The Institute of
Structural and Molecular Biology

ISMB 2018 Graduate Research Symposium

Programme & Abstracts

Monday 11th & Tuesday 12th June

Institute of Structural and Molecular Biology

ISMB 2018 Graduate Research Symposium

Programme & Abstracts

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Programme

Talks

Date: Monday 11th June, 9:30am to 5pm
Location: Room B33, Birkbeck College (see map on last page)

9:30 *Welcome Tea/Coffee* (15mins)
Room B62, Birkbeck (see map last page)

9:45 *Introduction from* Gabriel **WAKSMAN** – HoD (5 mins)

Session 1 – Chair: Nicholas KEEP

9:50 Emir **ACIYAN**
Characterisation of the NuA4 sub-complex: TINTIN - (20 mins)

10:10 Tolulope **ADEYELU**
Protein Network Analyses to Identify Drug Targets for Bladder Cancer (Final year talk) - (20 mins)

10:30 Sarah **ALAM**
Using in-cell NMR to study the protein folding and structural dynamics of TAR DNA binding protein-43 - (10 mins)

10:40 Georgina **BENN**
Investigating Gram-negative membrane attack using nanoscopic techniques - (10 mins)

10:50 Jennifer **BOOKER**
Structural studies of a voltage-gated sodium channel in open and closed states - (20 mins)

11:10 *Tea/coffee break*
Room B62, Birkbeck - (20 mins)

Talks

Date: Monday 11th June, 9:30am to 5pm
Location: Room B33, Birkbeck College (see map on last page)

Session 2 – Chair: Vitor BERNARDES PINHEIRO

11:30 Charles **BURRIDGE**
Dynamics of a globular protein during biosynthesis on the ribosome studied with NMR - (10 mins)

11:40 Alexander **COOK**
Cryo-EM structure of the kinesin-5 motor domain from the malaria-causing parasite (Third year talk) - (20 mins)

12:00 Emma **ELLISTON**
Structural studies into the pathological α1-antitrypsin polymer using single-particle electron microscopy - (20 mins)

12:20 Scott **GARDNER**
Mechanisms of chaperone-assisted protein folding - (10 mins)

12:30 Glen **GRONLAND**
Combinatorial protein-RNA recognition in the regulation of mRNA transport - (10 mins)

12:40 Paola **HANDAL MARQUEZ**
Mapping Sequence and Functional Landscapes of XNA Polymerases - (10 mins)

12:50 *Lunch* - (60 mins)

Talks

Date: Monday 11th June, 9:30am to 5pm
Location: Room B33, Birkbeck College (see map last page)

Session 3 – Chair: Giulia ZANETTI

13:50 Yan-Kay **HO**
Investigating genetic code plasticity in transient expression systems - (20 mins)

14:10 Harrison **O'BRIEN**
Structure, dynamics and interactions of the von Willebrand Factor C domains - (10 mins)

14:20 Alistair M **JAGGER**
Conformational dynamics of the disease-associated protein alpha-1-antitrypsin - (20 mins)

14:40 Juliette **JAMES**
Optimisation of a quadrupole time of flight mass spectrometer for the analysis of cross-linked peptides - (20 mins)

15:00 Evgenia **MARKOVA**
Reconstituting a mammalian intracellular transport system - (10 mins)

15:10 *Tea/coffee break*
Room B62, Birkbeck - (20 mins)

Talks

Date: Monday 11th June, 9:30am to 5pm
Location: Room B33, Birkbeck College (see map on last page)

Session 4 – Chair: Helen HAILES

15:30 Hyunah **LEE**
Kaposi's Sarcoma-associated Herpesvirus: Understanding host mRNA degradation by SOX and its potential inhibitors - (20 mins)

15:50 Arundhati **MAITRA**
Characterisation of cell-wall peptidoglycan recycling in Mycobacterium tuberculosis - (20 mins)

16:10 Faiza **JAVAID**
Appraisal of leucine-rich alpha-2-glycoprotein 1 (LRG1) as a target for a potential antibody–drug conjugate (ADC) - (10 mins)

16:20 Liam **MARTIN**
Synthesis of Isoquinoline Alkaloids as Anti-tubercular Agents - (10 mins)

16:30 Roxana J **MIRONSKA**
Fluorescence studies of the Nuclear Pore Complex - (20 mins)

16:50 Jasmine **MICHALOWSKA**
Single cell imaging comparing mechanical versus biochemical cell competition in epithelial monolayers - (10 mins)

Symposium ends 5pm

Talks

Date: Tuesday 12th June, 9:15am to 4:30pm
Location: Room B33, Birkbeck (see map on last page)

9:15 ***Welcome Tea/Coffee***
Room B62, Birkbeck (see map last page)

9:30 *Introduction from* Professor Ivan **GOUT** – DGT (5 mins)

Session 1 – Chair: Alethea TABOR

9:35 Joseph **NEWCOMBE**
Allosteric modulation of nicotinic acetylcholine receptors examined by computer docking studies with revised α7 homology models - (20 mins)

9:55 Povilas **NORVAIŠAS**
Metformin hijacks bacterial nutrient sensing system to extend host lifespan - (20 mins)

10:15 Sam M **IRELAND**
Predicting and Characterisng Zinc Metal Binding Sites in Proteins - (10 mins)

10:25 Jennifer **PRITCHARD**
Structural and functional studies of the BkaR regulon of Mycobacterium tuberculosis - (20 mins)

10:45 Leonor M **QUINTANEIRO**
Towards understanding host-microbe interactions - (20 mins)

11:05 *Tea/Coffee break*
Room B62, Birkbeck - (25 mins)

Talks

Date: Tuesday 12th June, 9:30am to 4:30pm
Location: Room B33, Birkbeck (see map last page)

Plenary Lecture - Host: Finn WERNER

11:30 – 12:30 ***Plenary Lecture***: Thorsten **ALLERS**
Associate Professor of Archaeal Genetics, Faculty of Medicine & Health Sciences, Nottingham University

Life without DNA Replication Origins - (60 mins)

Biography: Professor Thorsten Allers graduated from the University of Cambridge and his research career has focussed on DNA replication, recombination and repair. He has exploited model organisms from the three domains of life – Bacteria, Eukarya and Archaea. His PhD with David Leach (University of Edinburgh) was concerned with DNA repeats that are targeted by the Escherichia coli machinery for recombination. For his postdoctoral fellowship with Michael Lichten (NIH, USA), he switched to Saccharomyces cerevisiae and made the discovery that crossover and non-crossover products of homologous recombination are formed by different mechanisms. In 2001, he was awarded a Royal Society University Research Fellowship to establish his group at the University of Nottingham. His laboratory has developed a tractable genetic system for Archaea using the halophile Haloferax volcanii, which they have used to study how DNA replication, repair and recombination operate in the third domain of life.
http://www.nottingham.ac.uk/life-sciences/people/thorsten.allers

12:30 *Lunch* - (45 mins)

Date: Tuesday 12th June, 9:15am to 4:30pm
Location: Room B33, Birkbeck (see map on last page)

Session 2 – Chair: Konstantinos THALASSINOS

13:15 Katherine **RICHARDSON**
The effect of low pH on the aggregation of therapeutic human IgG4 antibody - (10 mins)

13:25 Rebecca **RODDAN**
Norcoclaurine synthase: Structural studies, enzyme engineering and the biocatalytic synthesis of novel alkaloids - (10 mins)

13:35 Harry **SCHOLES**
Gene function prediction in flies and yeast - (10 mins)

13:45 Haneesh **SIDHU**
How does the ribosome architecture influence co-translational protein folding? - (10 mins)

13:55 Matthew **SINNOTT**
Scoring structural models using mono-links from chemical crosslinking mass spectrometry - (10 mins)

14:05 Shaan **SUBRAMANIAM**
Identifying receptors internalised by a novel endocytic pathway using ion-mobility assisted proteomics - (20 mins)

14:30 *Poster Session* - (120 mins)
16:30 Room B62, Birkbeck - **Nibbles, snacks and drinks available**

Date: Tuesday 12th June, 2pm to 4:30pm
Location: Room B62, Birkbeck (see map on last page)

Poster Session

Snacks, nibbles and drinks available

Posters (alphabetical order) - A – H:

Joseph **BETON**	*The structure and action of the human Hsp70 disaggregase*
Joseph **BONELLO**	*Protein function scoring using homologues*
Gwenivere **CACKETT**	*The African Swine Fever Virus RNAP Transcription System*
Alessandra **CASAMENTO**	*Investigating the role of Bin1 in Fast Endophilin-Mediated Endocytosis (FEME)*
Saowalak **CHANGKO**	*Development of ptxD as a Crop Protection Tool and Chloroplast Selectable Marker for Chlamydomonas reinhardtii (Poster)*
Charles **ELDRID**	*Differential Collision Induced Unfolding Patterns for Alpha1-Antitrypsin Plasma Variants*
Eric **ESCRIVA**	*Tissue culture adaptation of human herpesvirus-6A defined by whole genome deep sequencing shows new reference genome and identifies virus entry complex changes*
Gil **FERREIRA HOBEN**	*Analysis and prediction of antibody stability*
Gar Kay **HUI**	*The solution structures of the full-length human antibody-receptor complexes*
Joshua **HUTCHINGS**	*Cryo-electron tomography and subtomogram averaging of COPII assembled on membranes*

Date: Tuesday 12th June, 2:30am to 4:30pm
Location: Room B33, Birkbeck (see map on last page)

Snacks, nibbles and drinks available

Poster Session

Posters (alphabetical order) - I – Z:

Hina **IQBAL**	*Solution structural differences between collagen in mannose binding lectin of complement and standard triple helices*
Harry **MACKENZIE**	*Hydrogen/Deuterium Exchange (HDX) in Arginine side-chains: Quantifying Intramolecular Interactions in Solution*
Stephen **MCCARTHY**	*Investigating the interactions of tarantula venom peptide Protoxin-II with Nav1.7*
Shomon **MIAH**	*Unravelling the role of CBF3 in point centromere function in S. cerevisiae*
Fiona **SHILLIDAY**	*Structure-function relationships of putative kinesin-8 molecular motors in Plasmodium parasites*
Lucas **SIEMONS**	*Determining isoleucine rotamer populations from chemical shift.*
Valentina **SPITERI**	*Unravelling the solution structures of therapeutic antibodies with and without glycans*
Lenka **STEJSKAL**	*Investigating the structure to function relationship of the Hepatitis C virus envelope glycoprotein 2 during host cell entry*
Trishant **UMREKAR**	*Insights into the function of MSP1, the major merozoite surface protein of the malaria parasite Plasmodium falciparum*
Hugo **VILLANUEVA**	*Can Bacillus phage Phi29 be re-engineered phage as a Clostridium phage?*
Henna **ZAHID**	*Site directed mutagenesis reveals plasticity of lipid binding to the groove of the fat depleting factor Zinc a2 glycoprotein*

Abstracts

Characterisation of the NuA4 sub-complex: TINTIN (3rd year talk)

Emir **ACIYAN**

Supervisors: Alan CHEUNG & Konstantinos THALASSINOS

ISMB, Division of Biosciences, Darwin building, University College London, Gower Street, London, WC1E 6BT

Email address: aciyan.emir@gmail.com

Abstract:
Histone acetylation plays a critical role in transcription regulation and DNA damage repair pathways in Eukaryotic systems. The process of acetylation is tightly regulated, involving large and highly dynamic protein complexes, that work within sophisticated signalling networks.

NuA4 (Nucleosome acetyltransferase of H4) is a histone acetyltransferase complex conserved throughout Eukaryotes, responsible for the acetylation of H4, H2A and the non-canonical H2A.Z. The complex has been implicated in transcriptional activation, chromatin remodelling and DNA damage repair mechanisms.
NuA4 subunits can form multiple sub-complexes that exist independently, or part of other chromatin modifying complexes, such as SAGA.

TINTIN (Trimer Independent of NuA4 for transcription Interactions with Nucleosomes) is a trimeric sub-complex of NuA4 and has been proposed to promote transcription elongation via interactions with the RNA Polymerase II CTD.

A high-resolution structure of TINTIN has yet to be produced and as a result, we have attempted to characterise TINTIN using a combination of crystallography, biochemistry and biophysical techniques.

Protein network analyses to identify drug targets for bladder cancer (3rd year talk)

Tolulope **ADEYELU**[1] Aurelio MOYA-GARCIA[2], Paul ASHFORD[1] & Christine ORENGO[1]

[1] Institute of Structural and Molecular Biology, Division of Biosciences, University College London, Darwin Building, Gower Street, London, WC1E 6BT
[2] Department of Molecular Biology and Biochemistry, Universidad de Malaga, 29071, Málaga Spain, CIBER de Enfermedades Raras (CIBERER), 29071, Málaga, Spain

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Abstract:
This study explored the molecular mechanism of bladder cancer by using known cancer genes to search for neighbours in a human protein network. A set of 123 cancer 'seed genes' was compiled from known and likely driver genes obtained from COSMIC, the cancer genome census (CGCs), and an in-house set of mutationally-enriched protein domain families. The expression profiles of 419 patients with bladder cancer were obtained from The Cancer Genome Atlas (TCGA) and differentially expressed genes identified by comparison with non-tumour samples. To obtain a comprehensive protein network, a gene co-expression network was constructed using the publicly-available WGCNA protocol and combined with the protein interaction network from Pathway Commons, using a commute-time kernel approach. This network was then analysed using the DIAMOnD algorithm, which links the seed genes to neighbours in the protein network. Some neighbouring genes identified in this way were already known to be implicated in bladder cancer, having relevant hallmarks and pathways such as myogenesis, p53-pathway, apical junction complex interaction. Novel genes were also identified, implicated in the same or related pathways to the known cancer genes. Drugs from ChEMBL were associated with the known and novel genes to suggest possible therapeutic strategies.

Using in-cell NMR to study the protein folding and structural dynamics of TAR DNA binding protein-43 (1st year talk)

Sarah M **ALAM**, Sammy H CHAN, Lisa D CABRITA & John CHRISTODOULOU

ISMB, Division of Biosciences, Darwin building, University College London, Gower Street, London, WC1E 6BT

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Abstract:
The presence of insoluble proteinaceous aggregates is a key pathological hallmark found in the motor neurones of both familial and sporadic Amyotrophic Lateral Sclerosis (ALS) patients. Trans-activation response DNA binding protein-43 (TDP-43) has been identified as the major pathological protein of ALS, but it is currently not known exactly how this protein is involved in the pathogenesis of the disease. TDP-43 is a 414 residue, multi-domain protein with a disordered C-terminal region ($CTD_{274-414}$) that propagates the aggregation of TDP-43 and hosts almost all disease- associated mutations. There is currently no 3D structural information of the TDP-43 protein or detailed understanding of how the CTD region mediates aggregation of the protein.

To initiate structural studies of TDP-43, we have developed a recombinant expression and purification strategy to produce the $CTD_{274-414,}$ and we apply NMR spectroscopy which is unique in its capacity to study dynamic systems at high resolution. In particular, we have been developing an in-cell NMR strategy which can observe proteins *in situ* within living cells. This approach enables us to study the structural and dynamical properties of TDP-43 as it exists within a native cellular environment.

Plenary Lecture: ***Life without DNA Replication Origins*** (60mins)

Thorsten **ALLERS**

University of Nottingham, Room D114 School of Life Sciences, Queen's Medical Centre, Nottingham, NG7 2UH, UK

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Abstract:
DNA replication is initiated at specific chromosomal sites called origins, which serve as binding sites for proteins that recruit the replicative enzymes. Replication origins are assumed to be an essential part of the DNA replication apparatus. We have shown that in the archaeon *Haloferax volcanii*, life without origins is not only possible but highly efficient.

The replication enzymes found in archaea and eukaryotes differ fundamentally from those in bacteria; due to their shared evolutionary history, the former two are highly similar. For example, the archaeo-eukaryotic replicative helicase MCM is distinct to the bacterial replicative helicase DnaB, and the two complexes migrate in different directions on DNA. By contrast, the key enzyme for homologous recombination – known as RecA in bacteria, RadA in archaea and Rad51 in eukaryotes – is conserved in all domains. If homologous recombination is an ancestral process that predates the split between bacteria, archaea and eukaryotes, and the evolution of their different machineries for DNA replication, could it have been used to initiate replication in the last common ancestor?

We have shown that in the archaeon *Haloferax volcanii*, deletion of all chromosomal origins leads to the initiation of all DNA replication by homologous recombination. Similar results have been obtained with deletion mutants lacking Orc1/Cdc6 replication initiator proteins, which are required for origin firing. Surprisingly, this leads to accelerated growth with no obvious defects, whereas deletion of origins (or initiator protein genes) in yeast or Escherichia coli leads to severe growth impairment. If homologous recombination alone can efficiently initiate the replication of an entire cellular genome, what purpose do replication origins serve and why they have evolved?

Investigating Gram-negative membrane attack using nanoscopic techniques (1st year talk)

Georgina **BENN**[1, 2], Edward PARSONS[2], Maxim RYADNOV[3] & Bart HOOGENBOOM[2,4]

[1] Institute of Structural and Molecular Biology, University College London and Birkbeck College, London, United Kingdom
[2] London Centre for Nanotechnology, University College London, London, United Kingdom
[3] National Physical Laboratory, Hampton Road, Teddington TW11 0LW, United Kingdom
[4] Department of Physics and Astronomy, University College London, London, United Kingdom

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Abstract:
The membrane of bacteria is particularly susceptible to attack, due to its exposure to the environment. Our immune system exploits this vulnerability via the membrane attack complex (MAC). The MAC is a key component of the complement pathway in the innate immune system. It is formed of 5 different proteins (C5b, C6, C7, C8 and C9) that circulate in the blood. Upon activation by the complement pathway, the subunits assemble sequentially; terminating with the addition of 18 copies of C9 to form a pore in the membranes of Gram-negative bacteria.

In this project we will use atomic force microscopy and fluorescence techniques to determine the kinetics and mechanism of the MAC as it forms in the membranes of bacteria. These techniques will allow us to track the formation of individual pores and the killing of individual cells, in real time and with high resolution, both on live bacteria and supported lipid bilayers. This will help to determine key questions for MAC formation: does the MAC traverse the peptidoglycan layer? How many MACs are required to kill a cell? And what is the rate limiting step in pore formation? Furthermore, these techniques can be applied to investigate membrane attack by designed antimicrobial peptides.

The structure and action of the human Hsp70 disaggregase (Poster)

Joseph **BETON**[1,2], Helen SAIBIL[1] (Supervisor), Bart HOOGENBOOM[2] (Co-supervisor) & Anthony ROBERTS[1] (Co-supervisor)

[1] ISMB, Department of Biological Sciences, Birkbeck College, University of London, Malet Street, London, WC1E 7HX
[2] London Centre for Nanotechnology, University College London, London, UK

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Abstract:
The accumulation of intracellular α-synuclein amyloid aggregates is associated with the onset and progression of Parkinson's Disease. *In vitro*, fibrillar α-synuclein aggregates are efficiently disassembled by a potent ATP dependent disaggregase machinery composed of human Hsp70 working in concert with a cognate J-protein (DNAJB1) and nucleotide exchange factor (Apg2). Disassembled fibrils have reduced cellular toxicity which suggests that the Hsp70 disaggregase has a role in protection from Parkinson's Disease. Despite this, the mechanism of Hsp70 mediated disaggregation remains undefined. We combine cryo EM and real-time atomic force microscopy (AFM) to study Hsp70 mediated α-synuclein disaggregation at high spatial and temporal resolution. By single particle cryo EM, we investigate the structure of DNAJB1, responsible for Hsp70 recruitment, in complex with α-synuclein fibrils. After 2D classification, bound DNAJB1 can be identified at the fibril surface, with further work aimed at producing a high-resolution reconstruction of the complex. Using AFM, we visualise the complete disaggregation of α-synuclein fibrils by the Hsp70 disaggregase machinery in real-time. This shows disaggregation proceeding over the course of several hours with initial fibril fragmentation in-to short (50 – 200 nm) segments which are then rapidly depolymerised.

Protein function scoring using homologues (Poster)

Joseph **BONELLO** & Christine ORENGO (Supervisor)

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Abstract:
Protein function prediction is a means of annotating proteins using computational techniques. These techniques can provide a quicker means of understanding the complex functions performed by proteins and can help in speeding up the experimentally-driven characterisation process.

This project uses set-based methods and a statistical model, exploiting homology, to create a set of scores that can be used to predict the possible functional domains that a protein might possess. Protein domains are conserved regions of a protein sequence and structure that can function independently from the rest of the protein chain. Homology-based methods take advantage of the evolutionary relationships that exist between individual domain sequences.

The method presented was evaluated against targets from the CAFA challenge, a community-wide endeavour whose aim is to assess the current state of the various methods available for protein function prediction. The method performed reasonably well on Prokaryotic targets when compared to the results of both CAFA 1 and CAFA 2, although further work is required to improve the scores. Predictions for Eukaryotic targets are currently being evaluated.

Structural studies of a voltage-gated sodium channel in open and closed states
(3rd year talk)

Jennifer **BOOKER**

Supervisor: Bonnie WALLACE

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Abstract:
Voltage-gated sodium channels (VGSCs) are important drug targets for the treatment of chronic pain and many other neurological and cardiovascular conditions. Many drugs show state-dependent block of these channels, and so rational design of new drugs will require detailed structural information about the structural changes taking place in the VGSC activation cycle. Due to the difficulty of obtaining purified eukaryotic VGSCs, bacterial VGSCs are used as models to probe VGSC structure and function, and some, such as the VGSC NavMs, have been shown to be excellent functional and structural models for human sodium channels. We have previously used X-ray crystallography to solve the structure of NavMs in the open, activated state to high resolution (2.45Å) (1), revealing novel features with functional importance. All crystals obtained were in the open state, so in order to study the channel in different stages of the activation cycle, we began single-particle cryo-electron microscopy (cryo-EM) studies. This talk will discuss the challenges of analysing a small membrane protein like NavMs by cryo-EM and summarise progress made so far, including analysis of preliminary maps, which indicate the protein is in a closed conformation.

[1] The Complete Structure Of An Activated Open Sodium Channel, Sula A.*; Booker J.*; Ng L.C.T.; Naylor C.E.; DeCaen P.G.; Wallace B.A., ***NATURE COMMUNICATIONS*** 8 : 14205 (2017)

* These authors contributed equally to this work.

Dynamics of a globular protein during biosynthesis on the ribosome studied with NMR (1st year talk)

Charles **BURRIDGE**[1], Chris WAUDBY[1], Lisa CABRITA[1] & John CHRISTODOULOU[1]

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Abstract:
Protein folding begins during biosynthesis on the ribosome. We have shown that co-translational folding is delayed for an Ig-like domain, ddFLN5 – the emergence of the entire domain from the ribosomal exit tunnel while tethered to the ribosome by its subsequent domain and its acquisition of native structure on the ribosome are offset[1]. Our NMR investigations of ribosome nascent chain complexes and NMR restrained MD simulations have revealed contacts between the nascent chain and ribosome surface as potentially a mechanism by which the ribosome could modulate protein folding as it occurs in the cell.

NMR spectroscopy has a unique ability to investigate the structure, dynamics and interactions of nascent polypeptides during their biosynthesis but requires significant development of strategies in terms of the preparative biochemistry and NMR methodology to optimally tease out mechanistic details[2]. Here the development of an isotopic labelling strategy for the methyl groups of nascent chains bound to isotopically silent ribosomes is described. The use of LC/ESI-MS to monitor the labelling efficiency and the measurements of cross-correlated relaxation processes using NMR are used to allow the probing of the interaction between the folded nascent chain and the ribosome surface in order to uncover the mechanistic basis of the delayed folding.

[1] Cabrita, L., et al. ***NSMB*** (2016)
[2] Cassaignau, A, et al. ***Nature protocols*** (2016)

The African Swine Fever Virus RNAP Transcription System (Poster)

Gwenivere **CACKETT**, Carol SHEPPARD, Raquel PORTUGAL, Michal MALECKI, Thomas FOUQUEAU, Linda DIXON, Jürg BÄHLER & Finn WERNER

ISMB, Division of Biosciences, Darwin building, University College London, Gower Street, London, WC1E 6BT

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Abstract:
African Swine Fever Virus (ASFV) is a nucleocytoplasmic large DNA virus that causes lethal and incurable haemorrhagic fever in domestic pigs. ASFV possesses a eukaryotic-like transcription system: consisting of an eight-subunit RNA polymerase (RNAP), and three transcription factors with homologs in the eukaryotic RNAPII system. Our work has focused on expressing recombinant ASFV transcription system proteins, with a central aim of resolving the recombinant ASFV-RNAP structure via CryoEM. We initially focused on using X-ray crystallography to structurally characterise the chimeric ASFV-RNAP assembly platform, which consists of only subunits ASFV-RPB3 and RPB10: containing an inbuilt RPB11 homolog within the ASFV-RPB3 sequence. We additionally utilised RNA-Seq to obtain a broader overview of how and when ASFV genes are expressed during the early and late stages of infection.

Investigating the role of Bin1 in Fast Endophilin-Mediated Endocytosis (FEME)
(Poster)

Alessandra **CASAMENTO** & Emmanuel BOUCROT

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Abstract:
Endocytosis is the process by which eukaryotic cells internalise extracellular material and transmembrane proteins. The best characterised endocytic pathway is Clathrin-Mediated Endocytosis (CME), which is constitutively active and regulates the uptake of the molecules required to sustain housekeeping functions in cells. In parallel to CME, other clathrin-independent endocytic pathways exist and among them is the recently discovered Fast Endophilin-Mediated Endocytosis (FEME). FEME is a rapid process (1-10 seconds) mediating the cellular internalisation of cell surface receptors, including some GPCRs, receptor tyrosine kinases, and bacterial toxins. FEME likely acts in physiological processes requiring quick internalisation of activated receptors from the plasma membrane, such as control of cell spreading and cell polarization. The main regulator of FEME is the N-BAR domain containing protein Endophilin. Screens for additional BAR domain containing proteins involved in FEME found Bridging integrator 1 (BIN1) colocalising with Endophilin at the plasma membrane and on FEME carriers. The aim of my project is to investigate the potential role of BIN1 in FEME, using a combination of biochemical, cellular and *in vivo* approaches.

Development of ptxD as a Crop Protection Tool and Chloroplast Selectable Marker for Chlamydomonas reinhardtii (Poster)

Saowalak **CHANGKO**, Rosanna YOUNG & Saul PURTON

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Abstract:
Microalgae have potential as a low-cost platform for production of therapeutic proteins and high-valued chemicals. However, there remains a problem of contamination when cultivating algae at large-scale, and therefore a need for sterilization of media and 'crop protection' strategies to mitigate invasion. To reduce such contamination problems, Loera-Quezada et al. (*Plant Biotechnol J.* 2016;14:2066) recently described a strain improvement strategy in which the alga is genetically engineered to allow metabolism of phosphite. Since the majority of organisms cannot use phosphite as a phosphorus source, cultivation using phosphite medium selects for growth of the engineered alga. Here, we have introduced the *ptxD* gene encoding an NAD(P)-dependent phosphite oxidoreductase from *Pseudomonas stutzeri* WM88 into the chloroplast genome of *Chlamydomonas reinhardtii*. We designed a codon-optimized version of the gene and generated transformants using restoration of the native *psbH* gene in a non-photosynthetic Δ*psbH* mutant, rather than using an antibiotic resistance marker. DNA analysis of the transformant lines confirmed the correct integration of *ptxD*, and western blot analysis showed that the recombinant PtxD accumulates in the chloroplast. To test the functionality of PtxD, we scored for growth on plates supplemented with phosphite as the sole phosphorus source. The transgenic lines were capable of growth, in contrast to a control transformant strain lacking *ptxD*, indicating that a functional PtxD allows the transgenic algae to oxidize phosphite to phosphate. On- going studies are testing whether this new phenotype allows growth in non-sterile phosphite medium without spoilage by bacteria or fungi. This would avoid costly sterilization of media and difficult aseptic procedure. The phosphite-based system also offers a promising direct selection for *Chlamydomonas* chloroplast transformation in which *ptxD* gene serves as a non-antibiotic resistance marker for genetic engineering wild-type strains. This would avoid regulatory issues related to horizontal gene transfer of antibiotic resistant markers to other microorganisms in the environment.

Cryo-EM structure of the kinesin-5 motor domain from the malaria-causing parasite (3rd year talk)

Alexander **COOK,** Maya TOPF & Carolyn MOORES

ISMB, Division of Biosciences, Darwin building, University College London, Gower Street, London, WC1E 6BT

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Abstract:
In 2017, 445,000 people died from malaria, a disease caused by the *Plasmodium* genus of intracellular parasites. Despite a recent reduction in malaria burden, the number of malaria cases began to rise again in 2017. Fresh resistance to frontline treatments means that novel therapeutic targets are urgently needed.

To this end, we have begun structural and biochemical characterisation of kinesin-5 from the deadliest of malaria parasite species - *Plasmodium falciparum*. Like other kinesin families, kinesin-5s contain an enzymatic module called the motor domain, which couples energy changes from its ATP hydrolysis cycle to mechanical work on the microtubule. Allosteric inhibition of the human kinesin-5 motor domain blocks cell division, making it a prime therapeutic target for cancer, with several inhibitors in clinical trials.

We hypothesise that the evolutionary difference between *Plasmodium* and human kinesin-5s could render the parasite kinesin-5 susceptible to selective inhibition over its homolog in the human host – offering a novel drug target. To assess this, we have used cryo-electron microscopy, to determine structures of *Pf*K5-MD on its microtubule track, at steps in its ATPase cycle at 6.5 Å resolution. So far, this data has revealed curious molecular behaviours unique to the *Pf*K5 motor domain. These structural insights do indeed reveal potential routes for selection inhibition of *Pf*K5, but also more broadly offer intriguing insights into kinesin motor mechanochemistry.

Differential Collision Induced Unfolding Patterns for Alpha1-Antitrypsin Plasma Variants (Poster)

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Abstract:
Alpha1-antitrypsin (AAT) is a metastable serine protease inhibitor with three N-glycosylation sites that is produced in the liver. Mutations such as E342K (Z variant) and E264V (S variant) are known to lead to AAT variants which are liable to misfolding and polymerisation. A precise structural model of unfolding has not yet been created for the wild type or any of the mutants, nor has there been confirmation that the M, Z and S variants unfold in the same manner. Ion mobility-mass spectrometry combined with collision induced unfolding has been used to elucidate the early unfolding stages of M, Z and S variants purified from human plasma.

Structural studies into the pathological α1-antitrypsin polymer using single-particle electron microscopy (3rd year talk)

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Abstract:
The hereditary disease α_1-antitrypsin deficiency, most prevalent in those with Northern European ancestry, is a cause of early-onset emphysema and liver cirrhosis. Currently the only effective treatment is a liver transplant. The disease is caused by mutations in the gene encoding α_1-antitrypsin (AAT) which make it misfold and aggregate into polymer chains. These polymers accumulate and cause cellular damage to hepatocytes, and the resultant lack of AAT in the circulation permits breakdown of lung tissue.

The mechanism of polymerisation and the linkage between individual subunits of the polymer found in affected individuals is unknown. Current structural models of polymerisation are based on artificially-induced *in vitro* polymers but their relevance to what is found *in vivo* is disputed. A structural understanding of the mechanism by which AAT polymerises will be crucial for the development and evaluation of therapeutics.

This project aims to determine the structure of polymers present in patient liver tissue using electron microscopy (EM). In preparation for this, polymers have been isolated and purified from liver explant samples following transplantation. To assist with single-particle analysis, each AAT subunit within the polymer chain has been labelled with antibody fragments (Fab) of known specificity. The labelled pathological polymer was imaged using negative-stain EM and 3 reconstructions of AAT/Fab dimers were obtained. Only the C-terminal model of polymerisation was consistent with the angular relationships between subunits. AAT/Fab dimers have also been studied using cryo-EM to try yield higher resolution information. Lack of definition in the second subunit of the dimer suggests greater angle variation between subunits in native conditions. The new approach to reveal the polymer linkage is to reconstruct a single subunit from within the polymer chain to high resolution.

Tissue culture adaptation of human herpesvirus-6A defined by whole genome deep sequencing shows new reference genome and identifies virus entry complex changes (Poster)

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Abstract:
Tissue-culture adaptation of viruses can modulate infection. Laboratory passage and BACmid cloning of human betaherpesvirus, cytomegalovirus, HCMV, have resulted in genomic alterations affecting virus infectious entry and cellular tropism with implications for pathology and vaccines. In this study we have examined tissue-culture adaptation in the related human betaherpesvirus, the Roselovirus reference genome from human herpesvirus 6A, HHV-6A, strain U1102. This virus also has an endogenous human form via 'cloning' within the germline subtelomeric chromosomal-integration in approximately 1% human populations. Therefore, understanding effects of laboratory passage are essential to understand relationships between exogenous and endogenous virus during infection and pathology. Using whole genome next-generation deep-sequencing Illumina-based methods, we compared the original clinical isolates to tissue-culture passaged, BACmid-cloned virus and endogenous 'cloned' virus genomes. This re-defined the reference virus genome and showed BACmid virus rearrangement. As shown for HCMV culture effects, SNPs accumulated during tissue culture passage, which affected the virus entry complex mediating cellular fusion and favouring cell associated spread. We constructed molecular models based on homologous tertiary structures and investigated effects on the multimeric fusion complex. Results show accumulated SNPs affecting the trimeric interfaces, which may influence the post-fusion structure for cell-associated spread affecting infection intervention and vaccine design.

Mechanisms of chaperone-assisted protein folding (1st year talk)

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Abstract:
In the cell proteins are synthesised as linear chains of amino acids. To carry out their biological functions proteins must fold into specific three-dimensional structures, referred to as their native states. Some human diseases, including late-onset neurodegenerative diseases such as Alzheimer's Disease, are related to the accumulation of non-native protein aggregates in cells. How proteins adopt their native states and how cells maintain proteostasis have been ongoing topics of biological and clinical relevance for over fifty years.

Many proteins require the assistance of molecular chaperones to reach their native state. The most well-studied chaperones are the chaperonins; ATP-driven containers that bind and encapsulate non-native protein, preventing their aggregation and enabling correct folding. How chaperonins accelerate protein folding remains unclear, do they act as passive aggregation inhibitors or do they play a more active role in protein folding?

We aim to apply the latest advances in cryogenic electron microscopy (cryoEM) and ion-mobility mass spectrometry (IM-MS) to determine the structures of protein folding intermediates interacting with chaperones. In doing this we hope to answer some of the outstanding questions regarding the mechanisms of chaperone-assisted folding.

Combinatorial protein-RNA recognition in the regulation of mRNA transport (1st year talk)

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Abstract:
Neuronal development represents a key step during embryogenesis that is crucial for healthy organismal growth. For development, core processes such as the branching of dendrites and axons, as well as the formation and differentiation of synapses require large, specific sets of mRNAs to be correctly localised and translated in a stage-specific manner.

The functionally related RNA-binding proteins IMP1 and Syncrip act as important co-ordinators of transport and translation of mRNAs necessary for neural development. Using their multiple RNA-binding domains they can recognise and interact with diverse ensembles of mRNAs, while also recruiting relevant cell machinery. Misregulation or dysfunction of these proteins is associated with severe neuro-degenerative disorders and several types of cancers. Understanding the structural and dynamic features that underpin specificity in protein-RNA recognition will allow us to engineer changes in said specificity, and manipulate functional contacts in the cell.

This project seeks to better understand how both IMP1 and Syncrip recognise specific RNA sequences via their RNA-binding domains, the role of each protein in the packing of RNA molecules, as well as IMP1's specific regulation by the kinase Src. To do this, we combine structural work on protein-RNA complexes using NMR and SAXS, with biophysical experiments such as biolayer interferometry to extract affinity and kinetic information.

Mapping Sequence and Functional Landscapes of XNA Polymerases (1st year talk)

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Abstract:
The chemical constitution of DNA and RNA, nature's only genetic polymers, enable information storage and replication and thus evolution and life. Nonetheless, it has been possible to replace their nucleobase, sugar and/or phosphodiester backbone with synthetic analogues giving rise to a range of synthetic nucleic acids (XNAs) that are also able to store and propagate genetic information. This discovery has not only expanded the central dogma but also the chemical diversity of genetic polymers and their potential applications in biotechnology. Still, XNA synthesis and replication are limited by the low efficiency and lack of selectivity for XNA synthesis by native replication machineries as well as currently available XNA polymerases. This project aims to further expand the XNA episome by sampling the functional sequence neighbourhood of polymerases through the construction of complex saturation mutagenesis libraries coupled to functional DNA- and XNA-based selection platforms. This will allow the identification of crucial functional networks in the polymerase and residues that enhance XNA activity and selectivity. In addition, the libraries will enable the study of epistasis, co-variation and synergism between sites to help improve future polymerase engineering.

Investigating genetic code plasticity in transient expression systems (3rd year talk)

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Abstract:
Life as we know it is dictated by the genetic code: a specific and unambiguous set of rules regarding the assignment between RNA triplet (genetic information) and amino acid (protein product). Aminoacyl-tRNA synthetases (aaRS) are central in that process as they create the physical link between amino acids and their cognate transfer RNAs (tRNAs). Likewise, tRNAs are essential components in bridging the messenger RNA triplet to amino acid gap. Engineering the identity elements of tRNAs or substrate specificity of aaRSs, however, allows the genetic code to be modified.

Such modification of the genetic code does not change biology but could conceivably create organisms unable to exchange genetic information with nature – a viable route towards biocontainment. Development and testing of these modified codes, particularly for sense-to-sense reassignments, is challenging. My goal has been to develop *in vivo* platforms to optimise engineered tRNA/aaRS pairs that can transiently reassign sense codons in *E. coli*, enabling us to systematically explore alternative genetic codes, whilst minimising toxicity to the host.

Using a H→L and L→H reassignment as a proof-of-principle, we are developing *in vivo* platforms, targeting codons that naturally rely on wobble base-pairing with their cognate tRNA, to transiently test for reassignment. As we expect a significant degree of toxicity, we are also developing a biological toggle circuit, based on serine recombinases, to produce a non-toxic permanent response to augment the initial transient signal of codon reassignment. Together, this platform will represent a first step towards the synthesis of semantically orthogonal organisms.

Analysis and prediction of antibody stability (Poster)

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Abstract:
Antibody therapies have been shown to be essential in the treatment of a range of diseases. In fact, half of the top grossing drugs in 2014 were mAb (monoclonal antibody) and it is estimated that this market will be worth 140 billion USD by 2024. This sharp increase in market size clearly reflects the huge potential of mAbs. The main advantage of antibody therapeutics over small drug molecules is their high specificity for their target antigen, which makes them ideal as therapeutics, in the treatment of diseases such as cancer, but also in a research environment. However, antibody therapeutics must also possess suitable biophysical properties in order to be developed as a biologic, including a low propensity for aggregation to reduce immunogenicity. Early detection of unsuitable mAbs during the antibody discovery process, and the identification of key residues involved in their stability, could enable the development of antibody therapeutics with improved biophysical properties.

The aim of this project is to develop a computational model that can predict antibody stability. To accomplish this over 900 Fabs (antigen-binding fragment) are being synthesised in partnership with UCB Celltech. These Fab are derived by pairing non-cognate heavy and light chains from an in-house library of antibody variable regions isolated from human IgG.

Currently, about half of the data has been obtained and is being used to build a variety of models to predict the thermostability of each pair. These models include simple one layer perceptrons, ensemble learners and deep autoencoders, which can learn linear and non-linear patterns in data. So far, the most successful models include features such as residue and atom hydrophobicity and surface accessibity. The best current model can predict antibody thermostability within a MSE of 33.2 and correlates well with the test and validation data. Additional data is being collected from other datasets to improve the current models.

The solution structures of the full-length human antibody-receptor complexes
(Poster)

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Abstract:
An antibody binds to foreign antigen through its two Fab regions and activates an immune response through its Fc region by binding to Fc receptors (FcγR). Human IgG antibody exists as four subclasses IgG1, IgG2, IgG3 and IgG4, which vary in structure and function even though they have ~95% sequence similarity. IgG1 activates immune responses because this binds to all three human FcγRs whereas IgG4 has reduced immune activity due to its weaker FcγR binding. X-ray small angle scattering (SAXS), analytical ultracentrifugation (AUC) and advanced atomistic scattering modelling methods were used to study the overall conformation of the full-length IgG antibody in its FcγR complex, which to date cannot be studied by protein crystallography. Both SAXS and AUC revealed that unbound IgG4 is more compact than IgG1. In the IgG1-FcγRI and IgG4-FcγRI complexes, AUC showed the complexes were monodisperse and revealed a 1:1 stoichiometry. By SAXS, the overall shapes of IgG1 and IgG4 changed upon binding of FcγRI to a more elongated conformation. Atomistic modelling of IgG1 and IgG4 starting from crystal structures and using molecular dynamics and Monte Carlo simulations to calculate the SAXS curves will reveal the atomistic structures of the full-length IgG antibody in their FcγR complexes.

Cryo-electron tomography and subtomogram averaging of COPII assembled on membranes (Poster)

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Abstract:
COPII is an archetypal coat complex of Eukaryotes that governs ER-to-Golgi transport of proteins. It assembles in a hierarchical manner to selectively package a diverse range of secretory substrates into vesicles, typically small soluble proteins such as insulin, but also fibrillar structures like procollagen that necessitate unconventionally large COPII carriers. Genetic diseases arising from mutants of COPII and its effectors cause secretory defects specific to large cargo, yet mechanisms for the formation of large carriers remain speculative (1). To address this, we sought to visualise COPII in the act of inducing membrane curvature using cryo-electron tomography. Cryo-EM tomography with subtomogram averaging has the unique advantage of achieving molecular resolution structures within native cellular contexts, yet its potential is only just being realised.

We reconstituted COPII activity by incubating purified proteins with giant unilamellar vesicles (GUVs). The reaction yields straight tubules wrapped in a helical lattice with dimensions commensurate with that of procollagen, as seen previously (2). We acquired a large tomography dataset of these tubules at high magnification. Subtomogram averaging was applied to attain a working resolution of 4.9 Å for COPII assembled on membranes. The structure reveals an unprecedented mechanism for the recruitment of coat components, prompting revisions of how COPII assembles and induces membrane curvature. Mutagenesis is currently being used to dissect the adaptable features of the coat needed to form large carriers. We propose how COPII effectors tap into this for regulation of ER-to-Golgi trafficking.

References:

1. 10.1038/ncb2434
2. 10.7554/eLife.00951

Solution structural differences between collagen in mannose binding lectin of complement and standard triple helices (Poster)

Hina **IQBAL**[1], Ka Wai FUNG[1], Jayesh GOR[1], Anthony C. BISHOP[2], George I. MAKHATADZE[2], Barbara BRODSKY[3] & Stephen J. PERKINS[1]

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Abstract:
The complement system, an important component of innate immunity, responds rapidly to invading pathogens. Mannan-binding lectin (MBL) is an oligomeric, primary recognition molecule of the lectin pathway of complement activation, and MBL deficiency is the most common human immunodeficiency identified to date. MBL contains multiple globular carbohydrate recognition domains linked by homotrimeric collagen triple helices. To follow our earlier work with a series of (Pro-Hyp-Gly)n collagen peptides, we performed similar experiments based on collagen peptides in MBL to evaluate its molecular flexibility and complement activation. To date we have data for three peptides (out of nine) based on variants of the MBL collagen sequence, plus (Pro-Hyp-Gly)10 as a benchmark for comparison. The thermal stabilities of the peptides by circular dichroism and differential scanning calorimetery revealed lower melting temperatures for the MBL peptides than for (POG)10. Analytical ultracentrifugation demonstrated their structural monodispersity in solution. Small angle X-ray scattering indicated flexibility in the MBL peptides. The X-ray scattering curves were fitted with 10,000-12,000 models of collagen helices from advanced molecular dynamics modelling of collagen helices. The best-fit structures showed bent collagen helices, indicating flexibility in the MBL collagen structures, and showing that these peptides are adapted to the function of MBL.

Predicting and Characterisng Zinc Metal Binding Sites in Proteins (1st year talk)

Sam M **IRELAND**

Andrew C. R. MARTIN (Supervisor), Stephen J. PERKINS (Co-Supervisor), Christine A. ORENGO (Thesis Chair)

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Abstract:
Zinc is the second-most abundant trace metal in Biological systems, behind only iron. It is catalytically essential to many enzymes, across all enzyme classes (the only such metal to be so widely represented), performs structural and stabilising roles in proteins (most famously in the zinc finger proteins), and 10% of the human proteome is thought to consist of zinc binding proteins.

Predicting and characterising zinc binding sites from genomic and proteomic data is therefore of considerable computational interest. Being able to annotate sequence or raw structural data with zinc binding residues will offer insights into protein function - particularly if the putative binding site can be classified.

An increased understanding of what characterises zinc binding sites will also aid in the ability to engineer zinc-binding into existing proteins, or even design *de novo* binding sites. Diseases such as Age-Related Macular Degeneration, in which pathological zinc-mediated aggregation seems to play a role, could also be treated by engineering zinc-binding *out* of a protein, or in designing drugs to inactivate the zinc binding site.

This talk will give an overview of the future aims of this project, as well as present the online database *ZincBind*, in which all known zinc binding sites are catalogued and classified.

Conformational dynamics of the disease-associated protein alpha-1-antitrypsin
(3rd year talk)

Alistair M **JAGGER[2],** Christopher A WAUDBY[1], Lisa D CABRITA[1], Bibek GOOPTU[1], John CHRISTODOULOU[1], James A IRVING[2] & David A LOMAS[3]

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Abstract:
α-1 antitrypsin (α1AT) is a 52 kDa serine protease inhibitor abundant in human plasma. Many naturally occurring single amino acid substitutions, the most common of which is the Z (Glu342Lys) mutation, promote misfolding and aggregation into long, ordered polymer chains at the site of synthesis in the liver endoplasmic reticulum. Accumulation of polymers leads to liver cirrhosis and the reduced protection of the lungs against proteolytic degradation predisposes individuals to early-onset emphysema. The molecular defect that promotes polymerisation remains incompletely understood: a crystal structure of the Z variant is essentially identical to that of wild-type (WT) α1AT and therefore fails to encapsulate this aberrant behaviour[1]. α1AT is intrinsically conformationally labile, and it is therefore the dynamic behaviour of the protein that is key to elucidating the molecular basis for the enhanced polymerisation. Here we have used NMR spectroscopy on patient-derived samples at natural isotopic abundance to investigate for the first time the effect of mutations and posttranslational modifications on the solution structure of α1AT. High-quality ^{1}H-^{13}C NMR correlation spectra have been acquired for two patient-derived aggregation-prone variants and these show long-range chemical shift perturbations, indicating that the solution-state structures of these variants are indeed different to that of the WT. Reference of these perturbations to the near complete assignment of the spectra of [^{2}H,$^{13}CH_3$–AILVM]-labelled WT α1AT also suggests that a long-range allosteric network may exist within the protein, and this is currently being investigated further using NMR measurements of dynamics across a range of timescales.

[1] Huang, X. et al. Molecular Mechanism of Z α1-Antitrypsin Deficiency. *J. Biol. Chem.* **291**, 15674-15686 (2016)

Optimisation of a quadrupole time of flight mass spectrometer for the analysis of cross-linked peptides (3rd year talk)

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Abstract:
Crosslinking Mass Spectrometry (XLMS) has emerged as an important technique for the structural elucidation of proteins that cannot be resolved by traditional methods. Almost exclusively, the analyser of choice for such an experiment has been the Orbitrap. Here we present for the first time an optimised protocol for the use of a QToF geometry to analyse cross-link data. We first tested six different energy ramps and analysed the fragmentation behaviour of cross-linked peptides identified by xQuest. By combining the most successful energy ramps, cross-link yield can be increased by up to 40%. When compared to previously published Orbitrap data, QToF data also offers improved fragmentation of the beta peptide.

Cross-link validation is a manual process with significant time requirements to inspect spectra. We have developed ValidateXL.py a computational solution that works with existing cross-link software to improve quality control and direct manual validation to where it is needed most.

Appraisal of leucine-rich alpha-2-glycoprotein 1 (LRG1) as a target for a potential antibody–drug conjugate (ADC) (1st year talk)

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Abstract:
Aberrant neovascularisation contributes to diseases such as cancer and is the consequence of inappropriate angiogenic signalling. In recent years, it has been shown that in the presence of the transforming growth factor β1 (TGFβ1), leucine-rich alpha-2-glycoprotein 1 (LRG1) is mitogenic to endothelial cells and promotes angiogenesis. LRG1 mediates this pro-angiogenic effect, in part, by modifying TGF-β signalling. For tumours to grow and survive they must build a vascular network. Tumour vessels, however, are often chaotic, dysfunctional and poorly perfused, characteristics that restrict the delivery of systemically administered drugs. Consequently, vascular normalization has become a highly sought after therapeutic objective. LRG1 antibody blockade inhibits this molecular machine whilst attenuating angiogenesis leading to vascular normalisation, and thus, could be exploited to inhibit cancer growth. We aim to combine the vascular normalisation achieved by LRG1 blockade with affecting cancer cell deterioration to form an antibody–drug conjugate (ADC). To achieve this, a function-blocking antibody against LRG1 called 15C4, and its humanised de-immunised variant; Magacizumab were site-selectively modified with a pyridazinedione (PD) linker that could bear a functional modality that could undergo "click" modification. These bioconjugation reactions were optimised to maximise homogeneity of the protein conjugate and to achieve a consistent loading of four PD molecules. The conjugates were then modified with AlexaFluor™ 488 azide to further corroborate the loading of four PD molecules and the ability to attach four modalities. The resultant fluorophore modified conjugates were shown to retain binding affinity by ELISA and showed excellent stability in blood plasma mimicking conditions by serum stability studies.

Kaposi's Sarcoma-associated Herpesvirus: Understanding host mRNA degradation by SOX and its potential inhibitors (3rd year talk)

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Abstract:

Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiologic agent of Kaposi's sarcoma (KS, the most common HIV-related cancer), primary effusion lymphoma and multicentric Castleman's disease. During the lytic phase of KSHV infection, the host shut-off exonuclease (SOX) plays a critical role in the global and rapid destruction of mRNA transcripts promoting evasion of host immune surveillance mechanisms whilst also enabling redeployment of the replicative machinery for the production of viral proteins. However, the mechanism in which how SOX recognizes and processes RNA transcripts remains incompletely understood.
Using X-ray crystallography, the first structure of a viral nuclease SOX productively bound to a viral pre-miRNA fragment (K2-31) implicated in anoikis was determined [1]. In conjunction with biochemical/biophysical studies, it has been revealed that despite targeting stem loop elements and bulges, SOX-mediated turnover appears nonsequence-specific and that there are no restrictions on the size of the loop/bulge elements incised. In addition, structural studies have highlighted a number of key residues involved in SOX-RNA interactions which are essential for switching SOX from an exo-to endo- RNase.

Finally, based on the interactions stabilizing the SOX-RNA complex and crude docking experiments identified phytic acid and pyranine as potential inhibitors. Following trials with both compounds, it has been possible to co-crystallise SOX with phytic acid and refine the structure at 2.3 Å . This complex reveals that phytic acid is favourably accommodated within the catalytic site region in a configuration that would block RNA binding whilst chelating to metal ions required for catalysis. This work therefore establishes a basis for the design of more potent inhibitors of KSHV-SOX aimed at the future treatment of Kaposi Sarcoma and related pathologies.

[1] Lee, H. et al. KSHV SOX mediated host shutoff: the molecular mechanism underlying mRNA transcript processing. ***Nucleic Acids Research*** (2017) doi: 10.1093lnarlgkw1340

Hydrogen/Deuterium Exchange (HDX) in Arginine side-chains: Quantifying Intramolecular Interactions in Solution (Poster)

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Abstract:
Arginine is an essential amino acid in nature and features in countless enzymatic active sites and protein interaction interfaces. As it bears a guanidinium group with a pK_a of around 12.5, it remains charged across the range of physiologically relevant pH values, essentially allowing nature to place a positive charge at any point in a protein structure. Further, this charge is delocalised over three nitrogen atoms and as such arginine is capable of an impressive range of hydrogen bonds and ionic interactions.
Here, an NMR method is presented to quantify the solvent exchange rates of arginine side-chain $^{1}H^{\varepsilon}$ protons and subsequently characterise the strength of possible interactions. The method employs ^{13}C-detection and utilises the large, one-bond ^{15}N deuterium isotope shift to generate two exchanging resonances in H_2O/D_2O mixtures. Applications to T4 Lysozyme will be shown, where there is a good correlation between the solvent exchange rates obtained and the interactions observed in the crystal structure. It is envisaged that the new method serves as a particularly valuable tool to characterise active sites in enzymes, protein-protein or protein-nucleic acid interactions.

Characterisation of cell-wall peptidoglycan recycling in Mycobacterium tuberculosis (3rd year talk)

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Abstract:

Novel therapeutic targets is the need of the hour to tackle tuberculosis (TB). The mycobacterial cell-wall is a unique structure composed of covalently linked peptidoglycan, arabinogalactan and mycolic acid. While the synthesis of peptidoglycan (PG) has been under investigation (1-3), its recycling, a phenomenon established in Gram-negative and positive organisms, is yet to be reported. Murein peptide ligase (Mpl) recycles recovered peptide stems into the PG monomer in Gram-negative bacteria but it is absent in Gram-positives. An *mpl* orthologue has been identified in the pseudo-Gram positive organism, *M. tuberculosis.* The gene is essential to the bacteria's survival and is conserved amongst other mycobacteria including *M. leprae*. A comparative analysis of the gene from clinical *M. tuberculosis* isolates with known resistance profiles showed natural variation of the gene which is not due to the focussed selective pressure induced by current anti-TB therapy, further validating it as a novel therapeutic target. An *in vivo* interaction network between Mtb-Mpl, regulatory proteins and cell-division proteins has been identified using a mycobacterial protein-fragment complementation assay. Recombinant, His-tagged Mtb-Mpl has been successfully co-expressed with mycobacterial chaperone TigA in *E. coli* and purified to its homogeneity. A non-radioactive, high-throughput, colorimetric assay developed in the lab revealed that Mtb-Mpl can ligate a range of peptides with different lengths. Blocking both *de novo* synthesis and the recycling of PG can severely debilitate the cell-wall metabolism leaving the infectious bacterial pathogen susceptible to immunological and therapeutic attack.

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2 Basavannacharya, C., Moody, P. R., Munshi, T., Cronin, N., Keep, N. H., & Bhakta, S. (2010). Essential residues for the enzyme activity of ATP-dependent MurE ligase from Mycobacterium tuberculosis. Protein & cell, 1(11), 1011-1022

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Reconstituting a mammalian intracellular transport system (1st year talk)

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Abstract:
Coat protein complex II (COPII) mediates the trafficking of a fascinating range of cargoes of different shapes and sizes from the endoplasmic reticulum in the direction of the Golgi apparatus. COPII proteins are highly conserved throughout eukaryotes, and studies in yeast have been instrumental to elucidating the fundamental assembly mechanisms. However, the needs of the mammalian cell are more complex, necessitating higher levels of regulation of COPII assembly. The transport of large cargo, for instance, requires additional protein components, such as the transmembrane proteins TANGO1 and cTAGE5.

Structures of individual COPII components and subcomplexes are available as obtained by X-ray crystallography, but questions remain open: how does the supramolecular arrangement of those building blocks incorporate the diversity of the mammalian cell? How do regulatory factors such as TANGO1 and cTAGE5 modulate COPII assembly?

Here we reconstitute COPII-mediated membrane budding *in vitro* using mammalian COPII proteins expressed and purified from insect cells and mammalian lipid models. Purified COPII components are capable of inducing membrane curvature and generate a range of morphologies as analysed by electron microscopy. We aim to elucidate the structure of COPII formations on membranes using cryo-electron tomography, providing insight into the transport of conventionally-sized cargo. In addition, we aim to structurally characterise the TANGO1 and cTAGE5 adapters and their complex with COPII by single-particle cryo-electron microscopy. Our studies will shed light on the mechanisms of versatile COPII carrier formation.

Synthesis of isoquinoline alkaloids as anti-tubercular agents (1st year talk)

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Abstract:
Tuberculosis (TB), an infection caused by the pathogenic bacillus *Mycobacterium tuberculosis*, is currently the largest infectious cause of death worldwide; an issue exacerbated by the spread of antimicrobial resistance.[1] With few novel compounds in the current drug development pipeline, new antibiotics with novel mechanisms of action are urgently required to keep pace with the emergence of extensively drug-resistant strains.

The isoquinoline scaffold is present in a range of pharmaceutical and biologically active compounds.[2] Recent work has established that several C-1-substituted tetrahydroisoquinolines have antimycobacterial properties.[3] We are currently establishing a structure-activity relationship (SAR) in order to further understand and optimise the observed antimycobacterial potency of this scaffold while minimising mammalian cell toxicity. Early work towards this goal has focussed on the influence of methoxy/hydroxy substitution, aromaticity and chirality. We are also investigating other structural motifs as potential antibacterials and their endogenous modes of action.

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Investigating the interactions of tarantula venom peptide Protoxin-II with $Na_v1.7$
(Poster)

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Abstract:
The voltage-gated sodium channel $Na_v1.7$ belongs to a class of membrane proteins responsible for the propagation of action potentials through the nervous system. It is under investigation as a target for the treatment of chronic pain after the discovery of a key mutation which renders its carriers unable to feel pain. However, inhibitors developed to date typically show low potency and are unable to discriminate between Na_v subtypes, leading to off-target activity and serious side-effects.
Protoxin-II, a peptide isolated from the venom of the Peruvian green velvet tarantula, is a potent inhibitor of $Na_v1.7$ and shows good selectivity over other subtypes – but how the peptide achieves this selectivity, and its mode of binding to $Na_v1.7$, are not yet established.

Our aim is to use synthetic chemistry, computational modelling and crosslinking mass spectrometry to investigate the interaction of Protoxin-II with $Na_v1.7$. In our work to date we have established a protocol for the solid-phase synthesis of Protoxin-II, confirmed the structure of the active form of the peptide and prepared analogues bearing functional handles. These peptides will be used for *in vivo* crosslinking mass spectrometry experiments to generate distance restraints which will inform computational models of the Protoxin-II/$Na_v1.7$ complex.

Unravelling the role of CBF3 in point centromere function in S. cerevisiae
(Poster)

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Abstract:
The kinetochore is a critical multi protein complex of the mitotic machinery, which binds both the centromere DNA of the chromosome and the microtubules from the miotitic spindle. In S. *cerevisiae,* the centromere DNA is short and conserved, and associates with a specialised non-canonical nucleosome, which includes a variant of histone H3 called Cse4. The centromere is specifically recognised by the kinetochore protein complex called CBF3.The CBF3 complex is composed of a homodimer of a zinc cluster protein, Cep3, a homodimer of a DNA binding protein, Ndc10 and a heterodimer of the F-box protein Ctf13 and F-box binding protein Skp1. Genetic studies have determined that the CBF3 is responsible for the recruitment of the specialised nucleosome to the point centromere, but the molecular mechanism of the recruitment is unknown.

We are using a single particle cryo-Electron Microscopy to determine the structure of the CBF3 in complex with the point centromere DNA. The structure will provide insights into how the individual subunits of CBF3 provide specificity for DNA binding and how the complex primes the point centromere for nucleosome recruitment. The structural studies will be complemented using Electrophoretic Mobility Shift Assays, to determine which components of the CBF3 provide affinity for centromere binding.

Single cell imaging comparing mechanical versus biochemical cell competition in epithelial monolayers (1st year talk)

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Abstract:
Cell competition is broadly defined as the elimination of less fit cells from a tissue through either long-range mechanical or short-range biochemical cues. Not much is currently known about how neighbouring cells influence the fate of individual cells at the single-cell level. A detailed characterisation of how cell-cell interactions scale and influence tissue-scale population changes can potentially lead to a greater understanding of field cancerization, and ultimately to the development of anti-cancer drugs.

Here, we develop and utilise long-term fluorescence time-lapse microscopy and machine learning, to quantitively compare mechanical and biochemical competition in MDCK epithelial cell monolayers. Using co-cultures of wild-type cells and cells depleted of the polarity protein scribble, or expressing RasV12, we determine how local density and cell identity affects the outcome of competition experiments. We track the proliferation of winner cells and apoptosis of loser cells in various seeding densities, and analyse, on a single-cell scale, how the neighbourhood in these mixed populations determines the outcome of competition.

Fluorescence studies of the Nuclear Pore Complex (3rd year talk)

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Abstract:
Nuclear pore complexes (NPCs) are large macromolecular machines that play a central role as gatekeepers of nucleo-cytoplasmic transport. Their large size and dynamic nature have impeded a comprehensive functional elucidation and their direct role in diverse diseases that frequently shorten human lifespan.
Optical techniques provided a powerful tool that allowed us to overcome some of those challenges. Hence, part of my PhD has focused on tackling nucleocytoplasmic transport using a statistical approach to observe single events in live cells. I successfully developed a highly sensitive single-molecule imaging assay using the photo-switchable protein mEos fused to ImpB. As this assay enables capturing multiple events in different cells, I acquired ample data monitoring spatial distribution and the turnover kinetics of individual ImpB molecules within intact NPCs in live cells. I observed high heterogeneity of ImpB distribution that strongly correlates with nuclear import efficiency in terms of rate and selectivity. By monitoring bulk import efficiency at the single cell level, I inferred that not all cells have the same functional phenotype due to differences in cell cycle stage.
Current and future work will lead to better understanding of how these essential macromolecular machines function *in vivo* as well as their assembly and disassembly process in real-time and in living cells.

Allosteric modulation of nicotinic acetylcholine receptors examined by computer docking studies with revised α7 homology models (3rd year talk)

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Abstract:
Nicotinic acetylcholine receptors (nAChRs) are pentameric neurotransmitter-gated ion channels that are activated by the binding of agonists to an extracellular orthosteric site. The nAChR α7 subunit forms functional homomeric receptors. Allosteric modulators of α7 nAChRs have attracted a lot of interest, in part due to the receptor's implication in a range of neurological and psychiatric disorders. The pharmacological properties of three positive allosteric modulators (PAMs) have been examined on the human α7 nAChR. The three PAMs selected for this study (A-867744, TBS-516 and TQS) share common features but are representative of chemically distinct families of α7 nAChR PAMs. We have obtained experimental evidence consistent with A-867744 acting *via* a mechanism that is distinct from that of other PAMs. Revised homology models of the human α7 nAChR have been constructed based on the structure of the *Torpedo* nAChR in closed and open conformations, after correcting the assignment of amino acids in the second and third transmembrane helices. Computer docking studies with these revised models suggest that all three PAMs bind at an inter-subunit site but also suggest that the binding site for A-867744 is distinct from that of TBS-516 and TQS. We conclude that the observed pharmacological differences between A-867744 and other PAMs may be a consequence of differences in their binding to the transmembrane region of the α7 nAChR.

Metformin hijacks bacterial nutrient sensing system to extend host lifespan (3rd year talk)

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Abstract:
Metformin is one of the most widely used drugs and the first line treatment of type-2 diabetes. But despite its wide use the mechanisms underlying metformin's action and its exact targets remain a matter of controversy. Recent studies suggest that metformin causes huge changes in patient's microbiota which significantly contribute to its efficacy. Moreover, is has also been shown to extend lifespan in rodents and nematodes in a microbiota dependent way. In Cabreiro lab at UCL we are investigating the interplay between host, its microbiota, diet and metformin using *C. elegans* - *E. coli* model. We have found that in bacteria metformin effects primarily result due to the activation of an ancient nutrient sensing transcription factor CRP. The resulting changes in bacterial metabolism lead to the dietary restriction–like response in the host and ultimately – lifespan extension. Currently, microbiota research focuses on the relative abundance of bacterial species, however, our results show that the effects of drugs can be more settle. Not only this reveals a completely novel mechanism of drug action through microbiota, but it also highlights a possibility of host therapy via microbiota state modulation.

Structure, dynamics and interactions of the von Willebrand Factor C domains
(1st year talk)

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Abstract:
The von Willebrand factor (VWF) is a large multimeric glycoprotein which plays two key roles in normal haemostasis, recruiting platelets to the site of vascular injury and acting as a carrier for blood clotting Factor VIII. The presented research focuses on the six C-terminal C domains of VWF. The structure and function of the C domains are relatively unknown, apart from the known binding site being for GPIIb/IIIa in C4, which aids platelet activation. Cysteine is the most abundant amino acid in these domains, accounting for 14% of residues with each cysteine appearing to be paired in both inter and intrasubunit disulphide bonds, likely making it a key contributor to the VWF structure. Due to their prominence, validating disulhpide bond formation will be a key factor in determining accurate structures of the C domains.

Nuclear Magnetic Resonance (NMR) spectroscopy is a useful technique for structural determination, particularly for proteins such as the C domains, which appear to be too flexible for X-ray crystallography. Through a series of triple resonance experiments, we can establish a secondary structure of the native state, and the tertiary structure by analysing interproton distances (NOEs). We are looking to express all the individual C domains in E. coli, and have so far successfully expressed a ^{13}C-^{15}N labelled C3 sample and conducted a number of double and triple resonance NMR experiments. This talk will outline our expression and purification strategy, how we validated the purification of our desired protein, and our plan going forward to determine the structure and dynamics of multiple C domains.

Structural and functional studies of the BkaR regulon of Mycobacterium tuberculosis (3rd year talk)

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Abstract:
Tuberculosis (TB) remains a major global health problem and there is an urgent need for novel therapies.

The tetracycline repressor (TetR) family regulators are abundant in the *Mycobacterium tuberculosis* genome and control the expression of genes involved in fatty acid metabolism, drug resistance and virulence. Many genes in TetR regulons have been implicated in the survival of the bacteria, making TetR regulators potential drug targets. TetR regulators bind to DNA to repress transcription and are only removed from DNA in the presence of a ligand.

One member of the TetR family in *M. tuberculosis* is the branched-chain keto acid regulator (BkaR), which is thought to control the expression of 12 genes in a regulon. The ligand for BkaR is unknown, but the genes of the BkaR regulon have been shown to be involved in the catabolism of branched-chain amino acids (BCAA); leucine, isoleucine and valine. This regulon has been shown to be important for the survival of the bacteria in macrophages. The physiological relevance of BCAA catabolism in *M. tuberculosis* is unknown, but it is thought that utilisation may be conditional.

The overall goal of this study is to determine the physiological role of the BkaR regulon in *M. tuberculosis*. We aim to use structural and biophysical approaches to characterise the regulation by bkaR and to determine the promoter binding properties. This project also aims to identify the ligand for the regulator and to determine the conditions under which the regulon is expressed.

Towards understanding host-microbe interactions (3rd year talk)

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Abstract:
The gut microbiota can be described as the large community of microorganisms that inhabit a host gastrointestinal tract. Host-microbe interactions have been linked to several aspects of host fitness and disease. However, very little is known about the effect of host-targeted drugs on gut-residing microbes and, consequentially, on the host metabolism. We use the nematode *C. elegans* and its gut microbe *E. coli* to unravel the mechanisms at play between the host, the gut microbe and the chemotherapeutic cancer drug 5-fluorouracil (5-FU). 5-FU is a fluoropyrimidine that inhibits cell division by acting as a uracil analogue that inhibits nucleotide synthesis[1]. Via our simple host-microbe-drug model, we performed high-throughput screens and used metabolomic approaches to reveal that microbes can affect fluoropyrimidine conversion and efficacy in the host[2].

The efficacy of fluoropyrimidines was found to be influenced by bacterial metabolism, including vitamin B_6, B_9 and ribonucleotide metabolism[2]. Furthermore, we have explored the role of dietary cues in host-microbe response to 5-FU. We utilised a novel high-throughput method that allows the screening of hundreds of different nutrients within the host-microbe-drug dynamic. This 4-way method enabled the identification of nutrients that decrease drug efficacy (e.g. sugar compounds, nucleotides). Going forward, we aim to characterise the bacterial dependent and independent mechanisms involved in regulating drug toxicity on the host in the context of these nutrients. Overall, this project reveals the complexity and importance of host-microbe interactions in response to drug treatment and nutrition.

References:
[1] Longley, D.B., et al. Nat. Rev. Cancer (2003)
[2] Scott, A.T., Quintaneiro, L. M., et al. Cell (2017)

The effect of low pH on the aggregation of therapeutic human IgG4 antibody (1st year talk)

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Abstract:
Monoclonal antibodies (mAb) are important therapeutics with sales of $90 billion in 2016. Engineered for efficacy against specific targets, they are used in diagnosis and treatment of increasing numbers of diseases including cancer and arthritis. The most frequently exploited antibody subclasses are human IgG1 and IgG4. During their manufacture, mAbs are susceptible to aggregation. Aggregates lead to loss of product, and may compromise safety, therefore increasing costs. mAb aggregation occurs readily under harsh bioprocessing condition, in particular during viral inactivation at low pH before being returned to a storage buffer. Commonly used storage buffers are phosphate buffer saline (PBS) pH 7 and histidine saline pH 6. Using size exclusion chromatography and analytical ultracentrifugation, we have investigated an IgG4 antibody in histidine saline buffer at pH 3 and then returning it to neutral pH. Aggregation was much reduced in histidine saline buffer compared to PBS. The longer IgG4 was held at low pH before neutralisation, the greater the aggregation. Having showed that buffer conditions affect aggregation rates, we will use small angle X-ray scattering and advanced atomistic modelling to investigate the effect of low pH and neutralisation on the IgG4 solution structure to better understand structural changes that may facilitate aggregation.

Norcoclaurine synthase: Structural studies, enzyme engineering and the biocatalytic synthesis of novel alkaloids (1st year talk)

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Abstract:
(*S*)-Norcoclaurine synthase (NCS), is a "Pictet- Spenglerase" enzyme which catalyses the production of (*S*)-norcoclaurine via the condensation of dopamine and 4-hydroxyphenylacetaldehyde (4-HPAA). (*S*)-Norcoclaurine is the first committed intermediate in the pathway to benzylisoquinoline alkaloids, many of which have pharmacological activities. Recently, recombinant NCS from *Thalictrum flavum* has been found to accept a wide range of aldehydes and ketones as substrates in place of 4-HPAA.[12] This results in the formation of a variety of molecules containing the tetrahydroisoquinoline moiety, a privileged drug scaffold, in the (*S*)-configuration with over 99% enantiomeric excess.

The aim of my project is to further probe the NCS mechanism of action and extend the substrate scope to enable the rational design of the enzyme via mutagenesis and thus the synthesis of novel alkaloids. This has involved the synthesis of transition state mimics for co-crystallisation with NCS and subsequent crystal trials.[3] In addition, investigation into the NCS substrate scope has led to the acceptance of novel substrate types.

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2 Ruff *et al.*, ***Tetrahedron Lett.***, 2012, **53**, 1071–1074.
3 Lichman, Sula *et al.*, ***Biochemistry***, 2017, **56**, 5274–5277.

Gene function prediction in flies and yeast (1st year talk)

Harry **SCHOLES,** Kostas THALASSINOS[1], Jürg BÄHLER[2] & Christine ORENGO[1]

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Abstract:

Longitudinal brain proteomics of a Drosophila melanogaster Alzheimer's disease model

My first project was a longitudinal study of the brain proteome in Alzheimer's disease (AD). Aggressive forms of AD can be inherited through the Arctic mutation in amyloid beta (Aβ42). We analysed the brains of a *Drosophila melanogaster* model of AD carrying Arctic mutant Aβ42 using label-free quantitative ion-mobility mass spectrometry. I identified 228 proteins that are significantly altered in AD, are enriched with AD processes, and have distinct properties in the brain protein interaction network. I have written a paper on this work and will submit it for publication soon.

Gene function prediction using growth phenotypes of fission yeast gene deletion mutants

My main project aims to assign functions to all genes in the fission yeast *Schizosaccharomyces pombe*. We are in the process of collecting quantitative growth phenotypes for all 3570 non-essential gene deletion mutants across 80 conditions. I will then use these phenotypes—alongside other heterogenous data sources, such as networks and gene expression—to train a machine learning model that will predict which Gene Ontology terms should be annotated to each gene.

Structure-function relationships of putative kinesin-8 molecular motors in Plasmodium parasites (Poster)

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Abstract:

Kinesin motor proteins have a variety of cellular functions and many are important during mitosis and cell division. Kinesin-8s have been found to regulate microtubule (MT) dynamics. Inhibition of human kinesins important in mitosis has been investigated as a treatment for cancer, for example kinesin-5 KIF11. In humans, kinesin-8 KIF18A localises to the mitotic spindle and disruption of kinesin-8 function causes disruption of mitotic progression.

The malaria-causing parasites, members of the *Plasmodium* genus, have a complex life cycle where there are stages of rapid and multiple nuclear and cellular divisions and kinesins may be expected to play an important role. Determining how these kinesins function and where and when they are expressed would be interesting to compare characteristics across species to the well-characterised eukaryotic kinesins. The genome of *Plasmodium* parasites only has a repertoire of nine kinesins, and two of these are putative kinesin-8s based on sequence similarity.

We have begun to characterise these two putative kinesin-8s – focusing on their conserved kinesin motor domains - using a variety of biophysical and biochemical assays to probe their activities, including MT-based motility and MT depolymerisation. Initial results indicate that both kinesins are capable of binding microtubules and exhibit depolymerisation activity, measured using co-sedimentation assays and total internal reflection fluorescence (TIRF) microscopy. We aim to study the near-atomic resolution structure of these putative kinesin-8s bound to MTs using cryo-electron microscopy. We have also begun to explore the function of these kinesins in vivo by looking at the expression profile across the parasite life cycle revealing potential splice variants and indications of higher expression during the sexual stages of the life cycle. We also aim to determine their cellular localisation and their knock-out phenotypes enabled by the recent developments in CRISPR-Cas9 for the parasite genome.

How does the ribosome architecture influence co-translational protein folding?
(1st year talk)

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Abstract:
Successful folding of proteins into their biologically functional structure is essential to cellular biological processes. *In vivo* proteins are synthesised on the ribosome and can fold co-translationally. The presence of the ribosome as well as the vectorial emergence of the nascent chain imposes major conformational restraints compared to protein folding in isolation. However, only few high resolution structural studies on co-translational folding exist. Our previous NMR studies have shown that the point at which a protein acquires its fold can be modified by the ribosome; how this occurs is not known.

We have successfully applied CRISPR-Cas9 strategies to generate ribosome variants that we are exploiting as a powerful tool to dissect the effect of the ribosome on nascent chain synthesis and folding. Using this tool, we have initially focused on editing ribosomal tunnel proteins uL23 and uL24, which we and others have previously shown to interact with the nascent chain during synthesis and folding. Both the nature and the role of these interactions are not known. In order to systematically assess these variants, we are developing a biochemical force-based assay that will enable us to rapidly evaluate ribosomal mutations and their impact on the onset of folding of the nascent chain. Ultimately, this rapid assay will allow us to screen and select a focused set of variants for our ongoing complementary high resolution structural studies by NMR and cryo-EM.

Determining isoleucine rotamer populations from chemical shift (Poster)

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Abstract:
We present a method for determining isoleucine rotamer population distributions from carbon chemical shifts. Here each rotamer is defined by both χ1 and χ2 angles providing a near-complete description of the side-chain. This gives a more detailed description of the side-chain dynamics than what is available using scalar-couplings. The readily available nature of chemical shifts allows this method to be applied in situations where long-range scalar-couplings are impossible to obtain. To demonstrate the method's utility isoleucine rotamer distributions were determined from chemical shifts for the DsbB–DsbA complex, a 41 kDa membrane protein, and the L24A FF domain's 'invisible' folding intermediate that exchanges with the ground state.

Scoring structural models using mono-links from chemical crosslinking mass spectrometry (1st year talk)

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Abstract:
Chemical Cross-Linking Mass Spectrometry (XL-MS) is a technique which provides solvent accessible distance restraints for modelling structure of proteins and protein complexes in solution. The experiment produces 4 different types of peptides: cross-links, mono-links, loop-links and unmodified peptides. While cross-links (two peptides joined by a crosslinking reagent) are the most information rich peptides in an experiment, as they convey both solvent accessibility and distance information between two residues, they are also the least abundant. Mono-links (one peptide where the crosslinking reagent has reacted to a single residue) provide only solvent accessibility information, yet they are far more abundant in an XL-MS experiment than cross-links. As yet, there are no defined approaches for utilising the information stored in mono-links to investigate protein structure. In this project, I have been investigating different approaches to scoring protein models using mono-links. Each of these approaches has been tested on a dataset of PDB structures with simulated mono-links.

Unravelling the solution structures of therapeutic antibodies with and without glycans (Poster)

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Abstract:
Immunoglobulin G (IgG) is the most abundant antibody in serum and encompasses four subclasses IgG1-IgG4. Human IgG is important as a biotherapeutic drug with an annual market of >$70 billion. IgG is a Y-shaped structure composed of two Fab regions, which interacts with antigen and the Fc region with two conserved glycans that interacts with FcγR receptors. In order to understand the molecular role of the two glycans in IgG, a multidisciplinary structural approach for monoclonal IgG1 and IgG4 was implemented using mass spectrometry (MS), analytical ultracentrifugation (AUC) and small angle neutron and x-ray scattering (SANS, SAXS). A enzymatic deglycosylation protocol using PNGase F using native MS confirmed the occurrence of non-denaturing deglycosylation. By AUC, the decrease in the sedimentation coefficient from 6.6S for native IgG1 and IgG4 to 6.2S after glycan removal indicated an elongation of the IgG1 and IgG4 structures. By SAXS, concentration dependences in the radius of gyration (Rg) data for IgG1 and IgG4 were observed, and the Rg values increased after glycan removal. Advanced molecular dynamic and Monte Carlo simulations will be used to account for these changes, thus providing a molecular description of the effect of glycan removal on the solution structure of IgG1 and IgG4.

Investigating the structure to function relationship of the Hepatitis C virus envelope glycoprotein 2 during host cell entry (Poster)

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Abstract:
Rational development of a prophylactic vaccine against HCV has been hampered by our poor understanding of the structure to function relationship of the immunodominant entry glycoprotein E2. Evidence suggests that the conserved antigenic core of E2 is shielded from the host adaptive immune response by two hypervariable regions. During virus entry, E2 is likely to go through sequential conformational changes that expose the antigenic core and prime the glycoprotein complex for fusion. These structural transitions are very poorly understood.

To investigate these processes, we are studying the evolution of E2 in the absence and presence of antibody selection. Without antibody selection HCV acquires mutations that alter the structural characteristics of E2, as evidenced by acute sensitivity to antibodies targeting various conserved neutralising epitopes. We hypothesise that these mutations lead to an increased exposure of the antigenic core of E2, which in turn, enhances receptor interactions and increases infectivity.

Starting with the recently published partial structure of E2, we have used Rosetta modelling software to create a complete model of the full E2 ectodomain. We then used molecular dynamic simulations to predict the impact of mutations on the structure and function of the E2 glycoprotein. We hope that a molecular understanding of the conformational states of E2 will further elucidate the mechanisms of HCV entry and aid the design of highly immunogenic vaccine candidates.

Identifying receptors internalised by a novel endocytic pathway using ion-mobility assisted proteomics (3rd year talk)

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Abstract:
Endocytic pathways regulate the uptake of extracellular micronutrients and turnover of plasma membrane components, thus acting as key mediators between the cell and its external environment. The fast endophilin-mediated endocytosis (FEME) pathway is a recently characterised clathrin-independent endocytic route notable for its speed and reliance on receptor-ligand interaction for activation. Active in a range of cell types, the pathway is known to be hijacked by bacterial toxins and is important in signalling and cell motility.

Incomplete knowledge of the receptors which trigger FEME uptake limits understanding of the pathway's functional roles. Thus far, a small number of receptors have been verified as FEME cargoes including G-protein coupled receptors, receptor tyrosine kinases and the cytokine receptor IL-2R, some of which are internalised through cytosolic adaptor complexes. Our work seeks to expand the catalogue of candidate FEME receptors and adaptors using affinity-purification mass spectrometry.

We present progress towards establishing a nanoLC-HDMS^E pipeline to analyse plasma-membrane enriched samples mammalian cell extracts on a Waters Synapt G2-Si instrument, comparing filter-aided, in-gel and on-bead digestion workflows.

Insights into the function of MSP1, the major merozoite surface protein of the malaria parasite Plasmodium falciparum (Poster)

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Abstract:
The protozoan parasite *Plasmodium falciparum* is responsible for over 90% of deaths due to malaria globally. The malarial asexual blood stage life cycle comprises recurring cycles of growth in red blood cells, followed by egress of invasive merozoites which immediately invade fresh cells. These processes involve multiple parasite-host cell interactions, many of which are poorly understood.

Merozoite surface protein 1 (MSP1) is a ~200 kDa GPI-anchored peripheral membrane protein and the most abundant protein on the merozoite surface, suggesting it has a role in host-cell interactions and parasite function. Despite this, the molecular function of MSP1 is unknown. Whilst MSP1 has been postulated to be involved in invasion, recent advances in the genetic manipulation of the parasite have shown that disruption of MSP1 causes an unexpected defect in egress. There is therefore a need to re-examine the function of this important parasite protein.

In this project, several diverse approaches are being used to examine the role of MSP1 in egress and invasion. Electron tomography has been used to examine the morphological state of parasites with and without MSP1, single particle electron microscopy analysis has shown novel structural characteristics of the MSP1 particle, and video light microscopy has provided the first visual demonstration that MSP1 is not essential for red blood cell invasion.

Can Bacillus phage Phi29 be re-engineered phage as a Clostridium phage?
(Poster)

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Abstract:
Overprescription of antibiotics and lack of strain-specificity is indirectly giving rise to antibiotic resistance in hospital and veterinarian environments, spreading to the environment in general. Phage therapy, a process during which bacteriophages are used to eradicate pathogen bacteria in a strain-specific manner, is a cheap and effective solution to the problem. The *Picovirinae* phage Phi29 which infects the Gram-positive bacteria, *Bacillus subtilis*, has the potential to be engineered to target human and animal pathogens such as *Clostridia perfringens*, *Staphylococcus aureus* and *Streptococcus pneumoniae*.

During prohead maturation in *Picovirinae*, capsid proteins assemble around a portal protein through which the terminally protein-tagged linear genome is then translocated whilst the tail then binds to the portal protein. The portal protein of Phi29 is composed of 12 subunits with 12-fold symmetry and makes contact with a structural pentameric RNA, as well as scaffold and capsid proteins during capsid maturation. Given the central role of portal proteins in the assembly and structure of phages, gaining further structural insights of similarities and differences between those of the *Picovirinae* would allow us to expand host specificity in the future through engineering to allow cross-phage chimera formation.

We have recombinantly expressed different homologous portal proteins from *Picovirinae* phages which normally infect different species of pathogenic bacteria. Using insights gleaned from structural studies and bioinformatics we were able to produce a fusion of two portal proteins and hope to create a chimeric phage *Phi29* that infects *C. perfringens* rather than *B. subtilis.*

Site directed mutagenesis reveals plasticity of lipid binding to the groove of the fat depleting factor Zinc a2 glycoprotein (Poster)

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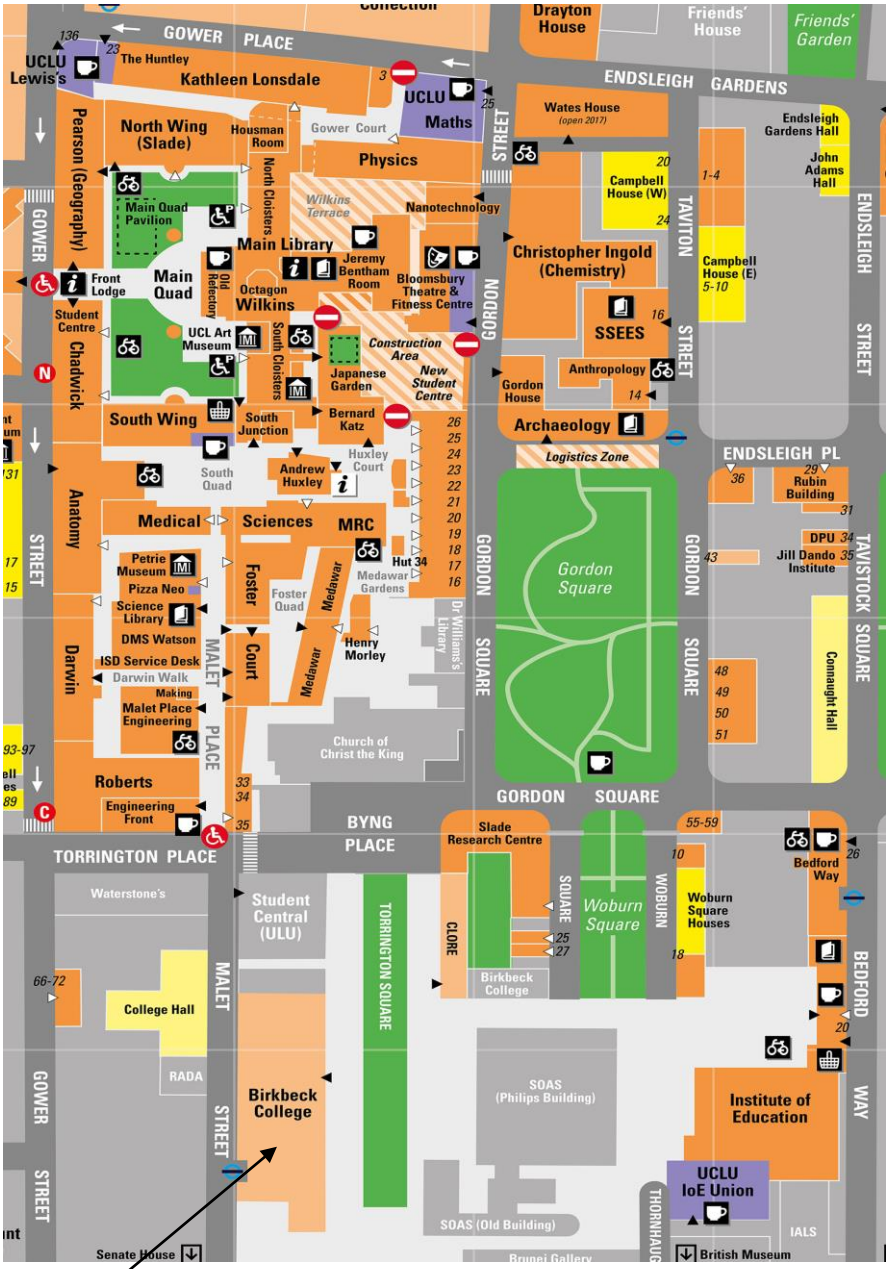
Abstract:
Zinc-α2-glycoprotein (ZAG) is a 42 kDa regulator of body fat mass, implicated in diabetes, obesity and cancer. ZAG has a class I MHC fold and its α1 and α2 domains form a lipid binding groove. The true ligand of ZAG remains unknown. We previously showed that ZAG has at least two distinct fatty acid binding sites with μM affinity for two fatty acid analogues DAUDA and C_{16}-BODIPY. Molecular docking of lipids to the ZAG groove revealed nine ligand contact residues R158, E166, K148, E80, L70, E62, T170, Y13, and A159 that are conserved across nine primate species. To test their involvement in DAUDA and C_{16}-BODIPY binding, we created eight alanine mutants, as well as A159G, R73E, R73A and R158E. All 12 mutants were expressed in E. coli, refolded from inclusion bodies and purified using size exclusion chromatography. Analytical ultracentrifugation and circular dichroism revealed that the mutants were monomeric and folded. Using fluorescence binding assays, we identified residues that enhanced or abolished DAUDA and C_{16}-BODIPY binding, particularly R73A that abolished all ligand binding. We conclude that the entire length of the ZAG groove is active in ligand binding and possesses a range of flexibility to accommodate ligands in more than one conformation.



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