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LMX: Large Molecule Single-Crystal Diffractometer at ISIS

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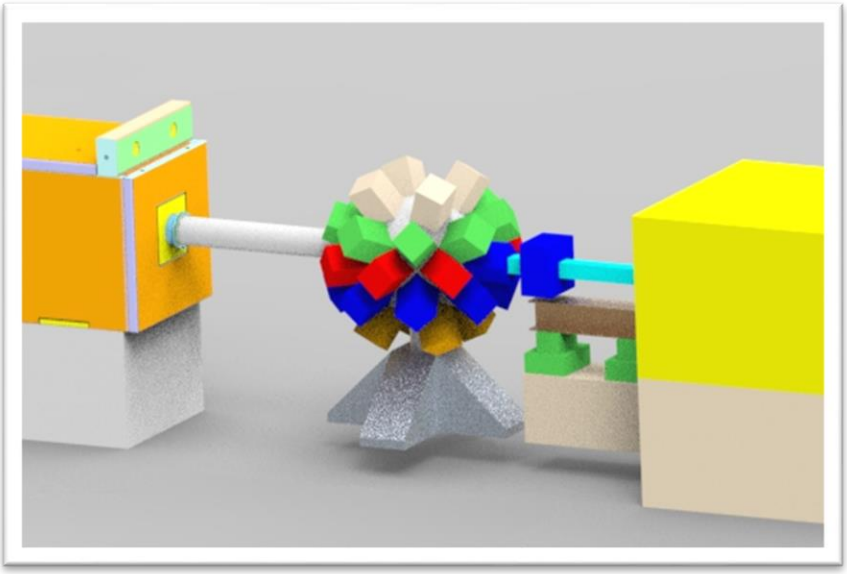

The Science and Business Case for the construction of a Large Molecule Single-Crystal Diffractometer, LMX, at the ISIS Facility

Nick Funnell, Silvia Capelli, Matthias Gutmann, Paul Henry, Steve Hull, David Keen, Michael Ritchie and Craig Bull

6th December 2022

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1. Introductory Remarks

This document presents the case for the construction of LMX; a new single-crystal diffractometer at the ISIS Neutron and Muon Source aimed at studying the crystal structures of large molecular assemblies. At the moment, the only instrument dedicated to single-crystal diffraction at ISIS is SXD – an instrument that was last upgraded and optimised for small-molecule crystallography about 20 years ago. The proposed LMX instrument will provide a step change in the single-crystal instrumentation capability at ISIS, as well as new abilities to measure:

- technologically-important functional materials with greater structural complexity than previously possible
- small proteins, exploiting the neutron's sensitivity to light atoms to further our knowledge of biologically-relevant systems
- Single-crystals with very small sample volume in order to meet the current requirements of the supramolecular/macromolecular science areas

In line with these goals, LMX will allow neutron single-crystal diffraction to be performed across many highly topical research areas, including supramolecular chemistry, hybrid organic-inorganic complexes that exhibit mixed properties, pharmaceuticals, framework materials, energy harvesting materials, catalysts, as well as biologically-relevant macromolecules such as proteins and nucleic acids. In particular, LMX will address new and important scientific challenges as defined by the UKRI and BEIS strategies[1] including life sciences and healthcare, energy and clean growth and advanced manufacturing and materials. This diverse range of new research opportunities provides a powerful motivation for the proposed LMX instrument, which will significantly expand the capabilities available at ISIS in the field of single crystal diffraction. The project has strong backing from existing ISIS users and significant potential to expand into new research communities who do not currently use the neutron diffraction facilities at ISIS.

Details of the LMX instrument design are provided in a separate Technical Design Document.

1.1. A brief history of the LMX project

Plans for an LMX instrument on the Second Target Station at ISIS have been discussed for well over a decade, with an outline design and a proposal submitted as part of the Phase-II instrument development suite in 2005. However, the project was not approved at that stage. Whilst the scientific case was acknowledged to be strong, there were technical concerns around the provision of suitable two-dimensional detector technology, as well as large financial costs associated with a long guide necessary for operation on the Second Target Station. In the past few years, significant advances have been made by the Detector Group at ISIS. A prototype of a two-dimensional position sensitive detector based on Wavelength Shifting Fibre (WSF) technology has been in use on the SXD instrument for the last two years, showing excellent performances both in terms of efficiency and spatial resolution (further

details are given in the LMX Technical Design Document). Furthermore, the science case presented in Section 2 will prove the requirements of the instrument can be fulfilled on the First Target Station with a shorter incident beam guide.

In this document, the ISIS Crystallography Group is revisiting the scientific and business cases for an LMX diffractometer including a re-evaluation of the technical design, taking advantage of the progress made in areas such as detector technology and neutron guides, to produce a new outline design.

1.2. Layout of the document

The document is divided between the Science and Business Cases:

- Section 2 describes the scientific drivers of the new instrument, which broadly fall within the topics of chemical crystallography and structural biology. Examples of current and future research topics to be addressed on LMX are provided, and their implications for the specification of the instrument are presented.
- Section 3 provides the business case for the construction of LMX, highlighting its likely scientific impact, comparison with other instruments worldwide and its complementarity with other facilities on the Harwell Campus (*e.g.* Diamond Light Source, Rosalind Franklin Institute and Research Complex at Harwell).

2. Science Case

"Why water boils at 100°C and methane at −161°C, why blood is red and grass is green, why diamond is hard and wax is soft, why glaciers flow and iron gets hard when you hammer it, how muscles contract, how sunlight makes plants grow and how living organisms have been able to evolve into ever more complex forms….the answers to all these problems have come from structural analysis."

Max F. Pertuz, 1996
Nobel Laureate in Chemistry, 1962

Single-crystal diffraction is the most definitive analytical method for determining the structure of materials in their solid state – containing structural information in three dimensions *cf*. one dimension for powder diffraction. Fundamentally, it provides a near-unambiguous quantitative model of the atomic arrangement in a given crystal. These models are then used to understand the *physical properties* and eventually *function* of the material. For instance, differences in atomic packing between graphite and diamond explain why they exhibit such different properties. In biological systems, the study of the active site of a protein, helps explain why only specific molecules are suitable to act as effective drugs in treating disease. It is no coincidence that some of the most important discoveries in materials science,[2] supramolecular chemistry,[3] and structural biology[4] are thanks to analysis of crystallographic models. Over 30 Nobel prizes are identified by the International Union of Crystallography as being associated with crystal structure analysis.[5]

Diffraction using neutrons[6] imparts specific benefits, unrivalled by other probes, thanks to the specific properties of neutrons, for example, their ability to distinguish between isotopes or electronically-similar elements, i.e. species that would have near-identical X-ray form factors, but quite different neutron scattering lengths.

The particular strength of neutrons with respect to this case are their ability to determine the positions of light atoms – in particular hydrogen and deuterium – in the presence of heavy ones. This is owed to the fact that neutrons are scattered by the atomic nuclei and therefore their scattering power is completely independent of their atomic number. This advantage is clear when investigating e.g. hydrogen transport mechanisms which are commonly organometallic species where the hydrogen atoms are located in close proximity to heavy-metal atoms. X-ray diffraction would be dominated by the scattering signature of the metal, whereas neutron diffraction is sensitive to the functionality-defining reaction pathway of the hydrogen atom. In biology, the structure of macromolecules is frequently dependent on hydrogen bonding and protonation states, both of which are most clearly identified through an unambiguous location of the hydrogen atoms.

The following subsections outline the wide-ranging, key, science areas over which the proposed LMX instrument would expand ISIS capability.

2.1. Chemical crystallography

Traditionally, 'chemical crystallography' describes the study of small-molecule compounds; typically organic/organometallic materials consisting of ca. 10–100 atoms per structural unit. This usually involves characterisation of molecular geometries and the nature of interactions between molecules. At ISIS, this community has been served by the single-crystal diffractometer SXD, which has been in operation since 1988, and was substantially rebuilt in 2000.[7] While SXD continues to serve this area of structural science, 'chemical crystallography' has expanded towards more complex materials that now also encompass framework materials[8] and supramolecular assemblies.[9,10] These are typically larger compounds, consisting of more atoms per formula unit which, invariably, have larger unit cell cells in order to describe the smallest-repeating unit in the crystal in full; unit cell axes typically exceed the 30 Å length that SXD was originally optimised to measure. Moreover, it is often prohibitively difficult to grow crystals with a volume (>1 mm^3) in line with the requirements for measurements on SXD, which are particularly stringent for weakly-diffracting organic samples. The former point is illustrated in Figure 2.1, showing the increasing unit cell volume by year of crystal structures published in the Cambridge Structural Database (CSD).

Faced with the new challenges of the evolving chemical crystallography field, SXD users are increasingly turning to other neutron facilities (such as the SNS in the USA,[11] hosting the three single-crystal diffractometers TOPAZ, MaNDi, and CORELLI), or to synchrotron X-ray measurements paired with theoretical calculations to overcome the difficulties in locating hydrogen atom positions. In many cases, as outlined over the following subsections, this structural information would be more accurately obtained from a neutron diffraction experiment – if such a facility were available at ISIS.

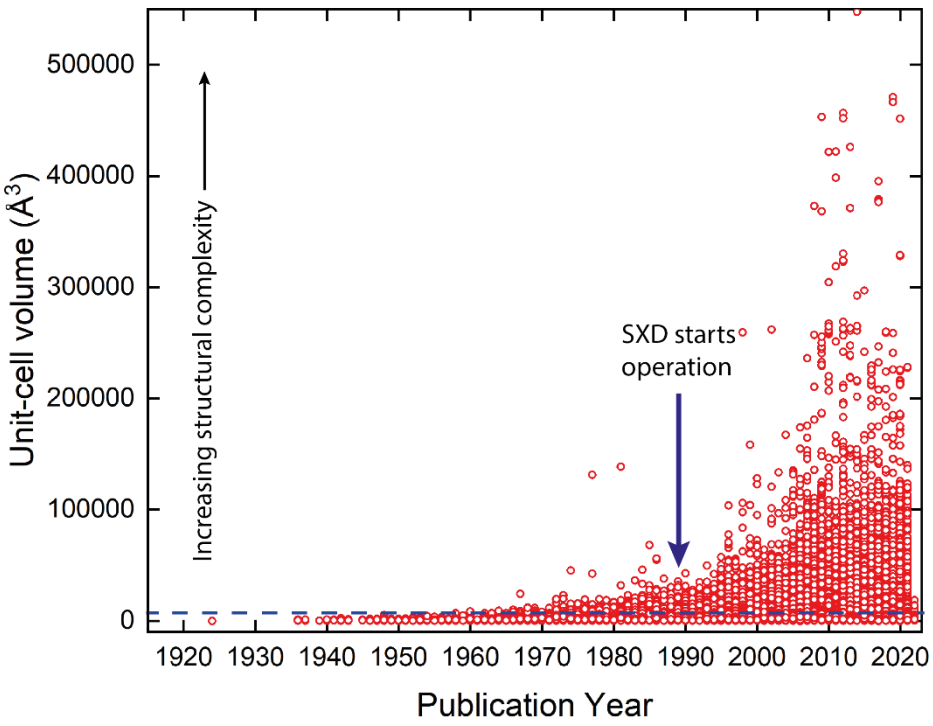


Figure 2.1. Unit cell volume versus publication year of crystal structures in the CSD. The blue dashed line indicates the upper limit of the unit cell volume that can currently be measured on SXD.

Studies of increasing structural complexity are nearly always motivated by the drive to develop materials with improved functionalities. These properties are usually tested by applying an external stimulus such as temperature, light, non-ambient atmosphere, electric field, or high pressure (or a combination of these). It is therefore critical that the LMX instrument offers non-ambient measurement environments for in-situ/operando studies (discussed in the Business Case).

2.1.1. Supramolecular chemistry/self-assemblies

Scientific relevance:
The combination of discrete molecular building units into larger assemblies via non-covalent interactions is the underpinning feature of supramolecular chemistry, where molecular recognition/complementarity dictates the final structural form. This phenomenon has long been observed to exist in nature (e.g. base-pairing in DNA[12–14]) and the chemistry community has since sought to exploit this principle in developing supramolecular systems with applications in e.g. green chemistry[15] and metal sequestration;[16] molecular drug delivery capsules;[17] molecule-specific sensing devices;[18] or even as nanoscale machines acting as artificial muscle,[19] or water-transport channels through membranes, mimicking biological processes[20] – so-called 'biomimetics'. In general, supramolecular materials have extremely wide-ranging practical applications[21] but they also represent an intellectual challenge in forming topologically-complex architectures, e.g. interpenetrating Borromean rings,[9] or materials that exhibit geometric frustration.[22]

Crystallography is an important analytical tool in the development of new supramolecular materials. Almost all these systems can only be understood (and further developed) by understanding how all their constituent parts are arranged spatially, once assembled. Common to almost all supramolecular assemblies is their ability to switch between multiple states, usually driven by some external stimulus. This can include gas adsorption and selectivity between competing species,[23] thermal and/or photochemical switching,[24–26] or host molecule dissolution.[27,28] In all cases, these are most straightforwardly confirmed by interrogation of a crystallographic model, often measured in-situ/operando during the diffraction measurement, in order to establish structure–property relationships.

Benefits of neutron diffraction:
When it comes to determining the most important structural aspects of a supramolecular assembly, neutron diffraction offers several distinct advantages, such as the ability to characterise and understand the role of influential H-bonding interactions in self-assembly processes. The directionality of hydrogen bonds is frequently exploited as an architectural motif in the targeted design of new materials[29] but only neutron diffraction can provide accurate hydrogen atom positions and anisotropic displacement parameters.[30] Knowledge of these is important for reliable assessment of molecular interaction energies.

The location of disordered guest molecules within supramolecular assemblies is another advantage of neutron diffraction. Identification of binding sites is key to understanding the mechanisms of guest capture and release. Disordered molecules may only have partial crystallographic occupancies, which makes their stable refinement challenging. Since

neutrons diffract from the well-defined atomic nucleus, rather than the more diffuse electron cloud, they offer a more precise means of locating a poorly-diffracting species.

In addition, the location of light organic species in the presence of heavy metals can be directly measured. Where the supramolecular assembly is built around metal-containing building blocks, these will dominate X-ray scattering patterns. Though it is clear that neutron diffraction is better-placed to study hydrogen atoms (e.g. measuring the incorporation of H_2 gas into supramolecular cavities), other relatively-poorly-scattering (by X-rays) organic species, such as CO_x and NO_x compounds are also often target guest materials,[31] and may occupy multiple possible binding sites. Their neutron scattering lengths are far more comparable to those of most metals, facilitating determination of their accurate location.

Case study: The structure of water in *p*-sulfonatocalix[4]arene
Ref. 32 – K. Fucke *et al*, *Chem. Eur. J.*, 2011, **17(37)**, 10259–10271

Calixarenes are formed of phenol units linked by carbon bridges to form macrocyclic compounds that possess small cavities in which guest species can be accommodated. The sulfonatocalixarene molecules reported in this study (see Figure 2.1.1) exist as hydrates containing 14 water molecules in the crystal structure and a guest water molecule located in the cavity. How these water molecules are oriented in the cavity is dependent on the intermolecular interactions they form; unambiguous assignment of hydrogen positions is necessary.
The authors measured single-crystal neutron diffraction data on a series of polymorphs in order to identify the binding geometries of the water molecules in each crystal form studied. Though three out of four polymorphs could be studied – on SXD at ISIS and D19 at the ILL, the authors noted particular difficulty in measuring 'Form III' owing to a very large cell axis (57.44 Å) that made its measurement 'extremely problematic'. To complete the study X-ray radiation was necessary but the authors additionally noted 'determining the hydrogen atoms position from X-ray data is extraordinarily difficult and generally not feasible.'

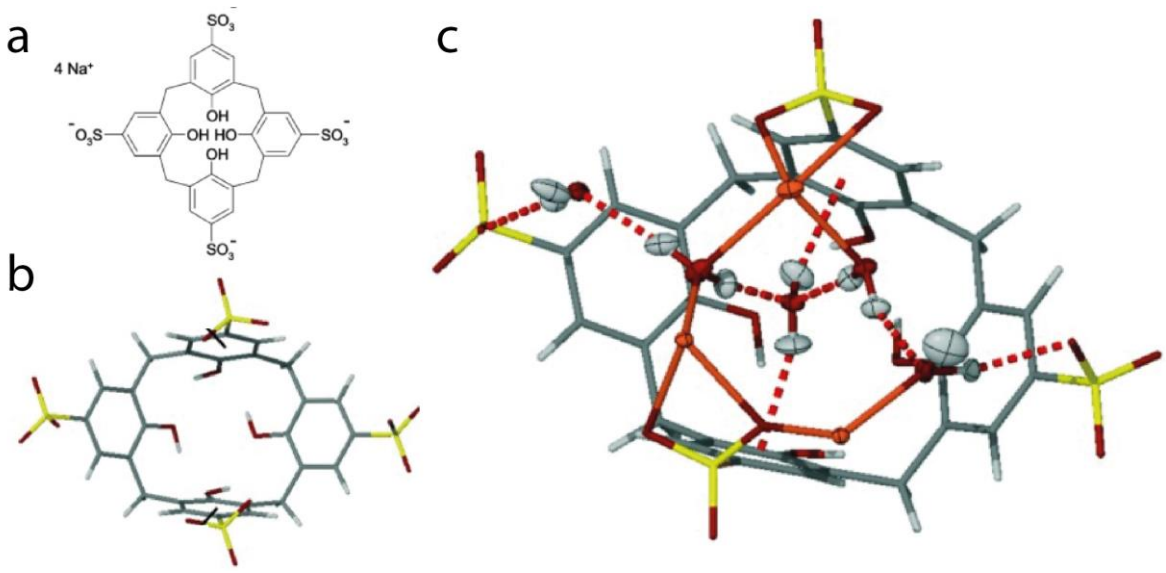


Figure 2.1.1. a) Chemical structure and **b)** neutron crystal structure of the unoccupied sulfonatocalixarene. **c)** Water molecule network, with hydrogen atom positions clearly defined. Figure adapted from manuscript.

2.1.2. Framework materials

Scientific relevance:
Over the past two decades, framework materials have become one of the most dominant research areas in chemical crystallography. Frameworks are comprised of repeated building blocks which, unlike supramolecular assemblies, form covalently-bonded, continuous, networks that possess porous cavities. As a consequence, crystallographic unit cells can be substantial with individual cell lengths commonly exceeding 20 Å and volumes $\gtrapprox$ 15,000 Å^3, and can be as large as 330,000 Å^3.[33]

Though there are too many subcategories of framework to cover here, the most prominent of these are 'metal–organic' frameworks (MOFs) and zeolites. Porosity, and chemical-specific interactions are key to the function of frameworks. Because of the many ways in which chemical composition can be varied (including post-synthetically[34]), and how pore shape and size can be tuned, MOFs have found wide-ranging application in areas such as: gas storage and capture, including toxic gases[35–37] and hydrocarbons;[38,39] confinement of explosive materials;[40] vapour sorption;[41,42] heterogeneous catalysis;[43] MRI contrast agents;[44] lung cancer drug delivery;[45] chemical sensors;[46] and ion conductors.[47]

The ability of framework materials to filter guest molecules on shape and size, mean they have demonstrable commercial application, with companies now being set up to bring this technology to the industrial sector.[48] Zeolites (aluminosilicates) have long been used as 'molecular sieves', perhaps most notably as water softeners in laundry detergent. MOFs present the opportunity to tune chemical specificity such that they are now considered promising solutions to real-world problems. This is perhaps most famously the case for the green energy economy where their capacity to uptake hydrogen makes them an appealing candidate for hydrogen storage, with only modest pressurisation.[49] In the life sciences, biologically-active MOFs ('bioMOFs')[50] used in drug delivery receive strong combined academic and industrial attention[51] and have now even been tested in phase I clinical trials.[52]

Benefits of neutron diffraction:
The main science drivers in framework crystallography are identifying the number of guest molecules that are retained in the framework and how, and where, they bind. Crystal structure analysis is therefore a very powerful means of achieving this. Neutron diffraction is clearly the superior technique when it comes to observing hydrogen (which forms a significant research focus),[8,53] or water-based guest molecules within the framework.[54] Moreover, the interactions of guest molecules with the host framework can be sufficiently weak that the guest species locations are partially ordered, benefitting from diffraction at the more-localised atomic nucleus, relative to the diffuse electron cloud. In the bioMOF field, the quantity of hydrogen present on drug molecules is normally substantial, and neutrons are important here for understanding how these interact with hydrogen bond receptors/donors on the framework, as this has implications for their bioavailability.

As their name implies, it is common for MOFs to contain metal atoms, often forming the nodes of the framework. With X-ray diffraction their presence can obfuscate the signature of the relatively-weak scattering from (usually) light-atom guest molecules. In fact, heavier metals are used with some MOFs deliberately because of the benefit they provide in terms of

radiation efficacy during cancer treatment.[55] This strong/weak scattering (by X-rays) scenario is relevant for a very large number of framework materials and it is clear that neutron diffraction has a distinctive, complementary, role in their analysis.

Case study: Water harvesting using metal-organic frameworks
Ref. 56 – N. Hanikel *et al*, *Science*, 2021, **374**, 454–459

A small number of porous materials are able to adsorb water vapour in conditions of low humidity; water that can be extracted in liquid form by subsequent heating. MOF-303 ($C_5H_3N_2O_5Al$) is exceptionally good at doing so, being able to extract its own weight in water over a 24 hour period, with near-perfect reversibility (Figure 2.1.2a).[56] It is also able to carry out a degree of de-salination.[57] The mechanism by which the pore volume is occupied by water follows a progressively-evolving sequence of hydrogen-bonding steps – crystal structures of intermediate bonding states proved crucial for understanding how the adsorption process proceeds (Figure 2.1.2b). Naturally this type of interaction is difficult to determine precisely using X-ray diffraction, and in-situ single crystal neutron diffraction would be the ideal experiment choice for these sorts of studies. Though unit cell sizes are suitably small, the crystal sample size is insufficient (15 × 15 × 20 µm) for study with the current single-crystal facilities at ISIS; the proposed LMX instrument will be capable of measuring smaller crystals than currently possible on SXD.

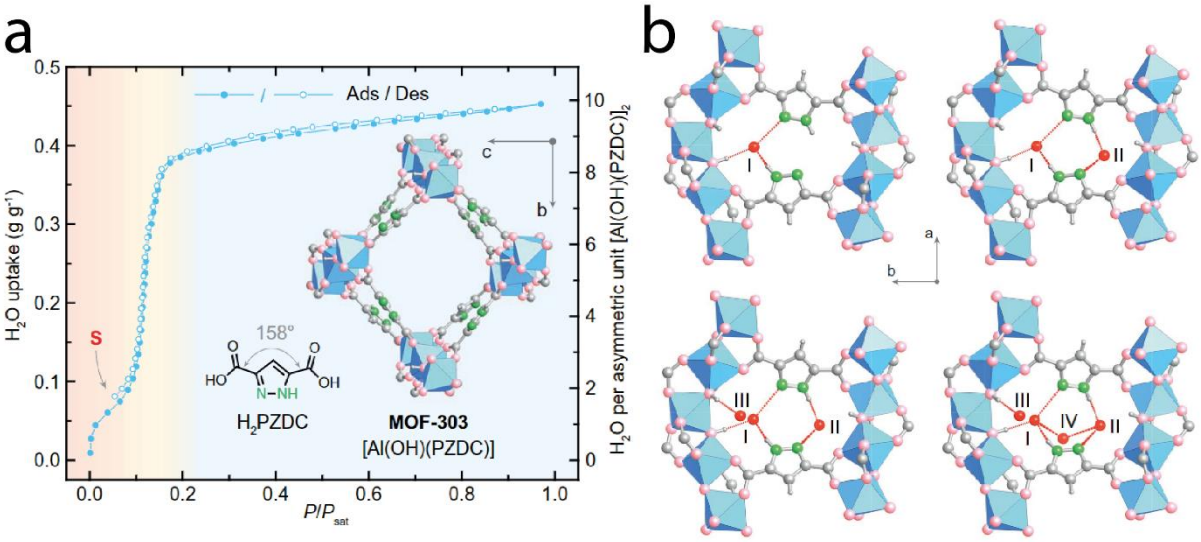


Figure 2.1.2. a) Adsorption/desorption isotherms for MOF-303, demonstrating excellent reversibility. **b)** Diagnostic crystal structures of progressive water uptake, demonstrating the mechanism by which pore volume is filled. Figure adapted from the original manuscript.

2.1.3 Catalysts

Scientific relevance:
It is difficult to identify industrial chemical processes that are not facilitated by catalysts. From automotive exhaust catalysts that prevent the release of harmful pollutant gases,[58,59] to nitrogen-fixing catalysts in fundamental ammonia chemistry,[60] and biologically-inspired materials for promoting the breakdown of synthetic dye contaminants in wastewater;[61] catalysts are pervasive in society. Although framework materials feature prominently in catalysis, transition metal-based molecular compounds form another area of scientific importance. They are influential in fundamental organic chemistry – e.g. Wilkinson's catalyst $(Ph_3P)_3RhCl$ which activates C=C bonds in hydrogenation processes[62] or a Ru-based pincer-ligand compound that reduces esters to alcohols.[63] Hydride complexes of late transition metals have a long history in hydrogen-transfer industrial processes.[64] The observation that such complexes, as well as Pincer complexes, could bind intact H_2 molecules to a metal centre,[65–68] has driven a tremendous effort in hydrogen research for applications in catalysis.

In order to understand how catalysts operate, it is necessary to trace the mechanistic pathway of the reactant species with which the catalyst is interacting. Even though the catalyst may operate in solution state, diffraction studies have still proved vital, where complexes at intermediate stages of the catalytic cycle have been isolated, and crystallised ('structure correlation'),[69] in order to locate (often) light atom species.[70]

Benefits of neutron diffraction:
Locating hydrogen atoms, and identifying their binding geometry, is critically important for understanding and improving the operation of industrially-relevant catalysts. As organometallic catalysts feature, by definition, a relatively-heavy metal atom (e.g. *d*- and *f*-block metals)[71,72] that is bound directly to the poorly-X-ray-scattering hydrogen atom, the hydrogen position is completely masked in structure determinations using X-ray diffraction. There are therefore numerous studies that exploit both X-ray and neutron diffraction in order to locate hydrogen at various stages of a catalytic cycle.[73,74] Furthermore, if the reactant molecule is bound in an unusual geometry, this means DFT/*ab initio* approaches[75] – a complementary means for determining hydrogen positions – are generally unsuitable for the task, and only neutron diffraction can provide the definitive answer.[76]

Though diffraction experiments benefit from lower incoherent backgrounds when hydrogen is substituted for deuterium, hydrogen and deuterium have clearly-distinguishable neutron scattering lengths. This can be exploited, using selective isotopic labelling to track reactant species of interest.[77] If intermediate species in a catalytic cycle can be isolated, it is then relatively straightforward to identify the movement of e.g. a lone hydrogen atom, yielding direct mechanistic insight. This same approach can also be exploited in the development of catalysts whose purpose is to promote hydrogen/deuterium exchange, which will directly aid other neutron experiments.[78,79]

Catalysts are a fundamental and industrially-important class of materials that benefit from particular research focus at ISIS, through collaboration with the catalysis hub.[80] There is already a strong link with prominent researchers in this area. Typical crystals of catalysts represent a system where ISIS has measurement capability only on occasion. While SXD has

successfully measured some catalyst crystal structures[81], there are numerous examples – such as the following case study – where the unit cell dimensions are prohibitively large.

Case study: Snapshots of concerted double element-hydrogen bond activation
Ref. 82 – J. A. B. Abdalla *et al*, *Nat. Chem.*, 2017, **9**, 1256–1262

Full mechanistic understanding of catalytic cycles is important for identifying *exactly* how reactant species bind and are transformed at a catalyst centre/surface. In this case study, a β-diketinimate-stabilised GaH_2 unit was reacted with a square planar Rh-phosphine complex, progressively activating the Ga-H bonds. Systematic variation of the ancillary metal-bound ligands, and a combined X-ray/neutron diffraction approach allowed a full structural determination of the intercepted, intermediate, complexes, shown in Figure 2.1.3a.

Starting from η^2-H,H σ-complex with little element-hydrogen (E-H) activation, the complex proceeds via an intermediate geometry featuring stretched E-H bonds, and compressed M-H/M-E bonds, through to a fully-activated metal dihydride. The authors' make particular note toward the value of neutron diffraction in locating the hydrogen atoms, which made it possible to determine the full reaction pathway (Figure 2.1.3b). Though the complexes could be grown as suitably-large crystals for neutron diffraction, some of the unit cell dimensions exceeded 40 Å. Separation of peaks along this axis would likely prove problematic on the SXD instrument; the neutron diffraction data in this study were measured on the KOALA instrument at ANSTO.

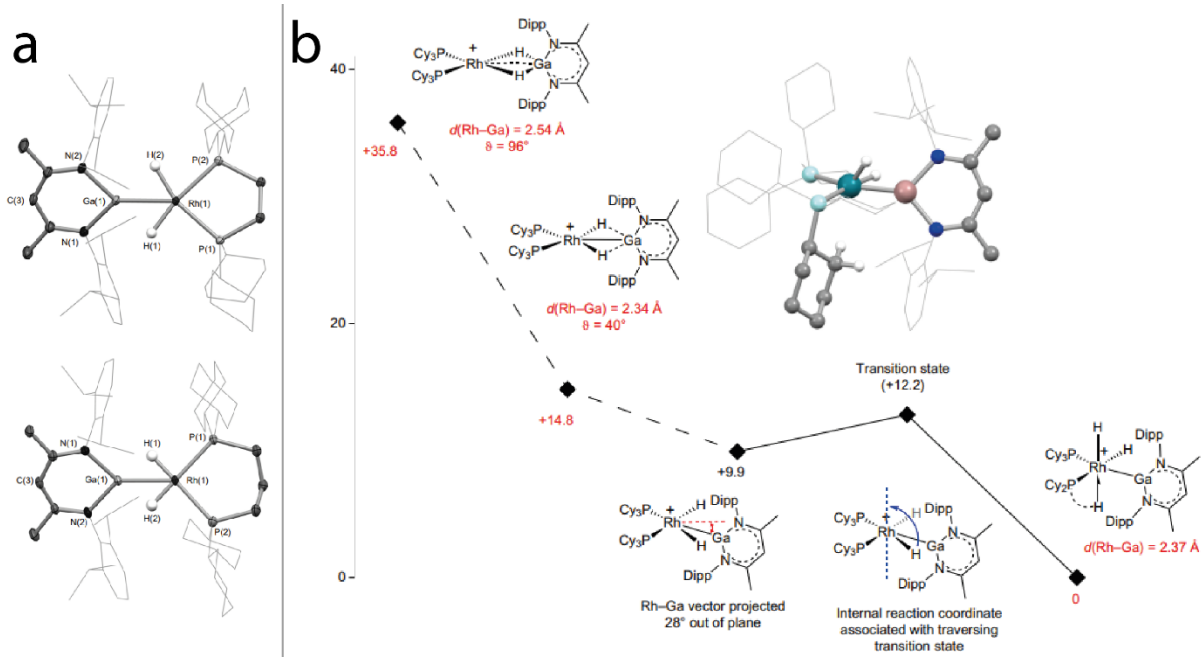


Figure 2.1.3. a) Crystal structures of key intermediate complexes where an unexpected five-coordinate Rh atom is evident and **b)** full mechanistic pathway of the E-H bond activation. Figure adapted from the original manuscript.

2.1.4. Pharmaceutical compounds

Scientific relevance:
The present and future health of populations depends on pharmaceutical innovation, but the health of the pharmaceutical industry depends on the amount of new drugs that can reach the market each year.[83] The range of chemical space that is explored leads to an enormous number of candidate compounds,[84] where analysis is supported by computer-aided drug design, combinatorial chemistry linked to high-throughput screening, genomics, and more recently on machine-learning techniques.[85] Active pharmaceutical ingredients (APIs) commonly take the form of small-to-medium sized organic molecules and crystallography has long been a fundamental technique for characterising their molecular structure and identifying geometric preferences of specific interactions of functional groups.[86]

One of the most important stages of drug development is solid-form structural characterisation, as the crystal structure – beyond the individual molecule – can have a direct effect on drug efficacy.[87] The most frequently cited example of this is that of Ritonavir, where its conversion to an unidentified crystal polymorph, post-commercial release, led to poor bioavailability.[88] Its temporary withdrawal from the market is estimated to have cost $250 million.[89] Thus the pharmaceutical industry now dedicates significant effort to understanding solid-state formulations.[90]

An increasingly-important component of pharmaceutical science is drug delivery. Again, crystallographic analysis is critical here to characterise e.g. engineered co-crystals that aim to simultaneously confer the benefits of multiple APIs,[91] binding mechanisms in supramolecular delivery capsules (see sections 2.1.1 and 2.1.2), or to understand deformation mechanisms, such as those induced by ‘slip planes’, during tabletting processes.[92,93]

Benefits of neutron diffraction:
Pharmaceuticals are typically ‘moderately-sized’ (ca. 10–100s of atoms), organic molecules which often crystallise in low-symmetry space groups with poorly-crystalline and weakly-diffracting samples. This means they are most routinely measured using intense synchrotron X-ray sources. However, the case for study by neutrons is quite straightforward: organic molecules usually have a high hydrogen content and thus hydrogen bonding is a key feature of the interactions between molecules. The differences in the aforementioned Ritonavir polymorphs can be ascribed to their respective networks of hydrogen-bonds. High-quality structural studies, e.g. to establish protonation states[94] or measure proton migration across intermolecular interations,[95] are those for which the information provided by neutron single-crystal diffraction experiments are vital.

Case study: Interaction energies of theophylline crystal forms
Ref. 96 – K. Fucke et al, *Cryst. Growth Des*., 2012, **12(3)**, 1395–1401

Theophylline is a small molecule used to treat bronchial asthma and is known to exist in three different anhydrous, solid, forms and as a monohydrate. The authors sought to measure the intermolecular interaction energies of the various crystal forms, which require high-precision atomic coordinates. For the monohydrate crystal form, they were able to collect single-crystal neutron data on VIVALDI (ILL) from a crystal measuring 2.0 × 0.5 × 0.4 mm. Crystals of the anhydrous compounds could not be grown sufficiently large for a neutron experiment. Forms I (0.31 × 0.18 × 0.09 mm) and II (0.98 × 0.14 × 0.08 mm) had to be measured using X-rays instead, and consequently energy calculations for these had to be treated with a little more caution. Figure 2.1.4a shows crystal structures of the anhydrous forms, and Figure 2.1.4b, the monohydrate. Neutron diffraction proved essential to this study as it revealed occupational disorder in the water hydrogen positions, dynamic disorder in methyl group orientations, and stronger H-bonding in form I than form II. Separately, the monohydrate was shown to have relatively weak water–theophylline interactions which rationalised its propensity to dehydrate.

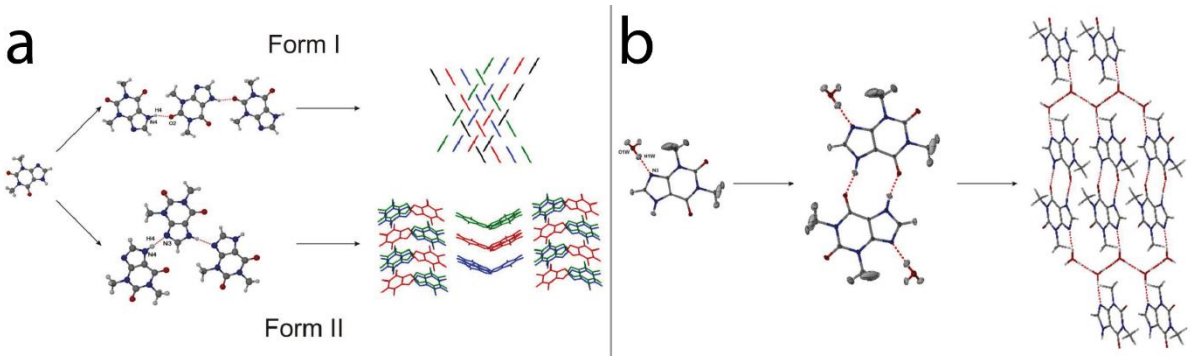


Figure 2.1.4. Crystal structures of **a)** the anhydrous forms of theophylline, and **b)** the monohydrate. The water molecules are disordered – all three partially-occupied deuterium sites are shown. Figure is adapted from the original manuscript.

2.1.5 Energy materials

Scientific relevance:
'Energy materials' is a catch-all term for compounds that address the societal challenge of clean energy generation and storage. It is an area of strategic priority, which is heavily-funded and consequently it attracts an active and vibrant research community.

Development of new battery materials is especially pressing given concerns that conventional lithium ion batteries[97] are drawing on a limited lithium reserve (ca. 14 million tons) and that its associated use with cobalt is problematic as these elements are often sourced using minor labour in geopolitically-unstable areas.[98] Alternatives are being sought in the form of nickel,[99] sodium,[100] potassium,[98] or even dual ion[101] batteries. Another source of clean energy is 'green' hydrogen, to be produced by renewable methods and combusted in fuel cells, of various types that are optimised to operate under specific working conditions.[102] In this case, the focus is not only on the development of the device itself but also on the source of the hydrogen fuel,[103] as well as its storage.[104] Lastly, the occurrence of solar cells that utilise the photovoltaic effect – i.e. the development of electrical properties from light exposure – rely on the information provided by crystallography on the relations between structural features and charge transport in their solid-state components.[105]

In-situ/operando studies are vital for helping identify these structure–property/ structure–function relationships and interrogation of crystal structures under some externally-applied stimulus is an ideal means for achieving this. This might include operation under gas pressure,[106] variable temperature,[107] or the study of ion mobility during charge/discharge cycling.[108] It is through these studies that continual improvements in functionality in these materials possible.[109]

Benefits of neutron diffraction:
Neutrons are sensitive to the location of light atoms, e.g. protons or dihydrogen that are integral to the material function as charge carriers or as a fuel source. Often, these light atoms are situated in the presence of relatively-heavier metals, distinguishable by neutron diffraction – e.g. local diffusion pathways for hydrogen absorption/desorption were identified in the $Ti_{25}Cr_{50}V_{20}Mo_5H_x$ metal hydride.[110] Lithium is also a light element, and neutrons were essential for measuring lithium and oxygen dynamics, as a function of lithium content, in the cathode material $Li(Li_{x/3}Ni_{(3/8-3x/8)}Co_{(1/4-x/4)}Mn_{(3/8+7x/24)})O_2$.[111] The presence of light, mobile, atoms around heavier metals is also a common issue for framework materials used as hydrogen storage devices (see Section 2.1.2).

Case study: Crystal structure determination of $Nd_8Sr_2Si_6O_{26}$ – a solid electrolyte prototype
Ref. 112 – T. An *et al*, *Inorg. Chem.*, 2014, **53**, 9416–9423

Lanthanide silicates have potential use as solid electrolytes in solid oxide fuel cells, for intermediate-temperature regime operation, showing greater oxygen mobility than yttria-stabilised zirconia alternatives which leads to superior performance. However, a detailed understanding of the influence of structural defects on oxygen transport had not been possible until now; powder diffraction measurements are hampered by grain boundaries, and suitably-sized single crystals had not previously been attainable. The authors used neutron single-crystal diffraction (KOALA, ANSTO) to fully characterise the structure (Figure 2.1.5a) and identify oxygen atoms in the presence of heavy elements. Moreover, they also observed partially-occupied interstitial oxygen sites (Figure 2.1.5b), revealing transport pathways through the crystal. Though a large single crystal, measuring 1.5 × 1.5 × 1.5 mm, of the apatite $Nd_8Sr_2Si_6O_{26}$ had been grown, it was noted that this had not been achieved previously. An ability to measure smaller crystals would have facilitated this study at an earlier stage.

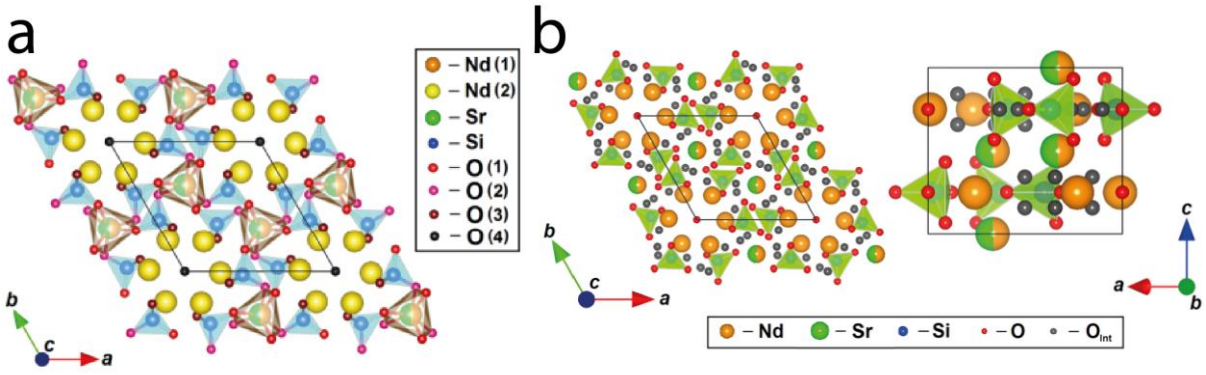


Figure 2.1.5. a) neutron-determined crystal structure of $Nd_8Sr_2Si_6O_{26}$ and **b)** distinction between the static (red spheres) and interstitial (grey spheres) oxygen sites. Figure is adapted from the original manuscript.

2.1.6. Chemical crystallography: the need for LMX

Currently, the chemical crystallography community at ISIS is served exclusively by the SXD diffractometer, though the sections above have highlighted topical research areas that it is unable to cater for. Structurally complex compounds such as supramolecular assemblies, frameworks, or large pharmaceuticals may have well-defined covalent and/or intermolecular interactions, but their size often permits a non-negligible degree of geometric flexibility leading to difficulties in growing single crystals of the size that would be required for measurement on SXD. The majority are << 1 mm^3 in volume, and new materials will likely have crystals that are smaller still, especially as techniques like electron diffraction become more accessible, reducing the need to grow larger-sized crystals. There can also be a practical need for small crystals as e.g. the efficiency of porous materials is directly related to their surface area. Thus most single-crystal studies of these materials are limited to X-ray radiation and/or neutron powder diffraction. There are several cases where neutron diffraction would have proved more useful than the X-ray experiment, e.g. establishing the orientation of water molecules in a confined ice, which exhibited geometric frustration.[113] However the crystal size was substantially smaller than 1 mm^3. To maintain crystallographic relevance in this wide variety of science areas, the capability to measure smaller samples is a necessity.

Conversely, the size of the crystal structures (and their associated unit cells) across all science areas is increasing, in direct correlation with the continually-evolving complexity of these compounds – see Figure 2.1.[114,115] Large unit cells lead to increased reflection overlap, which can ultimately make determining atomic coordinates problematic. The previous subsections presented numerous examples of materials that simply too complex to be measured on the SXD instrument. The LMX instrument, proposed here, will need to be capable of resolving closely-spaced reflections from large-unit-cell compounds from very small sample volumes.

Large unit cell structures are inherently weak at scattering either X-rays or neutrons. All the materials in the science subsections above would benefit from an improvement in neutron flux at longer wavelengths compared to the SXD instrument. Briefly considering the technical requirements of the instrument – this could be achieved using an alternative moderator, while still accepting a wide wavelength bandpath of neutrons to exploit the benefits of the Laue diffraction method, combined with time-of-flight peak separation. Weakly-scattering samples would need to be measured as efficiently as possible, in order to minimise count times, and this could be achieved by using the latest wavelength-shifting fibre detector technology. Large solid-angle coverage by the detectors would facilitate more rapid sample measurement, reducing the number of crystal orientations that would need to be sampled.

2.2. Structural biology

In contrast to a number of neutron facilities worldwide, there is currently no provision at all within the ISIS suite of diffraction instruments to address the field of structural biology. Consequently, the UK community of macromolecular crystallographers have no alternative to using European and international facilities for performing neutron measurements. Biological samples cannot be measured on SXD, in part, because their unit cell volumes are *significantly* larger than most of the chemical examples presented in the previous sections. A survey of the crystal structures deposited in the Protein Data Bank (PDB), shows an asymmetric distribution of longest unit-cell edges (Figure 2.2a). The largest cell edges can exceed 400 Å, but the peak distribution is centred about 120 Å. This represents the most commonly-studied unit cell size; its evolution with date of deposition shows that the mean remains at ca. 130 Å, despite the growth in studies with larger cells axes in more recent years.

The advent of third generation synchrotron sources has boosted protein crystallography in virtually every domain of structural biology and has made X-ray diffraction the ubiquitous technique in the field.[116] More recently, developments in X-ray free electron lasers (XFELs) and cryo-electron microscopy (cryo-EM) have seen an increasing number of macromolecular structures determined using these techniques. Though both XFELs and cryo-EM techniques are very powerful, the former is limited by expense and availability, and the latter by the requirement to perform measurements at cryogenic temperatures. Synchrotron X-rays

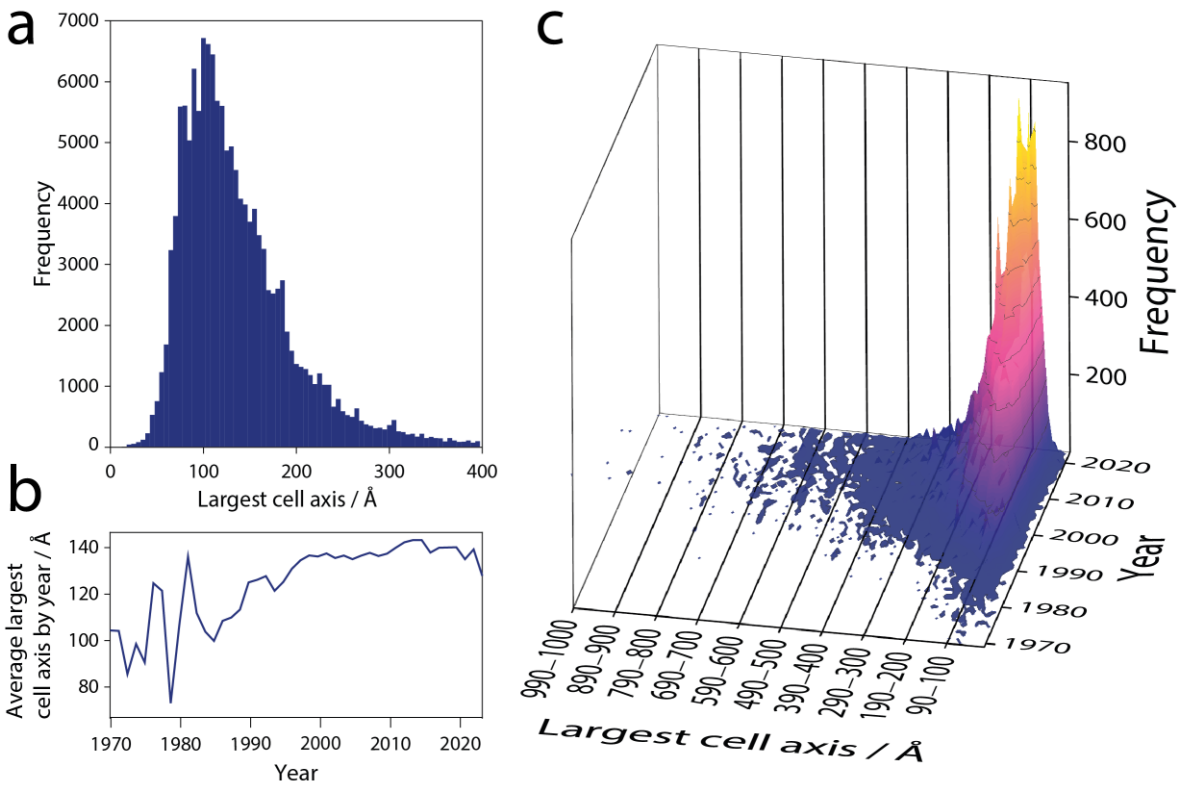


Figure 2.2. a) Histogram of the longest cell axes, per structure, across all recorded structures in the PDB. The largest value is recorded at 1933 Å – data are truncated for clarity; the frequency progressively decreases with largest cell axis size above 400 Å. The histogram maximum is located around 120 Å. **b)** The average largest cell axis, by year, shows the average value over the last three decades is ca. 130 Å. **c)** Three-dimensional heat map showing the by-year distribution of the largest cell lengths; the overwhelming majority of structures have cell lengths centred around 120-130 Å, even as capability to measure larger cells has increased.

remain the optimum choice for high-throughput measurements, but these are also constrained by the need to measure samples at cryogenic temperatures to avoid radiation damage.

Neutron protein crystallography has an unparalleled advantage in its ability to determine highly-detailed crystal structures at room temperature, providing an insight into biological mechanisms at physiologically-relevant conditions. Most biological processes – e.g. binding mechanisms within active sites in protein-ligand systems – are usually dependant on hydrogen bonding or other weak interactions involving hydrogen atoms or water molecules. The superior sensitivity of neutrons to both hydrogen and deuterium, compared to X-rays, means that they are required to describe fully the structure–function relationships of biotic systems.[117–120] Moreover, biological molecules typically contain substantial quantities of crystallised (and often, disordered) water, but only a small fraction of these can be characterised by X-rays. The respective negative and positive scattering lengths of hydrogen and deuterium are quite clearly distinguished by neutrons at very modest data resolution; comparison of ultra-high resolution synchrotron X-ray and neutron studies of concanavalin-A show that the latter is capable of identifying many more bound water coordinates, even at ca. 2.4 Å resolution.[121] Additionally, the relatively low-intensity of the neutron beam cf. synchrotron X-rays means that it rarely damages fragile crystal samples, avoiding this specific need to measure at low temperatures.

Neutron diffraction will never rival synchrotron X-ray experiments for high throughput structure determination, but it is vital for unveiling fine details. However, this alone does not account for the relatively small number of neutron studies. The current situation is best summarised by Förster and Schulze-Briese – "*neutron crystallography should be bigger than it is. This can largely be ascribed to the difficulty in obtaining sufficiently large samples, but also the lack of instrumentation available*".[122]

The following sections outline (non-exhaustively) some of the major biological science areas that would benefit from the LMX instrument.

2.2.1. Enzymes

Scientific relevance:
Enzymes are proteins that function as biological catalysts, showing highly-specific function and efficiency in facilitating particular chemical reactions – see Section 2.1.3. Like all proteins, they have a complex structure that directs their respective functions. The folding interactions give rise to active sites, and cavities, for substrate molecules to bind within.

The importance of enzymes in structural biology is such that entire databases are dedicated to curating enzyme data,[123] in addition to their crystal structures being stored in the PDB. Their exceptional catalytic ability means they are found at the forefront of biotechnology, where their functions are exploited to catalyse numerous industrial processes[124] – e.g. amylases are used in detergents to liquefy starches,[125] pectate lyase reduces water usage in textile processing,[126] and acylase is used in the synthesis of penicillin.[127]

They operate on an 'induced fit mechanism', where the active site continuously reshapes itself as it interacts with the substrate. Thus, much like chemical catalysis, *in-vitro* structural biology is predominantly concerned with characterising enzyme structure at isolated stages of its catalytic cycle, identifying the mechanisms by which substrate (or inhibitor/cofactor) binding occurs.[128,129] Recent work using extremely rapid time-resolved measurements, made possible with XFELs reported the direct observation of an ongoing reaction in real time in a β-lactamase.[130] Though the advances in rapid-timescale measurement are exciting, they remain expensive and relatively inaccessible options, and steady-state crystallography will continue to provide an important role in structural determination.

Benefits of neutron diffraction:
Enzymes, like all biological structures have a substantial hydrogen content, and feature numerous H-bonding interactions, between intra-protein regions, but also via intermediary water molecules, all helping in directing protein folding.[131] Additionally, several amino acid groups can exist in variable protonation states. Thus hydrogen atom location is an important challenge in all structural biology, and atomic-resolution X-ray crystallography cannot address this problem,[132] especially at room temperature where protons are more labile. It is now becoming increasingly common to combine several experimental approaches, e.g. neutron, XFEL, and synchrotron X-ray, yield complementary information that builds a more complete description of the structure.[133]

Neutron crystallography has demonstrable success in this area, particularly because it can take advantage of the scattering contrast between hydrogen and deuterium via isotopic substitution. This is an ideal experimental approach as both hydrogen and deuterium are visible at much lower data resolution than with X-rays.[134,135] This approach has been used to determine the protonation state of ferryl haem in peroxidase (a = 51.19, b = 75.83, c = 107.59 Å);[94] re-examine the reaction mechanism in D-xylose isomerase following unexpected protonation states (a = 94.64, b = 99.97, c = 103.97 Å);[136] and identified a change in hydrogen positions in the active site of HIV-I protease following protonation of a distant residue (a = 59.72, b = 87.26, c = 46.55 Å).[137]

Case study: Resolving an OH-bound resting state of a copper-containing nitrite reductase
Ref. 132 – Y. Fukuda *et al*, *Proc. Natl. Acad. Sci.*, 2020, **117**, 4071–4077

The copper-containing nitrite reductases (CuNIRs) are an important part of the nitrogen cycle, promoting the conversion of nitrite to nitric oxide. However, there remained some literature disagreement over the true electron transfer pathway; spectroscopic and computational data presented conflicting results (Figure 2.2.1a), on the location of hydrogen atoms. CuNIR structures are known to undergo photoreduction when irradiated by X-rays, which complicates the interpretation of the data. In order to definitively address this issue, the authors undertook a time-of-flight single-crystal neutron study to locate the proton positions. Using neutron data that exceeded 1.50 Å resolution (measured at 100 K on the iBIX instrument), hydrogen and deuterium atoms could be clearly located, a full hydrogen-bonding network established, and reassignment of an X-ray-determined water molecule as a sodium ion. The copper site was found to be bound via a free hydroxyl group (Figure 2.2.1b), supporting previous DFT calculations. Ultimately the structure showed that the electron transfer during the nitrite reduction proceeds via a hydrogen-bond jump pathway (route 'A' in the Figure).

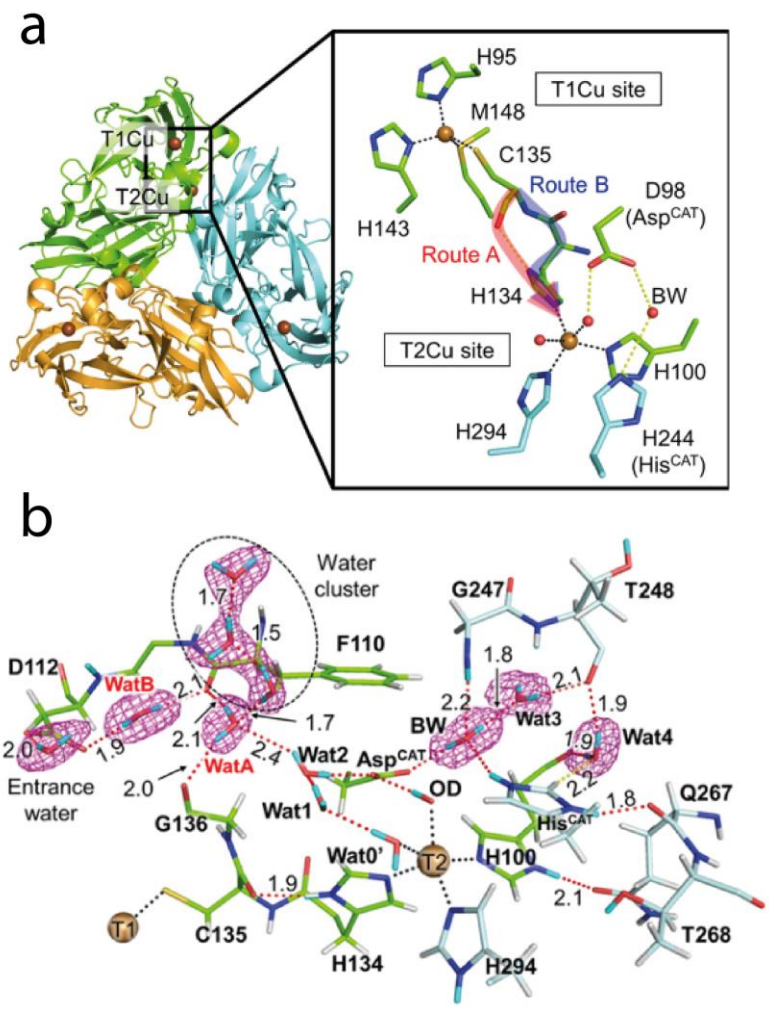


Figure 2.2.1. a) Crystal structure of CuNIR. The two possible intramolecular routes for electron transfer are shown, where route A (red) proceeds via a hydrogen-bond jump, and route B (blue) is mediated by covalent bonds only. **b)** Neutron structure determination showing the full hydrogen bonded network surrounding the copper cation ('T2' site), including the coordinated hydroxyl anion (OD). Figure adapted from original manuscript.

2.2.2. Drug delivery for disease treatment

Scientific relevance:
The study of diseases is one of the oldest scientific endeavours and in recent decades has, in part, depended on identifying the responsible macromolecular structure so that it might be effectively targeted during treatment. Crystallography has long played a pivotal role here – the 1946 Nobel prize in Chemistry was awarded for crystallisation of pure proteins and viruses.[138] Knowledge of structure has been important for drug development;[139] identifying and binding therapeutics to protein receptors is one of the most fundamental stages in treating diseases[140] such as cancer,[141] diabetes,[142] or pathogenically-transmitted cholera.[143] Even the recent efforts towards designing new vaccines for the SARS-Cov2 virus focussed on the structure of its spike protein and the interaction mechanism in protease receptors.[144]

This is a highly-applied research area and features strong engagement with the industrial sector.[145] Much like the case for enzymes, structures of viruses are in sufficient demand that they are curated in additional databases beyond those available in the PDB,[146] meaning that measuring structure, even without immediate application, has significant value. The drugs that bind to proteins are often small molecules, leading to synergies between the macromolecular and chemical worlds, with structural information passing back and forth to improve understanding within both.[147]

Benefits of neutron diffraction:
Like most problems in structural biology, neutrons are most useful for identifying the protonation states, and hydrogen bonding networks that form once a chemical ligand binds to a macromolecule receptor. For instance, the commercially-used acetazolamide (AZM) drug that binds to, and inhibits, carbonic anhydrases which regulate fluid secretion, already has several recorded X-ray crystal structures in its bound state.[148] However, only the neutron measurement provided scattering density with detail to show the protonation state and binding mode of AZM.[149]

A similar success story was reported for the crystal structure of the 'LecB' lectin that belongs to the pathogen responsible for lung infections in cystic fibrosis patients.[150] The avoidance of radiation damage with neutrons meant that structure determination could be performed at room temperature, giving a more accurate view of local flexibility and water mobility at biologically-relevant conditions. Perdeuterated crystal structures allowed detailed density maps to be obtained, showing that sugar and calcium binding mechanisms were mediated by a low-barrier hydrogen bond.

In some cases, like that of the HIV-1 protease inhibitor amprenavir in its bound state, X-ray measurements indicate structural differences to neutron data – namely in sidechain orientations, and subsequent alteration in hydrogen bonding networks.[151] It is necessary to collect synchrotron X-ray data at low temperature to avoid radiation damage, and in this case, measurement at 100 K induced this structural change, presenting a misleading picture of the binding mode of amprenavir. Only the room-temperature neutron data revealed this to be the case.

Case study: Suppression of SARS-CoV-2 replication

Ref. 152 – D. W. Kneller *et al*, *J. Bio. Chem.*, 2020, **295(50)**, 17365−17373
Ref. 153 – D. W. Kneller *et al*, *J. Med. Chem*., 2021, **64(8)**, 4991−5000

The SARS-CoV-2 virus is the cause of the COVID-19 pandemic which has led to millions of deaths worldwide in recent years. Its main protease is an essential enzyme for viral replication and, because it has no human equivalent, is an appealing target for small-molecule drug suppression of its catalytic action. Two recent room temperature neutron diffraction studies on IMAGINE at the SNS, using small single crystals (measuring 2.0 × 0.8 × 0.2 mm and 1.5 × 0.7 × 0.5 mm), have investigated the active site of this enzyme to determine its protonation state, the role of nearby water molecules and the prospects for optimising drug molecules that will fill these key active sites. The first study determined the ligand-free structure of the main protease of SARS CoV-2,[152] highlighting the locations of the hydrogen atoms in the enzyme and thus the important electrostatics of the enzyme's active-site cavity. The second reported the neutron structure of the same enzyme bound to telaprevir,[153] a possible protease inhibitor that is known to bind to the hepatitis C virus protease. The changes observed in the active site protonation states, when bound to telaprevir, provided important information for rational drug designers aiming to optimise the molecular shape and binding characteristics for strong inhibition of the SARS-CoV-2 virus function.

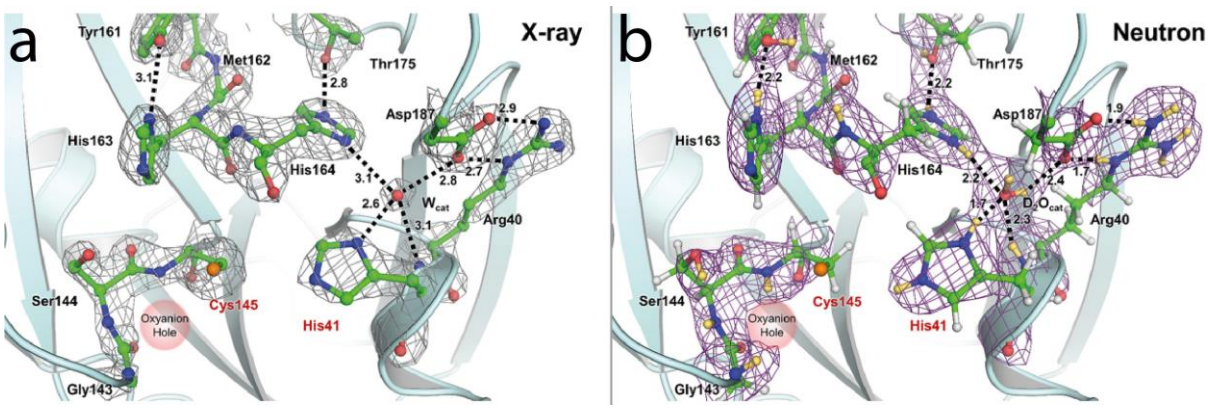


Figure 2.2.2. **a)** X-ray diffraction data of the SARS-CoV-2 3CL M^{pro} catalytic site – only 'backbone' non-H atoms are visible. **b)** Neutron density maps showing hydrogen atom positions, revealing protonation states of amino acid residues. Figure adapted from the original manuscript.

2.2.3. Transport proteins

Scientific relevance:
Transport proteins are responsible for moving other non-protein moieties around an organism. These can involve substances ranging in size from electrons/protons to macromolecular structures and impact almost all physiological functions, hence the interest in their study. Substances such as haemoglobin – a chromoprotein – responsible for circulating oxygen around the body via the iron-containing haem group,[154] or transferrin that moves iron through the blood plasma,[155] are among many proteins studied crystallographically to understand their binding processes.[156] There are approximately 20 crystallographic milestones that contributed to our knowledge of vitamin B_{12} and its transport in the body.[157] A further large sub-category of macromolecular transport systems are membrane proteins where they facilitate movement of chemicals across a cell membrane,[158] thereby regulating cell contents. They represent one of the most modern challenges in structural biology[159] – they have hydrophobic surfaces, and are extremely flexible, so they have proved difficult to crystallise. However, they account for approximately 25% of all proteins,[160] hence there are developments in effective methods for their expression,[161] purification,[162] and crystallisation.[163]

There are, however, several examples of successfully-measured transport proteins; crystal structures of the 'OmpF' porin membrane protein – which transmits nutrients and waste across the *E. coli* membrane – showed how it bound to detergent molecules,[164] which were in turn, determined from neutron data.[165] Crystal structures of a human membrane protein (LTC_4 synthase) with glutathione, obtained from a relatively large crystal ($0.2 \times 0.2 \times 0.05$ mm), showed how it exhibits high specificity for glutathione molecules, in contrast to other transferases.[166]

Benefits of neutron diffraction:
The extreme fragility of (most) transport protein crystals means that the lesser interaction with neutrons, comparted to synchrotron X-rays, offers a non-damaging means of measuring their structures. Like all biological structures, the movement of hydrogen, and orientation of water molecules is key to much of a protein's function. The biggest challenge is obtaining crystals of a large-enough size to be measured. Both the benefits and obstacles of neutron diffraction are recognised in the community and there are research efforts toward strategies for improving protein crystallisation, coupled with improvements in modern neutron instruments.[167,168] This points towards a need to support measurements at neutron facilities with onsite capability, dedicated toward crystal growth and handling.

This is an emerging area, but there are now neutron-determined crystal structures of transport proteins, representing a community desire to perform these experiments. These include carrier proteins, like that of sperm whale myoglobin,[169] but more notably channel proteins: a calcium transport protein (SERCA1)[167] and an E. col. ABC (ATP-binding cassette) phosphate transporter.[170]

Case study: Binding site asymmetry in human transthyretin
Ref. 171 – M. Haupt *et al*, *IUCrJ*, 2014, **1**, 429–438

Human transthyretin (TTR) is a transport protein responsible for moving thyroxine and retinol-binding protein in the blood and spinal fluid, but is also a suspected factor in amyloid disease. A specific serine residue (Ser117) is thought to play a central role in facilitating amyloid formation, along with a neighbouring water molecule, which may be influential in directing binding site availability. In order to fully establish these structural features, a joint X-ray and neutron single-crystal study was performed.

An orthorhombic crystal structure with a = 43.68, b = 86.26, c = 65.72 Å was determined from a large, perdeuterated, single crystal > 3 mm^3 at room temperature on the D19 instrument at the ILL. The crystal structure of TTR is shown in Figure 2.2.4a. The orientation of numerous water molecules, and protonation states were determined, including those found in the thyroxin binding pocket, shown in Figure 2.2.4b. The location of the critical serine hydroxyl–water molecule interaction at the thyroxine binding site (Figure 2.2.4c) shows that the water molecule is highly localised, rendering the two sites (Ser117A/Ser117A') inequivalent. This observation could be linked to proposed mechanisms which result in amyloidosis, suggesting that a proposed disassociation into monomer units is unlikely.

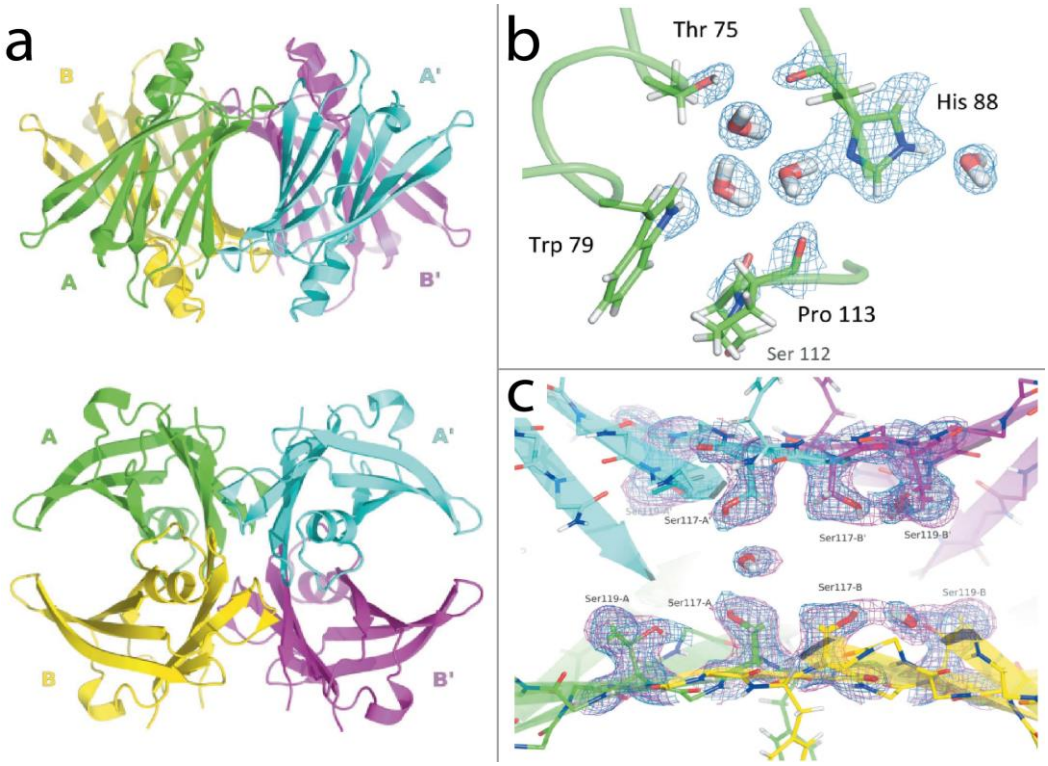


Figure 2.2.4. a) Crystal structure of TTR shown from two alternate orientations. Different subunits of the protein are labelled and colour-coded. **b)** Nuclear density maps showing histidine protonation states and water molecule orientations that stabilise the protein structure. **c)** Serine hydroxyl group orientations at the thyroxine binding pocket show an interaction is formed preferentially at the Ser117-A site with a nearby water molecule; its position is highly localised. Figure adapted from the original manuscript.

2.2.4. Nucleic acids

Scientific relevance:
Nucleic acids are long polynucleotide chains that store genetic information in all living organisms, They come in two main forms: ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). DNA has a double-helical structure in which the two strands interact via molecular complementarity, encoding genetic information, while the single-strand RNA has three forms (messenger, ribosomal, and transfer) which each have a specific function. The crystallographic study of nucleic acids is probably one of the most publicly-recognised areas of structural biology.[12,13] Though DNA and RNA are comprised of just a fructo-glucoside-phosphate backbone and five simple 'nucleotide bases' with highly-specific hydrogen-bonding preferences, these structures are complex, and flexible, yet have very specific structural features central to their function.[172] This flexibility enables the formation of higher-order quaternary structures between DNA molecules or DNA–protein complexes where, much like proteins, specific folding motifs emerge.[173] The latter assemblies play an important role in regulating how readily genetic information is transcribed.[174]

Crystallography is important for characterising the full 3D coordinates and identifying the key areas of interest with relevance to biological function. Crystal structures were invaluable for informing how peptide nucleic acids (synthetic DNA mimics) bind to DNA and RNA,[175] so that they can be developed as e.g. therapeutic agents[176] or genetic diagnostic tools.[177] In RNA, the so-called 'A-minor' interaction where unpaired adenosine nucleobases bind to the minor groove of the nucleic acid helical structure was found – via 3D coordinate determination – to be the underpinning feature that dictated the overall RNA tertiary structure.[178] Aside from function, it has elucidated more unusual architectures, such as DNA triplexes,[179] or the four-way DNA ('Holliday') junction.[180]

Currently, there are very few neutron studies reported on single crystals of nucleic acids, reflecting the particular difficulty in obtaining crystals of a size required for neutron diffraction. The majority of the reported structures are decameric sequences and have been published relatively recently, illustrating both improvements in instrument capability as well as an increased desire within the community to perform neutron measurements. Single crystal neutron diffraction will likely form an emerging area in nucleic acid research.

Benefits of neutron diffraction:
The function and biochemistry of nucleic acids is also dominated by hydrogen – neutron diffraction would provide all the same insights here as for proteins. In a few cases it has been used in DNA complexes to locate hydrogen, where it simply could not be located by any other means. Hydrogen bonding patterns and orientations of the first-shell water molecules surrounding a Z-DNA (left-handed) duplex were only measurable with neutron experiments,[181] revealing unexpected orientations. Another recent study reported making a small modification to a A-DNA duplex, tagging it with a $SeCH_3$ group to improve crystal growth,[182] which has also been demonstrated elsewhere.[183] Neutron diffraction revealed a previously-unknown protonation state on the phosphate backbone, and that this could be interchanged with Mg^{2+} at low temperature.

There are a greater number of neutron studies that use the fibre diffraction technique to measure the typically small (a few µm) and highly-anisotropic crystals. Hydration-driven conformational changes in DNA were observed with neutron fibre diffraction as function of relative humidity.[184]

Case study: Complicated water orientations in the minor groove of B-DNA d(CCATTAATGG)$_2$
Ref. 185 – S. Arai *et al*, *Nucleic Acids Res.*, 2005, **33(9)**, 3017–3024

The 'spine of hydration' – a chain of connected water molecules in the minor groove of B-DNA, is thought to be a structurally-important feature. Recent studies had identified a hexagonal arrangement of water molecules within this chain, however even with extremely high-resolution X-ray data (0.74 Å), the hydrogen atoms, and thus the water molecule orientations, could not be determined.[186]

The authors of the present study undertook a single crystal neutron diffraction measurement on a related decameric B-DNA duplex d(CCATTAATGG)$_2$ which exhibited the same hexagonal water motif. Neutron data from 2.8 and 1.6 mm^3 crystals (3 Å resolution), measured on the BIX-4 diffractometer, and combined with synchrotron X-ray data (2 Å resolution), found trigonal symmetry with $a = b = 32.9$, $c = 96.1$ Å. Difference density maps (Figures 2.2.3a and 2.2.3b) showed the superior sensitivity of the neutrons to the water hydrogen atoms, revealing several unusual, and unexpected, orientations. The determination of 27 water molecules revealed a far more extensive hydrogen bonding network that had previously been realised, comprising several interconnected hydration shells between separate DNA strands (Figure 2.2.3c).

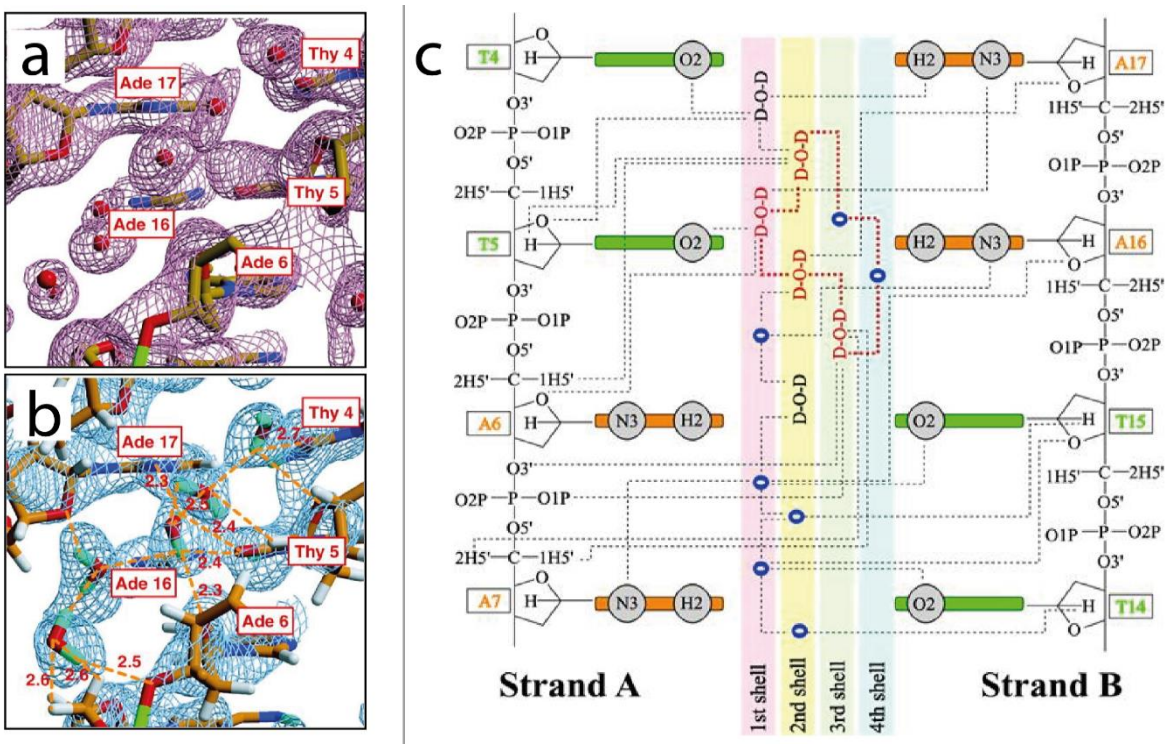


Figure 2.2.3. a) X-ray and **b)** neutron density maps showing water molecules in the 'spine of hydration', where the sensitivity of the neutrons to the water orientations is evident. **c)** Hydrogen bonding schematic showing hydration shells between DNA strands – dotted lines indicate hydrogen bonds. The water molecules partaking in the hexagonal motif is highlighted in red. Figure adapted from the original manuscript.

2.2.5. Structural biology: the need for LMX

The science sections above make the benefits of neutron diffraction abundantly clear – biological processes revolve around hydrogen and their accurate location is crucial to understanding function.

Unlike the chemical crystallography community, there is currently zero provision for structural biology among the crystallography instruments at ISIS. Unit cell sizes for macromolecular compounds are typically very large – the histograms given earlier, in Figure 2.2 show that cell axes > 100 Å are prevalent. As discussed in Section 2.1.6, the proposed LMX instrument will need to be capable of resolving the large-index *hkl* reflections that would be heavily-overlapped on SXD. A longer primary flight path is therefore a requirement.

The other insurmountable difficulty for SXD is the oft-cited barrier for all macromolecular neutron studies: the crystal size. Crystals measuring ca. 1 mm^3 or bigger are currently required on SXD, but in order to access a greater percentage of the biological community's samples, it will be necessary to measure crystals at least an order of magnitude smaller, i.e. 0.1 mm^3. This is the typical size range measured on other neutron diffractometers,[187] and should form one of the essential requirements of the LMX instrument.

Lastly, biological crystals are notoriously weakly-scattering with both neutrons and X-rays, because of their extremely large water content, leading to a very small size and poor crystallinity. An increase in neutron flux, relative to SXD, would offer an obvious improvement. Moreover, the larger unit cells of the samples described here would benefit from measurement using longer-wavelength neutrons than SXD receives from the water moderator on which it is situated. An important consideration is that hydrogen/deuterium atom positions can normally be elucidated from datasets with d_{min} = 1.5–2.5 Å,[188] so this region of reciprocal space should be accessible.

2.3. Instrument requirements

The scientific drivers justifying the proposed LMX instrument have been assessed from a range of sources, including:

- A thorough analysis of the information contained in structural databases for both chemical and biological crystal structures (see Figures 2.1 and 2.2 above), including current trends and likely future themes.
- A more detailed consideration of a number of high-profile scientific themes and specific studies, including those highlighted in the previous sections.
- Extensive consultation with the user community and staff at other neutron and synchrotron facilities (for further details, see section 3.8).

Macromolecular samples typically have larger unit cells, and smaller sample sizes than chemical samples, placing the most stringent restrictions on the instrument specifications. Drawing on the information sources given above, these requirements can be summarised as:

- The samples are likely to be weakly-scattering. This requires high neutron flux in order to measure the Bragg peaks with good statistics. In particular, the significant thermal vibrations of atoms typical in poorly-crystalline biological systems will suppress Bragg intensities at smaller *d*-spacing values.

- In combination with high neutron flux, the detector technology should provide the highest-efficiency available, resulting in good signal-to-noise ratios, with pixel sizes that are suitable for resolving reflections from large unit cells, given the beam divergence.

- The measurements will still be count-rate limited, thus data collections need to be efficient; a complete crystal structure should be determined from as few crystal orientations as possible. Therefore, a wide wavelength band should be used to exploit the advantages of the time-sorted Laue technique in full, coupling with a large detector coverage around the sample position, in order to maximise the information collected in a single measurement.

- The small sample volumes will benefit from a commensurate beam size, transporting a maximum number of neutrons, focussed on the sample. The beam size should be tuneable to accommodate any sample size. Consultation with the LMX Scientific Advisory Committee (SAC) led to the recommendation of a maximum beam aperture of 2.5 × 2.5 mm^3.

- The PDB deposition data shows that, overwhelmingly, the most commonly-occurring largest cell axis in macromolecular crystal structures is ca. 120 Å (Figure 2.2). On average, this is 130 Å, though this value is skewed by some very large recorded structures. Typically, a d_{min} of ca. 2 Å is required to observe hydrogen atom positions in neutron density maps of macromolecular samples.[188] In order to properly extract reflection intensities in this region of reciprocal space, the reflections must be sufficiently intense, but also distinguishable from each other; it will be necessary to resolve neighbouring reflections with *d*-spacing ca. 2 Å. For a cubic crystal with a = 120 Å, (0 k 0) reflections where k = 59–61 should be distinguishable in time-of-flight and the (0 60 1) and (1 60 1) reflections should be resolved from (0 60 0) spatially.

- For chemical systems with smaller unit cells *cf*. macromolecular structures, the most interesting structural features (e.g. weakly-bound light-atom species) require data measured to ca. d_{min} = 1.0 Å. X-ray experiments often require data measured to ca. 0.8 Å to extract these features, however, as neutron scattering length differences between the elements are, in general, less pronounced than for X-rays, it means that lighter atoms can be more easily distinguished from heavier atoms, even with lower-resolution data.

- Following consultation with colleagues at other neutron facilities, wavelengths > ca. 5 Å are not particularly beneficial to the sample measurement. Thus in order to best exploit a wide wavelength band, peak flux should be centred around 2-3 Å.

2.4. Technical specification

Based on the information presented in the previous subsection, we can define the following six key technical criteria for LMX:

- Measure data with d_{min} = 2 Å for macromolecular materials and 1 Å for other 'chemical' materials.
- Spatially, and temporally, resolve the (0 *k* 0), (0 *k* 1), (1 *k* 0), and (1 *k* 1) reflections where *k* = 59–61 for a cubic unit cell with *a* = 120 Å (*d*-spacing ≈ 2 Å; $\Delta d/d$ = 0.0165).
- Use a wide wavelength bandpath of 4 Å.
- Have high brilliance transfer across all wavelengths.
- Have high flux at wavelengths centred about 2-3 Å.
- 2.5 × 2.5 mm^2 beam size at the sample position.

These requirements place specific constraints on the technical design of the LMX instrument. The currently-proposed instrument design is outlined in the following section.

3. Business Case

The scientific drivers for LMX, as defined by the requirements of the single-crystal chemical crystallography and structural biology communities, were outlined in Section 2. Though the current SXD instrument maintains a strong presence in the small-molecule community, it is less well-placed to tackle the larger, more complex crystal structures that are now becoming more prevalent (see Section 2). Moreover, it has never had the ability to measure macromolecular crystals at all, having been optimised for far smaller unit cell sizes, and requiring a crystal volume that is unattainable for most biological compounds. The proposed LMX instrument would address this capability gap, and grow the existing user base at ISIS, forming an ideal complement to SXD. This section presents a detailed business case for the construction of LMX, including:

- its national and international context
- the need for in-situ studies and sample environment
- its complementarity with other experimental and computational techniques
- the user community
- potential cross-campus collaborations
- industrial partnership
- support infrastructure

3.1. Single-crystal diffraction: neutrons and X-rays

Structure characterisation by single-crystal neutron diffraction has proved to be a highly successful technique for many decades, originally at reactor-based facilities and then at spallation sources. As discussed in Section 2.1, while the SXD instrument at ISIS[7] has made significant contributions in the area of 'small-molecule' chemical crystallography (unit-cell volumes ca. < 10000 Å^3), the past 20 years or so has seen increased demand for the study of larger-volume molecular systems (see Figure 2.1). For biological systems, though there is no neutron capability at ISIS, the neighbouring Diamond Light Source has a suite of macromolecular beamlines dedicated to solving the crystal structures of large molecule systems using single-crystal diffraction.

Neutron sources have significantly lower flux than their X-ray counterparts so there are clear differences in the experiment objectives between the two approaches. Whereas X-rays are better suited for rapid time-resolved measurements (microsecond–second lifetimes),[189] or obtaining useable diffraction signals from very small sample volumes (ca. 10^{-5}–10^{-6} mm^3),[190] neutrons – as the earlier science examples show – offer the unrivalled ability to determine hydrogen/light atom positions, even in the presence of heavy metals (see Section 2). This is important structural information for understanding physiological and chemical processes. Following consultation with the research community (see Section 4.9), the remit of LMX will be to collect high-quality diffraction data with specific focus on addressing the scientific problems that neutron measurements are uniquely positioned to tackle. It is also worth noting that given the extra effort required by research groups to prepare a macromolecular sample suitable for a neutron experiment, the majority of experiments performed (elsewhere) are on significantly-important, topical, materials – the SARS-CoV-2 protease

presented in the Section 2.2.2 case study is a clear example of this. Consequently, the publication records of the major biological neutron instruments contain numerous papers published in high-impact journals including, but not limited to, Science,[94,191] Nat. Commun.,[119,144,150,192–194] Chem. Sci.,[195,196] Proc. Natl. Acad. Sci.,[197,198] and Nucleic Acids Res.[181,199]

In most cases, maximum information content on a sample is gained by performing both neutron and X-ray experiments – they are complementary approaches. Heavier elements, such as metal atoms in most functional materials, or sulphur in protein cysteine residues are most easily detected by X-rays, providing the bulk of the crystal structure. Neutrons are then vital for measuring the 'fine detail'; identifying light atom positions and giving complete insight into material functionality.

3.2. Computational techniques

The past few years have seen significant advances in the use of computational approaches to address problems in structural science and it is important to consider LMX in the context of the most significant, recent developments. For molecular compounds, the improvements in quantum crystallography – in particular the ability to compute aspherical form factors that describe the experimental electron density using the Hirshfeld atom refinement (HAR) method – has led to more accurate hydrogen atom positions from (lab-source) X-ray data, comparable to those determined in a neutron experiment.[75,200,201] Although such an approach could, in principle, be extended to larger molecules in the coming decades, the method is computationally expensive due to the wavefunction calculations involved and, in its current form, is not suited to large molecular assemblies.

However, despite the large improvements in refinement statistics that result from HAR, the hydrogen positions are not determined *a priori*; their coordinates are refined, not predicted. Initial placement of hydrogen atoms must be user-defined if they cannot be observed in the data. In some cases these are predictable, such as aliphatic CH_2 units, but for unusual and/or disordered geometries, there will always be a need for determination by experiment. The catalysis case study presented in Section 2.1.3 is a clear example of this point – the unusual (and unpredictable) hydrogen location on the catalyst intermediate led to an uncommon coordination geometry at the metal centre.[82] Though HAR has advanced the quality of X-ray single-crystal refinements significantly, it still has difficulty in clearly distinguishing hydrogen atoms in close proximity to heavy metals as a consequence of large Fourier truncation ripples in the X-ray data.[202] The results of HAR – the hydrogen atom placement – are often benchmarked against those determined from neutron diffraction in order to assess its performance and validity of the crystal structure; there is a clear need for neutron data here, and this will continue to be the case.

The leading computational tool in bioinformatics is AlphaFold – a predictor of protein folding, based on a given amino acid sequence.[203] Unlike other protein-folding algorithms, it is a machine-learning-based approach that uses crystal structures deposited in the PDB as the training data set, and has demonstrated impressive success.[204] Its aim to cover a large proportion of all catalogued proteins represents a major advance in the field.[205]

Like HAR, AlphaFold should be considered a complementary tool to neutron (and X-ray) diffraction; it is an important source of information, potentially to build a model for structure refinements.[206] However, having been trained almost exclusively on X-ray-determined structures, AlphaFold has little intuition toward accurate hydrogen location – this is especially the case where there are unexpected protonation states or unusual hydroxyl/water orientations or disorder. Moreover, because the majority of the training set will, by definition, represent 'standard' geometries it struggles to predict exceptional structures, such as those arising from mutations. The real-life implications of mutations (e.g. substitution of a single amino acid residue) can lead to pronounced functional differences, so an accurate determination of their effect on structure is important.

In summary, the most topical computational tools in both chemical and biological crystallography, do not directly deal with the science problems that neutrons are uniquely placed to tackle, but can provide complementary information. This scenario may change as a growing number of neutron structures are deposited in databases, but this would seem unlikely given the increasing rate at which X-ray structures can be measured. Neutrons have an unrivalled strength for locating hydrogen and this will continue to be case going forward. This will form one of the major drivers for the LMX instrument.

3.3. Sample environment

Neutrons are advantageous compared to X-rays when considering the design of new sample environment. Their highly-penetrating nature means that there are fewer geometry restrictions placed on the equipment – the neutrons will simply pass through most of its components. For instance, an aluminium window presents little obstacle to a neutron, however it would prove a much more significant contaminant (or even impassable object) for X-rays.

Consultation with the LMX scientific advisory committee (meeting summary available in Appendix 5) made clear that, for chemical crystallography, there is strong interest in establishing structure–property relationships, which typically means measuring crystals *in-situ/operando* under non-ambient conditions and observing the structural response. The need for variable environments is less urgent for macromolecular crystals, where the most common requirement is low-temperature measurement capability in the form of a nitrogen cryostream and cryostat. A number of sample environment possibilities are discussed in the following subsections.

3.3.1. Variable temperature

The ability to measure crystals at high and low temperatures is the most fundamental sample environment required. This can be achieved straightforwardly using simple, commercially-available, open-flow nitrogen (ca. 80–500 K) or helium (28–100 K) cryosystems, with minimal shadowing of detector coverage. High temperatures can be accessed with hot air blowers (ca. 1300 K). These options operate under ambient pressure environments; for samples where

vacuum is required, a compact Displex closed-cycle refrigerator (CCR) will allow access to a temperature range spanning ca. 12–500 K. Temperature changes allow some of the most fundamental physical properties of a crystal to be studied,[207] as well as driving phase transitions[208] and diffusion of labile, encapsulated guest molecules.[209] It is also important for replicating real-world working conditions, such as the above-ambient temperatures that automotive catalysts might experience.[210]

3.3.2. Gas flow

Many energy materials are concerned with the capture and/or storage of gaseous molecules, often using porous crystals (see Sections 2.1.2 and 2.1.5). Absorption/desorption studies are common in X-ray experiments where the sample is placed in an environmental gas cell and structural changes are recorded as a function of gas pressure (i.e. gas uptake). This forms a typical study of MOF materials.[211] Alternatively, variable humidity can be used to explore e.g. hydration-induced structural changes in proton conductors.[212] The ability of neutrons, in either example, to locate light atom species would provide highly-complementary data to X-ray measurements, where the structure of the parent compound would be the more evident feature. Though the neutron experiment would not detect changes occurring on a rapid timescale, as a single sample orientation is likely to take several hours to measure, any material that can be maintained in a steady state would be measurable.

3.3.3. Excitation

Equipment to explore structurally-excited states already exists at ISIS e.g. by photoactivation, or electric field application, and has been used on the SXD instrument, albeit on simpler compounds than LMX will study. It should be straightforward to make use of this equipment on LMX using a suitably-designed centre stick. Photochemical measurements in particular are becoming more routine – mostly on X-ray instruments[213,214] and induce short-lived structural changes in some materials. However, these (often disordered) structures can be thermally-stabilised, holding the material in a steady state, and thus allowing measurement by neutrons. A limiting factor with larger samples is the complete conversion within the crystal, but this will be aided by the ability to measure smaller crystal samples. Alternatively, the application of an electric field across the crystal can induce structural change, to the extent that it drives phase transitions.[215] Neutrons are ideally placed to observe e.g. the mobility of light atoms in the presence of heavy metals, when an external field is applied, and their subtle but significant effect on crystal symmetry. The cell used on SXD is shown in Figure 3.3.1

3.3.4. High pressure

Lastly, subjecting crystals to strain or hydrostatic pressure is a means for driving quite pronounced structural change in structure. Uniform pressure in particular causes a reduction in free volume, and can force crystals to undergo phase transitions. The capability to measure single crystal neutron diffraction data up to 16 GPa has been developed using ice, potassium dideuterium phosphate, and squaric acid,[216–220] (apparatus shown in Figure 3.3.1) however

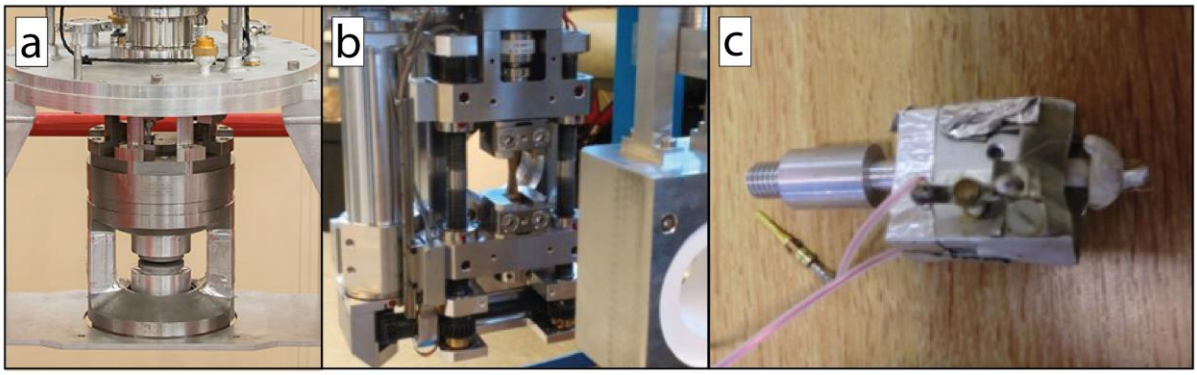


Figure 3.3.1. Single crystal sample environment – **a)** high pressure press equipment, **b)** uniaxial strain rig, and **c)** electric field cell.

the necessarily small sample volume required to attain high-pressure conditions, and the lower neutron flux, limits the range of science that can currently be performed. The ability to measure smaller samples with increased flux provides an increase in data quality, allowing measurement of materials not previously possible, e.g. H_2/D_2 for which there is very limited diffraction data available.[221] Recently the HAR technique[201] has been applied to synchrotron data, aiming to determine proton positions on ice VI (at 1.5 GPa) however, the accuracy of the measurement is an order of magnitude lower than that previously determined by neutron diffraction.[222] The capability to measure the effect of uniaxial force can also be explored through use of a dedicated strain rig, using an appropriately-aligned single crystal.

3.4. External context of LMX

In this section we consider the context of the proposed LMX instrument among existing single-crystal neutron instruments, both in the national (ISIS) and international arenas. For reference, a technical comparison between the predicted performance of LMX with existing large-molecule, single-crystal neutron instruments is available in Appendix 3.

3.4.1. ISIS / Crystallography Group context

The crystallography programme encompasses a range of sub-disciplines, with its instrumentation widely recognised as world-leading in areas including high resolution powder diffraction,[223] high pressures,[224] diffuse/total scattering[225] and complex magnetism.[226] However, at present, ISIS has only one dedicated single-crystal diffractometer, SXD, which has a scientific programme focussing predominantly on chemical crystallography, while attracting some interest from the physics and materials science communities (e.g. spin-ice,[227] incommensurate structures[228] and shape memory alloys[229]) as well as performing diffuse scattering studies of organic and inorganic materials.[230,231]

SXD will continue to serve these science areas; there is a programme to upgrade its suite of detectors that is anticipated to result in a six-fold improvement in count rate. However, even with the upgrade of the detector system, SXD will not be able to tackle molecular systems with unit cell volumes ca. >10000 Å^3, and will not be able to address the breadth of scientific

problems outlined in the LMX science case (Section 2). The LMX instrument will simultaneously expand the capabilities offered by the ISIS Crystallography Group – bringing new research groups into the user programme – as well as complementing the, predominantly, small-molecule research programme on SXD. There is additional single-crystal measurement capability on the WISH diffractometer, however, its experiment objectives are rather different to those of LMX and SXD. WISH only has partial detector coverage, meaning it is inefficient for obtaining 'complete' datasets, necessary for full single-crystal structure determination.

LMX would provide an ideal complement to the proposed WISH-II instrument on the ISIS Second Target Station. The current WISH instrument has proved to be highly successful, with an impressive publication output in high-profile journals, as well as a consistent, healthy, oversubscription rate of proposals submitted from the user community. As a consequence, a second instrument (WISH-II) is being designed, being optimised for single crystal studies of complex magnetic and nuclear structures. Though some of the design aspects of LMX and WISH-II are similar, such as the large solid angle detector coverage, the two are optimised for very different science requirements, leading to differences in guide geometry and wavelength band. Ultimately, LMX has been optimised to measure very small volumes of weakly-diffracting samples. In any case, a single instrument could not support the breadth of science that WISH-II and LMX seek to cover, especially with the forthcoming closure of the ILL and the anticipated growth in the ISIS user base.

In conclusion, the only option to meet the requirements of the science case presented in Section 2, and endorsed by the LMX SAC, is to design, build, and commission the LMX diffractometer at ISIS specifically optimised to meet the needs of the chemical crystallography and structural biology research communities. As the UK's only neutron source, LMX would represent the first neutron diffractometer in the country capable of measuring macromolecular samples and, as discussed in Section 3.1, form a complementary measurement technique to the suite of X-ray instruments at the neighbouring Diamond Light Source.

3.4.2. European / world context

Single-crystal diffractometers to study large-molecule and biological systems are currently operational, or under development, at most major neutron sources. Indeed, ISIS stands out as a facility lacking such an instrument. It is worth recalling the opinion of Förster and Schulze-Briese: "Neutron diffraction should thus be much more popular than it is. However, few neutron sources for [macromolecular] scientific use exist".[122] For the purposes of this case, the most relevant neutron facilities are those at the ILL and the ESS, as the UK is a member of both institutions.

There are several instruments worldwide with the capability to measure large-molecule crystal structures: LADI-III/DALI at ILL,[232] IMAGINE,[233] and MaNDi[187,234] at Oak Ridge, BIODIFF[235,236] at FRM-II, iBIX[237,238] at J-Parc and the planned NMX[239] at ESS. The characteristics are summarised in Appendix 3, including EWALD,[240] a potential future instrument for the

Second Target Station at SNS, but not selected in the first tranche of instruments, and the first dedicated neutron protein crystallography instrument PCS at Los Alamos.[241]

LMX has a number of advantages for single-crystal diffraction of large molecule systems, which give it clear selling points within the European landscape. In contrast to the ILL instruments LADI-III and DALI,[232] LMX will separate the Bragg peaks in time of flight, representing a significant advantage over the quasi-Laue instruments. This allows the full use of spatially-overlapped, harmonic reflections, which are indistinguishable in the quasi-Laue technique, tremendously increasing data completeness. The ability to measure more reflections with a single crystal orientation is an efficient use of beamtime, allowing for the fact that the measurements will be count-rate limited, which is important given the lower flux of the ISIS source.

The proposed NMX instrument[239] at the ESS will also exploit the ability to time resolve its Bragg peaks. However, its design is optimised for studies of very large protein structures (ca. 300 Å unit cell length); substantially bigger than envisaged for LMX (ca. 120 Å) which will focus on more regularly-encountered cell lengths – see Figure 2.2. As a consequence, NMX's broader resolution will be less suited to address the chemical crystallography questions outlined in the science case for LMX. In addition, the chosen detector technology for NMX (Gd-coated micropattern) has a significantly lower efficiency than the double-layer wavelength shifting fibre modules that will be used on LMX (ca. 11.8% and 75%, respectively, at 1.8 Å; 25% and 95% at 4 Å).[242]

In an international context, LMX would be a unique instrument in Europe and have comparable capabilities to the state-of-the-art instrumentation in the USA at the SNS and in Japan at J-PARC, as discussed in Section 3. The MaNDi instrument[234] at the SNS addresses similar science areas to those described in Section 2, probing unit-cell axis lengths between 30 and 300 Å, while providing higher neutron flux at the sample position. Its wavelength bandwidth $\Delta\lambda$ = 2.16 Å when operating at 60 Hz and can be doubled to 4.32 Å (with reduced resolution) by omitting every other neutron pulse (30 Hz). The LMX primary flight path has been set such that a wide wavelength band (ca. 4 Å) will be achievable using all available neutron pulses. The advantage of the wide wavelength band is that a larger volume of reciprocal space can be sampled in a single crystal orientation, increasing information content. The iBIX instrument[237,238] at J-PARC offers very similar capability to that envisaged for LMX, measuring unit cell lengths <135 Å, such that it can address both macromolecular and large-molecule science. Its $\Delta\lambda$ of 3.85 Å from a hydrogen moderator means that reciprocal space is sampled near equally-efficiently to LMX.

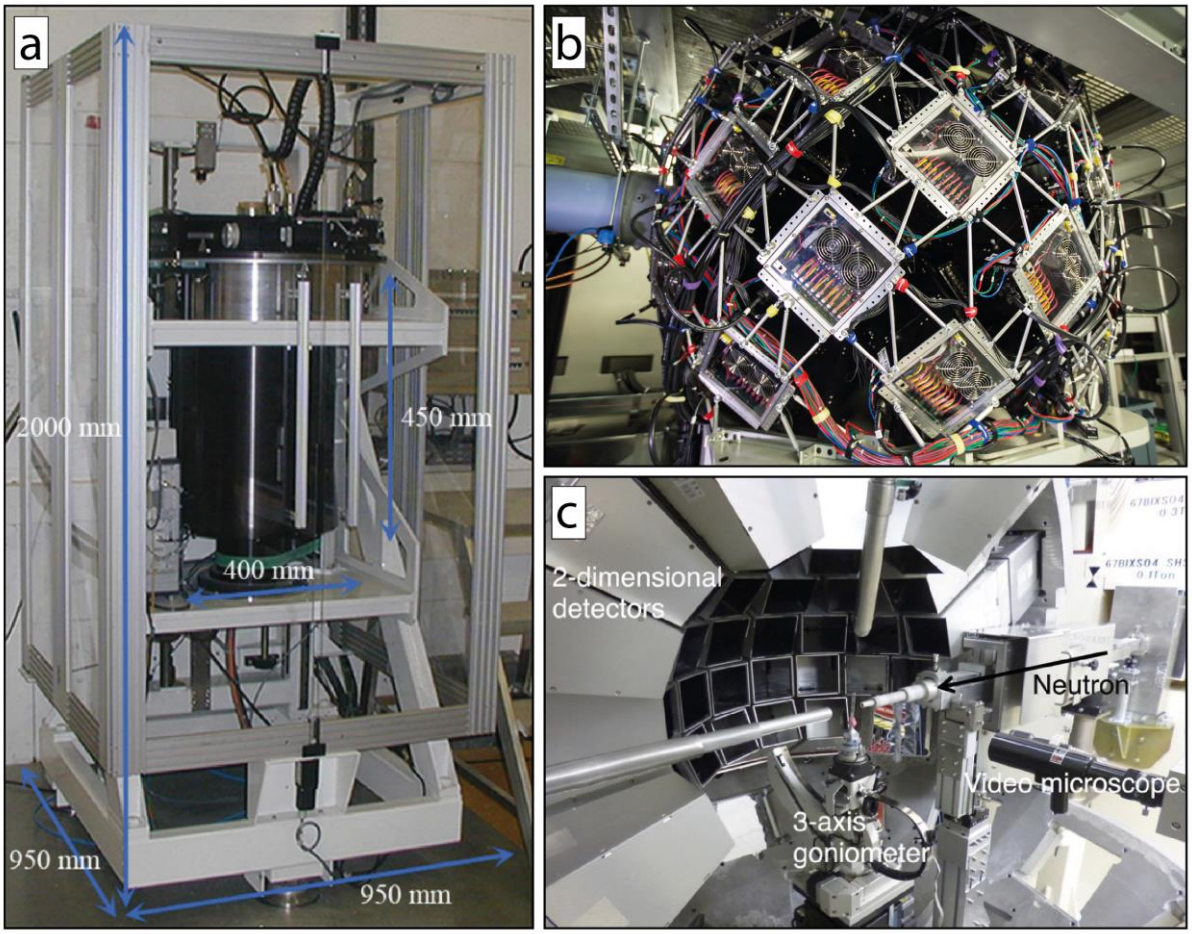


Figure 3.4.2. Images of selected neutron large-molecule diffractometers including **a)** LADI-III (ILL), **b)** MaNDi (SNS), and **c)** iBIX (JPARC).

3.5. UK user community

There is already a UK user community of structural biologists using neutron diffraction who, at present, are forced to travel abroad to perform their research. Clearly, they would benefit from a UK-based instrument optimised for their science programmes, especially considering the demand for the existing instruments in Europe, such as LADI-III at the ILL which has had an average oversubscription rate of 2.6 over the last decade, and 3.5 in more recent years (private communication, Matthew Blakely – LADI-III instrument scientist). As discussed earlier, there is already an established neutron chemical crystallography user base, as part of the SXD programme, however for more complex structures, these researchers must either look abroad (e.g. D19 at the ILL) or use synchrotron radiation. Both communities are the largest interest groups of the national body; the British Crystallographic Association (BCA), which has a total membership of ca. 500 researchers. The annual meetings of the chemical, and biological, structure groups, as well as the BCA main conference are ideal platforms to reach the audiences that would benefit most from LMX.

As outlined in the science case (Section 2), there is clear scope for the ISIS user communities in both the structural biology and chemical crystallography fields to expand significantly once LMX is operational. For example, there is a large structural biology community that regularly uses synchrotron radiation (Diamond Light Source has a strong user programme) and could benefit from neutron measurements, if a suitable instrument were available at ISIS. The appeal will be strengthened by a close synergy between the ISIS and Diamond facilities as well as the labs on the Harwell Campus (see section 3.7 below) – the complementarity between X-ray and neutrons has already been emphasised. Due to the (current) relatively low level of engagement between the UK neutron and biological communities, it will be critically-important to communicate the sample requirements of the instrument. Though growing a 0.1 mm^3 sample is still a non-negligible task, it is a far smaller size than what is often perceived among many structural biologists as being necessary for a neutron experiment.

Looking to the future, the European neutron landscape is going to see a large reduction in capacity as the ILL reactor source is scheduled to close over the coming decade. This is a significant loss for the whole neutron community; the structural biology field will lose two single-crystal diffractometers (LADI-III/DALI), and the closure of D19, which is dedicated to measurement of large-volume crystals, will reduce capability for chemical crystallographers. Though the ESS will start operations before this time, its two currently-planned single-crystal instruments cannot entirely fulfil the needs of the user community. Thus, we foresee that significant demand for LMX will be drawn not only from the UK community that presently uses the ILL for their experiments, but also from research groups outside the UK.

3.6. Industrial involvement

It is clear that many of the scientific problems described in the science case focus on technologically-relevant issues, with applications ranging from the design of new pharmaceuticals to understanding the behaviour of catalysts. There is, therefore, significant opportunity for LMX to attract industrial partners, e.g. the Wellcome Trust and Johnson Matthey – interest has already been expressed from AstraZeneca following preliminary discussions. However, it is important to stress that the large unit cells of the protein structures, supramolecular assemblies and framework structures to be studied on LMX will exhibit weak diffracted intensities and require longer data collections. Consequently, the instrument will not compete with the high-throughput measurements that (e.g.) pharmaceutical companies currently enjoy at synchrotron sources. Instead, LMX will be optimised to address more specific problems (mostly relating to the positions and/or behaviour of hydrogens) and it is important to highlight the implication for industry where fewer – but high quality – experiments are possible. There is clear potential for partnership with industry through the ISIS Collaborative R&D programme, where the benefits of the LMX instrument to UK industry can be communicated.

3.7. Local collaboration

As discussed above, and in the science case, X-ray diffraction is a widely used technique to study large molecular systems, but does not provide information on the location of light

atoms in the presence of heavy ones. The natural complementarity between crystallographic studies at ISIS and Diamond is clear – there already exists a strong collaboration between the respective crystallography groups, including a joint postdoctoral researcher in PDF methods (Polaris and I15-1). However potential partnership between large-molecule, single-crystal facilities is prevented by the lack of suitable instrumentation at ISIS.

There is considerable scope for additional collaborative links between organisations, beyond Diamond Light Source, on the Harwell campus, especially in structural biology field with colleagues at the Rosalind Franklin Institute and the Research Complex. To explore these possibilities a Scientific Advisory Committee for LMX has been established with representatives from academia and some of the relevant facilities at Harwell. There are clear opportunities across a number of areas which support the diffraction experiments on LMX, as described in Section 3.8.

3.8. Support facilities

Of all the new instruments and major upgrades planned as part of the ISIS Endeavour programme, LMX has arguably the greatest potential to attract new users who do not currently use ISIS, or even neutrons in general. However, as the meeting with the LMX SAC highlighted, successful engagement is highly dependent on there being minimal barriers to entry. It is essential that the overall LMX user programme is not just perceived as providing neutron measurements, but rather supporting the whole experiment process. The provision of suitable support facilities assisting crystal growth and deuteration, as well as accessible analysis software, will be essential. These requirements are discussed in more detail in the following sub-sections.

3.8.1. Sample preparation

The major bottleneck for structural biology diffraction measurements – especially neutron experiments – is sample crystallisation. To successfully engage with the structural biological community, for whom growing a suitably-sized, and possibly, perdeuterated crystal is a significant effort, it will be essential to provide facilities that support sample preparation/crystal growth. Synchrotron experiments typically require crystal screening before a sufficiently well-diffracting crystal is found, due to large sample variability. It is impractical to screen crystals on a feasible timescale, using a neutron instrument. For LMX, this would be performed on a lab X-ray diffractometer located in close proximity to the instrument, coupled with a means of transporting the crystals between the two, while avoiding damage to the sample.

Substitution of hydrogen with deuterium is desirable for when isotopic labelling of specific protons addresses a science question directly, but also to reduce the incoherent background scattering by hydrogen. This is significant for proteins where approximately 80% of their solvent content can be exchanged by mixing with D_2O. For deuteration of macromolecules themselves (or chemical compounds) a support laboratory would be required for LMX users, much like the current arrangement for the majority of other experiments at ISIS. The existing

deuteration laboratory, which has experience synthesising deuterated small molecules, will expand capability to include methods such as culturing microbes under deuterated conditions, producing biological samples. Discussions are already underway for new cross-campus partnership with the Research Complex, on the Harwell site, which already operates a protein-production facility for experiments at Diamond Light Source.[243] A combined crystal growth effort between the ISIS deuteration laboratory and the Research Complex would improve on-site capability enormously. Beyond local facilities, specific macromolecular projects would benefit from collaboration with existing large-molecule deuteration facilities at other neutron sources, such as the European Molecular Biology Laboratory (EMBL) in Grenoble, and the Deuteration and Macromolecular Crystallisation Laboratory (DEMAX) at the ESS. Representatives of the latter have already voiced an interest in collaborative partnership.

An expanded deuteration/crystallisation facility will also benefit the chemical crystallography community. Many topical materials contain significant quantities of hydrogen and, if significant synthetic effort is required, this could dissuade users from neutron measurements. Again, systematic screening of crystals is important and, whilst chemical crystal structures are relatively less complex, the potential chemical parameter space for crystallisation is far larger than for biological samples, which are largely limited to aqueous conditions only.

3.8.2. Software

Experience at ISIS during past instrument build/upgrade projects is that the timely provision of suitable data analysis software is essential to maximise the scientific exploitation of the new hardware. Otherwise, lack of accessible analysis routes quickly becomes a bottleneck to publication. In parallel with the LMX instrument design, we are already working on software projects which will be used to process data from the current SXD instrument and, in due course, LMX. Both are supported by funding from the Ada Lovelace Centre:

- A time-of-flight single crystal diffractometer with a large solid angle of detector coverage (such as SXD or LMX) collects diffraction data over large volumes of reciprocal space. Whilst this has a number of advantages over reactor-based instruments for studies of (*e.g.*) diffuse scattering, the quality of structure refinements based on the extracted intensities of the Bragg peaks is generally poorer. This is because the data are acquired from a wide range of neutron wavelengths, making corrections for the effects of absorption and extinction much more complex. New data correction routines for wavelength-dependent absorption from arbitrary-shape crystals have been produced, and software routines to treat wavelength-dependent extinction effects are currently under development.
- DIALS – the reflection intensity integration software for macromolecular crystallography – is widely used at macromolecular beamlines and the small molecule beamline I19 at Diamond Light Source. It is currently being adapted to work with time-of-flight neutron data. DIALS has the advantage of being familiar to the biological community, thus presenting a lower barrier to entry for neutron experiments.

3.9. User consultation

The scientific, business, and technical cases for a future LMX instrument were discussed during a European Neutron Diffraction single-crystal workshop, organised by the ISIS Crystallography Group at The Cosener's House, Abingdon in April 2017 (Figure 3.1). Instrument scientists and staff from the major world-wide neutron facilities, together with select expert members of the research community, engaged in discussion regarding the challenges (small sample size and increased structural complexity) that the user community now encounters more frequently.

The discussion identified the necessary direction of future instrumentation in order to keep pace with the evolving needs of this community. A meeting report is available,[244] but its strong recommendations for ISIS were to support the construction of the LMX instrument that will focus on complex structural problems in large chemical and biological materials, but also to upgrade the detector technology on the existing SXD instrument.

Figure 3.1. The participants at the European Neutron Diffraction single-crystal workshop in April, 2017

More recently, the Endeavour Programme at ISIS has been established, in order to update and develop the suite of neutron instruments at ISIS and fulfil the research needs of the neutron community. The programme is bidding for UK Government funding (ca. £91M) that would allow the upgrade of five existing ISIS instruments, and the construction of four new ones – including LMX.

A series of further user meetings was held in July 2021 to update the user community on the status of the Endeavour Programme; explore the scientific possibilities that the proposed instruments would provide; acquire community feedback on the various instrument projects, and their science cases, as well as identifying additional aspects (such as sample environment or software) that would be necessary to maximise the impact of the new instruments. The status of, and future plans for, LMX were presented at meetings devoted to the topics of Biosciences and Health (8/7/21) and Materials for the Future (15/7/21) by external representatives Peter Moody (University of Leicester) and Paul Saines (University of Kent), respectively. Both sessions were attended by members of the relevant user community who have strong engagement with the single crystal diffraction programme at ISIS. Their specific recommendations concerning the science programme, design of LMX, and need for support facilities have directed the content of this document.

The scientific, business, and technical cases for LMX have been developed in collaboration with a Scientific Advisory Committee (SAC), composed of researchers from UK Universities, industry, and organisations on the Harwell campus. The current membership is listed in Appendix 1.

The SAC met on 26th May 2022 and discussed a number of questions relating to the science case, technical specification, requirements for support facilities (sample preparation sample environment, software, etc.) and the complementarity of LMX with other neutron facilities and other experimental/computational techniques. These comments are incorporated in the content of this case.

4. Appendices

Appendix 1. LMX Scientific Advisory Committee

The current membership of the LMX Scientific Advisory Committee (SAC) includes representatives of both the chemical crystallography and structural biology fields:

- **Dave Allan**. Principal Beamline Scientist on the I19 small molecule single crystal diffraction beamline at Diamond.
- **Christine Beavers**. Rigaku US; formerly Principal Beamline Scientist on the I15 extreme conditions diffractometer at Diamond.
- **Stephen Carr**. Protein crystallographer at the Research Complex at Harwell (RCaH) and the Department of Chemistry, University of Oxford.
- **Simon Coles**. Professor of Structural Chemistry at the University of Southampton and Director of both the UK National Crystallography Service and the UK Physical Sciences Data-science Service.
- **Gwyndaf Evans**. Deputy Director of Life Science, Principal Beamline Scientist for the VMXm micro/nanofocus macromolecular crystallography beamline at Diamond, Diamond Research Fellow and Head of Technology at the Rosalind Franklin Institute.
- **Michael Hough**. Principal Beamline Scientist VMXI at Diamond.
- **Peter Moody**. Professor of Structural Biology, Department of Molecular Cell Biology, University of Leicester.
- **Stefan Norberg**. Associate Principal Scientist, Astra Zeneca, Gothenburg.
- **Paul Saines**. Senior Lecturer in Chemistry (Inorganic Materials) and head of the Materials for Energy and Electronics Group at the University of Kent.
- **Amber Thompson**. Chemical Crystallography Service Manager, Department of Chemistry, University of Oxford.
- **Armin Wagner**. Principal Beamline Scientist for the I23 macromolecular crystallography beamline at Diamond.

The SAC membership also includes several members of the ISIS Crystallography Group:

- Craig Bull
- Silvia Capelli
- Nick Funnell
- Matthias Gutmann
- Paul Henry
- Steve Hull
- David Keen
- Michael Ritchie

Appendix 2. Measurement details of macromolecular crystal structures

PDB code	d_{min} / Å	Crystal vol. / mm^3	Unit cell vol. / Å^3	Asym. unit vol. / Å^3	Space group	Time / days	Instrument
4C3Q	2.2	0.95	452000	75300	$P3_221$	7	BIODIFF
4BD1	2	2.7	452000	75300	$P3_221$	7	BIODIFF
4Q49	1.8	2	125000	62500	$P2_1$	8	BIODIFF
4CVJ	2.5	0.7	428000	107000	$P2_12_12_1$	22	BIODIFF
4AR4	1.38	2	51000	12750	$P2_12_12_1$	3	D19
3KCJ	1.8	28	984000	123000	$I222$	6	D19
4AR3	1.05	6.9	51000	12750	$P2_12_12_1$	8	D19
4PVN	2.3	3.4	248000	62000	$P2_12_12$	10	D19
4QDW	1.8	10	984000	123000	$I222$	14	D19
4DVO	2	50	984000	123000	$I222$	14	D19
3KCL	2	50	984000	123000	$I222$	14	D19
4QCD	1.93	2.7	299000	74750	$P2_12_12$	9	iBIX
3U2J	2	2.5	248000	62000	$P2_12_12$	30	iBIX
4K9F	1.75	0.7	51000	12750	$P2_12_12_1$	3	IMAGINE
4PDJ	2	3.6	155000	38750	$P2_12_12_1$	17	IMAGINE
3RZT	1.75	3.2	51000	12750	$P2_12_12_1$	0.5	LADI-III
3RZ6	1.75	3.9	51000	12750	$P2_12_12_1$	1.5	LADI-III
3SS2	1.75	3.9	51000	12750	$P2_12_12_1$	2	LADI-III
3KYY	1.66	4.1	51000	12750	$P2_12_12_1$	3	LADI-III
4PVM	2	3.4	248000	62000	$P2_12_12$	5	LADI-III
3RYG	1.75	3.9	51000	12750	$P2_12_12_1$	5	LADI-III
3KYX	1.68	3.9	51000	12750	$P2_12_12_1$	5	LADI-III
4N3M	1.9	4	815000	101870	$I222$	6	LADI-III
2XQZ	2.1	5	452000	75300	$P3_221$	6	LADI-III
AxCytCp	2.1	1.5	431000	35900	$P6_522$	10	LADI-III
2WYX	2.1	8.75	452000	75300	$P3_221$	12	LADI-III
AcNiR	2.3	0.3	893000	74400	$P2_13$	15	LADI-III
4JEC	2	0.2	240000	60000	$P2_12_12$	17	LADI-III
4QXK	2.2	0.95	249000	31120	$P4_12_12$	17	LADI-III
4CVI	2.4	1	428000	107000	$P2_12_12_1$	18	LADI-III
3QFS	1.85	0.13	59000	14750	$P2_12_12_1$	21	LADI-III
3Q3L	2.5	5	1141000	285250	$C2$	21	LADI-III
3R98	2.4	1	428000	107000	$P2_12_12_1$	25	LADI-III
3R99	2.4	1	428000	107000	$P2_12_12_1$	25	LADI-III
4N9M	2.3	1	815000	101870	$I222$	25	LADI-III
4NY6	1.85	0.23	59000	14750	$P2_12_12_1$	30	LADI-III
3QZA	2	9.4	984000	123000	$I222$	14	PCS
4QDP	2	10	984000	123000	$I222$	14	PCS
3KCO	1.8	50	984000	123000	$I222$	14	PCS
3TMJ	2	1.7	125000	62500	$P2_1$	20	PCS
3L45	1.8	2	45000	22500	$P2_1$	21	PCS
4GOC	2	2	125000	62500	$P2_1$	22	PCS
4YOJ	2	2	125000	62500	$P2_1$	22	PCS
4FC1	1.1	4	18000	9000	$P2_1$	22	PCS
3KMF	2	20	289000	144500	$P2_1$	23	PCS
3QBA	1.4	0.7	24000	6000	$P2_12_12_1$	25	PCS
3KKX	2	1.2	125000	62500	$P2_1$	55	PCS

Table A4.1. Measurement details of joint X-ray/neutron structures deposited in the PDB between 2010 and 2015. Reproduced from Ref. 245.

Appendix 3. Technical comparison of LMX with other instruments

Instrument	Moderator	L1 (m)	Band-width (Å)	Mean flux ($ns^{-1}mm^{-2}$)	Divergence (º)	L2 (m)	Pixel size (mm)	Max. unit cell (Å)
LADI-III	n/a	n/a	Ca. 1	1×10^{6}	Variable	0.2	0.125–0.5	150
DALI	n/a	n/a	Ca. 0.8	1×10^{6}	Variable	0.16	0.125–0.4	150
IMAGINE	n/a	n/a	Ca. 1	1×10^{5}	Variable	0.2	0.125–0.5	150
MaNDi*	Decoupled hydrogen	30	2.16	4.5×10^{5}	0.12–0.8	0.45	1	300
iBIX*	Coupled hydrogen	40	3.4	7×10^{5}	0.2	0.49	<1	135
BIODIFF	Deuterium	n/a	Mono.	$1–4\times10^{4}$	0.7–0.8	0.2	0.125–0.5	110
NMX*†	Hydrogen	158	1.7	2×10^{7}	0.4	0.2–1.0	0.2	300
EWALD*†	Coupled hydrogen	90	3	7×10^{6}	0.38	0.3	0.3	300
LMX (base)*	Hydrogen	19.5	Ca. 4	3.03×10^{5}	< 0.4	0.5	Ca. 1.5	>120
LMX (guide, m = 5)*	Hydrogen	19.5	Ca. 4	4.18×10^{5}	< 0.4–0.8	0.5	Ca. 1.5	>120
PCS*	Bespoke	28	5.5	Ca. 1×10^{5}	0.24	0.7	<1.5	Ca. 100
D19	n/a	n/a	Mono	10^{5} - 10^{6}	0.19(h), 0.12(v)	0.76	2.5×1.5	Ca. 50
TOPAZ*	Decoupled hydrogen	18	3.1	--	0.86–1.43	0.39–0.46	0.59	50
SENJU*	Hydrogen	34.8	4.0	6×10^{5} or 1.3×10^{6}	0.6 or 0.9	0.8	4	50
SXD*	Water	8.3	9.8	6×10^{5}	0.76	0.23–0.28	3	Ca. 40

Notes: *time-of-flight instrument; †proposed instrument

Table A4.2. A comparison of the proposed design and predicted performance of LMX with worldwide neutron single crystal diffraction instruments. Figures for the average flux on TOPAZ and D19 were not readily available. Mean flux numbers are not always directly comparably as they are often quoted for the instrument maximum divergence. The technical design of LMX is in progress and may be revised from the values given above.

Appendix 4. Outline costing

Item	Cost (£k)
Target shutter	90
Target insert	100
Neutron guide system	600
Chopper systems – disk, housing, bearing unit	400
Slits/jaws/aperture selectors (beam shaping)	100
Beamline snout/nozzle/exit systems	5
Beam monitors	10
Vacuum vessel	143
Beamstops	22
Detector system	1070.5
Beamline shielding	527
Block house	490
PPS	120
O_2 monitoring	3.1
Motion control system	76
Chopper control	20
Detector DAE	250
General power distribution	20
General network distribution	14
Vacuum	50
Mechanical services	79
Screened room/counting house	70
Materials handling	20
Access infrastructure	35
Sample environment/equipment	200
Capital sub-total (ex. VAT)	4514.6
Contingency (10%)	451.46
VAT (20%)	902.92
Capital sub-total (inc. VAT)	5868.98
Resource sub-total (labour)	1266.297
Total	**7135.277**

Table A4.3. An outline costing for the construction of the planned LMX instrument.

Appendix 5. Meeting summary with LMX Scientific Advisory Committee

The discussion with members of the LMX SAC is summarised below, under the headings of Science Case, Technical Design, and Business Case. This document was prepared ahead of the main LMX Case documentation and was used to inform the general direction of the LMX instrument development.

Science case

1) Is there any important field of science we are missing in the "science case" ? *(Or what do we consider to be the key scientific challenges in chemical and biological communities that LMX could address?)*

Structural biology

Hydrogen: Almost all (ca. 90%) the structural questions where neutrons will have an advantage concern *hydrogen*. More specifically, where do the hydrogen atoms move in proton transfer processes; what are the various protonation states across the material; which way are water molecules oriented?

Pharmaceuticals: Much pharmaceutical interest concerns binding of small molecules to active sites, so being able to characterise these, structurally, with sufficient resolution is important. Typically, for X-rays, this means d_{min}=1.5 Å, though for neutrons 2 Å should be adequate.

There is an inherent advantage provided by neutrons insofar as being a non-destructive probe – protein crystals are notoriously sensitive, and suffer radiation damage from X-rays.
The vast majority of biological samples are difficult to crystallise, at least with any appreciable size, a strong requirement would be capability to measure smaller crystals; 0.1-0.2 mm^3 should be considered as a sensible target. A typical crystal measured at a synchrotron might have dimensions ranging between 10-100 µm.
If we want to look at the materials that structural biologists are really interested in, we need the capability to measure unit cell lengths of ca. 200 Å.

Chemical crystallography

Framework/supramolecular-type materials: this field is moving away from simply recording new structures – there is now an increased focus on their functionality, namely what is occurring inside porous cavities on e.g. solvent or gas uptake. These materials often have significant hydrogen content. Molecular machines and covalent organic frameworks are representative materials of this field that have very large unit cells.

Mechanistic studies: there is a desire to understand how chemical processes occur, e.g. tracking labile/active species (like hydrogen) in catalytic processes, or *in-situ* hydrogenation of organometallic materials. Isotopic tagging is likely to be useful here, exploring differences between hydrogen and deuterium. This could be important for determining stereochemistry

where X–rays cannot discriminate between ^{1}H and ^{2}H. Locating hydrogen – specifically in non-standard bonding environments – is likely to be an always-unrivalled strength of neutron experiments.

Functional materials: the area more traditionally catered-for by neutrons – these might include perovskite-type materials where structures (and functionality) are heavily dependent on composition/dopants/holes. This area will continue to be important.

Small molecules: in general, structures with larger unit cells are being increasingly studied. The specific science case for these could vary quite significantly, but the capability to measure complex structures (e.g. modulated materials) is desirable. Small pharmaceutical molecules will be interesting to study under humid environments to explore moisture-induced degradation.

Overwhelmingly, in-situ/operando (dynamic) studies are likely to be key to most of the above, which will be heavily-dependent on sample environment. This doesn't necessarily mean making measurements on rapid timescales, but keeping materials in a steady state in order to measure structures representative of different stages of some process. This will place an emphasis on sample environment.

The ability to measure smaller crystals and larger unit cells are also requirements of the chemical community. Crystals will typically be ca. 0.1 mm^3 and smaller. Unit cell sizes can vary quite significantly, but the capability to measure 40 Å cell lengths is a sensible target. The small crystal size may actually be a requirement of the material functionality – it has implications for e.g. diffusion rates through the sample of gas/solvent molecules.

We should recognise that PhD students are now expected to be familiar with an increasingly-large number of characterisation techniques, so it is unlikely that neutron diffraction would provide all the data required for a single study. However, this also means that a neutron experiment need not provide a completely-conclusive structural determination; the complementarity of numerous techniques means they address each other's shortcomings. Collectively, a full structural picture is established.

2) Is this science case ambitious enough? Is it attainable?

There was no concern raised over any lack of scientific ambition. Certainly, a diverse range of materials were discussed. There were some concerns considering attainability – these are detailed in the next section

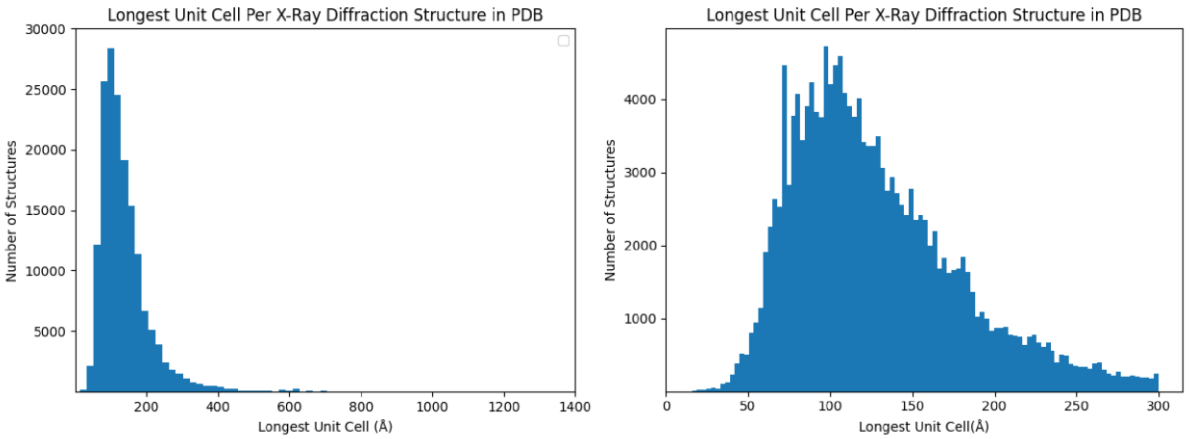
Technical design

3) Any comments on the current design?

The proposed capability to measure samples < 1 mm^3 needs to be improved. A common demand from both biological and chemical communities is the capability to measure crystals measuring ca. 0.1 mm^3 in size. However a maximum beam size of 1-2 mm was thought to be sensible.

However, concerns were raised over the maximum unit cell length that could be measured (120 Å proposed) – if we want to engage with the structural biological community, then 200 Å is should be considered.

If 200 Å should prove beyond the capability of LMX, histograms arranged by longest cell axis in the PDB show there is still reasonable interest < 200 Å, where ca. 110 Å is the most frequently encountered longest cell length.



Additionally there is some concern that d_{min} = 1.5 Å will be unattainable for typical biological samples; this level of resolution (to identify, and model, bound small-molecule drugs) will be key if we want to engage with the pharmaceutical industry. For protein structures, more generally, d_{min} = 2.0 Å should be considered the absolute minimum requirement. This would be sufficient to identify (well-behaved) hydrogen.
The resolution requirement for the chemical community is closer to 0.8-1.0 Å (e.g. to resolve disordered components) but this is considerably more attainable considering the smaller unit cell size, and therefore increased scattering power.

In order to best assess the complementarity between the proposed LMX design and the science case above, we need to simulate the time-of-flight neutron patterns we would see arising from examples of the materials listed in the science case.

4) What support facilities are required? (crystallisation lab/d-lab/other?)

To successfully engage with the structural biological community, for whom growing a suitable neutron-sized crystal would be a significant effort, provision of facilities to assist with crystal growth and deuteration will be essential. Perdeuteration will be necessary as ca. 50% of the protein structure consists of water hydrogen – this will lead to a significant incoherent background. There could be a campus-wide case for providing this sort of facility, as isotopic studies also have use beyond just neutron measurements.

The main bottleneck for protein experiments is sample production – this is expensive, so any assistance in this area should be considered.

Protein experiments typically require screening of tens-hundreds of crystals before a sufficiently high-quality crystal is found due to large sample variability. This could be performed on a lab X-ray diffractometer – we should consider having this capability available in close proximity to the LMX instrument, coupled with a means of transporting the crystals easily/quickly from one to the other in a stable environment.

A deuteration/crystallisation facility could prove quite useful for the chemical community as well. Many topical materials contain significant quantities of hydrogen and if significant synthetic effort is otherwise required, this could dissuade users from neutron measurements. This community typically starts from having some small/poorly-diffracting crystals. A means of improving on these to grow larger, higher-quality crystals would be useful. This would probably demand robotics for systematic screening, though this might prove difficult to design as the potential parameter space for crystallisations is far larger for chemical systems than biological (the latter being limited to aqueous conditions only).

5) What sample environments are needed?

Structural biology: Data collections from biological samples are greatly improved by cooling, as well as being important for stabilising the structure. However, this could all be done with nitrogen temperatures – there is no real need to cool to helium temperatures. So we should consider capability to measure at ambient and (modest) low temperature. Controlling the environment humidity will be crucial – as little as 3% change can cause degradation of the sample.

Chemical crystallography: Good sample environment is really going to be the key to the success of a chemical crystallography programme. The focus of the science case above is determining mechanisms/functionality via in-situ/operando measurements. This is not an exhaustive list, but temperature/gas pressure (<5 kbar)/electric field/humidity control/gas flow capability will all be important.

6) What software is needed?

The software needs to be accessible/have a low barrier to entry. This is particularly important if we are going to attract new users, who may have no prior experience with neutrons. This is particularly true for biological community, who would be most familiar with DIALS.

Reprocessing raw diffraction images is a more iterative process for single crystal measurements than for powders. It may be necessary to reprocess the data after the neutron experiment, thus it is important for users to have the capability to do so, with accessible software, that they can be easily trained to use.

Business case

7) What limits do you see (both science case and instrument)?

The biggest challenge is that it is unlikely LMX could tackle all the science scenarios listed here without serious compromise on data quality (requirements have been given elsewhere). However, something more targeted is entirely feasible.

In terms of structural determination, neutrons are never going to compete with X-ray/electron radiation on straightforward characterisation. So the focus should be on data quality; obtaining a strong structural understanding of the respective materials, and sticking to the scientific problems that neutron measurements are well-positioned to tackle.

If we position ourselves as providing 'quality' data (see section 10) this likely means longer data collections, and therefore fewer experiments. This reduction in throughput may make it difficult to engage with sectors of the biological community – pharmaceutical companies are significant synchrotron users and rely on high-throughput; 3 minute datasets are typical. A neutron source will never be able to handle this sort of sample quantity.

The large unit cells of typical protein structures, as well as supramolecular assemblies, frameworks, and molecular wire/machines mean these will likely exhibit weak diffracted intensities and require longer collections. However, the obvious industrial impact these all (potentially) exhibit should be emphasised in order to persuade funders that fewer, quality experiments are worth doing.

If we had to consider the trade-off between crystal size and data completeness, the completeness is more important in the structural biology world; anything < 90% is practically unpublishable. There are not necessarily the same requirements on completeness for chemical crystallography – as long as data are considered 'fit for purpose', then incomplete data can be tolerated. The more challenging in-situ/operando experiments often necessitate a reduction in data quality.

8) Do we have the community to support it?

There is a feeling that the respective wider communities do recognise the benefits in neutron measurements but we will still need to make efforts to engage with those who are not current users.

If we are going to encourage new users toward neutron science, they will need as many barriers to entry removed as possible. We should not view the LMX proposal as just providing neutron measurements for the biological community, but rather the whole experiment process. Thus the points on support facilities and software will prove quite important.

Among non-neutron users there is still a perception that enormous single crystal samples are required. Some effort needs to go into dispelling this view, as well offering help to grow larger crystals.

Part of the barrier to entry is that many groups have an established 'process' that may not include neutron measurements. Engagement with PhD students is likely important since they tend to be more open to new approaches, and they are typically the individuals performing the work. We need to consider the PhD timescale, particularly for structural biologists, and the significant time taken to grow/measure/analyse crystal structures in general – this is why a facility aiding crystal growth will be worthwhile.

ISIS scientists tend to offer a high level of support both pre- and post-experiment, however this may not be widely recognised in the community among non-neutron users. This is obviously a selling point for performing neutron measurements. However we will need to take care over how this is communicated, since the majority of this support is not formalised and mostly on an ad-hoc collaborative basis with ISIS instrument scientists.

9) What collaborations could be viable onsite?

There was a clear feeling that a shared, campus facility for crystal growth and deuteration would be highly beneficial for all communities, but particularly crucial for engaging structural biologists.

10) How does the instrument fit with those available at ISIS/ILL/ESS/elsewhere?

It would be informative to see a general benchmarking against competing instruments at the ILL and ESS.

Particularly in the structural biology world, there is not much awareness of the advantages of one neutron source over another (partly as a consequence of there being few groups working in this area). So it will be especially important to have a clear selling point for LMX. LMX will not compete with the ILL on flux, nor the ESS on speed of measurement. Given our anticipated low background, and therefore good signal-to-noise ratio, LMX would be positioned as providing high quality data.

The most probable gap is to target d_{min} resolution and improve on the maximum unit cell length size we can measure. The ability to time resolve our neutrons will help with peak resolution, compared to constant wavelength facilities.

11) Can everything be done with computational methods?

No – there is a general feeling that there will always be a place for neutron measurements as they will all be uniquely well-placed to tackle certain problems. In particular for dealing with

structural ‘peculiarities’ where hydrogen bonds in non-standard ways – this will likely always prove challenging for computational approaches.

However, we need to be aware of the improvements in quantum crystallography – in particular the ability to compute aspherical form factors. This has led to neutron-quality determination of hydrogen atom positions from X-ray data, and significantly-improved refinement statistics. Thus neutron diffraction no longer holds the advantage of being uniquely capable of determining hydrogen positions, where the bonding environment is ‘usual’ and/or ordered.

We should anticipate this approach being extended to use among the protein community within the next decade.

There will always be a need for experimental validation of computational predictions, though, and we should carefully consider how we focus the complementarity of LMX to the future of the X-ray world.

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