SCANZ — CRYSTAL 31 Abstracts — 2017

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SESSION 1: 1987 FUND LECTURE

Chair: Michael Landsberg

CryoEM of mitochondrial membrane protein complexes

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The advent of new direct electron detectors for electron cryo-microscopy (cryoEM) is having an enormous impact on the structure determination of large, flexible protein complexes that were previously out of reach. [5] Single-particle cryoEM of mitochondrial ATP synthase dimers revealed a pair of long, membrane-intrinsic helices in subunit *a* of two different mitochondrial ATP synthase dimers adjacent to the *c*-ring rotor [1, 4]. The helices are a fundamental feature of all rotary ATPases and play a key role in proton transfer. [6] By electron cryo-tomography (cryoET) of mitochondria from a wide range of organisms [3, 7, 8] we found that rows of ATP synthase dimers along cristae ridges are a conserved, universal feature of inner membrane organisation. By single-particle cryoEM we discovered an unexpected functional asymmetry in the respiratory supercomplex $I_1III_2IV_1$ from bovine mitochondria [9]. CryoET revealed that the interaction of complex I with the complex III dimer in the supercomplex is conserved across all species, and thus appears to be essential for effective energy conversion in mitochondria. Recently, we determined the cryoEM structure of the twin-pore protein translocase TOM that transports more than 1000 different pre-proteins from the cytoplasm into mitochondria [2]. In the TOM complex, two 19-strand beta barrels of the Tom40 subunit, which resemble the mitochondrial VDAC anion channel, are surrounded by small, trans-membrane Tom subunits and held together by the tilted helices of the pre-protein import receptor Tom22.

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SESSION 2: MATHIESON MEDAL LECTURE

Chair: Chris Ling

In situ and *in operando* neutron and X-ray studies of functional materials

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Functional materials are central to many technologically important devices and systems, including sensors, electronic devices, and energy storage and delivery. Such materials are often multifunctional and highly responsive. Developing experimental approaches that specifically target functional materials is essential to obtaining the atomic-level mechanistic understanding that leads directly to their advancement. This is particularly relevant given the increasing power and speed of characterisation instrumentation, allowing real-time and non-equilibrium measurement of the material during operation to be made. This presentation will give examples of the application of X-ray and neutron techniques of analysis, in combination with computational density-functional-theory-based methods, to understand materials function [1]. Example studies for a range of functional materials will be presented, covering experimental approaches that will include real-time studies of electrodes during battery operation (structural changes accompanying wide-range compositional variation) [2–3], as well as studies of porous framework materials applied as sorbents (gas-adsorption and temperature effects) and with interesting mechanical properties (pressure-dependent studies) [4–6].

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SESSION 3: KEYNOTE SPEAKER 1

Chair: Chris Richardson

Partially interpenetrated metal-organic frameworks

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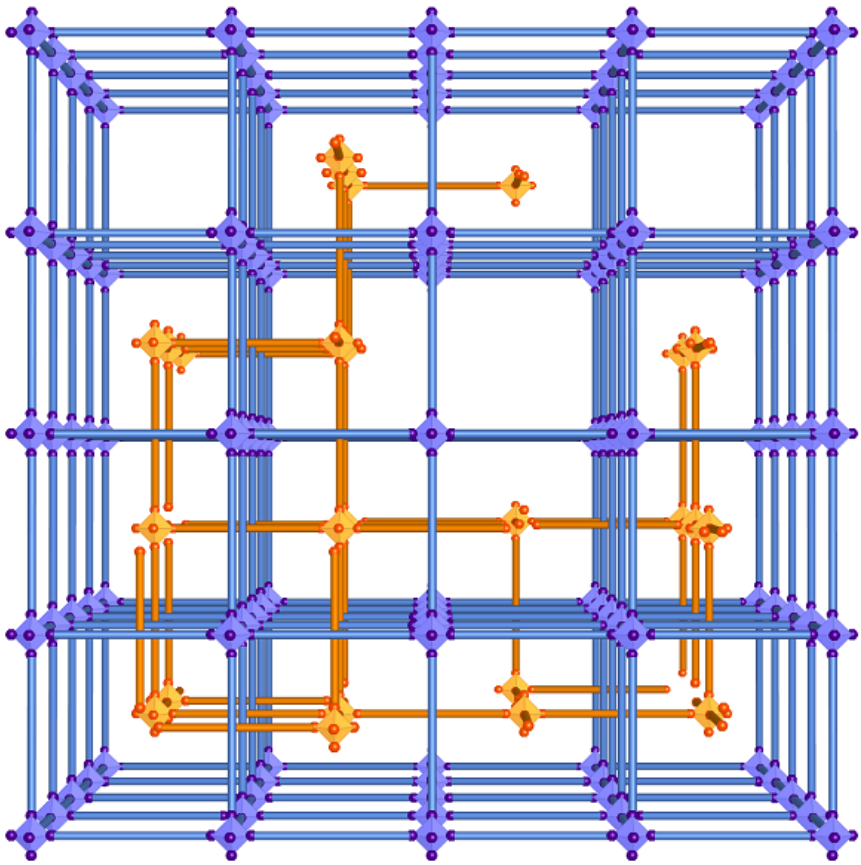
Metal-organic frameworks (MOFs) are often interpenetrated wherein a second copy of the lattice grows in the pores of the first. MOF interpenetration is typically a binary condition: an MOF is either interpenetrated or it is not. However, we have developed frameworks in which partial degrees of interpenetration can observed. Partial interpenetration can be induced and controlled either by direct growth from soluble precursors or by the single-crystal to single-crystal conversion of noninterpenetrated frameworks into their interpenetrated counterparts. These results cast new light on MOF growth mechanisms, structural properties, and functional applications. They also offer opportunities to synthesise interesting frameworks that have hitherto been inaccessible.



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CONCURRENT SESSION 4A: EXPLORATION OF MINERALS AND MATERIALS

Chair: Christine Beavers

Deciphering complex biogenic carbonates: The skeleton of coralline algae *Porolithon onkodes* viewed with synchrotron micro-X-ray diffraction

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INVITED SPEAKER

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Biogenic Ca-carbonates are precipitated by a range of calcifying marine organisms (e.g., corals, foraminifera, molluscs, crustaceans, and coralline algae) and — when deposited and preserved — form part of the massive limestone formations that we know today (e.g., the Dolomites). The Mg/Ca ratio of some of these shells and skeletons is related to temperature and may be used as paleo-climate indicators provided that the materials' mineralogy is well characterised and the mechanisms of mineralisation and Mg-partitioning well understood.

The identification of well-crystallised Ca-Mg carbonates (e.g., aragonite, calcite, Mg-calcite, dolomite and magnesite) with a combination of X-ray diffraction and chemical analysis is straightforward because their crystal structures are simple, and the relationships between unit-cell parameters and Mg-content is well-known [1], as is that between calcite d_{104} spacing and cation ordering [2]. In contrast to this, Ca-Mg-carbonates that form by biogenic processes are more challenging to characterise because they are typically very finely grained, poorly crystallised and contain abundant organic material, all of which contribute to XRD peak broadening and low intensities, making it particularly difficult to see weak reflections such as dolomite ordering peaks [3]. In addition, the biological materials frequently exhibit chemical inhomogeneity (Ca/Mg) on the nano-scale, causing peak overlap if the area sampled is larger than the domain size. In the case of crustose coralline algae *Porolithon onkodes* (Heydrich) Foslie, the mineralised skeleton is a bio-composite material, where up to five different carbonate phases are inter-grown on the micron- and nano-scales, posing challenges to analytical resolution and detection limits.

We present a detailed analysis of the complex carbonate skeleton of coralline algae using synchrotron micro-XRD in combination with microprobe analyses and scanning electron microscopy imaging. Five phases are identified (aragonite, Mg-calcite, dolomite, Ca-magnesite, and hydromagnesite), and we discuss their spatial distributions and the timing of their formation. The complexity of the skeletal structure of coralline algae suggests that the application of its Ca/Mg ratio as a temperature proxy might not be straightforward [4]. We propose a method to distinguish between Mg-calcite and dolomite that is not reliant on finding weak ordering peaks, but uses the position of the main d_{104} reflection in combination with composition data.

References

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Organic synthesis in a metal-organic framework — Insights from X-ray crystallography

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Chemoselective organic transformations are commonly required in synthesis. This presentation will outline our recent results utilising a bespoke metal-organic framework (MOF, **1** = $[Mn_3(L)_3]$ where L = bis(4-(4-carboxyphenyl)-1*H*-3,5-dimethyl-pyrazolyl)methane), in which metal-bound azide anions are precisely positioned within the pore network, to chemoselectively transform dialkynes into alkyne functionalised triazoles (Figure 1a). As illustration of this approach 1,9-decadiyne stoichiometrically furnishes a mono-"click" product without any bis-triazole side-product (Figure 1b). This chemistry builds upon our recent use of MOF **1** for gaining structural insight into catalytic transformations [1, 2].

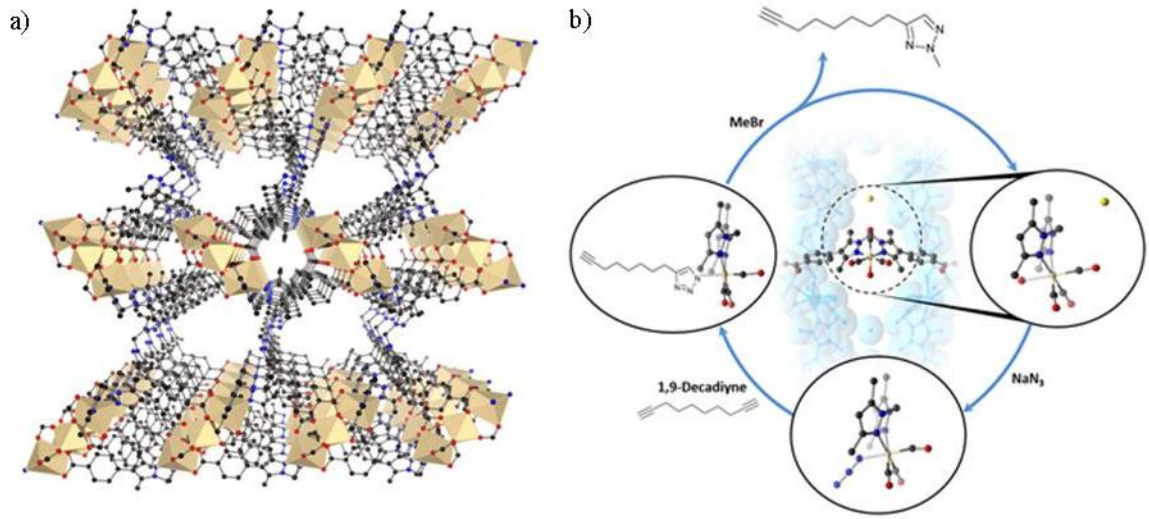

Figure 1. (a) The structure of MOF **1** and
(b) the organic transformations occurring within the material to form a mono-"Click" product.

To demonstrate the principles of the chemoselective transformation, single-crystal to single-crystal transformations of the Mn(I)-metalated material, **1**·$[Mn(CO)_3(H_2O)]Br$ to the corresponding azide species **1**·$[Mn(CO)_3N_3]$ with sodium azide, followed by a series of [3+2] azide-alkyne cycloaddition reactions and final liberation of the "click" products from the porous material by N-alkylation with MeBr to form **1**·$[Mn(CO)_3(H_2O)]Br$, were examined. X-ray crystallography confirms that the stepwise reaction cycle occurs at the metalation sites throughout the MOF. It appears that the physical isolation of the azide moieties is critical to the success of this strategy. This work suggests that carefully designed MOFs can act as physical protecting groups to facilitate other chemoselective transformations.

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Striped magnetic ground state of the ideal kagomé lattice compound Fe4Si2Sn7O16

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We have used representational symmetry analysis of neutron powder diffraction data to determine the magnetic ground state of $Fe_4Si_2Sn_7O_{16}$. We recently reported a long-range antiferromagnetic (AFM) Néel ordering transition in this compound at T_N = 3.0 K, based on magnetisation measurements [1]. The only magnetic ions present are layers of high-spin Fe^{2+} (d^6, S = 2) arranged on a perfect kagomé lattice (trigonal space group P-3m1). Below T_N = 3.0 K, the spins on 2/3 of these magnetic ions order into canted antiferromagnetic chains, separated by the remaining 1/3 which are geometrically frustrated and show no long-range order down to at least T = 0.1 K [2]. Moessbauer spectroscopy shows that there is no static order on the latter 1/3 of the magnetic ions — i.e., they are in a liquid-like rather than a frozen state – down to at least 1.65 K. A heavily Mn-doped sample $Fe_{1.45}Mn_{2.55}Si_2Sn_7O_{16}$ has the same ground state. Although the magnetic propagation vector k = (0, ½, ½) breaks hexagonal symmetry, we see no evidence for magnetostriction in the form of a lattice distortion within the resolution of our data. To the best of our knowledge, this type of magnetic order on a kagomé lattice has no precedent experimentally and has not been explicitly predicted theoretically. We will discuss the relationship between our experimental result and a number of theoretical models that predict symmetry-breaking ground states for perfect kagomé lattices.

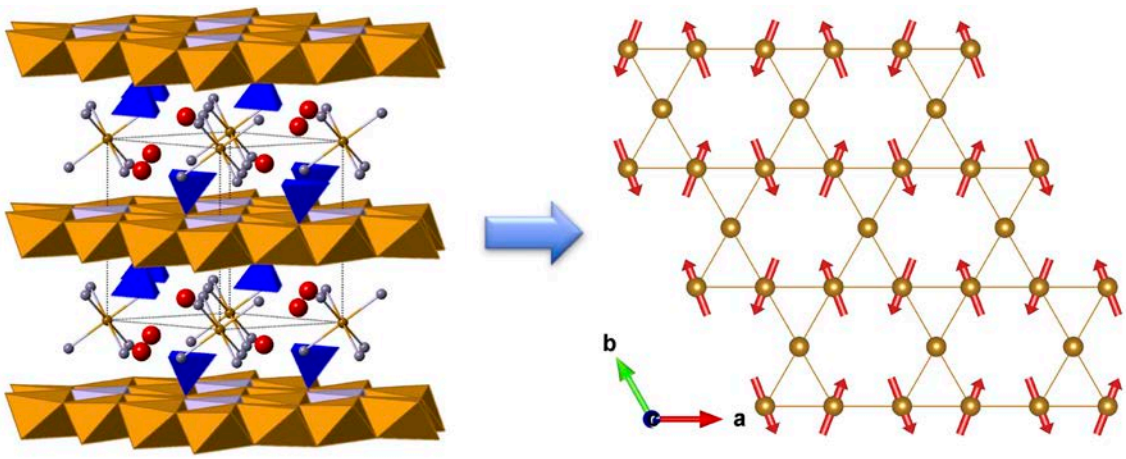

Figure 1.
(Left) P-3m1 structure of $Fe_4Si_2Sn_7O_{16}$, showing FeO_6 (gold) and SnO_6 (silver) octahedra in the oxide layer; Fe (gold), Sn (silver) and O (red) in the stannide layer; and SiO_4 tetrahedra (blue) between. (Right) Final refined magnetic structure, showing the Fe atoms in a single kagomé layer at z = ½.

References

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Chemical crystallography at the Australian Synchrotron MX Beamlines

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The macromolecular (MX) beamlines at the Australian synchrotron are mixed use between the structural biology and chemical crystallography (CX) communities. Since commissioning the high throughput MX1 bending magnet and the MX2 microfocus undulator beamlines have proven very successful for both communities.

The deployment of a 16M Eiger detector (funded by Australian Structural Biology laboratories and Australian Cancer Research Foundation) has changed the 'standard' MX2 collection for CX from 1° oscillation in 1 second over 360°, which takes **~15 min** with the beam attenuated to give a balance of resolution *vs* detector overloads to a new shutter less 360° oscillation yielding 3600 frames in **36 sec**.

This increase in data volume and experiment turnaround time has led to a number of challenges for the workflow for the users and highlighted the biggest dead time for beam is now: search and secure for hand mounting, and robot sample change time for automated sample handling including remote use. Indicative use of MX2 from completed search and secure in a 24-hour experiment with hand mounting (preferred by CX) was 188 completed searches. Maximum robot-mounted samples over the same duration is 288.

There is a robot upgrade under development to take sample change times from ~4 min to ~30 sec, and it is anticipated that MX1 will also receive a detector upgrade.

This increase in throughput is having a significant impact on our ability to return analysis on the experiment in real time, as well as deliver auto-processed data in a timely fashion (new computational hardware is on its way).

Given these dramatic increases in experimental throughput, what are the addition opportunities that may be embraced by the crystallographic community in Australia?

What is the future for chemical crystallography at the MX beamlines? A review of the current developments that are underway and some discussion of what may lie in the future will be presented.

CONCURRENT SESSION 4B: BIOLOGICAL ASSEMBLIES

Chair: Richard Birkinshaw

Phase plate cryo-EM analysis of chromatin

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Cryo-EM is a powerful technique for structure determination of isolated macromolecular complexes. Several important developments have contributed to the recent 'resolution revolution' in cryo-EM, including direct electron detection, correction of beam-induced motion, as well as improved classification and 3D reconstruction procedures. We have evaluated the potential of combining phase plate imaging and single particle analysis to determine the structure of a small protein–DNA complex. To test the method, we made use of a 200 kDa Nucleosome Core Particle (NCP) reconstituted with 601 DNA for which a high-resolution X-ray crystal structure is known [1]. We find that the phase plate provides a significant contrast enhancement that permits individual NCPs and DNA to be clearly identified in amorphous ice [2–3]. The refined structure from 26 060 particles has an overall resolution of 3.9 Å and the density map exhibits structural features consistent with the estimated resolution, including clear density for amino acid side chains and DNA features such as the phosphate backbone. Our results demonstrate that phase plate cryo-EM promises to become an important method to determine novel near-atomic resolution structures of small and challenging complexes.

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Characterisation of higher-order assembly signalling in Toll-like receptor pathways

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Toll-like receptors (TLRs) detect pathogen- and endogenous danger-associated molecules, initiating innate immune responses that lead to the production of pro-inflammatory cytokines. TLR signal transduction occurs through homotypic interaction of the TLR Toll/interleukin-1 receptor (TIR) domain with TIR domains of the TLR adaptor proteins MAL, MyD88, TRIF, and TRAM. Recruitment of these adaptors via TIR:TIR interactions orchestrates downstream signalling pathways, leading to the induction of immune responses.

Recent efforts directed at elucidating the structural basis of TIR domain self-association suggest signalling by TLR and adaptor protein TIR domains occurs through a higher-order assembly mechanism termed signalling by cooperative assembly formation (SCAF) [1]. SCAF explains many of the features of signalling in innate immunity pathways, including threshold-dose response, signal amplification, and reduction of biological noise. The TIR domain of MAL has been shown to spontaneously form filaments (helical assemblies) *in vitro*, form assemblies with other TIR domains (Figure 1), and induce the formation of large MyD88 (myddosome-like) assemblies [2].

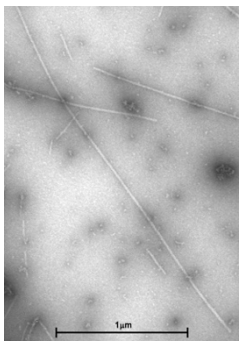

Figure 1. Negative stain micrograph of TLR4 TIR and MAL TIR co-filaments.

Here we present recent efforts directed at investigating the mechanism of TIR:TIR interactions in TLR pathways and elucidating the structural basis of TLR signalling by higher-order assembly formation.

References

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MR1 recognition by human γδ T cells

Jérôme Le Nours[1,2], Nicholas A. Gherardin[3,4], Sri H. Ramarathinam[1], W. Awad[1,2], J. Wubben[1,2], T. Praveena[1,2], Florian Wiede[1], Benjamin S. Gully[1,2], Richard Berry[1,2], Maria L. Sandoval-Romero[1], Shihan Li[3,4], Sidonia B.G. Eckle[3], Alexandra J. Corbett[3], Ligong Liu[5,6], David P. Fairlie[5,6], Tony Tiganis[1], James McCluskey[3], Daniel G. Pellicci[3,4], Adam P. Uldrich[3,4], Anthony W. Purcell[1], Dale I. Godfrey[3,4] and Jamie Rossjohn[1,2,7]

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The T lymphocytes repertoire is divided into two major lineages, αβ and γδ T cells, which are defined by their T cell receptor (TCR) gene-segment usage. The MHC-like molecule MR1 presents Vitamin-B derivatives to mucosal-associated invariant T-cells (MAIT). Using MR1 tetramers, we characterised a population of MR1-restricted human γδ T cells that included phenotypically diverse Vγ8-Vδ1, Vγ9-Vδ1 and Vγ8-Vδ3 subsets, all of which exhibited MR1 autoreactivity, independent on the nature of the bound ligand. The crystal structure of a γδTCR-MR1-antigen complex showed the γδTCR docked in a highly unusual manner that starkly contrasted all other TCR complex structures. The γδTCR bound under the MR1 antigen-binding cleft. Contacts were mediated largely by the TCR δ-chain and more surprisingly by the α3-domain of MR1. Our findings reshape our understanding of TCR recognition determinants and γδT-cells.

CryoEM structure of the Bluetongue virus core-like particle: A recombinantly engineered nano-delivery system

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Cryo-electron microscopy (cryoEM) has emerged as a promising structural biology technique that complements X-ray crystallography and NMR. Recent advances in imaging have dramatically improved the resolution of cryoEM structures, helping us to better understand the way multi-protein complexes are assembled. Here we present a 9.1 Å resolution cryoEM structure of the Bluetongue virus core-like particle (BTV-CLP) assembled by transiently expressing the capsid proteins VP3 and VP7 in plant leaves, and a 5.2 Å resolution structure of the modified core-like particle containing GFP fused to the N-terminus of VP3 (GFP-BTV) (Figure 1). The structure of BTV-CLP, which has been proposed as a nano-delivery particle, reveals a significant difference in assembly to the infection-derived viral particle [1]. Regardless, encapsidation of GFP at the N-terminus of VP3 does not alter the structure of the CLP, suggesting that incorporation of therapeutic peptides at the VP3 N-terminus might be a viable therapeutic delivery strategy.

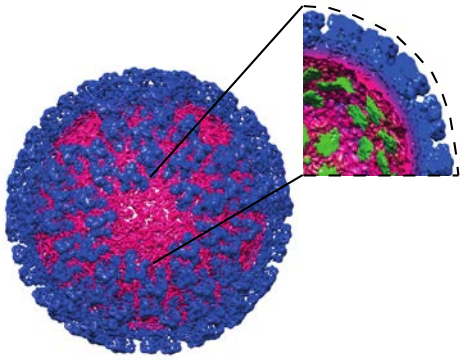

Figure 1. cryo-EM 3D map of GFP-BTV refined at 5.2 Å resolution. Green densities inside the capsid are thought to be the GFPs.

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Cryo-EM studies of *E. coli* ATP synthase

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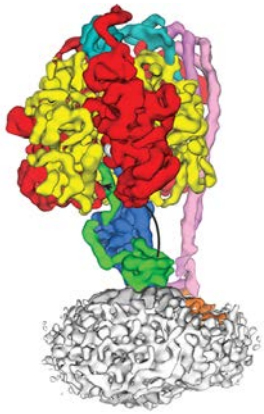

Here we present our cryo-EM maps of the intact ATP synthase complex from *Escherichia coli* [1]. This essential enzyme synthesises the bulk of cellular ATP, the energy currency of the cell. The structures highlight unique features of this ATP synthase complex, such as the bifurcation of the peripheral stalk homodimer and the position of the inhibitory subunit epsilon. Further studies on this complex reveal a possible partially active conformation, which points to the molecular events that may inhibit this marvellous motor.

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SESSION 5A: BACTERIA AND VIRUSES

Chair: Charlie Bond

Biology of bacterial sialic acid uptake

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INVITED SPEAKER

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Sialic acids comprise a varied group of nine-carbon amino sugars that are widely distributed among mammals and higher metazoans. Commensal and pathogenic bacteria that colonise heavily sialylated niches (e.g., the mammalian respiratory tract and gut) can scavenge sialic acid from their surrounding environment and use it as a carbon, nitrogen and energy source—that is, they eat your glycoconjugates for breakfast. Sequestration and degradation of sialic acid involves specific amino sugar transporters responsible for the import into the bacterial cell and five catabolic enzymes that successively degrade sialic acid. Regulation of this pathway is achieved at the transcription level by specific repressor proteins. In this talk I will present the first crystal structure of a sialic acid specific sodium solute symporter at 1.95 Å resolution in its outward-open conformation. Happily, this structure was determined in complex with sodium and sialic acid, providing insight into how this transporter mediates the movement of sialic acid across the membrane. Overall, the work provides new data that enriches our understanding of the import and degradation of sialic acid in clinically important human bacterial pathogens.

Structural characterisation of EutV interactions with anti-termination hairpins

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Enterococcus faecalis is a gram-positive commensal bacterium of the human intestinal tract and an opportunistic pathogen. The ability of *E. faecalis* to utilise ethanolamine as the sole source of nitrogen and carbon provides an advantage for survival and colonisation [1]. Ethanolamine metabolism in *E. faecalis* requires the ethanolamine utilisation operon (*eut*) that is under the TCS regulation of the EutW sensor kinase and EutV response regulator [2]. Phosphorylated EutV dimerises and binds dual mRNA hairpin sites upstream of multiple genes in the operon. EutV binding disrupts the intrinsic terminator hairpin allowing complete transcript production, gene expression and subsequent ethanolamine metabolism.

Limited structural information is available regarding EutV and the mechanisms by which it binds RNA. Here we present a series of crystal structures of the EutV as apo and bound with BeF_3 (phosphor-mimic) and in complex with its cognate anti-terminator RNA. The structures indicate specific features required for complex formation and allied with some biochemical and bioinformatics data point out the importance of key features of their interaction.

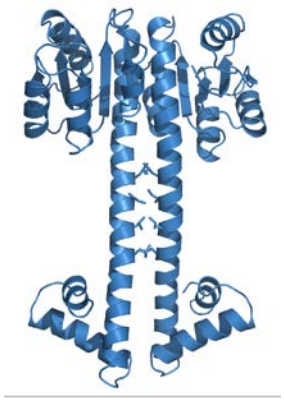

Figure 1.
Crystal structure of
EutV D54E dimer.

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MHC-I peptides get out of the groove and enable a novel mechanism of HIV-1 escape

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Major histocompatibility complex class I (MHC-I) molecules play a crucial role in both adaptive and innate immunity through the capture of intracellular peptides for presentation to T cells and natural killer (NK) cells. In MHC-I, the closed ends of the peptide binding groove result in bound peptides tethered at both their N and C termini. The peptide epitope is bound at the N-terminus through hydrogen bonding of the N-terminal backbone amine of the peptide with conserved residues in the A pocket of the MHC-I groove. Here we show that 20% of the HLA-B*57:01 peptide repertoire comprises N-terminally extended sets characterised by a common motif at position 1 (P1) to P2. Structures of HLA-B*57:01 presenting N-terminally extended peptides, including the immunodominant HIV-1 Gag epitope TW10 (TSTLQEQIGW), showed that the N-terminus protrudes from the peptide-binding groove.

The common CD8+ T cell response escape mutant TSNLQEQIGW bound HLA-B*57:01 canonically, adopting a dramatically different, register-shifted conformation compared with the TW10 peptide. This register shift of the T3N escape mutant epitope in the HLA binding groove was also shown to affect recognition by killer cell immunoglobulin-like receptor (KIR) 3DL1 expressed on NK cells. Structural characterisation of both KIR3DL1-HLA complexes allowed us to determine that this register shift primarily affects contacts between KIR3DL1 and the bound peptide.

We thus define a previously uncharacterised feature of the human leukocyte antigen class I (HLA-I) immunopeptidome, additionally highlighting the peptide dependence of KIR-HLA interactions and their implications for viral immune escape. We further suggest that recognition of the HLA-B*57:01-TW10 epitope is governed by a 'molecular tension' between the adaptive and innate immune systems.

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Grouper iridovirus GIV66 is a Bcl-2 protein that inhibits apoptosis by exclusively sequestering Bim

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Programmed cell death or apoptosis is a critical mechanism for the controlled removal of damaged or infected cells, and proteins of the Bcl-2 family are important arbiters of this process. Viruses have been shown to encode for functional and structural homologues of Bcl-2 to counter premature host cell apoptosis to ensure viral proliferation and/or survival. Grouper iridovirus (GIV) is a large DNA virus belonging to the iridoviridae family that harbours GIV66, a putative Bcl-2 like protein. GIV66 is a mitochondrially localised inhibitor of apoptosis, however, the molecular and structural basis of apoptosis inhibition is currently not understood. To gain insight into the mechanism of action we systematically evaluated the ability of GIV66 to bind peptides spanning the BH3 domain of pro-apoptotic Bcl-2 family members. Our data reveal that GIV66 harbours an unusually high level of specificity for pro-apoptotic Bcl-2, and only engages with Bim. We then determined crystal structures of both GIV66 on its own as well as bound to Bim BH3. Unexpectedly, GIV66 forms dimers via a novel interface that occludes access to the canonical Bcl-2 ligand binding groove, which break apart upon Bim binding. These data suggest that GIV66 dimerisation impacts on the ability of GIV66 to bind and select host pro-death Bcl-2 protein. Our findings provide a mechanistic understanding for the potent anti-apoptotic activity of GIV66 by identifying it as the first single specificity pro-survival Bcl-2 protein, and demonstrating the pivotal role of Bim for GIV mediated inhibition of apoptosis.

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The structure and function of KstR, the major regulator of cholesterol catabolism in *Mycobacterium tuberculosis*

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Tuberculosis (TB) remains a global threat to human health due to the emergence of extremely-drug-resistant (XDR) forms of *Mycobacterium tuberculosis* (*M. tb*) and co-infection with HIV [1]. Elucidation of metabolic networks essential to the pathogenesis of *M tb* is an important part of the quest to identify new drug target candidates that can be exploited to tackle XDR-TB. In particular, *M. tb* possesses an unusual ability to metabolise lipids, and its genome is heavily orientated towards this task, with the involvement of a remarkable number of genes: ~250, which constitutes around 6% of its genome [2].

Cholesterol can be a major carbon source for *M. tb* during infection, both at an early stage in the macrophage phagosome, and later within the necrotic granuloma [3]. KstR is a highly conserved TetR family transcriptional repressor that regulates a large set of genes responsible for cholesterol catabolism. Many genes in this regulon are either induced during infection or are essential for bacterial survival *in vivo* [4]. We identified two ligands for KstR, both of which are CoA thioester cholesterol metabolites with four intact steroid rings that strongly inhibit KstR-DNA binding [5].

Here we present crystal structures of the ligand-free, ligand-bound and DNA-bound forms of KstR. Structures of KstR□-ligand complexes demonstrate a position of the DNA-binding domain that is unfavourable for DNA binding. Comparison of ligand-bound and ligand-free structures identifies residues involved in ligand specificity and reveals a distinctive mechanism by which the ligand-induced conformational change mediates DNA release. The structure of the KstR-DNA complex suggests that a bend in the target DNA is structurally required for a snug fit with the two DNA-binding domains of the KstR dimer. Furthermore, data from molecular dynamics (MD) simulations strongly suggests that this signature DNA deformation is encoded in the nucleotide sequence.

As KstR controls the expression of a metabolic pathway that is essential to mycobacterial pathogenesis, it may present a novel opportunity for the development of new anti-TB therapeutics. It is possible that gratuitous inducers of KstR may be able to chemically reproduce the phenotype of the KstR knockout and prevent *M. tb* from growing *in vivo*. Conversely, compounds that lock KstR in a DNA-bound form and prevent it from responding to its natural ligands may be expected to prevent *M. tb* from regulating its cholesterol breakdown. Hence, we have identified small molecules ligands for KstR that are chemically distinct from the natural steroid metabolites using a fragment-screening approach, with the aim of assessing their ability to disrupt KstR activity.

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SESSION 5B: MEMBRANE PROTEINS

Chair: Sara Sandin

Specificity of the phosphate and sulphate initial receptors for ABC transporters: Never the twain shall meet

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INVITED SPEAKER

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One of the clearest examples of the high specificity of active transport systems (or ligand/substrate recognitions in general) is seen between phosphate and sulphate, two very similar tetrahedral oxyanions that are the principal sources of essential cellular phosphorus and sulphur, respectively. This is exceedingly important to ensure that one oxyanion does not inhibit the transport of the other.

The sulphate-binding protein (**SBP**) and phosphate-binding proteins (**PBP)**, members of the superfamily of proteins which serve as initial high affinity receptors ABC transport systems, remain the best model system for the study of sulphate and phosphate selectivity. Although the receptors are mostly found in bacterial cells, either anchored on the cell surface or free in the periplasm, many equivalent non-transporter PBPs (called DING PBPs) are found in all life kingdoms.

Although they vary significantly in size (~25 to ~60 kDa) and sequences, the roughly 50 different receptor X-ray structures are similar, consisting of two domains bisected by a cleft wherein the ligand is bound via a bending motion of the hinge connecting the two domains (the “Venus flytrap” model).

The non-overlapping selectivity of SBP (from *S. typhimurium*) and PBP (*E. coli*) is dictated by the protonation states for phosphate (monobasic or dibasic) or lack thereof for sulphate (a conjugate base of a strong acid). Astonishingly, as first discovered in the X-ray structure of the bound form, the receptor site for sulphate is buried and solvent-free and uses solely dipolar donor groups (NH/OH) with no formal charges for anion binding, selectivity and electrostatic balance. Although the SBP-sulphate interactions are charge-neutral hydrogen bonds, we ascribed them more generally as “ion-dipole” interactions. This ion-dipole recognition mechanism, requiring only dipolar groups, was recapitulated in the binding (or coordination) of Cl^- to its transporter/channel and K^+ to its channel as discovered by the McKinnon’s group. In fact the molecular features of sulphate and chloride binding are very similar.

The phosphate receptor’s high specificity is achieved largely by a carboxylate side chain hydrogen bonding the dibasic phosphate proton. The X-ray structures of about 10 prokaryotic PBPs and 1 eukaryotic DING PBP and 1 eukaryotic Pi major facilitator transporter (totally different from PBPs) have been determined (from PDB). All but one of these structures makes use of aspartate(s) in Pi recognition.

Paradoxically, although the sulphate in SBP is held in place by 7 hydrogen bonds and the phosphate in PBPs by 13–14 hydrogen bonds, the binding affinities are similar (in the low μM range). The N-termini of three α-helices contribute peptide backbone NH groups in sulphate and phosphate binding, but the concept of helix macrodipole plays little or no role in anion binding.

Cellular phosphate is often limiting so that transport of both monobasic and dibasic phosphate would be advantageous especially during starvation. Pi binding affinity to *E. coli* PBP are essentially unaffected by pH from 4.3 to 8.5, implying binding of both monobasic and dibasic Pi. We are using neutron diffraction to ascertain whether coli PBP is capable of binding monobasic Pi at pH 4.5.

Structural insights into the regulation and inhibition of bacterial aggregation and biofilm formation

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The autotransporter family of proteins is the largest group of secreted and outer-membrane proteins in Gram-negative bacteria. These proteins perform a vast array of functions linked to pathogenesis, from adhesion and invasion of human host cells to the formation of cell aggregates and biofilms on biotic and abiotic surfaces. The self-associating autotransporters (SAATs) are a sub-group of autotransporters widespread across pathogens [1]. These proteins promote the formation of aggregated communities and biofilms, which facilitate host colonisation and bacterial persistence in different environmental niches.

We previously elucidated the mechanism by which the SAAT Antigen43 from uropathogenic *E. coli* (UPEC) promotes bacterial aggregation/biofilm formation, by means of self-association between neighbouring cells [2]. We sought to determine if all SAATs shared a common mechanism for facilitating bacterial aggregation/biofilm formation, if this function was regulated and if it could be inhibited. TibA is a multifunctional SAAT from enterotoxigenic *E. coli* (ETEC), the leading bacterial cause of diarrhoea. This surface protein was known to be glycosylated by the cognate glycosyltransferase TibC. We determined the crystal structures of the glycosylated and unglycosylated forms of TibA and used this to inform further biophysical and phenotypic studies. We found that TibA self-associates in a head-to-tail manner with an extensive interface, to facilitate bacterial aggregation/biofilm formation. Glycosylation by TibC was found to physically block TibA self-association to reduce bacterial aggregation/biofilm formation. Our comprehensive structural and functional analysis provides a molecular understanding of how a post-translational modification switches the activity of TibA from an aggregative molecule to and adhesin and invasin. This may represent a general mechanism for bacteria to regulate the virulence functions of the vast number of SAAT expressed on their cell surface.

We have also developed an nM inhibitor of SAAT mediated aggregation/biofilm formation and have determined the first autotransporter-inhibitor crystal structure. Molecules that block bacterial cell clusters and biofilms could be used in synergy with antibiotics, detergents or anti-biofilm agents to improve their efficacy, which would impact environmental, industrial, and human medical microbiology.

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Cholesterol-dependent cytolysins: From water-soluble state to membrane pore

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Cholesterol-dependent cytolysins (CDCs) are a family of pore-forming toxins that punch holes in the outer membrane of eukaryotic cells. The CDCs exhibit a number of unique features amongst pore-forming toxins including an absolute dependence on the presence of cholesterol-rich membranes for their activity and the formation of oligomeric transmembrane pores greater than 150 Å in diameter. The first crystal structure of a CDC was that of perfringolysin O [1] and most of our understanding of CDC function is based on studies of this toxin [2, 3]. We have subsequently determined structures of other family members that have confirmed that the 3D fold first seen in PFO is shared by all family members [5–7]. We have now determined a number of new CDC structures that are providing valuable insights into the role of receptor binding, oligomerisation and prepore assembly [8]. The conversion from water-soluble monomer to pore is highly complex: it is essential that the pore does not form prematurely otherwise the target cell won't be successfully breached [9]. The crystal structures of the water-soluble states of these toxins, together with cryo-electron microscopy, small angle X-ray scattering data, fluorescence spectroscopy and molecular dynamics simulations have proved very useful for modelling their membrane pores.

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Protein conformation of C9 controls the final membrane complex assembly

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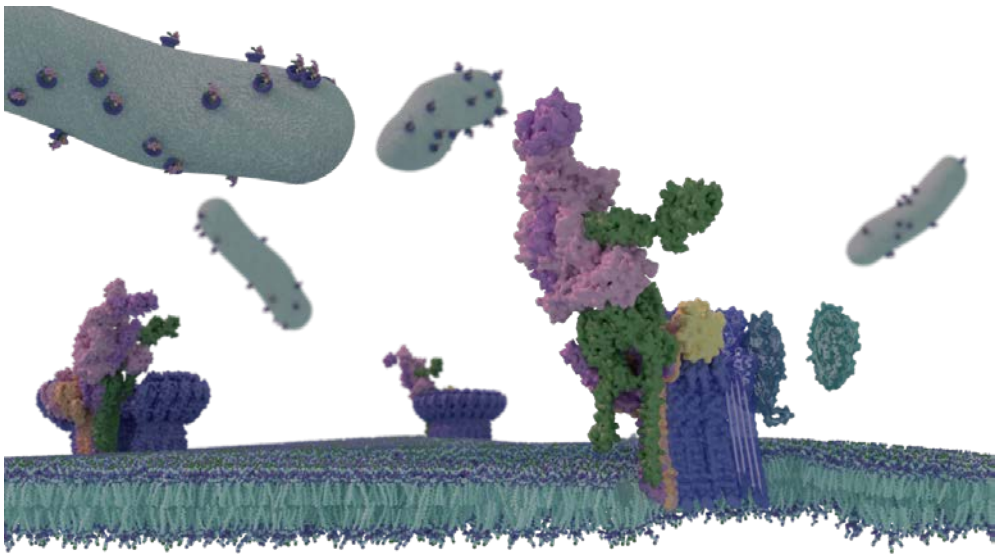
MACPF/CDC pore forming proteins show a unique ability to self-assemble from soluble monomeric proteins into oligomeric rings that change conformation and insert into the target cell membrane. The MACPF/CDC family has been shown to form giant beta-barrel pores that oligomerise, typically using the same unit, and are capable of passive transport of whole soluble proteins across lipid membranes.

The current dogma in the field suggests that oligomer assembly is mediated by: 1) increasing the effective concentration on a lipid membrane and, 2) planar diffusion upon the target membrane. However, to date, this model is only consistent with pore forming proteins, such as CDCs and perforin, which have dedicated membrane binding domains that facilitate binding to the membrane surface. In contrast, the proteins of the Membrane Attack Complex (MAC) lack any membrane binding region, which lends it to being able to target a wide range of eukaryotic and bacterial surfaces. However, this precludes the MAC using lateral diffusion for assembly and it is unknown how unwanted solution-based oligomer assembly is avoided.

Here we show the first X-ray structure of the soluble C9 component of the MAC and compare this to the near atomic single particle cryo-EM structure of the 22-subunit polyC9. Together these structures show that a 22 amino acid region within the TMH1 loop obstructs oligomer assembly at the oligomer interface. Disulphide trap mutants demonstrate that both TMH1 and TMH2 regions need to move position prior to binding of the next C9 unit in the oligomer assembly pathway.

These results challenge the existing dogma in MACPF/CDC pore assembly—that assembly is dependent on increasing the virtual concentration of the protein by membrane binding and requiring lateral diffusion. Instead, movements of the TMH1 and TMH2 of C9 drive MAC assembly. Accordingly, these results explain how the C9 component is able to self-oligomerise into the MAC without the need for lateral diffusion on a membrane and may explain the MAC's role in assembling on highly variable chemistries at the membrane surface of invading pathogens.



Structural characterisation reveals insights into substrate recognition by the glutamine transporter ASCT2 (SLC1A5)

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Cancer cells undergo a shift in metabolism where they become reliant on nutrients such as the amino acid glutamine. Glutamine enters the cell via transporters termed Alanine/Serine/Cysteine Transporter 2 (ASCT2) that are upregulated in several cancers to maintain an increased supply of this nutrient and are, therefore, an attractive target in cancer therapeutic development. ASCT2 belongs to the glutamate transporter (SLC1A) family but is the only transporter in this family able to transport glutamine and the structural basis for glutamine selectivity of ASCT2 is unknown. Here we have identified two amino acid residues in the substrate binding site that are responsible for conferring glutamine selectivity. We introduced corresponding mutations into a prokaryotic homologue of ASCT2 and solved several crystal structures, which reveal the structural basis for neutral amino acid and inhibitor binding in this family. This structural model of ASCT2 provides a basis for future development of selective ASCT2 inhibitors to treat glutamine-dependent cancers.

SESSION 6: NEW PEOPLE AND NEW RESULTS

Chair: Suzanne Neville

Functional superstructures of metal-organic frameworks

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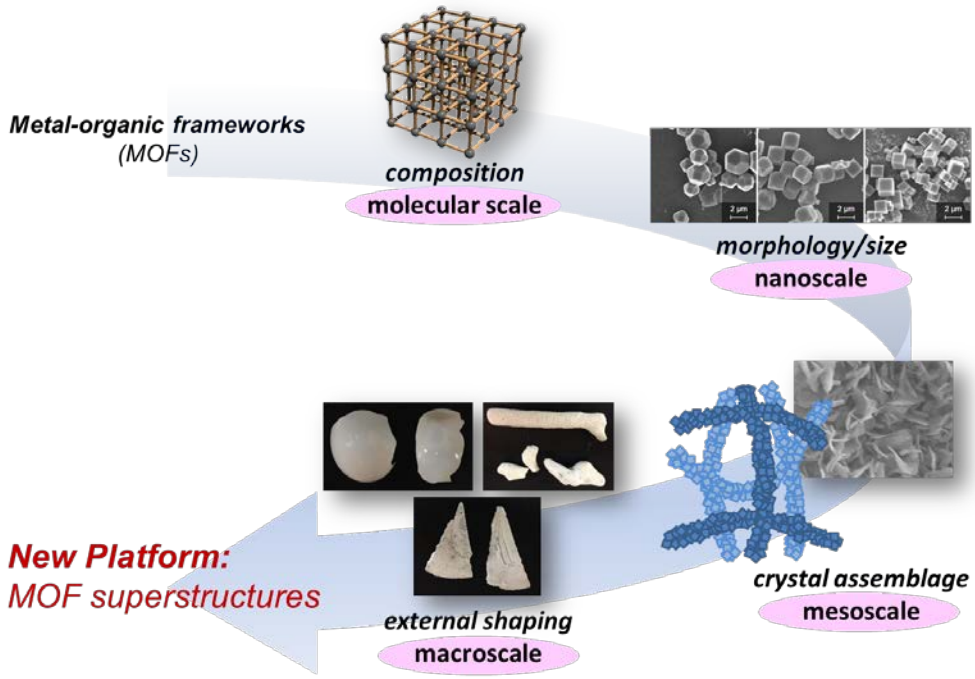
Metal-organic frameworks (MOFs) have received considerable investigation in recent years as a new class of porous materials that may supplant existing adsorbents in applications such as gas storage, molecular separations, and heterogeneous catalysis. The ability to rationally select the metal and organic components that construct these extended networks has led to an explosive growth in the number of compounds available to researchers. While most efforts in the field are still devoted to the preparation of new materials, the control of their physical form at the nano-, meso- and macroscales also presents new opportunities for enriching the properties of MOF-based systems [1, 2]. Here, recent developments related to the structuring of MOFs will be presented according to the various length scales. The properties of structuralised (monolithic) forms of MOFs prepared using the so-called *coordination replication* technique will be discussed [3–5], with a focus on the unique material properties that can be obtained *via* the immobilisation of the MOF crystals within higher-order architectures.



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Dissecting the chloride-nitrate anion transport assay

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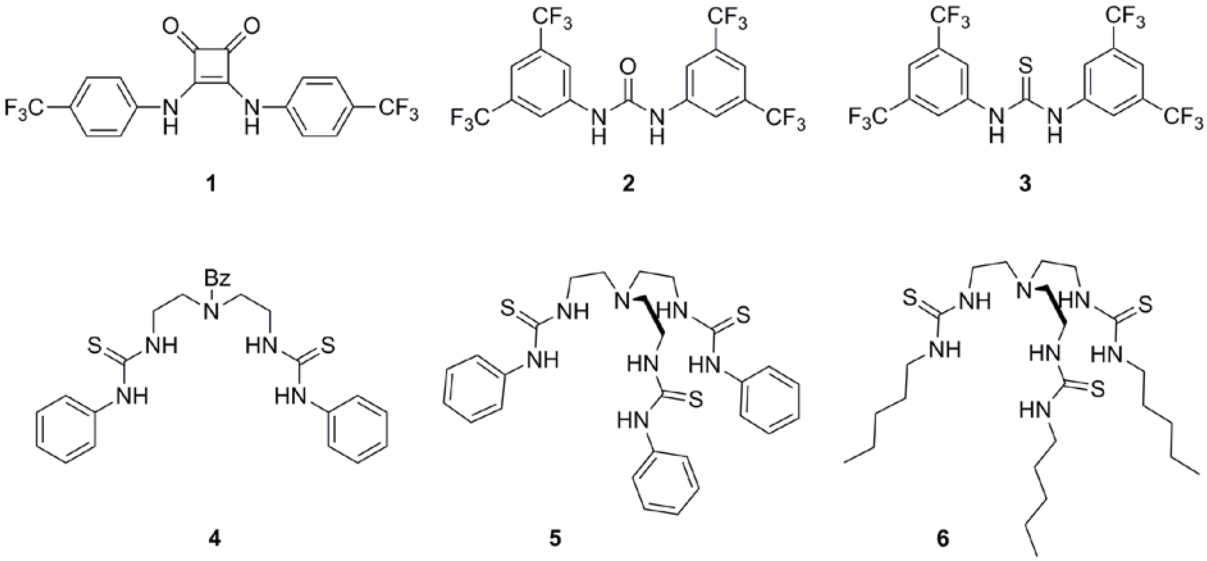
A systematic study of chloride *vs* nitrate selectivity across six anion transporters has revealed a good correlation between the selectivities of their anion binding and membrane transport properties. This work reveals the limitations of the chloride-nitrate exchange assay and shows how new approaches can be used to measure anion uniport [1].



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Recruiting the PAN2-PAN3 deadenylase complex to mRNA targets

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INVITED SPEAKER

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mRNA degradation is an essential component of the gene expression process. Cytoplasmic mRNA decay is typically initiated by the removal of poly(A) tails, a process termed deadenylation, which causes translational repression and, in most cases, triggers irreversible mRNA degradation. The PAN2-PAN3 deadenylase complex functions in both bulk and microRNA-mediated mRNA decay and is directly recruited to microRNA targets by GW182/ TNRC6 proteins. Taking a structural and functional approach, we have identified unusual features that mediate the interaction of PAN3 with PAN2 and TNRC6 proteins, as well as critical residues required for mRNA degradation *in vivo*. Collectively, our data describes the structural basis for the recruitment of the PAN2-PAN3 complex to microRNA targets by the TNRC6 proteins, and the essential role of PAN3 in coordinating deadenylation with downstream steps of the mRNA decay pathway.

Flexible crystals: Atomic resolution of the bending mechanism in [Cu(acac)$_2$]

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A crystal is normally thought of as *a homogenous solid formed by a periodically repeating, three-dimensional pattern of atoms, ions, or molecules.* Indeed, the regular arrangement of molecules in a single crystal lead to many useful characteristics (in addition to diffraction!) including unique optical and electrical properties, however, molecular crystals are not typically mechanically robust, particularly compared to crystals of network solids like diamond. Upon the application of stress or strain, these crystals generally irreversibly deform, crack or break resulting in the loss of single crystallinity.

We have recently discovered a class of metal-organic crystalline compounds that display the intriguing property of elastic flexibility — that is they are capable of reversibly bending without deforming, cracking or losing crystallinity. A number of these crystals are flexible enough to be tied into a knot! (See Figure 1.) We hypothesise that these intriguing properties stem from the nature and arrangement of intermolecular forces present between molecules in the crystal lattice, whereby weak interactions allow molecules to move sufficiently within a crystal lattice (resulting in flexibility) while stronger interactions between molecules maintain the crystallinity. This in turn leads to a loss of periodicity due to irregular compressions and expansions of intermolecular distances throughout the lattice, challenging the definition of a crystal. When the force is removed the crystals return to their perfectly ordered state. We have used micro-focused synchrotron radiation to map the changes in the crystal structure under strain and thus have determined the mechanism of flexibility with atomic precision [1].

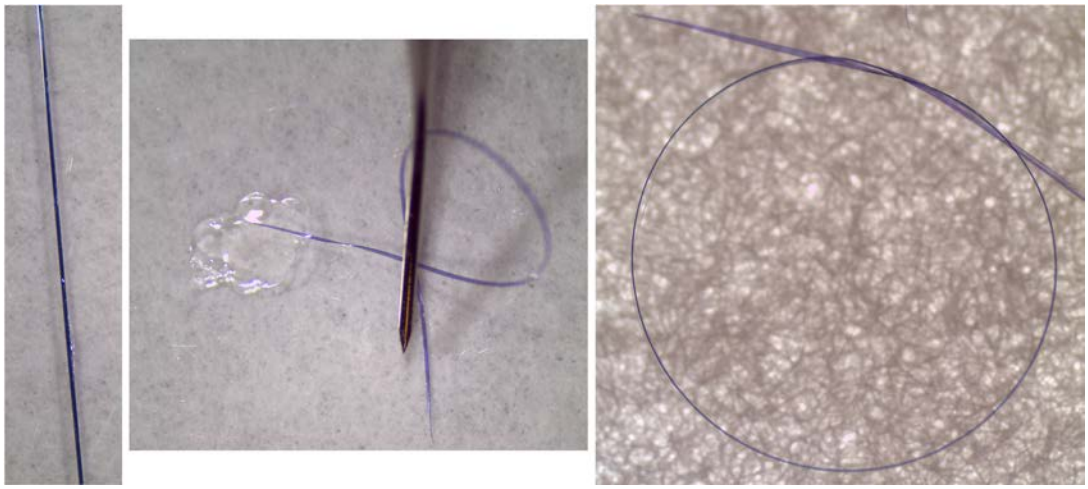

Figure 1: A crystal of [Cu(acac)$_2$] showing elastic flexibility.

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Structure of the AAA+ ATPase Vps4: A nightmare on EM Street

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The vacuolar protein sorting-associated protein 4 (Vps4) AAA+ ATPase is a key regulator of the function of a group of multifunctional complexes known as the ESCRTs (endosomal sorting complexes required for transport). ESCRTs classically control the formation of multivesicular bodies in eukaryotic cells and have subsequently been implicated in a variety of cellular membrane remodelling events that require the formation of an outward-facing bud, including cytokinetic abscission, viral budding and the repair of plasma membrane lesions. In these processes, Vps4 catalyses the recycling of membrane-associated ESCRT-III components away from the budding membrane — a step that is perquisite to membrane fission. The Vsp4 holoenzyme is a homo-oligomeric species, induced upon ATP binding. The structure of oligomeric Vps4 has been the subject of considerable controversy, largely due to the fact that the wild type enzyme forms only a transient oligomer while the use of a hydrolysis deficient point mutant ($Vps4^{E233Q}$) has been claimed to induce a structure that is non-native. Presented here will be recent structural studies that reveal two distinct conformations of $Vps4^{E233Q}$ using single particle cryo-EM. These structures indicate that Vps4, like other AAA ATPases, forms an asymmetric, hexameric ring resembling a “split washer”. Taken together with recent studies by others, these data suggest the EQ mutant does form a physiologically relevant structure. However, evidence from SAXS suggests that Vps4 also forms higher-order structures (>6mer), with the EQ mutant appearing to exhibit a greater propensity to form these. In the case of Vps4, the physiological relevance of these higher-orders structures remains to be established, highlighting the need for caution when employing the EQ mutant as model for the wild type holoenzyme. The presentation will highlight challenges encountered in characterisation of this recalcitrant system using cryo-EM and discuss how these were overcome.

SESSION 7: BRAGG MEDAL LECTURE

Chair: Alice Vrielink

New ways of thinking about molecules in crystals

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The title borrows from a rather wonderful quote, attributed to Lawrence Bragg, but for which there seems to be no authoritative source: *"The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them"* [1]. Many of my very good friends and colleagues disagree with this, and no doubt prefer Peter Kapitza's: *"Theory is a good thing but a good experiment lasts forever"* [2]. But surely both viewpoints are essential: high-quality experiments (and data) will always be invaluable, but science is more than observation, measurement, and collating "facts". We also need to think about what we have measured, in order to better understand what it is telling us, how it fits with our model of the world, and the insight it provides for subsequent experiments.

Almost ninety years ago, Kathleen Lonsdale used the pioneering technique of X-ray crystallography to firmly establish the planar structure of the benzene ring in a molecule [3]. In the section 'Statement of the Problem' she proposed nine questions that her analysis must try to answer. The very first question was: *"Does the organic molecule exist as a separate entity in the crystalline state?"* This was answered in the affirmative, citing the earlier X-ray structure of hexamethylenetetramine by Dickinson and Raymond [4], and adding: *"There is, in fact, very strong presumptive evidence for the separate existence of the molecule in many crystalline organic compounds."*

Of course today we don't ask questions like that. We take molecules in crystals for granted, focusing more on the number, nature and strength of the interactions between molecules, rather than the molecules themselves and how they may have been perturbed by crystallisation.

Just over twenty years ago we were interested in obtaining molecular dipole moments and their enhancement in the crystal, from the results of charge density analysis of X-ray diffraction data. This requires addressing the fundamental question of defining a molecule in a crystal: How do we partition a crystal into molecular fragments? After exploring several partitioning schemes we stumbled upon what we called at the time 'Hirshfeld partitioning with a surface' [5].

Amazingly, we have dedicated considerable research effort since then to exploring the ramifications of what was actually a very simple observation. The graphical and computational tools developed to exploit Hirshfeld surfaces in visualising and exploring intermolecular interactions in molecular crystals are now used by researchers in over fifty countries worldwide, and the simple concepts underlying these tools have been extended and developed far beyond what we originally thought possible [6].

This presentation will focus on some of the personal highlights of this journey — and the lessons learned along the way. It will touch on the fickleness of research funding and peer review, the key role of supportive mentors and colleagues, and the essential ingredients of serendipity and creativity.

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SESSION 8: KEYNOTE SPEAKER 2

Chair: Begoña Heras

A tale in two parts: How a search for antivirulence compounds led to the discovery of a shapeshifting copper resistance protein

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Protein disulphide bonds are covalent links formed between sulphurs of cysteine sidechains. There is now overwhelming evidence to show that these inter-residue bonds are critical for Gram-negative bacterial virulence [1].

This presentation describes how the structures of the bacterial machinery components that introduce disulphide bonds into folding proteins [2] have been used in the search for inhibitors [3, 4, 5]; and outlines the serendipitous discovery of a shape-shifting foldase [6] that is potentially useful for plug-and-play bionanotechnology.

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CONCURRENT SESSION 9A: APPLICATIONS OF POROUS MATERIALS

Chair: Dave Turner

Porous metal scaffolds for use in hydrogen storage

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Over the previous few years, hydrogen has attracted a great deal of interest as an energy storage medium for automobile applications [1]. Utilising hydrogen has multiple advantages, the most obvious being having near zero emissions when produced by electrolysis and converted to electricity in a fuel cell [2]. Many challenges remain to be faced in developing a hydrogen-based economy, especially in the production of a suitable on-board hydrogen storage material that will meet the hydrogen storage target (5.5 wt% H_2) set by the US Department of Energy [3].

The problem faced with many hydrogen-storage materials is that the temperatures and pressures under which they release and absorb hydrogen are not feasible for many applications. There are two main strategies for solving the challenges of accessible reversible hydrogenation. One method is thermodynamically destabilisation by the addition of a second phase such as a binary hydride, oxide, metal halide or a metal in order to reduce the enthalpy of dehydrogenation, decreasing of its decomposition temperature [2]. The second strategy is to improve the kinetics of hydrogenation by creating nanoparticles by confining the hydrogen storage material in a mesoporous scaffold [4].

We are pioneering the synthesis of porous metal frameworks which act to both thermodynamically destabilise and reduce the particle size of the hydrogen storage material contained therein. We have produced Al and Mg frameworks [5–7], and have successfully infiltrated $NaAlH_4$ and $LiBH_4$ as hydrogen-storage media. Porous Al scaffolds infiltrated with 7.3 and 13 wt% $NaAlH_4$ show superior H_2 release, with respect to thermodynamically destabilised $NaAlH_4$ in the temperature range 148–195 °C [8]. Porous Mg scaffolds infiltrated with $LiBH_4$ started desorbing H_2 at 100 °C, which is 250 °C lower than the accepted temperature of bulk $LiBH_4$ [7].

The techniques used in the characterisation of these materials will be discussed and, in particular, the use of small-angle X-ray scattering, both *ex situ* and *in situ* will be examined in the analysis of the porous structure of the scaffolds.

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Atomic-scale explorations of stimulus-responsive framework properties in an ultramicroporous gas sorbent

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Functional microporous materials capable of efficiently separating and/or storing gases at non-cryogenic temperatures are sought for a wide variety of important industrial applications, including pre- and post-combustion carbon capture, hydrogen fuel storage, and the purification of component gases from air. Understanding the atomic-scale interactions between the host material and guest species under variable operating conditions is essential for obtaining information about adsorption and separation mechanisms, which can in turn be used to design better sorbents targeted at specific applications.

The ultramicroporous metal-organic framework $[Cu_3(cdm)_4]$ (cdm = $C(CN)_2CONH_2^-$) was recently reported to exhibit moderately selective adsorption of CO_2 over CH_4, along with excellent exclusion of elemental gases such as H_2 and N_2 [1]. Although the very small pore diameter (3–4 Å) results in unpromisingly slow diffusion dynamics, its close similarity to the kinetic diameters of many small gas molecules [2] also raises the prospect of altering the gas sorption and selectivity characteristics of the material *via* minor structural modifications, such as might be introduced by changing the temperature and/or guest concentration during sorbent operation under industrially relevant conditions.

Using a combination of *in situ* neutron scattering experiments and density functional theory-based calculations, we examine in detail the interplay between lattice shape, pore size, temperature, and CO_2 concentration in $[Cu_3(cdm)_4]$. The rare and interesting fundamental property of areal negative thermal expansion (NTE) in $[Cu_3(cdm)_4]$ is attributed to a new variation of a well-known NTE mechanism, and is triggered by dynamic motions of the rigid cdm ligand within the constraints of the complicated framework topology. Although the thermal response of the pore diameter is surprisingly insignificant due to competition between multiple effects, the potential for similar materials to exhibit temperature-induced changes in adsorption properties is clearly demonstrated. This study illustrates the breadth and depth of information that can be obtained by combining the power of experimental and theoretical characterisation in an approach that is generally applicable to crystalline sorbent systems.

References

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Advanced characterisation methods applied to materials produced at CSIRO's Additive Manufacturing Centre

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New and emerging additive manufacturing technologies can offer significant advantages over traditional manufacturing methods in terms of product customisation and the ability to produce complex parts. However, the characterisation of additively manufactured products can be a significant challenge due to such shape complexity and also due to interface interactions, which can be exhibited at levels ranging from the nano- to micro-scale and above. The ability to characterise such has continued to grow in importance within CSIRO. In this presentation we discuss the application of X-ray diffraction (phase, residual stress and texture analyses), high-resolution imaging (including 3D tomography and low-kV X-ray mapping) and analytical spectroscopy (ICP-OES) as employed to characterise several examples of custom-engineered metal- and polymer-based products produced by CSIRO's Additive Manufacturing Centre and partners.

Development of a borane-loaded MOF reagent

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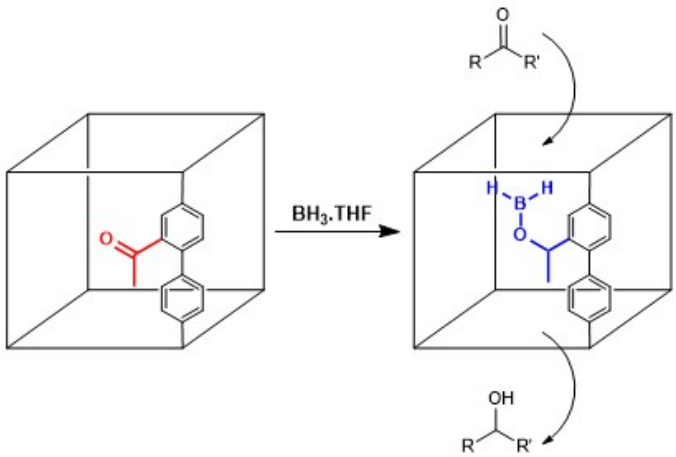
With their large surface areas and chemically tunable pores, metal-organic frameworks (MOFs) are well studied as heterogeneous catalysts [1, 2]. We believe that MOFs can also act as heterogeneous reagents that utilise their intrinsic size selectivity and their capability to induce transformations in controlled pore environments to provide benefits to the desired reactions, and would also have the advantage of easy recovery and reusability.

In this presentation, I will present my research into developing a heterogeneous borane-loaded MOF reagent via the post-synthetic modification of a methyl ketone-functionalised zinc MOF. The aim of this study was to develop a post-synthetic method for installing borane and then applying this material as a heterogeneous reductant for ketones and aldehydes in solution. Borane MOF functionalisation within an MOF is rare, with only one other borane functionalisation having been reported in the literature [3]. Such a solid-state reagent could through additional pore tuning be developed further to impart size- and/or stereo-selectivity onto this process. The chemistry to accomplish functionalisation and the analysis of the MOF materials will be presented.



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Porous coordination polymers of alkylamine ligands

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We have been investigating the use of alkylamine ligands in the synthesis of porous coordination polymers [1–10]. The amine groups form part of the ligand backbones, and are designed to improve the selectivity of carbon dioxide capture over other gases. More than 50 new ligands have been made, and more than a dozen porous frameworks identified and tested. The ligands investigated fall into three different categories: (i) azamacrocycles, (ii) piperazines, and (iii) linear alkyl amines. Good carbon dioxide capacities and selectivities have been observed, as well as high stability to moisture, unusual structural transformations and interesting structural features. Related work has also looked at the use of these materials for the separation of complex aromatic hydrocarbon mixtures, and the incorporation of metal carbonyl species into the ligand backbones, with a view to creating new heterogeneous catalysts.

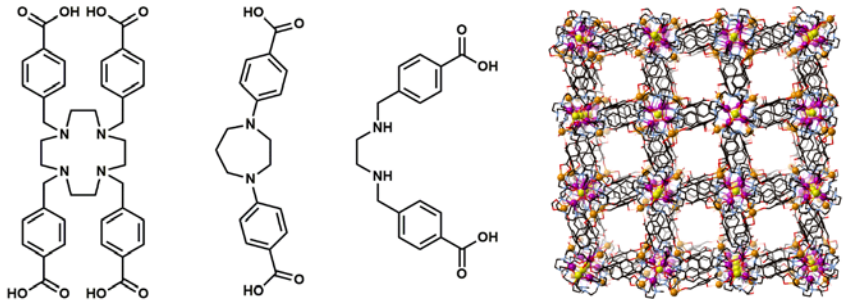

Figure 1. Example alkylamine ligands and porous coordination polymer.

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CONCURRENT SESSION 9B: SIGNALLING AND REGULATION

Chair: Bostjan Kobe

Assembly and function of two interacting oncogenic pseudokinase scaffolds

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The mammalian pseudokinase SgK223 and its structurally related homologue SgK269, are oncogenic scaffolds that nucleate the assembly of specific signalling complexes and regulate tyrosine kinase signalling. Both scaffolds are implicated in specific human malignancies: SgK223 in pancreatic ductal adenocarcinoma (PDAC) progression and SgK269 in colon and breast cancer as well as PDAC**.** Recently, we demonstrated that these proteins form homo- and hetero-oligomers *in vitro* and in cells, a mechanism that underpins a diversity of signalling outputs [1]. However, how these two scaffolds organise specific signalling complexes to regulate contrasting cellular responses remains largely uncharacterised.

To gain mechanistic insights into how these enzymatically-dead pseudokinases regulate oncogenic signal transduction networks, we determined the structure of SgK223 pseudokinase domain and its adjacent N- and C-terminal helices. Our structure uncovers how the N- and C-regulatory helices engage in a novel fold to mediate the assembly of a high-affinity dimer. In addition, we identified regulatory interfaces on the pseudokinase domain required for the self-assembly of large open-ended oligomers. This study highlights the remarkable diversity in how the kinase fold mediates non-catalytic functions and provides mechanistic insights into how the assembly of these two oncogenic scaffolds is achieved in order to regulate signalling output. The latest data will be presented.

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A generic mechanism for poly-γ-glutamylation in biomolecules

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Poly-γ-glutamate tails are found on a limited number of biomolecules, including two cofactors, folate (and its derivatives) and F_{420}. Tetrahydrofolates (THFs), a family of cofactors that are collectively called folates, are essential for cell growth and replication, primarily due to their central role in one-carbon metabolism. Poly-γ-glutamylation plays an important role in folate homeostasis through promoting intracellular retention of these essential cofactors. In addition, folate-dependent enzymes have a higher affinity for poly-γ-glutamylated cofactors. F_{420} is a flavin derivative that is sporadically distributed among microorganisms, mainly archaea and some bacteria. In a similar context, the F_{420} poly-γ-glutamate tail modulates catalysis in bacterial F_{420}-dependent oxidoreductases, with long-chain F_{420} resulting in higher-affinity enzyme-cofactor interactions.

Folylpolyglutamate synthase (FPGS) and F_{420}:γ-glutamyl ligase (FbiB) catalyse poly-γ-glutamate incorporation on folates and F_{420}, respectively. The chemical mechanism of poly-γ-glutamylation has been assumed to be similar for both cofactors, involving activation of the carboxylic acid on the elongated substrate in a nucleotide-dependent manner (ATP in the folates and GTP in F_{420}), formation of an acyl phosphate intermediate, and finally nucleophilic attack by the incoming L-glutamate. However, there have been no studies that definitively show how successive glutamate residues are added. The current assumption in the literature, especially for folates, is that the formation of the tail takes place through an “extension” mechanism, where the enzyme “walks along” the tail and additional glutamate residues are added to the growing terminus.

We have solved crystal structures for both FPGS and FbiB [1–2]. These structures fail to show how an extension mechanism might work, and instead suggest that an alternative ‘insertion’ mechanism might apply. To test this idea, we have used HPLC and mass spectrometry (LC/MS and LC/MS-MS), to show that archaeal, bacterial and human enzymes all do in fact use a novel insertion mechanism to form the poly-γ-glutamate tail in both F_{420} and the folates. Using radiolabelled L-glutamate as the substrate in functional experiments, we show that the incoming glutamate is inserted immediately before the first glutamate residue in the tail. This insertion mechanism resolves a long-standing mystery as to how these cofactor modifications take place.

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The human sliding clamp as a therapeutic target

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The human sliding clamp (also known as PCNA) controls access to DNA of many of the proteins involved in essential processes such as DNA replication, DNA repair, and cell cycle control. Proteins compete for interaction with the PCNA surface by means of a short, conserved peptide sequence known as the PCNA-Interacting Protein motif (or PIP-box). Binding to PCNA via the PIP-box allows access to DNA. For example, the major replicative polymerase, pol delta, requires PCNA for processive DNA synthesis, without interaction with PCNA the polymerase dissociates from DNA and is incapable of processive DNA synthesis. As such, many groups have proposed the usefulness of PIP-box mimetics for use as cancer therapeutics given they would block upregulated PCNA form allowing interaction with pol delta and hence would inhibit DNA replication. However, no peptide mimetics of PCNA have been forthcoming to date. Here we describe the design and synthesis of the first PCNA peptidomimetic. Our mimetic, ACR2, was designed through synthetic lactam chemistry to constrain the secondary structure of the peptide for optimised binding to PCNA. NMR solution studies show that the wild-type p21 peptide from which ACR2 was designed adopts no defined secondary structure in solution, while our mimetic adopts a 310 helix in solution, which has been shown in previous studies to be essential for PIP-box binding to PCNA. Binding experiments determined a Kd of 200nM of ACR2 for PCNA, which is higher than the wild-type peptide. A co-crystal structure of ACR2 bound to hPCNA revealed the mechanism of interaction of this mimetic with PCNA.

A viral immunoevasin controls innate immunity by targeting a prototypical Natural Killer cell receptor

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Natural killer (NK) cells play a key role in innate immunity by detecting alterations in self and non-self ligands via paired NK cell receptors (NKR). Despite identification of numerous NKR-ligand interactions, physiological ligands for the prototypical NK1.1orphan receptor remain elusive. Here, we identify a viral ligand for the inhibitory and activating NKRP1 (NK1.1) receptors. This murine cytomegalovirus (MCMV)-encoded protein, m12, restrains NK effector function by directly engaging the inhibitory NKR-P1B receptor. However, m12 also interacts with the activating NKR-P1A/C receptors to counterbalance m12 decoy function. The structure of the m12:NKR-P1B complex revealed that m12 sequesters a large NKR-P1 surface area via a ‘‘polar claw’’ mechanism. Polymorphisms in, and ablation of, the viral m12 protein and host NKR-P1B/C alleles impact NK cell responses in vivo. Thus, we identify the long-sought foreign ligand for this key immunoregulatory NK cell receptor family and reveal how it controls the evolutionary balance of immune recognition during host-pathogen interplay.

Structural basis of TIR domain assembly formation in the Toll-like receptor TRIF-dependent pathway

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Toll-like receptors (TLRs) are membrane-bound sensor proteins of the innate immune system that detect pathogen- and damage-associated molecular patterns and induce an initial innate immune response. TLRs can signal through either the MyD88-dependent signalling pathway or through the TRIF-dependent (MyD88-independent) pathway [1].

The MyD88-dependent pathway is the more characterised of the two. This pathway is utilised by all TLRs except for TLR3. MyD88 signalling is important for broad-spectrum activation of the early NF-κB response, CREB, AP-1 and IRF51. TRIF-dependent signalling is utilised by TLR3 and endosomal TLR4 and, while still very broad acting, is more specific to viral infections leading to activation of type-1 inteferons though IRF3 and IRF7 and the late NF-κB response [2].

Previous work in our lab has shown that the adaptor protein MAL (PDB ID 5UZB) of the MyD88-dependent pathway undergoes assembly formation into a filament *in vitro*. The filament itself is 30 nm wide with a C6 symmetry composed of 12, 2 stranded proto-filaments. Mutations in the inter-strand interface between subunits generally disrupt the function of MAL *in vivo*. These interfaces have also been observed in the crystal structure of TRR-2 (PDB ID 4W8G) and from the crystal packing in the MyD88 microcrystals [3].

Modelling of the interface of TRAM into the interfaces of MAL shows that the interfaces may be conserved between the two proteins [3]. Using electron microscopy, we have shown that TRAM is able to undergo assembly formation into a filament. TRAM filament, unlike MAL, does not seem to be able to form a tube and rather takes on an F-actin like structure.

Initial processing of TRAM filament structure was done using a single particle-like approach, where the filament was divided into many segments and treated as individual single particles. Averaging of these segments revealed the presence of layer lines; from this we have estimated the helical rise. 2D classification of the segments revealed that the TRAM subunit structure and helical radius similar to that of MAL proto-filament. Further processing is required to determine the helical rotation per subunit (Azimuthal angle) and to reliable perform the 3D reconstruction.

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The structure-function relationship of transcriptional activators and antiactivators controlling quorum sensing and horizontal gene transfer

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Bacterial cell-cell communication, or "quorum sensing" facilitates coordinated regulation of phenotypes at a multi-cell level. Quorum-sensing regulation systems are prone to spontaneous autoinduction and therefore several systems have evolved "antiactivator" components that prevent spurious activation of critical processes. The DUF2285 domain is present in proteins encoded throughout the proteobacteria but has not been characterised at a structural level. Our work has shown DUF2285 proteins encode both transcriptional activators and antiactivators. The antiactivator QseM binds and inhibits both a DUF2285 gene-transfer activator FseA and the structurally unrelated quorum-sensing activator TraR.

We are investigating the molecular processes driving QseM binding and inhibition to these two structurally distinct proteins by investigating QseM and QseM mutant structures via X-ray crystallography and nuclear magnetic resonance (NMR). QseM crystals were generated with and without the incorporation of L-selenomethionine, and currently show low-resolution diffraction (both native and anomalous), at the Australian Synchrotron. We have successfully acquired NMR data from unlabelled, single (15N) and double-labelled (13C and 15N) samples of QseM at a range of concentrations up to 1 mM. Purified FseA protein is currently being tested for interactions with QseM and DNA using both NMR and surface plasmon resonance. Current data supports QseM as a stable, monomeric, mostly alpha-helical protein, which still functions as an antiactivator with its recombinant N-terminal hexahistidine tag present. QseM is an excellent candidate for continued NMR and X-ray crystallography experiments due to its monomeric molecular weight of 10kDa.

Structural information gained from this work combined with information from mutagenesis work will provide insight into QseM antiactivation mechanism and, more broadly, provide insight into the roles of DUF2285 domains in other bacteria. We are currently investigating the potential for exploitation of these activator-antiactivator pairs in synthetic molecular circuits.

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SESSION 10: CHALLENGES IN CRYSTALLOGRAPHY

Chair: Chris Sumby

Recent and future developments on the Australian Synchrotron MX2 beamline driven by the Eiger 16M detector deployment

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INVITED SPEAKER

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The new pixel array detector — Eiger 16M — deployed on MX2 in February 2017 has now generated more than 152 Tb of data compared with 18 Tb in the same period last year using a CCD based detector. This has not only revolutionised the speed that datasets are collected but also put challenges in the way we collect, take notes, process and store data. Here we will present how some of these challenges have been tackled and what are the future developments already being worked on for deployment in the next 12 months. We will also briefly describe one of the most common traps on collecting data on the Eiger 16M.

Taking our raw data to the next level

James Hester[1] and Sydney Hall[2]
INVITED SPEAKER

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The advent of computers and consequent high-volume data generation has led to a chronic separation of scientific results from the underlying evidence. Efforts are underway to both preserve the raw data in repositories and to resurrect the link between the measurements and the results. These raw data repositories would be much more valuable if they presented a uniform interface that allows third-party software and web-based tools to access and manipulate arbitrary raw data without requiring local or ad-hoc knowledge. Technical barriers to this simple-sounding objective include heterogeneous file formats and storage layouts, ill-defined notions of 'dataset', inconsistent metadata, and an underdeveloped system for data citation.

Recent developments in the CIF world suggest that these technical issues are manageable within a relatively simple framework, and this opens the way for an intelligent raw data repository. The unification of the Australian Synchrotron and OPAL under the ANSTO umbrella provides a new opportunity for a significant proportion of Australia's raw crystallographic data to be made available in this way, if there is sufficient support within the scientific community.

Practical uncertainty in protein crystallography: It's a monomer. Or is it?

Jason W. Schmidberger[1], Brady Johnston[1] and Charles S. Bond[1]
INVITED SPEAKER

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Crystallography is an excellent tool for investigating the quaternary structure of biomolecular complexes. However, because of crystallographic details, such as symmetry elements or even unusual crystal pathologies, the actual quaternary state of a structure may not be clear unless carefully analysed and annotated. Databases such as the protein databank attempt to help by assigning a "biological assembly" which can be compared with the actual protein stoichiometry and symmetry. Unfortunately the assignment is not always right. In this talk I will explain the background and discuss some examples from the literature, and then focus on the example of crystal structures of designer pentatricopeptide repeat proteins and their complexes with RNA. These structures appear to be of infinite polypeptide and nucleotide chains, and thus defy the available descriptors.

Prospects for organic minerals on Saturn's moon Titan

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Titan, the largest moon of Saturn, contains a vast inventory of organic molecules and is considered a prebiotic chemical laboratory on a planetary scale. Active photochemistry in the atmosphere via solar radiation and energy from Saturn's magnetosphere causes N_2 and CH_4 to dissociate and recombine, generating organics ranging from simple (ethane, acetylene, HCN) to complex (>10,000 Da) molecules. These molecules continue to react as they move through Titan's atmosphere, forming aerosol haze layers and eventually depositing on the surface [1].

Additionally, the Cassini spacecraft revealed that Titan has standing bodies of liquid on its surface, in the form of lakes and seas. This is a remarkable discovery, as it makes Titan only the second planetary body known to have such features (after our own Earth). These lakes, which are evidenced to contain mainly methane and ethane, could dissolve many of the molecules that were generated in Titan's atmosphere. These could subsequently form precipitates and create evaporite deposits similar to those observed by the Cassini Visual and Infrared Mapping Spectrometer (VIMS) and Synthetic Aperture Radar (SAR) around some of the northern lakes [2]. Previous work has demonstrated [3] that two common organic molecules on Titan, ethane and benzene, form a unique and stable co-crystalline structure at Titan surface temperatures, which could comprise these evaporite deposits.

Influenced by the discovery of a new solid phase for Titan, a survey has been undertaken outlining the current structural understanding of molecular solids under Titan conditions. Using the Cambridge Structural Database (CSD) a number of possible minerals 'types' that would be expected on the surface of Titan have been identified. The subsequent classification of possible Titan minerals is done on the basis of intermolecular interactions, with the materials organised into 'Molecular solids', 'Molecular co-crystals' and 'Hydrates' grouping. This classification is designed to aid future work in determining how a number of the features on Titan may have formed.

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Has crystallography lost the plot on gender equity, or has it been penalised because of its historically greater equity?

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Crystallography as a science has a history that predates the discovery of the phenomenon of X-ray diffraction, having been an issue for students of mathematics, chemistry, biology, geology and physics—for example, Pasteur's key observation of the chirality of crystals [1]. Could it be that this scientifically broadly based activity has contributed to the remarkable success of crystallographic studies in modern science? Is it this broad-based interest that saw the atypical evolution of crystallography in the 20th century as a science based in mathematics and physics in which women were welcome and, indeed, became key players as the science expanded into molecular and macromolecular studies?

Whether by accident, or virtue, the prominence of women in crystallography compared to other physical sciences in the late 20th century was a fact clearly observed by many of our members—some of us still practising in the field today. At the most recent International Union of Crystallography (IUCr) meeting, it was notable that the representation of women at the conference, in particular the numbers of women presenting plenary lectures (none) and keynote and invited lectures, appeared to be diminished with respect to what appear to be healthy numbers of women practitioners in crystallography.

It is currently fashionable in STEM subjects (Science, Technology, Engineering and Mathematics) for significant attention to be paid to gender balance within educational and scientific organisations. Concepts such as "male champions of change" are being advanced as a "solution" to the perceived "problem". This author questions the validity of such an approach where an acknowledged outlier field is undergoing an apparent reversal from a situation of greater to one of lesser gender balance and indeed equity. Is something else at play here?

Could it be that in the atypical gender balance evident in crystallography in the late 20th century, in the "primary subject areas" across which crystallographic endeavours are distributed, a perceived (whether real or not) level of feminisation of crystallography has actually led to the diminution of respect and career prospects for practitioners of crystallography for either gender? The relegation of crystallographic appointments in university chemistry departments to the status of service or peripheral activity, delivered in many instances by practitioners of limited experience and subject to limited tenure, has occurred widely in chemistry departments and anecdotally is now reported to be underway to some extent in the biological areas of crystallography. Is our science actually being diminished and downplayed by the primary subject areas because it has been "feminised" to a modest extent?

It is up to us as the professional scientific crystallographers to understand what the drivers are that may have been reversing what was our more desirable gender balance. It is especially important to enquire and understand why our science itself has been so diminished as to be in many cases only a handmaiden to the main game. It is critical for the future of our science that we reflect on these questions of status and equity and act collectively to ensure our science is properly valued.

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SESSION 11: RISING STARS

Chair: Jenny Martin

Structure of a lipid A phosphoethanolamine transferase, an endotoxin modifying enzyme from Gram-negative bacteria

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Multi-drug resistance in Gram-negative bacteria is a serious threat to public health. The bacteria gains resistance to current antimicrobial treatments by modifying the endotoxins (lipid A) present on the outer membrane. One such modification is brought about by the enzyme lipid A phosphoethanolamine transferase (EptA), which adds phosphoethanolamine from phosphatidylethanolamine to lipid A at 1 and 4' head group positions [1]. This modification prevents the clearance of bacteria by the innate immune system [2] and increases the bacterial resistance to cationic antimicrobial peptides such as colistin [3].

The 3D structure of EptA was determined to 2.75 Å resolution and shows a helical transmembrane domain linked to a periplasmic facing soluble domain. The structure also shows the active site of the enzyme comprising residues from both domains. Molecular dynamics and intrinsic fluorescence studies suggest the protein adopts multiple conformational states, which may be required for the binding of two different sized substrates. These studies provide insights into the mechanism of endotoxin modification and will aid in the design of novel therapeutic agents for the treatment of multidrug-resistant bacterial infections.

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The first crystal structures of Bak in complex with lipid offer novel insights into oligomerisation and membrane permeabilisation

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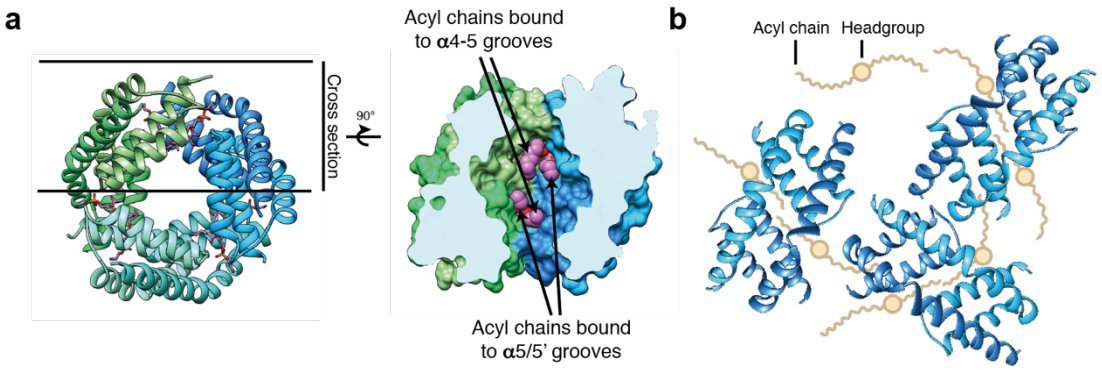
The Bcl-2 protein family regulates the intrinsic apoptotic pathway through an intricate network of protein:protein and protein:membrane interactions. The pathway culminates in the permeabilisation of the mitochondrial outer membrane by the pro-apoptotic effector proteins Bak and Bax, an event that commits a cell to death. To facilitate membrane permeabilisation, Bak and Bax undergo a series of conformational changes to convert from inert monomers to membrane-embedded homodimers that nucleate and propagate apoptotic oligomers. One important change is the separation of the protein into "core" and "latch" domains [1, 2]. Freed core domains then form homodimers, these then oligomerise into larger structures that permeabilise the membrane.

While great strides have been made in understanding how Bak and Bax are activated and form homodimers, questions remain surrounding subsequent events including homodimer interactions with the membrane, homodimer oligomerisation, and the mechanism of membrane disruption/permeabilisation. In an attempt to address these questions, we have solved several X-ray crystal structures of lipids bound to Bak core homodimers. These are the first structures of any Bcl-2 family protein in complex with lipid. The protein:lipid interface involves both polar and non-polar interactions with lipids headgroups and acyl chains. Acyl chains bound primarily to two symmetric hydrophobic grooves on a planar hydrophobic surface present on the Bak dimer. In one structure, adjacent homodimers were cross-linked by phospholipids, with each acyl chain of the diacyl lipids binding to a different homodimer (Figure a). Despite intense investigation, a requisite protein:protein interface between Bak homodimers within larger oligomers has not been identified. This structure suggests lipids may play a direct role in facilitating Bak oligomerisation (Figure b). Bak oligomers could be dissociated with phospholipase A2 treatment, supporting a role for lipid in oligomer stability. Collectively, the structures suggest a lipid-mediated model for Bak oligomerisation and provide clues in the search for the elusive mechanism of membrane disruption by Bak and Bax.



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Structural and functional analysis of two *Proteus mirabilis* copper resistance proteins reveals an unusual redox relay system

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Proteus mirabilis is an important human pathogen that employs copper resistance as a key virulence trait. We are investigating the *P. mirabilis* suppressor of copper sensitivity (PmScs) proteins to better understand copper resistance and its role in bacterial virulence. Using three crystal structures, SAXS and biochemical data we showed that PmScsC is a highly dynamic trimeric disulphide isomerase [1]. Until then, bacterial disulphide isomerases — essential for folding many secreted proteins — were all thought to be dimeric [2]. The dimeric bacterial isomerases rely on a partner protein to keep them reduced. We hypothesised that, similarly, trimeric PmScsC would have a redox partner and our research shows that this is indeed the case. The periplasmic N-terminal domain of the membrane protein PmScsB (PmScsBα) reduces PmScsC. We report the high-resolution crystal structure of PmScsBα, unexpectedly revealing two immunoglobulin-like (Ig) folds, one of which includes a putative redox active site CXXXC. By contrast, the redox partners of dimeric bacterial disulphides have just one immunoglobulin fold [3]. We confirmed the functional importance of the PmScsBα cysteines, and used cysteine mutants to generate a 3:1 PmScsC:PmScsBα complex. The low-resolution structure of PmScsC:PmScsBα was determined using SANS. The data show that upon PmScsBα binding, the normally highly dynamic PmScsC becomes much more ordered. The SANS model (Figure 1) has the catalytic Ig1 sub-domain of PmScsBα bound to one PmScsC subunit and the non-catalytic Ig2 sub-domain seems to interact with two PmScsC subunits. These findings expand our understanding of the poorly characterised Scs system and provide new details of redox folding processes employed by bacterial pathogens.

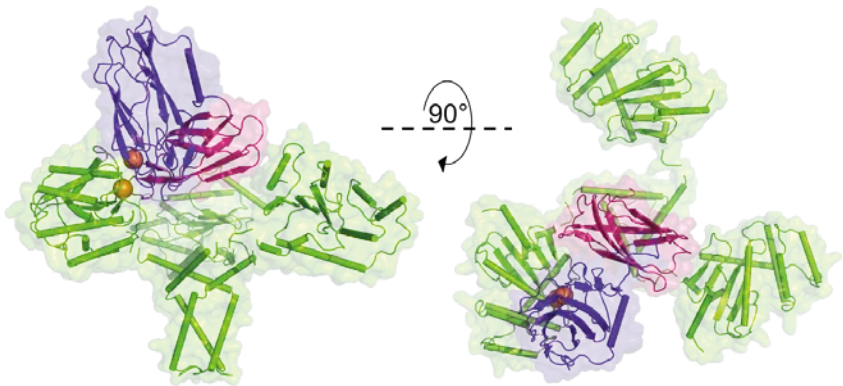

Figure 1. Backbone and surface of PmScsC:PmScsBα model.
Green: PmScsC. Purple and magenta: Ig1 and Ig2 of PmScsBα, respectively.
Orange spheres: Cysteines of the intermolecular disulphide bond.

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The unusual structural chemistry of uranium: Controlling phase transformations in ternary uranium oxides

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Structural investigations of ternary uranium oxides are pertinent to the management of spent nuclear fuel since such environmentally hazardous and potentially dangerous secondary phases may form during the process and/or storage of the material [1]. They further allow for the exploration of the peculiar, exotic and poorly known properties of materials containing, or which can access *5f* electrons. The rhombohedral oxides AUO_4 for A = α-Sr or Ca in space group $R\bar{3}m$ exemplifies this. We have found through a combination of *in situ* synchrotron X-ray powder diffraction and X-ray absorption spectroscopy, that α-$SrUO_4$ undergoes a fascinating first-order phase transformation under oxidising conditions [2]. This involves the synergetic loss of lattice oxygen resulting in oxygen vacancy defect formation and reduction of the uranium cations, which seemingly reduces the activation energy barrier for transformation to its orthorhombic form, β-$SrUO_4$. Under similar conditions, $CaUO_4$ does not display any transformative behaviour, however, defects can be engineered through the substitution of Ca^{2+} for Sr^{2+} in the solid solution α-$Sr_xCa_{1-x}UO_4$ when heated to high temperature under oxidising conditions. The introduction of Sr^{2+} cations in α-$Sr_xCa_{1-x}UO_4$ was found to decrease the temperature at which oxygen vacancy defects form. This phenomenon was rationalised as a consequence of the introduction of Sr^{2+} cations leading to lattice expansion, which causes the proximity of defects to increase. This subsequently reduces free energy increasing defect-defect interactions, allowing defects to form at lower temperature. Remarkably, when heated under reducing conditions, the disordered oxygen defect containing rhombohedral α-$SrUO_{4-x}$ structure undergoes a reversible first-order phase transformation that involves the ordering of the oxygen defects resulting in lowering of the crystallographic symmetry to triclinic in space group $P\bar{1}$ denoted δ-$SrUO_{4-x}$. This remarkable transformation, which implies entropy is being decreased as temperature increases, could be replicated in $CaUO_4$ and also in α-$Sr_{0.4}Ca_{0.6}UO_4$ where the transformation temperature is reduced by increasing the Sr^{2+} content, consistent with the effects of reducing defect-defect interactions. The S-XRD data shows the structure of δ-$CaUO_{4-x}$ to be incommensurate whereas δ-$SrUO_{4-x}$ is commensurate. This implies a miscibility gap may exist between the isostructural $CaUO_4$ and α-$SrUO_4$ related to short-range order. This investigation demonstrates the rich and fascinating crystal chemistry present in uranium oxides, which, in some cases, may have profound societal importance if it can either be safely used or if associated properties can be replicated into non-actinide materials.

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Cryo-EM structure of a type-II ABC toxin complex provides new clues to the mechanism of cell surface recognition

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ABC toxins are tripartite, pore-forming toxins found in a range of bacterial pathogens of insects and humans. The A subunit perforates cell membranes, enabling translocation of a cytotoxin (subunit C) and is strongly implicated in the specific recognition of host cell surfaces prior to (or upon) pore formation. It has remained unclear whether mechanisms of pore formation and cell surface recognition are shared across the ABC toxin family. Here we present a 4.4 Å resolution cryo-EM structure of the A subunit from the *Yersinia entomophaga* type-II ABC toxin, YenTc. We were able to show that YenTc is a pore-forming toxin and used *in silico* analyses to find structural evidence supporting a conformational change from the inactive pre-pore form to the biologically active pore form. The pre-pore form features a central α-helical bundle, the tip of which is particularly hydrophobic and protrudes from the complex so that it can be inserted into a lipid membrane in the pore form (Figure 1). Preliminary cryo-EM reconstructions of the YenTc pore form provide insights into this conformational change, particularly into the rearrangement of domains implicated in cell surface recognition. Overall, our results suggest that while the structural basis of pore formation is conserved across ABC toxin subtypes, the motifs involved in cell surface recognition are diverse, and provide insights into the mechanisms that ABC toxin-producing bacteria utilise to target different hosts.

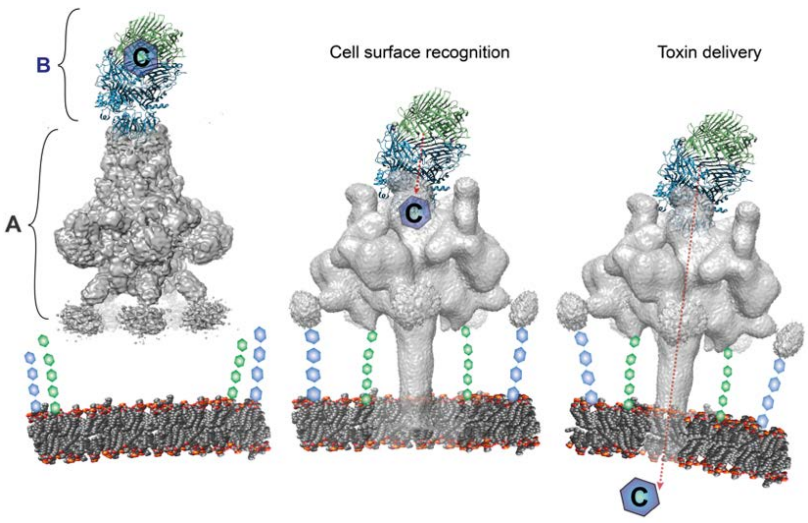

Figure 1. Schematic of YenTc conformational change and host recognition from available pre-pore (left) and pore form (middle and right) cryo-EM maps (grey).

Development of potent and selective bicyclic peptide inhibitors of the Grb7 cancer target

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Grb7 is an intracellular signalling protein, with critical roles in tumour cell proliferation and migration, and is established as a therapeutic target for both HER2+ and triple negative breast cancer. Grb7 interacts with the phosphorylated tyrosine kinases via its C-terminal SH2 domain leading to migratory and proliferative signalling, and it is this domain that is a therapeutic target for the development of novel anti-cancer agents.

We have discovered and developed a new bicyclic peptide scaffold incorporating thioether and lactam linkers that binds with nanomolar affinity whilst maintaining specificity for Grb7-SH2 over the closely related SH2 domains of Grb2 and Grb10. The X-ray crystal structure of the Grb7-SH2/bicyclic peptide complex revealed an unexpected binding orientation as well as the basis for the binding selectivity. To further improve on the binding affinity, we incorporated phosphotyrosine mimetics into the bicyclic peptide scaffold and arrived at an optimised inhibitor that binds potently to the Grb7 SH2 domain (K_D=130 nM). The X-ray crystal structures of these Grb7-SH2/peptide complexes revealed the molecular basis for the most potent inhibitors of Grb7 developed to date. Lastly, we have shown that that cell permeable versions of these peptides successfully block Grb7 mediated interactions in a HER2+ breast cancer cell line.

It is envisaged that the development of these specific and potent Grb7 targeting peptides will both provide tools to further explore the role of Grb7 in healthy and cancerous cells, and also provide proof-of-concept evidence that Grb7 can be successfully targeted for the development of novel anti-cancer agents.

SESSION 12A: PROPERTIES THROUGH MATERIALS DESIGN

Chair: Josie Auckett

Understanding correlated disorder within an MOF-5 analogue

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INVITED SPEAKER

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An important principle underlies the development of functional materials: atomic structure drives function. Diffraction techniques have become adept at describing periodicity in many materials and understanding atomic structure. However, deviation from perfect order occurs in most real materials, be it through defects, chemical inhomogeneity, and/or microstructural strain. Traditional crystallographic techniques fail to describe such deviations from periodicity, and while we understand the importance of disorder we are yet to understand how to describe, characterise and control disordered states.

A very specific disordered state is correlated disorder. This state arises when dominant interactions — i.e., chemical bonding — dictate fixed local atomic arrangements that need not result in long-range 3D order but may result in aspects of the structure being correlated. These correlations can produce signature patterns in the measured diffraction pattern in the form of diffuse scattering. In a class of materials known as metal-organic frameworks (MOFs), the local interactions driving formation usually result in long-range periodic arrangements. However, we can introduce disorder in MOFs through asymmetry in the linker, which acts in an analogous way to cyanide in transition-metal cyanides [1], second-order Jahn-Teller displacements in $BaTiO_3$ [2] and hydrogen bonding in square ice [3].

We substitute terephthalate linkers with asymmetric pyrazole-carboxylate and show that while powder and single crystal diffraction data suggest the linkers are disordered, the highly structured diffuse scattering visible in single-crystal X-ray patterns indicates correlated disorder along the linker rows. By comparing experiment with models calculated using simple local rules, we are able to understand the specific type of correlations giving rise to the diffuse, and prove the presence of correlated disorder in an MOF-5 analogue. The fixed local arrangements in the structure results in specific pore chemistry and binding sites important for adsorption and catalytic applications. More generally, these results contribute to the ability to identify specific types of correlated disorder within analogous systems, and therefore, understand the nature and location of defects.

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Bis(amino-acid) ligands in crystal engineering — Finding the steric Goldilocks zone

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Bis(amino-acid) derivatives of naphthalenediimide have been explored for use in the formation of robust and reproducible motifs in the solid state. Steric limitations have been found that limit the applicability of this class of ligand.

Our previous results, using ligands derived from alanine, leucine and phenylalanine have shown the formation of an $\{M_2L_2\}$ macrocyclic motif in more than 70% of cases [1–3]. This motif allows for interpenetration, by either catenation or the formation of rotaxanes, supported by face-to-face pi-interactions when aromatic guests pass through the macrocycle. To explore the limitations of this motif, new ligands were synthesised that are derived from glycine or isobutyric acid, i.e., containing either one fewer or one more methyl group that the alanine analogue. Surprisingly, these small changes in steric bulk appear to completely disfavour the formation of the $\{M_2L_2\}$ macrocycle, with no instances observed in our studies to date. This observation can be sterically rationalised by the manner in which the ligands pack with each other, showing that the original family of ligands was in a steric Goldilocks zone.

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Improving hydrophobicity of MOFs using aliphatic linkers

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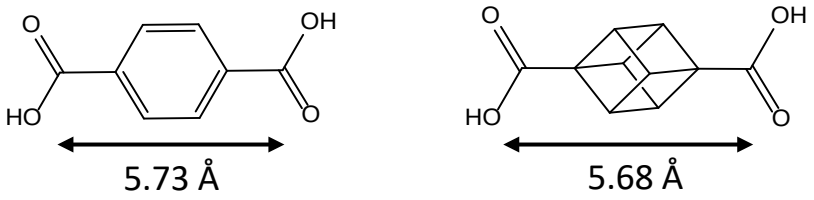
Metal-organic frameworks (MOFs) have become renowned throughout the chemistry and materials communities as an exciting suite of porous material, capable of being designed and adapted to suit various objectives and applications [1]. Aromatic ligands with carboxylate functionalities are a commonly selected organic linker when synthesising MOFs due to their commercial availability and the numerous variable coordination modes exhibited by these functionalities, consequently leading to a high degree of framework connectivity [2]. Structurally similar to 1,4-dicarboxybenzene, frequently used as an organic linker in MOF synthesis, is 1,4-dicarboxycubane due to its *para* coordination capabilities and carboxylate functionalities. Unlike 1,4-dicarboxybenzene, 1,4-dicarboxycubane is non-planar in nature, features a higher steric bulk and is void of conjugation within the ring (Figure 1). These differing properties can be exploited to modify the local environment within the pores of resultant MOFs to be more hydrophobic.

We have substituted 1,4-dicarboxybenzene with 1,4-dicarboxycubane during the synthesis of known and well-characterised MOFs with the aim of modifying the pore environment within the framework. These MOFs were structurally characterised using single crystal X-ray diffraction and Rietveld refinement of synchrotron X-ray diffraction data collected at the MX and PD beamlines at the Australian Synchrotron. Here we will discuss the structural and behavioural differences between the MOF species, pertaining to their selective gas sorption, water retention and thermal stability properties.



1,4-dicarboxybenzene *1,4-dicarboxycubane*

Figure 1. Ligand structures of 1,4-dicarboxybenzene (left) and 1,4-dicarboxycubane (right).

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Modulating organic reactivity in the solid-state through employing coordination polymer assemblies

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Isophthalic acids are well used as ligands in discrete and polymeric metallosupramolecular assemblies. We have been exploring the structures and chemistries of coordination polymers formed from isopththalic acids tagged with thermoresponsive allyloxy and propargyloxy groups (Figure 1, top). In this talk I will share our findings on the structural variety observed in coordination polymers with zinc, cobalt and copper, which include dense 2-D sheet structures and pro-porous 3-D structures (Figure 1, bottom), and comment on differences and similarities in solid-state compared to solution-based reactivity.

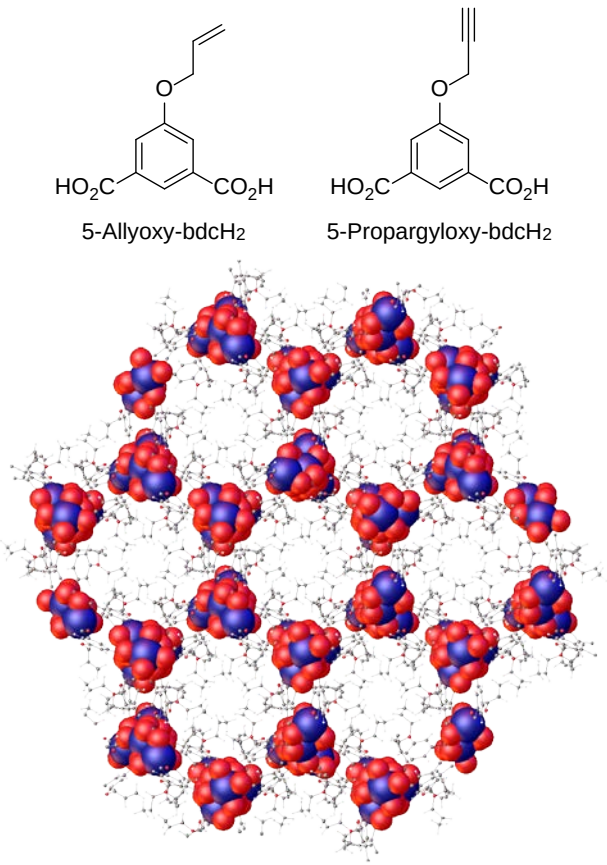

Figure 1. The molecular structures of thermoresponsive isophthalic acids (top);
Part of the 3-D structure of the zinc complex formed with 5-Allyloxy-bdcH_2 (bottom).

A crystal structure that contains regions with different orientations, different origins and different space groups

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The crystal structure of 3,4-dibromo-5,5-dimethoxy-1,5-dihydro-2*H*-pyrrol-2-one, C6H7NO2Br2, was initially solved in space group *Pca*2_1, *a* 16.479, *b* 7.958, *c* 14.198 Å, T -123 C, with two molecules in the asymmetric unit, using separate scales for *h* odd and *h* even reflections, Kodd / Keven = 0.871. Layers perpendicular to **c*** are pseudo centro-symmetric causing *h* even data to see a 1:1 disordered structure of approximate *Pcmn* symmetry in the cell **a" = ½ a, b" = b**, **c" = c**, implying ordered layers of approximate *P*.2_1/*a*. symmetry have alternative origins ½ **a** apart. Weak extra reflections consistent with an ordered structure of *B*.2_1/*a*. symmetry for a cell **a' = a, b' = b**, **c' = 2 c** were observed and reflections were then processed for this cell.

The space group *Pcmn* has an interface of *P*2_1*mn* symmetry at *z*" = ¼ and ordering doubles the *a* axis and lowers the symmetry to *P.a*. for an interface with two molecules per asymmetric unit. Structures with different orientations, different origins and different space groups, *Pca*2_1 or *B*.2_1/*a*. are then the result of different stackings of this interface.

Observed reflections with *h* odd see either the *Pca*2_1 or the *B*.2_1/*a*. component whereas reflections with *h* even see both enabling a correlation coefficient to be evaluated.

All twinning implies a correlation coefficient of 0, i.e., sample regions see only a single structure and intensities are a sum of component intensities. A correlation coefficient of 1.0 implies sample regions all see the same average disordered structure.

A correlation coefficient between 0 and 1.0 can be estimated for this structure by comparing the scales of different subsets of reflections.

SESSION 12B: BIOMOLECULAR RECOGNITION

Chair: Isabelle Lucet

Molecular basis of the assembly of COMMD proteins into the CCC/Retriever complex

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Internalised cargo proteins, lipids and nutrients enter intracellular sorting stations called endosomes, and perturbations in endosomal trafficking and lysosomal degradation are implicated in the onset of debilitating diseases, including hypercholesterolemia, Wilson's disease, and neurodegenerative and neurodevelopmental disorders. Recently, we and others have identified an evolutionarily conserved endosomal protein trafficking machinery called the CCC/Retriever complex (Fig. 1). This complex is comprised of approximately 20 subunits of which ten belong to the family of Copper Metabolism MURR1 Domain-containing (COMMD) proteins. As a first, we have resolved the crystal structures of both the conserved modules of COMMD proteins. Our structural characterisation demonstrates the mechanism underpinning the COMMD dimerisation. Using a hybrid structural biology approach, we have also deduced the overall architecture of several members of the COMMD proteins. We show that COMMD proteins interact with each other forming hetero-complexes. Importantly, the structure of COMMD proteins is not similar to any protein structure belonging to the metazoan family; instead the overall architecture of some of the COMMD members resembles proteins associated with the type III secretion system of *Chlamydia* bacterium. Such structural similarity suggests that Chlamydial proteins have evolved to possess similar structures as COMMDs to hijack the trafficking pathways controlled by COMMD proteins.

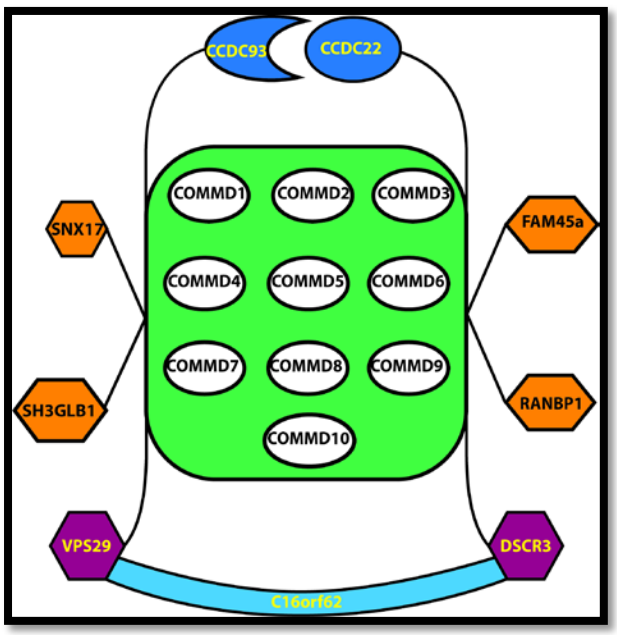

Figure 1. Schematic of the CCC/Retriever complex.

Pre-empting BCL2 mutational tolerance to Venetoclax, insights from structural biology

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Cancer treatment remains one of the great health challenges. Recently, Venetoclax (ABT-199) was approved for the treatment of chronic lymphocytic leukaemia (CLL) harbouring the 17p chromosomal deletion, with trials ongoing for treatment of other cancers. Venetoclax is the first approved BH3 mimetic (drugs targeting BCL2 proteins), promoting apoptosis in resistant cells and revolutionising outcomes for CLL 17p sufferers.

To date ABT-199 resistance has not been encountered in patients, however, it is likely to occur in the future. A common resistance mechanism is mutation of the target protein, as typified by the ABL kinase T315I mutation [1]. ABT-199 tolerance has been induced in a mouse tumour model and human cell lines, revealing mutation of phenylalanine 104 in BCL2 to either leucine or cysteine as a mechanism for ABT-199 resistance [2].

We have solved the first crystal structure of Venetoclax bound to BCL2. This structure has revealed subtle differences in Venetoclax binding compared with prediction models using structures based of BCL2 with ABT-199 analogues [3]. We have applied molecular dynamics to the diffraction data to model conformational dynamics within the BCL-2–ABT-199 interface. This has revealed large movements in the protein backbone proximal to the ABT-199 binding site, indicating potential for expansion of the ABT-199 scaffold in this region.

To understand the F104 mutations we have measured the affinity of ABT-199 for the BCL2 mutants by surface plasmon resonance, revealing a reduction of affinity upon mutation. We have solved the structures of both mutants and the wild type BCL2 protein with ABT-199 and an ABT-199 analogue. These structures have revealed minimal changes in binding conformations of the ABT-199 analogue to both mutants, indicating the reduction in affinity is due to a loss of shape complementarity. These insights combined with the potential to expand the ABT-199 scaffold, provides an exciting opportunity to develop a novel therapeutic that overcomes mutation of F104.

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Plant TIR domains as NADases: Missing link in plant innate immune signalling?

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Plant NLRs (Nucleotide binding [NB], Leucine-rich repeat [LRR] Receptors) provide resistance to a range of biotrophic pathogens, by recognition of avirulence factors, and proceeded by initiation of downstream immune responses. NLR signalling is transduced through the N-terminal TIR (Toll/interleukin-1 receptor) domain, and resistance is often characterised by localised cell death around the site of infection. How plant TIR domains transduce this signal is still unknown, however, like TIR domains from mammalian NLRs, plant TIR domains form homo- and heterotypic dimers, and this dimerisation is required for signalling.

Recently, a TIR domain from the mammalian TLR (Toll-Like Receptor) adaptor family, SARM (Sterile Alpha and TIR motif-containing 1), was shown to possess NADase activity, the first report of a TIR domain displaying enzymatic activity [1]. The NADase activity of SARM was shown to promote Wallerian degeneration of axons, with SARM induced depletion of NAD leading to axon degeneration [1, 2]. Given the structural similarities between SARM TIR and plant TIR domains, the requirement of SARM dimerisation for NADase activity, and the lack of downstream binding partners identified for plant NLRs, we hypothesise that some plant TIR domains also possess NADase activity.

Here we present experiments that demonstrate L6 (from flax) and Run1 (from grapevine) TIR domains possess NADase activity, by both 1-D NMR and utilisation of a fluorescent analogue of NAD+. The ability of plant TIR domains to hydrolyse other nucleotides was also tested. We also report the NADase activity of plant TIR domains with mutations in the hypothesised NAD+ binding pocket, as well as present some preliminary data on the structure of a plant TIR domain with a bound ligand.

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Development of a Bak inhibitory peptide based on the crystal structure of the Bak:BimBH3 complex

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Crystal and NMR structures have provided novel insights into BH3-only induced Bax and Bak activation and oligomerisation [1, 2, 3]. In particular, this work has revealed the nature of the activation sites, an ensuing separation of the Bax and Bak core and latch domains and subsequent dimerisation of the released cores. Here we present the first crystal structure of Bak in complex with an activating BH3 domain [4]. This reveals insights into differences in interactions of BH3-only proteins with Bak compared to pro-survival relatives and provides possible mechanisms towards the unlatching of the Bak globular fold. Based on the structure we have made a novel modification that changes the activity of the BH3 peptide dramatically. No longer does it bind transiently to the target and induce conformational change, instead it binds with measurable affinity to Bak and inhibits conformational change. As a consequence this peptide acts as a Bak inhibitor in permeabilisation experiments; to our knowledge this is the first example of an agent that can directly bind to and inhibit Bak. These findings provide novel insights into Bak activation and new strategies for developing therapeutics capable of inhibiting Bak activity.

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Structural insights into the killer-cell immunoglobulin-like receptor family

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Natural Killer (NK) cells play a crucial role in the innate control of infection and the elimination of tumours. There are a remarkably diverse set of mechanisms by which NK cells detect infected or transformed cells including receptor/ligand interactions between cell surface receptors expressed by NK cells and target cell ligands. Of central importance to NK function is the receptor/ligand pairing of antigen-presenting HLA molecules with killer cell immunoglobulin-like receptors (KIRs). The KIR family comprises 14 members that can be either inhibitory or activating. The inhibitory KIRs are associated with viral and tumour clearance through recognition of decreases in cell surface expression of HLA molecules ("missing self") to escape immune pressure mediated by T cells. The role of activating KIR is currently unknown. The KIR gene family, and their HLA encoded ligands, are the most polymorphic in the human genome and are functionally diverse. Thus, our ability to fully harness this system in the clinic is limited by our incomplete knowledge of which allelic pairings are favourable in the context of disease progression and organ transplantation. To address this, we provide binding data from single-antigen bead arrays of 100 HLA alleles for each of the 14 KIR members and allelic variants therein. Further, we provide a structural perspective on what makes a good KIR-HLA pairing, in the context of binding affinity, through discussion of recent published and unpublished crystal structures we have obtained for the inhibitory KIR2DL2, 2DL3, 2DL4 and 3DL1 and the activating KIR2DS2. Further, through the use of hydrogen-deuterium exchange assays we show that allelic differences in KIR3DL1 affinity correlate with differences in the dynamics of the alleles. The structural and dynamics data is complemented with functional data from primary NK cells to build the most complete picture available of the molecular nuances underlying the differences between inhibitory and activating KIR and provide a framework for decoding the constellation of allelic variation within this receptor family.

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SESSION 13A: MAGNETISM AND PHASE TRANSITIONS

Chair: Emily Reynolds

The symmetry-mode decomposition for better understanding of the structural evolution presented in polar functional materials

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INVITED SPEAKER

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The phase and structure evolution of the (1-x)AgNbO3-xLiTaO3 solid solution is investigated by the neutron diffraction, dielectric and ferroelectric measurements. The symmetry-mode decomposition of the distorted AgNbO3 structure defined on the experimental space group, Pmc21 has been conducted. The four main modes, T4+, H2, Λ3 and Γ4-, exhibit large distorted amplitude to stabilise the Pmc21 structure. The mode refinement with referring to the Pmc21 was adopted to (1-x)AgNbO3-xLiTaO3 material system. It is found that with the increasing LiTaO3 concentration, the orthorhombic phase partially transfers to the rhombohedral R3c phase and the fraction of the R3c phase gradually increases. Correspondingly, the mode amplitudes of the H2 and Λ3 drop abruptly. The hidden structural correlation between H2 and Λ3 modes facilitates the understanding of the antiferroelectric nature observed in the AgNbO3. The variation of the main modes rationally bridges the Pmc21 and R3c phases, revealing the underlying phase transition mechanism of these two phases. Additionally, the evolution of the R3c phase fraction and corresponded mode amplitude in both Pmc21 and R3c phases provides a clear picture to explain the additional peak observed in the temperature-dependent dielectric spectra and composition-dependent polarisation-electric field hysteresis loops.

Structural studies of the monoclinic fergusonite to tetragonal scheelite structure in lanthanoid orthoniobates

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Lanthanoid orthoniobates (LnNbO$_4$) are of considerable interest for their potential use in applications ranging from phosphors in solid-state lightening to ion conductors in solid-oxide fuel cells and humidity sensors. The LnNbO$_4$ orthoniobates generally adopt the monoclinic (space group $I2/a$) fergusonite type structure, which is often described as a distortion of the tetragonal ($I4_1/a$) scheelite structure. Both structures are built on unlinked NbO$_4$ tetrahedra and LnO$_8$ dodecahedra (reflecting the 12 triangular faces). In the tetragonal structure the NbO$_4$ tetrahedra are undistorted with all four Nb-O distances being equal whereas in the monoclinic structure the NbO$_4$ tetrahedra have two pairs of Nb-O distances. Tetrahedral coordinated Nb^V cations are relatively rare since the Nb^V cation is generally considered to be too large for this. Typically Nb^V cations are octahedrally coordinated in oxides.

In the present work we have prepared 13 members of the series LnNbO$_4$ using solid-state methods and characterised their structures using the powder diffractometer at the Australian Synchrotron. For selected examples the structures have been studied as a function of temperature. The data was of sufficient quality that, in addition to providing very precise values of the unit cell parameters, Rietveld refinements provided precise coordinates for the anion positions, and hence *Ln*-O and Nb-O bond distances. Bond valence sums calculated for the Nb cation coordinated to four oxygen anions result in unrealistically small effective bond valences (range 4.1–4.6) for pentavalent niobium. Examination of the structure reveals two longer Nb-O contacts near 2.5 Å, and if these are included in the BVS calculations the values are much more reasonable (4.6–4.9).

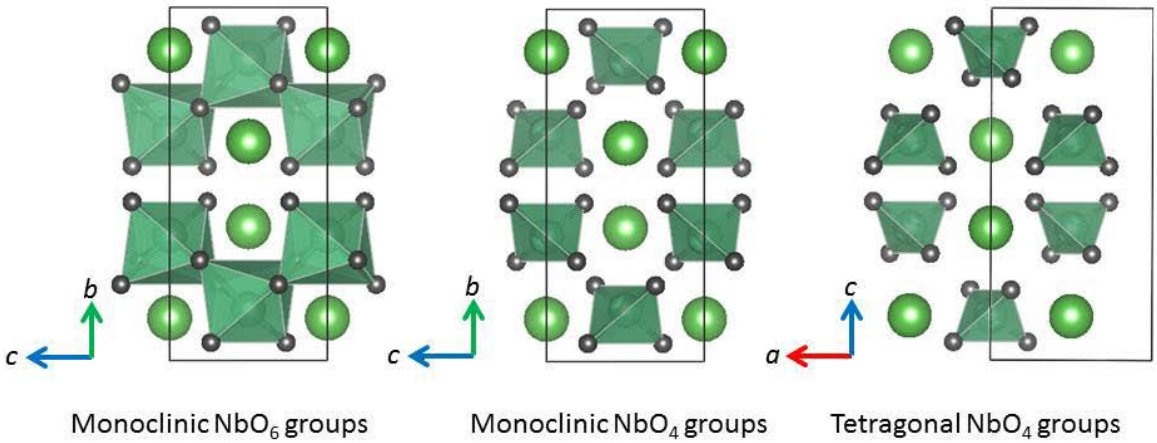

Figure 1. Representation of the NbO$_n$ polyhedra in the monoclinic and tetragonal LnNbO$_4$ structures. Both octahedral and tetrahedral polyhedral are illustrated for the monoclinic structure.

This presentation will discuss the systematics of the structures and illustrate that the fergusonite scheelite transition in the lanthanoid orthoniobates involves a progressive weakening and ultimately breaking of the long Nb-O bond.

Tailoring elastic frustration in spin crossover networks

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Spin crossover (SCO) materials can be converted between two local spin states (high and low spin) with differing magnetic, optical, electrical and structural properties [1]. Iron(II) complexes show a particularly assorted set of SCO behaviours, encompassing gradual, abrupt, hysteretic, and multi-step spin transitions. Materials that display stepwise transitions are particularly sought after as they lead to high-order data-storage possibilities such as ternary and quaternary processing but are comparatively rare in existence.

We have generated a structural platform of 2-D framework materials that intrinsically support the structural distortions requisite of multi-stepped SCO. Through tailoring host-host and host-guest interactions in these materials we have produced a diverse range of multi-stepped (two- [2], three- [3] and four-stepped [4]) and guest-modulated [5] spin transitions (Figure 1). Detailed structure–function studies on these materials have provided important information on the relative importance of weak and strong interactions and structural distortions on lattice cooperativity (i.e., elastic versus frustrated elastic states).

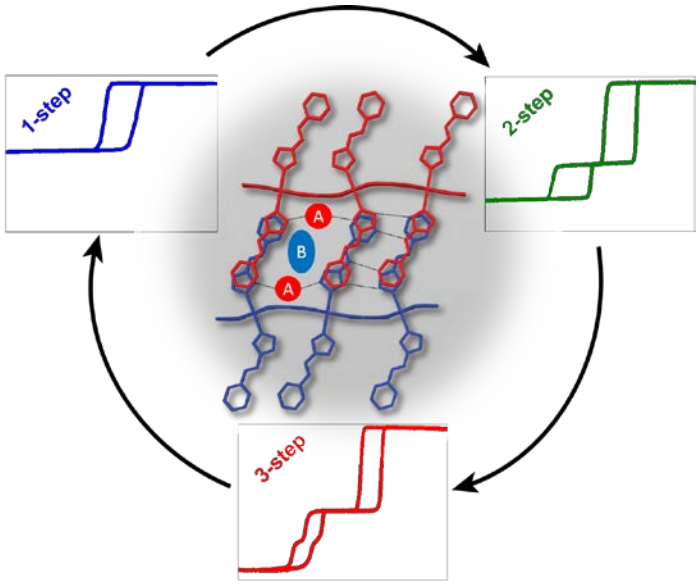

Figure 1. Guest-modulated one-, two-, three-step SCO.

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New electrode materials for lithium- and sodium-ion batteries

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As a result of increased energy demand, energy storage has become a growing global concern over the past decade. Electrochemical energy storage (EES) technologies based on batteries are beginning to show considerable promise as a result of many breakthroughs in the last few years due to their appealing features including high round-trip efficiency, flexible power, energy characteristics to meet different grid functions, long cycle life, and low maintenance [1, 2]. My project focuses on the discovery, characterisation and optimisation of electrode and solid electrolyte materials in both lithium-ion batteries and sodium-ion batteries, in which the investigation of nuclear materials and magnetic structures and the dynamics of Li/Na ion are key issues.

In this presentation three techniques below that have been heavily utilised to theoretically and experimentally characterise new electrode materials will be systematically discussed.

1. Ab initio calculation—It is employed to identify and compare the energies of framework structures with hosting Li/Na from materials data mining, which give an improved understanding of how the experimentally determined structures arise and how they will evolve with mobile ion concentration under electrochemical cycling. Knowledge of the ground-state magnetic structure also permits the accurate calculation of redox potentials, in conjunction with electrochemical measurements.

2. Neutron scattering—It concerns new crystalline materials for light metal-ion batteries in several ways. Neutron diffraction reveals the location and occupancies of Li/Na sites in the crystal lattice and, hence, conduction pathways. In situ experiments explicitly reveal Li/Na ion mobility, as well as phase changes under operating conditions that undermine long-term stability. Inelastic and quasielastic neutron scattering probe the dynamics of the mobile ions and the supporting lattice. Besides, low-temperature neutron diffraction reveals the spin-ordered ground states of the transition metal counter-cations, which are not only fundamentally fascinating due to their complex super-super-exchange pathways, but also characteristic of their electrochemical states in batteries.

3. In-situ TEM characterisation—It is performed to study how materials degrade on a larger scale over repeated cycling: nanocrystallisation, and changes in the roughness of the interfaces. The information of the materials failure collected by virtue of this technique will help to effectively design accurate ways to optimise the materials.

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Structural trends and single electron magnetism in Ru/Os scheelite type oxides

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Several novel scheelite type oxides containing Ru and Os have been synthesised and their structures and magnetic properties determined. The oxides take the general form AMO_4 where A represents a 1+ cation (K, Rb, Cs) and M = Ru, Os. This results in a 7+ charge of the metal cation resulting in an S = ½ magnetic moment. Room temperature crystal structures of these materials have been determined via Rietveld refinement carried out against neutron and synchrotron X-ray powder diffraction. Most compounds were found to crystallise in the archetypal tetragonal scheelite $I4_1/a$ structure with the exception of $RbRuO_4$ and the two Cs compounds which are orthorhombic in *Pnma*, consistent with previous reports [1, 2]. This distortion is likely a result of the larger ionic radius of the Rb and Cs cations [3]. Variable temperature X-ray diffraction data were collected at the Australian Synchrotron between 90 K and 750 K. 1st order phase transitions from *Pnma* to $I4_1/a$ scheelite structures were observed for $RbRuO_4$ (450 K) and $CsOsO_4$ (420 K). A *Pnma* to $I4_1/amd$ (zircon structure) 1st order transition for $CsRuO_4$ (480 K) was observed. $KOsO_4$ and $RbOsO_4$ were found to remain in $I4_1/a$ for all measured temperatures. Variable temperature magnetic susceptibility data indicates antiferromagnetic ordering at low temperatures (60 K – 10 K) for all structures, with the exception of $CsOsO_4$ which shows no magnetic order to 2 K. Effective magnetic moments determined were consistent with a spin-only S=1/2 model ($\mu_{SO} = 1.76\mu_B$) for the Ru compounds, ($\mu_{eff} = 1.4\mu_B – 1.7\mu_B$), however, the Os compounds showed appreciably lower magnetic moments ($\mu_{eff} = 1.2\mu_B – 1.4\mu_B$), likely as a result of a stronger spin orbit coupling [4]. Heat capacity data collected for magnetic compounds does show a feature consistent with long-range magnetic order occurring slightly below the compounds' Neel temperatures. Unfortunately, neutron powder diffraction data collected below the magnetic ordering temperature does not show addition reflections or appreciable increases in intensity on Bragg reflections arising from long-range magnetic order. The recent report by C.A. Marjerrison et al. [2] does indicate long-range AFM order in $KRuO_4$ below the Neel temperature, however, it is noted in this report that the largest magnetic intensity represents approximately 0.25% of the total intensity. This study proposes that long-range AFM order is present in these compounds, however, owing to the very small magnetic moment observed for these compounds, superior instrumentation is required to observe magnetic scattering in neutron powder diffraction data.

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SESSION 13B: METHODS

Chair: Mitchell Guss

A structural analysis of an entire enzymatic pathway

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First synthesised in the 1800s, the triazines are a family of anthropogenic compounds that share the same core structure, an aromatic ring of three carbon atoms and three nitrogen atoms arranged alternately to provide three-fold rotational symmetry. This basic core is used in agriculture as non-selective herbicides (e.g., atrazine), as monomers in plastics and polymers (e.g., melamine), in explosives (e.g., RDX) and as carriers of disinfectants (e.g., cyanuric acid). Although most triazines are synthetic and their introduction to the environment is relatively recent, related compounds, such as pyrimidines (e.g., uracil and thymine), are abundant and have been so since the early stages of life on Earth, forming the basis of our genetic material. The introduction of relatively high concentrations of these herbicides into the environment has also introduced new selection pressure for exposed organisms. Those microorganisms exposed have taken advantage of an abundant nutrient source and have developed catabolic pathways to do so. I will discuss our endeavour to fully characterise one of these catabolic pathways both structurally and functionally.

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Establishing micro electron diffraction as new tool for structural biology

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X-ray crystallography has been the dominant method for protein structure determination since the first structure of myoglobin was solved in the 1950s. The requirement for large, well-formed, micron-sized crystals can be a limiting factor in obtaining these structures. Consequently, projects that fail to meet these requirements often rely on X-ray Free Electron Lasers (XFELs), a method that require copious amounts of small crystals which is something that cannot be readily achieved for many proteins. In addition, access to free-electron lasers is prohibitive with only a handful currently operating. Given these challenges, an alternative method is required to determine high-resolution protein structures from small crystals.

Recent advances in cryo electron microscopy have allowed for the development of a technique called micro electron diffraction (MED) where an electron microscope is used to collect electron diffraction patterns from cryogenically frozen sub-micron (< 500 nm) sized crystals. This technique is becoming increasingly popular with several structures solved including the core peptide of Tau filaments to a resolution of 1.1Å, the first novel structure solved by MED. Additionally, numerous crystallisation conditions that are typically overlooked for use by conventional methods have been shown to contain nano-crystals when analysed by electron microscopy, presenting new opportunities for MED.

In collaboration with Thermo Fisher (formerly FEI), we are working to establish MED as a viable alternative to both X-ray crystallography and XFELs for protein structure determination here at Monash University. Preliminary data collection techniques have been developed, along with pre-processing software to help streamline indexing, merging, and analysis of electron diffraction data. More recently, we have successfully collected test datasets of lysozyme that have yielded structures with a resolution of 2.8Å. Together, we have demonstrated the feasibility of this technique and now look toward applying it to novel protein samples.

Microseed matrix-screening (rMMS): Introduction, theory, practice and a new technique for membrane protein crystallisation in LCP

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Random Microseed Matrix-Screening (rMMS), where seed crystals are added automatically to random crystallisation screens, is a significant recent breakthrough in protein crystallisation [1]. During the eight years since the method was published, theoretical understanding of the method has increased [2–4], and several important practical variations of the basic method have emerged [5, 6]. We will briefly describe some of these variations, including cross-seeding, and introduce a novel method of making LCP seed stocks by scaling up LCP crystallisation conditions.

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Structural studies of MLKL's interaction with the plasma membrane using liposomes as a model system

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Necroptosis is a form of programmed cell death characterised by lack of caspase activity and a loss of plasma membrane integrity. Morphologically similar to necrosis, in the act of necroptosis, the plasma membrane is disrupted, causing release of cellular components to the extracellular fluid and an ensuing inflammatory response. Necroptosis proceeds via a regulated kinase cascade involving Receptor Interacting Protein Kinases RIPK1 and RIPK3. Mixed Lineage Kinase domain-Like protein (MLKL), a pseudokinase, is the final known obligate effector essential for the execution of necroptosis. Whilst the MLKL pseudokinase domain is incapable of catalysing phosphotransfer reactions, it is the site of RIPK3 phosphorylation. This phosphorylation event is thought to be integral to flipping a molecular switch regulated by the pseudokinase domain, resulting in activation of MLKL. Upon activation, MLKL oligomerises and translocates to the plasma membrane, and is there thought to play a destabilising role. Details of MLKL's molecular mechanism of action, such as the stoichiometry of oligomerisation and how it interacts with the plasma membrane, remain unknown.

We have used a variety of techniques to examine the structural details of MLKL's interactions with membranes. Direct visualisation of recombinant MLKL's interaction with small uni-lamellar vesicles using cryo-electron microscopy has revealed that MLKL stably binds to the membrane and forms small pores. Further experiments employing cryo-electron tomography will illuminate any structures that MLKL forms on the membrane. In a complementary manner, we have studied MLKL's interaction with giant uni-lamellar vesicles using confocal microscopy, yielding kinetic and binding data that in concert with cryo-EM results suggests a model for MLKL oligomerisation and pore formation in the plasma membrane.

Isoniazid-oleanolic acid co-crystal system: Synthesis, anti-TB and toxicological effect on the Human Embryonic Kidney (HEK293) and Human Hepatocellular Carcinoma (HepG2) cell lines

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Tuberculosis (TB) is highly endemic disease worldwide. One of the commonly used first-line drugs for anti-TB therapy (ATT) is isoniazid (INH). Isoniazid is known to be majorly metabolised and detoxified in liver by both phase I and phase II group of drug metabolising enzymes. The drug along with its metabolite are toxic and during its assimilation process cause injury to liver. The work presented here involves investigation of co-crystals involving isoniazid and oleanolic acid. A 1:1 co-crystal involving isoniazid, a foremost first-line drug recommended by the World Health Organization for the treatment of tuberculosis, which causes damage to the liver and oleanolic acid, a hepatotoxicity naturally occurring compound, have been synthesised for the first time. Considering drug combination perspective, this is an interesting pharmaceutical co-crystal because of the known side effect of isoniazid therapy, which might be improved upon by the presence of the oleanolic acid. The co-crystal compound was characterised using PXRD, TGA and SEM, which were further evaluated for *in vitro* anti-TB and cytotoxicity index, using Human Embryonic Kidney (HEK 293) and Human Hepatocellular carcinoma (HepG2) cells. The PXRD of the synthesised co-crystal compound maintained crystalline nature like isoniazid for the three methods, TGA for all the three methods have cleavage values from 220^0 C–360^O C, and the SEM images obtained from the three synthetic methods appear rod-like in nature. The co-crystal of OA with INH, increased the anti-TB MIC values for the three synthetic methods used as follows: (a) Solvent evaporation(1.06µM), (b) Solvent drop (0.50µM), and (c) Direct grinding (0.61 µM). The cytotoxicity test of the co-crystal system on the two human cell lines (HEK 293 and HepG2) were found to be $IC_{50} \geq 300$ug/ml. The finding of the current work is that co-crystal compound of OA with INH is probable and could be utilised to design better treatment for tuberculosis disease, which can further be extended to the treatment of HIV/TB co-infection.

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SESSION 14: KEYNOTE SPEAKER 3

Chair: Helen Maynard-Casely

High-pressure crystallography: Not just for mineralogists anymore!

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Crystallography's favourite thermodynamic variable, temperature, is often manipulated: sometimes as a means to a higher quality data set [1], and other times as a probe for material properties [2]. Pressure is a less likely variable to manipulate in a crystallographic experiment, and for many years, was mostly appreciated in the mineral physics community [3]. Many improvements in instrumentation have made moderate- to high-pressure experiments more accessible and applicable to chemistry, biology and materials science. Within this presentation, I plan to share the evolution of high-pressure crystallography to its modern state and to give the audience a feel for what is entailed in a high-pressure experiment, as well as describing the rewarding information that these experiments can yield.

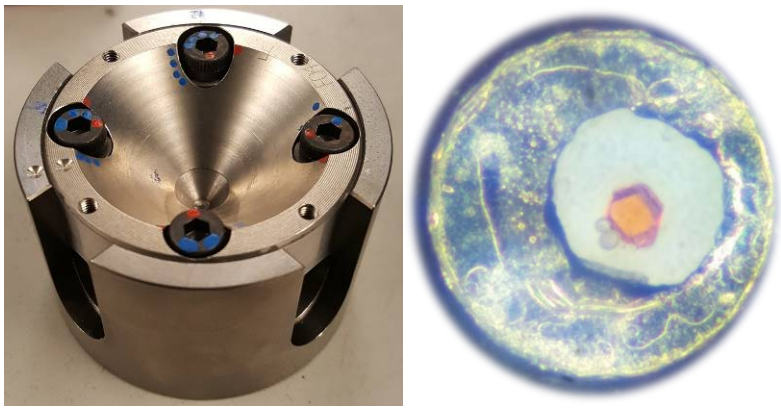

Figure 1. (left) a BX90 diamond anvil cell (DAC), (right) a DAC sample chamber at pressure, containing an orange single crystal and two ruby spheres.

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SESSION 15: KEYNOTE SPEAKER 4 — THE EMBO LECTURE

Chair: Jade Forwood

Crystallographic insights into the molecular mechanism of nucleocytoplasmic transport

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A double membrane nuclear envelope (NE) separates Eukaryotic cell nuclei from the cytoplasm and generates the separation of transcription from translation that enables key modifications (including splicing, capping and polyadenylation) to be made to mRNA in the nucleus before it is exported to the cytoplasm for translation. Moreover, many of the processes that occur in the nucleus require the presence of proteins that are synthesised in the cytoplasm and which must then be imported across the nuclear envelope. Passage of proteins and RNAs across the NE is an active, energy-requiring process that is facilitated by nuclear pores (NPCs), huge macromolecular assemblies that perforate the NE [1]. NPCs have 8-fold rotational symmetry, are ~1500Å in diameter and ~700 Å thick, are constructed from multiple copies of ~30 different proteins called collectively nucleoporins or "Nups", and have a ~300 Å central transport channel in which natively unfolded regions of Nups that contain characteristic FG (Phe-Gly) repeats generate a densely-packed permeability barrier. A range of transport factors or carriers bind macromolecular cargoes in one compartment and then, through interactions with the FG-nups, facilitate movement through the pores, after which the cargo is released into the other compartment. Movement through NPCs is passive and mediated by diffusion, with energy used instead to mediate cargo:carrier complex assembly/disassembly, somewhat like Maxwell's demon. Proteins and some RNAs are transported by members of the karyopherin-β family, such as importin-β, and interactions the cargo are orchestrated by the Ran GTPase that is GTP-bound in the nucleus but GDP-bound in the cytoplasm, by virtue of its guanine nucleotide exchange factor being located in the nucleus, whereas its GTPase activating protein is cytoplasmic [1]. mRNA is instead transported by NXF1:NXT1, to which binding and dissociation are orchestrated by helicases.

Crystal structures of the molecules and complexes involved in nucleocytoplasmic transport have given considerable insight into the mechanism of the process and enable mutant proteins to be engineered to test functional hypotheses. β-karyophenins are solenoid proteins built from a series of HEAT repeats that results in there being flexible. However, binding RanGTP locks them into a more rigid conformation and alters their affinity for cargoes, generating low affinity for import cargoes, but high affinity for export cargoes [2]. Consequently, import cargo:carrier complexes are dissociated in the nucleus, whereas export complexes are assembled. Conversely, in the cytoplasm, hydrolysis of Ran GTP results in dissociation of export complexes and assembly of import complexes. NPCs can also function as interaction platforms to orchestrate gene expression and the assembly/disassembly of cargo:carrier complexes. In yeast, Nup1, for example, facilitates RanGTP binding to importin-β, the TREX2 complex that also binds to Nup1 facilitates assembly of export-competent mRNPs together with localising several key genes, including *GAL1*, to the nuclear envelope when they are activated [3], and Nab2 that binds to Mlp1 orchestrates polyA tail length [4].

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Quantifying intermolecular interaction in crystals using Roby-Gould bond indices

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Identifying the nature of intermolecular interactions in crystals and quantifying their relative strengths is significant in the context of crystal engineering. The question whether these interactions are formed as a result of molecule⋯⋯molecule close packing or localised atom⋯⋯atom interactions is a matter of debate. In this study, we evaluate the covalent, ionic and total Roby-Gould bond index for 'σ-hole' interactions (halogen bonding and chalcogen bonding) in comparison with well-known classical hydrogen bonds. The Roby-Gould bond indices have been analysed for a dataset of 97 crystal systems comprising 42 hydrogen bonds, 31 halogen bonding and 33 chalcogen bonding. Our method provides insights into the nature of these interactions by separately estimating the bond indices for molecule⋯⋯molecule and atom⋯⋯atom. Hirshfeld charge for these interactions has been also reported with a trend of charge transfer from bond acceptor to donor. A conservation law of the bond order involving the interacting atoms has been found with our Roby-Gould bond index.

Investigating the role of conformational change in gating and conduction of K_{IR} K^+ channels

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Control over potassium (K^+) levels is essential to tissue homeostasis and physiological processes such as electrolyte balance, cell signalling, and electrical activity. Potassium channels are highly selective tetrameric pores that allow K^+ diffusion across cell membranes. Like other ion channels, they open and close in a controlled manner, a process termed 'gating'. It is thought that K^+ channels adopt alternative conformational states, with 'open' channels having a sufficiently wide opening to accommodate a fully hydrated K^+ ion (~8 Å), while 'closed' channels are too constricted to allow them through.

My work investigates the role of conformational change in gating and conduction of K_{IR} K^+ channels. The underlying hypothesis of this study is that if conformational change is essential for gating, preventing it will result in non-conducting channels. Conversely, if change is not an essential factor in gating, preventing it will not impact on function.

Kir channels have a narrow pore in all crystal structures reported to date. It is widely accepted that this conformation represents a 'closed' state of the pore. Our approach was to chemically lock the conformation in place to constrain relative movement of the inner helices so that pore widening is not possible and to evaluate the function of locked, relative to non-locked, channels. A caveat was that the four subunits be linked together around the periphery of the pore so the ion conduction path is not directly blocked by crosslinkers.

The project involved expression, purification, point mutagenesis and crosslinking of K^+ channels. Evaluation of the crosslinks and conformations was by crystal structure and native mass spectrometry. The ability of all channels to conduct ions was assessed by fluorescent liposome flux assays, and later by bilayer recordings.

Crystal engineering of alkylamine-based coordination polymers for carbon dioxide capture

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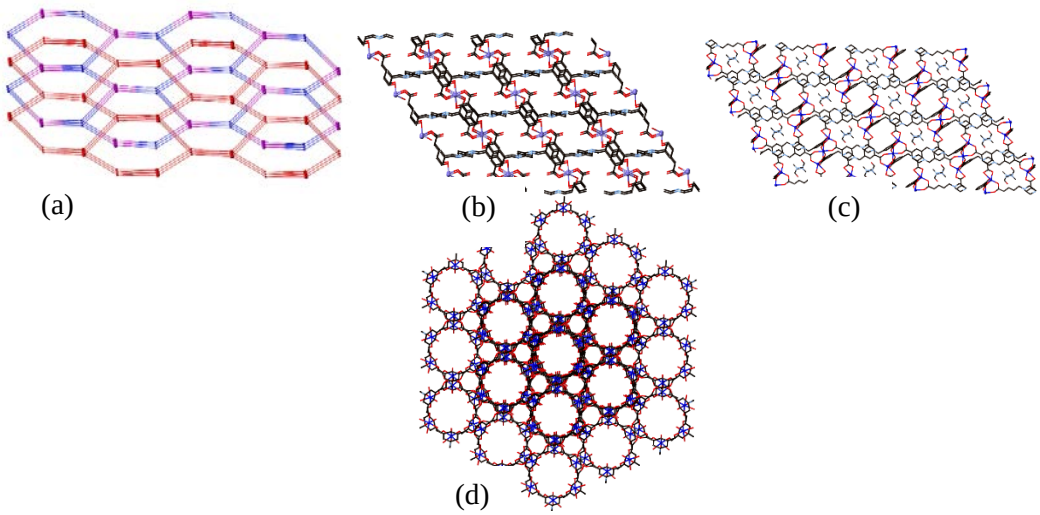
Our research group focuses on the synthesis of porous coordination polymers using flexible linear alkylamine ligands. Emerson et al. recently reported three new coordination polymers: poly-[Cu_2(L1)$(OH_2)_2$]·6DMF·3H_2O 1, poly-[Zn(H_2L1)$(OH_2)_2$]·DMF·4H_2O and poly-[Cd(H_2L1)$(OH_2)_2$]·DMF·3H_2O where H_4L1 is N,N,N',N'-tetra(4-carboxybenzyl)-1,3-diaminopropane dihydrochloride trihydrate. 1 exhibited 23 cm^3/ g of CO_2 at 273 K and atmospheric pressure [1]. In addition, similar frameworks previously published have also exhibited selective CO_2 adsorption [2–5]. We report here the synthesis of four potentially porous metal-organic frameworks using two new different ligands: L6 and L7. The first MOF synthesised using the L7 ligand and cadmium chloride or nitrate has the formula poly-[Cd(L7)]·9H_2O (Figure a). The three other porous MOFs were synthesised using the H_4L_6 ligand that coordinated with three different metals (Cd, Cu, Zn) to form three different MOFs with the following formulas: poly-[Cd(L6)]·2H_2O (Figure b), [Zn(L6)]·1DMF·4H_2O (Figure c), [Cu2(L6)(H_2O)2]·5H_2O·4DMF (Figure d), where H_4L6 is N,N'-bis(3,5-dicarboxyphenylmethyelene) piperazine and H4L7 is N,N'-bis(3,5-dicarboxyphenylmethyelene) homopiperazine. The exploration of the CO_2 adsorption of these new materials is ongoing.



Figures a, b, c and d.

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Crystal structure of a novel membrane protein essential for cell wall lipoglycan synthesis in *Mycobacteria*

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The bacterial suborder *Corynebacterineae* includes important pathogens, most notably *Mycobacterium tuberculosis*, which infects one-third of the entire human population, resulting in around 1.4 million deaths each year [1]. These bacteria synthesise a unique, complex, multi-layered cell wall, which confers intrinsic resistance to host antibacterial factors, antibiotics and adverse environmental conditions [2]. A diverse range of non-covalently linked lipoglycans and waxes form the cell wall layer and mediate interactions between pathogen and host. One class of abundant glycolipids synthesised by all *Corynebacterineae* is the phosphatidyl-*myo*-inositol mannosides (PIM). The PIMs also serve as membrane anchors for hyperglycosylated species, the lipomannans (LM) and lipoarabinomannans (LAM). These complex surface lipoglycans are essential for both the viability and *in vivo* survival of pathogenic mycobacterial species, due to their capacity to modulate host immune responses during infection.

While many enzymatic steps of the PIM-LM-LAM biosynthetic pathway are now known, the mechanisms by which the pathway is regulated and how intermediates are transported through the cell wall layers remain poorly understood. Here we describe a new membrane protein, highly conserved in all *Corynebacterineae*, which is required for synthesis of full-length LM and LAM. Deletion of the *NCgl2760* gene in *Corynebacterium glutamicum*, a useful model organism for the study of *Corynebacterineae* cell wall synthesis, resulted in a complete loss of mature LM/LAM and the appearance of a novel truncated LM (t-LM). Lipid structural studies indicated that the Δ*NCgl2760* t-LM comprised a series of short LM species containing a truncated α1-6 linked mannose backbone with greatly reduced α1-2 mannose side chains, similar to the LM species produced by a *C. glutamicum* mutant lacking the MptA mannosyltransferase that extends the α1-6 mannan backbone of LM intermediates. Attempts to delete the NCgl2760 orthologue in *Mycobacterium smegmatis* (MSMEG_0317) have been unsuccessful and our analysis of a *MSMEG_0317* knockdown strain confirmed that the gene is essential for bacterial growth, as is the *M. tuberculosis* orthologue, Rv0227c.

This protein lacks motifs or homology to other proteins of known function. It contains a C-terminal transmembrane domain and has been shown to be surface-located in *M. tuberculosis* [3]. To gain insights into function, we have solved the crystal structure of MSMEG_0317 to 2 Å resolution. The structure revealed a novel fold consisting of 12 antiparallel β-sheets arranged in a V-shape and capped by a single α-helix. The most striking feature is the presence of a narrow hydrophobic channel that could accommodate a single fatty acyl chain. Collectively, our data suggest that MSMEG_0317/NCgl2760 has a novel lipid binding function essential for the elongation of the mannan backbone of LM. These findings further highlight the complexity of lipoglycan synthesis pathways of the *Corynebacterineae*.

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Exploring the programmability of synthetic PPR proteins to target specific RNA sequences

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PentatricoPeptide Repeat (PPR) proteins are prime candidates for biotechnology applications due to their structure and roles. Appearing all eukaryotes, PPRs are most prevalent in land plants, specifically angiosperms, forming superfamilies of more than 450 proteins. PPR proteins are encoded in the nuclear genome and mainly localise to the mitochondria and plastids. PPRs are implicated in a wide range of post-transcriptional organelle processes, such as C-U RNA editing, splicing, stability and initiation of translation.

PPR proteins bind RNA transcripts in a sequence specific modular fashion with great potential for genetic engineering. PPR proteins are modular tandem repeat proteins, each repeat motif consisting of ~35 amino acids forming two anti-parallel α-helices. Multiple repeats (2–30) yield a super helical solenoid. They bind single stranded target RNA using a clear one nucleotide, one motif association known as the PPR code, defined by amino acids at certain positions in the repeat. Their ability to recognize primary RNA sequences and their modular structure has prompted research into their viability as engineered RNA editing tools. Key to designing these fully synthetic designer PPR proteins is understanding exactly how the PPR code specifies a certain base and any affinity a certain repeat may have for other bases that could affect targeted binding.

Previous work in designer PPRs have focused on positions 5 and 35 for determining specificity in the PPR code. Soluble designer PPRs were successfully made, however, their ability to target specific RNA sequences needs improvement. Recent work in designer PPRs [2] has shed more light on RNA binding and opens the door to exploring the roles of other PPR residues in guiding RNA binding specificity, specifically a third amino acid at position 2 in the repeat. Agene library of 25 mutants for a 10-mer PPR has been designed and synthesised with the preliminary aim to explore the effect of position 2 on specificity of binding to RNA target molecules.

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Rational targeting of the signal recognition particle receptor

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The signal recognition particle (SRP) is an essential ribonucleoprotein complex responsible for the co-translational delivery of membrane and secretory proteins to the plasma membrane in bacteria and to the endoplasmic reticulum in eukaryotes [1]. In *Eubacteria*, the SRP complex consists of one protein (SRP, Ffh), one receptor (SRP, FtsY) and a 4.5S RNA. The SRP cycle involves recognition of the signal peptide (SP) on the translating ribosome (RNC) by the SRP followed by complex formation with SRP Receptor (SR) upon GTP binding and finally the delivery of the RNC to the translocon and GTP hydrolysis. The process is controlled by a series of coordinated conformational changes in each step that leads to the GTPase activity by SRP and SR [2].

Truncations or mutations on any of the bacterial components of the SRP system have proven to be either lethal or to severely impact cell viability, indicating that disruption of this system could be a suitable target for drug discovery [3]. An interesting point identified in previous crystal structures of the bacterial SRP complex with SR (FtsY) indicated new and specific interactions of SR with SRP RNA that are essential for early complex formation [4, 5].

In order to investigate how cofactors such as GTP modulate FtsY structure and its interactions with RNA, we have analysed the binding of FtsY to GTP analogues, such as GMPPNP and GCPPNP as well as to GDP and 4.5S RNA using Nuclear Magnetic Resonance (NMR) spectroscopy and crystallography. We have crystallised a longer construct of *E. coli* FtsY than the ones published before (termed NG+1 which comprises the NG domain with an extra Phe at the N-terminus that stabilises the first helix in the structure) in an apo form that diffracts to much higher resolution at 1.38 Å. We have tested soaking condition for GTP analogues and GDP and have solved structures of these bound to NG+1 at 1.6 Å. We now have a robust methodology that yields crystals that diffract to high resolution enabling us to study in more detail the SRP-SR complex formation.

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Ebony C-terminal domain is an arylalkylamine *n*-acetyltransferase

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In *Drosophila*, the regulation of the neurotransmitter histamine is ensured by a mechanism in which it is inactivated by Ebony through the formation of carcinine (β-alanyl-histamine) and regenerated by Tan through hydrolysis of carcinine [1] (Figure 1). Ebony is an unusual type of Non-Ribosomal Peptide Synthetase (NRPS) as it has been reported to function much faster than typical NRPS machineries [2] and does not contain all catalytic domains typically observed in a functional assembly line. Indeed, with a single A-domain involved in amino acid activation and a peptidyl carrier protein domain (PCP), Ebony lacks a condensation (C) domain that is usually required to catalyse peptide bond formation between β-alanine and histamine. In place of this missing C-domain, the primary sequence of Ebony shows an uncharacterised C-terminal domain of roughly 27kDa.

In order to gain insight into this uncharacterised domain and thus understand the mechanism of carcinine formation by Ebony, we cloned, purified and determined the crystal structure of the C-terminus domain of Ebony. Crystals belonged to the $P2_1$ space group and diffracted to 2.8Å. The structure was solved by molecular replacement using a model generated by Robetta server [3] from an arylalkylamine N-acetyltransferase (AANAT) [4]. Despite a low sequence identity (less than 18%) Ebony C-terminal domain shares a well-conserved fold with other AANATs.

This finding unravels a new type of NRPS architecture and unveils how, without a condensation domain, insects evolved a fast and efficient way of making peptide bonds using an NRPS machinery.

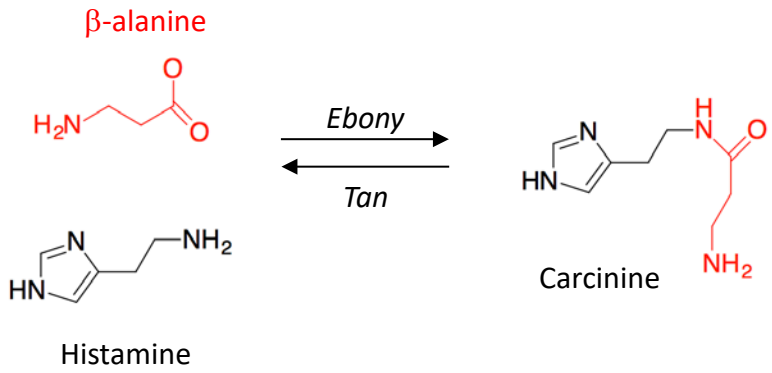

Figure 1. The regulation of the neurotransmitter histamine in *Drosophila*.

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Designer pentatricopeptide repeat proteins, a molecular spring in action

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Designer nucleic-acid-binding proteins show potential in their ability to be reprogrammed in both binding target and overall protein function. The family of pentatricopeptide repeat (PPR) proteins is found widely in land plants with over 400 members, but only seven are currently known in human cells [1, 2]. PPR proteins are characterised by degenerate 35 amino acid repeats that form repetitive helix-turn-helix motifs that are involved in binding individual RNA bases. Amino acids at specific residues are known to confer binding specificity of each repeat for target RNA bases [3]. These proteins are found to be involved in inhibition of nuclease activity, altering the secondary structure of mRNA, and participation in RNA editing and maturation [2].

Designer PPR proteins have already successfully been targeted to novel RNA sequences (*in vitro*), thereby highlighting their potential use as biotechnological tools. Following our previously published structure of a designer PPR protein [3], we have now solved the structure of a complex with RNA. Comparison of the structures of these designer proteins in their bound and unbound states reveals insight into the RNA-binding mechanisms of the PPR family of proteins. Interestingly, while the width and cylindrical gap inside remain almost unchanged between bound and unbound forms, a change of the pitch between alpha-helices of each repeat results in a significant change to the overall conformation, analogous to the compression of a spring. With this additional structural and mechanistic information, we draw closer to fully understanding the PPR binding code and how to effectively target them to new RNA sequences and for new purposes.

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Extending CINDER to let users score for themselves

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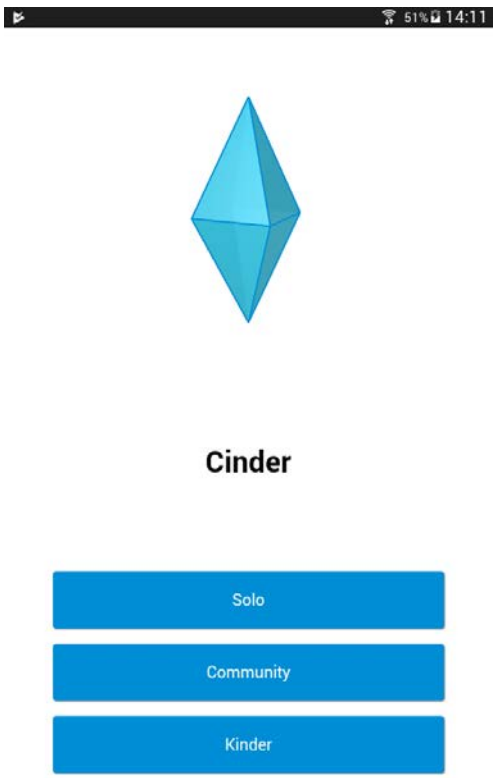
There is a wealth of information in all crystallisation experiments, not just those that produce crystals. In order to make use of these data, there needs to be available both well-curated details of the input parameters of each experiment and a description of the outcome of the experiment. With the advent of robotic imagers, collating information about the experimental setup is relatively straightforward, however, consistent and complete annotation of the outcomes has proven elusive.

In C3, we have been working towards using machine learning approaches to classify crystallisation images. Most recently, we have been using convolutional networks (‘deep learning’) approaches, and have implemented one of these into the C3 workflow. The DeepCrystal AI returns around 80% accuracy for the ‘crystal’ and ‘clear’ classes.

Improving the DeepCrystal AI currently running in C3 requires additional well-scored images to be available to extend the training set. We have modified our CINDER application to allow users to score their own images, and will use these user scores to find images most appropriate for the online retraining of the autoscoring algorithm.

CINDER now has three sections — ‘Cinder Solo’, where users are presented with their latest images collected in C3; ‘Cinder Community’, where citizen scientists can contribute scoring of anonymous images; and ‘Cinder Kinder’, which is a teaching tool for instructing novice users on what to look for when scoring crystallisation images.



Targeting TIR domain assemblies in TLR signalling pathways to design anti-inflammatory drugs

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Toll-like receptors (TLRs) are central components of host innate immunity. Among the ten human TLRs, TLR2 and TLR4 are activated by host-derived ligands, even in the absence of microbial challenge. Upon activation, their cytosolic TIR (Toll/interleukin-1 receptor) domains recruit the TIR-domain containing adaptor proteins MyD88 (myeloid differentiation primary response gene 88) and MAL (MyD88 adaptor-like protein) via TIR:TIR interactions, which trigger downstream activation of the transcription factor NF-κB, to induce anti-pathogen responses. MAL works as a bridging adaptor between MyD88 and the TLR2/4 receptors.

Excessive or prolonged activation of this signalosome may lead to chronic inflammatory diseases, so a broad-spectrum anti-inflammatory drug can be designed by targeting TLR2/4-signalosome. TLR-antagonist design against the extracellular domains of the receptors has been explored, but with limited outcome due to lack of specificity. Targeting downstream adaptor protein-protein interactions can provide a better specificity.

We have recently shown that the MAL TIR domain spontaneously and reversibly forms filaments *in vitro* and cryo-EM; mutational and cell-based studies have identified a conserved open-ended mode of TIR domain interaction that is important for the formation of a functional TLR2/4 signalosomes and NF-κB activation. In order to identify small molecules that can inhibit the formation of these TIR domain assemblies, we have adopted a fragment-based drug design (FBDD) approach and screened a library of 400 fluorinated fragments for binding to MAL using ^{19}F NMR spectroscopy. Seventeen hits were identified from the ^{19}F NMR screen, which has been validated by ^{15}N-heteronuclear single quantum coherence (HSQC) and surface plasmon resonance (SPR). Further biochemical and structural studies can help to establish the shortlisted fragments as potential anti-inflammatory drugs.

Structural determination of phosphatidylinositol-synthesising engineered phospholipase D from *Streptomyces antibioticus*

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Phosphatidylinositol (PI) is one of the major structural lipids that builds eukaryotic cell membranes. Derivatives of PI also play important roles in various cellular processes. A defect in PI metabolic pathways or a lack of PI will result in various diseases. On the other hand, some reports also show various therapeutical values of PI [1, 2]. Therefore, the use of PI as dietary supplements to improve health or treat specific medical conditions is gaining interest [3].

Chemical extraction of PI from natural resources produces a heterogenous mixture of PI molecular species, which is not ideal if PI is intended to be used as dietary supplement [4]. Therefore, production of specific PIs through enzymatic synthesis is preferable. *Streptomyces antibioticus* phospholipase D (SaPLD) can synthesise most phospholipids, but not PI. This is possibly due to steric hindrance in the active site towards bulky molecules such as *myo*-inositol [5].

Although engineered SaPLD enzymes that can accept *myo*-inositol and synthesise PI have been obtained by site-directed saturation mutagenesis [2, 4], the positional specificity of the enzymes was insufficient. To boost the specificity of SaPLD towards the production of 1-PI, the only natural PI isomer, an engineered variant of the enzyme was finally obtained by several rounds of mutagenesis. This mutant: 186T, 187N, 191Y, 385R can specifically produce 1-PI with isomeric purity of >97% [6].

Crystallographic structure determination of the mutant PLD with and without *myo*-inositol will explain the mechanism that underlies the accommodation of this bulky acceptor molecule and the resulting positional specificity of the phospholipid product. This work will support further strategies to optimise PLD for the production of structured and pure 1-PI suitable for various industrial purposes.

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Biophysical and structural characterisation of the *Neisserial* capsule export machinery

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Many pathogenic bacteria produce outer polysaccharide capsules, which play a major role in disease progression. The capsule provides protection against host immune responses such as opsonisation, phagocytosis and complement mediated lysis [1]. Certain capsules also act to mask the bacterium from the host immune system through molecular mimicry. Despite the importance in disease progression, very little is known about the formation of the capsules.

The polysaccharide components of the capsule are synthesised within the cell and exported to the exterior by a large cell envelope spanning protein complex. This export is coordinated by one of two systems: the Wzy dependent system and the ABC-transporter dependent system. The Wzy dependent system has been characterised to some extent while the ABC-transporter dependent system remains largely unstudied [2].

Neisseria meningitidis utilises an ABC-transporter dependent system involving four proteins: Capsule transporter A (CtrA), CtrB, CtrC and CtrD. CtrC and CtrD collectively form an ABC transporter that initiates export across the inner membrane. The translocation across the periplasm and peptidoglycan layer is then thought to be assisted by CtrB. Finally, CtrA completes the export across the cell envelope and the polysaccharides are anchored into the outer membrane. My project aims to elucidate the three-dimensional structures of CtrA, CtrC and CtrD. In addition, biophysical techniques will be used to determine the oligomeric state of each protein in a single complex, as well as the binding affinity between them.

Here I will present my progress on the recombinant expression, purification and biophysical analyses of CtrA, CtrB, CtrC and CtrD.

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Structural analysis of the type E pseudomurein peptide ligase from methanogenic archaea

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The pseudomurein in methanogenic archaeal cell walls shares an overall structure that is similar to the murein in bacterial cell walls in that it contains a glycan backbone with a peptide cross-link [1]. The cross-linking peptides have similarities except that pseudomurein contains only L-amino acids and involves isopeptide bonds (γ, Ɛ). The *in vitro* synthesis of the UDP-activated pentapeptide of pseudomurein suggests a stepwise addition of amino acids similar to that of murein [2], thereby indicating that similar enzymes are required for the role. We performed sequence and gene cluster analyses of the murein peptide ligase genes, which suggested analogous putative pseudomurein peptide ligases exist in methanogens and could share a direct evolutionary history.

We identified that pseudomurein peptide ligase type E (pMurE) is analogous to murein peptide ligase type E (MurE) and it is therefore proposed to add L-lysine to the cross-linking peptide chain of pseudomurein. We present the structures of pMurE, the first of any archaeal pseudomurein peptide ligase, and compare them to bacterial MurE. The structures of pMurE (Mfer762 and Mth734) from methanogens *Methanothermus fervidus* and *Methanothermobacter thermautotrophicus* ΔH, have been determined to a resolution of 1.8 and 2.9 Å, respectively. The structures show high similarity to one another and share a three-domain arrangement like that observed for bacterial peptide ligases [3]. We identified a UDP binding site at the exterior surface of the Mfer762 structure unique to archaea. This binding site is formed through an amino acid insertion (relative to bacterial MurE) and is well conserved in pseudomurein-containing methanogens. A different crystal form of Mfer762 revealed a second UDP-binding site present at the inner cavity at the interface of the three domains. The UDP binding at the inner cavity of Mfer762 is required to incorporate the expected large product (N^{α}-UDP-Glu^{γ}-Ala-$^{\varepsilon}$Lys) formed by the enzyme. Although ATP is not bound to any of the structures, we have identified that the residues likely involved in ATP binding for MurE are conserved in pMurE, suggesting a similar mode of ATP binding in pMurE. The findings, with regard to the Mfer762 structure, are similar for Mth734. Moreover, the Mth734 structure reveals two different conformational states with a rigid-body rotation of the C-terminal domain. Similar conformational states are adopted by the murein peptide ligases during substrate or product binding [4, 5], suggesting that a similar mechanism may exist for pMurE. The study shows structural homology of pMurE with MurE, thereby supporting the proposed role of the enzyme and suggestive of an evolutionary connection to a common ancestral gene.

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Cell envelope biosynthetic pathways as targets for novel antibacterial drug design against *Burkholderia pseudomallei* and *Neisseria meningitidis*

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The bacterial cell envelope is the first line of defence against antibacterial agents and is the target of several existing antibiotics. Antimicrobial therapy against Gram-negative organisms such as *Burkholderia pseudomallei* and *Neisseria meningitidis* is hindered by both their dual-layer cell envelope structure and by acquired resistance to previously effective antibiotics. Proteins responsible for synthesis and assembly of the cell envelope represent promising targets for development of novel antimicrobials, as they may increase membrane permeability and impair bacterial viability [1]. Transposon mutagenesis of *B. pseudomallei* K96243 has identified of a number of putatively essential proteins associated with cell envelope biosynthesis, outlined in Table 1 [2]. This project will investigate these proteins and their orthologues as potential drug targets in *B. pseudomallei* and *N. meningitidis.* Targets will be characterised through a combination of structural and molecular biology approaches. Pure recombinant protein of each target will be produced, facilitating both structural studies and inhibitor testing. In addition, unmarked deletion mutagenesis will verify target essentiality in each organism. Pure recombinant protein will be used to investigate inhibitor efficacy through binding studies and enzymatic testing. Crystallographic studies will be performed in order to better inform future structure-based drug design. Progress towards these aims will be presented.

Name	**Function**	**Cellular Location**
UppS	Synthesises the glycan lipid carrier Und-P, which is essential for peptidoglycan formation	Cytoplasm
LptD	One member of the multi protein Lpt complex which mediates transport and selective insertion of lipopolysaccharide into the external leaflet of the outer membrane	Outer membrane
LspA	Cleaves the signal peptides of prolipoproteins, an essential step towards formation of mature lipoproteins	Inner membrane
ArnC	One enzyme within the ArnBCADTEF operon encoding for L-Ara4N synthesis and its transfer to Lipid A, resulting in resistance to cationic antimicrobial peptides	Inner membrane

Table 1. Function and cellular location of targets of interest.

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Screening and crystallographic analysis of intramembrane helix interactions using lipidic cubic phase (LCP) techniques

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The mechanisms of assembly and function for many important type I/II (single-pass) transmembrane (TM) receptors are proposed to involve the formation and/or alteration of specific interfaces among their membrane-embedded alpha-helical TM domains. What these intramembrane interfaces look like and how they gain specificity is only poorly understood because of substantial technical challenges regarding their structural characterisation. Isolated TM fragments are generally considered poor candidates for crystallisation due to their high hydrophobicity, flexible non-helical flanking sequences, low abundance of potential crystal contacts and the requirement for high lipid or detergent content in samples for screening. However, our lab has recently determined structures of TM complexes derived from glycophorin A [1] and the immunoreceptor signalling adaptor DAP12 [2] in monoolein lipidic cubic phase (LCP), demonstrating that TM complexes can be crystallised in a lipid bilayer and that native TM helix-helix interfaces are faithfully recapitulated under these conditions [3]. Since this provides compelling support for the wider application of LCP media for structural analysis of small TM complexes, we are currently using the technique in combination with LCP-FRAP (Fluorescence Recovery After Photobleaching) analysis to obtain structural information about other cell-surface receptors and related regulatory proteins that act via TM-TM interactions.

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Single crystal diffuse scattering using neutrons

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There is now a wealth of evidence to show that the more local structure is investigated the more we are obliged to reassess our understanding of crystalline structure and behaviour [1]. Diffuse scattering from single crystals is a prime source of such local structural information. Bragg scattering can only reveal average structures and it is important to realise that average structures are just that; and, as materials' properties often depend on the local structure, it is important to understand of what they are averages. Developing, optimising and critically assessing the tools that we have available to uncover local structure in crystals is now a vital part of crystallography [1]. Although diffuse X-ray scattering is of prime importance, diffuse neutron scattering also plays an important role. In this paper we review the developments that have taken place in the last 10–12 years in making diffuse neutron scattering a viable research tool. In particular we compare experiments made on the molecular material *d*-benzil on SXD at the ISIS spallation neutron source [3] with more recent experiments made at SNS using the dedicated diffuse scattering instrument *Corelli* [4]. In addition we show what can be achieved at the Australian Opal reactor source using the *Wombat* powder beamline.

It might be supposed that obtaining diffuse scattering data using neutrons is simply not a viable proposition since the neutron fluxes available at the best neutron facilities in the world are so low in comparison to the photon fluxes available for X-ray sources. For example, the neutron flux on SXD [2] at ISIS is ~ 7×10^4 neutrons per sec per mm^2 whereas the X-ray flux at the 1-ID beamline at APS is ~1×10^{12} photons per sec per mm^2. However, in the case of X-rays, measuring diffuse scattering requires the use of a monochromatic beam and so most of the available photons are discarded. In contrast, for experiments at a spallation neutron source, it is possible to use the whole spectrum of incident neutron wavelengths since their contribution to the scattering can be separated by time-of-flight spectroscopy. If this is coupled with the use of extensive detector banks that cover a large fraction of the scattering solid angle, a large enough enhancement factor relative to the X-ray case is obtained such that recording diffuse scattering becomes viable for a suitable sized sample. While SXD uses the spallation pulse itself to allow time-of-flight resolution of the energy of each detected neutron, the Corelli instrument at SNS uses a statistical chopper together with a cross-correlation analysis technique.

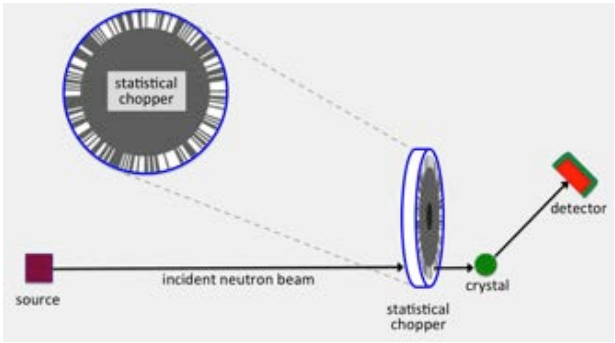

Figure 1. Schematic drawing of the Corelli cross-correlation spectrometer.

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Expression, purification and biophysical characterisation of enzymes from the lipid A biosynthesis pathway

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The outer membrane of Gram-negative bacteria possesses an inner leaflet composed primarily of phospholipids while the outer leaflet contains both phospholipids and lipopolysaccharide (LPS). LPS acts as a structural barrier that protects Gram-negative bacteria from antibiotics and other environmental stress [1]. LPS contains a lipid A moiety that is a glucosamine-based saccharolipid. Lipid A is the active component of the bacterial endotoxin and strongly modulates the human immune response.

Lipid A is synthesised in the cytosol of Gram-negative bacteria by nine constitutive enzymes. LpxH (UDP-diacylglucosamine pyrophosphohydrolase) and LpxB (lipid A disaccharide synthase) are two peripheral membrane proteins that catalyse the 4th and 5th steps of the lipid A biosynthesis pathway (Fig. 1). LpxB is an inverting glycosyl transferase of the GT-B superfamily and a member of CAZy database family 19. LpxB is a target for the development of new antibiotics, but no member of LpxB orthologues have been characterised mechanistically or structurally. LpxH catalyses the first membrane-associated step of the lipid A biosynthetic pathway [2] and occurs in 70% of clinically important Gram-negative pathogens. Here we present the expression, purification and biophysical characterisation of both LpxB and LpxH from *N. meningitides*. We also detail our preliminary studies towards structural characterisation of LpxB.

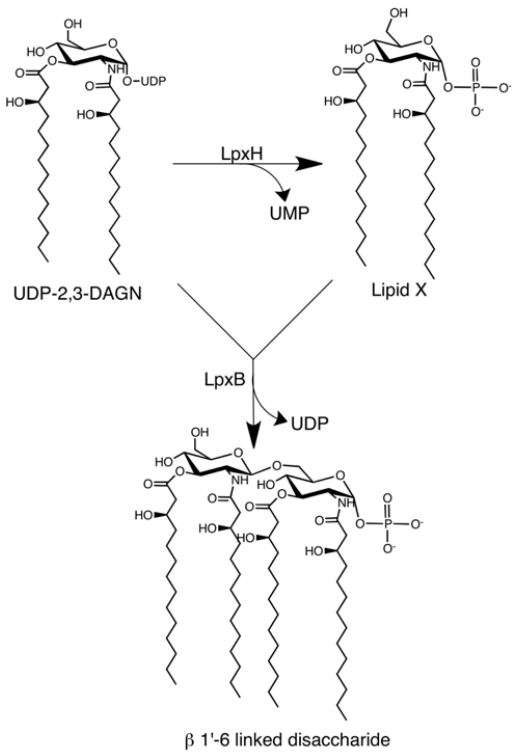

Figure 1. Enzymatic reactions catalysed by LpxH and LpxB

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DNA specificity of ribbon-helix-helix proteins controlling mobilisation of antimicrobial-resistance plasmids in *Staphylococcus aureus*

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The diverse ribbon-helix-helix (RHH) DNA-binding protein family contains an N-terminal anti-parallel β-sheet that binds DNA in the major groove, directly decoding the DNA site [1]. Recently the SmpO RHH-protein was discovered to be critical for DNA sequence recognition by pWBG749-family conjugative plasmids in *Staphylococcus aureus*. pWBG749 conjugative plasmids are able to mobilise antimicrobial-resistance plasmids carrying specific 'origin-of-transfer' DNA sequences (*oriT*). These *oriT* are carried by 53% of non-conjugative *S. aureus* antimicrobial-resistance plasmids and, therefore, are likely a dominant mechanism of resistance transfer in *S. aureus*. pWBG749-family *oriT*s share a common arrangement of inverted-repeat (IR) sequence motifs, but distinct subfamily members each carry a unique inverted-repeat-2 (IR2) sequence [2]. The most prevalent conjugative plasmid subfamilies, pWBG749 and pWBG745, are almost identical over their entire sequence but carry distinct *oriT*s named OT49 and OT45, which are also the most prevalent *oriT* mimics on non-conjugative plasmids. pWBG749 and pWBG745 specifically mobilise plasmids carrying mimics of their cognate *oriT*, but they can be made to mobilise plasmids carrying each other's *oriT* in the presence of the other's relaxosome specificity factor gene $smpO_{49}$ or $smpO_{45}$ [2]. Thus the encoded SmpO RHH-proteins enable the specific discrimination between OT49 and OT45 *oriT* sites. Here we present our most recent work showing that single amino-acid substitutions in the predicted DNA-binding regions of $SmpO_{49}$ or $SmpO_{45}$ (F7K or K7F respectively) reverse the *oriT* specificity for each conjugative plasmid in mobilisation experiments. Furthermore, we present surface-plasmon resonance based DNA-binding data for each SmpO variant to these *oriT* sequences. Minimal dsDNA oligonucleotides were also used in SmpO-DNA co-crystallisation experiments. These findings expand our understanding of the capacity for antimicrobial-resistance plasmid transfer in *S. aureus*. The structure of SmpO proteins and SmpO-DNA complexes will provide insight into how conjugative plasmids can distinguish distinct *oriT* sequences and how rapidly they can evolve to facilitate horizontal transfer of new sequences.

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