VMX

A versatile micro-focus and in-situ diffraction facility for macromolecular crystallography

A proposal prepared for Diamond SAC October 2011

Acknowledgements

This proposal has been prepared by a user community working group and members of the Diamond MX village that has been chaired by Dave Brown (Cangenix Ltd.). The contributors are listed below. The contributions of Diamonds optics and insertion device groups is also gratefully acknowledged.

Name	Affilliation	Role
Dave Brown	Cangenixs Ltd.	Chair & co-proposer
David Barford	ICR, London	Co-proposer
Philip Evans	LMB, Cambridge	Co-proposer
Jonathan Grimes	U. Oxford and Diamond	Co-proposer
Ben Luisi	U. Cambridge	Co-proposer
Peter Moody	U. Leicester	Co-proposer
Ehmke Pohl	U. Durham	Co-proposer
Pierre Rizkallah	U. Cardiff	Co-proposer
Roberto Steiner	Kings College, London	Co-proposer
Ivo Tews	U. Southampton	Co-proposer
Johan Turkenburg	U. York	Co-proposer
Frank von Delft	SGC, Oxford	Co-proposer
Roger Williams	LMB, Cambridge	Co-proposer
Keith Wilson	U. York	Co-proposer
Snezana Djordjevic	UCL	Co-proposer
	Diamond co-proposers	
Gwyndaf Evans, Dave Hall, Katherine McAuley, Thomas Sorensen, Armin Wagner and Martin Walsh		

1. Summary

Macromolecular crystallography (MX) is the most powerful technique at our disposal to visualize biological structure at the atomic level – its impact on our understanding of biological processes is unquestionable. The UK crystallographic community has pioneered the field from the first atomic pictures of DNA, myoglobin, haemoglobin and lysozyme to the recent landmark structures of the first membrane-containing viruses and the 30S ribosomal subunit. Molecular interactions studied by crystallography encompass protein-protein, protein-nucleic acid, protein-carbohydrate, protein-lipid; and the interaction of macromolecules with small molecules, enzyme substrates and regulators. These structures and interactions make up the molecular machinery of the living cell and the impact of the work has been recognised with 7 Nobel Prize winners in recent years of which two are from the UK.

The VMX proposal, aims to provide a high flux density, submicron variable focus beam for the most demanding crystals samples. It will also provide an entirely automated facility for characterisation of, and data collection directly from, crystallisation experiments *in situ*. The VMX beamline will plug significant gaps in the Diamond MX Village portfolio, to enable the elucidation of structures beyond the reach of the existing infrastructure.

VMX will extend existing automation to (i) include tomographic-enabled matching of beam to crystal and (ii) include and develop *in situ* analysis seamlessly within existing crystallisation pipelines. The provision of both sub-micron X-ray beams and new methods of sample handing and data collection will enable data to be measured from crystals on the order of, or less than, a micron in size. The facility will deliver major benefits to the entire UK MX community, both academic and industrial, for whom the generation of diffraction capable crystals is the major bottleneck on their productivity.

2. Scientific case

2.1 Introduction

Structure underpins our understanding of cellular function, for example through the study of enzyme mechanisms a vast number of metabolic processes including cell signalling, cell motility and division have been revealed. Macromolecular crystallography (MX) has been the key technique to provide atomic resolution for biological structures for the past four decades. During this period significant advances in molecular biology and crystallographic methods and at synchrotron beamlines have made the solution of many crystallographic problems routine. There has been an explosion in the application of crystallography to the study of biological systems in the human genome era, with a huge increase in the number of scientists utilising MX and also a number of Structural Genomics/Proteomics Initiatives, including, in the UK, the Scottish Protein Production Facility, OPPF-UK, located adjacent to Diamond, and the Structural Genomics Consortium. MX beamlines have become highly automated and increasingly efficient, with scheduling carefully managed to ensure that as much of the valuable synchrotron time as possible is productively used exposing samples. There have been significant productivity improvements through standardisation of sample handling, data collection and structural solution. These advances and initiatives have also extended the reach of the method in terms of both sample quality and the size and nature of the crystallographic problems that are tractable.

The current microfocus beamline (I24) has pioneered microfocus experiments at Diamond and highlighted the potential of what can be achieved with samples of a few microns in size. Additionally I24 has been utilised to explore the possibilities of *in situ* diffraction (collecting data on crystals in the same environment in which they were grown). These initial experiments, some of which will be exemplified in the following scientific cases, have highlighted both the potential value of a sub micron microfocus endstation and also the additional benefits of integration of a dedicated automated *in situ* diffraction mini-hutch. The complementarity that will be offered by the facility is aptly illustrated by the structure determination of the polyhedrin protein from cytoplasmic polyhedrosis virus (CPV15).

2.2 Science enabled by submicron variable focus capabilities

Obtaining high quality atomic structural information about important biological molecules, complexes and systems is currently the domain, principally, of X-ray crystallography. In turn the quality of the results is critically dependent on the quality (order and homogeneity) and size of the crystals that can be prepared.

Often in science the more interesting the problem, the more scientifically challenging it is. This is frequently true in biological crystallography; the more biologically relevant and important a molecule or macromolecular complex is, the more difficult it can be to produce well ordered, large and homogeneous crystals. The inherent flexibility of many important classes of macromolecules inhibits the production of a crystal lattice with the required long range order. Indeed some important biological systems only yield small crystal as a result of restrictions in crystal growth due either to induced lattice strain from molecular geometry [1] or because the crystals have natural inclusion bodies that again induce lattice strain thereby inhibiting growth [2].

Since the late 90's, when the first monochromatic microbeams were used to solve protein crystal structures [3], their use has become routine. Indeed beamlines with beams of between 5 and 20μm in size, and photon fluxes exceeding, 10^{12} ph/s, are now available at most 3rd generation synchrotron sources. Their impact has been profound since they have enabled high quality data to

be measured from small, poorly ordered, inhomogenous and weakly diffracting crystals of membrane proteins, large macromolecular complexes and viruses. Along with the instruments themselves, techniques for rapidly characterizing crystal quality immediately before data collection have been devised, that enable scientists to perform the best possible experiments.

There are several recent examples of the structural elucidation of members of the large and hugely important family of G-protein coupled receptors (GPCRs) which could only be solved using

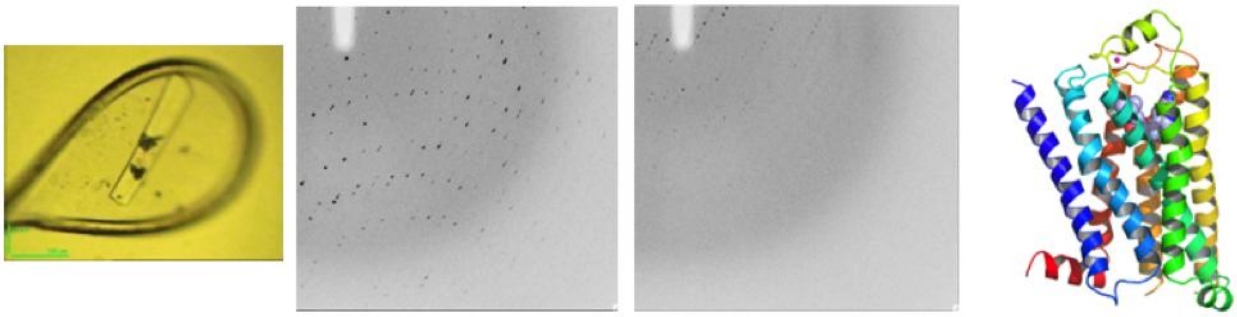


Figure 1. β1 adrenergic receptor [4] (left panel) a crystal of cryo-cooled in a loop, showing two regions of X-ray exposure (centre-left) diffraction pattern from a good part of the crystal (centre-right) from a bad part (right) schematic diagram of the structure.

microfocus beam lines. This family of proteins are of interest both because of their crucial role as gatekeepers in the peripheral membrane of cells, and because they are major targets for many important medical drugs. For example, the β_1-adrenergic receptor was solved [4] from a single crystal using 3 sweeps of data, selected from the 7 positions collected from the thin plate crystals. As can be seen from Figure 1, there was a great difference in the quality of diffraction between the best and worst parts of the same crystal.

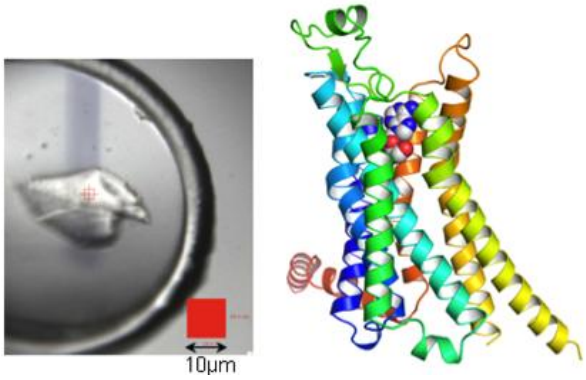


Figure 2. Adenosine A2a receptor, collected on Diamond beamline I24 with a 10µm beam (left) from 4 crystals, with up to 4 positions on each crystal, (right) structure with bound adenosine

Similarly another GPCR, the A2a receptor

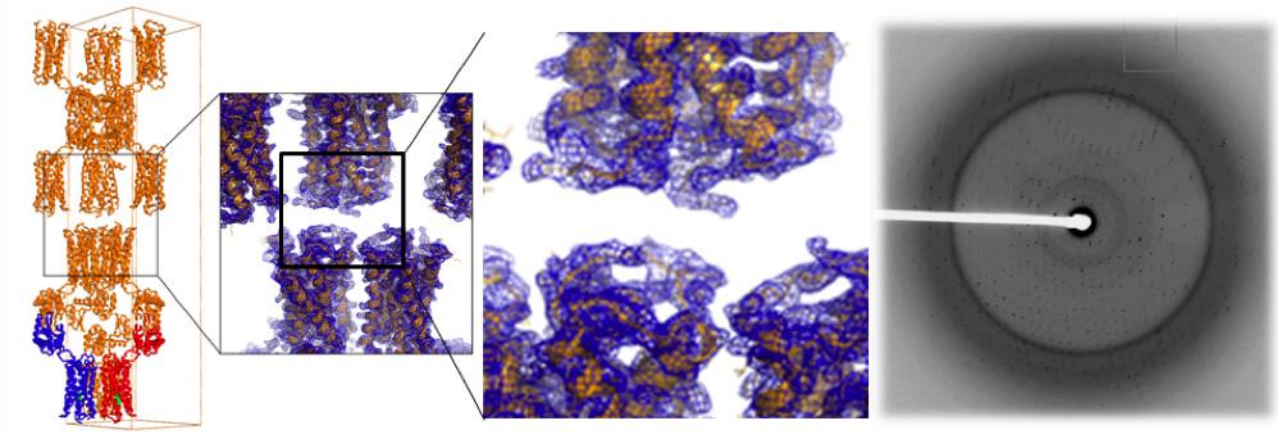
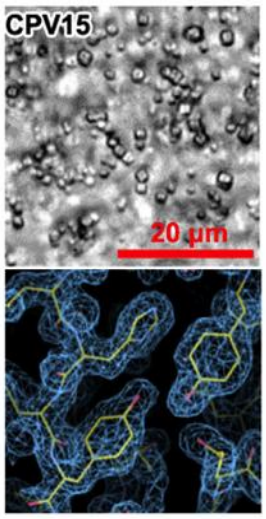


Figure 3. Electron density (left) from the final refined model of the Histamine H1 receptor along with the diffraction (right)obtained from the best crystal (30 x 10 x 5 µm). Several hundred crystals were screened over many months before finding a good crystal diffracting to beyond 3.0Å. The central figure illustrates the poor contacts between molecules forming the crystal lattice and is the principal cause of the challenges associated with membrane proteins.

(Figure 2), needed a microfocus beam to measure data from crystals which showed a substantial variation in quality within the each crystal [5]. The structure of the A2a receptor is now being exploited by the pharmaceutical company Heptares in a search for medically useful ligands [6]. More recently at the Diamond I24 microfocus beamline the structure of another medically relevant GPCR protein Histamine H1 receptor in complex with a 1st generation anti-histamine drug Doxepin has been solved [7] (see Figure 3). Diffraction data were recorded from a single crystal but in 8 positions across its length. Data from 5 positions were scaled to provide the final data set. The elucidation of this structure combined with other bound ligand structures can now assist in the development of advanced 2nd generation drug compounds against severe allergic reactions.

The power of microfocus beams for recording data from micron sized crystals has also been demonstrated at I24 with the structure solution of CPV15 polyhedrin (spacegroup I23; a=b=c=103 Å). The crystal size varied between 1.3 and 1.5 µm and data were recorded with a 5 µm × 5 µm FWHM beam size. A total of 40 native crystals and 112 Se-Met crystals were used for phasing to 1.8 Å by the Single Isomorphous Replacement with Anomalous Scattering (SIRAS) method. The crystals and the resulting high quality electron density are shown in Figure 4. This case is considered in more detail in § 2.2.1. (Figure 5).



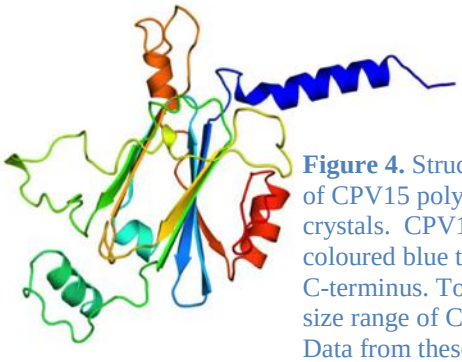


Figure 4. Structure determination of CPV15 polyhedrin from 1.5µm crystals. CPV15 polyhedrin is coloured blue to red from the N to C-terminus. Top left panel shows size range of CPV15 polyhedra. Data from these crystals were collected to 1.8 Å resolution. Bottom left panel shows a representative portion of the 1.8 Å density map.

All of the above examples have been enabled by microbeam capability; moreover the ability to vary the beamsize has been a critical part of optimising data collection strategy to accommodate crystal variability. The membrane protein examples shown have all suffered from poor crystal homogeneity, difficulties in obtaining large, ordered crystals and weak diffraction. The combination of high flux, variable microbeams and large, low noise X-ray detectors has yielded high quality structural information about medically important targets. The provision of additional microbeam capability with a beamsize range an order of magnitude smaller than I24 will open doors to even more challenging biology by broadening the ability of the MX Village to serve the UK community.

2.2.1 Pushing the limits with micron sized beam

Aside from crystal quality, size and diffracting power, the major factors limiting data quality are X-ray background and radiation damage. Microbeams provide an obvious route to reduce the X-ray background for small crystals through the ability to match beam size to crystal size. Radiation damage however is always present, even for crystals at 100K. 'Real-world' calculations performed by Holton and Frankel [8] have shown that for two extremes of crystal samples, lysozyme (14 Da per Asymmetric Unit or A.U.) crystals yielding 2 Å data and 10 MDa per A.U. crystals (4× larger than the 70S ribosome) yielding 3.5 Å data, the crystal diameters (assuming them to be spherical) would need to be 8.3µm and 43 µm respectively in order to yield complete data sets. This calculation assumes a fairly poor detection limit of 100 photons per diffraction spot determined assuming an X-ray background per image of ~100 photons per pixel. The crystal dose limit is assumed to be 30 MGy [9]. The CPV system mentioned above (which has a molecular mass ~2x

that of lysozyme) has already broken this theoretical size barrier and the VMX proposal will allow us to push these limits further.

Predicted limits using multiple crystals

It is already common-place when solving structures using microcrystals to accumulate data from multiple crystals and using data sets comprising of data sets from more than 100 crystals to determine novel structures (Geoff Sutton, personal communication). We can calculate that accumulating a data set from 100 crystals would reduce the crystal size required for the two exemplar cases above to 3.6 µm and 18.5 µm respectively. If we were able to reduce the recorded X-ray background to zero then crystal sizes of 1.2 µm and 6.5 µm could be used (see Table 1). At present real experiments at I24 can already achieve a significant fraction of this advantage, since by combining a microbeam and large, low noise Pilatus 6M detector. For example, as already mentioned, data have been recorded and a structure solved for CPV15 polyhedrin (28 kDa per A.U.) using crystals of between 1.3 and 1.5 µm linear dimension (volume range from 2.2 μm^3 to 3.4 μm^3) using a non-optimal beamsize of 5 µm × 5 µm (FWHM). Diffraction from one of these crystals is shown in Figure 5. The observed background in this case is already low (~6 counts per pixel), far lower than that assumed in the Holton and Frankel estimates.

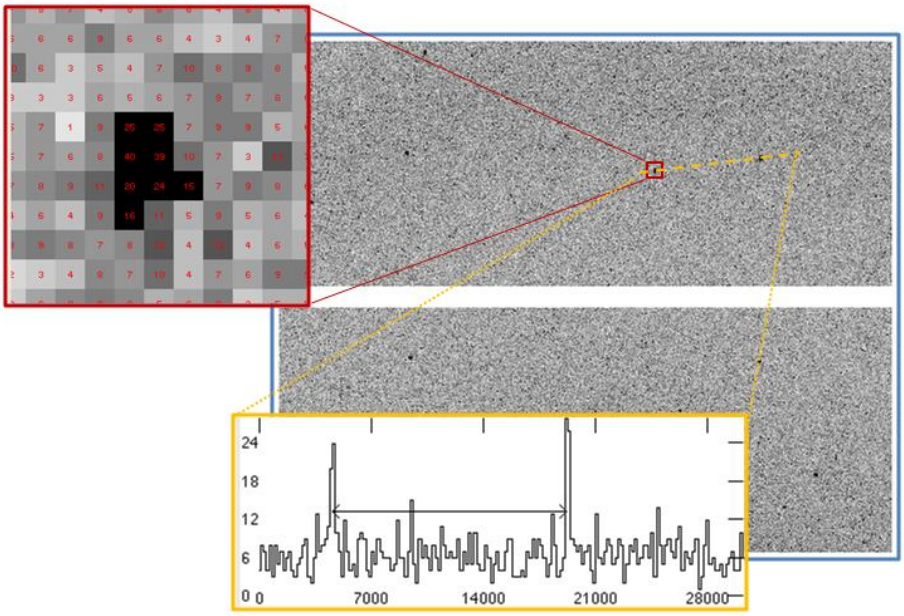


Figure 5. A region of a diffraction pattern from a single crystal of CPV15 polyhedrin (max. dimension 1.5 µm). The average background in this region is 6 counts per pixel on average.

Table 1 summarises the arguments given above and shows where the I24 example of polyhedrin sits in the context of the Holton and Frankel calculations. I24 is not optimised for crystals < 5 µm, nor was it designed to be, and the gains in reduced X-ray background that might be achieved using beams of ~1µm FWHM would be significant. To a rough approximation (calculated using MOSFLM style summation integration [10]) the reduction in background by a factor of 10, that would be achieved by using a 1.5µm beam (instead of 5 µm), would immediately result in a factor of 2 improvement in the signal to noise for the weakest measurable spots[1]. The implication of this is that such a reduction in X-ray background, achieved simply by matching the size of the X-ray

[1] Calculation is performed using a MOSFLM style summation integration [10] and assumes a diffraction spot containing 10 counts extending over 9 pixels sitting on a background of 6 counts per pixel and a 9 × 9 integration box size. This gives a resulting I/σ=1.2. A further reduction in background to 0.6 counts per pixel increases I/σ to 2.5.

beam to that of the crystal, would enable the crystal volume to be further reduced by a factor of ~2.5 whilst yielding data of equivalent statistical quality.

		Av. BG (counts/pixel)	ph/*hkl* (at highest resolution)	high resolution limit (Å)	Diameter for complete data set	Diameter for 100 crystal dataset
Realistic data	14 kDa	100	100	2.0	8.3	3.6
	10 MDa	100	100	3.5	43	18.5
Ideal data	14 kDa	0	4	2.0	2.8	1.2
	10 MDa	0	4	3.5	15	6.5
I24 example (polyhedrin)	28 kDa	6		1.8		1.6 (spherical equivalent)

Table 1 Calculations from Holton and Frankel for 'realistic' and 'ideal' data sets from a small well diffracting 14 kDa protein crystal and a larger 10MDa protein crystal. Using crystal volumes 100 times smaller (assumption of 100 crystals per complete data sets) we calculate predicted usable crystal diameters for data collection. In these calculations all crystals are assumed spherical and their diameter is given.

Expected gains with microcrystals and microbeams due to photoelectron escape

Much radiation damage is assumed to be caused by photoelectrons and is deposited a little distance from the site of the initial photon interaction. Thus if the beam size is comparable to the mean path length of the photoelectrons much of the damage might be inflicted on a region outside the irradiated volume. The effects of photoelectron escape have not been considered in the above analysis, but recent work by Sanishvilli *et al* [11] suggests strongly that for X-ray beams and samples of ~1µm in size and X-ray energies above 18 keV that the decrease in observed radiation damage might be as much as ×3. This suggests that the minimum crystal volume that might be measured would therefore be reduced by a factor of 3. In aggregate, with the X-ray background gain and the resultant 7.5 fold reduction in crystal volume required, (to 0.3 μm^3) would mean that high resolution structure determination could be achieved for a CPV cubic crystal of edge length 0.67 µm.

In summary we believe that for systems of molecular mass up to several MDa, using optimised low noise detectors and versatile micro beams would allow useful multicrystal data sets to be recorded from crystals of dimensions between 0.5 and 5 µm. It would be essential to have high flux X-ray beams in the same size range with energies of 18 - 30 keV [11] to take advantage of the photoelectron escape effect.

Recent exciting work published by Chapman et al. [12] has demonstrated the feasibility of recording diffraction data using FEL radiation on samples of between 0.3 µm and 0.7 µm, albeit to low resolution (8.5 Å). The 70 fs FEL pulse delivered a sample dose of 700 MGy to the crystals and a total of 15000 crystals were required to generate sufficient data to allow the calculation of an electron density map using known phases. Although the dose of 700 MGy is much greater than the 30 MGy limit which applies for a continuous source, the FEL experiments were also non ideal and used a beam cross section approximately 100 times greater than that of the crystal. Optimisation of the beam to the crystal, whilst being extremely challenging, would enable a much lower background for the FEL case as well. The FEL experiments, like the I24 experiments on

polyhedrin, demonstrate that useful data can be collected on very small crystals. Taken together recent results strongly suggest that structure determination from submicron crystals of macromolcules can be turned into a reality with the proposed versatile microfocus beamline.

2.3 Science enabled by *in situ* diffraction capabilities

The *in situ* facility addresses three related current needs in macromolecular crystallography (MX):

- identifying diffraction quality crystals for biological systems that are difficult and expensive to crystallize (*membrane proteins; large complexes*)
- obtaining diffraction datasets for the biological systems of which crystals are routinely resistant to cryogenic harvesting (*large complexes; viruses; membrane proteins*)
- characterizing protein-ligand interactions rapidly by collecting large numbers of datasets from similar crystals with different compounds added (*industrial drug development*)

Currently most macromolecular structure determinations based on synchrotron data rely on cryogenically protected frozen crystals, primarily because of the dramatic (~100 fold) reduction in radiation damage that this usually achieves. Although the choice of cryo-protectant remains largely *ad hoc* there are now standard procedures for the cryo-cooloing of macro-molecular crystals, which are used routinely on the suite of beamlines within the Diamond MX village. The great success of cryo-data collection has masked two ever-present challenges that are addressed by the *in situ* HTP side station proposed here.

Crystallisation is often a very long and iterative process where initial *promising* conditions are optimised until crystals suitable for the diffraction experiments are obtained. But, crystallisation is in almost all cases separated from the diffraction experiment. Crystals are more often than not optimised on the basis of their appearance, due to the difficulty to freeze and ship every potential sample to the synchrotron. Secondly standard procedures when preparing crystals for cryo-cooled data collection involve the manual physical manipulation of individual crystals, soaking them in cryo-protectants and subsequent cryo-cooling. Inevitably the mechanical and osmotic stress that occurs during these steps will often significantly reduce the diffraction capabilities of the crystal.

There are four obvious advantages of *in situ* diffraction: (i) experiments can be carried out without any manipulation of individual crystals, thus preserving the crystal integrity, in contrast to the standard cryo-protection procedures; (ii) it provides immediate feedback on the diffraction, crystal quality and, in many cases, unit-cell parameters, space group, even in the case of micro-crystals (2-5 μm); (iii) the method can be fully automated with high reliability; (iv) complete datasets can be collected rapidly from crystals that consistently lose all diffraction when conventionally harvested, or else are hazardous and may not be harvested for safety reasons.

At Diamond a directed approach has explored the benefits of *in situ* analysis, providing exemplars and proof of principle experiments at I24 that confirm the feasibility of the technique for performing non-standard and challenging diffraction experiments using 10 to 40 μm beams. These are discussed in detail below along with a discussion of the advantages that an *in situ* facility would offer to industry.

2.3.1 Identifying diffraction quality crystals

Characterisation of membrane protein crystals.

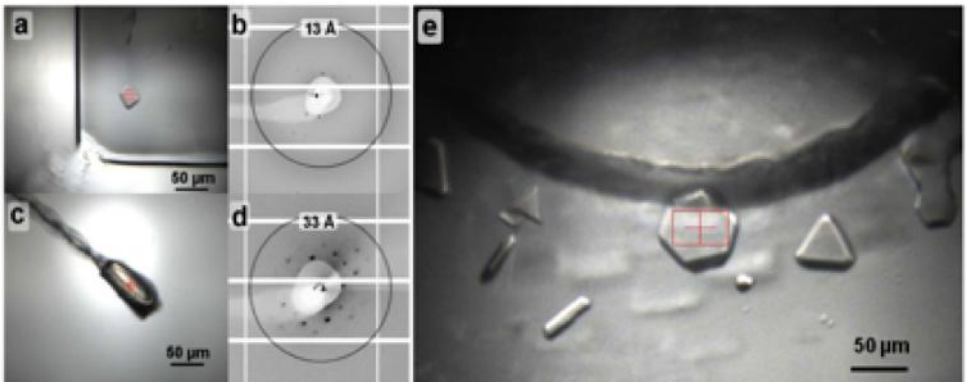


Figure 6. Inserts a, b, c and d show the detrimental impact of manipulating and freezing crystals on diffraction quality in cases where samples are particularly sensitive. *In situ* diffraction (a and b) from crystals of a large multiprotein complex (courtesy of the Membrane Protein Laboratory) shows diffraction to ~10 Å whereas frozen samples (c and d) are limited in diffraction to ~30 Å: a considerable improvent enable rational decisions about impact of crysallizaton conditions to be made without concerns about the rôle of other factors. Insert e) illustrates preliminary hits from lipid cubic phase crystallization experiments for a membrane protein (courtesy of the MPL). In this particular case using crystal morphology and size alone as a guide to crystal optimisation could have misled scientist and wasted time and money. Diffraction from these samples showed that every hit was in fact a crystal of detergent.

The ability to rapidly characterise the diffraction properties of potential crystals using *in situ* diffraction will provide a particular competitive edge for the analysis of unstable large macromolecular complexes, membrane proteins, and for the assessment of initial micro-crystal hits. A facility to test without manual intervention whether your "micro crystal" hits are protein and diffract, will mean that immediate feedback can be given, so that either crystals can be further optimised or, equally importantly, false positives can be rapidly discarded, saving both time and money. This is particularly valuable in the case of membrane protein crystals grown in lipidic cubic phase where the harvesting is extremely problematic. The importance of using *in situ* diffraction analysis in the optimisation of crystallization conditions is illustrated in Figure 6 which demonstrates the deleterious effects that freezing can have on some sensitive samples and how easy it is to be misled by the visual cues given by crystal size and morphology in the absence of hard evidence about diffraction properties and quality. This will be generally important not only in the specific case of membrane proteins but also more generally for 'sensitive-to-manipulation' crystals. This was a key motive for the creation of the Wellcome Trust funded Membrane Protein Lab (MPL) adjacent to the microfocus beamline I24 at Diamond.

Characterisation of micro- crystals

We have recently conducted test experiments to show that we can screen ~3-5 µm crystals (in this case of CPV polyhedra) for diffraction at room temperature using *in situ* diffraction plates. A drop of solution containing polyhedra crystals was placed in a Greiner CrystalQuick™ X plate (designed for *in situ* experiments), and the drop scanned for diffraction, using a 5x5 µm beam on I24. As Figure 5 shows, spots to beyond 3 Å were readily observed, even though optical visualization of the sample was not possible. To be able to observe diffraction from ~3-5 µm crystals at room temperature is quite remarkable and shows the potential of this method for rapidly and reliably assessing the diffraction of even micro-crystals.

Existing MX beamlines at Diamond are optimized through the use of the robotic handling of individually prepared crystals, for data collection with medium-scale crystal screening of selected samples that have been selected by eye for potential diffraction quality. Expanding the beamline infrastructure to include dedicated *in situ* data collection capabilities will allow the routine, systematic and objective assessment of the diffraction potential of all types of crystals grown to find the best samples for subsequent data collection experiments.

Structure determination as part of a high-throughput pipeline

As part of our efforts to validate the assumption that it is possible to collect and solve novel protein structures given only limited amounts of challenging samples we tested crystals grown as an on-going collaboration between the OPPF-UK and Prof. A. Morgan (Leeds University). This first experiment used crystals of the immunoglobulin receptor FcγRIIIa (CD16) which is distributed on natural killer (NK) cells, macrophages, and γδ T cells. The initial crystallization screen yielded hits in 3 wells. The crystals in these wells were typically ~30 × 30 × 30 μm^3 (Figure 8). The crystals were grown in standard Greiner SBS plates. These 3 wells were analysed *in situ* on I24, eliminating the highly laborious and time consuming step of harvesting crystals for data collection. The data collected from this first crystal screen led to the structure being successfully determined at 2.4 Å with relative ease. Diffraction data were collected from 66 crystals from these three drops using 12.68 keV X-rays with a beam size at the sample of 20 × 20 μm^2 and a photon flux of ~5×10^{11} $ph.s^{-1}$. Each sweep of data consisted of approximately 10 0.5° oscillation images each of 0.1s duration. Integrated data from all diffraction wedges were merged and scaled to produce an optimized scaled data-set (Table 2). The structure was solved by molecular replacement and refinement is currently in progress (Table 2 and Figure 7). A key to this successful analysis has been the in-house development of methods at Diamond to handle data acquired from multiple crystals; in this case procedures to automatically assess the data sets to scale and the optimal combination of datasets to produce the final dataset for structure solution and refinement. These devlopments, which are continuing, are aimed at rendering such a strategy of structure determination routine.

	FcγRIIIa
Spacegroup	$P6_122$
Unit cell (a,b,c) (Å)	65.34, 65.34, 178.31
Number of crystals used	44
Initial number of images integrated	527
Number of images forming final	87
Number of scaling cycles required	18
Mn(I)/sd	5.3 (1.9)
Rmeas	0.201 (0.870)
Rpim	0.086 (0.36)
Completeness (%)	95.5% (97.0%)
Number of unique observations	8941 (1290)
Total number of observations	41038 (6187)
R_{work}/ R_{free}	0.24 /0.28

Table 2. Data processing and initial structure determination statistics for FcγRIIIa. Quantities for outermost resolution shell (2.53 - 2.4 Å) given in parentheses

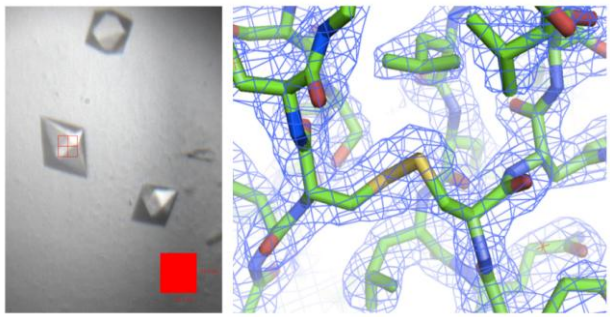


Figure 7. Crystals of FcγRIIIa (CD16) and electron density around a disulphide bridge

Structure determination of Bovine enterovirus

Virus crystals in particular are notoriously difficult to cryocool. This is due in a large part to the decrease in forces holding the crystal together relative to the particle mass. Historically, virus data collection has frequently involved the manual mounting of many hundreds of virus crystals in capillaries and the provision of a platform to eliminate this step would significantly accelerate the rate of virus structure determination. Bovine enterovirus (BEV) is a picornavirus, endemic in cattle

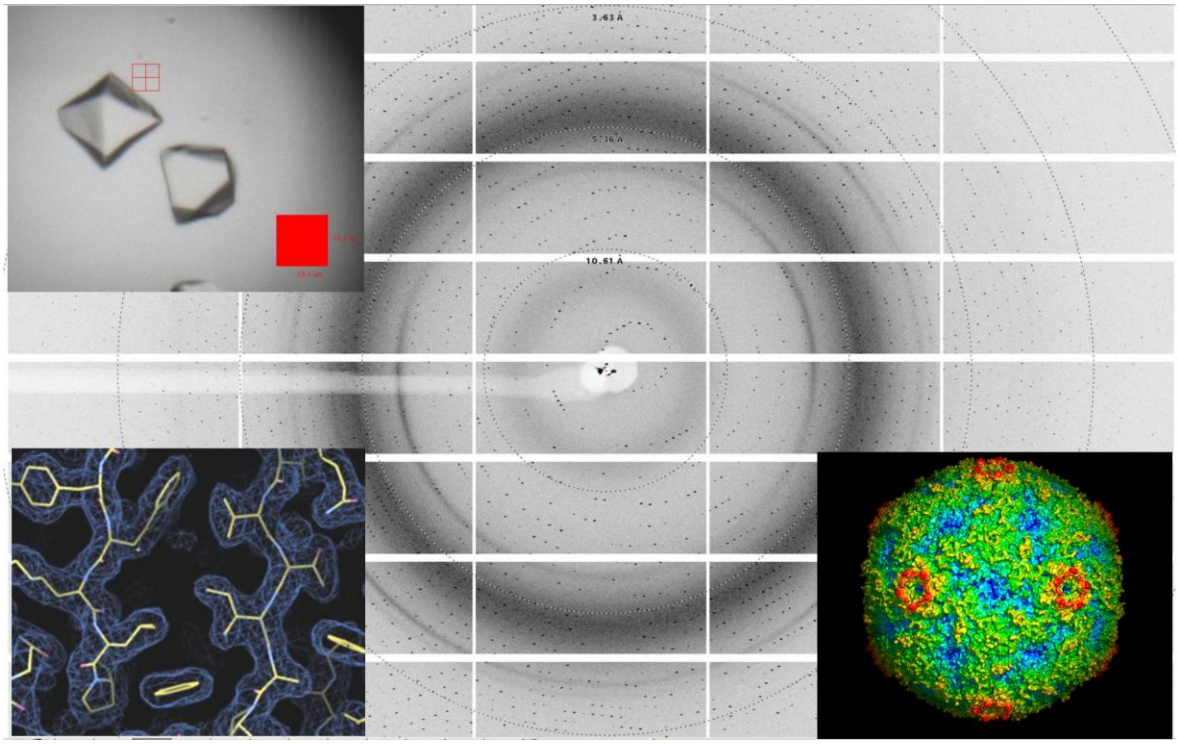


Figure 8. Diffraction pattern for BEV-2 crystals. Typical crystals are shown in the top left panel. The beam size is defined by the red cross-hairs and outlined box. A representative portion of the five-fold averaged electron density map is shown in the bottom left panel. The overall view of the virus is shown in the bottom right panel, coloured according to the radial distribution of structure.

populations where it can cause reproductive, respiratory or enteric disease. Crystals of serotype BEV-2 (a virus of unknown structure) were grown in Greiner CrystalQuick™ X plates, using standard nano-drop crystallisation techniques [16] after optimisation from initial crystallisation hits. Crystals had, typically, a maximum linear dimension of 50 µm. Using a beamsize of 20x20 μm^2, 76 exposure positions on 30 crystals were used to collect diffraction data, to a maximal resolution of 2.1 Å. 326 frames of 0.1 degree oscillation each were used to assemble a 66% complete data set to 2.1 Å resolution (> 80% to 2.6 Å), with an overall R_{merge} of 19.6%. The crystals belong to space group F23, with unit cell parameters a=b=c=434 Å. The structure has been solved and refined with residuals R=18% R_{free} = 19% (Figure 8). In the last year, with limited access to the prototype *in situ* setup at I24, nine virus structures have been determined using this *in situ* protocol. We note that dedicated access to an *in situ* data collection facility will, for the first time. enable the use of rapid and routine structural analysis of whole viruses in drug discovery (for example for the optimisation of capsid binders which are active against enteroviruses).

2.3.2 Characterizing protein-ligand interactions

Benefits to industry: rapid ligand/fragment based screening

The importance of structure based drug design is clearly illustrated by the success of the viral neuraminidase inhibitors Zanimir (Relenza™) and Oseltamivir (Tamiflu™), and is recognized in the commitment of all major pharmaceutical companies to structure aided design as a fully embedded process in their early stage their drug discovery programs. Industrial MX is geared up to high throughput studies on large numbers of similar samples per therapeutic target. Turnaround time is a critical factor in order for MX to have an impact in the drug design cycle. This has driven industrial experimenters to use high multiples (which comes with overheads in terms of sample setup and mounting) of each sample to ensure successful structure determination on the first synchrotron visit; thus eliminating the need to go through the expensive (in terms of time and consumables) reiteration of crystal optimisation and data collection.

Since industrial users have many hundreds of very similar samples, this can involve many hours of manual intervention soaking and/or co-crystallisation of the target protein with ligands/fragments. These hundreds of crystals then need to be physically mounted up in loops and cryo-cooled. The development of ligand screening by MX has led to an urgent need for efficient and possibly fully automated methods for co-crystallization or soaking and subsequent structure determination with little crystal handling. Proof of principle experiments have been carried out by Jean-Luc Ferrer and colleagues at the FIP beamline (ESRF) [13,15] which demonstrate that ligands can be easily identified (and refined) in electron density maps calculated from data collected at room temperature from ligand soaked crystals (in this case of a kinase).

Fully automated *in situ* data collection, as proposed for VMX, alongside parallel developments in simple automated procedures for soaking or co-crystallisation offers huge potential to deliver experimental data with reduced time and material costs. Indeed as costs per structure have reduced with the advent of improved automated conventional beamlines and automated refinement methods the drive to provide data on more ligands has increased. As such initial discussions with industrial users of MX at DIAMOND have highlighted the importance of *in situ* capabilities for development of these applications to industry.

2.3.3 Challenges and Developments

Future developments

There are a number of facilities offering some limited form of *in situ* diffraction experiments. *In situ* plate diffraction experiments were initiated on beamline FIP-BM30A which has provided a focus for the developments to improve and validate the technique. Very recent developments at the SLS, with a plate storage hotel, integrated with the beamline, have begun to enable users to screen crystals for diffraction by remote access, and data collections with test model systems such as lysozyme have been reported [14]. As reported, experiments at I24 have already provided ample evidence that data collection is feasible from crystals as small as 3 - 5 μm at room temperature *in situ*. Moreover, data collection at frame rates above 15 Hz is shown to significantly extend the crystal lifetime at room temperature (R. Owen *et al*., manuscript in prep.). Building on these on-going developments, such as integrated tray hotels and tray-capable goniometers has the potential to have a major impact on MX. Besides opening up the possibility of a fully automated pipeline to structure determination (from protein purification to crystallographic data with manual intervention) it makes the high-throughput diffraction screening of micro-crystals a reality. Advances in robotics, liquid handling, crystal recognition as well as data processing will make analysis of diffraction data from such crystals (without the bottle-necks of sample manipulation and handling) a standard crystallographic method. These hardware and software developments will be key in transforming what is currently a niche method used by a small but growing number of groups to one which is widely taken up with the availability of dedicated facilities. Now that the requirements for an unmanned automatic *in situ* facility are clear it is timely, and will be cost-effective, to design a simple dedicated and efficient facility.

2.4 User community – needs and requirements

The growth of the UK and European MX user community continues to show no sign of abating. The provision of ultra-reliable synchrotron sources, coupled to extremely robust and intense tuneable beamlines, as exemplified by the ESRF and more recently by the medium energy 3rd generation sources, has fuelled this continued growth. Close to 90% of the 7,910 protein structures determined and deposited in the PDB in 2010 used synchrotron radiation, illustrating that synchrotrons are the new 'home' source. The availability of a spectrum of highly specialized yet reliable beamlines and the maturation of the methodology has opened up macromolecular crystallography as a technique to the wider community of biologists, whilst also enabling more problematic crystallographic projects to be successfully addressed. This has contributed to the growth and evolution of the structural biology user community and its diversification from hard-core 'crystallographers' to those users from a broad range of scientific disciplines where structural information is fundamental to an understanding of the system(s) under investigation – hence users are now typically spread between biochemists, microbiologists, molecular biologists, cell biologists, neurobiologists, botanists, chemists, industrialists and physicists who require an atomic view of the biological molecule or system under study.

The drivers for the continued growth of MX are the significant developments that have been made in the technologies employed to grow high quality macromolecular crystals, coupled with dramatic advances in sample preparation and purification. These improvements have opened up whole new vistas of biology to macromolecular analysis. These changes, together with advances in many aspects of synchrotron sources and beamlines, mean that an arsenal of specialized beamlines is now required to fully serve the needs of this burgeoning community as well to provide mechanisms that allow highly fluid access to these beamlines. The number of beamlines currently dedicated to macro-molecular crystallography worldwide now exceeds 100; this number, however, only tells half the story, since there is a wide variation in the productivity of the beamlines. As Diamond has learnt, efficient use and flexible access is greatly facilitated by providing time to a block of users (in some cases disparate) who can bring the large number of samples to efficiently use typically blocks of 24 hours of beamtime.

Although only 5 of the 20 operational beamlines at Diamond are MX, currently over 50% of Diamond's users originate from the MX community. The beamlines are used in an extremely efficient manner and are pushed to their limits by the users who typically need to evaluate large numbers of samples over numerous repeat visits to complete an experiment. The MX BAG system (where a group of users, usually from the same department, apply for blocks of beamtime) has enabled this efficient use of beamtime by providing a regular, yet limited, amount of time for each BAG member. Although a systematic approach to the implementation of high-throughput pipelines continues to increase capacity, the methodological improvements in protein molecular biology (from cloning through to crystallisation) coupled with the tremendous developments in beamlines (beams and detectors) has meant that far more challenging biological samples can now be tackled (such as membrane proteins targets which are of high medical interest). These more challenging projects require more time for screening of crystals (a typical novel membrane protein analysis often requires the diffraction analysis of ~1000 crystals). Thus significant pressure remains on the facilities to augment access to beamlines and improve data acquisition speeds, whilst also improving the quality of the data that can be measured. Since 2000 a three-fold increase in the number of structures being deposited annually with the PDB has been achieved, in the most part, due to a concerted effort by facilities to increase the percentage of time the X-ray shutter is open and X-rays being used to collect data – an impressive achievement that allows facilities such as the ESRF to now routinely evaluate in excess of 100,000 samples/annum, whilst at Diamond in excess of 50,000 samples have been screened to date in 2011 alone. Diamond is

thus acutely aware of the need to keep pace with the growth and demands of the life science community.

The UK MX community is currently served by five MX beamlines at Diamond each with a unique focus. The suite caters for state of the art MAD experiments and has led the way in delivering the first fully dedicated tuneable variable microfocus MX capability worldwide (I24). Alongside these operational beamlines, a beamline designed to operate at long wavelengths is currently under development. To complete the needs of the user community and maintain the UK's leading role in structural biology, the extension of the microfocusing capabilities of the village to sub-micron size beams together with a dedicated facility for *in situ* diffraction data collection from crystals at room temperature will complete this comprehensive suite of beamlines and provide capabilities to the UK community that are currently (and will be) unmatched elsewhere. The support for completing this suite of distinct and unique set of beamlines at Diamond is reflected in the letters of support (over 300) received from essentially every University and Industrial structural biology group in the UK and the significant support and interest provided by colleagues in Europe.

2.5 Industrial and technological applications

MX has been the application of choice for revealing in atomic detail how drug molecules interact with their target. Since the late 1980's Pharmaceutical companies have invested in the technology and over the last decade the approach has become fully integrated into the drug discovery process. The application of structure based drug design has underpinned the discovery of several marketed drugs for the treatment of Cancer, Glaucoma, Hypertension, Thrombosis, Inflammation, as well as anti-virals (HIV, HCV and Influenza) and anti-bacterials,

Advances in molecular biology and the industrialisation of parts of the crystallographic process, alongside improved access to state of the art synchrotron facilities, have lowered the cost and increased the turnaround of the structural biology process making the technology affordable and accessible to Biotechs and SME's. An approach to compound hit discovery, known as fragment screening was pioneered by industrial X-ray crystallographers, in collaboration with NMR spectroscopy, to provide weak by highly efficient starting points for a new drug program. A number of Biotechs, such as Astex and Plexikon employ such approaches as part of their core technology. A number of other SME's and specialised Contract Research Organisations offer crystallography as a service.

Beamline developments over the last decade have been key to allowing this industrial application of MX to impact in the pharmaceutical environment. The automation of endstations, availability of microfocus beams and improvements in detector technology alongside improvements to synchrotron computing infrastructure have made it possible to work on larger numbers of smaller samples than in the past.

To highlight this point, as noted above, the use of microfocus beamlines has enabled the recent structure determination of G protein-coupled receptors (GPCRs) which has in turn resulted in two new companies Heptares and Receptos being formed to exploit the structural data obtained on GPCR's to guide drug design against this target class (GPCRs represent of 50% of the current druggable targets)

2.6 Comparison to other facilities

The exploitation of microfocus beams for MX was pioneered by C. Riekel and his team at the ESRF beamline ID13 in the late 1990's [3], which led to the first dedicated microfocus MX beamline ID23-2 at the end of 2005. The success of these beamlines quickly led other synchrotrons to follow suit. Currently there are six fully dedicated MX beamlines with microfocusing capabilities operational worldwide, and a similar number currently under

commision or in construction phase. All these beamlines are operating with the smallest routine beam size in the range of 5-10 μm^2 (with the exception of BL32XU at Spring 8, which came online in 2010 and can provide a beam at the sample down to 1 x 1 μm^2. At present there is no operational beamline we are aware of that offers sub-micron focused beam capabilities for MX. At Diamond I24 provides a variable focus microbeam from 5 x 5 μm^2 to 50 x 50 μm^2. The beamline started user operations in late 2008 and provided the first dedicated tuneable and variable microfocus beamline for MX worldwide. The high quality of data being measured from crystals on the order of 5-10 microns and the advantages of using micron sized beams on significantly larger samples (where internal crystal heterogeneity can be a problem) is the main driver for complementing this beamline with a sub-micron capable dedicated facility that will allow the UK community to continue to lead the field and provides a village that can offer beam sizes to the community spanning the 0.5 – 100 μm range.

In situ room temperature diffraction analysis was first developed on the French CRG beamline FIP-BM30A at the ESRF, using their automated sample robot system (CATS). The beamline provided the impetus for development of diffraction capable crystallization plates and other crystallisation technologies (such as microfluidics). The beamline is limited in its capabilities due to the large beam size at the sample (300 x 300 μm^2) and limited photon flux. Nevertheless the facility has allowed both characterisation of, and data collection to be collected from, well diffracting optimised crystals. These early proof of principle experiments at FIP-BM30A resulted in the SLS building the first and only dedicated in-situ X-ray diffraction screening facility (on the superbend magnet X06DA). The beamline is integrated with the crystallization facility of SLS and its primary aim is to aid crystal optimisation, but has the potential to collect data from a significant range of crystal sizes with a beam size of 80 x 40 microns delivering 5 $x10^{11}$ ph/s/400mA at 12.4 keV. At Diamond proof of principle experiments on I24 show the clear potential of collecting diffraction data from initial-hit crystals, in addition to significantly aiding in the initial characterisation and optimisation of microcrystals for diffraction analysis. A high-flux ID sidestation with a focused beam of 5 - 50 μm in linear dimensions and delivering 1 x 10^{12}/phs/s/300mA at ~14 keV is proposed to enable a fully integrated diffraction screening and data collection facility that will be run in an automated non-attended fashion enabling the full potential of *in situ* diffraction to be realized.

2.7 Combined impact of the *in situ* facility and other on-site activities

The presence of a dedicated *in situ* capability at Diamond, as well as providing a unique tool for the UK MX community as a whole, will multiply the value of on-site protein expression and crystallization activity at the Wellcome Trust Membrane Protein Laboratory (MPL), the Oxford Protein Production Facility (OPPF-UK) and the many UK research groups based in the Research Complex at Harwell (RC@H). Both the MPL and OPPF-UK serve as user facilities and between them a total of 86 user projects are currently benefiting from access to their state-of-the-art facilities. Complementing and enhancing these activities with dedicated *in situ* characterization and data collection within Diamond will ensure that UK structural biology remains in a position to address the most important questions in fundamental biology and human and animal health.

3. Beamline specification

The beamline requirements are for a sub-micron variable focus endstation with a fixed wavelength variable focus *in situ* mini-hutch. A summary of the main performance specifications are listed in Table 3.

Table 3. Summary of the key performance parameters for VMX

Endstation	Sub microfocus (VMX_{μ})	*In situ* (VMX_i)
Energy range (keV)	5 – 30	Nominally ~14 keV
Beamsize (μm)	0.5 – 5 (300)*	5 – 50**
Flux (phs/s)	~ 10^{12} at 20 keV	>10^{12}

*The focused beam size at the sample will be continuously variable independently in the horizontal and vertical directions. In addition the beamline optical design will allow the user to change to a fixed large beam configuration of at least 300 μm for tomographic applications in near real time.

** The focused beam size at the sample will be continuously variable independently in the horizontal and vertical directions.

4. Beamline requirements

4.1 Source

To provide the high brightness requirements for the microfocus beamline and *in situ* mini-hutch facility two undulator sources are essential. Modification of one of Diamond's standard 5 m straight sections has shown it is feasible to constructively use this space to incorporate two 2 m long insertion devices or combinations thereof. This will necessitate the reorganisation of the MX village.

In the current proposal, the sub microfocus endstation the higher flux requirements above 20 keV can be accommodated by the use of a 3 m Cryogenic Permanent Magnet Undulator (CPMU) with a period length of 21 mm for which the flux output is displayed in Figure 9. This device provides more than ample flux in the 12-15 keV range and would still provide approximately 10^{12}/photons/s/300mA above 20 keV at the sample position. Recent and on-going developments in undulator technology (e.g. the use of superconducting undulators) should provide more flux at higher energies and these possibilities will be considered in more detail at the technical design stage of the project.

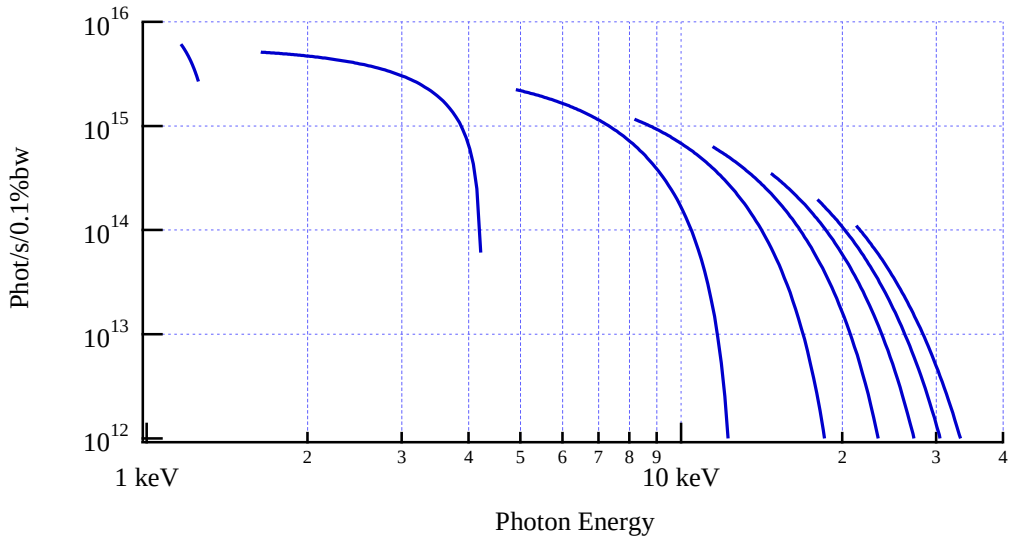


Figure 9. Calculated flux for a 3 m CPMU (21 mm period, 7mm minimum gap, 300mA ring current),

For the *in situ* mini-hutch, there is the possibility to install a device upto 1 m in length which, together with the ability to accommodate a smaller gap than that of the canted I04 straight, will easily achieve the photon flux requirements for the sidestation. Here we have the opportunity for further increasing the flux by a factor of at least 10 with the use of a multilayer monochromator

will be considered during the design phase that would lead to photon fluxes of >10^{13}/ph/s/300mA at the sample position. The high flux density delivered will be exploited by a high frame rate detector to extend crystal lifetimes at room temperature (R. Owen *et al*., manuscript in prep). Figure 11 compares the performance of this device to that currently installed on beamline I04-1 which delivers 3.5 x 10^{11}/ph/s/300mA in an approximately 60 (h) x 40 (v) μm beam.

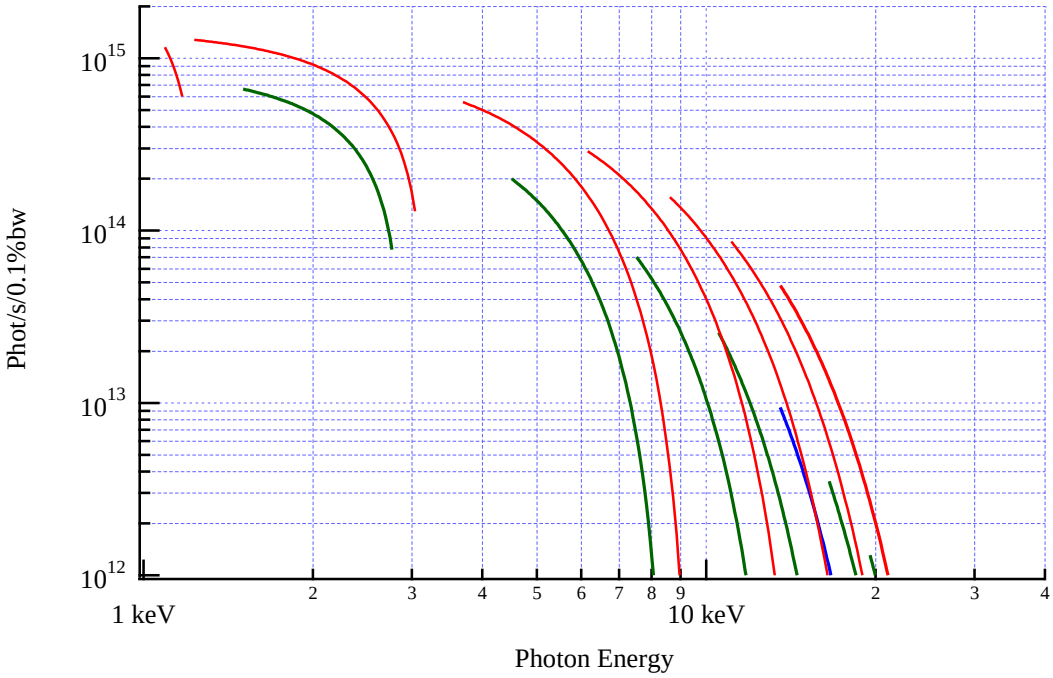


Figure 10. Comparison of calculated flux delivered by a 0.7 m ID (Period = 28 mm, gap 11.18 mm) represented in red when compared to a similar insertion device installed on beamline I04-1 (Period = 30.8 mm, gap = 15.84) represented in green and blue, (blue: 9th harmonic, flux at 13.53 keV =9.4 x10^{12} ph/s/0.1%bw/300mA).The thick red line is the 11th harmonic of the new device (13.53 keV =4.8 x10^{13} ph/s/0.1%bw/300mA)

4.2 Beamline Optics

Diamond is a low-emmittance third generation synchrotron source that is well suited for the provision of micro-focusing beamlines. Within the MX village, I24 provides a variable focus X-ray beam in the 5-50 μm range. The aim of the present proposal is to go below 5 μm, and to provide a continuously variable focused beam size from 0.5 μm to 5 μm at the sample as well as an integrated mini-hutch dedicated to *in situ*. The beam size will be independently adjustable in the two orthogonal directions. An outline beamline scheme is shown in Figure 11. To avoid confusion, the submicron variable focus endstation is designated as VMXμ while the mini-hutch is VMX_i.

4.2.1 Sub-micron focus endstation (VMXμ)

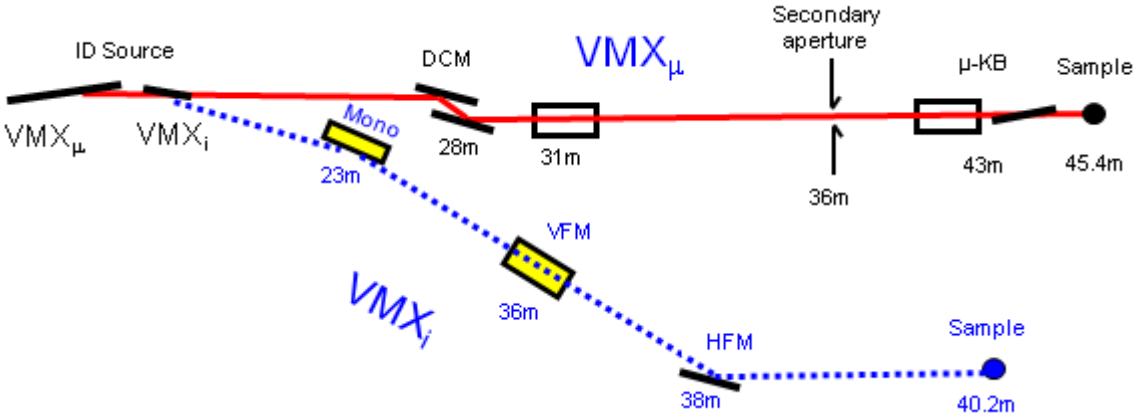


Figure 12. A schematic layout of the proposed beamline (not to scale). Distances are shown relative to the source position.

The proposed scheme employs a two-stage focusing in the horizontal direction and a one-stage focusing in the vertical direction. This scheme is similar to the approach proposed by NSLS-II and ALBA for their micro-focusing MX beamlines but is different from Spring-8 and Petra-III proposals that are pursuing a one-stage focusing. The two-stage focusing offers the usual advantages of having a secondary source (immunity against source movement or the upstream monochromator vibrations, flexibility of tailoring the final beam size by adjusting the secondary aperture).

As illustrated in Figure 11 the first optical element is a Si 111 DCM located at 28m from the source which is followed by a horizontally deflecting meridionally bent cylindrical mirror nominally placed at 31 m from the source. This HFM will focus at the secondary source position. The micro-focusing optics placed beyond the secondary source directly images the source in the vertical direction and images the secondary source in the horizontal direction. The most promising micro-focusing optics to date are still the KB mirror optics and we base our feasibility design on this. It is clear that several competing technologies are fast evolving, and these options will be carefully evaluated for their suitability closer to the implementation of the beamline (at the TDR stage). Refractive optics in particular are very promising – be it the transfocators based on Be lenses [17] or micro-fabricated Kinoform lenses. Refractive optics is attractive as it suffers much less from imperfections like roughness and slope errors, unlike mirror optics. To achieve sub-micron focusing with mirrors, the focused beam size is mostly limited by the slope error of the mirrors. However, significant improvements in mirror polishing have been made and mirrors with the required slope errors of 0.1 μrad rms are already available commercially. In addition, Diamond's Optics group is pursuing a development project to produce super-polished bimorph mirrors. A 150 mm long super- polished bimorph has been produced with excellent surface figure (<2nm peak to valley) [18]. This super-polished adaptive mirror provides variable focal distance and local figure control in the sub-nm range. The optic has the potential to generate distortion-free beams. Detailed characterisation with the synchrotron beam is currently in progress. Similarly, the Diamond Optics group is also developing Kinoform lenses, and have already produced high efficiency lenses that provide ~200 nm one dimensional focused beam sizes [19].

The proposed optics scheme here illustrates the tractability of the project and will already provide a beam with dimension as small as ~0.5 μm fwhm in the vertical and <5 μm fwhm in the horizontal directions. Smaller beam sizes will be achieved through use of the secondary aperture to reduce the size of the beam in the horizontal direction. To get variable beam size up to 5 μm in the vertical direction, the KB mirrors will be slightly defocused. The range of radii required for this variation is feasible to achieve. A photon flux of 1 x 10^{12} ph/sec is expected at the sample

position at high photon energies (>20 keV). The mirrors will be coated with two stripes Rh and Pt to get smooth reflectivity all through the energy range of interest.

A requirement of the beamline is to also provide a relatively large beam size (~200-300 μm) at the sample position to exploit the use of tomography to assist in sample visualization and location. This is accommodated for in the current conceptual design and could be achieved, for example, by introducing a plane mirror to replace the HFM of the micro-focusing KB mirror. The geometry would be used in conjunction with a 5keV incident X-ray energy to yield sufficient absorption contrast in the tomograms.

The sample environment will consist of a high precision vertically oriented goniometer axis and sample positioning system plus high quality on-axis visualization for sample alignment. Alignment using UV and tomographic methods will be adopted for microcrystals and samples embedded in opaque materials. Cooling to <100 K will be available. A low-noise, large format, high-frame rate (>50Hz) detector optimised across the beamlines energy range will be used.

Building on the experience of I24, I18 and the test beamline B16 at Diamond the high stability requirements of the end-station can be met. The development of tomography and its applications to MX is on-going within Diamond and preliminary experiments show the potential for the technique to be used to aid visualization of micro-crystals which currently remains challenging. To obtain adequate contrast from protein samples we propose to use the 3rd harmonic radiation at around 5 keV for tomography.

4.2.2 In situ diffraction end station and mini-hutch (VMXi)

The VMXi endstation will operate at a fixed energy and will be fed by a canted-undulator of up to 1.0m length placed in the same straight as the VMXμ undulator. The first optical component may be a single bounce horizontally-deflecting Si 111 monochromator that produces a deflection of 16.2°, which is sufficient to separate the branch line from the main line to install subsequent optics and the sample and its environment. The option of a multilayer monochromator will also be explored. A similar fixed-wavelength beamline is already in operation at Diamond as I04-1. This beamline uses a toroidal mirror to focus in the two orthogonal planes. For VMXi, we propose instead a KB mirror system for focusing, as it allows adjusting the magnification in the two orthogonal planes independently, although we will explore refractive options. The relative positions of the optical components are given in Figure 12. The ray tracing results for the current feasibility study indicates that a ~5 μm x 5 μm beam size is readily achieved at the sample position with the desired flux.

The sample environment will consist of a high precision goniometer with a crystallization plate holder and plate positioner capable of allowing any point within the plate to be centered to the goniometer rotation centre (Figure 12). On-axis visualization will assist remote users to see their samples and make key experimental decisions. Both X-ray diffraction and UV methods will be employed to locate and centre crystals for characterisation or data collection. Temperature regulation to maintain samples at their crystallization temperatures will be essential within the range 4 - 18 °C. Proven in-house solutions for the sample environment and beam conditioning will be deployed. The detector will be low-noise, large format and high frame-rate (>50 Hz) optimised for operation at 14 keV.

Crystallisation Plates will be loaded from nearby plate storage facilities onto the goniometer automatically and experiments during user operation will be performed exclusively from remote locations. The facility will operate unattended 24/7. Largely commercial robotics will transfer the plates from outside the mini hutch. The plates will be accepted outside the hutch either from a

shuttle allowing direct loading of plates (in the order of 6-12 at a time), or most commonly from a commercially available turn-key plate storage solution. These devices (for example the Formulatrix Rock Imager 1000) are relatively inexpensive, and highly reliable. The imager will incorporate both visible and UV imaging of the crystallisation plates, with regular imaging sessions and web-based remote viewing of images (e.g. via existing xtalPiMS software). Initially one 1000 plate imager at a single temperature (probably 20º C) will be provided, with the option of additional storage at a different temperature at a later date, if this is useful for the user community. A significant software effort will be required to integrate the well developed plate storage software with the Diamond ISPyB and GDA software to allow reliable remote crystal selection and task scheduling.

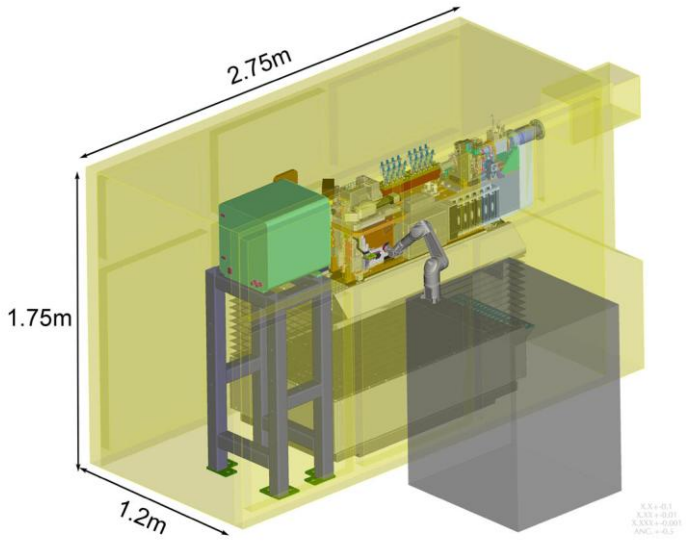


Figure 12. Schematic of the in situ mini-hutch that will complement the sub micron endstation capabilities.

The *in situ* mini-hutch will complete the complex by allowing both evaluation of and data collection from microcrystals through a two-pronged approach of submicron beams and *in situ* data collections at ambient temperatures.

.

5. Costs

Description	Budget (£K)
Beam pipes, slits, diagnostics	
Vacuum components & controls	
Monochromators	
Optics (mirrors and lenses)*	
Experimental table and Beam conditioning	
Sample environment (goniometer, robotics, visualization, auxiliary equipment, cryo, stages etc)	
In situ automation	
Diagnostic equipment	
Detectors	
Safety (PSS etc)	
Hutches	
Cabins & Services	
Computing and data acquisition	
Miscellaneous	
Total	

*Options to be investigated in detail

6. References

1. Diaz-Avalos, R., et al., *Cross-beta Order and Diversity in Nanocrystals of an Amyloid-forming Peptide.* Journal of Molecular Biology, 2003. **330**(5): p. 1165-1175.
2. Ji, X., et al., *How baculovirus polyhedra fit square pegs into round holes to robustly package viruses.* EMBO Journal., 2010. **29**(2): p. 505-514.
3. Perrakis, A., et al., *Protein microcrystals and the design of a microdiffractometer: current experience and plans at EMBL and ESRF/ID13.* Acta Cryst., 1999. **D55**(10): p. 1765-1770.
4. Warne, T., et al., *Structure of a [bgr]1-adrenergic G-protein-coupled receptor.* Nature, 2008. **454**(7203): p. 486-491.
5. Lebon, G., et al., *Agonist-bound adenosine A2A receptor structures reveal common features of GPCR activation.* Nature, 2011. **474**(7352): p. 521-525.
6. Doré, Andrew S., et al., *Structure of the Adenosine A2A Receptor in Complex with ZM241385 and the Xanthines XAC and Caffeine.* Structure (London, England : 1993), 2011. **19**(9): p. 1283-1293.
7. Shimamura, T., et al., *Structure of the human histamine H1 receptor complex with doxepin.* Nature, 2011. **475**(7354): p. 65-70.
8. Holton, J.M. and K.A. Frankel, *The minimum crystal size needed for a complete diffraction data set.* Acta, 2010. **D66**(4): p. 393-408.
9. Owen, R.L., E. Rudino-Pinera, and E.F. Garman, *Experimental determination of the radiation dose limit for cryocooled protein crystals.* Proc. Natl. Acad. Sci. USA, 2006. **103**(13): p. 4912-4917.
10. Leslie, A., *The integration of macromolecular diffraction data.* Acta Cryst., 2006. **D62**: p. 48-57.
11. Sanishvili, R., et al., *Radiation damage in protein crystals is reduced with a micron-sized X-ray beam.* PNAS, 2011. **108**(15): p. 6127-6132.
12. Chapman, H.N., et al., *Femtosecond X-ray protein nanocrystallography.* Nature, 2011. **470**(7332): p. 73-77.
13. Jacquamet, L., et al., *Automated analysis of vapor diffusion crystallization drops with an X-ray beam.* Structure, 2004. **12**(7): p. 1219-25.
14. Bingel-Erlenmeyer, R., et al., *SLS Crystallization Platform at Beamline X06DA – A Fully Automated Pipeline Enabling in Situ X-ray Diffraction Screening.* Crystal Growth & Design, 2011. **11**(4): p. 916-923.
15. le Maire, A., et al., *In-plate protein crystallization, in situ ligand soaking and X-ray diffraction.* Acta Crystallogr D Biol Crystallogr, 2011. **67**(Pt 9): p. 747-55.
16. Walter, T.S., et al., *A procedure for setting up high-throughput nanolitre crystallization experiments. Crystallization workflow for initial screening, automated storage, imaging and optimization.* Acta Crystallogr D Biol Crystallogr, 2005. **61**(Pt 6): p. 651-7.
17. Vaughan, G.B.M., et al., *X-ray transfocators: focusing devices based on compound refractive lenses.* Journal of Synchrotron Radiation, 2011. **18**(2): p. 125-133.
18. Sawhney, K.J.S., Alcock, S.G. and Signorato, R., *A novel adaptive bimorph focusing mirror and wavefront corrector with sub-nanometre dynamical figure control.* Proc. SPIE, 2010. **7803**: p. 780303.
19. Alianelli, L., et al., *High efficiency nano-focusing kinoform optics for synchrotron radiation.* Opt. Express, 2011. **19**(12): p. 11120-11127.

7. Expressions of interest & support from the community

The full support of the UK community is clearly seen from the letters of support received for the VMX beamline. A total of 307 letters of support for the proposal has been received representing virtually all academic and Industrial groups in the UK. Of these letters, 20 were received from non UK based groups mainly from Europe, but letters from the USA and Asia were also received. A full list of the support is given in Appendix I.

Appendix I Letters of Support for VMX

	First Name	Last Name	Organisation
1	chris	Abell	university of cambridge
2	Jan-Pieter	Abrahams	Leiden University
3	Patrizia	Abrusci	University of Oxford
4	Ravi	Acharya	University of Bath
5	Christopher	Agnew	University of Bristol
6	Maruf	Ali	Imperial College London
7	Sharff	Andrew	Global Phasing Ltd
8	Maxwell	Anthony	John Innes Centre
9	Fred	Antson	University of York
10	Margarida	Archer	Institudo Tecnologia Quimica e Biologica
11	Alexandru	Aricescu	University of Oxford
12	Antonio	Ariza	University of Leeds
13	P J	Artymiuk	University of Sheffield
14	Frances	Ashcroft	University of Oxford
15	Ming	Attarataya	University of Bristol
16	Mohammad	Bahar	University of Oxford
17	Patrick	Baker	University of Sheffield
18	Steve	Baldwin	University of Leeds
19	Mark	Banfield	John Innes Centre
20	John	Barr	University of Leeds
21	Tracey	Barrett	Birkbeck College
22	Richard	Bayliss	Institute of Cancer Research
23	Konstantinos	Beis	Imperial College London
24	Christian	Bell	University of Oxford
25	Andrew	Bent	St Andrews
26	Alex	Berndt	MRC Laboratory of Molecular Biology
27	John	Berrisford	University of Newcastle
28	Benjamin	Bishop	University of Oxford
29	Wulf	Blankenfeldt	Universität Bayreuth
30	Lynda	Blayney	Cardiff University
31	Tom	Blundell	Cambridge University
32	Matthias	Bochtler	Cardiff University
33	Victor	Bolanos-Garcia	University of Cambridge
34	Martino	Bolognesi	University of Milano
35	Kieron	Brown	Vertex Pharmaceuticals
36	Tom	Brown	University of Southampton
37	Marek	Brzozowski	University of York
38	Doryen	Bubeck	University of Oxford
39	Martin	BUCK	ICL
40	Simon	Bullock	MRC Laboratory of Molecular Biology
41	Alex	Bullock	University of Oxford
42	Karen	Bunting	University of Nottingham
43	Nick	Burton	University of Bristol
44	Carina	Büttner	University of York/YSBL
45	Bernadette	Byrne	Imperial College London
46	Martin	Caffrey	Trinity College Dublin

47	Rosaleen	Calvert	KCL
48	Alex	Cameron	Imperial College London
49	Iain	Campbell	Biochemistry, University of Oxford
50	Liz	Carpenter	Structural Genomics Consortium
51	Stephen	Carr	The Research Complex at Harwell
52	Andrew	Carter	MRC
53	Stephen	Carter	University of Leeds
54	Tom	Ceska	UCB Celltech
55	Shus	Chen	University of Oxford
56	Dima	Chirgadze	University of Cambridge
57	Alessio	Ciulli	University of Cambridge
58	Jordan	Clay	University of Oxford
59	Amber	Clayton	University of Oxford
60	Anne	Cleasby	AstexTherapeutics
61	Alun	Coker	UCL Division of Medicine
62	Charlotte	Coles	University of Oxford
63	Atlanta	Cook	University of Edinburgh
64	Jon	Cooper	UCL
65	Kevin	Cowtan	YSBL, University of York
66	Georgina	Cox	University of Leeds
67	Paul	Crichton	MRC Mitochondrial Biology Unit
68	Nora	Cronin	ICR
69	Stephen	Curry	Imperial College London
70	Luigi	de Colibus	University of Oxford
71	Isabel	De Moraes	Imperial College London
72	Janet	Deane	University of Cambridge
73	Jeremy	Derrick	University of Manchester
74	Balvinder	Dhaliwal	King's College London
75	Joao	Dias	Heptares Therapeutics
76	Kay	Diederichs	University of Konstanz, Germany
77	Bauke	Dijkstra	University of Groningen
78	Mike	DiMattia	University of Oxford
79	Sarah	Dobson	CRUK London Research Institute
80	Eleanor	Dodson	University of York
81	guy	dodson	NIMR; University of York
82	Pawel	Dokurno	Vernalis (PLC)LTD
83	Changjiang	Dong	University of St.Andrews
84	Andrew	Dore	Heptares Therapeutics
85	Jacqueline	Dornan	I.S.M.B., University of Edinburgh
86	Ingrid	Dreveny	University of Nottingham
87	Steven	Ealick	Cornell University
88	Thomas	Edwards	University of Leeds
89	Kamel	El Omari	University of Oxford
90	Conti	Elena	Max Planck Institute of Biochemistry
91	Jonas	Emsley	University of Nottingham
92	Jane	Endicott	Oxford University
93	Francisco	Enguita	Instituto de Medicina Molecular, Lisbon
94	Lars-Oliver	Essen	Philipps University
95	Neil	Fairweather	Imperial College London
96	Susan	Firbank	University of Newcastle Upon Tyne
97	Andreas	Förster	Imperial College London
98	Keith	Fox	University of Southampton
99	Annika	Frank	University of York

100	Liz	Fry	University of Oxford
101	Vilmos	Fulop	University of Warwick
102	Klaus	Futterer	University of Birmingham
103	Mads	Gabrielsen	University of Glasgow
104	Stephan	Gadola	University of Southampton
105	Steven	Gamblin	MRC, NIMR
106	Javier	Garcia	University of York
107	Elspeth	Garman	University of Oxford
108	Christopher	George	Cardiff University
109	Ermanno	Gherardi	MRC Centre, Cambridge
110	Robert	Gilbert	University of Oxford
111	Opher	Gileadi	University of Oxford
112	Alex	Gingras	University of Leicester
113	Tracey	Gloster	University of York
114	Lata	Govada	Imperial Coleege London
115	Stephen	Graham	University of Cambridge
116	Trevor	Greenhough	Keele University
117	Ingo	Gregor	MRC Laboratory of Molecular Biology
118	Gideon	Grogan	York Structural Biology Laboratory
119	Piet	Gros	Utrecht University
120	Tim	Gruene	Georg-August-University Goettingen
121	Yvonne	Hackmann	Cambridge Institute for Medical Research
122	Lesley	Haire	MRC,NIMR
123	mike	hann	gsk
124	Karl	Harlos	University of Oxford
125	Jenny	Harmer	University of Southampton
126	Nic	Harmer	University of Exeter
127	Claire	Harris	Cardiff University
128	Andrew	Hemmings	University of East Anglia
129	Glyn	Hemsworth	University of York
130	Richard	Henderson	MRC Laboratory of Molecular Biology
131	Matthew	Higgins	University of Oxford
132	Charlotte	Hodson	CR UK London Research Institute
133	Erhard	Hohenester	Imperial College London
134	Peter	Holt	MRC MBU
135	Doug	Houston	University of Edinburgh
136	Roderick	Hubbard	University of York
137	William	Hunter	University of Dundee
138	Marko	Hyvonen	University of Cambridge
139	Rivka	Isaacson	Imperial College London
140	Misha	Isupov	University of Exeter
141	Momi	Iwata	Imperial College London
142	Lauren	Jackson	Cambridge Institute for Medical Research
143	Bert	Janssen	University of Oxford
144	Steven	Johnson	University of Oxford
145	Yvonne	Jones	University of Oxford
146	Amar	Joshi	Imperial College London
147	Nicholas	Keep	Birkbeck College
148	Bernie	Kelly	University of Cambridge
149	Amir	Khan	Trinity College Dublin
150	Mairi	Kilkenny	University of Cambridge

151	Stefan	Knapp	University of Oxford
152	David	Komander	MRC Laboratory of Molecular Biology
153	Olga	Kotik	Imperial College London
154	Norbert	Krauss	Queen Mary, University of London
155	Tobias	Krojer	University of Oxford
156	Edmund	Kunji	MRC
		Kutti	
157	Vinothkumar	Ragunath	MRC Laboratory of Molecular Biology
158	F.Anthony	Lai	Cardiff University
159	Meindert	Lamers	MRC Laboratory of Molecular Biology
160	Roy	Lancaster	Saarland University
161	Ivan	Laponogov	King's College London
162	David	Lawson	John Innes Centre
163	Susan	Lea	University of Oxford
164	wei	lee	STRUBI (University of Oxford)
165	Yang	Lee	MRC Mitochondrial Biology Unit
166	Eoin	Leen	Imperial College MSF
167	Rick	Lewis	University of Newcastle Upon Tyne
168	CEDRIC	LEYRAT	STRUBI
169	James	Lillington	University of Oxford
170	Jenny	Littlechild	University of Exeter
171	Edward	Lowe	University of Oxford
172	Jan	Lowe	MRC Laboratory of Molecular Biology
173	Ben	Luisi	University of Cambridge
174	Timm	Maier	Biozentrum, Basel, Switzerland
175	Tomas	Malinauskas	University of Oxford
176	Erika	Mancini	University of Oxford
		Marles-	
177	Jon	Wright	Newcastle University
178	Steve	Matthews	Imperial College London
179	Melanie	McDowell	University of Oxford
180	Iain	McNae	University of Edinburgh
181	Philippe	Meyer	CNRS
182	Halina	Mikolajek	University of Southampton
183	John	Miles	Cardiff University
184	Paul	Miller	STRUBI
185	Matthew	Mold	Keele University
186	Martin	Montgomery	MRC Mitocondrial Biology Unit
187	Guillermo	Montoya	CNIO
188	Jenny	Moran	Keele University
189	Olga	Moroz	University of York
190	Jens Preben	Morth	University of Oslo
191	Chris	Mowat	University of Edinburgh
192	James	Murray	Imperial College London
193	Tom	Murray-Rust	Cambridge University
194	Andrea	Musacchio	Max-Planck Dortmund
195	Kiyoshi	Nagai	MRC Laboratory of Molecular Biology
196	Martin	Noble	Dept. Biochemistry, Oxford University
197	Ravi	Nookala	University of Cambridge
198	Chris	O'Callaghan	University of Oxford
199	Takashi	Ochi	University of Cambridge
200	Derek	Ogg	AstraZeneca
201	Udo	Oppermann	University of Oxford

202	David	Owen	University of Cambridge
203	Raymond	Owens	University of Oxford
204	Guido	Paesen	Strubi WTCHG Oxford
205	Andrew	Pannifer	Beatson Institute for Cancer Research
206	James	Parker	University of Oxford
207	Laurence	Pearl	University of Sussex
208	Xue-Yuan	Pei	University of Cambridge
209	Luca	Pellegrini	University of Cambridge, UK
210	Simon	Phillips	The Research Complex at Harwell
211	Chris	Phillips	Pfizer
212	Richard	Pickersgill	Queen Mary University of London
213	Ashley	Pike	University of Oxford
214	Jennifer	Potts	University of York
215	David	Price	Cardiff University School of Medicine
216	Stephen	Prince	University of Manchester
217	Andy	Purkiss-Trew	CR UK London Research Institute
218	Valerie	Pye	Trinity College Dublin
219	J B	Rafferty	University of Sheffield
220	Venki	Ramakrishnan	MRC
221	Randy	Read	University of Cambridge
222	Jingshan	Ren	University of Oxford
223	David	Rice	University of Sheffield
224	Julia	Richardson	University of Edinburgh
225	Katrin	Rittinger	MRC, NIMR London
226	Shirley	Roberts	University of York
227	Ross	Robinson	University of Oxford
228	Hubbard	Roderick	Vernalis
229	David	Roper	University of Warwick
230	Pietro	Roversi	University of Oxford
231	Karen	Ruane	University of Warwick
232	Jonathan	Ruprecht	MRC Mitocondrial Biology Unit
233	Rupert	Russell	University of St Andrews
234	Neil	Rzechorzek	University of Cambridge
235	Mark	Sanderson	King's College London
236	Renos	Savva	ISMB, Birkbeck, London
237	Christopher	Sayer	University of Exeter
238	Leonid	Sazanov	MRC Mitochondrial Biology Unit
239	Axel	Scheidig	Christian-Albrechts University of Kiel
240	Helgo	Schmidt	MRC Laboratory of Molecular Biology
241	John	Schwabe	University of Leicester
242	Elena	Seiradake	University of Oxford
243	Devon	Sheppard	University of Oxford
244	Annette	Shrive	Keele University
245	Christian	Siebold	University of Oxford
246	Martin	Singleton	CR UK London Research Institute
247	Carrie	Smallcombe	Keele University
248	Stephen	Smerdon	MRC, NIMR London
249	Peter	Smith	University of Southampton
250	Myron	Smith	Evotec
251	Callum	Smits	Dept. Chemistry, University of York
252	McKenna	Sophie	YSBL
253	Thilo	Stehle	Unversity of Tuebingen, Germany
254	Ron	Stenkamp	University of Washington

255	Clare	Stevenson	John Innes Centre
256	Murray	Stewart	MRC Laboratory of Molecular Biology
257	Nicola	Stonehouse	University of Leeds
258	Se Won	SUH	Seoul National University
259	Brian	Sutton	King's College London
260	Geoff	Sutton	University of Oxford
261	Michael	Swan	The Institute of Cancer Research
262	Chris	Tate	MRC Laboratory of Molecular Biology
263	Edward	Taylor	University of York
264	Garry	Taylor	University of St Andrews
265	Ian	Taylor	MRC,NIMR, London
266	Paul	Taylor	University of Edinburgh
267	Jonathan	Taylor	Imperial College London
268	Nethaji	Thiyagarajan	University of Bath
269	Andrew	Thompson	University of York
270	andrew	thompson	synchrotron SOLEIL
271	Andy-Mark	Thunnissen	University of Groningen, the Netherlands
272	Ian	Tickle	Astex Therapeutics Ltd.
273	Philip	Townsend	University of Durham
274	Chi Hung	Trinh	University of Leeds
275	Stephen	Tucker	University of Oxford
276	John	Vakonakis	University of Oxford
277	Eugene	Valkov	University of Cambridge
278	Daan	van Aalten	University of Dundee
279	Mark	van Breugel	MRC Laboratory of Molecular Biology
280	Rob	van Montfort	The Institute of Cancer Research
281	Cara	Vaughan	Birkbeck University of London
282	Gabriel	Waksman	Birkbeck and UCL
283	Miriam	Walden	University of Leeds
284	John	Walker	MRC Mitocondrial Biology Unit
285	Malcolm	Walkinshaw	University of Edinburgh
286	Kim	Watson	University of Reading
287	Ian	Watt	MRC Mitocondrial Biology Unit
288	Johan	Weigelt	Structural Genomics Consortium
289	Joern	Werner	University of Southampton
290	Isaac	Westwood	Institute of Cancer Research
291	Daniel	Whalen	University of Oxford
292	Fiona	Whelan	University of York
293	Scott	White	University of Birmingham
294	Jean	Whittingham	Dept. Chemistry, University of York
295	Dale	Wigley	Institute of Cancer Research
296	Klemens	Wild	Heidelberg University Biochemistry Center
297	Roger	Williams	MRC Laboratory of Molecular Biology
298	Philip	Williamson	University of Southampton
299	Jon	Wilson	Insitute of Cancer Research
300	Steve	Wood	UCL Medicine
301	Linda	Wooldridge	Cardiff University
302	Luke	Yates	University of Oxford
303	Mark	Young	Cardiff University
304	Philippe	Young	Simpleware
305	Daopeng	Yuan	King's College London

306	Wyatt	Yue	University of Oxford
307	Xiaodong	Zhang	Imperial College London