Diamond Light Source

Outline Proposal for a Phase III beamline

Beamline for Advanced Structure-Function Studies in Life and Physical Sciences[1].

Document to be no longer than 4 pages
(not including cover page)

Times New Roman 12 pt

[1] This is a consolidated proposal originating from two EOIs, 12 (Picosecond Time Resolved X-ray Crystallography @ Diamond) and 13 (BARCLA: Structural Biology Beamline for Redox Systems including Metalloproteins). It also includes components of a proposed Flexible MX beamline. LOS for each of the individual beamlines should therefore be included together for this consolidated proposal.

Beamline for Advanced Structure-Function Studies in Life and Physical Sciences

Case prepared by:

(a) Samar Hasnain, Michael Hough and Richard Strange (University of Liverpool)
(b) Arwen R Pearson (Astbury Centre, University of Leeds)
(c) David Allan, Robin L Owen, Thomas Sorensen (Diamond Light Source)
(d) John McGeehan (Biophysics Laboratories, University of Portsmouth)
(e) Paul Raithby (Department of Chemistry, University of Bath)

Summary

This beamline proposal brings together researchers from the life and physical sciences with the common aim of understanding molecular processes at atomistic resolution in well defined functional states. The proposed beamline will provide, in one place, a wide range of spectroscopic techniques, bright stable monochromatic and narrow band-pass Laue capabilities together with a flexible sample environment to provide a state-of-the-art facility for time-resolved and functionally-validated structure determination at atomistic resolution. The science drivers for this proposal can be broadly summarised as follows:

The need to determine spectroscopically validated structures of redox systems and to structurally characterise spectroscopically defined intermediates with lifetimes from ps to s at atomistic resolution in order to understand the structural basis of the molecular properties of the complex systems.

The beamline will provide a unique array of resources that will enable the systematic structural characterisation of a wide range of chemical and biological systems in spectroscopically defined states. We have taken a purposely flexible design approach to ensure that as new tools emerge novel technologies can be incorporated. This will enable UK's community to maintain the facility at the cutting edge of structural science over the next 10-15 years.

Scientific Case

The fundamental aim of both physical and life scientists engaged in structural studies is to understand how structure leads to function. Whilst a single resting-state structure can provide a wealth of information about a given molecular system, the value of this information is greatly increased if it is spectroscopically validated in real time and assigned to a particular functional state of a biological or chemical system. In other cases, for a more complete understanding of how systems function it is vital to be able to watch and understand chemical reactions and biological processes as they occur, and an explicit time resolved (TR) structural description is essential. In the proposed beamline, we are aiming to satisfy the requirements for both time resolved structural studies and for spectroscopic validation of molecular states with very high resolutions. Specifically, the beamline will combine recent developments in automation with a novel multi-crystal narrow band-pass Laue experimental approach, permitting structural changes to be followed down to ps time resolution in non-cyclical biological and chemical systems. For longer lived species, such as those stabilised by classical cryo-trapping approaches or generated by X-ray radiolysis, the beamline will have a highly stable and tuneable monochromatic data collection configuration, suitable for measuring very high resolution diffraction data and X-ray absorption data.

TRLaue. Key events occur on sub μs timescales and, until now, have been predominantly elucidated using indirect spectroscopic probes. Functionally related atomic motions can, however, be directly observed by X-ray crystallography if the majority of the molecules within the crystalline sample behave in a concerted manner and the experiment has the time resolution to determine various structures along the reaction coordinate. This is, however, non-trivial as several factors can make it extremely difficult to determine the structures of meta-stable or transient states. The three major factors that limit these studies are a) the experimental time-resolution required to determine the structure of short-lived, but often extremely interesting, intermediates, b) X-radiation induced changes that occur from the first moment of X-ray exposure as a result of inelastic scattering events and c) the ambiguities in interpreting electron density that is consistent with multiple isosteric but chemically distinct species.

Studies of relatively long lived (>ms) intermediate species have already shed light on some fascinating structure-function relationships. For example, cryo-trapping techniques have proved successful in the observation of low temperature metastable stable states in macromolecular systems[2], while X-rays may be used to drive or 'pump' reactions in crystals in order to generate functionally-relevant intermediates[3]. However, these methods are limited by non-isomorphous behaviour between crystals and the difficulty in "catching" intermediates. In molecular systems, there has been success in obtaining detailed structural data both on meta-stable systems and on systems with lifetimes down to the μs regime[4]. In order to obtain greater time resolution, pump-probe Laue diffraction TR crystallographic techniques have been developed at the APS and ESRF[5] to observe functionally related atomic motion in light activated systems with time-resolutions as fine as 100 picoseconds[6]. However, only a handful of systems have been studied with this approach. This is due to the limited nature of current Laue methodology, in which, as the functionally related structural changes are of the same order of magnitude as those due to non-isomorphism between crystals, a single crystal must be used to collect the entire dataset, requiring the system to be cyclical, i.e. it must return to its initial ground state after photoactivation, ready for the next pump-probe. Figure 1 illustrates the novel multi-crystal TR Laue approach that we are proposing for non-cyclic systems, which vastly expands the applicability of the method using the new beamline.

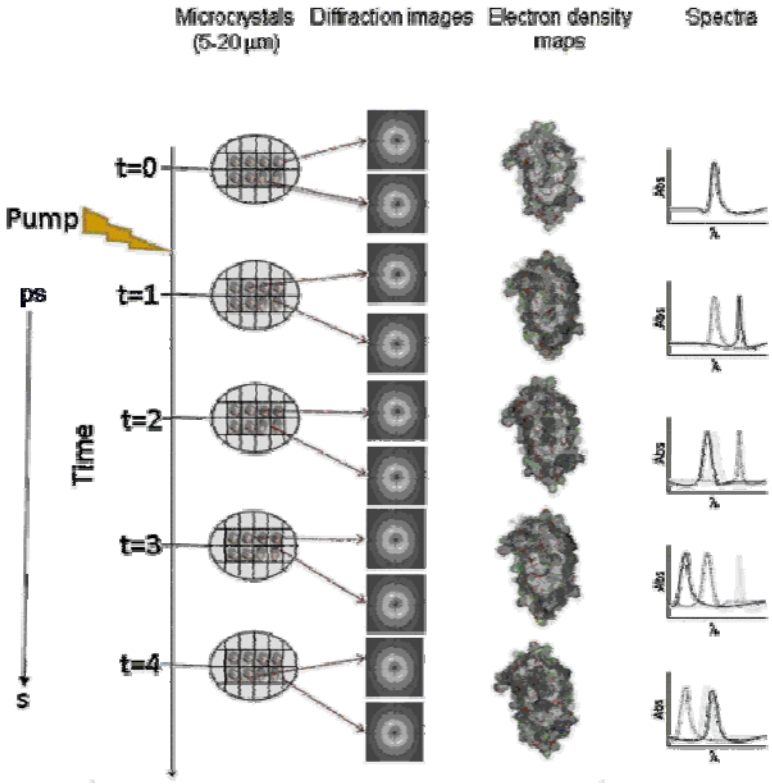

Figure 1: A schematic representation of a time resolved experiment. Mesh mounts containing many microcrystals (5-10μm), mounted using a sample changer, are stabilised using a vapour stream (> 0 °C) or cryostream. The ability to carry out experiments at a range of temperatures above the dynamical transition will reduce reaction rates, enhancing our ability to trap short-lived species. Single diffraction images (100 ps exposure) are recorded from each crystal at the desired time delay after the co-incident laser pulse has photoactivated the system. Spectra are recorded *simultaneously* from the X-ray/Laser exposed volume to assist in a) determining the degree of photoactivation and b) precisely place each image along the reaction coordinate. Images are indexed, integrated and combined to yield a complete dataset at each time point. Statistical approaches are then applied to extract coherent (functionally related) from non-coherent (noise) structural changes.

Spectroscopic validation. A key requirement in structural analysis of functionally relevant species is that the structures determined are as representative as possible of the physiological or chemical pathway under study. This is particularly important when a structure is used as the central evidence for proposing a functional mechanism, and becomes crucial if the proposed mechanism has implications for pharmaceutical or technological developments. Because of this, when determining the structures of short-lived or metastable species or X-ray sensitive redox systems, X-radiation induced changes are a major problem. This has long been recognised for biological samples[7], where radiation damage severely limits the diffracting life-time of macromolecular crystals, even at cryo-temperatures. At a 3rd generation source such as Diamond, diffraction limiting doses such as that

[2] Hajdu *et al.*, (2000) *Nature Struct. Mol. Biol.* **7** 1006-12
[3] Berglund *et al.*, (2002) *Nature* **417** 463-8
[4] Warren *et al.*, (2009) *Angew. Chem. Int. Ed.* **48** 5711-4, Coppens *et al.*, (2004) *Chem. Comm.* 2144-5
[5] Schotte *et al.*, (2003) *Science* **300** 1944-7
[6] Coppens *et al.*, (2008) *Z. Kristall.* **223** 265-71, Kaminski *et al.*, (2010) *J. Synch. Rad.* **17** 479-85
[7] Garman (2010) *Acta Cryst.* **D66** 339-51

proposed by Henderson[8] can be reached in less than 4s of exposure on beamline I24[9]. While it is normally possible to collect a complete dataset from a single crystal, radiation damage becomes an even more pressing concern when studying X-ray labile species such as oxidised redox centres. These are very rapidly reduced in the X-ray beam at a fraction of the dose required to affect the diffracting power of the crystal[10]. Indeed, that atomic co-ordinates alone are insufficient to fully describe the state of macromolecular systems containing redox centres has recently been recognised with the introduction of a complementary spectroscopy field in PDB entries. Very recently, radiation damage has also been recognised as a significant problem for the study of molecular systems where high levels of solvent are present in the crystals, for example in metal organic framework systems, where X-ray damage has been observed after only 0.5s exposures on beamline I19. In order to detect these X-ray induced changes, as well as guide the generation and trapping of reaction intermediates, a wide range of single crystal spectroscopic tools have been developed[11] that we will integrate into the beamline to enable the monitoring of electronic and structural changes during X-ray exposure. These include optical (UV/Visible and Fluorescence) and vibrational (Raman and IR) spectroscopies, as well as XANES and XRF. The optical and vibrational spectroscopies will be carried out "on-axis" ensuring that the spectroscopy is probing the same volume as the incident X-rays[12]. This will enable users to design data collection strategies to obtain undamaged structures, as well as identify the nature of any intermediates arising from X-ray illumination that may represent interesting species that are difficult or impossible to generate in other ways. This will ensure that the data that users take home from the beamline are known to be from a specific, functionally-relevant state of the system under study, thus preventing post-experimental misidentification of these states and the misinterpretation of the catalytic and functional mechanisms involved.

The availability of this wide range of spectroscopic instrumentation on the beamline will also allow users to address another common problem, especially in macromolecular crystallography, that of ambiguous electron density at or close to catalytically active sites. This can range from unidentified "blobs" of density at mid to low resolution or high resolution data that could be equally well assigned to multiple "isosteric" but chemically distinct species (i.e. -OH vs. =O). Single crystal spectroscopy provides additional data that can be the key to resolving these problems and this approach of identifying and spectroscopically ‘fingerprinting’ states is proving a powerful tool.

We note that there are many systems where the structural changes associated with function cannot be observed using crystallographic approaches, for example where large structural changes disrupt the lattice or crystals cannot be obtained. However, very useful information can be derived from X-ray scattering approaches such as SAXS and WAXS for solution state systems. This beamline will provide the capacity for time-resolved SAXS and WAXS experiments to yield complementary structure data to that obtained by crystallography.

As we keep pushing the boundaries for what is possible in structural science it has become clear that one-size fits all beamlines don't always cater for some challenging experiments requiring actual experimentation rather than simply doing a data collection. For example, many projects that require non-standard data collection conditions (darkness, anaerobic environment, flow cells, furnaces) that cannot be implemented in a standard 2-3 shift session. Rather, 3-4 days are usually required to set up the equipment, optimise the conditions and run the experiments. The proposed beamline will be a flexible and uncrowded end-station where users can assemble the required specialist equipment for their experiments, while the majority of the spectroscopic "on-axis" instrumentation, XRF detectors, cryo and vapour streams etc are automatically positioned. This flexibility will allow users to progress projects that otherwise would stall for a long time or be abandoned altogether as impractical. The beamline design anticipates future scientific demands and technical developments, placing us in a strong position to respond to new scientific ideas and approaches.

---

[8] Henderson (1990) *Proc. R. Soc. Lond. B.* **241** 6-8

[9] Evans *et al.*, (2010). *Crystallography Reviews* in press

[10] Beitlich *et al*., (2007) *J. Synch. Rad*. **14** 11-21; Ellis *et al*,. (2008) *J. Synch. Rad*. **15**, 433-9. Hough *et al.* (2008) *J. Mol. Biol*. **378** 353-61.

[11] Pearson and Owen (2009) *Biochem. Soc. Trans*. **37** 378-81

[12] Owen *et al*., (2009) *J. Synch. Rad*. **16** 173-82

Outline specification

A. Source & Optics The beamline will run in two alternate modes. A narrow band pass Laue mode for ps-ms time resolved studies and a monochromatic mode for the study of cryo-trapped intermediates generated chemically or radiolytically, and XANES/XRF measurements. Accurate monitoring of the X-ray flux at varying wavelengths will allow accurate dose estimation. A flexible X-ray beam size ($5\mu m^2$ - $200\mu m^2$) is required to match the range of sample sizes.

Narrow band-pass Laue mode: The band pass provided by an ID is typically 1% which with focussing optics can provide the brightest micro-focus beams. Additional focussing and narrowness of band-pass can be achieved via the use of focussing lens and/or capillary optics and a multilayer monochromator. A full ray tracing with appropriate source will need to be undertaken before the final choice is made. Optical layout will need to ensure that optimum position is chosen for the ultra-fast chopper shutter system in order to achieve pico-second time resolution with minimum of maintenance requirements. Note, 8 to 24 bunch ring modes will be required for ps time-resolved experiments.

Monochromatic mode: Highly tuneable beamline optics (e.g. a rapid scanning double crystal monochromator), providing monochromatic X-rays with a stable beam position over a range of absorption edges with an energy resolution $\Delta E/E \sim 10^{-4}$ (such as a Si111 double crystal monochromator). Higher order harmonics will be suppressed by the use of mirrors and monochromator crystal's de-tuning. Wavelength range of 0.6 – 2.3 Å, allowing high resolution data collection with short wavelengths and access to a large number of absorption edges (primarily first row transition metal K-edges from V to Mo), for XANES and XRF measurements.

B. Detectors The X-ray crystallographic/scattering detectors should allow very rapid measurement of very high resolution diffraction (up to 0.8Å) in a single pass, have a large area and low noise level to facilitate low dose experiments. Currently a large Pilatus area detector satisfies these requirements reasonably well but we expect that improved detectors would become available in the time frame of this proposal for similar costs. For SAXS/WAXS, again a photon counting detector of the Pilatus type will be appropriate. An alternate integrating detector may be needed for ps time-resolved experiments. A high resolution (high count rate), energy resolving X-ray fluorescence detector is required for collecting XANES data and this should have a 'low profile', i.e. a small volume, to allow positioning close to the sample without obstructing other instrumentation

C. Spectroscopic Equipment Integration of the *in situ* spectroscopy equipment is an essential design component. The sample (crystal/goniometer) environment will be designed to enable on-axis measurements of UV-vis absorption in the range ~280-1000nm, covering the majority of relevant LMCT bands as well as chromophores, fluorescence , Raman (non-resonant and resonant) and IR spectroscopy. X-ray absorption spectroscopy (XAS) is another essential spectroscopic-structural probe as the near-edge X-ray absorption (XANES) region is a strong indicator of both the oxidation state and the coordination environment of a metal centre. This implementation will build on the successful on-axis design in operation at the SLS[12] and under development at I24.

D. Sample Environment and Automation: Efficient use of the beamline will require a flexible endstation design to allow us to seamlessly change mode of operation and sample environment configuration. This should as far as possible be automated, including automatic insertion/alignment of ancillary equipment at the crystal position and then removal to a safe parking space, with synchronised movement of other beamline components to accommodate this procedure. A robotic sample changer, cryo-stream and humidity controlled vapour stream are also required. We expect that flow-cells, furnaces etc. will be provided, as required, by users.

Community

This proposal aims to take a significant step towards maximising the structurally and functionally relevant information that can be obtained using synchrotron radiation. It will positively impact a wide range of science across the physical and life sciences community (see letters of support from the community). In addition, it will provide a unique venue for close interaction between life and physical scientists with the common aim of understanding how structure relates to function. This will facilitate the cross-pollination of best practices from both fields, as well as providing an unparalleled environment for the training of early career scientists.

Beamline for Advanced Structure Function Studies in the Life and Physical Sciences

Proposers:

(a) **Samar Hasnain, Michael Hough** and **Richard Strange** (University of Liverpool)
(b) **Arwen R Pearson** (Astbury Centre, University of Leeds)
(c) **David Allan, Robin L Owen, Thomas Sorensen** (Diamond Light Source)
(d) **John McGeehan** (Biophysics Laboratories, University of Portsmouth)
(e) **Paul Raithby** (Department of Chemistry, University of Bath)

Appendix: List of Supporters

Last Name	First Name	Institution
Banfield	Mark	John Innes Centre, Dept. of Biological Chemistry
Barber	James	Imperial College London, Division of Molecular Science
Beddard	Godfrey	University of Leeds, School of Chemistry
Blanford	Christopher	University of Oxford, Inorganic Chemistry Laboratory
Butt	Julea	University of East Anglia, School of Chemistry
Conway	Stuart	University of Oxford, Chemistry Research Laboratory
Crennell	Susan	University of Bath, Dept. of Biology and Biochemistry
Edwards	Thomas	University of Leeds, Astbury Centre for Structural Molecular Biology
Evans	Phil	MRC Laboratory of Molecular Biology
Evans	Robert	Brunel University London, School of Health Sciences & Social Sciences
Fernig	David	University of Liverpool, Dept. of Chemistry an Structural Biology
Fritz	Guenter	Universitaets Klinikum Freiburg, Germany, Protein Biophysics and Biochemistry
Fulop	Vilmos	University of Warwick, Dept. of Biological Sciences
Garman	Elspeth	University of Oxford, Laboratory of Molecular Biophysics
Hadju	Janos	Uppsala University, Sweden, Laboratory of Molecular Biophysics
Hardie	Michaele	University of Leeds, School of Chemistry
Harmer	Nicholas	University of Exeter, Dept. of Biosciences
Henderson	Peter	University of Leeds, Institute of Membrane and Systems Biology
Holton	James	University of California, San Francisco, USA, Advanced Light Source
Hunte	Carola	University of Freiburg, Germany, Institute for Biochemistry and Molecular Biology
Katona	Gergely	Gothenburg University, Sweden, Dept. of Chemistry
Kneale	Geoff	University of Portsmouth, Institute of Biomedical and Biomolecular Sciences
Lawson	David	John Innes Centre, Dept. of Biological Chemistry
Le Brun	Nick	University of East Anglia, School of Chemistry
Littlechild	Jenny	University of Exeter, School of Biosciences
Neutze	Richard	Gothenburg University, Sweden, Dept. of Chemistry
Ramakrishnan	Venki	MRC Laboratory of Molecular Biology
Raven	Emma	University of Leicester, Dept. of Chemistry
Rizkallah	Pierre	Cardiff University, Wales Heart Research Institute
Schofield	Christopher	University of Oxford, Chemistry Research Laboratory
Scrutton	Nigel	University of Manchester, Interdisciplinary Biocentre

Snell	Eddie	Hauptman-Woodward Medical Institute, Buffalo, USA
Steiner	Roberto	Kings College London, Division of Cell and Molecular Physics
Stocker	Achim	Universitaet Bern, Switzerland, Dept. of Chemistry
Tromp	Moniek	Technische Universitaet Muenchen, Germany, Inorganic Chemistry
Turnbull	Bruce	University of Leeds, School of Chemistry
Walden	Helen	Cancer Research UK, Protein Structure Function Laboratory
Webb	Michael	University of Leeds, School of Chemistry
Wilson	Chick	University of Glasgow, School of Chemistry
Worral	Johnathan	University of Essex, Dept. of Biological Sciences