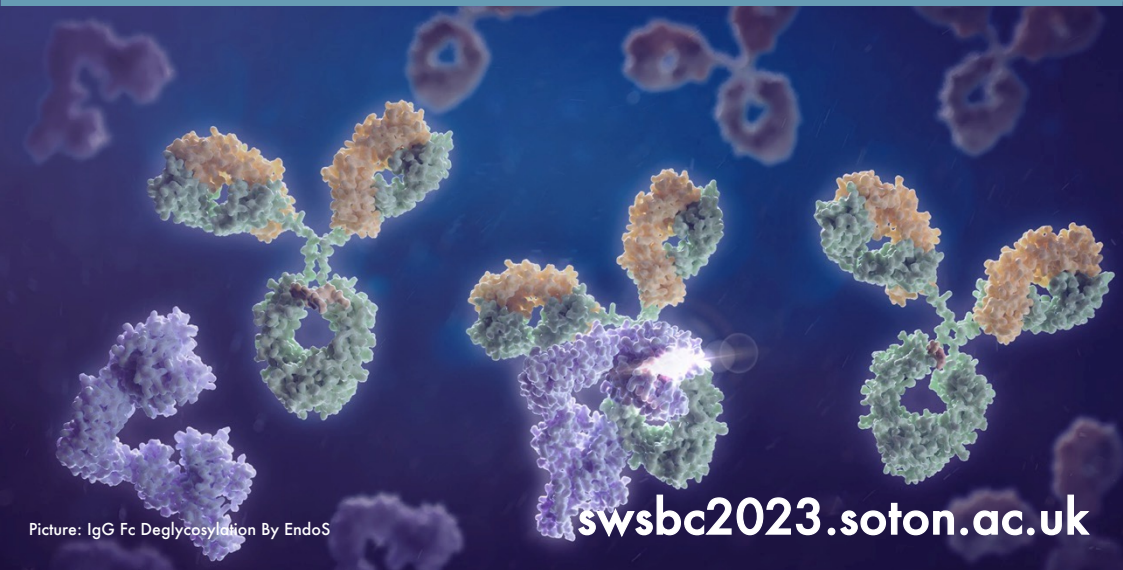
SOUTH WEST STRUCTURAL BIOLOGY CONSORTIUM 2023

Highfield Campus, Southampton

10th & 11th July 2023

CONFERENCE PROGRAMME

University of Southampton



Picture: IgG Fc Deglycosylation By EndoS

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Welcome to the 2023 South West Structural Biology Consortium meeting hosted at the University of Southampton. We have listed important event information here. If you have any further queries, please do not hesitate to contact any of the organisers for assistance.

Organising committee

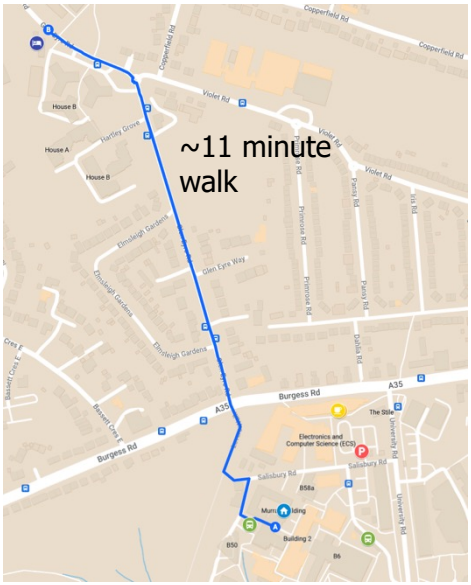
Abigail Sudol, Charlotte Cordery, Jack Stubbs, Martin Malý, Chris Holes

Registration

Please go to Building 58 entrance

Accommodation info for those in halls

If you have booked halls accommodation you will be staying in Chamberlain Halls, SO16 3UD. It takes around 11 minutes to walk



Lunches and refreshments

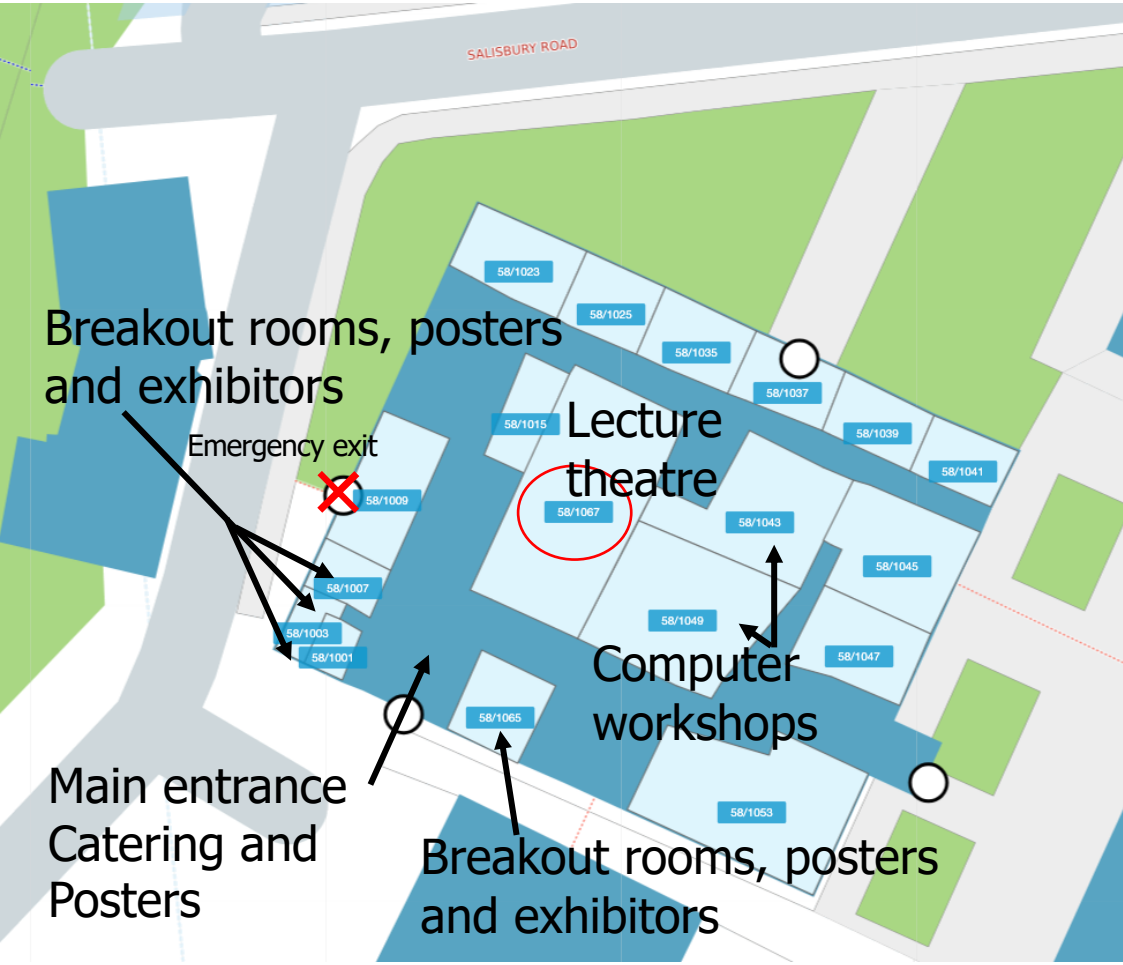
Lunches and coffee breaks will be held in the B58 entrance

Conference dinner and transport

- The conference dinner will be held in the Dancing Man Brewery, SO14 2AR
- The **U1C** bus departs from Highfield Interchange at 18:10, 18:22, 18:34, 18:49 and arrive at the Telephone House stop (what3words is ///letter.like.settle). From there, it is a ~3 minute walk.
- If you have any queries or wish to get a taxi instead please either speak to one of the organising committee or there are taxi options that you can book yourself;
  - Uber
  - Radio Taxis (023 80 666 666)
  - West Quay cars (023 80 999 999)

Speaker abstracts
swsbc2023.soton.ac.uk/talks

Conference Location



Poster abstracts
swsbc2023.soton.ac.uk/posters

SWSBC 2023 Programme

Monday 10th July 2023

9:30-10:00	**Registration & Coffee – Murray building, B58**		
9:55-10:00	**Welcome words**		
10:00 - 10:30	**Opening presentation**	Max Crispin	South-ampton
10:30 - 12:00	**Session 1**		
	Cross-recognition of bacterial and preproinsulin peptides by HLA A*24:02-restricted T-cell receptors in type 1 diabetes	Aaron Wall	Cardiff
	Structural Investigations into Antibiotic Hydrolysis by the KPC-2 β-Lactamase	Catherine Tooke	Bristol
	Allosteric activation and inhibition of glycogen phosphorylase share common transient structural features	Dylan Ivory	Exeter
	Biophysics to Bioreactors: Using Small Scale Experiments to Predict Enzyme Activity on a Large Scale	Matilda Clark	Ports-mouth
12:00 - 13:45	**Lunch and posters**		
13:45 - 15:50	**Session 2**		
	In situ structure of a dimeric hibernating ribosome from a eukaryotic intracellular pathogen	Mathew Mclaren	Exeter
	Cryo-EM structure of the R2TP chaperone and its role in macromolecular assembly	Mohinder Pal	Sussex

SWSBC 2023 Programme

Monday 10th July 2023

14:00-15:45	Cryo-ET for investigating the role of respiratory chain organisation in health and disease	Emma Buzzard	Exeter
	CryoEM structure and Alphafold modelling of slipper limpet hemocyanin	Mark Young	Cardiff
	Exploring the catalytic core of yeast DNA-polymerase ε	Anil Kumar Jamithireddy	Exeter
	New and Exciting Updates from Molecular Dimensions	Hassan Akram-Sheikh	Molecular Dimensions
15.45-16.30	**Coffee and posters**		
16:30-18:15	**Session 3**		
	Time-resolved Serial Crystallography at I24	Sam Horrell	DLS
	The Diamond Standard: Exploring Unique Beamline Resources at Diamond Light Source	Christian Orr	DLS
	Sample delivery techniques to perform time-resolved serial crystallography at the XFEL Hub of Diamond Light Source	Anastasya Shilova	DLS
	The High-energy Electron Xtallography Instrument: a tool for macromolecular structure determination	Pedro Nunes	DLS
	CCP4 overview	Ivo Tews	Southampton
19:00	**Conference dinner** – Dancing Man Brewery		

DLS = Diamond Light Source

SWSBC 2023 Programme

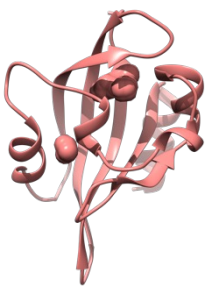
Tuesday 11th July 2023

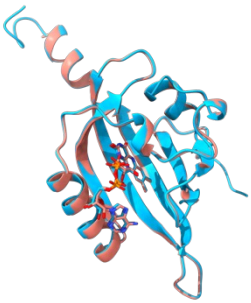
9:30-11.00	**Session 4**		
	High Resolution Structure of HAdV-D10 Determined by Cryo-Electron Microscopy	Rosie Mundy	Cardiff
	Cryo-electron microscopy of the f1 filamentous phage reveals insights into viral infection and assembly	Becky Conners	Exeter
	Understanding the breakdown of enmetazobactam by the extended spectrum beta-lactamase GES-1	Michael Beer	Bristol
	Serendipity in crystallography	Kyle Gregory	Bath
11.00-11.15	**Coffee and posters**		
11.15-12.15	**Computer workshops – PAIREF (CCP4)** with Martin Malý **or CCP-EM** with Tom Burnley		
12:15-13:00	**Lunch and posters**		
13:00-14:30	**Session 5**		
	Sample preparation for routine and advanced structural biology, including serial data collection, microED, and cryoEM	Patrick Shaw-Stewart	Douglas Instruments
	Enzymatic Degradation of Crystalline Polyethylene Terephthalate (PET): Discovery, Engineering, and Structural Characterization of Novel Plastic-Degrading Enzymes	Brooke Wain	Portsmouth

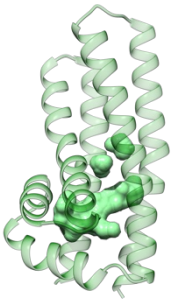
SWSBC 2023 Programme

Tuesday 11th July 2023

	Understanding the HLA class-II peptide presentation platform and its role in anti-SARS-CoV-2 T cell memory	Bruce MacLachlan	Cardiff
	Orthogonal Spectrometry - Systems for analysis of protein stability and structural changes	Jenna Roberts	Applied Photophysics
14:30 - 15:15	**Coffee and posters**		
15:15 - 16:30	**Electron Diffraction**		
	Electron Diffraction at the National Crystallography Centre	Simon Coles	Southampton
	Software development for ED in macromolecular crystallography	Tarik Devron	STFC
	Advances in the use of microED and serial crystallography methods, an enzyme user case	Nicholas Harmer	Exeter
16:30 - 17:15	**Closing lecture, prizes and awards**	Phil Williamson	Southampton
17:15	**Departure**		







Speaker abstracts

Aaron Wall – University of Cardiff

Introduction: Bacillus thuringiensis (Bt) is a bacterium that produces a broad range of insecticidal proteins, many of which have resolved structures, including Tpp80Aa1,(1) Cry11Aa1,(2) and Mpp75Aa1.(3) Of particular interest are the vegetative insecticidal proteins (Vips) which exhibit insecticidal properties against Lepidoptera. Members of Lepidoptera, particularly from the genus Spodoptera, are agricultural pests requiring decisive measures to manage the significant economic risks they pose.(4,5) Currently, Vip3Aa16 and Vip3Bc1 have protoxin and activated structures available.(6,7) However, the activated forms lack resolved structures for the extended Domain 1 assembly. Additionally, the exact mechanism through which Vips exert their toxic effect is poorly understood. This project aims to elucidate these mechanisms. Methods: ColabFold was employed to produce predicted structures of the extended Domain 1 assembly, which were integrated into the existing crystal/Cryo-EM structures to provide complete Vip models. GROMACS was employed to conduct molecular dynamics simulations to explore the interactions of Vips with membranes and how Vips lead to cell/insect death. Membrane:protein systems were generated using the CHARMM-GUI interface. Results: Vips demonstrate an extended (~200 nm) coiled-coil composed of 4 α-helices, with Vip3A variants exhibiting an unstructured region near the N-terminus which could convey flexibility or promote rotational movements of the terminal residues. The putative transmembrane region displays a hydrophilic exterior and hydrophobic interior. Vip3Bc1 also displays an unstructured region. Molecular dynamics simulations demonstrate the ability of water to move through the extended Domain 1 assembly, suggesting cell death is mediated via pore formation and cell leakage. Conclusion: Evidence suggests Vips exert toxicity via pore formation mediated by a 200 nm needle-like structure which embeds into target membranes.

1. Best HL, Williamson LJ, Lipka-Lloyd M, Waller-Evans H, Lloyd-Evans E, Rizkallah PJ, et al. The Crystal Structure of Bacillus thuringiensis Tpp80Aa1 and Its Interaction with Galactose-Containing Glycolipids. Toxins (Basel). 2022 Dec 1;14(12):863.
2. Tetreau G, Sawaya MR, De Zitter E, Andreeva EA, Banneville AS, Schibrowsky NA, et al. De novo determination of mosquitocidal Cry11Aa and Cry11Ba structures from naturally-occurring nanocrystals. Nature Communications 2022 13:1. 2022 Jul 28;13(1):1–18.
3. Kouadio JL, Duff S, Aikins M, Zheng M, Rydel T, Chen D, et al. Structural and functional characterization of Mpp75Aa1.1, a putative beta-pore forming protein from Brevibacillus laterosporus active against the western corn rootworm. PLoS One. 2021 Oct 1;16(10):e0258052.
4. Lázaro-Berenguer M, Quan Y, Hernández-Martínez P, Ferré J. In vivo competition assays between Vip3 proteins confirm the occurrence of shared binding sites in Spodoptera littoralis. Nature Scientific Reports. 2022;12(4578).
5. Goergen G, Kumar PL, Sankung SB, Togola A, Tamò M. First Report of Outbreaks of the Fall Armyworm Spodoptera frugiperda (Lepidoptera, Noctuidae), a New Alien Invasive Pest in West and Central Africa. PLoS One. 2016;11(10).
6. Núñez-Ramírez R, Huesa J, Bel Y, Ferré J, Casino P, Arias-Palomo E. Molecular architecture and activation of the insecticidal protein Vip3Aa from Bacillus thuringiensis. Nat Commun. 2020 Dec 1;11(1).
7. Byrne MJ, Iadanza MG, Perez MA, Maskell DP, George RM, Hesketh EL, et al. Cryo-EM structures of an insecticidal Bt toxin reveal its mechanism of action on the membrane. Nat Commun. 2021 Dec 1;12(1:2791).

Speaker abstracts

Catherine Tooke – University of Bristol

Bacterial antimicrobial resistance (AMR) is a global health threat, associated with 4.95 million deaths in 2019. Within Gram-negative pathogens, β-lactamase production is the major resistance mechanism to β-lactams, the most prescribed antibiotic class. KPC-2 (Klebsiella pneumoniae carbapenemase-2) is a globally disseminated serine-β-lactamase (SBL) responsible for clinical antibiotic failure. SBLs bind and break down β-lactam antibiotics in an acylation-deacylation mechanism via a covalent acyl-enzyme intermediate. We implement a multidisciplinary approach of enzyme kinetics, X-ray crystallography and simulation to investigate the versatile, broad-spectrum activity of KPC-2. We present high-resolution (1.25-1.4 Å) crystal structures of KPC-2 with representative penicillins, cephalosporins, and carba(penems). Mobility of an active-site loop was found to negatively correlate with antibiotic turnover rates (kcat), highlighting this region's role in positioning catalytic residues for efficient hydrolysis of different β-lactams. Further, using our crystallographic data as accurate starting structures, we explore loop mobility with molecular dynamics and the significance of carbapenem tautomerization with hybrid quantum mechanics/molecular mechanics (QM/MM) simulations. These simulations identify loop rearrangements align with both crystallographic data and kinetic activity. QM/MM calculations reveal tautomer-specific carbapenem deacylation (the Δ1-(2R) isomer has a 7 kcal/mol higher barrier than the Δ2 tautomer) alongside exclusive electrostatic interactions and stabilization of transition states to promote Δ2 acyl-enzyme deacylation. Taken together, our data show loop plasticity confers broad-spectrum KPC-2 activity, while carbapenemase activity stems from the ability for KPC-2 to accommodate both isomers, yet efficiently deacylate the Δ2-enamine acyl-enzyme. Understanding KPC-2 activity is essential for inhibitor discovery and development to counter β-lactam resistance.

Speaker abstracts

Dylan Ivory – University of Exeter

Determining the specific structurally dynamic changes that underpin protein functional switching remains a major challenge. High resolution models of protein structure need to be complemented with the determination of how these structures change as proteins function. Glycogen phosphorylase (GlyP), which catalyzes the release of glucose from glycogen, is regulated both by phosphorylation and a myriad of allosteric effectors. The exploitation of GlyP as a drug target for type II diabetes and metastatic cancer is hindered by a lack of understanding of the dynamic structural changes that mediate its complex regulation. Using time-resolved non-equilibrium millisecond hydrogen/deuterium mass spectrometry (HDX-MS), we have precisely located dynamic structural changes of GlyP during allosteric regulation. This includes transient local dynamics common to both allosteric activation by AMP and inhibition by caffeine, which bind at separate sites. This approach has broad applications to determine structural kinetic mechanisms underpinning protein function and regulation.

Matilda Clark – University of Portsmouth

Over the last 50 years plastics have become a major source of pollution in a wide variety of habitats due to a lack of end of life solutions for these materials. Recently a number of enzymes have been discovered and engineered to degrade these plastics. However, many of these enzymes have only been tested on a small scale that does not reflect the much larger scale that would be required to tackle the scale of the problem. The upscaling of these enzymes to an industrial scale is the next step in using biorecycling to create a circular plastic economy. In order to scale up these enzymes the conditions must be optimised but this requires large amounts of enzyme which is costly and time consuming to produce. Here, we have used a small scale biophysical approach to understand how the enzymes act at different temperatures and how their structures can unfold leading to a loss of activity. Differential scanning calorimetry (DSC) and isothermal calorimetry (ITC) were used to understand the thermal stability of the enzymes, before they were scaled up into bioreactors to simulate industrial conditions. We have used these techniques to calculate how long the enzymes will be active at different temperatures in order to make an informed decision about what temperature to use for the large scale reaction. We hope to use this technique to scale up other enzymes in future to save time and enzyme and speed up the process of getting these enzymes into industry.

Speaker abstracts

Mathew Mclaren - University of Exeter

Translational control is an essential process for the cell to adapt to varying physiological or environmental conditions. To survive adverse conditions such as low nutrient levels, translation can be shut down almost entirely by inhibiting ribosomal function[1]. Here we investigated eukaryotic hibernating ribosomes from the microsporidian parasite Spraguea lophii in situ by a combination of electron cryo-tomography (cryoET) and single particle cryoEM. Microsporidia begin their life cycle as dormant spores that need to enter and exploit the energy metabolism of a host cell in order to proliferate. They achieve entry into host cells via a preformed, tightly coiled 'polar tube' (PT) that is rapidly expelled from the dormant spore on germination[2]. The PT then penetrates the host cell membrane, and the spore content (sporoplasm) is rapidly transported down the tube into the host cell. We show that microsporidian spores contain hibernating ribosomes that are locked in the 100 S dimeric state, which is formed by a unique dimerisation mechanism involving the beak region[3]. This provides structural evidence for dimerisation acting as a mechanism for ribosomal hibernation in eukaryotes. The ribosomes within the dimer are fully assembled, suggesting that they are ready to be activated once the host cell is invaded.
[1] Trosch, R. & Willmund, F., Biol Chem 400, 879-893 (2019).
[2] Xu, Y. J. & Weiss, L. M., Int J Parasitol 35, 941-953 (2005).
[3] McLaren, M., Gil-Diez, P., Isupov, M. N., Conners, R., Gambelli, L., Gold, V., Walter, A., Connell, S. R., Williams, B. & Daum, B. bioRxiv: 2022.04.29.490036 (2022), preprint

Mohinder Pal - University of Sussex

R2TP is a multi-protein complex that consists of RUVBL1 (RuVB-like helicase 1), RUVBL2 (RuVB-like helicase 2), Tah1p/RPAP3 (TPR-containing protein Associated with Hsp90) and PIH1D1 (Protein Interacting with Hsp90). R2TP is a specialised Hsp90 co-chaperone essential for the assembly and maturation of multi-subunit complexes, including the small nucleolar ribonucleoproteins, RNA polymerase II and mTOR of phosphatidylinositol-3-kinase-like kinases (PIKK). This work aims to determine the role of R2TP in the assembly and maturation of mTOR kinase using structural biology techniques. In this study, we have determined the structures of the yeast and human R2TP complex using X-ray crystallography and single particle cryo-electron microscopy (cryo-EM) together with biochemistry data providing mechanistic insight into how R2TP functions. After determining the crystal structures of the smaller sub-complexes, our cryo-EM data has revealed the architecture of the full-length R2TP complex (Mr ~500kDa). Additionally, we determined cryo-EM structures showing how R2TP binds TELO2-TTI1-TTI2 (TTT) adaptors in forming the R2TP-TTT complex (Mr ~800kDa), which acts as a bridge connecting mTOR to HSP90. This structural data, complemented with our biochemistry work, also show how R2TP is switched on by its Tah1p/RPAP3-PIH1D1 components and switched off by TTT adaptors to recruit mTOR client protein to the HSP90 chaperone. Together, our work has become the basis for understanding the role of HSP90 and R2TP-TTT chaperones in assembling and regulating multi-protein complexes.

Speaker abstracts

Emma Buzzard - University of Exeter

Mitochondrial respiratory chain proteins can be organised into supercomplexes, such as ATP synthase dimers and respirasomes (comprised of complexes I, III2 and IV) [1,2]. Dissociation of both ATP synthase dimers and respirasomes into monomeric complexes is associated with ageing, and the latter with age-related disorders such as Parkinson's disease [3]. Unfortunately, it has thus far been impossible to exploit this known relationship for drug development due to lack of knowledge regarding the biological role of respiratory supercomplexes [4]. We have used electron cryo-tomography supported by biochemistry to study the effect of reducing respirasome stability on mitochondrial morphology and respiratory chain organisation in the model organism C. elegans. RNAi technology was used to reduce respirasome stability by knocking down an accessory subunit of CI, called NDUFA11, required for interaction with CIII. Mitochondria were then isolated from NDUFA11 knockdown and empty vector control animals for analysis. Mitochondrial morphology was studied through segmentation and quantification of tomograms of whole mitochondria, and respiratory chain organisation by sub-tomogram averaging of densities corresponding to the ATP synthase and complex I. We found that reduced respirasome stability was associated with aberrant crista membrane morphology, impaired respiration and severely inhibited reproduction [5], and are currently exploring the effect on supercomplex abundance and stoichiometry. This study also reveals a novel ATP synthase architecture unique to C. elegans. Further investigation revealed a relationship between ATP synthase architecture and crista shape, when we compared sub-tomogram averages and segmentations with those from other organisms [6]. We speculate that a range of dimer angles may have evolved to alter crista diameter and thus suit bespoke energetic needs.

[1] I Arnold et al., EMBO 17 (1998), p. 7170-7178. doi: 10.1093/emboj/17.24.7170
[2] H Shagger and K Pfeiffer, EMBO J 19 (2000), p. 1777-1783. doi:10.1093/emboj/19.8.1777
[3] CR Arthur et al., Mol. Neurodegener. [Online] 4, 37 (2009), https://molecularneurodegeneration.biomedcentral.com/articles/10.1186/1750-1326-4-37 (accessed 02/03/23)
[4] M Blaza et al., Cell Metab. 25 (2017), p. 765-776. doi:10.1016/j.cmet.2017.03.009
[5] A Knapp-Wilson et al., J. Cell Sci. 134 (2021), p. jcs258399. doi:10.1242/jcs.258399
[6] E Buzzard et al., BioRxiv (2023). doi: 10.1101/2023.02.02.526626

Speaker abstracts

Mark Young - University of Cardiff

Hemocyanins are oxygen transport proteins present in the blood of arthropods and molluscs. Each 350 kDa subunit contains up to 8 oxygen-binding functional units, and in molluscs, hemocyanins are assembled in decamer building blocks formed of 5 dimer plates, routinely forming didecamer or higher-order assemblies with d5 or c5 symmetry. Their large size and high degree of symmetry makes hemocyanins ideal targets for 3D-structure determination by cryoEM. Slipper limpets (Crepidula fornicata) are an invasive species of molluscs which destroy coastal ecosystems in the UK. Their hemocyanins are similar to those of keyhole limpet but they have not previously been characterised. We isolated and purified slipper limpet hemocyanin (SLH), which displayed substantial sample heterogeneity, forming both 20-subunit didecamers and a range of higher-order oligomers. Using cryoEM, we determined the 3D-structures of the didecamer (20-mer) and tridecamer (30-mer) forms of SLH at 7.0 and 4.7 Å resolution respectively. We showed that two decamers assembled in a 'tail-tail' configuration, forming a partially capped cylinder, with an additional decamer adding on in 'head-tail' configuration to make the tridecamer. We suggested that higher-order structures could assemble by successive addition of decamers in head-tail configuration. The assembly of the full-length sequence for SLH from publicly available transcriptome data enabled us to make a molecular model of SLH using Alphafold, which showed an excellent fit with the cryoEM density. This showed an overall arrangement of functional units within the dimer plate where the final two functional units of the subunit (FU-g and FU-h) form the partial cap at one end of the decamer and permitted analysis of the subunit interfaces governing the assembly of tail-tail and head-tail decamer interactions as well as potential sites for N-glycosylation. Our work has contributed to the understanding of higher-order oligomer formation in molluscan hemocyanins and has demonstrated the utility of Alphafold for building accurate structural models of large oligomeric proteins.

Speaker abstracts

Anil Kumar Jamithireddy - University of Exeter

The eukaryotic major leading-strand DNA polymerase, DNA polymerase ε, has a catalytic subunit and three non-catalytic subunits. The N-terminal domain of the catalytic subunit (Pol2CORE) harbours both polymerase and exonuclease activities. There are three cysteine rich motifs in the catalytic subunit, one in Pol2CORE and two in the C-terminal domain. The former constitutes an Fe-S cluster. The catalytic core has an extra domain (P-domain), unlike other polymerases, that allows Pol ε to encircle the nascent double stranded DNA and thereby increase processivity. The Fe-S cluster is located at the base of the P-domain joining it with "finger and palm domains" Mutational removal of this Fe-S cluster severely affects the polymerase activity but not exonuclease activity. This work is aimed at understanding the catalytic core of DNA polymerase ε.

Hogg, M., et. al., Structural basis for processive DNA synthesis by yeast DNA polymerase ε. Nat. Struct. Mol. Biol. 2014, 21, 49–55.
Ganai, R.A., et. al., Switching between polymerase and exonuclease sites in DNA polymerase ε. Nucleic Acids Res. 2015, 43, 932–942.
Ter Beek, J. et. al., Structural Evidence for an Essential Fe-S Cluster in the Catalytic Core Domain of DNA Polymerase. Nucleic Acids Res. 2019, 47, 5712−5722.

Hassan Akram-Sheikh – Molecular Dimensions

New and Exciting Updates from Molecular Dimensions

Molecular Dimensions

Speaker abstracts

Sam Horrell – Diamond Light Source

I24 is a microfocus macromolecular crystallography beamline capable of performing standard cryogenic rotation data collection and more complex room temperature time-resolved serial crystallography experiments with a variety of pump-probe methods. The microfocus beam provides up to 8x10^12 photons per second at 12.4 keV, which can be defocused to a 50-micron beam with the same jnumber of photons per second for use with larger samples. I24 has recently commissioned a new CdTe Eiger detector to facilitate high energy data collection (>20 keV), which reduces background and increases mean diffracted intensity per unit dose with no downside to data collection [1]. High energy data collection is particularly beneficial when using micro crystals (<10 µm), as samples benefit from photoelectron escape, reducing the onset of radiation damage [2]. Users can switch between detectors and wavelengths seamlessly during their beamtime to perform their diffraction experiments on I24.

Room temperature serial crystallography plays a major role in the future development of I24, with more and more beamtime on I24 being dedicated to the technique. Serial experiments can begin with a little as one drop of microcrystals between two thin films of mylar, with the end goal typically being a molecular movie of protein dynamics working in real time [3]. I24 has a variety of sample delivery methods for serial crystallography (fixed targets [4], viscous jets, thin films) and pumps for time-resolved experiments (femtosecond laser, rapid mixing, dose series) to facilitate your science, and I24 staff are happy to advise users how best to move from single crystal rotation data collection to time-resolved serial experiments on I24. This talk will give a detailed overview of what can be done at I24 and the exiting science coming from the I24 user community.

[1] Storm SLS, Axford D, Owen RL. Experimental evidence for the benefits of higher X-ray energies for macromolecular crystallography. IUCrJ. 2021 Sep 9;8(Pt 6):896-904.

[2] Nave C, Hill MA. Will reduced radiation damage occur with very small crystals? J Synchrotron Radiat. 2005 May;12(Pt 3):299-303.

[3] Baxter JM, Hutchison CDM, Maghlaoui K, Cordon-Preciado V, Morgan RML, Aller P, Butryn A, Axford D, Horrell S, Owen RL, Storm SLS, Devenish NE, van Thor JJ. Observation of Cation Chromophore Photoisomerization of a Fluorescent Protein Using Millisecond Synchrotron Serial Crystallography and Infrared Vibrational and Visible Spectroscopy. J Phys Chem B. 2022 Nov 17;126(45):9288-9296.

[4] Horrell S, Axford D, Devenish NE, Ebrahim A, Hough MA, Sherrell DA, Storm SLS, Tews I, Worrall JAR, Owen RL. Fixed Target Serial Data Collection at Diamond Light Source. J Vis Exp. 2021 Feb 26;(168).

Speaker abstracts

Christian Orr – Diamond Light Source

Diamond Light Source is a globally recognised facility, offering a plethora of cutting-edge beamlines and groups engineered for the detailed study and understanding of complex molecular structures. This presentation will explore the capabilities of some of these resources.

I23, the long-wavelength macromolecular crystallography beamline, is critical for crystallographic phase problem solution and identifying biologically important lighter atoms such as sulfur, phosphorous, chlorine, potassium, and calcium. The precise location of these can also be ascertained. Ongoing research aims to detect even lighter elements.

The VMXm beamline is a micro/nanofocus macromolecular crystallography beamline. It collects data from crystals as small as 1 µm and aligns these tiny protein crystals *in-vacuo*for high-precision, low background X-ray diffraction data.

HeXI, a project employing MeV electrons for macromolecular structure determination, is still in development. Building on VMXm's expertise, it targets small molecules and macromolecular crystal structures of pharmaceutical relevance.

I24, a microfocus macromolecular crystallography beamline, performs cryogenic rotation data collection and room temperature time-resolved serial crystallography experiments. Recent enhancements allow high-energy data collection benefiting from increased diffraction intensity, reduced background scattering, and photoelectron escape.

The VMXi (Versatile Macromolecular Xtallography in-situ) beamline, fully automated, facilitates rapid and routine determination of high-quality room temperature structures. It provides essential feedback on diffraction, crystal quality, and unit-cell parameters, even for micro-crystals.

The XFEL Hub at Diamond supports time-resolved serial crystallography experiments at synchrotrons and XFELs. It accommodates different sample presentation methods, such as fixed target supports, high-viscosity extrusion injecton, and on-demand acoustic droplet ejection, and offers extensive training and expertise to academic and industry users.

In summary, Diamond Light Source hosts a range of advanced beamlines and facilities that contribute to the advancement of structural biology research. These enhance the fragment-based drug discovery capabilities of XChem and automated data collection services of beamlines I03, I04, and I04-1.

Speaker abstracts

Anastasya Shilova – Diamond Light Source

Serial crystallography experiments have been continuously performed and developed at X-ray free-electron lasers (XFELs) and synchrotron beamlines over the last decade. In this method, a complete diffraction dataset is collected from a large number of microcrystals serially delivered and exposed to an X-ray beam in random orientations at room-temperature (RT). Short X-ray exposure minimizes radiation damage, and data collection at RT provides more biologically reliable data on structural dynamics in proteins compared to the data collection at cryo-temperatures. It also allows to perform time-resolved studies and to create dynamic molecular movies.

The XFEL Hub at Diamond is developing several techniques to enable time-resolved serial crystallography experiments at synchrotrons and XFELs. This development relies on a growing variety of sample presentation methods each with unique requirements, including different fixed target supports (1), injection methods using high-viscosity extrusion injectors, and on-demand acoustic droplet ejection or piezoelectric injection of nanolitre to picolitre (2,3) droplets either into the X-ray beam or onto a tape drive. Some of the drop-on-demand methods are also compatible with complementary X-ray emission spectroscopy (XES) measurements from the same sample and X-ray pulse. This allows to follow the oxidation/spin states changes of biologically relevant metals during catalysis. During the first synchrotron SSX +XES experiments we were able to identify copper and iron state from different protein microcrystals. The XFEL-hub goals include promoting the efficient use of samples and accessibility of serial crystallography experiments for academic and industry users, in part, by transferring/ adapting appropriate technology between different facilities.

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Speaker abstracts

Pedro Nunes – Diamond Light Source

Funded by the Wellcome Trust “Electrifying Life Sciences” grant, the High-energy Electron Xtallography (HeXI) project aims to build an electron diffraction optimized instrument to investigate the use of Mega-electron-volt (MeV) electrons for macromolecular structure determination. This instrument will leverage the increased penetration of MeV electrons to bridge the crystal size gap between electrons and X-ray scattering and determine structures from crystals with sizes ranging between 300 nm and 3 µm (see Figure 1).

The HeXI instrument will enable rapid high-resolution structure determination from crystal sizes which are inaccessible to both conventional electron diffraction in transmission electron microscopes (TEM) which requires sample sizes below 300 nm, and X-ray scattering methods which need crystals over 1 µm in size. This novel instrument will marry the unique sensitivity of electrons to structural information with the performance and fidelity of goniometry developed at Diamond for macromolecular crystallography beamlines. Users will be able to determine structures inaccessible to X-rays and generate additional structural information as the Coulomb potential maps obtained by electron diffraction provide complementary information to electron density maps. For example, the intrinsic sensitivity of electron diffraction to hydrogen atoms has been used to identify extensive hydrogen-bond networks in small peptides and proteins [1]. Moreover, electron diffraction Coulomb potential maps reveal the charge states of atoms which are particularly relevant in ligand binding studies and drug development [2].

The HeXI instrument at Diamond will offer data collection under three different modalities:

•Three-dimensional electron diffraction (3DED) of small molecules - Enables the structures of small molecules to be determined from synthesis products directly, without further purification or crystallization, thus offering users a vial-to-structure workflow [3].
•Cryo-3DED of macromolecules – Enables structure determination from frozen-hydrated protein crystals with sizes too small for MX beamlines and too large for 3DED on TEMs [4].
•SerialED – Enables time-resolved (ms to µs) structure determination of macromolecules [5].

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Speaker abstracts

Rosie Mundy - University of Cardiff

Human adenoviruses have garnered interest as therapeutic vectors. Vectors using human adenovirus type 5 (HAdV-C5) face challenges due to high levels of pre-existing immunity, hampering clinical efficacy. Species D adenoviruses have lower seroprevalence within the population and are a potential alternative platform to overcome this problem. We developed adenovirus type 10 (HAdV-D10), isolated from patients with keratoconjunctivitis, as a low seroprevalence vector platform for vaccine and oncology applications. Structural insights have proved critical in detailing vector: host interactions that may affect a vector's clinical use. Advances in cryo-electron microscopy (cryo-EM) have led to the technique being routinely used for detailing molecular structures at near atomic resolution. Here, we provide preliminary structural information for the HAdV-D10 capsid using negative stain EM and cryo-EM. We previously developed an HAdV-D10 vector which is targeted to αvβ6 integrin via an insertion of the A20 peptide from Foot-and-Mouth Disease Virus into the fiber-knob protein. The structure was solved via cryo-EM using RELION to process a high resolution data set. Conservation of the traditional adenovirus capsid structure was observed with hexon proteins making up the icosahedron facets, a penton base at each vertex and the fiber protein extending away from the capsid. The cryo-EM density map was of sufficient quality to provide experimental data for typically flexible regions of the hexon, such as the hypervariable loops, allowing atomic model building. There is also density available for residues in the minor proteins in the capsid, with the exception of minor protein VI, and symmetry mismatch techniques are being used to investigate the shaft of the fiber protein. The map is at 3.3Å by gold standard FSC calculations. This project assessed the first data set available for the HAdV-D10 capsid, providing a high resolution reconstruction. Analysis of this map will allow future investigation of potential interactions with host proteins, which may induce adverse events, such as the recent investigations into instances of thrombosis with thrombocytopenia syndrome (TTS) following vaccination with adenoviral SARS-CoV-2 vaccines.

Speaker abstracts

Becky Conners - University of Exeter

Phages are viruses that infect bacteria. They dominate every ecosystem on our planet and are exploited as tools in molecular biology and biotechnology. The Ff (f1, fd or M13) phages represent a widely distributed group of filamentous viruses(1). The complete structure of the phage capsid and consequently their mechanisms of infection and assembly remain largely mysterious. We use cryo-electron microscopy and a highly efficient system for production of short virus nanorods to determine a structure of a filamentous virus including the tips. We show that structure combined with mutagenesis can identify phage domains that are important in bacterial attack and for release of new progeny, allowing new models to be proposed for the phage lifecycle (2).

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Speaker abstracts

Michael Beer – University of Bristol

β-lactamase mediated resistance is the primary form of β-lactam antibiotic resistance in Gram-negative bacteria1. Enmetazobactam is a penicillanic acid sulfone (PAS) β-lactamase inhibitor that differs from the clinically used tazobactam by an additional methyl group, which enhances bacterial uptake2. Past work has shown that enmetazobactam and tazobactam can inhibit an extended spectrum β-lactamase (ESBL) by forming a lysinoalanine cross-link within the active site3. However, this cross-link does not form in class A carbapenem-hydrolysing β-lactamases, such as KPC-2. The GES family of enzymes contains 57 variants that range from ESBLs to carbapenemases, although all contain an active site disulfide bridge, more commonly associated with carbapenemases. We present here multiple sub-2 Å resolution crystal structures of the globally disseminated ESBL GES-1 in complex with enmetazobactam, at exposure times ranging from 1 minute to 2.5 hours, that show the presence of both covalently and non-covalently bound products. Our structures identify either the imine or trans-enamine as covalently bound to the catalytically active Ser70 at both 1 minute and 2.5 hour time points. We further identify accumulation of a non-covalent product, 3,3-dihydroxypropanoic acid, that is not present after 1 minute, but is resolved after 2.5 hours exposure to enmetazobactam. These data support conclusions that the covalently attached imine/trans-enamine can further break down to non-covalent products. In contrast to previous findings for other ESBLs, mass spectrometry and crystallographic evidence provide no evidence for lysinoalanine cross link formation, and indicate that GES-1 can return to the uncomplexed state 24 hours after incubation. These are properties previously associated with carbapenem-hydrolysing class A β-lactamases, but not with ESBLs. Taken together, this work further establishes the basis for enmetazobactam inhibition of diverse class A β-lactamases, and may guide the development of future related β-lactamase inhibitors

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Speaker abstracts

Kyle Gregory - University of Bath

Serendipity is defined as 'the occurrence and development of events by chance in a happy or beneficial way', with perhaps the most famous example being the discovery of penicillin by Alexander Fleming. In this talk we will explore some examples where we experienced serendipity in our own research, which involves the use of x-ray crystallography to study the therapeutically important molecules- Botulinum neurotoxin (BoNT) and Angiotensin I-converting enzyme (ACE). BoNT is currently used to treat a range of neuromuscular disorders through inhibition of acetylcholine release at the neuromuscular junction, and ACE inhibitors are widely used to treat high blood pressure. In two cases, we observed different crystal morphologies in the same drop which, to our surprise, revealed insights into the dynamics of both BoNT and ACE.

Brooke Wain - University of Portsmouth

The rapid accumulation of Polyethylene Terephthalate (PET) waste in the environment has become a pressing global concern, necessitating the development of efficient recycling strategies. The enzymatic degradation of plastic offers a promising approach, however known PET degrading enzymes have an affinity for amorphous PET, which is a challenge due to the high crystalline PET content in most polymer materials. Therefore, the discovery and engineering of novel plastic degrading enzymes that are tolerant of crystalline PET is important and subsequently understanding their structural biology is crucial for optimising their efficiency and viability for industrial applications.

From a DNA screening, 23 candidate proteins have been identified, with 6 showing high expression yields and promising activity. These enzymes will be further characterised and engineered to increase their activity, thermostability and durability. Although the structures of the candidate enzymes have been predicted using the DeepMind developed AI system AlphaFold, the structures of those displaying significant activity against PET will be determined using X-ray crystallography.

Speaker abstracts

Patrick Shaw Stewart – Douglas Instruments

Douglas Instruments
Success in protein crystallization

Serial data collection usually requires relatively small crystals that are well-ordered. Microseeding is an effective way to generate such samples. During the ten years since the random microseed matrix-screening (rMMS) method was published, understanding of the theoretical advantages of the method has increased [2 - 4], and several practical variations of the method have emerged. Moreover seeding can be carried out in a microbatch-under-oil setup, which is easy to scale up, volume-wise, and allows easy interpretation of phase diagrams. By combining these techniques, control can be increased and sample quality for both routine and advanced data collection improved. Protein structure determination by cryoEM requires expensive equipment that has low throughput. It is therefore wasteful to examine samples that can be shown in advance to be aggregated, since such samples are unlikely to be suitable. We used a high-throughput screening approach with dynamic light scattering to explore 96 chemical conditions with as little as 10 µL of protein solution to identify conditions with reduced aggregation.

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Speaker abstracts

Bruce J MacLachlan - University of Cardiff

CD4+ T cells recognise a broad range of peptide epitopes of SARS-CoV-2 which contribute to immune memory and limit COVID-19 disease. The selection of which peptides are presented on HLA class-II molecules for interrogation by T cell receptors (TCRs) on CD4+ T cells is determined by the biochemical compatibility of peptide to the HLA class II heterodimer. We demonstrate the immunogenicity of SARS-CoV-2 peptides, in the context of the model HLA class-II allotype HLA-DR1, does not correlate with their binding affinity to the HLA heterodimer. Analysing six epitopes, some with very low binding affinity, we solve x-ray crystallographic structures of each bound to HLA-DR1. Further structural definitions revealed the precise molecular impact of viral variant mutations on epitope presentation. Omicron escaped ancestral SARS-CoV-2 immunity to two epitopes through two distinct mechanisms: i) through mutations to TCR-facing epitope positions and ii) through a mechanism whereby a single amino acid substitution caused a register shift within the HLA binding groove – completely altering the peptide-HLA structure. This HLA-II specific paradigm of immune escape highlights how CD4+ T cell memory is finely poised at the level of peptide-HLA-II presentation.

Speaker abstracts

Jemma Roberts – Applied Photophysics

Spectroscopic techniques to study protein stability and structure are well established. Circular Dichroism detects the difference in absorbance of left and right circularly polarised light in the presence of a chiral molecule. Chromophores from proteins that can be detected include peptide carbonyl bonds, aromatic residues, and disulphide bonds, allowing for comparison of protein secondary structure and individual amino acids. The Chirascan CD spectrometers from Applied Photophysics contribute to a deeper understanding of biomolecular characteristics, mechanisms, and interactions. Our system can be used to gain insight, detect changes in secondary and tertiary structures, and study folding and unfolding mechanisms during altered physiological parameters.

Differential Scanning Fluorimetry (DSF) is a valuable and widely used technique that monitors protein unfolding by detecting changes in fluorescence as temperature increases. DSF can be used to optimise protein buffer composition rapidly and effectively before downstream structural analysis or crystallography screening. A primary buffer screen of large global parameters (buffer system, pH, salt, concentration) can be used to optimise protein homogeneity, increase solubility and stability, prevent protein unfolding and aggregation, and minimise consumable costs. The stability of a protein in its initial buffer condition has been directly linked with crystal formation success in subsequent crystallography screens. The SUPR-DSFs 384 well plate format allows simple screening of 96 condition buffer screens, including all replicates, in one thermal ramp experiment, no proprietary consumables required. The conventional workflow for DSF uses extrinsic dyes that may influence the protein's thermal stability under investigation. This can affect the quality of your data by generating false positives or negatives during screening. The SUPR-DSF system from Protein Stable measures the full spectrum intrinsic fluorescence of proteins in a small volume whilst avoiding the use of additional dyes. SUPR-DSF reduces operator time and minimises the risk of errors in multi-step sample preparation while also bringing down the cost of consumables and sample consumption without compromising data quality.

AppliedPhotophysics

Speaker abstracts

Simon Coles - University of Southampton

Electron crystallography has held much promise for some time now and is about to be embraced by the chemical crystallography community. It has been possible to solve structures using TEM instruments for a number of years, but its very time consuming and not the principle purpose of the instrument. Dedicated electron diffractometers are just beginning to hit the market with hardware and software configurations that mean this technique will become much more accessible.
This talk will outline how the UK National Crystallography Service is currently setting up a national facility to determine structures by electron diffraction, based on two of the first instruments of this nature (being installed in the universities of Southampton and Warwick). It will then outline the kind of results that can currently be acquired and then comment on quality of these results and the sort of developments that need to occur in order to bring them in line with the standard of results the community is used to seeing with analagous X-ray methods.

Tarik Devron - STFC

Electron diffraction has gained popularity over the past few years as an alternative way to X-ray diffraction for molecular structure determination. This technique leverages the strength of the electron-atom Coulomb interaction enabling experiments to be carried out using nano crystals at low radiation dose. This is of great interest for biological samples and in particular macromolecules which are sensitive to radiation damage and can be hard to grow to large enough dimensions traditionally necessary for X-ray diffraction.
By adapting the X-ray density atomic form factors to electrostatic potential through the Mott-Bethe formula, it has been demonstrated that structures can be solved from electron diffraction experiments using the standard kinematic approximation. However, the predicted intensities and resulting Rfactors tend to be significantly worse than those obtained from X-ray experiments. It has in fact been established both theoretically and experimentally that the kinematic approximation is too crude and should be replaced by rigorous dynamical diffraction theory.
For small molecules, it has been shown that taking into account dynamical effects not only consistently improves Rfactors but also allows to resolve information such as hydrogen positions, absolute structure determination and local charges which are otherwise not typically accessible through standard X-ray diffraction experiments.
Although techniques and software targeted at small molecules have been well developed, both theoretical and computational work is still required to tackle macromolecular crystals. The presence of defects, solvent scattering and inelastic scattering significantly complicate the coherent dynamical diffraction picture while the very large unit cells and number of atoms in macromolecular crystals make it a computational challenge.

Speaker abstracts

Nicholas Harmer - University of Exeter

The eukaryotic major leading-strand DNA polymerase, DNA polymerase ε, has a catalytic subunit and three non-catalytic subunits. The N-terminal domain of the catalytic subunit (Pol2CORE) harbours both polymerase and exonuclease activities. There are three cysteine rich motifs in the catalytic subunit, one in Pol2CORE and two in the C-terminal domain. The former constitutes an Fe-S cluster. The catalytic core has an extra domain (P-domain), unlike other polymerases, that allows Pol ε to encircle the nascent double stranded DNA and thereby increase processivity. The Fe-S cluster is located at the base of the P-domain joining it with “finger and palm domains” Mutational removal of this Fe-S cluster severely affects the polymerase activity but not exonuclease activity. This work is aimed at understanding the catalytic core of DNA polymerase ε.

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Closing Lecture

Phillip Williamson – University of Southampton

The cellular milieux represents a complex matrix in which proteins are required to fold, and in the case of proteinopathies the miss-regulation of this process results in the formation of fibrillar aggregates such as those found in Alzheimer's and Parkinson's Disease. The onset and progression of these diseases is accompanied by significant changes in cellular environment, including elevated levels of neuroinflammatory proteins and ionic dyshomeostasis. One such protein is S100A9, which is released by neutrophils as part of a neuroinflammatory response. S100A9 has been shown to be intrinsically amyloidogenic, readily forming amyloid fibrils both in-vitro and in-vivo. Interestingly, S100A9 in both its soluble and fibrillar forms has been shown to promote the deposition of amyloid beta-peptide and alpha-synuclein fibrils, the hallmarks of Alzheimer's and Parkinson's Disease respectively.

As a calcium binding protein, S100A9 is sensitive to the calcium dyshomeostasis that accompanies many neurodegenerative diseases. Using a combination of synchrotron radiation circular dichroism and solution-state NMR, we show that despite the persistence of secondary structure at low calcium ion concentrations, there are significant increases the conformational plasticity and a reduction in protein stability that promotes the formation of S100A9 fibrils. Despite this conformational flexibility, solid-state NMR studies indicate that irrespective of the calcium concentration, the S100A9 fibrils adopt a single conformational state.

In the case of synucleinopathies, such as Parkinson's Disease, recent cryo-EM studies have indicated that the strain of amyloid fibril formed may be a key discriminator in determining the pathology presented. Given S100A9's demonstrated ability to promote alpha-synuclein deposition, we have used proton detected ultra-high field solid-state NMR experiments to ascertain how S100A9 influences alpha-synuclein fibrils aggregation. Our studies reveal that S100A9 not only enhances the rate of alpha-synuclein deposition but plays an important role in determining the conformation alpha-synuclein adopts within the fibril. Preliminary paramagnetic relaxation enhancement studies indicate that these variations in molecular structure, may influence the interaction of alpha-synuclein fibrils with cellular receptors implicated in the propagation of the fibrils through the brain and the progression of the disease.


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Parking (Hampton)
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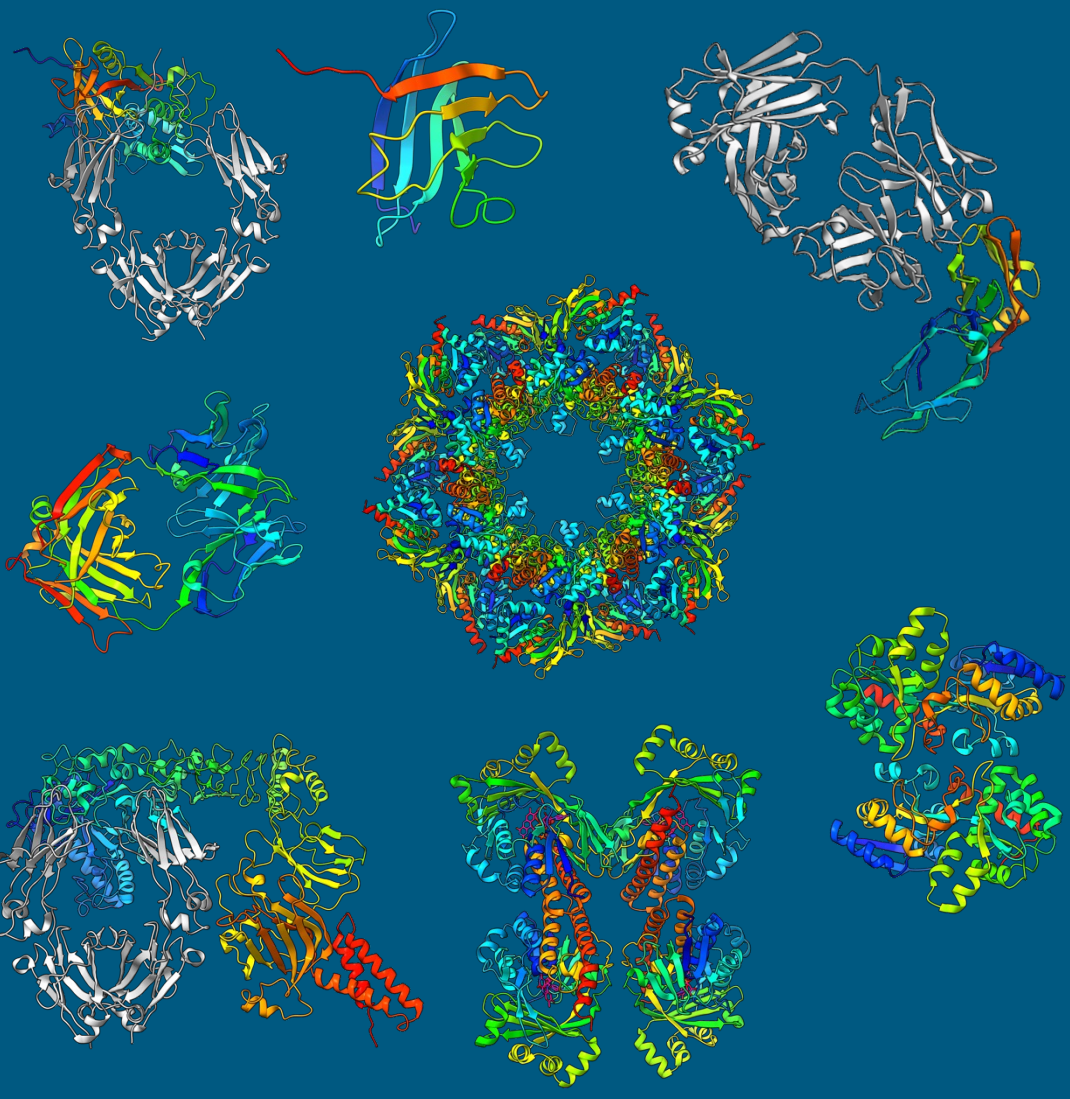

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