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Modern structural biology would essentially be unthinkable without synchrotron radiation. In particular, the large macromolecular complexes and machines that are part of the cell can currently only be visualized at the atomic level by synchrotron based X-ray crystallography.

It allows us to obtain information from very small crystals that would otherwise be impossible to do. Finally, outside my own field there are a host of applications in physics and chemistry that require these powerful sources of X-rays. Given this increasing and crucial need, it has been extremely useful to have the Diamond Light Source, a state-of-the-art facility that is easily accessible to users in the UK. I also think that having allied institutions nearby for structural biology and other fields will have a synergistic effect by improving both the user facilities and the science that will be done at Diamond. This will benefit both the pursuit of applied knowledge and applied research.

This has been another year in which the performance and scientific and technological output of Diamond has strongly advanced.

The facility now rivals the most advanced and successful synchrotron facilities in the world in terms of reliability and output. Close links have also been established with industry to ensure that we capitalise on the contributions that Diamond can make to product development and enhancement. If academic research in collaboration with industry is included then more than 20% of beamtime is now directly connected with industry.

In addition to the growth in the use of the facility we have been pressing ahead with the completion of the Phase II beamlines and ramping up our work on Phase III. The final set of ten Phase III beamlines was determined through a process of detailed engagement with the user community. The first of these beamlines will commence operation in 2014 and the final beamline in 2018. As in the past we are being fortunate in being able to attract outstanding scientists and engineers to work on this final phase of the project so we are confident that Diamond will continue to meet and exceed expectations.
This year Diamond Light Source Ltd is celebrating its 10th Anniversary. The company was officially formed on the 27th March 2002 by the signing of the Joint Venture Agreement between the UK Government and the Wellcome Trust. This milestone provides an opportunity to reflect on the challenges and achievements this anniversary celebrates. We built Diamond during the initial five years and have in the following five years established a very successful operation programme, while still building the new beamlines of Phases II and III. There are many facets to this success – the commitment and dedication of our staff and contractors, the continued support of our user community and the ongoing financial backing of our shareholders. These ingredients are each filled with their many challenges, and in overcoming these, there lies success!

The confirmation in 2010 that Phase III funding was granted allows for 10 additional beamlines to be constructed and will bring the total number of beamlines to 32 by 2018. This maximises the initial capital investment made in the facility. The selection of these beamlines required the initial proposal from the science community, the evaluation by the Scientific and Industrial Advisory Committees (SAC and DISCo), the inclusion of the funders through the Large Facilities Steering Group (LFSG) and the final approval by Diamond’s Board of Directors and the Shareholders, namely the Science and Technology Facilities Council (STFC) and the Wellcome Trust. I would like to thank the members of these various groups and committees for their active and positive engagement, making sure that Diamond is well positioned to provide the most sought-after and leading edge instruments for the UK and international communities.

With 20 beamlines now operational, there has been an increase in science delivered by the facility. Over the past financial year, a total of 4651 user visits were serviced by Diamond, with 398 from outside the UK, conducting experiments over a broad range of scientific fields. This means that 6349 shifts were allocated enabling users to follow their scientific goals formulated in 475 successful proposals. Users from 281 academic institutes have visited Diamond for beamtime with 142 coming from outside the UK.

By April 2012, Diamond’s activities had led to over 1700 scientific publications with more than 420 reported so far in 2011/12. In 2011 just over 200 protein structures from Diamond were deposited in the World Protein Databank, reaching 825 since our start of operations.

Diamond supports the delivery of the research portfolio of the UK Research Councils but has also a major role to play with Industry. We close the financial year with our 50th industrial customer and we have increased our support of industrial research, with overall 6% proprietary access across 17 beamlines, rising to 19% on a single beamline. In addition 15% of non-MX (Macromolecular Crystallography) academic proposals were also directly related to industrial partners and 32% of the MX BAGs (Block Allocation Groups) reported direct industrial involvement.

This user success has only become possible through the excellent reliability and stability of the accelerators which is reflected in 98% uptime and an increase of the mean time between failures by a factor of two, bringing it to now 56 hours.

Our vision for Diamond remains bold: “Throughout its lifetime, Diamond endeavours to be a leading edge facility for scientific research, supporting a wide range of users from both academia and industry, thereby delivering benefits to the UK society and economy. Diamond will strive to respond to the 21st century scientific challenges through the successful management of the facility, the high quality science it delivers and encourage its wide dissemination”.

Diamond will continue to play a major role in the UK scientific landscape for decades to come. Being part of the Diamond team has been a very rewarding experience for me. I have been grateful for my staff’s commitment and support and I thank them for their continued efforts and hard work to make Diamond a true user facility.

I would finally like to thank our shareholders, once again, together with the UK Research Councils and all our partners and stakeholders for their outstanding support over the past ten years. I am sure that the team will remain focused on delivering science of the highest quality with a strong impact.
Diamond now has 20 operational beamlines, with the final Phase II beamlines in optimisation mode within 2012. The first six approved Phase III beamlines are underway. The last four beamlines have now been selected by the Science Advisory Committee and taken forward.

The beamlines are organised into six villages as described below. Phase III beamlines are shown in grey.

**Diamond’s beamlines: current operation status April 2012**

<table>
<thead>
<tr>
<th>Beamline Name and Number</th>
<th>Main Techniques</th>
<th>Energy / Wavelength Range</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>I03 - Macromolecular Crystallography</td>
<td>Macromolecular Crystallography, Multiwavelength Anomalous Diffraction (MAD)</td>
<td>5 - 25 keV</td>
<td>Operational</td>
</tr>
<tr>
<td>I04 - Macromolecular Crystallography</td>
<td>Macromolecular Crystallography, MAD</td>
<td>5 - 25 keV</td>
<td>Operational</td>
</tr>
<tr>
<td>I04-1 - Monochromatic MX</td>
<td>Macromolecular Crystallography</td>
<td>3.53 keV to 150 Å</td>
<td>Operational</td>
</tr>
<tr>
<td>I05 - ARCES</td>
<td>Angle-resolved Photoelectron Spectroscopy (ARPES)</td>
<td>15 - 240 eV</td>
<td>Phase II under construction</td>
</tr>
<tr>
<td>I06 - Nanoscience</td>
<td>X-ray absorption spectroscopy, X-ray photonemission microscopy and X-ray Micronage, Circular and Linear Dichroism</td>
<td>First harmonic range 100 - 1500 eV</td>
<td>Linear Horizontal: 80 - 2100 eV, Linear Vertical: 100 - 1500 eV</td>
</tr>
<tr>
<td>I07 - Surface and Interface Diffraction</td>
<td>Surface X-ray Diffraction, Grazing Incidence X-ray Diffraction (GIXD), Grazing Incidence Small Angle X-ray Scattering (GISAXS), X-ray Reflectivity (XRR)</td>
<td>0.25 - 100 Å</td>
<td>Operational</td>
</tr>
<tr>
<td>I07 - VERSOK</td>
<td>Versatile Soft X-ray beamline</td>
<td>Spectroscopic and scanned-probe imaging</td>
<td>Phase II approved</td>
</tr>
<tr>
<td>I08 - Soft X-ray Microscopy</td>
<td>X-ray scanning microscopy</td>
<td>0.25 - 10 Å</td>
<td>Phase II under construction</td>
</tr>
<tr>
<td>I09 - SISA</td>
<td>Surface and Interface Structural Analysis</td>
<td>Reflection - X-ray Absorption Spectroscopy (XAS), X-ray Photoelectron Diffraction, X-ray Reflectivity</td>
<td>Phase II approved</td>
</tr>
<tr>
<td>I09 - SISA</td>
<td>Surface and Interface Structural Analysis</td>
<td>Reflection - X-ray Absorption Spectroscopy (XAS), X-ray Photoelectron Diffraction, X-ray Reflectivity</td>
<td>Phase II approved</td>
</tr>
<tr>
<td>I11 - High resolution powder diffraction</td>
<td>X-ray powder diffraction</td>
<td>5 - 10 Å</td>
<td>Operational</td>
</tr>
<tr>
<td>I13 - X-ray Imaging and Coherence</td>
<td>Phase contrast imaging, Tomography</td>
<td>8 - 35 keV</td>
<td>Operational</td>
</tr>
<tr>
<td>I14 - A Hard X-ray Nanoprobe for Complex Systems</td>
<td>X-ray fluorescence, X-ray spectroscopy and diffraction, X-ray and wide angle X-ray scattering</td>
<td>3.5 - 30 keV</td>
<td>Phase III under construction</td>
</tr>
<tr>
<td>I15 - Extreme Conditions</td>
<td>Powder diffraction, single crystal diffraction</td>
<td>20 - 70 Å</td>
<td>Operational</td>
</tr>
<tr>
<td>I15 - EPR</td>
<td>X-ray Pair Scattering Distribution Function (XPDF)</td>
<td>0.5 - 2 Å</td>
<td>Operational</td>
</tr>
<tr>
<td>I16 - Materials and Magnetism</td>
<td>Diffraction / Scattering, Spectroscopy</td>
<td>3.5 - 25 keV</td>
<td>Operational</td>
</tr>
<tr>
<td>I16 - Test beamline</td>
<td>Diffraction, Imaging, Reflectometry</td>
<td>4 - 20 keV monochromatic focused</td>
<td>Operational</td>
</tr>
<tr>
<td>I16 - Test beamline</td>
<td>Diffraction, Imaging, Reflectometry</td>
<td>4 - 20 keV monochromatic focused</td>
<td>Operational</td>
</tr>
<tr>
<td>I17 - Nonlinear Spectroscopy</td>
<td>X-ray absorption spectroscopy (XAS), Extended X-ray Absorption Fine Structure (EXAFS), Fluorescence microscopy</td>
<td>2 - 20 keV</td>
<td>Operational</td>
</tr>
<tr>
<td>I18 - Core EXAFS</td>
<td>X-ray absorption spectroscopy</td>
<td>2.05 - 35 keV</td>
<td>Operational</td>
</tr>
<tr>
<td>I19 - Small molecule single crystal diffraction</td>
<td>Small Molecular Single Crystal Diffraction</td>
<td>2 to 25 keV</td>
<td>Oct 2012</td>
</tr>
<tr>
<td>I20 - LOLA</td>
<td>X-ray spectroscopy</td>
<td>X-ray Absorption Spectroscopy (XAS), Energy Dispersive EXAFS (ED EXAFS), X-ray Emission Spectroscopy</td>
<td>Commissioning</td>
</tr>
<tr>
<td>I21 - High throughput SAXS</td>
<td>Small Angle X-ray Scattering &amp; Diffraction</td>
<td>0.2 - 10 Å</td>
<td>Phase II under construction</td>
</tr>
<tr>
<td>I22 - Inelastic X-ray Imaging</td>
<td>Inelastic X-ray Imaging</td>
<td>0.25 - 10 Å</td>
<td>Phase II under construction</td>
</tr>
<tr>
<td>I23 - Long wavelength MX</td>
<td>X-ray Absorption Spectroscopy, X-ray Photoelectron Diffraction, X-ray Reflectivity</td>
<td>Phase II under construction</td>
<td></td>
</tr>
<tr>
<td>I23 - Circular Dichroism</td>
<td>Circular Dichroism</td>
<td>Operational Module 4, 120 - 300 Å</td>
<td>Operational</td>
</tr>
<tr>
<td>I24 - Cryo-TM</td>
<td>Full field X-ray imaging</td>
<td>Up to 2 Å</td>
<td>Phase II under construction</td>
</tr>
<tr>
<td>I25 - Microfocus Crystallography, MAD</td>
<td>Microfocus Crystallography, MAD</td>
<td>4 - 20 keV</td>
<td>Operational</td>
</tr>
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</table>

With Phase II almost completed and funding confirmation for Phase III in October 2010, Diamond Light Source issued a call for ideas for new beamlines as part of the final prioritisation procedure for Phase III.

Outline Proposals were considered by the SAC and DISCs in June 2011, and full proposals considered at an Open Meeting of the SAC on 11th October 2011. The final recommendations from SAC and DISCs to complete the Diamond Phase III beamline portfolio were considered and approved by the Diamond Board of Directors in December 2011. This will now give Diamond 32 operational beamlines by 2016.
**Spectroscopy Village**
Fred Musselmann, Village Coordinator

It is an exciting time in the Spectroscopy Village as two of the new Phase III beamlines currently being designed will provide nanofocus spectroscopic imaging on dedicated beamlines in the soft and hard X-ray regimes. The two first XAS beamlines, I18 microfocus spectroscopy and B18 core EXAFS, continue to progress with both now running reliably in the new style generic data acquisition (GDA) software. I20 LOLA is beginning to see light at the end of the beampipe as solutions have been developed for its difficult technical challenges.

Furthermore, the new radionuclide laboratory facility has had its first users: the BIGRAD (biogeochemical gradients and radionuclide transport) consortium, which is funded by NERC, the Natural Environment Research Council. This facility enables some low level radionuclides to be handled onsite before they are sealed securely, allowing the possibility for delicate radionuclide species to be examined at Diamond.

The range of science on spectroscopy beamlines is diverse, with much biological imaging continuing on I18 such as: Jon Dobson and his colleagues’ work investigating Alzheimer’s Disease, the University of Kent’s insalubrable Mary Rose research informing the strategy for the conservation of archaeological marine wood, and Rob Dyke and collaborators looking at the formation of gold nanoparticles at the interface between organic and aqueous phases with a microfocus beam. On B18, users are often chemists and include the catalysis studies by Moniek Tomp’s team, where they are trying to capture information on short lived intermediates, and Semma Corr and associates’ work in understanding lithium batteries. Environmental scientists are frequent visitors to the beamlines, Guarnierino Cibin and co-workers have studied arborescent mineral dust from ice cores, which can inform the climate change debate.

B18 has now been open for nearly two years in March 2012. The number of user groups using the beamline has continued to grow, and many positive comments about the quality of data have been received. Commissioning time was mainly spent improving the IEXAFS capability of the beamline and the software. After the replacement of the stepper motor Bragg axis with a servo motor in August 2011, the reliability of IEXAFS improved dramatically and it is now the default mode of operation providing a factor of two improvement in throughput. Whilst the beamline was not intended for ultra-dilute EXAFS, it has been possible to measure 1 nm³ Cu protein and few hundred ppm absorbed species on a mineral in typically 6-8 hours.

On I18, the optical table of the end station was replaced with two large granite blocks in November 2011, at the same time the opportunity was taken to optimise the table top layout. This new gives more space for additional optics, such as zone plates, and in October a zone plate trial in collaboration with the Optics group produced the first sub 100 nm hard X-ray beam measured on Diamond. The granite blocks should provide better stability as we aim to open a sub-micron XRF mapping option on I18 for 2013. We are also beginning to develop XANES mapping on the beamline and performed the first trial experiment in February 2012. The new GDA gui combined with sample stage rastering has substantially increased the mapping speeds feasible on the beamline. We are looking to reduce the time per point still further in summer 2012 when we aim for 100 Hz sampling frequency.

After solving the vacuum problems and the thermal management issues of the I20 four bounce monochromator in 2010-2011, further commissioning brought to light time and energy dependent intensity fluctuations in its operation. These effects prevented the collection of good quality EXAFS spectra and were tracked down to factors related with the mechanical flexibility of the monochromator crystal cages. To solve these issues a complete re-design of the crystal cages of the two monochromator axes was required. This project began in June 2011 and the installation of the newly designed components is scheduled for the June 2012 shutdown. The rest of the beamline components have now been commissioned, taking advantage of the time needed to solve the monochromator problems. The emission spectrometer assembly has been completed and is now ready to take X-ray beam.

Over the past year, significant progress in building of the dispersive branch of the beamline has been achieved. First light in the Optics Hutch was seen in early 2012. The polychromator, a Diamond in-house design, is scheduled for installation in May 2012, and this will complete the installation of components in the Polychromator Hutch. Furthermore the assembly of the spectrometer in the Experimental Hutch is also very advanced and we expect to be ready for X-ray commissioning of this branch at I20 in the summer of 2012.

I08-SXM will be filling the gap on international level by providing unique capabilities in its energy range for a scanning X-ray microscope (SXM). The instrument covers a 250-4200 eV photon energy range from soft X-rays providing access to major K- and L- absorption edges for elemental and chemical analysis with lateral resolutions better than 20 nm. This is complemented by versatile X-ray transmission contrast and exploring new imaging techniques, including new 3D imaging by depth profiling approaches, for example by structured illumination. The central objective of the I08-SXM beamline is the ability to obtain morphological and chemically-specific information on a broad range of organic and inorganic matter under in-situ conditions, mimicking real environments. The technical design review for I08 took place in October 2011 and it is on track to open for users in 2014.

The central objective of the I08-SXM beamline is the ability to obtain morphological and chemically-specific information on a broad range of organic and inorganic matter under in-situ conditions, mimicking real environments. The technical design review for I08 took place in October 2011 and it is on track to open for users in 2014.

114 Hard X-ray nanoprobe will be an instrument to provide X-ray beam sizes from 1um to 25nm and beyond. The beamline will be over 185m long and will consist of two endstations. The first will provide beam sizes from 1um to 100nm and will target on SXFS/WAXS and elemental analysis with ample space for a wide range of sample environments. The second endstation is a nanoprobe dedicated to delivering the smallest possible beam size for elemental, spectroscopic and diffraction studies, with beam sizes down to 25nm. The ability to study elemental and structural variations on such a wide scale has attracted a broad user community with a wide range of applications in biological, environmental and material science. The beamline had its conceptual design review in February 2012 and is scheduled to open in 2016.
Application of microfocus X-ray beams from synchrotrons in heritage conservation


A major cause of the degradation of heritage and museum objects is attack by acid, including the attack by acids caused by atmospheric pollution or acidic species generated from within the artefact. In the case of the Mary Rose, the warship of Henry VIII that was raised in 1545 and is currently being prepared for exhibition in a new museum in Portsmouth Harbour, the acid is sulfuric acid which originates from the oxidation of sulfur compounds deposited in the wood by bacteria on the seabed. Treatment of objects with mild alkali is one method of neutralising the acids in artefacts; however this can lead to the growth of salt deposits which can disrupt the structure due to the pressures that are generated. A relatively recent approach to the problem is to impregnate the object with nanoparticles of an insoluble alkaline earth oxide, hydroxide or carbonate which will only form small particles with acids and act as a de-acidification reservoir. We have been using nanoparticles of strontium carbonate to treat Mary Rose timbers and using X-ray absorption spectroscopy to monitor the effectiveness of the treatment, to understand the chemistry involved and to measure the penetration in the wood.

The Mary Rose was a favourite warship of King Henry VIII of England. It sank in July 1545 whilst on route to confront ships of the French fleet outside Portsmouth Harbour. It remained on the seabed for over 400 years before it was unreeved and then raised in 1982. Approximately half of the ship survived as a consequence of being buried by silt, and thereby protected from the currents and from the organisms that eroded away the exposed timbers. However, the surviving timbers now exhibit potentially damaging sulfur salt precipitates. The problem was first identified in 2000 in the conserved Swedish shipwreck, Vasa1. The origin of the precipitates is the formation of H2S in the polluted sea of the harbour by sulfur reducing bacteria which diffuses into the wood and reacts to form a range of reduced sulfur compounds. In the anaerobic conditions of the sea-bed these materials are not a problem, but in the raised timbers air causes oxidation, the formation of sulfuric acid with degradation of the cellulose and formation of the mineral deposits. The growth of these deposits causes further deterioration of the timber. The problem is further exacerbated by the presence of iron species in the wood (from original fittings, bolts and nails) which catalyse the formation of sulfuric acid. The effect is illustrated in Fig. 1, which shows sulfate mineral aggregates on a knife handle. At Diamond Light Source we have been using X-ray absorption spectroscopy (XAS) to monitor the iron and sulfur speciation in the Mary Rose timber and artefacts to measure the effectiveness of various preservation techniques. In the case of sulfur XAS is particularly powerful as the shift in the K-edge absorption edge in the X-ray absorption near edge structure (XANES) can distinguish all the sulfur oxidation states, i.e. from -1 to +6. This is illustrated in Fig. 2, which shows the sulfur K-edge XANES of a Mary Rose timber and compounds known to be present in the wood. We have used the microfocus XAS station, I18, to characterise the composition of the Mary Rose timbers and artefacts2 and used bulk XAS measurements to monitor the effectiveness of a number of standard non-invasive techniques (EDXRF, D0RAS, ammonium citrate) in the removal of iron compounds. In addition, we have monitored the effect of chelating agents on timbers which have been impregnated with polyethylene glycol (PEG), a polymer used to fill the holes in degraded water-logged wood and give them mechanical stability. This blocks the entrance of the chelating agents.

It has been estimated that the Mary Rose timbers contain some 2 tonnes of sulfur in various chemical forms, both inorganic (pyrites and other sulfates) and organic (cystene, cysteine, methionine and thios) and many of them can potentially oxidise to sulfuric acid. Therefore treatments that provide a long-term protection from acid attack are desirable. In a series of sulfur K-edge XAS experiments undertaken at beamline 118, SLSR and Diamond beamline 11B, we have explored treatment with alkaline nanoparticles to provide de-acidification reservoirs, a method that has been used in other heritage artefacts. Timbers and artefacts were impregnated with 20-50 nm diameter particles of strontium carbonate (SrCO3) prepared by high energy ball-milling. We have impregnated timbers and artefacts with 20-50 nm diameter particles of strontium carbonate, SrCO3, prepared by high energy ball-milling. SrCO3 was chosen as other alkaline earths (magnesium and calcium) are present in the Mary Rose and could unnecessarily complicate the analysis. Wood samples were sonicated in 2-prepared dispersions of the nanoparticles for various periods of time and then examined by XAS. The sulfur K-edge results for Mary Rose wood treated for three days are shown in Fig. 3. The treatment leads to dramatic changes in the spectra, most notably bringing the growth of sulfur after the treatment. Further analysis was possible by simulating the wood spectra as the linear combination of components of sulfur compounds known to be in the wood. This not only shows the formation of strontium sulfate but also a reduction in the concentration of the organic sulfur compounds. Thus there is an additional advantage of using the nanoparticles.

Recently we have studied simulated Mary Rose timbers prepared from fresh oak and impregnated with iron sulfate. These were treated with SrCO3 nanoparticles and the iron and strontium K-edge XAS collected on B18. These measurements confirmed the formation of sulfate to form inactive strontium sulfate. In addition, they provided information on the penetration of the nanoparticles into the wood. This work showed that water dispersions gave a greater penetration depth for the nanoparticles, compared to 2-prepared dispersions and that the penetration is hindered in PEG treated samples. This information, along with the sulfur K-edge results now allows us to design the optimum nanoparticle treatment regimes for timber and artefacts.

References

Funding Acknowledgements
We thank the Heritage Lottery Fund for continued support of the conservation of the Mary Rose and the grant which funded this project. AOC would like to thank the Leverhulme Trust for an Emeritus Grant to support this research.

Research carried out at Diamond Light Source on beamline 11B and at the SSRRL on beamline 4-3.
Nucleation and growth processes of gold nanoparticles at the liquid-liquid interface


Gold nanoparticles have attracted enormous interest recently because of their unique optical, electrical, and chemical properties. The properties of these particles, which have dimensions in the range 1 to 100 nm, are very promising for medical applications in biotechnology and catalysis. However, their performance depends on their size, structure and composition, hence much effort has been invested to develop methods to control the properties of nanoparticles. One promising way to make nanoparticles is using the ‘two-phase’ methods, where a metal ion (gold, in this case) is dissolved in an ‘oil’ phase and the agent, that reduces the ion to form the metal particles, is dissolved in water. We have combined this two-phase method with in situ X-ray absorption spectroscopy on beamline I18 which allows us to examine the local environment of the Au ion, and gain insight in this important particle formation process.

Nanoparticles formed in 1,2-dichloroethane via the reduction of Au(III) using aqueous phase borohydride. XANES spectrum of the tetrachloroaurate ion in 1,2-dichloroethane.

Differential XANES was used to show that the tetrachloroaurate ion retained its electronic structure in both phases (in the absence of ferrocyanide, see Fig. 3). Significantly, in the presence of ferrocyanide, reduction to form metallic gold was not seen. Metallic gold formation, which had been described in the earlier report by Schliffrin and Cheng1, occurred only in the presence of (added) metallic nuclei, see Fig. 4. This indicates that there is a high activation barrier to particle formation at the liquid-liquid interface, which could be attributed to the energetics of phase formation.

We aim to improve the time and spatial resolution of these processes to obtain more information on the intermediates involved in the deposition process. We also seek to modify our chemical system to probe a reaction closer to the spontaneous chemical processes used to form Au NPs using, via the Brust-Schiffrin approach.

References

Funding Acknowledgements

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High field magnetic resonance microscopy of the human hippocampus in Alzheimer’s disease: Quantitative imaging and correlation with iron

Antharam, V., Collingwood, J.F., Bullivant, J.P., Davidson, M., Chandra, S., Mikiyaula, A., Finneghan, M., Batch, C., Forde, J. & Dobson, J.


The accumulation of iron compounds in the brain has been known to be associated with Alzheimer’s disease (AD) since the early 1950s. Only recently, however, have we begun to develop and refine techniques that enable us to map and characterise these iron compounds. By mapping the distribution of these iron compounds in AD brain tissue and by characterising their chemical nature - which significantly impacts their magnetic properties - we hope to build a better understanding of iron’s potential role in AD pathology as well as explore the effects of the magnetic properties of iron compounds on Magnetic Resonance Imaging (MRI) signal parameters. At beamline I18 we have used X-ray fluorescence (XRF) techniques to examine human brain tissue and characterise the nature and spatial distribution of anomalous iron compounds associated with Alzheimer’s disease. By analysing adjacent tissue sections with XRF and magnetic resonance imaging (MRI), we have been able to identify specific iron compounds in a region of the brain, which is critical to AD pathology. We have correlated, for the first time, brain iron distribution in hippocampal sub-regions with quantifiable effects of MRI signal intensity and identified image parameters that enable us to distinguish between AD and control tissue. The goal of this work is to use iron as a biomarker to develop a new, MRI-based diagnostic technique for this disease.

The association between iron and Alzheimer’s disease was first reported nearly 60 years ago; however, until recently, very little was known about the form of iron, its distribution and whether it plays a role in the disease or is simply a by-product, perhaps a potential biomarker. While the last question has proven more difficult to unravel, we have been able to shed some light on the form and distribution of iron in the affected brain using X-ray fluorescence X-ray techniques. Regardless of the role it plays in the disease, iron compounds are generally strongly magnetic, understanding the form and spatial distribution has implications for the development of MRI-based diagnostic techniques due to iron’s effect on proton spin relaxation rates in nearby tissue.

The relaxations of iron-bearing compounds that form iron oxides are characteristic for MRI applications, as they affect quantifiable parameters including the transverse relaxation rate (R2) and the susceptibility-related parameter R2*. Characterising the iron compounds – particularly iron oxides -- present in AD tissue is important as some, such as magnetite (Fe3O4), are about 100 times more magnetic than the normal form of stored iron in the body, ferritin. The outstanding sensitivity and specificity of synchronon XRF is used to obtain maps of iron distribution in brain tissue sections. XRF images are then obtained from localized iron concentrations revealed in the maps, and by performing linear combination fits with standards, we are able to identify the various iron compounds present and their distribution in relation to tissue structure, pathological inclusions, and brain region. This information permits direct investigation of the influence of iron on MRI at the microscopic scale, to establish the source of iron-related contrast in healthy and diseased brain tissue.

The relationship between iron distribution in AD and MRI contrast is not a straightforward one. Current clinical studies of brain iron as a potential biomarker of neurodegeneration rely on assumptions that signal changes in MRI are due to iron, where interpretation of data is often based on post-mortem measures of iron in tissues. As Alzheimer’s is a highly histrionic group, these factors have contributed to the difficulty of developing efficient MRI algorithms that are able to track iron in AD.

In our recent study at beamline I18 we mapped the iron distribution in tissue sections cut from blocks in which the R2 and R2* parameters had already been measured using magnetic resonance microscopy with a similar in-plane spatial resolution of 60 μm. The region investigated was the hippocampus, a critical region for AD pathology. The hippocampus contains distinct subfields which are differently affected in AD, and contain different iron concentrations of iron as revealed in the synchronon XRF maps. Analysis of the individual sections provided direct evidence of the predicted positive correlation between iron and the R2 and R2* parameters, and statistically significant differences in iron concentration were observed as a function of hippocampal sub-field (Fig. 2). While significant differences in iron concentration were not observed between AD and controls in the small number of cases studied here, we did observe that the variance in the susceptibility-related MRI parameter, R2*, is significantly different between affected and control groups (p < 0.001). This difference was clearly revealed in histogram plots of the signal, and may provide a useful measure of altered iron distribution within the tissue, allowing differences in tissue iron between AD and healthy brains to be observed even though the absolute concentration may not vary significantly in the hippocampus. The application of µXRF to enable direct quantification of the relationship between iron concentration and MRI contrast has confirmed the relationship between iron and specific MRI parameters at high spatial resolution, and provided a potential starting point in our search for an iron-based, clinically relevant Alzheimer’s disease biomarker.

References


Funding Acknowledgements

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Insights in the Mechanism of Selective Olefin Oligomerisation Catalysis using Stop-flow Techniques: a Mo K edge EXAFS Study


Polyethylene (PE) is the most widely used plastic, with its primary use in packaging. The polymer is classified into several different categories based on its density and branching, properties which govern its mechanical properties. Linear low density PE (LLDPE) is an overall linear polymer, with significant numbers of short branches, and therefore has a narrower molecular weight distribution and different melt flow properties from other PE's. It is less shear-sensitive and has a low viscosity at all strain rates. In contrast to other PE's, very thin LLDPE films can therefore be produced easily, while maintaining high strength and toughness, making it perfect for applications in flexible tubing as well as plastic bags, sheets, films and membranes. The selective trimerisation and tetramerisation of ethene to produce the linear alpha-olefins (LAs) 1-heptene and 1-octene respectively are of major significance due to the importance of these chemicals in the production of (LLDPE), which accounts for around 50% of the LAG co-monomers produced industrially. A variety of transition metal catalysts, most of which are based on early transition metals, can facilitate this type of selective oligomerisation. By using a Cr(III) pre-catalyst with ethene in the presence of methylaluminoxane co-catalyst, an extremely high selectivity can be obtained. This process is thought to occur via a microcyclic mechanism based upon a metallocyclic intermediate formed through reaction of Cr(III) pre-catalyst with ethene in the presence of methylaluminoxane co-catalyst. The pursuit of higher performance catalysts requires a detailed understanding of the individual stages of the catalytic cycle, the dependence upon metal, promoter and/or co-catalyst. However, developing such catalyst systems is hindered by characterisation difficulties due to the paramagnetism of the majority of the Cr complexes.

Stopped flow systems have recently been developed to probe homogeneous catalysis in situ and time-resolved (down to milliseconds) using X-ray Absorption Spectroscopy (XAS) in energy dispersive or quick EXAFS mode. However, the methods have been proven for homogeneous Cr and Co systems, the Cr edge at a lower energy at which solvent, reactant and even a few cm of air are highly absorbing and severely hamper the transmission experiment.

We have pursued two methods to allow characterisation of these catalytic systems: (i) substituting Mo(III) for the Cr(III) to allow the use of time-resolved XAS to probe the early stages of the reaction, and (ii) modification of the stopped flow system in order to maintain the time resolution advantages, but trap the intermediates to allow for long XAS data acquisition for low X-ray energy systems.

We have prepared the analogous Mo(III) trichloro complexes, [MoCl3(E)(SNS)] (E=S with R=CH2-C6H4-p-C(CH3)3 or E=N with R=n-C10H21). [25 mM in toluene] and [MoCl3(SBz)] (E=S with R=CH2-C6H4-p-C(CH3)3 or E=N with R=n-C10H21). [25 mM in toluene] and 20 equivalents of AlMe3 (E=S with R=CH2-C6H4-p-C(CH3)3 or E=N with R=n-C10H21). [25 mM in toluene]. The reaction was freeze-quenched after 5 seconds and 5 minutes of reaction. EXAFS analysis showed that the partial removal of Br, without dissociation of the Sb ligand (Fig. 3). In addition, the set-up as developed now gives access to good quality EXAFS data 5 seconds after mixing, something which could not be obtained using QEXAFS as performed (QEXAFS time-resolution at our beamtimes: 20-30 s/spectrum, or 1-3 minutes/spectrum).

The results demonstrate that treatment of the Mo complex solution with excess AlMe3 (not X-ray beam induced) results in the formation of a new species which has strong evidence that dimers with direct Mo-Mo bonds or halide-bridged dimers are not present under the conditions examined (1:20 Mo:Al). At the high Al:Mo ratios used, with the complexes investigated, the reaction mechanism cannot be resolved with the present technique, especially for the trichloride complexes, providing insights into the low overall catalytic activity and deactivation which has been reported for these systems under real catalytic conditions. The new freeze-quench attachment allows reaction, short-lived intermediate species to be trapped (≤ 1 s), and provides strong evidence that dimers with direct Mo-Mo bonds or halide-bridged dimers are not present under the conditions examined (1:20 Mo:Al). At the high Al:Mo ratios used, with the complexes investigated, the reaction mechanism cannot be resolved with the present technique, especially for the trichloride complexes, providing insights into the low overall catalytic activity and deactivation which has been reported for these systems under real catalytic conditions.

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Acknowledgements
The authors gratefully acknowledge EPSRC grant (EPSRC/EP/G054100/1) for the support of the UK Diamond Light Source beamline B18. The EPSRC IMW project and the contribution of the Diamond Light Source beamline B18. The EPSRC IMW project and the contribution of the Diamond Light Source beamline B18.
An XRF-XANES characterization of deep ice core dust


Ice formed in old glaciers is among the most important sources of paleo-proxy data. Ice cores contain information on ancient variations of Earth’s climate, some reaching back almost a million years. The knowledge of the mineralogy of the airborne dust in particular, allows the reconstruction of the aerosol trajectories and consequently the patterns of transport in the last climatic cycles. In this research, we have used TXRF and XANES to analyze the composition of the insoluble fraction extracted from deep ice cores from Antarctica, on samples dating across the last glacial transition, and compared these with samples from a glacier in the Italian Alps. We were able to detect and follow the variations in the composition patterns with time. The results show that such synchrotron radiation-based combined analysis complements XRD and traditional aerosol analysis techniques. They give clear element-specific insights into the local structure of specific fractions of the analyte, adding invaluable information on the dust composition and aerosol transport mechanisms that can lead to the recognition of the dust source areas.

Climate changes, natural or anthropogenic, may have a large impact on the Earth in the next decades. As a consequence, paleoclimatology is a new emerging research area. While meteorological instruments may show trends over the past century, paleo-proxy data provide information on climatic variability regarding thousands and even millions of years. However, proxy records are not direct measurements and information has to be extracted and calibrated to have reliable estimates.

A vertical timeline of the past climate is stored in ice sheets and mountain glaciers, as in marine sediments and other proxies. Deep ice cores cover relatively short periods, but with a high temporal resolution. As an example, the longest chronological record from ice cores comes from the EPICA perforation in Antarctica, and covers the last 120,000 years, providing information about the temperature, precipitation levels, the atmospheric composition and volcanic activity. Antarctic dust archived in polar and mid-latitude ice cores represents a particularly precious proxy for assessing environmental and atmospheric circulation variability, with special emphasis on the regional-to-global climate changes at different time scales. Results from drilling sites around the world help distinguish the local climate (i.e. the Alps, Andes, and Himalayas) from global climate trends (i.e. in Antarctica and Greenland).

One of the major challenges of this research is the extremely small amount (in the µg range) of the available ice core insoluble fraction, containing small crystallites, poorly crystalline and often including glass and tephra contributions from volcanic events. In case, the low amount of dust available required clean procedures to collect, prepare and store samples. In our experiments, after filtration, part of the insoluble fraction was deposited on thin membranes and the remaining aliquot was suspended in water evaporated on high purity Si wafer substrates.

We applied the spectroscopic techniques Total-Reflection X-ray Fluorescence (TXRF) and XANES to the mineralogical analysis of such challenging samples. We combined elemental and spectroscopic information with data obtained by other techniques such as XRD, transmission electron microscopy and Particle Induced X-ray and Gamma-ray Emission, to achieve a clear element-specific insight into the local structure of specific fractions of the analyte.

Some of the experiments were performed at the Stanford Synchrotron Radiation Lightsource (SSRL) at beamline 10-2, using a dedicated TXRF set-up, while a custom clean sample chamber was used at the Core XAS beamline (B18) at Diamond. Silicon Drift Detectors were used allowing detection of low Z elements, down to Al. XAS spectra have been collected at the Fe and the Ti K edges.

Dust from Antarctic and Alpine samples is qualitatively composed of a mixture of silicates: clays, quartz, and feldspars, with minor contributions of pyroxenes, amphiboles, metal oxides, and volcanic glasses. XRF and XANES results are compared with rock and soil geochemical data, as possible dust source areas, from the available literature. In Fig. 1 we show a comparison between our samples with reference rocks and minerals from the possible dust source areas (Australia, South America). Alpine samples in particular are a close group separate from the other datasets.

Moreover, the distribution of the Antarctic samples lies closely in the region of the Patagonian samples. Fig. 2 shows the iron oxide concentration in mineral dust vs. time for samples collected in Saisi Dome, Antarctica. It represents a partial-time evolution of the iron oxide concentration in mineral dust from Holocene to the 4th Marine isotope stage (MIS4) period and its trend evidences the minimum typically observed in the interglacial periods. The Fe K edge position moves up with lower temperatures and quickly descends to a sharp minimum in correspondence of a strong volcanic event (see the right side).

Figure 1: Vertical timeline of the past climate is stored in ice sheets and mountain glaciers as in marine sediments and other proxies. Deep ice cores cover relatively short periods, but with a high temporal resolution. As an example, the longest chronological record from ice cores comes from the EPICA perforation in Antarctica, and covers the last 120,000 years, providing information about the temperature, precipitation levels, the atmospheric composition and volcanic activity. Antarctic dust archived in polar and mid-latitude ice cores represents a particularly precious proxy for assessing environmental and atmospheric circulation variability, with special emphasis on the regional-to-global climate changes at different time scales. Results from drilling sites around the world help distinguish the local climate (i.e. the Alps, Andes, and Himalayas) from global climate trends (i.e. in Antarctica and Greenland).

Figure 2: Dust concentration (µg/L) of the GLACE Dome ice core (former experiment). XRF detection limits (µg/L) are for Fe, concentration high in depth is 5 vs. 1000. Dots data cover the last marine isotope period (Miocene, MIS 5) and the last interglacial period, just before the last glaciation. While the Fe/Z concentration ratio is relatively insensitive to the temperature (there is a clear descending trend towards the glacial period), the Fe K edge position moves up with lower temperatures and quickly descends to a sharp minimum in correspondence of a strong volcanic event (see the right side).

Figure 3: Comparison among Fe K-edge XANES spectra of a sample belonging to a glacial period (corresponding to a volcanic event); reference phyllosilicate samples (i.e. muscovite having Fe in different local environments); and a sample from a volcanic period (top). The characteristic Fe K edge XANES spectra of Antarctic samples shown in Fig. 3 indicate the presence of clay minerals in the dust. The spectra show that iron is octahedrally coordinated, with variable oxidation state. The observed absorption edge shift (middle panel in Fig.) demonstrates that the amounts of Fe(3+) changes with time (i.e. vs. depth). The highest Fe(2+) concentration point demonstrates the occurrence of a volcanic event.

Combining XANES information from spectra at different edges improves the mineralogical characterization of dust. As an example, significant differences among Antarctic and Alpine samples appear in the XANES (540 eV) and in the pre-edge (right) regions at Ti K-edge (Fig. 4). So the composition differences highlighted in the ternary plot of Fig. 1 are also related to different concentrations of Ti-bearing minerals. Continental and oceanic materials are characterized by having different Fe/Ti ratios. In our case, comparing Fe and Ti allows identification of samples containing mineral fractions coming from the nearby Antarctic coast.

In conclusion, our research, although still limited in terms of samples analyzed, has demonstrated that the combined use of synchrotron radiation-based techniques to obtain both elemental and species-selective information on the dust mineralogy, can be a valuable resource for the analysis of the aerosol transport mechanisms and of the climatic variability during the last climatic cycles.

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Research carried out at Diamond Light Source on beamline B18 and at the SSRL on beamline 10-2.

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Investigation of the lithium ion-battery cycling mechanism in a 3.90 V iron-based fluorosulphate cathode material


The diminishing supply of fossil fuels, together with a desire to reduce greenhouse gas emissions, has propelled electroweathering storage to the forefront of modern research. In particular, lithium ion batteries, which possess high power capabilities, have been identified for future use in hybrid electric vehicles, where high power densities become essential. To understand the intimate relationship between structure, property, and function in materials as applications for positive insertion electrodes, we have employed a broad range of characterisation methods including local structure studies performed at the B18 Core EXAFS beamline at the Diamond Light Source. Of particular interest is the mechanism through which the insertion and deinsertion of lithium ions occurs. In the present study, we have investigated the triplite-phase of Li[(Fe1−δMnδ)]SO4·H2O which exhibits the highest Fe3+/Fe2+ redox voltage of any inorganic compound to date.

The search for new positive cathodes with enhanced electrochemical performance continues, with a focus on the role of the polyanion as a way to manipulate the position of the transition metal redox couple. An example of this is the tavorite-phase of LiFeSO4F, where the potential of the Fe3+/Fe2+ redox couple is 150 mV higher than that of LiFePO4 due to the increased electronegativity of the SO3 groups compared to PO4. Interestingly, the manganese-based homologue, LiMnSO4F, does not adopt the tavorite structure but instead is found to crystallise in the triplite structure and shows no discernable electrochemical activity. The triplite structure is characterized by edge-sharing chains of MO4F2 octahedra, which exhibit a significant degree of site-mixing between manganese and lithium and can be contrasted against the tavorite phase, which displays corner-sharing chains that are invariant against the tavorite phase, which displays corner-sharing chains that are invariant against the tetragonal symmetry of the triplite structure but instead is found to crystallise in the triplite structure and shows no discernable electrochemical activity.

We observe a linear increase in the unit cell volume of triplite with increasing substitution of manganese for iron, as should be expected given that the ionic radius of Mn2+ is larger than that of the Fe3+. Mössbauer spectroscopy, which provides a direct probe of the local iron environment, revealed significant differences between the tavorite and triplite phases. The triplite phase, which has two distinct crystallographic sites for iron, displays a corresponding two sharp sets of peaks. In contrast, the triplite phase gives non-resolved, broad peaks, indicating multiple iron environments and an increase in disorder in the triplite phase. This inherent disorder may be the reason for the absence of electrochemical activity in this phase, without a coherent pathway to follow, lithium ion diffusion would prove difficult in such a phase.

The solid solution Li[(Fe1−δMnδ)]SO4·H2O was prepared by reacting stoichiometric amounts of a mixed-metal sulphate monohydrate precursor (Fe3+Mn3+SO4·H2O) with LiF at 295 °C using a traditional solid-state synthesis method. A broad range of techniques, including synchrotron X-ray diffraction, Mössbauer spectroscopy, synchrotron X-ray absorption spectroscopy and electrochemical cycling, were characterised by a broad range of techniques, including synchrotron X-ray diffraction, Mössbauer spectroscopy, synchrotron X-ray absorption spectroscopy and electrochemical cycling.

We have performed in-situ experiment to monitor the changes in the X-ray absorption spectra as lithium is inserted and deinserted from the triplite phase. X-Ray Absorption Spectroscopy (XAS) is an innovative method for following in-situ the changes occurring in materials while undergoing electrochemical cycling.

Figures 1 and 2 show the voltage-composition curve for the triplite phase, Li[(Fe1−δMnδ)]SO4·H2O, showing a 3.90 V redox potential, the highest recorded for the Fe3+/Fe2+ couple to date for an inorganic compound.

Of particular note in this study has been the electrochemical performance of the triplite phase Li[(Fe1−δMnδ)]SO4·H2O, which, at 3.90 V, displays the highest Fe3+/Fe2+ redox voltage reported to date for an inorganic compound (Fig. 2). Power cycle stability and Li-ion diffusion measurements were collected in-situ on a sample during electrochemical cycling to monitor the changes occurring and both confirmed the reversibility of the lithium insertion/extraction process in the samples which contained iron. Also of interest has been the observation that the total volume change between the lithiated and delithiated species is a mere 0.6%. This is in contrast to what is seen for the tavorite phase, where a volume change of 10.4% is observed.

This is an important observation for commercial applications, where large volume changes on extended cycling give rise to mechanical stress which can cause dewetting from current collectors and thereby result in device failure.

The practical importance of this material and the unprecedentedly high redox potential, we wanted to study more closely the roles of iron and manganese during cycling. Vital to this exploration and in an effort to elucidate the lithium insertion in the triplite phase Li[(Fe1−δMnδ)]SO4·H2O, we made use of the B18 Core EXAFS beamline to conduct in-situ studies to monitor the changes in the X-ray absorption spectra as lithium is inserted and deinserted from the triplite phase. X-Ray Absorption Spectroscopy (XAS) is an innovative method for following in-situ the changes occurring in materials while undergoing electrochemical cycling. Normalized XANES (X-ray absorption near structure) spectra recorded at the iron and manganese K-edges during lithium desorption in an operating battery cell revealed that the iron undergoes a change from Fe2+ to Fe3+ upon deinsertion, while there is no discernable change in the oxidation state of manganese (Fig. 3). From these XANES measurements at the B18 beamline, it is clear that the role of the manganese ion is that of a spectator – complicit in the structural transformation to the triplite structure, but itself electrochemically inactive.

It is clear that these kinds of local structure studies shed light on reactions occurring in battery materials and could be useful in determining the mechanisms these compounds undergo during the intercalation process.

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Figure 1: Crystal structure of (a) tavorite characterized by chains of corner-sharing octahedra and (b) triplite, which consists of chains of edge-sharing octahedra.
Soft Condensed Matter Village

Nick Terrill, Village Coordinator

The scientific output of the Soft Condensed Matter village covers a very diverse range of scientific communities: from cultural heritage and archaeology, through biology and chemistry to energy and engineering, and it is also reflected in the range of scientific papers that have been published this year and represented by the articles that appear in this section.

With its final name of MIRIAM (Multimode InfraRed Imaging And Microspectroscopy), the IR beamline B22 completed its optimisation period this year and now joins I22 and B23 in full user operations. Using the Infrared generated by the synchrotron, B22 offers two complete Fourier Transform interferometers (FTIR) coupled to IR microscopes as end stations enabling molecular analysis at microscopic scale. Meanwhile installation and commissioning for B21, HATSAXS (Highly Automated Throughput Small Angle X-ray Scattering) the second SANS beamline for Diamond, is progressing well. The beamline team expects to take first light later in 2012 leading up to first user experiments in the first half of 2013.

Mid-IR ‘fingerprint’ analysis on the B22 MIRIAM beamline has been successfully utilised by more than 20 research groups from the UK and EU in a wide range of fields including mainly the life sciences and biomedical communities, some cultural heritage and archaeology as well as environmental research and materials science.

With particular focus on cancer, histological tissue analysis and microbiology at the single cell level, the IR microprobe on B22 is extremely bright and non-destructive which makes it ideal for the fingerprint analysis of biological systems. In the case of ex vivo cells, spectromicroscopy on B22 allowed researchers to monitor via FTIR the biochemical events within spatially different compartments of an individual living cell at the micron scale without the need for fixing, staining, or labelling. A collaboration between Diamond and Manchester University has led to the development of a microfluidic device for an in vivo study of single cells in an aqueous environment.

Even before MIRIAM began operation, its user working group considered the surrounding facilities and recognised the need for a cell culture lab close to the IR facility to enable live cell work. During 2011, the cell culture lab has been made operational under Containment Level II and it is now available for users while transporting from the laboratory to the IR beamline.

I22, the Circular Dichroism (CD) beamline, which produces high intensity collimated UV beams, enables the measurement of small volumes of sample solutions with high signal-to-noise ratios. I22 had an extremely busy and exciting 2011. It has been used by researchers from biological, biochemical, chemical, pharmaceutical, and crystallographic sciences to examine a range of biological macromolecules and drug complexes. In July, more than 150 delegates from around the world, including leading experts in the field of CD spectroscopy, gathered in Oxford for the 13th International Conference on Chiroptical Spectroscopy (CD2011). Delegates at the conference presented new theoretical and practical advances in the field including: the fundamental forces influencing chiral transitions, the origins of homochirality in the universe, nano and meta-materials and single particle spectroscopy, as well as emerging techniques such as vibrational CD and Raman Optical Activity.

The conference was organised by Prof Giokas Siligardi and Dr Rohanah Hussain from Diamond, together with Dr George Tranter (Chiralab) and Prof Laurence Barron (University of Glasgow). Prof Siligardi was appointed onto the International Scientific Advisory Committee during the meeting, thus ensuring that Diamond continues to be a very active contributor to this important international conference. The B23 team also achieved a major technical milestone in 2011 by delivering a collimated microbeam allowing the detection of protein structures with low concentration of proteins (from 0.5-1ppm) with low volume requirement. This development is now available for research into nanoparticles and particularly understanding nanotoxicology.

B23 continues to develop its science portfolio exemplified by the science contributions presented later in this section. The Moggridge group from Cambridge examined potential alternatives to mechanical or bioprosthetic heart valves on the beamline. Both former types have their associated problems, whereas polymeric heart valves have the potential to overcome these limitations and be a useful alternative in surgery. The examination of the mechanical properties of these polymeric artificial heart valves exemplifies what is possible with the RAPID detector system, uniquely available on I22. While the data described was collected on the millisecond timescale, proof of principle measurements were carried out on the tens of microsecond timescales and provided evidence that this would also be feasible for experiments that could be cycled. Continuing the medical theme, researchers from Cardiff have been looking at the collagen structure contained in the cornea and have some cautionary advice to offer on the use of a hypo-osmolar riboflavin/UVA cross-linking for the treatment of very thin keratoconus corneas.

Biological solution scattering is playing an ever-increasing role on all SANS beamlines. The article by the Brady group from Bristol shows how I22 is being used to provide valuable additional information to structural biology on complicated multi domain macromolecules. At Diamond, we are developing a range of sample delivery systems to meet the needs of this ever growing community, including a BioSAXS robot for automated sample delivery and an online HPLC for aggregating samples. These are described in greater detail later in the technical developments section. Other upgrades to I22 sees a Pilatus 2M now performing routinely as the default SANS detector.
Polymeric materials for application in a novel prosthetic heart valve


Although artificial heart valves have been used for over half a century and some improvements have been made, no significant change in their clinical outcome has been achieved. At present, commercially available heart valves are either mechanical or biological. The main complication of the mechanical valves is thrombogenicity, which is a tendency to coagulation of the blood caused by artificial material. Bioprosthetic valves, usually made from porcine tissue, have limited durability and are prone to calcification. Polymeric heart valves could be an alternative for heart valve replacement, overcoming the limitations of mechanical and bioprosthetic valves. Among other desired characteristics, a potential polymeric material should have good mechanical properties such as tensile strength and durability. These, as we have demonstrated, can be enhanced by ordering the microstructure of phase separated block copolymers such as styrenic elastomers. Long term stability of these properties depends on how the microstructure responds to fast cyclical mechanical stress. The facilities available at the I22 beamline at Diamond Light Source allowed monitoring of microstructural evolution in real time while applying deformation mimicking valve opening and closing in a real heart beat cycle. X-ray measurements in millisecond timescales have been performed to track microstructural changes. The dynamic measurements were used to evaluate the response of materials to mechanical stress and the reversibility of the response over 10,000 cycles.

The heart valves consist of thin flaps of flexible, tough, fibrous tissue firmly attached at the base to the valve rings. The orientation of the cardiac valves is responsible for unidirectional flow of blood by opening and closing during the contractions of the heart. Besides the biometric shape (shown in Fig. 1), and hemocompatibility, a good prosthetic heart valve should demonstrate extended durability. Therefore an important step in the use of new materials for the design of such biomedical devices is the acquisition of basic understanding of their short and long term mechanical and fatigue properties, as well as their performance under realistic conditions of cyclic loading and unloading. These tests aim to identify any hysteretic response in the behaviour of the material while loading and unloading is applied, as well as the maximum number of preconditioning cycles required to obtain steady state properties.

Block copolymers with cylindrical morphology allow molecules to arrange in evenly dispersed fibrous composite materials at the nanoscale. A flexible matrix, reinforced with the rigid one, allows combination of flexibility with strength. Moreover, long range alignment of the cylindrical microstructures result in orthotropic mechanical properties of the material. Such a fibrous structure is present in natural heart valve, where collagen fibres, arranged in a specific orientation, add strength to elastic tissue.

For advanced polymeric nano-composites, which mimic the natural fibrous structure, microstructural arrangement has a significant impact on mechanical performance. Hence dynamic mechanical studies of the materials in real time during deformation are of great importance.

Figure 1: A prototype of polymeric heart valve.

Three cylinder-forming block copolymers were examined: polystyrene-block-polyisobutylene-block-polyisoprene, containing 30% wt styrene (SIS30); polystyrene-block-polyisoprene-block-polyisobutylene containing 18% wt styrene (SIP18); and polystyrene-block-polysobutylene-block-polyisoprene having 30% wt styrene (SBIS30). Samples of oriented microstructure were prepared by compression moulding in a die. Stretching of samples with cylinders oriented both parallel and perpendicular to the direction of loading was carried out for each material.

One heartbeat lasts about 800 ms, valve opening and closure each occurring in ten milliseconds. To mimic the natural loading cycle, the materials were stretched and relaxed in a cycle based on a nominal rate of 67 beats per minute. To achieve such fast loading and unloading, a deformation rate of 200 mm s$^{-1}$ was applied. Under load, the polymer morphology undergoes continuous change during constant strain-rate deformation (Fig. 2). The higher the time resolution of the X-ray pattern recorded, the more accurate information can be obtained about the nanoscale X-ray scattering. On beamline I22 of Diamond Light Source, we were able to collect good quality SAXS data for each material, over one stretching cycle. The microstructural changes exactly followed the macroscopic deformation; the dynamics of macro and micro changes were indistinguishable.

Long term stretching tests provided an effective comparison between polymers, while also representing the scenario of conditions that would be experienced by the polymers in applications such as a heart valve leaflet. Mechanical properties of these polymers are governed not by the direction of stretching with respect to microstructural orientation, and second by the hard and soft segment composition. Microstructural stability over an extended time (10,000 cycles) was evaluated by tracking the amplitude of intensity changes observed between the loaded and unloaded conditions. The inherent stability has also been confirmed by monitoring domain spacing, which in this case is the distance between cylinders. Within the range investigated, the inverse of domain spacing was proportional to the deformation of the sample, consistent with affine deformation. Cyclic stretching over a long period of time does not significantly affect the degree of deformation during each cycle. As shown in Fig. 4, both at the early stage of cyclic deformation, and later after 9000 cycles, the distance between cylinders changed proportionally to the stress.

This study links orientation to mechanical properties, tracking the evolution of the morphology from the initial state as the deformation proceeded, unveiling the nanoscale influences on the long term cyclical strain behaviour. All materials showed good long term stability over 10,000 cycles, the best performance being exhibited by SBS18 (followed by SIS18), with no discernible structural changes per cycle. This is an encouraging result for potential applications such as a prosthetic heart valve, where great structural stability over very long periods of use is of paramount importance.

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Structural implications of a new therapy for corneal disease


The cornea is the precisely curved, transparent window at the front of the eye. It is responsible for over two-thirds of the eye’s refractive power and variations in its shape are detrimental to the quality of the image formed on the retina. As its essential properties are largely governed by a unique arrangement of collagen, abnormalities in the structural organisation of collagen have been implicated in keratoconus (Fig. 1’), a condition characterised by progressive corneal thinning and steepening and severe, irregular astigmatism. Keratoconus is an area of great scientific and economic importance as it affects up to 200 people per 100,000 and is a leading cause of corneal transplant surgery.

Collagen cross-linking therapy, an in vivo technique for increasing corneal stiffness, has recently been deemed an effective therapy for keratoconus1. In this treatment, the cornea is exposed to UVA light for 30-minutes, in the presence of riboflavin. The riboflavin acts as a photosensitizer to encourage the production of collagen cross-links. Furthermore, as an absorber of UVA irradiation, it has the added benefit of helping to prevent damage to the sensitive endothelial cells lining the back of the cornea, as well as deeper ocular structures such as the lens and retina. Despite the clinical success of the technique, little is known about the specific nature of the cross-links that are formed during cross-linking, or about their location (either within collagen fibrils or in the interfibrillar matrix) and, although guidelines exist regarding the safe use of crosslinking, variations of the technique are being continually developed. One such variation is the substitution of the standard iso-osmolar riboflavin solution (containing the dehydrating agent dextran) with a hypo-osmolar riboflavin solution which allows the cornea to swell. This facilitates the treatment of very thin corneas (<0.4 mm) which were previously regarded as unsuitable for cross-linking due to the potential for damage to deeper ocular structures3. The safety of this modification, which increases the thickness of thin corneas by up to 30% to reach the safe threshold for riboflavin/UVA cross-linking, has caused much controversy amongst ophthalmologists and vision scientists, largely because of the effect of this treatment on corneal structural stability.

In order to investigate the effect of hypo-osmolar and iso-osmolar cross-linking on an collagen organisation in the corneal stroma, small-angle X-ray scattering data was collected on beamline I22 from post-mortem donor corneas and two keratoconus corneas (donated following corneal transplantation) before and after cross-linking with either an iso-osmolar or hypo-osmolar solution. As collagen interfibrillar spacing is sensitive to changes in corneal hydration, the water content of the tissue was calculated before and after treatment and the corneas classified as ‘swollen’ (≥76%) or ‘unswollen’ (water content ≤76%) (Table 1).

Table 1: Tissue hydration and collagen parameters measured before (CXL-) and after (CXL+) riboflavin/UVA collagen cross-linking. Average values (+/- SEM) of collagen parameters for all normal (N) and keratoconus (K) corneas were calculated using 3-5 measurements recorded from the central inner region of the corneas before (CXL-) and after cross-linking (CXL+). Table shows iso-osmolar and hypo-osmolar cross-linking as a percentage of CXL-.

Before treatment After treatment

<table>
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<th>Sample</th>
<th>Water content (%)</th>
<th>Fibril Diameter (nm)</th>
<th>Interfibrillar Spacing (nm)</th>
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<tr>
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The corneas were wrapped in low density polyethylene to prevent tissue dehydration and mounted in a sealed Perspex chamber with Mylar windows. X-ray scattering patterns, resulting from a 10s exposure to a 0.1nm wavelength X-ray beam focussed to measure 0.2x0.2 mm at the specimen were collected at 30kV and 22mA with a Pilatus detector system positioned 3m behind the specimen.

Measurements of collagen axial D-period (Table 1) show that riboflavin/UVA induced cross-linking does not have any measurable effect on the axial spacing (Table 1 and Figure 2), due to the dehydrating effect of the dextran within the riboflavin solution. One would expect the ordering to increase as fibrils approach each other and this is consistent with the reported rise in transparency of hen corneas following iso-osmolar riboflavin/UVA collagen cross-linking5. In the keratoconus corneas and the unswollen normal corneas, inter-fibrillar spacing increased significantly after hypo-osmolar riboflavin/UVA treatment (Table 1 and Figure 2).

The use of a hypo-osmolar riboflavin/UVA cross-linking for the treatment of keratoconus and normal corneas may be justified if the change in fibril spacing is at least 10mm. Since the majority of the tissue swelling occurs between fibrils it is foreseeable that this therapy will reduce the effective thickness of the protective riboflavin film. Since the hypo-osmolar solution also has a lower viscosity and lower UVA absorption coefficient than that of the iso-osmolar solution and becomes unstable after only 90 seconds, we suggest that this therapy is used with caution in a clinical setting and that this procedure warrants further scientific investigation.

References

Funding Acknowledgements
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Figure 1: The normal curvature of the cornea (highlighted by a broken white line) is gradually lost in keratoconus (B) resulting in the development of severe, irregular astigmatism.

Figure 2: Changes in collagen interfibrillar spacing (IFS) during riboflavin/UVA collagen cross-linking. The centre maps show collagen interfibrillar spacing (IFS) in an unswollen normal cornea (N1) and a slightly swollen keratoconus cornea (K2) before and after hypo-osmolar riboflavin/UVA cross-linking (Iso-R CXL) and a normal unswollen cornea (N1) before and after iso-osmolar riboflavin/UVA cross-linking (Iso-O CXL). The centre of each corneal button corresponds approximately with the centre of each map.

Table 1: Tissue hydration and collagen parameters measured before (CXL-) and after (CXL+) riboflavin/UVA collagen cross-linking. Average values (+/- SEM) of collagen parameters for all normal (N) and keratoconus (K) corneas were calculated using 3-5 measurements recorded from the central inner region of the corneas before (CXL-) and after cross-linking (CXL+).
Mechanosensitive responses of a bacterial adhesin at the cell surface


Bacteria are the source of countless human diseases. Understanding how bacteria infect humans is crucial to preventing such diseases. Traditional approaches to understanding infection have concentrated on either studies of whole bacteria and their host organisms, or dissection of the molecules present within these cells. In this study a novel method has been developed for bridging these, until now, separate approaches. The target of these studies is the common bacterium, Moraxella catarrhalis, which causes middle ear infections in young children, exacerbates common obstructive pulmonary disease and is frequently a major cause of morbidity in those with heart disease. The Moraxella cell surface is decorated with a forest-like layer formed from multiple copies of a large adhesin protein, termed UspA1. Dominating the bacterial surface, these proteins are the first point of contact with host organisms (humans) and infection proceeds via UspA1 binding specifically to a range of receptors. However, the function of the UspA1 protein is also affected by its dense packing within the cell surface layer, and hence its study has therefore required a unique combination of techniques to probe its workings both as isolated molecules and in situ at the cell surface. This has required the development of new technologies. Diamond Light Source has been central to several of these approaches.

Firstly, electron microscopy was used to show UspA1 proteins (heterodimers of 3–1,000 amino acids) form a densely packed annular layer at the Moraxella surface. At about 800 Å in length, UspA1 provides an extended surface along which a range of receptor binding regions are strewn: extracellular proteins such as fibronectin and laminin bind towards the Moraxella surface. At about 800 Å in length, UspA1 provides more amenable to crystallographic techniques. As there was very limited information of these sites is inaccessible in the densely packed arrangements of UspA1 could be obtained, we instead determined their structures via SAXS studies using the I22 beamline at Diamond Light Source. Distinctive molecular envelopes for each complex were calculated from the scattering data, and models of the complexes could be reliably assembled from the crystal structures of each component (Fig. 2). What was evident in both cases was the fully extended and tightly packed UspA1 structures observed at the bacteria surface did not seem compatible with the angular receptor-ligand pair associations we observed using the isolated molecular fragments. This therefore stressed the importance of measuring UspA1 mechanics directly at the cell surface, and how this might change on exposure to its various receptors.

To achieve this, we exploited the recent development1 of a specialised AFM with a transverse cantilever combined with Total Internal Reflection Fluorescence (TIRF) detection (Fig. 3). The Lateral Molecular Force Microscope (LMFM) differs from more conventional atomic force microscopes in laterally tapping samples (in this case, individual cells) against an extremely fine oscillating lever, rather than fluctuating the lever vertically across the surface of a fixed sample as is more usually the case. Fabrication of extremely thin but stiff cantilevers, together with exceptionally fine motor movements and a specialised visualisation system, has all been combined in the device to tremendous effect. The result has been a machine that can measure exquisitely fine molecular changes and forces in individual molecules directly on a living cell surface without deforming the bacterial membrane. This has permitted measurement of adhesion forces and detection of changes in the thickness of the annular UspA1 layer, and hence the degree of bending of extended molecular structures. Once the technology was perfected, AFM data was obtained that indicated, at the Moraxella cell surface, the adhesion properties and thickness of the UspA11 annular layer decrease significantly in the presence of either the fibronectin or CEACAM1 receptors, but not with a control protein. Adhesion events also decreased after saturation with either receptor, and individual single-molecule-binding events could be observed. The decrease in thickness of the adhesin layer could be measured with sufficient accuracy to correlate these in situ changes with the expected dimensions produced by discrete bending of UspA1 molecules. This receptor-induced distortion of the extended UspA1 molecules could be correlated with the UspA1 model derived from both the crystallographic and SAXS studies. This study therefore provides a rare demonstration of substantial conformational changes in proteins directly at the cell surface, and correlates this with the instigating molecular structures. These molecular changes lead to significant changes in the physical properties of the bacterial cell surface and are believed to relate to its permissiveness for receptor binding. They are therefore integral to the infection process. To date conformational changes in proteins have usually only been observed in isolated structures, not in situ within their native cellular environment. This study demonstrates the feasibility of visualising such changes and may prove equally useful for the study of many other biological processes directly within their natural environment, something that has long been needed in molecular medicine.

References


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Figure 1: Surface view (left) and top view (right) of the UspA1 trimers complex from crystal structures of two fragments. The trimers are intertwined and assembled with highcharge complementarity.

Figure 2: Molecular envelope for the UspA1 head domain-receptor (domains 12-13) complex as determined by SAXS.

Figure 3: Bending of a single UspA1 molecule (left) as detected with TIRF (top right) which measures displacement of the stylus (bottom right, red box deflection corresponds to a single molecular event).
A step towards true quantitative analysis in infrared microscopy: from models to real samples


Accurate detection and classification of materials, such as diseased tissues or illicit substances, is critical and misclassification can sometimes have life threatening consequences. Infrared (IR) absorption spectroscopy has been widely adopted as a simple but powerful characterisation tool, effectively producing a fingerprint of the sample's molecular composition and aiding classification. IR absorption spectroscopy is quantitative and highly sensitive, but some measurement configurations, particularly in microspectroscopy, can suffer from optical artefacts. Misleading results can be obtained if changes in the IR absorption spectrum are not directly associated with chemical changes in the sample. Whether caused by the optical arrangement of the measurement or something intrinsic to the sample morphology itself, it is important to understand, and ideally correct for, any source of variance in the spectrum which is not due to the molecular composition. In this work a severe artefact is addressed which is present for samples on IR reflective slides due to the drop in IR intensity near a metallic surface.

There is a linear relationship between the IR light absorbed and the amount of absorbing material or chemical component in the sampled volume, which is expressed in the well known Beer-Lambert law. Molecular composition can, for example, help determine whether a biological tissue sample is healthy or diseased. In order to correctly and consistently classify samples it is crucial that the quantitative accuracy of the IR absorption spectra is maintained in all measurement configurations, especially as there is no standard measurement protocol.

The most common modes of measuring IR absorption spectra with a microscope are transmission and transflection. In transmission, the quantitative nature of IR spectroscopy is maintained, samples are mounted on a transparent window and the amount of light that passes through the sample is measured. In the transflection geometry, samples are mounted on reflective substrates, the IR light passes through the sample, is reflected off the substrate and passes back out through the sample, doubling the absorption path length. This configuration is popular in the biomedical sciences because it produces the strongest absorption signal for thin transparent samples such as cytology or histology specimens, it works over a broad spectral range and the substrates are relatively inexpensive.

In this work, it is shown that despite its advantages, the transflection measurement geometry induces complex optical effects which destroy the quantitative linear relationship between IR absorption and sample composition in a way which varies strongly with the IR wavelength.

The first investigation used a standard, homogenous material, (Bovine Serum Albumin, BSA) with a well characterised IR absorption spectrum, to determine the relationship between absorbance and sample thickness. Measurement of several samples of BSA gel with different thicknesses in transmission showed the material was indeed homogeneous and that the transmission configuration maintains the Beer-Lambert relationship. In the transflection geometry, however, clear non-linear behaviour is observed.

Transfection spectra for BSA gels of thickness 200-1200 nm are shown in Fig. 1(a) with absorbance normalised to the N-H stretching peak at 3300 cm\(^{-1}\). If the absorbance was linear with thickness in transflection these spectra would all be identical. The non-linearity is illustrated by integrating two spatial regions: the C-H stretching band (Fig. 1 (b), 2830-3010 cm\(^{-1}\)), and the amide I+II bands (Fig. 1 (c), 1480-1760 cm\(^{-1}\)), and plotting them against the sample thickness determined by atomic force microscopy (AFM).

The non-linearity of absorbance with thickness is caused by heterogeneity in the IR intensity at the reflective surface. When light is reflected from a perfect conductor, the reflected wave undergoes a phase shift of -180°, causing the incident and reflected light to interfere with each other. This interference creates a sinusoidal standing wave in the light intensity which drops close to zero at the reflective surface. This spacing between intensity maxima and minima depends on the refractive index of the sample and the wavelength of the light. The dashed lines in Fig. 1 (d) and (e) show the predicted variation of the absorbance with sample thickness based on a new phenomenological model of the electric field standing wave (EFSW):

\[ A(\lambda, g, l) = a_0 \left( \frac{1}{\lambda} \right)^2 \left( \frac{1}{\rho_1} - \frac{1}{\rho_2} \right) x \times \left[ 1 + \left( 1 - \frac{\lambda}{\rho_1} \right) \left( 1 - \frac{\lambda}{\rho_2} \right) \frac{2g}{l} \right] \]

where \( A \) is the absorbance of light of wavelength \( \lambda \) by a sample of thickness \( l \) and refractive index \( n \) in the presence of the EFSW, \( g \) is a scaling parameter for the optical geometry, \( \rho \) is a spectrum of scaling coefficients for the absorption – proportional to the true absorbance coefficients, \( x \) is the distance from the reflective surface and \( R \) is a positive valued interference coefficient at each wavelength which is strongly dependent on the reflectivity of the sample/air interface. The integral term describes how much of the sinusoidal EFSW interacts with the sample and the term in the square brackets describes optical interference effects due to internal reflections.

Having used the BSA standard material to quantify the relationship between absorbance and thickness for a homogenous sample, the results were compared with a heterogeneous biological material, of the kind commonly studied with IR microscopy.

Breast adenocarcinoma cells, MCF-7, were grown on IR transmission and transflection substrates (Fig. 2 (a) and (b)) and analysed using an IR microscope equipped with a Focal Plane Array (FPA) imaging detector. The FPA has 64x64 pixels for simultaneous collection of 4096 IR spectra. The IR absorbance image of six cells was measured, three in transmission and three in transflection (Fig. 2 (b)). AFM topography images (Fig. 2 (c)) were measured to determine the thickness of the cells at each pixel in the FPA image. The C-H stretch and amide I+II bands of the IR spectra were again integrated and plotted against the sample thickness (Fig. 2(d) and (g)). The integrated absorbance values were segregated into 100 nm thickness bins and averaged for plotting. Sample regions over 1 µm thick were rejected from the analysis because the spectra were few in number and corresponded to small, localised regions below the spatial resolution of the IR measurement. Integrated absorbance vs. thickness plots for the cells are shown at the bottom of Fig. 2. The transmission data (Fig. 2(b) and (d)) shows an linear relationship between the sample thickness and absorbance, i.e. it obeys the Beer-Lambert law, demonstrating also that there is a negligible change in component concentration in different cell regions. The cell transflection data (Fig. 2(g) and (j)) shows a very similar trend to the BSA standard transflection data (Fig. 1(b) and (c)), especially the almost quadratic relationship between the absorbance and thickness below 600 nm.

The results of the full analysis of these six cells confirm that the EFSW artefact has a profound effect on the transflection spectra of such materials, and that the main spectral variations can be related to the sample thickness rather than any chemical differences. Understanding to what extent the EFSW artefact has affected the conclusions of previous investigations using the transflection geometry (of which there are many) and whether it can be corrected is the focus of ongoing work.

References

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Since the middle of the 20th century, infrared (IR) spectroscopy coupled to microscopy has been used as a non-destructive, label-free, highly sensitive and specific analytical method to reveal molecular structure. Nowadays, synchrotron based IR microspectroscopy offers a signal-to-noise spectral quality unreachable by other broadband sources, and achieves the highest optically attainable IR spatial resolution on microscopic scale samples. This is particularly relevant in Life Sciences, with a significant progression of applications in biomedical research and in particular cancer studies. In view of the validation of the IR fingerprint region as a spectral marker of cancer and anticancer therapy follow up, we have recently performed a set of key experiments on leukemic blasts at the IR beamline B22 ‘MIRIAM’. This results in identification and cross-validation of IR markers of drug actions in the spectra of K562 leukemic blasts are in the following report.

The underlying principle of IR spectroscopy is that the molecular structure can be revealed by exciting the vibrational modes in materials. The absorption bands in an IR spectrum are thus a fingerprint of the molecular composition. Within the linear range of Lambert-Beer law, the absorbance is a quantitative measure of the molecular species concentration.

Modern IR spectrometers are based on Fourier Transform (FT) interferometer and they are commonly coupled to all-reflector IR microscopes enabling the acquisition of spatially resolved IR information, for instance, in an individual cell. When the microscope aperture defining the IR imaging spot at the sample is reduced to 20 µm or less, the photon throughput towards the detector significantly reduces while the detector noise remains constant, thus the signal to noise ratio (S/N) is strongly decreased. Moreover, when working with microbeams approaching in size the wavelength of mid-IR radiation (above 2.5 to 25 µm wavelength), the diffraction limit becomes dominant in the IR spectral interpretation.

By exploiting Synchrotron Radiation (SR) as an IR source, the brightness of the photon flux density reaching the sample is no more a limitation in the illumination of microscopic sample features. High quality spectra of individual cells with a diameter around 15 µm, which is the average diameter of a granulocyte, were obtained at beamline B22. For reference, samples composed of very homogeneous cell populations were measured and usable spectra with acceptable S/N values were achieved with conventional source from larger areas (e.g. 50 µm), which demonstrated the complementarity of microFT-IR with conventional lab instruments.

The IR spectrum of a cell usually contains a large number of absorption bands, many of them can be confidently assigned to the molecular vibrations of a particular group, in particular analysing the ‘Fingerprint region’. The unequivocal interpretation of pre-assign specific vibrational mode is not straightforward because the common modes of different molecular components within a cell may overlap and the spectrum may reflect only the average biochemical composition. The development of the so-called molecular medicine requires the identification of biomarkers that can be associated to disease-specific molecular pathways. In an attempt to extend the biomedical applications of microFT-IR, we applied mid-IR analysis to compose to very homogeneous cell populations were measured and usable spectra with acceptable S/N values were achieved with conventional source from larger areas (e.g. 50 µm), which demonstrated the complementarity of microFT-IR with conventional lab instruments.

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Analysing functional DNA nanomaterials with SRCD spectroscopy


DNA is, besides displaying its central biological role as bearer of the genetic material, an extremely versatile construction material. DNA has become very attractive for the creation of novel nanosized objects. We are studying the influence of modifications of DNA with the aim to create functional DNA based nano-materials. Our modifications generally consist of large chromophores, which show specific light induced activity such as energy or electron transfer. We are using modifications which are derived from naturally occurring molecules, such as porphyrins. Both DNA and the porphyrins show specific response when measured using circular dichroism (CD) spectroscopy, where the UV part gives insight into the structure of the DNA, and the visible part displays important electronic information on the porphyrin. By making use of the sensitivity of the beamline B23 SRCD spectroscopy station, we have analysed a number of different DNA strands. The CD spectra are also extremely valuable in determining the presence of different metals within the porphyrins; metallation of the porphyrin macrocycle and they can easily be attached to a DNA scaffold by coordination to a metal ion. The electronic difference in the visible range, which arises from both different metal ions and the structure of the DNA, and the visible part displays important electronic information on the porphyrin. By making use of the sensitivity of the beamline B23 SRCD spectroscopy station, we have analysed a number of different DNA strands. The CD spectra are also extremely valuable in determining the presence of different metals within the porphyrins; metallation of the porphyrin macrocycle and they can easily be attached to a DNA scaffold by coordination to a metal ion.

Alongside the porphyrins, transition metal complexes based on terpyridine have become of increasing interest. Terpyridine is a ligand that is covalently attached by a flexible propargyl amide base connection. Molecular modelling shows an even distribution of the propargyl amide bases along the major groove of the DNA (Fig. 1). CD spectroscopy has proven an invaluable tool to determine the structural influence of the newly added group within the DNA. Of particular importance was, in this case, the large electronic difference in the visible range, which arises from different metal ion coordination and different metalation states of the DNA. These features are not easily detectable using standard UV–vis spectroscopy.

Alongside the porphyrins, transition metal complexes based on terpyridine are inherently interesting to study due to their large diversity in both electrochemistry and photophysics. We have recently synthesized the corresponding nuclease-terpy DNA building block and incorporated it site specifically into DNA. To probe their efficiency as metal chelators, we have designed a supramolecular system which makes use of orthogonal binding modes to assemble nano-sized DNA structures (Fig. 3). First, the DNA strands were designed to be in part self-complementary with overhanging ‘sticky ends’. Those sticky ends, which are again complementary to each other, will selectively anneal to form the DNA duplex according to the highly selective and specific base pairing. The result is that the strands, once mixed, will spontaneously form an almost infinitely long DNA strand. Or so we thought. AFM images revealed that the linear terpy-DNA actually folds into well-defined nano-rods of 50 to 100 nm diameter. By strategically placing terpy units into the strands, the metal binding units can now be used to connect different strands, and with it form an extended array of DNA. Again, CD spectroscopy has proven very valuable to follow the assembly event. Furthermore, since the optical window of the chelator is outside that of the DNA, and seems very sensitive to the environment of the structure, it will provide a structural handle for further exploration. However, the combination of the base pairing and selective metal binding leads to the formation of nano-tubes, which are 50-200 nm wide and 2-50 nm high, and have a length of several micrometers. This result can, to a certain extent, be explained by the fact that the terpy units are not positioned at exactly 180° in the DNA duplex, but rather form an angle of about 130°, thus facilitating the formation of tubular structures rather than flat sheets. Nevertheless, the direct formation of DNA nano-arrays through orthogonal self-assembly is still a very promising approach to obtain new functional materials.

The modifications, which we attach to the DNA and successfully characterise using the excellent facilities available at Diamond Light Source, are now under further investigation as optical handles to analyse DNA in more biological environments. As an example, we have designed molecular rulers based on porphyrin-DNA, which allows the analysis of DNA on a nano-meter resolution; this ruler is now being applied to the study of DNA protein interactions.

References

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Peptoids are peptidomimetics, where the side chain is moved from the α-carbon to the adjacent amide nitrogen, generating a tertiary amide. This chemical modification is responsible for desirable chemical, physical and biological properties, e.g. different interactions along the backbone, flexibility, resistance to proteases and higher cell permeability. The presence of tertiary amides in the peptoid backbone results in decreased hydrogen-bonding potential of the peptoid backbone, linear and β-peptoid residues. In our previous work we have shown that, despite the cis/trans isomerism of the amide bond, thus complicating NMR spectra. As a result, interpretation of NMR spectra is generally complicated, and clinical techniques, such as circular dichroism, become essential tools for the conformational investigation of peptoids.

The use of SRCD was essential for the conformational analysis of these novel compounds as the far UV below 200 nm was the most characteristic region and the high signal/noise ratio in this spectral region was essential for the understanding of the data.

Our research has focused on investigation of the conformational preference of novel α,β-peptoids, which are composed of alternating α- and β-peptide residues. In our previous work we have shown that, despite the decreased hydrogen-bonding potential of the peptide backbone, linear and cyclic α,β-peptoids can adopt more than one ordered conformation and their sequence composition as well as to their three dimensional structure. Therefore, when generating synthetic peptidomimetics, their ability to fold into a three dimensional structure (i.e. conformation) needs to be considered and their conformational preference investigated. However, the conformational investigation of peptoids is complicated by their inherent flexibility due to the presence of tertiary amide bonds which are devoid of H-bonding donors. Thus the H-bonding potential of the peptide backbone is significantly reduced. Also, the energy barrier for cis/trans isomerisation of the tertiary amide is decreased relative to the secondary amide in peptides. As a result, interpretation of NMR spectra is generally complicated, and clinical techniques, such as circular dichroism, become essential tools for the conformational investigation of peptoids.

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Our research has focused on investigation of the conformational preference of novel α,β-peptoids, which are composed of alternating α- and β-peptide residues. In our previous work we have shown that, despite the decreased hydrogen-bonding potential of the peptide backbone, linear and cyclic α,β-peptoids can adopt more than one ordered conformation and their sequence composition as well as to their three dimensional structure. Therefore, when generating synthetic peptidomimetics, their ability to fold into a three dimensional structure (i.e. conformation) needs to be considered and their conformational preference investigated. However, the conformational investigation of peptoids is complicated by their inherent flexibility due to the presence of tertiary amide bonds which are devoid of H-bonding donors. Thus the H-bonding potential of the peptide backbone is significantly reduced. Also, the energy barrier for cis/trans isomerisation of the tertiary amide is decreased relative to the secondary amide in peptides. As a result, interpretation of NMR spectra is generally complicated, and clinical techniques, such as circular dichroism, become essential tools for the conformational investigation of peptoids.
Innovative methods for protein-nanoparticles interactions using synchrotron radiation circular dichroism


Nanoparticles (NP) are used in different applications such as cosmetics and medicine. To assess potential toxic effects and to design NP-based drug delivery systems it is critical to understand what happens to proteins upon interaction with these special particles. This information is difficult to obtain, but for the first time we have shown that using the B23 beamline, it is possible to detect and analyze structural changes of proteins in protein-metallic nanoparticle complexes. By using synchrotron radiation circular dichroism (SRCD), we have measured the structure and stability changes of proteins upon their interaction with nanoparticles at nanomolar concentration. In particular, we measured a decrease of 6°C in the thermal unfolding of human serum albumin upon interaction with silver nanoparticles. This effect does not emerge with gold nanoparticles. SRCD allows the measurement of critical parameters for protein-nanoparticle interactions by using only a few micrograms of proteins, providing the relative stability of key proteins. This information will help understanding and predicting the potential toxicity of nanomaterials. In addition it may contribute to the design of the next generation of non-toxic nanoparticle-based drug delivery systems.

Figure 1: CD spectra of HSA 20nM (black line) and HSA 20nM+AgNP (red line).

Nanotechnology is gaining more and more interest in many industrial activity fields. Nanoparticles are already extensively used in widely different applications such as cosmetic sunscreens, in diagnostics and in medicine as drug delivery systems. When nanoparticles enter a biological system, they become coated with a complex mixture of proteins (the so-called protein corona) and this interaction can both alter the properties of the nanoparticles and of the interacting proteins. In particular it has been shown that this interaction can change enzymatic activity, alter protein conformation, expose previously hidden epitopes, and all these alterations can induce unexpected biological reactions and lead to toxicity. In order to investigate possible toxic effects of nanoparticles and to design the next generation of delivery systems, it is essential to understand what happens to the structure and stability of relevant proteins upon interaction with it. Unfortunately, this kind of information is very difficult to obtain due to the very complex nature of the system (solid/liquid interface) and the experimental constraints, usually the amount of protein-NP is very low.

Circular dichroism (CD) is an excellent and sensitive method for rapidly evaluating the secondary structure, folding and binding properties of proteins, and recently it has also been used to detect structural changes of proteins interacting with nanoparticles. The use of synchrotron radiation to perform CD experiments presents several advantages with respect to the conventional CD technique. The major advantage is the high flux provided by synchrotron radiation that allows CD data to be measured both with very low amounts of proteins and in the presence of highly absorbing chemicals such as suspensions of gold and silver nanoparticles. It enables the detection of the secondary structure of proteins with only a few hundred nanograms in the spectral range of 195-250 nm.

Using beamline B23 at Diamond Light Source we have shown that, using a 10 cm cell with total volume under 0.8 ml, it is possible to detect and analyze structural changes of proteins in the low nanomolar concentration range when forming stable non-covalent protein-metallic nanoparticle complexes. In particular, we measured the secondary structure of human serum albumin (HSA) in a well-defined stoichiometric complex with silver (AgNP) and gold (AuNP) nanoparticles at nanomolar concentrations. An example of these studies is illustrated in Fig. 1, where the SRCD data of the HSA-AgNP system are reported.

In addition we have been also able to follow the changes in the thermal stability of human serum albumin when complexed to metallic nanoparticles. Fig. 2 shows the thermal unfolding of free HSA and the fitting of the data used to calculate the melting temperature (Tm) of the protein. By collecting the CD spectra of the protein at variable temperatures between 20°C and 90°C, and fitting the data of the protein unfolding with a sigmoidal equation, we could calculate a melting temperature of 75.1°C for the free HSA protein.

By repeating this experiment with HSA-AuNP and HSA-AgNP we could calculate the melting temperature of the human serum albumin protein when interacting with AuNP (Tm = 74.8°C) and with AgNP (Tm = 69.1°C). The comparison of the unfolding of HSA in the three conditions (alone, -AgNP, -AuNP), partially shown in Fig. 2, indicates that upon interaction with silver nanoparticles the protein changes its melting temperature from 75.1°C to 69.1°C. This decrease of 6°C in the melting temperature indicates that upon interaction with AgNP the serum albumin significantly reduces its thermal stability. On the other hand a similar destabilisation is not observed in the case of HSA-AuNP complexes, which have a melting temperature of 74.8°C, experimentally indistinguishable from the Tm value for the protein alone.

Our work shows that by using synchrotron radiation circular dichroism, it is possible to analyse the secondary structure and stability of proteins in the low nanomolar concentration range, thus providing a unique method for detecting the relative stability of key biological proteins interacting with nanoparticles. In particular, this extreme sensitivity has allowed us to show that human serum albumin is significantly destabilised when interacting with silver nanoparticles, while its stability is not affected when interacting with gold nanoparticles. The high sensitivity provides structural information on protein-nanoparticles complexes at near equimolar ratios and allows access to detailed information that has been very difficult to obtain until now.

References

DOI 10.1021/nl202099s
Technical Developments

BioSAXS at Diamond

BioSAXS, solution scattering from proteins and other macromolecules, is an important and growing aspect of Small Angle X-ray Scattering (SAXS) worldwide. Based on the needs of the user community, Diamond has invested in state of the art delivery systems. The technologies described in this article are either available at Diamond on I22 now or will be available from October 2012 onwards.

Development of a micro-droplet flow system for BioSAXS

The development of a micro-droplet flow system for BioSAXS has been the subject of a collaboration between Diamond Scattering Group and University of Reading started in 2009. Essentially, the micro-droplet flow system uses a capillary cell which is connected to a syringe pump via fluorinated tubing and is capable of delivering microlitre volume droplets. Full automation of droplet flow and data collection has been achieved by exploiting the significant change in transmission between air and water-based sample droplets. It was shown that a minimum sample volume of 5µl was sufficient to measure a useful scattering pattern (Fig 1). Another advantage of this system is its simplicity of setting up and therefore this system would also suit the high throughput concept. After commissioning with a range of standard proteins, this system was successfully used to study a range of challenging biological systems. One of the most successful experiments was carried out in collaboration with Dr. Waterton (University of Reading) to investigate a single monomer of a plasma membrane protein.

Solution SAXS experiments with inline HPLC system

High-performance liquid chromatography (HPLC) is a chromatographic technique used to separate a mixture of compounds in analytical chemistry and biochemistry with the purpose of identifying, quantifying and purifying the individual components of the mixture. A specialised online sample environment for solution SAXS experiments, combining a commercially available HPLC system (Agilent 1200 Series) with a specially designed capillary sample cell has been built and commissioned for I22. Between the separating column and the end fraction collector, the separated components of the solution pass through a capillary cell, allowing SAXS measurements to be made. During commissioning a range of cells were tested. Developed through the collaboration between Reading University and Diamond, a shared design for the HPLC and micro-droplet cell has been established with appropriate adaptors for each sample environment (Fig 3). The flow cell consists of a 0.2mm-thick capillary, consequently giving a higher signal to noise ratio for BioSAXS samples. Test experiments with standard protein samples, such as lysozyme, bovine serum albumin and ovalbumin, have confirmed that the HPLC system works well. Moreover, a trial experiment with the size exclusion column was carried out on E. coli chlorophyll, GeSi, successfully obtaining SAXS data (Fig 4). It has been established that approximately 100 µl of 10 mg/ml will be needed to get optimal data for further analysis from this system. It should be noted that a whole concentration series SAXS data can be obtained from just one injection with these conditions. This principle will allow reduced total quantities of protein to be used and as a result will fit the high throughput concept for the systems. We expect to offer the HPLC system for users after some final commissioning experiments on the in situ separation of protein mixture samples in April 2011.

BioSAXS robot

The EML-Grenoble designed BIOMAX robot, now used at both the ESRF and PETRA III (DESY), was purchased to promote high-throughput studies in the BioSAXS field and is now in final manufacture by MAXIEM. The basic concept of the BioSAXS robot is the same as the micro-deep apparatus described above, but additionally combines a temperature-controlled auto-sampler connecting with the high performance pump system (Fig 5). Protein samples which are located on a 96-well plate in the auto-sampler can be injected via a hypodermic needle into the flow cell to allow SAXS measurements. All experimental sequences including SAXS data acquisition can be performed automatically. Moreover, the robot is capable of a mixing procedure to make different concentrations of protein solutions, thus concentration series experiments which are essential for solution SAXS studies can be carried out in a fully automated manner. The robot has a capability to perform with a minimum sample volume of 5µl at a maximum rate of 20 measurements an hour. The robot will be delivered to Diamond in mid June 2012 and is planned to be commissioned on I22 before its ultimate home, B21, is available. We aim to ensure all sample environments described are compatible across the two 96K SAXS beamlines at Diamond.

Beamline B22 – Overview of the IR beamline

The design of IR beamline B22 (IRAM) allows two separate end stations to be operational from the same beamline mirror optics and front end by using, respectively, the bending and edge synchrotron radiation available from the B22 bending magnet source. A unique feature of B22, with respect to other synchrotron IR beamlines worldwide, is its in-vacuum environment along the complete length of the beam path. The two end stations can be operated independently and, by a detector upgrade also implemented in 2011, they are both capable of point-by-point IR microspectroscopy and mapping via single detector (scanning mode), and IR imaging by a parallaxed Focal Plane Array detector (full-field mode). This last mode is still a novelty among the set up found routinely at other facilities, especially in combination with the 7K4 magnification objective/condenser at the microscope.

Preliminary tests are in progress on a microfluidic device for in vivo experiments of single cell in aqueous environment in collaboration between B22 staff and Manchester University. The beamline B22 was conceived as a research tool to perform IR absorption microscopy and spectroscopy at the maximum spatial resolution, highest sensitivity and signal to noise ratio attainable.

References

The broadband far-IR coherent synchrotron radiation from beamline B22 in low α-mode: a new scientific tool for experiments in the THz gap at Diamond


The beamline B22 at Diamond is a Multimode Infrared (IR) Imaging And Microspectroscopy beamline known as MIRIAM. This beamline has been operational since late 2009 for diffraction limited microscopy and imaging in the mid-IR range. Since its design, the broadest IR regime was also foreseen for general Fourier Transform IR (FTIR) spectroscopy. This beamline has been operational since late 2009 for diffraction limited microscopy and imaging in the mid-IR range. Since its design, the broadest IR regime was also foreseen for general Fourier Transform IR (FTIR) spectroscopy.

The scientist driver for using far-IR radiation in the millimeter wavelength range, also known as the ‘Terahertz gap’, has grown with the technical availability of tunable or broadband intense THz sources. In particular, access to the Terahertz gap allows the study of folding/twisting of large molecules or biological complexes, DNA inter- and intra-molecular modes enzyme activity, conformational changes as well as hydrogen tunneling. In condensed matter physics straightforward applications concern far-IR optoelectronic/photonics devices, including new IR detector materials, superconductor characterization, inter-/intra-band transitions in structured semiconductors, phonon band observation in solid IR detector materials, superconductor characterization, inter-/intra-band transitions in structured semiconductors, phonon band observation in solid IR detector materials, superconductor characterization, inter-/intra-band transitions in structured semiconductors, phonon band observation in solid...
Engineering and Environment Village

Chiu Tang, Village Coordinator

In the Engineering and Environment Village, we now have three operational beamlines: I15 Extreme Conditions, I11 High Resolution Powder Diffraction and I12 Joint Engineering, Environmental and Processing, JEEP. The first two beamlines have maintained their operational effectiveness, supporting thousands of user hours and performing many routine experiments, as well as challenging experiments. The operational time (user beamtime) of our newest beamline, I12 has increased in the reporting year, offering multiple X-ray techniques such as imaging, tomography, single crystal and powder diffraction and small angle scattering (SAXS). The new second experimental hutch in the external building is now fully operational, supporting sample environments up to 2 tonnes in weight, using similar techniques to those already available in the first experimental hutch. With this additional facility, high energy SAXS experiments with a sample-detector distance of 30 m are now possible. As all the beamlines’ hardware and software have been improved, their performance and reliability have also increased. In addition, new instrumental developments or upgrades have further enhanced their capabilities. As a consequence, our users, together with beamline staff, have continued to publish many papers from the results using these facilities. The articles here are representative contributions reflecting the interesting work performed in this village. We also have contributions describing the commissioning of recent upgrades.

The high pressure capability of I15 using high-energy angular-dispersive diffraction has continued to produce interesting new science. The common theme of the user contributions is structural changes under strong compression, achieved using small volume diamond anvil cells. The high pressure (HP) structural behaviour of mineral biotite (KbiO3) was studied by David Santos-Andrés and co-workers. Although the mineral is of great interest for Earth and material sciences, the behaviour under compression was poorly understood before this study. The research group have therefore accurately measured the structural sequence and compressibility of the mineral from ambient to high pressure. The effect of pressure on biomass atomic displacement in pyrochlore structured materials at non-ambient temperatures has been investigated by Andrew Hector and his colleagues. They examined the interaction between Bi atoms that are responsible for structural changes in the crystal lattices of Bi–Ti and Bi–Sn oxide pyrochlores. Complex melting behaviours under compression are commonly observed in alkali metals. Considering the similarities in HP behaviour of potassium with that of the other alkali metals, its apparent simple or normal melting curve was poorly understood. Before this study, the research group have therefore re-measured the melting curve of K using I15 and the results presented are very different from previously reported. They conclude that the complex behaviour closely resembles that of Na, and is related to structural and electronic changes in the solid/liquid phase.

The high resolution (HR) mode of beamline I11 using multi-analyzing crystals (MAC detectors) has continued to work reliably and produced the bulk of the publications by our users. Andrew Cooper and his research group contributed the latest research on microporous crystalline cages, which are promising framework materials for gas storage, carbon capture and sequestration. The new porous crystals they have synthesised show exceptionally high gas uptakes for this class of material. Another research group (Alvaro Mayoral et al.) also studied a microporous solid (Ag-zeolite A) using the I11 HR capability. Complementary results were obtained from atomic resolution electron microscopy, and the detailed crystalline structure of the zeolite was described, including the locations of catalytic Ag atoms in the framework. With high quality powder diffraction data obtained from multilayer thin films at non-ambient temperatures, Philip Lightfoot describes how his team have observed unexpected and complex phase behaviour in these materials. The new results could help with the interpretation of the electrical and magnetic behaviour reported in the literature.

The contributions from the JEEP beamline I12 show the versatility of this instrument, which uses high energy X-rays (50-150 keV). Firstly, the mapping of the orientation of crystalline grains and strain in thick (several mm) engineering samples were performed using transmission Laue micro-beam diffraction. Felix Hofmann, his colleagues and beamline staff have developed two novel setups for the mapping of microstructure, lattice orientation and elastic strain in individual grains of polycrystalline engineering materials. The other two contributions used in situ energy dispersive powder diffraction with two different sample environments. Using a specifically designed electrowinning cell and furnace, Matthew Rowles and co-workers studied the structural and chemical changes of an inert-electrolysis anode at a high temperature. As molten-salt electrolysis is used extensively in the production of light metals, an inert probe, if operated reliably, is more cost effective and has a much lower environmental impact compared with a conventional reactive carbon anode. The final research group (Gareth Williams et al.) studied the intercalation chemistry of the layered double hydroxide, AL(OH)_3, using an in-situ reaction cell. The results obtained have revealed the interesting insights into the reaction mechanisms.
Atomic resolution analysis of silver ion-exchanged zeolite A


Electron microscopy is currently one of the most powerful techniques for the characterisation of inorganic solids, which unfortunately presents some difficulties with respect to zeolites due to their strong beam sensitivity. By controlling the beam current, we have successfully managed to image the most beam sensitive zeolite: zeolite A (LTA structure type) and capture the distribution of silver within the cages, observing directly for the first time silver octahedra composed of 6 atoms. These results were compared to those obtained through Rietveld refinement of the structure against powder synchrotron X-ray diffraction data collected on I11, corroborating the formation of such species.

Zeolites are microporous silicate solids first noted by a Swedish mineralogist, Baron Axel Fredrik Cronstedt, in 1756, who discovered examples in Iceland and in a mine in the north of Sweden. The first zeolite synthesis was carried out in 1940 with Barret’s synthesis of montmorillonite. Industrial applications became apparent in the 1950s with zeolites finding usage as dryers for gases and as molecular sieves. In 1962, zeolite X was introduced as a cracking catalyst. About ten years later, zeolite Y started to replace phosphates in detergents.

Zeolites have a pore size below 20 Å and their structure is based on infinitely extending three-dimensional, four-connected frameworks of 4Al and 5Si tetrahedra, linked to each other by sharing oxygens. The combination of these tetrahedra can lead to at least 176 different zeolitic types. As a result they can be employed as desiccants, molecular sieves (water/air treatment) and more recently as bifunctional catalysts carrying a catalytically active metal.

In the present work we report a detailed study, performed on silver ion-exchanged zeolite A (LTA structure type) with \( \text{Si/Al} = 1 \). The characterization was done by means of aberration (Cs) corrected scanning transmission electron microscopy (STEM) in combination with high resolution powder XRD.

Sodium zeolite A (NaA) was prepared using a verified standard synthesis procedure, i.e. exchanging Na+ with Li+ with no silver in the structure and b) with silver atoms displayed in grey. Sodium zeolite A (NaA) was exchanged with Ag+ ions and then dehydrated. Rietveld refinement against powder synchrotron XRD data was performed to determine the atomic parameters of the dehydrated silver-exchanged zeolite A, followed by silver ion exchange and complete dehydration.

Al and only 4 or 9 atomic columns corresponding to Ag are observed (Fig. 3a). In the current case, the zeolitic framework is virtually invisible due to the high difference between Ag and Si/Al. The experimentally measured distances between Ag are in good agreement with the values obtained by powder XRD refinement, respectively, 4.72 Å and 4.76 Å between the centers of the cages and 7.41 Å and 7.57 Å between two adjacent cages (Fig. 3b and 3c). The agreement of comparing the nature of the species observed in the sodalite cages, intensity profile analyses were performed over the atomic columns marked 1 to 6. The intensity profile observed was, in this case, consistent with an octahedral surrounded by 8 Ag cations. The atomic positions obtained from the Rietveld refinement were used for theoretical simulations (Fig. 3f), which also showed good agreement, with respect to atomic positions and intensities, with the experimental data.

In conclusion, the present results demonstrate the ability of aberration-corrected STEM–HAADF to characterize, at atomic resolution, zeolite frameworks with different metal cations incorporated in the structure. With these results, we have been able to image for the first time thesilver atom distribution in dehydrated silver-exchanged zeolite A, which was first described by Kim and Seif in 1977. Two crystal structures of partially decompensated vacancy-dehydrated fully silver(1) ion-exchanged zeolite (X) were obtained, one in the center of a 6-ring. Many important bifunctional catalysts consist of noble metals incorporated into the zeolite structure, and this work, represents a considerable step forward in the characterisation of these and other solids, as we have demonstrated the feasibility of imaging even the most beam-sensitive materials at a resolution as high as one single atom.

Table 1: Refined atomic parameters obtained from Rietveld analysis of dehydrated silver zeolite A.

<table>
<thead>
<tr>
<th>Atom</th>
<th>Wyckoff position</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Occupancy</th>
<th>B</th>
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<tr>
<td>Si (1)</td>
<td>96</td>
<td>0</td>
<td>0.0919 (3)</td>
<td>0.1841 (3)</td>
<td>1</td>
<td>0.70 (4)</td>
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<tr>
<td>Al (1)</td>
<td>96</td>
<td>0.1853 (3)</td>
<td>0.0914 (3)</td>
<td>1</td>
<td>0.70 (4)</td>
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<tr>
<td>O (1)</td>
<td>96</td>
<td>0.1109 (2)</td>
<td>0.2079 (7)</td>
<td>1</td>
<td>1.38 (9)</td>
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<tr>
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<td>1</td>
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<tr>
<td>O (1)</td>
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<td>Ag (1)</td>
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<td>Ag (3)</td>
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<td>0.1692</td>
<td>2.90 (4)</td>
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Using high-resolution powder diffraction to unravel the unexpected phase behaviour of multiferroic fluorides

Resinger, S. A., Leblanc, M., Mercenier, A.-M., Tang, C. C., Parker, J. E., Morrison, F. D. and Lightfoot, P. Phase separation and phase transitions in multiferroic K$_{0.58}$FeF$_3$, with the tetragonal tungsten bronze structure, Chem. Mater. 23, 5440-5445 (2011)

Multiferroic materials display two or more ‘ferroic’ properties simultaneously, usually ferroelectric and ferromagnetic ordering. Such materials may form the basis for future information storage devices. However they are rare, since the two ordering phenomena are often intrinsically mutually exclusive. Understanding of these materials relies on detailed knowledge of crystal structure and its relationship to the two physical properties. In this experiment, the high resolution available on beamline I11 has been used to reveal an unexpected and complex phase separation phenomenon in the multiferroic fluoride K$_{0.58}$FeF$_3$. At ambient temperature, the as-made material is shown to consist of a mixture of two very similar phases, one tetragonal and one orthorhombic. Heating above 340 K causes the phase separation to disappear, but this returns on cooling and persists below room temperature, whence the tetragonal phase transforms to a second, distinct orthorhombic phase. The two orthorhombic phases continue to co-exist down to at least 125 K. This previously unobserved behaviour will help in interpretation of the structural, electrical and magnetic properties reported by other workers.

During the recent resurgence of interest in this field, the vast majority of multiferries studied have been oxides. Amongst the non-oxides which have been studied, the mixed metal fluorides based on the ‘tetragonal tungsten bronze’ (TTB) structure (Fig. 1) have been some of the most promising. Compositions of the type K$_{0.6-x}$FeF$_x$ have been well studied, but there have been conflicting reports of their crystallographic nature, as well as their electrical and magnetic behaviour. Previous structural studies have generally been carried out on either single crystals or by electron microscopy, both of which may suffer from seeing only an unrepresentative portion of a bulk, ceramic sample. The aim of our study was therefore to exploit the advantages of powder diffraction in this respect, to look at the whole sample, and also to ensure that key crystalline sublattices could be seen by obtaining the highest resolution possible, on beamline I11.

The beamline was used in high-resolution mode (MAC detectors) and data were collected at 34 temperatures between 100 and 500 K, each data collection lasting 30 minutes. At ambient temperature a good Rietveld fit could be obtained using the standard tetragonal TTB aristotype model (P4/mmm, a $\approx$ 12.68 Å, c $\approx$ 3.98 Å). However, on closer inspection, this model can be seen to be inadequate (Fig. 2). Subtle shoulders on certain peaks cannot be explained by a simple symmetry-lowering of the tetragonal structure, and are in fact best modelled by invoking a phase separation, with ca. 10% of a second, very similar but orthorhombic phase with a $\approx$ 12.66 Å, $b \approx$ 12.70 Å co-existing with the parent phase. On further cooling below 300 K the tetragonal phase transforms to a second type of orthorhombic phase (this one having an enlarged unit cell, at least $\sqrt{2} \times \sqrt{2}$ bigger than the aristotype). This is evidenced by splitting of a different set of Bragg peaks, as shown in Fig. 3. These two distinct orthorhombic phases continue to co-exist down to the lowest temperatures studied, as summarised by the lattice parameter trends presented in Fig 4. Such subtle features cannot be seen on a conventional lab-based powder diffractometer.

The previously reported ferroelectric $T_c$ for this composition is around 490 K, whereas the ferromagnetic ordering temperature is $\approx$ 120 K. The significance of our work lies especially, therefore, in the interpretation of previous magnetic data, where single phase behaviour has previously been assumed, despite more than one ‘magnetic’ event apparently occurring below 125 K, in some studies.

We also carried out a follow-up experiment on a closely-related composition with somewhat lower potassium content, again we find a systematic and reproducible phase separation phenomena, this time of a slightly different nature. It seems that competing instabilities in these systems lead to intrinsic phase separation. From this study it is not possible to pinpoint the precise difference between the two co-existing phases, or indeed the reasons behind the phase separation, so subtle are the details we observe. Only approximate structural models can be used, due to the severe overlap of inequivalent Bragg peaks, although the differences in unit cell metrics can be clearly seen, and refine robustly. The phase separation may be due, inter alia, to a miscibility gap in potassium content, competing Fe$^{3+}$ / Fe$^{2+}$ charge-ordering preferences, or competing ‘tetrahedral’ tilt’ instabilities. These possibilities require further study using neutron diffraction and other techniques; nevertheless, this study provides a clear new insight into the structural behaviour of these materials, which may have important repercussions in the interpretation of their electrical and magnetic properties.

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Porous organic cages: gas trapping in modular crystals


Porous materials are useful for catalysis and separations, and have gained recent prominence in technologies such as hydrogen storage and carbon capture and storage (CCS). Beside the well-known inorganic examples, such as zeolites or metal oxides, new materials comprised entirely of light, abundant atoms offer to extend the range of properties and applications. Organic polymer networks, in particular, allow broad control over composition because of the wide range of chemistry that is available. However, porous polymer networks have some limitations: for example, they cannot dissolve and are hence not solution processable. It is therefore desirable to synthesise porous molecular solids which combine inherent porosity with the processability and modularity associated with single molecules. In this contribution, we present functional porous materials and the underlying design-principles for their assembly from inherently porous organic cages, along with the potential advantages of these self-assembled, extended solids. The synthetic strategies for organic cages and their assembly into crystalline porous materials are relevant to several challenges in research, such as the generation of free volume in solids via 'badly packing' molecular building blocks, and this gives rise to new tools for the chemist to rationally design adsorptive surfaces.

Recently, microporous and mesoporous organic polymers' and covalent organic frameworks (COFs), in particular, have extended the range of possible materials and applications for porous, crystalline organic materials because their constituent building blocks can be readily diversified using organic synthesis. The surface area in such networks is generated typically via templating or scaffolding approaches: as such, the porosity is a consequence of the solid-state packing, or enforced by the binding motif of the respective building blocks, and is 'intrinsic' in nature—that is, between the building blocks. Other organic materials exhibit 'intrinsically' porosity that results from the shape of the isolated molecules themselves, for example in the form of synthetically prefabricated pores, cavities, or windows. Examples of such 'porous molecules' include calixarenes and a range of other small organic synthetically prefabricated pores, cavities, or windows. These 'intrinsically' porous molecules with shape-persistent covalent cavities. In this context, cage-like 'porous molecules' include calixarenes and a range of other small organic synthetically prefabricated pores, cavities, or windows. Examples of such shape of the isolated molecules themselves, for example in the form of state packing, or enforced by the binding-motif of the respective building chemistry that is available. However, porous polymer networks have some limitations: for example, they cannot dissolve and are hence not solution processable. It is therefore desirable to synthesise porous molecular solids which combine inherent porosity with the processability and modularity associated with single molecules. In this contribution, we present functional porous materials and the underlying design-principles for their assembly from inherently porous organic cages, along with the potential advantages of these self-assembled, extended solids. The synthetic strategies for organic cages and their assembly into crystalline porous materials are relevant to several challenges in research, such as the generation of free volume in solids via 'badly packing' molecular building blocks, and this gives rise to new tools for the chemist to rationally design adsorptive surfaces.

![Image](image.png)

**Figure 1:** Building blocks for porous organic crystals. Porous organic cages, 3, 8, and 9 are obtained via (4+6) Schiff-base condensation of 1,3,5-triformylbenzene (yellow box) with L-2-ethylenediamine (II), L-2-diaminocyclohexane (III), and L-2,2'-diaminobiphenyl (aliphatic box), respectively. Accessible surface area (as seen by a BET sorptometer) vary according to packing mode and bulkiness of cage vertex functionalities.

For one of these extrinsic pore channels, aryl groups (coloured green and yellow in Figure 3) from three neighboring cage stacks engage in a pattern of cooperative C-H ••• F bonds (Fig. 3. E and F). This pattern has been observed previously—and exclusively—for small organos-fluoranes, and has been defined as a rare supramolecular synthons of fluorobenzene. The other set of 1-D channels in cage 10, by contrast, show no sign of the influence of anisotropic electrostatic interactions. Here, the aryl units (marked red in Fig. 3. G and H) are sufficiently flat apart to give rise to a helical 1-D pore channel. Interestingly, both the hydrogen bonded and the π-stacked aryl red and green in Fig. 3. B and C assume orientations close to or identical to the most favourable packing observed for smaller, 'free' molecular entities,4,5 thus enhancing the pore volume—two cages, cage 9 and cage 10, were synthesised by cyclodimerisation of 1,3,5-triformylbenzene with (V)-2,2'-biphenyl (2-formylphenothane/1,2- diimide, respectively, in dichloromethane using trifluoroacetic acid as a catalyst. Two different polymorphs are observed for the resulting desolvated cage 9 (space groups R3 and P3), while cage 10 forms an R22(1) polymorph only (Fig. 2). These crystallographic differences have an immediate impact on the quantities of gas that the desolvated molecular solids can physabsorb. The Langmuir surface areas calculated from the Type I nitrogen sorption isotherms were 952 m² g⁻¹ (SA₉ = 854 m² g⁻¹) for cage 9 (P), 575 m² g⁻¹ (SA₁₀ = 501 m² g⁻¹) for cage 9 (R1), and 533 m² g⁻¹ (SA₁₀ = 460 m² g⁻¹) for cage 10.

The apparent drop-off in accessible surface area for cage 10 can be rationalised by closer inspection of the crystal structure in this molecular solid. Unlike the 1-D gas transport channels in cage 9 polymorphs, the extrinsic pore channels in cage 10 vary by the presence of hydrogen bonds.

**Figure 2:** Connolly surface plots for cage 9 (R3) (A), cage 9 (P3) (D), and cage 10 (G) with probe radius of 1.4 Å (blue) for the crystal structures for the desolvated molecular solids shown along the c-axes with (left) and without (right) the cage framework. Schematic of cage-cage packing in the molecular solid is determined by C-H ••• F interaction for desolvated cage 9 (R1), cage 9 (P), and cage 10 (shown top down, E, F, and C, G, and D, and B, respectively). Carbon, nitrogen, hydrogens and fluorine atoms are colored blue, white, red, and yellow, respectively. Nearest aryl groups with the same orientation with respect to the cage unit are indicated in the same color, and surrounding red, yellow and purple and yellow and green forming a set of neighboring aryl groups in the same cage.

**Figure 3:** Scheme illustrating solid-state packing for cage 10. Arrows with the same orientation with respects to the cage unit are shown to guide the eye, as in Figure 2. Arrows shown (B), the red and green arrows to represent cronic cage-cage red, respectively, in a window in molecular interactions that formly displayed from the orientation, between cage conoery and other cage—cage axes, C—C bonds are marked in dashed green. Top-down (G) and perspective (H) view of the helical 1-D intrinsic pore channel in cage 10, spanning vertical aryl groups.
New insights into the intercalation chemistry of Al(OH)$_3$


Aluminium hydroxide, Al(OH)$_3$, is a lamellar material with octahedral vacancies in its layers. It has long been known that it can ‘imbibe’ LiX salts to form layered double hydroxides (LDHs) of the form [LiAl(OH)$_6$X·H$_2$O], where X is a generic anion. LDHs are important ion-exchangers, with applications in catalysis, biomedicine, and polymer science. Although size considerations should not be other metal ions to be in the octahedral vacancies in Al(OH)$_3$? is challenging, and was not reported until 2004 when Zn$^2+$, Cu$^{2+}$, Ni$^{2+}$ and Co$^{2+}$ nitrates were successfully intercalated to give novel [MAl(OH)$_3$(NO$_3$)$_y$·yH$_2$O] LDHs. In this work, we report recent developments in the intercalation chemistry of Al(OH)$_3$.

These include a detailed structural study, the synthesis of new LDHs containing mixtures of M$^{2+}$ cations, and a comprehensive in situ study of their intercalation reactions. Through use of the high-quality energy-dispersive diffraction capabilities of beamline I12, we were able to continuously record diffraction patterns in real-time as reactions proceeded, and from these data derive information on the reaction mechanisms. These results are crucially important in our quest to understand more about how solid state and heterogeneous solid/liquid reactions proceed.

Layered double hydroxides are a widely studied class of materials comprising positively charged layers, with charge-balancing anions located in the interlayer space. They have the generic formula [M$^2+$M$'^{2+}$Al$_8$(OH)$_{24}$]X$^{n-}$, where M$^2+$, M$'^{2+}$, Al$^{3+}$ and X$^{n-}$ are the divalent cations, trivalent cations and anions, respectively. LDHs have a range of potential applications, including in catalysis, biomedicine, and polymer science. They display a diverse ion-exchange intercalation chemistry, in which the initial interlayer anion is replaced by another. Generally, LDHs contain M$^2+$ and M$'^{2+}$ metal ions, with the M$^2+$M$'^{2+}$ ratio in the range 1–1.5. The LDH [LiAl(OH)$_3$(NO$_3$)$_y$·yH$_2$O] is prepared through the mixing of LiX salts with Al(OH)$_3$, at around 90 °C, and is the only LDH containing monos- and trivalent metal cations. Although Li$^+$ is similar in size to Zn$^{2+}$, Cu$^{2+}$, Ni$^{2+}$ and Co$^{2+}$, it is much harder to intercalate the latter into Al(OH)$_3$. Successful reaction could only be achieved by first grinding γ-Al(OH)$_3$, in a ball mill, and then subjecting it to an extended hydrothermal treatment. The LDHs thereby generated are highly novel synthetic materials with formula [MAl(OH)$_3$(NO$_3$)$_y$·yH$_2$O] (M = Zn, Cu, Ni, Co, MAH).

![Diagram of a schematic representation of intercalation into the LDH (CoZnAl$_8$(NO$_3$)$_y$·yH$_2$O).](https://via.placeholder.com/150)

The study of reaction mechanisms and kinetics has been neglected for the solid state, largely because there are few non-invasive probes available. A powerful non-invasive probe is in-situ energy-dispersive X-ray diffraction (EDXD). The exceptional EDXD capabilities of beamline I12 provide an unparalleled opportunity to extend our knowledge in this area, and the present study forms part of work to explore the nanoscopic processes involved in a range of solid state reaction processes.

In this study, we sought to expand our earlier work in a number of ways. First, we used Rietveld refinement methods to propose a structure for the new MAI, NO$_3$, materials. It was found that M$^2+$ cations occupy the octahedral vacancies in the Al(OH)$_3$ layers, with almost complete ordering of M$^2+$ cations. Next, we explored the possibility of incorporating salts other than nitrates, and of further reacting the MAI, NO$_3$, materials to fill the remaining octahedral holes. Beyond this, experiments were undertaken to prepare three-metal LDHs by reacting Al(OH)$_3$, with mixtures of M$^2+$ nitrates. The idealised formula of these materials is [MAl$_x$(OH)$_{24}$X$_n$·nH$_2$O]. However, it was found that Al(OH)$_3$, exhibits selective intercalation chemistry, in which metal species preferentially in the order Li$^+$ > Mg$^{2+}$ > Ca$^{2+}$ = Zn$^{2+}$. By varying the ratio of metals in the reaction gel, it proved possible to control the stoichiometry of the LDHs produced. For instance, a 1:1 mixture of Zn and Co nitrates yields the LDH [CoZnAl$_8$(NO$_3$)$_y$·yH$_2$O].

In order to prove definitively that the new materials prepared are layered double hydroxides, it was necessary to demonstrate that the interlayer nitrate anion could be replaced with other anions, and hence reactions were undertaken using a range of simple organic anions (see Fig. 1). It proved facile to replace the nitrate anion with a range of organic species such as phthalate (C$_4$H$_6$O$_4$). These experiments proved that the new materials were indeed LDHs. As they are highly crystalline, with distinct and non-overlapping sets of Bragg reflections for each phase, they were chosen as ideal candidates for the first time-resolved study of a chemical reaction system to be undertaken at Diamond Light Source. The existing Oxford University in situ reaction cell was refurbished, commissioned onto beamline I12, and a detailed agreement with the I12 optics undertaken. Photographic and schematic representations of the reaction cell are given in Fig. 2.

We were pleased that, with very little optimisation, it proved possible to record high-quality diffraction patterns in as little as 5 – 10 s. Unfortunately, the LDH intercalation reactions were observed to be very rapid (< 30s); hence, a syringe pump was used to add a solution of the guest to a LDH intercalation reaction cell at an offset position. This required removal of a collimator, in order to introduce extra noise into the diffraction patterns. Nevertheless, very high quality data were obtained. Data for the reaction between [CoZnAl$_8$(NO$_3$)$_y$·yH$_2$O] and phthalate to give CoZnAl$_8$[C$_4$H$_6$O$_4$] are shown in Fig. 3. A plot showing the variation of diffracted intensity with time is depicted in Fig. 3a. This contains only two phases: CoZnAl$_8$-NO$_3$ and CoZnAl$_8$-phthalate. No crystalline intermediates may be observed.

The observed Bragg reflections were integrated and converted into the extent of reaction, as α = I$_hkl$(t)/I$_hkl$(max), where α is the integrated intensity of the hkl reflection. The extent of reaction vs. time plots given in Fig. 3b show the product and reaction curves cross very close to α = 0.5, suggesting that loss in diffracted intensity from the host is exactly matched by the increase in intensity from the product, and confirming a one-step reaction process (if intermediates were involved, then the curves would cross at α = 0.5). This is in agreement with previously reported results for the MAl$_x$(OH)$_3$(NO$_3$)$_y$·yH$_2$O LDHs.

As a result of these preliminary experiments, we have now developed an optimised set-up for the study of chemical reactions on I12, and are actively working with Diamond Light Source to develop a new furnace with improved capabilities, as well as to reduce the noise introduced to the system when the detectors are moved to an offset position to access the high d-spacing region. This research forms part of a programme of investigations seeking to elucidate more detail on the nanoscopic processes involved in solid state reaction mechanisms, and the outstanding capabilities of I12 will be profoundly important in allowing us to drive this work forward.

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References


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Probing inert anodes with high-energy X-rays


The development of inert anode materials relies on the characterisation of samples that have been removed from their operational environment at various pre-defined points in time.

This ex situ approach can be problematic, as conventional analysis techniques typically require some form of sample preparation, ranging from simply allowing the sample to cool, to more invasive procedures such as cutting and polishing. While any material studied outside of its operational environment will be altered to some extent, friable surface layers, crucial to the understanding of reaction mechanisms, are at particular risk. Obtaining clear information about how these layers evolve during cell operation, without disrupting their fragile structure, requires a quantitative in situ characterisation method.

Molten-salt electrolysis is used extensively in the production of light metals such as aluminium, lithium and magnesium, and is being investigated as a potential replacement for the Kroll process for titanium production. Currently, reactive carbon anodes are used in titanium electrowinning research with many unwanted outcomes, such as the emission of greenhouse gases, reaction of carbon with the electrolyte, and carbon contamination of electrowon metal. Conversely, an ideal inert anode is not consumed during the electrolysis, does not react with the electrolyte and produces only oxygen, therefore having a much lower impact on process control and the environment. However, inert anodes are prone to failure as, in practice, they are attacked by both the electrolyte and the oxygen evolved at the anode. In order to develop these new anode materials further, a detailed understanding of the structural and chemical changes that lead to their failure is needed.

Due to the aggressive sample environment, molten calcium chloride at 950 °C, the in situ characterisation of inert anodes employed in molten-salt electrochemistry is difficult. A ‘see-through’ cell, developed by McGregor et al., which uses a transparent quartz crucible for the direct observation of both the anode and cathode, has given many insights into physical aspects of anode behaviour. However, determining chemical and structural changes in an operational anode requires a different approach. In order to study structural changes in materials with respect to time, synchronisation and neutron diffraction techniques are often used. The intensity of such advanced radiation sources allows data to be collected on the time scales necessary to observe reaction changes and investigate their kinetics.

This experiment, performed at beamline I12, aimed to look at the formation of surface layers on the anodes whilst they were operating in molten salt at 950 °C, required: (i) highly penetrating radiation in order to pass through the furnace walls and several centimetres of electrolyte, (ii) high spatial resolution in order to selectively study only the anode surface in a complex sample environment and (iii) high temporal resolution in order to study the changes in the anode on a minute by minute basis. Synchrotron-based energy-dispersive X-ray diffraction (EDXRD) is uniquely suited to fulfil these experimental requirements as its characteristics include: (i) penetrating high-energy X-rays, (ii) a fixed detector position, allowing tight collimation of the diffracted beam, providing good spatial resolution, and (iii) a very high intensity beam allowing for short data acquisition times. In order to carry out this work, an electrowinning cell and furnace, Fig. 1 was designed to allow for the operation of a molten-salt electrochemical cell in a beamline environment whilst still allowing for valid data collection to be obtained. The guiding principle behind the design of the cell and furnace was to enable in situ characterisation of an inert anode using EDXRD, without compromising the electrochemical reactions at either the anode or cathode.

The electrowinning cell was designed to minimise the X-ray beam path through the electrolyte, while still allowing sufficient electrolyte for electrolysing to occur. The furnace was designed to reach a maximum of 1100°C, while only using a standard 240 V 10 A power supply.

The anodes used in this study were a Magneli-phase material (Donnay\textsuperscript{4}), containing Ti\textsubscript{0.8}O\textsubscript{2.2}, n = 4-6. This material oxidises to rutile (TiO\textsubscript{2}) due to the evolution of oxygen at the anode surface, producing a passivation layer which ultimately causes the anode to fail. Donnay\textsuperscript{4} was used as a model anode as, (i) the phase changes that occur in this material during electrolysis have been substantially characterised ex situ\textsuperscript{4}; allowing findings made during in situ experimentation to be corroborated by ex situ data, (ii) it does not contaminate the electrolyte or cathode, and (iii) it remains dimensionally stable.

In separate experiments, in situ EDXRD data were collected above or below the surface of the electrolyte to observe the effect of electrolyte absorption on the diffraction intensities; electrolysis is possible slightly above the surface of the anode as it creeps up the surface of the anode. Datasets were collected for 60 sec, with a 5 sec delay between consecutive datasets, for the duration of the experiment, typically 6-7 hours. Accumulated diffraction patterns collected centrally on the anode are shown in Fig. 2, top and bottom of the surface of the electrolyte. The evolution of the Magnéli-phase material, Magnéli-phases to rutile can be seen clearly. For the data collected above the electrolyte, the diffraction peaks for each phase are clearly visible. For the data collected below, the diffraction peaks for each phase are less visible due to the absorption of lower energy X-rays by the electrolyte.

The data were analysed quantitatively using the Rietveld\textsuperscript{1} method to track the evolution of the various phases present in the anode. Scarlett\textsuperscript{1} had previously developed this methodology for the quantitative analysis of EDXRD data which uses the energy spectrum of the incident beam, sample absorption, and the crystal structures of the anode materials in order to calculate a model diffraction pattern. Such a model was then refined against the EDXRD data in order to extract quantitative phase analysis. Fig. 3 shows the results of this quantitative phase analysis. It can be seen that in all systems, rutile forms at the expense of both Magnéli phases equally.

The similarities in the quantitative phase analysis both above and below the electrolyte surface suggest that it is possible to obtain meaningful in situ diffraction data with the incident beam just above the electrolyte, mitigating the deleterious effect of X-ray absorption on the resultant diffraction patterns. Initial kinetics modelling suggests that the limiting factor in the growth of the rutile layer is the rate of solid-state diffusion of oxygen within the anode structure. This continual monitoring of the rutile phase fraction throughout the duration of the experiment revealed the way in which the layer grows, something that would be very difficult to do accurately ex situ experimentation. It also confirms the absence of any intermediate phases which would not be observed using traditional post mortem methodologies.

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References

High-energy transmission Laue (HETL) diffraction: a tool for mapping grain-level orientation and strain in thicker metallic polycrystals


Micro-beam Laue diffraction is a versatile probe for orientation and plastic strain in individual grains of metallic polycrystals. It can help elucidate the dependence of macroscopic material behaviour (deformation response, fatigue, fracture etc.) on microstructure, defect population, macro- to microscopic load redistribution, etc. Furthermore, it can provide quantitative validation for the crystal plasticity models used to study structural engineering alloys. The application of micro-beam Laue diffraction to real-life engineering components is limited by the absorption of the probing beam. To improve penetration into thicker samples we developed a high-energy transmission Laue (HETL) micro-beam diffraction setup on I12. The use of higher photon energies (50 – 150 keV) permits measurements in several-millimetre-thick polycrystalline samples. We established two different approaches to extend this method and allow resolution in three dimensions. The first approach relies on the application of tomographic reconstruction principles to orientation-specific scattered intensity. The second uses wire scans and triangulation to determine the volume of origin of each scattered contribution recorded on the detector.

For safety-critical aerospace applications, the ability to obtain accurate predictions of the grain-level response to deformation of polycrystalline alloy is essential. This is particularly the case since material failure is governed by ‘weakest link’ type mechanisms such as grain boundary creep and cracking, void nucleation, etc. that strongly depend on local inter- and intra-granular interactions and on the partitioning of macroscopic loading at the micro-scale. Computationally these phenomena can be studied by crystal plasticity simulations that take into account local morphology, evolution of crystal slip, lattice rotation and dislocation density. For model calibration and validation, experimental measurements at the same (micro-)scale are essential. Micro-beam Laue synchronised X-ray diffraction is ideally suited to this purpose. Conventionally, a polychromatic, micro-focused X-ray beam probe (2-25 keV) is used to illuminate intra-granular scattering volumes. The resulting diffraction patterns consist of a number of Laue spots and are recorded on an area detector positioned in reflection geometry. By indexing the spots and refining their positions, lattice orientation and elastic strain in the scattering volume can be computed. Orientation spread in the illuminated volume causes streaking of reflections that can be interpreted in terms of the underlying dislocation structure and active slip systems.

The shallow penetration of the probing beam (~70 µm in nickel) precludes the application of micro-beam Laue diffraction to the study of deformation in the bulk of real-life engineering components. To increase penetration to several millimetres, we extended the technique to significantly higher photon energies (50 to 150 keV). Transmission geometry is the natural choice since Bragg’s law dictates that at higher photon energies, the stronger, lower order reflections are forward scattered. We used this new technique to study the evolution of lattice orientation and strain in individual grains of polycrystalline nickel samples during in-situ deformation. The results highlighted that, even for uniform macroscopic loading, significant heterogeneities exist at the grain-scale. Furthermore the local stress state is strongly influenced by the morphology of the grain neighborhood.

At beamline I12 we developed two novel techniques to extend HETL to 3D grain-level resolution. The first, Laue Orientation Tomography (LOT), relies on the application of tomographic reconstruction principles to orientation-specific scattered intensity. The sample is mounted in transmission geometry (Fig. 1a.). Laue patterns are collected at x-positions covering the entire width of the sample for 91 φ-rotations between 0° and 180°. Indication of the Laue patterns acts as an orientation-sensitive filter. Similar to absorption tomography, lattice-orientation-specific sinograms (‘origrams’) are then established as functions of φ and θ, considering only the scattered intensity that corresponds to a certain orientation. The shapes of individual grains in the illuminated sample slice are reconstructed by inverting these ‘origrams’ using filtered back projection algorithms. Fig. 1b. shows the grains present in the slice of a polycrystalline nickel sample reconstructed on I12. To map the full 3D microstructure, slices at different heights of the sample are reconstructed. Further work is under way to obtain maps of intra-granular mis-orientation within individual crystallites.

The second technique, High Energy Differential Aperture X-ray Microscopy (HEDAXM), is an extension of the corresponding low energy technique. Tungsten wires are scanned across the scattered beams immediately downstream of the sample (Fig. 2a.). When a given reflection is obscured by a scanning wire it triangulates from the reflection position on the detector, via the wire, to the incident beam. Hence the through-thickness position of the coherently scattering volume that gives rise to the reflection can be determined. On I12 we studied a three-layer silicon wafer sample as a proof of principle. Fig. 2b., shows the complete diffraction pattern arising from this sample. Using HEDAXM we successfully separated this pattern into the individual contribution from each layer (Fig. 2c.-e.).

These successful first results demonstrate the promise of LOT and HEDAXM for the 3D mapping of microstructure, lattice orientation and elastic strain in individual grains of polycrystalline engineering samples.

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The effect of pressure on bismuth displacements in pyrochlore materials

Pyrochlore-structured materials containing bismuth are useful electroceramics, and they have also been studied for their interesting crystal structures and polymorphic phase transformations. This study applies pressure to two of these materials and used synchrotron X-ray diffraction on beamline I15, combined with further studies at the ESRF and ILL, to observe how the structures change. Examining the way in which the environment around bismuth changes gives insight into the interactions between bismuth atoms that are responsible for structural change, and also the factors that are responsible for the asymmetric environments around bismuth. In the latter case our results suggest that the bismuth sites are underbonded and that this has a more significant effect on the Bi³⁺ cation than the possible active lone pair.

Interest in bismuth-containing pyrochlore materials is derived from their complex structural chemistry in which bismuth ions are displaced from the centre of the coordination site normally occupied in pyrochlores by large cations including group 2 or 3 metals. The displacements have been linked to the high dielectric constants and other desirable electrical properties of pyrochlores including useful materials such as Bi₂Ti₂O₇ and Bi₂(Zn₂/3Nb₁/3)₂O₇ (Bi₂ZNO₂). The latter compound is now being incorporated into capacitors for microwave applications and thin film devices. In Bi₂Ti₂O₇, the Bi³⁺ ions are displaced in a disordered manner and so the off-centre displacements do not cause any significant change in the overall cubic symmetry of the structure. However, Bi₂Sn₂O₇ and Bi₂Hf₂O₇ exhibit displacements that are cooperative with the metal centres and the distortion is seen to cause a significant overall change to the lattice, resulting in a monoclinic symmetry with a four-fold increase in unit cell volume. Local environments around the large 'A' cation are shown in Fig. 1.

Bi₂Ti₂O₇ is known to remain stable between 2 and 710 K above which point it starts to decompose into Bi₂O₃, TiO₂, and Bi₂O₃ from high pressure and high temperature studies. Inorg. Chem. 11905-11913 (2011)

Prior to this work the structures of the α- (<470 K) and γ- (>903 K) phases had been determined, with γ-Bi₂Sn₂O₇ isostructural with Bi₂Ti₂O₇. Our Diamond Light Source experiment was conceived around this interest in bismuth-containing pyrochlores: new insights into the interactions between bismuth atoms that are responsible for structural change, and also the factors that are responsible for the asymmetric environments around bismuth. In the latter case our results suggest that the bismuth sites are underbonded and that this has a more significant effect on the Bi³⁺ cation than the possible active lone pair.

Figure 1: La or Bi environment (yellow) in La₂Ti₂O₇ (left) with La at the centre of the coordination sphere, Bi₂Ti₂O₇ (middle) with disordered Bi displacements and Bi₂Sn₂O₇ (right) with ordered Bi displacements. Red spheres are the Nd, Ti, Sn, and O atoms and the larger yellow spheres are oxygen atoms.

Figure 2: Le Bail fits to XRD patterns of Bi₂Sn₂O₇ collected at the pressures shown in a LiF pressure medium. Data points are shown as black crosses, the fit is the blue line and the difference is the blue line. Black tick marks indicate allowed reflection positions for Bi₂Sn₂O₇ and red tick marks the positions for the standard tetrahedral oxygen sites, and there are further lines via other oxygen atoms.

α-Bi₂Sn₂O₇ has displacements within the ring of 6 O' atoms that coordinate it, and also a further displacement toward one of the O' atoms that results in an overall ice-like network in which every O' atom has two longer and two shorter Bi-O' bonds. β-Bi₂Sn₂O₇ seems to have only the displacements within the ring, and in γ-Bi₂Sn₂O₇ these become disordered (Fig. 4). Interactions within the tetrahedral network would be expected to be subject to some degree of frustration, and the phase changes can be understood in reference to the different strengths of the correlations via one or two-ring networks. Interaction of α-Bi₂Sn₂O₇ with oxygen atoms in red.

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Melting curve of potassium to 22 GPa


Since the work of Zha and Boehler in 1985, potassium ‘K’ has been believed to have a ‘simple’ melting curve, characterised by the continuous increase of the melting temperature with pressure, at least to 14.5 GPa. This ‘normal’ melting is in contrast to that observed in the other alkali metals Li, Na, Rb and Cs, where one or more maxima in the melting curve are found to be of substantial decreases in the melting temperature to minimum values, above which the melting curve recovers a large positive slope. Considering the strong similarities in high-pressure behaviour of K with that of the other alkali metals, its simple melting curve seems to be rather surprising. Using X-ray diffraction facilities of I15 beamline, we have shown that the melting behaviour of K is far more complex than was previously reported. A melting maximum is found in the stability field of the K-I phase at ~4 GPa, followed by a decrease in the melting temperature down to a melting minimum at the phase transition from K-I to K-III at ~19 GPa. Above 19 GPa, the melting curve recovers a positive slope, increasing rapidly with pressure, changing by some 65(5) K/GPa. The re-measured K melting curve closely resembles those of Na, but we have come to the conclusion that K is believed to be related to structural/electronic changes either in the solid or liquid phase, or both. Similarities between the melting curves of these two alkali metals suggest that the same mechanisms might explain the melting curve of K.

Complex melting behaviour is one of the common features among alkali metals. The appearance of one or more melting curve maxima has been observed in Li, Na, Rb and Cs, but not, however, in K, at least not up to 14.5 GPa. Using optical observations of a sample enclosed in a diamond anvil cell (DAC) Zhou & Boehler reported that the melting temperature continuously increases in both the K-I (which has a body-centred cubic (bcc) crystal structure) and K (with a face-centred cubic (fcc) crystal structure) phases, reaching a value of 649 K at 14.5 GPa. However, theoretical calculations of the melting temperature of K have suggested the presence of a melting maximum between 4 and 8 GPa. To resolve this apparent discrepancy between experimental and theoretical results, and to extend the measurements of the potassium melting curve into the incipient K-III phase (with the fcc-host-guest crystal structure) above 19 GPa, we have performed an in situ synchrotron X-ray diffraction (XRD) study of the melting curve of K at pressures up to 22 GPa. DAC, XRD measurements were performed principally on beamline I15 at the Diamond Light Source, and also on beamline D15 at the ESRF.

A representative set of high-pressure high-temperature 20XD images, and their integrated XRD profiles (Fig. 1), demonstrates the sharp melting transition from fcc-K to liquid-K at 18.5(3) GPa, induced by the small increment increase of only 4 K. A subsequent pressure increase of about 2 GPa at the same temperature, 408(10) K, brings the sample into the stability field of the incipient fcc phase. By varying pressure and temperature conditions we tracked the melting curve of potassium at pressures from ambient to 22 GPa, as shown in Fig. 2.

Our data, combined with all the previous melting curve measurements up to 3 GPa, suggest the presence of a melting maximum in the bcc phase, the position of which was estimated using the Kechin equation. The obtained value of 530(3) K and 5.6(5) GPa is in good agreement with previous theoretical calculations. At pressures above this maximum, the melting temperature decreases to 466(10) K at the bcc-fcc-liquid triple point at 13.6 GPa, and then remains almost constant up to 15.6 GPa, where the melting curve again changes: its behaviour, descending to a clear minimum at 190(10) K and 19.0(5) GPa at the fcc-liquid triple point. The K-fcc phase boundary is found to be almost vertical. In the fcc phase, the melting temperature increases very rapidly with pressure, changing by some 65(5) K/GPa (Fig. 2).

The behaviour is very similar to that observed in Na, where the melting temperature, after passing through a deep minimum at 300 K and 118 GPa, increases again after the transition to the fcc phase at 125 GPa. The close resemblance of the melting curves of these two alkali metals might suggest that similar structural mechanisms lie behind the melting behaviour of K and Na. Currently there are two theoretical approaches to explain the complex nature of the Na melting curve: (i) softening of the elastic modulus of solid Na above 30 GPa and a corresponding decrease in the melting temperature, which, according to the Lindemann criterion, results in a negative slope of a melting curve; (ii) structural and electronic transitions in liquid-Na at about 30 and 80 GPa, which resemble the transitions occurring in solid-Na at higher pressures.

In attempting to explain the melting curve of K (Fig. 2) we have considered both approaches. The theoretical work by Kinet & et al. did show softening of elastic constants in both bcc- and fcc-K. Using the Lindemann criterion they also predicted a melting curve maximum at about 5 GPa (in a good agreement with the 5.6 GPa presented here). However, the same calculation underestimated the melting temperature over a large pressure range (dash-dotted line in Fig. 2). The possible softening of elastic moduli in bcc- and fcc-K, and its effect on the melting curve of K, is yet to be investigated.

In the framework of the second approach, the melting maximum at about 6 GPa by decrease of the melting temperature, decrease of the fcc-liquid triple point, would suggest a transition in liquid-K from a bcc-like to a more dense fcc-like liquid at about 6 GPa. Above the bcc-like liquid-tripoint, where the solid-K itself assumes the fcc-structure, the melting temperature is almost constant. The following decrease of the melting temperature between 15.6 GPa and 19 GPa would suggest a second transition in liquid-K, from fcc-like to Na-like. To confirm these predictions further, experimental studies, as well as computer simulations of the properties of both solid and liquid dense K, are required. However, our data do indeed show some structural changes in liquid-K (Fig. 3), thus providing evidence in the favour of the approach suggested by Raty et al. Below 5 GPa the position of the first diffraction peak of liquid-K is in good agreement with the bcc-like equation of state, while between 5 and 14 GPa it closely follows the fcc-equation of state. This might be related to a bcc- to fcc-like liquid transition in liquid-K. Above 14 GPa there is another change in the structural behaviour of liquid-K, which again could be related to a structural transition, this time from fcc-like to Na-like. We hope to use high-pressure facilities of I15 beamline again in the near future to investigate these changes further and in more detail.

To conclude, the re-visited melting curve of K appears to be very different from that previously reported. In the stability field of bcc-K there is a very shallow maximum, like that observed previously in Na, Rb and Cs. The melting behaviour of fcc-K resembles that for Na. Using similar arguments put forward previously to explain the melting curve of Na, we suggest that the behaviour of the K melting curve is likely to be due to structural and electronic changes in liquid-K from 4–6 and 14 GPa, which mirror those that occur in the solid phase at 13.6 and 19 GPa, respectively. However, the anomalous melting of bcc- and fcc-K may also suggest a softening of elastic moduli of these phases as pressure increases. Further work is required to understand the complex nature of dense K.
Structural behaviour of mineral barite under strong compression


Research on the mineral barite, BaSO₄, is of great interest for Earth and material sciences. Its unique properties, the high density and chemically inertness, make barite useful for instance for oil and gas drilling. Particularly, this compound has recently been of renewed interest regarding its high-pressure behaviour. Previous reports present some controversial results on the behaviour of this mineral under strong compression. Some authors observed small changes in the X-ray diffraction patterns and they inferred a phase transition below 13 GPa, whereas others did not observe any transition up to 21.5 GPa. In this study, we have made use of the high-energy synchrotron radiation available in the I15 beamline of Diamond Light Source and in the ID27 beamline of the ESRF to accurately determine the structural sequence and compressibility of BaSO₄.

Mineral barite, BaSO₄, crystallizes at ambient conditions in an orthorhombic structure (space group: Pnma, Z = 4) with lattice parameters:

\[
\begin{align*}
\alpha &= 8.821(4) \text{Å}, \\
\beta &= 5.622(2) \text{Å}, \\
\gamma &= 7.191(3) \text{Å} \quad (V = 245.586(7) \text{Å}^3).
\end{align*}
\]

Its structure can be easily described in terms of the cation subarray Ba₄ which is of the Fe₄-type (B2T), the structure consisting of triangular prisms of Ba atoms that share faces along the b direction and corners in the other two directions, with the SO₄ groups inserted into these metal prisms (see Fig. 1). Barite had been studied under pressure using Raman spectroscopy and energy-dispersive X-ray diffraction. Lee et al. observed small changes in the diffraction patterns and a subtle variation of the lattice parameters at about 13 GPa and they inferred a phase transition. The high-pressure (HP) phase was tentatively determined to be triclinic. More recently, however, Crichton et al. carried out Raman and angle-dispersive X-ray diffraction (XRD) measurements using a diamond anvil cell (DAC). Silicone oil and He were used as pressure transmitting media. They did not observe any phase transition in BaSO₄, the barite-type structure remaining to the highest investigated pressure.

Therefore, in order to better understand the poorly known behavior of this mineral under compression, we performed high-pressure XRD measurements using a diamond anvil cell (DAC). Silicone oil and He were used as pressure-transmitting media. The pressure was estimated using the ruby fluorescence technique.

Fig. 2 shows some XRD patterns at selected pressures using He as pressure medium. In good agreement with Crichton’s data, high-pressure X-ray patterns could be indexed in the orthorhombic phase which is stable at room conditions, up to 27 GPa. Above this pressure, new peaks appear in the XRD pattern, indicating the onset of a phase transition in BaSO₄. At 40.5 GPa, the diffraction peaks of the low-pressure phase have almost disappeared completely. This transition was also observed using silicon oil as pressure medium, but the lack of hydrostaticity led the phase transition to occur at lower pressures, at 19 GPa. These structural changes were found to be reversible.

From the analysis of the XRD patterns, we obtained the pressure evolution of the unit-cell volume and lattice parameters of the low-pressure phase. The contraction of the lattice constants is rather isotropic. The pressure-volume curve was analyzed using a third-order Birch-Murnaghan equation of state (EOS). By fixing the zero-pressure volume (V₀) to its measured value, we obtained the bulk modulus, B₀ = 58.6(2) GPa, and its pressure derivative, B’₀ = 4.8(1). The values of these characteristic parameters are in excellent agreement with those theoretically calculated.

We have also proposed a crystalline structure for the high-pressure phase. As it can be seen in Figs. 2 and 3, the diffraction pattern of the high-pressure phase (40.5 GPa) obtained with He as pressure medium still has very well defined peaks. They could be indexed in an orthorhombic cell with lattice constants: a = 6.55(5) Å, b = 5.87(4) Å, and c = 6.33(4) Å \(V = 243.3(8) \text{Å}^3\). At 4 GPa, a high figure of merit \(M(20) = 21.2\) was obtained in the orthorhombic phase \(P2_12_12_1\) structure with \(Z = 4\]. Therefore, this structure implies a volume change of about 2% at the transition. The systematic

The high-pressure phase has a very strong distortion of the initial barite phase (see Fig. 1). The a axis contracts approximately 18.1%, the b axis expands approximately 20% and the c axis remains nearly constant at the transition pressure. This lattice transformation entails a small displacement and tilting movement of the \(\text{SO}_4\) tetrahedra and the elimination of the a axis implies that we can not consider the existence of trigonal prisms anymore.

The variation of the unit-cell volumes of the high-pressure phase of BaSO₄ with pressure could be fitted to a third-order Birch-Murnaghan EOS where the values of the bulk modulus \(B_0\) and cell volume at zero pressure \(V_0\) are left to vary freely and \(B'_0\) is fixed to 4. The characteristic parameters for the \(P2_12_12_1\) phase are: \(B_0 = 325(3)\) GPa and \(B'_0 = 78(6)\) GPa.

The HP structure of barite is very interesting regarding the position of this compound on the Bastide’s diagram. This diagram has proven useful in classifying and predicting phase transitions in ABX₄ compounds. The location of BaSO₄ in the lower-right quadrant, due to its cation-to-anion radius ratio \(r_A/r_X\), did not allow for the prediction of a post-barite structure, which was to be described. Thus, the post-barite structure found in this study, the \(P2_12_12_1\) phase, turns out to be one of the missing structural types in the Bastide’s diagram and gives, therefore, further insight on the structural sequences of the ABO₄ anides under compression.

**References**


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One of the most exciting and rapidly evolving fields in modern materials is the study of how gases interact with solids, particularly under non-ambient conditions of pressure and temperature. Typical areas of interest include the study of catalysts under realistic working conditions, host-guest interactions in gas loaded frameworks and porous materials for gas storage or pollution scrubbing, absorption/desorption kinetics in novel hydrogen storage systems for future energy storage technologies and in the development of materials tailored to CO2 sequestration for greenhouse gas remediation. In order to allow the user community of beamline I11 to meet these fast moving scientific challenges, a recent addition to the beamline’s non-ambient capabilities has been the commissioning of a gas control system to allow the in-situ dosing of powder samples with gases such as hydrogen, methane and carbon dioxide.

The gas control system has been designed to handle gases over a wide pressure range (mbar – 100 bar), be simple for user operation, allow in-situ gas dosing and connect easily to both beamline and user sample environments. To compliment this system, low and high pressure capillary sample cells have also been constructed and, together with the gas delivery system, are now in routine use during user experiments. The cells are based on a simple to operate design and have the flexibility to be used with non-contact, non-ambient, sample environments, such as cryostream or hot-air blower, to allow a wide-range of temperatures (80 – 1273 K) to be applied to the sample under investigation, while a connection to a turbo pump allows vacuum pressures in the region of 10^-3-10^-4 mbar to be applied prior to dosing. The gas control system (Fig. 1) is located outside the experimental hutch, allowing sample dosing to be controlled while data collection is underway, and is configured to enable the use of either one low pressure gas, or two high pressure gases, during any one experiment. The outlet from the system is transferred to the sample cells in the experimental hutch via a stainless steel line, which is terminated with a self-sealing ‘quick-connect’ fitting (Swagelok) for ease of cell mounting and dismounting.

The low and high pressure capillary gas cells can be used with glass, quartz or sapphire capillaries, and mount on a goniometer at the centre of the I11 diffractometer using a custom mounting plate, allowing diffraction data to be collected with either the high resolution multiple analysing crystal (MAC) system for detailed structural investigations, or the position sensitive detector (PSD) for time resolved kinetic studies. During data collection the cells can be oscillated by rocking the θ circle, e.g. by ± 10°, to improve particle statistics. Figure 2 shows one of the gas cells for high pressure experiments. Figure 3 shows the diffraction pattern of a clathrate hydrate which was produced by injecting CO2 gas at 20 bar of pressure into water ice held at 220 K inside the high pressure cell. The original hexagonal ice structure has been transformed into a cubic hydrate structure, where CO2 molecules reside within a large H2O cage structure. The new facility opens up new possibilities for users at beamline I11, in particular for the study of gas adsorption in microporous materials, e.g. zeolites and metal-organic frameworks, where structural investigations using I11 have already produced a number of high profile publications.
Over the last year the Surfaces and Interfaces Village beamlines have continued to explore the fundamental properties of a wide range of materials, revealing intriguing hidden properties of matter and develop new methods with which to explore nanostructures. The village now has three operational beamlines (I06, I07 and I10) with another two (I09, I05) in an advanced stage of construction. Furthermore, a new Phase III beamline (VERSOS) dedicated to photoemission spectroscopy under ambient conditions will start its detailed planning and design phase in the coming year. In this section we have selected some exciting new results covering time-resolved diffraction to X-ray holography to self-organisation in organic overlayers.

Modern devices exploit not only the charge of an electron, but also its intrinsic spin—leading to incredible advances in data storage capacity. In recent years new materials have been engineered with novel properties such as perpendicular magnetic anisotropy. Nistor et al. (Ecole Polytechnique, France) have taken this a step further and combined perpendicular magnetic anisotropy (PMA) with the Rashba effect in heterostructures lacking inversion symmetry. Angle-dependent X-ray Magnetic Circular Dichroism (XMCD) was then used to study the photoinduced melting of electronic ordering in magnetic materials. Resonant soft X-ray scattering has been used to understand the orbital and magnetic ordering in many materials in recent years, but on I06 the technique has been extended to the ultraviolet timescale to probe the photoinduced melting of orbital and magnetic order in a half-doped manganite. The long-lived phase, existing several hundred picoseconds after photoexcitation, was found to retain the lattice distortions, but without any magnetic ordering demonstrating a previously hidden phase of this material. This work was extended to even faster timescales by developing the Diamond synchrotron for a low-background measurement of the complicated magnetic ordering in many materials. In the coming months I10 will embark on a fast polarisation switching mode of operation that should allow much faster acquisition of dichroism spectra.

Beamline I07 continues to provide accurate surface structure information for a wide range of materials and sample environments. The double crystal deflector, which enables X-ray reflectivity from liquid interfaces, has been used to study nanoparticle formation at liquid-liquid interfaces, incorporation of proteins into lipid bilayers and self-assembled molecular systems. A second experimental end-station for surface X-ray diffraction has been developed allowing in situ sample preparation and characterisation. The surface X-ray diffraction results can be correlated with other structural probes, available on the same system, such as low-energy electron diffraction, X-ray photoelectron spectroscopy and Auger electron spectroscopy to provide a comprehensive set of structural data. Amongst the first to use the system was the group of Bennett (University of Reading), who studied the internal atomic structure of Cr nanostructures grown on W surfaces. In February 2012, beamline I07 received first light and we will continue commissioning the beamline in 2012 with first users planned for the end of the year. In addition, I09 will also proceed with building a separate soft X-ray branchline covering the energy range 50 eV to 2100 eV. Beamline I05 is ready to start the installation phase of the beamline components and is on course to start user operation next year. In the past year, also I06 and I10 have also benefitted from a high-precision soft X-ray polarimeter and is on course to start user operation next year. The laboratory allows samples to be grown, self-organised and studied by Scanning Tunnelling Microscopy, and easily transferred to the beamlines under UHV conditions for X-ray studies.

Electrochemistry is a key element in many energy-related applications, such as fuel cells and batteries, making an understanding of the complex interface processes vital. On beamline I07 Lucas et al. (University of Liverpool) have used surface X-ray diffraction to study the effects of interacting electrtytes on Ag surfaces. Structural changes of the Ag surface and cation-substrate distance were found to change dramatically depending on the electrode potential, which has important implications for the understanding of electrochemical reactions.

Surfaces and Interfaces Village
Samjeet Dhesi, Village Coordinator
Promising candidates for spintronics - vector measurement of the orbital moment anisotropy of Pt/Co/AlO$_x$ heterostructures with a strong Rashba interaction


Orbital moment anisotropy of Pt/Co/AlO$_x$ heterostructures with strong Rashba interaction. Phys. Rev. B. 84, 054464 (2011)

Ultra thin magnetic structures with surface-induced perpendicular magnetic anisotropy (PMA) are promising candidates for novel spintronic systems such as non-volatile high-density memory devices and magnetic tunnel junctions with perpendicular magnetisation, which should be much faster than their electronic counterparts. Of particular interest are Pt/Co/AlO$_x$ heterostructures, owing to their PMA and large coercivity, which allows stable encoding of information over long periods of time. Moreover, the strong Rashba spin-orbit interaction exhibited by these trilayers enables perpendicular magnetic switching via in-plane current injection, which is a simple and efficient method for magnetic encoding\(^1\). We present here a study of PMA in Pt/Co/AlO$_x$ heterostructures, which we show to be related to the Rashba interaction. By combining transverse and collinear x-ray magnetic circular dichroism (XMCD) on beamline I06, we achieve a vector measurement of the Co spin and orbital moments. We derive the anisotropy of the Co orbital moment and demonstrate its connection with the macroscopic magnetic anisotropy of the heterostructure, as predicted theoretically.

Following early predictions of interface-induced PMA by Néel, a host of layered systems with surface-induced anisotropy has been proposed and studied. New phenomena were discovered in these systems, such as enhanced spin and orbital moments. It was shown that PMA is a consequence of the orbital moment anisotropy, which couples the crystallographic symmetry axes of the system to the spin magnetic moment\(^1\). Although the orbital magnetic moment is only a small fraction of the order of 10% of the total magnetisation, it couples simultaneously to the crystal field and to the spin magnetic moment through spin-orbit coupling and consequently mediates an interaction between the spin moment and the lattice.

The coupling between spin and orbital moments is of great interest in structures lacking inversion symmetry, such as Pt/Co/AlO$_x$ trilayers. In such systems, PMA combines with non-equilibrium current-induced spin-orbit torques, which provides effective means to control the magnetisation of high-curvature ferromagnetic layers\(^2\). However, few studies of systems displaying such effects have been performed, and several outstanding questions remain open. In particular, the magnitude of the orbital moment anisotropy defining the PMA and, likely, the current-induced spin-orbit torques need to be studied together with its dependence on the thickness of the magnetic layer.

In order to address these points, a set of four Pt (3 nm)/Co (0.6–2.2 nm) heterostructures with Co (2 nm) samples at the Co L$_2$,3 edges are shown in Fig. 2. From these spectra, we can extract the Co orbital and spin magnetic moments by application of the XMCD sum rules.

The measured Co orbital and spin magnetic moments in vector representation are shown in Fig. 3 (a) for all four Co film thicknesses. We see that in the thinnest Co film (0.6 nm) the orbital and spin moments are pulled away from the field direction and towards the sample normal. This indicates a strong magnetic anisotropy in the out-of-plane direction. As the Co film thickness increases, the spin and orbital moments gradually rotate towards the in-plane direction. This behaviour can be explained as a competition between the out-of-plane anisotropy which pulls the orbital moment out-of-plane and the shape anisotropy which pulls the magnetisation towards the sample plane. We also notice that the spin and Co moments are non-collinear in the thinnest Co (0.6 nm) film, which suggests that the anisotropy of the spin moment is indeed driven by the orbital moment, which ties the Co magnetisation to the out-of-plane direction.

In order to quantify the orbital moment anisotropy, we propose a simple model which assumes that the orbital moment of the Co film is composed of the anisotropically orbital moment of the two interface Co monolayers and the orbital moment of the Co multilayers that do not belong to either interface. This simple model gives for the Co (0.6 nm) sample an orbital moment anisotropy \(\Delta E_{\text{MCA}}\) of \(-0.045\) μeV/atom. A tight-binding model which relates the magnetocrystalline anisotropy \(E_{\text{MCA}}\) to the orbital moment anisotropy yields a value \(\Delta E_{\text{MCA}}\) of \(0.11\) mJ/m\(^2\) for the Co (0.6 nm) sample. We compare this theoretical value for \(E_{\text{MCA}}\) with the experimental \(E_{\text{MCA}}\) that we derive by setting the effective field \(H_{\text{eff}}\) parallel to the Co magnetisation. Depending on the degree of oxidation of the top Co monolayer, we obtain for the experimental \(E_{\text{MCA}}\) of Co (0.6 nm) values in the range (0.7 meV/\(10^{-12}\) m\(^2\)), which are reasonably close to the theoretical value of 0.11 meV. This agreement provides further evidence that the spin moment anisotropy is a consequence of orbital moment anisotropy in this class of samples.

Finally, although the absolute values for both spin and orbital magnetic moments increase with Co thickness, we find that the orbital-to-spin ratio of the magnetic moments are considerably larger in the thinnest Co layers, as shown in Fig. 3b. Our data indicate that the interactions responsible for PMA, the Rashba effect, and the generation of spin-orbit torques are stronger in the ultrathin limit (t < 1 nm), and provide clues on how to optimise such effects in spintronic devices.

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Holographic imaging of magnetic nanostructures using extended references


Magnetic microscopy forms an important part of today’s science because it provides scientists with a powerful tool to study the magnetic structure on a submicron scale. We have used state-of-the-art Fourier transform holography (FTH) techniques to produce high-resolution images of multilayered magnetic samples with different magnetic properties. For this we have used a novel imaging method known as ‘holography with extended reference by autocorrelation linear differential operator’ (HERALDO), which allowed us to investigate magnetic coupling effects in systems which are of tremendous interest for advancing spintronic technologies.

We used the Nanoservice beamline I06 to perform a series of soft x-ray HERALDO experiments to study the detailed magnetic structure of thin magnetic films. We found that the HERALDO method significantly reduced the required counting time to record a hologram in comparison with conventional magnetic FTH, which relies upon small-diameter pinholes to provide the holographic reference waves. This is due to the increased photon flux that passes through the extended reference structure.

The sample substrate was an X-ray transparent silicon nitride membrane holding the magnetic film on one side. On the opposite side of the membrane, a thick film of gold was deposited in order to block all incident x-ray beams passing through when the sample is exposed to the beam. Focused ion beam milling was then used to pattern a viewing aperture into the gold which was milled down to the membrane substrate and allowed the radiation to transverse through the magnetic layers. Close to the viewing aperture we milled an extended holographic reference slit through the entire gold-sample ensemble. In these experiments magnetic contrast is obtained using x-ray magnetic circular dichroism (XMCD), which relies upon small-diameter pinholes to provide the holographic reference waves. This is due to the increased photon flux that passes through the extended reference structure.

We performed the initial experiments on a magnetic film containing a stack of 30 Co/Pt bilayers with induced perpendicular anisotropy. Fig. 2a shows the resulting hologram after a differential filter was digitally applied to the recorded interference pattern on the CCD. The corresponding Fourier transform of this outcome is shown in Fig. 2b and reveals the reconstructed magnetic domain structure contained within the viewing aperture (an enlargement of one of the cross correlated is shown in Fig. 2c).

Next, we used this technique to study a similar sample which contained the required counting time to record a hologram in comparison with conventional FTH. This is quick and straightforward to apply, and all the information required is readily available from the recorded interference pattern.

To independently measure the scattering from the different magnetic layers the X-ray energy was tuned to the absorption edges of the specific elements. A reconstruction of the domain structure in the Co/Pt multilayer with the sample normal to the beam direction was recorded (Fig. 3a) before the sample was rotated to an angle of 45º. Here the X-ray energy was tuned to the Co L3 absorption edge. The domain structure in the Co/Pt was imaged again in the off-normal geometry (Fig. 3b), shortly followed by an image of the permalloy (Fig. 3c) recorded with a beam energy corresponding to the nickel L2,L3 absorption edge.

We found that the domains in the permalloy layer were closely correlated with the contrast in the Co/Pt multilayer. We also found that applying a magnetic field removed the induced domain structure and that returning to the remanence state did not review the domain structure in the permalloy (results not shown here). It is speculated that after magnetising with the applied field, the permalloy layer forms larger domains which are not affected by the dipolar field from the Co/Pt due to high exchange coupling within the domains.

References
Light-induced melting of magnetic order in a manganite probed with ultrafast resonant soft X-ray diffraction


Photo-excitation in a manganite at near-infrared or visible wavelengths transfers charges across semiconductor bonds, drastically perturbing spin and orbital order to enable optical control of magnetism. However, a comprehensive understanding of the underlying physics is still missing. This is particularly elusive because charge, spin, and orbital arrangements are interdependent degrees of freedom. They evolve on sub-picosecond time scales and nanometer length scales, and can only be disentangled with ultrafast techniques sensitive to nanometer-scale modulations of charge and spin densities.

In the past, static electronic order in manganites has been probed with resonant soft X-ray diffraction (RSXD). This technique is directly sensitive to electronic states close to the Fermi level using photon energies resonant with the $2p \rightarrow 3d$ dipole transitions ($Mn_{3+}$). The energy dependence of the RSXD intensity can be used to understand the competing interactions leading to spin and orbital ordering.

Here, we have extended RSXD to the ultrafast time scale in order to investigate photo-induced dynamics in the single-layer, half-doped manganite $La_{0.5}Sr_{1.5}MnO_4$. Time-resolved RSXD was measured in a pump-probe scheme. To this end, trains of 100 fs, 800 nm wavelength (1.5 eV photon energy) laser pulses at a repetition rate of 22 kHz were synchronized to soft-X-ray pulses at beamline I06. The time delay between laser pump and X-ray probe was set by a mechanical delay stage. Separation of transient spin and orbital order is achieved by detecting changes in the time-dependent intensity at two different diffraction peaks. Single-bunch X-ray pulses were resolved using photon counting and gating electronics. The time resolution of this experiment was limited by the X-ray pulse duration to about $\sim 50$ ps in hybrid mode and $\sim 110$ ps in low-mode.

In the low-temperature $C$-type antiferromagnetic phase of $La_{0.5}Sr_{1.5}MnO_4$, charge, spins, and orbitals form a characteristic pattern, well visualized as a set of antiferromagnetically coupled ferromagnetic zigzag chains of $3d^3$ ($3y^2-r^2$) orbitals at the Mn sites. Figure 1a and 1c show the time-resolved photo-induced changes in the diffraction intensity of the (1/4 1/4 1/2) and (1/4 1/4 0) peaks, measured near the Mn $L_3$ edge at 640.15 eV and 640.25 eV, respectively. Antiferromagnetic order along the $c$-axis, proportional to the (1/4 1/4 1/2) diffusion signal, is completely melted for an excitation fluence of 5.5 mJ/cm$^2$, while in-plane orbital order, measured at the (1/4 1/4 0) peak, is reduced by about 25% only. At 200 ps time delay, the energy-resolved diffusion intensity at the magnetic wave vector decreases, while the structureless fluorescence signal (see Fig. 1b), shows the energy dependence of the magnetic peak intensity.

The temporal evolution of our experiments was improved by operating Diamond Light Source in low-x mode. Here, shorter X-ray pulses ($\sim 9$ ps) were generated by compressing the electron bunches in the ring at the expense of total current and X-ray flux. Already on the sub-10 ps time scale the magnetic (1/4 1/4 1/2) diffusion peak promptly disappears (see Fig. 2). Finally, we measured the dynamics on longer time scales by electronically controlling the delay between pump and probe pulses. The diffusion intensity of the magnetic peak recovers back to the static value with double-exponential time constants of 10 and 110 ps.

Comparing the time-resolved intensity changes of the different diffraction peaks to static temperature dependence, the optically induced rearrangement of spin and orbital order cannot be explained by laser induced heating effects. This is also confirmed by a calculation of the heat in the sample generated through the laser pulse. Thus, the magnetic and orbital order melting must be strongly influenced by the non-thermal physics occurring immediately after optical excitation.

We interpret our observations along the following lines: Due to strong on-site Hund’s coupling, excitation with the linearly polarised 800 nm pulses results in charge transfer along the ferromagnetically aligned zigzag chains. This photo-excitation can be thought of as the injection of sparse defects into the static electronic structure of the manganite, perturbing the orbital occupancy of various orbitals. According to the Goodenough-Kanamori-Anderson rules, these charge defects effectively couple to the magnetic order, which is stabilized by short-range exchange interactions. Furthermore, this excitation triggers long-range distortions of the lattice by rearranging the lattice-Teller effect. Obviously, the long-range lattice order is more robust against the optical excitation than the short-range exchange interactions. It is then possible that the long-lived product phase reduces to a metastable spin-disordered or even ferromagnetic phase or a mixture of the two.

This work was continued using time-resolved soft X-ray diffraction experiments at a 4$^{th}$ generation light source, the free electron X-ray laser LCLS, where optically induced changes in the magnetic ordering were studied on the femtosecond timescale.

References

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Probing the atomic structure of the electrochemical double layer


Discovering the fundamental principles that govern electrochemical reactivity is key to the design of new materials for a range of scientific applications, particularly in energy-related technologies. Such information can only be obtained from model systems with well-defined elemental reaction sites using state-of-the-art instrumental probes. In this report we describe the application of in situ surface X-ray scattering (SXS) to study the structure of the electrochemical double layer at the interface between a Ag(111) electrode and alkaline electrolyte. Detailed modelling of the SXS data at negative potential is consistent with the presence of a hydrated K+ cation layer at a distance of 4.1 Å from the Ag surface and, at positive potential, indicates that the presence of OH− stabilizes the hydrated K+ cations through a non-covalent interaction forming a compact double layer structure in which the Ag−K+ distance is reduced to 3.6 Å. Knowledge of the double layer structure is key to understanding electrochemical reactivity, as solution species must traverse the structure at the interface in order to react.

The design and synthesis of energy efficient and stable electrochemical interfaces (materials and double layer components) for accelerating and directing chemical transformations, is key to developing new alternative energy systems – fuel cells, electrolyzers and batteries. Two types of interactions have traditionally been considered in aqueous electrolytes, depending on the nature of the reacting species, the supporting electrolyte and the metal electrodes: (i) direct –covalent bond formation between adsorbrates and electrodes, involving chemisorption, electron transfer and release of the ion hydration shell; and (ii) relatively weak non-covalent metal-ion forces that may affect the concentration of ions in the vicinity of the electrode but do not involve direct metal-adsortate bonding. The latter type of interactions are characteristic for the alkaline environment and it has been shown that the interaction of covalently bonded OH and alkal metal ions on Pt electrodes, leads to the formation of OH− cations complexes with the metal centered in the compact part of the double layer. Surface X-ray scattering (SXS) is an ideal technique for probing the atomic structure at the electrochemical interface. By combining specular crystal truncation rod (CTR) results (where the momentum transfer, Q0, is entirely along the surface normal direction) with non-specular CTR results (where Q0 has an additional in-plane component), it is possible to probe both the termination of the crystalline lattice and layer ordering above the interface, i.e. in the electrolyte layer, where the species are incommensurate with the underlying crystal lattice. X-ray reflectometry (XR) measurements, in which the scattered X-ray intensity at structural-sensitive reciprocal lattice positions is measured as a function of the applied electrode potential, are shown in Fig. 1 (b) an ‘anti-Bragg’ position on the specular CTR sensitive to any layered ordering at the interface, (c) and (d) ‘anti-Bragg’ positions on the non-specular CTR which is sensitive to interfacial atomic positions that are commensurate with the Ag lattice. The fact that the changes at the non-specular CTR positions are very small, ~4%, indicates that there are only subtle changes to the surface. In contrast, the changes at the specular CTR position are relatively large, ~50% change in the scattered intensity. The intensity along the specular CTR is sensitive to ordering normal to the interface, including species that are not necessarily commensurate with the Ag lattice; this implies that there are significant structural changes on the electrolyte side of the interface.

In order to derive a structural model for the interface, CTR data at three potentials (-1.0 V and -0.2 V) were measured by performing rocking scans around the surface normal at successive L values to obtain background-subtracted integrated intensities4. A potential of -1.0 V corresponds to the double layer region and no species are chemically bonded to the surface. At this potential, the best fit to the non-specular CTR data indicated that the surface Ag layer has a slightly reduced occupancy with a small inward relaxation of ~0.7% of the Ag(111) layer spacing (dAg=2.36 Å). Although this structural model gave a good fit to the [0, 0, L] CTR data, it did not give a good fit to the specular CTR. In order to get a good fit to the specular CTR data it was necessary to include a layer in the electrolyte at a distance of 4.1 Å from the topmost Ag layer. Given that there is no specific adsorption at negative electrode potentials, we assign this layer to hydrated K+ cations in the outer part of the double layer. The best fit to the data has a cation layer with a surface coverage, θK+=0.25±0.1 ML at a distance, dAg-K+=4.1±0.3 Å above the Ag surface.

As shown by the XRR data in Fig. 1, scanning the electrode potential from -1.0 V to -0.2 V substantially changes the specular CTR data. CTR data were measured at -0.2 V and a ratio of the two CTR data sets (I-0.2V/I-1.0V) showed clear systematic changes consistent with the potential-dynamic measurements presented in Fig. 1. The non-specular CTR ratio data was modeled by a change of relaxation that occurred at the inward surface relaxation of the topmost Ag atomic layer has increased to ~1.1% of the Ag(111) lattice spacing. In order to model the specular CTR ratio data set, however, it was necessary to include two layers in the electrolyte, one representing OH− and the other which we assign to hydrated K+ cations.

A schematic illustration of the interface structure determined from the CTR measurements is shown in Fig. 2 and summarises the results. At E=−1.0 V, where there is no chemisorbed species on the surface, the CTR measurements are consistent with the presence of a hydrated cation adlayer at a distance of 4.1 Å above the Ag surface. At E=−0.2 V, however, OH− stabilizes the hydrated K+ cations, at a distance of 3.6 Å above the Ag surface, through a non-covalent (van der Waals) interaction. Although the results presented here are specific to the Ag(111) electrode in alkaline solution, both the methodology and results are important in developing an understanding of the role that the compact electrolyte layer at the interface (and the specific cation in solution) plays in determining the kinetics of electrochemical reactions that occur in this potential range. Recent measurements, performed on beamline I07, have probed the structure of thin metal Ag and Cu films electrodeposited onto Au(111) surfaces. These systems have allowed the influence of the double layer structure on sacrificial metal oxidation to be studied in detail.

References

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Electroactive organic materials offer tunable functionality afforded through facile synthetic preparation balanced with good charge-transport properties arising from a highly conjugated polymer backbone. One well-studied example is PANI, which demonstrates a variety of oxidation states with associated colour changes, in addition to relatively high levels of conductivity. The polymer, however, is by nature poorly soluble, which inhibits solution processing and leads to a range of structural defects, thus limiting the observed conductivity. A solution to these issues is to use short- or well-defined oligomeric species, which afford tunable architectures and unlock new processing routes. Oligomers also offer enhanced supramolecular self-assembly as a means to overcome the disorder inherent in the polymeric analogue. This has been shown to be an effective strategy in the bulk, when combining oligomers with an acid-surfactant using the so-called ionic self-assembly (ISA) approach. ISA serves both to dope the oligomer to the conducting state and facilitate the self-assembly into well-ordered columnar structures through the secondary π–π conducting state and facilitate the self-assembly into well-ordered columnar self-assembly.

Despite the wide range of research into such materials, very little is known of the thin film properties. Such thin films are important as electronic devices and are often manufactured with an active organic layer of approximately 100 nm. Within this regime, interfacial effects can lead to a range of microstructures and crystalline orientations, thus giving rise to varied and unpredictable behavior. In this work we have prepared nanofilms of aniline oligomers and oligomer-surfactant complexes by drop casting onto silicon followed by solvent annealing both steps using tetrahydrofuran (THF) solvent. When dry, the structural details of the films were examined using synchrotron surface diffraction performed in the X-ray reflectivity (XRR) geometry. The effect of oligomeric molecular architecture and self-assembly behavior was studied by considering two oligomers: four aniline monomers (TANI) and eight monomers (OANI), the structures of which are shown in Fig. 1. Additionally, the thickness of the films was controlled by varying the concentration of the initial solution, allowing two regimes to be examined: thick film samples (~350 nm) and thin film samples (~35 nm).

Surface diffraction data revealed that in the native EB oxidation state the oligomers did not self-assemble into any long-range order. When complexed with the acid-surfactant BEHP, however, all thick film samples exhibited Bragg peaks in the reflectivity curves, indicating well-ordered lamellar layering parallel to the underlying substrate (Fig. 2a). In the case of TANI(BEHP)0.5, the d-spacing was found to be 2.15 nm. In addition, two higher order Bragg peaks can be seen corresponding to n = 2 and 3 reflections, indicating a high degree of long-range order. Using the interpretation of the bulk scattering data from this complex we can infer that the lamellar structure is made up of a bilayer of TANI molecules separated by interdigitated alkyl tails as shown in Fig. 3. This arrangement allows efficient packing of alkyl tails into a thermodynamically stable structure with optimal van der Waals interactions stabilized by n–n stacking. As XRR measures the electron density profile perpendicular to the substrate it is clear that the lamellae must be aligned approximately parallel to the substrate.

With a greater doping ratio, the multilayer spacing of TANI(BEHP)0.5 increased slightly by ~0.03 nm to 2.18 nm. This is not a significant departure from the value for TANI(BEHP)0.5, and suggests that the similar lamellar structure is retained despite doubling the volume of alkyl tails. There are two possible explanations for this observation: either the TANI(BEHP)0.5 complex has not been fully formed, or the crystal structure has expanded under optimal lattice. When the oligomer chain-length is doubled (i.e., in the OANI system) we found that the reflectivity curves retained the Bragg diffraction peaks, however, they were much broader with lower intensity.

The d-spacing increased to 2.35 nm but the overall level of order within the system was lower. The shape of Bragg diffraction peaks was considered to further characterize the degree of order in these films. The full-width at half-maximum (FWHM) is inversely proportional to the size of the ordered domain or coherence length \( L_z \), which for the TANI(BEHP)1.0 thick film was extremely large at around 100 nm. This value is especially significant since the thickness of the film was only around 150 nm. This \( L_z \) value was roughly halved to 48 nm for the TANI(BEHP)0.5 complex, possibly due to the greater volume of surfactant, giving the complex a softer nature and indicating greater disorder. For OANI(BEHP)0.5, \( L_z \) was only ~7 nm. This result can be explained in light of the different molecular architectures of TANI and OANI. With more monomer units in the chain, OANI is more flexible than TANI owing to the increased number of phenyl–nitrogen–phenyl bonds about which rotation can occur. This results in a greater entropic barrier to ordered structure formation. A summary of structural information is presented in Table 1.

Moreover, the Bragg angle \( \theta \) is related to the interfacial energy at the air-film interface. These results demonstrate that highly-ordered nanofilms of aniline oligomer-surfactant complexes with well-defined lamellar morphologies can be generated through drop casting and solvent annealing. We have found that, the presence of the surfactant dopant is necessary to induce self-assembly, the nature of organization within the film depends on the molecular architecture of the constituent components and the formation of structure is dependent on the thickness of the film, a property which is related to the interfacial energy at the air-film interface. These results provide a platform for us to correlate the self-assembled, ordered thin film structures with the enhanced conductive properties that they promise to mediate.

Reference


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Table 1: Summary of structural parameters for oligoaniline-surfactant complexes.
Materials Village
Steve Collins, Village Coordinator

The Materials Village has continued to thrive, with the established beamlines – I19, I16 and B16 – producing an exciting array of scientific highlights and developments. The most exciting newcomer to the village is the coherence and imaging beamline I13, which will be operated in collaboration with the University of Manchester. It is now available for users and is set to provide a world-leading resource for the future.

The beamline I13 is one of Diamond’s long beamlines, dedicated to hard X-ray imaging on the macro and nano length scale and coherence related experiments. It comprises two independent branches: the coherence branch, which welcomed its first users in October, and the imaging branch will followed out in April 2012.

The funding from the University of Manchester includes capital staff and operational costs towards the I13 imaging branch beamline in return for dedicated access. The staff financed through this collaboration have accelerated the completion of the I13 imaging branch and ensured its operation for the next seven years. The effort is further supported by a team from The University of Manchester, based at the Research Complex at Harwell, to drive forward the research.

Over the last year the construction of the I13 beamline has forged ahead, implementing novel concepts for the optical elements, such as a horizontally deflecting monochromator and mirror; and a robot arm for coherent diffraction X-ray experiments. The ‘mir’ beta-modification to the storage ring has proved extremely successful in enhancing the undulator brilliance, and has now been adapted for beamline I19.

To complement these advances in electron and X-ray optics, state-of-the-art imaging detector technology is essential. For the short-term, a MaxiPixII array has proved extremely successful in enhancing the undulator brilliance, and has now been adopted for beamline I19.

In addition an X-ray chopper and laser system have been successfully commissioned for time-resolved studies and the first successful study of a photo excited species, with a millisecond lifetime, was conducted at the start of the operation for the next seven years. The effort is further supported by a team from The University of Manchester, based at the Research Complex at Harwell, to drive forward the research.

The core science output of the beamline remains focused on electronic structure, magnetic and fundamental material properties and structure. The primary mandate of I16, Diamond’s Test beamline, is to facilitate advanced scientific experiments in the fields of diffraction and topography utilizing synchrotron radiation at all wavelengths. The primary mandate of I16 is to facilitate advanced scientific experiments in the fields of diffraction and topography utilizing synchrotron radiation at all wavelengths.

Considerable progress has been made in the commissioning of the Pilatus 300 K detector on the I19 X-ray diffractometer in Experimental Hutch 2, and a GDA software development is under test prior to making the detector available to tolerant and experienced users. Experimental Hutch 1 at beamline I10 continues to support the majority of users of the beamline and an increasing number are benefitting from the use of the robotic sample changer. A fluorescence detector for studies probing anomalous scattering effects due to absorption edges has recently been commissioned.

Over the last year the beamline has been used to determine crystal structures that have been included in a number of high-profile publications which are well represented in this report. Highlights include: the structure determination of a molecular perfluoroalkyl chain, which was synthesised using self-assembly techniques (Leigh et al.); the structure determination of a high-nickelity metal-organic nanosphere (Champness et al.); and the structural pressure dependence of a metal-organic framework system (Moghadam et al.).

The primary mandate of B16, Diamond’s Test beamline, is to facilitate technical developments in optics, detectors and instrumentation. This is complemented by a disparate and varied range of science based experiments. This test beamline facilitates research in many areas, from material science, to radiation physics and biology. In the past year, a range of experiments based on diffraction, reflectivity, imaging and topography have been progressed, making B16 an ideal platform to progress such experiments.

The radiation physics/biology research led by Fred Currell of Queen’s University, included in this section, is a superb example of cross-disciplinary research involving international collaborations and the use of complementary techniques across international facilities to understand the nanoscale mechanisms underlying a new approach to cancer therapy.

An interesting example of a technique development on B16 applied to an important science area is reported by Kukushkin et al. The micro-focused X-ray reciprocal space mapping was established to provide invaluable information about microstructure of the nitride alloy epilayers. Commercial use of nitrides spans a wide field from optoelectronics to microwaves.

The capability and functionality of B16 is continuously being evolved, and several important developments have been made in the last year. One of which was the establishment of techniques for at-wavelength (i.e. using X-rays) metrology on B16 and employing them to characterise several important X-ray optics, as it is demonstrated in the development section. I16 (Materials & Magnets), the most mature of the Village beamlines, continues to pursue an active development programme. A cryogen-free 2K cryostat, mounted on the six-circle kappa diffractometer, has recently been tested very successfully.

While scattering is restricted to the horizontal plane, this geometry is proving increasingly popular for studies of thin-film samples, and as a means of obtaining high quality resonant magnetic scattering with enhanced sensitivity to magnetic moment directions.

The more onwards and ever-increasing use of state-of-the-art area detectors continues, with two- and three-dimensional reciprocal space mappings finding their way to high quality publications. For example, the work by Gorfman et al. on the structure of an important ferroelectret material, PdB2Ti3O10 – or P21. The majority of this work has exploited the Pilatus 100K detector, but routine availability of the larger Pilatus 2M, mounted on its dedicated support stage, has prompted considerable activity in software development, with the short-term goal of using this large detector to prepare developments on the Pilatus. Continuous scanning has been tested with Pilatus detectors to provide data which is not only collected faster, but also with improved angular accuracy.

The core science output of the beamline remains focused on electronic ordering in novel materials, as you will see from this year’s science contributions. Pascut et al., used beamline 116 to investigate a novel symmetry-breaking in AgK, by resonant X-ray scattering, where it becomes energetically favourable to create a ‘charge-honeycomb metal’. Non-resonant magnetic scattering, on the other hand, was found by Johnson et al. to be the technique of choice for elucidating the unusual helical magnetic structure of LiCu4Al2S4, suggesting an unusual form of magnetic coupling to explain the obtained electric polarization in this multiferroic material.
Single-crystal resonant X-ray scattering was used on beamline I16 to reveal a novel form of spontaneous electronic order in a two-dimensional triangular metal described as a ‘charge-ordered honeycomb metal’. This phase occurs in the hexagonal metallic magnet AgNiO$_2$, which contains orbitally-degenerate electrons in the cross-over region close to the Mott transition. The electronic ordering in such materials has been intensively investigated, in order to understand related phenomena such as e.g. colossal magnetoresistance, and possibly high-temperature superconductivity. The charge ordered phase occurs as a surprising alternative to the conventional Jahn-Teller orbital order, ubiquitous in more localised systems. This radically different electronic metallic phase, which is charge- but not orbitally-ordered, was predicted theoretically based on indirect structural evidence that observed a small oxygen breathing distortion leading to expanded and contracted octahedra around Ni sites. Here we use resonant single-crystal X-ray scattering to be directly sensitive to changes in the electronic structure at the Ni sites themselves, and observe direct and quantitative evidence for charge order. By tuning close to a Ni resonance, we observe large enhancements of the supercell reflections and a rich spectrum as a function of energy. This we quantitatively explain in terms of interference scattering from Ni sites with energy-shifted atomic form factors, a characteristic signature of charge order.

Orbital ordering whereby orbital degeneracy is lifted either by a spontaneous lattice distortion driven by the cooperative Jahn-Teller (JT) effect, or by similar orbital physics, has been considered ubiquitous in both band and Mott insulators containing JT-active ions. For this reason, the recent proposal of a radically different type of electronic ordering in the weakly metallic hexagonal 2H-AgNiO$_2$ was surprising. In this scenario, orbital degeneracy at JT-active low-spin Ni$^{2+}$ would be lifted through charge disproportionation and charge ordering (CO) rather than orbital ordering (OO), in sharp contrast with the closely related but insulating nickelate 2H-NiO$_2$(gray balls). The dashed rhombus is the CO supercell. a) Oxygen breathing mode around electron-rich Ni sites. b) Ni$^{2+}$, Ni$^{3+}$ and Ni$^{4+}$ (electrons 0, 8/3, 2) in NiO$_3$ plane (top) and Ni$^{2+}$, Ni$^{3+}$, Ni$^{4+}$, Ni$^{5+}$, Ni$^{6+}$ (electrons 0, 8/3, 2, 4, 5) in NiO$_5$ pyramids (bottom). The dashed rhombus is the CO supercell. (gray balls). a) Oxygen breathing mode around electron-rich Ni sites. b) Ni$^{2+}$, Ni$^{3+}$ and Ni$^{4+}$ (electrons 0, 8/3, 2) in NiO$_3$ plane (top) and Ni$^{2+}$, Ni$^{3+}$, Ni$^{4+}$, Ni$^{5+}$, Ni$^{6+}$ (electrons 0, 8/3, 2, 4, 5) in NiO$_5$ pyramids (bottom). The dashed rhombus is the CO supercell.

Here we present resonant X-ray scattering data to probe directly the electronic order at the Ni sites. In particular, Ni K-edge resonant scattering probes primarily dipole-allowed transitions from the core 1s level to the valence $p_{3/2}$ ($1s^22p^6\rightarrow1s^22p^53s$) final states, which is strongly sensitive to changes in the coordination environment. From our data, we extract in an unambiguous way the anomalous scattering factors of the different Ni sites. By comparing the experimental results with LDA bandstructure calculations, we show that the 4p-level shift accounts for just over half of the edge shift, implying a core-level shift of 1 eV that provides direct evidence of CO. We also show that the results are quantitatively consistent with the amount of charge disproportionation predicted by band-structure calculations which prove that in weakly-metals close to the Mott transition, CO can be an energetically favourable way to lift orbital degeneracy that is driven by electron and hole transfer.

Experiments were performed on a small single crystal of 2H-AgNiO$_2$ (70 µm diameter in all planes) using the I16 beamline operated with a Si (111) double-crystal monochromator (ΔE/Emax=10$^{-4}$ at 8.35 keV). Fig. 2a shows the energy-dependent Bragg peak intensity, after correction for absorption and double-crystal monochromator (ΔE/Emax=10$^{-4}$ at 8.35 keV). Fig. 2a shows the energy-dependent Bragg peak intensity, after correction for absorption and conversion to absolute units, for three representative supercell reflections.

Figure 2: a) Energy-dependent intensity of three supercell reflections at 300 K near the Ni K-edge (solid lines) are model calculations using the atomic scattering functions shown in Fig. 3. b) Energy dependence of the empirically-extracted real and imaginary parts of the atomic scattering factors for Ni$^{2+}$ (solid line) and Ni$^{3+}$ (dashed line) obtained from the best fit to the data in a). The data points are shown by a square. a) Energy-dependent intensity of three supercell reflections at 300 K near the Ni K-edge (solid lines) are model calculations using the atomic scattering functions shown in Fig. 3. b) Energy dependence of the empirically-extracted real and imaginary parts of the atomic scattering factors for Ni$^{2+}$ (solid line) and Ni$^{3+}$ (dashed line) obtained from the best fit to the data in a). The data points are shown by a square.

The fact that the K-edge absorption energy of the electron-rich Ni$^{2+}$ is slightly different from that of electron-depleted Ni$^{2+}$, providing a strong energy-dependent contrast between the two sites. The spectra can be reproduced quantitatively in absolute units by a model (solid line) based on an unbiased reconstruction of the atomic scattering factors for the Ni$^{2+}$ (solid line) and Ni$^{2+}$ (dashed line) obtained from the best fit to the data in a).

The edge shift of ~2.5 eV suggests that there is a disproportionation of ~1.65 electrons, in very good agreement with the expected CO scenario of Ni$^{2+}$ and Ni$^{3+}$. However, the initial- and final-state contributions to the edge shift are typically of similar magnitudes, while only the former are directly related to charge ordering. The shift in the final state due to hybridisation with the oxygens, are calculated using LDA to be ~1.5 eV, which can account for only half of the total observed shift of 2.5 eV. This implies a shift of the core level of ~1 eV between Ni$^{2+}$ and Ni$^{3+}$, attributed to the different charge states of the distinct Ni sites. Furthermore, this extracted core level shift of ~1 eV is also in quantitative agreement with LDA calculations extended to calculate the core levels of the distinct Ni sites.

In summary, we have reported Ni K-edge resonant X-ray scattering measurements on a single-crystal of the orbitally-degenerate triangular-lattice metal 2H-AgNiO$_2$, to investigate the transition to a triply hexagonal unit cell. We have observed a large resonant effect on the superstructure reflections with a risk energy-dependent structure that can be quantitatively accounted for by interference scattering from the electron-rich and -depleted Ni sites with energy-shifted atomic scattering factors. By considering the various contributions to this edge shift we have determined a core-level shift of ~1 eV between Ni sites, in quantitative agreement with first principles electronic structure calculations, thus providing clear, direct and quantitative evidence of charge order in the triply hexagonal layers. Charge order as an alternative to orbital ordering in systems with Jahn-Teller active ions was recently brought into the spotlight in connection with 1D pronkite nickelates RNiO$_2$, and is likely controlling the essential physics of ferrates Ca$_2$FeO$_4$ and Fe$_2$O$_4$ and other orbitally-degenerate systems located in the crossover region between local Mott insulator and interlayer behaviour.

References
High-resolution X-ray diffraction study of single crystals of lead zirconate titanate ‘PZT’


L ead zirconate titanate, PbZr\(_1-x\)Ti\(_x\)O\(_3\) (PZT), is a perovskite-based ferroelectric which possesses exceptionally good piezoelectric properties and is currently the most important material in the piezoelectric industry. Although a number of studies of PZT have been performed to date, the reason for the remarkably piezoactivity is not yet understood. The piezoelectric response of PZT is particularly enhanced in the vicinity of the so-called morphotropic phase boundary (MPB), originally defined as a nearly vertical line on the \(x\)-\(\beta\) phase diagram, separating the Zr-rich \(\beta\) phase and the titanium rich \(\alpha\) [0] phase. Since these space groups are not group-subgroup-related, either a two phase coexistence region, or an intermediate phase should be present. The symmetry of any intermediate phase is particularly important, predefining such macroscopic properties as the allowed crystallographic direction of the polarisation, the non-zero piezoelectric tensor coefficients, type of domain pattern, etc. In particular the atomic structure, domain distribution and disorder parameters of PZT, especially for the composition ranges around the MPB, are matters of strenuous scientific debate.

As single crystals of PZT were not available until very recently, the most current conclusions regarding the structure and symmetry of this ferroelectric were drawn from powder-diffraction data, where the results are often ambiguous. The so-called monoclinic PZT phase, suggested by Noheda et al., is often described by the space group \(P2_1\) with \(P2_1\) symmetry of the same order. The analysis was followed by a least-squares fit of all the observed reflections, assuming different models for the symmetry of a single ferroelastic domain, and supposing that the twinning originates from the high-temperature prototypic phase of point group \(P\overline{4}\) [0]

The first successful synthesis of PZT single crystals for the composition close to the MPB region was only recently achieved. The aim of the work described here is to perform detailed X-ray diffraction studies of PZT single crystals to obtain the most precise current information about the average structure. Two single crystals of PZT (PZT31 and PZT46) were attached to a specially designed holder to be transferrable between diffractometers while maintaining the orientation of the crystal. The orientation of the crystal was determined and large areas of reciprocal space were explored using a Gemini I16 diffractometer (Agilent Technologies). The crystals were then transferred to a high-resolution X’ Pert Powder diffractometer (PANalytical) to collect high-resolution reciprocal-space maps around a series of selected Bragg reflections.

Finally, the crystals were taken to beamline I16 to measure representative reciprocal space maps for (00l), (10l) and (11l) type reflections at higher temperatures. The wavelength selected was 0.96 Å, corresponding to an X-ray absorption coefficient 3 times lower than for the laboratory X-ray source: the synchrotron measurements therefore provide information more distant from the bulk of the crystal, rather than from a surface layer.

Figure 1: Room-temperature reciprocal space maps for different \{hkl\} reflections collected for PZT46 (a), (b), (c) and PZT31 (d), (e), (f).

In conclusion, we have performed a series of high-resolution X-ray diffraction studies of twinned PZT crystals of two different compositions (PZT31 and PZT46). Analysing the angular positions of reflections lends credence to the key splitings, important in determining crystal symmetry, do not arise from the presence of adaptive phases. This provides further proof of the existence of the low-symmetry monoclinic phases in PZT. Furthermore, we have shown that the average structures of PZT of both investigated compositions are best described by a combination of at least rhombohedral and monoclinic symmetries.

References

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A new twist for multiferroics


Multiferroics are materials that are simultaneously magnetic and ferroelectric. The possibility to use them to develop an electrical read-and-write technology for magnetic information storage has stimulated an intense research activity in the past few years. Through electric polarisation and bulk magnetisation measurements, we show that a new complex oxide, Cu$_3$Nb$_2$O$_8$, is multiferroic below 24 K, the electric polarisation occurring concomitantly with a magnetically ordered phase. By using neutron powder diffraction and non-resonant X-ray diffraction measurements we demonstrate that the ferroelectric polarisation is induced by a long-range generalised helicoidal magnetic structure. Unusually, the electric polarisation was found to lie perpendicular to the plane of rotation of the magnetic moments. This observation cannot be reconciled with conventional theories developed for cycloidal multiferroics, in which the electric polarisation must strictly lie within the plane of rotation. Our results are consistent with a new multiferroic mechanism of ferroaxial coupling between a macroscopic structural rotation allowed in the paramagnetic point group, and magnetically induced structural chirality.

The research in this field has been driven by the discovery of a new class of materials, such as TbMnO$_3$. In this material the electrical polarisation is directly induced by magnetic symmetry breaking, and couples very strongly to the magnetism. Most materials of this kind possess the so-called cycloidal magnetic structure, where all the spins rotate in a plane containing the propagation vector of the magnetic wave. A comprehensive theory has been developed to account for the magneto-electric coupling in these cycloidal magnets, a strong prediction of this theory is that the electrical polarisation is constrained to lie in the plane of rotation of the spins. Here, we describe a previously unreported multiferroic compound, Cu$_3$Nb$_2$O$_8$, where the electrical polarisation is almost perpendicular to the plane of spin rotation, leading us to postulate a new magneto-elastic coupling mechanism.

High quality single crystals of Cu$_3$Nb$_2$O$_8$ were grown in an optical floating zone furnace, giving samples with approximate dimensions of 2 x 2 x 1 mm$^3$. The room temperature crystal structure was determined through lab-based X-ray diffraction and was found to adopt the centrosymmetric triclinic space group $P\bar{1}$. As shown in Fig. 1, there are two distinct Cu$^{2+}$ sites in the unit cell, located at Wyckoff positions $a$ (Cu$_1$, on an inversion centre and in square planar coordination) and $b$ (Cu$_2$ in a general position), which form sawtooth chains parallel to the a-axis. The chains are composed of Cu$_2$-$Cu_1$-$Cu_2$ steps linked by Cu$_2$-$Cu_2$ rows, and are separated along the b-axis by a layer of non-magnetic Nb$^{5+}$ ions (Fig. 1).

Heat capacity measurements (Fig. 2a) displayed two anomalies at 26.5 K and 24.2 K. Neutron powder diffraction experiments showed that the first anomaly was coincident with the onset of long-range antiferromagnetic order, as shown in Fig. 2b. Indexing the powder diffraction data indicated that the magnetic order had the propagation vector $Q = (0.487, 0.283, 0.0299)$ incommensurate in all reciprocal space directions. Given that the R$^3$m crystal structure has $P$$_1$ symmetry, any low temperature phase transitions were likely to be associated with the breaking of inversion symmetry (the only symmetry element except E) and hence result in a polar phase. Indeed, pyroelectric measurements confirmed the development of a ferroelectric polarisation below 24.2 K (Fig. 2c). The electric polarisation was measured in four independent directions, as shown in Fig. 2a, in order to determine the direction of the polarization, $P$$_1$. The polarisation vector $P$$_1$ was normal to the basal plane and parallel to the a-axis, consistent with a helicoidal magnetic structure.

To understand the multiferroic properties of Cu$_3$Nb$_2$O$_8$ it was necessary to determine the microscopic magnetic structure that induces the ferroelectric polarisation below $T_c$. Initial fits to the neutron powder diffraction data showed that the Cu$^{2+}$ magnetic moments form a rotating spin structure, however, it was not possible to uniquely determine the plane of rotation of the spins, a crucial ingredient in the theoretical interpretation of the multiferroicity. We therefore performed a non-resonant magnetic X-ray diffraction (NRMXD) experiment at 116 K, in which we exploit the dependence of the NRMXD cross section on the direction of the magnetic moment with respect to the incident and scattered directions of light and the x-ray polarisation. To reduce the fluorescent background the incident beam energy was tuned to 7.855 keV, well below the copper and niobium K absorption edges. Three magnetic diffraction peaks were located, and at each diffraction intensity (in both (001) and (002) channels) was measured whilst rotating the sample around the scattering vector (azimuthal scan). The data is plotted in Fig. 3. Initial fits to the azimuthal dependences showed that all spins rotated in a common plane within experimental error. The final fits, shown in Fig. 3, were therefore constrained to just four free parameters: the vector normal to the rotation plane $\mathbf{b}$ and two scale factors for each copper site, $F_1$ and $F_2$. These were found to be $\mathbf{b} = (75.52 \text{\degree}, 54.92 \text{\degree}, 1.000 \text{\degree})$. The above parameters were then fed back into the neutron powder diffraction data refinement to quantitatively determine the magnitude of the magnetic moments, $m_{\text{Cu}1} = 0.89(2) \mu_B$, $m_{\text{Cu}2} = 0.69(1) \mu_B$, and the relative phase of the two Cu$_2$ ions, $\phi = 75.5(2) \text{\degree}$. The magnetic structure comprises an essentially ferromagnetic coupling within the Cu$_2$-$Cu_1$-$Cu_2$ steps of the copper chains, with a predominantly antiferromagnetic coupling through the rings, as shown in Fig. 1. The incommensurate propagation vector then gives rise to a slow rotation parallel to the a-axis, and a fast rotation in the b- and c- directions. The most significant result of this study comes from comparing the direction of the electric polarisation with respect to the plane of rotation of the spins. $P$$_1$ was found to be almost perpendicular to the rotation plane (14° from the normal).

These results cannot be explained by the theory developed for cycloidal magnets such as TbMnO$_3$, which requires that the electrical polarisation be constrained to lie within the plane of rotation of the spins. We propose an alternative mechanism, which couples the chiral component of the magnetic structure to an axial rotation of the crystal structure that is allowed in the $P$$_1$ space group. This ‘ferroaxial’ coupling of spins to a ring of spins will lead to a small induced polarisation. However, the conceptual framework developed in this study has recently led us to the discovery of ‘giant’ antiferroelectricity at rather high temperature in the ferromagnetic manganite CaMn$_2$O$_4$.

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Microdiffraction X-ray reciprocal space mapping of nitride alloy epilayers


The III-nitride semiconductors, GaN, InN, AlN and their alloys, have become the subject of intense research in the past fifteen years due to their huge commercial utility and unique physical properties. These include a wide direct bandgap, considerable mechanical strength and high melting temperatures. The technological breakthrough in the epitaxial growth of GaN structures has revolutionised the optoelectronic devices, leading recently to robust and compact all-solid state light sources covering the broad spectral range from infra-red to ultraviolet. However, further advances in nitride technology, for example nitrides emitting in the green, red and ultra-violet spectral regions, require a deeper understanding of the microstructure and nanostructure of InN, GaN and AlN alloy films. X-ray Reciprocal Space Mapping (RSM) is a powerful tool to structure the materials. However, RSMs are usually measured in two dimensions ignoring the third dimension of diffraction space volume. The idea of full three-dimensional diffraction space mapping to obtain information on the structure of materials was first introduced by Fewster.

The samples studied were two InN epilayers, labelled A and B, and one InAlN, grown by Metalorganic Chemical Vapor Deposition (MOCVD) on GaN/N(100) substrates. The thickness of the InAlN and InN epilayers was 250 nm and 120 nm respectively. The X-ray beam with energy of 12000 eV (1 Å) was focused by Byrdram Chemical Refractive Lens (CRL) to a spot size with fwhm of 1.2 μm ×1.6 μm (horizontal × vertical). The measurements of the full 3D shape of the (10-13) RLP were done in skew symmetric geometry by changing the incident angle ω to the diffracting plane and recording the 2D diffraction pattern with a Pilatus100k area detector, which is estimated to be ~250 nm after demagnification by CRLs and change of beam footprint. The speckles is due to partial coherence of the incident X-ray beam. The width of the InGaN RLP is increasing when going from 20% to 9% of InN. As the substrate surface is covered, the structural disorder increases, thus, the breadth of the InGaN RLP is increasing when going from 20% to 9% of InN. Rutherford Backscattering Spectrometry (RBS) experiments confirmed the existence of composition gradient as shown in Fig. 5(b). Simultaneous fitting of experimental RBS spectra using NDF code [4] indicated by the breadth of the InGaN RLP.

Heteroepitaxial films with large lattice mismatch and exhibiting a high density of dilatations are best described as a collection of mosaic crystals, tilted and twisted with respect to each other. In the geometry of the experimental, a tilt φ along the [001] direction corresponds mainly to a twist with respect to the [010] direction through the following relation:

(1) \( \tan \varphi = \frac{\tan \Theta}{\sin \chi} \)

where \( \varphi \) is the angle between the (010) and (001) planes. A speckle pattern of diffracted X-ray intensity is observed for the InGaN ‘seed’ in both samples, as shown in Fig. 4. The appearance of the speckles is due to partial coherence of the incident X-ray beam. In this case of sample B there is only one sharp RLP on the Qx-Qz plane for the same samples. The inclined and vertical red lines in (a) and (b) indicate lattice constants for relaxed InGaN alloys and a lattice constant (3.189 Å) for InN. The X-ray beam with a twist of ω=20.251°, (b) Simultaneous fitting of experimental RBS spectra using NDF code [4], signal the existence of composition gradient as shown in Fig. 5(b). Simultaneous fitting of experimental RBS spectra using NDF code [4], signal the existence of composition gradient as shown in Fig. 5(b). Simultaneous fitting of experimental RBS spectra using NDF code [4], signal the existence of composition gradient as shown in Fig. 5(b). Simultaneous fitting of experimental RBS spectra using NDF code [4], signal the existence of composition gradient as shown in Fig. 5(b). Simultaneous fitting of experimental RBS spectra using NDF code [4], signal the existence of composition gradient as shown in Fig. 5(b). Simultaneous fitting of experimental RBS spectra using NDF code [4], signal the existence of composition gradient as shown in Fig. 5(b). Simultaneous fitting of experimental RBS spectra using NDF code [4], signal the existence of composition gradient as shown in Fig. 5(b).

The compositions estimated from RBS are in very good agreement with those estimated from RSM.

In summary, 3D RSM with a microfocused X-ray beam reveals a complex structure of InGaN and InAlN epilayers on the microscale and smaller. Strain-free and twisted InAlN nanocrystallites, less than ~250 nm in lateral size, are observed at the lower end of composition gradient. Formation of strain-free InAlN islands during the initial phase of growth was also observed by in-situ 2D RSM. Most importantly it was observed that structural and compositional disorder increases with growth time, thus supporting suggestion that the islands observed in our in-situ experiments correspond to the beginning of epitaxial growth. The twist of InAlN nanocrystallites is caused by the twist of the underlying GaN mosaic blocks which, in the case of 11-11 nitrides, was found to be related to a type edge dilatations. The lattice mismatch between InGaN and GaN may be relieved by the microstrain associated with a type-edge dislocations. The lattice mismatch between InGaN and GaN may be relieved by the microstrain associated with a type-edge dislocations. The lattice mismatch between InGaN and GaN may be relieved by the microstrain associated with a type-edge dislocations. The lattice mismatch between InGaN and GaN may be relieved by the microstrain associated with a type-edge dislocations.

References

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**Radioenhancement of gold nanoparticle doped biological systems: understanding the nanoscale mechanisms underpinning a new approach to cancer therapy**


**Figure 1.** Results of Monte Carlo radiation transport simulation showing d-shell ionisation by a 50 MeV proton near the surface of a 20-nm diameter gold nanoparticle imaged at the same magnification. The plasmonic track is shown in green while the electron tracks are shown in red. The phototransformation and the first Auger electrons depart with high energy and are responsible for p1 ionisation, producing fairly straight tracks. These latter energy electrons give rise to multiple interactions disloacting all of their energy near the nanoparticles so is essential for the sample and localized track structure they produce.  

**Figure 2.** Measured energy dependence of the enhancement of DNA single strand break induction, compared to a model that considers the nanoscale energy dose due to the absorption of the gold.

**Figure 3.** Spatial microdosimetry of energy deposition in DNA damage induced by gold nanoparticles. The data show that the gold nanoparticles are acting as radiation enhancers with a maximum localised enhancement effect occurring near the nanoparticle where the energy deposition is highest. The data show that the gold nanoparticles are acting as radiation enhancers with a maximum localised enhancement effect occurring near the nanoparticle where the energy deposition is highest.

**Figure 4.** Diagram showing the cross-section of the cell, the DNA damage and the repairs that take place. Since these images are collected using confocal microscopy it is possible to build up a three-dimensional representation of the cell, the DNA damage and the gold nanoparticles. Such rich data sets contain lots of information about the relationship between the gold and the DNA damage. We are currently developing data-analysis tools to mine this data with a view to extracting this relationship.

**References**


High-nuclearity metal-organic nanospheres


The synthesis of high-nuclearity transition metal co-ordination clusters has been the focus of much research over recent years due to interest in tailoring the properties of nanoscale objects. Such molecules are a target for bottom-up nanotechnology fabrication techniques and, despite many potential applications, large clusters are often most admired for their attractive architectures and topologies that are reminiscent of Platonian and Archimedean solids and even viruses.1 We report herein the synthesis and full characterisation of a highly unusual Cd6 cluster, a high-nuclearity system which comprises a central inorganic Cd-oxo-hydroxy-nitrate cluster core surrounded by an organic shell of ligands. This highly unusual molecular compound has a molecular weight of about 23,800 Daltons, has an external diameter of 3.18 nm, an internal diameter of 1.22 nm, and thus, can be considered a metal-organic nanosphere.

As a development of our research into metal-organic frameworks12 we have initiated a research program to synthesize high-nuclearity transition metal clusters. Our strategy employs a carboxylate-based ligand H3L which was designed as an ambivalent non-planar building-block that can bind to metal centers via three carboxylates on the same face to stabilise high-nuclearity clusters, or via carboxylate donors that point in opposing directions to generate co-ordination polymers.

Reaction of H3L and Cd(NO3)2·4H2O in dimethylformamide (DMF) solution affords single crystals of a compound as colourless parallelepipeds. Single crystal X-ray analysis on Diamond beamline I19 revealed the resulting compound to be a highly complex cluster composed of a spherical shell of 66 Cd(II) cations bridged by 28 μ3-hydroxide, 16 μ3-oxygen and 5 μ5-nitrate anions, surrounded by a shell of 20 triadipod capping ligands (L3-) and 12 DMF ligands, [Cd66(µ3-OH)]12[µ3-OH][µ3-O]-[µ5-N]2[µ3-OH][µ3-Cd(II)]12(L)20(µ2-DMF)12 (Fig. 1). The cluster crystallizes in the trigonal space group R3 with the cluster having C3v crystallographic symmetry and idealised chiral tetrahedral T symmetry. However, each crystal is a racemate with chiral molecules of both hands found in the unit cell.

A dual-shell structure for the cluster can readily be observed (Fig. 2) in which a central inorganic core is covered by an organic layer of ligands L3-. The 66 Cd(II) cations can be divided into six groups of centres with the same approximate symmetry; five groups of twelve centres show a distorted octahedral co-ordination geometry, and one group of six centres have bio-capped square pyramidal geometry. The NO3- anions lie parallel to the surface of the inorganic core with each anion bridging five Cd(II) centres (Fig. 3). Considering the idealised T symmetry of the cluster, there are two ligand environments (Fig. 2); eight ligands sit at the corners of a cubic array on positions of three-fold symmetry, bridging a triangle of three symmetry-related Cd(II) cations; twelve ligands sit along the edges of the cubic array bridging to one central Cd(II) via their three-carboxylate groups. These two types of ligand have opposite twist i.e. eight (three-fold symmetric) ligands have a clockwise twist, with the remaining twelve ligands having an anticlockwise twist.

Some electron density peaks in the centre of the inorganic shell could not be modelled as discrete atomic sites, but this void of 861 Å3 is large enough to accommodate up to nine small solvent molecules, assigned as DMF.

If the 66 Cd(II) centres and 12 NO3- anions are considered as vertices, a polyhedral framework can be constructed (Fig. 4). The 66 vertices are all six-connected with Cd6 separations ranging from 3.49 to 3.99 Å, with the NO3- anions five-connected to Cd(II) centres (66 N centers ranging from 2.74 to 3.31 Å). Importantly, the 12 NO3- vertices are the precise number of five-co-ordinate defects necessary to allow the 66 six-co-ordinate vertices to enclose a sphere as stated by Euler’s theorem. Thus, the convex polyhedron has 78 vertices (V), 120 edges (E) and 152 triangular faces (F) giving it a dodecahedron Euler characteristic of χ = 2 (V+E−F).

Although the Cd66 nanosphere is only sparingly soluble in organic solvents the compound can be characterised by XMR spectroscopy, MALDI mass spectrometry and Dynamic Light Scattering (DLS) measurements, the last indicating a species with a diameter of 2.9(±1.1) nm, consistent with the structurally characterised molecule.

In conclusion, we have successfully demonstrated the synthesis and characterisation of a highly unusual, high-nuclearity Cd6 metal-organic nanosphere. Our strategy leads to the formation of a dual-shell cluster defined by an inorganic shell built from Cd(II) cations, O2-, HO-, and NO3- anions, capped by an organic shell of tricarboxylate and DMF ligands. The cluster forms a unique polyhedra made of hexagonal prisms, icosahedrons and icosidodecahedrons which encompass a large void space over 1.2 nm in diameter. Future work seeks to develop and investigate new methodologies for the synthesis of complex high-nuclearity metal nanoclusters and aggregates.

References

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Figure 1: View of ligand H3L.

Figure 2: View of the Cd66 nanosphere. Atom colours: cadmium dark blue; oxygen red; ligands of different symmetry coloured light blue and purple. Ligand at front of ball removed to aid clarity.

Figure 3: View of the co-ordination environment of nitrate anions in the Cd66 nanosphere. Atom colours: nitogen blue; oxygen red; cadmium green.
Squeezing porous materials


Porous materials have been used, amongst other applications, for storing gases such as hydrogen to be used as a clean energy source, with one of the most studied materials being metal organic frameworks (MOFs). MOFs are special because we can chemically tune their pore size and selectivity, thereby tuning its application for specific target guests. To date, chemical modification has been the primary route to change pore size, with chemicals utilising temperature as the main variable in their manufacture. In our group, we have been using extreme pressures to modify the pores of MOFs. Applying high pressures may seem impractical for pore modification; however e.g. guanacol is already manufactured at 8000 atmospheres in the food processing industry. Here, we apply pressure by loading our sample into a diamond anvil cell. We then surround the sample with a liquid (or medium) to make sure that pressure is applied evenly. In this study, applying pressure to a MOF called Cu-btc caused the medium to be squeezed into the pores. This caused Cu-btc to expand on initially increasing pressure. On increasing pressure further, more material was then squeezed into Cu-btc until a gassing pressure of 50,000 atmosphere was reached. Above this threshold, the liquid comes out of the pores due to pressure, the sudden decrease and increase in length of the Cu-O bonds. The ability to tune pore content with pressure has numerous applications. Synchrotron radiation allows us to study this behaviour in detail, which is essential if we are to use this technology.

A large body of scientific research is directed towards the design and synthesis of materials of ever more varied and complex variety of porous metal-organic framework materials. MOFs have been designed for a number of different applications, including molecular sensing, gas separation and storage, drug transport and as high surface area catalysis. Whatever the application, the primary factor that makes MOFs so appealing is the ability to tune their pore size and shape, and therefore selectivity. To date, there is a large body of literature where these properties have been altered via ‘chemical’ means, modifying the rigid organic unit, metal linker, or both. More recently, the ability to tune the pore size and shape of MOFs, including guest content, has been achieved by applying high pressures (>70,000 atmosphere).

In this study, a high pressure single crystal X-ray diffraction study was performed on the porous framework Cu-btc ([Cu₃(BTC)₂(H₂O)₃]·8H₂O) at pressures up to 5.0 GPa (Table 1). Prior to our pressure experiment, an ambient pressure single crystal data, it was clear that there were some discrepancies in reported structural details for Cu-btc (Fig. 1). From our high pressure X-ray data set we collected on a crystal equipped with 600 µm culets and a tungsten gasket using a 16:3:1 mixture of Cu-btc in order to provide data for comparison with the high pressure studies (which were also performed at ambient temperature). The same crystal was then loaded into a Merrill-Bassett Diamond Anvil Cell (DAC) equipped with 600 µm culets and a tungsten gasket using a 60:1:3 mixture of methanol-water-ethanol-water (20:3:5:2) as the hydrostatic liquid. High pressure data were collected at (approximately) 0.5 GPa steps between ambient pressure and 5.0 GPa (Table 1).

Cu-btc crystallizes in the cubic space group Fm-3m (a = 26.434 (6) Å, Vol. = 18471(12) Å³). Pairs of Cu(II) ions, bridged by four carboxylate (μ 2-OCO) groups on discrete 1,3,5-benzenetricarboxylate (btc) ligands, form Cu(btc)₃ dimers coordinate to water molecules to form a Jahn-Teller distorted axis creating a distorted octahedral environment around the Cu(II) centres (Fig. 2). Cu-btc crystallises in the cubic space group Fm-3m (a = 26.434 (6) Å, Vol. = 18471(12) Å³). Pairs of Cu(II) ions, bridged by four carboxylate (μ 2-OCO) groups on discrete 1,3,5-benzenetricarboxylate (btc) ligands, form Cu(btc)₃ dimers coordinate to water molecules to form a Jahn-Teller distorted axis creating a distorted octahedral environment around the Cu(II) centres (Fig. 2). The unit cell compression of Cu-btc is therefore likely to be mediated through the Cu-btc bonding interactions within the Cu-btc-paddlewheel. In Cu-btc, there are two symmetry independent Cu-btc bonding interactions, the axial Cu-O bond (Cu-O2) which points into the pores of the guest-accessible cavities at [0,0,0] and the equatorial Cu-O bond (Cu-O1, Fig. 2). All four Cu-O1 bonds are symmetry equivalent. On initially increasing pressure to 0.5 GPa, no reduction in the equatorial Cu-O1 bond, while the axial Cu-O2 bond actually increases in length (Fig. 1). This coincides nicely with the increase in unit cell volume observed on increasing pressure to 0.5 GPa, which suggests that the swelling phenomena observed here is driven by the elongation of the Cu-O2 bonds. We postulate that increasing the pore content with NEV at 0.5 GPa increases h-bonding interactions with the O2 water ligand that points into the large central pore volume at [0,0,0], weakening the Cu-O2 bonding to cause it to increase in length. It is striking to see that, upon increasing pressure further to 3.9 GPa, the axial Cu-O2 bond continuously decreases in length, even though the pore content increases, while the equatorial Cu-O1 bond remains unchanged (Fig. 3). It would therefore appear that after the initial swelling, the overdriving increase in length again, while the volume decreases continuously. The data collected shows that on initial application of pressure, the pores are filled with the NEV medium. On increasing pressure further to 5.0 GPa, a sudden and marked decrease in volume occurs. This region again represents a pore emptying mechanism. However, although a decrease in pore content is observed on increasing pressure from 3.9 GPa to 5.0 GPa, the pore content is not reduced substantially (~16% decrease), and equates to approximately the same pore content as observed at 3.1 GPa on increasing pressure. The sudden decrease in volume can therefore not be solely due to the pores emptying above 5.0 GPa.

In our previous work on amino acids, compression of covalent bond length is not expected within this pressure regime, rather the compression of much weaker intermolecular interactions takes place. By contrast, metal-ligand bond distances are more flexible and compliant in nature, for example, pressure induced c-ordination changes have been observed. The unit cell compression of Cu-btc is therefore likely to be mediated through the Cu-btc bonding interactions within the Cu-btc-paddlewheel. In Cu-btc, there are two symmetry independent Cu-btc bonding interactions, the axial Cu-O bond (Cu-O2) which points into the pores of the guest-accessible cavities at [0,0,0] and the equatorial Cu-O bond (Cu-O1, Fig. 2). All four Cu-O1 bonds are symmetry equivalent. On initially increasing pressure to 0.5 GPa, no reduction in the equatorial Cu-O1 bond, while the axial Cu-O2 bond actually increases in length (Fig. 1). This coincides nicely with the increase in unit cell volume observed on increasing pressure to 0.5 GPa, which suggests that the swelling phenomena observed here is driven by the elongation of the Cu-O2 bonds. We postulate that increasing the pore content with NEV at 0.5 GPa increases h-bonding interactions with the O2 water ligand that points into the large central pore volume at [0,0,0], weakening the Cu-O2 bonding to cause it to increase in length. It is striking to see that, upon increasing pressure further to 3.9 GPa, the axial Cu-O2 bond continuously decreases in length, even though the pore content increases, while the equatorial Cu-O1 bond remains unchanged (Fig. 3). It would therefore appear that after the initial swelling, the overdriving increase in length again, while the volume decreases continuously. The data collected shows that on initial application of pressure, the pores are filled with the NEV medium. On increasing pressure further to 5.0 GPa, a sudden and marked decrease in volume occurs. This region again represents a pore emptying mechanism. However, although a decrease in pore content is observed on increasing pressure from 3.9 GPa to 5.0 GPa, the pore content is not reduced substantially (~16% decrease), and equates to approximately the same pore content as observed at 3.1 GPa on increasing pressure. The sudden decrease in volume can therefore not be solely due to the pores emptying above 5.0 GPa.

In summary, we have shown that by applying pressure to Cu-btc we can force the hydrostatic medium to enter the pore, initially causing the sample to expand. On increasing pressure further to 3.9 GPa, the unit cell volume and the axial Cu-O2 bond contrast, even though more solvents enter the pore. On increasing pressure further to 5.0 GPa, we enter a pore emptying region. The volume decreases quite dramatically here, however we discover that the transition from a pore filling to a pore emptying mechanism is in fact associated with the sudden compressibility of the stiffer equatorial Cu-O1 bonds. The Cu-O1 bonds are actually very resilient to any compression to 3.9 GPa, and only contract on increasing pressure to 5.0 GPa.
Knots are found in DNA and proteins and even in the molecules that make up natural and man-made polymers, where they can play an important role in the substance’s properties. For example, up to 85% of the elasticity of natural rubber is thought to be due to knot-like entanglements in the rubber molecules chains. However, deliberately tying molecules into knots so that these effects can be studied is extremely difficult. Up to now only the simplest types of knot, the trefoil knot with three crossing points and the topologically-trivial unknot without any (zero) crossing points, have succumbed to chemical synthesis using non-DNA building blocks. Here we describe the first small-molecule pentafoil knot, which is also known as a cinquefoil knot or a Solomon’s seal knot — a knot with five crossing points that looks like a five-pointed star. The structure of the knot was determined using data collected on I19 through the Engineering and Physical Sciences Research Council (EPSRC) National Crystallography Service. Making knotted structures from simple chemical building blocks should make it easier to understand why entanglements and knots have such important effects on material properties and may also help scientists to make new materials with improved properties based on knotted molecular architectures.

Figure 1: The topologies of the four simplest prime knots: (a) unknot (zero crossing points); (b) trefoil knot (three crossing points); (c) figure-of-eight knot (four crossing points); (d) pentafoil knot (five crossing points).

Knots are important structural features in DNA. They are found in some proteins and play a significant role in the physical properties of both natural and synthetic polymers. Although billions of prime knots are known to mathematicians, the topologically-trivial unknot, i.e. a simple closed loop without any crossing points (Fig. 1a) and the next simplest knot, featuring three crossing points, the trefoil knot (Fig. 1b) are of particular interest. A pentafoil knot (Fig. 1d) or cinquefoil knot (the 5 1 knot in Alexander-Briggs notation) — a torus knot* with five crossing points, is inherently chiral, and is the fourth prime knot (following the unknot, trefoil knot and figure-of-eight knot (Fig. 1c)) in terms of number of crossing points and complexity.

The synthesis of a molecular pentafoil knot was achieved in a one-pot, 16-component self-assembly reaction by combining the use of metal helicates to create crossover points, anion template assembly to form a cyclic array of the correct size, and the joining of the metal complexes by reversible imine bond formation, aided by the gauche effect to make the continuous 160-atom-long covalent backbone of the molecular knot (Fig. 2).

Single crystals of the molecular pentafoil knot were obtained by slow diffusion of diethyl ether vapour into a solution of the knot in acetonitrile:toluene (3:2) and the solid-state structure determined by X-ray crystallography on station I19. The crystal structure (Fig. 3) confirmed the topology and symmetry of the molecular pentafoil knot. The single organic ligand weaves a continuous path about the five co-planar iron centres, the loop passing over and under itself each time it wraps around a metal ion.

The pentafoil knot has symbolic significance in many ancient and modern cultures and religions (as does its two-dimensional projection, the pentagram) and features as the central emblem on the present day flags of both Morocco and Ethiopia. The practical significance of its preparation in molecular form includes the lessons learned from the multitude of different structural design features used in its assembly and the potential for the synthesis of higher order structures with precisely defined knotted architectures that may enable the role of entanglements in molecular materials to be elucidated and exploited.

References

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Figure 2: Synthesis of a molecular pentafoil knot.

Figure 3: X-ray crystal structure of a molecular pentafoil knot. (Image credit: Robert W. McGregor, www.mcgregorfineart.com).
Development of at-wavelength metrology on B16

Hongchang Wang, Sébastien Berujon and Kavil Sawhney

One challenge of the research with synchrotron radiation is to achieve coherence preserving and diffraction-limited hard X-ray optics. This requires development of precise, accurate and repeatable metrology. In this respect, in-situ at-wavelength, i.e. using X-rays, methods are considered a major pathway. Two of these methods have been established and further developed on B16, Diamond’s Test beamline. One is based on a grating shearing interferometer whereas the other is based on the X-ray speckle tracking technique. Both at-wavelength metrology methods have opened up new possibilities for an optics characterization.

Grating interferometer

Grating interferometers are very attractive for performing at-wavelength metrology as they are able to measure wavefront gradients with tens of nanoradian accuracy and with a low sensitivity to mechanical vibrations. Furthermore, they have a low demand on longitudinal and transverse coherence. The interferometer consists of a one dimensional (1D) phase grating as a beam splitter and an absorption grating as a transmission mask for the detector. The two gratings produce Moiré fringes, and the calculation of their distortion from the straight lines allows one to measure wavefront aberration of the order of the wavelength. Such an interferometer has been established on B16 that works at hard X-ray energies (8 -20 keV). Both the Moiré fringes method and the phase stepping method can be used to determine the wavefront distortions. The method has been enhanced by developing a rotating shearing interferometer technique, in which one of the two gratings of the shearing interferometer is rotated to derive the X-ray wavefront radius of curvature and its distortions from a single image. The method has been successfully used to derive the wavefront distortions caused by reflecting mirrors and thin compound refractive lenses.

Recently, a 2D grating interferometer has been introduced into the X-ray regime which allows, simultaneously, the recovery of both the horizontal and vertical beam phase gradient, eliminating most of the common reconstruction artifacts usually encountered with the 1D interferometer. We have demonstrated that it is also possible to perform sub-micron phase microscopy using a Fresnel zone plate (FZP) and a 2D grating interferometer. The technique provides the spatial resolution of absorption-contrast microscopy, and also provides the wavefront gradient profile of the specimen with a high sensitivity, independent of the orientation of the sample features.

A sketch and a photo of the experiment setup are shown in Fig. 1 and Fig. 2 respectively. A bunch of carbon fibres has been used as a representative specimen. The two reconstructed orthogonal wavefront gradient maps are shown in Fig. 3. It can be seen that the vertical features in the horizontal wavefront gradient map have very low visibility, and vice versa: this demonstrates the advantage of the 2D grating interferometer over the more traditionally used 1D interferometer. When working with a 1D grating interferometer, the gradient map is lost in one direction because of the insensitivity of the device to objects oriented parallel to the lines of the grating. This problem can be solved by using simultaneously the vertical and horizontal gradients, the final reconstructed wavefront is shown in Fig. 4. For comparison, absorption and dark field images are also shown. The sensitivity of our FZP-2D grating microscope is <100 nrad, and this high and quantitative sensitivity thus makes it a promising candidate for sub-micron analysis of complex samples made of light materials and different feature orientations.

X-ray Speckle Tracking Technique

Sébastien Berujon, who is a joint PhD student of the ESRF and of the Diamond Light Source, has developed a new method to analyse quantitatively the wavefront of a partially coherent X-ray beam using two-dimensional speckle patterns. The X-ray speckle tracking (XST) technique is based on the use of 2D speckle patterns combined with digital image correlation algorithms, and offers a pixel size resolution, providing a 2D wavefront gradient. This technique has a low sensitivity to mechanical vibrations and the requirements on transverse and longitudinal coherence are also low.

The setup is quite simple, requiring only the random phase object and a 2D detector to resolve the high-spatial frequency features contained in the object. A solid membrane, easy to align and with low sensitivity to vibrations, produces a random intensity speckle pattern that is static. The simplicity of the setup of the XST technique also avoids any tedious calibrations. By recording this random pattern two times in planes located at two different distances from the membrane (Fig. 5) or in the same plane at two different time intervals, the ray paths or their evolution can be tracked using a digital image correlation algorithm capable of sub-pixel accuracy.

The XST method can be understood as a high spatial frequency intensity modulation of the wavefront using motionless speckle to trace the geometrical path of the light passing through each pixel of the detector. To demonstrate the application of the XST technique, a 2D parabolic, rotationally symmetric compound refractive lens (CRL) has been characterized. The reconstructed wavefront influence function of the CRL is shown in Fig. 6. The corresponding aberrations of the lens can then be retrieved from the wavefront profile, which are otherwise difficult to measure.

Summary and Outlook

The grating interferometer and the X-ray speckle tracking technique have both been established and commissioned on B16, and user-friendly software has been developed on the IODL platform for complete data analysis. Both techniques are compact, robust and have low requirements on longitudinal and transverse coherence. The representative examples given above show that sub-microradian accuracy in determining the wavefront gradient is achieved. These at-wavelength metrology methods will enable determination of the wavefront distortions caused by the various beamline components like mirrors, monochromators, die windows, refractive lenses, Fresnel Zone Plate, KB mirrors etc.

References

2011 has been a year of intense development in the MX Village, in particular it has seen the completion of a two year project to design, manufacture and install new end-stations for three beamlines; the beginning of an upgrade program of mirror motion systems and the mirrors themselves for beamlines I02, I03 and I04; the upgrade of motion stages in I24's microfocusing mirror assembly; and the completion of an upgrade to I04-1 and I124's sample changer. These developments have been keenly balanced against minimising downtime and allowing the high impact science detailed below to be delivered by our user community. Since the last report at least another 300 structures have been added to the Protein Data Bank.

Looking to the future it is a pleasure to report that the final beamline to be approved for construction in Diamond Phase III program is the versatile microfocus and in situ beamline proposal (VMX) that was submitted on the behalf of the MX community by Dave Brown et al. The VMX beamline will consist of a submicron tuneable end-station and a dedicated in situ data collection end-station. The beamline and its two end-stations will bring the final count of MX Village end-stations to seven by 2017 and enable a broad range of unique capabilities while maintaining its core ability of satisfying the UK user demand for high quality and high throughput structure solution.

In September 2011 the Village hosted the 2nd MX user workshop at the Diamond Synchrotron Users meeting. The workshop had about 50 attendees and comprised sessions on 'Spectroscopy', 'Crystal dehydration and room temperature data collection', 'using microscopes' and 'getting the most out of your beamtime'. Each session saw one staff and two user presentations where experiences of using beamlines and equipment effectively for science were shared.

New end-stations for I02, I03 and I04

In the past year a team of Diamond beamline scientists, engineers and technicians led by Dave Hall (picture) has been delivering new end-stations to beamlines I02, I03 and I04. The new end-stations incorporate the best of the previous sample environments whilst providing a versatile chassis for adding new features which have been in parallel development within the MX Village. Several novel features are already available to users, including microfocus beams (in I04), minibeam apertures, improved sample viewing and positioning, easy switching between cryogenic to room temperature or humidity controlled environments and in situ data collection. Current developments that will be rolled out for routine use in 2012 include the integration of microspectrophotometry, mini-kappa geometry and further improvements to microfocus capabilities and variable beam sizes. This platform is in the handover to the MX team at Diamond to develop and deliver new functionality to our users. The new end-stations are now in routine use on beamlines I02, I03 and I04 and are supporting the user programme with aplomb.

Mirror polishing to achieve improved beam quality and beam size versatility on I02 and I03

A programme of work to refurbish the horizontally and vertically focusing bimorph mirrors on I02, I03 and I04 has been running and towards the end of 2011 the repolished mirrors were reinstalled on I02 and I03. Beam sizes at the sample down to 17 µm (v) × 70 µm (h) are now routinely delivered with fluxes in excess of 1.2×10^12 ph/s. The high quality of the polish is illustrated in Figure 1 and this results in the ability to defocus the beam away from the sample plane thereby increasing the vertical beam size up to 80 µm while preserving the near-perfect Gaussian profile of the beam. This ability to vary the beam size while preserving its quality is vitally important to users for optimising signal to noise in their diffraction data and will be the subject of ongoing collaboration with the Optics Group at Diamond throughout 2012. The final set of mirrors for I04 are now being repolished and will be reinstalled later in 2012 making the three Phase I MX beamlines an immensely competitive set of instruments. The important capability of flexible beam size has been a key factor in the success of I04 to date and has also been recently introduced to I04-1 through the use of defining apertures ranging in size from 10 - 70 µm.

Microfocusing on I04 using CRLs (Compound Refractive Lenses)

Since July 2011 I04 has been delivering beam focused using compound refractive lenses rather than the more conventional mirror set-up. The mirror systems are currently undergoing repolishing to provide improved beam profile and flux and are expected to return later in 2012. By using a carefully selected CRL setup I04 has continued to operate a highly successful user programme in the absence of mirrors and has been able to deliver beams down to 2 µm (v) x 8 µm (h) in size at two specific energies. Intuitive beamline software has enabled users to select which beam size they would like to use and have the beamline set itself up accordingly. The use of CRLs in combination with focusing mirrors is currently under investigation on I02 and I03 with a view to delivering a highly versatile focusing arrangement on I02, I03 and I04 towards the end of 2012.

Faster sample changers for I04-1 and I124

The end-effectors for the CARS sample changer systems on I04-1 and I124 were upgraded during 2011 and now deliver exchange times of 45 seconds for the users. This upgrade follows a similar upgrade to the ACTOR sample changers on I02, I03 and I04 from the previous year and now ensures that all beamlines can operate robustly to the highest levels of throughput. On average the combination of fast exchange sample changers and high frame rate Pilatus 6M and 2M detectors can double the data throughput of users.

I23 team begins assembly of beamline components

The lead batches of the long-wavelength MX beamline I23 started to appear in the experimental hall during summer 2011, with the cabins following before the end of the year. In parallel, all contracts for the major beamline components were signed, with the semi-cylindrical Pilatus 12M being the most outstanding one. Design of the I23 in-vacuum end station will have priority in the coming months, while the components for the optics hutch will be assembled and installed to receive first light in autumn 2012. So far all milestones have been met and the beamline is on track for first users in autumn 2013.
How DNA is kinked by ruthenium complexes and by dehydration


Ruthenium is a rare element, a heavier analogue of iron, and a key sensitizer in some solar energy conversion systems. This rarity may prove to be a handicap in that application, but is less so in the more value-added applications of DNA sensing and photodynamic therapy. Both applications make use of the valuable photochemistry and synthetic versatility of ruthenium complexes, which, unlike iron, keep their enantiomeric purity in solution. Although ruthenium polypyridyl complexes have been known to bind to DNA since the 1980s, most famously giving the so-called ‘light switch’ effect (fluorescence on binding), there has been no crystallographic evidence for any of the suggested binding models. Recently we were successful in obtaining crystals of two ruthenium complexes, one of which is photooxidising, bound to several DNA decamer sequences. Unexpectedly, the structures show intercalation by one ligand of the complex into one DNA duplex, though shallow enough to permit a second ligand to kink a second duplex, a mode of binding known as semintcalation. The net result is that two duplexes are noncovalently crosslinked. In a second structure, we see three binding modes, the new one being a classical symmetrical perpendicular intercalation from the minor groove. All three modes of binding appear to be DNA sequence-specific, and involve the recognition of a specific basepair step. The kinking of DNA by small molecules turns out to be a rather unusual phenomenon, though well known in the area of transcriptional regulation by regulator proteins. By controlling crystal humidity at room temperature, we have shown the reversible dehydration of this crystal, which is accompanied by further kinking of the DNA.

Despite the enormous amount of work on DNA and its binding by small molecules, there are comparatively few atomic resolution structure determinations showing distinct binding modes. ‘Classical’ intercalation is well documented, for example by daunomycin, which intercalates from the minor groove. Such structures have illuminated the field of anticancer drug design for many years. The binding of the platinum anticancer drugs to DNA, when such a complex was eventually crystallized, showed covalent linkage between platinum and two adjacent guanine residues in the major groove, resulting in a 53° kink at that site, constricting the major groove and expanding the minor groove. Subsequent studies showed that the therapeutic effect of platinum arose from its effect on proteins which could then bind in the expanded minor groove. Such an arrangement is strongly reminiscent of the stacking of the planar aromatic ligands of ruthenium polypyridyl complexes on DNA, first demonstrated by our recent key paper. In both cases, the kinking is seen specifically at a G-G step in a duplex (Fig. 1).

Although what we thought we were looking for was a definitive characterisation of the intercalation mode of these complexes (major vs minor groove, parallel vs perpendicular vs some other orientation), this rare very characteristic of semintcalation may yet turn out to be the key finding! Our work suggests that there may be therapeutic applications, which, as with platinum, could be connected to a facilitation of, or blocking out adenine at the end of the chain means that the intercalation is not unambiguously, and to the same ruthenium complex (Fig. 2). The flipped-out adenine at the end of the chain means that the intercalation is not classical, because it is a symmetry-related adenine which stacks onto the dipyridophenazine (dppz) chromophore. This work has opened many scientific doors. The photoactivation of some of the complexes is a property we are still exploring, and is very relevant to questions of DNA damage mechanisms, and possible applications in photodynamic therapy. Analysis of the sequence-specificity of the binding, and the extension to other compounds, will clearly occupy us for some time to come. We have already shown that classical intercalation can indeed be seen, by making small changes to the decamer sequence. More unexpectedly, the crystal in Fig. 2 can be reversibly dehydrated at room temperature, using Diamond Light Source’s own controlled humidity device, giving a second crystal form. In this second form, the reversible movement of water in and out of the solvent channel in the crystal means that we can observe an increased bending of the DNA towards the major groove, in the form of an additional 53° kink. Remarkably, the adenine flipping at the end of the duplex is also reversible, so that in the dehydrated form the intercalated dppz chromophore is tightly sandwiched between two purine bases from the same DNA strand, rather than between two symmetry related strands, as shown in Fig. 3.

This result is a tribute to the power of stacking interactions, and to the stabilising and stiffening effect of the ruthenium complex on our famously flexible friend, the DNA duplex.

References

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Mechanisms of up-regulating a tumourigenic lipid kinase
Hoon, W.-C., Berndt, A. & Williams, R. L. Regulation of lipid binding underlies the activation mechanism of class IA PI3-kinases. Oncogene, (online 28 November 2011) in press

The phosphoinositide 3-kinases (PI3Ks) are a family of lipid kinases, which can be found in animals. These enzymes have a key role in various cell functions. They transduce growth factor signalling by phosphorylating the lipid substrate phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) to produce PI(3,4,5)P3, which modifies cell surface receptors and activates intracellular signaling pathways.

PI(3,4,5)P3, acts as a second messenger and activates downstream effectors, including Akt and mTOR, which are key regulators of cell growth, proliferation, survival, and metabolism.

The PI3K catalytic subunit p110α binds to its regulatory subunit p85α, which inhibits its lipid kinase activity. This inhibition is relieved by various mechanisms, including activation of p110α or inactivation of p85α by mutations. These mechanisms are crucial for the activation of PI3Ks in various cellular processes.

Activated PI3Ks can be divided into two classes: type I and type II. Type I mutants are characterized by a gain of function, where the enzyme is activated by a mutation in the activation loop or by a mutation in the C-terminal tail. Type II mutants are characterized by a loss of function, where the enzyme is inactivated by a mutation in the C-terminal tail.

Figure 1: Structure of the kinase domain in WT and mutant PI3Kα complexes. The structure of the kinase domain of PI3Kα was determined by cryo-electron microscopy and density functional theory. The structure was solved at 2.6 Å resolution and contains both the catalytic and regulatory subunits. The structure shows the lipid kinase activity of PI3Kα and the activation of the enzyme through mutations in the activation loop.

Figure 2: Lipid kinase and lipid binding activities of WT and cancer-linked mutants of p110α. The activities are measured using liposomes and purified enzymes. The activities are compared to the activity of the WT enzyme.

The class I PI3Ks phosphorylate the lipid substrate PI(4,5)P2 in membrane bilayers (made from long-chain lipids) or in micelles (made from short-chain lipids) and are not active towards soluble substrate with very short acyl chains.

Figure 3: Summary and model of membrane binding by the p110α/PI3Kα complex. The model shows the interaction of the p110α/PI3Kα complex with the membrane bilayer and the orientation of the PI(4,5)P2 and phosphatidylinositol phosphates. The model is based on a combination of crystallographic and biochemical data.
While many organisms require oxygen to breathe, bacteria are more versatile and use alternative chemicals such as nitrate, sulphate, or even solid iron or manganese minerals. One example of these mineral breathing bacteria is the organism Shewanella oneidensis. These bacteria generate energy by breaking down organic molecules, releasing electricity inside the cell that is conducted through biological wires to the outside surface of the cell where it is discharged into the mineral. To do this the surfaces of these bacteria are covered in red iron-containing proteins known as cytochromes that act as the surface electrode terminals and are at the centre of the interface between microbe and mineral. This year, we solved the crystal structure of one of these cell surface cytochromes. The protein was shaped roughly like a disc, with ten iron atoms arranged in a staggered cross through the disc. Each iron is bound in an organic cofactor called a haem.

Figure 1: Mineral respiring electron transport chain of Shewanella oneidensis. Electrons from flavin electron shuttles or participation in extracellular inter-cytochrome interface is the source of much debate in recent literature. There is evidence for direct electron transfer between MtrC/F and the insoluble mineral interface. Each of these four sites will accept electrons from the MtrD-MtrE complex, the other three could serve as electron egress sites. The most likely site for electron ingress is either heme 5 or heme 10, at the opposite ends of the pentaheme domains, leaving the opposing heme to be potentially available for direct electron transfer to the surface of a mineral. The other two sites are unlikely to be involved in direct electron transfer, but the pentaheme domains could potentially serve as association sites for flavins and soluble electron acceptors. MtrF was shown to be able to serve as an electron donor to a variety of iron chelates and flavin acceptors. The discovery of the MtrF structure has provided a rationale for previous studies that show S. oneidensis is able to use multiple routes of electron egress, and has for the first time given biochemists and molecular modellers the opportunity to understand and predict how organisms can associate with the surface of an outer membrane cytochrome.

References

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A neutralizing antibody that binds to all influenza A haemagglutinins


Antibodies that neutralise influenza virus infectivity bind to the haemagglutinin virus membrane glycoprotein (HA), recognising in particular its virus membrane-distal, sialic acid receptor binding subdomain. The majority of antibodies characterised are strain specific, and the antigenic variants that they select are able to re-infect individuals previously infected even with related viruses. As a consequence, this variation requires influenza vaccines to be frequently updated so that they contain HAs closely related to those of viruses in current circulation. To do this, and because there is a continuous threat of influenza viruses being introduced into humans from avian species that serve as reservoirs for viruses containing all sixteen subtypes of HA, attempts have been made to obtain antibodies that would block virus infection irrespective of strain and subtype variation.

The Fab binds into a shallow groove in the F subdomain of HA (Fig. 2), the sides of which are formed by residues from helix 4 of HA2 and from two strands of HA1 (residues 38–32 and 130–120) and the base of which is formed by the HA2 turn containing residues 18–21 of the ‘Fusion Peptide’. The HCDR3 loop crosses helix A at an angle of about 45°, making Leu-100A, Tyr-100C, Phe-100D, and Trp-100F to make hydrophobic contacts with residues in the groove. Tyr-100C and Trp-100F potentially also make hydrogen bonds with the side chain of Thr-49 of HA1 and the main chain carbonyl of residue 19 of HA1, respectively. Two additional polar interactions are formed by main chain carbonyls at residues 98 and 99 of HA1 with His 53 of the HA1 helix. Overall, the interactions fit makes with the hydrophobic groove in H1 and H3 HAs, are remarkably similar. The LCRD1 loop of FAB makes two contacts with the side of helix A, opposite to the side that contributes to the hydrophobic groove. Phe-27D makes hydrophobic contact with the aliphatic part of Lys-39, and Asn-28 hydrophobic bonds to Arg-43.

Two differences between the FI6-H3 and FI6-H1 complexes are notable. The first is that H3 HA is glycosylated at HA1 Asn-38, but H1 HA, in common with all Group 1 HAs, is not. In the unbound structure of H3 HA, the carbohydrate side chain projects towards helix A of the same subunit, overlapping the site of FAb binding. However, FAb binding to H3 HA has made possible by orientisation of the carbohydrate side chain that involves its rotation through about 87°, away from the surface of HA (Fig. 3).

The second difference involves the Group-specific environment and orientation of HA2-Trp-21. In Group 1 HA, Trp-21 is approximately 24 deeper in the hydrophobic groove than in H3 HA, contributing to the Group1 HA complexes, making a similar contact distance between Trp-21 and Phe-100D in both groups. In previously published structures of complexes formed by Group 1-specific antibodies it appears that there is less flexibility than in FI6 complexes for the contact residues to move to the hydrophobic groove to accommodate the Group2 orientation of HA2-Trp-21.

Attempts to derive information on the mechanism of infectivity neutralisation by FAb by selecting resistant antigen variants from cells infected in vitro in the presence of antibody or from lungs of antigen-treated, infected mice, were not successful. However, from consideration of the structures of the antibody complexes, a number of suggestions can be made. In the first stages of infection, bound influenza virus are taken into endosomes from where the virus genome-transcriptase complex is released into the cell as a result of low pH-activated, HA-mediated fusion of virus and endosomal membranes. Assuming that FAb-H3 complexes are taken into endosomes, it is possible that FAb could block the mechanism of membrane fusion, through HCDR3 interactions with the ‘Fusion Peptide’ and LCDR1 interacting with the C-terminal residues of HA1 from the neighbouring subunit, to crosslink the HA trimers and prevent the conformational changes in HA required for fusion. Membrane fusion might also be blocked by bound antibodies restricting the formation of complexes of activated HAs that have been proposed to be involved in the fusion process.

The efficiency of these mechanisms of neutralisation would obviously be influenced by the affinity of FAB for HAs on viruses and this would depend on the accessibility of the binding site. Accessibility is presumably related to the spacing of HAs on the virus surface relative to the size of the antibody molecule. Estimates of between 20% and 50% of virus surface coverage by HA have been made from different measurements, and at either edge of HA’s binding site to the membrane proximal region of HA is likely to be comparatively restricted.

It is also likely that HA is a target for infection-blocking antibodies in the final stages of infection on the surfaces of infected cells, where the interaction of FAB with newly-made HA is less likely to be restricted by spacing considerations. At its site, HA-bound antibody might prevent virus assembly by crosslinking HAs or by more specifically blocking functions or associations in assembly which have not as yet been defined. In addition, infected-cell lysis as a result of FAB-HA complex recognition at the cell surface by Fc-receptor-bearing cells may, as our negative results with FI6-HA complexes, have an obvious advantage. The main incentives for our studies of cross-reactive antibodies are the unpredictability of the occurrence of influenza epidemics, the wide range of antigenically novel viruses that might cause them, and the need for new anti-viral agents for treatment of severe influenza infections. The results of prophylaxis and therapy experiments that we report with these studies of FAb antibody structure, provide justification for adding FAB to the arsenal of monoclonal antibodies that is accumulating for potential use against influenza. Among the antibodies available to date, FAB, because of its pan-influenza cross-reactivity, would have an obvious advantage.

References


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Figure 1: 3 Fabs from SI6 human monoclonal antibody bind to the Fab subdomain of the H3 HA trimer (pdb 3ZTJ) represented in Ribbons. One HA monomer is coloured blue for HA1 and red for HA2 respectively, shows that each monomer of the HA trimers binds one Fab, binding to the F subdomain of H1 and H3 HAs, from Group 1 and Group 2 respectively, shows that each monomer of the HA trimers binds one Fab, binding to the F subdomain of H1 and H3 HAs, from Group 1 and Group 2 respectively.
Helping to lower cholesterol levels - the crystal structure of a bacterial homologue of the bile acid sodium symporter ASBT


Although cholesterol is an essential component of the plasma membrane, excessively high cholesterol levels greatly increase the risk of heart disease. Approximately half of the daily amount of cholesterol that is eliminated from the body is through its conversion to bile acids. Bile acids are secreted into the intestine where they help emulsify dietary lipids. Rather than being excreted from the intestine and so eliminated from the body by 95% of the bile acids are reabsorbed and taken back to the liver. The Apical Sodium-dependent Bile acid Transporter (ASBT) actively transports bile acids across the apical membrane of the ileum using an inwardly-directed sodium gradient. As this is the rate-limiting step for bile acid reabsorption, ASBT is a target for drugs aimed at lowering cholesterol levels with some successful animal models. To further understand the molecular basis for bile acid transport and to aid drug discovery we have determined the structure of an ASBT bacterial homologue in complex with sodium ions and bile acid at 2.2Å resolution. The structure should aid the rational-based design of new inhibitors against ASBT, with the goal of treating hypercholesterolaemia.

ASBT is a secondary transporter that uses the energy stored in the sodium motive force to drive the uphill transport of bile acids across the apical membrane of the ileum. It is a member of the SLC10 family of secondary transporters, with several members indentified to date. Very little structural information has been available for this family and there has been some disagreement in the proposed topology. ASBT is a pharmacological target for drugs aimed at lowering cholesterol, so knowledge of the bile acid binding-site would be beneficial to drug discovery. Using fluorescence based screening methods we identified a bacterial homologue of ASBT that was tractable to crystallography. More specifically, the protein is stable in short chain detergents, which is important to obtaining well-ordered crystals, especially for membrane proteins with short loops like ASBT. ASBT is from N. meningitidis and 26% identical to human ASBT with many of the residues known to be critical for function conserved. Through transport assays we were able to confirm that ASBT is a sodium-dependent bile acid transporter.

The structure of ASBT was solved to 2.2Å resolution at the ESRF using data collected on I02 and I03 beamlines. The structure was refined in the space group P2_12_12_1 with four molecules in the asymmetric unit and had z-values of 2.4. The structure was refined at a resolution of 2.2Å to an R-factor of 19.3% and a free R-factor of 22.9%. Important to obtaining high resolution data for this membrane protein was the use of dehydration. Controlled dehydration of the crystals was carried out using the HCT device at Diamond Light Source.

The structure of ASBT is shown in Fig. 1. The key features of the 10 transmembrane helix TM structure are: 1.) It is made of two 5-TM segments that are structural repeats related by a pseudo 2-fold axis running through the centre of the membrane. 2.) The fourth TM in each domain is discontinuous, a helix-breaking TMs (TM 4, 7, 9). 3.) The two 5-TM repeats interweave to form two domains, a core domain (TM 3-5 and 9-10) and a panel domain (TM 1-2 and 6-7), and 4.) The discontinuous helices cross over to create two pseudo-helices formed from the amino-terminal and the carboxy-terminal halves of these helices respectively (Fig. 1a).

We solved the structure ASBT by SAD from a mercury derivatised crystal using data collected on I02 and I03 beamlines. The structure was refined at a resolution of 2.2Å to an R-factor of 19.3% and an R-free of 22.9%. Important to obtaining high resolution data for this membrane protein was the use of dehydration. Controlled dehydration of the crystals was carried out using the HCT device at Diamond Light Source.

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ASBT is a sodium-dependent bile acid transporter and it is involved in the absorption of bile acids across the apical membrane of the ileum. Using fluorescence based screening methods we identified a bacterial homologue of ASBT that was tractable to crystallography. More specifically, the protein is stable in short chain detergents, which is important to obtaining well-ordered crystals, especially for membrane proteins with short loops like ASBT. ASBT is from N. meningitidis and 26% identical to human ASBT with many of the residues known to be critical for function conserved. Through transport assays we were able to confirm that ASBT is a sodium-dependent bile acid transporter.

The three-dimensional structure of ASBT shows two different conformations of the N and C-terminal domains, a model of the outward-facing state can be generated from the inward-facing state and vice versa. By applying this methodology we generated a plausible model of ASBT in an outward-facing state and observed that the largest conformational change between model and outward-facing state is the movement of the panel domain across the core domain. We propose that sodium binding at critical locations in ASBT controls the conformation of the core domain, which in turn drives the movement of the panel domain (Fig. 3). This large conformational change of the panel domain relative to the core domain is required to allow the accessibility of the substrate-binding pocket.

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All eukaryotic cells, from yeast through to humans, are characterised by their compartmentalisation: membrane-bound organelles separate the distinct chemical environments within the cell that sustain its various biological functions. Membrane-bound transport vesicles transfer components between these intracellular organelles and the extracellular milieu in a highly directed manner. Components are delivered when a cargo-containing vesicle that buds off one organelle, or the plasma membrane at the cell surface, fuses with another organelle into which its contents mix. Much of the energy and specificity involved in the fusion of transport vesicles with their target organelles in eukaryotic cells is provided by a large family of proteins termed SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors). These small membrane-anchored proteins have distinct steady state organelle localisations, although the individual SNARE proteins are constantly in motion as they bud off from, or fuse with, source/target organelles. Three Q-SNAREs from one membrane form a complex with one R-SNARE from the other membrane, their 16-turn helical SNARE motifs wrap around each other and thus provide the energy to pull the two membranes together to facilitate their fusion.

Previous studies have shown that only limited combinations of the 38 SNARE proteins present in mammalian cells form complexes, confirming specificity to vesicle/organelle and organelle-organelle membrane fusion events. However, the mechanisms by which the correct SNAREs are selected for incorporation into forming vesicles remain poorly understood. Data collected on beamlines 103 and 104 demonstrate that the endocytic clathrin-coated vesicle (CCV) component CALM (Clathrin Assembly Lymphoid Myeloid-leukemia protein) binds directly to the R-SNARE VAMP8, selecting this SNARE for incorporation into endosomes thereby ensuring their ability to fuse with the correct target organelle.

The process of clathrin-mediated endocytosis from the plasma membrane is the main mechanism by which the complement of transmembrane proteins on the surface of the cell is regulated and as such plays a critical role in a vast array of processes including, cell/cell and cell/substrate recognition, transduction of external signals, and the uptake of nutrients and other small molecules. It is also the route by which many pathogens gain entry into cells. Endocytic CCVs are surrounded by a polyhedral clathrin scaffold, which is linked to the membrane by a group of architecturally similar proteins termed clathrin adaptors. These bind directly to both clathrin through a newly unstructured region and to the plasma-membrane enriched phospholipid PtdIns4P, which also exists in cells. A subset of clathrin adaptors are responsible for selecting the cargo that is to be incorporated into CCVs through their binding to a variety of specific determinants on the cargo cytoplasmic region. In addition to general transmembrane protein cargoes, an endocytic CCV must also contain the R-SNAREs that will allow it to fuse with its target organelle, the early endosome. In mammalian cells three R-SNAREs are either VAMP8, VAMP3 or the neuronal-specific VAMP2. How these R-SNAREs are sorted into endocytic CCVs was unknown, however, studies in D. melanogaster (C. elegans) and S. cerevisiae suggested that these organisms’ single ANTH domain containing clathrin adaptor was somehow involved.

Biochemical studies allowed us to show that the ANTH domain of ubiquitously-expressed mammalian clathrin adaptor CALM bound directly to the SNARE motifs of VAMP8, VAMP3 and VAMP2 with a Kd of around 20μM. Extensive attempts to crystallise CALM’s ANTH domain in the presence of VAMP8 yielded only structures of the unliganded ANTH domain. However, inspection of these structures revealed that the final helix of this domain, helix α11 (Fig. 1), was often poorly ordered and assumed different positions on top of helices α9 and α10 which form a hydrophobic trough (Fig 1). As this was a potential binding site for the long amphipathic SNARE motif of VAMPs, helix α11 was deleted. Surprisingly, rather than potentiating VAMP binding, deletion of helix α11 blocked VAMP binding completely. Structure determination and MALDI-MS analysis showed that truncated ANTH domain lacking helix α11 form tight dimers, the buried dimer interface being the proposed VAMP binding site. However, point mutations of hydrophobic residues lining the potential SNARE-binding trough of the full-length ANTH domain resulted in strong inhibition of VAMP binding in biochemical assay without affecting the field of the ANTH domain. A chimeric construct was thus created in which the SNARE motif of VAMP8 was appended to the end of helix α10 of the CALM ANTH domain. The crystal structure of this chimeric protein showed that VAMP8 does indeed bind in the hydrophobic trough between helices α9 and α10 of the CALM ANTH domain with excellent spatial and chemical compatibility (Fig 1). We therefore propose that helix α11 binds loosely to the CALM ANTH domain to protect the hydrophobic interface from the polar solvent until it encounters a SHARE. The residues which form the interaction between VAMP8 and CALM are well conserved in VAMP1 and VAMP2, allowing us to conclude that these other R-SNAREs will bind the CALM ANTH domain in an identical manner. The CALM binding region of VAMP8 adopts a helical conformation and its interaction with the CALM ANTH domain is reminiscent of its participation in an assembled SHARE complex (Fig 2). While binding of VAMP8 to CALM is mutually exclusive with SHARE complex formation, since the same binding interface is utilised for both interactions, pre-assembly of the CALM binding region of VAMP8 into helices is compatible with its identification as the ‘trigger’ region of the R-SNARE that potentiates SHARE complex formation (Fig 2). CALM thus incorporates fusion-ready R-SNAREs into nascent vesicles. Point mutations of key residues in the interface of either partner as indicated by the chimeric structure abolished the interaction between CALM and VAMP8 in vitro and, when transfected into the genes in vivo, blocked endocytosis of VAMP8 without significantly affecting the endocytosis of standard cargos (Fig 3).
Polynucleotide Kinase 3’ Phosphatase: revealing the molecular details of substrate and co-factor recognition


Genetic material, within each cell of the human body, is under attack from a seemingly continuous barrage of DNA-damaging agents, arising from a wide range of both internal and external sources. One of the most commonly occurring forms of damage to DNA are single-strand breaks (SSBs) where one strand of the DNA duplex is interrupted or broken; appearing with a frequency of around 1000 per cell, per day. If these breaks are not quickly repaired, they can be converted to the potentially more harmful double-strand breaks (DSBs) during the process of DNA replication, leading to genetic instability and even to the onset of cancer. Additionally, the damage processes that generate SSBs often do not leave free 3’ hydroxyls or 5’ phosphates at the margins of the gap, both of which are essential for subsequent template-directed repair. Polynucleotide Kinase 3’ Phosphatase (PNK) is an unusual dual function enzyme, which can restore DNA ends, by the removal of 3’ phosphate blocking lesions and/or by phosphorylating 5’ hydroxyls, thereby facilitating the subsequent gap-filling and ligation steps through the respective actions of DNA polymerase β and DNA ligase III. Previous structural studies had defined the overall architecture of PNK but provided little insight into how the enzyme actually recognises its different DNA substrates. With diffraction data principally collected on beamlines I02 and I03, we were able to determine structures of the catalytic region of PNK in complex with DNA, bound to the active sites of both the 3’-phosphatase and 5’-kinase domains, thereby providing a comprehensive picture of DNA end recognition by this enzyme.

With an Escherichia coli expression construct encoding a truncated form of the murine protein (PNKΔFHA), we were able to produce initial crystals of the 3’-phosphatase form of the enzyme. Diffraction data were collected on beamline I03 to a resolution of 1.65 Å, and an apo form of the enzyme. Diffraction of the murine protein (PNKΔFHA), we were able to produce initial or broken; appearing with a frequency of around 1000, per cell, per day. If these breaks are not quickly resolved, they can thereby providing a comprehensive picture of DNA end recognition by this enzyme.

The ssDNA was found to sit in a narrow channel traversing the surface of the phosphatase domain. In close proximity to the DNA, was a well-ordered magnesium ion coordinated by the side-chains of amino acids Asp170 and Asp288, as well as the backbone carbonyl of Asp172 (Fig. 1A). The location and coordination of the ion identified it as the essential cofactor required for the phosphatase activity of the enzyme. Of the five bases comprising the co-crystallised ssDNA (5’-GTCAC-3’), only three were clearly resolved in maps. The bases of C3 and A4 are stacked against each other, and are themselves sandwiched between the side-chains of Lys225 and Phe184 (Fig. 1B). The orientation of the base pairs both 5’ and 3’ to the nick can stack (Fig. 3B). Interestingly, the asymmetric unit of these crystals contains a single copy of PNK, associated with five copies of the 5’-GTCAC-3’ oligonucleotide in the asymmetric unit, builds the crystal lattice in the PNK-DNA2 complex.

3’-terminal phosphate would, however, be unencumbered, and readily hydrolysed. The dimensions of the DNA binding channel would also preclude binding of duplex DNA, therefore providing an explanation for the in vivo observations that PNK preferentially dephosphorylates either ss- or DNA with at least a 3 nucleotide 3’ overhang.

Despite exhaustive trials with the wild-type protein and conventional nicks or gapped double-stranded DNA substrates (i.e. those generated by annealing of three separate oligonucleotides), we were unable to obtain crystals with DNA bound to the kinase domain. We therefore tried a number of alternative strategies, with success ultimately resulting from incubation of a phosphate-inactive version of the enzyme (D170N) with Mn2+-ATP and a 10-fold excess of ssDNA. Diffraction data for this crystal form were collected on beamline I02, to a resolution of 2.15 Å.

The nick or discontinuity in the DNA backbone is recognised through insertion of a helix from the kinase domain (α13) into the path of the DNA duplex, causing a substantial bend (~ 70°), which provides access to the incoming substrate 5’ hydroxyl (Fig. 3A). The side-chains of Met476 and Val477 from helix 13 form a pseudo-continuous nicked DNA substrate. The side-chains of Met476 and Val477 from helix 13 form a pseudo-continuous nicked DNA substrate. The side-chains of Met476 and Val477 from helix 13 form a pseudo-continuous nicked DNA substrate.
Understanding HIV cellular defence mechanism by determining the structure of deoxyxynucleotidetriphosphatase triphosphohydrolase SAMHD1


Lentiviruses are associated with chronic disease states in a variety of mammals. In humans the HIV-1 lentivirus is the aetiological agent of AIDS, a disease that affects 34 million people worldwide and is responsible for 1.8 million annual deaths. In order to combat lentiviruses and other retroviruses, cells have evolved restriction factors that in some cases are able to inhibit infection. SAMHD1 is a protein originally associated with the human infantile disease Aicardi-Goutières syndrome (AGS). Additionally, it is the latest of the human restriction factors to be discovered and is responsible for the restriction of myeloid derived dendritic cells (MDDCs) and macrophages (MDMs). Using the triphosphoryl derived derivatized cells (MDDCs) and macrophages (MDMs). Using the triphosphoryl derivative, the active site of SAMHD1 is dimeric in solution (Kd ~5µM). The active site of the molecule is located at the intersection of helices α4, α5, α7 and α11 in a deep cavity and contains tightly bound zinc and magnesium ions. The histidine, aspartate and arginine side chains that coordinate the zinc and phosphate ions. The histidine, aspartate and arginine residues that line the cavity and coordinate the zinc and phosphate are shown in stick representation. Hydrogen bonding interactions are displayed as dashed lines.

Figure 1: A ribbon representation of the SAMHD1 dimer showing the active and allosteric sites. Each monomer is colored uniformly and the secondary structure elements are labeled sequentially from the N- to C- terminus on a single chain.

The active site of SAMHD1 is characterized by two catalytic histidine and aspartate residues and defines a superfamily of metal-dependent phosphohydrolases. The crystal structure shows that the active site of SAMHD1 is located at the intersection of helices α4, α5, α7 and α11 in a deep cavity and contains tightly bound zinc and magnesium ions. The histidine, aspartate and arginine side chains that coordinate the zinc and phosphate ions. The histidine, aspartate and arginine residues that line the cavity and coordinate the zinc and phosphate are shown in stick representation. Hydrogen bonding interactions are displayed as dashed lines.

Figure 2: SAMHD1 active and allosteric sites. (A) The active site of SAMHD1 is located at the intersection of helices α4, α5, α7 and α11 in a deep cavity and contains tightly bound zinc and magnesium ions. The histidine, aspartate and arginine side chains that coordinate the zinc and phosphate are shown in stick representation. Hydrogen bonding interactions are displayed as dashed lines. (B) The allosteric site is located at the dimer interface and contains residues mainly from helix α2. Hydrogen bonds from the side chains of Asp 137, Glu 416 and Arg 145, located on α2, to the functional groups on the guanine base are shown as dashed lines. Fig. 1A is located at the opposite face of α2 with the guanine base orientation the back of the active site. The residues highlighted with asterisks are those whose mutations are hard to all PLs.

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Structural and mechanistic insights into PAR hydrolysis by the PARG enzyme poly(ADP-ribose) glycohydrolase


A key role in many critical cellular pathways, from DNA repair and chromatin stability to mitotic progression and caspase-independent cell death, plays poly(ADP-ribosyl)ation, which is a reversible protein post-translational modification. While poly(ADP-ribose)polymerases (PARPs) are responsible for catalysing poly ADP-ribosylation using NAD as a substrate, poly(ADP-ribose) glycohydrolase (PARG) reverses the modification through hydrolysis of the unique ribose-ribose PAR bond to produce free ADP-ribose. These enzymes are vital for many biological processes – the absence of PARG activity leads to the accumulation of PAR and ultimately cell death. We were able to solve the first crystal structure of a PARG enzyme, revealing it is a distant member of the ubiquitous ADP-ribose-binding macro domain family. PARG structures complexed with ADP-ribose have allowed us to propose a detailed model for PAR binding and catalysis by PARG. These structural insights provide a framework for understanding PARG activity in reversible protein poly(ADP-ribosyl)ation and for further study and therapeutic intervention.

The failure to maintain a stable genome has been linked to many disease states including cancer, neurodegeneration, immunological disorders and numerous developmental syndromes. The poly(ADP-ribosyl)ation of proteins is an early step in the initiation of DNA damage repair in cells. The activation of nuclear poly(ADP-ribosyl)ation enzymes such as poly(ADP-ribose) polymerase (PARP) and poly(ADP-ribose) glycohydrolase (PARG) mediates the formation and degradation of the poly-ADP-ribose (PAR) modification. PARPs signal the presence of DNA damage and facilitate DNA repair. While PARP catalyses the addition of ribose units to DNA, histones, and various DNA repair enzymes, PARG is the key enzyme involved in the specific and efficient hydrolysis of the PAR ribose-ribose bond. Given the link between DNA repair and cancer, targeting poly(ADP-ribosyl)ation has attracted considerable attention in recent years and PARP inhibitors have already been shown to exhibit striking efficacy against hereditary breast and ovarian cancers. PARG plays a key role in DNA repair and appears to possess a protective role within the cell, as the build up of PAR leads to apoptotic cell death. Moreover, increased PARP activity has been linked to uncontrolled cell proliferation and differentiation in glioma tumor dividing cells suggesting that PARP regulation may also have a crucial role in cancer. Although the first PARG enzyme was discovered more than 40 years ago, until now there has been no structural data available for any PARG enzyme, hindering development of effective PARG-specific inhibitors.

To address this, we solved a bacterial PARG crystal structure (from Thermotoga maritima) both with and without ADP-ribose. The PARG structures reveal a core ADP-ribose-binding macrodomain fold with an additional ~30 amino acid C-terminal extension (Fig. 1A). The diphosphate-binding loop that flanks one side of the ADP-ribose-binding cavity is highly conserved between PARG and other macrodomain structures, with ADP-ribose bound in a similar manner. In contrast, the opposite side of the PARG-ADP-ribose-binding cavity is lined by a stretch of amino acids containing the PARG-specific GGQ motif. The GGQ signature sequence (Fig. 1A). The introduction of this ‘PARG-specific’ loop would seem the reason why only PARPs, and not other macrodomain proteins, are catalytically active. We speculate that the PARG-specific loop has been introduced into the macrodomain fold to position the C115 side chain into the PAR active site to catalyse hydrolysis (Fig 1B).

To provide further insight into PAR binding, an el115→21 G-lycosidic linkage with an additional ADP-ribose group was modelled into the PARG active site (with P. Luther, Université d’Orléans, Orléans), creating a model of PARP in complex with di(ADP-ribose). Our models suggest that the structure also reveals this enzyme is likely to be an exohydrolase, as the protein structure does not provide sufficient space for any substituent on the bound ADP-ribose 2′-OH group. The E. curvata PARP complex with ADP-ribose and the corresponding PAR→PARG model allow us to propose a mechanism for PARG catalysis. The key ribose→ribose PAR O-glycosidic linkage is in direct hydrogen-bonding contact with Gu 115. This supports the formation of a putative positively charged oxocarbenium intermediate concomitant with protonation of the (n − 1) PAR 2′-OH leaving group by Gu 115. The barrier to oxocarbenium formation is lowered by electrostatic interaction with the diphosphate group (a contact enforced by the conserved Phe227). A water molecule present in the active site is ideally positioned to attack the oxocarbenium intermediate, activated through covalent deprotonation by Gu 115, leading to the release of ADP→ribose and (n − 1) PAR (Fig. 2).

We tested our proposed mechanism by analysing the properties of PARG active site mutants (with I. Ahel, Patterson Institute, Manchester). Both Glu114Ala and Glu115Ala mutants were inactive, and binding studies revealed Glu115Ala had no effect on ligand binding, while Glu114Ala led to an approximate tenfold decrease in binding affinity. Mutations of Phe 227 implicated in positioning the terminal ribose also render the enzyme inactive. Mutations of Ser 98 and Val 226, implicated by our model in binding the (n − 1) ADP-ribose, greatly diminish E. curvata PARP activity. Importantly, mutations of the corresponding catalytic residues in human PARP (a more complex enzyme) have a very similar effect on the enzymatic activity, suggesting a universal structural and catalytic mechanism for bacterial and mammalian PARPs.

Our recent findings offer the first detailed structural and mechanistic insights into this intriguing protein family. These findings should help lay the framework to unravel the biochemical strategies regulating reversible poly(ADP-ribosyl)ation. We believe that our findings will provide the necessary tools for future studies that might ultimately lead to the development of small, cell-permeable PARP inhibitors and the potential to manipulate the physiology of health and disease by interfering with PAR metabolism.

References

Figure 1: (a) Overall fold of PARG with the core macro domain depicted in blue, and the additional N-terminal extension in red. The bound ADP-ribose is shown in green. (b) o-2Fc (omit map) density contoured at 1.2σ in (u,v) space. The PARG-specific catalytic loop is shown in yellow, and the diphosphate-binding loop in magenta. Additional interactions for the additional phosphate and ribose moiety are shown in green. (c) Detailed view of ADP-ribose bound in the PARG active site coloured as in (a).

Figure 2: Proposed mechanism for PAR hydrolysis, (a) and (b) (Adapted from Slade et al. Nature 477(7366), 616-20 (2011).)
The structure of the protein BLF1: understanding the mechanism of the Vietnam time bomb


The structure of BPSL1549, a protein of unknown function from Burkholderia pseudomallei, was determined to high resolution by a combination of biochemical analysis, functional studies and X-ray crystallography. This strongly suggested that BLF1 catalysed the deamidation of Gln 339 of eIF4A to glutamate, a modification that was confirmed by mass spectrometry. BLF1 is therefore a new member of the superfamily of GTPases that use GTP to hydrolyse Gln to Glu, thereby affecting actin cytoskeleton assembly. The conservation of the fold and key catalytic residues with CNF1 immediately suggested BPSL1549 might also be cytosolic via a glutamine deamidase activity. However, given that the molecular surface around the active site of BPSL1549 is different from that of CNF1, BLF1 was suggested to be a glutamine deamidase.


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Structural insight into the attachment of anionic polymers to the Gram positive cell wall


The bacterial cell wall maintains the structural integrity of the cell and enables bacteria to survive in a wide range of environments. The Gram-positive bacteria include many important human pathogens, including *Streptococcus pneumoniae*, the causative agent of invasive pneumococcal diseases. In these bacteria, the cell wall contains two major components: peptidoglycan, the target of many successful antibiotics and anionic cell wall polymers, which are attached to the peptidoglycan. These polymers include wall teichoic acids (WTAs) and acidic capsular polysaccharides which play a wide range of roles within the cell, including the control of autolytic activity, antigenicity and innate immune recognition, and the resistance to antibiotics. The addition of WTAs to peptidoglycan is vital to cell wall architecture and function. We identified the widespread LytR-Cps2A-Psr (LCP) protein family as the most likely candidates for the enzymes required for anionic polymer attachment. In order to gain insight into the function of this enzyme class, we determined the crystal structure of *S. pneumoniae* Cps2A. This structure, along with functional data, confirms that this family of proteins attach anionic polymers to peptidoglycan. The knowledge gained of this protein family provides a foundation for the exploration of these enzymes as novel targets for antibiotic development.

The cell wall is crucial for the maintenance of the structural integrity and the characteristic shape of bacterial cells. In Gram-positive bacteria, the cell wall has two major components: peptidoglycan (PG), whose synthesis is the target for the highly successful β-lactam and glycopeptide antibiotics and the PG-attached anionic cell wall polymers (APs), which include wall teichoic acids (WTAs) and acidic capsular polysaccharides. The WTAs and their lipid-linked variant, the lipoteichoic acids, have a wide range of important roles in the cell, including the control of autolytic activity, antigenicity and innate immune recognition, pathogenicity, cation homeostasis, and cell elongation and division. Most of the steps involved in the synthesis of WTA are known; the polymer is synthesised in the cytoplasm and then translocated across the membrane by an ABC transporter before catalytic attachment to the PG outside the cell by a phosphotransferase enzyme. The physical connection to the cell wall is essential for the proper function of the WTA, but the enzyme catalysing this final step has thus far not been identified.

Deletion of different components of the gene cluster responsible for WTA synthesis in the Gram-positive model organism, *Bacillus subtilis*, has various effects, from severe growth defects to lethality. A number of genes encoding the poorly characterised LytR-Cps2A-Psr (LCP) family of proteins have been identified within the WTA locus in *B. subtilis*, with homologues widely distributed across Gram-positive bacterial phytoplasms. These proteins possess an N-terminal trans-membrane (TM) region of one to three TM helices and a major C-terminal catalytic domain. To gain insight into the role the LCP protein family plays in WTA synthesis, we determined the structure of the soluble portion of the Cps2A protein from *S. pneumoniae* by x-ray crystallography and biochemical purification of homologues from other species. The structure of Cps2A comprises two distinct domains: domain 1, the accessory domain, and domain 2, the LCP domain (Fig. 1). The accessory domain has a three-layered α-β-α fold that has weak secondary structure homology with a large variety of other proteins, including the monoo- and dioxygenases, and protein residues shown as coloured sticks. (B) The lipid binding pocket is lined with solvent exposed.

In summary, the structural and functional characterisation of the LCP protein family has identified the proteins responsible for the final step in the anionic polymer synthesis pathway. The identification of its active site and its catalytic mechanism will allow directed drug discovery, particularly because the active site of this enzyme is not located within the cytoplasmic membrane, any inhibitor will not have to pass across the cytoplasmic membrane thus rendering the LCP family an excellent target for the development of much needed novel antibiotics.

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Dotting the ‘i’ in complement: the crystal structure of human complement factor I

Roversi, P., Johnson, S., Caesar, J.J., McLean, F., Morgan, B.P., Harris, C.L., Sim, R.B. & Lea, S.M.

Diffractive data enabled us to determine the crystal structure of the 88 KDa serine protease human complement Factor I (fI), a key regulator of the complement system, which is a part of the immune system. The project was hampered by the combined effects of sample heterogeneity, intrinsic conformational plasticity of the serine protease domain, lack of reactivity of the enzyme to small molecule inhibitors, and by tetartohedral twinning. The fI crystal structure, together with mapping of fI and cofactors’ point mutations with altered C3b/C4b proteolytic activity, supports the idea that only upon encountering cofactor and substrate does the serine protease domain fold into its fully active conformation. The structure also reveals that in the native enzyme, the fI heavy chain behaves as a non-competitive inhibitor of the serine protease domain. Disruption of the fI heavy-chain/light-chain interface, either effected by the physiological binding of fI to the cofactor:substrate complex, or by mutations of interface residues, releases the inhibition.

Many biological processes involve amplification loops, which in turn need to be controlled. By using diffractive data from beamlines I02 and I03, we determined the crystal structure of a protein essential to keep one such biological amplification loop in check: the 88 KDa serine protease complement factor I, a key regulator of the complement system. This 500 million years old arm of innate immunity is conserved throughout evolution in the plasma of vertebrates, and has evolved to function as a quick-acting machinery to target foreign tissues for destruction if they come into contact with the plasma. After surveillance, complement molecules make initial contact with the enemy, a sequence of proteolytic events is triggered, converging onto proteins C3b and C4b. These molecules enter complexes (‘C3convertases’) amplifying the cascade, generating many more copies of C3b on the targeted cellular surfaces and in the plasma fluid phase. By cleaving C3b and C4b, fI offers the only means of keeping complement activation in check, both in plasma fluid phase; on cell surfaces, fI-mediated inactivation of C4b/C3b provides extra complement control, in addition to the C3–convertase decay acceleration warranted by other self-cell anchored regulators. When complement regulators go wrong, self-tissues are harmed, and a number of auto-immune conditions ensue. Some of the naturally occurring fI polymorphisms are associated with diseases ranging from renal pathologies to arthritis-like syndromes. We undertook the fI structural determination with the aim of gaining insight on the molecular basis for fI pathologies to arthritis-like syndromes. We undertook the fI structural determination with the aim of gaining insight on the molecular basis for fI pathologies to arthritis-like syndromes. We undertook the fI structural determination with the aim of gaining insight on the molecular basis for fI pathologies to arthritis-like syndromes. We undertook the fI structural determination with the aim of gaining insight on the molecular basis for fI pathologies to arthritis-like syndromes.

The human fI structure determination required about 300 96-well crystallisation plates, diffraction measurements from about a hundred crystals, and thousands of data-processing and phase-determination computing jobs. The project was played by the combined effects of sample heterogeneity, intrinsic conformational plasticity of the serine protease domain, partial reactivity to inhibitors, and by a relatively rare and not trivial to detect/overcome type of crystal twinning.

With hindsight, and in the light of the structure (Fig. 2), a few of the difficulties mentioned above resolved in properties of the fI molecule that are essential for its function. For example, the active site of native fI in our crystals turns out to be partially disordered; a property fI shares with many plasma serine proteases, which circulate in zymogen form until a transition to the enzymatically competent form takes place. Thus, the fI structure, together with mapping of fI and cofactors’ point mutations showing altered C3b/C4b proteolytic activity, supports the idea that only upon encountering cofactor and substrate the serine protease domain become fully active. Structures of inhibitor-bound fI and/or of fI with substrate and cofactor (e.g. a C3b-CF10 ternary complex) will be needed to fully appreciate the details of this zymogen-to-enzyme conformational transition.

It is also possible that our early attempts at rigidifying/ordering the SP domain by mutating it with covalent and non covalent inhibitors of serine proteases (e.g. PEFABOC, benzamidine) created a mixture of inhibitor-bound and inhibitor-free molecules, perhaps explaining the initial failure to reproducibly grow large, well-ordered crystals. Indeed, human fI was demonstrated to be fully inactivated by the covalent serine protease inhibitor DFOP only when treated in presence of one of its cofactors. As soon as serine protease inhibitors were omitted, we obtained crystals in which the SP domain formed most of the contacts, ordering itself well enough to grow the crystals. The same crystal contacts may unfortunately have prevented twinning. The SP A,B and C-D interfaces are slightly different, and yet similar enough that a twin domain boundary may be formed whenever either of molecules B,C or D, are substitues for A.

The fI SP domain was shown to possess weak activity for small molecule substrates even in absence of cofactor, and kinetic measurements on several point mutants of fI gene reveal of V_c’s significantly higher than the native. Our structure reveals that the fI mutations that give increased catalytic activity, all map to the interface between the heavy chain and the SP domain. We therefore propose that in the native enzyme, the heavy chain behaves as a non-competitive inhibitor of the SP domain (Fig. 3). Disruption of this interface, either effected by the physiological binding of fI to the cofactor:substrate complex, or by the known point mutations to the heavy-chain-light-chain interface residues, allosterically releases the inhibition.

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Figure 1: Three different crystal forms of human factor I. (A) Space group I432, 10% solvent content, tetartohedrally twinned. (B) Space group P2_12_1, 20% solvent content. (C) Space group I432, 20% solvent content, tetartohedrally twinned.

Figure 2: The crystal structure of human factor I. On the left hand side, the light chain (SP domain), painted yellow to red. On the right hand side, the heavy chain, painted blue to light green.

Figure 3: The heavy chain acts as an allosteric inhibitor of human SP domain. The cartoon illustrates the proposed opening (red arrow) of the interface between the heavy chain (blue, green, orange and yellow domains) and the light chain (red domain), leading to allosteric activation of the SP domain (green, blue, red and yellow). White star = Residues bound to the C3b/C4b domains.
Type 1 diabetes can occur as a result of the body’s own immune system attacking and destroying the cells in the pancreas that manufacture the hormone insulin. Insulin controls blood sugar levels and a lack of insulin is fatal if untreated.

The mechanism by which the body attacks its own insulin producing cells in the pancreas is not fully understood. Our findings show how T-cells might play an important role in autoimmune diseases—like diabetes—and we have caught the first-ever glimpse of the mechanism by which killer T-cells can attack our own body’s cells to cause disease. This first sight of how killer T-cells make contact with the cells that make insulin is very enlightening, and increases our understanding of how Type 1 diabetes may arise. This knowledge will be used in the future to help us predict who might get the disease and also to develop new approaches to prevent it. Our aim is to catch the disease early before too many insulin-producing cells have been damaged. The team now hope that by gaining a deeper insight in this process it will put them in a much stronger position to devise new ways to prevent or even halt the disease.

T-cells are part of the adaptive immune system and mediate responses to diseases and infections. In order to perform this role, T-cells have a polyclonal receptor on their surface, the T-cell receptor (TCR), that can bind to disease markers, usually peptide oligomers, bound by the major histocompatibility complex (pMHC) on the surface of Antigen Presenting Cells (APC). The initial recognition event triggers a cascade of secondary interactions, resulting in direct killing of the APC, or recruiting other immune cells to effect programmed cell death or apoptosis. The genome can produce up to 100 million TCRs, but the disease marker peptides, 9 residues or longer, may have >1015 (thousand trillion) possible sequences. The gap is up to 100 million TCRs, but the disease marker peptides, 9 residues or longer, may have >1015 (thousand trillion) possible sequences. The gap is

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Structure of a novel single-stranded DNA binding protein


Biology requires a single-stranded DNA (ssDNA) binding protein and all organisms from humans to bacteria have one. Yet despite this, the archaeal organism Thermoproteus tenax, which can live under extreme conditions such as high temperatures, lacked the consensus sequence for ssDNA binding. Bioinformatics had identified a candidate and the structure and biochemistry of this protein was evaluated. The structure determined revealed a novel fold and a new member of the ssDNA binding superfamily. This has important implications for the evolution of life.

The *oligonucleotide-binding (OB) fold* is the hallmark of ssDNA binding proteins (SSB). In fact the OB fold plays a wider role and is found in proteins which bind other biopolymers. The amino acid sequence homology varies enormously amongst the OB superfamily but modern bioinformatics tools are able to detect OB fold-containing proteins in all living organisms, with one exception, the Thermoproteales family of organisms. The puzzle is that all forms of life that we know of require to replicate and repair DNA. Thus all forms of life must have an SSB protein, and it follows they should have an OB-fold.

As bioinformatics failed to identify a candidate gene for the SSB function, 7 mmor cell extracts were prepared and passed over affinity columns. This led to the identification of two new proteins, Ttx2090 and Ttx1576, both of which were candidates for the role of SSB. Ttx2090 did not express in a soluble form, is similar to proteins Ttx2090 and Ttx1576, both of which were candidates for the role of SSB function, and was once again grown with protease present in the well.

A number of these crystals were screened at the beamline and all diffracted poorly, with a lower resolution limit and higher mosaic spread than native. The crystal we collected, judged by us to be the ‘best’ based on diffraction resolution, spot shape and mosaic spread, was indeed a good quality reproducible native crystal (denoted cTtx1756). Using beamline I03 we collected a native data set to 2.9 Å. As the protein was predicted to contain a new fold, we resorted to SeMet and anomalous phasing. Crystals were once again grown with protease present in the well.

Despite the apparent low quality of the data which would normally be expected to preclude solution, we were able to identify selenium positions and proceed with phasing in a relatively straightforward manner. The highly redundant data (20) may have been responsible for our success. We traced amino acids 24–139 and identified a compact folded domain. We did not know, since mass spectrometry on the crystals was inconclusive, whether the missing residues (amino acids 1–23 and 140–196) were disordered or simply reflected batch to batch variability. We have not established whether differences between native and SeMet crystal were systematic or simply reflected batch to batch variability.

The oligonucleotide-binding (OB) fold is the hallmark of ssDNA binding proteins (SSB).

<table>
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<tr>
<td>PDB</td>
<td>3TEK</td>
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</tbody>
</table>

Table 1.

**Figure 2:** The open cleft of Ttx1576 shows aromatic residues which are likely to bind ssDNA.

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**Funding Acknowledgements**

Funded by Biotechnology and Biological Sciences Research Council Grant BB/S5/B44545. K.S. Makarova and E.V. Koonin are supported by intramural funds of the US Department of Health and Human Services to the National Library of Medicine, National Institutes of Health. Mass spectrometry which was used in the work is supported by the Wellcome Trust.
Small bacterial haemoprotein reveals a novel mechanism of protection against CO toxicity


H em is one of the most versatile protein cofactors with involvements in gas transport, electron transfer, catalysis and signalling. Preventing the irreversible binding of Carbon monoxide (CO) to haem is a critical aspect of its biological function. CO binding to hemoglobin can lead to eventual death in environments with excess CO, and more general poisoning of haemoproteins by CO can have fatal consequences for cellular function and metabolism. CO is a normal metabolic product and is now recognized as having a variety of physiological functions in immune regulation and antioxidant defence mechanisms. The toxicity of CO results from its very high affinity for haem. Competing hypotheses have sought to explain why myoglobin (Mb) is able to lower the ratio of CO to O2 affinity to ~25 as compared to ~2000 in bare haem. Because of the inherent geometric preferences of Fe-C-O (linear) and Fe-O-O (bent) moieties it was initially proposed that Mb discriminated against O2 due to distal pocket steric hindrance, which created a significant energetic cost for binding the CO-O2 unit. This hypothesis has since been downplayed for Mb in favour of preferential H-bonding to haem-bound O2. Variations in haem conformation and proximate interactions have also been suggested as key factors that modulate haem reactivity with diatomic gases. Using single amino acid mutations, reaction kinetics, Raman spectroscopy, X-ray crystallography and energy measurements and calculations, we provide a paradigm shift in our understanding of how CO poisoning is prevented in haem proteins due to the energy costs associated with conformational changes.

In biology, substrates have distinctive sizes, shapes, and charge distributions that are specifically recognised by their target biological partner proteins. The discrimination between the gases O2, NO and CO by haem proteins is a remarkable example of biological specificity because these molecules are apolar and of very similar size. Molecular recognition of these gases is essential for respiration, cell-signaling, aero/chemotaxis, and/or NO shuttling during denitrification. The best characterized CYTcp belongs to a family of pentaaaza (5c) haem proteins that discriminate between these diatomic gases efficiently protecting bacteria from nitrosative stress and/or NO shuttling during denitrification. The best characterized CYTcp is from the denitrifying bacterium Alcaligenes xylosoxidans (Ax), which does not form a stable complex with NO, binds CO weakly as a distal six-coordinate (6c) haem-carboxyl (6c-CO), and reacts with NO to form a unique proximal 6c-haem-nitrosyl (6c-CO) via a distal 6c-haem-nitrosyl (6c-N2O) intermediate. This utilisation of both faces of the haem in ligand binding is unprecedented.

The crowded distal haem pockets of all CYTcp proteins contain a non-polar residue (Leu, Phe, or Met) close to the Fe, which enforces selectivity in the propionate, which remained on the distal haem face. Resonance Raman (RR) spectroscopy confirmed the presence of both conformations at cryogenic temperatures, but only the linear conformation was detected at room temperature. Typical RR spectroscopy data for L16A are shown in Fig. 2.

Mutation of Leu16 to Gly or Ala resulted in a linear CO binding conformation that did not require movement of the haem propionate. In the bent conformation, a smaller shift in the position of Leu16 was accompanied by a considerably smaller shift in the propionate, which remained on the distal haem face. Resonance Raman (RR) spectroscopy confirmed the presence of both conformations at cryogenic temperatures, but only the linear conformation was detected at room temperature. Typical RR spectroscopy data for L16A are shown in Fig. 2.

Redox titration of WT AxCYTc led to release of CO to yield the free ferrous protein at -200 to 0 mV, followed by oxidation of the ferrous protein to 0 to 200 mV. In contrast, the L16A protein released CO concomitantly with oxidation to the ferrous state at 300 to 600 mV. Thus, the requirement for movement of Leu16 and the haem propionate upon CO binding reduces the potential of the haem-CO adduct of WT AxCYTc to bind CO strongly, allowing reversibility of CO binding. Energy calculations using Density Functional Theory (DFT) approach provided consistent values to these measured values.

We propose that these new findings, coupling the energetic of structural changes with gas release, may have broad implications for the functioning of a wide variety of haem systems including haem-based sensors.
Insights in the regulation of cell-to-cell interactions - structural and functional analysis of the LDL-receptor-related protein 6 (LRP6)


Structural and Functional Studies of LRP6 Ectodomain Reveal a Platform for Wnt Signalling, Dev. Cell. 21, 848-861 (2011)

Within a multi cellular organism, whether fly, fish, mouse or human, gradients in concentration of secreted signalling molecules, termed morphogens, provide one of the fundamental mechanisms determining the development of the basic body plan. The Wingless-Type MMTV Integration Site Family (Wnts) form one of the major morphogen families. As well as playing a central role during embryogenesis, the development process that enables the formation of a multicellular organism from an egg cell, Wnts are involved in many aspects of adult physiology and aberrant Wnt signalling is profoundly implicated in cancer biology. Classically, Wnts trigger their signalling pathway within a cell by binding to two types of receptors on its surface. The first type is the signalling receptor, and, according to the specificity of the Wnt molecule, is one of ten members of the rather evolutionarily named Frizzled (Fz) family. The second type is one or other of two members of the LDL-receptor-related protein (LRP) family, either LRP5 or LRP6. The extracellular region of LRP5/6, which protrudes from the cell surface to bind Wnts, was predicted to contain four repeated units, each a six-bladed β-propeller plus epidermal growth factor (EGF)-like module, together they form the PE repeat. We used X-ray diffraction data collected on beamline 124 to determine the crystal structure of a Wnt-binding portion of the LRP5 extracellular region comprising the third and fourth PE repeats. Guided by this detailed structural information we were then able to use electron microscopy, cellular and binding assays to build up a model in which LRP5/6 functions as platform structure supporting an interplay of ligands through multiple interaction sites.

Wnts have a central role in control of embryonic development and adult tissue homeostasis. Conventionally, Wnt signalling is implicated in a broad range of human diseases including cancer and osteoporosis. Therefore, there is considerable biomedical interest in this mechanism of Wnt signalling and its inhibition. Several physiological inhibitors have been identified, including Dickkopf 1 (DKK1), a secreted protein which acts through binding to the co-receptor for Wnt signalling, LRP5/6. In mammals LRP5 and LRP6 can have overlapping roles, but in general LRP5 takes the dominant role in development. LRP6 is a type 1 transmembrane protein (i.e. with N-terminal extracellular region preceding a single plasma membrane spanning helix). Sequence homologies with the low-density lipoprotein receptor (LDLR) suggest that the extracellular region of LRP5/6 contains four WTD β- propeller-EGF-like domain (PE) repeats (P1E–P4E) in tandem followed by three LDLR type A (0.1–1.3). Prior data from a number of laboratories indicated that P3E3 plus P4E4 (i.e. P3E3P4E4) acts as a single functional unit and provides the primary binding site for Wnt3a. We therefore set out to determine the structure of this basic building block of the Wnt signalling system.

In order to produce soluble LRP6EHEP for structural and functional studies we needed to use a resurrection (mammalian) cell-based expression system. Human LRP6H (I24w (22–1244) was transiently expressed in HEK293 cell cultures HEK293G (for crystallisation) or HEK293T (for functional analysis). A specialised chaperone, Meso (Medos development), is known to be important for LRP6 biogenesis and so we co-expressed our LRP6H with human Meso to boost the levels of secreted protein. The purified LRP6H EHEP crystallised at 21 °C in 0.1 M sodium hydrogen carbonate and, after optimisation, yielded thin, long plate-like crystals. Initial x-ray diffraction characterisation and data collection used the microfocus beam on beamline I03-2 at the European Synchrotron Radiation Facility. X-ray diffraction data of sufficient quality for a high resolution structure determination were collected on beamline I24 of the Diamond Light Source. The optimal data set was achieved by collecting at 100 K in wedges of 30° from multiple positions along a single crystal using the Pilatus 6M detector. These data allowed us to refine the crystal structure of LRP6EHEP at a resolution of 1.9 Å (Rwork 18%, Rfree 21%).

The structure reveals the detailed architecture of the tandem P3E3P4E4 arrangement, defining the domain interfaces and the surfaces exposed for potential Wnt binding (Fig. 1). The β-propellers P3 and P4 are classic examples of the six-bladed WTD class with main chain topology that superpose well on the crystal structures of β-propeller domains from LDL and nidogen (each propeller to propeller superposition giving root mean square deviations in the range 1.13–1.35 Å for some 200–220 Cα pairs). As expected E3 and E4 have the standard EGF-like fold and form apparently rigid interfaces with their respective β-propeller domains. The two repeat units, P3E3 and P4E4, abut side by side forming an extensive and tight interface. When viewed in the orientation shown in Fig. 1, upper panel, the EGF-like domains can be described as packing against the bases of the two β-propellers while the tops of the β-propellers form a continuous, curved surface. Guided by this structural information we used site directed mutagenesis in combination with cellular and binding assays to locate the Wnt3a binding site on the ‘top’ surface of P3E3, primarily β-propeller P3. We used similar assays to demonstrate overlap between this Wnt binding site and the binding site of a protein, Dickkopf 1 (DKK1), known to inhibit Wnt signalling (Fig. 2). Gratifyingly, we published our results simultaneously with structural reports from two other laboratories (those of Prof Weng Xiu, University of Washington, and Prof Bill Wei, Stanford University) that confirmed this footprint for Dkk1 binding.

In order to provide some structural insight into the overall architecture of the LRP6 extracellular region we also produced a second form of the full LRP6 ectodomain. We could only generate rather low yields of protein but these were sufficient for us to carry out negative stain electron microscopy (EM). This allowed us to produce a low resolution (25 Å) single-particle EM reconstruction of the LRP6 ectodomain which revealed a relatively simple homo-oligomeric structure. This observation combined with our cryo-electron microscopy and functional analyses, led us to propose that the LRP5/6 forms a tray-like platform at the cell surface. Data from ourselves and others suggest that the various members of the Wnt family may vary as to which PE unit they use as their primary binding site. A platform structure would allow a variety of Wnt-LRP complexes to have similar spatial relationships to the plasma membrane and to the Frizzled signaling receptors. Furthermore, this platform construction could support a relatively complex interplay of interactions between LRP5/6 and its agonists or antagonists. These insights can now be taken forward into further structural and functional studies.

References
Allergies are widespread amongst human beings. Allergic reactions occur to usually harmless environmental substances such as dust, pollen, animal hair or food. Treatments for allergies include the use of antihistamines, which bind to the respective receptor. The human Histamine H1 receptor belongs to the family of G protein-coupled receptors (GPCRs). Activation of this receptor increases vascular permeability causing fluid to escape from capillaries into the tissues, which leads to the common symptoms of allergic reactions. Antihistamines are inverse agonists (inhibitors) of the Histamine H1 receptor. They suppress the histamine-induced response by blocking the binding of histamine to this receptor. Although antihistamines are commonly used safely, they have some side effects including: sedation, drowsiness or even more major symptoms such as arrythmia, which are caused by non-specific binding to various other receptors in the body. Therefore, the high resolution structure of the human Histamine H1 receptor with a commercially available antihistamine bound in the active site, helps to understand the ligand binding and activation of this important membrane protein. Although there are more than 1,000 GPCRs in the human body, which are major drug targets, only about a handful of drug-binding GPCR structures have been known so far. The structure of the Histamine H1 receptor also provides invaluable information for GPCR studies and more rational drug discovery.

Figure 1. X-ray diffraction pattern of the Histamine H1 receptor complex. (A) the microfocus beamline I24, the microcrystals were flash frozen in liquid nitrogen and shipped to the 2.

The human Histamine H1 receptor belongs to the family of GPCRs which share a common feature of 7 helices spanning the membrane (Fig. 2). The well-conserved pocket of mostly hydrophobic amino acids plays a key role in the first-generation drugs. This pocket is associated with an anion-binding region occupied by a phosphate ion and the docking of various anions. This site is essential for the high resolution structure of the human Histamine H1 receptor with a commercially available antihistamine bound in the active site, helps to understand the ligand binding and activation of this important membrane protein. Although there are more than 1,000 GPCRs in the human body, which are major drug targets, only about a handful of drug-binding GPCR structures have been known so far. The structure of the Histamine H1 receptor also provides invaluable information for GPCR studies and more rational drug discovery.

The ligand is primarily surrounded by highly conserved residues among all of the receptors, which make the major interactions with the protein. A novel feature of our complex structure is the presence of an anion-binding site at the entrance to the binding pocket. A phosphate ion, which is part of the crystallization buffer, is placed into the observed strong density in the electron density map. The presence of phosphate at this position is likely because a phosphate ion affects the binding of some ligands and also the stability of the receptor.

The ionic interaction between the phosphate ion and the ligand Doxepin suggest that a phosphate ion may serve as a positive modulator of ligand binding. This has been supported by comparing the thermostability and ligand affinity with and without phosphate present. The detailed analysis of the structure gives deeper insights into the protein-drug relationship of this protein for the first time and enables more rational drug design.

References

Funding Acknowledgements
This work was supported by the ERATO Human Receptor Crystallography Project from the Japan Science and Technology Agency and by the Targeted Proteins Research Program of MEXT (S.I.), Japan, NIH Common Fund grant P50 GM071872. The work was also partly funded by the Biotechnology and Biological Sciences Research Council (BBSRC) BB/I032452/1 (S.I.), Grant-in-aid for challenging Exploratory Research (T.K.), the Mochida Memorial Foundation for Medical and Pharmaceutical Research (T.S. and T.K.), the Biotechnology and Biological Sciences Research Council (BBSRC) BB/I032452/1 (S.I.), Grant-in-aid for challenging Exploratory Research (T.K.), the Mochida Memorial Foundation for Medical and Pharmaceutical Research (T.S. and T.K.), Takeda Scientific Foundation (M.S.) and the Sumitomo Foundation (T.K.). A part of the work was performed in the Membrane Protein Laboratory funded by the Wellcome Trust (grant 061264/2/00/2) and at the Scripps Research Institute, Y. Zhang, The Ohio State University and M. Caffrey, Trinity College Dublin, Ireland, for the generous loan of the m6nu robot.

DOI 10.1038/nature10236
Intermolecular ‘glue’ – the role of inositol tetraphosphate in the HDAC3 co-repressor complex


Histone deacetylase complexes play a central role in the regulation of gene expression through the regulation of the acetylation state of histones and hence chromatin structure. The enzymes ‘class 1 histone deacetylases’ (HDACs) are recruited to chromatin, by repressive transcription factors, as complexes with cognate co-repressor proteins. These complexes are essential for cell viability and are particularly important for determination of cell fate and lineage commitment during development. They are particularly important for determination of cell fate and lineage commitment during development. Importantly, most of the class 1 HDACs are only enzymatically active when recruited to their respective large multi-subunit co-repressor complexes. To understand the structural and functional basis for this activation, we determined the structure of HDAC3, in complex with the interacting portion of the SMRT co-repressor. This is the first structure of an HDAC-co-repressor complex and it reveals the molecular basis for the specificity of assembly and provides clues as to the activation mechanism. Most surprisingly a small molecule, inositol-(1,4,5,6)-tetraphosphate (IP4), was observed at the interface between HDAC3 and the SMRT co-repressor. The IP4 appears to act as an ‘intermolecular glue’ between the two proteins. Functional studies showed that the IP4 is essential for both co-repressor interaction and for HDAC3 activation. HDACs are emerging cancer drug targets, and these results offer potential new therapeutic opportunities.

The most surprising finding from the structure was the presence of an unexpected small molecule bound in a highly basic pocket at the interface between the HDAC3 and SMRT-DAD, which had co-purified with the proteins from the mammalian cells. The small molecule was first identified in the early stages of refinement and the high quality of the electron density map meant that it could be unambiguously assigned as D-myo-inositol-1,4,5,6-tetraphosphate, based on the axial orientation of the hexose group on carbon 2 (Fig. 2). The Ins(1,4,5,6)P4 is coordinated by ten residues, five each from HDAC3 and SMRT-DAD (Fig. 3). If the Ins(1,4,5,6)P4 were not present then the many basic residues on either side of the pocket would repel each other electrostatically and likely prevent complex formation. The fact that the Ins(1,4,5,6)P4 remained tightly bound through out purification suggests that the Ins(1,4,5,6)P4 is essential for complex formation and acts as an 'intermolecular glue' holding the complex together. The presence of Ins(1,4,5,6)P4 in the structure also provides an explanation as to why proteins expressed in bacteria do not interact, since bacteria probably do not contain sufficient Ins(1,4,5,6)P4 to support complex formation.

Mutation of residues on either the SMRT-DAD or on HDAC3 that are involved in Ins(1,4,5,6)P4 binding resulted in the loss of both HDAC3-SMRT-DAD interaction and HDAC3 activity. This suggests that both the SMRT-DAD and the Ins(1,4,5,6)P4 are required for activation of HDAC3. To test this, we established an in vitro reconstitution assay in which bacterially expressed SMRT-DAD was able to activate mammalian expressed HDAC3 in the presence of Ins(1,4,5,6)P4. Other inositol phosphates (Ins(1,4,5,6)P4, Ins(1,2,3,4,5,6)P6) were able to support reconstitution but only at 10-fold higher concentrations than observed with Ins(1,4,5,6)P4, thus confirming that Ins(1,4,5,6)P4 is the physiologically relevant ligand. Of course this was expected, since both IP6 and IP5 are much more abundant in cells than IP4, yet the complex selected and co-purified with the tetraphosphate.

The role of inositol phosphate in HDAC3-co-repressor interaction/activation appears to be common feature with other HDAC-co-repressor complexes since the residues which mediate Ins(1,4,5,6)P4 binding on HDAC3 and the SMRT-DAD are conserved in HDAC1 and 2 and their co-repressor partners (HDAC1-3) and CBX7/8/11. This conservation extends to the yeast HDAC Rpd3 and the Srt1 co-repressor, suggesting that the mechanism is conserved through evolution.

The structure of the HDAC3-SMRT-DAD complex also provides insights into the mechanism of activation of HDAC3 upon binding SMRT-DAD. The binding of the SMRT-DAD and Ins(1,4,5,6)P4 to HDAC3 probably stabilises a number of loops and helices which contribute to one side of the active site surface. As such, we propose that changes in both conformation and dynamics occur when the SMRT-DAD and Ins(1,4,5,6)P4 bind to HDAC3 and that these facilitate substrate access to the active site resulting in enhanced enzyme activity.

The presence of Ins(1,4,5,6)P4 at the interface of the HDAC3-SMRT-DAD complex raises the question of whether it is simply an essential structural co-factor or whether it has a regulatory role. Previous studies have linked phosphoinositols and their kinases with transcriptional regulation, suggesting that Ins(1,4,5,6)P4 probably plays a regulatory role. The yeast protein Arg82, which is an inositol phosphatase kinase that converts Ins(1,4,5,6)P4 to Ins(1,4,5,6)P3, has been shown to be required for chromatin remodelling, and mutation of this protein resulted in changes in the regulation of many genes. Ins(1,4,5,6)P4 itself has been shown to modulate the activity of KIF-dependent chromatin remodelling complexes and consequently stimulate nucleosome remodelling.

The HDAC3-SMRT-DAD structure is the first structure of a HDAC with its co-repressor partner. This structure has provided insights into the activation of HDAC3 by the SMRT domain of SMRT and revealed an unexpected small molecule Ins(1,4,5,6)P4, which bridges the interface between HDAC3 and the SMRT-DAD. The requirement of a phosphoinositol for complex assembly and activation is likely to be conserved to other HDAC-co-repressor complexes, and presents a novel opportunity to target HDACs for anti-cancer and other therapies.

References:

Funding Acknowledgements
The Wellcome Trust (grant WT085408).

Figure 1: Structure of the HDAC3-SMRT-DAD complex, HDAC3 is shown as a grey cartoon, and the Ins(1,4,5,6)P4 as a stick representation. Side chains which are important for the interaction of the SMRT-DAD with HDAC3 and Ins(1,4,5,6)P4 are shown as cyan sticks. Insert panel - 15 μM crystal in loop.
**Structure determination and crystal characterisation in situ**

Danny Axford, Robin Owen, Alice Douangamath, Jose Brandao-Neto, Katherine McAuley, Dave Stuart, Gwynnaf Evans

X-ray data collection from protein crystals within crystallisation plates - in situ data collection - is quickly growing in popularity at Diamond owing to the ease with which crystals can be characterised for their diffraction properties in a 'non-invasive' way and the efficiency with which data can be accumulated from multiple crystals to yield novel structures. Three beamlines, I24, I04-1 and I03 within the MX Village, now offer this powerful analysis technique to users.

I24 was the first beamline at Diamond to introduce in situ macromolecular crystallography and utilizes an adaptation of standard goniometry to hold crystallisation trays (Figure 1a). Development of the method on beamline I24 has culminated in the solution and publication of six virus structures, in addition to a publication detailing the new methodology in which the determination of two other human protein structures is described. The ability to obtain information on the diffraction quality of crystallisation hits is particularly valuable in the challenging task of membrane protein crystallisation. In situ characterisation on I24 has become a routine part of the crystallisation optimisation operations of the Diamond Membrane Protein Laboratory. This method of data collection reduces the risk of false negatives and offers a faster and more sensitive way to compare different crystallisation conditions.

For virus crystallography the virtue of in situ data collection is the elimination of sample handling, allowing a safe working environment for pathogenic samples and removing the potentially destructive effects of cryoprotection. To this end I24 can now operate at biological containment level 2, moderate risk to personnel and the environment, thereby broadening the range of virus samples that can be analysed at the beamline. This work has shown how it is possible to collect enough data to solve a virus structure in situ from crystals no larger than 50 µm using only a few hours of beamtime (Figure 2). During 2011 the availability of in situ data collection and crystal screening has now been extended with I03 and I04-1 also offering the capability.

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I04-1 provides users with a four position plate hotel and utilizes the Rigaku ACTOR sample changer to automatically mount plates kept in a rack (see Figure 1b) onto the sample goniometer. This system allows analysis of many plates under CL3 conditions without users or staff having to enter the experimental hutch. To facilitate this, plates are supported in a metal frame prior to mounting in the rack.

The Generic Data Acquisition (GDA) beamline control software has now been developed on each beamline to allow users to navigate around the plate so that each crystallisation drop can be easily aligned to the beam (Figure 3). The evolution of in situ methods and software will continue at Diamond in the coming years as the beamline teams and software developers offer closer integration between the crystallisation facilities and the beamline. Together with the installation of plate hotels and plate mounting systems this will allow for the automation and full remote control of in situ crystal characterisation and data collection.

**References**

In our 5th year of operation (2011/2012) we saw an increase both in scheduled beam delivery and reliability. A total of 211 days, which corresponds to 5064 hours, were scheduled for users, the majority being either in standard multibunch mode (900 bunch train) or hybrid mode (666 bunch train and single bunch) with top-up operation.

Because of the lack of a spare RF cavity, a cautious approach has been taken to increasing the beam current, which was raised to 225 mA in May 2011 and 250 mA in July 2011. In addition, there were two periods of four days’ ‘Special Beam Conditions’ in September 2011 and February 2012, when the machine was operated in low-alpha mode to produce short bunches (3.5 ps rms) for both time-resolved experiments and coherent TlZ radiography. The uptimes, which is the beam delivered as a percentage of scheduled standard user mode hours, was 97.3 %. This is a slight increase when compared to the previous year (97.5 %), however the average time between failures increased significantly from 28.5 hours last year to 55.4 hours. In July 2011 we also achieved our longest period of beam delivery without a trip, 12.6 days, interrupted only by scheduled weekly Machine Development periods.

Further problems have unfortunately been experienced with the superconducting cavity that was sent back to the supplier for repair following its removal from the storage ring in January 2010. The cavity was returned to Diamond in June 2011, but subsequently failed during cool-down, with a third attempt required to further lengthen the repair. The cavity has just been returned to Diamond in March 2012 and testing and conditioning is about to start. Meanwhile, to ensure the long-term operability of the RF cavities, the go-ahead has been given for the purchase of a fourth cavity, which is due to be delivered at the end of 2013.

Another restriction on increasing beam current in the past was the excessive helium consumption of the two superconducting wigglers which supply beamlines I12 and I15. This was resolved following an intervention to increase the beam current in the March/April 2011 shutdown to install a new liner in the I15 supply beamlines I12 and I15. This was resolved following an intervention to increase the beam current in the March/April 2011 shutdown to install a new liner in the I15 supply beamlines I12 and I15. This was resolved following an intervention to increase the beam current in the March/April 2011 shutdown to install a new liner in the I15 supply beamlines I12 and I15. Studies performed during the May 2011 shutdown indicated that increasing the beam current to 250 mA would result in significant savings in helium consumption.

Phase II & III Beamlines

It was another busy year in carrying out installations and modifications to the storage ring for the remaining Phase II and initial Phase III beamlines. Front-ends were installed for beamline I09 (4.2T) and I13 and insertion devices were installed for I10 (second APPLE II undulator, I13 (in-vacuum undulator) and I99 (APPLE II undulator). In addition, extensive changes have been made to the storage ring itself. In March/April 2011 a major modification of the Storage Ring was carried out for the I09 beamline, similar to that carried out for I13 in December last year. This involved swapping out the two main electrodes, one on either side of the insertion device straight section, with modified ones containing an extra quadrupole magnet. In addition, a short section with two extra quadrupoles was installed in the middle of the straight. The additional quadrupoles allow the machine optics to be altered to produce two minima of the beam envelope in the vertical plane, allowing the installation of two narrow-gap undulators. Commissioning of the I13 optics in late 2010, with a shift of vertical tune from 12.36 to 12.86, proved to be much more difficult than expected; however, the second modification for I09 allowed the vertical tune to be increased to 13.6, which, because it differs by a whole integer from the original value, results in very similar beam dynamics performance. As a result, commissioning the new 09/113 optics took only a few days and was in regular use by the end of April 2011.

Further significant changes have also taken place for the implementation of the second phase of operation of the I10 beamline, namely in ‘polarisation switching’ mode, see Fig. 2. This involved, in August 2011, swapping the two adjacent order grinds with modified ones, which would later allow the required kickermagnets to be installed. The new kicker magnets themselves were installed in January 2012.

The five kicker magnets allow the polarisation of the light received by the beamline to be switched, using the scheme illustrated in Fig. 3. The upper part of the diagram shows the situation when the electron beam is made to travel through an angle at an angle through the upstream undulator (B) such that the beamline only receives radiation from the downstream undulator (A). The lower part shows the reverse situation when the beamline only receives radiation from the upstream undulator (B). This switching from one undulator to the other takes place at a frequency of 10Hz or every 100 msec. Thus, if the undulators are set to opposite polarisation (e.g. right-circular and left-circular) the light that the beamline receives is switched between these two states, so allowing experiments which measure small effects due to differences in polarisation to be carried out.

Accelerator Physics Studies

As well as underpinning the current operation of the machine and the developments that are needed for future beamlines, some of which are described above, a vigorous programme of accelerator physics studies has been carried out, aimed at achieving a better understanding of the machine behaviour, and which might lead to performance improvements in the future. These include: studies of non-linear beam dynamics (affecting dynamic aperture and lifetime), collective effects and instability behaviour, and operation at a reduced vertical emittance etc. At the same time, improved diagnostics are also being developed to permit different beam characteristics to be measured.

One example of recent work has been the successful absolute measurement of the machine energy using a technique called ‘resonant spin depolarisation’. This measurement has proved very elusive in previous attempts, and success might lead to performance improvements in the future. Figure 2 shows a typical measurement waveform that is applied to the kickers while a combination of harmonic waveforms are applied to the rest of the ring. This measurement has proved very elusive in previous attempts. The successful measurement shown in Fig. 4 reveals that the true machine energy is 3.0147 GeV. As well as being of interest in itself, such measurements also allowed other basic machine parameters, e.g. chirp and the momentum compaction factor, to be determined and compared with the machine model.

A second example is the measurement of microbunching, as evidenced by bursts of emission of coherent mm-waves. Early measurements were reported in the 2009/10 Annual Report using a Schottky barrier diode installed on the output port originally developed for visible-light extraction. During the last year a dedicated extraction port has been installed which has resulted in significantly greater sensitivity, and enabling finer features to be observed. Figure 5, for example, shows the bursts of mm-wave emission in low-alpha mode at two different RF voltages. The higher voltage produces a shorter bunch which shows regular bursts of mm-wave emission and a lower overall threshold for bunching, shown by the white dashed lines. Such measurements are useful to compare with the predictions of instability theory and detailed particle tracking simulations, allowing a deeper understanding of the complex interaction of the beam with its environment via the ring ‘impedance’ and with itself via coherent radiation interactions.

Efficiency Saving Measures

The Installation and Facilities Management group has been progressively introducing various utility saving measures over the past few years (see Table 2). Areas of particular focus related to savings on electrical and liquid nitrogen consumptions. Savings were achieved mainly by modifications to the lighting system (i.e. installing light sensors, removing unnecessary fittings, etc.), a reduction in the number of Air Handling Units in operation, as well as the installation of variable-speed drivers on a number of pumps. A programme of reconditioning the whole liquid nitrogen system started in June 2010. By restoring the vacuum insulation, the nitrogen losses were reduced while at the same time the quality of the liquid nitrogen supply to the end users was also improved.

Further savings have been achieved following the decision in 2011 to bring in house the maintenance activities. A review indicated that reducing the frequency of certain maintenance tasks and re-arranging the team structure, could bring about a significant reduction in maintenance costs.

Table 2: Predicted annual savings for the utilities and maintenance in 2012.
The Optics and Metrology Group support all of Diamond's beamlines in a number of key areas including: optical simulation and design, specification and procurement, acceptance testing and optimisation of beamline optics and mechanics. To extend Diamond's capability and achieve world leading performance, the group is also actively involved in a range of diverse research projects including development of next-generation optics, instrumentation, and techniques1.

Over the past year, the Optics and Metrology group have moved beyond simple acceptance testing of synchrotron optics, and progressed to investigate routinely and optimise complex optical systems in a configuration which closely replicates beamline conditions. (Fig. 1).

Industrial scale mechanics have been developed to manipulate and test large (>1.5 m) and heavy (>100 kg) optical assemblies in the metrology cleanroom laboratory. Using feedback from the metrology instruments, the Diamond Nomadon Optical Metrology – known as the Diamond-NOM – and the Fizeau interferometer, the metrology and beamline teams are able to interactively the clamping configuration, or physically modify the mechanics, until an acceptable compromise is achieved between mechanical stability, cooling rates, and optical performance. This improved procedure gives a much clearer understanding of how optics will ultimately perform on Diamond's beamlines.

Recent investigations have provided several alarming examples of how clamping, or attaching water cooling manifolds, can seriously deform the optical surface, which dramatically distorts the reflected or diffracted X-ray beam. In one case, a state-of-the-art mirror for the Surface and Interface Structural Analysis (SIA) beamline was significantly degraded. Using the Diamond-NOM, the minor mechanics and clamps were iteratively adjusted to an acceptable level (Fig. 2). Without this insight, this mirror, and other such optics, would have been installed on the beamlines in a distorted state causing degrading performance. Currently, the major limiting factor for synchrotron optics is not the quality of optical substrates, but how the optics are mechanically clamped. In collaboration with the engineers and optics suppliers, it is hoped that metrology data can be used to help design and implement improved mechanics for synchrotron optics, resulting in world class performance and scientific output for Diamond's beamlines.

Figure 1: An example of optimisation of a complete optical system in the Metrology lab (I13). Coherence branchline mirror investigated using the Fizeau interferometer. Before optimisation, it was found that the clamping and cooling pipework were significantly distorting the mirror surface. Characterisation of the metrology cleanroom, prior to beamline installation, can save significant amounts of X-ray commissioning time.

Figure 2: Prior to clamping, a Diamond-NOM shape error map (blue curve) showed that the mirror for the UVF plane giving monochromatic radiation, was not particularly good. After optical clamping, the shape error of the mirror was significantly improved (red curve). Using feedback from the Diamond-NOM, the clamps were iteratively adjusted to minimise mechanical stress (green curve) and provide good optical performance.

Over the past year, the Optics and Metrology group have moved beyond simple acceptance testing of synchrotron optics, and progressed to investigate routinely and optimise complex optical systems in a configuration which closely replicates beamline conditions. Using this apparatus and commercial Fresnel zone plates, sub-100nm foci have recently been achieved for the first time at Diamond on the Microfocus Spectroscopy beamline I18. Further trials are planned to provide such beams routinely to Diamond users.

A series of X-ray and ex-situ metrology tests revealed surface defects on several bimorph mirrors at Diamond, which were degrading the beamline performance. To investigate and correct these issues, the optics group worked together with teams from I02, I03 and I04. Four bimorph mirrors were removed from the beamlines and returned to the manufacturers. After successful repolishing at SESI (Société Européenne de Systèmes Optiques), and optimisation in the Metrology lab using the Diamond-NOM and Fizeau interferometer, the mirrors were safely reinstalled on their beamlines. X-ray tests have recently shown that the re-polished, high quality mirrors have significantly improved the size and shape of the focal X-ray beam (Fig. 3), extending the impact of science performed on the beamlines.

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Side bounce
Fizeau interferometer
Optics and Metrology Group

Figure 4: Stokes-Poincaré parameters (P1, P2 and P3) of the polarised light on I10 beamline from an excited device, as a function of the linear arbitrary polarization angle.

Linear Arbitrary Polarisation Angle (°)

Side bounce
Fizeau interferometer
Optics and Metrology Group

Figure 5: The optical beam profile before (left) and after (right) in polishing a pair of bimorph mirrors on an MX beamline. The improved surface quality has significantly improved the size and shape of the X-ray beam profile.

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In the past year, the detector group of Diamond has contributed to a number of projects in collaboration with STFC and other institutions. The detector group was a partner of the EU funded joint research activity HIP2PAD where it contributed to the characterisation beam tests of the hybrid CdTe detector assemblies produced by other partners. The detector group collaborated with the Daresbury Laboratory of STFC in the development of a germanium strip detector for dispersive EXAFS, and with Rutherford Appleton Laboratory (RAL) in the development of a detector for high energy diffraction based on CMOS sensors previously developed at RAL. Two of these detectors will be delivered in 2012 to Diamond.

Diamond is a member of the CERN-led MEDIPIX3 consortium, and through the detector group contributes very actively to the characterisation, development, and scientific exploitation of the MEDIPIX3 ASIC. A contract with the University of Manchester was recently agreed to develop a beam profile diagnostic based on MEDIPIX3 technology.

The majority of the work of the detector group in detector development has been devoted to two development projects: EXCALIBUR and MERLIN.

**EXCALIBUR detector development**

The EXCALIBUR project has its origins in the survey on future detector needs undertaken in 2008. From this, it emerged that the major detector need for the beamlines of Diamond was a photon counting area detector with a pixel size of 50 micron. The detector group then promoted the use of the MEDIPIX3 ASIC to build a first prototype of large area detector with photon counting characteristics.

The detailed specifications that came from beamline I13 - which had been chosen as the test case - were for a matrix of 3 million pixels, with at least 2000 pixels with no dead areas in one direction. The detector should be capable of storing data continuously at a speed of 100 frames per second or to local memory at a speed of 1,000 frames per second for a few seconds.

The plan was then to build the detector from three monolithic silicon sensors with a matrix of 2048 x 512 pixels each. Each sensor is hybridised to 16 MEDIPIX3 ASICs leading to a total of 48 ASICs for the entire detector. These specifications were a big challenge, pushing the boundaries of the production technology of hybrids and of the data acquisition electronics. The sensors are the longest monolithic sensors built for this kind of applications and the largest wafers available from the manufacturer had to be used to produce them. A big challenge for the manufacturer was to keep the wafer bow over the entire wafer to acceptable levels. Similarly the frame rate stipulated in the specifications, required the ASICs to operate in full parallel read-out mode, leading to an extremely high density of electronics tracks in the interconnections boards and a huge data volume to be managed by the back-end of the detector.

The EXCALIBUR project was pursued in the framework of an organic collaboration with STFC. The team took the responsibility for the development and production of hybrid assemblies, including the design and production of carrier and interconnection boards. We coordinated the design and hybridisation of the silicon sensors by liaising actively with the developers of the read-out chips as well as with the manufacturers of silicon sensors and hybrid assemblies. A class-10,000 clean room was set up in our laboratories to provide a clean environment for the test and bonding facility which was developed for assembly and quality control of EXCALIBUR modules. This facility includes a probe station to test, electrically and with radiocative sources, the hybrids before they are bonded to the carrier board, assembly jigs to bond the hybrid to the cold-finger and the carrier board (Fig.1c), visual inspection instruments for alignment and documentation. At the same time, we carried out the design of carrier and flexi-rigid interconnection boards needed to interface MEDIPIX3 ASICs to the read-out FPGA boards developed by STFC. The design was sent to manufacturers and the boards were used to produce test assemblies such as the one shown in Fig.1b). These test assemblies were delivered to STFC, where they are used to develop the firmware of the read-out FPGA boards.

The work on EXCALIBUR is ongoing - initial results with the first hybrid produced are encouraging, and no show stoppers emerged with the interconnection boards and the module assembly procedures developed by the detector group. The EXCALIBUR detector will be delivered to I13 in 2012 and we look forward to seeing our detector in operation.

**MERLIN detector project**

The MERLIN project is carried out entirely at Diamond and consists of the development of a single MEDIPIX3 ASIC read-out system. The ASIC is driven by a National Instruments FPGA board through an adapter board designed by us and a 1 m to 10 m long cable that connects the adapter board to the ASIC.

The origin of this project is in the pilot read-out system that the detector group built to understand the challenges related to driving the MEDIPIX3 ASIC. It was then realised that this system could be a very compact and high performance detector operating a single MEDIPIX3 ASIC, which could be easily located in places such as a diffractometer arm. A project was then started to develop and build two systems for I16 and B16, consolidating and expanding the capabilities of our pilot system (Fig.2). The MERLIN project is carried out with the support of a contractor for the National Instruments card programming.

The MERLIN project will deliver detectors to I16 and B16 in 2012 and will become the major development, test, and evaluation platform of the detector group for MEDIPIX3 ASICs. This system is presently the fastest available data acquisition single ASIC system within the MEDIPIX3 consortium and we will offer it to the other members when a high performance system is required.

**References**

The Data Acquisition and Scientific Computing group provides a number of software tools namely the Generic Data Acquisition (GDA) for customised data acquisition and the Scientific Data Analysis workbench (SDA) for data analysis. In combination with highly evolved software workflows, customised parallelised applications and powerful underlying architecture of processing systems and networks, some of the most advanced acquisition and analysis systems are now available for scientists.

The efficient storage of all data resulting from experiments at the beamlines is an important task of the computing group. In the year 2011, a total of 1726 experimental visits took place. Together with preceding years, the measured data volumes sum up to ~22TB and 95,000,000 files on the ICAST data archive.

There have been many advances during the past year; highlights include the full use of I13 for tomography and imaging within the standard user interface, a common user interface and scripting system for all spectroscopy beamlines, implementation of the MX end station upgrades with full fast integration of Pilatus detectors up to 6M and the implementation of ptychography on the I13 coherence beamline.

The GDA and SDA technology

GDA and SDA are written in JAVA within the Eclipse Rich Client Platform. The common underlying architecture virtually eliminates duplication of software by using common plug-ins that are highly configurable within an industry standard framework. Moreover the architecture facilitates beamline customised configurations and allows specific scientific analyses to be built as plug-ins while encouraging collaboration in their implementation.

The GDA highly evolved powerful python based scripting interface and associated graphics. User interfaces are used for the acquisition part of the underlying EPICS software control system or for systems such as TANGO (Tao Next Generation Object) or even bespoke controllers, e.g. the Medipix2 detector on the I11 Coherence beamline is TANGO interfaced. It is important also to note that GDA includes native support for the continuous scanning mode which is faster and more capable than stepping mode further enabling advanced experimentation. GDA is an open-source framework (link: www.opengda.org).

The SDA has an integrated Python and Cython scripting interface for these programming languages, to enable both simple and complex data evaluation and analysis. Additional options include support for ICAST, linux browsing, remote data download and interfaces with tools such as EDNA, a framework for developing plug-in based applications, and Fasermaler workflow tool. Mechanisms can be provided for the complex workflows necessary for automating data analysis or even for defining interspersed acquisition scans with complex analysis. The SDA is available on all Diamond beamlines, however it can also have science specific plug-ins that are specialised for certain beamlines and are in routine use, see table 1.

GDA – used a powerful and flexible beamline alignment tool

In configuring a beamline for user services it is usually necessary to optimize the X-ray insertion device in order to optimize the photon flux and the cross-sectional area of the beam. The extraordinary flexibility of GDA scanning allows this to be performed very accurately and easily in minutes. In the case of the I13 undulator, for instance, this was done in less than two minutes and all with just one scripting command that even enabled the user to view step-graphically.

GDA enabling the PE Loop Environment - a new tool for measurement of X-ray diffraction and ferroelectric polarization at I11

The PE Loop Environment software allows the simultaneous measurement of X-ray diffraction and ferroelectric polarization data as a function of the applied electric field. The goal was to investigate the electric polarization of polycrystalline materials as a function of frequency in order to determine the microstructural origin of the piezoelectric effects within these materials by probing their dynamic ferroelectric response. Stroboscopic and real time data can be collected by using the time frame generator (TFG) with a continuous display of collected data.

GDA Difficall – an improvement for diffractometry at Diamond

The enhanced Difficall II program includes a new calculation engine that offers hundreds of ways to constrain the extra degrees of freedom provided by many diffractometers and their geometries. The application allows a much wider range of experiments to be performed at Diamond using several different diffractometer types. In addition the new functionality offered supports the full range of features for conventional step scans, but also extends them to continuous or trajectory scans in any mix on the command line.

Diamond’s Materials and Magnetism beamline 116 exploits this new functionality and can now continuously scan its 6-circle diffractometer through reciprocal lattice space while collecting and processing images from a Pilatus 2M detector at 10 Hz. Acquiring 2d data while the sample and detector are moved continuously rather than stepped from image to image allows data to be collected orders of magnitude faster and in some cases capture information that step scanning cannot. Difficall is currently available and is being used on the following beamlines, each with different hardware and scientific disciplines to support: 106 (Nanoscience), 107 (Surfaces), 110 (Advanced Dicsohrism), 113 (Imaging/Coherence), 116 (Testing) and 116 (Materials and Magnetism).

High Speed automated data processing for MX

Raw diffraction data can be recorded in less than three minutes on Diamond 106 beamline i02, as part of ongoing research into DNA ligand structures. The automatically calculated map, derived purely from experimental information, requires a search over a number of parameters. This automated analysis enables scientists to get the results less than two minutes after data collection, a step which would once have taken hours. The whole process from data acquisition to an electron density map of the sample was performed in less than five minutes.

Further activities

Examples for the SDA - Integrating detailed sample viewing, evaluation and archiving for i22

Examples for the SDA - Graphical sample analysis for Nanostructures (i06)

Table 1: Examples of beamlines where SDA is in use at Diamond.

<table>
<thead>
<tr>
<th>Beamline</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I02</td>
<td>Magnetic Materials and Magnetism</td>
</tr>
<tr>
<td>I03</td>
<td>Nanocrystals and Nanomaterials</td>
</tr>
<tr>
<td>I10</td>
<td>Advanced Dichroism</td>
</tr>
<tr>
<td>I13</td>
<td>Imaging and Coherence</td>
</tr>
<tr>
<td>B16</td>
<td>Materials and Magnetism (Testing)</td>
</tr>
<tr>
<td>I22</td>
<td>Non Crystalline Diffraction</td>
</tr>
</tbody>
</table>

References

Key Facts and Figures

Facility usage
In our fifth year of operations (April 2011 to end of March 2012), we received 881 proposals for experiments, which requested a total of 11,568 shifts. After peer-review, 475 proposals were awarded beamtime. This resulted in 6,349 experimental shifts being allocated, spread across 20 beamlines. We welcomed 4851 user visits throughout the year.

User shifts requested, awarded and delivered by village and beamline 2011/12

Research outputs
The Diamond publications database contains all user publications based on synchrotron data gathered wholly or in part at Diamond Light Source and scientific papers published by our staff. Further improvements have been made to simplify the submission of publications. Another key indicator of our performance is the number of protein structures solved on the Macromolecular Crystallography beamlines and deposited in the Protein Data Bank.

Cumulative number of publications by our scientists and users and cumulative number of protein structures solved

Machine performance

To date

Archaeological and Cultural Heritage
Biology and Bio-materials
Chemistry
Energy
Engineering
Food Science
Materials
Medicine
Physics
Technique Development
With over 50 companies participating in experiments across 17 beamlines, industrial research and development activities continue to be of great importance throughout the facility. The Industrial Liaison Office has developed a flexible approach to working with industrial partners, recognising that providing a tailored research solution may require varying levels of support at different stages of the project. Diamond offers a wide variety of access mechanisms ranging from collaboration and full data collection and analysis services, through to mail-in data collection, remote access, and beamtime only access for experienced users who prefer to perform their own experiments.

With industrial partners from across the globe and just around the corner, from large organisations through to SMEs and start-up, the range of types of companies that use Diamond is often surprising. Heptares, a Herfordshire-based biotechnology SME, have solved the structure of a protein involved in Parkinson’s disease and other neurological disorders. The team used the microfocus macromolecular crystallography beamline U4 to reveal the complex structure of the adenosine A2A receptor and show how xanthine-based drugs such as caffeine bind to their target. Their findings, published in the journal Structure, could pave the way for a new generation of targeted drug treatments. Dr Fiona Marshall, Chief Scientific Officer at Heptares, explains “these co-structures of xanthines in complex with the adenosine A2A receptor advance our understanding of what is happening at the molecular level when the drug binds to its target and blocks the receptor’s response. Along with novel chemotypes discovered by our team, the structural data we collected at Diamond is enabling us to develop highly-optimised next-generation drug candidates for Parkinson’s disease and other neurological disorders.”

BioFocus, a Cambridge-based drug discovery provider, is working with OIL! Foundation, Inc., a biomedical research organisation exclusively focused on developing disease-modifying therapies for Huntington’s Disease, a dominantly-inherited neuropsychiatric disorder. It has been known since 1993 that the disease is due to mutation of a single gene coding for huntingtin (HTT) that extends the poly-glutamine (poly-Q) repeats in the protein. Inhibiting the enzyme responsible for the cleavage of mutant HTT - thereby reducing the generation of the poly-Q fragments - could be one way of preventing neurodegeneration. Caspase-6 has been suggested to be this processing enzyme, but, while structures of other caspase enzymes have been solved, caspase-6 had proven resistant to structural studies, with only the crystal structure of the apo-enzyme in a pH-inactivated state solved. BioFocus identified crystallisation conditions for apo-caspase-6 in the active state, in complex with the irreversible inhibitor as well as in complex with the reversible inhibitor. They found that, surprisingly unlike the reversible inhibitor, the irreversible inhibitor binds to caspase-6 in the inactivated conformational state, and in a binding mode not previously observed for any other caspase-inhibitor complex. “The rapid access to the MX stations at the Diamond synchrotron helps us to deliver final structures to our clients within a few weeks of obtaining the first crystals,” said Dr Ilka Müller, Senior Scientist at BioFocus.

Johnson Matthey is a speciality Chemicals Company with key research areas focusing on catalysis, precious metals, fine chemicals and process technologies. They access our facilities via the proprietary route and also apply for peer-reviewed beamtime in collaboration with leading UK academics, publishing their findings from these experiments. “Non-proprietary work allows, amongst other aspects, an opportunity for methodology development in the areas of in situ characterisation while working with academically relevant catalysts and materials,” explains Dr Tim Hyde, Principal Scientist at Johnson Matthey. “This is a great way to work as we help to develop techniques that everyone can use through the peer review system. Once these techniques are available we may be in a position to apply them to our materials under the proprietary arrangement for industry.”

Large scale research challenges facing an entire industry sometimes require a large scale collaborative approach. In the past decade, the world has experienced a widening gap between the predicted demand for oil and known reserves, fuelled particularly by the growth of new economies like China and India. High oil price may particularly affect the competitiveness of the chemical industry in Europe, relying on imported oil for more than 70% of supplies. In a global environment, with the higher cost of naphtha from crude oil and the higher cost of CO2, the chemical industry may need to turn to novel feeds such as natural gas, coal and biomass to stay competitive. Technologies that are able to use light alkanes (C1 – C4) and CO2 as feedstocks are needed. However, light alkanes and CO2, in contrast to long-chain hydrocarbons from oil, are stable molecules that are difficult to activate and transform directly and selectively into added value products. Radical scientific and technological improvements are thus required to enable efficient and competitive routes for their use. In June 2011, the Industrial Liaison Office started work on the CARENA project (Catalytic Reactors based on New Materials), a large EU-funded collaborative project to create technologies enabling efficient conversion of light alkanes and CO2 into higher value chemicals. CARENA brings together a strong European consortium with top level universities, R&D centres, industrial technology providers, chemical producers and innovative SMEs, with the Industrial Liaison Office at Diamond as one of the British partners involved in the collaboration. Diamond’s role in the project focuses on the development of cells for structural properties studies of catalysts under operating conditions. Additionally, spectroscopic and diffraction techniques will be exploited to characterise the novel catalytic materials, membranes, metal organic framework materials and proton conductors prepared by the other members of the consortium. Collaboration in the CARENA project will allow Diamond to continue to develop its capabilities as a centre of excellence for catalysis research and in providing additional support for Diamond’s industry and academic users.

In the past year three new members joined the team. Dr Alexandre Dias and Dr Jilka Waterman, experienced structural biologists, work closely with our industrial partners to provide research solutions to the pharmaceutical and biotechnology industries, and Dr Leigh Connor takes responsibility for industrial engineering and diffraction experiments at Diamond. To find out more about the work done by all members of the team and ways in which we may be able to help a wide range of industries solve their research problems, please visit our webpages at www.diamond.ac.uk/industry.
Engaging the public
We aim to engage the public both on and off our site, and to work with organisations around the country to underline our role as a national facility. In addition to visiting science festivals in London, Bradford, Oxford and Cheltenham, we have launched new engagement projects aimed at the online community.

Light Reading was a short story writing competition, which produced works of fiction across all genres, inspired by our science and technology. Following promotion through social media, the competition attracted over 70 entries, which were judged by Jenny Rohn, founder of LabLit, and Anjana Ahuja, freelance science journalist. The entries demonstrated deep engagement with the science and technology of Diamond, the writers produced stories of incredibly high quality both in a scientific and literary sense. The project continued our long standing relationship with the arts, which will continue into 2012 through collaborations with Oxford Inspires. We intend to extend the project in 2012 to local schools, creating a new cross curricular project linking science with the arts in the classroom, and in the process, have become partners in the bid for Oxford to be the UNESCO 2014 World Book Capital.

We also produced a new series of films focusing on applications in engineering, environment, health and structural biology. These are available to everyone, and have been created to promote the breadth of science being undertaken at Diamond. In the coming year, we will promote these resources for teaching in schools and to undergraduates. We also worked with STFC on their ‘Backstage Science’ programme aimed at a younger audience, with contributions from Diamond scientists and engineers.

Inside Diamond was as popular as ever, with the four Saturday events in the past year being full to capacity, which means in total almost 1,000 people got the chance to visit through these sessions. Being able to talk directly with our scientists and engineers about the work being carried out here is incredibly important, and is inspiring for our staff and visitors alike.

Away from Diamond, the past year saw us visiting the Big Bang Fair, Cheltenham Science Festival, the British Science Festival and the Oxfordshire Science Festival, meeting members of the public and encouraging engagement with our science and technology. Festivals are an important means of engagement, and we will continue to take our science on the road throughout 2012, starting with the Edinburgh Science Festival in April. The Oxfordshire Science Festival also hosted a celebration exhibition of images from our construction and operations at Oxford Castle complex throughout the duration of the festival.

Engaging schools
Our series of events for A-level students, based on the format for Inside Diamond continued to grow in 2011-2012, welcoming 720 students. In addition, we continued our successful partnership with STFC to deliver expanded Particle Physics Master classes and the Engineering your Future event by welcoming 420 pupils to the facility to joint events.

We carried out our first continuing professional development for teachers event in collaboration with the examinations body OCR (Oxford Cambridge and RSA Examinations), and we will continue to develop resources for A-level teaching throughout the coming year, delivering training for teachers in schools around the region. For teachers, we have also continued to host celebration events with STEMnet, bringing together our STEM ambassadors with local teachers to encourage collaborative working.

Engaging through our scientific user community
Our role in supporting the user community in engagement is growing, and we are committed to providing opportunities for engagement, resources and training for users.

In the past year, we hosted our first dedicated student event as part of the user meeting, bringing 30 PhD students together for training in communication skills and public engagement. The student workshop will become a regular event, linked to the user meeting, and we will implement a coordinated training scheme for all PhD students linked to Diamond.

We introduced a new scheme - the Young Investigators Award - launched at the user meeting 2011. The first winner of the award will be invited to speak at the 2012 meeting, and we hope that the competition will become an important means of recognizing achievements made by our younger users.

We welcomed over 1,000 scientists through our programme of workshops and scientific conferences.

Some of the highlights include CD2011 which saw 150 members of the circular dichroism community come together for a successful conference hosted at the Said Business School, Oxford. We also hosted international meetings for the optics community (ACTOP), and in radiation damage (RDT).

The 2011 user meeting welcomed Nobel Prize winner and Diamond user Venki Ramakrishnan as a keynote speaker, with over 250 delegates attending. The user meeting continues to grow in its new format, with the 2012 meeting celebrating our 10th anniversary.
Governance and Management

Diamond Light Source Ltd was established in 2002 as a joint venture limited company funded by the UK Government via the Science and Technology Facilities Council (STFC) and by the Wellcome Trust, owning 86% and 14% of the shares respectively. Diamond now employs over 400 scientists, engineers, technicians and support staff from over 40 countries worldwide. The Chief Executive and Directors are advised by committees representing key stakeholder groups, including the Science Advisory Committee, Diamond Users’ Committee, and Diamond Industrial Science Committee (DISCs).

Diamond is free at the point of access for researchers, provided the results are in the public domain. Allocation of beam time is via a peer-review process to select proposals on the basis of scientific merit. Six peer-review panels meet twice a year to assess the proposals submitted for each six-month allocation period. Diamond also welcomes industrial researchers through a range of access modes including proprietary research.

Board of Directors

Lord Alex Dearnley (Chairman)
House of Lords Science & Technology Select Committee

Prof Gerhard Materlik
Chief Executive Officer, Diamond Light Source

Andy Akerman
Director Finance & Corporate Services, Diamond Light Source

Prof Jim Naismith, FRSE
Director Finance & Corporate Services, Diamond Light Source

Prof Gerhard Materlik
FRS, CBE, FInstP
was appointed CEO of Diamond Light Source in 2001. He was previously Director of the Hamburg Synchrotron Radiation Laboratory and a member of the Scientific Directorate of the German Electron Synchrotron DESY, and also Professor of Physics at Hamburg University.

Prof Trevor Rayment joined Diamond as Director of Physical Sciences in 2008. He is also Professor of Physical Chemistry at the University of Birmingham, where his research focuses on development of novel techniques for studying interfaces and their applications in nanoscience, solid-liquid interface of electrodes and biomolecular interactions at interfaces.

Prof David Stuart
FRS is MRC Professor of Structural Biology at the University of Oxford, and Head of the Division of Structural Biology at the Department of Clinical Medicine. He was appointed Director of Life Sciences at Diamond in 2009. His principal research interests include the structure of viruses and viral proteins as well as cellular proteins, especially those that interact with viruses.

Prof Richard Walker joined Diamond as Technical Director in January 2002. He was previously Director of the Light Source Division at Sincrotrone Trieste in Italy, and prior to that he was a key member of the Daresbury Laboratory SRS team. He is a visiting Professor of Physics at the University of Oxford.

Andy Akerman joined Diamond as Director of Finance and Corporate Services in 2009. With over 30 years experience in financial management, he was most recently Finance Director of the Defence Aviation Repair Agency.

Summary of Financial Data

<table>
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<tr>
<td>Near Cash Operating Costs £m</td>
<td>23.5</td>
<td>28.4</td>
<td>30.5</td>
<td>33.5</td>
<td>36.5</td>
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<tr>
<td>Total Staff (Year End)</td>
<td>326</td>
<td>369</td>
<td>401</td>
<td>419</td>
<td>438</td>
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<tr>
<td>Capital Expenditure – Operations £m</td>
<td>1.0</td>
<td>4.5</td>
<td>5.7</td>
<td>8.6</td>
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<tr>
<td>Phase II £m</td>
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<td>25.5</td>
<td>22.0</td>
<td>16.2</td>
<td>9.9</td>
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<tr>
<td>Phase III £m</td>
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<td>0.1</td>
<td>0.3</td>
<td>3.0</td>
<td>10.3</td>
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</table>
The Scientific Advisory Committee (SAC) advises the CEO and the Science Directors on the scientific and technical questions impacting on the specification, design, commissioning and operation; experimental and user support facilities, and opportunities for scientific exploitation.

Prof Alexander Korsunsky (Chair)  
University of Oxford (UK)

Prof Paul Freemont (Vice Chair)  
Imperial College London (UK)

Prof Jesper Andersen  
University of Lund (Sweden)

Dr John Barker  
Evotec (UK) (DISCo Representative)

Prof Anthony Cheetham  
University of Cambridge (UK)

Prof Chi-Chang Kao  
SSNL (USA)

Prof Giacomo Ghiringhelli  
Poliemica di Milano (Italy)

Prof Chris Jacobson  
Argonne National Lab (USA)

Prof Sean Langridge  
ISI, STFC (UK)

Dr Andrew Leslie  
MRC Laboratory of Molecular Biology  
Cambridge (UK)

Prof Des McMorrow  
UCL (UK)

Prof Richard Patrick  
University of Manchester (UK)

Prof Rasmita Raval  
Univ. of Liverpool (UK)

Prof Janet Smith  
University of Michigan (USA)

Prof Mark Spearing  
University of Southampton (UK)

The Diamond Industrial Science Committee (DISCo) advises the CEO and Directors on opportunities for industry to be engaged in research at Diamond, industrial research priorities that will help shape operational strategy, including the best way to exploit the current suite of beamlines and to develop the case for investment in future beamlines, and to develop best practice for industrial engagement.

Dr Malcolm Skingle (Chairman)  
GlaxoSmithKline

Dr Peter Ash  
Johnson Matthey

Dr John Barker  
Evotec

Prof David Brown  
Cangenta Ltd

Dr Rob Cooke  
Heptares Therapeutics

Dr Jonathan Hyde  
National Nuclear Laboratory (NNL)

Dr Matthew Johnson  
GlaxoSmithKline

Dr Anne Kavanagh  
AstraZeneca

Dr Ken Lewtas  
Infinum

Prof Dave Rugg  
Rolls Royce

Emily Nott  
Technology Strategy Board

The Diamond User Committee (DUC) has been set as a platform for discussion between Diamond and the user community of matters relating to the operation and strategy of Diamond.

Prof Bill Clegg (Chair)  
University of Newcastle

Dr Joanna Collingwood  
University of Warwick

Dr David Dye  
Imperial College London

Prof Peter Lee  
University of Manchester

Dr Karen Edler  
University of Bath

Dr Karen Hudson-Edwards  
Birkbeck, University of London

Dr David Lawson  
John Innes Centre

Prof Malcolm McMahon  
University of Edinburgh

Prof Keith Meek  
University of Cardiff

Dr Peter Moody  
University of Leicester

Prof Pagona Papakonstantinou  
University of Ulster

Dr Josep Sureda-Suso  
Keele University

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