Luminescent Multi-functional Imidazole Based Cu and Cd MOFs for Sorption, Sensing and Catalytic Application

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The initial reports on the synthesis and structural characterizations of coordination polymers (CPs) and metal organic frameworks (MOFs) in late 1990’s and in 1995’s respectively and subsequent introduction of its applications in the fields of material science and catalysis have stimulated the design and synthesis of functional materials of prototype MOFs using the different organic linkers, and metal ions, as nodes. Some of the notable MOF materials which have multiple applications in the fields of gas storage, separations, sensing and catalysis are MOF-5, MOF-74 and HKUST-1 to name a few. Recently, the use of N-heterocyclic based ligands have been studied in order to develop the MOFs containing free nitrogen donor on its surface of the channels which potentially captures gaseous molecules and also to carry out Lewis base assisted organic synthesis. Keeping this in view, we have synthesized isomeric 1-methyl-imidazole-dibenzoic acid which has an inherent properties of luminescence, and used as a precursors for preparation of copper and cadmium based MOFs. In this presentation, the solid state structures of these MOFs and its properties on sorption and sensing of gaseous molecules and anions will be discussed.

Figure 1. Cu-MOFs, viewing direction c.

5. Sachan, S. K.; Tripathi, S.; Avinash, I.; Anantharaman, G. (manuscript under preparation.)
Structural phase transition in hydrogen-bonded co-crystals of 1,4-diazabicyclo[2.2.2]octane and some naphthalenediols

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Some studies that 1,4-diazabicyclo[2.2.2]octane (DABCO) forms co-crystals with molecules of hydrogen bond donor were reported. It was found that the co-crystal shows structural phase transition about rotating motion of the DABCO molecule at low temperature.

In our study, DABCO cocrystallized with some naphthalenediols, and crystal structures for the co-crystals were determined. It was found that the co-crystal between DABCO and naphthalene-1,5-diol shows disorder for orientation of the DABCO with partial occupancy ratio about two orientations at room temperature, and the DABCO orientation are ordered to one side at low temperature. This co-crystal undergoes a polymorphic phase transition at ca. 325 K. The space groups in low temperature phase (LT) and high temperature phase (HT) are the same $P1_1$, and the unit-cell volume in HT reduces to half of that in LT. Small thermal anomaly was found on DSC measurement data around the temperature for the structural change on X-ray measurement. DABCO—naphthalene-2,7-diol co-crystal shows similar structural phase transition at ca. 294 K, but symmetry change of crystal structure for the co-crystal is different to that for 1,5-diol co-crystal. The space group in HT is $C2/c$ for 2,7-diol co-crystal, and that in LT is $P1_1$. On this phase transition from HT to LT, crystal become twin. The thermal anomaly on DSC measurement for 2,7-diol co-crystal is sharper than that for 1,5-diol co-crystal. Both phase transitions for 1,5-diol co-crystal and 2,7-diol co-crystal are same order-disorder transition, but those symmetry changes are different each other.

Figure 1. DABCO—Naphthalene-1,5-diol (1:1) at 335 K (HT).
Covalent bonding character of $d$-electron of molybdenum from synchrotron X-ray diffraction

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Electrons in $d$-orbitals of transition metals and their complexes govern their properties and functions. The spatial and energetic structures of $d$-electrons have been largely investigated both experimentally and theoretically in the case of 3$d$-system. The spatial structure of 4$d$- and 5$d$-system have never been revealed experimentally since the contribution of 4$d$- and 5$d$-electrons to X-ray diffraction is much lower than that of 3$d$-system. In this study, we determined the occupancies and spatial distribution of 4$d$-electrons in pure molybdenum by a charge density study from synchrotron radiation X-ray diffraction. The accuracy and precision of the determined $d$-orbital structure have been evaluated by a comparison of a density functional theoretical calculation by WIEN2k packages. There are valence charge density maxima indicating bond formation in the observed valence charge density. The electron deficiencies of $\Gamma_{12}$ orbitals were visualized in the observed static deformation density. Approximately 0.5 electrons deficiency was observed from the orbital population analysis through a multipole refinement. Observed features were well agreed with theoretical study.

Figure 1. Valence charge densities of molybdenum in 110 plane for (a) experiment and (b) theory.
Control of solid state photochromism utilizing multiple step crystalline photoisomerization

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Salicylideneaminopyridine shows photochromism in the crystalline-state. The cyanoalkyl cobaloxime complex undergoes crystalline-state photoisomerization by irradiation with visible light. In the present study, a δ-cyanobutyl cobaloxime complex was selected as a cobaloxime complex, and the complex with N-3,5-tert-butylsalicylidene-3-aminopyridine (SAP derivative) as a ligand was synthesized (Figure 1). The δ-cyanobutyl group has possibility of multistep isomerization because of its bulkiness. We tried to control the rate of photochromism of SAP derivatives dynamically using the change of intermolecular interaction in the crystal by the multistep crystalline-state photoisomerization of δ-cyanobutyl cobaloxime complexes.

The three-dimensional structure was determined by single crystal X-ray analysis of the complex. Subsequently, the photoreactivity in the crystalline-state was investigated for the complex. As a result, the photochromic reaction of the SAP derivative by ultraviolet light irradiation and the photoisomerization of the δ-cyanobutyl cobaloxime complex by visible light irradiation respectively proceeded in the crystalline-state for this novel complex. Therefore, we succeeded in obtaining a dual photoreactive complex. Furthermore, the X-ray crystal structure analyses were performed for each visible light irradiation time. The X-ray results show that δ-form isomerized to γ-form and α-form, that is, the δ-cyanobutyl group isomerized in multistep way. Next, the thermal fading rate of the colored species by photochromism of SAP derivative was measured before, after 50 h, and 300 h visible light irradiation, respectively. After 50h irradiation, the fading rate became slower than initial one, and after 300h irradiation, the rate became faster again. We succeeded to control the fading rate by isomerization of δ-cyanobutyl group of cobaloxime complex dynamically.

Fig. 1. Crystalline-state photoreaction of a new dual photoreactive complex
In situ Control of Photochromism in Spiropyran Derivatives by Crystalline-State Photoreaction

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Spiropyran (SP), which is one of the well-known organic photochromic compounds, shows photochromism by converting to merocyanine in solution upon UV light irradiation. So far, only a few solid-state photochromic SP derivatives have been reported in the literature in spite of their importance for materials chemistry. Recently, we found a new series of solid-state photochromic SP derivatives.

In this work, we synthesized a β-cyanoethyl cobaloxime complex (β-Cob) with an SP derivative (SP3) as an axial ligand. The complex showed β-α crystalline-state photoisomerization reaction under visible light irradiation, and both before and after the isomerization, SP3 moiety displayed photochromic reaction by UV light irradiation. Interestingly, after the photoisomerization of β-Cob complex, the colour fading rate of SP3 photochromism decreased significantly. The detailed analysis of the crystal structures indicated that the reaction cavity around the SP3 moiety became smaller by photoisomerization of the complex, which caused the fading rate change. This indicated that the complex is a novel dual photoreactive complex, and it will be possible to control the crystalline-state reaction by using two different wavelengths.

Figure 1. Photochromism of SP3.

References
Unusual 2D→3D Single-Crystal to Single-Crystal Transformation and Multifarious Techniques of Characterization

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The single crystal to single crystal transformation is an exceedingly powerful tool to correlate the progress of physical or chemical reaction in the solid state. This provides direct insight of the many subtle changes that occurs as a result of any external stimulation. Here, we report an unprecedented 2D→3D transformation of a trinuclear secondary building unit changing to a mononuclear node. The solvothermal reaction of Zn(NO₃)₂·6H₂O and a linear dicarboxylate ligand H₂L, in the presence of urotrope in N,N′-dimethylformamide (DMF), gives rise to a new porous two-dimensional (2D) coordination network, \{
Zn₃(L)_3(urotropine)₂·2DMF·3H₂O\}_n (1), with hxl topology. The 2D framework of 1 allows replacement of urotrope molecules by 4,4′-azopyridine (azp) linker resulting in a three-dimensional (3D) metal–organic framework, \{Zn(L)(azp)]·4DMF·2H₂O\}_n (2). The 1→2 transformation takes place in single-crystal-to-single crystal (Sc-SC) manner supported by powder X-ray diffraction (PXRD), atomic force microscopy (AFM), high-resolution transmission electron microscopy (HRTEM) and morphological studies. This study furnishes the usefulness of different characterizations which one can be follow into such type of unusual changes.

Figure.1. SC-SC conversion of 2D MOF into a 3D MOF
Crystal structure and aqueous solubility of norfloxacin treated by high-pressure CO$_2$

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Crystal structure and aqueous solubility of norfloxacin treated in high-pressure CO$_2$ were investigated at various temperature, pressure and depressurization rate. Norfloxacin was treated in high-pressure CO$_2$ at 30 to 60 °C and 5.0 to 20.0 MPa for 2 h. The treated norfloxacin was characterized by XRD, DSC and FT-IR. The solubility of norfloxacin treated in high-pressure CO$_2$ was measured by using ultraviolet-visible spectroscopy. In Figure 1, it can be seen that the XRD pattern of norfloxacin is changed by the treatment in high-pressure CO$_2$ at 40 °C and depressurization rate 0.1MPa min$^{-1}$ under different pressures. The results indicate the crystal structure of norfloxacin can be changed by high-pressure CO$_2$. The XRD patterns of the treated norfloxacin is divided into the two kinds. (a) 0.1 and 5.0 MPa treatment with different crystal structure form untreated sample at low angle and (b) 10.0 to 20.0 MPa treatment with totally different at 6 to 36 degree. The effect of pressure on the aqueous solubility of norfloxacin was also investigated. The solubility increases with the pressure in high-pressure CO$_2$ treatment. The maximum solubility of treated norfloxacin is about 1.6 times higher than that of the untreated one.

![Figure 1. XRD patterns of norfloxacin treated in CO$_2$](image-url)
In-situ CO$_2$ gas sorption and SCSC transformation of new azamacrocyclic complexes

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Coordination polymers based on azamacrocyclic compounds as building blocks with organic linkers have been widely studied because of structural stabilities related with heat and pressure and potential applications such as gas storage or separation, homo-/hetero-catalysis, magnetism, as well as molecular sensing.

Recently, we designed and prepared two significant azamacrocyclic coordination polymers with nickel(II) and copper(II) metal ions and 2,7-naphthalenedicarboxylic acid (2,7-NDC), [Ni(C$_{16}$H$_{38}$N$_6$)(2,7-NDC)]$_n$ (1) and [(Cu(C$_{16}$H$_{38}$N$_6$)(H$_2$O)$_2$)][2,7-NDC] (2), respectively. Significant CO$_2$ sorption properties and behaviors of 1 were determined by in-situ single crystallography. 2 shows interesting phenomena related with specific solvent-induced single crystal to single crystal (SCSC) transformation from the 1D to 0D and 0D to 1D, reversibly.

Here, we will present the detailed preparation, crystals structures, and characteristic behaviors of each compounds.

Figure 1. CO$_2$ gas adsorption behaviors of 1.
Decomposition products of the spherical Keplerate type polyoxometalate

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The spherical Keplerate type polyoxometalate, $\text{[Mo}^{VI}_{72}\text{Mo}^{V}_{60}\text{O}_{372}(\text{CH}_3\text{COO})_{30}(\text{H}_2\text{O})_{72}]^{42-}$ (Mo\textsubscript{132}), is a fullerene-like metal oxide cluster \cite{1}. We observed at least two substances when tracking the decomposition process of Mo\textsubscript{132} in water (50 µmol/L, 80 °C) by time-dependent UV-vis measurements \cite{2}. At early stages, a substance showing absorption at 325 nm was observed (Fig. 1, 9 hour, referred to this substance as A hereafter). With prolonged heating, the solution turns blue and a substance showing absorption around 750 nm was observed (Fig. 1, 25 hour, referred to this substance as B hereafter). In this work, we tried to analyze their structures by crystallizing with various cations.

The substance A was selectively generated by boiling Mo\textsubscript{132} aqueous solution under basic condition (pH 11). It can be isolated by adding sodium chloride.

The substance B was generated by boiling Mo\textsubscript{132} aqueous solution. Deep blue crystals precipitated by the vapor phase diffusion of chloroform into the acetonitrile solution of its tetrabutylammonium salt. Single crystal X-ray diffraction revealed that the crystal contains a 102-nuclear spherical Keplerate type polyoxometalate.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig1.png}
\caption{UV-vis spectra of Mo\textsubscript{132} aqueous solution}
\end{figure}

\textsuperscript{1} Achim Müller, Erich Krickemeyer, Hartmut Bögge, Marc Schmidtmann, Frank Peters, \textit{Angew. Chem. Int. Ed.} \textbf{1998}, 37, 3359
\textsuperscript{2} Yuichi Shiokawa, Kenji Ohashi, Tomoji Ozeki, the 68th Conference of Japan Society of Coordination Chemistry, \textbf{2018}, 1C-07
Stuctures of butt-fusion joint in HDPE pipes as studied by wide and small angle X-ray scattering

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Polyethylene pipes in gas and water applications have proven to be reliable over their 70 years of successful use. The obvious advantage of polyethylene over the conventional metal-based pressure pipes is that they maintain exceptional resistance to corrosion and chemical attacks. In recent times, the application of polyethylene pipes has extended into areas where their safe performance is critical to maintaining the structure integrity of the piping system. Such applications include safety class pipes in nuclear power plants and large diameter pipes for conveying and/or storing greater scale volume of water. In designing a sound polyethylene piping system, the structural integrity of the joints between pipes is of importance. There are variety of joining methods available for polyethylene pipes that include butt-fusion, electro-fusion, mechanical joint, etc. Among them the butt-fusion method is the most widely used for joining larger diameter pipes. During butt fusion, a melt fusion zone (MFZ) is formed, which contains various morphologies due to the difference in flow behavior within MFZ. It has been reported that in polyethylene and polypropylene pipe butt-fusion joints, cracks were seen to initiate in the zone between the oriented crystallinity and fine spherulites. Through various studies it was observed that the ordering between crystal lamella and amorphous region as well as molecular orientation are crucial in giving rise to butt-fusion joint integrity. In this work for the first time 2D wide and small angle X-ray scattering techniques were applied to investigate the microstructure of MFZ in butt-fusion joint of HDPE pipe.

Figure 1. Schematics of wide and small angle X-ray scattering in melt-fused zone of HDPE pipe
Reduction of dopant atoms and enhancement of ferromagnetism by x-ray and UV irradiation in Ce-doped TiO2

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Extended x-ray absorption fine structure (EXAFS) and x-ray absorption near-edge structure (XANES) techniques have been employed to probe the local structure and valence of Ce dopant atoms in Ce-doped TiO₂ nanocrystals, respectively. As revealed by the EXAFS analysis, the Ce dopant atoms were found to substitute for Ti atoms in the TiO₂ host with local structural distortion and formation of oxygen vacancy defects. The XANES data also show that subvalent Ce³⁺ ions coexist with Ce⁴⁺ ions in the as-made Ce-doped TiO₂ sample. As a result of x-ray or UV irradiation, the number of oxygen vacancy defects, as well as the Ce³⁺ concentration, progressively increases with the irradiation time. Furthermore, these materials exhibit room-temperature ferromagnetism that appears to be enhanced due to UV light exposure. To explain the experimentally observed changes of magnetic properties due to oxygen vacancy formation, first-principle density functional theory (DFT) calculations have also been performed by using the Vienna ab initio simulation package (VASP). The Ce-doped TiO₂ diluted magnetic semiconductor (DMS) with room-temperature ferromagnetism enhanced by UV irradiation may lead to important technological applications.
Template Synthesis and Structure of Eight-Membered Cu$_4$I$_4$ Ring Crown Motif from Dithiolate Complex

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The construction of coordination polymers based on metal ions and bridging organic ligands is one of the most attractive areas in the field of materials research. In our previous studies, we have reported the synthesis and structures of mixed metal coordination polymers and monomer $A_2[Ni(mnt)$_2$Cu$_4$I$_4]$ ($A = n$-Bu$_4$N, Na(THF)$_4$, Na(18-crown-8)(THF)$_2$; mnt = 1,2-dicyanoethylene-1,2-dithiolate)$^1$. The crown motif in these complexes consists of an eight-membered Cu$_4$I$_4$ ring, which is supported by four sulfur atoms of the Ni(mnt)$_2$ moiety. This type of crown motif containing the Cu$_4$I$_4$ ring was synthesized using a template based on dithiolate complexes, [M(mnt)$_2$]$^{2+}$. We assumed the isomer metal dithiolate complexes, [M(i-mnt)$_2$]$^{2+}$ (i-mnt = 2,2-dicyanoethylene-1,1-dithiolate), are available for template synthesis of such crown motifs. In this work, we have successfully synthesized ($n$-Bu$_4$N)$_2[Ni(i-mnt)$_2$Cu$_4$I$_4]$ building unit with the crown motif, as shown in Figure 1. X-ray diffraction analysis of tiny single crystals was performed at the BL02B1 beamline at the SPring-8 synchrotron radiation facility. Copper and iodine atoms are alternately connected within the eight-membered ring structure. The copper atom is coordinated to the nitrogen atom of the cyano group of the neighboring molecule, which is a doubly bridged anionic 1-D polymer consisting of [Ni(mnt)$_2$Cu$_4$I$_4$]$^{2-}$ units as in ($n$-Bu$_4$N)$_2[Ni(mnt)$_2$Cu$_4$I$_4]$. The details of the molecular structure and possible applications of the eight-membered Cu$_4$I$_4$ ring crown motif will be presented.

![Figure 1. ORTEP diagram illustrating (a) top and (b) side view of [Ni(i-mnt)$_2$Cu$_4$I$_4$]$^{2-}$.](image)

Crystallinity and orientation of micro-crystals in ultrathin biodegradable polymer blend films

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Many physical properties of ultrathin polymers are different from those in the bulk, such as glass transition temperature, average size of crystallites, and crystallinity. Preferred orientation of crystallites dispersed in ultrathin polymers can be another feature which often brings useful anisotropic properties for applications. Polymer blend is an effective method to create new desired physical properties. As a promising biodegradable polymer, we investigate crystalline Poly(3-hydroxybutyrate) [PHB] which has often been blended with other polymers to reduce its high crystallinity so as to improve unfavourable properties, i.e., brittleness and poor processability. Poly(L-lactic acid) [PLLA] is a biodegradable polyester. Thus, blend of PHB and PLLA should be beneficial for expanding the range of applications. In this study, we studied molecular weight (Mw) dependence of PLLA on crystallization and preferred orientation of PHB films using surface-sensitive X-ray scatterings. In 30-nm-thick films, crystallization of PHB was reduced in the blends with PLLAs (23,000>Mw>13,000 g/mol), although the higher and lower molecular weight PLLAs did not affect the crystallization significantly. In contrast, in 13-nm-thick films, crystallization is completely inhibited in the blends with PLLAs having Mw>7,000 g/mol, while the lower molecular weight PLLA hardly affected the crystallinity. Our results showed that the ability of PLLA for controlling the crystallinity of PHB ultrathin films strongly depends on the molecular weight and film thickness.
Dehydration mechanism of drug crystal: A new classification of hydrates by kinetic behavior

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Clarifying the dehydration mechanism through the intermolecular interaction energy of crystalline water in crystal is important in drug development because it strongly influences the stability of drug product. The classification rule of dehydration process has been cumbersome because of the lack of an unambiguous comparison parameter. In this study, the activation energy of dehydration and rehydration transition of hydrate crystals were quantitively evaluated by differential scanning calorimetry, dynamic vapor sorption, and thermal gravimetric measurements, and powder X-ray diffraction analyses. The activation energy and rehydration behaviour were used to classify the hydrate crystals from kinetic behaviour point of view, which can be related to the crystal structure. Seven pharmaceutical hydrate crystals were classified into three classes. “Class 1” had low activation energy and rehydrated easily to transform to its original hydrate. “Class 2” also had low activation energy but did not rehydrate due to destruction in the dehydration process followed to form amorphous phase. “Class 3” had high activation energy owing to rearrange into new anhydrous form and resulted in no rehydration. Moreover, these dehydration and rehydration characters agreed with their crystal structures analysed by single crystal X-ray diffraction. Specifically, water molecules were arranged i) one-dimensionally in the crystal structure like channel hydrates in Class 1, ii) two-dimensionally like layer hydrates in Class 2, iii) without contact to each other like isolated site hydrates in Class 3. Therefore, activation energy offers useful measure to specify a variety of dehydration states and, furthermore, to predict characterization of crystal packing in hydrates.
Structural Instability and Improper Ferroelectricity in Aluminate-Sodalite-type Oxides

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The aluminate-sodalite-type oxide $M_8[AlO_2]_{12}(XO_4)_2$ incorporates the $[AlO_2]_{12}$ framework whose structural voids are filled by $M$-cations and $XO_4$-anions. (Fig. 1) In the present study, the ferroelectricity in $M_8[AlO_2]_{12}(XO_4)_2$ ($M =$ Ca, Sr and $X =$ Mo, W) is demonstrated by dielectric and pyroelectric measurements. [1, 2] The ferroelectricity in $M_8[AlO_2]_{12}(XO_4)_2$ ($M =$ Ca, Sr and $X =$ Mo, W) is found to be of improper-type, which is driven by structural fluctuation unlike the case of proper ferroelectricity that is induced by polarization fluctuation. Systematic investigations with first-principles calculations and structural analyses using synchrotron powder x-ray diffraction clarify a mechanism of the improper ferroelectricity in the $M_8[AlO_2]_{12}(XO_4)_2$ ($M =$ Ca, Sr and $X =$ Mo, W). An excellent Figure-of-Merit for the pyroelectric energy harvesting is suggested in the aluminate-sodalite-type oxides with the improper ferroelectricity.

References:
Chirality Control of Triglycine sulfate Crystals by Amino acids

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Chiral crystals are widely applied such as medicine, absolute asymmetric synthesis, optical devices and so on. Also, it is essential to be determined chirality of chiral crystals when we use them in industry. However, when we grow chiral crystals composed of achiral molecules, we obtain the same amount of left-handed crystals and right-handed crystals [1]. Because among crystals composed of achiral molecules about 8% of them are chiral crystals, it is very important to grow chiral crystals that have particular chirality. In the past, although a method to synthesize particular enantiomer and grow chiral crystals has been established [2], there are no methods to grow chiral crystals from achiral molecules. In this study, we aim to grow chiral crystals from achiral molecules.

We have focused on Triglycine sulfate (TGS) crystals as chiral crystals from achiral molecules (Fig.1(a)). We grew TGS crystals by doping it with chiral amino acids, alanine or threonine, then obtained L-alanine-doped TGS (LATGS) crystals, D-alanine-doped TGS (DATGS) crystals, L-threonine-doped TGS (LTTGS) crystals and D-threonine-doped TGS (DTTGS) crystals. By single X-ray crystal structure analysis of those crystals, we found that chirality of ATGS crystals were uniquely determined by chirality of alanine. LATGS crystals were left-handed crystals and DATGS crystals were right-handed crystals. On the other hand, TTGS crystals were not uniquely determined by chirality of threonine. Both LTTGS crystals and DTTGS crystals were left-handed crystals or right-handed crystals. Furthermore, TGS crystals are ferroelectricity, we measured P-E hysteresis loop. In TGS crystals, internal bias field (E_{i.b.}) did not arise. In LATGS crystals and DATGS crystals E_{i.b.} arised, on the other hand, in LTTGS crystals and DTTGS crystals E_{i.b.} did not arise. Also, LATGS crystals showed positive E_{i.b.}, and DATGS crystals showed negative E_{i.b.}. Because we can consider that these results showed there is a relationship between chirality of crystals and E_{i.b.}, we discussed their relationship and mechanism of controlling chirality of TGS crystals by doping it with alanine.

References
The Hierarchical Self-Assembly of a Functionalised M₄L₆ Tetrahedral Cage
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Hierarchical self-assembly provides higher control over MOF formation compared to the standard "one-pot" synthetic approach. This is a direct result of the preformation and isolation of a repeating metallosupramolecular structural motif. The main requirement for this synthetic approach is the presence of groups which contain further coordinating functionality beyond what is required for the assembly of the initial structure. Accordingly, we have investigated the coordination chemistry of functionalised 2,2′:5′,5″:2″,2″′ quaterpyridine ligands. As an example, ligand 1 has been synthesised and reacted with iron(II) to yield an M₄L₆ tetrahedron by self-assembly, the structure of which was verified by synchrotron analysis. The presence of the ethyl ester groups provide a scaffold for further chemistry to take place, demonstrating the significance of this structure. They also impart the potential for the metallosupramolecular cage to be used in the hierarchical self-assembly of MOFs through deprotection and further coordination. Investigations into further self-assembly will be presented, along with detailed studies on the properties of the metallosupramolecular components. In-depth knowledge on the chemical properties of the cage are particularly significant, as they ideally remain unchanged with further self-assembly. As a result, well-defined properties such as host-guest relationships in a single cage can be translated into elegantly designed MOFs.

Figure 1. Crystal structure of ester functionalized quaterpyridine 1, self-assembled into a Fe₄1₄⁺⁺ tetrahedron with FeCl⁺ bound inside
Quantification of amorphous content in mixtures using the direct derivation method

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The direct derivation (DD) method is a new technique, recently proposed for the quantitative phase analysis (QPA) using the X-ray powder diffraction method1–3. Squared structure factors, when they are summed up, can be replaced with the sum of squared numbers of electrons belonging to the atoms in the unit cell. Therefore, the DD method can conduct the QPA of Rietveld equivalent without referencing to structural parameters but only from chemical composition data of individual phases.

Quantification of amorphous content in multi-component materials is a problem, which can be found anywhere in the fields of research and development as well as the quality control on production lines. In the DD method, the sum of integrated intensities, corrected for the Lorentz-polarization factor, or the integrated profile intensities for individual phases, measured in a wide 2θ range, are used as observed data. In the whole-powder-pattern fitting, a halo pattern from a targeted amorphous component can be used as one of the component model patterns composing the total diffraction pattern. Weight fraction of amorphous component in the mixture can directly be derived, as one of the constituent materials in the mixture, without using internal/external standard reference material.

In the case of crystalline α-SiO2 and glass SiO2 system with various weight ratios, as one of the test examples, errors, defined as a difference between derived weight fraction and weighed value, were in the range of 0 – 1.5 wt. (%) in the optimized conditions. Other test results will be presented in this report.

References)


Analogous nickel(II) and cobalt(II) metal–organic frameworks showing influence of framework metal ion on the dye removal properties from water

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Complexes \([\text{M}_4(\text{d-cam})_4(\text{bpzpip})_3(\text{H}_2\text{O})]\cdot\text{DMAc} \cdot 3\text{H}_2\text{O} \ (1, \ M = \text{Ni}; \ 2, \ M = \text{Co})\) were hydro(solvo)thermally prepared from the reactions of \(\text{MCl}_2\cdot 6\text{H}_2\text{O} \ (M = \text{Ni, Co}), \ \text{d-camphoric acid (d-H}_2\text{cam}), \text{ and } N,N'\text{-bis(pyraz-2-yl)piperazine (bpzpip)} \) in \(N,N'\text{-dimethylacetamide (DMAc)} - \text{H}_2\text{O} \) at 150 °C. Complexes 1 and 2 are isomorphous and isostructural, both of which adopt a 3D (4,6)-connected framework with \((4^2.5^2.7^2)(4^2.5^2.7^2.8)\) topology. Dye removal performances of 1 and 2 toward methyl orange (MO), malachite green (MG), and methyl blue (MyB) from water were explored in dark at room temperature. Among the three selected organic dyes, 1 adsorb only MyB from aqueous solution with saturated adsorption capacity of 216 mg g\(^{-1}\), while 2 capture not only MyB but also MG from aqueous solution with saturated adsorption capacities of 671 and 405 mg g\(^{-1}\), respectively. The isotherm and kinetic studies revealed that adsorption data for MyB over 1 and 2 and MG over 2 all were well fitted to the Langmuir isotherm and the pseudo-second-order kinetics models. Furthermore, 1 can selectively bind MyB over MO from a MyB/MO mixture while 2 can selectively bind MyB and MG over MO from a MyB/MO mixture and a MG/MO mixture, respectively. These findings demonstrate that the two analogous MOFs are promising adsorbents for efficient removal of dyes and show the influence of framework metal ion (Ni\(^{2+}\) and Co\(^{2+}\)) on the dye removal properties including adsorption rate and capacity as well as adsorbate from water.
Structural study of sustained release of bisphenol inclusion compounds with fragrant monoterpenic alcohols

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Inclusion crystals incorporating guest molecules into host frameworks are functional crystalline materials offering protection or sustained release of the guest molecules. In this study, novel functional inclusion crystals that release fragrant molecules were designed by using bisphenol host BHC with monoterpenic alcohols guests (L-menthol, α-terpineol, terpinen-4-ol, linalool and (−)-carveol). In addition, their crystal structures, and the guest desorption rates of inclusion crystals were examined to reveal structural factors of the sustained release function.

The inclusion crystals had similar structures with a host-guest ratio of 1:1 (Fig.1). Between the layered host frameworks, the guest monoterpenic alcohols were trapped by accepting and donating O-H...O hydrogen bonds and formed sandwich-like inclusion structures. Although the high crystal structure similarity, the sustained guest release rates were significantly different, and the order was L-menthol > α-terpineol > terpinen-4-ol. This order would be explained by the difference of the volume of the guest-occupied region in each inclusion crystal. The volume of the regions were different due to the positions of the OH and molecular shapes of the guests. Actually, the volume order was L-menthol > α-terpineol > terpinen-4-ol, which corresponded to the guest release rates. Therefore, guest molecules in a small solvent region are strongly retained by the host framework and difficult to be desorbed. Interestingly, after the complete desorption, the crystal structure transformed to one polymorph of BHC, which went back to the inclusion crystal form by absorbing the guest vapors.

**Figure 1** Guest molecule region of inclusion crystals: (a) L-menthol-BHC, (b) α-terpineol-BHC and (c) terpinen-4-ol-BHC.
Structural Studies of the interaction between PTP1B and the Janus Kinases

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The highly coordinated regulation of JAK-STAT signalling is critical for maintaining homeostasis, as aberrant signalling can result in a number of haematological malignancies. Protein Tyrosine Phosphatase 1B (PTP1B) is a negative regulator of cytokine signalling that functions by directly removing phosphate groups from tyrosine residues on the Janus Kinase (JAK) proteins, thereby inactivating them. Previous studies of PTP1B bound to phosphorylated peptide substrates revealed two phosphotyrosine binding pockets which bind tandem phosphotyrosine residues, with a preference for the first phosphotyrosine in the catalytic site. Despite the important role PTP1B plays in the negative regulation of the JAK proteins, little is known about the interaction between these molecules, thus we sought to investigate how PTP1B interacts with, and subsequently dephosphorylates JAK2. We have determined the co-crystal structures of the PTP1B phosphatase domain bound to JAK activation loop peptides. NMR and biochemical assays were used to support structural data and allowed us to shed light onto the mechanism of substrate dephosphorylation. Our PTP1B and JAK activation loop peptide co-crystal structures revealed that for JAK2, the second phosphotyrosine is the preferred substrate for the PTP1B catalytic site. NMR studies further supported the crystal structures and showed that the second phosphotyrosine is likewise the preferred substrate for dephosphorylation. These data suggest a mechanism for PTP1B catalysed dephosphorylation of the JAK proteins. Our findings provide useful information regarding the mechanism of ligand recognition and dephosphorylation by PTP1B and may aid design of novel small molecules to either inhibit PTP1B for the treatment of diseases.
Phactr1 contains an atypical RVxF motif competitively binding to PP1 or G-actin

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Phosphatase and actin regulators (Phactr) are a family of four proteins that are highly expressed in the nervous system. They have been described as PP1 and G-actin binding proteins, but their function is still not very well understood. Phactr1 has been implicated in reorganisation of actin cytoskeleton, angiogenesis or cell migration, however the signalling pathways affected by Phactr1 activity has not yet been elucidated. Phactr1 contains a G-actin binding domain formed by a repeat of three RPEL motifs. Actin binding regulates Phactr1 intracellular localization as well as interaction with PP1. PP1 is a ubiquitously expressed Ser/Thr phosphatase which generally lack substrate specificity and associate with a large array of targeting subunits (~200) to achieve the requisite selectivity. We suggest, that Phactr1 works as a regulatory subunit of PP1.

Phactr1 contains an atypical RVxF motif (⁵¹⁵LIRF⁵²²) located within the overlapping region between the G-actin binding domain and the PP1 binding domain. We have demonstrated that PP1 and G-actin compete to bind to the atypical RVxF motif of Phactr1. Using X-ray crystallography we solved the structure of Phactr1 bound to three G-actin molecules as well as the structure of Phactr1 bound to PP1α. These crystal structures shed light on the molecular mechanism allowing Phactr1 atypical RVxF motif to either bind to G-actin or PP1. Also, the structure of Phactr1:PP1α complex was solved at two different pH showing different binding modes.
POSTER BOARD 23

Toward elucidation of substrate recognition mechanism of PAD isozymes

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Peptidylarginine deiminase is a post-translational modification enzyme that catalyzes the conversion of arginine residue in protein to citrulline in the presence of Ca²⁺ ion. In human, there are five PAD isozymes (PAD1-4, PAD6) and they are related to a variety of diseases such as rheumatoid arthritis, dermatosis, and multiple sclerosis and so on. In the hair follicle, expression of three isozymes (PAD1, PAD2, and PAD3) is known and the X-ray crystal structures of PAD1 and PAD2 have been reported. These three PAD isozymes convert arginine residues of Ca²⁺-binding protein S100A3, which has four arginine residues (R3, R22, R51, R77), to citrulline residues. In vitro, PAD3 specifically citrullinates R51, whereas other two isozymes PAD1 and PAD2 citrullinate all four arginine of S100A3. The goal is to elucidate the structural differences of substrate recognition by PADs. Using the crystal that obtained under the condition (0.1-0.2M HEPES, 0.1-0.2M KCl, 35-45% 5/4 PO/OH), soaking to the solution containing the BAEE (Nα-Benzoyl-L-arginine ethyl ester hydrochloride) was carried out by gradually increasing its concentration. As a result, the BAEE-PAD3(C646A)-complex structure was obtained at 2.54 Å resolution, and it was able to confirm the electron density of BAEE. The Crystals belonged to space group R32, with unit-cell parameters a=b=115.711, c=330.207 Å. We will report the details of the crystal structure of the BAEE-PAD3(C646A)-complex. In addition to the detailed mechanisms of substrate recognition by PAD isozymes will be discussed in this conference.

References
Decameric and dodecameric structures of archaeal peroxiredoxins

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Peroxiredoxins (Prxs) are thiol-dependent peroxidases that are widespread in all biological kingdoms. Prx has a variety of quaternary structures involving monomers, dimers, decamers, and dodecamers. Decameric and dodecameric Prxs consist of ring-type arrangements of five and six homodimers, respectively. Here, we present the crystal structures of Prxs from the two hyperthermophilic archaea *Pyrococcus horikoshii* and *Thermococcus kodakaraensis* (PhPrx and TkPrx, respectively). These Prxs have high sequence identity: among the 216 residues, 196 are common. PhPrx was a decamer as we have previously reported [1]. However, TkPrx was shown to be a dodecamer. Since oxidized PhPrx can form a dodecamer [2], we compared PhPrx and TkPrx mutants in which all Cys residues were converted to Ser. As a result, the mutants exhibited the same crystal structure as the wild type, and the differences in the PhPrx and TkPrx quaternary structures were not affected by the mutation. By gel-filtration chromatography, TkPrx was eluted from the column with a slightly smaller elution volume than PhPrx. Since these Prxs are highly homologous, the gel-filtration chromatography supports higher-order molecular assembly of TkPrx in solution. The gel-filtration peaks of PhPrx and TkPrx shifted to higher elution volumes. This shift was considered to be a consequence of fast equilibration between the decamer/dodecamer and dimers rather than reflecting the lower-order assembly of these Prxs.

Characterisation of the UIM domain in RNF114

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Attachment of ubiquitin molecule(s) to proteins, termed ubiquitylation, regulates many cellular processes such as proliferation, cell growth and DNA repair. In the process of ubiquitylation, the key player is an E3 enzyme which facilitates the transfer of ubiquitin from an E2 ubiquitin-conjugating enzyme to substrate proteins. RNF114 is a RING E3 ligase involved in ubiquitin-mediated proteasomal degradation of MAVS (mitochondrial antiviral-signalling) protein in the RLH signalling pathway (Lin et al., 2017). This study aims to characterise RNF114 RING E3 activity and the role of its UIM (ubiquitin interacting motif) domain.

SEC-MALS analysis demonstrated that RNF114 is a monomer in solution. Biochemical assays showed that this RING E3 canonically interacts with UbcH5b and Ube2K and promotes ubiquitylation in vitro. Intriguingly, the C-terminal helical UIM domain significantly enhanced the processivity of RNF114. Remarkably, this domain promotes poly-ubiquitin chain assembly by UbcH5b, an aspect that has not been previously reported. Pull-down assays suggested that the UIM domain not only significantly increased the affinity for the E2~Ub conjugates but also for ubiquitin chains as acceptor ubiquitin (UbA). Mutations targeting the residues in the UIM domain significantly decreased the affinity for poly-ubiquitin chains that resulted in the loss of RNF114 E3 ligase activity. Collectively, the findings suggest that the UIM might bring UbA in proximity of the E2 active site, thereby facilitating Ub transfer. It could be postulated that other domains additional to the RING domain (e.g. the UIM domain) could enhanced ubiquitylation of RING E3 ligases.

Reference

Regulation of ubiquitin transfer by Ark2C

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Ubiquitylation is a post-translational modification executed by a cascade of enzymes that result in attachment of ubiquitin to a substrate lysine residue. The E2 conjugating enzymes determine the topology of ubiquitin signal, whereas the E3 ligases specify the substrate and enable its modification by ubiquitin. In recent years, it has become apparent that regulation of E3 ligases is important for proper cell function, and is carried out in different ways\(^1\). Notably, research in our laboratory revealed that the RING domains of Arkadia and Ark2C, a subclass of E3 ligases, bind ubiquitin and that this molecule regulates activity\(^2\).

Our aim was to reveal the molecular details of key interactions between Ark2C bound regulatory ubiquitin (Ub\(^R\)) and E2 conjugated donor ubiquitin (Ub\(^D\)). We crystallized and solved the structure of Ub-Ark2C:UbcH5b~Ub in a catalytically primed conformation (Fig.1), and this structure provided critical information on how Ub\(^R\):Ub\(^D\) interactions drive ubiquitylation by Ark2C.

Figure 1. Catalytic complex of Ub\(^R\)(green) bound to Ark2C(red) and UbcH5b(blue) conjugated with Ub\(^D\)(orange).

As we have shown that Arkadia and Ark2C interact with two E2s, UbcH5b and Ubc13, resulting in distinct biological outcomes, we investigated what defines E2 specificity. We have successfully identified two key features of Ark2C that contribute to activity regulation and E2 specificity. These features will be characterized on the poster.

We hypothesise that the Ub\(^R\) binding by RING domain of Ark2C drives ubiquitylation, whereas the residues N-terminal to the RING domain confer E2 specificity.


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 signaling 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. Previously, 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 recently 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 signaling output [2].


Assembly and function of two
New approaches to synchrotron X-ray diffraction data collection

Paterson, Neil

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Despite the advances in synchrotron hardware over the years, particularly in the detector field, the way we approach data collection still largely follows the old paradigm of screening images > strategy determination > data collection for each individual sample. This ‘maximum dose/minimum completeness’ approach risks radiation damage to samples that are more sensitive than average and the resulting dataset can be inadequate for weaker experimental phasing cases. The advent of fast photon-counting detectors; widespread availability of multi axis goniometry; high-speed and precise stages; and robust automatic processing of both full and partial data sets mean that this traditional approach can also be quite inefficient in the use of beamtime due to the overheads associated with each step.

At Diamond, we are exploring the idea of using low dose, high multiplicity measurements to build up each dataset and relying on counting statistics to improve signal/noise. This involves the collection of multiple weak sweeps of data that are combined to yield a dataset with comparable intensity to the traditional approach but with higher precision and a more even radiation damage spread. For sensitive samples, radiation damaged data can be excluded without compromising overall completeness. This removes the need for indexing and lifetime estimation prior to data collection. In combination with automated X-ray centring of samples, this approach provides a path to providing fast and efficient, fully automatic data collection.
Developing selective inhibitors to MYST histone acetyltransferases

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Histone acetylation by lysine acetyltransferases (KATs) is an important biological process for the function and regulation of chromatin. There are a number of important lysine acetyltransferases found in the cell and one class of these is the MYST family of KATs (KAT5 – KAT8). When dysregulated, these proteins may become oncogenic (e.g. KAT6A also known as MOZ). This family of proteins has been investigated by the CRC for Cancer Therapeutics as potential targets for cancer therapeutics. Structure based drug design using SPR and X-ray crystallography was an important component of the campaign to develop specific inhibitors to these targets. I will describe the methods used and some of the results from this program of work.

References:

Phototoxic Orange Fluorescent Proteins

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Phototoxic fluorescent proteins represent a sparse group of genetically encoded photosensitizers that could be used for precise light-induced inactivation of target proteins, DNA damage, and cell killing. Only two such GFP-based fluorescent proteins (FPs), KillerRed and its monomeric variant SuperNova, were described up to date. We present a crystallographic study of their two orange successors, dimeric KillerOrange and monomeric mKillerOrange, at 1.81 and 1.57 Å resolution, respectively. They are the first orange-emitting protein photosensitizers with a tryptophan-based chromophore (Gln65-Trp66-Gly67). Same as their red progenitors, both orange photosensitizers have a water-filled channel connecting the chromophore to the β-barrel exterior and enabling transport of reactive oxygen species (ROS). In both proteins, Trp66 of the chromophore adopts an unusual trans-cis conformation stabilized by H-bond with the nearby Gln159. This trans-cis conformation along with the water channel was shown to be a key structural feature providing bright orange emission and phototoxicity of both examined orange photosensitizers.
Targeting TIR domain assemblies in TLR signalling pathways to design anti-inflammatory compounds

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Toll-like receptors (TLRs) are central components of host innate immunity. Upon activation, their cytosolic TIR (Toll/interleukin-1 receptor) domains recruit the TIR-domain containing adaptor proteins MyD88 and MAL via TIR: TIR interactions, which triggers downstream activation of the transcription factor NF-κB to induce anti-pathogen responses. MAL works as a bridging adaptor for the association of MyD88 with TLR2 and 4.

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 the TLR2/4 pathways. TLR-antagonist designed against the extracellular domains of the receptors has had limited outcomes. Targeting protein-protein interactions of the downstream adaptors provides an alternative approach.

We have recently shown that the MAL^{TIR} 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 TLR4 signalosomes and NF-κB activation. To identify small molecules that can inhibit the formation of these TIR domain assemblies, a library of 400 fluorinated fragments was screened by ^19F-NMR spectroscopy, and 17 hits were identified that bind with MAL^{TIR}. Hits were further validated by HSQC and SPR. SAR by catalogue and molecular docking have been adopted to identify commercial compounds that carry the scaffolds of these hits. Moreover, a number of small molecules have been identified that inhibit MAL^{TIR} assembly in vitro at μM concentrations. They also significantly inhibit TLR-mediated (MAL^{TIR}-dependent) IL-6 production in human PBMCs and are promising leads for further evaluation as anti-inflammatory drugs.
Biochemical and structural characterisation of a SGNH hydrolase from *Anabaena sp.*

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The SGNH hydrolase superfamily is a large, recently discovered group of α/β proteins. The superfamily is named after the conserved catalytic residues: Ser, His constituting the classical triad with Asp and the oxyanion hole residues, Gly and Asn. Though the superfamily members share poor sequence homology, they possess a highly conserved flavodoxin-like core fold. Sequence and structural variations within the superfamily has resulted in diverse enzyme families with broad substrate specificities and functions. In order to better understand the structural basis of substrate specificity in this superfamily, we chose AnAEst, a SGNH hydrolase from the cyanobacterium *Anabaena sp.* AnAEst exhibits a natural variation where the conserved Gly is replaced by Arg, making it a SRNH hydrolase. Further, in a case of correlated mutation, the Arg54 is stabilised by a salt-bridge with Glu92 present in a structurally variable region. AnAEst displayed arylesterase and thioesterase activities, with specificity for aryl esters of short chain carboxylic acids (~C3). Interestingly, unlike the wild-type, the mutant R54G demonstrated an enhanced specificity for phenyl esters over naphthyl esters, suggesting a role for Arg54 in substrate binding and specificity. The crystal structures of wild-type (1.75 Å) and two mutants, R54G (1.78 Å) and E92A (1.80 Å) were determined. A comparison of the wild-type and mutant structures revealed changes in non-bonded interactions in the active site showing how a single switch residue can modulate substrate specificity in SGNH hydrolases.

![Figure 1. Ribbon diagram of AnAEst monomer with the catalytic residues represented as sticks. The salt-bridge between Arg54 and Glu92 is indicated by a dashed line (PDB ID 1Z8H).](image)
MenJ, a novel oxidoreductase involved in menaquinone biosynthesis that is essential for pathogenesis in *Mycobacterium tuberculosis*

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According to the World Health Organisation, tuberculosis (TB) has recently become the leading cause of death due to infectious disease, surpassing HIV. Challenges to the treatment of TB include drug resistant strains of the causative pathogen, *Mycobacterium tuberculosis* (*Mtb*), and the latency of the bacterium that allows it to persist in a non-replicative, drug-insensitive state. To address these challenges, new therapeutics must be designed that target novel mechanisms essential for *Mtb*’s survival. One such mechanism is the partial saturation of menaquinone, the lipoquinone that functions to ferry electrons across the electron transport chain during oxidative phosphorylation. The enzyme catalysing this reaction, MenJ, is non-essential for *in vitro* growth but is conditionally essential for *Mtb*’s survival within host macrophages. Thus, inhibition of MenJ would potentially prevent *Mtb* from surviving within macrophages. MenJ is a FAD-containing protein, homologous to bacterial geranylgeranyl reductases. Despite *Mtb* utilising menaquinone as the sole lipoquinone *in vivo*, MenJ has been shown to recognise both menaquinone and ubiquinone *in vitro*. Understanding substrate recognition and regiospecificity would be enabled by a high-resolution crystal structure of MenJ. The primary aim of this project is to produce protein crystals of MenJ from *Mtb* or *Mycobacterium smegmatis* suitable for X-ray diffraction analysis, which would be the first step in a target-based drug discovery approach to the design of MenJ inhibitors.
Investigation of lysozyme-like proteins from *Trichomonas vaginalis*

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*Trichomonas vaginalis* is the most common non-viral STI in the world, with more than 143 million new infections each year, infecting 5% of women and 0.6% of men worldwide causing trichomoniasis¹. Infection requires adherence of the protozoa to the epithelial tissue of the urogenital tract, which is inhibited by the native microbiota. Symptoms include urethritis, and vaginal pruritus. Most infections are asymptomatic but confer complications such as increased cervical and prostate cancer risk, and a 2-3-fold increased risk of HIV acquisition. Treatment, limited to metronidazole and tinidazole, is becoming less frequently successful as drug resistant strains are on the rise.

The *T. vaginalis* genome was published in 2007, which identified a highly expanded degradome². Previous work from our group characterised nine NlpC proteins, which demonstrate activity against bacterial cell wall peptidoglycan by cleaving the peptide cross links.

To further explore the repertoire of peptidoglycan degrading enzymes in *T. vaginalis*, we searched the *T. vaginalis* genome for lysozyme-like proteins. Lysozymes are peptidoglycan degrading enzymes which hydrolyse the glycosidic bond between the N-acetylmuramic acid and N-acetyl-D-glucosamine subunits of peptidoglycan. We identified eight candidate lysozyme-like genes. Sequence analysis revealed five contained arginine rich signal sequences like those seen in NlpC proteins. Cloning and expression revealed that removal of signal sequences was required for soluble expression. Turbidity assays are in progress to determine the ability of *T. vaginalis* lysozymes to degrade peptidoglycan extracted from *Escherichia coli* and *Bacillus subtilis*. One lysozyme-like protein has successfully been crystallised and its structure solved to 1.4Å.
Crystal structure of inositol 1,3,4,5,6-pentakisphosphate 2-kinase from *Cryptococcus neoformans*

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The fungal pathogen *Cryptococcus neoformans* is a causative agent of meningoencephalitis in humans. For its pathogenicity, the inositol polyphosphate biosynthetic pathway plays critical roles. In particular, an inositol 1,3,4,5,6-pentakisphosphate 2-kinase (Ipk1) catalyzes the phosphorylation of IP\(_5\) to form IP\(_6\), a substrate for subsequent reaction to produce inositol pyrophosphates, such as PP-IP\(_5\)/IP\(_7\). In fact, deletion of *IPK1* significantly reduces the virulence of *C. neoformans*, indicating that Ipk1 from *C. neoformans* (CnIpk1) is a major virulence contributor. We initiated structural analysis of CnIpk1 to provide structural information for the possible development of drug design for treatment of cryptococcosis. A crystal structure of the unliganded CnIpk1, the first structure for a fungal Ipk1, will be presented at 2.35 Å resolution. Structure comparisons of CnIpk1 with those from *Arabidopsis thaliana* and *Mus musculus* suggest structural similarities and discrepancies for fungal Ipk1 among members of the Ipk1 family. This work was supported by Next Generation BioGreen 21 program of Rural Development Administration (Plant Molecular Breeding Center) of Republic of KOREA.

Figure 1. Overall structure of CnIPK1
Ensemble properties of BAX determine its function

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BAX and BAK are essential mediators of intrinsic apoptosis that permeabilise the mitochondrial outer membrane (MOM). BAX activation requires its translocation from cytosol to mitochondria where conformational changes cause its oligomerisation. Translocation is dependent on the accessibility of a transmembrane domain (α9) that is either exposed or protected (folded in a hydrophobic groove at the surface of the protein). To better understand the critical step of translocation, we examined its blockade by several mutations or by antibody-binding near the N-terminus.

Structural comparisons between the wild type BAX, its mutants or the antibody bound BAX do not explain the translocation defect. Complementary biophysical and biochemical studies, including hydrogen-deuterium exchange (HDX) by mass spectrometry or limited proteolysis revealed evidence of allosteric mechanisms controlling BAX targeting to the MOM. Cytosolic BAX exists as an ensemble of conformers. This ensemble is shifted to conformations in which α9 is sequestered in inhibitory conditions.

Interestingly, the same antibody that blocks cytosolic BAX translocation can activate BAX anchored at the MOM through its α9. In that case, the unfolding of certain helices seen upon binding of the antibody to BAX is able to active BAX as the α9 lock is no more present.

Our data suggest that BAX displays allosteric properties that derive not from differences in equilibrium structure but rather from differences in its ensemble of structures, from which ensues allostery without apparent conformational change (Figure 1).

Figure 1. The ensemble of conformers of BAX can be shifted by mutations or antibody binding at different sites (Robin, Iyer et al. 2018, Structure, 26, 1-14).
Crystal structure of the H3.3 histone chaperone complex subunit HIRA and its functional role

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Inheritance, expression, and repair of eukaryotic genetic materials all involves chromatin assembly. The assembly involves histones and a group of proteins called histone chaperones. The HIRA histone chaperone complex of histone variant H3.3 allowing for its incorporation at transcribed regions and DNA repair sites. The HIRA complex comprises three identified subunits, HIRA, UBN1 and CABIN1. In order to reveal the functional oligomerization state of the HIRA complex, we conducted biochemical and crystallographic analysis.

For crystallization analysis we focused the C-terminal region (aa 644-1017) of human HIRA. We obtained crystal of HIRA(644-1017) after buffer optimization based on differential scanning fluorimetry, and then determined its structure at 2.45 Å resolution. The HIRA(644-1017) formed homotrimer in the crystal as well as in solution according to the SEC-MALS results. The oligomeric state of HIRA C-terminal region was investigated using sedimentation equilibrium analysis with its partner protein CABIN1. This revealed that the HIRA (aa 661-1017)-CABIN1 complex contains three HIRA and two CABIN1 molecules.

Site-directed mutagenesis, and the following cell biological experiments confirmed that (1) HIRA subunit homooligomerizes in the human cell, (2) Trp799 and/or Asp800 are critical residues for homotrimer formation, and (3) HIRA homotrimerization is required for its localization at UV damage sites. These observations strongly suggest that functional oligomeric state of HIRA is homotrimer. We speculate that homotrimeric HIRA might act as a platform for multiple players of transcription or repair machineries present at DNA bubble structures (Ray-Gallet, D et al., Nature Commun. 9, 3013 (2018)).

Figure 1. Crystal structure of homotrimeric HIRA(644-1017)
Molecular dynamics insights into KstR transcriptional regulator function

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The TetR family of transcriptional regulators is widespread in bacteria. They regulate biological processes such as multidrug resistance, biofilm formation, biosynthesis of antibiotics, catabolic pathways, nitrogen fixation, and stress responses amongst others, and as such are often important for pathogenicity. KstR and KstR2 are two TetR family repressors that regulate cholesterol metabolism in Mycobacterium tuberculosis and other actinomycetes. KstR is essential for pathogenesis in M. tuberculosis and is a candidate for drug development.

Molecular dynamics simulations have provided insight into which regions of KstR change conformation, and the extent of conformational deviation that can occur between different states of KstR. This demonstrated distinct conformational paths between apo KstR, DNA-bound KstR, and ligand-bound KstR. Here, we use dynamical network analysis to identify the communication pathways that propagate the allosteric changes that occur in KstR upon ligand and DNA binding. This mechanistic insight will be useful for the design of drugs to selectively inhibit KstR.
Structural-based inhibition studies of phosphatidylcholinespecific phospholipase C

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Phosphatidylcholine-specific phospholipase C (PC-PLC) is an enzyme that catalyses the hydrolysis of phosphatidylcholine (PC), which is a phospholipid, into phosphocholine and diacylglycerol (Figure 1). PC-PLC has emerged as a novel target for the development of new anti-cancer drugs, as enhanced PC-PLC activity was observed during tumour progression. Interestingly, however, the identity of mammalian PC-PLC is not known to date. By using PC-PLC from Bacillus cereus (PC-PLC\textsubscript{Bc}) as a model system, we report our progress in the development of new PC-PLC inhibitors by using a combined structural and biophysicalbased approach. We hope our compounds will enable the development of new anti-cancer agents, as well as facilitating our quest to identify mammalian PC-PLC (e.g. as chemical probe).

Figure 1. PC-PLC catalyses the conversion of phosphatidylcholine to phosphocholine and diacylglycerol.
Structural basis of RhoA recognition by unique guanine nucleotide exchange factor SmgGDS

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SmgGDS has dual functions in cells and regulates small G proteins as both a guanine nucleotide exchange factor (GEF) for the Rho family and a molecular chaperone for small GTPases possessing a C-terminal polybasic region (PBR) followed by four C-terminal residues called the CaaX motif, which is posttranslationally prenylated at its cysteine residue. Our recent structural work revealed that SmgGDS folds into tandem copies of armadillo-repeat motifs (ARMs) that are not present in other GEFs. However, the precise mechanism of GEF activity and recognition mechanism for the prenylated CaaX motif remain unknown because SmgGDS does not have a typical GEF catalytic domain and lacks a pocket to accommodate a prenyl group. Here, we aimed to determine the crystal structure of SmgGDS/farnesylated RhoA complex (Figure. 1). We found that SmgGDS induces a significant conformational change in the switch I and II region that opens up the nucleotide-binding site, with the prenyl group fitting into the cryptic pocket in the N-terminal ARMs. Taken together, our findings could advance the understanding of the role of SmgGDS and enable new drug design strategies for targeting SmgGDS and small GTPases.

Yersinia pseudotuberculosis is a gram-negative bacterium causing yersiniosis. HddC (d-glycero-α-d-manno-heptose-1-phosphate guanylyltransferase) is the fourth enzyme of the GDP-d-glycero-α-d-manno-heptose biosynthesis pathway which is important for the virulence of the microorganism. Therefore, HddC is a potential target of antibiotics against yersiniosis. In this study, HddC from the synthesized HddC gene of Y. pseudotuberculosis (YpHddC) has been expressed, purified, crystallized. Synchrotron X-ray data from a selenomethionine-substituted YpHddC crystal were also collected and its structure was determined at 2.0Å resolution. Structure analyses revealed that it belongs to the glycosyltransferase A type superfamily members with the signature motif GXGXR for nucleotide binding. Despite of remarkable structural similarity, YpHddC uses GTP for catalysis instead of CTP and UTP which are used for other major family members, cytidylyltransferase and uridylyltransferase, respectively. We suggest that EXXPLGTGGA and L(S/A/G)X(S/G) motifs are probably essential to bind with GTP and a FSFE motif with substrate.
Structure determination of HldC from *Burkholderia pseudomallei*


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The crystal structure of D-glycero-b-D-manno-heptose-1-phosphate adenylyltransferase from *B. pseudomallei* (BpHldC), the fourth enzyme of the heptose biosynthesis pathway, has been determined. BpHldC converts ATP and D-glycero-β-D-manno-heptose-1-phosphate into ADP-D-glycero-β-D-manno-heptose and pyrophosphate. The crystal structure of BpHldC belongs to the nucleotidyltransferase α/β phosphodiesterase superfamily sharing a common Rossmann-like α/β fold with a conserved T/HXGH sequence motif. The invariant catalytic key residues of BpHldC indicate that the core catalytic mechanism of BpHldC may be similar to that of other closest homologues. Intriguingly, a reorientation of the C-terminal helix seems to guide open and close states of the active site for the catalytic reaction.
Applying Nanodisc Technology and EM to determine the structures of Odorant Receptors

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Insects have the ability to distinguish among thousands of different odorants at a wide range of concentrations due to their extremely sensitive odorant receptors (ORs). These ORs are housed in the odorant sensory neurons (OSN) which express at least one OR as well as the conserved coreceptor Orco. Insect ORs are a novel family of heptahelical transmembrane proteins that form a distinct sub-class of heteromeric cation channels. Most insect species have one Orco alongside a distinct complement of ORs ranging from 10-300. Orco is known to play a role in regulating the cation channel for olfactory transduction. A recent study has reported a low-resolution cryo-EM structure of Orco existing as a homotetramer. However, it is not known how the Orco subunit interacts with widely divergent ORs to form distinct cation channels for olfactory transduction. A key question remains in regards to the interacting pattern and stoichiometry between Orco and ORs. Answering this question will help us understand how the cation channel is activated by the ORs upon ligand-binding thus regulating the olfactory signal transduction pathway. These ORs are membrane proteins and as such require a phospholipid bilayer to function. This makes structural investigations difficult, hence the lack of structural information to date. Integration of the ORs into a lipid bilayer mimic such as nanodiscs would make the protein soluble and would make structural study by EM manageable. A Nanodisc is a phospholipid bilayer which is encircled by a pair of amphipathic helical proteins. Here we aim to integrate insect ORs in lipid nanodiscs to maintain them in a near-native state, and use electron microscopy to investigate their structure. Understanding the structure of insect ORs will enhance our knowledge about its usage in biosensor technology and pest management strategies.
Non-ribosomal peptide synthetases (NRPSs) are multi-modular enzymes which function as molecular assembly lines to synthesize a wide range of structurally and functionally diverse peptides. A minimum module of an NRPS consists of an adenylation, peptidyl carrier protein (PCP) and a condensation domain. The adenylation domain ‘activates’ a specific amino acid by adenylyating and transferring it to the 4’-phosphopantetheine arm of the downstream PCP domain. The condensation domain catalyzes the formation of a peptide bond between the activated amino acids in two adjacent modules. The resulting peptide is then transferred to the PCP domain of the downstream module. The peptide product is finally released from the terminal PCP domain by a termination domain usually present in the C-terminus of the last module.

X-ray crystallography has been a valuable tool in elucidating the mechanism of NRPSs, which are characterized by significant domain movements resulting in several intra and inter-domain interactions during different steps of peptide synthesis. One such unexplored interaction is of the termination domain with its upstream PCP domain occurring during the product release step of peptide synthesis. Reductase domains are termination domains known to catalyse NAD(P)H dependent reductive release of their peptide products. In this work, we present the first crystal structure of an NRPS reductase domain along with its upstream PCP domain of an NRPS enzyme (mru_351) from Methanobrevibacter ruminantium which will contribute to our understanding of inter-domain interactions of the product release domains.

Figure 1. Ribbon diagram of the PCP-reductase di-domain structure of the NRPS mru_351 from Methanobrevibacter ruminantium. The PCP domain is shown in red, reductase domain in cyan and 4’-phosphopantetheine in green.
Structural studies on carbapenem-hydrolyzing class D serine β-lactamases from *Acinetobacter baumannii*

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The class D serine β-lactamases comprise a superfamily of over 660 enzymes capable of conferring high-level resistance to β-lactam antibiotics, predominantly the narrow-spectrum penicillins including oxacillin and cloxacillin. Recently it was discovered that some members of the class D superfamily have evolved the ability to deactivate carbapenems, “last resort” β-lactam antibiotics generally held in reserve for highly drug resistant bacterial infections. These enzymes are collectively known as Carbapenem-Hydrolyzing Class D serine β-Lactamases or CHDLs. The mechanism of β-lactam deactivation by the class D serine β-lactamases involves the covalent binding of the antibiotic to an active site serine to form an acyl-enzyme intermediate (acylation). This is followed by hydrolysis of the covalent bond (deacylation), catalyzed by a water molecule activated by a carboxylated lysine residue. It was initially thought that the carbapenems acted as potent inhibitors of the class D enzymes since the formation of the covalent acyl-enzyme intermediate effectively expelled all water molecules from the active site, thus preventing the deacylation step. Our structural studies on two CHDLs have indicated that their carbapenem hydrolyzing ability may be due to two surface hydrophobic residues which allow for the transient opening and closing of a channel through which water molecules from the milieu can enter the binding site to facilitate the deacylation reaction. Although the hydrophobic residues responsible for the channel formation are present in all class D β-lactamases, sequence and structural differences nearby may be responsible for the evolution of carbapenemase activity in the CHDLs.

Figure 1. The surface of OXA-143 calculated with Val130 in the open conformation, showing a hole which opens into the active site.
Battling resistance to the last-line of defence antibiotics by targeting MCR-1

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The emergence of a novel mobile colistin resistance gene (mcr-1) has jeopardised the use of polymyxins, last resort antibiotics that are used increasingly to treat infections caused by multidrug-resistant (MDR) Gram-negative pathogens. The gene encodes a 60.1-kDa cytoplasmic transmembrane protein known as MCR-1 that is predicted to be a phosphoethanolamine (pEtN) transferase by multiple sequence alignment. The activity of MCR-1 as a pEtN transferase was recently confirmed by both in vitro and in vivo experiments where the protein was shown to attach a pEtN moiety to the lipid A head groups of lipopolysaccharide (LPS), one of the major constituents of the Gram-negative bacterial membrane.

The primary focus of this research is to design and develop molecular inhibitors against MCR-1, guided by virtual screening, differential scanning fluorimetry, and WaterLOGSY. We also incorporate crystallographic methods to elucidate the soluble domain structure of MCR-1 and aim to characterise the protein-inhibitor interaction.

Figure 1. Full-length model of MCR-1 produced from crystal structure and homology models¹,²

Crystal structure of TAZ-TEAD complex reveals a distinct interaction mode from that of YAP-TEAD complex

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The Hippo pathway is a tumor suppressor pathway that is implicated in the regulation of organ size. The pathway has three components: the upstream regulatory factors, the kinase core, and the downstream transcriptional machinery, which consists of YAP, TAZ (transcription co-activators) and TEAD (transcription factor). Formation of YAP/TAZ-TEAD complexes leads to the transcription of growth-promoting genes. Herein, we report the crystal structure of TAZ-TEAD4 complex, which reveals two binding modes. The first is similar to the published YAP-TEAD structure. The second is a unique binding mode, whereby two molecules of TAZ bind to and bridge two molecules of TEAD4. We validated the latter using cross-linking and multi-angle light scattering. Using siRNA, we showed that TAZ knockdown leads to a decrease in TEAD4 dimerization. Moreover, the results from luciferase assays, using YAP/TAZ transfected or knockdown cells, give support to the non-redundancy of YAP/TAZ co-activators in regulating gene expression in the Hippo pathway.
Analysis of the filamentous type II Chaperonin from *Sulfolobus solfataricus* by crystallography and cryo-EM.

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Thermophilic Factor 55 (TF55, rosettasome) is a type II chaperonin occurring in Sulfolobus. Filaments composed of TF55 have previously been observed *in vivo* and *in vitro*, but the nature of the interactions between successive subunits has not been established. We have used cryo-EM and crystallography to solve the filamentous structure of TF55 \(\beta\) isolated from *Sulfolobus solfataricus*. Crystallographic studies show a possible filament arrangement, which we have confirmed using averaging of cryo-EM micrographs taken on individual filaments. The oligomerisation surface relies on overlap of the helical protrusions implying that these filaments may play a role in the regulation of chaperonin activity.
Structural studies of viroplasm major component protein Pns12 from Rice Dwarf Virus

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Rice Dwarf Virus (RDV), the causal agent of rice dwarf disease, infects rice plants and negatively affects rice production in East Asia. RDV is transmitted to rice plants by vector insects, leafhoppers. RDV is a member of the genus Phytoreovirus of the family Reoviridae. The Reoviridae has a wide host range, including human, animals and plants. RDV has an icosahedral double-layered shell with the diameter of approximately 70 nm and contains 12-segmented double stranded RNAs (S1 to S12) as the genome. The genome encodes 7 structural (P1, P2, P3, P5, P7, P8, P9) and 5 nonstructural (Pns4, Pns6, Pns10, Pns11, Pns12) proteins. The nonstructural proteins Pns6, Pns11 and Pns12 of RDV were reported as constituents of a viral factory, called viroplasm. The viroplasm assembles in the cytoplasm of infected cells. The replication and assembly of RDV inner capsid occur inside viroplasms and the outer capsid assembles at periphery of the viroplasms. In addition, Pns12 forms viroplasm like inclusions in non-host insect Sf9 cells (Wei et al., 2006) and Pns12 silenced rice plants are resistant against the RDV infection (Shimizu et al., 2009). Pns12 has a critical role in the virus replication. To reveal the assembly mechanism of RDV in the viroplasm, we determined the structure of viroplasm major component protein Pns12 by the X-ray crystallography and the phase contrast cryo-electron microscopy. The structure revealed the unique multimer of the C-terminal domain and disordered regions derived from the N-terminal.
Structural basis of ascorbate-dependent iron reduction by human duodenal cytochrome b (Dcyt b) involved in intestinal iron absorption

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Dietary iron absorption is regulated by duodenal cytochrome b (Dcyt b), an integral membrane protein that catalyzes reduction of non-heme Fe³⁺ by electron transfer form ascorbate (Asc) across the membrane. This step is essential to enable iron uptake by the divalent metal transporter. Here we report the crystallographic structures of human Dcyt b and its complex with Asc and zinc ion. Each monomer of the homodimeric protein possesses cytoplasmic and apical heme groups, as well as cytoplasmic and apical Asc binding sites located adjacent to each heme. Zn²⁺ coordinates to two hydroxyl groups of the apical Asc and to a histidine residue. Biochemical analysis indicates that Fe³⁺ competes with Zn²⁺ for this binding site. These results provide a structural basis for the mechanism by which Fe³⁺ uptake is promoted by reducing agents and should facilitate structure-based development of improved agents for absorption of orally administered iron.

Figure 1. Overall structure of human Dcyt b in complex with ascorbate and zinc ion.
Organometallic Complexes and their Side Chain Specific Reactions with a Model Protein

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New Zealand suffers from one of the highest rates of cancer incidents per year, bearing a huge strain on the health system. Platinum based anticancer agents are currently used in 50% of cancer treatments.³ However, the interaction with multiple binding partners beyond the major target DNA, patients treated with these platinum based therapies suffer a large number of side effects. To improve the pharmacological properties there has been a fervent search for the next ‘blockbuster’ metal-based anticancer drug. This search led to the advent of organometallic piano-stool complexes. This scaffold holds a number of favourable properties and through relatively small changes to the ligand structures, significant changes in the biological activity are observed.²

To better understand the structure reactivity relationships that these compounds have with proteins we have investigated the interactions between different organometallic compounds and the model protein hen egg white lysozyme (HEWL). In particular we have focussed on the impact of metalation on protein.³ Using protein crystallography, the interaction of HEWL and [Ru(cymene)(NHC)Cl₂] ligand exchange was observed with the Ru centre coordinating to His15 and Arg14 (Figure). In addition, the p-cymene ligand was cleaved which was concomitant with an oxidation of the ruthenium metal centre from RuII to RuIII, as confirmed by electron paramagnetic resonance spectroscopy (EPR).⁴ Changes in the metal centre alter binding characteristics and the site of interaction. These studies demonstrate that small changes in organometallic compounds can have significant effects on both their ability to bind and the mode of binding to a protein substrate.

Figure: Ru II(η⁶-p-cymene)(1,3-dimethylbenzimidazol-2-ylidene)Cl₂ interacts with HEWL, upon an binding an oxidation occurs forming a [Ru III(1,3-dimethylbenzimidazol-2-ylidene)(H₂O)Cl₂] fragment attached to Arg14 and His15 of HEWL.

References:

Insights into the chromosome partitioning system, ParABS
Chromosome segregation is regulated by the ParABS system that is an important biological process in all domains of life. ParABS system includes ParA (an ATPase), ParB (a parS binding protein) and parS (a centromere-like dsDNA). The homologous proteins of ParA and ParB in Helicobacter pylori are HpSoj and HpSpo0J, respectively. We characterized the binding of parS and HpSpo0J and solved the crystal structure of the HpSpo0J-parS complex, with two HpSpo0J molecules bind with one parS. HpSpo0J interacts vertically and horizontally with its neighbors through the N-terminal domain to form an oligomer. These adjacent and transverse interactions might be needed for molecular assembly of a high order nucleoprotein complex and for ParB spreading. In addition, the ATPase activity of HpSoj was determined and the non-specific DNA binding of HpSoj dimer was detected. Based on these findings, we propose a structural model for the HpSoj and HpSpo0J complex in the ParABS partitioning system.
Interactions at the centre of TRIM28 targeted transcriptional silencing of retroviral pathogens
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TRIM28 (KAP1/TIF1β) acts as the universal co-repressor for the single largest family of transcription factors in mammals, namely the Krüpple-associated box containing-Zinc Finger Proteins (Krab-ZFPs). Krab-ZFPs recognise a specific DNA sequence via their zinc-finger domains and recruit TRIM28 via their conserved Krab domain. Once recruited TRIM28 undergoes auto-SUMOylation and the SUMOylated TRIM28 acts as a scaffold to recruit repressive chromatin modifiers including histone methyltransferase SETDB1, the NuRD histone deacetylase complex, heterochromatin protein 1 (HP1) family, and DNA methyltransferases.

TRIM28 belongs to the TRIM protein family with over 80 members in humans. They share a conserved N-terminal tripartite motif (TRIM), also known as the RBCC as it consists of a RING domain, one or two B-box domains and an antiparallel coiled-coil. Using biophysical techniques we have identified the domain responsible for higher order assembly in TRIM28, and determined the crystal structure of the assembly interface. We have also performed structure-based mutagenesis to investigate the functional importance of TRIM28 self-assembly. We have determined the binding affinity and stoichiometry of the TRIM28/Krab-ZFP interaction, and undertaken Small Angle X-ray Scattering (SAXS) to determine the molecular envelope of the TRIM28 RBCC/MBP-Krab complex. This model suggests the Krab domain binds to the central part of the TRIM28 coiled-coil, consistent with our results from domain mapping using a series of recombinant TRIM28 truncation constructs.
A structural insight into NRZ mediated apoptosis regulation in zebrafish

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Bcl-2 family proteins play a crucial role in regulating apoptosis, a process critical for development, eliminating damaged or infected cells, host-pathogen interactions and in disease. Dysregulation of Bcl-2 proteins elicits an expansive cell survival mechanism promoting cell migration, invasion and metastasis. Through a network of intra-family protein-protein interactions Bcl-2 family members regulate the release of cell death factors from mitochondria. NRZ is a novel zebrafish pro-survival Bcl-2 orthologue resident on mitochondria and the endoplasmic reticulum (ER). However, the mechanism of NRZ apoptosis inhibition has not yet been clarified. Here, we examined the interactions of NRZ with pro-apoptotic members of the Bcl-2 family. We show that NRZ binds almost all zebrafish pro-apoptotic proteins and displays a broad range of affinities. Furthermore, we define the structural basis for apoptosis inhibition of NRZ by solving the crystal structure of both apo-NRZ and a holo form bound to a peptide spanning the binding motif of the proapoptotic zBad, a BH3-only protein orthologous to mammalian Bad. The crystal structure of NRZ revealed that it adopts the conserved Bcl-2 like fold observed for other cellular pro-survival Bcl-2 proteins and employs the canonical ligand binding groove to bind Bad BH3 peptide. Our findings provide a detailed mechanistic understanding for NRZ mediated antiapoptotic activity in zebrafish and suggest that NRZ likely occupies a unique mechanistic role in zebrafish apoptosis regulation.

References

Characterising the interaction between Fv1 and members of the mammalian ATG8 family

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The co-evolution of retroviral pathogens and mammals has led to development of intracellular defence systems to combat retroviruses. Retroviral replication can be restricted at multiple stages of the retroviral life-cycle by anti-viral proteins termed restriction factors. Fv1 is the prototype restriction factor that was first discovered to determine susceptibility of mouse cells to MLV. Fv1 has phenotypic similarities to the unrelated restriction factor TRIM5α, both recognise the viral capsid lattice and block replication prior to integration of the provirus into the host genome. The similarity extends to the functional domain architecture of an N-terminal dimerization domain and a C-terminal recognition domain. TRIM5α has been proposed to act as an autophagic receptor for recruitment of HIV-1 capsid to the autophagosome by interacting with the mammalian ATG8 protein family. The ATG8 family interact with proteins containing a well characterised LIR binding motif principally consisting of two hydrophobic residues that bind conserved hydrophobic pockets in the ATG8 proteins. Recently, the Fv1 N-terminal domain (20-200) was demonstrated to bind to the ATG8 protein LC3B. We investigated binding of Fv1 to the ATG8 family by sedimentation analytical centrifugation (SV-AUC) experiments, demonstrating Fv1 (20-200) bound all 6 members. A saturation binding experiment was undertaken on 5 ATG8 proteins showing the binding affinity for Fv1 is very weak. To identify the Fv1 binding site we made a series of Fv1 (20-200) constructs containing single aromatic-to-alanine mutations and investigated binding to LC3B by SV-AUC.
Improving the low-temperature activity of β-mannanase based on its structural information

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Cold adaptation of enzymes is a favorable character for their industrial application because it can reduce heating costs to reach the optimal temperature for enzymatic activities. Structural feature of cold-adapted enzymes is regarded as high flexibility. On the other hand, the high flexibility can cause structural instability, resulting in lower enzymatic activity. Therefore, the good balance between structural flexibility and stability is crucially important for industrial application of enzymes. We focused the regulation of salt bridges in protein structures, which contribute to structural stabilities. Endo-1,4-β-mannanase have been used for various bioprocesses such as bleaching of softwood pulps, declining viscosity of feeds and foods, and clarifying beverages. We have already reported that the earthworm Eisenia fetida has some cold-adapted enzymes, however, mannanase from E. fetida (Ef-Man) showed only weak activity at lower temperatures (~30 ºC). We determined the crystal structure of Ef-Man at 1.7 Å resolution (Fig. 1(a)). The overall structure of Ef-Man is similar to those of the glycoside family 5 family proteins, and tertiary structures around the active site are conserved among endo-1,4-β-mannanase families. Ef-Man has 12 salt bridges, and we focused three salt bridges which have pairwise and bifurcated hydrogen bonding interaction between side chains (Fig.1(b)). We designed and produced three Ef-Man mutants, R125K, R213K, and R302K. Enzymatic activities in all mutants improved at lower temperatures, especially the activity of R302K was about three times higher than that of wild type at 20 ºC.

Figure 1. Crystal structure of Endo-1,4-β-mannanase from Eisenia fetida. (a) Overall structure. (b) Pairwise and bifurcated hydrogen bonding interaction in salt bridge
Structural basis for modulating ERp44 chaperone activity with designed peptides
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Type 2 diabetes is a growing, worldwide epidemic. Adiponectin, a hormone naturally produced by adipocytes, confers insulin sensitivity and other metabolic properties that suppress the onset of diabetes. However, secreted levels of adiponectin decrease under oxidative stress conditions, e.g. obesity, due to aberrant sequestration by the chaperone ER resident protein 44 (ERp44). We aim to increase circulating levels of adiponectin by introducing competitive peptides to disrupt the ERp44-adiponectin interactions with an aim to mitigate obesity-related metabolic syndrome.

ERp44 is a protein that modulates disulphide bond formation in its client proteins: adiponectin, IgM antibodies, serotonin transporter, and peroxiredoxin 4 (Prx4). Previous in vivo studies showed altered levels of adiponectin secretion when peptides derived from adiponectin and IgM sequences were introduced to mice: the adiponectin-derived peptide decreased adiponectin secretion while the IgM-derived peptide increased adiponectin secretion.

Structural information on the ERp44 active site (Figure 1) binding to its client proteins could shed light onto why particular sequences affect secretion, but the current information is sparse: only one structure is available on the ERp44-Prx4 complex and suggested it is a backbone-mediated interaction. However, results from our group showed sequence-dependent effects.

Additional peptides combining sequence features from both the adiponectin and IgM sequences have been subjected to in vivo studies to determine the role of particular residues in the peptide sequence. Efforts to crystallise ERp44 in its apo and peptide-bound forms with sufficient resolution to characterize protein-peptide interactions will be presented in conjunction with computational docking calculations, leading to new insights on ERp44 mechanism.

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Figure 1. Close-up view of the ERp44 active site showing the key Cys29 residue (magenta). There are two states to ERp44: the “off” state where the C-terminal tail (orange) folds over and protects the active site (shown), and the “on” state where the tail moves away and ERp44 is ready to accept its client protein.
The aldehyde dehydrogenase (ALDH) superfamily is a large group of enzymes that catalyze the NAD$^+$-dependent oxidation of aldehydes to carboxylic acids. Humans have 19 different ALDHs, which process a diverse array of substrates, including small aldehydes, amino acid derivatives, and lipids. ALDHs are important for detoxification of reactive aldehydes, amino acid metabolism, embryogenesis and development, neurotransmission, oxidative stress, and cancer. Consistent with the diversity of substrates and widespread tissue distribution of ALDHs, mutations in ALDH genes are associated with numerous inherited metabolic disorders. ALDH16 is the least understood member of its superfamily. Although found in organisms ranging from bacteria to man, its physiological substrates and biological functions are poorly understood. Notably, ALDH16 differs from other ALDHs in having a ~250-residue domain of unknown function not found in other ALDHs. Another remarkable feature is that some ALDH16 enzymes lack the catalytic Cys residue found in all other ALDHs, prompting speculation that they are pseudo-enzymes that function in protein-protein interactions. To better understand the molecular function of this enigmatic enzyme and provide a foundation for exploring its biological functions, we have determined the first crystal structure of an ALDH16 enzyme. Structures of ALDH16 in various states of ligation were determined at resolution limits ranging from 1.5 Å to 2.3 Å, with refined R-factors of 15-19%. The oligomeric structure of ALDH16 in solution was studied using small-angle X-ray scattering. The structural data not only reveal the fold and function of the domain of unknown function, but also uncover a fascinating example of trans-hierarchy structural similarity in which the tertiary structure of one protein mimics the quaternary structure of another.
Structural insights into the allosteric binding sites of the M5 muscarinic acetylcholine receptor

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The five muscarinic acetylcholine receptors (mAChRs M1-M5) are an important family of class A G protein-coupled receptors that are widely expressed throughout the central and peripheral nervous systems. Alterations in mAChR levels and activity, particularly concerning the M1, M4, and M5 mAChR subtypes, have been implicated in the pathophysiology of major neurological and psychiatric diseases, including Alzheimer's disease, schizophrenia, and drug addiction. As such, mAChRs have remained essential targets for drug discovery, despite the difficulty associated with designing molecules to target the highly conserved orthosteric binding site selectively. While crystal structures of the M1-M4 mAChRs have been determined, there are only a few ligand-bound structures that exist, including no structures of the M5 receptor. Here we report the first crystal structure of the M5 mAChR allowing a full comparison of all five subfamily members bound to structurally similar ligands. In addition, recently discovered highly selective M5 allosteric modulators have provided the opportunity for a deeper understanding of the receptor's physiological and therapeutic relevance; however, questions remain about how such ligands interact with the receptor. In an attempt to address these questions, we utilized structural, biophysical, and pharmacological experiments to examine how different types of allosteric modulators bind to the M5 receptors. Overall, our results indicate that the highly selective M5 allosteric modulators do not bind to the prototypical mAChR allosteric site.

Figure 1. Structure of the M5 mAChR.
Crystal structure of PCAF-homology domain of GCN5 reveals its unique structure as ubiquitin E3 ligase

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The acetyltransferase is one of post-translational enzymes and transfers acetyl group to target enzyme. GCN5 is well known as a histone acetyltransferase to promote transcriptional activity. GCN5 is composed of three domains: PCAF-homology domain (PCAF-HD), acetyltransferase (AT) domain and Bromo domain. Both AT and Bromo domains are highly conserved among protist and many structural studies relating to their histone acetyltransferase activity have been reported. However, PCAF-HD is only conserved among vertabrate and its structure and function have not been reported. Previous study indicated that the PCAF-HD would have novel Ubiquitin E3 ligase folding. In this study, we focused on uncharacterized PCAF-HD of GCN5 and performed structural based study for elucidating its function as Ubiquitin E3 ligase. We succeeded to determine the crystal structure of PCAF-HD of GCN5 at 1.8Å resolution. PCAF-HD consists of helices only and the whole folding is completely distinct from already reported Ubiquitin E3 ligase proteins. Interestingly the two clear and strong electron densities were observed, and they were identified as Zn atom by using XAFS analysis. This result showed that this PCAF-HD has unique binuclear Zn finger motif (Zn2Cys5His2). We prepared several mutants at the Zn finger domain and revealed that the Zn finger domain is responsible for Ubiquitin E3 ligase activity of GCN5. In this study, we revealed that PCAF-HD has Ubiquitin E3 ligase activity with unique binuclear Zn finger domain. This result opens new avenues for both functional study of GCN5 and ubiquitin biology.

Figure 1. Crystal structure of PCAF-HD of GCN5
Smallpox virus chemokine-binding protein: a potential therapeutic approach

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Smallpox virus, the deadliest poxvirus, infected humans for thousands of years before its eradication through vaccination. The compelling virulence of smallpox virus was aided by the utilization of immunomodulatory proteins. Among which, chemokine binding proteins (CKBPs) play a crucial role as they empowered the virus to evade host immune cells detection. However, the overexpression of chemokines, which takes place following microbial infection, also occurs during autoimmune and inflammatory diseases and during cancer. Smallpox virus CKBPs, which have been adapted by evolution for targeting human hosts, might have a role in the treatment of conditions associated with chemokine overexpression.

In this project, one smallpox CKBP, known as G3R, was expressed in HEK293E, purified and crystallized. The G3R protein crystallized in P2₁ and was solved at 2.5 Å. It adopts a β-sandwich fold, consisting of two stacked β-sheets one with a positive electrostatic surface and one with a negative electrostatic surface.

Structural work on G3R was supported by studies of its binding affinity to chemokines from different classes. These measurements were conducted using ELISA, SPR and ITC methods. Trans well migration assays was also done. These affinity assays demonstrate that G3R binds primarily to CC class chemokines but also to some other classes with lower affinity. The migration assays confirmed the inhibitory effect of G3R on the migration of leukocytes.

Co-expression of G3R and hCCL2 in HEK293E has also been undertaken and purification of this complex has been confirmed using mass spectrophotometry, DLS and MALS. Crystallization of G3R with CCL2 is under process as well as other biological assays.
Novel antigen-binding chimeric proteins as tolls in crystallography and cryo-EM.

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Next to X-ray crystallography, single particle cryo-EM has become a versatile technique for the structural analysis of macromolecular complexes. Although instrumentation and methods for data analysis improve steadily, the tools are still missing to analyse small size, low symmetry, and highly flexible particles to high resolution. In addition to the homogeneity of a given sample, the highest achievable resolution of the 3D reconstruction is greatly dependent on the ability to iteratively refining the orientation parameters of each individual particle to high accuracy. Whereas large molecules are relatively easy to recognize in noisy low-dose images of frozen hydrated samples, and these particles have sufficient structural features to facilitate accurate determination of their orientation parameters, the process of collecting and processing images of small particles is much more difficult. Preferred particle orientation due to the surface properties of the macromolecules that cause specific regions to preferentially adhere to the air–water interface or substrate support also represents a recurring problem in cryo-EM.

To overcome these inherent performance barriers of cryo-EM and in order to improve crystal contact a crystal lattice, we designed rigid antibody chimera we call Megabodies (Mbs). These Mbs are built from Nanobodies (Nbs) that are grafted onto large scaffold proteins via two short peptide linkers to produce stable and rigid monomers. Nbs are the small (15kDa) and stable single domain fragments harbouring the full antigen-binding capacity of the original heavy chain only antibodies that naturally occur in Camelids. The useful applications of Nbs in structural biology are numerous but on their own they are too small for common applications in single particle cryo-EM. We anticipated that megabodies would add enough mass and defined features, even to relatively small target proteins, for accurate image alignment of the vitrified particles in cryo-EM.

Figure 1. Novel Nanobody-based chimeric antigen binding proteins. A Crystal structure of β2 adrenergic receptor (β2AR, green) in a complex with Nb80 (purple). B Models of two different Nb80-derived rigid chimeric proteins, called megabodies.

Copper is an essential trace element required for all living organisms, however, it is potentially toxic when in excess due to its redox activity and ability to disrupt protein-bound iron sulfur clusters. Copper has long been used as an antibacterial, however diminished effectiveness of this agent has increasingly been observed with the evolution of copper resistant bacteria. This is of particular significance to agriculture, where reagents such as copper sulfate provide affordable solutions to the control of pathogenic organisms. Typically, copper tolerant organisms possess plasmid-encoded copper resistance systems, termed Pco/Cop (plasmid-borne copper resistance/copper resistance) that enable survival at millimolar concentrations of copper.

The plant growth-promoting bacterium *Pseudomonas fluorescens* SBW25 features a chromosomal *cop* operon encoding two Cop proteins CopCD whose expression is sensitive to environmental copper levels. Unlike previously characterized CopC proteins, that include both Cu(I) and Cu(II) binding sites, *Pf*-CopC lacks the side-chains anticipated for a Cu(I) site and has an altered sequence for a ‘typical’ Cu(II) site. In addition, *Pf*-CopC binds Cu(II) with significantly tighter affinity than other bacterial CopC proteins characterized to date.

In order to understand how CopC proteins contribute to bacterial copper resistance, and the molecular details of their metal binding properties, we have crystallized and determined the structure of the *Pf*-CopC protein in its native, Cu(II)bound form. The structure reveals that the metal binding site is formed by ligands provided by histidine and aspartic acid side chains and the N-terminus of the protein and suggests structural motifs, which contribute to the tight copper binding property of this protein. The structure also places *Pf*-CopC within a large, predominant class of copper resistance proteins, with relevance to a wide variety of bacterial pathogens.
Deciphering how bacteria eat algae - structural and functional characterization of ulvan degrading lyases

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Marine green algae represents a huge potential biomass for the biorefinery processes. Polysaccharides present in their cell wall play a structural role and are major contributors to their biomass. Bacteria cohabiting with green algae produce a cascade of enzymes to depolymerize the polysaccharide for nutrient source. These enzymes potentiate biotechnological processing of algal biomass. Ulvan a major cell wall polysaccharide of green algae and its enzymatic depolymerization has been less explored. Genes encoding ulvan degrading lyases have been recently discovered in several marine bacteria. This study contributes to the first structure and the detailed molecular mechanism of ulvan degradation. Using X-ray crystallography, we solved the structure and biochemically characterized three ulvan lyases, PLSV3936, LOR107 and NLR48. All three enzymes share very low sequence identity and act differentially on substrate. The ulvan lyases LOR107 and PLSV3936 share the 7-bladed β-propeller fold and NLR48 has a β-jelly roll fold. Despite different structural scaffold, the β-elimination catalytic machinery is conserved among these enzymes. However, the complex structure with the bound tetrasaccharide substrate reveals the difference in the active site and their mode of substrate cleavage. This reflect the plasticity adopted by these enzymes to accommodate the heterogeneity of ulvan. Overall, our structural data will serve as a template and aid in engineering ulvan lyases for industrial applications.
Crystal structure of the substrate bound form of biliverdin reductase with unpredicted substrate-stacked geometry revealed unique reaction mechanism

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Biliverdin reductase (BVR), the enzyme responsible for the last step in heme degradation, produces the major lipophilic antioxidant bilirubin (BR). BVR reduces the γ-methene bridge of biliverdin (BV) using NAD(P)H as a cofactor. The molecular mechanism of the BVR-mediated reaction, in particular the BV binding site and catalytic residues, remains undetermined. To elucidate these details, we determined the crystal structure of cyanobacterial BVR in complex with substrate and cofactor (\textit{Nat. Commun}, 8:14397 (2017)).

The structure reveals an unprecedented mode of substrate binding: two BV molecules (proximal and distal relative to the bound NADP\textsuperscript{+}) bind with stacked geometry in the active site. The substrate binding stoichiometry of cyanobacterial BVR in solution determined by the titration experiment agreed closely with the crystal structure. In addition, the titration curve also indicated that two BV molecules bound in both human and rat BVRs. Therefore, the mode of substrate binding is common among all BVRs.

We investigated the catalytic residues based on this structure; mutagenesis and steady-state kinetics demonstrated that Arg185 in the active pocket is the proton donor. This arginine residue is completely conserved among BVRs, but is located far (~6 Å) from the reducing site in the bound BV. Instead, the Arg185 interacts with the propionate side chain of the proximal BV. The geometry of the bound BVs suggests that the proton is transferred from Arg185 via the propionate side chains of the stacked BVs. We will discuss the reaction mechanism of BVR based on these structural features.
Molecular architecture of the *E. faecalis* anti-termination protein EutV bound to RNA

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Regulated transcription termination provides an efficient and responsive means to control gene expression. Rho-independent termination occurs through the formation of a RNA stem-loop which disrupts the RNA polymerase elongation complex. In anti-termination, a mutually exclusive RNA structure is formed, and in some cases stabilised by ANTAR domains of proteins, preventing termination. We have determined the novel 3.8 Å crystal structure of the stabilising anti-terminator protein EutV, bound to RNA. Our studies highlight the key interactions between conserved EutV residues and the RNA, as well as protein conformational changes undergone upon RNA binding. This has allowed us to propose a broad model for ANTAR domain anti-termination.

Figure 1. Structure of the dimeric EutV from *E. faecalis*
Structure studies of the Fam20 kinases

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The family with sequence similarity 20 (Fam20) kinases function in the secretory pathway to phosphorylate proteins and proteoglycans. The human genome encodes three Fam20 paralogues: Fam20A, Fam20B, and Fam20C. Fam20C is the physiological casein kinase and phosphorylates hundreds of proteins involved in biomineralization, phosphate metabolism, cell adhesion and migration, and cardiac function. Diminished activity of Fam20C causes Raine syndrome, an incurable malady associated with severe calcification disorders. Fam20A functions as a pseudokinase to enhance Fam20C activity and mutations in FAM20A lead to dental and renal abnormalities. Fam20B regulates the biosynthesis of proteoglycans by phosphorylating a xylose residue within the tetrasaccharide linker region of heparin and chondroitin sulfate proteoglycans. Here we systematically investigate the structure and function of the Fam20 kinases. We show that Fam20C activation results from the formation of an evolutionarily conserved homodimer or heterodimer with Fam20A. Compared to Fam20C itself, Fam20A has an optimized Fam20C-binding surface and is therefore a specialized Fam20C-allosteric activator. We further show that the monomeric Fam20B xylosylkinase activity preceded the appearance of the dimeric Fam20C protein kinase in animals. The crystal structure of a Fam20B orthologue in complex with the Galβ1-4Xylβ1 disaccharide reveals the substrate recognition mechanism of Fam20B. Further structural analyses suggest that the dimerization trait of Fam20C has emerged concomitantly with a change in substrate specificity. These results provide comprehensive insights into the function of this unique and biomedically important family of kinases and shed light on their evolutionary history.
Structural basis for reactivating the mutant TERT promoter by cooperative binding of p52 and ETS1

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Transcriptional factors ETS1/2 and p52 synergize downstream of non-canonical NF-κB signaling to drive reactivation of the −146C>T mutant TERT promoter in multiple cancer types, but the mechanism underlying this cooperativity remains unknown. Here we report the crystal structure of a ternary p52/ETS1/−146C>T TERT promoter complex. While p52 needs to associate with consensus κB sites on the DNA to function during non-canonical NF-κB signaling, we show that p52 can activate the −146C>T TERT promoter without binding DNA. Instead, p52 interacts with ETS1 to form a heterotetramer, counteracting autoinhibition of ETS1. Analogous to observations with the GABPA/GABPB heterotetramer, the native flanking ETS motifs are required for sustained activation of the −146C>T TERT promoter by the p52/ETS1 heterotetramer. These observations provide a unifying mechanism for transcriptional activation by GABP and ETS1, and suggest that genome-wide targets of non-canonical NF-κB signaling are not limited to those driven by consensus κB sequences.

Figure 1. A ribbon diagram showing a p52/ETS1 heterotetramer bound to −146C>T TERT promoter DNA observed in the crystal lattice.
Comprehensive structural studies reveal the evolution of strigolactone/karrikin selectivity of HTL and D14 in *Striga*

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*Striga hermonthica* is a weed that is causing severe crop yield loss in sub-Saharan Africa. The germination of *Striga* is induced by a host-secreted terpenoid called strigolactone (SL). Recently it has been showed that the perception of SL was mediated by ShHTL proteins. Eleven members of ShHTL in *Striga* have been identified and categorized into 3 phylogenetic clades: conserved-, intermediate- and divergent-clades. ShHTLs belonging to different clades showed different responses to SL and KAR (another germination stimulator; putative ligand of HTL in *Arabidopsis*).

We first determined the crystal structures of different clades of ShHTLs and ShD14 (a paralog of ShHTLs). Structural overlay of ShHTLs and ShD14 showed that these structures were highly similar to one another there were apparent structural differences in helix αD1, which was located at the entrance of the ligand-binding pocket. The structural differences in the cap domain appeared to arise from residue 150 on loop αD1-αD2. It is reasoned that Y150 was evolved to F150 in a subgroup of the divergent clade, thus resulting in the larger ligand-binding pockets of ShHTLs of divergent clade. Moreover, ShHTLs of divergent clade have more unbulky residues and thus larger ligand-binding pockets. Consequently, ShHTLs of divergent clade can recognize various SLs while ShHTLs of conserved and intermediate clades with smaller pocket can bind only the smaller molecule KAR. These results provide structural insights into the evolution of ligand specificity of ShHTLs and ShD14 and will provide information for the design of *Striga* germination stimulants.
Status of neutron time-of-flight single-crystal diffraction data processing software STARGazer

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STARGazer is a data processing software for neutron time-of-flight (TOF) single-crystal diffraction data collected by the IBARAKI Biological Crystal Diffractometer (iBIX) at the Japan Proton Accelerator Research Complex (J-PARC). This software creates hkl intensity data from three-dimensional (x, y, TOF) diffraction data. STARGazer is composed of both a data processing component and a data visualization component. The former is used to calculate the hkl intensity data. The latter displays the three-dimensional diffraction data with searched or predicted peak positions and is used to determine and confirm integration regions. STARGazer has been developed to make it easier to use and obtain more accurate intensity data. For example, a profile-fitting method for peak integration was developed and data statistics were improved. STARGazer and its manual composed of installation and data processing components have been prepared and contributed to iBIX users. This poster describes the status of data processing software STARGazer.

Figure 1. Graphical user interface of STARGazer.
Teleocidin B is a toxin against aquatic organisms produced by *Streptomyces*. It also shows potent tumor-promoting activity via protein kinase C pathway, and therefore it is considered as an important natural product in the pharmaceutical field. Naturally purified teleocidin B is a mixture of four stereoisomers (Teleocidin B1, B2, B3 and B4). The enzyme TleD catalyzes teleocidin A1 to teleocidin B1, teleocidin B4 and des-O-methyl-olivoretin C, which contains a monoterpenoid cyclization step. TleD is an S-adenosyl-L-methionine dependent methyltransferase and acts as one of the key enzymes in the teleocidin B biosynthesis pathway. Surprisingly, besides the methyl transferring, TleD also rearranges the geranyl and indole moiety of the precursor to form a six-membered ring, but does not show homologies to any known terpenoid cyclases. To clarify the TleD catalytic mechanism, we determined the complex crystal structures of TleD and the cofactor analogue S-adenosyl-L-homocysteine with substrate teleocidin A1. A domain-swapped pattern via an additional N-terminal α-helix is observed in TleD hexamers. The structural comparison and alignment shows this additional N-terminal α-helix is the common feature of SAM-MTase-like cyclases TleD and SpnF. The residue Tyr21 anchors the additional N-terminal α-helix to “core SAM-MT fold” and is a key residue for catalytic activity. Molecular dynamic simulation results suggest that the dihedral angle C23-C24-C25-C26 of teleocidin A1 is preferred to 60° - 90° in the TleD and substrate complex structure, which tend to adopt a Re-face stereocenter at C25 position after reaction and is according to *in vitro* enzyme reaction experiments. Our results also demonstrate methyl transfer can be a new chemical strategy for carbocation formation in the terpene cyclization, which is the key initial step.
Structural basis for Ragulator and EGO-TC functioning as scaffolds in membrane-anchoring of Rag/GTR GTPases

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The mechanistic Target Of Rapamycin Complex 1 (mTORC1) coordinates cell growth, proliferation, and differentiation in response to environmental conditions of energy, nutrients, and extracellular growth factors. Dysregulation of mTORC1 is often implicated in pathophysiological conditions such as tumorigenesis and diabetes. The mechanism by which this signal is transduced to TORC1 is largely conserved and requires the Rag/Gtr family GTPases in both yeast and mammals. Amino acid-dependent activation of mTORC1 is mediated by Rag GTPases, which are recruited to the lysosome by the Ragulator complex consisting of p18, MP1, p14, HBXIP and C7orf59. In budding yeast, EGO-TC complex, consisting of Ego1, Ego2 and Ego3, localizes to the endosomal and vacuolar membranes and plays a pivotal role in cell growth and autophagy regulation through relaying amino acid signals to activate TORC1. We report the crystal structure of EGO-TC complex in which Ego2 and Ego3 form a heterodimer flanked along one side by Ego1. We also determined the structure of Ragulator and in which p18 wraps around the MP1-p14 and C7orf59-HBXIP heterodimers and the interactions of p18 with MP1, C7orf59, and HBXIP are essential for the assembly of Ragulator. These results suggest that the Ragulator and EGO-TC are structurally conserved and might exert similar function(s) via similar mechanisms in the mTORC1 signaling that Ragulator and EGO-TC functions as scaffolds to recruit Rag/Gtr GTPases to lysosomal/vacuole membrane in mTORC1 signaling.

Figure.1. The Ragulator functions as a scaffold to recruit the Rag GTPases to the lysosomal membrane in mTORC1 signaling. (a) Working model for the functional role of the Ragulator complex in targeting the Rag GTPases to the lysosome. (b) Superposition of the structures of the Ragulator and EGO-TC complexes. (c) The Ragulator and EGO-TC complexes share similar structural and functional features in mTORC1 signaling. Ego1, Ego2, and Ego3 of the EGO-TC appear to be the counterparts of p18, HBXIP, and p14 of the Ragulator.
Recognition mechanism and inhibition of DNA targeting by CRISPR-Cas systems

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The CRISPR-Cas systems were found in almost all archaea and about 50% of bacteria and function as RNA-guided adaptive defense systems. The CRISPR-Cas systems can be grouped into two classes and subdivided into 6 types and 19 subtypes: Class 1 systems (Type I, III and IV) rely on multi-subunit surveillance ribonucleoprotein complexes termed Cascade (CRISPR-associated complex for antiviral defense), while Class 2 systems (Type II, V and VI) rely on single Cas protein involved ribonucleoprotein complexes. In turn, phages and mobile genetic elements have developed divergent strategies to overcome CRISPR-Cas systems in host via a series of anti-CRISPR proteins through repressing the activity of effector proteins. To date, CRISPR-Cas systems are successfully applied to genome edit in both eukaryote and prokaryote. Moreover, anti-CRISPR proteins provide opportunities to act as ‘off-switches’ tools for spatially and temporarily or conditionally controlling activity of effector proteins. Further knowledge is required to minimize side effects resulting from alternate cleavage patterns, thereby insuring effective and safe genome editing in the clinic. The understanding of cleavage and inhibition mechanisms provide clues for goals to improve fidelity and efficiency, reduce off-target rate, and spatially and temporally control activity of CRISPR-Cas systems. We focus on precise recognition and cleavage mechanisms of effector complexes in CRISPR-Cas systems, as well as the inhibition and regulation of these effector complexes. Our findings provide clues and structural information for related applications of these CRISPR-Cas systems.

Figure 1. Structural basis of recognition and inhibition mechanisms of CRISPR-Cas systems. (A) Recognition and cleavage of dsDNA by Type V-B Cas12b system. (B) Inhibition mechanism of Type II-A SpyCas9 by phage encoded anti-CRISPR protein. (C) Assembly, recognition of dsDNA by Type I-F Csy complex and inhibition by phage encoded anti-CRISPR proteins.
Structure analysis of seven disease-causing mutants of human lipoamide dehydrogenase

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(Dihydro)lipoamide dehydrogenase (E3) is a homodimeric flavin-disulfide oxidoreductase that catalyzes the oxidation of dihydrolipoamide cofactors covalently attached to the E2 subunits of the mitochondrial alpha-keto acid dehydrogenase complexes. As a common third subunit to the above multienzyme complexes, the human (h) E3 plays pivotal roles in metabolism; pathogenic hE3 variants affect several central metabolic pathways simultaneously and lead to E3 deficiency. Clinical manifestations of E3 deficiency are diverse, generally very severe, and do not correlate well with the loss in E3 activity. This implies that other auxiliary biochemical mechanisms, presumably the elevated reactive oxygen species (ROS) generating activities of certain pathogenic variants and/or impaired interactions among the subunits of the relevant multienzyme complexes, might also contribute to the pathogenesis.

High-resolution crystal structures of the wild type hE3 and seven of its disease-causing mutants at the resolution range of 1.4 to 2.3 Å have been determined. The pathogenic amino acid substitutions are located in either the dimer interface (G426E, D444V, I445M, R447G, and R460G), the active site (P453L), or the cofactor binding region (G194C). Analyses of the crystal structures enabled us to propose individual molecular pathomechanisms, of the compromised catalytic activities and altered capacities for ROS generation, to the respective disease-causing hE3 mutants.
Structural Evidence for Transient Binding of Water Molecules in the Active Site of Bacillus sp. TB-90 Urate Oxidase.

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There are many experimental evidences that the introduction of mutation in a surface loop of an enzyme has often increased its activity. Nonetheless, a detailed understanding of the relationship between the changes of surface loop plasticity and the catalytic process is currently lacking.

Bacillus sp. TB-90 urate oxidase (BTUO) is a thermostable homotetrameric enzyme (molecular mass, 38 kDa×4) catalyzing the oxidative opening of the urate purine ring. Gly substitution of Pro298 in the interface loop II (residue 277-300) of BTUO led a 1.7-fold increase in the turnover number. Calorimetric and kinetic analyses suggested that this activation of BTUO mutant P287G resulted from the entropic decrease of the activation energy via the enthalpic destabilization of the ES complex. Crystal structures of the ES-complex mimics of the wild-type BTUO and the mutant P287G were determined, suggesting that four water molecules played an important role in the formation of hydrogen-bonding network between a general base residue Lys13 and a substrate uric acid (Fig. 1). When their crystal structures were determined after dehydration using the humid air and glue-coating (HAG) crystal-mounting method, three and two water molecules were lost from the four in the active sites of the wild-type BTUO and the mutant P287G, respectively. These results suggested that the transient binding of the four water molecules were involved in the activation of the enzyme, and the plasticity of the interface loop II helped to stabilize the hydrogen-bonding network of water molecules in the active site.

Fig. 1 A close view of the active-site pocket of BTUO
Anion exchanger 1 (AE1), known as Band 3, mediates the Cl\(^-\) and HCO\(_3\)^- exchange across the red blood cell membrane by “Ping Pong Mechanism”. Band 3 mediates the anion exchange from inside to outside by conformational change from inward conformation to outward conformation, and vice versa from outside to inside. It plays a pivotal role for delivering oxygen appropriately to metabolically active tissues. For understanding molecular mechanisms, it is essential to know the relationship between structure and function. The outward conformation of Band 3 in complex with the Fab fragment was already solved and reported at 3.5 Å resolution (Arakawa, T, et al., Science, 350, (2015)). In terrestrial environments, however, nobody could make good quality crystals of Band 3 that diffracted to over 8 Å resolution. To understand more detailed mechanisms of Band 3, we have tried crystallization of Band 3 without the Fab fragment. To improve crystal quality, in this study, we purified the transmembrane domain of Band 3 from human red blood cells and crystallized without the Fab fragment under microgravity environments at the Japanese experiment module “KIBO” on the International Space Station. Crystallization condition has been also customized and optimized for crystallization under microgravity. Obtained crystals diffracted over 5 Å resolution. These results indicated that crystallization of Band 3 under microgravity was effective for improvement of crystal quality.
EXPLORING THE LANDSCAPE OF BIOLOGICAL SOLUTIONS WITH THE BioSAXS-2000\textsuperscript{nano}

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Small angle X-ray scattering (SAXS) is a useful technique for extracting structural information from biological samples in solution. Most biological SAXS samples are aqueous solutions with proteins present in low concentration. In other cases, it may be of interest to study macromolecules at high concentration, at high viscosity or at conditions in which macromolecules are expected to crystallize. In such cases, SAXS can provide useful information about the inherent structure and phase of macromolecules.

Rigaku Oxford Diffraction’s BioSAXS-2000\textsuperscript{nano} system is well suited for analysis of all types of biological samples, independent of concentration, viscosity and phase. The BioSAXS-2000\textsuperscript{nano} system uses 2D Kratky collimation with confocal optics to achieve maximum X-ray flux on the samples. The system includes Rigaku’s hybrid photon counting detector (HPC), the HyPix-3000. The HyPix-3000 is ideal for measuring scattering from biological solutions because the detector combines ultra-low noise, high dynamic range and direct detection of X-ray photons. New features of the BioSAXS-2000\textsuperscript{nano} add further capabilities, such as variable q-range, measurement of anisotropic samples and grazing incidence (GI) compatibility. These capabilities come in the form of hot swappable attachments and use auto-detection methods, including capillary and flow cell support with the automatic sample changer (ASC).

In this study, we describe the versatility offered by the new BioSAXS-2000\textsuperscript{nano} system for biological SAXS and demonstrate the capabilities have been extended for samples types other than dilute protein solutions. In particular, we describe experiments with non-typical macromolecular samples, whether for the purposes of optimization of biological pharmaceuticals, for characterizing crystallization suspensions prior to measurement at an XFEL and for characterizing structural changes in response to solution conditions.
Structural study of translocation domain of tetanus neurotoxin.
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Tetanus neurotoxin (TeNT) is one of the most toxic substance known to human and causes strong paralysis called tetanus. TeNT consists of three functionally different domains, a receptor binding domain (Hc), a translocation domain (Hn), and a zinc protease domain (LC), which is surrounded by the belt region in Hn. It is known that Hn domain undergoes conformational change in response to the acidic endocytic environment resulting in the formation of an LC protein conducting channel. However, the molecular mechanism by which TeNT translocate LC across lipid membrane has been elusive. The Hn domain has characteristic region called "belt region", which surrounds LC. It has been suggested that the belt region play a role upon translocation mechanism. To investigate how this region behaves in the absence of LC, we conducted X-ray crystal structure analysis and small angle X-ray scattering analysis of the Hn domain. Our investigation revealed that in the absence of LC, the belt region swings away and exposes the putative channel forming motif, which was thought to initially insert into endosomal membrane. Furthermore, the exposed region displayed increased B-factor, suggesting improved mobility. From these results, we propose that in the initial event of translocation mechanism, flexible movement of belt region starts concerted conformational change of LC and Hn domain of TeNT.
Determination of dopant induced lattice deformations in polymer composites: Variation co-efficients as a function of concentration

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Considering a polymeric system of interest, we have computed lattice parameters and their variation with dopant concentration, which is on similar lines of thermal expansion studies; here concentration of dopant is treated as a variable of study. For this, a program TEV [1] is made used, which runs on JAVA platform and freely available online. To understand the dopant induced changes in polymer composites, we have studied two polymeric systems whose microstructural and lattice parameters are evaluated and verified. Hydroxypropyl methyl cellulose was chosen as polymer matrix, to which various weight percentages of Nickel and Cobalt chloride were doped and studied individually. Results shows the changes encountered and the variation of lattice coefficients, which are represented and visualized using TEV. Changes in the lattice expansion tensor components, eigenvalues, eigenvectors and their angles with the crystallographic axes with concentration can be evaluated from this program. Here we have used the concentration as the variable of dependency instead of Temperature, which is used to study thermal expansion. Besides, variation of lattice strain in these composites are evaluated for supportive justification and a plot of degree of distortion is drawn for better understanding.

Figure.1. Visualization of expansion co-efficient using TEV.

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Mixed valence iron-chloranilate coordination polymers incorporating redox active cations

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Electrically conductive coordination polymers, particularly those which exhibit porosity, have diverse potential application. 1 The dianion of 2,5-dihydroxy-1,4-benzoquinone (dhbq^-2, X=H) and the halogenated analogues (Xan^-2, X=F, Cl, Br), have been used extensively to generate coordination polymers, 2 exhibit three readily accessible redox states: a dianion, a paramagnetic radical trianion species, and an aromatic tetraanion. This characteristic may facilitate charge transport and engender the materials with bulk electronic conductivity. 3–5 Viologens are a class of cationic molecules which can also exist in multiple redox states. 6 We investigated influence upon structure and physical properties, such as conductivity, upon incorporation of various viologen dications into semi-conducting iron-chloranilate coordination polymers. The resulting materials were examined using X-ray diffraction, as well as a range of spectroscopic and bulk property measurements.

Figure 1. Structure of a 2D mixed-valence iron-chloranilate coordination polymer.

Supramolecular architectures of the Cu(II) and Cu(I) halopyrazine complexes
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Properties of crystalline solids are closely related to the manner in which building units are linked together in the solid state. Metal-containing systems can be composed of monomeric units mutually linked via non-covalent interactions (in particular hydrogen and/or halogen bonds), or their entire architecture can primarily rely on coordination bonds, only supported by much weaker non-covalent interactions (polymeric structures).\(^1,2\) We report here on the series of copper(II) and copper(I) complexes, namely \([\text{CuCl}_2(\text{I-pz})_2]\) (1), \([\text{CuBr}_2(\text{I-pz})_2]\) (2), \([\text{CuBr}(\text{Br-pz})_n]\) (3) and \([\text{CuBr}(\text{I-pz})_n]\) (4), equipped with a set of charge compensating and small aromatic ligands bearing closely related supramolecular functionalities and designed to play with the architecture of the crystal packings.

In the monomeric structures 1 and 2, Cu(II) forms square planar coordination spheres, while Cu(I), in the polymeric structures of 3 and 4, sits in the center of a tetrahedral environment. In the monomeric structure of 1, crystal packing is guided by a halogen interaction: C3–I1 \(\cdots\) N4\(^i\) \((-1/2+x,1/2-y,1/2+z\) which connects the complex units into the endless two-dimensional waves, “oscillating” along the 002 plane. Furthermore, pseudo-octahedron formed around Cu1, with Cl\(ii\) \((-1+x,y,z\) and Cl\(iii\) \((3-x,-y,1-z\) in the apical positions \([\text{Cu – Cl} \text{ distance of 3.051(1) Å vs. 2.285 (1) Å for the in-square coordination bonds}]\) connects the molecular waves (\textit{vide supra}) into a three-dimensional network. In the structure of 2, a halogen interaction, C5 – I1 \(\cdots\) Br1\((2-x,-y,-z\) connects the complex molecules into double chains along the crystallographic c axis. Crystal arrangements in 3 and 4 are isostructural and characterized by the \([\text{Cu – pz - }]\) polymeric chains disposed along the crystallographic c axis, and interconnected by the Cu-Br coordination bonds which lie along the crystallographic a axis. As a result, two-dimensional molecular networks are aligned parallel to the 010 plane. Within these two-dimensional networks, the \(ls\) planes of the analogous pyrazine rings are parallel. The Cu - Br1 - Cu distances are 3.935 (4) Å in 3 and 4.077 (3) Å in 4, while the interplanar distance between the parallel \(ls\) planes calculated through the atoms of the stacked pyrazine rings is 3.593 (3) Å in 3 and 3.666 (2) Å in 4. The stacking is affected by an offset of 1.597 Å in 3, and 1.781 Å in 4.

References:

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From Functional Interpenetrating Polymer Networks to Guests Interpenetration/Impregnation in Metal-organic Frameworks

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Interpenetrating polymer networks (IPNs) can have two or more polymers with different chemistries interweaving/entangling together. They have no chemical bonding between each other but they are physically inseparable. Such unique structural feature gives rise to the multi-functionality of IPNs, as they can exhibit properties from their different polymer components. In this presentation, I will start from our recent progress in multifunctional IPNs which demonstrates their usefulness as actuator/sensor and supercapacitor. I will then bridge the concepts of IPNs and guests formed in metal-organic frameworks (guest@MOFs) by showing the interpenetration of electrically conducting polymer, poly(3,4-ethylenedioxythiophene) (PEDOT), in a MOF. The PEDOT@MOF is significantly more conductive than the insulating MOF host. Finally, as related to the further exploration of guest@MOF systems, the recently found metamorphosis during the process of guest@MOF carbonization will be introduced.

Figure 1. Schematic presentations for interpenetrating polymer networks (IPNs) and guests interpenetration/impregnation in metal-organic frameworks (guest@MOF systems). The typical dimensions of interpenetrations are also given as labelled.
Simulation of diffuse scattering in DL-norleucine

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With the advent of single crystal diffractometers that utilize area detectors it is now a simple matter to use the raw data frames not only to obtain integrated intensities of Bragg peaks for crystal structure determination but also to construct reciprocal space plots of any desired reciprocal lattice section. Such plots are useful for checking crystal quality and the possible presence of twinning but can also reveal diffuse scattering due to the presence of disorder. For data collected using a single counter diffractometer such information was not available so the presence of diffuse scattering and disorder was largely undetected and its study was confined to a relatively small number of specialist groups. In this paper we describe how diffuse scattering observed in a standard crystal structure determination experiment has been used to investigate details of the structural disorder in the amino acid DL-norleucine, \(\text{C}_6\text{H}_{13}\text{NO}_2\).

Figure 1. View of the a-axis projection of the structure.
The Hierarchical Self-Assembly of Metal-Organic Frameworks from an M₄L₆ Tetrahedron Cage

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The ability for chemists to replicate the efficiency and selectivity exhibited by Nature in binding substrates to enzymes is still a coveted objective. Understanding how to artificially reproduce important biological pathways will lead to complex synthetic systems. Designing metal-organic systems which form with predictable, predetermined geometries, properties and functions is, therefore, an important goal in self-assembly. Metal-organic frameworks (MOFs) are a class of porous material which consists of metal ions coordinatively linked to multidentate organic ligands. These complex architectures typically demonstrate large thermal and mechanical robustness, increased surface areas and large pore volumes which are useful for the encapsulation of smaller guest molecules. The predictability of the resulting MOF structure and its properties have always been serendipitous largely due to the “one-pot” reaction possessing numerous uncontrolled factors.

Using a new approach termed hierarchical self-assembly, the predictable design and synthesis of porous MOFs with tailored functionalities has been reported. This hierarchical self-assembly approach uses previously synthesised metallo-supramolecular architectures as molecular building blocks for the construction of MOFs.

Here we report the synthesis of a new multidentate organic ligand and resulting M₄L₆ tetrahedron cage. This anionic cage is a prime candidate for the hierarchical self-assembly of a MOF due to the ligating functionalities at each corner. In addition to the internal cavity, this tetrahedron possesses some interesting and distinctly different binding pockets located externally to the cage which may lead to allosteric effects in guest ingress and egress.

Figure 1. An X-ray crystal structure of the T-symmetric gallium M₄L₆ tetrahedron with an encapsulated cationic guest of tetraethylammonium.
Efficient CO\textsubscript{2} Separation Using Mixed-Matrix Membranes with Metal-Organic Polyhedra

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The metal-organic polyhedra (MOP) with triethylene glycol pendant groups was synthesized by the self-assembly of 5-tri(ethylene glycol) monomethyl ether isophthalic acid and Cu(II). The single-crystal X-ray diffraction analysis revealed that EG\textsubscript{3}-MOP has a cuboctahedron core structure assembled by 12 copper paddle wheels and 24 IPA linkers with (Figure 1). EG\textsubscript{3}-MOP has 24 TEGME tails at the periphery of the MOP. The axial positions of the copper paddle wheels in the MOP are occupied by H\textsubscript{2}O and DMF molecules, which are located in the inner and outer cavities of the MOP, respectively. The coordinated water molecules in the copper paddle wheels represent the hydrophilic environment of the cavity with a size of 13.8 Å. As shown in Figure 1, such hydrophilic cavity is accessible through 8 triangle and 6 square apertures with diameters of 4.0 Å and 6.6 Å, respectively. The tail-to-tail diameter of EG\textsubscript{3}-MOPs is approximately 4.3 nm. This MOP was uniformly dispersed in XLPEO without particle agglomeration. Comparing with conventional neat XLPEO, the homogenous dispersion of EG\textsubscript{3}-MOPs in XLPEO enhanced CO\textsubscript{2} permeability of MMMs. Upon increasing the amount of EG\textsubscript{3}-MOPs, the membrane performance such as CO\textsubscript{2}/N\textsubscript{2} selectivity was steadily improved because of unsaturated Cu(II) sites at paddle-wheel units, which was confirmed by Cu K-edge XANES analysis. Details of the work will be presented.

Figure 1. X-ray crystal structure of EG\textsubscript{3}-MOPs
Hydration mechanisms and proton conduction in the mixed ionic-electronic conductors Ba$_4$Nb$_2$O$_9$ and Ba$_4$Ta$_2$O$_9$

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Mixed conductors – materials that exhibit significant mobility of more than one type of charge carrier such as oxide ions, protons and electrons – have a range of important applications including solid oxide fuel cell membranes, electrodes, batteries and sensors. We recently studied the behaviour of hydrogen in the mixed ionic-electronic conductors γ-Ba$_4$Nb$_2$O$_9$ and 6H-Ba$_4$Ta$_2$O$_9$, using a combination of experimental (neutron diffraction and inelastic neutron scattering) and computational ($ab$ initio molecular dynamics) methods. While these compounds have isostructural low-temperature polymorphs, they adopt distinct forms in the high-temperature conducting regime. We found that they also have distinct mechanisms for hydration and ionic conduction. Hydration of γ-Ba$_4$Nb$_2$O$_9$ is localised to 2-D layers in the structure that contain a 1:1 ratio of isolated but adjacent NbO$_4$ and NbO$_5$ polyhedra. OH$^-$ and H$^+$ ions combine with two polyhedra respectively to form complete layers of NbO$_4$OH polyhedra, giving rise to a stoichiometric hydrated form γ-III-Ba$_4$Nb$_2$O$_9$.1/3H$_2$O. Protons then diffuse through these 2-D layers by “hopping” between oxygen atoms on adjacent polyhedra. In the case of 6H-Ba$_4$Ta$_2$O$_9$, hydration occurs by intercalating intact water molecules into the structure up to a maximum of ~0.375 H$_2$O per formula unit. This explains the unusual local and long-range structural distortions in the hydrated form observed by neutron diffraction. Diffusion then occurs by water molecules moving between neighboring symmetry equivalent positions. These fundamentally different hydration and proton conduction mechanisms explain why 6H-Ba$_4$Ta$_2$O$_9$ has the less well-defined and higher maximum water content, while γ-Ba$_4$Nb$_2$O$_9$ has the higher proton conductivity.

![Figure 1](image-url)
Tandem Crystallization Strategies for Resolution of Trifluorolactic Acid [CF$_3$CH(OH)COOH] by Chiral Benzylamines

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The successful resolution of rac-3,3,3-trifluorolactic acid by diastereomeric salt formation was reinvestigated using inexpensive chiral benzylamines, such as phenylethylamine and phenylglycinol. A previous report indicated that S-phenylethylamine was inefficient.¹ We have found this is due to coprecipitation of two diastereomeric phases, 1 S-[NH$_3$CH(CH$_3$)Ph] S-[CF$_3$CH(OH)COO] (Fig 1, left) and 2 S-[NH$_3$CH(CH$_3$)Ph] R-[CF$_3$CH(OH)COO].H$_2$O. Furthermore, phase 2 shows some R/S-disorder at the anion site. Phase pure 1 which is enantiopure may be obtained by removal of water in the reaction using molecular sieves. Seebach et al. have previously reported an effective resolution of trifluorolactate with S,S-2-amino-1-phenyl-propan-1,3-diol.² However we find there are two problems with this system, firstly the resolving amine is more expensive and secondly the product salt S,S-[NH$_3$CH(CH$_2$OH)CHOHPh] R-[CF$_3$CH(OH)COO] is complicated by formation of two structurally related polymorphs, 3 which is monoclinic but not enantiopure, and 4 which is orthorhombic and clean. Herein we report an efficient resolution using inexpensive R- or S-phenylglycinol. Phase pure 5 S-[NH$_3$CH(CH$_2$OH)Ph] S-[CF$_3$CH(OH)COO] (Fig 1, right) is produced in good yield (>98%ee in a single step compared to its S-R diastereomer 6). This is supported by chiral chromatography and single crystal X-ray structure determinations. Overall the most efficient resolution of the acid can be achieved by step-wise ‘tandem’ crystallization, isolating a diastereomeric salt first from one base and then a different one, thereby avoiding contamination from racemic crystals.

Figure 1. Ion pairs in structures of S,S-salts 1 (left) & 5 (right). These phases show little enantio-contamination & can be used sequentially to effect a high yield tandem resolution if one of the cations is switched to the R-form.

References
Fluorescent cadmium bipillared-layer open frameworks: synthesis, structures, sensing of nitro compounds, capture of volatile iodine, and CO₂ uptake

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Fluorescent Cd metal–organic frameworks (MOFs), [Cd₂(dicarboxylate)₃(Ni-bpy-44)₂] (dicarboxylate = 1,4-bdc (1), Br-1,4-bdc (2), NO₂-1,4-bdc (3), bpdc (4)), featuring non- and twofold interpenetrating pcu-type bipillared-layer open structures with sufficient free voids of 58.4, 51.4, 51.5, and 41.4%, respectively, have been hydro(solvo)thermally synthesized. MOFs 1–4 emitted ligand-centered blue or cyan fluorescence emissions at 447±7 nm in solid-state, which are solvent-dependent. After immersing the crystalline samples in different solvents, H₂O (1 and 2), DMSO (1 and 2), NB (nitrobenzene) (1–4), and PhOH (phenol) (1–4) exhibited remarkable quenching effect, while o-Xyl (o-xylene) and p-Xyl (p-xylene) (4) caused significant fluorescence enhancement. The sensing ability of 1–4 toward nitro compounds carried out in the vapor phase showed that NB, 2-NP (2-nitrophenol), and 4-NT (4-nitrotoluene) are effective quenchers because of their electron-deficient aromatic rings and higher vapor pressures. Moreover, 1–4 are highly reusable for quick capture of volatile iodine, I₂(g), as supported by obvious crystal color change from colorless, pale-yellow, or pale-orange to brown, and also by immense fluorescence quenching responses due to donor–acceptor interaction. Carbon dioxide adsorption isotherms indicate that activated materials 1’–4’ are inefficient at taking up CO₂, with an uptake capacity of only 16.7, 15.2, 23.4, and 30.5 cm³ g⁻¹, respectively, at 195 K and P/P₀ = 1.
Micro-crystal X-ray Diffraction Beamline - Advanced and Non-ambient Crystallography

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A dedicated X-ray diffraction beamline "Micro-crystal X-ray diffraction beamline (μ-XRD)" for advanced and non-ambient crystallography of chemical crystal research are scheduled at TPS 15A, a Phase-II beamline at TPS (Taiwan Photon Source). The beamline consists of a tapered cryogenic undulator source (CUT15), a coupled Double Crystal Monochromator (DCM) / Double Multilayer Monochromator (DMM) system, three focusing mirrors (HFM1, VFM, and HFM2) and two end-stations (ES1 and ES2). CUT15 will generate high brilliance X-ray for the designed available energy range 9 – 35 keV. The X-ray beam is focused by HFM1 and delivered to ES1 with adjustable beam size 100 – 200 μm²; then X-ray is focused again by a pair of Kirkpatrick-Baez (KB) mirror (VFM and HFM2) down to ~ 2×2 μm² (FWHM) in diameter at the sample position of ES2. Experiments can be conducted in either monochromatic (by DCM) or pink (by DMM width bandwidth 3-5 %) beam mode in both end-stations. A set of instrument, including the high-heat-load chopper, millisecond shutter, and a high-speed Jülich chopper,¹ are synchronized to the storage ring clock, which will be used to isolate a single X-ray pulse (< 100 ps) and deliver to the sample at ES2. Both end-stations will equip with a high precision kappa geometry goniometer and a large and fast area detector for data collection.

Scientific Opportunities of TPS 15A

Figure 1. Scientific opportunities of TPS 15A.
Magnetic Phase Transitions of New Sodium-ion Battery Cathode Na₄Ni₇(PO₄)₆

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Sodium-ion batteries (NIB) are of intense current interest as alternatives to lithium-ion batteries for large-scale applications in which kinetics and weight are not the primary consideration. However, the jury is still out on the ultimate competitiveness of NIB versus other energy storage solutions, with one major uncertainty being a lack of Na electrode materials. [1] Monoclinic Na₄Ni₇(PO₄)₆ has demonstrated a great potential to be a new electrode candidate in our recent study. In addition, its complex magnetic properties also attracted our interest. [2] In this presentation, we mainly focus on investigation of the magnetic phase structures of Na₄Ni₇(PO₄)₆. According to the magnetic susceptibility analysis and variable temperature neutron diffraction measurements, Na₄Ni₇(PO₄)₆ presents three successive antiferromagnetic (AFM) ordered phases (Phase I, Phase II and Phase III) at 9.1-17K, 4.6-9.1K and <4.6K with the magnetic ordering vector [0, 1, 1/2], [0, 2/3, 1/2], and [0.076, 2/3, 1/2], refer to the nuclear structure (Figure 1). The magnetic ordering shows distinct ferromagnetic (FM) Ni²⁺ strips and antiferromagnetic arrangements between FM strips. The moment amplitude of all strips is equivalent in Phase I but varies in Phase II. Phase III is an incommensurate structure and should have a similar spin arrangement with Phase II.

Figure 1 Magnetic structures of Na₄Ni₇(PO₄)₆ from the Rietveld refinements: [NiO₆] and [PO₄] are shown as wireframes; Na⁺ ions are not displayed; the magnetic moment are shown as arrows; the dash rectangles indicate layers of [NiO₆] - [PO₄].

Non-3d Metal Modulation of Cobalt Imidazolate Framework

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A non-3d metal modulation of Cobalt imidazolate frameworks \([\text{Co}_4(\text{MO}_4)(\text{eim})_6]\) (Heim = 2-ethylimidazole, MAF-69-Mo) is reported here. Relative to the RHO-[Co(eim)_2] (MAF-6-Co), MAF-69-Mo exhibits the best electrocatalytic performance (e.g. 1 mA cm\(^{-2}\) at overpotential of 210 mV in CO\(_2\)-saturated 0.5 M KHCO\(_3\) electrolyte and 2/10/22 mA cm\(^{-2}\) at overpotential of 388/490/570 mV in phosphate buffer solution) for the oxygen evolution reaction (OER) among non-precious metal catalysts and even outperforming RuO\(_2\).
Coordination Polymers Based on Linear Benzobisimidazole Ligands: Structure and Porous Properties

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Coordination polymers, also known as metal-organic frameworks (MOF), are a new class of porous materials because of their high porosity, high surface area, easily synthesized, variable size of the framework, chemical modification for target requirements, rich structure, etc., has been in the gas adsorption, catalysis, optoelectronic materials and other fields by people’s attention.

In this work, two coordination polymers, Cd$_2$(OAc)$_4$(H$_2$bbim)(DMA)$_2$(1) and Cd(OAc)(H$_2$bbmim) (2), were obtained by the reaction of the linear benzimidazole ligand H$_2$bdim and the methyl modified H$_2$bbmim with Cd(OAc)$_2$•2H$_2$O. The single crystal structure shows that the one-dimensional chain structure in compound 1 is interlaced by the hydrogen bonds between the chains, thus exhibiting a one-dimensional pore with a porosity of about 35.8%. Compound 2 is a closely packed three-dimensional structure due to the greater steric hindrance of ligands by methyl modification.

Figure. 1. Structure of coordination polymer (a) 1, (b) 2.
Study on the co-crystal of donepezil and valsartan

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Donepezil is an anti-Alzheimer's drug commonly used in clinical practice and belongs to acetylcholinesterase inhibitors. Valsartan is a non-peptide, orally effective angiotensin II (AT) receptor antagonist, and is currently used as a first-line antihypertensive drug. Both valsartan and irbesartan are angiotensin receptor blockers, and they also have similar hydrogen bonding sites in structure. Valsartan also has a carboxyl structure, which is more conducive to eutectic binding. Therefore, it is considered to prepare a co-crystal of donepezil and valsartan. The valsartan sample is easy to form an amorphous state, so it is considered to design a variety of eutectic preparation methods to prepare the co-crystal of donepezil and valsartan. The co-crystal preparation of donepezil and valsartan was carried out by various physicochemical methods to form different eutectic solid states. The analytical analysis was carried out by PXRD, ss-NMR, DSC and FTIR. By studying the preparation of donepezil and valsartan into a eutectic solid state with specific non-covalent forces, a new substance different from donepezil, valsartan and the simple combination of the two was formed. The bioactivity study found that the new co-crystal of donepezil and valsartan have special advantages in the prevention and treatment of cardiovascular and neurological disorders such as heart failure, myocardial ischemia, hypertension, and atherosclerosis.

Figure 1. AUC of co-crystal of donepezil and valsartan
A New Co-crystal of Berberine Hydrochloride

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Berberine is a quaternary ammonium salt that found in some plants such as huanglian (Coptidis Rhizoma), huangbo (Phellodendri Chinensis Cortex), sankezhen (Berberidis Radix). Berberine hydrochloride has extensive pharmacological effects, such as anti-bacterial, anti-viral, anti-inflammatory, analgesic and anti-cancer, hyperglycemic, anti-lipidemic, anti-hypertension, arrhythmia, heart failure and so on. Experimental study and clinical reports demonstrates that berberine has therapeutic effect on the endocrine system, circulatory system, nervous system, digestive system, respiratory system and other diseases. However, it isn't approved as drug because of its poor solubility and bioavailability. To improve the solubility and bioavailability, co-crystals of berberine hydrochloride have been screened and studied. A new co-crystal of berberine hydrochloride and fumaric acid was obtained. The yellow needle crystals were obtained from the methanol solvent, and the SXRD analysis showed that the API and CCF were formed as the ratio 2:1. Furthermore, the chloride ion acted as the hydrogen bond receptor and linked the API and CCF molecules. The two berberine hydrochloride molecules and one fumaric acid molecules made a “Z” type in the asymmetric unit. Besides SXRD, PXRD, IR, DSC and Raman analysis were used to identify the new co-crystal. The solubility of the co-crystal in vitro was evaluated. The results showed that the new co-crystal of berberine hydrochloride and fumaric acid were superior to berberine hydrochloride in water, hydrochloric acid, acetate and phosphate systems. This study is of great significance for the development of new drug of berberine hydrochloride.

Figure 1. 2-dimensional hydrogen bond network of co-crystal of berberine hydrochloride and fumaric acid (2:1)
Thiocarbamate complexes: Au…O/π and Arene-C-H…π(quasi chelate ring) interactions

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Thiocarbamides, of general molecular formula ROC(=S)N(H)R’ (R, R’ = alkyl, aryl), present fascinating properties upon complexation with coinage metals. For example, these organic molecules, when coordinated to gold(I) and copper(I), form complexes that exhibit very promising anti-bacterial and anti-cancer properties. In their crystals, non-standard intermolecular interactions are often observed. Thus, in the crystals of phosphanegold(I) thiolate complexes, that is with the deprotonated form of the ligand, the presence of intra- and inter-molecular Au−O and Au−π interactions are evident. DFT calculations suggested that Au−π interactions are more favourable than the corresponding Au−O interactions. On the other hand, the crystals of copper(I) thiocarbamide congeners, where the ligand coordinates in its neutral form, feature unanticipated C−H…π(quasi chelate ring) interactions following a ring closure mediated by N−H…Cl hydrogen bonding, that is, a {CuCl…HNCS} S(6) loop. When occurring intramolecularly, these C−H…π(quasi chelate ring) interactions afford energies of attraction of approximate 3.5 kcal mol⁻¹. A subsequent review of analogous copper(I) complexes revealed that intermolecular C−H…π(quasi chelate ring) interactions also imparted stabilisation to their crystal structures, resulting in the formation of zero-, one-, two- and three-dimensional aggregation patterns/architectures. It transpires that C−H…π(quasi chelate ring) interactions occurred, intra- and inter-molecularly, in about one-third of structures where they could potentially form, at least for these copper(I) complexes.
Fast ionic conduction in solid electrolytes plays a key role in feasibility of the all-solid-state battery system. Kanno et al. had developed new super lithium-ion conductors, LGPS (Li$_{12}$GeP$_2$S$_{12}$) system [1,2]. Among the lithium ion conductors, the LGPS and LSPSC (shows the conductivity comparable to organic liquid electrolytes. This higher conductivity makes a possibility of all-solid-state battery which solid-state electrolyte was used. The crystal structures of the solid solution were studied using neutron diffraction technique. Their conduction pathway was estimated using Maximum Entropy Method (MEM) based on their structure information. These results indicate the lithium conduction pathway in LGPS is one-dimensional pathway along c axis at room temperature, and three-dimensional one at higher temperature [3]. However, the results of MEM indicated only the space of possible lithium ion conduction. Then, the study of lithium diffusive behaviors is necessary to understand the lithium-ion conduction mechanism. The dynamics of lithium ion diffusions can be obtained directly by using the Quasielastic Neutron Scattering (QENS) technique because the quasielastic scattering spectrum is a broadening of elastic peak caused by the diffusion of atoms or ions within a material. When ions cause diffusion motions on a fixed sublattice, the quasielastic scattering spectra can exhibit a $Q$-dependence, which provides information on the dynamical structure of the ion diffusion. In this study, the QENS measurements were performed using the high-resolution Si crystal analyzer TOF type near-backscattering spectrometer, DNA, at MLF/J-PARC, Tokai, Ibaraki, Japan, at the energy resolution of 3.6 $\mu$eV [4]. $S(Q,\omega)$ spectra from 150 to 640 K were collected. After the analyzed those using jump-diffuse model, self-diffusion constants, jump length and mean residence time of conduction lithium ions in LGPS system were determined. The jump length was 1.73 Å at 473K and 2.72 Å at 640 K respectively. The jump length at 473 K was consistent with conduction along c axis. The jump length at 640 K also corresponded to the three-dimensional conduction model. In this presentation, the mechanism of lithium ion diffusion in LGPS system will be discussed.

Acknowledgement
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References
Crystal chemistry of pyrite-type compounds and single crystal structure analyses of FeS₂, MnS₂, MnSe₂ and MnTe₂

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The crystal structures of MnTe₂ [a= 6.9513(1) Å; u= 0.38554(2); space group Pa3; Z=4], MnSe₂ [a= 6.4275(2) Å; u= 0.39358(2)], MnS₂ [a= 6.1013(1) Å; u= 0.40105(4) from Osorezan, Aomori] and FeS₂ [a= 5.4190(1) Å; u= 0.38484(5) from Kawarokoba, Nagasaki] have been analyzed by the single-crystal X-ray diffraction and refined yielding the R1 values of 0.0113, 0.0160, 0.0189 and 0.0181 using 354, 278, 318 and 165 unique reflections with Fo>2σ(Fo), respectively. There were no evidences of lower space group symmetry. The mean square displacement U₁₁ for S (0.00896(19) Å²) is smaller than that for Mn (0.0112(2) Å²) in MnS₂.

Thermal vibration characteristics for the manganese system are significantly different from the tendency in FeS₂ pyrite. The S–S distance of 2.0914(8) Å in MnS₂ is significantly shorter than that of 2.1618(9) Å in FeS₂. Obtained Θ₀ for Fe and S in FeS₂ pyrite are 493 and 587 K, respectively. Our obtained values are consistent with the Θ₀ of 605 K determined by specific heat measurement. The estimated Debye characteristic temperatures, Θ₀= 229 K (Mn) and 176 K (Te) in MnTe₂, Θ₀ = 244 K (Mn) and 233 K (Se) in MnSe₂ and Θ₀ = 263 K (Mn) and 384 K (S) in MnS₂) increase from Te to S. The 15th group (pnictogen) and 16th group (chalcogen) elements in same period (S:P, Se:As and Te:Sb) take similar X–X values in pyrite structures. The anion-anion distances tend to shrink when the cation-anion distances extend. Most pyrite type compounds have u-parameters in the range of 0.37 to 0.40. The pyrite type structures have only two degrees of freedom of lattice constant, a, and u-parameter. In order to maintain high symmetry, adjustment of chemical bonding and transfer of electrons will be carried out. The lattice constant of most pyrite type compounds ranges from 5.4 to 7.0 Å. MnS₂ and MnTe₂ have the maximum u-parameter and lattice constant, respectively. It is important that the X–X bonds of the pnictogen MX₂ are close to those of the chalcogen one in the pyrite-type compounds. This comes from the similar bond character of the X–X. The X-X in MnS₂ (2.0915(8) Å) is longer than S-S double bond (1.89 Å) and is equivalent to S-S single bond (2.06 Å) in S₂ disulfur molecule. The X-X in FeS₂ (2.1618(9) Å) is even longer than the distance in MnS₂. This elongation of S-S bonds shows an increase in ionicity and a decrease in covalent bonding character. Fe in pyrite is a low spin state with short distance and electronic polarization of iron in pyrite is increasing.
The New Chromium Thiophosphate Cluster via Two-Step Solid and Solution State Reactions.

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The new quaternary chromium thiophosphate, Cs₂CrPS₆ has been prepared with alkali metal halide flux at 873K and the structure has been investigated with X-ray diffraction data. The compound consists of Cs⁺ and anionic [CrPS₆²⁻]. Two anionic units form a binuclear cluster, [Cr₂P₂S₁₂⁴⁻] through a pair of edge-sharing Cr-centered octahedra and two P-centered tetrahedra. Due to the ionic nature of Cs₂CrPS₆, we were able to dissolve the compound in various polar solvents such as water and NMF. The monovalent salt, PPh₄Cl has been added into the solution and green needle-shaped crystals were obtained at room temperature. The crystal structure has been determined by X-ray diffraction techniques. The crystal is proved to be [PPh₄⁺][Cr₂P₂S₆(H₂O)₄][Cl]₂[H₂O]₁₀, where four terminal S atoms of [Cr₂P₂S₁₂⁴⁻] are replaced by O atoms of the water molecules. Organic cations [PPh₄⁺], chloride anions, and water molecules are included in the crystal via ionic interactions or hydrogen bonds. The oxidation state of Cr⁵⁺ in the precursor has been reduced to Cr³⁺, which is supported by the colour change. In this study we propose that a productive route to some of the soluble transition metal thiophosphates is the solubilization of materials prepared by solid state reactions at high temperature.

Figure.1. Structure of [Cr₂P₂S₁₂⁴⁻] and [Cr₂P₂S₆(H₂O)₄].
Nano-materials spark tremendous interest owing to their intriguing mechanical, physical and chemical features. Here, combining both the electron microscopy with crystallographic group theory, we present the atomistic characterizations of domain structures in different nano-materials. The orientation relationships of domain structures and atomic-scale configurations of the various interfaces are studied by applying electron diffraction and aberration-corrected transmission electron microscopy. Moreover, the growth processes of nanostructures are directly monitored. For instance, in-situ high resolution transmission electron microscopy reveals the atomistic oscillatory dissolution and precipitation of zinc blende (ZB)-structure InAs at the liquid-solid interface. Recently, the vapor-solid-liquid growth processes of Sn-catalyzed wurtzite (WZ) and ZB ZnO are directly observed (Fig. 1). The growth kinetics of WZ and ZB crystal phases in ZnO appear markedly different in terms of the NW-droplet interface, whereas the nucleation site as determined by the contact angle between the seed particle and the NW is found to be crucial for tuning the NW structure through combined experimental and theoretical investigations. These results have implications for the phase-controllable synthesis of compounds and heterostructures with tunable band structures.

Figure 1. The Sn-catalyzed growth of WZ and ZB ZnO nanowires.
MOF Isomerism due to Guest Molecules: 
Three Distinct Forms of [Cd(BzIm)$_2$] All with ABW-Topology

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ZIFs are a new class of porous material with three-dimensional structures constructed from tetrahedral metal dications (e.g., Cadmium, Zinc and Cobalt) bridged by bent imidazolate type anions. Their frameworks are structurally analogous to SiO$_2$ and Aluminosilicate zeolites [1]. Unlike SiO$_2$, the imidazole linkers can be chemically varied. Deliberate control of crystallization processes has produced many materials not known in nature [2].

![Image](image_url)

Figure 1. View of plane of (100) of [Cd(BzIm)$_2$] •0.5 Solvent (Solvents from left to right: Dioxane, DEF, DMSO)

Three new ZIF phases of [Cd(BzIm)$_2$] were obtained by solvothermal reactions of benzimidazole with Cd$^{2+}$. These all have the ABW zeolite topology, but with major difference in the porous channels and cavity structure. It illustrates surprisingly that knowledge of the metal, ligand and topology is insufficient to fully define a MOF, which is a quite different case from SiO$_2$ zeolites.

References:


Engineering tailored fluorescent Zn-based pillared-layer frameworks: synthesis, structures, CO$_2$ uptake, and nitro-analyte sensing

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Through dual-ligand synthetic approach, the isoreticular pcu-type pillared-layer open MOFs [Zn$_2$(1,4-bdc)$_2$(NI-bpy-44)] (1), [Zn$_2$(Br-1,4-bdc)$_2$(NI-bpy-44)] (2), [Zn$_2$(1,4-bdc-NH$_2$)$_2$(NI-bpy-44)] (3), [Zn$_2$(2,6-ndc)$_2$(NI-bpy-44)] (4), and [Zn$_2$(bpdc)$_2$(NI-bpy-44)] (5) have been prepared from the hydro(solvo)thermal reactions of Zn(NO$_3$)$_2$·6H$_2$O or ZnSO$_4$·7H$_2$O, N-(pyridin-4-yl)-4-(pyridin-4-yl)-1,8-naphthalimide (NI-bpy-44), and benzene-1,4-dicarboxylic acid (1,4-H$_2$bdc), 2-bromobenzene-1,4-dicarboxylic acid (Br-1,4-H$_2$bdc), 2-aminobenzene-1,4-dicarboxylic acid (NH$_2$-1,4-H$_2$bdc), naphthalene-2,6-dicarboxylic acid (2,6-H$_2$ndc), and biphenyl-4,4′-dicarboxylic acid (H$_2$bpdc), respectively. PLATON calculations suggest 27.0%, 62.1%, 52.2%, 37.0%, and 33.6%, extra-framework voids for MOFs 1–5, respectively. Low pressure CO$_2$ physisorption experiments have been performed on thermally-activated materials of MOFs 1–5 at 195, 273, and 298 K, displaying type-I microporous adsorption isotherms with adsorption capacities of 111.9, 89.8, 68.2, 58.7, and 45.7 cm$^3$ g$^{-1}$ at 195 K and $P/P_0 = 1$. MOFs 1–5 emit ligand-originated solid-state fluorescence centered at around 420–475 nm, of which MOFs 1–4 are served as fluorescence platforms to selectively detect nitro aromatic explosives such as NB, 1,4-DNB, 4-NT, 2,4-DNT, 2-NP, 3-NP, and 4-NP over nitro aliphatic analytes such as NM and DMNB.
Effects of Seeding Polymorphs and Environmental Factors on the Crystallization of Celecoxib

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Amorphous pharmaceuticals can have advantages over crystalline pharmaceuticals such as high solubility, fast dissolution rate and/or improved bioavailability. Amorphous materials have short range order and thermodynamically unstable relative to the crystalline state. The poor physical stability of amorphous materials hinders usage of amorphous pharmaceuticals in drug products since amorphous materials will not possess advantages any more after crystallization. It is important to understand how the amorphous active drug crystallizes under external stress conditions and how the seeding or starting materials affect the physical stability, and to fully understand fundamental properties of amorphous materials. The purpose of this study was to investigate the crystallization behavior of amorphous celecoxib exposed to various external conditions such as temperature, pressure, and/or humidity. Amorphous Celecoxib was prepared by quench-cooling three different starting forms, including celecoxib form III, celecoxib-DMA solvate, and celecoxib-DMF solvate. Ground and intact amorphous celecoxib were then exposed to various stressed conditions. Amorphous forms were characterized by PXRD, TGA, and DSC. Crystallization behavior of amorphous celecoxib was investigated. The glass transition temperatures ($T_g$) and the crystallization behaviors of amorphous celecoxib obtained from the starting forms were different. The crystallization of amorphous celecoxib was found to be much faster at higher temperatures (100°C and 80°C) and comparatively slower at lower temperatures (60°C, 40°C, 40°C/75%RH, 25°C/52%RH). The recrystallized forms obtained from the amorphous forms prepared from celecoxib form III, celecoxib DMA, celecoxib DMF, were different. We were able to obtain polymorphs I, II, and III which are difficult to obtain by solution crystallization. It can be concluded that the environmental factors as well as the starting materials can affect the physical stability of amorphous celecoxib and the polymorph selections of celecoxib after verification.
Hierarchically porous adamantane-shaped carbon nanoframes

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Hollow carbons have emerged as a new class of porous carbon materials, showing promise in a variety of areas. However, their morphology has been limited to spherical shapes, with the carbon shell possibly limiting access to their inner surfaces and utilization. Herein, we report a new type of hollow carbon material consisting of non-spherical, adamantane-shaped, hierarchically micro- and macro-porous, N-doped carbon nanoframes (mM-NCs) by exploiting selective etching and pseudomorphic thermal conversion of zeolitic imidazolate framework-8 (ZIF-8). The mM-NCs showed superior performance as adsorbents for large dye molecules and as catalysts for the oxygen reduction reaction relative to macropore-free N-doped carbons, which can be attributed to the presence of macropores, fully utilisable pore surfaces, and nitrogen species.

Figure 1. Schematic illustration (top) and high magnification SEM images (bottom) of the preparation of porous adamantane-shaped carbon nanoframes from ZIF-8.
Crystal Engineering of Chiral Space using Assemblies of Tartramide-based Spiroborate Anions [B(L-TarNH$_2$)$_2$]$^-$

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Recently we prepared a spiroborate anion bis(mandelato)borate [B(Man)$_2$]$^-$ [1,2] and bis(N,N'-diphenyltartramido)borate [B(L-Tar(NHPh)$_2$)$_2$] [3] for the efficient resolution of a variety of chiral cations. Many of these gave > 90%ee in the first isolation step via one pot reaction or metathesis reaction. In seeking to extend to a family of spiroborate anions with application to resolution we have found novel anionic assemblies using [B(L-TarNH$_2$)$_2$]$^-$. These systems can offer prospects for the dual resolution of both cations and/or chiral solvents. The array of structure types and nets formed by [B(L-TarNH$_2$)$_2$]$^-$ are dependent on counter cation, solvent and crystallization conditions such as temperature and time.

Self-aggregation between [B(L-TarNH$_2$)$_2$]$^-$ via inter-amide NH---O=C R$_2^2$(8) synthons are typically forms preserved anion layers, which are separated by chiral layers of cations and solvent. More complex assemblies such as the example shown (Fig 1, left) can demonstrate high levels of resolution as for R-NH$_3$CHMePh cations. Alternative nets where the [B(L-TarNH$_2$)$_2$]$^-$ assembly provides cavities for cations within the layer can also be found (Fig 1, right).

We are grateful to the Research Grants Council of Hong Kong for funding of this work (grant 16306515).

Figure 1. Anionic assemblies from [B(L-TarNH$_2$)$_2$]$^-$ incorporate cations and solvent in chiral channels or cavities.

References

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Crystal Engineering the Resolution of a Chiral Amine using A Family of Spiroborate Anions

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We have recently reported the utilization of cheap bis(mandelato)borate anions \([\text{B(Man)}_2^-]\) for effective resolution of a range of cation types.\(^1,2\) According to the success and limitations of \([\text{B(Man)}_2^-]\), we decided to develop a wider family of spiroborate anions \([\text{B(L-Tar(NH}_{\text{Ar}})_2]^2^-\) using diaryl-L-tartramide diols. The application of the parent ion of this family \([\text{B(L-Tar(NHPh)}_2]^2^-\) to chiral resolutions via diastereomeric salt formation was initially explored for five challenging cases; sec-butylamine, 2-pentylamine, 1-phenylethylamine, 1-phenylpropylamine and phenylglycinol. These were resolved in facile one-pot reactions with enantiomeric excesses of 34%-94%.\(^3\) Encouraged by this, we then extended the use to further aryltartramide spiroborate anions (-\[\text{NH}_{\text{Ar}}/-\text{NHCH}_2\text{Ar}, \text{Ar} = \text{Ph}, 4\text{FPh}, 4\text{-ClPh and 4-BrPh}\) to optimize resolution in one of the five examples: \textit{rac}-phenylethylamine as a typical test system. This is challenging since there is a single chiral centre with strong shape similarity between enantiomers of the racemic pair and both CH/Me and re-pyramidalization can occur to disorder the cation site. We have found some structural similarity of the resulting salts with preserved packing motifs, including an \(\text{NH}_3\text{R}\) binding site and a short 5.5Å axis. Importantly modification of the cation pockets in these related crystalline salts can lead to successful optimization of the \(R/S\) selectivity, with effective enantiopurity in at least two cases, improving on the 76% ee found for the salt from the parent \([\text{B(L-Tar(NHPh)}_2]^2^-\).

\[\text{References}\]

Insights into the catalytic activity of post-synthetically metalated Zr-based Metal-organic Frameworks

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Catalysis by metal-organic frameworks (MOFs) has attracted increasing attention in recent years. The modular synthesis of MOFs, from organic linkers and metal nodes, affords porous materials with features that are highly desirable for catalysis including: large internal surface areas and chemical tuneability with respect to their pore environment.2 As such, MOFs can act as heterogeneous catalysts via intrinsic activity (metal nodes/organic ligands) or by providing a support for known homogeneous catalytic moieties.3,4 Whilst this is a rapidly growing area of research, there has been minimal focus on determining the location of catalytic activity, either at the surface or within the pores for the MOFs currently reported.5 As such, identifying and precisely characterising the active species within MOFs, determining the location of catalytic activity, and analysing how pore confinement can affect catalysis with MOFs are of upmost importance to further this field of research.

This contribution will present our recent efforts to characterise reactive complexes and catalytically active species within derivatives of the zirconium-based framework UiO-67 (containing 5,5′-dicarboxy-2,2′-bipyridine [bpydc] and 5,5′-dicarboxy-6,6′-dimethyl-2,2′-bipyridine [Mesbpydc]). These types of MOF can be post-synthetically metalated with transition metal catalysts and the structures of the metal-centred catalyst revealed by single crystal X-ray diffraction.5 Additionally, we will discuss the difference in catalytic activity at the crystal surface and within the pores for ethylene oligomerization.

References


PROXIMA 2A: Striking a Balance between Automation and Human intervention or How to Treat your Precious Crystals with TLC

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PROXIMA 2A is a high performance 3rd generation synchrotron beamline dedicated to X-ray micro-crystallography on biological macromolecules. Since its opening in March 2013, a large number of users have collected vast amounts of X-ray diffraction images from literally thousands of crystals. The beamline delivers a high flux, finely focused X-ray beam (10 µm * 5 µm) tunable over a wide energy range. The experimental station is equipped with a robotic sample changer, a kappa geometry micro-diffractometer, and an EIGER X 9M detector (750 Hz). The experimental functionalities include X-ray fluorescence scans, routine SAD and MAD experiments, automatic loop centering, X-ray centering and raster scans of the sample, helical scans for needle-shaped crystals, and kappa geometry strategies for sulfur SAD experiments. Our efforts to automate and streamline the throughput of the beamline include parallel data processing on a 144-core server, a molecular replacement pipeline and the use of artificial intelligence for sample detection.

Many structural biologists work hard to obtain well-diffracting crystals of their biological molecule for structure determination, but it is ironic that many automatically collect their X-ray data in a “fast and furious” mode rather than with some careful thought and a little “tender loving care”. Despite the high level of automation, radiation damage and systematic errors can “kill” an experiment and waste a precious crystal. On PROXIMA 2A we help users to strike a balance between automation and human intervention in order to provide the best results from their hard won crystals.
Acquirement and measurement of Micro-focused beam for MX at SSRF

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The macromolecular crystallography beamlines at third-generation synchrotron facilities play a central role in solving macromolecular crystal structures and also in understanding the biological function at molecular levels. There is some benefit of using micro-focused beam in MX data collection. It is very difficult to acquire one micron beam, and also for its measurement due to some environmental factor. A new macromolecular crystallography beamline with two-stage focusing scheme is designed at Shanghai Synchrotron Radiation Facility. The beam with a directly focused size around 4 µm × 3 µm (H × V, FWHM) could be conveniently down to 1 µm × 1 µm by adjusting secondary source slit, and up to around 10 µm × 10 µm by inserting compound refractive lens. The performance of beamline is simulated by OASYS. The flux is expected as around 10^{11} phs/s for 1 µm × 1 µm beam at 12 keV. One-knife scanning and image identification is used to measure the one micron beam.
Application of Virtual Screening with Crystallography

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Heat shock protein 90 (Hsp90) is a dimeric molecular chaperone that is crucial for the folding and stabilisation of client proteins. The Hsp90 protomer is composed of three structurally distinct domains: the N-terminal domain (NTD), which binds ATP, the middle domain (MD), which contains the co-chaperone binding site, and the C-terminal dimerization domain (CTD). In cancerous cells, the client proteins of Hsp90 also include oncoproteins that promote cancer cell development. Hence, the inhibition of Hsp90 may be a useful strategy towards the treatment of cancers. Many inhibitors were reported for the NTP of Hsp90. However, as these compounds typically target the ATP binding pocket, they lack selectivity. The development of MD and CTD inhibitors is challenging due to a lack of a defined binding pocket. Here, by using the crystal structures of Hsp90 MD and CTD, we apply molecular modelling to define potential inhibitor binding pocket(s) on Hsp90, and use virtual high throughput screening (vHTS) to identify new Hsp90 inhibitors.
CrystalDirect-to-Beam: Opening the shortest path from crystal to data

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CrystalDirect™ (CD) is a fully automated harvesting system that mounts crystals grown on an ultra-thin film directly compatible with X-ray data collection on SPINE compliant sample holders [1, 2]. The CD harvester at the EMBL Grenoble HTX lab is now integrated with a FlexHCD [3, 4], which allows 40 crystals per hour to be harvested. The Crystallization Information Management System (CRIMS) (https://htxlab.embl.fr/) allows for the pre-location of crystals in CD plates, and their subsequent tracking via the ISPyB experimental database (https://exi.esrf.fr/mx). In tandem with the fully automated ESRF MX beamline workflows [4, 5] this system is now extensively used to support high throughput fragment screening applications. CD harvesting is also applicable for difficult projects, such as those involving fragile or micro crystals, opening a new way to supply MX beamlines with crystals. Here, we describe a CrystalDirect-to-Beam installation made at the ESRF beamline ID30B [4]. Preliminary results are encouraging, enabling data collection at cryo-cooled or room temperature, as well as humidity control experiments [6]. These results show how a CD could eventually become a standard beamline component at synchrotron sites with crystallisation facilities. This concept can also be exploited for more advanced data collection methods, such as serial data collection at 4th generation synchrotrons like the ESRF Extremely Brilliant Source.

Figure 1. CrystalDirect™ harvester installed on ID30B at the ESRF.

PHOTON III: Mixed Mode Pixel Array Detector for the XFEL and Home Laboratories

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X-ray free electron lasers present extreme demands to detector performance. In particular, conventional pixel array detectors (HPADs, PADs) suffer from count rate saturation due to the extremely high instantaneous intensity. Charge-integrating Pixel Array Detectors (CPADs) have therefore been developed to cope with these challenging requirements.\textsuperscript{1)}

The PHOTON III is a new CPAD which features ‘mixed mode’ (simultaneous photon counting & integration) operation to achieve Poisson-limited statistics without count rate saturation at count rates of up to $10^{15}$ X-rays/pixel-sec.

At the same time modern chemical and biological crystallography continuously pushes the limits to ever smaller samples with typically weaker diffraction properties. Its high count rate linearity make the PHOTON III also for home laboratory systems the perfectly choice as it additionally avoids charge-sharing, another disadvantage of conventional pixel array detectors.

Bruker’s in-house solutions, the D8 QUEST and D8 VENTURE both take advantage of the new PHOTON III detector technology, leading to a previously unknown level of performance.

Here we will describe the principles of CPADs and mixed-mode operation and the design and characteristics of the PHOTON III for XFEL applications. We also present experimental data obtained from extremely challenging samples collected on an in-house system to illustrate the strength of the new PHOTON III X-ray detector in that segment.

Neutron Diffraction Information from Standard X-ray Data: Hirshfeld Atom Refinement and Olex2
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"Hirshfeld atom refinement (HAR) is a novel X-ray structure refinement technique that employs aspherical atomic scattering actors obtained from stockholder partitioning of a theoretically determined tailor-made static electron density. HAR overcomes many of the known limitations of independent atom modelling (IAM), such as too short element-hydrogen distances, or too large atomic displacement parameters (ADPs)" [1]

HAR is now available in the general release version of Olex2 [2]. Given a standard X-ray dataset of good quality, information that could so far only be obtained by neutron diffraction can now be determined using HAR: accurate hydrogen atom positions. The residual electron density maps derived from this quantum crystallographic technique will also be much 'cleaner' than those obtained from standard Independent Atom Model (IAM) refinements. HAR is easy to run from Olex2, and in this contribution, I will introduce what HAR is, how it is implemented in Olex2 and how you run it. I will also discuss the current limitations of the technique. I invite you to try this at home: this presentation is available online and contains everything you need to know to get started [3].

Olex2 is open-source, available for Windows, MacOS and Linux and is free of charge.

[3] This presentation: https://goo.gl/CFZ8ar
A new On-Axis Video microscope for high-throughput MX-Crystallography

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*Arinax Scientific Instrumentation*

Over the last two decades video microscopes evolved to a standard tool for sample visualization in MX-crystallography. This is true for synchrotron MX-crystallography as well as for MX-crystallography on home-laboratory x-ray sources.

MicroDiffractometers (MD2, MD2S and MD3), used for precise crystal alignment and orientation, today widely used on highly automated synchrotron MX-beamlines, make extensive use of a video microscope with a variable zoom level. Today, such type of video microscope is still the predominant tool for sample alignment on MX-beamlines.

Each crystal alignment procedure makes it necessary to cycle the zoom level from minimum zoom (large field of view) to maximum zoom (small field of view) and back (for the next alignment procedure). This is achieved by means of a motorized zoom column with moving optics.

With the characterization of 200+ crystals per day on a high-throughput MX-beamline the wear-out time of the motorized zoom column mechanics cannot be neglected anymore. Furthermore, with approximately 10 s for a full zoom cycle the cycling time becomes a limiting factor for the general beamline performance, e.g. measured by the number of crystals characterized per day.

To overcome this bottleneck we developed a new On-Axis Video microscope, called B-Zoom, with a motor-less zoom and a high-speed video server for easy integration into modern MX-beamlines. The B-zoom’s cycling time is reduced to only several milliseconds. We present quantitative data which demonstrate the high-quality of the images delivered by the system.
Shine Bright Like a Diamond: Microfocus X-ray Sealed Tube Sources with Diamond Hybrid Anode Technology

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Diamond exhibits several unique properties, such as high thermal conductivity, low thermal expansion, extreme hardness and chemical inertness. The thermal conductivity of diamond is about 5 times higher than that of copper and the highest known conductivity of all bulk materials.

Therefore, diamond, being a non-toxic material, is increasingly replacing traditional materials for the thermal management in challenging applications where a high local heat load needs to be dissipated, such as in heat sinks for high power microelectronic devices. In X-ray sources diamond, can be used as a transparent heat sink directly coupled to the anode material. This improves the heat dissipation considerably and allows for an increase in tube brilliance by applying a higher power load on the anode.

The IµS DIAMOND provides a new class of microfocus sealed tubes based on a unique anode technology, the diamond hybrid anode. It takes advantage of the exceptional high thermal conductivity of diamond by using a bulk industrial diamond as a heat sink, which is coated with a layer of the target material, such as Cu, Mo or Ag. The diamond heat sink makes the heat dissipation in a diamond hybrid anode significantly more efficient. Consequently, the IµS DIAMOND can accept a higher power density in the focal spot on the anode, resulting in a higher intensity, without damaging the surface of the target layer. The balanced heat management in the source assures that the intensity loss over time is only a few percent over 10,000 h of full power operation. This makes the IµS DIAMOND significantly more stable than typical microfocus rotating anode sources, where under the same conditions a more than ten times higher intensity degradation is observed. As a consequence, the intensity of the IµS DIAMOND is about 20% higher than the average intensity output of a modern microfocus rotating anode. The IµS DIAMOND maintains all well-appreciated advantages of a standard IµS with respect to comfort, long lifetime and low maintenance.

In this presentation, we will present results demonstrating the impact of this new class of X-ray sources on the data quality for applications in protein and small molecule crystallography along the main new features of the IµS DIAMOND.
Chemical Crystallography at the Australian Synchrotron MX Beamlines

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Since commissioning, the Macromolecular (MX1) and Microfocus (MX2) beamlines at the Australian Synchrotron have proven successful at catering to both the structural biology and chemical crystallography (CX) communities.

The recent deployment of a Dectris 16M Eiger detector (funded by a consortia of Australian Structural Biology laboratories and the Australian Cancer Research Foundation) on MX2 has changed the ‘standard’ data collection protocol for CX work to a new shutterless 360° oscillation yielding 3600 frames in 36 seconds.

This step change in sample and data throughput has led to challenges in user workflow and highlights that the biggest dead time during beamtime arises during manual sample handling with the need to search and secure the endstation (robotic mounting does allow sample changes to occur in less than 40 seconds).

The dynamic range of the Eiger is substantially greater than a CCD detector, however ‘overloaded’ pixels can occur. These ‘overflows’ are not immediately obvious in the frames, but can have a significant effect during count rate correction of the Eiger output. New tools are being developed to better quantify data quality prior to structure solution. Other software tools are being developed to aid in data processing.

Future upgrades are underway to further improve MX1 with goniometer modifications and a Dectris 9M Eiger detector scheduled for early 2019. Given the dramatic increase in experimental throughput, what additional opportunities can be embraced by the Australian Synchrotron’s chemical crystallographic community? A review of current developments and discussion of future directions will be presented.
Fully automated data collection system at the Photon Factory macromolecular crystallography beamlines

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Structural Biology Research Center in the Photon Factory, Japan, has five macromolecular crystallography beamlines in two synchrotron radiation facilities in PF and PF-AR. We carry out to develop experimental techniques with these beamlines and provide them to academic and industrial users in Japan as well as the world. Among these developments, automation of experiments at the beamlines is one of the most important subjects, and we have developed automation techniques so far [1,2,3]. Very recently we have improved our automated crystal centering by combining two new techniques, a robust loop recognition and protein crystal detection with X-ray diffraction scanning. We have implemented this centering to our fully automated data collection system originally developed for pharmaceutical researches [3]. From last May, A new beamline usage scheme coupled with our fully automated data collection system will be opened for foreign users in 2019 April. In this scheme, users who have a valid proposal send their samples to our beamlines, and diffraction data collection is performed in a fully automated manner. After the experiment, the samples and data are sent back to users.

IDEAL - Invariom Derived Electron AnaLysis for APEX3

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With the introduction of large shutterless detectors, data quality to high resolution has dramatically improved over the last few years. Data to 0.5 Å and beyond can be collected with only one detector setting, with short exposure times, and short overall experiment times. Traditionally structures are refined using an Independent Atom Model (IAM), although good data beyond the traditional 0.83 Å reveals additional electron density that cannot be modeled appropriately. Often practitioners choose to cut data to preserve a structure reliability criteria R\(^1\), sacrificing additional information and overall structure quality.

IDEAL uses a database of bond-oriented deformation density parameters derived from the invariom\(^1\) database of \textit{ab initio} calculations of model compounds. IDEAL generates BEDE (Bond Electron Density) and LONE (Lone Pair Electron density) for refinement with an extended version of SHELXL. SHELXL uses IAM scattering factors and in addition, models scattering contributions of bonds and lone pairs.

IDEAL delivers structures with increased model accuracy and provides access to more detailed model properties. IDEAL is easy to use and seamlessly integrated with APEX3 and SHELXle.

Development of Automated Home-Lab Beamlines

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Abstract:

For over a decade high flux synchrotron beamlines have focused intensely on automating their macromolecular crystallography beamlines in to increase throughput, reduce sample handling errors and improve data quality. This focus has been driven by the fact that highly intense beams coupled with fast detectors now enable complete data sets to be collected in only minutes. As a result the dead time attributed to sample mounting and centering consumed the vast majority of the total experiment time. The pressure to mount and center crystals quickly rapidly increased the number of samples lost due to handling errors.

Bruker integrated the ultra-high brilliance METALJET source into their D8 VENTURE platform over five years ago, and subsequently developed the first CPAD detector designed specifically for compatibility with such X-ray sources. Users of such an in-house beamline have found that they face similar difficulties to those experienced at synchrotron beamlines before modern automation was introduced.

Here, we will introduce new developments made by Bruker in automating their high-brilliance D8 VENTURE systems, namely AGH, SCOUT, and ISX STAGE.

The AUTOMATED GONIOMETER HEAD is a highly robust goniometer head designed for fast crystal centering in combination with a KAPPA goniometer. In addition to optical crystal centering, the AGH features, for the first time in-house, the ability to center crystals using their X-ray diffraction. This enables the most accurate centering of small crystals by eliminating errors associated with optical centering. It also enables the reliable centering of crystals embedded in opaque LCP, the ability to center on the “diffraction hotspots” of larger crystals provides a further route to obtaining the best quality data from the crystals available.

The SCOUT Automated Sample Changer is a six-axis robotic system designed for the automated sample mounting, centering and retrieval of crystal under cryogenic conditions. Unlike earlier such robots, SCOUT has been conceived and designed specifically to meet the demands of in-house crystallography. The priority has been on maximizing the reliability of sample mounting and retrieval, and minimizing icing. This ability to retrieve crystals and return them to storage in LN2 without ice accumulation is an area that is much more demanding than in synchrotron crystallography, were crystals are typically discarded after exposure. An advanced software GUI enables automated crystal centering, scoring and data collection.

The ISX STAGE is the only automated plate-stage compatible with a KAPPA goniometer. The methods development associated with room temperature in situ crystallography over recent years have been mirrored in the popularity of the ISX STAGE. Here, we will review the key features of the stage and highlight the key applications.
Upgrades on NFPS BL19U1: a protein Complex crystallographic beamline at the SSRF

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The beamline BL19U1 is located at the Shanghai Synchrotron Radiation Facility (SSRF) and it is dedicated for protein complex crystallography. Based on a small-gap in-vacuum undulator, it is designed for large protein complexes and assemblies with large cell dimensions. BL19U1 has a working energy range of 7-15 keV for accessing the absorption edges of commonly used heavy atoms for phasing. A fixed-exit double-crystal monochromator [Si(111)] and a pair of rhodium-coated X-ray mirrors are used for beam monochromatization and manipulation, respectively. This beam line is employed with Pilatus 6M detector, MD2 goniometer, fluorescence detector, cryogenic sample cooler and automated sample changer. The BL19U1 beamline has officially opened to users since March 2015. To date, over 300 research groups have collected data using this beamline, and more than 200 pdbs was deposited to the Protein Data Bank (pdb.org).
Small molecule X ray Diffraction Data Acquisition in NFPS BL17B1 at the SSRF

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BL17B1 is a protein crystallographic beamline station affiliated with NFPS in Shanghai Synchrotron Radiation Facility (SSRF). It can carry out SAD, MAD studies with S, Se, Pt, Hg for macromolecular structural solution. Besides, BL17B1 also open for small molecule data acquisition and a series of significant structures have already been resolved. BL17B1 has a working energy range of 5-20 keV. This beamline is equipped with a MD2 diffractometer, a Rayonix MX300 detector, and a CATS sample changer. The size of the small molecule crystals could be as small as 10μm in dimension, and still useful diffraction data could be collected and the structures could be solved.

Figure. 1. The BL17B1 station
TPS 07A Micro-focus Protein Crystallography Beamline at the National Synchrotron Radiation Research Center

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TPS 07A is an in-vacuum undulator-based, high-intensity and variable beam size (0.7-30 μm) crystallography beamline at the 3 GeV Taiwan Photon Source (TPS). This beamline is specially designed for significant but difficult projects with protein crystals of small size (1-30 μm) and weak diffraction, such as membrane proteins and protein/nucleic acid complexes. In addition, the beamline will also support the high-throughput data collection for pharmaceutical drug design. TPS 07A covers the energy range 5.7-20 keV (2.18-0.62 Å) for accessing the absorption edges of common heavy elements used for phasing. At 12.4 keV the flux at the sample is $1.4 \times 10^{12}$ photons s$^{-1}$ and beam size at focal point is 1.76 x 0.67 μm FWHM (H x V). The beamline endstation will be equipped with a micro-diffractometer (MD3-Up), a photon-counting pixel area detector (EIGER2 X 18M), fluorescence detector, cryogenic sample cooler and automated sample changer (ISARA robotic system) for automatically sample mounting and centering, making the data acquisition more efficient. Substantial user-support, remote access and mail-in service are also provided. The design and constructing status are given in this article.