Diamond-II
Proposal for flagship project:
I24: Kinetic Micro Crystallography

Science Group: Macromolecular Crystallography

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I24: Kinetic Micro Crystallography

1. Summary/Impact statement

KMX is a flagship beamline upgrade to I24 that will enhance its capability in the measurement of high quality, low-dose, microsecond time-resolved MX data from cryo-cooled and room temperature 1 – 10 micron-sized crystals. Critically it will build upon I24's strong track record in delivering high quality microfocus capability by further optimising the beam delivery systems to improve signal to noise. In the area of TR crystallography KMX will provide well-integrated tools for initiating and tracking reactions in the microsecond to second time domain, thereby complementing the femto- to nano-second time resolution that can be achieved at XFELs. The project goal will be to enable routine microsecond, microbeam SSX capability within the envelope of a world-class microfocus structural biology beamline.

2. Scientific Case

I24 is the highest impact life science beamline at Diamond. For more than a decade it has provided users with state-of-the-art microfocus capability that is high throughput, delivers high quality data and is very easy to use. It has been in very high demand from industry and has helped expand our understanding, for instance, of a range of therapeutically relevant GPCR structures [1-6] as well as providing insight into foot and mouth disease virus, malaria, Parkinson's disease and antibiotic resistance [7-10]. Its ability to provide *in situ* screening and data collection alongside cryo-crystallography was a unique innovation at a microfocus beamline [11]. In recent years the portfolio of capability has been broadened to include fixed target and extruder serial synchrotron crystallography (SSX): capability that provides users with very low dose snapshots of radiation sensitive samples, retaining biological and chemical relevance of the structures, and is beginning to provide time resolved data for some proteins on the millisecond timescale [12, 13].

Time resolved macromolecular crystallography has enjoyed a renaissance in recent years in no small part due to the promise of femtosecond, and slower, time resolution promised by X-ray free electron lasers using serial femtosecond crystallography (SFX) [14]. Together advances in sources and sample delivery have opened the door to following irreversible processes *in crystallo*. More recently these serial sample delivery techniques have been exploited at, and optimised for, data collection at synchrotrons – so-called Serial Synchrotron Crystallography (SSX) [15] - a field in which I24 and the XFEL Hub are world leaders. Time-resolved SSX at I24 can already reach the millisecond timescale and the combination of Diamond II and KMX upgrades will facilitate a push into the microsecond time domain - much faster than the average 60 millisecond turnover time of enzymes – enabling capture of short-lived and radiation sensitive intermediates.

Despite the dramatic advances in cryo-EM, MX remains the leading tool for visualisation of enzymes on an atomic scale. It provides an essential and very rapid means of determining the shape of enzymes in detail, and reveals how they interact both with each other and with small molecules such as drug fragments. The ability to collect a series of structures of an enzyme along a catalytic reaction coordinate will enable molecular stop-motion movies to be produced, providing direct evidence of how protein structure relates to function. Moreover, simultaneous collection of complementary spectroscopic data (*e.g.* X-ray emission and/or electronic absorption, fluorescence, or Raman spectroscopies), will provide the experimenter with both atomic and electronic information, reducing model ambiguity and significantly increasing functional insight.

An example of where such an approach could have a significant impact is in understanding the sequential acylation and diacylation of the ß-lactam ring that occurs when ß-lactams interact with penicillin binding proteins and with ß-lactamase enzymes. ß-lactamases provide resistance to antibiotics – a significant risk to human health – and determination of their atomic structure can provide insight into compounds that inhibit their function [16]. Some of the first experiments at the European XFEL determined the structure of a ß-

lactamase providing a first step to revealing transition states during inhibitor binding [17]. A second key area is the structural biology of membrane proteins such as GPCRs. In a striking contrast, 60% of drugs target membrane proteins yet they comprise less than 5% of structures in the protein databank. X-ray crystallography has the power to visualise unusual binding modes in GPCRs [18] through provision of a snapshot of a ground, or bound, state. GPCRs are dynamic molecules, however, where ligand binding produces a conformational change that ultimately triggers a cellular response. KMX will build on the first steps of serial [19, 20] and microfocus high throughput GPCR crystallography [21] to provide visualisation of conformational changes such as these complementing other Diamond-II capabilities such as time-resolved SAXS.

Reactions and catalysis can be triggered in crystals by several means, the fastest being laser illumination. Laser excitation allows fast biological processes such as carbon monoxide binding in myoglobin [22] and isomerization of photoactive proteins [23] to be probed in crystals. The use of photocages can expand this approach so that it becomes applicable to enzymes that are not light dependent [24]. For slower processes, such as changes in conformational modes or ligand binding, substrate can be mixed with [25] or ejected [26] onto crystals. For substrate binding, and larger conformational changes, a key premise is that data should be collected from microcrystals (less than ~10 microns in size). Microcrystals allow efficient soaking of substrates into crystals, meaning that diffusion times do not limit the time resolution that can be realised [27].

Due to the extremely high flux densities that will be realised, and a desire to also routinely work at room as well as cryo temperatures, crystals will have an extremely short lifetime at KMX and it will not be possible to obtain a complete dataset from a single crystal. On-line tracking of *in crystallo* reactions and cross-validation of where a crystal is on the catalytic pathway will aid in merging, processing and interpretation of diffraction data.

A critical focus for the beamline should be on ensuring the serial approaches offered are sample efficient, since this currently limits the impact of the method. Currently data from many hundreds or thousands of crystals are merged to obtain an SSX dataset: hugely daunting numbers for the majority of crystallographers. The use of a wide bandpass multilayer helps address this challenge, significantly reducing the number of crystals required to form a dataset. The gain in flux provided by the multilayer also provides access to the microsecond time domain by reducing the time required to obtain an interpretable diffraction pattern.

The proposed major upgrade to I24 will ensure that microfocus MX at Diamond remains world-leading for data collection from small, challenging crystals with significant improvements to the optics, endstation and detector. This will encompass upgrades to undervalued components such as slits, apertures, scatterguards and the beamstop which have a large impact on signal to noise and hence the quality of data that can be obtained from small crystals [28]. The full integration of serial approaches and time-resolved crystallography builds on this providing a pathway from static single crystal structures to a molecular movie. Provision of both by KMX makes the feedback loop more efficient, allowing iteration towards the ultimate goal of many time-resolved data points along a reaction co-ordinate. Automated cryo-MX capability will ensure the beamline continues to offer high throughput microfocus MX of the highest standard and latest technologies in-between demanding dynamic and microfocus experiments balancing the load across the other beamlines, retaining Diamond's overall capacity and meeting the UK's demand for protein crystallography.

3. Benefit to the Diamond research community

A major I24 upgrade will improve the delivery of high impact science through microfocus cryo-data collection capability and will provide additional routine access to low dose datasets from challenging radiation sensitive protein samples where the interpretation of biological function can be compromised by X-ray damage. KMX will enable Diamond to build upon its competitive position in SSX by developing dynamic crystallography at Diamond to create an exciting and highly relevant asset for the already vibrant XFEL-Hub community. Far from merely providing a pathway towards XFEL experiments however, KMX will be able to probe key

biological timescales, provide physiologically relevant low-dose room temperature structures and improve upon its high-throughput cryo-MX capability for both academic and industrial users.

Industrial use of serial techniques is in its infancy. Due to the potential of room temperature SSX to provide a complete assessment of protein-drug kinetics and enable optimisation of drugs targeted to transition states, this use can be expected to increase and this is reflected by ongoing collaborative experiments between I24 and multiple industrial groups. Application of key synchrotron attributes such as throughput, exploitability by non-expert users, and automated processing pipelines will drive uptake by both the academic and industrial user communities.

4. Outline Specification

We propose a repurposing/redesign of I24 which already provides and has expertise in some of the key functionality proposed. The key aspects of the beamline are defined by the science case above. Current beamline parameters are summarised in table 1.

- The Diamond-II lattice together with CPMU and a DMM will offer high flux (>10^{14} ph s^{-1}) at both ~12.4 keV and 25 keV plus.
- Variable focus of ~1 - 30 micron (though would anticipate predominately operating at the smaller end of this scale).
- Highly flexible sample environment. This will build on the existing model developed at I24 where micro-crystallography and serial approaches make use of the same sample position exploiting identical core instrumentation such as sample visualisation and automation.
    - High precision retractable goniometer equipped with cold nitrogen stream.
    - Full integration of established serial approaches such as fixed targets, pL – nL drop on demand sample delivery, and viscous media extruder.
    - Ability to accommodate new developmental approaches. SSX is a rapidly evolving field so the ability to 'empty' the sample environment to accommodate novel approaches is extremely valuable and, crucially, does not compromise core functionality if built into the initial design.
- Fast shuttering
    - Chopper for accessing fast time points that is fully synchronised with sample delivery and detectors as well as the storage ring clock.
    - Complex shuttering, exploiting for example Hadamard encoding, offers access to shorter time domains [29]. It may also be possible to achieve this by electronic detector gating or use of a detector such as the Timepix.
- Light delivery to the sample position should be built into the initial design
    - fs, ns, and/or cw laser illumination options from the UV through IR region.
    - If feasible decouple laser commissioning/setup from need for X-ray shutter to be closed perhaps by provision of laser hutch adjacent to beamline.
    - Protocols for a permanently dark or selective wavelength lighting of the sample environment (including during hutch search) should be incorporated.
- Multiple detectors built into initial design. Parallel (tagged) readout so different data types can be cross correlated.
    - Diffraction (large area, high frame rate, integrating, forward direction)
    - Multi-crystal wavelength dispersive X-ray spectrometer in von Hamos geometry to collect XES from first row transition elements.
    - *in situ* spectroscopies such as Raman, UV-Vis absorption.
- The large area detector will need to be integrating due to the extremely high count-rates that will be realised and a high Z sensor (CdTe or CZT) is essential if energies above ~17 keV are to be exploited. These key characteristics are provided by the proposed SFTC Dynamix detector.

- State-of-the-art automated sample exchange for both cryogenic and room temperature samples allowing high throughput cryo-MX and remote SSX for less complex experiments.
- Offline sample preparation in close proximity to beamline is essential.

5. State of the art benchmark

Beamline	Focus μm²	Flux × 10¹³ ph s⁻¹	Energy keV	Detector	Sample delivery	Other key features
I24	6×6 to 50×50	0.8 (12.4 keV)	7-30	Pilatus3 6M CdTe Eiger2 9M	cryo pin, tray, fixed target, extruder	Two goniometers PORTO
VMXi	5×5 to 30×30	~0.2 (DCM) ~1.5 (DMM)	10-25	Eiger2 4M	Tray	Highly automated, unattended data collection
KMX	1×1 to 30×30	~1.5 (DCM) ~15 (DMM)	7-30	CdTe Eiger2 9M Jungfrau/Dynamix tbc	Cryo pin, fixed target, viscous extruder, drop on demand	XES + spectroscopy Dual DCM DMM PORTO. Chopper shuttering
MicroMAX, MaxLab	1×1 to 10×10	0.8 (DCM) 100 (DMM)	5 - 20	Integrating tbc	Cryo pin. range of serial tbc	Dual DCM DMM. Chopper shuttering Initial SSX will be fixed target
ID29, ESRF	0.5×0.5	100* (10 keV)	10-25 and 35	Jungfrau 4M	Fixed targets and extruders.	Multilayer. Chopper shuttering Two experimental hutches
FMX, NSLS-II	1×1.5 to 10×10	0.35 (12.4 keV)	5 – 30	Eiger 16M	Cryo pin. Serial in development	Two goniometers
P14-EH2, PETRA-III	15×10	0.02 (12.7 keV)	12.7	Pilatus3 6M	Cryo pin (EH1) Fixed targets (EH2)	Parameters are for EH2. Laser excitation available

Table 1. Vital statistics of I24, VMXi and KMX with comparable existing and upcoming beamlines at other sources. Note that these beamlines are in varying states of design and commissioning so numbers may be estimates, hardware subject to change, and capabilities somewhat aspirational.

Table 1 summarises comparable synchrotron beamlines: MicroMAX[1] MAX-IV, Lund); ID29[2] (ESRF, Grenoble); FMX[3] (NSLS-II, Brookhaven); and P14-EH2[4] (PETRA III, Hamburg). Key to KMX is parallel readout of different detectors so both atomic and electronic structural data can be simultaneously obtained from crystals. A complementary focus on room temperature data collection from modest numbers of crystals, exploiting for example the showers of crystals often obtained during crystallisation, is also key and bridges the gap to serial experiments for non-expert users who comprise the vast majority of the UK's structural biologists. This would complement the massively automated capabilities of VMXi to collect room temperature data from 1000s of crystals per day in crystallisation media. There is a synergy in building a room temperature community around I24, VMXi, and the XFEL-Hub to develop current and emerging methods. Together, and working with the XChem facility, a range of opportunities to provide complementary information at near physiological temperatures will be enabled, particularly the area of drug discovery.

I24 KMX would also compare to XFEL beamlines in terms of its scientific focus although, due to the nature of the source, it primarily targets slower timescales. Serial X-ray diffraction MX methods are also complementary to the emerging fields of serial electron diffraction [30, 31] and time resolved cryoEM [32]. Both of these approaches suffer from limitations however, for example a restriction on crystal thickness to a few hundred nanometres or the need to cryo-cool. While both will certainly evolve in the coming years, serial X-ray diffraction sits in a sweet spot, with a common theme between all fields being sample delivery: approaches developed for KMX can have a reach well beyond the beamline and SSX.

[1] https://www.maxiv.lu.se/accelerators-beamlines/beamlines/micromax/
[2] https://www.esrf.eu/cms/live/live/en/sites/www/home/UsersAndScience/Experiments/MX/About_our_beamlines/id29.html
[3] https://www.bnl.gov/ps/beamlines/beamline.php?r=17-ID-2
[4] https://www.embl-hamburg.de/services/mx/P14_EH2/

6. Community engagement

Workshops will be led by DLS and XFEL Hub aiming to expand the current user base of serial techniques at Diamond. Close collaboration with a small number of groups (as currently ongoing with Hough, Tews, Schofield and others) on serial techniques at Diamond and XFELs is essential for developing methodology and to provide exemplar use cases. The beamline working group will be made up from a cross section of academia and industry.

7. References

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