Analysing IRF4 interactions to ISRE motifs in Multiple Myeloma (Oral)

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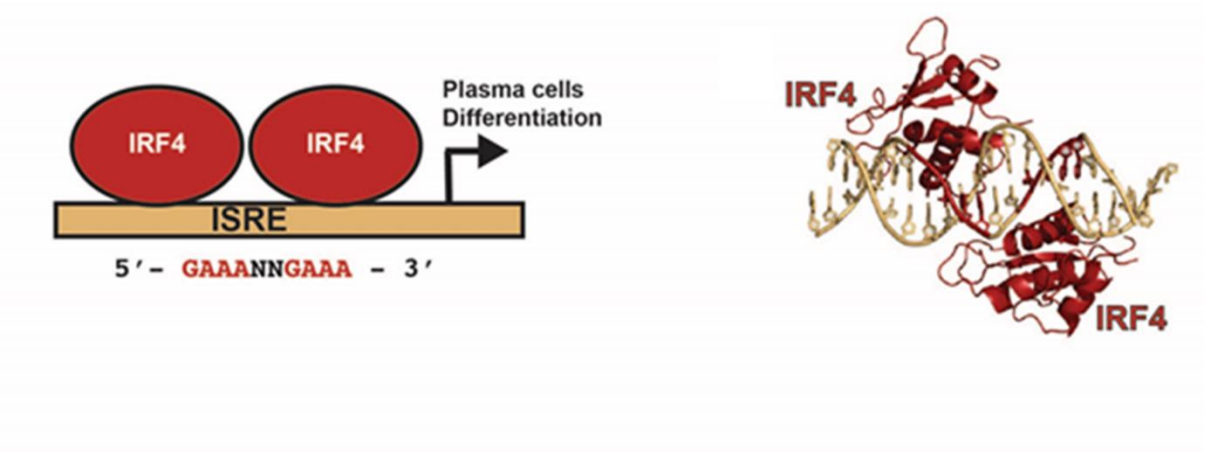
Multiple Myeloma (MM) is an incurable hematologic malignancy characterized by abnormal proliferation of plasma cells [1,2]. Interferon Regulatory Factor 4 (IRF4), a member of the interferon regulatory family of transcription factors, is central to the genesis of MM [2,3]. Data suggest that in MM IRF4 binds as a homodimer to the interferon sequence response element (ISRE) DNA motifs, therefore targeting its homodimerization ability to bind DNA would constitute a valid approach to MM subversion [1]. So far the mechanism of IRF4 homodimerization and binding to DNA has not been elucidated. These data would be key to small-molecules drug discovery programmes aimed at disrupting the IRF4-DNA binding interface. We solved the structure of the IRF4 DNA binding domain in complex with various ISRE sequences. These data provides key insights into the ISRE binding specificity and affinity as well as IRF4 homodimerization in the context of MM.



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Synthetic Cannabinoid Receptor Agonists are Monoamine Oxidase-A Specific Inhibitors; Implications for Harm Reduction Strategies in Using Communities (Poster)

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SCRA consumption frequently leads to severe and adverse health effects compared to those seen from cannabis usage.[1-4] These include tachycardia, hypertension, myocardial infarction, and cardiac arrest to name a few. The origin of such side effects is not well understood, as there is a distinct lack of evidence around the pharmacological and toxicological effects of these compounds. Given that the pressor response, associated with MAO-A, can give rise to symptoms similar to some of the 'unexplained' symptoms of SCRA use including hypertension and stroke, and SCRAs have structural similarity to known MAO inhibitors (MAO-Is), we test the hypothesis that SCRAs act as MAO-Is. We investigate this inhibition in both MAO-A and MAO-B using a combination of in-vitro and in-silico techniques. Well-known SCRA compounds have been paired with a series of four synthesised compounds, all containing an indole or indazole core. Flexible docking studies using Autodock Vina have been carried out and compared to experimental kinetic inhibition studies.

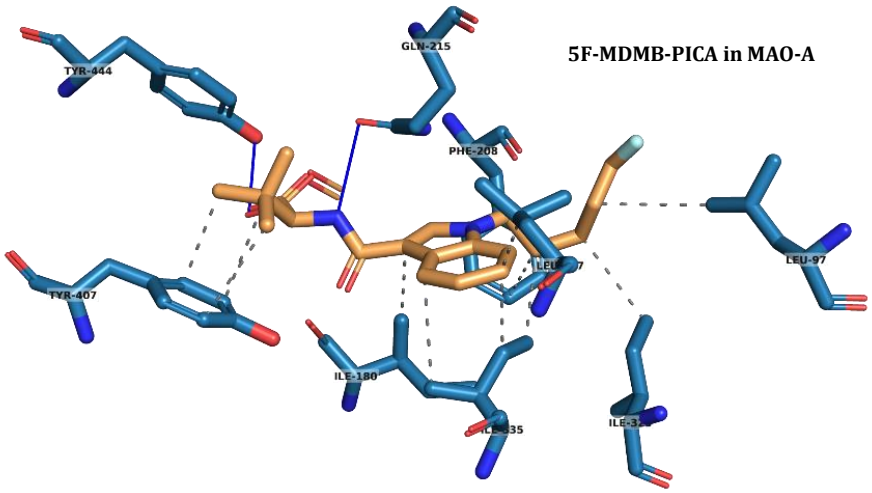

Figure: The mode of interaction of 5F-MDMB-PICA, a common synthetic cannabinoid, with the binding pocket of human MAO-B. Hydrophobic interactions are represented with a dotted grey line and hydrogen bonds with a solid blue line.

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Measuring energy-dependent photoelectron escape in microcrystals (Poster)

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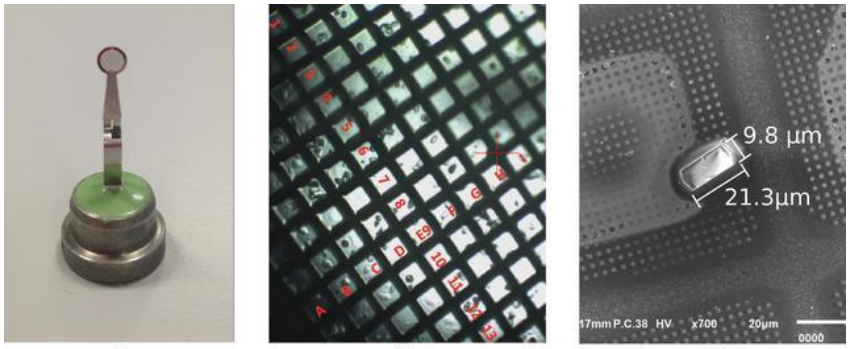
With the increasing trend of using microcrystals and intense microbeams at synchrotron X-ray beamlines, radiation damage becomes a more pressing problem. Theoretical calculations by Nave and Hill [1] show that the photoelectrons primarily causing damage can escape microcrystals. This effect would become more pronounced with decreasing crystal size as well as at higher energies [2, 3]. To prove this effect, data from cryo-cooled lysozyme crystals of dimensions 5 x 3 x 3 μm^3 and 20 x 8 x 8 μm^3 mounted on cryo-transmission electron microscopy (TEM) grids were collected at 13.5 keV and 20.1 keV using a 2M CdTe Pilatus detector, which has similar quantum efficiency at both energies. Accurate absorbed doses were calculated with RADDOSE3D [4] through direct measurement of individual crystal sizes using scanning electron microscopy after the experiment and characterization of the X-ray microbeam. The data were processed with DIALS [5] and crystal lifetime was then quantified based on the $D_{1/2}$ metric. In this first systematic study, a longer crystal lifetime for smaller crystals was observed and crystal lifetime increased at higher X-ray energies supporting the theoretical predictions of photoelectron escape. The use of detector technologies specifically optimised for data collection at energies above 20 keV allows the theoretically predicted photoelectron escape to be quantified and exploited, guiding future microfocus beamline design choices.



(a) (b) (c)

Primary Reference

Storm, S. L. S., Crawshaw, A. D., Devenish, N. E., Bolton, R., Hall, D. R., Tews, I. & Evans, G. (2020). IUCrJ 7, 129-135. https://doi.org/10.1107/S2052252519016178

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Uncovering an allosteric mode of action for a selective inhibitor of human Bloom syndrome protein
(Oral or Poster)

Xiangrong Chen[1,2], Yusuf I Ali[2,3], Charlotte EL Fisher[1] , Raquel Arribas-Bosacoma[1] , Mohan B Rajasekaran[3] , Gareth Williams[3] , Sarah Walker[3] , Jessica R Booth[3] , Jessica JR Hudson[3] , S Mark Roe[4] , Laurence H Pearl[1] , Simon E Ward[3,5]*, Frances MG Pearl[2] *, Antony W Oliver[1] *

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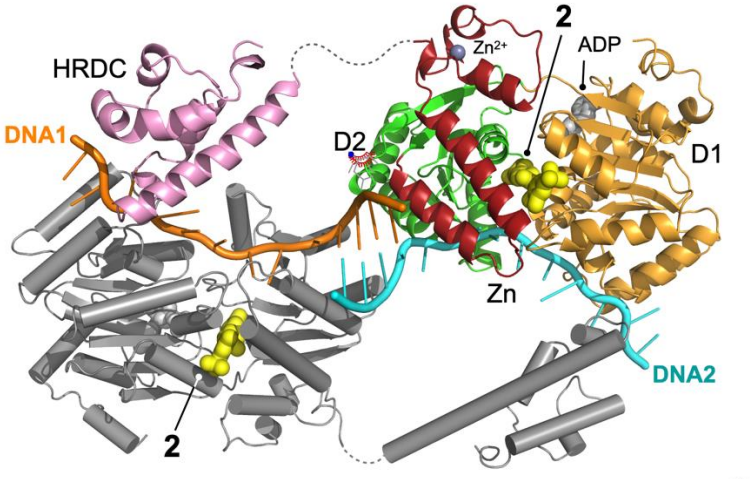
BLM (Bloom syndrome protein) is a RECQ-family helicase involved in the dissolution of complex DNA structures and repair intermediates. Synthetic lethality analysis implicates BLM as a promising target in a range of cancers with defects in the DNA damage response; however, selective small molecule inhibitors of defined mechanism are currently lacking.

Here, we identify and characterise a specific inhibitor of BLM's ATPase-coupled DNA helicase activity, by allosteric trapping of a DNA-bound translocation intermediate. Crystallographic structures of BLM-DNA-ADP-inhibitor complexes identify a hitherto unknown interdomain interface, whose opening and closing are integral to translocation of ssDNA, and which provides a highly selective pocket for drug discovery. Comparison with structures of other RECQ helicases provides a model for branch migration of Holliday junctions by BLM.



Primary citation (make sure to link to the online publication)
Chen, X., Y. I. Ali, C. E. L. Fisher, R. Arribas-Bosacoma, M. B. Rajasekaran, G. Williams, S. Walker, J. R. Booth, J. J. R. Hudson, S. M. Roe, L. H. Pearl, S. E. Ward, F. M. G. Pearl and A. W. Oliver (2021). "Uncovering an allosteric mode of action for a selective inhibitor of human Bloom syndrome protein." eLife **10**: e65339. https://elifesciences.org/articles/65339

Biomolecular docking studies of the farnesoid X receptor (FXR) DNA-binding domain reveal structural reasons underpinning isoform-specific binding of different DNA motifs
(Poster)

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Research into bile acid signalling via the nuclear receptor farnesoid X receptor (FXR) has gained substantial traction following its deorphanisation in the late 1990s. Despite this, research efforts focused primarily on the ligand-binding domain, with very little attention directed towards the DNA-binding domain (DBD). FXR binds to the bipartite FXR response element containing the 5'-AGGTCA-3' consensus sequence, which can be arranged into direct (DR), inverted (IR) and everted (ER) repeats. Previous research has identified that FXR isoforms -α2 and -α4 bind everted hexamer repeats spaced by two nucleotides (ER-2) with significantly higher affinity compared to isoforms -α1 and -α3, which preferentially bind inverted hexamer repeats spaced by one nucleotide (IR-1). However, no structural reasons have been identified which could account for this difference in binding selectivity. Using molecular docking, idealised IR-1 and ER-2 motifs were docked to FXRα1 and -α2 homology models, first as a monomer, then an FXR homodimer, and lastly an FXR/RXR heterodimer. Both the monomers and the dimers were shown to possess an intrinsic ability to differentiate between the relative orientations of the 5'-AGGTCA-3' consensus sequence. This work also demonstrates that the absence of a four amino acid-long MYTG insert located in the C-terminal region of the FXRα2 and -α4 DNA-binding domains facilitates this effect by inducing a sequence-independent geometrical conformation that is more conducive to stronger interactions between the FXR-DBD and DNA. Furthermore, the spacer region separating the consensus half-sites was found to be directly implicated in dictating the dimerisation properties of the receptors, which may prove consequential in allosteric control and DNA-binding cooperativity. Previously, the role of the MYTG insert in FXR function was merely speculative. The present study outlines a plausible structure-based rationale for MYTG-mediated DNA binding specificity in FXR. These results demonstrate that the MYTG insert can confer isoform-specific binding properties to FXR and can thus serve as the basis for further experimental approaches investigating DNA-binding modulation in FXR.

Novel bile acid derivatives as potential drugs for neurodegenerative disease (Oral or Poster)

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2. NZP UK Ltd

Substantial evidence now suggests that mitochondria play a pivotal role in health and disease. With mitochondrial dysfunction and the disruption of normal mitochondrial dynamics acting as underlying features in most diseases, it stands as a potential target for many different fields of therapy research. This project stands to review some of the mechanisms by which mitochondrial function is regulated, particularly via a mitochondrial lipid transporter, and how this could be targeted for neurodegenerative disease therapies, including Parkinson's disease and Alzheimer's disease. A further aim of this project is to study the mechanism of how bile acids might be altering the levels of lipids within mitochondria. By determining this mechanism could we discover a new therapy on how to safeguard the cells in the brain and reverse some of the damage done by these debilitating diseases?

CryoEM structure of the outer membrane secretin channel pIV from the f1 filamentous bacteriophage
(Oral and Poster)

Becky Conners[1,2], Mathew McLaren[1,2], Urszula Lapinska[1,2], Kelly Sanders[1,2], M. Rhia L. Stone[3], Mark A. T. Blaskovich[3], Stefano Pagliara[1,2], Bertram Daum[1,2], Jasna Rakonjac[4] and Vicki A.M. Gold[1,2]

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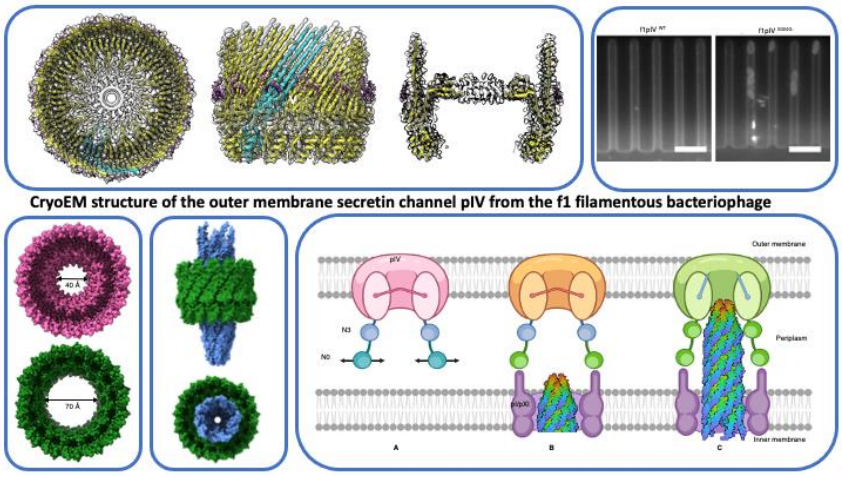
The Ff family of filamentous bacteriophages infect gram-negative bacteria, but do not cause lysis of their host cell. Instead, new virions are extruded via the phage-encoded pIV protein, which has homology with bacterial secretins. Here, we have determined the structure of pIV from the f1 filamentous phage at 2.7 Å resolution by cryo-electron microscopy, the first near-atomic structure of a phage secretin. Fifteen f1 pIV monomers assemble to form a gated channel in the bacterial outer membrane, with associated soluble domains projecting into the periplasm. We model channel opening and propose a mechanism for phage-mediated gate movement. By single-cell microfluidics experiments, we demonstrate the potential for secretins such as pIV to be used as adjuvants to increase the uptake and efficacy of antibiotics in bacteria. Finally, we compare the f1pIV structure to its homologues to reveal similarities and differences between phage and bacterial secretins.



Conners R, McLaren M, Lapinska U, Sanders K, Stone MRL, Blaskovich MAT, Pagliara S, Daum B, Rakonjac J and Gold VAM. CryoEM structure of the outer membrane secretin channel pIV from the f1 filamentous bacteriophage. In preparation

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The X-ray structure of juvenile hormone diol kinase from the silk worm *Bombyx mori*. (Poster)

Jingxu Guo,[a,b] Ronan M. Keegan,[c,d] Daniel J. Rigden,[d] Peter T. Erskine,[a,e] Steve P. Wood,[a,f] Sheng Li[g] & Jonathan B. Cooper.[a]

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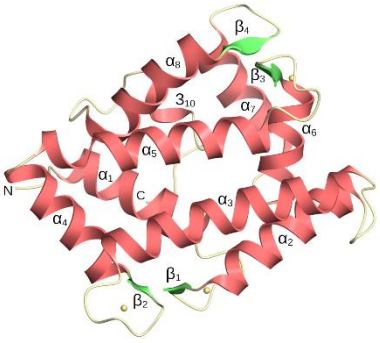
Abstract
Insect juvenile hormones (JH's) are a family of sesquiterpenoid molecules that are secreted into the haemolymph by the corpora allata. JH's have multiple roles in insect development, metamorphosis and sexual maturation. A number of pesticides work by chemically mimicking JH, thus preventing insects from developing and reproducing normally. The haemolymph levels of JH are governed by the rates of its biosynthesis and degradation. One enzyme involved in JH catabolism is JH diol kinase (JHDK) which uses ATP (or GTP) to phosphorylate JH diol to JH diol phosphate which can be excreted [1]. We have determined the X-ray structure of juvenile hormone diol kinase (JHDK) from silk worm *Bombyx mori* at a resolution of 2.0 Å with an R-factor of 19.0 % and an R-free of 24.7 %. There are 6 molecules in the asymmetric unit and four of them form covalent dimers linked by SS bridges involving the C-terminal residue, Cys 183. The enzyme consists of two domains (N- and C-terminal) of approximately 90 amino acids each. The structure possesses three EF-hand motifs which are occupied by calcium ions. This is in contrast to the recently reported structure of the JHDK-like-2 protein [2] from *B. mori* (6kth) which possessed only one calcium ion. Since JHDK is known to be inhibited by calcium ions, it is likely that our structure represents the calcium-inhibited form of the enzyme. Proteins with sequence similarity to JHDK demonstrate strong conservation of the metal-binding ligands in the first 3 EF-hand motifs.



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CCPNmr Analysis Version-3: modern software for integrated NMR analysis (Poster or Oral)

Edward J. Brooksbank[1], Luca G. Mureddu[1], Eliza Płoskoń-Arthur[1], Victoria A. Higman[1], Gary Thompson[2], Timothy J. Ragan[1] and Geerten W. Vuister[1]

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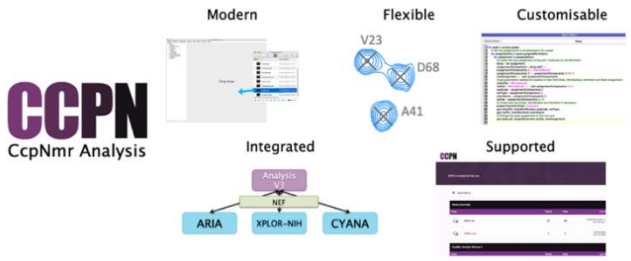
CcpNmr Analysis Version-3 is the latest NMR data analysis software from the Collaborate Computational Project for NMR (CCPN). It caters to biomolecular solution and solid-state NMR scientists, by developing applications, including those for resonance assignment, the analysis of chemical shift perturbation data[1], and the analysis of structural, metabolomics and screening[2] data. The software is built on modern concepts that enable users easy, fast and flexible ways of conducting routine and complicated tasks. Interfacing with structure calculation programs is achieved via the new NMR Exchange Format (NEF)[3]. Exported NEF files can also be used for the deposition of chemical shifts and structural restraints with the BMRB and wwPDB. A Python console which echoes commands from the graphical user interface provides an accessible way for users to start writing their own macros for bespoke tasks.



Primary citation

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Phylogenetic analysis with prediction of cofactor binding for *Pseudomonas aeruginosa* PAS domains. (Oral and Poster)

Andrew Hutchin, Charlotte Cordery, Martin Walsh, Jeremy Webb, Ivo Tews

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National Biofilms Innovation Centre (NBIC), University of Southampton, Southampton, SO17 1BJ
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Bacteria possess a variety of PAS domains in intracellular proteins and the related CACHE domains in periplasmic or extracellular proteins. PAS/CACHE domains are predominant in sensory systems, often carry cofactors, and serve as dimerization domains in protein association. We analyzed the proteome of *Pseudomonas aeruginosa* PAO1 *in silico*. The ability of this bacterium to survive under different environmental conditions, to switch between planktonic and sessile/biofilm lifestyle, or evade stresses notably involves c-di-GMP regulatory proteins or depends on sensory pathways involving multi-domain proteins that possess PAS/CACHE domains. Maximum likelihood phylogeny was used to group PAS/CACHE domains on the basis of amino acid sequence. Conservation of cofactor or ligand coordinating amino acids aided by structure-based comparison was used to inform function. The analysis predicts novel functions for sensory proteins and sheds light on functional diversification in a large set of proteins with similar architecture

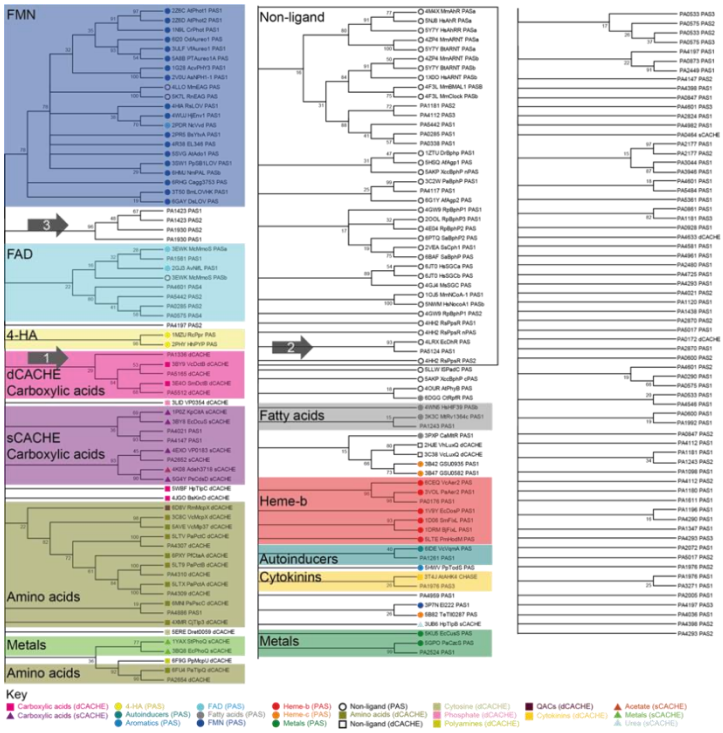

Figure 1Maximum Likelihood Phylogenetic Analysis of Pseudomonas aeruginosa *PAO1 PAS domains with the reference set of structurally characterised PAS domains.*

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Structure of the ChAdOx1 Virus Vaccine (Oral and Poster)

A. Teijeira Crespo*, Magdalena Lipka-Lloyd*, Alexander T. Baker, Alan L.Parker, , Pierre J. Rizkallah

* *Joint presentation - these authors contributed equally to the work.*

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Covid-19 has become a big burden, in the past year, affecting millions of people around the world, emerging as a global health crisis. As there is no cure for this disease caused by a coronavirus, scientists across the world have focused their effort on the development of a vaccine capable to combat SARS-CoV-2. One of these vaccines is an adenovirus-based vaccine developed by AstraZeneca-Oxford University. Although this vaccine has gone through different studies showing its potential as a covid vaccine, its structure has never been studied. Thus, what we show here is the structure of the ChAdOx1 fiber-knob receptor (PDB 7OP2) which is responsible for the primary virus-cell interaction during the infection.

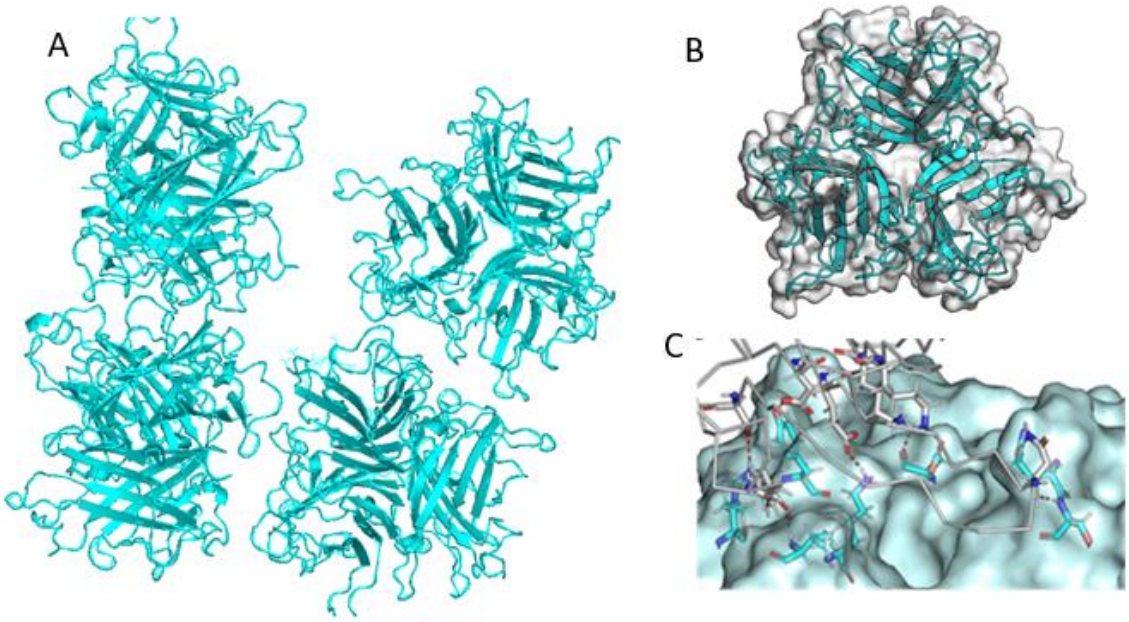

Figure 2 **ChAdOx1 fiber knob structure**. Asymmetric unit (A) showing 4 trimeric copies of an homotrimer with a 3-fold symmetry which contain three monomers (B). Prediction of the possible polar contacts (red dashes) of ChAdOx1 with CAR (white ribbon)

The study has been extended to examine the mechanism of vaccine induce clot formation, and to identify possible virus receptors in the human body, based on an EM structure determination of the whole vaccine virus (Figure 2). The manuscript for this work has been published in bioRxiv (**doi:** https://doi.org/10.1101/2021.05.19.444882)

HotSolute: *Thermus thermophilus* as a Whole Cell Factory for the Production of Extremolytes (Poster)

Simone Antonio De Rose, Michail Isupov and Jennifer Littlechild

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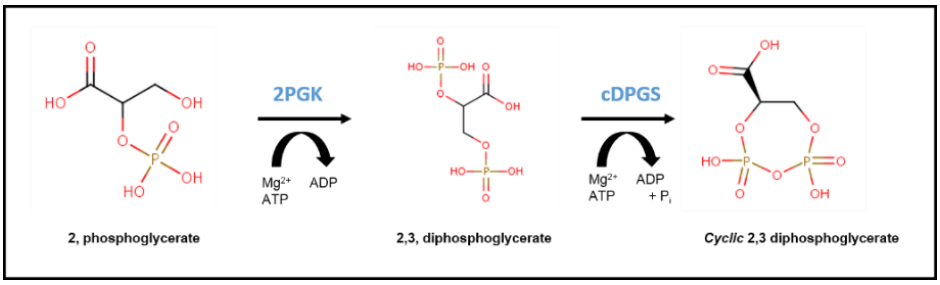
Extremolytes are found naturally in the cells of hyperthermophilic microorganisms who accumulate them in response to environmental and endogenous stresses. These small molecules have great potential for applications in the food, health care, consumer care and cosmetics markets.

Cyclic 2,3 di-phosphoglycerate (cDPG) has been found in hyperthermophilic methanogens in concentrations up to 1.1 M. cDPG is formed by a **two-step synthesis** from 2-phosphoglycerate via phosphorylation by 2-phosphoglycerate kinase (2PGK) and cyclisation by di-phosphoglycerate synthetase (cDPGS). It is thought to protect proteins and DNA against thermal and oxidative damage and to function as a superoxide scavenger.



The production of cDPG in a mesophilic host (yeast or *Escherichia coli*) is currently hampered by the production of active enzymes at the host growth temperature. This study has shown that the thermophilic bacterium ***Thermus thermophilus*** can act as a cell factory for the cDPG synthetic pathway.

The two enzymes involved in the pathway for synthesis of cDPG are structurally novel. The crystal structure of the cDPGS has been solved by experimental phasing, while the 2PGK has also been crystallised but is yet to be solved.

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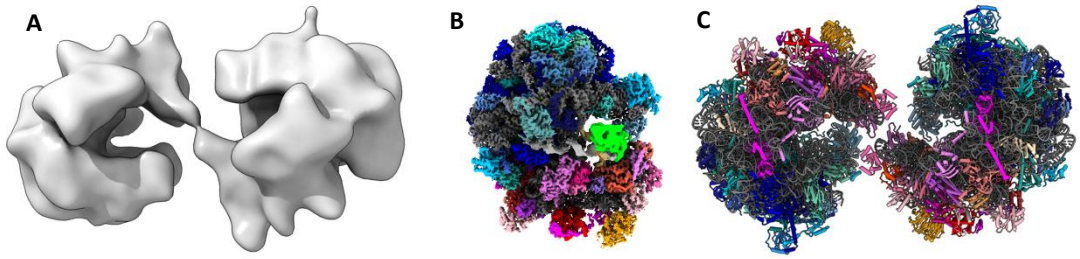
Structure of the eukaryotic hibernating ribosome dimer from the microsporidia *Spraguea lophii*
(Oral or Poster)

Patricia Gil-Diez, Mathew McLaren, Misha Isupov, Lavinia Gambelli, Bryony Williams & Bertram Daum

Living Systems Institute, University of Exeter, Exeter, EX4 4QD

Microsporidia are fungal intracellular pathogens that infect a broad range of animals, including humans. During the infective stage of their life cycle, the microsporidia form dormant and environmentally resistant spores. After entering a host, the infection process is triggered and the spores eject their cellular content through a long cellular appendage called polar tube (1). We employed electron cryo-tomography to investigate the macromolecular organisation of microsporidian cells traversing the polar tubes. Surprisingly, we found that microsporidian ribosomes form 100 S dimers inside these polar tubes. While ribosome dimerisation is a ~~well-known~~well-known mechanism to shut down protein translation during adverse environmental conditions in bacteria (2) its prevalence in eukaryotes was so far not very well established (3). By sub-tomogram averaging, we obtained a 20 Å structure of the ribosome dimers in the native state (A) and by single particle analysis of isolated ribosomes, we solved the structure of the *S. lophii* ribosome at 2.3 – 2.6 Å resolution (B). By combining both datasets, we obtained a first atomic model of a eukaryotic ribosome dimer (C), shedding new light on the molecular basis of ribosome hibernation in eukaryotic organisms.



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The Structural Basis of +ssRNA Virus Replication (Poster)

Kain van den Elsen[a,b], Luo Dahai[b], and Bertram Daum[a,d]

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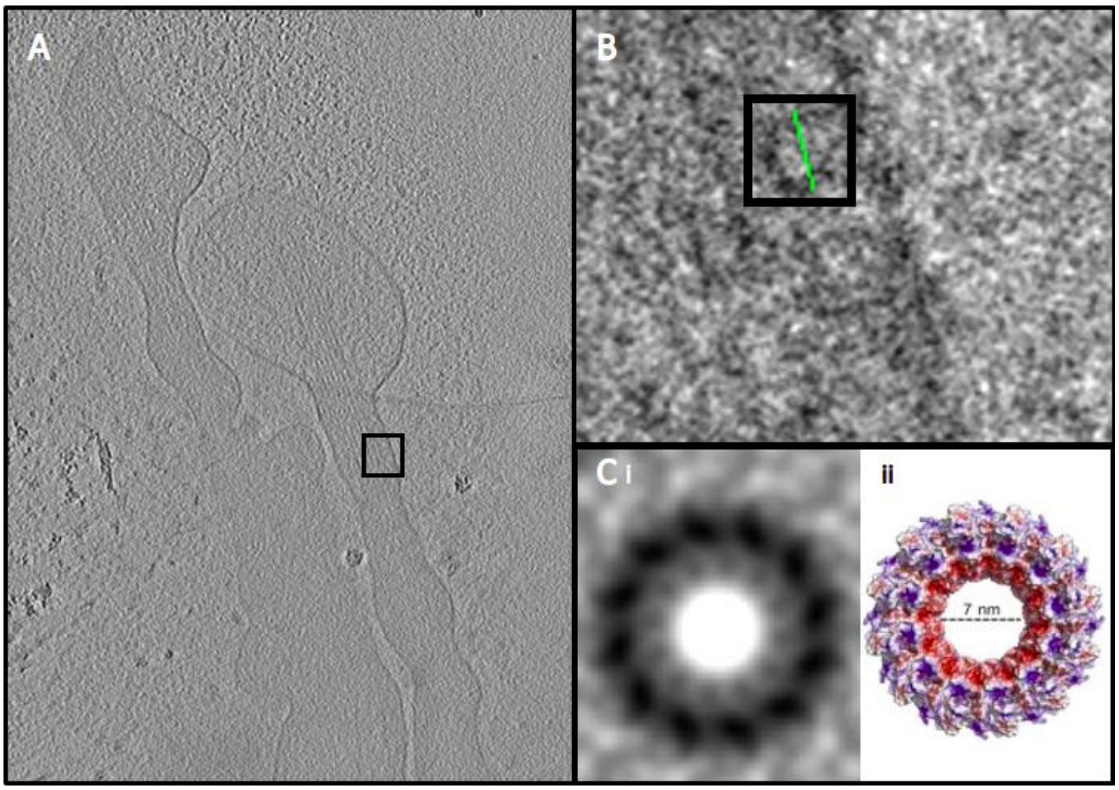
[b] Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore 636921, Singapore
[d] College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4QD, UK

Many +ssRNA viruses are well-known and clinically important human pathogens, including rhinoviruses, coronaviruses, flaviviruses and alphaviruses such as Chikungunya virus. During infection, these viruses induce formation of mini-organelles associated with host-cell organelles, which act as viral replication factories. Within these replication complexes (RCs), multiple viral and host factors combine to form the RNA-replicase machinery. These proteins perform crucial functions during viral replication, making them enticing drug targets. Nevertheless, effective drug development has been hampered, as the inner workings of RCs are inadequately understood. A comprehensive understanding of the complex interplay between viral and host factors, and the RC molecular architecture is required to effectively target viral replication. We aim to reveal the structure of the CHIKV RC-pore, NSP1, through a high-resolution structural approach. By employing cryo-electron tomography, we aim to observe this molecular machine *in situ* and to elucidate the intricate mechanisms and interactions pivotal to its function. This work will provide fresh insights into our understanding of the replication of +ssRNA viruses inside host cells and will aid structure-based drug design and the production of effective vaccines and anti-virals.



A) Tomograph of actin-containing filipodia induced by NSP1, with an enlarged image of the 18nm diameter rings in the membrane (B), presumably NSP1. A rotationally averaged sub-tomogram average (from 25 particles) of the rings, with 12-fold symmetry applied (Ci) compared to a published ex-situ structure of the complex (Cii).

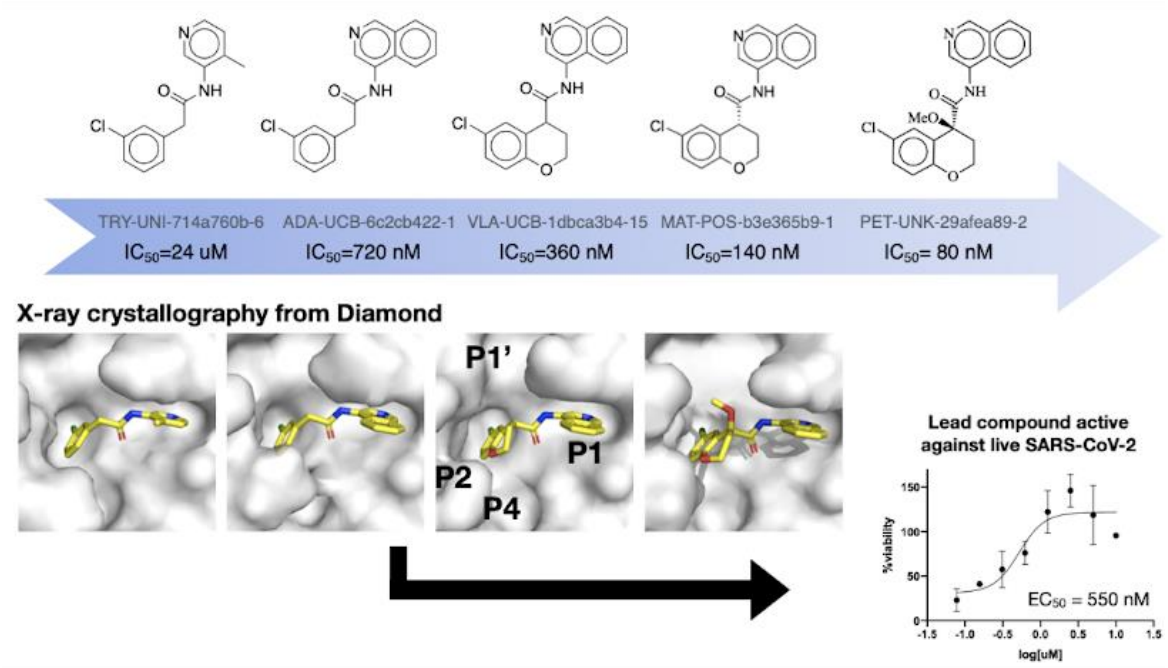
Crystallographic fragment screening with the XChem platform at Diamond Light Source and hit-to-lead progression of novel SARS-CoV-2 Main protease inhibitors
(Oral or Poster)

Daren Fearon, Anthony Aimon, Jose Brandao-Neto, Patrick Collins, Alex Dias, Alice Douangamath, Louise Dunnett, Tyler Gorrie-Stone, Tobias Krojer, Nick Pearce, Ailsa Powell, Rachel Skyner, Romain Talon, Warren Thompson, Conor Wild, Frank von Delft. (Oral or Poster)

Diamond Light Source Ltd, Harwell Science & Innovation Campus, Didcot, Oxfordshire OX11 0DE
Research Complex at Harwell, Harwell Science & Innovation Campus, Didcot, Oxfordshire OX11 0FA, UK

Fragment-based drug discovery is a well-established method for the identification of chemical starting points which can be developed into drugs for use in clinic. Historically X-ray fragment screening using traditional crystal soaking methods was tedious, low-throughput and time consuming. However, thanks to advances in synchrotron capabilities and the introduction of streamlined crystal soaking facilities, such as the XChem platform at Diamond Light Source, there has been substantial improvements in throughput and integration between sample preparation, data collection and hit identification.

To identify starting points for developing therapeutics which target SARS-CoV-2, the XChem team at Diamond Light Source, in collaboration with various international colleagues, performed large crystallographic fragment screens against 7 key SARS-CoV-2 proteins. We will present the tools and technologies that have enabled this work to be carried out at a rapid pace and for the effective development of lead-like inhibitors of the SARS-CoV-2 Main protease.



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Identification and development of novel prebiotic targets in the gut microbiome
(Poster)

Anatol Gawrzak, Ged Baltulionis, Alexandra Maruszak, Lucien Harthoorn and Kim Watson

1. School Biological Sciences, University of Reading
2. Clasado Biosciences, UK

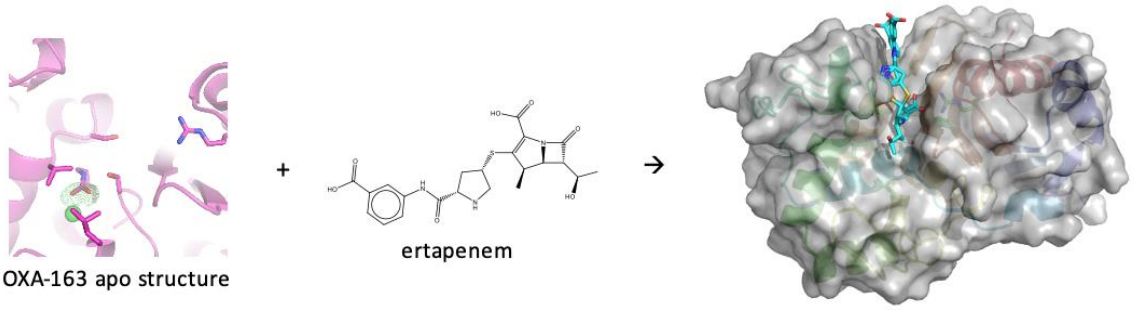
The human gut microbiome is an extensively research topic due to its potential role in not only influencing the immediate integrity of the intestinal tract, but also having a systemic effect on various metabolic functions throughout the organism. Prebiotics, indigestible substances that are metabolised by the gut microbiota to have various effects on gut population and metabolite production, are of growing interest as potential target for therapeutic development. This study aims to produce novel prebiotics, specifically prebiotic gGalactooligosaccharides (GOS), which would have a unique effect on bacterial culture. Specifically, it investigates the catalytic mechanism behind Bifidobacterium bifidum β-galactosidase hydrolysis of lactose derivatives and aims to create constructs of said enzymes with higher synthetic potential and unique GOS profile composition. Additionally, other novel prebiotics with be evaluated for their prebiotic potential.

Substrate and Inhibitor Interactions of Class D OXA β-lactamases (Oral and Poster)

Kirsty E. Goudar, Catherine L. Tooke, Phillip Hinchcliffe, James Spencer

School of Cellular and Molecular Medicine, Biomedical Sciences Building, University of Bristol, Bristol, BS8 1TD

Class D (OXA) β-lactamases are a diverse group that utilize a serine nucleophile to inactivate β-lactams, in a covalent acylation-deacylation reaction involving a carbamylated lysine general base. Some hydrolyse the most potent β-lactam class – carbapenems, and most escape clinically available inhibitors, making them targets for new therapies to counter resistance. Using X-ray crystallography, we investigated binding of enzymes from the OXA-48 group (OXA-163, OXA-405) to carbapenems and β-lactamase inhibitors such as the diazabicyclooctane (DBO) avibactam. Our structures reveal diversity in carbapenem and DBO binding, identifying both R- and S-stereomers of the OXA-163:ertapenem acylenzyme in the D1 (imine) tautomer and a decarbamylated lysine general base in both DBO and carbapenem complexes. Our data demonstrate OXA-163 and OXA-405 to be amenable systems for characterisation of ligand complexes, while simultaneous presence of both stereomers may indicate exchange between acylenzyme forms. Future work will explore additional time points to investigate this.



OXA-163 apo structure

OXA-163:ertapenem acylenzyme complex with Δ1 tautomer in dual stereomers

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Engineering a cytochrome P450 for demethylation of lignin-derived aromatic aldehydes
(Oral or Poster)

Emerald S. Ellis,a‡ **Daniel J. Hinchen,b‡** Alissa Bleem,c,d‡ Lintao Bu,c Sam J.B. Mallinson,b† Mark D. Allen,b Bennett R. Streit,a Melodie M. Machovina,a¶ Quinlan V. Doolin,a William E. Michener,c Christopher W. Johnson,c Brandon C. Knott,c Gregg T. Beckham,c,d* John E. McGeehan,b* Jennifer L. DuBois,a*

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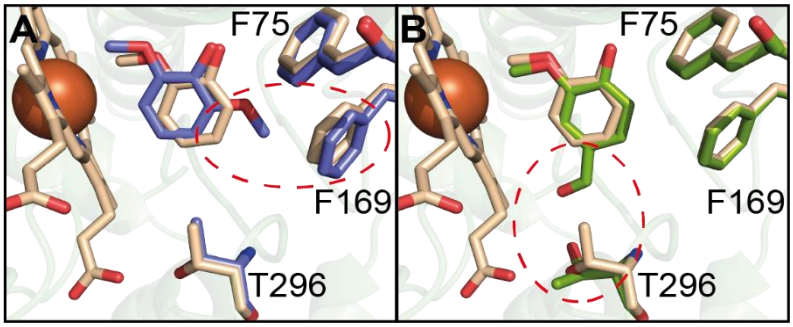
* Corresponding authors ‡ Denotes equal contribution

Biological funneling of lignin-derived aromatic compounds is a promising approach for valorizing its catalytic depolymerization products. Industrial processes for aromatic bioconversion will require efficient enzymes for key reactions, including demethylation of O-methoxy-aryl groups, an essential and often rate-limiting step. The recently characterized GcoAB cytochrome P450 system comprises a coupled monoxygenase (GcoA) and reductase (GcoB) that catalyzes oxidative demethylation of the O-methoxy-aryl group in guaiacol. Here, we evaluate a series of engineered GcoA variants for their ability to demethylate o-and p-vanillin, which are abundant lignin depolymerization products. Two rationally designed, single amino acid substitutions, F169S and T296S, are required to convert GcoA into an efficient catalyst toward the o- and p-isomers of vanillin, respectively. Ten novel x-ray structures solved at the Centre for Enzyme Innovation and Diamond Light Source, with kinetics, molecular dynamic data, and in-vivo studies, from our US collaborators, reveal we have expanded the known aromatic O-demethylation capacity of cytochrome P450 enzymes towards an important class lignin-derived aromatic monomers, the aldehydes.



Primary citation:
Engineering a Cytochrome P450 for Demethylation of Lignin-Derived Aromatic Aldehydes
Emerald S. Ellis, Daniel J. Hinchen, Alissa Bleem, Lintao Bu, Sam J. B. Mallinson, Mark D. Allen, Bennett R. Streit, Melodie M. Machovina, Quinlan V. Doolin, William E. Michener, Christopher W. Johnson, Brandon C. Knott, Gregg T. Beckham, John E. McGeehan, and Jennifer L. DuBois
JACS Au 2021 1 (3), 252-261
DOI: https://doi.org/10.1021/jacsau.0c00103

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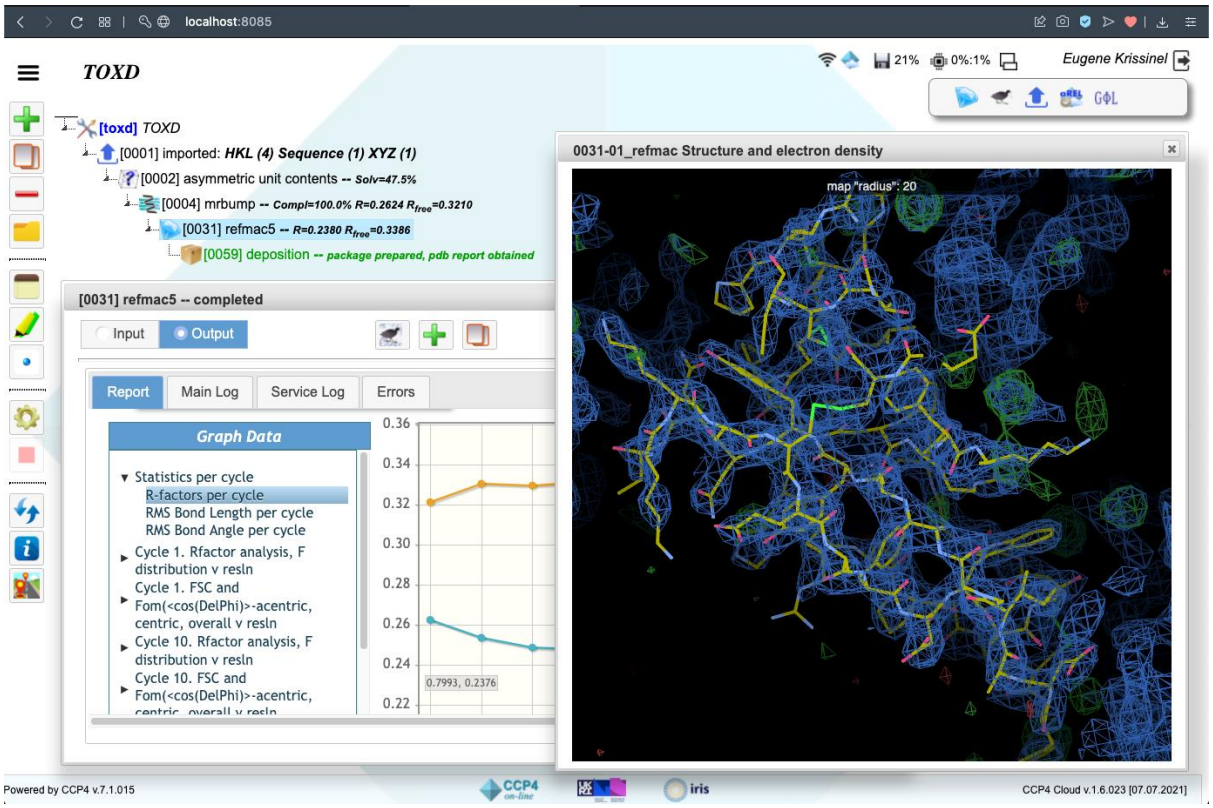
Solving Structures Online with CCP4 Cloud
(Oral or Poster)

Eugene Krissinel, Andrey Lebedev, Oleg Kovalevskyi, Ville Uski, Ronan Keegan and Charles Ballard

CCP4, Scientific Computing Department, STFC, Rutherford Appleton Laboratory, Harwell, OX11 0FA

The demonstration will present principal structure solution scenarios in CCP4 Cloud, the newest CCP4 interface for solving structures online, as well as in desktop mode. CCP4 Cloud was developed for delivering computing power, centralised software and data services, as well as modern interface concepts, to structural biologists worldwide. CCP4 Cloud can be used with any modern web-browser, on a range of devices including tablets and smartphones. There are several good reasons for exploiting the distributed computing paradigm in crystallography. Firstly, they are convenient for delivering computing power and keeping databases, necessary for automated structure solvers. Secondly, researchers are relieved from the burden of maintaining complex software locally. Thirdly, cloud computing streamlines data management and logistics and can be shared in real time between a team of co-workers. Finally, Cloud approach provides an infrastructure for keeping data and structure solution projects, as important complementing materials to scientific publications, in long-time perspective.



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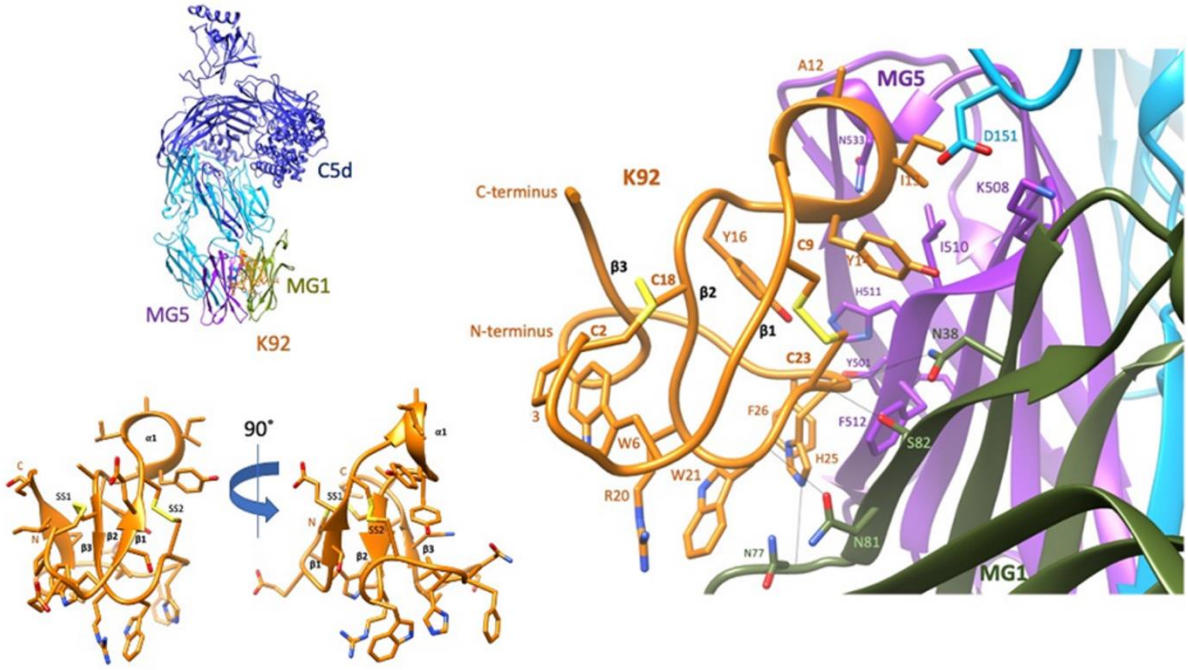
The allosteric modulation of C5 by knob domain peptides (Oral and Poster)

Alex Macpherson[1,2], Alastair Lawson[2] and Jean van den Elsen[1]

[1]Department of Biology and Biochemistry, University of Bath, Bath, U.K.
[2]UCB Pharma, Slough, U.K.

We have previously described the isolation of disulphide-rich knob domains, found within the ultralong CDRH3 of a subset of bovine antibodies, as functional antibody fragments[1]. This exploits the bovine immune system to optimise cystine-rich peptides *in vivo,* creating binding fragments roughly one third of the size of a camelid VHH[1]. Here, we describe how knob domains achieve allosteric modulation of the therapeutic drug target Complement C5[2]. We present the first co-crystal structures of isolated knob domain peptides in complex with antigen, in concert with two solution biophysics techniques, small angle X-ray scattering and hydrogen-deuterium exchange-mass spectrometry, which enable the visualisation of allosteric effects in solution[2].



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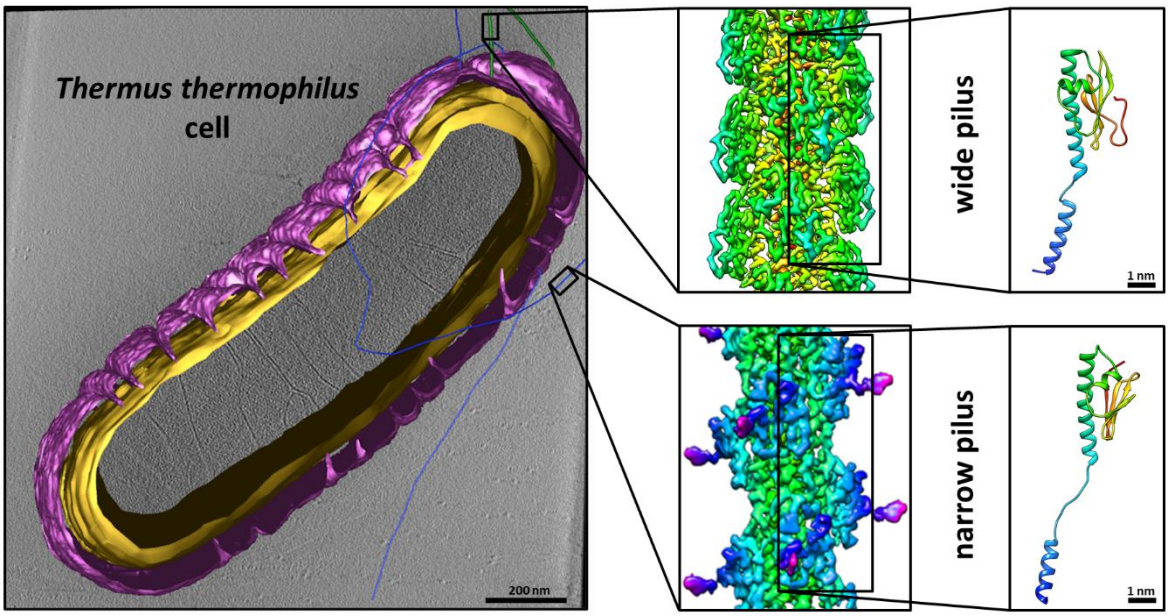
Cryo-electron microscopy reveals two distinct type IV pili assembled by the same bacterium
(Oral or Poster)

Alexander Neuhaus, Muniyandi Selvaraj, Ralf Salzer, Julian D. Langer, Kerstin Kruse,Lennart Kirchner, Kelly Sanders, Bertram Daum, Beate Averhoff & Vicki A. M. Gold

Living Systems Institute, University of Exeter, Stocker Road, Exeter EX4 4QD, UK

Type IV pili (T4P) are flexible filaments on the surface of bacteria, consisting of a helical assembly of pilin proteins. T4P are the Swiss Army knives of bacteria and can be retracted quickly with a force 20-fold higher than that generated by muscle myosin. They are involved in bacterial motility (twitching), surface adhesion, biofilm formation and DNA uptake (natural transformation). Here, we use cryo-electron microscopy and mass spectrometry to show that the bacterium *Thermus thermophilus* produces two forms of type IV pilus ('wide' and 'narrow'), differing in structure and protein composition. Wide pili are composed of the major pilin PilA4, while narrow pili are composed of a so-far uncharacterized pilin which we name PilA5. Functional experiments indicate that PilA4 is required for natural transformation, while PilA5 is important for twitching motility.



Primary citation (make sure to link to the online publication)

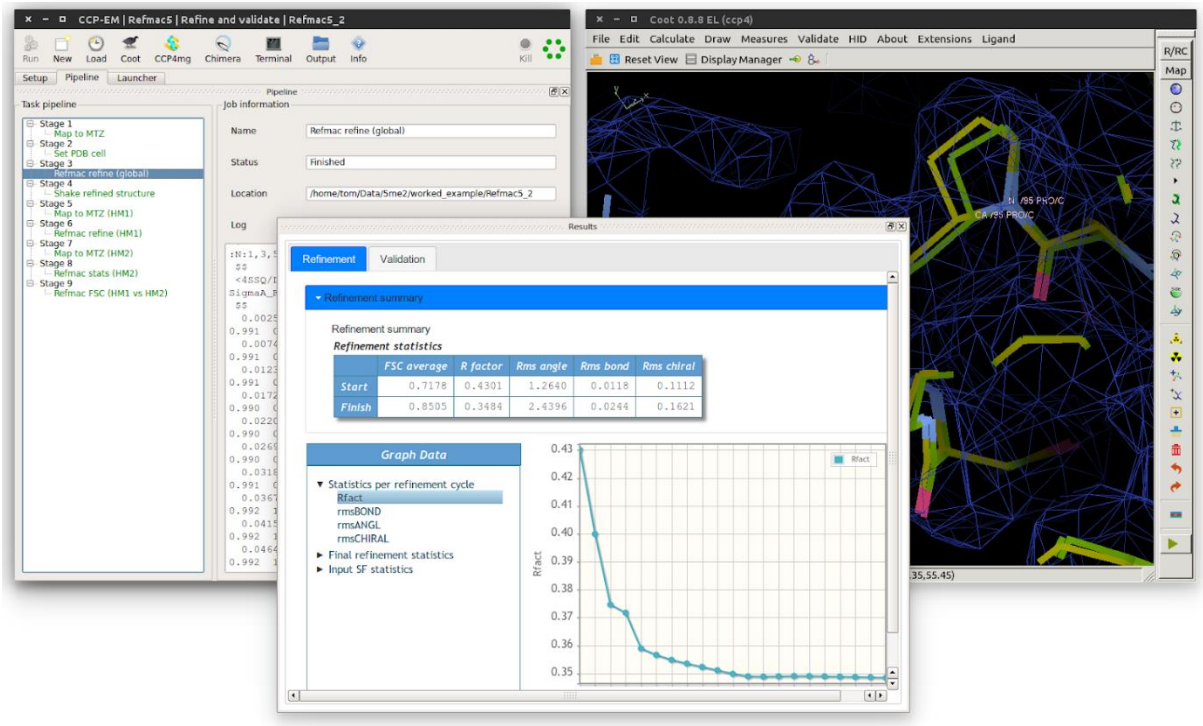
- Neuhaus, A., Selvaraj, M., Salzer, R. et al. Cryo-electron microscopy reveals two distinct type IV pili assembled by the same bacterium. Nat Commun 11, 2231 (2020). https://doi.org/10.1038/s41467-020-15650-w

The CCP-EM software suite for CryoEM
(Oral or Poster)

Colin Palmer, Tom Burnley, Agnel Joseph, Jola Mirecka, Matt Iadanza, Alister Burt and Martyn Winn

CCP-EM, Scientific Computing Department, STFC, Rutherford Appleton Laboratory, Harwell, OX11 0FA

CCP-EM provides a software suite for cryoEM data processing, with a current focus on single particle reconstruction. As for CCP4, there is a wide network of external developers providing particular tools. A unifying framework and GUI is developed and maintained by the CCP-EM core team at STFC. The core team also work closely with the national microscope facility at eBIC. The suite can be downloaded from ccpem.ac.uk
This talk will describe the overall features of the CCP-EM software suite, and give a walk-through of the main workflow.
The main engine for single particle reconstruction is the RELION package, which CCP-EM includes and for which it is developing a new integration layer. CCP-EM provides tools for optimising the resulting map, in terms of local sharpening, denoising and assessing confidence in specific regions. There is then a choice of approaches for building, refining and validating atomic models, using metrics appropriate to cryoEM. Finally, the talk will cover recent development projects applying AI to cryoEM, and applying cryoEM validation tools to structures determined from SARS-CoV-2.



Reference

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Structural and functional insights into PabB – the cellular target of abyssomicin C
(Oral or Poster)

Lynden Rooms

Biochemistry Dept., University of Bristol, University Walk, Bristol, BS8 1TD

With anti-microbial resistance deaths predicted to surpass 10 million annually by 2050[1], there is an urgent global need for new antibiotics. The shikimate pathway produces chorismate from phosphoenolpyruvate and erythrose-4-phoshate, and is only found in bacterial, fungal and plant metabolism[2]. The shikimate pathway, and subsequent chorismate utilising enzymes (CUEs), are therefore possible druggable targets. The *atrop*- isomer of abyssomicin C, isolated from *Micromonospara maris* in 2004, has proven efficacy against Gram positive bacteria by binding to PabB (a CUE) and inhibiting folate biosynthesis[3]. Here, structural data for PabB and TrpE (a similar CUE) is presented, and their striking structural similarity yet differing catalytic capabilities discussed. Activity data also proves the enzymes to be active and points to a possible moonlighting effect between these pathways. These efforts may drive future rational drug design efforts and inform about the next generation of folate inhibitors.

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Links below:
1 https://www.who.int/news/item/29-04-2019-new-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis
2 https://pubmed.ncbi.nlm.nih.gov/29882815/
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A structural and mechanism insight into the mechanism of ROK kinases (Oral)

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Carbohydrates (sugars) are very important for all organisms. They provide energy, intermediates for biosynthesis, and signals within and between cells. To conserve imported sugars within the cell, most organisms use a simple chemical modification called phosphorylation, performed by enzymes called carbohydrate kinases. Understanding the mechanism of carbohydrate kinases is important as defects in them are linked with diseases and are also being targeted for development of novel therapeutics. We determined the structure of *N*-acetylglucosamine kinase (NagK), an enzyme of the poorly understood ROK kinase class, from the putative human pathogen *Plesiomonas shigelloides.* This enzyme phosphorylates the sugar *N*-acetylglucosamine (GlcNAc), a major component of bacterial cell wall structure and contributes towards bacterial pathogenicity. For the first time, our combination of structural, functional and kinetic studies provides a detailed characterisation of the function of a ROK kinase and demonstrating how ligand binding leads to conformational changes that assembles the active site.

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Synuclein plasticity: the Achilles heel to the onset of Parkinson's disease (Oral or Poster)

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Alpha-synuclein (aSN) is an intrinsically disordered protein whose aggregates are a primary component of neuronal Lewy bodies that form in Parkinson's disease. We hypothesise that trafficking of aSN between presynaptic cellular environments alters its biological and pathological function by inducing changes in molecular structure from the different chemical compositions. By developing novel instrumentation and software, we were able to measure the hydrogen exchange rates of aSN at amino acid resolution under various sub-cellular conditions, mimicking those in the extracellular, intracellular, and lysosomal compartments of a dopaminergic neuron. We correlate these structural changes with two measures of pathological phenotype: aggregation kinetics and morphology of the resulting fibrils. Therefore, we propose local structural dynamics that underpin pathophysiological behaviour of this key protein in the Parkinson's disease process.

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Guide to the Offline Graphical Interfaces of CCP4 (Oral or Poster)

CCP4i2 Team, Kyle Stevenson

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CCP4i2 is one of the main graphical interfaces to the CCP4 software suite and is designed to help structural biologists navigate the process of structure determination, with a focus on streamlining the solution process through the use of pipelines and a dedicated report system for results.

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Characterising the structural and biophysical interactions between nonsense-mediated mRNA decay factors UPF2, UPF3B and RNA (Oral and Poster)

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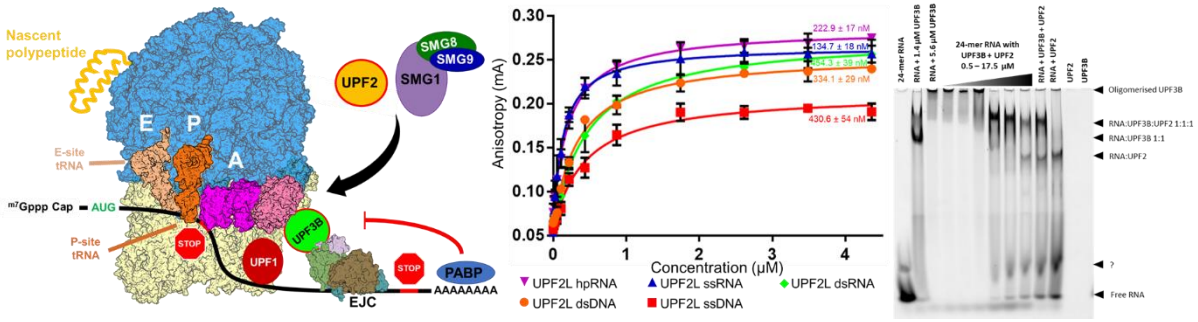
Eukaryotes must maintain a high quality of messenger RNA (**mRNA**) in order to preserve normal cell function. Nonsense-mediated mRNA decay (**NMD**) functions as a quality control mechanism to identify and degrade mRNAs contain premature stop codons (**PTC**), preventing the formation of truncated proteins that are potentially harmful to the cell. Mutations in NMD factors can cause neurological disorders such as schizophrenia and autism. Three up-frameshift proteins (**UPF1-3**) play central roles through their interactions with translating ribosomes and other NMD factors. UPF2 and UPF3B have been shown to interact with nucleic acids as well as each other, however the molecular mechanisms facilitating this interaction remains unknown. Here, we show the binding preferences of UPF2 with nucleic acids, the interplay between UPF2 and UPF3B complexed with RNA using electromobility shift assays and fluorescence anisotropy, and attempts to structurally characterise the interplay between UPF2:RNA and UPF2:UPF3B:RNA complexes using X-ray crystallography and EM respectively.



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Crystal structure of *Lysinibacillus sphaericus* Tpp49 solved using serial femtosecond crystallography (Poster)

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Lysinibacillus sphaericus is an entomopathogenic bacterium with the ability to produce crystal proteins which exhibit mosquitocidal activity. The two-part Cry48/Tpp49 pair is composed of Cry48, belonging to the 3-domain family of Cry proteins, and Tpp49 (formerly Cry49), belonging to the family of Toxin-10 Pesticidal Proteins. Importantly, Cry48/Tpp49 exhibits toxicity against Tpp1/Tpp2 (BinA/B)-resistant larvae. Tpp1/Tpp2 is the major insecticidal factor used in mosquito biolarvicides, highlighting the potential of Cry48/Tpp49 for managing mosquito resistance.

Here, we use serial femtosecond crystallography combined with an X-ray free electron laser to determine the Tpp49 structure from natural crystals isolated from spores. Diffraction data extended to 2.2 Å and revealed the presence of a homodimer with a large intermolecular interface. Within each monomer, two distinct domains exist: An N-terminal β-trefoil domain and C-terminal domain with a topology characteristic of the Aerolysin family of pore forming toxins.

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HIV-1 capsid curvature and its interaction with host cell factor (Oral or Poster)

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Mature HIV-1 particles contain a conical-shaped capsid that encloses the viral RNA genome and performs essential functions in the virus life cycle. In mature virion, the assembled capsid structure is best described by a fullerene cone model that is made up from a hexameric lattice containing hexameric and pentameric capsid protein (CA). We have determined structures of curved HIV-1 capsid assemblies and in complex with the host protein cyclophilin A (CypA) at near-atomic resolutions. The cryoEM CA hexamer is intrinsically curved, flexible and asymmetric, revealing the curved capsomere as the key contributor to capsid curvature. CypA recognizes specific geometries of the curved CA lattice, simultaneously interacting with three CA protomers from adjacent hexamers via two additional non-canonical interfaces, thus stabilizing the capsid. By determining cryoEM structures of various curved assemblies, we further revealed the essential plasticity of the CA molecule that allows formation of continuously curved conical capsids and the mechanism of capsid pattern-sensing by CypA.