whole energy range with beam sizes as low as 10 x 10 mm (> 5 x 5 mm by using apertures). A new Pilatus 6M detector as part of the upgrade has a high quantum efficiency at lower energies and allows the accurate measurement of very weak X-ray diffraction thus allowing the exploitation of weak anomalous signals.

1.2 SCIENTIFIC CASE

Much of the scientific case for the upgrade/refurbishment of the ESRF MX facilities has been outlined in the CDR for UPBL10 and will not be repeated here. However, it is worth reiterating that the ever more complex systems being studied by Structural Biologists are now placing increasing demands on the facilities currently used for MX experiments at synchrotron sources. Nowhere is this more true than in the exploitation of anomalous diffraction (AD) techniques such as MAD (Multi-wavelength Anomalous Dispersion; Ogata, 1998) and SAD (Single-wavelength Anomalous Dispersion, Dauter, Dauter, & Dodson 2002; Dodson, 2003).

The ESRF has always been at the forefront in the provision of AD resources for MX and was also the first synchrotron radiation source to provide another facility crucial to the field of MX – an automated, user friendly microfocus beamline (ID23-2).

However, Structural Biology is constantly evolving. The size of the biological complexes now being studied means that anomalous signals available for structure solution exploiting AD are often smaller than those usually exploited and very small crystals (≤50 µm, smallest dimension) are now the norm rather than the exception. Additionally, there is a blossoming interest in solving the phase problem in MX by collecting diffraction data at wavelengths longer than those currently routinely accessible. There is also a need to ensure that the diffraction data required for successful phasing using AD is collected from as few crystals as possible, particularly in cases where radiation damage is a significant problem. A glaring omission in the ESRF's portfolio of MX resources is thus a facility that fully addresses this new reality. This can be rectified by the medium term development of ID29 as described in this document.

Micron beam sizes in macromolecular crystallography

The idea of using microfocus beams in MX is not new and was pioneered on ID13 of the ESRF where, in the late 1990s, high quality medium diffraction data from microcrystals (~30 x 30 x 5 μm^3) of bacteriorhodopsin were collected and the micro/mini-diffractometer now installed on ID13 and the MX Group's beamlines was first developed (Pebay-Peyroula et al, 1997; Cusack et al, 1998; Figure 1).

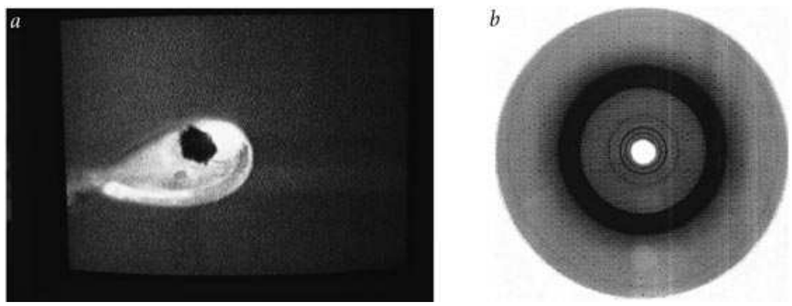

Figure 1. The diffraction pattern (right, d_{min} ~2.0 Å) obtained from a microcrystal of bacteriorhodopsin (left) on the ESRF microfocus beamline ID13. The X-ray beam was focussed to 30 μm at the sample position with a flux of ~10^{12} photons s^{-1} at λ = 0.68 Å. Figure from Cusack et al, 1998.

Bacteriorhodopsin was not the only system studied and these initial experiments also highlighted the benefits of microcrystallography when faced with large but very thin crystals. Here, microbeams can be used to successively expose new sections of a crystal in order to increase crystal lifetime in the X-ray beam or to pick out regions of a crystal displaying better diffraction properties than surrounding ones (Figure 2a). Other experiments, also suggest that using microbeams can be beneficial for data collection from larger crystals (Figure 2b). The observations above provided the impetus for the construction and commissioning – in November 2005 - at the ESRF of a fixed-wavelength (λ = 0.88 Å) microfocus beamline (ID23-2) dedicated to MX. This beamline was the first of its type and it has proved highly successful with 23 PDB depositions to date.

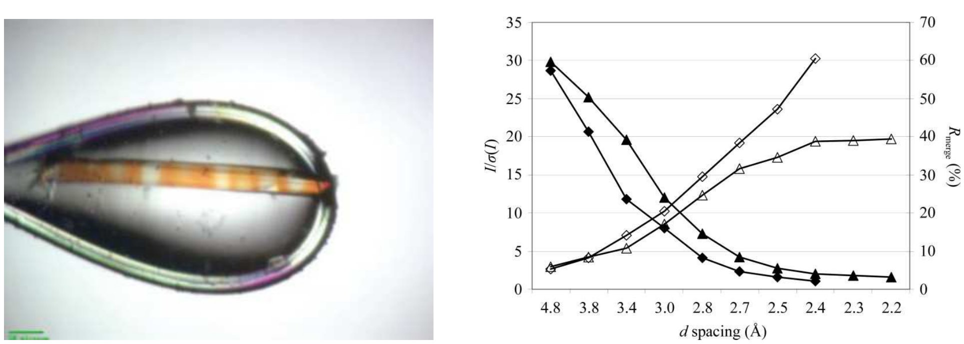

Figure 2. Left: An illustration using a micro beam to successively expose new sections of a crystal. Right: Comparison (figure from Sanishvili, R., Nagarajan, V., Yoder, D., Becker, M., Xu, S., Corcoran, S., Akey, D. L., Smith, J. L. & Fischetti, R. F. (2008). ***Acta Cryst.,*** **D64, 425-**

435) of diffraction data collected from a large crystal using either a large (♦) or a mini-beam (▲). This figure was published with permission of IUCr (http://journals.iucr.org/).

Subsequent experience has shown the limitations of a microfocus beamline not dedicated to solving the crystal structures of biological macromolecules using AD techniques. Whilst a microbeam had proved crucial for the collection of diffraction data from native microcrystals of bovine rhodopsin, all attempts to obtain phasing information using heavy atom derivatives were unsuccessful (Edwards et al, 2004). In particular, attempts at SAD phasing using diffraction data collected at energies 50–100 eV above the L_{III} absorption edges of the heavy elements introduced into crystals have failed, but may have been successful if the rapid scanning of absorption edges had been possible. The increased anomalous signal available for data collected at the peak of an absorption edge, coupled with the enhanced data collection strategies currently available using software such as BEST (Popov & Bourenkov, 2003), may have allowed structure determination to be accomplished before coordinates of the structure of bovine rhodopsin in a second crystal form became available for use as a search model in molecular replacement calculations (Li et al, 2004). Rapidly tuneable, microbeam facilities are thus essential if the structure solution of some of the most important biological structures is to become more tractable.

Long wavelength and macromolecular crystallography

The routinely accessible energy range accessible on the MX Group's tuneable beamlines is between ~20.0 keV (λ = 0.62 Å) and 6.5 keV (λ = 1.9 Å). In recent years attention has shifted towards the lower energy part of this spectrum for the following reasons:

1. There is a growing interest in exploiting the anomalous signal from sulphur and phosphorous in macromolecular structure determination (Dauter et al, 1999; Gordon et al, 2001; Micossi et al, 2002; Schuermann & Tanner, 2003; Mueller-Dieckmann et al, 2007; Ramagopal et al, 2003). While the K-absorption edges of S and P (λ= 5.01 Å and 5.78 Å respectively) are likely to remain inaccessible to macromolecular crystallographers, f" for both these elements is significant at wavelengths that are more accessible, ranging from ~0.7 $^{e-}$ at 7 keV (λ ~ 1.8 Å) to ~1.3 $^{e-}$ at 5 keV (λ ~ 2.5 Å). Carrying out experiments at the longer wavelength could, provided experimental difficulties can be overcome, make structure solution easier.

2. The derivation of protein crystals with elements such as Xe (Schiltz et al, 1994; Sauer et al, 1997) and I (Evans & Bricogne, 2002) is becoming more common. Experiments targeting the K-edges of these elements, though possible, would yield f" of ~5-6 $^{e-}$. In contrast, experiments at the LI- or LII- absorption edges (λ = ~2.3 – 2.6 Å) of these elements would yield f" ~13 $^{e-}$ with, for MAD, the possibility of inducing a maximum Δf' of ~ -14 $^{e-}$. Again, to ease structure solution, the use of longer wavelengths is essential.

It should be noted that phasing experiments using X-rays of λ ~2.5 Å are, in principle, currently possible on both ID29 and ID23-1. However, the limited flux available at the sample position makes such experiments impractical. It should also be noted that methods of addressing the problems associated with experiments at long wavelengths – principally due to absorption - are currently being investigated (Leal et al, 2008; Brockhauser et al, 2008).

ID29 in the context of UPBL10 and ID23

The plans proposed below for the medium term upgrade of ID29 are complementary to those proposed in the CDRs for UPBL10 and ID23. In particular, the beam sizes available on the MX Group's tuneable facilities will cover the entire range from 200 – 1 µm. 'Outsourcing' sample evaluation to the MASSIF section of UPBL10 will mean the availability of more beam time (i.e. longer-term access) on the new 'data collection stations' and allow researchers to obtain the best data possible from the best samples identified by MASSIF. Such a paradigm for the analysis of single crystals of biological macromolecules is planned at no other synchrotron source. The proposed upgrade/refurbishment of the MX Group's resources will thus provide an unparalleled resource for Structural Biologists (both in academia and industry) in Europe and elsewhere.

Although data collection from crystals of macromolecules using a 1 µm beam has been shown to be feasible (Moukhametzianov et al, 2008), experiments on non-model systems are likely to be highly complicated. Advanced 'helical' data collection[1] strategies based on feedback from on-going collections will need to be developed, as will real time data analysis and comparison of data collected from different segments of the same crystal. As is currently the case, the ESRF will be in the vanguard when developing these methods which will benefit, and be carried out in collaboration with, the broader European MX community both at synchrotron facilities and elsewhere.

1.3 PROJECT HISTORY

The last review of the ESRF's MX beamlines (ID14 A and B, ID23-1 and 2; ID29) carried out in November 2006 recommended that the MX Group initiate an appraisal of the technical capabilities of all the Group's beamlines. The ideas contained in the proposals for UPBL10 were based on this review and followed considerable consultation with the ESRF's MX User Community (see Annex, UPBL10 CDR).

Other common themes that emerged during the consultation process were the need for more (automated) microfocus resources for MX and the desire that the ESRF play a leading role in developing new protocols and providing new facilities for the exploitation of weak anomalous signals in MX. The medium term development proposed here for ID29 thus anticipates a resource combining, under normal working conditions, a microfocus beam (~10 x 10 m2, collimatable to give 5 x 5 m2 with an extended wavelength range at both ends of the spectrum currently available. For larger crystals, bigger beam sizes will become available by removing the microfocusing element from the path of the X-ray beam. The resulting facility will be state-of-the art.

[1] See (http://www.esrf.fr/UsersAndScience/Experiments/MX/About_our_beamlines/ID29/helical-data-collection)

1.4 BASIC TECHNICAL CONSIDERATIONS

The scientific case defines ID29 as a microbeam MAD facility with an extended energy range (5–20 keV):

X-ray source and undulators

Existing source
The photon flux currently available at the position of the primary slits (27 metres) with an aperture of 3 x 1 mm^2 (H x V) and a ring current of 200 mA are $5.7x10^{14}$ ph/s/0.1%bw at 14 keV and $3.5x10^{14}$ ph/s/0.1%bw at 5 keV.

Future source modifications
The foreseen 6 m straight sections allow the optimisation of the undulator configuration to provide additional flux over the whole of the wavelength range desired. Two possible configurations were considered: a) 1 in-vacuum u21 plus 2 x in-air u35; b) 1 in-vacuum u21 plus 2 x in-air revolver u26/u32 combination. Configuration a) gives $7.6x10^{14}$ and $7.0x10^{14}$ ph/s/0.1%bw at 14 and 5 keV respectively and is preferred because it maintains the rapid tunability of the beamline.

Front end:
The front end should be capable of transmitting the photon flux from the undulators defined above. It allows an unrestricted opening of the primary slits of up to 4 x 2 mm^2 (H x V). To improve the photon flux available at the sample position at long wavelengths/low energies it will be necessary to convert the front end to a UHV configuration (see below).

Beamline layout

During phase 1 of the ESRF Upgrade Programme no major infrastructure changes are foreseen. However, within the UPBL10 CDR provision is made for the conversion of ID29 CC4 and CC5 to house the cryobench facility.

Refurbishment projects

Micro-focus MAD
In 2006 an initial study to reduce the focus spot of ID29 to 5 x 5 μm^2 was carried out by O. Hignette (Optics Group). The configuration proposed comprised using the existing toroidal mirror as a pre-focusing device and inserting a 170/300 mm KB pair at a free working distance of 800 mm from the sample. The new requirement of a larger 10 x 10 μm^2 focal spot would require a new study. However, we can foresee that the free working distance would be increased. Care would have to be taken to ensure a pragmatic approach to operation at a variable focus that does not require long alignment times.

Long Wavelength Optimisation

The flux at the sample position at low energies will need to be increased. The beamline was initially designed with a number of beryllium windows to separate vacuum sections. Additionally, there are a number of scattering foils used to give diagnostics of the beamline performance.

In 2004 the beryllium windows were removed to increase flux at low energies. Nonetheless, under the current configuration only ~15% of photons produced at 6 keV are transmitted to the sample. Major gains in sample position flux, however, can be made by removing the diamond window in the front end which absorbs about 60 % of the photons at 6 keV. Additionally, the path between the exit window of the beamline and the sample should be in helium or rough vacuum rather than air to decrease absorption and air scattering. Thus an increase in photon flux at the sample of a factor of five can be achieved.

To make the beamline compatible with an UHV front end, it would be necessary to modify certain vacuum chambers. In conclusion, a number of pumps will be added, limited conductance tubes substituted for existing large diameter tubes, interlocks on the experimental hutch would trigger valves and equipment such as attenuators and slits would be made compatible with a UHV environment at the beamline/machine interface.

Detectors

A major part of the medium term development proposed for ID29 is the purchase of a new detector. Currently the beamline has a ADSC Q315r system installed. This detector is more than suitable for most applications in MX. However this, and other tiled 3x3 CCD detectors, suffer from one major drawback. The process of cutting and bonding the fibre optic tapers to the CCD chips results in a non-uniform point-spread function where corners of the CCD chips abut. Data quality in these regions is degraded, sometimes seriously (Figure 3) and this can seriously hamper phasing experiments where the anomalous signal is small. Despite extensive efforts to find a method to correct this problem, it remains a serious issue for such detectors. *In order to maximise the chances of success for the type of projects we anticipate carrying out on a refurbished ID29, the purchase of a more modern, improved detector is essential.*

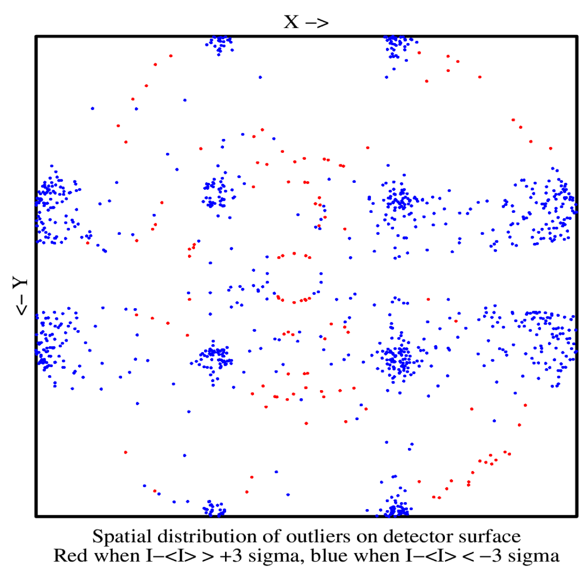

Figure 3. The spatial distribution of data outliers on the surface of a 3x3 tiled CCD detector. The regions mostly blue are close to the areas where the corners of adjacent CCD chips abut.

There are several such detectors already on the market. Currently the most obvious choice is a Pilatus 6M pixel detector. These are single photon counting detectors with Detector Quantum Efficiencies of 100% at 8 keV, 75% at 12 keV, ~60% at 14.0 keV, a 20 bit dynamic range and 'noiseless' readout. The biggest of these detectors has an active area of 431 x 448 mm^2 and,although the pixel size is rather large (172 μm^2), the point-spread function of one pixel results in a very high resolving power.

The detector also has a very fast readout time (~3 ms) and can be electronically gated at a rate of 12 Hz. This allows 'continuous rotation' data collection, eliminating a source of error in current data collection protocols. The ability to electronically gate the detector may also provide a rapid means of centring a microcrystal in the X-ray beam. Using either diffraction or (in some cases) fluorescence as a monitor, sample containing loops could be rapidly scanned in one, two or three dimensions to allow the location of a crystal to be discerned. A further interesting feature of the detector is that it has an adjustable energy threshold. In AD experiments, this could be used to suppress noise due to fluorescence on diffraction patterns and provide a further means of reducing measurement error.

Although a full assessment as to the suitability of the Pilatus 6M has yet to be made, the advertised specifications allows for the accurate measurement of very weak X-ray diffraction signals and should allow the exploitation of even the weakest anomalous signals (i.e. $\Delta F \leq 1\%$) in structure determination.

1.5 REFERENCES

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