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Diamond Light Source

Outline Proposal for a Phase III Beamline

AMALFI
A Multipurpose Automated Laue μ-Focus Instrument

A combination of the original proposals:
a) LaTR: A microfocus Laue beamline for time-resolved X-ray crystallography at Diamond
& b) High-throughput small molecule single crystal Laue diffraction

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1. Summary

We propose an interdisciplinary microfocus Laue beamline at Diamond Light Source (DLS) using a revolutionary multi-crystal methodology that will permit time-resolved structural studies of both reversible and irreversible chemical and biological processes over a range of timescales from picoseconds to resting state. In addition, this multi-crystal approach coupled with the intrinsic speed of the Laue method and a microfocus beam will allow us to:

- Visualise structures of intermediates in both small molecule and macromolecular systems in a time-resolved manner, including key events in chemical and biochemical processes that occur on timescales down to picoseconds and can, at present, only be routinely elucidated using indirect spectroscopic probes;
- Engage in the challenging area of dynamic structure determination with the ultimate aim of watching molecular processes as they occur as a function of time or external stimuli including hydration, pressure, temperature etc;
- Study microcrystalline powders one crystallite at a time, enabling rapid sample screening and phase identification with a resolution not possible with current crystallographic techniques;
- Carry out analysis of framework materials for gas storage and catalysis, nanomaterials, molecular magnets, and pharmaceutical and supramolecular compounds where structure determination is beyond the capabilities of current DLS instruments.

2. Scientific Case

The fundamental aim of both physical and life scientists engaged in structural studies is to understand how structure leads to function. To understand how systems function it is vital to be able to watch chemical reactions and biological processes as they occur, and an explicit time resolved structural description at atomic resolution is therefore essential.

X-ray crystallography (XRD) is a well established technique that enables the direct visualisation of atomic structure at high resolution. Although often seen as providing time and space averaged structures, direct observation of functionally relevant atomic motions during a reaction can be achieved using XRD. However, for this to be possible the majority of the population within the crystal must behave in a concerted manner *and* the time resolution of the experiment must be sufficiently fine to determine multiple structures along the reaction coordinate.

Time Resolved Crystallography: Relatively long lived species (lifetimes > s) are experimentally easy to stabilise and study. Indeed, in macromolecular systems many reactions can be stopped by simply rapidly cooling them to cryo-temperatures (100K).[1] This relies somewhat on serendipity to "catch" specific intermediates. An alternate approach is to use mechanistic trapping, where the reaction conditions are such that the reaction cannot proceed beyond a certain point, allowing build-up of the intermediate that precedes the block.[2] It is also possible to trap small molecules and structurally characterise their metastable intermediates at low temperatures.[3] For intermediates with lifetimes in the ms – s range, it is often possible to maintain a detectable equilibrium "excited state" population by continuously illuminating the sample.[4] These approaches have been used to obtain detailed information about catalysis and mechanism. However, even in systems where some intermediates can be stabilised, there are many transient species that cannot be "captured" or that are labile in the X-ray beam. To directly observe these, the crystallographic experiment itself must be altered to record diffraction data with a time resolution matched to the lifetime of the species.

The time-resolution of a XRD experiment is fundamentally determined by the number of incident X-ray photons required to produce a measurable diffraction pattern which is related to both the intensity of the incident X-ray beam and the scattering power of the sample. Even on the modern high brilliance monochromatic XRD beamlines at DLS, (I24 & I19), the available flux density limits the time-resolution achievable to only ~60-70 μs (best case scenario) for macromolecular systems and ~ 10 μs for more strongly diffracting molecular systems.[5] This is insufficient to probe fast chemical and photochemical processes (Figure 1). Pioneering work at the ESRF and APS has developed the pump-probe Laue XRD approach to overcome this problem.[6] Here, instead of a monochromatic beam, a narrow bandpass polychromatic beam, or pink beam is used providing a considerable increase in flux (~10^{10} photons / 100 ps pulse at APS) yielding sub ns time-resolution.

In time-resolved Laue XRD the reaction is usually initiated with a fs-ps pulse of laser light (the pump) and probed after a given time delay by recording a diffraction image, or series of diffraction

images at increasing time delays. To observe the fine structural changes associated with function the molecules within the X-ray illuminated volume must be reacting synchronously and, for fast time-scales, this can only be achieved with photoactivation. The photo-excited state is then allowed to decay back to its starting point after which the crystal is reoriented and the process is repeated until a complete dataset has been obtained. Although indisputably powerful, this approach has remained a niche activity due to the requirement for the system to relax back to its starting state after photoactivation to allow the accumulation of sufficient diffracted intensity in each image. This requirement for a state-reversible or cyclic process is probably the major limiting factor that has prevented the wider application of the pump-probe Laue XRD approach in molecular and macromolecular structure determinations of short lifetime species.

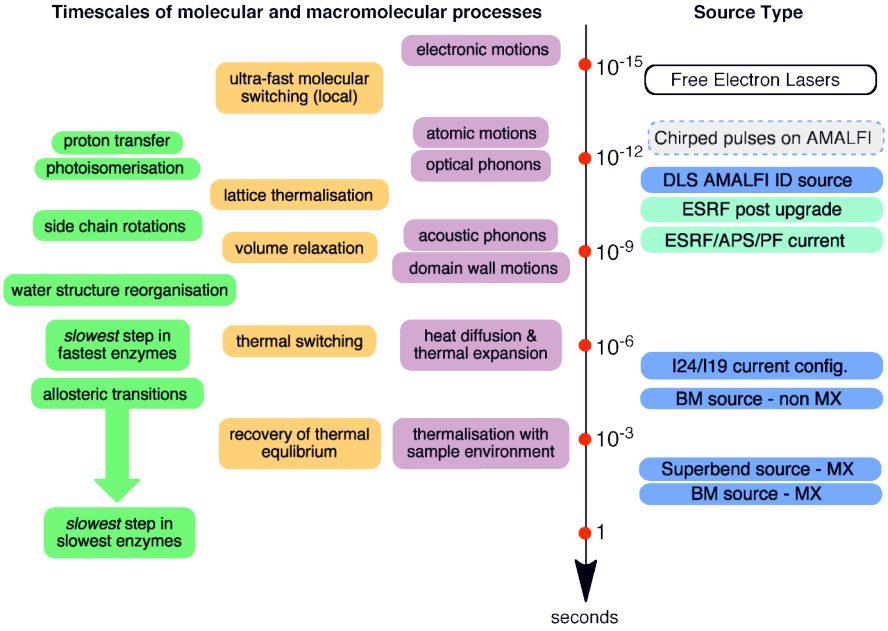

Figure 1 Schematic showing time-scales for molecular and macromolecular processes of interest to members of the AMALFI working group (left) and how these map onto the time-resolutions attainable with different source configurations (right). Green boxes highlight biological, orange boxes chemical and purple boxes physical processes. The time-resolution attainable with different sources at DLS is indicated in blue boxes. For reference, the time resolution achievable at existing time-resolved Laue beamlines and the new FEL sources are shown in pale green boxes. The dotted blue box indicates the possibility of extending the time-resolution of AMALFI using chirped X-ray pulses.

Phase Resolution & Dynamic Studies: We have developed a new generally applicable approach to time-resolved Laue XRD that removes the requirement for reversibility and, for the first time, will enable the study of any molecular system for which crystals can be obtained. This revolutionary multi-crystal approach, coupled with the use of mechanical and electronic choppers, opens up new horizons for studying reversible and irreversible chemical and biological process as they occur *in real time*. In our approach, we collect single diffraction images from many (10^2-10^3) micro-crystals (5 x 5 x 5 – 20 x 20 x 20 μm^3) at each time-point and merge these to give a full data set. We have already shown in test experiments on I24 that we can determine protein structures to 1.8 Å using this approach and have recently demonstrated photoactivation and turnover for the non-reversible reaction of L-aspartate-α-decarboxylase.

This multi-crystal approach also enables the rapid screening of large numbers of microcrystals of industrial importance in a manner that is currently not possible. Coupling the Laue method with a microfocus beam makes it possible to perform single crystal diffraction experiments on a bulk microcrystalline powder, allowing individual crystallites within a sample to be rapidly tested in order to detect miniscule levels of impurities. This effectively removes the current detection limit for such studies by powder XRD (0.1% on synchrotron sources and 1-5% on laboratory X-ray sources). In addition to phase purity analysis, this has a wide range of applications as it effectively allows materials to be studied in the absence of radiation effects. Transient species generated as a function of temperature, solvation, solvent/gas pressure etc are highly sensitive to sample heating and damage caused by prolonged radiation exposure, but since our microfocus Laue method will enable many (10^2-10^3) micro-crystals to be placed in the same environment, new crystallites can be

examined at each step. This has direct implications for the study of, for example, gas storage in Metal-Organic Frameworks (MOFs); desolvation in pharmaceuticals and catalytic function, as well as facilitating analysis of samples that are too small/weak to examine using laboratory equipment, but are too fragile to study on I19.

Impact: Single-crystal X-ray diffraction provides the definitive structural description on which our knowledge of (commercially) important processes in solids is built, leading to an understanding of the mechanisms involved in physical and chemical transformations, catalysis and diffusion, and thereby affording control over material properties. The addition of sub ns time-resolution to this high-accuracy spatial information will have a tremendous impact across the molecular sciences. There are many important chemical processes where a detailed knowledge of the reaction pathway or of the structural details of a charge-transfer complex in its activated state would have fundamental implications, for example in the design of new, efficient optical sensors, energy storage devices and magnetic materials. The combination of time-resolution with tailored environmental cells will allow the rapid acquisition of the data necessary to follow processes with short timescales. This will enable the mapping of processes and trends for catalytic reactions, phase transitions, host-guest complexes and solvation reactions, including in candidate pharmaceuticals where it will be possible to identify and determine the structures of different polymorphs. Similarly, in the life-sciences detailed time-resolved structural information will be invaluable in providing an understanding of how biological catalysts are able to activate molecular processes and how the wider protein matrix contributes to the regulation and precision of the reaction progress. As well as providing increased insights into the chemistry of life, this has the potential to aid in the design and creation of new biocatalysts and therapeutics that currently rely largely on empirical rule-based approaches as well as a fair degree of serendipity.

Many of the classes of materials that can be studied by these revolutionary techniques map closely onto key industrial and research council priority areas, including the RCUK priorities of *energy*, *healthcare*, *manufacturing*, *digital economy*, *bioenergy*, *animal health*, *ageing, bionanotechnology*, and *technology development for bioscience* and will help the UK communities to meet the developing Grand Challenges over the next five decades.

3. Outline Specifications

The fundamental time limit of a time-resolved XRD experiment is determined by the number of photons that can be delivered to the crystal per unit time. The state-of-the-art Laue beamlines at the APS, ESRF and Photon Factory are able to deliver sufficient photons in ~10-100 pulses (100 ps/pulse) to obtain usable diffraction data.[7-9] The planned ESRF upgrade includes improvements that would enable usable data to be acquired from a single 100 ps pulse (beam size at sample of 40x40 μm^2).[10] It should be noted, however, that the beamline designs at the APS, ESRF and Photon Factory are not suitable for the experimental approach we intend to use as we require not only a smaller (< 10x10 μm^2) beam size but also access to high throughput sample changing robotics.

Source and Optics: As is summarised in Figure 1, the nature of the X-ray source dictates the time resolution that can be achieved by the beamline. For excited-state experiments on short-lived species (sub μs) a single bunch or hybrid ring mode is required. Single bunches can be isolated using supersonic rotating choppers (already successfully implemented at the ESRF and APS). In hybrid mode it is possible to mechanically chop out the multi-bunch component of 600 2ns bunches (1.2μs) opposite the desired single bunch. The medium energy of the Diamond synchrotron is optimal for yielding one of the shortest time-structures (30–40 ps) worldwide whilst maintaining a high current (*cf.* 100 ps minimum limit at the ESRF, owing to the greater electron-electron repulsion resulting from having double the GeV). Additional time-resolution could be obtained by chirping the 100 ps X-ray pulse in order to vary the energy with time. Indexing of the resulting Laue diffraction pattern allows precise assignment of each reflection to a specific incident energy, potentially providing sub-ps time resolution within a diffraction image.[11] Obviously to obtain complete datasets at each time-point many more crystals would be required, however, our automated multi-crystal approach is fully compatible with such an experiment.

Our ideal beamline would comprise a double undulator source able to provide ~ 10^{10} ph/40ps pulse into a beam focal spot of < 10 x10 μm^2 at the sample position with an energy range of 15-25 keV and a bandwidth of 1-3 x 10^{-2} $\Delta E/E$. We are keenly aware of the limited availability of straight

sections for the Phase III beamlines and so Figure 1 highlights the time resolution, and hence the scientific questions that could be addressed, using a bending magnet or superbend source instead. Our calculations indicate that on a bending magnet *with currently available optics* we would be limited to only ~30 ms time-resolution for macromolecular samples. This time-resolution is similar to that achievable on I24 or I19 in their current monochromatic configurations with a fast (50 ms) rotating shutter. Due to their increased scattering power, the time-resolution for molecular crystals is 1-3 orders of magnitude greater than that of macromolecular crystals of the same volume.[12] Hence a time-resolution of ~30 μs could be achieved with a bending magnet source for a strongly diffracting molecular sample. Figure 1 clearly demonstrates the considerable scientific interest and benefit in being able to access sub μs time-resolutions, enabling the study of molecular processes that, due to their irreversibility, are *inaccessible* using the current Laue beamlines available at the ESRF, APS and the Photon Factory. For ultrafast (fs) time-resolution the developing Free Electron Lasers (FEL) are a fantastic but limited resource, and are unlikely to be used for slower (ps-μs) studies. AMALFI, able to access this time-resolution range, is therefore an essential resource and a bridge between the FEL and other synchrotron facilities worldwide.

End station*:* The diffractometer will have a relatively unrestrictive geometry to allow access at the sample position for an open-flow cryostat and robotic sample changer or environmental sample cell. The goniostat will be simple with a 3-circle fixed-chi geometry, preferably with an air-bearing for the omega axis. A large area, fast readout detector will support the high throughput needed. The experimental hutch will also be equipped with a fluorescence detector for anomalous dispersion and excited state experiments, a reliable incident-beam monitor for Bragg peak intensity normalisation, single crystal spectroscopic (optical and vibrational) instrumentation and lasers for photoexcitation.

Operation: The timescales of interest to the time-resolved community span from ps to s, and in a single project users will collect data across several orders of magnitude in time. We anticipate, therefore, that AMALFI will run in ultra-fast mode (requiring the DLS ring to operate in hybrid or single bunch mode) for only a small portion of each run with minimal impact on other beamlines, during which time users will collect their ps-μs time-resolved data. For the majority of the run (with the DLS ring operating in normal top-up mode) AMALFI will operate conventionally, appropriate for μs-s time-resolved studies and the other experiments detailed herein.

4. Community

The strategic importance and potentially enormous impact of this proposal are evidenced by the extremely high levels of support shown for the original proposals (> 200 Principal Investigators, plus their collaborators within academia and industry from the UK, mainland Europe, USA and Japan). There is also strong interest in AMALFI from groups wishing to exploit the unprecedented time resolution possible to study irreversible molecular and macromolecular systems or use the revolutionary multi-crystal approach for rapid screening of key industrially relevant materials. Strong support from overseas research groups confirms the significant likely international impact of the facility, while direct and indirect industrial support attests to its potential to bolster wealth creation in fields such as human health, energy and manufacturing. AMALFI will be unique in terms of new experimental methods, the range of sample quality that can be accommodated, and the high throughput it will deliver. By enabling experiments that are impossible elsewhere, it will provide a major stimulus to, amongst others, the large and vibrant UK chemical and macromolecular crystallography communities. These are amongst the most diverse and productive in the world, in part due to the platform provided by previous facilities at Daresbury and now at Diamond. In fact, the process of preparing this bid has already identified several exciting new interdisciplinary projects which could be hosted on AMALFI, which will be a unique resource that supports both the biological and chemical communities, providing a focus for the exchange of best practice as well as stimulating the development of new cross-disciplinary science and technology.

5. References

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