SAC		Doc No: DLS-SACDISCo-K04 Issue: 1 Date: Nov 2020

Beamline K04 for Ultra-XChem

Rebuilding I04-1 for Ultra-High Throughput MX and XChem Fragment Screening to Meet Demand and Expand Science

Prepared for Diamond SAC/DISCO

November 2020

1. Acknowledgements

List of members of the working group, lead external champion, DUC representative, Diamond's lead and other key community contributors to proposal including all Diamond staff involved. List meeting dates (UWG meetings and community engagement (webinar))

User Working Group

- John Barker (*Evotec*) – **Chair; Lead External Champion; Webinar speaker**
- David Briggs (*Francis Crick Institute*) – **DUC representative**
- Christoph Müller-Dieckmann (*ESRF*)
- Katherine McAuley (*Swiss Light Source, PSI*)
- Jane Endicott (*Newcastle University*) – **Webinar speaker**
- Chun-wa Chung (*GSK*)
- Rod Hubbard (*University of York; Vernalis*)
- Simon Ward (*University of Cardiff*)
- Anke Müller-Fahrnow (*Nuvisan ICB*)
- Chris Phillips (*AstraZeneca*)
- Manfred Weiss (*BESSY*)

Diamond staff

- Frank von Delft (*PBS: I04-1/XChem*) – **Diamond Lead; Webinar speaker**
- Dave Hall (*Science Group Leader: MX*)
- Jose Brandao-Neto (*Senior Beamline Scientist*)
- Alice Douangamath (*Senior Beamline Scientist*)
- Rachael Skyner (*Postdoctoral Research Fellow*)
- Louise Dunnett (*Senior Support Scientist*)
- Daren Fearon (*Senior Support Scientist*)

Meeting dates

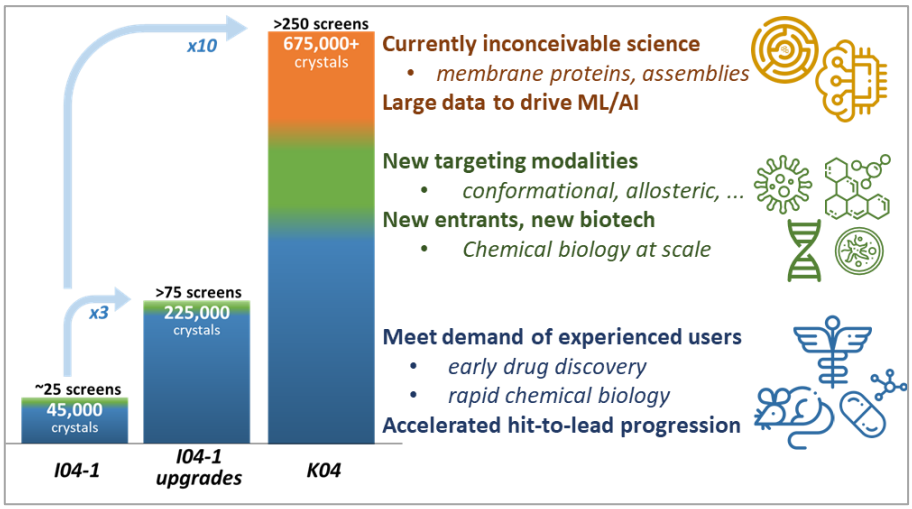
- User Working Group, meeting 1: Monday, Nov 9th
- User Working Group, meeting 2: Friday, Nov 13th
- Community engagement Webinar: Monday, Nov 16th

2. Executive Summary

XChem co-evolved with automation of beamline I04-1 and its user programme into a unique and world-leading facility for accelerating structure-based drug design (SBDD), heavily oversubscribed by academic and industrial users. Diamond-II presents an opportunity for a step-change in the impact of this asset. This proposal is to construct a replacement beamline, K04, to provide transformed throughput and capability immediately on Diamond-II start-up, while allowing I04-1 to meet demand up to the dark period, when it must be decommissioned. K04 will extend the MX user programme and vastly expand the scale and scope of the XChem programme.

This will help transform rational drug discovery and chemical biology, speeding up the route to clinic and simplifying the discovery and deconvolution of molecular biological mechanisms and processes. It will democratise the entry point for drug discovery, providing capability for academic and SME users and enhancing productivity for large pharmaceutical companies. Potential drug targets and drugging modalities will be de-risked more effectively, accelerating translation of biological insights into the clinic and enhancing initiatives for pandemic preparedness.

K04 will achieve this by exploiting the flux and beam properties of Diamond-II, and XFEL technology for fully automated sample preparation, to implement extremely high-throughput fragment screening. This will not only support 10-fold more users and harder scientific problems, but will ultimately allow each experiment to produce sufficient data to allow the downstream work to be simplified into a routine and reliable process, within budget and reach of many labs. The vision is to use developments in AI and automated chemistry at the Rosalind Franklin Institute and around the world, to commodify the production of chemical tools, molecules that potently block or modify the function of target proteins, and thereby to trigger an explosion of biological research.



3. Scientific case

3.1 Introduction

3.1.1 Capacity in MX drives Structure-based drug design

The power and popularity of SBDD lies in its ability to accelerate the process of developing compounds into potent inhibitors of the target protein, by revealing in 3D the precise interactions by which the protein binds the compound, and showing how it might be modified to be bound better. This has been recognised and exploited for half a century, underpinned by technical transformations spanning molecular biology, protein chemistry, synchrotron science, algorithms, software and automation. It has fuelled huge investments at synchrotrons, to meet an expanding demand for rapid turn-around of protein-ligand structures by protein crystallography. The charge was led by the pharmaceutical industry, but the methodology has been embraced widely, expanding the horizons of biotech and academic drug discovery[1].

Beyond that, industrialised SBDD has fundamentally shaped thinking in the larger field of chemical biology, which seeks to dissect biological processes using chemical approaches. In particular, "chemical probes", also known as "tool compounds", are developed (or discovered) to inhibit specific proteins potently and selectively; they thus allow dialling those proteins' activity in a highly controlled way, leading to deeply insightful cellular and physiological experiments[1]. Though in general prohibitively difficult to achieve, they are highly prized in pharmaceutical research to validate drugging strategies[2], and a steady stream have become publicly available in recent years, quickly becoming heavily used[3]. Industrialised SBDD has been key, now even making it conceivable to develop such probes at genomic scale[4], with enormous impact across the full spectrum biological research.

Despite recently ceding the headlines to cryoEM, this transformation in the infrastructure of SBDD has been no less dramatic. Indeed, testament to its success is that is it now largely taken for granted as part of the infrastructure of modern drug discovery, invisible even in publicly available statistics such as PDB depositions, which likely underestimate just the academic output by at least an order of magnitude.

It has not of course been invisible at synchrotrons, with Diamond continually investing in high-throughput and automated MX, including: beamline I04-1 in 2008, to add capacity to the MX village especially for SBDD; the XChem facility from 2013 onwards, to implement crystal-based fragment screening[5]; and flux and detector upgrades in 2020-2022 to expand capacity.

3.1.2 XChem achieves routine and fast fragment screening

Fragment-Based Drug Design (FBDD) is a complement to SBDD that entails testing a collection ("library") of small compounds ("fragments") to identify any that bind to the protein of interest, however weakly; those hits provide starting points that are developed into potent molecules. Over two decades the approach has become firmly established in drug development, leading to over 30 clinical drug candidates and 3 FDA-approved drugs in oncology[6], and an array of powerful biophysical tools for identifying fragments that bind to a given target.

Over the last half-decade Diamond's XChem facility has shifted the paradigm for FBDD, by making it possible to routinely perform the initial identification of fragments by high-throughput crystallography. Such an experiment involves: generating 100s of repeat protein crystals; soaking the crystals with as many as possible of the fragment compounds in the library (individually or as cocktails); scooping and storing the crystals in liquid nitrogen; completing diffraction experiments on each; converting the reflection data to electron density maps; and inspecting them for evidence of compound bound to the protein – most will have none. Historically this was prohibitively difficult, available only to a few specialised, typically industrial groups.

XChem changed this by implementing (along with the other MX beamlines) fully automated data collection on I04-1, as well as developing lab infrastructure, new robust methods for crystal preparation and data analysis, and an experienced support team. Even with the limited flux of I04-1 (over 3x lower than I03), the facility achieves an order-of-magnitude increase in speed and scale of the overall experiment (compared to published state-of-the-art[7]). This transformed the take-up of fragment screening by X-ray crystallography, democratising FBDD in academia and small companies, and streamlining it for large pharmaceutical customers. The accessibility and ease of use of XChem has enabled over 200 experiments since 2016, from academic, industrial and in-house groups. Academic access to XChem is 1.5x - 3x oversubscribed (Figure 1) and industrial demand is equally high with waiting times for access of over 6 months (section 3.5).

Beamline I04-1 continues to serve the overall MX user programme, but around a third of beamtime is allocated to XChem. The facility's XChem capacity is a function of other factors besides beamtime, because the steps outside of the beamline are only semi-automated. It has therefore been vital to establish lab space and instrumentation, set up a dedicated support team (expanded in 2018) and build an experienced user community that can prepare and complete experiments independently. This finally made it possible in 2019 to invite proposals for a Block Allocation Groups (BAG) access route.

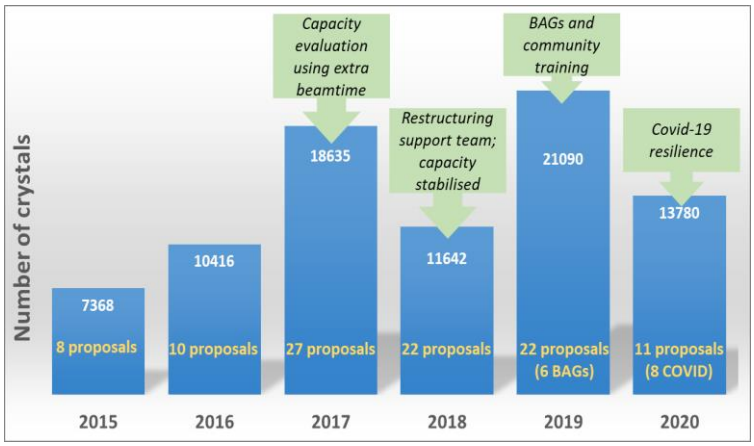


Figure 1: Uptake, development and expansion of the XChem user programme. Capacity was initially overestimated (2017), but usage stabilised once staff time and lab capacity were properly factored in (2018). Productivity went up sharply with formalised user training and empowerment of superusers via BAGs (2019); and in COVID lockdown the facility demonstrated its resilience (2020).

Refining the peer review process and criteria has also been key: not only are scientific excellence and technical feasibility assessed, but also proposers' capacity to advance hits to potency and biology insights. This narrowed the user base, and severely limited the opportunities for technically risky or even scientifically exploratory projects – deliberate outcomes in view of the capacity constraints.

The proposed Diamond-II lattice makes it unfeasible to retain beamline I04-1 in its current position, as the I04 and I04-1 undulators will no longer be able to occupy the same straight section in their current canted configuration. Moreover, the layout of beamline I04-1 precludes rebuilding it *in situ* at its current position (the future K03 straight), as it would be neither cost- nor time-effective: the level of demand necessitates running the facility right up to the dark period.

3.1.3 Highlights

The XChem experiment stands at the start of a complex process of compound development and subsequent chemical biology, that is typically longer than the current age of the facility. Such projects are often competitive or else aimed at commercialisation, with data therefore not disclosed until publication. Therefore, few outcomes are yet expected in the public domain, and no drugs. Nevertheless, multiple papers have now emerged (15 by users, 11 by collaborators), especially from early XChem experiments such as a lead inhibitor of the Alzheimer's target Notum (Atkinson *et al.*, 2019) (Figure 3); the startup and patent mentioned in the statements of support (section 7) suggest additional commercialisation of outputs; and the industrial Webinar speaker (Barker) described the profitable acquisition of a client for whom they had completed a successful XChem experiment.

A 2019 survey of academic XChem users gauged the impact of XChem experiments through the funding raised to progress hits towards potency (Figure 2). The 30% of users who responded to the question had collectively raised over £5m, a 10-fold leverage of the XChem investment calculated at commercial rates. Moreover, over a quarter had raised funds for a full-blown discovery campaign, with a further quarter raising enough for meaningful exploratory medicinal chemistry. It is anticipated that the experimental flexibility enabled by BAGs will similarly allow such funding to be raised and allocated more efficiency.

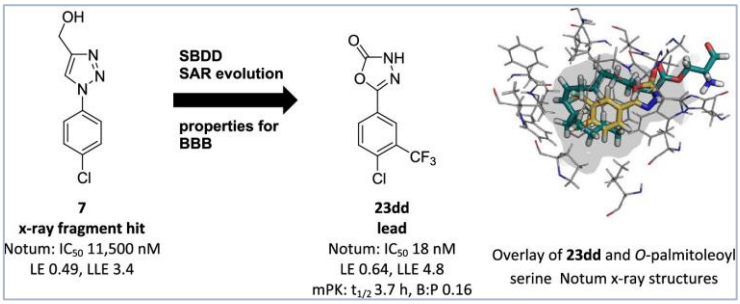


Figure 3: Progression of XChem fragment hit to potent drug lead (**23dd**) targeting the Alzheimers target Notum. Here, the final molecule is no larger than the fragment, and potency was achieved was achieved by adjusting the electronics around the molecule; more typically, the fragment is expanded to effect new interactions with the protein.

An unprecedented number of publicly available, high-quality 3D protein-ligand structures have been produced by the combined user and in-house research programmes: at least[a] 1100 structures are deposited in the PDB, and 2500 (a further 1400) are available from Diamond's Fragalysis server[b]. Overall, at least 10,000 modelled fragment-protein structures from historic experiments will become available once users release them, a process facilitated by tools developed at XChem.

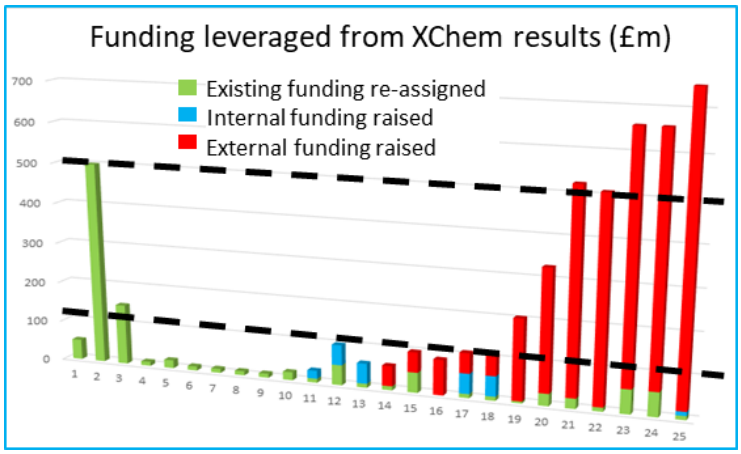


Figure 2: XChem experiments trigger further funding, as shown in responses to 2019 survey of academic users.

To further develop its offering, the XChem facility participated in securing 6 large grants (total value £67m), and was involved in 11 in total (8 ongoing). These variously fund the facilitation of access, development of methodologies and reagents, integration of capabilities with active compound discovery projects, and the harnessing or development of data science tools.

3.1.4 XChem has incubated new methodologies and best practice

To establish the user programme, the facility also had to: develop new technologies within the XChem experiment; explore and establish best practice (Figure 4); and set up initiatives to resolve upstream and downstream bottlenecks highlighted by the user programme. This involved a close and ongoing partnership with the Protein Crystallography group, also run by the I04-1 PBS, of the Structural Genomics Consortium (now Centre for Medicines Discovery) at University of Oxford. Key contributions also came from PhD students recruited from the SABS Centre for Doctoral Training, Oxford.

The work resulted in new approaches to crystal identification[8], soaking[9] and harvesting[10], as well as data management software[11] and an algorithmic approach to identifying fragments[12] that is now widely used in

[a] The estimate assumes users have followed voluntary naming and working conventions.
[b] https://fragalysis.diamond.ac.uk and https://diamondlightsource.atlassian.net/wiki/spaces/FRAG/overview

the community. The crystal harvesting technology is now sold by a startup [c], and the open availability of the tools has allowed other synchrotrons to adapt the them to set up equivalent platforms[13]. Ongoing projects address data analysis, model completion, and data dissemination through the Fragalysis platform[b].

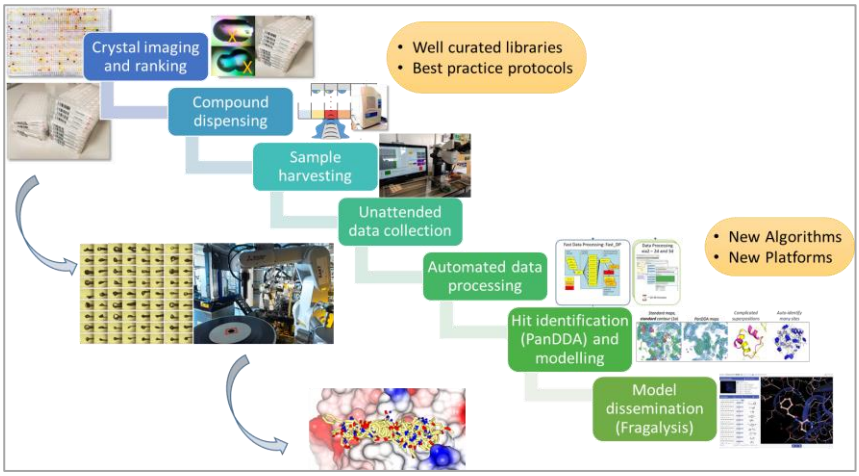


Figure 4: Overview of the XChem experiment. The picture inserts illustrate the new methods that had to be developed.

Experience from the many projects was distilled into best practice for crystal preparation[14], while a fragment library was evolved that implemented the "poised" concept for aiding fragment progression[15], also helping establish the practice of making library composition public. The platform has demonstrated the importance of well-maintained infrastructure and documented processes *(manuscript in preparation)*, and made it possible to evaluate other fragment libraries[16,17], to compare libraries[18], and to inform the design of the collaborative EUOpenscreen-DRIVE library[19].

Ongoing developments target the upstream challenge of establishing robust and consistent crystals: a grant was secured (BBSRC) to engineer generic protein crystal lattices ("crysalins") that reliably crystallize and tether the protein of interest. Downstream, there is a lack of processes and tools for progressing XChem hits towards potent molecules; therefore, projects and collaborations were initiated to develop the premise that XChem data, combined with large-scale simulations and unbiased machine learning, allows autonomous design of molecules that are both immediately potent, and synthesizable with a narrow repertoire of robot-compatible reactions. The work forms part of the Next Generation Chemistry theme of the Rosalind Franklin Institute.

3.1.5 Learnings for K04: large experiments are effective and necessary

The COVID-19 lockdown and suspension of the normal user programme enabled us to test the long-standing suggestion that larger experiments might be more effective. The result was that a large screen of 1,200 compounds against the SARS-CoV-2 main protease (Mpro)[18], achieved a drug lead in only 6 months during challenging working conditions (vs 12 months standard in industry). The dense set of hits (Figure 5) provided many ideas for merging binding motifs from separate fragments. Those that were synthetically tractable were tested in the COVID Moonshot[20], which discovered at least one that was rapidly progressed to potencies suitable for lead optimisation and initial *in vivo* experiments (Figure 5b). That this merge also closely recapitulates the originally observed interactions (Figure 5c) is unlikely to be a coincidence, pointing to algorithmic approaches to enumerating merges from fragment screens, to generate compounds that can be made and tested rapidly at very modest cost (discussed below), and would be starting points for successful compound series.

A second project demonstrated an orthogonal need for capacity, namely the importance of screening additional crystal forms of the same protein. The macrodomain of SARS-CoV-2 NSP3 was quickly solved by multiple groups internationally, and two variants were screened with fragments[19], one at XChem (Ivan Ahel)

[c] https://oxfordlabtech.com/

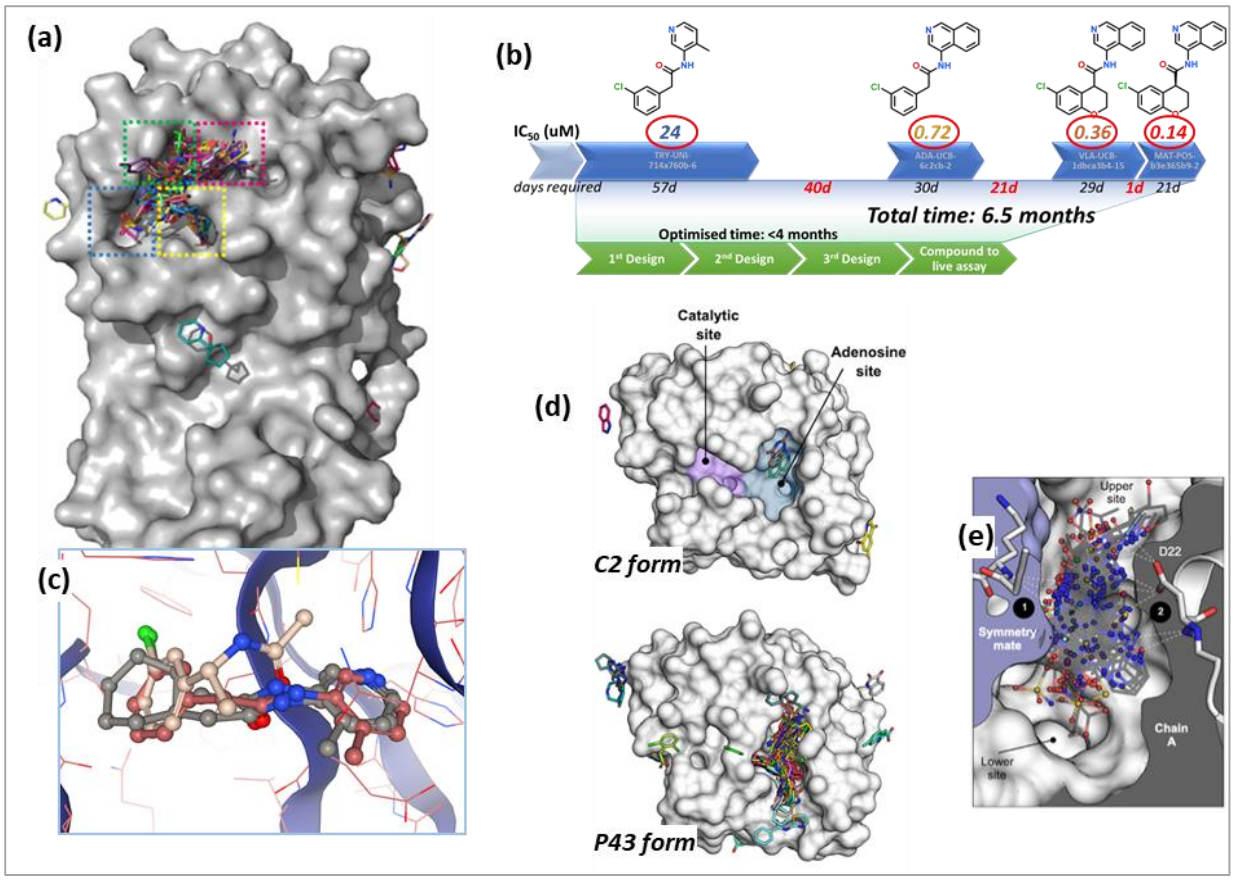


Figure 5: Evidence for the need for large fragment screens on multiple crystal forms. (a) Ensemble overview of the non-covalent hits in the active site of SARS-CoV-2 Mpro. (b) One fragment "merge", TRY-UNI-714a760b-6, could be progressed rapidly to a 200x increase in potency (red circles). (c) The experimentally observed pose of the "merge" (teal), overlaid on those of the fragments that inspired the "fragment merge" (grey, beige), demonstrates how the merge closely recapitulates the originally observed interactions. (d) Diverse compound design opportunities afforded by two views of the active site of Mac1, the macrodomain of SARS-CoV-2 NSP3, screened as two different crystal forms but with the same fragment library (UCSF). While the C2 form better revealed the catalytic site, the P43 form had an extremely rich set of hits. (e) During compound design on the P43 hits, it was crucial to take into account that interactions with the crystallographic symmetry copy had increased the hit rate.

and the other at UCSF and SSRL (James Fraser). The screens yielded significantly divergent views of the active site (Figure 5d), both of which appear relevant to (ongoing) compound design, including allowing rationalising the role of crystal contacts (Figure 5e).

These learnings have informed major upgrades of I04-1 in progress in 2021 (new Eiger2 detector) and 2022 (new HPMU insertion device, providing a 3.5x flux increase), which will improve throughput by up to 5x and will be ready for reuse on the new beamline. These are unlikely to allow both appropriately bigger experiments and a significant expansion of the user base.

3.2 Science enabled by project

This proposal identifies seven high-level science drivers for reinventing I04-1 by rebuilding it on the K04 straight, fully exploiting Diamond-II properties to increase throughput by 15-fold or more, and thus hugely increasing XChem capacity and simultaneously supporting demand for MX assuming the beamtime allocation remains as now with 1/3rd going to XChem experiments.

3.2.1 Business continuity: minimise loss of ongoing science with Diamond-II

The ongoing user programme is in high demand and the science is stringently selected by peer review, or prioritised by industrial users; consequently, any loss of capacity would greatly slow down science and discovery. This is true for all scenarios that do not involve building a new beamline elsewhere (K04): rebuilding I04-1 *in situ* would mean down-time before or after the Diamond-II dark period; and not rebuilding it at all would move the entire burden on the rest of the MX village, placing increasing pressure on the MX user programme. Since the new beamline will be tunable, it will have comparable capabilities to I03 and I04, ensuring there is capacity, robustness and resilience across the whole suite of MX beamlines; this allows all beamlines to pursue their orthogonal science programmes aggressively (XChem in the case of K04).

3.2.2 Accelerate progression of XChem results through routinely large experiments

It is now clear (3.1.5) that routinely large XChem screens will greatly speed up progress to potency and biological impact, and that screening additional protein conformations in different crystal forms has great added value, not least to ensure what is being targeted is a biologically relevant conformation. The current schedule rarely allows experiments larger than ~500 crystals, whereas the goal should be >1500. This can only be achieved by building K04 in the Diamond-II upgrade.

3.2.3 Enable and accelerate far more drug discovery in established and new groups

As the XChem approach becomes more powerful, overall demand will increase, from both established drug discovery groups and new entrants into the field, whether academic or industrial. Consequently, achievable throughput must be significantly higher than what is currently available on I04-1 and in the MX group. K04 is projected to support 10x more projects, ensuring that more scientifically excellent proposals can be accommodated.

3.2.4 Enable exploratory tools for investigating novel biology and validating drugging modalities

A widely shared concern in drug discovery is the rarity of good, validated targets; and conversely of effective ways of drugging proteins known to be key, but where direct inhibition generates excessive toxicity, or leads to resistance.

In contrast, the intersection of structural biology and biological chemistry continues to fruitfully produce specific hypotheses around functions of specific proteins/genes, along with general concepts of how biological or clinical function may be modulated. Ideas include allosteric binding, protein-protein interactions, PROTACS, or modulating large conformational changes. Most XChem experiments already reveal many putative pockets through clusters of hits, yet their biochemical relevance is near-impossible to validate without tool compounds. Accelerating their development through K04/XChem would unlock a large spectrum of biology and clinical impact. This complements one of the scientific foci of the Rosalind Franklin Institute.

3.2.5 Extend screening to previously intractable systems (membrane, complexes)

A large number of proteins remain out of reach of small molecule investigations, often because the crystals are too poor for the I04-1 pipeline. The K04 beamline will have the flux needed to measure such crystals reliably with high throughput. This will turn difficult systems into tractable ones, including membrane proteins or protein-protein complexes, allowing an in-depth understanding of their chemical biology in the first instance, and subsequently their validation as druggable targets.

3.2.6 Provide key infrastructure for rapid response to future pandemics

K04 and its promise of accelerating screen-to-potency through generic approaches would form a critical part of pandemic planning, in anticipation of widely predicted emergence of further pandemics.

3.2.7 Generate the vast datasets needed to develop robust in silico *drug discovery*

A horizon prospect in SBDD is the 4th industrial revolution, with artificial intelligence and automation achieving the design *in silico* of clinic-ready compounds, and tool compounds becoming a commodity in biological research. Evolving methods that factor out the human will require very large quantities of data, which K04/XChem will be uniquely positioned to deliver, working closely with the Rosalind Franklin Institute.

3.3 Diamond-II portfolio

The K04/XChem proposal directly addresses multiple aspects of the Diamond-II vision:

- Retain a world leading research facility status
- Enable a step change in generating ideas to solve 21st century challenges
- Increase the capacity to serve the user community
- Improve the efficiency and productivity of industrial partner

Diamond-II enables this proposal most directly by opening up a new straight section for a beamline to be built from the ground up, optimised for the flux and extreme automation needed to achieve the throughput that will deliver the primary science case.

Beyond that, the specific properties of the electron beam are what will make XChem screening viable on the weakly diffracting, radiation sensitive and inhomogeneous crystals typical of membrane proteins and large complexes. The high brilliance will provide the necessary flux in small beams for measuring locally-ordered crystal volumes, while the low emittance furthermore allows routine exploitation of photon escape[21], the effect enhanced by the higher energies (25keV) achievable. Collectively, this increases the yield of diffracting photons relative to radiation decay[22], bringing weak crystals into reach of high-throughput experiments.

The K04/XChem proposal fits precisely into the science group roadmap by ensuring that the UK continues setting the pace in SBDD, a field in which both academic and industrial sectors have been foundational and where it has remained world leading. Moreover, the photons and specialisation required to deliver the science case cannot be provided by the other MX beamlines: it was stressed in the 2018 user consultation that Diamond's existing offering of high-quality, high-throughput MX analysis must if anything be expanded, and certainly not be compromised by the development of new experimental modalities; the current impact on biological discovery is enormous and should continue that way.

The new offering has close synergy throughout the science group. Most straightforwardly, beyond what I04-1 does today, K04 will provide comparable capabilities to **I03 and I04**, ensuring seamless delivery of standard MX experiments, thus ensuring village-wide capacity increases with Diamond-II. This creates space on all 3 beamlines to develop orthogonal modalities (e.g. research at BSL3 at I03, phasing specialisms and complementary information on I04), the way XChem is established on K04, and building resilience in the user programme by high peak capacity. For fragment screening itself, future XChem experiments will routinely call on the room temperature beamline **VMXi**, since crystal structures and fragment hits obtained at room temperature provide highly complementary information to the cryogenic XChem data that will be generated on K04, as it is now on I04-1. Moreover, VMXi will help identify variations in protein conformation, providing alternative crystals for additional cryogenic screening.

Techniques for small-crystal data collection will be directly adopted **from I24**, where they are being pioneered and the serial approaches of the I24 KMX upgrade and XFEL-Hub can add kinetic information to the drug discovery project for critical targets; and the **MPL** will be able to assist users with challenging proteins. Other science groups host strengths that should be invaluable for historically difficult targeting modalities, including blocking protein-protein interactions (PPIs) or disrupting or stabilising conformational

changes. The sensitivity of SAXS to changes of shape suggests that it would be excellent approach for detecting even weak binding effects, so that the upgraded **B21** would be a powerful tool for identifying fragments; while rapid turn-around of cryoEM structures at **eBIC** would help validate hits, not only through complementary confirmation of structural effects, but conceivably to estimate potency if the population of different conformational states can be quantified through particle classes.

3.4 Academic user community and beneficiaries

Demand for XChem currently vastly exceeds capacity, at two levels: calls for proposals are oversubscribed (1.5-3x); and the experiments themselves are far smaller than necessary. Experienced FBDD users requested larger screens from the start, but since even smaller experiments yield valuable hits, the bias has been to accommodates more experiments, rather than few large ones.

The beamtime available for academic XChem measurements within the overall I04-1 user programme provides for approximately 700 crystals a week, which must also accommodate crucial crystal characterisation experiments. Screens thus tend to comprise ~500 datasets, well below the 1000-2000 now evidently necessary. Since 2019, most capacity is allocated to BAGs, as they were judged to maximise impact; this has necessarily limited the size of the user community.

UK users account for 60-70% of experiments, with the remainder recruited from Europe (funded by the iNEXT grant) and North America, along with individual users from South Africa (funded by the GCRF-START grant) and Brazil.

Well over 100 researchers have visited the facility, most of whom once or twice. Thanks to 10 designated superusers, who are able to work fully independently and train their own users in turn, the number of users involved has been growing faster than the team's capacity to train new users.

Growth of the community will be driven by two developments. The first is that major capital upgrades of the existing beamline I04-1 are approved and in progress (fast EIGER2 detector, 3-5x flux increase through a new undulator; both to be transferred to K04) that will support up to 3x more projects The second is Diamond's participation in two large Europe-funded projects that started in 2020, and which collectively reach very large audiences: iNEXT-Discovery[d] (Horizon2020) funds trans-national access to facilities across Europe for translational research, including the four facilities offering fragment screening (Diamond, EMBL/ESRF, BESSY and MAX IV); and EUbOpen[e] (IMI2) is developing large chemogenomic compound collections, including through crowdsourcing experimenters through XChem.

Finally, the community of European developers and providers of crystal-based fragment screening is brought together through the iNEXT-Discovery grant, with a Joint Research Activity aimed at improving infrastructure for data analysis and harmonising mechanisms of dissemination.

Overall, the user community will be thoroughly primed for the dramatic increase in capability provided by the K04/XChem facility, as will be the ultimate target community, which comprises all practitioners of SBDD and is very large indeed. It has grown through initiatives like the recent COVID Moonshot[23], almost ~400 people around the globe took up an invitation, broadcast on social media, to evaluate the SARS-CoV-2 Mpro fragment hits, submitting >4000 compound designs within 2 weeks.

3.5 Industrial user community and beneficiaries - impact on UK PLC

The industrial XChem user base ranges from large pharmaceutical companies, through contract research organisations (CROs) and SMEs down to small start-ups, with usage and demand growing strongly year-on-

[d] https://inext-discovery.eu/
[e] https://www.eubopen.org/

year (Figure 6). This leads to the conclusion that for smaller companies, the facility (like the whole of Diamond) represents a *"world-class enabling innovation"* [Chris Phillips, UWG] that is presumably at least partially baked into their business plans, because it allows them to function at the level of a large pharmaceutical. In the latter sector, companies that had not yet established their own in-house platforms, now appear to judge that they no longer need to do so either.

In the UK, there is a noteworthy alignment of requirements and expectations between start-ups and the various Drug Discovery Units (DDUs) based in academic centres and heavily funded by charities. The latter therefore straddle the industrial and academic user bases, similar to spin-outs, and for both the facility makes it possible to work on challenging targets.

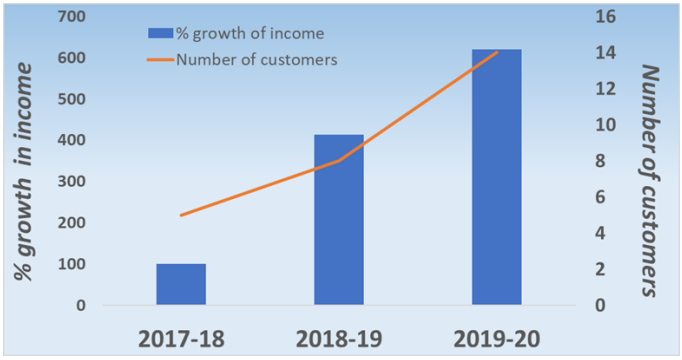


Figure 6: Growth of Industrial usage of XChem

Collectively, these activities mean that the XChem facility currently impacts on UK Plc most strongly via SMEs and start-ups; but that the step-change K04 will achieve will likely make large pharmaceuticals re-evaluate their sunk costs and also come to see K04/XChem as part of their external infrastructure, as they already do for the MX village as a whole.

Meanwhile, industrial users have if anything been clamouring even more loudly for increased capacity than the academics. Waiting times for industrial users approach 6 months, even after the industry allocation on I04-1 was doubled in 2019. Such long delays are highly problematic for active discovery projects, which tend to need vital data far quicker than that.

Nevertheless, growth of industrial use has allowed Diamond to invest in increasing capacity of both user programmes, through new lab space, equipment, and above all staffing of the support teams. This collectively did resolve the non-beamtime bottlenecks, so that industrial XChem beamtime is now again invariably fully booked.

A final impact of XChem on UK Plc is indirect: by enabling academic groups to engage in active discovery projects, it helps train the next generation of scientists in drug discovery, increasing the pool of expertise from which companies can recruit.

3.6 Comparison to other synchrotron facilities, current and planned

Since the beginning of the XChem operations in 2016, I04-1 has continually evolved to ensure fast, reliable and fully unattended data collection. Feasibility had previously been demonstrated at the Lilly-CAT[f] beamline (APS), which ran an unattended programme since the early 2000s. The fully automated beamline MASSIF-1 (ESRF)[g] evolved over time to encompass broader capabilities, initially screening and collecting from user generated crystals, and now offering a crystallisation-to-structure service linked with the HTX platform[h] of EMBL-Grenoble.

I04-1 supports the regular MX user programme, alongside the high-throughput XChem user programme. The high throughput MX beamlines at Diamond will collectively offer a rapid response service: thus for

[f] http://lrlcat.lilly.com/
[g] https://www.esrf.eu/MASSIF1
[h] https://www.embl.fr/services/ht_crystallisation/

instance, in the current COVID-19 restrictions, they were able to mix fully-automated collection with interactive remote experiments. The K04/XChem facility will be positioned to take the developments driven from this to another level for FBDD.

Diamond's XChem development has been mirrored by independent initiatives at EMBL-Grenoble/ESRF and BESSY. Other synchrotrons exploited XChem's open technologies to also set up such offerings, either complete (SLS, MAX-IV) or in development (SSRS, PLS); and one of the very first experiments at Sirius (Brazil) was a fragment screen in 2020, partly enabled through XChem's PanDDA software[12]. Diamond however stands alone in the size of its offering, enabled by dedicated weekly beamtime supported by the overall capacity of the MX village, and by the funding of a focused user support team along with the necessary advanced lab equipment, duplicated for redundancy.

An orthogonal development in structural biology, the emergence and rapid growth of cryoEM, presents a tremendous opportunity for repositioning of crystallography[24,25]. The transformation in throughput delivered by MX beamlines such as K04, linked to the unique capabilities of the XChem facility, opens possibilities not currently available, increasing the relevance of expanded capacity and rapid response to existing and emerging health needs. A significant expansion of output from K04 will be transformative and cannot be matched by any other structural biology technique. This is an opportunity also embraced by the new FragMAX[i] facility (MAX IV); what will be unique however at Diamond-II is that full capacity can be sustained for other MX experiments, thanks to the breadth of the MX group at Diamond.

I04-1/XChem is now unique in its capabilities for joined up FBLD activities for proprietary and non-proprietary research and recognised as world-leading[25]. The delivery of beamline K04 will ensure continued pre-eminence in this field for Diamond and the UK.

3.7 Combined impact of project and added value in relation to activities on the Harwell Campus and beyond.

A key part of expanding both the community and the impact of experiments, is establishing avenues for progressing hits to potency. A 2019 survey (Figure 3) confirmed what had been assumed all along, that a significant proportion of academic users, unless they were part of a drug discovery ecosystem, faced tremendous challenges in building even on highly successful XChem experiments. This is wholly unsurprising, since it requires both highly specialised knowledge and experience, and high levels of sustained funding – both of which are notoriously difficult to acquire outside of industry.

However, the real answer must lie in closing the huge and self-evident technology gaps (Section 3.2), and the Harwell Campus provides an exceptional opportunity to do so: the Rosalind Franklin Institute was founded specifically to address such questions, and its Next Generation Chemistry for Medicine theme encompasses methodology of medicinal chemistry.

The Research Complex at Harwell has similarly provided excellent synergies, and will continue to do so. To date, its office and laboratory space and crystallization facilities have been pivotal to developing, maturing and offering the XChem facility, and it will continue to do so. Moreover, as and when follow-up technologies mature at the Rosalind Franklin, it will be because of the RCaH that it will be conceivable to convert them to accessible user facilities.

Such ambitions were also a key driver of Diamond joining the EUbOpen project, but it was specifically the context of the Harwell Campus that made them credible when Diamond was debating the business case.

[i] https://www.maxiv.lu.se/fragmax/

4. Beamline performance specification and requirements (1 page)

For the beamline, the throughput and data quality can be achieved initially with refinement of existing technology, although ultimately aiming at a standard energy of ~25keV will necessitate the arrival of cost-effective high Z, large area integrating detectors.

Flux in excess of ~10^{13} ph/sec in a 1-50µm beam with a narrow bandpass being acceptable, and a highspeed, integrating detector along with a fast, high-density sample changer will enable the aims of K04/XChem. Development of fully automated sample preparation technology, to ensure the necessary large numbers of samples can be generated for large numbers of experiments annually, should run in parallel to development of the beamline.

- Coupling a 1.5m HPMU (transferred from I04-1) in straight section K04 with a DMM/DCM and the new Diamond-II lattice will provide at least two orders of magnitude higher flux (>10^{13} ph/sec) than currently available at the fixed energy I04-1, over a broad energy range (10 – 27 keV). This provides a flexible platform for experiment adaptability and provisions for future exploitation of high energy data collection.
- Variable focussing options (~1-50µm) should be available to adapt to crystal properties of the target campaign.
- Diagnostic feedback systems and beam position control loops should be built in from the ground up for continuous beam delivery and alignment.
- High capacity, ultra-high-throughput end-station capable of 5,000 data collections per day (developed for MXBridge and refined for K04 and ultimately I03 and I04)
    - High speed goniometer equipped with cold nitrogen stream.
    - High speed robot exchange.
    - High capacity cryogenic sample storage system able to exchange large numbers of samples rapidly to minimise hutch open time.
- A silicon integrating detector will be appropriate initially but longer term a high Z sensor detector will be needed if routine use above 20 keV is shown to be effective for the smallest crystals.
- Data analysis pipelines will be in place to cope with the ultra-high data rates. Reduction of diffraction data on the fly will be essential for both x-ray centring and integration. Consideration of data model for storage will need to be assessed and implemented. Cloud based capabilities will need to be baked in at the outset for downstream XChem analysis pipelines.

4.1 Additional developments required

K04 itself will implement the ability to routinely screen membrane protein systems by having at its disposal at least 100x more flux than I04-1 in 2020 (3x10^{13}ph/s @ 25keV from an undulator to be delivered in 2022) focussed as a microbeam (1-5um). This will require adopting methodologies developed on I24.

New automation will be required to increase the shutter-open time: robust multi-crystal sample holders, offline sample pre-centring in parallel with the experiment, and a double gripper robotic sample changer.

New fully automated, high-speed (acoustic) crystal harvesting with in-line compound (acoustic) dispensing will be required, coupled to high-volume crystal production and streamlined compound library management.

With such capacity, the compute power will be broadened as well given the overall requirement for processing 15000 samples within 40 hours per weekly run (375 samples per hour). All that data served through an expansion of the XChem Fragalysis cloud compute infrastructure.

5. Schematic outline of beamline or project (3 pages max)

The overall K04 concept is that it must guide and support users to reliably deliver and measure what is today a very large number of samples and analysis results; and it should do so for samples classed as challenging (*e.g.* membrane proteins) because of their need for small beams and high flux, and strategies to mitigate radiation damage. Crystals will feed into a new, highly automated sample preparation and data collection workflow that in turn relies on a bright and stable beam delivery from a source designed to deliver that.

5.1 Fast queues and on-the-fly analyses

XChem queues are envisaged to run 3 days per week. For challenging crystal systems (16s/dataset), the rate will be 225 datasets per hour (80s per multi-sample pin), yielding up to 9000 datasets per week, to accommodate multiple datasets for the same compound. Crystals can be as small as 1um, to exploit photon escape at high energies.

For well-behaved, routine crystals, weekly throughput will be higher (15,000 or more), allowing 3-5 distinct screens, either for different crystal forms or else different samples

This data rate will require extremely high-performance compute infrastructure, which approaches what is currently available across all of Diamond.

5.2 Sample preparation

A key challenge for big screening campaigns by X-Ray crystallography is to obtain a reproducible, robust crystal system for the target of interest. This is generally protein-dependent, but might be addressed by developments in the community of molecular technologies that address this (e.g. crysalins, section 3.1.4).

As libraries will now larger, engineered solutions for compound logistics will be necessary, including an argon-purged storage unit and plate sealer. In addition, a compound management database will be required to track usage of individual compounds from the libraries.

Crystal harvesting itself will need to be a newly engineered solution to achieve fully automated harvesting (Figure 7) to deliver 3000 crystals/day, as the current semi-automated solution (300/day) will not scale. Multi-sample pins holding 5 or more crystals have already been shown to be feasible, with no cross-contamination of compounds in separate crystals (Figure 7d,e). Both compound and crystal dispensing can be achieved by acoustic dispensing, but likely from different sources simultaneously, with two ultrasonic transponders (Figure 7f); extensive experience is available from the XFEL hub, as well as the literature[26], not least for how to dispense 1um crystals. Finally, freezing of samples will need to be automated, which has been shown to be feasible (e.g CrystalDirect[27] or Nanuq[28]).

5.3 End-station

As the end-station currently in I04-1 has evolved by an artisanal process to achieve 30-60 datasets per hour, it will not be appropriate for the K04 requirement of 3000 samples per day. However, it does contain a key lesson, which is that it will have to be engineered to be dedicated to ultra-high throughput. Diamond has extensive experience with end-station design, most recently I24, VMXi and VMXm; these designs will therefore be closely studied for crucial learnings, in particular how to optimise signal from very small (1um) samples.

What is already clear is that it will be crucial to break the sequential nature of the current process, where any step in the process precludes another from proceeding (e.g. data collection and crystal centring). A thorough technical review will examine options such as orthogonal goniometers, which can drive into the beam with high accuracy, allowing sample exchange and optical centring to proceed on one, while data collection proceeds on the other.

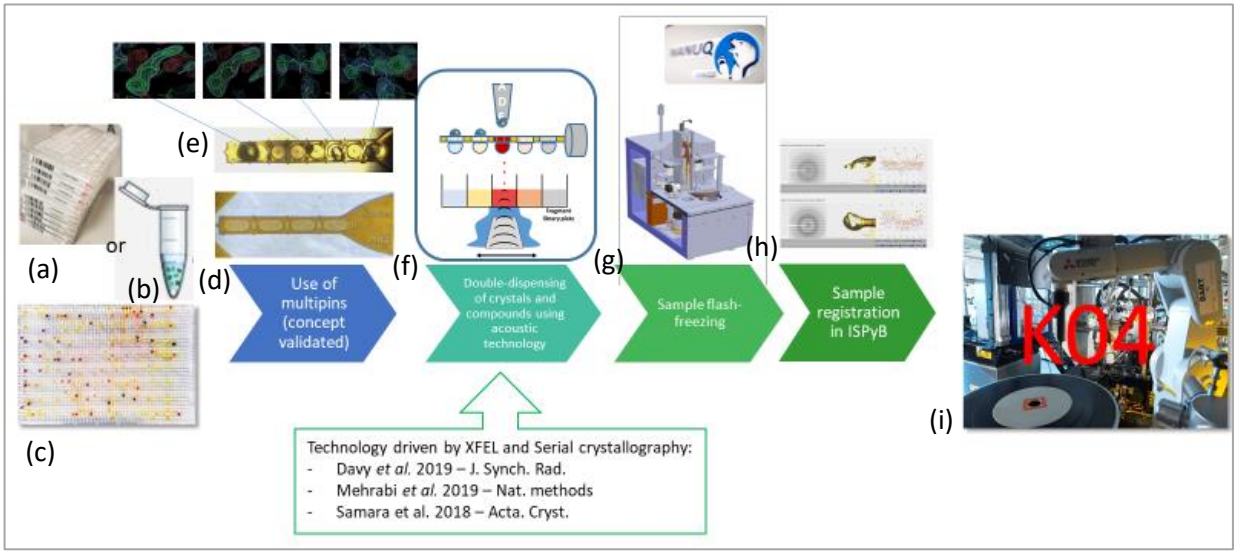


Figure 7: Concepts for fast automated sample harvesting, combining multipin and acoustic dispensing technology. (a)Vapour diffusion tray; (b) Batch crystallisation vial; (c) Compound stock tray (a.k.a. compound library); (d) Multipin holder; (e) e-density for each crystal in a multipin; (f)Acoustic droplet ejection for compound and crystal; (g) Nanuq sample flash cooler; (h)Sample registration; (i) Samples reach beamline K04

The sample changer will also need to be tightly integrated with the process, and will include a double-gripper. To ensure it remains manageable to move the envisioned large numbers of samples from lab to beamline, the density of the holders ("pucks") will likely need to be increased as well. Full automation of sample preparation (section 5.2) opens engineering opportunities that will be carefully assessed during conceptual and technical design reviews.

5.4 Optics

Current models of flux and throughput assume that a high band pass monochromating system (Dual Multilayer Monochromator, DMM) will be combined with compound refractive lenses (CRLs). A possible arrangement is shown in Figure 8, which achieves a focussed beam around 5 µm, and coupled with X-Ray beam position monitoring stabilisation systems (XBPMs), has proven to deliver highly reproducible, stable, intense and bright X-Ray fluxes in several Diamond beamlines. However, the required beam might also be achieved with a shorter beamline, saving cost and leaving room for laboratory space; this will be assessed in-depth during conceptual design review.

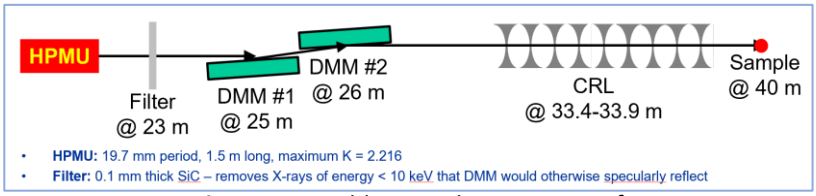


Figure 8: Possible optical arrangement for K04.

5.5 Source

The new insertion device for I04-1 that is being commissioned for installation in 2022, was specified also to be used as the K04 source. It therefore complies with both D-I and D-II machine requirements (heat load, geometry and services), and was optimised to deliver its highest flux in Diamond II. It is a 1.5m in-vacuum hybrid permanent magnet undulator (HPMU), and the primary energy for routine operation was chosen as 25 keV, to maximise photon escape for extending sample lifetime.

Combined with the dual DMM and CRL systems (section 5.4), it will deliver to the sample a flux of 6.0x10^{13} ph/s in D-II (a nominal 170x increase over the 3.5x10^{11} ph/s currently delivered to I04--1). It thus forms the cornerstone of the envisaged increase in capacity.

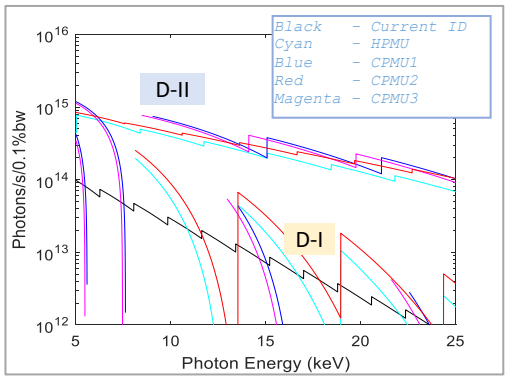


Figure 9: Flux delivered by the proposed HPMU (cyan) compared to the current ID (black) and CPMU models, in Diamond-I and Diamond-II scenarios.

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7. Expressions of interest & support from the community

The User Working Group, half of its members representing industry, contributed expertise spanning the multidisciplinary scope of the proposal: beamlines, automation, chemical biology and drug discovery. UWG discussions fundamentally shaped both science case and webinar agenda, which included one academic and one industrial speaker (Endicott, Barker). Webinar announcements appeared to be widely recirculated on social media, and the webinar attracted 159 attendees, generating insightful questions. The 118 statements of support (collated in Appendix I) were submitted by individuals, institutes, drug discovery units, doctoral training centres, SMEs and companies with broad international reach. Another 175 letters of support from life science researchers were submitted for the Wellcome Trust application to fund the Diamond-II project (UK academics: 97; industry: 12; international: 66). Those singling out the K04 flagship project are also included in Appendix I.

Clear themes emerged from the letters and statements of support, but most prominent (28 respondents) was the need for, but also difficulty in, accessing the current XChem facility (*"the pipeline is a victim of its popularity"* – Yann-Vaï le Bihan, ICR). Another theme was the need to address challenging problems (19 respondents); and thought leaders highlighted the opportunities for chemical biology (18 respondents):

- *"The XChem facility is a remarkable resource that … is absolutely critical to the realisation of this global goal [Target2035] for biomedical science"* Prof **Adam Nelson, U. Leeds**
- "*By enabling the development of site-specific modulators of macromolecular function, this beam line can be expected to have a fundamental impact on the entire field."* **Prof Martin Noble, U. Newcastle**

The need for accelerated drug discovery was mentioned in many contexts, along with several examples of XChem results leading towards commercialisation, including:

- "*Based on data collected using the K04/XChem facility … [we] have attracted substantial series A venture capital investment of £12.4 M to establish a newCo ... Fair to say that the XChem facility has been transformational for the work of our lab*" **Prof Alan Warren, U. Cambridge**
- *"… the XChem platform at Diamond has been a triumph - it jump-started a structure guided drug design campaign that […] has already resulted in one published patent application"* **Prof Yvonne Jones FRS, U. Oxford**
- "*… XChem … in securing new grant income for my spin-out and … molecules designed from … XChem … were able to kill Neisseria gonorrhoea*" **Jon Sayers, DeFENition Ltd**

Industry respondents spoke to integrating XChem in their business plans:

- "*… would enable Exscientia to continue to pioneer productive drug discovery and provide large additional value to the company and to the UK economy*" **Anthony Bradley, Exscientia**
- "*… will be a key component of the UK Life Science infrastructure … upgrade will mean that we and others might actually get on it!*" **Chris Phillips, AstraZeneca**

Finally, several respondents highlighted the strategic possibilities:

- *"… peer-reviewed access to high-throughput in-crystallo fragment screening... was an extraordinary breakthrough in the field, … Large scale facilities of this sort harness decades of engineering and scientific expertise and have led to many novel discoveries. … highly strategic objectives which are perfectly timed*" **Jon Cooper, UCL**
- *"I think this is a marvellous example of a central scientific facility supporting innovative biotech and academic groups to identify perturbants for biological systems"* **Jonathan Read, AstraZeneca**
- "*I cannot imagine a structure-guided project that in the future would not go through this pathway."* **Marko Hyvonen, U. Cambridge**
- "*Securing the upgrade to the Beamline will help maintain the nation's leading position in both industrial and academic small molecule drug discovery*" **Prof Mike Waring, Director of MoSMed CDT, U. Newcastle**
- "*The majority of data [of protein-fragment interactions] is locked away in industrial organisations … XChem [will place] fragment structures in the public domain to facilitate methods development*" **Jason Cole, CDC**
- *[Mine] was the first Brazilian project to be accepted to use this pipeline … projects coming from developing countries are more likely to have a chance of being accepted."* **Victor Rangel, U. Saõ Paolo, Brazil**

Statements of support summary

Total number of submissions: 118

Key for statements in Appendix I	Respondent's primary field of research	Percentage of respondents
	Life Sciences & Biotech	91.5%
	Chemistry	8.5%

Respondent location	Percentage of respondents
UK	69.5%
International	30.5%

Type of organisation supporting	Percentage of respondents
Academic	78.0%
Industry	14.4%
Charity	2.5%
Government	2.5%
Other	2.6%

Diamond user status	Percentage of respondents
Not currently a user at Diamond	11.9%
Currently a user at Diamond	88.1%

Engagement webinar summary

Date of webinar: Monday 16th November 2020
Number of attendees: 159

Attendee location	Percentage of attendees
UK	68.8%
International	31.2%

8. Appendix I

List of letters of support