The Institute of Structural and Molecular Biology

ISMB 2024 Postgraduate Research Symposium

Programme & Abstracts

Tuesday 11th and Wednesday 12th June

Institute of Structural and Molecular Biology

ISMB 2024 Postgraduate Research Symposium

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Date: Tuesday 11th June, 9:15am – 2:30pm
Location: JZ Young LT map
G29, Anatomy Building, Gower St., London WC1E 6BT

Talks

9:15 Welcome Tea/Coffee -15 mins – *Available in Gavin de Beer LT*

9:30 Introduction from ISMB Director: Professor Franca **FRATERNALI** - (5 mins)

Session 1: Chair - Dr Kish **ADONI**

9:35 Kirsten **SILVEY**
Characterisation of monomeric isoforms of amyloid-β using solvation-based Nuclear Magnetic Resonance techniques - (10 mins)

9:45 Li **CHENG**
Discovery and rational engineering of phage depolymerases with expanded host ranges - (10 mins)

9:55 Bolaji **COKER**
Quantifying the impact of mutations on the protein-protein interaction network of Mycobacterium tuberculosis using in silico tools - (10 mins)

10:05 Zijie **DAI**
Probing the structural dynamics of human islet amyloid polypeptide using cyclic ion-mobility mass spectrometry - (10 mins)

10:15 Ireno **DEMMANGNGEWA**
Structural characterisation of co-translational folding H-Ras intermediates by 19F NMR - (10 mins)

10:25 Christopher **DULSON**
Targeting the RNA polymerase of African Swine Fever Virus for Drug Discovery - (20 mins)

10:45 Tea/Coffe Break -15 mins - *Available in Gavin de Beer LT*

Date: Tuesday 11th June, 9:15am – 2:30pm
Location: JZ Young LT map
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Talks

Session 2: Chair – Dr Dorothy ABOAGYE-MENSAH

11:00 Ailsa **GAVAN MCHARG**
Structural and Functional Characterisation of the Replication Complex of Hepatitis C Virus - (10 mins)

11:10 Trupti **GORE**
Using Deep Learning for Sequence-based TCR-MHC Binding Prediction - (20 mins)

11:30 Pokchut **KUSOLKUMBOT**
Production in the algal chloroplast of oral subunit vaccines and bacteriophage lytic proteins against pathogens in the aquaculture and poultry industry - (20 mins)

11:50 Comfort break - (10 mins)

GUEST SPEAKER

12pm-1pm Tanmay **BHARAT**
Structural Cell Biology of Prokaryotic Cell Surfaces Using Correlative Imaging Techniques - (60 mins)
Hosted by: Magnus **BLOCH** and Alana **COWELL**, Birkbeck postdoctoral researchers.

1pm- 2:30pm
Poster session/Lunch - (90 mins) in Gavin de Beer LT

Date: Tuesday 11th June, 9:15am – 2:30pm
Location: Anatomy Gavin de Beer LT map
G04, Anatomy Building, Gower St., London WC1E 6BT

Poster Session

(Lunch available)

Rinad **ALHEDAITHY**	*Synthesising ncAA-containing recombinant proteins in the chloroplast of Chlamydomonas*
Charalampos **BOUTSELAKIS**	*Missense variants in the Catalogue of Somatic Mutations (COSMIC) reveal recurrent neoantigens in a multitude of cancer types*
Samadrita **CHATTERJEE**	*Developing a kinase optimized meta-predictor to detect the implication of pathogenic mutations in diseases*
Chris **DANIEL**	*Investigating carprofen's interference with regulatory mechanisms of iron acquisition and homeostasis in mycobacteria*
Julian **HERRERA BRAGA**	*Towards the next generation of variant impact predictors using deep learning and multiomics data*
Charity **HORNBY**	*Predicting Alpha-1-Antitrypsin co-translational intermediates*
Yuting **LI**	*How do genetic variants in complement factor H and complement C3 lead to rare kidney inflammatory diseases*
Reuben **MARTIN**	*Quantum Mechanics for High-Throughput Drug Discovery*
Peter **POLGAR**	*Investigating regulatory mechanisms in mycobacteria*
Daowen **YIN**	*Self-assembly and Partner-protein Interactions Regulating Clathrin Functions*

Date: Wednesday 12th June, 9:15am 1:30pm
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Talks

9:15 Welcome Tea/Coffee **-**15 mins – *Available in Gavin de Beer LT*

9:30 Introduction from HoD Structural Molecular Biology:
Prof Snezana **DJORDJEVIC** - (5 mins)

Session 1: Chair - Dr Elena **PLESSA**

9:35 Terence **HO**
Investigation of the Mechanism of Relaxase in Type IV Secretion System of Bacterial Conjugation - (10 mins)

9:45 Augusto **LI**
How the Archaeal Virus SSRV1 Hijacks the Host RNA Polymerase - (10 mins)

9:55 Hao **LUO**
Observing and manipulating R-loop formation at the single-molecule level - (10 mins)

10:05 Katherine **MORLING**
Design, synthesis and characterisation of cofactor-mimicking HIV-1 capsid inhibitors - (20 mins)

10:25 Alice Jane **PETTITT**
An integrative characterisation of proline cis and trans conformers in a disordered peptide - (20 mins)

10:45 Sarah **LOWEN**
Characterising the alpha-1-antitrypsin pathological polymer by NMR spectroscopy - (20 mins)

11:05 Jennifer **STIENS**
Making 'antisense' of the PhoPR two-component system in the Mycobacterium tuberculosis complex – (20 mins)

11:25 Tea/Coffee break - (20 mins) – Available in Gavin de Beer LT

Date: Wednesday 12th June, 9:15am 1:30pm
Location: JZ Young LT map
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Talks

Session 2: Chair - Dr Oriol Gracia I CARMONA

11:45 James **SWEET-JONES**
Computational Developability Triaging of Antibody Libraries for Discovery of New Therapeutics - (20 mins)

12:05 Mollie **VIRGO**
Different modes of interbacterial competition elicit distinct host responses and health outcomes - (20 mins)

12:25 Tina **WRIGHT**
A comparison of human and mammalian Surfactant Protein B, sequences and structures as predicted by AlphaFold 2 - (10 mins)

12:35 Songlin **XUE**
Investigation of Conjugation Pilus Biogenesis - (10 mins)

12:45 Luyao **YANG**
Oral vaccines by design: chloroplast engineering in the green alga Chlamydomonas for production of double-stranded RNAs - (20 mins)

13:05 Tracy **ZHAO**
Identification and characterisation of CoA S-transferase(s) - (10 mins)

13:15 Prizes/Closing comments - (5 mins)

13:20 **Symposium Ends**

Abstracts
Last name order – A-Z

Synthesising ncAA-containing recombinant proteins in the chloroplast of Chlamydomonas (Poster)

Rinad **ALHEDAITHY**[1], Harry JACKSON[1] and Saul PURTON[1]

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Lab: Saul Purton Lab

Abstract:
Genetic code expansion aims to reassign codons to incorporate non-canonical amino acids (ncAA) at specific sites in a polypeptide chain for the chemical diversification of the target protein. In nature, the full potential of protein functionality has not been unlocked due to the limited set of 20 proteogenic amino acids; however, ncAAs offer a diverse set of building blocks to broaden a protein's chemical repertoire. The incorporation of a ncAA at a specific site in a target protein relies on a genetic system that has an unused codon that can be reassigned to the ncAA, an orthogonal pair comprising a tRNA specifically for the ncAA that recognises the codon together with the cognate aminoacyl tRNA synthetase (aaRS), and the presence of the ncAA itself within the system.

Chlamydomonas reinhardtii is a model microalga that is genetically tractable, allowing it to be used as a light-driven platform for the manufacturing of recombinant proteins. The chloroplast is particularly suited for genetic engineering using ncAAs due to its tiny chloroplast genome where none of the endogenous genes overlap or undergo RNA editing, and DNA integration occurs via homologous recombination. Importantly, the chloroplast genome does not use the UGA stop codon, allowing for its reassignment. Using synthetic biology techniques, elements of the ncAA-incorporating machineries can be engineered into the chloroplast to synthesise novel recombinant proteins in C. *reinhardtii*. I will present my research into the expression of different ncAA tRNA/aaRS pairs in the chloroplast, and the engineering of a pathway for biosynthesis *in situ* of the 22nd amino acid, pyrrolysine.

GUEST SPEAKER*: Structural Cell Biology of Prokaryotic Cell Surfaces Using Correlative Imaging Techniques* – (60 mins)

Tanmay **BHARAT**

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Abstract:
Our laboratory studies cell surfaces of prokaryotes across scales at the atomic, molecular, cellular and multicellular levels. Surface molecules mediate cellular interactions with the environment and play important roles in key processes including cell adhesion, biofilm formation and antibiotic resistance of pathogenic bacteria. In my talk, I will show how we are using sub-tomogram averaging to derive high(3 Å)-resolution structures of cell surface molecules from whole cells, as well as FIB-milled multicellular communities. Time permitting, I will also show how we are combining cryo-EM with mass spectrometry to derive detailed chemical information about our target molecules, leading to biological insights about how prokaryotic cell surfaces are organised, thus illuminating their role in shaping interactions of cells with their surrounding environment.

Missense variants in the Catalogue of Somatic Mutations (COSMIC) reveal recurrent neoantigens in a multitude of cancer types (Poster)

Charalampos **BOUTSELAKIS**

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Lab: Irene Nobeli Lab

Abstract:

Neoantigens are tumour specific peptides, that are presented on the surface of cancer cells by the major histocompatibility complex and can act as potential biomarkers for tumour immunotherapy. In recent years neoantigens have been utilised in a personalised therapy fashion in many clinical trials against cancer; the process is computationally expensive and the selection of successful candidates difficult to achieve. In this pilot project we use the large set of mutations in COSMIC to extract mutant peptides and predict which of them will bind a set of representative HLA alleles. We have devised a computational pipeline to predict these putative shared neoantigens to create a list based on mutation recurrence and mutation prevalence in the wider population. This narrow list can act as a guidance, supplement and facilitate further research into the subject.

References

Blanc, E., Holtgrewe, M., Dhamodaran, A., Messerschmidt, C., Willimsky, G., Blankenstein, T., & Beule, D. (2019). Identification and ranking of recurrent neo-epitopes in cancer. *BMC Medical Genomics*, *12*(1), 171. https://doi.org/10.1186/s12920-019-0611-7

Cafri, G., Yossef, R., Pasetto, A., Deniger, D. C., Lu, Y.-C., Parkhurst, M., … Rosenberg, S. A. (2019). Memory T cells targeting oncogenic mutations detected in peripheral blood of epithelial cancer patients. *Nature Communications*, *10*(1), 449. https://doi.org/10.1038/s41467-019-08304-z

Fouad, Y. A., & Aanei, C. (2017). Revisiting the hallmarks of cancer. *American Journal of Cancer Research*, *7*(5), 1016–1036. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/28560055

HLA allele frequencies and reference sets with maximal population coverage – IEDB Solutions Center. (n.d.-b). Retrieved November 16, 2020, from https://help.iedb.org/hc/en-us/articles/114094151851-HLA-allele-frequencies-and-reference-sets-with-maximal-population-coverage

Jiang, T., Shi, T., Zhang, H., Hu, J., Song, Y., Wei, J., … Zhou, C. (2019, September 6). Tumor neoantigens: From basic research to clinical applications. *Journal of Hematology and Oncology*. BioMed Central Ltd. https://doi.org/10.1186/s13045-019-0787-5

Developing a kinase optimized meta-predictor to detect the implication of pathogenic mutations in diseases (Poster)

Samadrita **CHATTERJEE[1],** Paul ASHFORD[1] , Vaishali P. WAMAN[1], Andrew C.R. MARTIN[1] & Christine ORENGO[1]

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Lab: CATH Lab

Abstract:
Mutations in kinases play a significant role in the development and prognosis of cancer. A study has shown that family-based predictors outperform generic predictors. Our study is aimed at developing a kinase optimized meta-predictor to determine the pathogenicity of mutations in kinases of human proteome. VariPred, an in-house predictor exploits protein sequence embeddings derived from protein language models and has been shown to outperform other state-of-the-art pathogenicity predictor whilst depending only on a single sequence feature. We developed a meta-predictor by combining other state-of-the-art predictors namely Alphamissense and Polyphen-2 with VariPred and testing on a dataset from Humsavar. Logistic Regression, Multi Layer Perceptron, Random Forest, CatBoost, XGBoost are the 5 classifiers that have been used to build the model and are tested on the dataset. It is found that Alphamissense combined with the original VariPred gave MCC of **0.6815** with XGBoost and an MCC of **0.6728** with CatBoost as the classifier. This is higher than individual performance of Alphamissense**(MCC=0.6656)** and VariPred**(MCC=0.610)** when tested on the dataset. Further, VariPred shall be optimized for kinases and combined with other state-of-the-art predictors namely Alphamissense, 3CNet,Polyphen-2 for building a kinase optimized meta-predictor. This predictor shall be applied to detect the mutations involved in renal cancer by using TRACERx data from Dr. Charles Swanton's group.

Discovery and rational engineering of phage depolymerases with expanded host ranges (1st year talk)

Li **CHENG**[1], Yunlong HUO[1], Shaun KANDATHIL[1], David JONES[1] & Joanne Santini[1]

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Lab: Jo Santini Lab

Abstract:
Klebsiella pneumoniae is classified as a critical multidrug-resistant (MDR) pathogen, capable of producing diverse protective polysaccharides (e.g. capsule polysaccharides) that are crucial for its pathogenicity and multidrug resistance. To tackle these protective barriers, the phage-derived depolymerase – polysaccharide degrading enzyme, has been regarded as a promising therapeutic agent. However, the natural phage depolymerase exhibits high specificity for certain types of polysaccharides, which limits their effectiveness against a broad spectrum of bacterial infections. Here, this research aims to rationally engineer the phage depolymerases with expanded host ranges by taking advantage of computational tools including machine learning (ML). To achieve this, depolymerases from publicly available *Klebsiella* phage genomes will be collected together with data from our lab phage collection as input for ML. A high-throughput screening platform using *Escherichia coli* will be developed. It is anticipated that this research will bring new insights into phage depolymerase function providing the groundwork for their therapeutic use.

Quantifying the impact of mutations on the protein-protein interaction network of Mycobacterium tuberculosis using in silico tools (1st year talk)

Bolaji **COKER**[1] & Irilenia NOBELI[1]

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Lab: Irilenia Nobeli Lab

Abstract:
An estimated 1.3 million people died from tuberculosis in 2022, making it the second most common infectious disease-related cause of death after COVID-19 [1]. The capacity of the bacteria to mutate while remaining highly infectious has limited the efficacy of TB medications and the one vaccine, Bacille Calmette-Guérin [2]. In addition, mutations in the Mycobacterium tuberculosis complex are responsible for differences in pathogenicity, host specificity and adaptation to ecological niches. Although mutations may affect directly a single protein, their effect is propagated through interaction networks. In this project, we aim to explore publicly available sequence and structural information using in silico approaches to shed light on how observed mutations may be affecting the protein-protein interaction networks of M. tuberculosis.

Here, I will present the first steps towards achieving this goal. I will describe the creation of a database of dimers using data from the STRING database, the prediction of three dimensional structures of the dimers using Colabfold and the mapping of Single Nucleotide Polymorphisms (SNPs) from the CRyPTIC consortium of tuberculosis mutations onto these structures. I will also present some preliminary results on the assessment of these predicted structures using homologue information from the PDB [3]. Finally, I will present my plans for analysing the impact of SNPs on the dimers and the PPI network as a whole.

References:
1. WHO website. https://www.who.int/news-room/fact-sheets/detail/tuberculosis. Accessed 14th May 2024.
2. Grange JM, Gibson J, Osborn TW, Collins CH, Yates MD. What is BCG? Tubercle 1983;64:129-39.
3. H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, The Protein Data Bank (2000) *Nucleic Acids Research* 28: 235-242 https://doi.org/10.1093/nar/28.1.235.

Probing the structural dynamics of human islet amyloid polypeptide using cyclic ion-mobility mass spectrometry (1st year talk)

Zijie **DAI**[1], Konstantinos THALASSINOS[1, 2] & Daniel RALEIGH[1, 3]

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Lab: Kostas Thalassinos Lab

Abstract:
Human islet amyloid polypeptide (hIAPP), a 37-residue pancreatic hormone, plays an adaptive role in metabolic regulation under normal physiological conditions but converts through an unknown mechanism into insoluble extracellular pancreatic amyloid fibrils, significantly implicating it in the pathogenesis of Type-2 Diabetes (T2D). It is now widely accepted that the early oligomeric states of hIAPP are key to further hIAPP aggregation and cytotoxicity. The hIAPP aggregation mechanism, the structures of the cytotoxic oligomers and the origin of the unique amyloidogenicity and toxic effects of hIAPP variants, however, remain poorly understood. This knowledge gap is primarily due to the limitations of conventional techniques in characterising the heterogeneous and transient nature of early oligomeric species.

Here we employ state-of-the-art Cyclic Ion Mobility Mass Spectrometry (cIMMS) combined with electron-capture dissociation to address these challenges. This methodology enables a detailed examination of the effects of hIAPP sequence variants on protein conformation, dimerisation interface, and oligomeric stability. We compare the amyloidogenicity and propensity of different variants to form hetero-oligomers, which are key factors in understanding the earliest oligomeric intermediates that form on-pathway to hIAPP fibril. Our findings offer new insights into the molecular underpinnings of hIAPP aggregation dynamics and illuminate pathways that may contribute to the amyloid-associated pathology in T2D.

Investigating carprofen's interference with regulatory mechanisms of iron acquisition and homeostasis in mycobacteria (Poster)

Chris **DANIEL**[1], Sam WILLCOCKS[1,2], Helen C. HAILES[3] & Sanjib BHAKTA[1]*

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Abstract:
Antimicrobial resistance (AMR) is a global concern, with an estimated death toll of 10 million people per year by 2050[1]. *Mycobacterium tuberculosis* is the causative agent for tuberculosis and is one of the biggest contributors to AMR. *Mycobacterium abscessus* is an intrinsically resistant non-tuberculous mycobacteria gaining attention for causing severe respiratory infections in immunocompromised patients with diseases such as cystic fibrosis, asthma and COPD. However, infections in immunocompetent patients are now being reported[2]. The development of new drugs and/or repurposing of approved drugs is a priority. Carprofen is a non-steroidal anti-inflammatory carbazole approved for use in humans and shows bactericidal against *M. tuberculosis*[3]. Research has shown that carprofen disrupts the membrane potential in both *Mycobacterium smegmatis*[4] in *Staphylococcus pseudintermedius*[5] resulting in death. Carbazoles have also been found to interfere with iron acquisition in *Plasmodium*[6]. We have identified that carprofen also kills *M. abscessus* at higher concentrations than *M. tuberculosis* but is 8 times more potent in iron-deficient conditions *in vitro*. Three regulatory genes, *tetR*, *lsr2* and *ideR*, associated with iron acquisition and homeostasis have been silenced using CRISPRi to evaluate their drug susceptibility profiles. Interestingly, *Δlsr2* mutant has shown to be 4 times more susceptible to carprofen. This could be due to *lsr2* protecting mycobacteria from reactive oxygen intermediates[7] which NSAIDs and carbazoles have been reported to induce. However, the molecular interactions have to be investigated further to validate carprofen's interference with Lsr2-mediated regulatory mechanisms of iron homeostasis in mycobacteria. This research further endorses *lsr2* to be a promising therapeutic target in mycobacteria.

References:

[**1**] Murray, C. J. *et al. The Lancet* **6736**, (2022).
[**2**] Qin, L. *et al. Frontiers in Cellular and Infection Microbiology* **13**, 1229298 (2023).
[**3**] Guzman, J. D. *et al. BMJ Open* **3**, 1–13 (2013).
[**4**] Maitra, A. *et al. Journal of Antimicrobial Chemotherapy* **75**, 3194–3201 (2020).
[**5**] Magnowska, Z. *et al. Scientific Reports* **9**, 1–14 (2019).
[**6**] Wang, W. *et al. International Journal for Parasitology: Drugs and Drug Resistance* **7**, 191–199 (2017).
[**7**] Colangeli, R. *et al. Proceedings of the National Academy of Sciences* **106**, 4414–4418 (2009).

Structural characterisation of co-translational folding H-Ras intermediates by 19F NMR (1st year talk)

Ireno **DEMMANGNGEWA,** Shahzad BASHIR, Julian STREIT, Lisa CABRITA, John CHRISTODOULOU & Sammy CHAN

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Lab: Co-translational folding protein group

Abstract:

Co-translational folding can occur during protein synthesis, presenting a complex interplay of effects between the ribosome and the nascent polypeptides chain that gradually emerges from the ribosome exit tunnel[1]. The folding pathway, energetics, and outcomes are therefore distinct to those found in *in vitro* refolding studies[2]. We have found that the oncogenic protein HRAS fails to refold to its functionally active conformation in vitro, indicating that co-translational folding is critical to its activity. To examine its co-translational folding, we have examined site-specifically labelled ribosome-nascent chain complexes of HRAS by ^{19}F NMR spectroscopy and identified the population of two stable folding intermediates that are not found in isolation and only detectable by ^{19}F-labelling. A structural characterisation of intermediates requires multiple probes that report on the local structure; we have found the ^{19}F chemical shift to be dominated by ring current effects. We are therefore using protein engineering and molecular dynamics simulations to introduce ^{19}F-labelling sites that report on local ring currents, permitting the acquisition of valuable information on the structures of the folding intermediates on the ribosome, including the formation of secondary structure (i.e.. α-helix, β-strand) during co-translational folding. A detailed characterisation of the co-translational folding intermediates will provide insights into how the ribosome regulates the formation of functionally active protein.

References:

1. Chan, S.H.S., Włodarski, T., Streit, J.O. et al. The ribosome stabilizes partially folded intermediates of a nascent multi-domain protein. Nat. Chem. 14, 1165–1173 (2022).
2. Waudby, C. A., Dobson, C. M., & Christodoulou, J. (2019). Nature and Regulation of Protein Folding on the Ribosome. In Trends in Biochemical Sciences (Vol. 44, Issue 11, pp. 914–926). Elsevier Ltd. https://doi.org/10.1016/j.tibs.2019.06.008

Targeting the RNA polymerase of African Swine Fever Virus for Drug Discovery (Final year talk)

Christopher **DULSON,** Gwenny CACKETT, Simona PILOTTO, Michal SYKORA & Finn WERNER

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Lab: Finn Werner Lab

Abstract:
African Swine Fever Virus (ASFV) causes a lethal haemorrhagic fever in domestic pigs and has been reported in 80 countries since 2005. There are no approved antiviral drugs or effective vaccines, therefore infections continue to threaten global food security. ASFV produces its own 8-subunit viral RNA polymerase (RNAP) that is required for viral transcription within the cytoplasm of the infected cell. The essential role provided by the RNAP makes it a key target for drug discovery. To facilitate the discovery of specific inhibitors from within a low biosafety containment laboratory, the RNAP was produced recombinantly via a baculovirus expression system. The purified recombinant RNAP was transcriptionally active in vitro and the optimal performance over ranges of temperature, pH, ionic strength, divalent cation concentration and with different DNA templates, were determined by quantification of α-^{32}P-UTP incorporation into the RNA transcription product. The optimised conditions were then used to develop a high-throughput assay for the detection of the RNA product by interaction with RiboGreen dye which induces fluorescence. A range of compounds that inhibit polymerases and ATPases were screened for inhibition of ASFV RNA polymerase, which led to the discovery of novel inhibitors for the ASFV RNA polymerase.

Structural and Functional Characterisation of the Replication Complex of Hepatitis C Virus (1st year talk)

Ailsa **GAVAN MCHARG[1],** Gabriella HELLER[1], Greg TOWERS[2] & Jerome GOUGE[1]

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Lab: Gouge Lab and the XDT Lab

Abstract:
Hepatitis C virus is a major public health burden, with chronic infections leading to cirrhosis and hepatocellular carcinoma. The development of direct-acting antiviral agents has paved the way for major advances in HCV treatment; available drugs inhibit specific proteins vital for viral replication. Despite this success, resistant-associated mutations are emerging, and the treatment costs remain. With a limited understanding of the mechanisms governing RNA replication in HCV, the exact molecular mechanism of many of these inhibitors remain elusive. This project aims to assemble and characterise two critical components of the HCV replication complex: the nonstructural protein 5B (NS5B) RNA polymerase and its partner, the highly dynamic nonstructural protein 5A (NS5A). Molecular reports support a direct interaction that influences HCV replication but the underpinnings of how these proteins interact in the replication complex remains unknown. To investigate this, the NS5B-NS5A complex was recombinantly expressed and purified in insect cells using the BigBAC-baculovirus expression system. A successful purification protocol was developed that demonstrated NS5A-NS5B could be isolated as a complex, verifying a critical HCV protein interaction. Ongoing work utilising cryogenic-electron microscopy (cryo-EM) will provide a detailed structural map of the NS5B-NS5A replication complex, towards a molecular understanding of RNA synthesis in HCV.

Using Deep Learning for Sequence-based TCR-MHC Binding Prediction (Final year talk)

Trupti **GORE**[1], Justin BARTON[1], Michele MISHTO[2,3] & Adrian SHEPHERD[1]

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[2] Centre for Inflammation Biology and Cancer Immunology (CIBCI) & Peter Gorer, Department of Immunobiology, King's College London (KCL), London, UK
[3] Francis Crick Institute, London, UK

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Lab: The Shepherd Group

Abstract:
CD8+ T cells are key components of the adaptive immune system, playing crucial roles in targeting intracellular pathogens and in tumour surveillance. T cell immune function is mediated by the T cell receptors (TCRs) on their surface, which bind to complexes formed between antigenic peptides and MHC molecules. An individual's TCRs are highly diverse; many millions of unique TCR sequences have been identified (e.g. via single-cell sequencing experiments), but the antigenic targets of such TCRs are unknown in all but a tiny fraction of cases.

The aim of this research is to predict the potential antigenic targets of T cells from their TCR sequences. This can be decomposed into two complementary sub-tasks that focus on TCR binding to the antigenic peptide and to the MHC molecule respectively. Here the focus is on the latter.

MHC molecules are highly polymorphic in humans, and analysis of TCR-peptide-MHC structural complexes indicates that there is considerable variation in the pattern of contacts formed between MHC molecules and the six variable (CDR) loops of a TCR. In this research, this challenging function prediction task has been addressed using state-of-the-art deep learning methods, notably protein language models with transformer architectures.

Towards the next generation of variant impact predictors using deep learning and multiomics data (Poster)

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Abstract:
The classification of missense variants regarding their consequences to pathogenicity is a complex task. The presence of variants does not necessarily indicate pathogenicity. Typically, sequence conservation and variant incidence are features used in variant impact predictors, but these are often inconclusive. Features derived from other levels of biological complexity such as transcription, protein structure, dynamics, protein abundance and interactions could be informative, but they have not been systematically integrated and applied in variant impact prediction. The aim of this research is to investigate the application of machine learning to a multiomic database covering these new features to achieve predictive capabilities of missense variant impact. I am currently building an ontology-based multiomics graph database in Neo4J using BioCypher framework with the aim to integrate common variations data from gnomAD and pathogenic variations from ClinVar, and investigate the contribution of transcriptomics, proteomics and interactomics data in discerning variant impact. I have investigated, as a case study, protein structural data, by extracting features which distinguish physiological protein dimeric complexes from artefacts of X-ray crystallographic experiments. Using a simple logistic regression model, I found that hydrophobicity of residues alone contains good signal (AUC = 0.745) to identify physiological interfaces. Recently, I have improved the integration pipeline and added more data sources, such as transcriptomics data from GTex, protein models from AlphaFold and protein residue contact networks. In parallel, I am exploring machine learning models applied to the multiomics database with the goal of classifying damaging variations.

Investigation of the Mechanism of Relaxase in Type IV Secretion System of Bacterial Conjugation (1st year talk)

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Abstract:
The project aims to elucidate the complex processes involved in bacterial genetic exchange via conjugation. This biological phenomenon significantly impacts bacterial evolution by enabling the transfer of genetic elements, such as antibiotic resistance genes. Central to this project is the investigation of the relaxase enzyme, which plays a crucial role in initiating and mediating the transfer of DNA during conjugation. Relaxase interacts with the origin of transfer within the plasmid DNA, executing a nicking reaction to initiate the transfer and remaining attached to the DNA throughout the process.

This study employs a combination of molecular biology, biophysical, and structural techniques to explore the interactions of relaxase within the Type IV Secretion System (T4SS). The project focuses particularly on how relaxase is processed to and after entry into the recipient cell, an area that remains poorly understood despite its importance in the conjugation process. By using advanced methods such as cloning, crosslinking mass spectrometry, and cryo-electron microscopy, the project seeks to provide detailed insights into the structural and functional mechanisms of relaxase, including its interactions with the T4SS and other conjugative proteins.

Predicting Alpha-1-Antitrypsin co-translational intermediates (Poster)

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Abstract:
Nascent polypeptides can begin to fold during translation on the ribosome, but this process can be imperfect and lead to misfolding which for alpha-1-antitrypsin (AAT) when it misfolds can result in self-assembly and is implicated in the human disease, alpha-1-antitrypsin deficiency (1). However, a high-resolution structure of AAT on the ribosome has not been solved through experimental techniques due to its size and flexibility so using molecular dynamics to simulate a ribosome-nascent chain complex of AAT we can predict the structures of the intermediates that AAT forms on the ribosome. We validate the ensemble against protection factor data predicted from PEG-accessibility experiments. These intermediates are implicated in assembly of AAT polymers on the ribosome and so molecular dynamics will also be used to model the assembly of an AAT polymer on the ribosome to better understand the earliest stages of polymerisation.

References:
Plessa, E., Chu, L.P., Chan, S.H.S. *et al.* Nascent chains can form co-translational folding intermediates that promote post-translational folding outcomes in a disease-causing protein. *Nat Commun* **12**, 6447 (2021). https://doi.org/10.1038/s41467-021-26531-1

Production in the algal chloroplast of oral subunit vaccines and bacteriophage lytic proteins against pathogens in the aquaculture and poultry industry (Final year talk)

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Abstract:

The chloroplast of the green microalga *Chlamydomonas reinhardtii* offers various advantages for the sustainable production of recombinant proteins. The alga is Generally Recognized as Safe (GRAS), and the chloroplast compartment represents an enclosed system that allows transgene expression to a high level and correct protein folding. Two viral pathogens were selected for the production of subunit vaccines in the chloroplast: 1. Infectious Spleen and Kidney Necrosis Virus (ISKNV) causes a severe pandemic in fish. ISKNV infection results in epidermal lesions with significant petechial haemorrhages and induces fish cell death by causing a pro-apoptotic process. 2. Infectious Bursal Disease Virus (IBDV) is an acute and severe immunosuppressive disease that infects 3- and 6-week-old birds. IBDV infects and damages IgM-containing B-lymphocytes in the Bursa of Fabricius (BF). In addition, a novel bacteriolytic enzyme (endolysin) was selected that targets *Streptococcus suis* and *Streptococcus agalactiae*; two bacteria that are lethal to livestock. All three proteins were successfully produced in the algal chloroplast. The algal strain used in these studies is *C. reinhardtii* CC-4033: ptxD which is engineered to contain a bacterial gene that allows selective growth of the strain in media containing phosphite. The strain also loses chlorophyll when cultures are transferred from the light to the dark. These two features were chosen to reduce scale-up costs and improve palatability. Furthermore, a novel method to resolve downstream processing costs is to use the whole algal cell as the product, allowing vaccine encapsulation in the dried algae and formulation into aquaculture or poultry feed. Moreover, a codon reassignment strategy results in the biocontainment of the transgenes in the chloroplast and prevents their functional transfer to other microorganisms. The effectiveness of these strains for oral delivery of vaccines and anti-bacterials is currently being explored in collaboration with research groups in Thailand.

How the Archaeal Virus SSRV1 Hijacks the Host RNA Polymerase (1st year talk)

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Abstract:
Archaeal viruses are unusual and distinct from bacteriophages and eukaryotic viruses; they do not encode viral RNA polymerases (RNAPs) and rely on host RNAPs and factors (TBP, TFB, TFE) to transcribe viral genes[1].

This provides an intriguing *conundrum*: How do archaeal viruses usurp host transcription, and what is archaeal viruses' overall gene expression strategy?

Saccharolobus solfataricus rod-shaped virus 1 (SSRV1) encodes a viral homolog of (v)TFB[3]. Studying the function of vTFB provides insights into mechanisms of how SSRV1 persuades the host RNAP to transcribe the virus instead of host promoters.
To shed light on vTFB, we follow a multidimensional approach: First, we characterised the transcriptome of SSRV1 using RNA-seq and capable-seq. *Secondly*, we have prepared recombinant vTFB and using Electrophoretic Mobility Shift Assays (EMSA) and *in vitro* transcription assays tested the formation and activity of transcription pre-initiation complexes (PICs) on a selection of viral SSRV1 and host promoters. *Thirdly*, we have overexpressed vTFB in *Sulfolobus acidocaldarius*, which significantly impacts cell growth.

Our results demonstrate a limited degree of vTFB promoter specificity and a surprising level of redundancy with host TFB1. We propose a model by which vTFB supports virus promoter transcription while inhibiting host transcription by forming inactive complexes.

References

1. Pilotto, S. & Werner, F. How to Shut Down Transcription in Archaea during Virus Infection. *Microorganisms* 10(2022).
2. Sheppard, C. & Werner, F. Structure and mechanisms of viral transcription factors in archaea. *Extremophiles* 21, 829-838 (2017).
3. Baquero, D.P. et al. New virus isolates from Italian hydrothermal environments underscore the biogeographic pattern in archaeal virus communities. *ISME J* 14, 1821-1833 (2020).

How do genetic variants in complement factor H and complement C3 lead to rare kidney inflammatory diseases (Poster)

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Abstract:
Genetic variants in the complement proteins of innate immunity are associated with the development of inflammatory diseases in the kidney (termed aHUS and C3G), in particular those variants in complement factor H (CFH) and complement C3. The same also applies to age-related macular degeneration (AMD) in the eye. The mechanistic link of these variants with these diseases remains unclear. We maintain a successful interactive web database for genetic variants in CFH. In this project, we will use our most recently upgraded interactive web database from 2021 to investigate the complement variants associated with aHUS and C3G, and also AMD in order to plan and perform rational mutagenesis experiments on the interaction between CFH and C3 fragments. The mutants will be used to test the effect on the binding affinity between C3 and CFH and their stabilities, and compare these with structural predictions. This work will establish the distinct structural locations of the complement genetic variants in CFH and C3 that influence the development of either aHUS, C3G, or AMD, and will determine experimentally the effect of the variants on their molecular interactions that lead to these three different inflammatory diseases. By performing these experimental studies, we aim to clarify the molecular mechanism leading to these kidney and eye diseases, and in turn inform the targeted development of improved bioinformatics prediction methods and clinical therapeutics.

Characterising the alpha-1-antitrypsin pathological polymer by NMR spectroscopy (Final year talk)

Sarah M. **LOWEN**[1,2†], Christopher A. WAUDBY[3†], Alistair M. JAGGER[1†], Ibrahim ALDOBIYAN[1,2], John CHRISTODOULOU[2§], James A. IRVING[1,2§] & David A. LOMAS[1,2§]

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Abstract:
Alpha1-antitrypsin (AAT) is a 52 kDa serine protease inhibitor found at high concentrations in human plasma. The Z mutation (E342K) occurs in 1 in 1700 Northern Europeans and promotes ordered aggregation ('polymerisation') leading to liver cirrhosis and early-onset emphysema. Solution NMR investigations of the monomeric states of the wild-type and Z variants purified from patients have allowed us to probe structural and dynamic features at the earliest stages of misfolding (Jagger, Nat Commun 2020). However, the structure of the polymer itself is currently unknown, yet critical to a full understanding of the polymerisation mechanism and application to drug development efforts.
Polymerisation of isotopically enriched AAT was induced artificially to yield the first high-resolution spectrum of AAT polymers. As it is not certain that recombinant samples accurately recapitulate what is found within patients, we purified pathological polymers from liver explant for comparison. We overlayed the high-field methyl resonances of ex vivo polymers with the artificial polymer standards. These showed resonance equivalence that explain the mechanism of aberrant polymer formation in Z AAT deficiency and is the first direct elucidation of the internal structure of the AAT polymer subunit. Further work is being completed on 2D CH solid state spectrum of the liver-derived AAT polymers.

Observing and manipulating R-loop formation at the single-molecule level (1st year talk)

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Lab: Graeme King Lab

Abstract:
RNA plays essential roles in many cellular processes, from gene regulation to protein production. In order to perform these roles, RNA must adopt a wide range of structures and conformations. One example is an R-loop, which is a three-stranded nucleic acid structure consisting of a DNA-RNA hybrid and a non-template single-stranded DNA. The aim of this project is to directly observe and manipulate R-loop formation during transcription at the single-molecule level, using a combination of optical tweezers and fluorescence imaging. The results from these experiments will improve our mechanistic understanding of how R-loops are formed and regulated in the cell. The first stage in this project was to design a DNA template containing the T7 RNA polymerase (RNAp) promoter. To this end, I designed suitable primers to amplify selected regions of Lambda DNA, which were then ligated together. In this way, I obtained a DNA template that is 10kb in length and contains the RNAp promoter. I have also successfully performed bulk in vitro transcription using this construct. The next step will be to create a longer DNA template (at least 25 kb in length) and then to perform in vitro transcription using this template in the optical tweezers.

Quantum Mechanics for High-Throughput Drug Discovery (Poster)

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Supervisor: Andrea Townsend-Nicholson

Abstract:
Structure-based drug design (SBDD) is rapidly evolving to be a fundamental tool for faster and more cost-effective methods of lead drug discovery. SBDD aims to offer a computational replacement to traditional high throughput screening (HTS) methods of drug discovery. This 'virtual screening' technique utilises the structural data of a target protein in conjunction with large databases of potential drug candidates and then applies a range of different computational techniques to determine which potential candidates are likely to bind with high affinity and efficacy. It is proposed that high throughput SBDD (HT-SBDD) will significantly enrich the success rate of HTS methods, which currently fluctuates around ~1%. This project is centered around the design and implementation of quantum mechanics into the current drug discovery pathway. Here I plan to investigate and develop automated tools for the application of Fragment Molecular Orbital (FMO) techniques for early-stage high throughput Structure-based drug design (SBDD) as an alternative to traditional HTS methods.

During the FMO process, a computational replica of the target biological system is fragmented into groups of atoms which are treated as one, due to the nature of the calculations being performed this fragmentation is the only way that allows quantum mechanical calculations to be done on a system that is large enough to be biologically interesting. For protein:drug complexes, the drug candidate is typically treated as one fragment, and individual residues of the target protein as other fragments. The output is a series of interaction energies, where the total interaction energy for any given drug candidate is equal to the sum of interactions between the individual fragments. I have also applied Molecular Dynamics simulations to study the dynamic relationship of biological systems. Here I present results of implementing these techniques with the output of identifying significant binding patches and an explanation of a mutation study first identified in 1994.'

Design, synthesis and characterisation of cofactor-mimicking HIV-1 capsid inhibitors (Final year talk)

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Lab: Selwood Lab

Abstract:
HIV combination therapy has been hugely successful at suppressing viral replication and increasing life expectancy. However, resistance remains a major issue, demonstrating the need for drugs with novel mechanisms. The conical HIV-1 capsid is formed of around 250 hexamers and 12 pentamers of capsid protein. Guided by interactions with host cofactors, capsid is responsible for delivering the viral genome to the host chromatin. Blocking or mimicking cofactor interactions interferes with capsid activity and therefore infection. An early capsid inhibitor, PF74, blocks DNA synthesis and prevents cofactor binding, but is not potent enough for clinical use. Ultrapotent capsid inhibitor Lenacapavir is the first-in-class drug to be approved by the FDA. However, resistance to Lenacapavir rapidly arises.

We used rational design to derive PF74 derivatives termed allosteres. Structural studies show that allosteres bind the same site as cofactors Sec24C, Nup153 and CPSF6 and inhibit HIV-1 infection with nanomolar potency. We have shown that allosteres activate innate immune sensing during HIV-1 infection, through release of viral DNA into the cytoplasm, and linked this to their ability to affect capsid stability. We have investigated whether allosteres are active against the Lenacapavir resistance mutations, finding similar resistance profiles, and further characterised N74D and Q67H to understand resistance mechanism. These results will guide design of new allosteres with increased potency and reduced sensitivity to resistance mutations.

An integrative characterisation of proline cis and trans conformers in a disordered peptide (Final year talk)

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Abstract:
Intrinsically disordered proteins (IDPs) often contain proline residues, which undergo *cis/trans* isomerisation. While molecular dynamics (MD) simulations have the potential to fully characterise the proline *cis* and *trans* sub-ensembles, they are limited by the slow timescales of isomerisation and force field inaccuracies. Nuclear magnetic resonance spectroscopy (NMR) can report on ensemble-averaged observables for both the *cis* and *trans* proline states, but a full atomistic characterisation of these sub-ensembles is challenging. Given the importance of proline *cis/trans* isomerisation for influencing the conformational sampling of disordered proteins, we employed a combination of all-atom MD simulations with enhanced sampling (metadynamics), NMR, and small-angle X-ray scattering (SAXS) to characterise the two sub-ensembles of the ORF6 C-terminal region ($ORF6_{CTR}$) from SARS-CoV-2 corresponding to the proline-57 (P57) *cis* and *trans* states. A good agreement between the *cis*-P57 populations predicted from MD simulations in AMBER03ws was observed with populations obtained from experimental NMR data. Moreover, we observed good agreement between the radius of gyration predicted from the MD simulations in the AMBER03ws force field and that measured using SAXS. Our findings suggest that both the *cis*-P57 and *trans*-P57 conformations of $ORF6_{CTR}$ are extremely dynamic and that interdisciplinary approaches combining both multi-scale computations and experiments offer avenues to explore highly dynamic states that cannot be reliably characterised by either approach in isolation.

Investigating regulatory mechanisms in mycobacteria (Poster)

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Abstract:
Mechanisms coupling transcriptional and translational control remain incompletely understood in Mycobacterium tuberculosis (*Mtb)*. We have found that premature termination of transcription is widespread and mediated by Rho and moreover, inversely correlated with translation of overlapping open reading frames, which are abundant in *Mtb*. These overlaps are dominated by four-nucleotide start-stop (NUGA) overlaps facilitating Shine-Dalgarno independent translational coupling via termination- reinitiation or stop codon suppression leading to fusion proteins. Further analysis of sequences surrounding these overlaps suggests mycobacterium-specific codon enrichment upstream of the UGA stop codon. We are currently investigating the potential causes and effects of these overlaps in relation to *Mtb*'s lifestyle.

Characterisation of monomeric isoforms of amyloid-β using solvation-based Nuclear Magnetic Resonance techniques (1st year talk)

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Abstract:
Alzheimer's disease is a fatal neurological condition characterised by plaques of aggregated amyloid-β (Aβ) found in patient's brains. Aβ aggregates in a complex, multistage process from an intrinsically disordered monomeric peptide to highly ordered fibril. The two most common forms of Aβ (Aβ40 and Aβ42) have strikingly different aggregation rates, seen in the earliest stages of aggregation, with Aβ42 more commonly associated with toxicity. The differences in these early-stage aggregation rates hint at structural differences in the monomeric forms of the peptides. Despite extensive structural studies into monomeric Aβ, there have been no significant differences identified in their monomeric states, suggesting the differences in aggregation rates are driven by the two additional C-terminal hydrophobic amino acids in Aβ42 or in early aggregated species. Using CLEANEX Nuclear Magnetic Resonance (NMR) to monitor residue-specific hydrogen exchange rates, I have identified differences between the monomeric forms of Aβ40 versus Aβ42 upstream of the hydrophobic C-terminal region, hinting at differences in solvent protection between the two peptides. I have implemented key controls for pH and aggregation into my experimental workflow, making it a more reproducible and reliable platform. These results highlight the sensitivity of using CLEANEX as a method to study challenging intrinsically disordered systems in contrast to more traditional NMR approaches such as chemical shift perturbations.

Making 'antisense' of the PhoPR two-component system in the Mycobacterium tuberculosis complex (Final year talk)

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Abstract:
Mycobacterium tuberculosis (*M.tb*) and *Mycobacterium bovis* (*M.bovis*) have nearly identical genomes but different host specificities and pathogenic profiles. Both lineages of the *Mycobacterium tuberculosis complex* (MTBC) demonstrate pervasive antisense transcription which increases in stress conditions; however, comparative transcriptomics reveal differences in the use of transcription start sites and expression of regions outside of protein-coding genes, including antisense RNA. We propose that these understudied non-coding elements have an influence on transcription and/or translation in a host-specific manner. We used computational methods with publicly-available *M.tb* RNA-seq data to identify and cluster all expressed transcripts in a range of conditions and identified an antisense transcript found opposite the *phoR* gene which is highly expressed in acid and stationary growth conditions. The PhoR sensor-kinase is part of a two-component system that controls the cell response to acid stress by activating the PhoP transcription factor and is active and essential for virulence in both *M.tb* and *M.bovis* despite a potentially deleterious SNP in *M.bovis phoR*. We used a CRISPR-interference system to create a *M.tb* strain with inhibited expression of the *phoR*-antisense and evaluated the effects of this inhibition on the transcriptome using RNA-seq.

Computational Developability Triaging of Antibody Libraries for Discovery of New Therapeutics (Final year talk)

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Abstract:
Therapeutic monoclonal antibodies (mAbs) are a successful class of biologics in the treatment of cancers and autoimmune diseases. However, while the first clinically approved mAbs were approved 40 years ago, only about 120 mAbs have been approved since. This is because their discovery pipelines and clinical trials can be subject to late-stage failures due to developability features, which, in brief is a mAb's intrinsic ability to be produced at scale and tolerated in the body.

By using antibody language models, it has become possible to compare the profiles of clinically approved mAbs and naive antibodies which have been sequenced from human patients or genetically engineered mice in order to predict their developability profiles. As a result, a triaging pipeline has been constructed where a library of antibody sequences are input, and a selection of antibodies which are predicted to be successful in clinical trials is output. This pipeline hopes to improve the chances of a given mAb to reach the clinic through identifying candidates with good developability profiles early in the selection process.

Different modes of interbacterial competition elicit distinct host responses and health outcomes (Final year talk)

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Lab: Brian Ho Lab

Abstract:
Interbacterial competition is known to shape the microbial communities found in the host, however the interplay between this competition and host defense are less clear. Here, we use the zebrafish hindbrain ventricle (HBV) as an *in vivo* platform to investigate host responses to defined bacterial communities with distinct forms of interbacterial competition. We found that antibacterial activity of the type VI secretion system (T6SS) from both *Vibrio cholerae* and *Acinetobacter baylyi* can induce host inflammation and sensitize the host to infection independent of any individual effector. Chemical suppression of inflammation could resolve T6SS-dependent differences in host survival, but the mechanism by which this occurred differed between the two bacterial species. By contrast, colicin-mediated antagonism induced a negligible host response despite being a more potent bacterial killer. Altogether, these results provide insight into how different modes of interbacterial competition *in vivo* affect the host in distinct ways.

A comparison of human and mammalian Surfactant Protein B, sequences and structures as predicted by AlphaFold 2 (1st year talk)

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Supervisor: Katherine Thompson

Abstract:
The composition of lipids and the residues of surfactant protein B found in lung surfactant, varies among mammals, and depends upon characteristics and behaviours such as hibernation. SP-B functions in the lipid insertion for the packing and re-extension of the monolayer at the Air-Water interface, facilitating the expansion of the lungs and therefore, is deemed crucial for survival. Human SP-B is a 79-residue protein, featuring an insertion sequence, 7 Cys residues forming intramolecular disulfide bonds and a single intermolecular disulfide bridge, which alongside R52 and E51, were postulated to linking together monomer units to create a homodimeric structure. Mammalian SP-B residue composition varies. The residues more readily susceptible to oxidation via the pollutant ozone were compared, together with the impact they have on SP-B structure. It has been found that human SP-B lacks His, there is a single Trp residue throughout all mammals, Cys residues are highly conserved but differ in pigs and the Yangtze River Dolphin, but Met is highly variable and is lacking in small diving mammals, in addition to R52 and E51 variation. The predicted structures were discerned using AlphaFold 2, to observe how the structure of SP-B changes. AlphaFold 2 also revealed that the R52 and E51 sidechains are positioned facing outwards, supporting recent research that claims that SP-B exists as a hexameric structure. The biological significance of these differences in Met in diving mammals will be observed by undertaking molecular dynamics simulations, for potential further work on synthetic surfactants for divers.

Investigation of Conjugation Pilus Biogenesis (1st year talk)

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Abstract:
Bacterial conjugation is a major mechanism mediating horizontal gene transfer in bacteria and plays a crucial role in the spread of antibiotic resistance. During conjugation, donor cells produce a conjugative pilus that connects the donor cell to a recipient. We have developed a fluorescence-based strategy to visualize the conjugative pili of live cells. Using time lapse microscopy, we were able to measure the pilus dynamics of different conjugative systems in *Escherichia coli*, including those encoded by plasmids RP4, and R388. Interestingly, for both systems, pilus biogenesis appeared to require the presence of potential recipient cells. We are now following up on these preliminary observations to uncover the mechanisms controlling conjugative pili biogenesis. We found that cell-free supernatant of recipient cells is sufficient to induce R388 pilus biogenesis and are now analysing the composition of the supernatant to identify the recipient cell signal (RCS). We are also trying to identify the plasmid factors that prevent donor cells from producing this signal. To do this, we are generating a library of cloned R388 plasmid fragments. We will then screen the supernatants of *E. coli* cells carrying these plasmid fragments for loss of the RCS. Lastly, recent structural analysis of the R388 conjugation apparatus has revealed a regulatory role for the protein VirB10 in pilus biogenesis. Using mutational analysis of this protein, we will try to determine how RCS sensing leads to pilus biogenesis. By studying the processes controlling conjugative pilus biogenesis, we aim to gain insight into the broader mechanisms controlling horizontal gene transfer within bacterial communities.

Oral vaccines by design: chloroplast engineering in the green alga Chlamydomonas for production of double-stranded RNAs (Final year talk)

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Abstract:

Aquaculture faces significant challenges in controlling viral disease amongst farmed fish or shellfish under intensive production conditions. These challenges could be mitigated by oral delivery of affordable therapeutics designed to block the infection process. The edible microalga *Chlamydomonas reinhardtii* has emerged as a promising platform for producing such recombinant therapeutics. Our research focuses on engineering the chloroplast of *C. reinhardtii* to produce double-stranded RNA (dsRNA) molecules designed to target key viral genes. These dsRNAs can trigger RNA interference (RNAi) in animals, producing small interfering RNA (siRNA) that silences viral genes. Traditional therapeutic delivery in aquaculture, involving purification, cold chain storage, and manual injection, is technically challenging and expensive, limiting its widespread use. Our study aims to develop a system for whole-cell bio-encapsulation and oral delivery of dsRNA via *C. reinhardtii*. Using shrimp as a model, we optimized dsRNA administration doses, evaluated shrimp growth and viral challenge performance, and developed a low-cost 'hanging bag' photobioreactor system for large-scale algae production. This system successfully produced sufficient dried biomass for shrimp feeding trials. Moreover, the dsRNA expression system can be adapted for other research purposes, such as studies aimed at silencing key endogenous genes in animals that naturally feed on microalgae, such as the larvae of mosquitoes and other insect vectors.

Self-assembly and Partner-protein Interactions Regulating Clathrin Functions
(Poster)

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Abstract:
Intracellular membrane traffic is an essential process for cell function, and clathrin is a key player in this process. There are two types of clathrin in humans, formed from clathrin heavy chains CHC17 and CHC22, with distinct functions. CHC17 plays a role in synaptic vesicle formation in neurons and mediates general clathrin sorting pathways. While CHC22 traffics the glucose transporter GLUT4 to an intracellular storage compartment in muscle, facilitating its insulin-stimulated translocation to the plasma membrane. Clathrin needs to be recruited to a target membrane and self-assemble into a basket to transport cargo. These processes are regulated by molecular interactions between CHCs and partner proteins and between CHCs. The studies to be presented have defined a specific CHC22-SNX5/6 interaction that facilitates CHC22 clathrin recruitment to its site of specialised GLUT4 transport by *in vitro* pulldown assays. The second part of this project investigates the key residues involved in the self-assembly of CHC17 clathrin and assesses the effects of disease-associated mutations on clathrin assembly. This was accomplished by using recombinantly expressed CHC17 to produce clathrin baskets *in vitro*. The assembly properties and clathrin basket morphology of CHC17 mutants were assessed by light-scattering assay and electron microscopy, respectively. Disease-associated mutations in CHC17 are linked to intellectual disability and dysfunction of CHC22 is implicated in Type 2 diabetes. Therefore, defining these molecular mechanisms regulating both clathrins has relevance to neurological and metabolic diseases.

Identification and characterisation of CoA S-transferase(s) (1st year talk)

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Abstract:
Coenzyme A (CoA) is a conserved metabolite that has established role in cell metabolism, energy conversion and gene expression regulation. Recently our group has found a novel role of CoA in anti-stress response through a process termed 'protein CoAlation'. Protein CoAlation describes the attachment of CoA onto the cysteine residue of proteins, and change in protein CoAlation pattern is implied in many pathophysiological conditions. So far, little is known about the molecular mechanism of protein CoAlation. To better understand its role in both physiological and disease settings, it is important to identify and characterise enzymes implicated in the CoAlation cycle, including CoA S-transferase which mediates protein CoAlation in cell. With a list of candidate proteins identified in previous mass spectrometry analysis, the identification of CoA S-transferase will be carried out via machine learning prediction, *in vitro* CoA transferase assay, mutagenesis study and molecular dynamics simulation. Finding CoA S-transferase will lay the foundation for studying protein CoAlation, opening up new questions and possibilities towards the physiological impact of this phenomenon. The association between protein CoAlation and complex diseases like cancer, once well studied, may lead to the discovery of new biomarkers and potential therapeutic drugs.