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Diamond Light Source

Outline Proposal for a Phase III beamline

Coherent and sub micron focus x-rays for macromolecular crystallography

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1. Summary

The beamline will allow improved protein structure determination particularly from marginal crystals such as those from membrane proteins and multi-protein complexes, which play a central role in cellular operation. The aim of the beamline is to obtain data which is limited by the quality of the crystal rather than the instrument performance. Discrimination between regions with different structure should also allow better structural models to be obtained. These developments will be of benefit for academic bio-medical research and for the pharmaceutical industry.

Coherent and sub micron focus x-rays combined with a detector having 5-10 times better angular resolution will give the following advantages.

- Better discrimination of signal to background and therefore higher resolution data. This should give significant benefit for all protein crystallography projects.
- Better handling of disorder (e.g. different unit cell lengths, twinning, poorly diffracting regions, very high orientational mosacity).
- Novel phasing methods from the speckle around and between diffraction spots.
- Imaging of crystals which have an intrinsic biological function
- Possible exploitation of photo-electron escape to reduce radiation damage

The main requirements are an undulator to provide bright radiation, beamline optics which will provide a coherent and/or microfocus beam and a detector with an angular coverage and resolution matched to the properties of the crystal and the beam.

2. Scientific Case

The letters of support for this beamline describe a variety of structural biology projects in both academic research laboratories and industry. The emphasis is on membrane proteins or multi-protein complexes but individual soluble proteins are also included. Examples include inner membrane transport proteins, growth factor signalling pathways, complexes involved in chromatin mediated cell regulation, development of novel agents for anti-cancer therapy, novel GPCR structures and co-structures relevant to drug design and various metabolic enzymes.

Projects such as these have benefited from previous developments in brighter undulator beamlines and larger, faster detectors. Each time a significantly improved beamline or detector has become available, the benefits have become apparent, typically with an improvement in the resolution obtainable from poorly diffracting crystals as the incident x-ray beam, the detector and consequent data collection protocols become better matched to the intrinsic properties of the crystal. However, at the limiting resolution, there are still at least two limitations. These are

- X-ray background. The resolution of the data is typically defined as the shell in which the average value of I/sigma (I) = 2. In the absence of background, a value of I/sigma(I) =2 can be obtained with only 4 photons in a diffraction spot. Typically tens or hundreds of background photons per pixel occur in spots occupying 25 pixels on the detector. These figures are consistent with a 15-700 fold difference between the required scattering power of crystals on present beamlines and the theoretical limit (Holton and Frankel, Acta Cryst, 2010, D66,393-408).
- Inability to properly model the disorder (different structures of protein and solvent within a crystal). This leads to uncertainties or errors in the average or representative structures which are published and results in high crystallographic R factors for

refined structures. These typically have values of around 20% whereas, for chemical crystallography values of 3% are normal.

These factors limit virtually all macromolecular crystallography projects. They cause particular difficulties for the most challenging projects of the type mentioned above. The proposed beamline will give advantages for the following types of crystals

a) Large well ordered crystal. The large high resolution detector will retain small diffraction spots as it is moved further from the sample whereas the background will decrease due to the inverse square law. As the resolution is limited by the background, higher resolution data should therefore be obtained. Room temperature data collection would particularly benefit from this as the "mosaic spread" is often lower in this case.

b) Large disordered crystal, particularly those at cryo-temperatures. The new beamline will produce beams down to 0.5 microns in size (compared with 5 microns on I24), enabling the best diffracting regions to be selected. This could include use of diffraction cartography (e.g. Bowler et. al. *Acta Cryst.* 2010, D66, 855-864; Aishima et. al. *Acta Cryst.* 2010, D66, 1032-1035;) which systematically maps the best regions of the crystal. This method has been proven to work but does have limitations as the crystal is a 3 dimensional object and the best regions cannot be completely selected by a 2 dimensional scan. If the crystal is fully illuminated, the diffraction spots will contain intrinsically sharp features corresponding to domains or mosaic blocks within the crystal. These have been observed with a suitable high resolution diffraction set up (e.g. Kriminski, et.al., 2002, *Acta Cryst.* D58, 459-471; J. Lovelace, et. al. *J. Appl. Cryst.* 2006. 39, 425-432) and individual domain rocking widths down to 0.01 degrees measured (fig. 1). With a set up of the type proposed, these sharp features will be observed above the x-ray background giving higher resolution data. It should be possible to produce a domain model of the crystal from the lower resolution higher intensity data and apply this to the analysis of the higher resolution lower intensity diffraction spots. Exploiting this can be considered as an extension of present methods, involving recording and using more detailed 3 dimensional spot profiles. It should also be possible to pick out the best diffracting region in 3 dimensions with a set up like this. It was observed some time ago (C. Nave 1998, Acta Cryst., Vol. D54, pp. 848 - 853) that different cell dimensions are present in crystals at cryotemperatures and these can be observed with a suitable diffraction set up. These crystals will have different structures for both the protein and solvent and being able to separate them should lead to more accurate structures.

c) Small crystals. The microfocus capabilities will give optimum diffraction from small crystals by matching the beam size to the crystal size and thus reducing the x-ray background. The low divergence beam and matched detector will also provide lower background images as for the case of the larger crystals. A more speculative possibility is to illuminate the small crystal coherently and record diffraction fringes round each spot corresponding to the shape of the crystal and its' internal structure. These diffraction fringes can be used to reconstruct the crystal in 3 dimensions using the technique of coherent diffraction imaging. Such a reconstruction would provide even higher resolution images of the crystal enabling smaller domains to be imaged than is possible with direct imaging and consequent advantages as described in b) above. The diffraction fringes can extend between diffraction spots and therefore provide information about the phase relationship between the spots. Ultimately it should be possible to reconstruct an image of the crystal where each unit cell would be resolved. This would enable averaging of the images of the protein in real space with the ability to resolve regions with different structure and separate out the protein component from other material. Fringes around the diffraction spots and reconstructions of the crystal shape have already been recorded for the protein ferritin (Boutet, S. & Robinson, I. K. (2008) *J. Synchrotron Rad.* **15**, 576-583). Recent experiments at the LCLS (John Spence, private communication) have successfully recorded coherent diffraction data from photosystem 1. Strong fringes were observed extending between diffraction spots and, by

counting these, one can determine the number of unit cells across the crystal facets (results to be published in Science after which images should be available). A continuous source would require larger crystals to get sufficient signal. It would give the advantage that one should be able to fully record the diffraction spots (including the fringes). For larger crystals, there would not be enough photons in a single FEL pulse to reach a radiation damage threshold for a continuous source. There will therefore be a crystal size cross over point between a FEL and continuous source where, for a larger crystal, the continuous source should give more data per crystal. Although these ideas are somewhat speculative they offer the possibility of novel structure determination methods for very small crystals.

d) Twinned Crystals
Crystals with twinning of various types (non-merohedral, pseudo-merohedral and merohedral) are a common occurrence in macromolecular crystallography. Various statistical and other procedures have been developed to identify twinning and "detwin" the data. Such procedures work well but can break down in certain cases. Examples include cases of pseudo-merohedral twinning where there is a high degree of overlap between diffraction spots or merohedral twinning with a twin fractions of 50%. The detwinning algorithms usually require separate measurements of reflections related by the twinning law and this is not always possible with radiation sensitive crystals. A coherent beam should enable the individual components to be separated out and their contribution measured directly. Experiments in this direction (see fig. 2) have started on Diamond Beamline I16 using manganite crystals as a test case (Aranda, et. al. submitted to J. Synchrotron Rad.).

e) Biologically relevant crystals. In some cases, crystals of proteins can occur naturally and have a biological function. An example is the occurrence of polyhedric crystals within cells infected by a virus (e.g. Anduleit et. al. Protein Science (2005), 14:2741–2743; Xiaoyun Ji, et. al. 2010, EMBO Journal 29, 505 - 514). The virus particles are protected by the polyhedra and the virus particles only released in the appropriate environment. These structures are also of interest for the development of protein nanocontainers relevant to pharmaceutical and other developments. It would therefore be of interest to obtain high resolution images of these crystals using coherent diffraction techniques.

f) Crystals suffering radiation damage. This is a serious problem in all structural investigations of biological material using x-rays both at ambient and cryotemperatures. The microfocus properties could provide reduced radiation damage by exploiting photo-electron escape whereby reduced radiation damage occurs when the crystal size or illuminated region is less than the path length of the photo-electron. These possibilities are still being investigated at a number of synchrotron facilities. If it emerges that significant benefits occur, this beamline will be ideal for exploiting the effects.

Although some of the above possibilities are more speculative than others, some (such as reduced background, better profile fitting and selection of the best diffracting regions) are clear. The ability to either use coherent illumination or microfocus illumination (or both together) will be a special property of this beamline enabling optimum diffraction data to be obtained from a wide range of specimens.

3. Outline specification

The beamline will require an undulator source to enable the production of both microfocus and coherent beams of sufficient intensity. The beamline will need to select the coherent portion of the beam and produce focal spots down to 0.5 microns in size. These requirements are compatible in the sense that small focal spots are easier to produce with a low divergence beam and one would not in any case want to focus the full undulator beam down to a 0.5 micron spot for radiation damage reasons. Other requirements can be defined as follows. In simple terms, the crystal size (or other feature) divided by resolution (e.g. 10,000 for a 3 micron crystal at 0.3nm resolution) can be used to define the required resolving power of the

monochromator (e.g. 10,000) and the number of resolution elements on the detector (e.g. greater than 20,000 in each direction for a centred detector). Development of a detector with sufficient angular resolution elements matched to the coherence requirements is the main technical challenge for a beamline of this type. It would require either new technology or the cost benefits resulting from high volume production using present technology. If this can not be achieved initially, any improvement in detector resolution would still give improvements in terms of improved signal to background. It would also allow the recording of fringes/speckles for part of the complete pattern, for example to provide some phase information.

4. Community

It is envisaged that all the macromolecular crystallography community will benefit from the beamline. This includes over 270 macromolecular crystallographers on the Diamond mailing list and BAG groups from 23 different academic institutions in the UK. Letters of support have also been obtained from users supported by the EU. The expressions of interest received so far cover a wide range of types of projects with an emphasis on the most challenging problems including those which link structural biology at the molecular and cellular level.

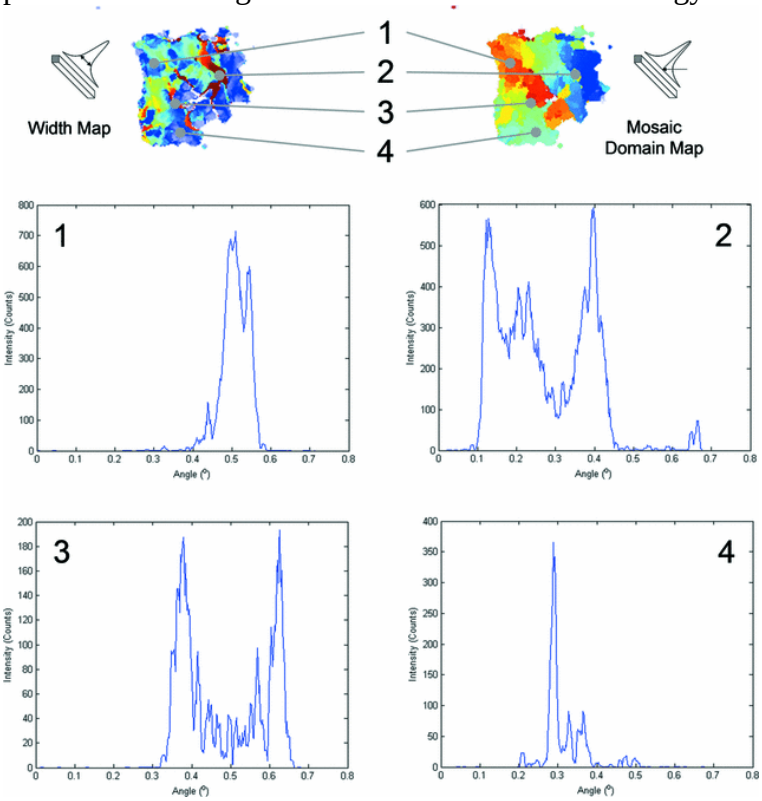

Fig. 1 Rocking widths of individual domains within a protein crystal. . J. Lovelace, C. R. Murphy, R. Pahl, K. Brister and G. E. O. Borgstahl J. Appl. Cryst. (2006). 39, 425-432

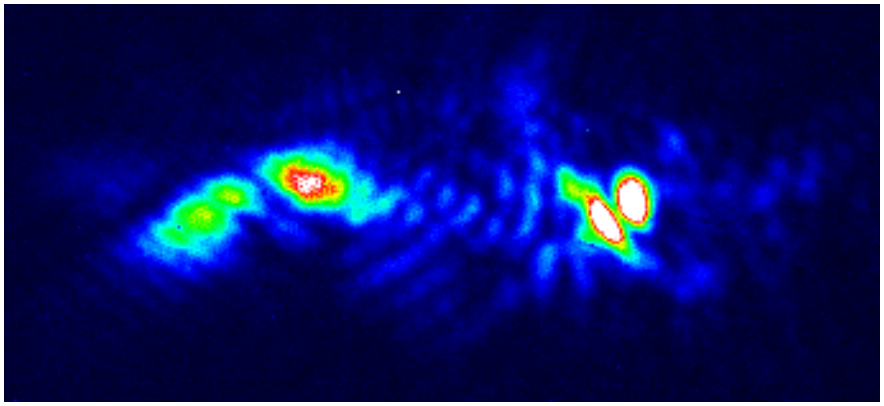

Fig. 2 Single image coherent diffraction pattern for PCMO3-7 microcrystal showing speckles extending between two centres of a split Bragg peak due to twinning of (121) reflection Aranda, et. al. submitted to J. Synchrotron Rad

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