SWSBC 2020

This year's **South West Structural Biology Consortium (SWSBC)** annual meeting includes five sessions with talks and one poster session. The program additionally offers two plenary lectures and CCP4 and CCPN roadshows.

P R O G R A M

SWSBC2020, rather than being a physical event, is an online meeting. To join, click on the session that will redirect you to the MS Teams site. The primary role of our meeting is to allow PhD students, PostDocs and ECRs to present their work, and educate and teach good practice in Structural Biology.

SWSBC holds an annual meeting as a forum for the South West of England to come together, present research, and build networks amongst each other. Founded in 2001, participating universities are presently Bath, Bristol, Cardiff, Exeter, Reading, Portsmouth, Southampton, Sussex and UCL.

Day 1: Monday 20.07.20

Session 1 - CryoEM of molecular machines and assemblies

Hosted by Exeter (Vicki Gold, Bertram Daum and Simone de Rose)

08:45-09:00 Welcome

09:00-09:10 Patricia Gil Diez* - Structure of a eukaryotic hibernating ribosome dimer from the fungal parasite *Spraguea lophii* (Abstract 1)

09:15-09:25 Alexander Neuhaus - Cryo-electron microscopy reveals two distinct type IV pili assembled by the same bacterium (Abstract 2)

09:30-09:40 Lavinia Gambelli - Architecture and modular assembly of *Sulfolobus acidocaldarius* S-layer revealed by electron cryomicroscopy (Abstract 3)

09:45-09:55 Kapil Gupta* - Unexpected free fatty acid binding pocket in SARS-CoV-2 spike: path to new antivirals? (Abstract 4)

10:00-10:10 Kaïn van den Elsen - The Structural Basis of the Flavivirus Replication Process (Abstract 5)

10:15-10:25 Nik Harmer - A Micro-ED facility for SWSBC (Abstract 6)

10:30-10:45 Ufuk Borucu - The GW4 Regional Facility for High-Resolution cryoEM (Abstract 7)

Session 2 – Enzymes 1

Hosted by Bristol (Jim Spencer and Leo Brady)

11:00-11:20 Greg Pollard – Lysyl oxidase: self-healing with biocatalysts (Abstract 8)

11:30-11:40 Rachel Bolton - Data Collection Strategies for the Radiation Sensitive, Ferric Iron Binding Protein, FutA (Abstract 9)

11:45-11:55 Charlie Collingham - The Link between Mitochondrial Dysfunction, Bile Acids and Neurodegenerative Disease (Abstract 10)

12:00-12:10 Simone Antonio De Rose* - HotSolute: Thermus thermophilus as a Whole Cell Factory for the Production of Extremolytes (Abstract 11)

12:15-12:25 Geoffrey Masuyer - Crystal Structure of Exotoxin A from *Aeromonas* Pathogenic Species (Abstract 12)

12:30-12:45 General Q&A

CCP4 ROADSHOW

13:00-13:30 Setting up CCP4 on your computer

* Also presents a poster

Day 1: Monday 20.07.20

PLENARY 1

14:00-15:00 Alan Cheung - Structure and function of chromatin modifying complexes SAGA and NuA4

Session 3 – Protein interactions and disease

Hosted by Cardiff (Dafydd Jones)

15:15-15:25 Hayden Fisher – Hinge region disulfide patterns dictate activity in human IgG2 antibodies through conformational restriction (Abstract 13)

15:30-15:40 Rhys Dunphy* - Elucidating the N-terminal strand swapping mechanism exhibited by C3d dimers in the presence of a *Staphylococcus aureus* immune evasion protein (Abstract 14)

15:45-15:55 Rory Munro* - What is the role of S100A9 in the onset of neurodegenerative disease? (Abstract 15)

16:00-16:10 Alessandro Agnarelli* - Analysing IRF4 interactions to ISRE motifs in Multiple Myeloma (Abstract 16)

16:15-16:40 Daren Fearon – Crystallographic fragment screening of the SARS-CoV-2 main protease and crowdsourcing the development of antiviral drugs (Abstract 17)

POSTER SESSION

Hosted by Southampton (Phil Williamson and Ivo Tews)

18:00-18:05 Brief introduction to online posters

18:00-20:00 Visit the poster inn MS teams, just select the channel with the poster you are interested in, watch the clip, and ask the presenter questions

18:30-19:30 Poster pitches, 2.5 mins each

SOCIAL

Hosted by Reading (Charlie Collingham & many others, Kim Watson)

20:00-??? Main event and breakout rooms

* Also presents a poster

Day 2: Tuesday 21.07.20

CCP4 ROADSHOW

09:00-11:45 MR, Phasing, Refinement (Ed Lowe & Stuart McNicholas)

CCPN ROADSHOW

09:30-11:30 Introduction, Assignments and Titrations (Vicky Higman)

PLENARY 2

12:00-13:00 Allen Orville - Time-resolved serial femtosecond crystallography of the early intermediates in the isopenicillin N synthase reaction with ACV and O2

Session 4 – Biotechnology

Hosted by Portsmouth (John Mc Geehan)

13:15-13:25 Erik Landin - The Aminotriazole Antagonist Cmpd-1 Stabilises a Distinct Inactive State of the Adenosine 2A Receptor (Abstract 18)

13:30-13:40 Alexander J Lander - Total Chemical Synthesis and Racemic Protein Crystallography of Bacteriocins (Abstract 19)

13:45-13:55 Sam Robson - Sequencing and Tracking of Phylogeny in COVID-19: A Genomic Epidemiological Approach to the COVID-19 Pandemic (Abstract 20)

14:00-14:10 Maria Concistrè - 1Strategies for 1H-detected dynamic nuclear polarization magic-angle spinning NMR (Abstract 21)

14:15-14:25 Emiliana De Santis* - Peptide virus-like particles: from synthetic biologics to reference standards for advanced therapies (Abstract 22)

14:30-15:00 General Q&A

Session 5 – Enzymes 2

Hosted by Bath (Susan Crennell)

15:15-15:25 Charlotte Colenso - Simulation strategies for zinc metalloenzymes applied to metallo-β-lactamases (Abstract 23)

15:30-15:40 Daniel Mitchell* - A novel sensor for measuring femto-Newton forces in enzyme turnover and the importance of good crystallographic data (Abst. 24)

15:45-15:55 Paul James - A Novel 'Split-gene' transketolase from the hyper-thermophilic bacterium *Carboxydothermus hydrogenoformans*: structure and biochemical characterisation (Abstract 25)

16:00-16:10 Éilís Bragginton - Insight into the structure and function of OXA-57; a class-D β-lactamase from *Burkholderia pseudomallei* (Abstract 26)

16:15-17:00 PRIZES & CLOSING (Kim Watson, John Mc Geehan)

* Also presents a poster

Structure and function of chromatin modifying complexes SAGA and NuA4.

Alan Cheung
School of Biochemistry, University of Bristol

Complex programmes of transcription define all aspects of cellular function and their misregulation leads to the widest spectrum of human disease, from cancer to neurological and autoimmune disorders. In eukaryotes, RNA polymerase II (Pol II) synthesises messenger RNAs that are translated into proteins. However, Pol II requires dozens of additional large protein complexes that carefully regulate its activity and allow it to transcribe through chromatin. Our goal is to understand the interplay between transcription factors, Pol II and chromatin coactivator complexes, and uncover fundamental molecular mechanisms of transcription and its regulation. This seminar will focus on the coactivators SAGA and NuA4. These large complexes are histone acetyltransferases required for transcription through chromatin. They are also related by their common incorporation of Tra1, a large protein that accounts for almost one-third of each complex and enables their recruitment to specific genes during transcription activation. We use a multi-disciplinary approach covering crystallography, cryo-electron microscopy, biochemistry, biophysics and genetics to study these large complexes required for transcription.

Wang, H., Dienemann, C., Stützer, A., Urlaub, H., Cheung, A. C. M., and Cramer, P. (2020). **Structure of the transcription coactivator SAGA.** *Nature,* 10.1038/s41586-020-1933-5.

Díaz-Santín, L. M., Lukoyanova, N., Aciyan, E. and Cheung, A. C. M. (2017). **Cryo-EM structure of the SAGA and NuA4 coactivator subunit Tra1 at 3.7 angstrom resolution.** *eLife*, *6*:e28384

Time-resolved serial femtosecond crystallography of the early intermediates in the isopenicillin N synthase reaction with ACV and O_2

Patrick Rabe[1], Jos J. A. G. Kamps[1], Cindy Pham [2], Michael A. McDonough[1], Thomas M. Leissing[1], Jurgen Brem[1], Pierre Aller[3], Agata Butryn[3], Franklin D. Fuller[2,4], Alexander Batyuk[4], Mark S. Hunter[4], Roberto Alonso-Mori[4], Sheraz Gul[2], Iris Young[2], In-Sik Kim[2], Kyle Sutherlin[2], Asmit Bhowmick[2], Aaron S Brewster[2], Nicholas K. Sauter[2], Vittal Yachandra[2], Junko Yano[2], Jan F. Kern[2], Allen M. Orville[3] and Christopher J. Schofield[1]

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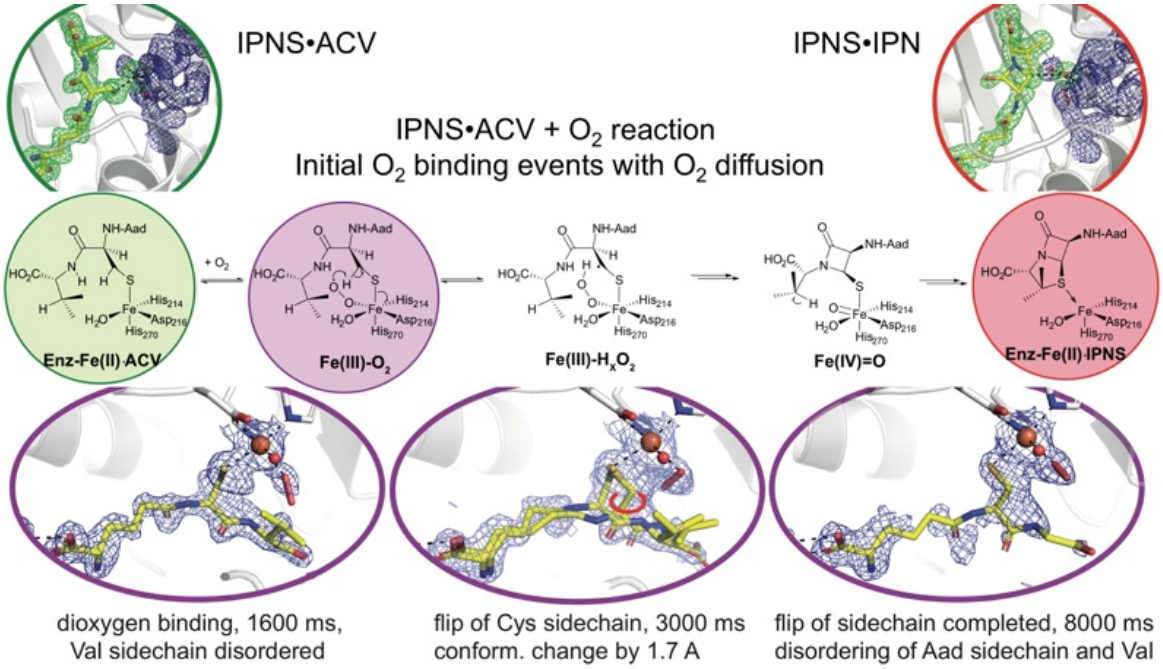
[2] Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, CA 94720, Berkeley, CA, USA.
[3] Diamond Light Source, Harwell Science and Innovation Campus, OX11 0DE, Didcot, UK.
[4] Linac Coherent Light Source, SLAC National Accelerator Laboratory, Menlo Park, CA 94025, USA

The femtosecond pulses at X-ray free electron lasers allow experimental access to enzyme reaction cycles and to reveal time-resolved atomic and electronic structures, without X-ray radiation-induced changes to sensitive sites such as an active site metal centre. We have developed a drop-on-demand sample delivery system that enables simultaneous collection and correlation of time-resolved femtosecond crystallography data with X-ray emission spectroscopy data.[1] Isopenicillin N synthase (IPNS) catalyses the nonheme iron-dependant, four electron oxidation of the linear tripeptide δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine (ACV) into isopenicillin N.[2] A unique feature of the proposed reaction mechanism is the role of two reactive iron species -- an Fe(III)-superoxo and a high-spin Fe(IV)=O species -- that promote the first and second ring closures of the β-lactam, respectively. We present results for the early reaction intermediates obtained during O_2-catalysed turnover of the IPNS•Fe(II)•ACV complex. Our results indicate several dynamic and catalytic roles for O_2 that ultimately impact the reaction coordinate.



[1] Fuller, F. D., Gul, S., Chatterjee, R., Burgie, E. S., Young, I. D., Lebrette, H., et al, Yano, J. (2017) Drop-on-demand sample delivery for studying biocatalysts in action at X-ray free-electron lasers, *Nat Methods 14*, 443-449 doi: 10.1038/nmeth.4195

[2] Rabe, P., Kamps, J., Schofield, C. J., and Lohans, C. T. (2018) Roles of 2-oxoglutarate oxygenases and isopenicillin N synthase in beta-lactam biosynthesis, *Nat Prod Rep 35*, 735-756 doi: 10.1039/c8np00002f

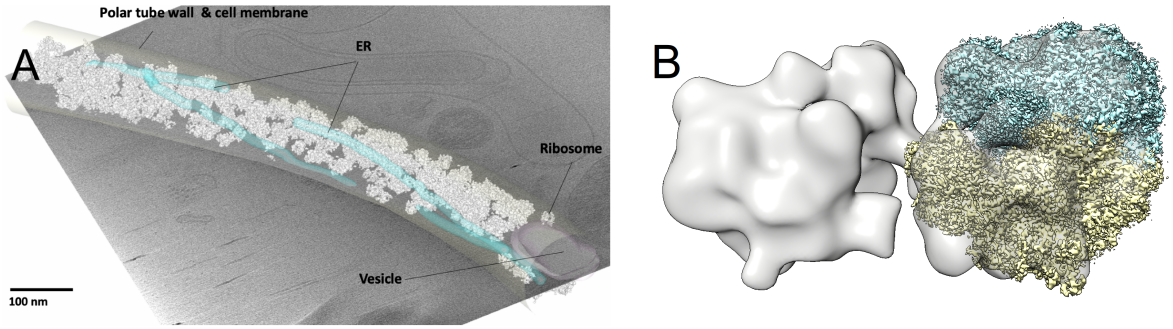
Partially supported by a Wellcome Trust Investigator Award in Science (210734/Z/18/Z) and a Royal Society Wolfson Fellowship (RSWF\R2\182017) to AMO.

Structure of a eukaryotic hibernating ribosome dimer from the fungal parasite *Spraguea lophii*.

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

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

Microsporidia are unicellular, eukaryotic intracellular pathogens that can infect almost all animal lineages, including humans. Microsporidian life cycles undergo two stages: a vegetative phase inside the host cell and an infective stage in the form of a dormant and environmentally resistant spore. During infection, spores eject their cellular content though a long cellular appendage called a polar tube (1). We used electron cryo-tomography to investigate polar tubes from the microsporidian species *Spraguea lophii (A)*. By sub-tomogram averaging, we found that microsporidian ribosomes form dimers at the spore stage (B). Ribosome dimerisation is a ubiquitous mechanism in spore-forming bacteria to silence ribosomes (2), however it is not well established if this mechanism is also present in eukaryotes (3). We are currently investigating the structure of silenced microsporidian ribosomes at high resolution by single particle cryoEM (B), aiming to understand the molecular basis of ribosome dimerisation and hibernation in eukaryotic organisms.



1. Vávra, J., & Lukeš, J. (2013). Microsporidia and 'the art of living together'. *Advances in parasitology*, *82*, 253–319. https://doi.org/10.1016/B978-0-12-407706-5.00004-6

2. Trösch, R., & Willmund, F. (2019). The conserved theme of ribosome hibernation: from bacteria to chloroplasts of plants. *Biological chemistry*, *400*(7), 879–893. https://doi.org/10.1515/hsz-2018-0436

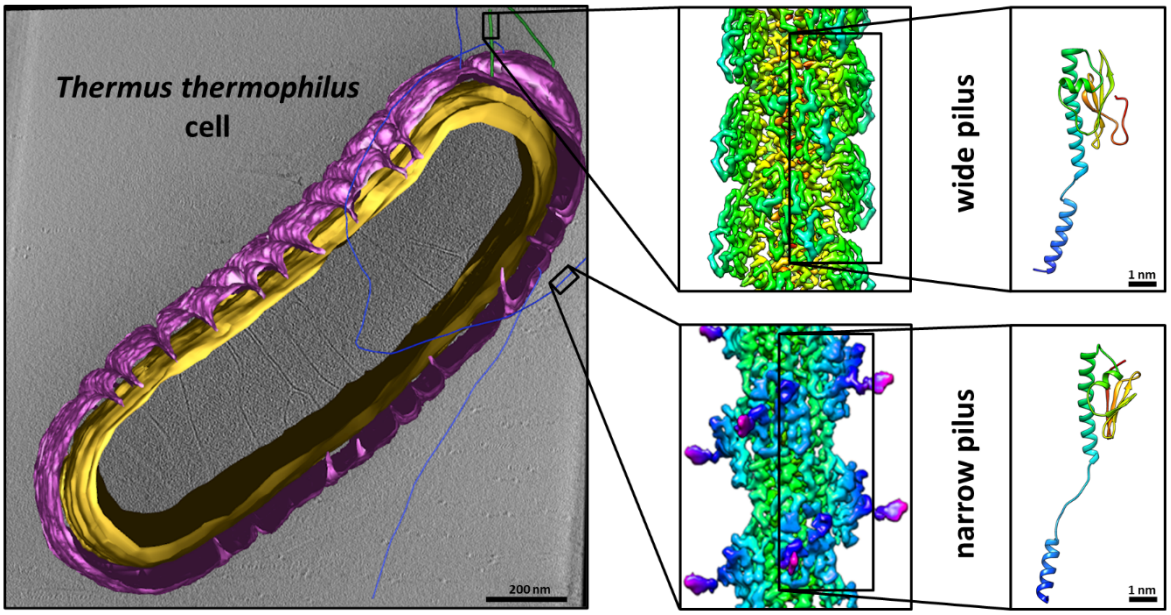
3. Krokowski, D., Gaccioli, F., Majumder, M., Mullins, M. R., Yuan, C. L., Papadopoulou, B., Merrick, W. C., Komar, A. A., Taylor, D., & Hatzoglou, M. (2011). Characterization of hibernating ribosomes in mammalian cells. *Cell cycle (Georgetown, Tex.)*, *10*(16), 2691–2702. https://doi.org/10.4161/cc.10.16.16844

Cryo-electron microscopy reveals two distinct type IV pili assembled by the same bacterium

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.



- 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

Architecture and modular assembly of *Sulfolobus acidocaldarius* S-layer revealed by electron cryomicroscopy

Lavinia Gambelli[a,b], Benjamin H. Meyer[c], Mathew McLaren[a,b], Kelly Sanders[a,d], Tessa E. F. Quax[e], Vicki A. M. Gold[a,d], Sonja-Verena Albers[e], and Bertram Daum[a,d]

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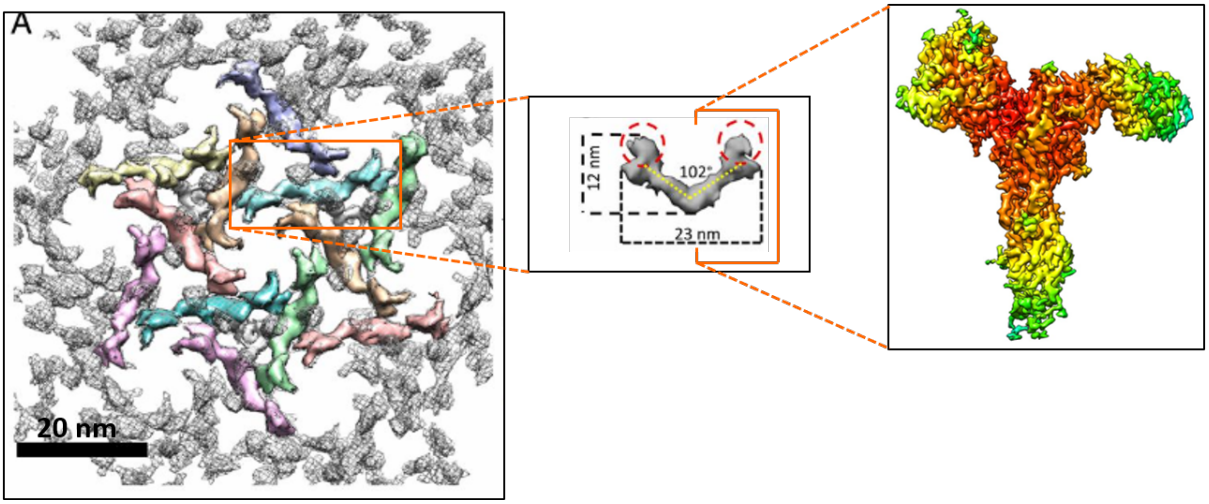
[e] Institute of Biology II, Molecular Biology of Archaea, University of Freiburg, 79104 Freiburg, DE

Surface protein layers (S-layers) are resilient para-crystalline protein arrays that often constitute the only component of the archaeal cell wall. S-layers are are important for cell survival and serve a plethora of cellular functions, including maintenance of cell shape, osmotic and mechanical stability, and cell–cell/surface interaction and adhesion. Despite their importance for archaeal life, their architecture is poorly understood. Here we present unprecedented structural insights into the archaeal S-layer from *Sulfolobus acidocaldarius*[1]. Using electron cryo-tomography, we visualise how the two component subunits SlaA and SlaB self-assemble into the S-layer and with the aim to build an atomic model, we have recently obtained a structure of SlaA at 3.2 Å resolution. We find that SlaA determines the unit cell size and topology of the S-layer, while SlaB anchors the S-layer in the plasma membrane and defines a pseudoperiplasmic space.



Primary citation

- Uwe B. Sleytr, Bernhard Schuster, Eva-Maria Egelseer, Dietmar Pum. "S-layers: principles and applications". FEMS Microbiology Reviews. 2014. Vol. 38 (5), 823–864. https://doi.org/10.1111/1574-6976.12063.

References

[1] Lavinia Gambelli, Benjamin H. Meyer, Mathew McLaren, Kelly Sanders, Tessa E. F. Quax, Vicki A. M. Gold, Sonja-Verena Albers, Bertram Daum. "Architecture and modular assembly of *Sulfolobus* S-layer revealed by electron cryotomography". PNAS. 2019. Vol. 116 (50), 25278-25286. https://doi.org/10.1073/pnas.1911262116

Unexpected free fatty acid binding pocket in SARS-CoV-2 spike: path to new antivirals?

Toelzer Christine[1,2], Gupta Kapil[1,2], Yadav Sathish K.N., Borucu Ufuk, Garzoni Frederic, Staufer Oskar, Capin Julien, Spatz Joachim, Fitzgerald Daniel, Berger Imre and Schaffitzel Christiane

[1]School of Biochemistry, University of Bristol, 1 Tankard's Close, Bristol BS8 1TD, UK. [2]Bristol Synthetic Biology Centre BrisSynBio, 24 Tyndall Ave, Bristol BS8 1TQ, UK.

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) has been responsible for COVID-19 creating a global crisis. Understanding of the mechanisms driving high infectivity, broad tissue tropism and severe pathology of SARS-CoV-2 is needed for therapeutic developments. Here we present our cryo-EM structure of SARS-CoV-2 spike (S) glycoprotein trimer, which reveals that the receptor binding domain of each S subunit binds tightly and specifically to one copy of the essential free fatty acid (FFA) linoleic acid (LA) in a composite binding pocket. The key residues of this pocket are conserved in other highly pathogenic coronaviruses, SARS-CoV and MERS-CoV, suggesting a broad druggable potential. Coronavirus infection remodels the lipid metabolome, and the LA metabolic pathway is central to inflammation, immune modulation and membrane fluidity. Here we provide a direct structural link between LA and the SARS-CoV-2 spike glycoprotein as a starting point for interventions targeting LA binding and metabolic remodelling by SARS-CoV-2.

• Toelzer C, Gupta K, Yadav SKN, Borucu U, Garzoni F, Staufer O, Capin J, Spatz J, Fitzgerald D, Berger I and Schaffitzel C. "Unexpected free fatty acid binding pocket in the cryo-EM structure of SARS-CoV-2 spike protein." BioRxiv. doi: 10.1101/2020.06.18.158584.
https://www.biorxiv.org/content/10.1101/2020.06.18.158584v1

The Structural Basis of the Flavivirus Replication Process

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

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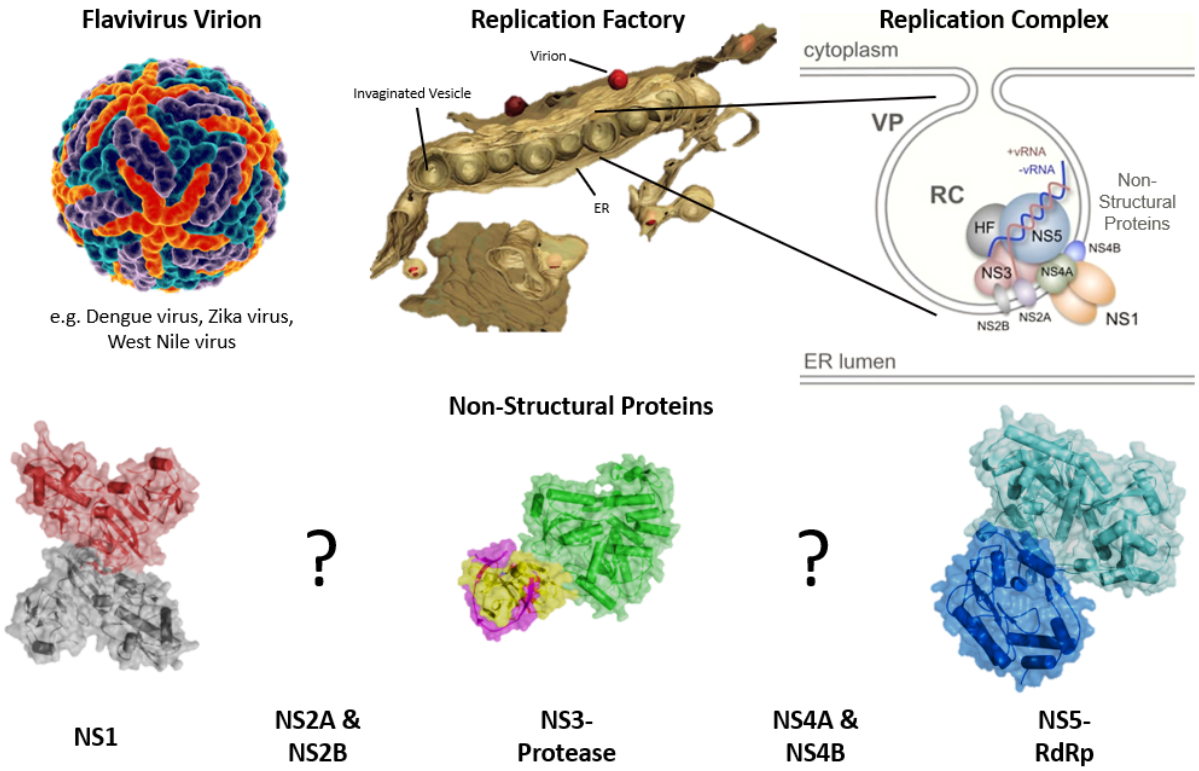
[d] College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4QD, UK

Many flaviviruses are well-known and clinically important human pathogens. During infection, these viruses induce the formation of mini-organelles associated with the endoplasmic reticulum, 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. This study aims to reveal the RC structure through a high resolution structural approach, employing cryo-electron tomography to observe this molecular machine and elucidate the intricate mechanisms and interactions pivotal to its function. These advances in our understanding will aid structure-based drug design and accelerate the production of effective vaccines and anti-virals.



A Micro-ED facility for SWSBC

Nicholas Harmer

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

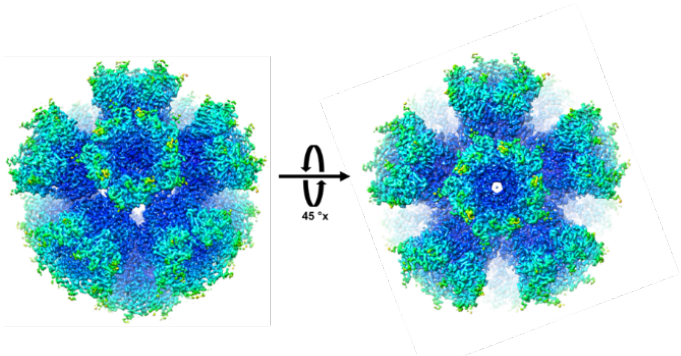
Micro-electron diffraction is an electron microscopy technique that is becoming accessible for a broad user base. High level facilities are available at Diamond and other "Hub" sites. However, most of these facilities require prior screening of samples for suitability. It would be very valuable for SWSBC members to have access to facilities for screening and collection of data in ideal cases. This session is designed to briefly introduce the concept for micro-electron diffraction, and to establish a conversation about the level of interest in our community. Likely next steps would be identifying individuals to lead a bid for equipment, others who would contribute to this, and how to ensure that expertise in sample and data handling can be obtained.

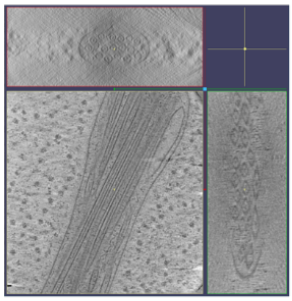
Visualising molecular details with the GW4 Facility for High-Resolution Electron Cryo-Microscopy

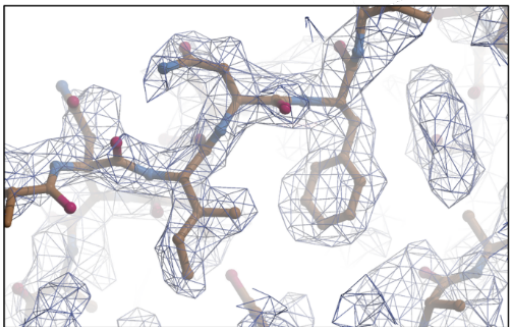
Ufuk Borucu

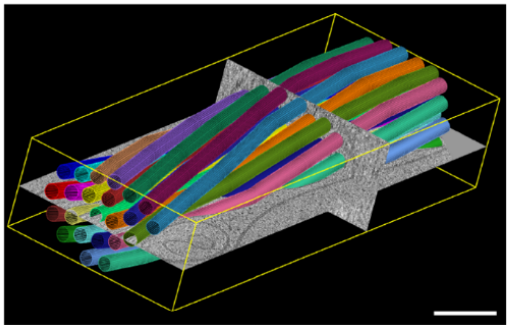
University of Bristol

The GW4 Facility for High-Resolution Electron Cryo-Microscopy was opened in a joint effort between Universities of Bath, Bristol, Cardiff, and Exeter. The facility provides access to cryo-microscopy and analysis tools to researchers from diverse disciplines across the Great West region and beyond, enabling them to study the molecular processes responsible for cell function or malfunction using single particle cryo-EM or cryo-tomography. Located at the University of Bristol, it houses an FEI Talos Arctica equipped with a Gatan K2 DED and Gatan GIF Quantum LS energy filter, sample preparation equipment and a data analysis suite. Almost three years since its start of operation, the GW4 Facility for High-Resolution Electron Cryo-Microscopy has undergone transformative changes enabling record 2.2 Å resolution single-particle structures and ground-breaking cryo-tomographic results.









- Paul, DM. et al. In situ cryo-electron tomography reveals filamentous actin within the microtubule lumen. J Cell Biol 7 (2020). https://doi.org/10.1083/jcb.201911154
- 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
- Vragniau, C., Bufton, J. C., Garzoni, F. et al. Synthetic self-assembling ADDomer platform for highly efficient vaccination by genetically encoded multiepitope display. Science advances, 5(9), eaaw2853 (2019). https://doi.org/10.1126/sciadv.aaw2853

Lysyl oxidase: self-healing with biocatalysts

Gregory J. Pollard, Nicolette G. Moreau & Paul R. Race

School of Biochemistry, Faculty of Life Sciences, University of Bristol, Bristol, BS8 1TD.

Intermolecular cross-linking is one of the most important techniques that can be used to fundamentally alter the material properties of a polymer. The introduction of covalent bonds between individual polymer chains creates 3D macromolecular assemblies with enhanced mechanical properties and greater chemical or thermal tolerances. In contrast to many chemical cross-linking reactions, which are the basis of thermoset plastics, enzyme catalysed processes offer a complimentary paradigm for the assembly of cross-linked polymer networks through their predictability and high levels of control. Additionally, enzyme catalysed reactions offer an inherently 'greener' and more biocompatible approach to covalent bond formation, which could include the use of aqueous solvents, ambient temperatures, and heavy metal-free reagents. This talk focuses on lysyl oxidase as part of an important class of enzymes that provide covalent crosslinks *in vitro* and how its kinetic characterisation provides insight into its role as a biocatalytic crosslinker.

- Maddock, R. M. A., Pollard, G. J., Moreau, N. G., Perry, J. J., & Race, P. R. (2020). *Enzyme-catalysed polymer cross-linking: Biocatalytic tools for chemical biology, materials science and beyond. Biopolymers.* **https://doi.org/10.1002/bip.23390**

Data Collection Strategies for the Radiation Sensitive, Ferric Iron Binding Protein, FutA

R. Bolton[1,2], M. Machelett[1], D. Axford[2], J. Beale[2], S. Storm[2], R. Owen[2], J. Trincao[2], G. Evans[2], I. Tews[1]

[1]Department of Biological Sciences, Institute for Life Science, University of Southampton, Highfield Campus, Southampton SO17 1BJ, United Kingdom
[2]Diamond Light Source, Harwell Science & Innovation Campus, Didcot OX11 0DE, United Kingdom

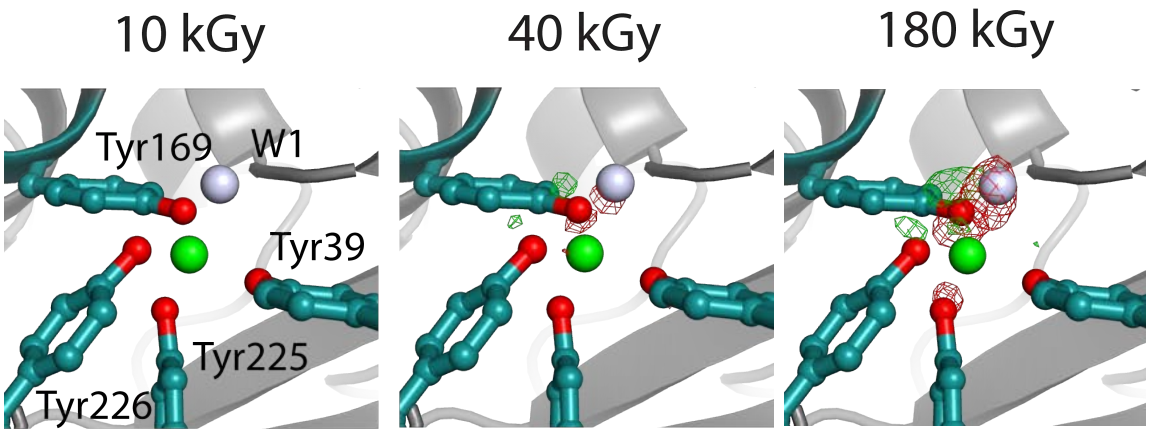
Specific radiation damage is a pervasive problem in X-ray crystallography, often occurring before a complete dataset can be collected, at doses as low as tens of kGy [1]. Metalloproteins are particularly susceptible to specific radiation damage as the metal ions are rapidly reduced by solvated electrons [2].

The FutA-proteins found in cyanobacterium are characterised as both, ferric Fe(III) and ferrous Fe(II) iron binders. FutA2 is a periplasmic ferric iron binding protein associated with the Fut ABC transporter which mediates iron uptake, whilst FutA1 is a cytoplasmic ferrous iron binding protein suggested to protect photosystem II against oxidative stress [3].

We currently study FutA from the cyanobacterium *Prochlorococcus MED4,* which contains only a single homologue of FutA known to bind ferric iron. The sensitivity of this FutA protein to radiation damage is demonstrated by the complete reduction of the ferric iron to ferrous iron after a dose of 200 kGy at 100K, as measured by absorbance spectroscopy.

Using *Prochlorococcus MED4* FutA, various data collection strategies were employed to study the iron binding site of FutA. Single crystal rotation data revealed the damaged state of FutA, which may relate to a biologically relevant ferrous iron binding state. Furthermore, serial synchrotron and serial femtosecond crystallography revealed the progression of radiation damage within the protein and the undamaged state of FutA.

Overall, this work aims for the successful characterisation of the FutA iron binding site and to offer insight into the advantages and disadvantages of different data collection techniques.



Primary citation

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The Link between Mitochondrial Dysfunction, Bile Acids and Neurodegenerative Disease

Collingham, Charlie [1]; Watson, Kimberly [1]; Weymouth-Wilson, Alex [2]

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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, and in some cases, not regulated, and how this could be targeted for many disease therapies, including Parkinson's disease and Alzheimer's disease. A further aim of this project is to determine how bile acids and lipids may play a part and by concentrating on the 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?

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

Simone Antonio De Rose, Michail Isupov and Jennifer Littlechild

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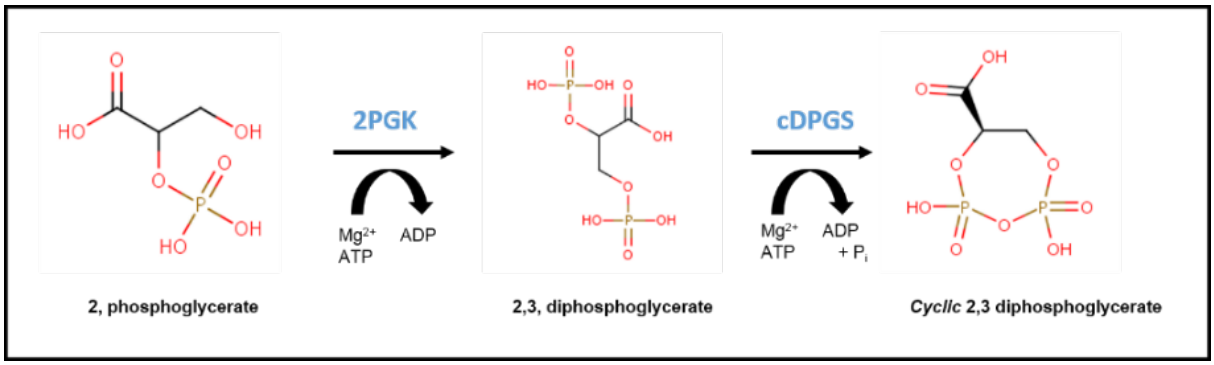
Extremolytes are found naturally in the cells of hyperthermophilic microorganisms who accumulate them in response to environmental and endogenous stresses. These small molecules have great potential for applications in the food, health care, consumer care and cosmetics markets.

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 recently the 2PGK has also been crystallised

Visit http://hotsolute.com/ for more information

Crystal Structure of Exotoxin A from *Aeromonas* Pathogenic Species

Geoffrey Masuyer

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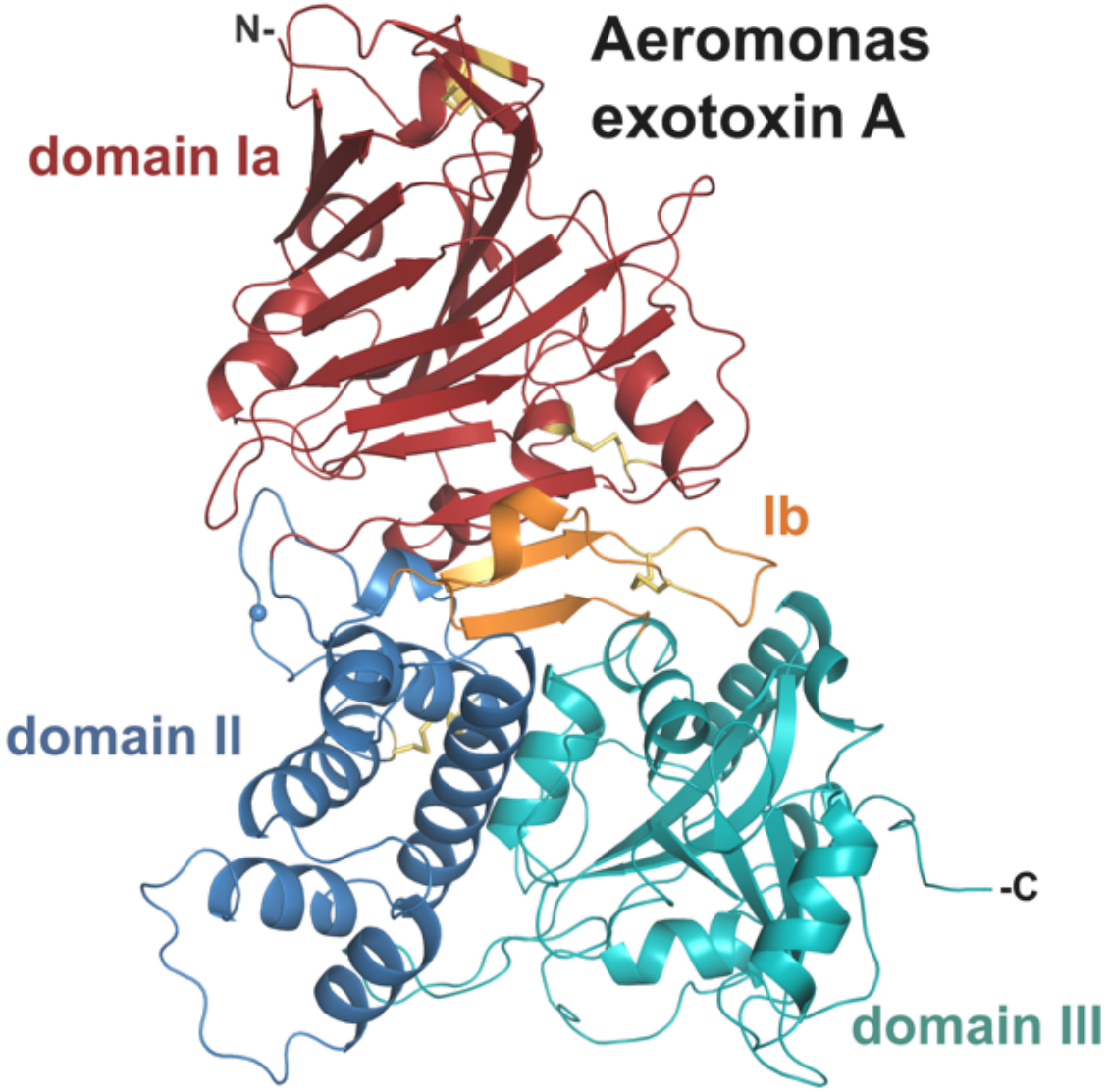
Aeromonas exotoxin A (AE) is a bacterial virulence factor recently discovered in a clinical case of necrotising fasciitis caused by the flesh-eating *Aeromonas hydrophila*. Here, database mining shows that AE is present in the genome of several emerging *Aeromonas* pathogenic species. The X-ray crystal structure of AE was solved at 2.3 Å and presents all the hallmarks common to diphthamide-specific mono-ADP-ribosylating toxins, suggesting AE is a fourth member of this family alongside the diphtheria toxin, *Pseudomonas* exotoxin A and cholix. Structural homology indicates AE may use a similar mechanism of cytotoxicity that targets eukaryotic elongation factor 2 and thus inhibition of protein synthesis. The structure of AE also highlights unique features including a metal binding site, and a negatively charged cleft that could play a role in interdomain interactions and may affect toxicity. This study raises new opportunities to engineer alternative toxin-based molecules with pharmaceutical potential.



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Hinge region disulfide patterns dicate activity in human IgG2 antibodies through conformational restriction

Hayden Fisher, Chris Orr, Claude Chan, Patrick Duriez, Tatyana Inzhelevskaya, Ian Mockridge, Martin Glennie, Ann White, Mark Cragg, Jon Essex, Ivo Tews.

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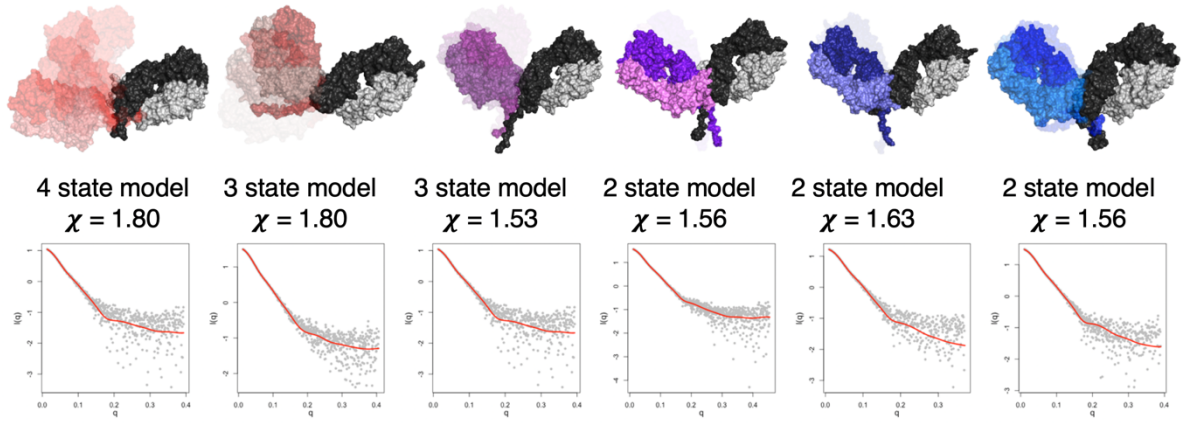
Antibody and Vaccine group, Centre for Cancer Immunology, Cancer Sciences, Faculty of Medicine, University of Southampton, Southampton General Hospital, Southampton, SO16 6YD

Monoclonal antibodies (mAb) are now used widely in the treatment of disease, with particular prominence in cancer immunotherapy. Of particular interest are mAb of the human IgG2 isotype, which have the unique ability to undergo hinge region disulfide shuffling, existing as either inactive IgG2A or active IgG2B. How this disulfide shuffling mediates differences in activity has not been previously determined. A range of hinge Cys-Ser mutants were generated with varying activities to investigate this further. Fitting of models extracted from molecular dynamics simulations against SAXS data using multi-state and ensemble methods demonstrated that IgG2B-like variants adopt a more compact conformation in solution compared to IgG2A-like variants. This allows us to propose a mechanism of activity regulation dictated by the conformation of the hinge region disulfide bonds. This mechanism may be more broadly applicable against other members of the TNFRSF or could be used to further 'tune' the activity of mAb.



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Elucidating the N-terminal strand swapping mechanism exhibited by C3d dimers in the presence of a Staphylococcus aureus immune evasion protein.

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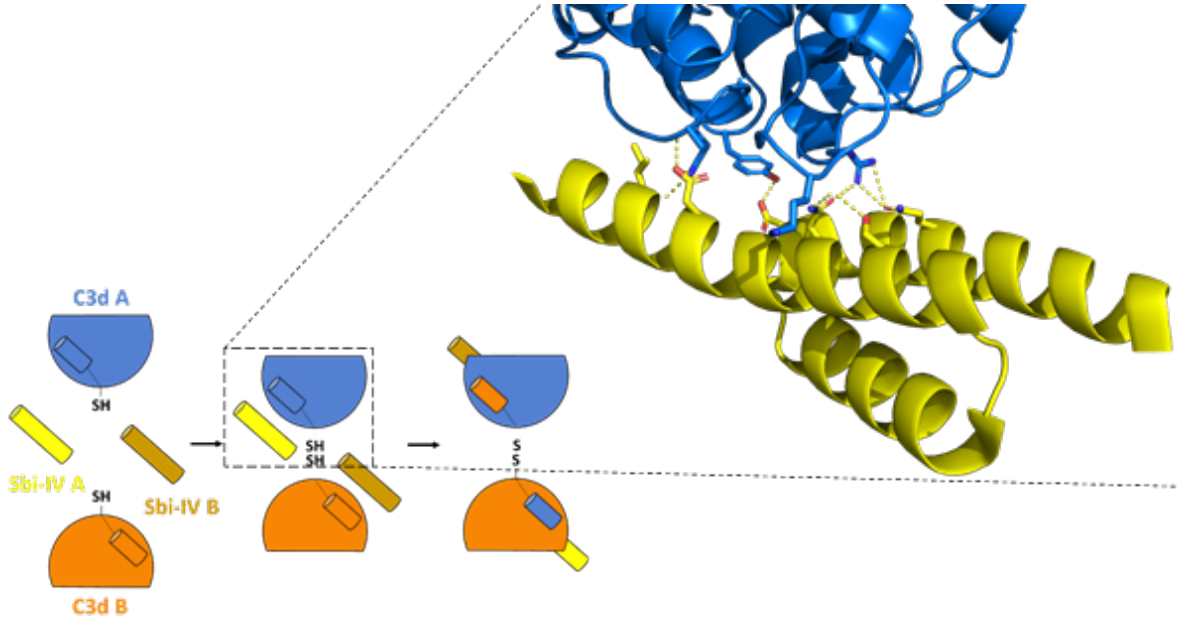
Recently the structures of dimeric C3d in the presence and absence of *S. aureus* immune evasion protein Sbi were determined. These structures show that Sbi induces the N-terminal helices of C3d to swap over, making the dimer more stable. In addition, these dimers of C3d were shown to crosslink complement receptor 2 and thereby modulate B cell activation, inducing immune tolerance. To understand the molecular mechanism controlling the ligand-induced 3D strand swap, we screened single amino acid mutants and truncations of Sbi, based on key interactions observed in an alternative binding mode between Sbi and C3d. The effect of these mutations on the Sbi's ability to cause C3d to strand swap was determined by near-UV thermal melt circular dichroism. Subsequently, promising mutants were analysed using X-ray crystallography. Stabilisation of strand swapped C3d dimers suggests a novel *S. aureus* immune evasion mechanism whereby the increased stability alters B cell activation.



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What is the role of S100A9 in the onset of neurodegenerative disease?

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Neurodegenerative diseases such as Parkinson's and Alzheimer's Disease are becoming increasingly prevalent in our ageing population, rendering sufferers cognitively and physically impaired. One of the hallmarks of these diseases is the formation of insoluble deposits rich in fibrillar species composed of proteins characteristic of that disease. Recently however it has been shown that the elevated levels of the protein S100A9, itself intrinsically amyloidogenic, can enhance the rates at which other amyloidogenic proteins such as amyloid-beta peptide[1] or alpha-synuclein[2] are deposited; and influence their appearance. Our current studies have sought to characterise the structure of S100A9 amyloid fibrils and determine how its presence may influence the types of amyloid-beta and alpha-synuclein polymorphs formed. To do this we have undertaken a series of solid-state NMR investigations which are beginning to resolve the basic architecture of the S100A9 fibrils and highlight features which may impact on their interactions with alpha-synuclein and amyloid-beta peptide. Building on these studies a range of alpha-synuclein polymorphs have been characterised as a prelude to understanding how S100A9 may influence the structures and toxicity of the species formed.

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Analysing IRF4 interactions to ISRE motifs in Multiple Myeloma

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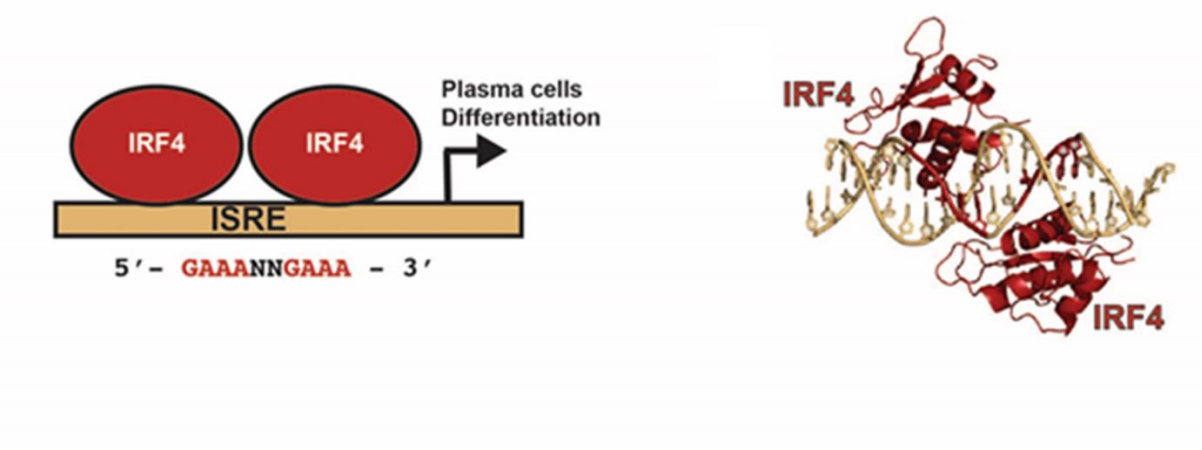
Multiple Myeloma (MM) is an incurable hematologic malignancy characterized by abnormal proliferation of plasma cells[1,2]. Interferon Regulatory Factor 4 (IRF4), a member of the interferon regulatory family of transcription factors, is central to the genesis of MM [2,3]. Data suggest that in MM IRF4 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 have solved the structure of the IRF4 DNA binding domains bound to a variety of ISRE sequences. Our data provides insights into the DNA binding specificity and affinity as well as IRF4 homodimerization.



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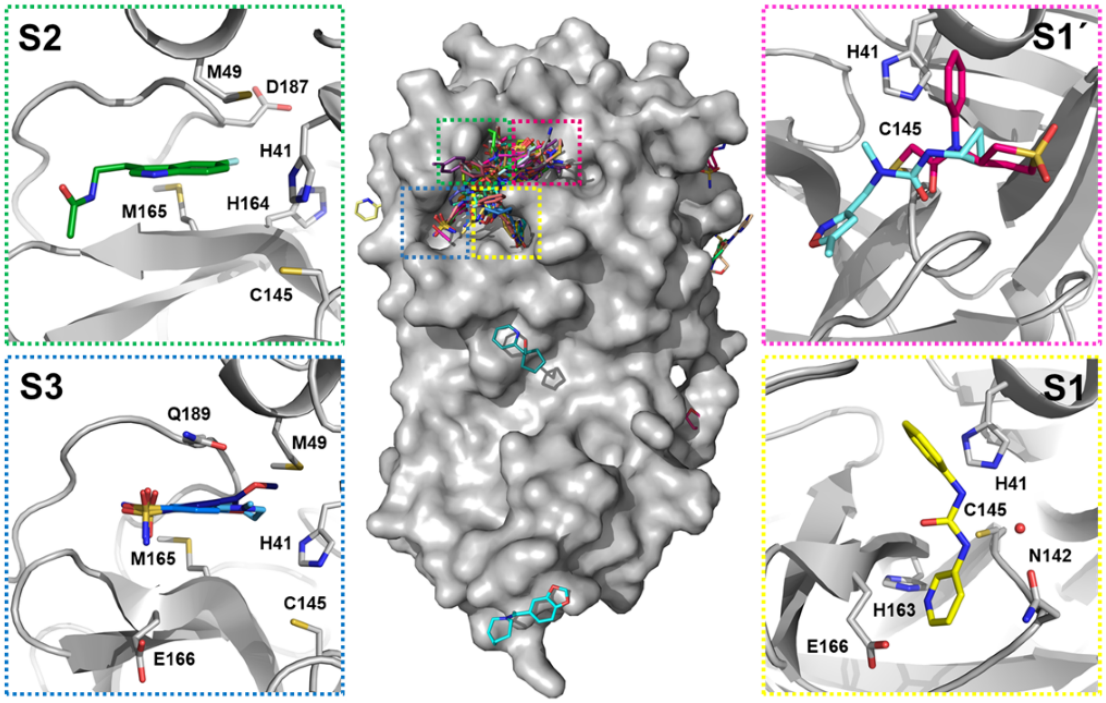
Crystallographic fragment screening of the SARS-CoV-2 main protease and crowdsourcing the development of antiviral drugs

Daren Fearon, Alice Douangamath, Paul Gehrtz, Tobias Krojer, Petra Lukacik, C. David Owen, Efrat Resnick, Claire Strain-Damerell, Anthony Aimon, Péter Ábrányi Balogh, José Brandaõ-Neto, Anna Carbery, Gemma Davison, Alexandre Dias, Thomas D Downes, Louise Dunnett, Michael Fairhead, James D. Firth, S. Paul Jones, Aaron Keely, György M. Keserü, Hanna F Klein, Mathew P. Martin, Martin E. M. Noble, Peter O'Brien, Ailsa Powell, Rambabu Reddi, Rachael Skyner, Matthew Snee, Michael J. Waring, Conor Wild, Nir London, Frank von Delft & Martin A. Walsh

Diamond Light Source, Harwell Science and Innovation Campus, Didcot, OX11 0DE, United Kingdom

To contribute to the global effort against COVID-19, Diamond Light Source is currently performing multiple crystallographic fragment screens against various potential therapeutic targets. One promising target is the SARS-CoV-2 main protease (MPro). After solving the structure of Mpro at high resolution, we rapidly completed a large XChem crystallographic fragment screen which yielded an exceptionally rich set of structural information. Rapid release of this data led to the creation of the COVID Moonshot – an international crowdsourced initiative to combat COVID-19. The goal of the Moonshot is to develop a synthetically simple, potent antiviral, with a clear mechanism-of-action and is unencumbered by IP. Within 4 months of the release of our initial data more than 800 compounds have been designed, synthesised and tested with 15 compounds displaying sub µM activity. The ongoing optimization of these compounds is being guided by routine X-ray crystallography.



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The Aminotriazole Antagonist Cmpd-1 Stabilises a Distinct Inactive State of the Adenosine 2A Receptor

Landin, Erik; Lovera, Silvia; de Fabritiis, Gianni; Kelm, Sebastian; Mercier, Joël; McMillan, David; Sessions, Richard; Taylor, Richard; Sands, Zara; Joedicke, Lisa; Crump, Matthew

School of Chemistry, Cantock's Close, University of Bristol, Bristol, BS8 1TS

G-protein coupled receptors are both drug targets of high interest and some of the most challenging proteins to study. One member of this family is the adenosine 2A receptor. A crystal structure of this protein in complex with a novel antagonist was published in 2017[1]. It was inferred from this structure that the novel antagonist may cause previously unseen conformational changes by binding to a novel allosteric pocket. Here molecular dynamics simulations have been used to select a site for cysteine mutagenesis and tagging on helix V that was hypothesised to show differentiable chemical shift perturbations in response to the binding of different ligand classes. This A2A mutant has been expressed, purified and tagged with ^{19}F. The NMR responses to several ligand classes were measured and demonstrate that the ^{19}F tag on helix V of the adenosine A2A receptor can discriminate between this novel antagonist and previously identified ligands.

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Total Chemical Synthesis and Racemic Protein Crystallography of Bacteriocins

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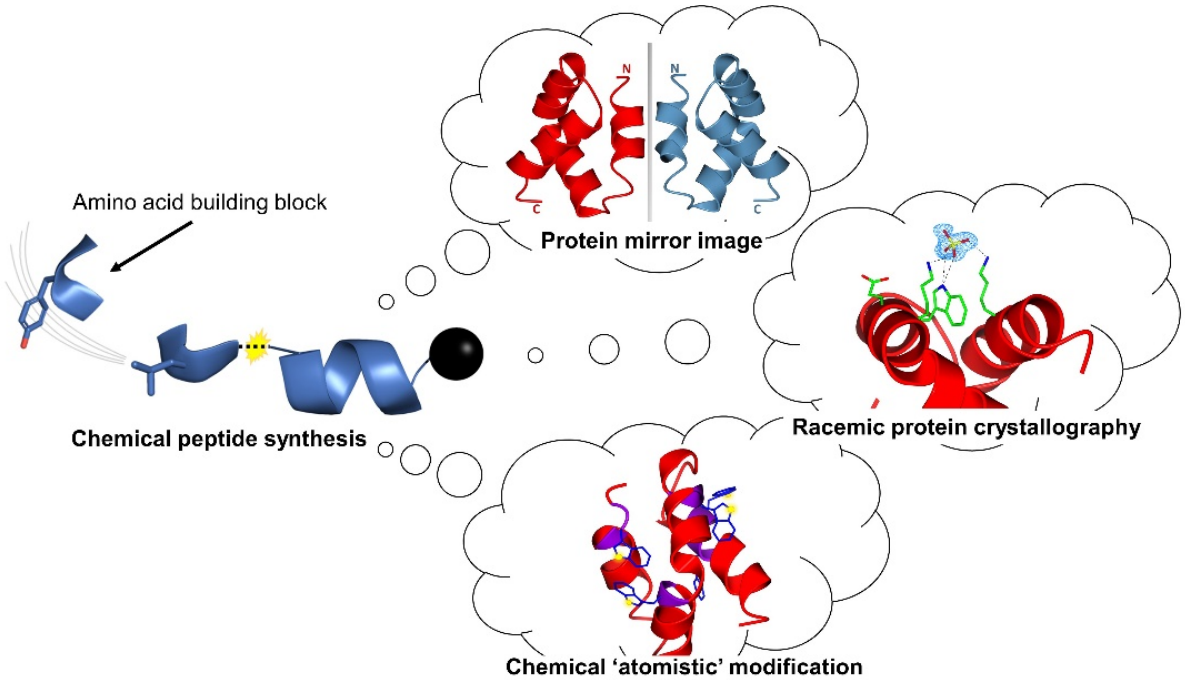
School of Chemistry, Main Building, Cardiff University, Park Place, Cardiff. CF10 3AT

Aureocin A53 (AucA) and lacticin Q (LnqQ) are class IId bacteriocins that display broad-spectrum activity against Gram-positive bacteria in the nanomolar range, of which their modes of action are unclear due to the lack of structural insight raised from the difficulty of crystallisation. Here, we reported the chemical synthetic routes of AucA and LnqQ, enabled through the technique of native chemical ligation. To demonstrate the versatility of the syntheses, we prepared their enantiomeric counterparts, D-AucA and D-LnqQ, comprised of entirely D-amino acids. X-ray crystal structure of AucA obtained through racemic protein crystallography at 1.13 Å indicates a unique Lys-Trp-sulfate network. The reported interactions may mimic binding to the isosteric phosphate of the lipid head group in the bacterial cell membrane. This work lays foundations for gaining further mechanistic insights into these potent bacteriocins through total chemical synthesis and racemic protein crystallography.



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Sequencing and Tracking of Phylogeny in COVID-19: A Genomic Epidemiological Approach to the COVID-19 Pandemic

Angela Beckett[1], Yann Bourgeois[2], Garry Scarlett[2], Katie Loveson[1], Sharon Glaysher[3], Scott Elliott[3], Kelly Bicknell[3], Ethan Butcher[3], Kelly Bicknell[3], Sarah Wyllie[3], Allyson Lloyd[3], Anoop Chauhan[3], Sam Robson[1,2] and The COVID-19 Genomics UK COG-UK consortium

1. Centre for Enzyme Innovation, University of Portsmouth, Portsmouth, PO1 2DT
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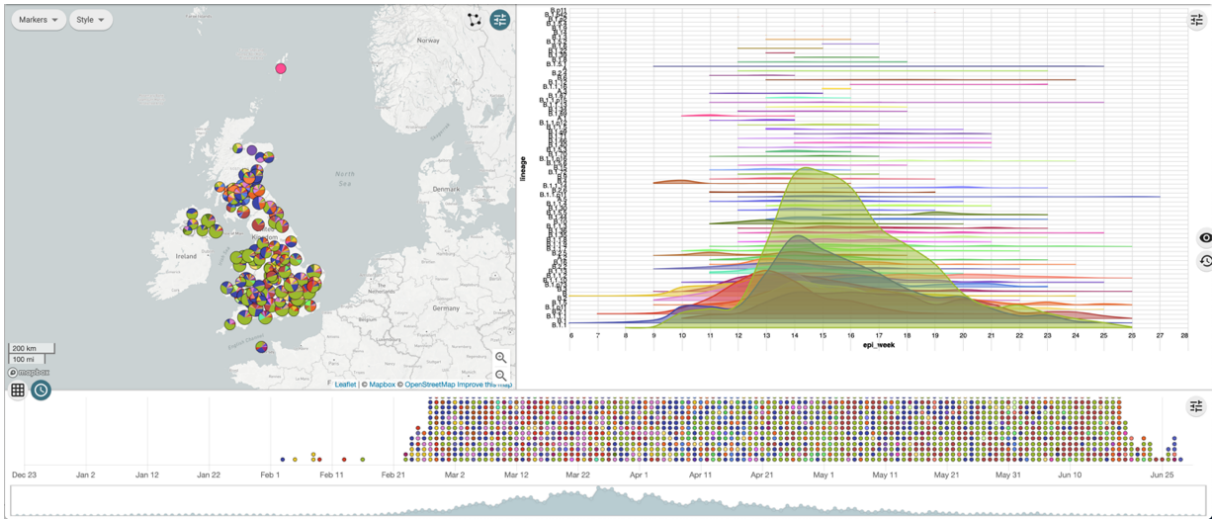
The COVID-19 pandemic has had an unprecedented effect on our way of life. COVID-19 is a severe acute respiratory syndrome caused by the coronavirus SARS-CoV-2. The University of Portsmouth is a member of the COVID-19 Genomics UK Consortium (COG-UK; https://www.cogconsortium.uk/); a national consortium working directly with NHS Trusts and Public Health Agencies to provide genomic surveillance of the virus throughout the pandemic. As the virus moves from person to person, it can undergo mutations, which can be used to model transmission dynamics and track how the virus has spread. Through Nanopore sequencing of genomic RNA extracted from COVID-19 positive tests, we genotype viral lineages and utilise this information to understand the spread of the virus and deconvolute transmission chains in complex infection clusters to help inform infection control procedures. As we begin to see easing of the lockdown measures, surveillance of the viral transmission will be essential to ensure that we prevent a second wave.



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Strategies for ^{1}H-detected dynamic nuclear polarization magic-angle spinning NMR

Maria Concistrè[1], Subhradip Paul[2] and Philip T. F. Williamson[3]

[1] *Chemistry, University of Southampton, Southampton, UK.*
[2]*Mansfield Imaging Centre, University of Nottingham, Nottingham, UK.*
[3]*Biological Sciences/Institute of Life Sciences, University of Southampton, Southampton, UK.*

Remarkable advances have been done in solid-state NMR (SSNMR) experiments for the characterization of protein structure and function. However, some applications are still complicated, impaired or made impossible by the low sensitivity of the technique.

To overcome these limitations, advances are being made in two areas: dynamic nuclear polarization (DNP)[1] and proton-detect fast-MAS NMR[2]. Extending these approaches to large systems, complex biomaterials and unlabeled samples require a combination of these two methodologies. This is the ultimate aim of this work that will allow NMR investigations of samples where labelling may prove intractable, a major step for the structural investigation of complex biological assemblies or drug formulations where labelling is costly or intractable.

In this contribution we show advances done to identify optimal strategies for combining the two methodologies. Working on a model sample, we also demonstrate that ^{1}H-detected MAS-DNP may be used to study biomaterials in 'widely' available commercial instruments.

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Peptide virus-like particles: from synthetic biologics to reference standards for advanced therapies

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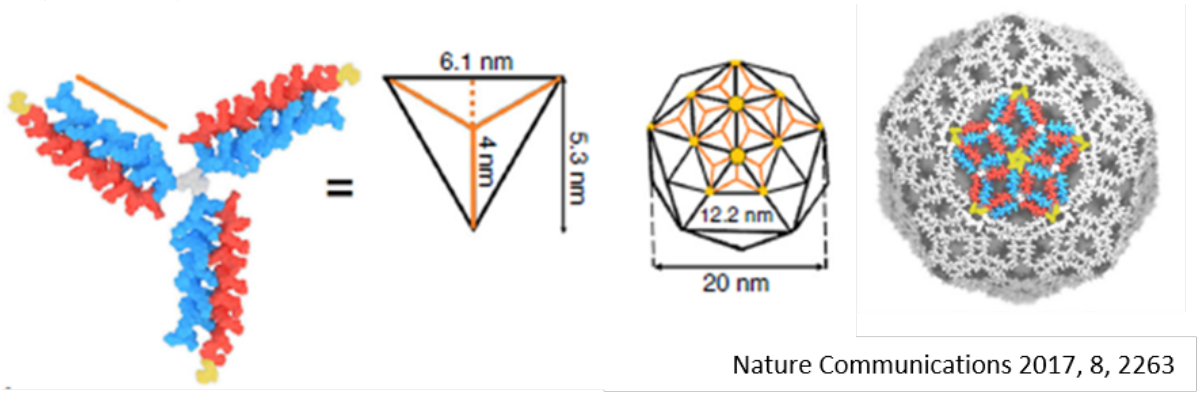
Nanoscale biofunctional materials hold promise as bottom-up synthetic biologics, functional components for advanced therapies and structural platforms for vaccine development. Non-viral virus-like particles (VLPs) assembled from artificial proteins constitute an important type of such materials.
Here, we discuss a repertoire of VLPs designed in our lab together with their biophysical, biological and structural characterisation including high resolution imaging. We highlight the application of these structures as antimicrobial viruses and gene delivery vectors and discuss their relevance and potential as reference standards for gene delivery and vaccine development.



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SIMULATION STRATEGIES FOR ZINC METALLOENZYMES APPLIED TO METALLO-β-LACTAMASES

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[1] School of Cellular and Molecular Medicine, [2] School of Biochemistry, [3] School of Chemistry, University of Bristol, Bristol.
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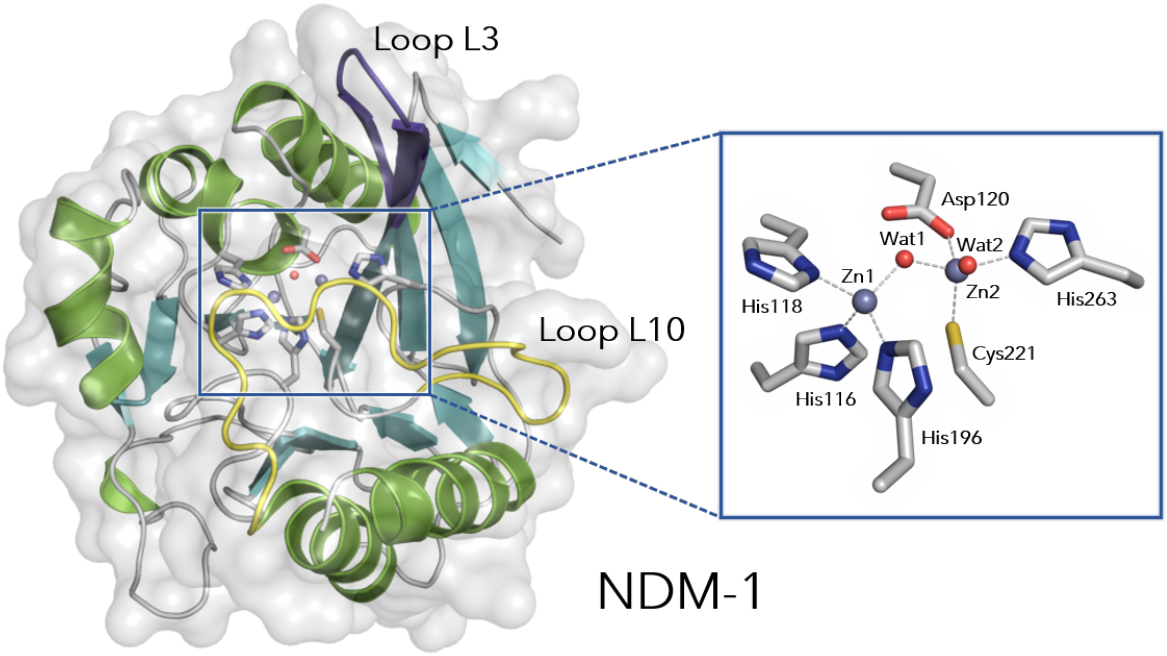
Metallo-β-lactamase (MβL) enzymes contain one or two active site zinc ions intrinsic to the catalytic mechanism. Computational simulation of metal-dependent proteins has proven historically challenging due to the uncertainty in parameterizing the metal ions. Here, we evaluate current metal ion modelling strategies, namely the purely nonbonded, 12-6-4 Lennard-Jones-type nonbonded and cationic dummy atom models to simulate native NDM-1 (and, subsequently VIM-2 and IMP-1) by performing 5-fold replica 100 ns molecular dynamics simulations (totalling 500 ns). Subsequently we develop a novel method, the restrained dummy atom model (RDA), which combines the use of dummy atoms and harmonic restraints to zinc coordinating atoms, and demonstrate that it outperforms the existing methods, maintaining correct active site coordination, a near neutral active site net charge and a hydrogen bond network involving active site residues. This is the first study to evaluate the different modelling strategies for simulating class B1 β-lactamases over long timescales.



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A novel sensor for measuring femto-Newton forces in enzyme turnover and the importance of good crystallographic data

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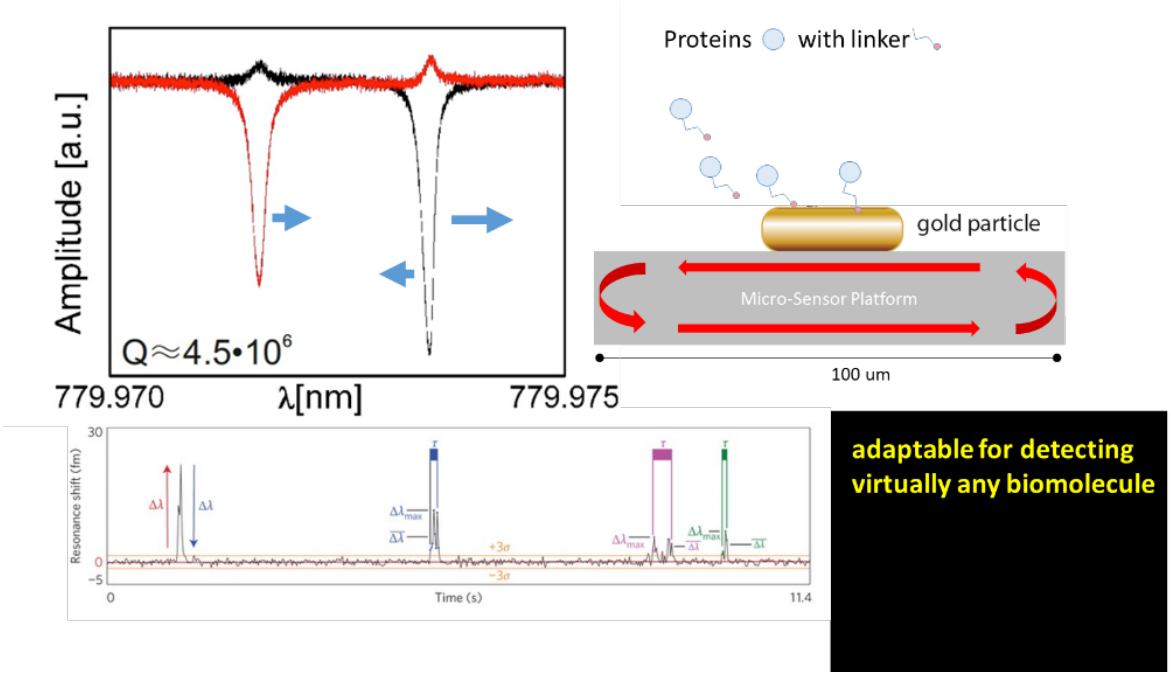
The femto-Newton forces exerted by enzymes associated with their catalytic activity which lead to conformational change in 3D structure have previously been very difficult to measure since current techniques do not have a high enough sensitivity. We are able to overcome this sensitivity issue by employing optoplasmonic sensors, consisting of a microsphere resonator and an attached gold nanoparticle that acts as the sensor. By attaching an enzyme to the gold, we can create a very sensitive system in which small changes in the enzyme conformation affect the resonance frequency of microsphere, allowing very tiny forces to be measured.

In order to understand the data produced by the optoplasmonic sensor, it is highly useful to have accurate crystal structures in a range of conformations, with ligands bound and without. From this we can assign the signal observed on the sensor to the conformational change seen upon ligand binding. To this end, two transaminase enzymes from *Sulfolobus solfataricus* and *Chromobacterium violaceum* and one glucanase enzyme from *Candida albicans* with previously solved structures were used as test enzymes for our gold sensor, with the conformational change seen in the x-ray crystallographic data used for guidance.



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A Novel 'Split-gene' transketolase from the hyper-thermophilic bacterium Carboxydothermus hydrogenoformans: structure and biochemical characterisation

Paul James, Michail Isupov, Simone Antonio De Rose, Christopher Sayer, Isobel Cole and Jennifer Littlechild

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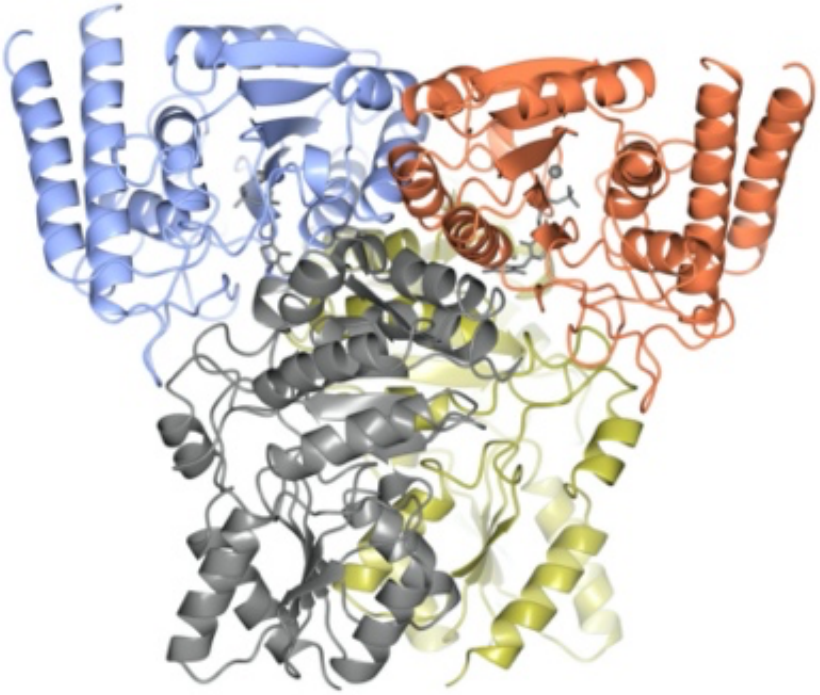
The genes encoding the two parts of a novel split transketolase were identified from the genome of the hyperthermophilic *Carboxydothermus hydrogenoformans*. These have been cloned and over-expressed in *Escherichia coli* before being reconstituted and purified by size exclusion chromatography. This novel enzyme is the first TK to be reconstituted and characterised both biochemically and structurally with the enzyme active using hydroxypyruvate as the ketol donor and different aldehyde acceptors. The ability of thiamine pyrophosphate (TPP) enzymes such as transketolase to form carbon-carbon bonds is a valuable feature used in the synthesis of high value chiral compounds and there is an interest to extend the range of substrates and products of these reactions. 1-Deoxy-D-xylulose 5-phosphate synthase (DXP synthase) is another TPP-dependent catalyst which transfers a two-carbon unit from pyruvate onto specific aldose D-glyceraldehyde 3-phosphate but is able to use the cheaper and more stable pyruvate as the ketol donor. A structural comparison of the split *Carboxythermus* transketolase, the *Escherichia coli* full length transketolase and the DXP synthase has been carried out in an attempt to rationalise the substrate specificity differences between the three enzymes.



Primary citation

- James, P., Isupov, M., De Rose, S., Sayer, C., Cole, I., and Littlechild, J. "A Novel 'Split-gene' transketolase from the hyper-thermophilic bacterium Carboxydothermus hydrogenoformans: structure and biochemical characterisation." Frontiers in Microbiology. Manuscript in Preparation

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Insight into the structure and function of OXA-57; a class-D β-lactamase from *Burkholderia pseudomallei*

Éilís Bragginton*, Philip Hinchliffe*, Karina Calvopiña[§], Yuiko Takebayashi*, Charlotte Colenso*, Christopher Schofield[§], James Spencer*

*School of Cellular and Molecular Medicine, University of Bristol, Bristol, BS8 1TD
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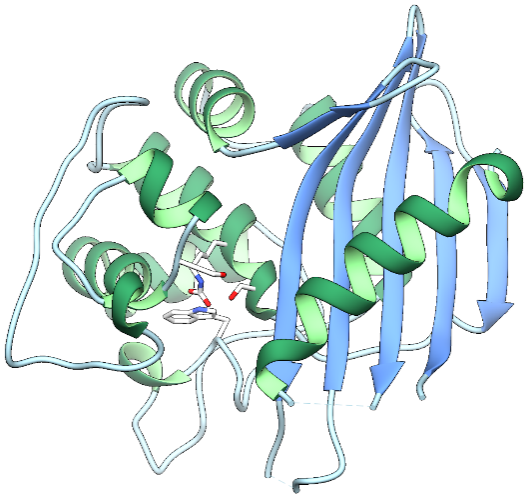
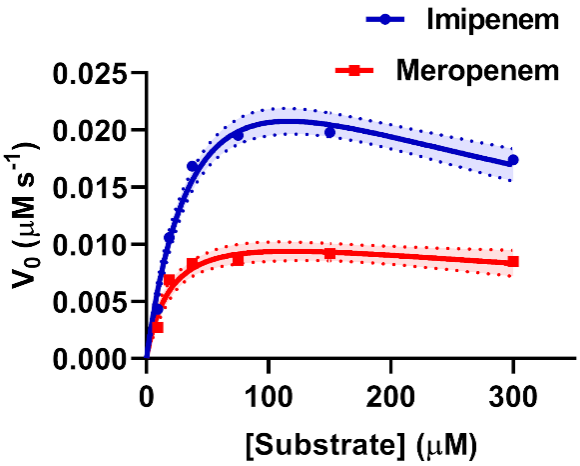
Burkholderia pseudomallei is the causative agent of melioidosis, a disease resulting in severe morbidity and mortality endemic to South East Asia and Northern Australia. Resistance to β-lactams that are used to treat melioidosis is an increasing clinical problem due to β-lactamases encoded on the genome. The primary β-lactams used for treatment of melioidosis are ceftazidime and carbapenems. The establishment of ceftazidime resistance through the expression of a genomic class A β-lactamase is broadly understood. However, the effects of the class-D β-lactamase OXA-57 and its implication in resistance phenotypes is unknown. We have shown that OXA-57 is capable of hydrolysing carbapenems and is inhibited by avibactam. Using X-ray crystallography, we have determined the structure of *apo* OXA-57 and in complex with meropenem (a carbapenem) and avibactam. These data emphasize the potential importance of OXA-57 in carbapenem resistance and highlight potential treatment combinations for the treatment of carbapenem resistant melioidosis.





Primary citation

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Day 1: Monday 20.07.20

POSTER SESSION

Hosted by Southampton (Phil Williamson and Ivo Tews)

18:00-18:05	Brief introduction to online posters
18:00-20:00	Visit the poster inn MS teams, just select the channel with the poster you are interested in, watch the clip, and ask the presenter questions

We have selected 12 posters for poster pitches; please keep to 2.5 mins each:

18:30	Poster 11: Feliciotti	19:00	Poster 25: Williamson
18:35	Poster 15: Gregory	19:05	Poster 8: Cordery
18:40	Poster 26: Zalaite/Munro	19:10	Poster 2: Andrews
18:45	Poster 16: Guo/Cooper	19:15	Poster 6: Buzzard
18:50	Poster 5: Bochel	19:20	Poster 13: Gaines
18:55	Poster 7: Connners	19:25	Poster 17: Hinchen

All poster abstracts:

Poster 1: Agnarelli, see abstract (16)
Poster 2: Andrews
Poster 3: Aksakal
Poster 4: Baltulionis
Poster 5: Bochel
Poster 6: Buzzard
Poster 7: Conners
Poster 8: Cordery
Poster 9: Diez, see abstract (1)
Poster 10: Dunphy, see abstract (14)
Poster 11: Feliciotti
Poster 12: Fenn
Poster 13: Gaines
Poster 14: Graham
Poster 15: Gregory
Poster 16: Guo/Cooper
Poster 17: Hinchen
Poster 18: Holes
Poster 19: May
Poster 20: Mitchell, see abstract (24)
Poster 21: Munro/ Zalaite, see abstract (15)
Poster 22: de Rose, see abstract (11)
Poster 23: de Santis, see abstract (22)
Poster 24: Toelzer/Gupta, see abstract (4)
Poster 25: Williamson
Poster 26: Zalaite/Munro

Poster 1: Agnarelli, see abstract (16)

Investigation into the Sensitivity of Fluorescence Spectral Fingerprinting as a Method for Synthetic Cannabinoid Detection

Rachael Andrews, Benedict May, Christopher Pudney, Dave Carbery.

Department of Biology & Biochemistry, University of Bath, Claverton Down, Bath, BA2 7AY
Department of Chemistry, University of Bath, Claverton Down, Bath, BA2 7AY
Centre of Sustainable and Circular Technologies (CSCT), University of Bath, Claverton Down, Bath, BA2 7AY

Synthetic cannabinoids (SCs) are a class of recreational drugs commonly taken to mimic the effects of tetrahydrocannabinol (THC). Currently, there are 169 SC compounds known to the European Monitoring Centre for Drugs and Drug Detection.[1] This poses a challenge in drug detection due to the vast number of possible compound found in SC samples. Fluorescence spectral fingerprinting (FSF) is a detection method that can indicate the presence of SCs in a sample.[2] In order to investigate the sensitivity of this method, a QSAR series of compounds have been synthesised with small structural differences, and FSFs were calculated for each. FSFs were also calculated for photodegraded samples of the series, indicating structural changes to the compounds following photodegradation. Our results demonstrate that this detection method can be highly sensitive to small structural differences. Additionally, sample photodegradation has provided an additional level of information to assist in detection of the SC present.

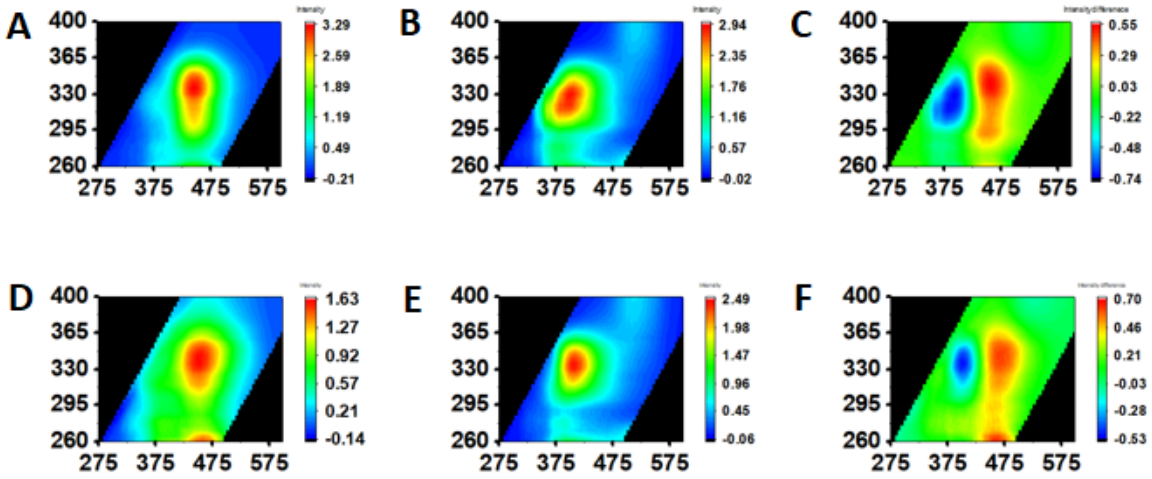

Figure: A-C: FSFs of undegraded (A) and degraded (B) 2-(2-bromophenyl)-1-(1H-indol-3-yl)ethanone, and a difference map between the two (C).
D-F: FSFs of undegraded (D) and degraded (E) 2-(2-chlorophenyl)-1-(1H-indol-3-yl)ethanone, and a difference map between the two (F).

Up to three references

1 EMCDDA, *Perspectives on drugs: Synthetic cannabinoids in Europe*, Lisbon, 2017.
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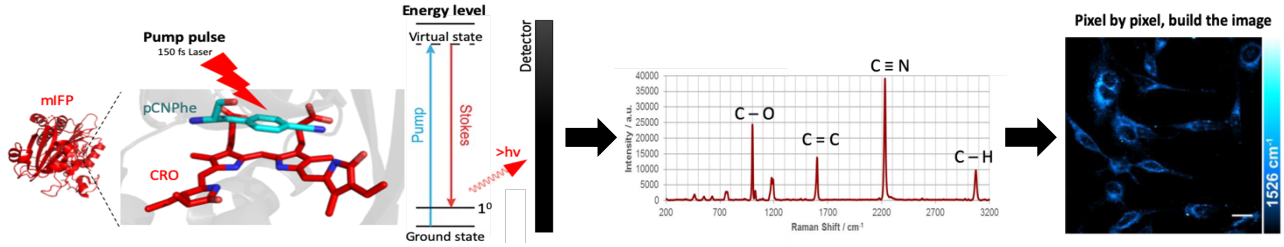
In Silico determination of Raman-active fluorescent proteins for next-generation live cell imaging.

Ozan Aksakal

DDJ lab, School of Biosciences, Cardiff University, CF10 3AX

Fluorescent microscopy has been an incredible servant to Cell and Molecular Biology, however, the limitations of multiplexing various fluorescent proteins (FPs) has restricted researchers in their pursuit of observing the 'full picture'. Raman microscopy is a next-generation live-cell imaging method that aims improve multiplexing capabilities as we can observe sharp spectral peaks within the amplified biologically-silent Raman window, situated within IR spectrum. Our project aims to identify suitable variants of mCherry to incorporate Raman-active bonds at different positions within the chromophores' environment via an *in silico* approach. We assessed the stability of these variants by undergoing molecular dynamics (MD) simulations and analysing the average structures, RMSD, RMSF, rate of gyration and the difference in free energy of each variant. This approach also required the parametrisation of novel compounds, including non-canonical amino acids and chromophores. We have now been able to identify suitable variants to take forward into wet-lab studies.

Raman Microscopy



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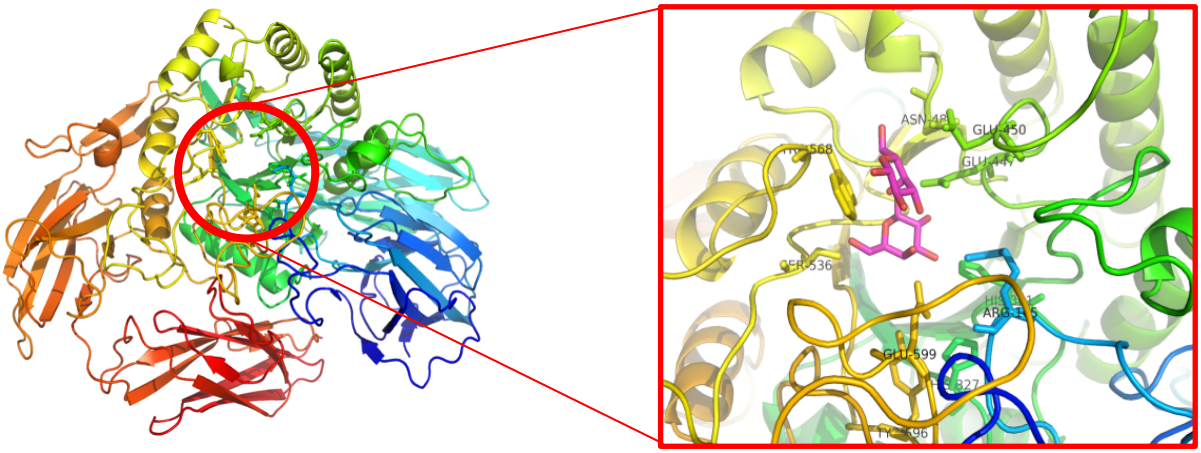
Bioengineering a synthetic enzyme for production of functional prebiotic oligosaccharides

G Baltulionis[1], A Chatzifragkou[1], D Charalampopoulos[1], G Gibson[1] and KA Watson[1]

[1]University of Reading, Whiteknights Campus, Reading, RG6 6UR

Breast-fed infants have a *Bifidobacterium*-rich gut microbiota compared to those who are formula fed. Fucosylated oligosaccharides (FucOS) are major components of human milk oligosaccharide (HMO) thought to confer a prebiotic effect and protection from pathogens. It has been shown that some β-galactosidases are able to utilise fucose monosaccharides generating FucOS. Our previous work, using predictive and experimental tools to assess the impact of domain truncations and site-directed mutagenesis on *Bifidobacterium* β-galactosidase III (BbgIII), led to the development of improved BbgIII enzyme variants that gave galactooligosaccharides (GOS) with high purity mixtures. We used protein engineered β-galactosidase enzymes to produce FucOS. In addition, we identified other synthetic bifidobacterial enzymes producing complex high DP mixtures starting from lactose and other substrates. Also, the oligosaccharide product range in our synthesis reactions was greatly expanded by utilising additional acceptor substrates. These synthesis mixtures will be assessed for their prebiotic effect using gut model systems.



Primary citation
Osman, A., Symeou, S., Trisse, V., Watson, A., K., Tzortzis, G. (2014). "Synthesis of prebiotic galactooligosaccharides from lactose using bifidobacterial β-galactosidase (BbgIV) immobilised on DEAE-Cellulose, Q-Sepharose and amino-ethyl agarose." Biochemical Engineering Journal **82**: 188-199. https://doi.org/10.1016/j.bej.2013.11.020

Studying sugar recognition by the Cation-Independent Mannose 6-phosphate receptor

Alice Bochel,[1] Dr Chris Williams, [1] Professor Bass Hassan[2] and Professor Matt Crump[1]
[1]*School of Chemistry, University of Bristol, Cantock's close, BS8 1TS,* [2] *Oxford Molecular Pathology Institute, Sir William Dunn School of Pathology, University of Oxford, Oxford, OX1 SRE*

The Cation-Independent Mannose 6-phosphate Receptor (CI-MPR), a transmembrane glycoprotein, plays a crucial role in intracellular protein trafficking, lysosome biogenesis and cell growth regulation. The extracellular region of the CI-MPR is approximately 250 kDa and consists of 15 homologous domains - four of which bind mannosylated proteins. Although several multi-domain fragments have been solved, several regions are completely uncharacterised and the domain arrangement and oligomeric state of the full extracellular region of human CI-MPR is poorly understood. A modular approach has been adopted to express and purify single and multi-domain constructs containing the elusive carbohydrate binding domain 9, with the ultimate goal of isolating the full-length extracellular region.

A second project takes the stable, well-studied, IGF2 binding domain - domain 11 – and aims to engineer in sugar binding. Initial promising results combining the use of structure led mutagenesis, crystallography and high-resolution NMR are presented here.

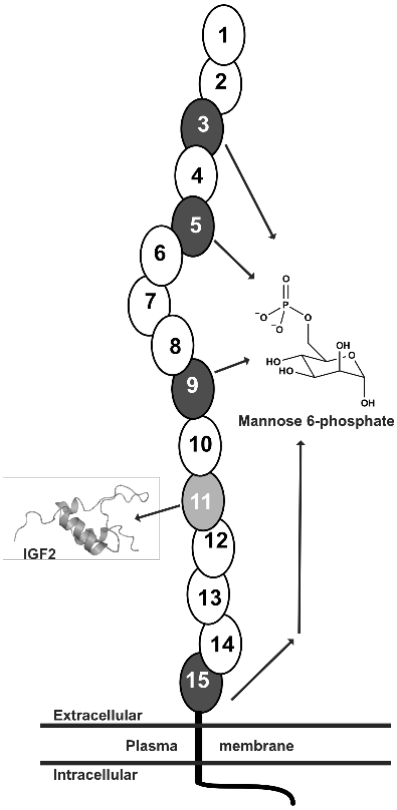


Figure 1: Schematic of CI-MPR extracellular region. Mannose 6-phosphate binding domains (Domains 3, 5, 9 and 15) coloured grey. IGF2 binding domain (Domain 11) coloured light grey.

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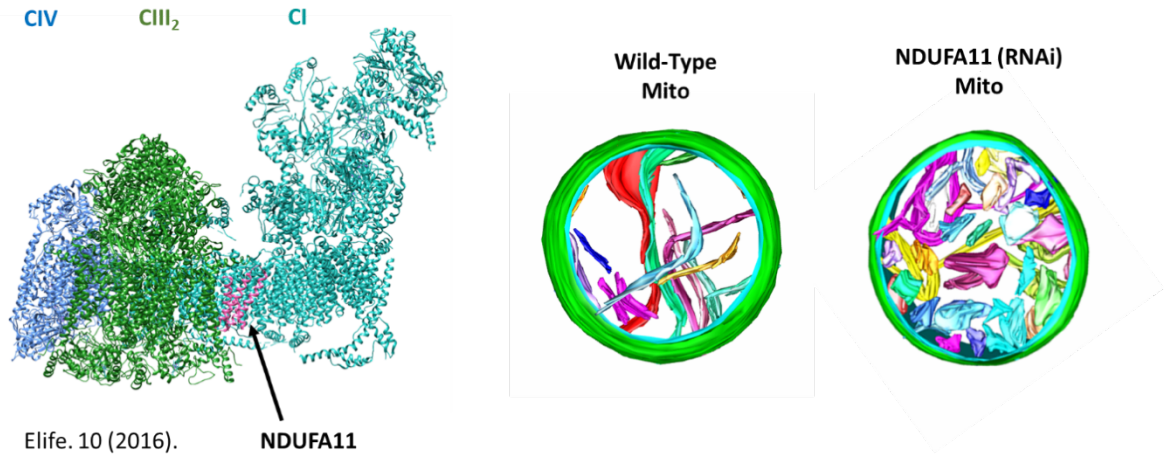
CryoET for investigating mitochondrial respirasome stability in health and disease

Emma Buzzard[1], Amber-Knapp Wilson[2], Patricia Kuwabara[2], Ian Collinson[2] & Vicki Gold[1]

[1] College of Life and Environmental Sciences, Living Systems Institute, University of Exeter, Exeter, EX4 4QD
[2] School of Biochemistry, Biomedical Sciences Building (M floor), University of Bristol, Bristol, BS8 1TD

The mitochondrial respirasome (a $I_1III_2IV_1$ supercomplex) functions to pump protons into the intermembrane space, which flow back into the matrix through ATPase to generate ATP. Although there are many unanswered questions concerning respirasome function (1), it is known that loss of respirasome stability is associated with ageing (2). Using the nematode worm *C. elegans* as a model system, I aim to investigate the role that respirasome stability plays in ageing and related diseases, through knocking down an accessory protein of complex I (NDUFA11) known to be crucial for respirasome assembly (3). Using electron cryo-tomography and sub-tomogram averaging, I will assess how loss of respirasome stability in the knockdown influences both respirasome structure and mitochondrial morphology. Alongside biochemical assays to assess how knockdown influences oxygen consumption, this study will further our understanding of the fundamental mechanisms of ageing, and could form the basis for future interventions designed to modify or slow the ageing process.



Left: Atomic model of the bovine mitochondrial respirasome (Sousa *et al.*) with the location of critical assembly subunit NDUFA11 highlighted in pink. Right: The effect of knocking down the *C. elegans* NUDFA11 homologue on mitochondrial membrane morphology.

Up to three references

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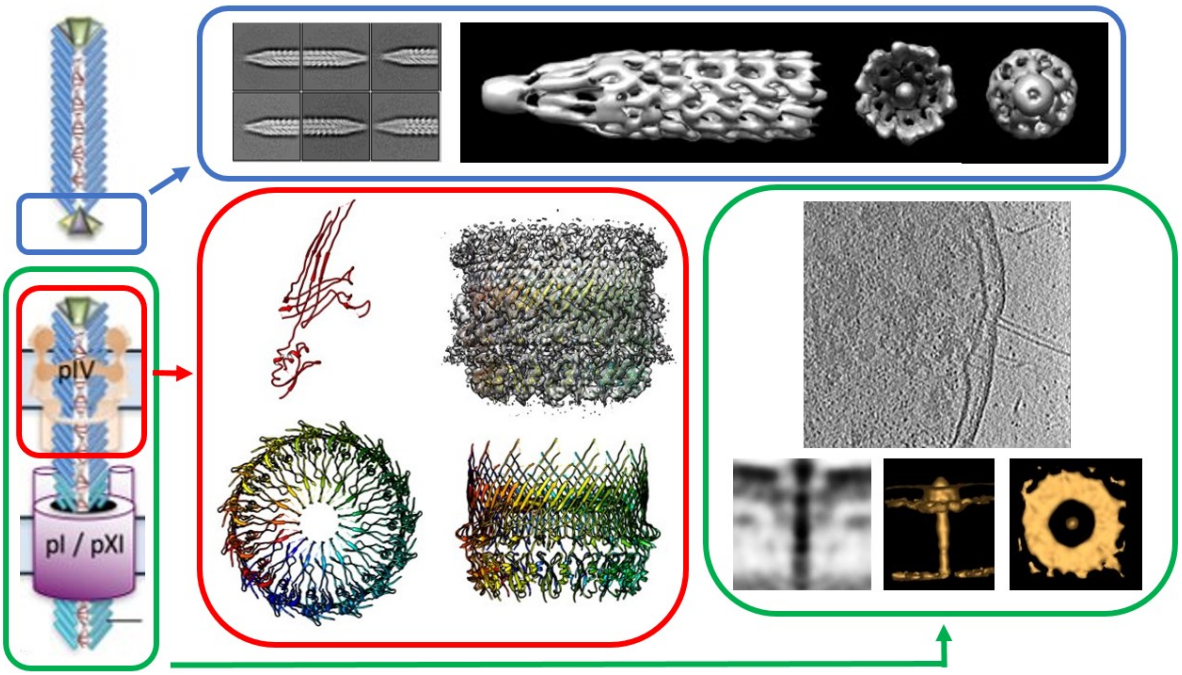
The filamentous phage assembly system

Becky Conners[1], Rayen Leon[2], Mat Mclaren[1], Jasna Rakonjac[2] & Vicki Gold[1]

[1]Living Systems Institute, University of Exeter, Stocker Road, Exeter, EX4 4QD and [2]Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand

Filamentous phages (Ff, fd, M13) are viruses that infect predominantly Gram-negative bacteria. Interestingly, the appearance and mode of assembly of these phages share many similarities with bacterial pili, which drive cell motility. The phage infects the bacterial host cell without causing cell lysis, and has many potential applications in biotechnology and nanotechnology; including phage display, production of nanowires and nanorods, bio-templated lithium ion batteries, as a vaccine carrier and as a biosensor.

We aim to enhance our understanding of a number of different stages of the viral life cycle using a combination of methods. We have used single particle cryo-electron microscopy to look at different parts of the viral capsid, and also the secretion pore the virus uses to exit the host cell. We aim to combine these higher resolution structures with an *in situ* study of the entire viral assembly complex determined by cryo-electron tomography.



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Understanding the regulation of intracellular c-di-GMP levels using *Pseudomonas aeruginosa* phosphodiesterase protein RbdA (Regulator of Biofilm Dispersal)

Jack Craddock, Charlotte Cordery, Martin Walsh, Jeremy Webb, Ivo Tews

Biological Sciences, Institute for Life Sciences B85, University of Southampton, Southampton, SO17 1BJ
National Biofilms Innovation Centre (NBIC), University of Southampton, Southampton, SO17 1BJ
Diamond Light Source, Harwell Science and Innovation Campus, Didcot, Oxfordshire, OX11 0DE

Pseudomonas aeruginosa is an opportunistic pathogen which readily forms biofilms complicating infections and causes the fatality of many Cystic Fibrosis sufferers. C-di-GMP controls bacterial biofilm formation and motility, intracellular levels are controlled by Diguanylate cyclases (DGCs) and Phosphodiesterases (PDEs). Influenced by environmental factors, these are multi-domain proteins which include sensory and regulatory domains. Manipulating intracellular levels of c-di-GMP may provide anti-biofilm therapies. RbdA (regulator of biofilm dispersal A) is composed of PAS-DGC-PDE and displays dimerization dependent PDE activity despite containing domains of opposing catalytic activity. Knockout studies of RbdA revealed RbdA is involved in the NO dispersal response and mutant biofilms showed elevated c-di-GMP levels, decreased motility and greater biomass formation. The DGC domain negatively feeds back to influence activity of the PDE domain. Structural studies informed that RbdA's PDE domain is unique, and upon activation forms a dimeric state rather than an auto-inhibitory conformation.

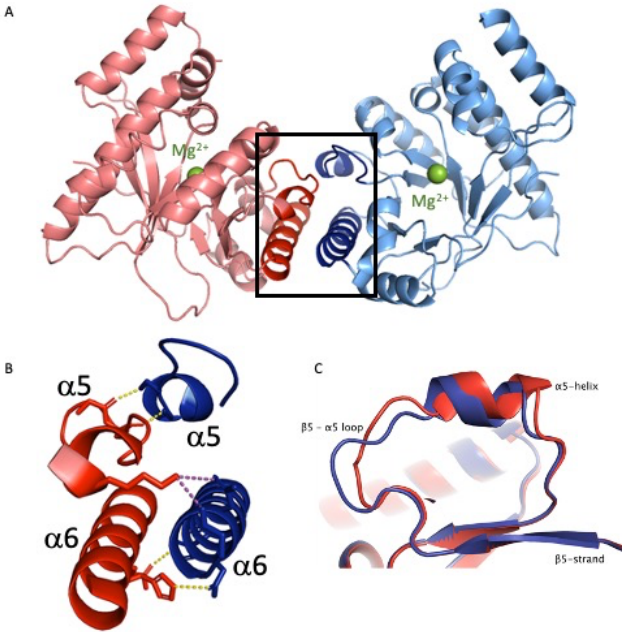

Figure 1 RbdA EAL dimer, figure by Jack Craddock. Hydrogen bonds are yellow and salt bridges are purple.

Primary citation

Cai, Y., Hutchin, A., Craddock, J., Walsh, M.A., Webb, J.S. and Tews, I. Differential impact on motility and biofilm dispersal of closely related phosphodiesterases in *Pseudomonas aeruginosa*. *Sci rep*. 10, 6232 (2020). 10.1038/s41598-020-63008-5

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Poster 9: Diez, see abstract (1)

Poster 10: Dunphy, see abstract (14)

Structure-function characterisation of novel galactosidases from *Lactobacillus plantarum*: exploring the link between gut bacteria and lipid levels

Feliciotti Irene[a], Kolida Sofia[c], Rastall Bob[b], Watson Kimberly[a]

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The human gut microbiome is a complex ecosystem, which plays a crucial role in human health. However, the gut microbiome equilibrium is delicate and as such different strategies to modulate it have been proposed. One approach involves the use of prebiotics and probiotics as supplements able to alter the microbial composition in the gut and increase its beneficial effects.

This project focuses on *Lactobacillus plantarum*, a probiotic strain already used for its beneficial effects in lipid metabolism. Recent sequencing of Lactobacillus plantarum has revealed potential novel beta-galactosidase sequences responsible for galacto-oligosaccharide (GOS) synthesis. Select enzymes have been targeted for detailed structure-function studies to elucidate their functional role in GOS production. Bioinformatics tools have been utilized to identify important functional and structural domains and X-ray crystallography will be used for their structural characterisation. We anticipate that this work will be valuable for understanding detailed mechanisms of action of specific galactosidase enzymes in the synthesis of oligosaccharides.

Evaluating radiation damage to a selenium-sulfur bond in a 1.47Å ebselen Impase crystal structure.

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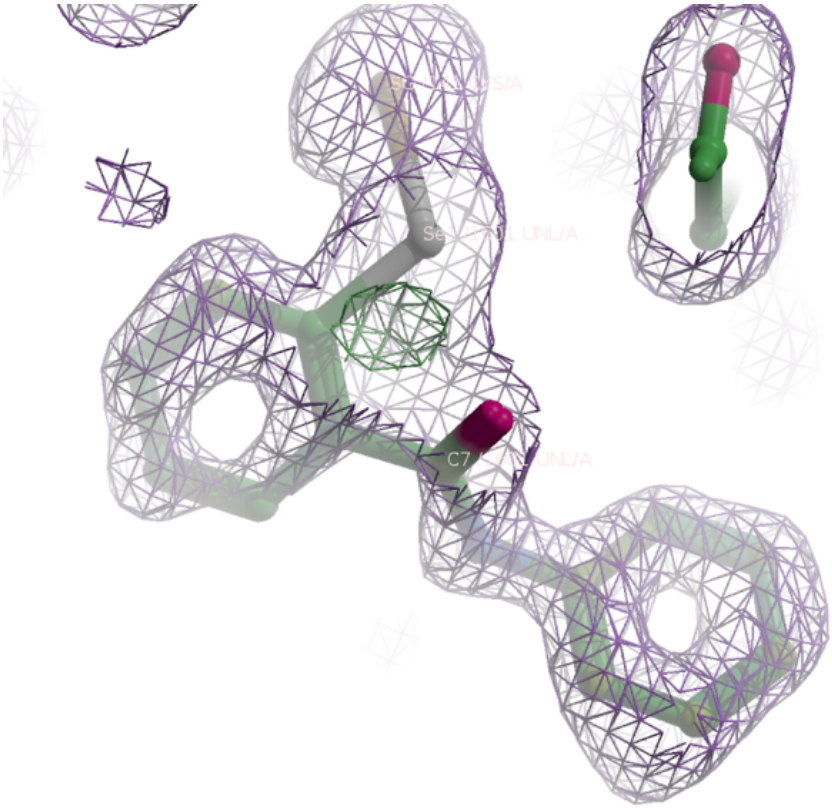
Inositol monophosphatase (IMPase) is a drug target for the treatment of bipolar disorder; it is inhibited by lithium. Bipolar disorder affects approximately 2% of the population (1). IMPase inhibitors aiming to replace lithium tend to be polar and lack the ability to penetrate the blood-brain barrier (2). However, one promising candidate is ebselen, a selenium-containing antioxidant, which has been demonstrated to produce lithium-like effects, both in a murine model and in clinical trials. The primary target for ebselen for bipolar disorder is believed to be IMPase, but like lithium ebselen displays polypharmacology. Ebselen and derivatives also have potential use in treating motor neuron disease; they can affect the folding of SOD-1 (3). A 1.47Å dataset on a co-crystal of ebselen with IMPase shows ebselen covalently attached to a cysteine residue. The selenylsulphide bond shows signs of radiation damage. Refinement strategies for refining this selenylsulphide bond will be discussed.



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Towards solving the structure of the *Bacillus sphaericus* S-layer

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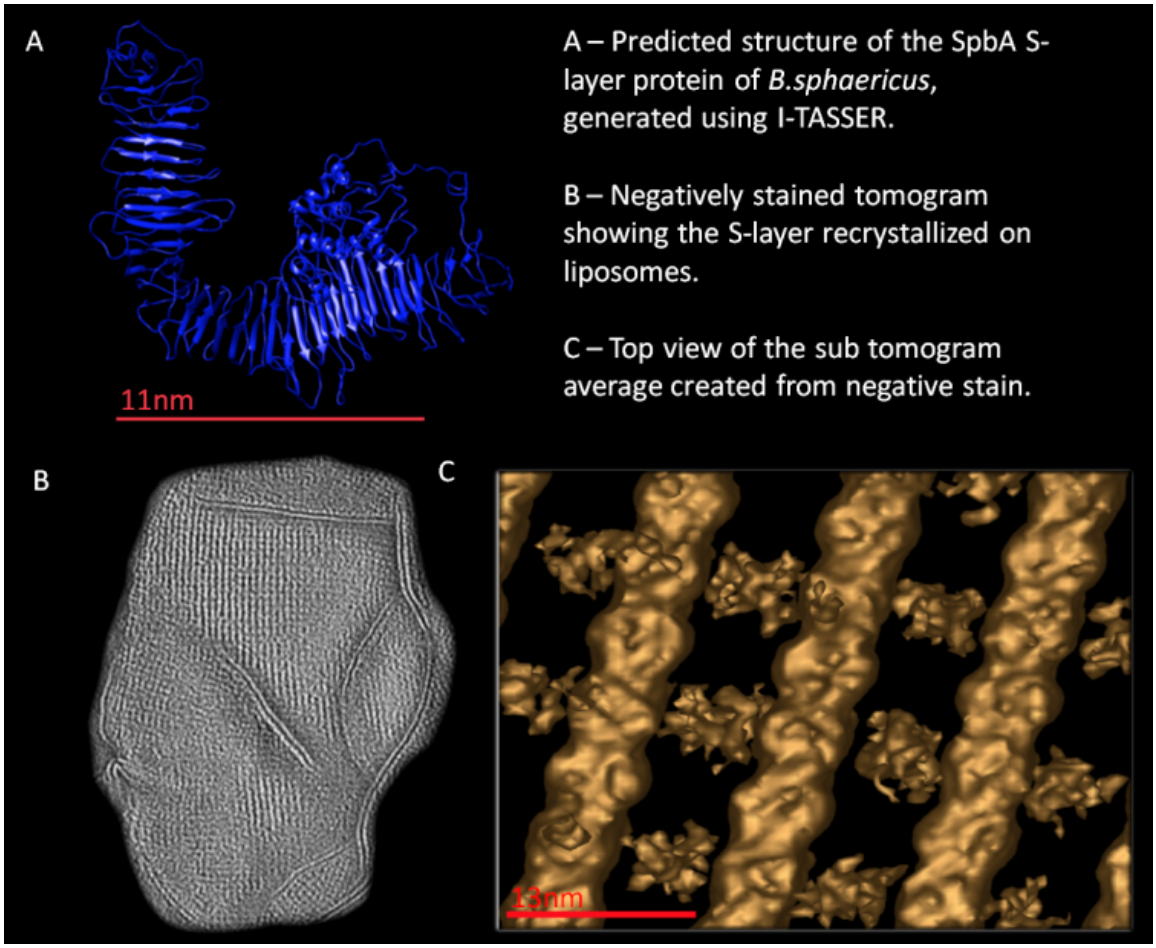
S-layers are para-crystalline structures that comprise important cell envelope components of many Bacteria and Archaea. Some S-layers readily self-assemble into highly stable protein matrices on various organic and inorganic surfaces and thus show great promise as nanomaterials for synthetic biology and drug delivery. The aim of this PhD project is to explore the suitability *Bacillus sphaericus* S-layer for such applications – initially by solving its structure. To this end, we overexpressed the 125 kDa S-layer subunit protein SbpA in *E. coli,* purified it by size exclusion chromatography, and re-crystallised it on liposomes. S-layers were imaged using negative stain electron microscopy and a first 3D map was generated by electron tomography and sub-tomogram averaging. Future work will focus on obtaining samples suitable for cryoEM and solving the structure of the S-layer below <4Å to build an atomic model. The structure will inform new synthetic biology and drug delivery approaches based on S-layers.



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Designing chimeric fusion proteins for improved PET hydrolysis

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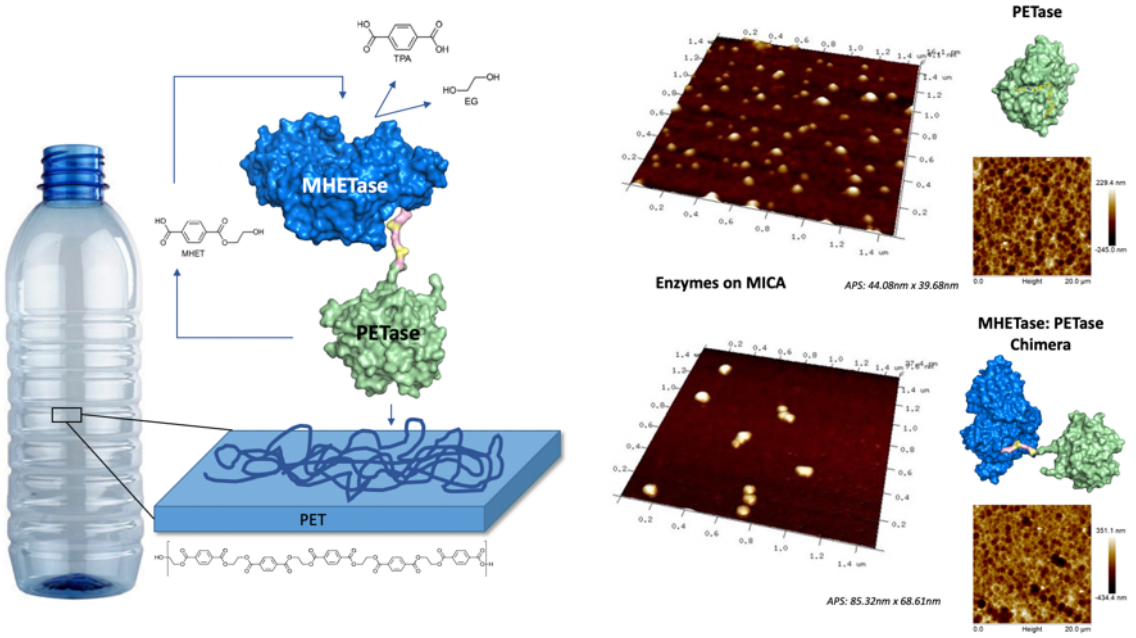
The deconstruction of recalcitrant polymers such as cellulose or chitin is accomplished in nature by synergistic enzyme cocktails where soluble oligomeric intermediates are released via interfacial biocatalysis, and additional enzymes further process to monomers for microbial uptake. One example of this is the recent discovery of a two- enzyme system for PET deconstruction, which employs MHETase and PETase to synergistically work to produce the constituent PET monomers, this suggests that nature is evolving similar deconstruction strategies for synthetic plastics. In the current study we compare the performance of MHETase:PETase chimeric proteins of varying linker lengths with data shown here using HPLC to compare PET monomer release upon incubation with enzyme and Microscopy to characterise surface degradation. Chimeric constructs exhibit improved PET and MHET turnover relative to the free enzymes and this may inform industrial enzyme cocktail-based strategies for plastics upcycling.



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Study of Ganglioside binding in Botulinum Neurotoxin subtype A3 by X-ray Crystallography

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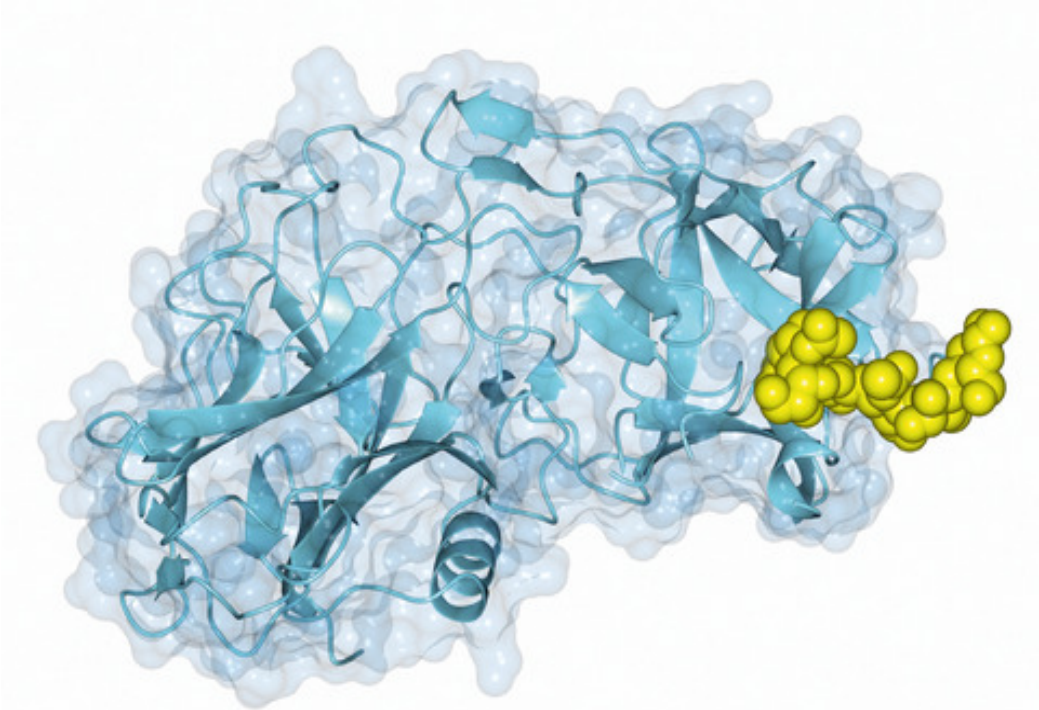
Botulinum Neurotoxin (BoNTs) can be utilized as a drug or bioweapon. Structural characterisation of these molecules is therefore essential to aid both drug development and defend against acts of bioterrorism. BoNTs consist of three essential domains: A cell binding domain (H_C), translocation domain (H_N) and catalytic domain (LC). The H_C binds to the neuromuscular junction *via* a dual-receptor complex involving both a protein and ganglioside receptor. Variation in activity amongst BoNT serotypes/subtypes has been attributed to differences in protein and ganglioside binding. Here we present a detailed analysis of the crystal structure of H_C/A3 in complex with GD1a ganglioside. H_C/A3 forms a total of seven hydrogen bonds with GD1a and displays subtle changes in conformation upon binding.



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In crystallo-screening for discovery of human norovirus 3C-like protease inhibitors

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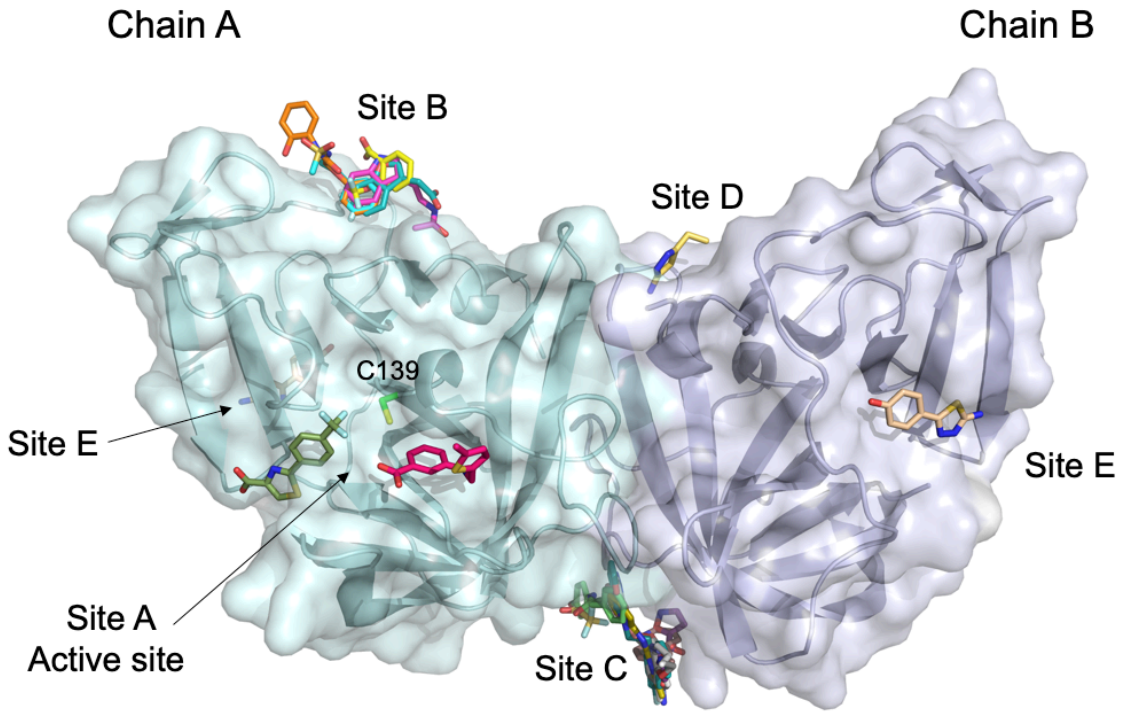
Outbreaks of human epidemic nonbacterial gastroenteritis are mainly caused by noroviruses. Viral replication requires a 3C-like cysteine protease (3CLpro) which processes the 200 kDa viral polyprotein into six functional proteins. 3CLpro has attracted much interest as a potential target for antiviral drugs. Building on our prior studies of Southampton virus 3CLpro[1], a new crystal form has allowed the native structure to be determined at near-atomic resolution (1.3 Å). This crystal form was also suitable for fragment screening studies at the DLS XChem facility. A total of 19 fragments were found to bind to the protease out of the 844 which were screened. Two of the hits were located at the active site of the enzyme and showed inhibitory activity in kinetic assays. Another 5 were found at the enzyme's RNA-binding site and a further 10 were located in the central cavity of the putative tetramer observed in the crystal structure.



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A manuscript with the same title and authorship as this abstract has been submitted to Journal of Structural Biology: X.

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Engineering a Biocatalyst for Demethylating Lignin-Derived Aromatics

Daniel J. Hinchen[a], Emerald S. Ellis[b], Alissa Bleem[c], Sam J.B. Mallinson[a], Mark D. Allen[a], Melodie M. Machovina[b], Christopher W. Johnson[c], Gregg T. Beckham*[c], Jennifer L. DuBois*[b] and John E. McGeehan*[a]

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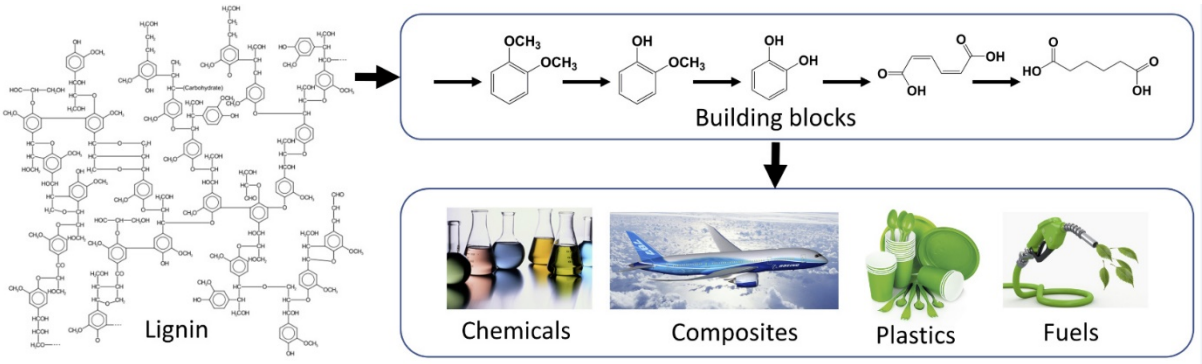
Depolymerisation products from lignin can be productively funnelled into biosynthetic pathways, an attractive method for lignin valorisation. While this approach provides new avenues for the processing of heterogenous mixtures, a rate limiting step for efficient bioconversion of aromatic products is the removal of O-methyl groups, which decorate many of the monomers produced by depolymerisation. A Cytochrome P450 system (GcoAB) was characterised and shown to be capable of demethylating guaiacol and subsequent work demonstrated substrate specificity could be enhanced by incorporating a single amino acid change in the active site to accommodate the larger monomer, syringol. Here we show, through a combination of x-ray crystallography and structure-led design we can further extend the substrate range of this system to include a new class of aromatic monomers. This work adds to the toolbox of enzymes required for efficient biological lignin conversion into multiple value-added products.



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The Macromolecular Crystallography Facility at the University of Southampton

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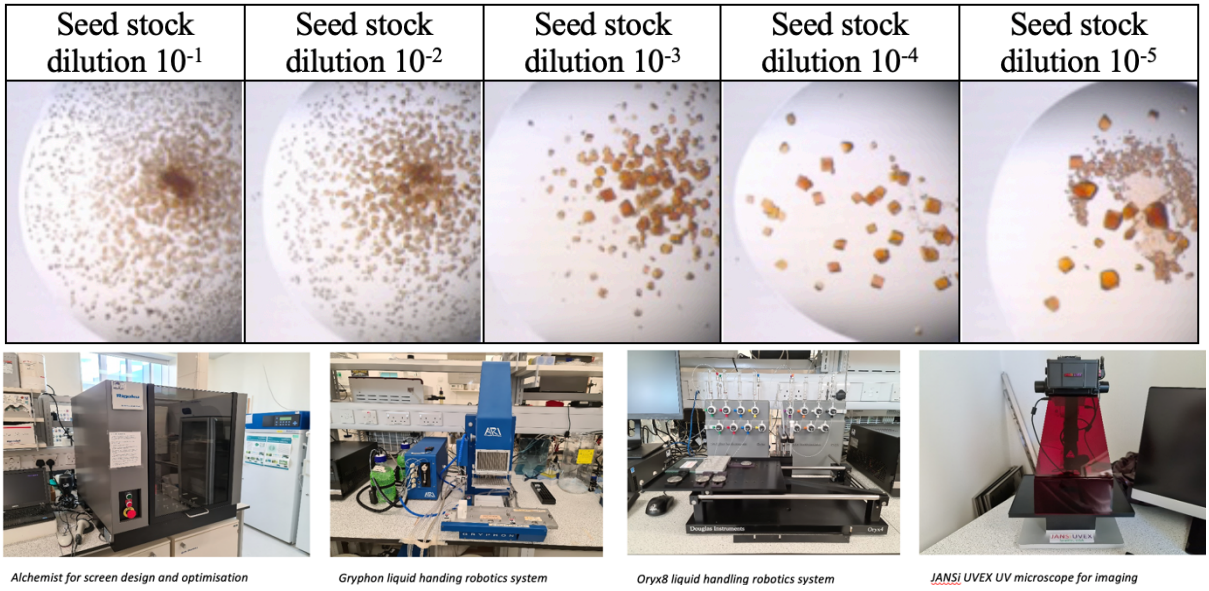
The University of Southampton's Macromolecular Crystallisation Facility, housed at the Institute for Life Sciences (IfLS), together with facilities at the Southampton Diffraction Centre, offers all steps in protein crystallography: from purified sample to 3D structure. It is home to a fully integrated pipeline and includes the Rigaku Alchemist for screen design and optimisation, the Art Robbins Instruments Gryphon for liquid handing and plate dispensing, JANSi UVEX and Rigaku Minstrel ultra-violet and Leica M165C light microscopy, as well as plate storage and different temperatures. Recent addition of a Douglas Instruments' Oryx8 liquid handling robotics system (the Oryx8) has added protocols for optimising nano-crystallisation by "seeding" useful in ongoing XFEL research. Seeding additionally vastly improved the success and quality of crystallisation experiments, opening up previously inaccessible crystallisation conditions for many protein targets.



Alchemist for screen design and optimisation *Gryphon liquid handing robotics system* *Oryx8 liquid handling robotics system* *JANSi UVEX UV microscope for imaging*

Primary citation

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Detecting Synthetic Cannabinoid Receptor Agonists using Fluorescence Spectral Fingerprinting

Benedict May (PhD student University of Bath Biology and Biochemistry), Husain A Naqi , Jenny A Scott, Ian S Blagbrough , Stephen M Husbands, Christopher R Pudney

Synthetic cannabinoid receptor agonists (SCRAs) are molecules that emulate the psychoactive compounds in marijuana. Their abuse is highly prevalent in the U.K. prison system and among homeless populations. With no reliable way to determine dosage, overdoses are common. Their effects include psychosis, strokes, seizures, and death. The diversity of SCRA structures presents a challenge in developing detection modalities. Typically, GC-MS is used for identification; however, this cannot be in place in settings where detection is critical, e.g., Emergency Departments, custody suites/prisons, or among homeless communities. Ideally, real time, point-of-care identification of SCRAs is desirable to direct the care pathway of overdoses and assist with informed consent. Herein, we show that fluorescence spectral fingerprints (FSFs) can be used to identify SCRAs and provide information on their structure and concentration (~1 μg per mL). We demonstrate that FSFs detect combusted and uncombusted material and such fingerprinting is practical for detection in oral fluids. Our study suggests that the approach could be useful in a range of capacities, notably in harm reduction for users of Spice/K2.

Primary citation
Benedict May, Husain A. Naqi, Michael Tipping, Jenny Scott, Stephen M. Husbands, Ian S. Blagbrough, and Christopher R. Pudney “Synthetic Cannabinoid Receptor Agonists Detection Using Fluorescence Spectral Fingerprinting” *Analytical Chemistry* 2019 *91* (20), 12971-12979 DOI: 10.1021/acs.analchem.9b03037

Poster 20: Mitchell, see abstract (24)

Poster 21: Munro/ Zalaite, see abstract (15)

Poster 22: de Rose, see abstract (11)

Poster 23: de Santis, see abstract (22)

Poster 24: Toelzer/Gupta, see abstract (4)

Molecular analysis of the interaction of the Bin pesticidal protein with its mosquito receptor

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Bacillus and *Lysinibacillus* bacteria are able to produce a range of insecticidal toxins that are highly specific for narrow ranges of target insects. This makes them attractive agents for use as biopesticides for the control of pest insects. Specificity is determined by recognition of receptors present on target cells, but the molecular details of toxin-receptor interactions have not been elucidated for any of these toxins. The Bin toxin of *Lysinibacillus sphaericus* is selectively active against *Culex* and *Anopheles* mosquitoes and the receptor protein, an alpha glycosidase, has been identified. Recognition of this receptor is mediated by the BinB protein and structures for both the receptor and BinB protein have been elucidated. By utilising computational modelling, mutagenesis and X-ray crystallography techniques, we aim to elucidate the toxin-receptor complex structure and characterise toxin-receptor interactions. Our early results support the hypothesis that toxin-receptor binding occurs via the N-terminal region of the BinB protein.

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Using Solid-State NMR to Investigate S100A9's Influence in Alpha-Synuclein Polymorphism and Parkinson's Disease.

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One of the hallmarks of neurodegenerative diseases is the formation of insoluble deposits rich in fibrillar species composed of proteins characteristic of that disease. Recently however it's been shown that elevated levels of the amyloidogenic protein S100A9 can enhance the rates at which other amyloidogenic proteins such as amyloid-beta peptide or alpha-synuclein are deposited; and influence their appearance. Our current studies have sought to characterise the structure of S100A9 amyloid fibrils and determine how its presence may influence the types of amyloid-beta and alpha-synuclein polymorphs formed. We have undertaken a series of solid-state NMR investigations which are beginning to resolve the basic architecture of the S100A9 fibrils and highlight features which may impact on their interactions with alpha-synuclein and amyloid-beta peptide. Building on these studies a range of alpha-synuclein polymorphs have been characterised as a prelude to understanding how S100A9 may influence the structures and toxicity of the species formed.

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