QRW Programme

Biomolecular Interactions and Engineering QMB Satellite Symposium

1 September – 2 September, 2019, Rydges Hotel, Queenstown, New Zealand

Sunday 1 September

Time	Details	Room
9:00-9:10am	**Opening remarks:** Ren Dobson	**Rees Room**
	Session 1 Chair: Volker Nock and Adele Williamson	
9:10–9:40am	**Keynote (K1) Olwyn Byron** University of Glasgow, Scotland ***The spins: bacterial aldehyde-alcohol dehydrogenase forms spiral complexes critical for activity***	**Rees Room**
9:40–10:00am	**Invited (I1) Jenny Malmstrom** University of Auckland, New Zealand ***Protein driven iron mineralisation: self-assembly towards functional nanostructures***	**Rees Room**
10:00–10:30am	**Keynote (K2) Tim Cooper** Massey University, New Zealand ***Evolvability and its basis: adaptation of experimentally evolved bacteria***	**Rees Room**
10:30–11:00am	**Morning Tea**	**Trade Area**
	Session 2 Chairs: Margie Sunde and Paul Gardner	
11:00–11:30am	**Keynote (K3) Laura Domigan** University of Auckland, New Zealand ***Lens protein biomaterials for use in ocular surgery***	**Rees Room**
11:30–11:50pm	**Invited (I2) Michael Griffin** University of Melbourne, Australia ***Cryo-EM of the malaria parasite PA28/20S proteasome complex reveals an unusual activation mechanism with implications for artemisinin sensitivity***	**Rees Room**
11:50–12:10pm	**Invited (I3) Brendon Green** ADVANCED BIOTECH NZ, New Zealand ***ABNZ - Bovine collagen for wound care***	**Rees Room**
12:10–12:30pm	**Selected (S1) Michal Bernach** University of Canterbury, New Zealand ***Artificial leaf surfaces and fluorescently labelled bacteria to investigate phyllosphere microbiology***	**Rees Room**
12:30–1:30pm	**Lunch**	
	Session 3 Chairs: Karen Fleming and Peter Mace	
1:30–2:00pm	**Keynote (K4) Margaret Sunde** University of Sydney, Australia ***Viral proteins that mimic host protein interactions to undermine antimicrobial defenses***	**Rees Room**
2:00–2:20pm	**Invited (I5) Vanessa Morris** University of Canterbury, New Zealand ***Cysteine oxidation triggers amyloid fibril formation by the tumour suppressor p16***	**Rees Room**
2:20–2:35 pm	**Selected (S2) Mihnea Bostina** University of Otago, New Zealand ***Using Cryo-EM to understand seneca valley virus specific tropism for cancer cells***	**Rees Room**
2:35–3:00pm	**Keynote (K5) Dominika Elmlund** Monash University, Australia ***The TAFs of TFIID bind and rearrange the topology of the TATA-less RPS5 promoter***	**Rees Room**
3:00–3:30pm	**Afternoon Tea**	
	Session 4 Chairs: Tim Cooper and Emma Petrie	
3:30–3:50pm	**Invited (I6) Paul Gardner**	**Rees Room**

	University of Otago, New Zealand ***Protein expression is controlled by the accessibility of translation initiation sites***	
3:50–4:10pm	**Invited (I7) Will Barker** Mint Innovation, New Zealand ***A biometallurgical approach to recovering gold from electronic waste***	**Rees Room**
4:10–4:30pm	**Poster Plugs**	**Rees Room**
4:30–6:30pm	**Poster Session (with beer/wine)**	**Rees Room**
7:00pm – late	**Conference Dinner – Winnies (shared with Infectious Diseases)**	

Monday 2 September

Time	Details	Room
	Session 5: Membrane Protein Structure and Function (shared with Infectious Diseases) Chair: Jane Allison	
9:00–9:35am	**Keynote (K6) Karen Fleming** *sponsored by Maurice Wilkins Centre* John Hopkins University, USA ***From Chaperones to the Membrane with a BAM!***	**Queenstown Room**
9:35–9:55am	**Invited (I8) Ren Dobson** University of Canterbury, New Zealand ***Insane in the membrane: Biology of bacterial sialic acid metabolism.***	**Queenstown Room**
9:55–10:30am	**Keynote (K7) Michelle Dunstone** Monash University, Australia ***Pore forming proteins of the immune system: What happens when there are no target-recognition domains?***	**Queenstown Room**
10:30am – 11:00am	**Morning Tea**	
	Session 6 Chair: Laura Domigan and Michael Griffin	
11:00–11:20am	**Keynote (K8) Juliet Gerrard** University of Auckland, New Zealand ***Protein nanotechnology: towards applications***	**Rees Room**
11:20–11:40pm	**Invited (I9) Volker Nock** University of Canterbury, New Zealand ***Using Lab-on-a-Chip technology to reduce complexity in plant-fungi interaction studies***	**Rees Room**
11:40–11:55pm	**Selected (S3) Akash Bhattacharya** Beckman Coulter, USA ***How does a "scorched earth" enzyme work? Experimental and computational studies on the human antiretroviral restriction factor SAMHD1.***	**Rees Room**
11:55–12:10pm	**Selected (S4) Esteban Cruz** University of Sydney, Australia ***Multifunctional gold nanoparticles targeted against HER2-amplified cells for selective delivery of cytotoxic payloads***	**Rees Room**
12:10–12:30pm	**Keynote (K9) Elizabeth Ostrowski** Massey University, New Zealand ***Population genetics of allorecognition in the social amoeba***	**Rees Room**
12:30–2:00pm	**Lunch**	
	Session 7 Chair: Olwyn Byron and Dominika Elmlund	
2:00–2:30pm	**Keynote (K10) Tom Laue** *sponsored by Lab Supply Ltd* University of New Hampshire, USA ***High concentration protein solutions: insights from analytical ultracentrifugation and analytical electrophoresis***	**Rees Room**
2:30–2:50pm	**Selected (S5) Adele Williamson** University of Waikato, New Zealand	**Rees Room**

	Repair outside the box? Structural and functional diversity of bacterial ATP-dependent DNA ligases	
2:50–3:10pm	**Invited (S6) Alexander McLellan** University of Otago, New Zealand ***Promoters to drive Chimeric Antigen Receptor (CAR) T cell therapy***	**Rees Room**
3:10–3:30pm	**Invited (I8) Emma Petrie** Walter + Eliza Hall Institute, Australia ***Learning from viral inhibitory proteins to block the pathway to necroptotic cell death***	**Rees Room**
3:30–4:00pm	**Afternoon Tea**	
Session 8: Chair: James Murphy and Vanessa Morris		
4:00–4:25pm	**Keynote (K11) Jane Allison** University of Auckland, New Zealand ***Elucidation of allosteric mechanism via network analysis of molecular dynamics simulation***	**Rees Room**
4:25–4:40pm	**Invited (S7) Adam Middleton** University of Otago, New Zealand ***Discovery of two inhibitors of ubiquitin chain growth and their future in cells***	**Rees Room**
4:40–5:00pm	**Invited (I9) Peter Mace** *sponsored by Beckman Coulter Life Sciences* University of Otago, New Zealand ***Using helices to cope with stress***	**Rees Room**
5:00–5:30pm	**Keynote (K12) Tuomas Knowles** *sponsored by Thermo Fisher Scientific* University of Cambridge, England ***Protein self-assembly and misassembly***	**Rees Room**
5:30–5:35pm	**Concluding remarks**	**Rees Room**

	Gender		**Level**		
	Female	**Male**	**Student**	**Emerging**	**Established**
Invited Speakers	54% (12)	46% (10)			
Keynote Speakers	75% (9)	25% (3)	0	2	10
Invited Speakers	30% (3)	70% (7)	0	3	7
Abstracts submitted for talks	11% (1)	89% (8)			
Selected speakers	14% (1)	86% (6)	3	3	1
Session chairs	60% (9)	40% (6)	0	6	9

K1 - The spins: bacterial aldehyde-alcohol dehydrogenase forms spiral complexes critical for activity

Byron, O.[1], Kim, G.[2], Azmi, L.[1], Jang, S.[2], Jung, T.[2,3,4], Hebert, H.[3,4], Roe, A.[1], Song, J.[2]

[1]University of Glasgow, Glasgow G12 8QQ, Scotland, UK, [2]Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Korea, [3]KTH Royal Institute of Technology, Novum, Sweden, [4]Karolinska Institutet, Huddinge, Sweden.

Escherichia coli O157:H7 (EHEC) is a pathogenic strain of *E. coli* and the causative agent of bloody diarrhoea, severe colitis and haemolytic uraemic syndrome. These conditions arise owing to the production of Shiga toxin, which is released upon exposure to wrongly prescribed antibiotic treatment. In fact, supportive care is the only current treatment for EHEC. This unmet need, together with the rise in antimicrobial resistant (AMR) bacteria, is driving an effort towards the discovery antibiotic alternatives to treat EHEC.

As part of this effort, high-throughput screening of salicylidine acylhydrazide (SA) compounds was previously shown [1] to suppress the expression of the type three secretion system (T3SS) and disable EHEC motility. To understand the mode of action of these compounds, their cellular targets were identified and characterised. Only the deletion of *adhE* resulted in T3SS down-regulation and overexpression of non-functional flagella, replicating the phenotype observed in the presence of SA compounds. However, the molecular basis underlying this phenotype is unknown.

AdhE (aldehyde-alcohol dehydrogenase) is a key enzyme in bacterial fermentation, converting acetyl-CoA to ethanol, via two consecutive catalytic reactions. In order to be able to pursue structure-based design of compounds suitable as potential drugs, the high-resolution structure of AdhE was sought. However, AdhE is known to form heterogeneous spiral super-complexes (spirosomes) that preclude crystallisation of the monomeric protein. In this talk I will describe how we have used our favourite biophysical tools (AUC and SAXS) for the structural study of AdhE and its complexes, and report on a recent fortuitous, fruitful and ongoing collaboration with colleagues who have determined the high-resolution structure of AdhE within the spirosome and identified a single mutation that significantly alters spirosome assembly [2].

1. Wang, D., Zetterström, C., Gabrielsen, M., Beckham, K., Tree, J., Macdonald, S., Byron, O., Mitchell, T., Gally, D., Herzyk, P., Mahajan, A., Uvell, H., Burchmore, R., Smith, B., Elofsson, M. & Roe, A. (2011). *Identification of bacterial target proteins for the salicylidene acylhydrazide class of virulence blocking compounds*. Journal of Biological Chemistry 286, 29922-29931.

2. Kim, G., Azmi, L., Jang, S., Jung, T., Hebert, H., Roe, A., Byron, O. & Song, J. (2019). *Aldehyde-alcohol dehydrogenase forms a high-order spirosome architecture critical for its activity*. *(*under review*)*.

I1 - Protein driven iron mineralisation: self-assembly towards functional nanostructures

Jenny Malmström,[1,2] Sesha Manuguri,[1,2,3] Laura Domigan[1,2],David E. Williams,[2,3] and Juliet A Gerrard[2,3,4]

[1]Department of Chemical and Materials Engineering, University of Auckland, NZ

[2] MacDiarmid Institute for Advanced Materials and Nanotechnology, Wellington, NZ

[3] School of Chemical Sciences, University of Auckland, NZ

[4] School of Biological Sciences, University of Auckland, NZ

Structured or organised surfaces with nanoscale features are important in a range of fields ranging from energy and computing to controlling cellular adhesion or differentiation. The precise organisation of proteins at surfaces is one route to creating such engineered interfaces. Proteins exist with an enormous structural and chemical versatility and lend themselves well to be functionalized with different moieties. The ability to rationally engineer proteins enables the use of proteins as carefully designed nanometer sized building blocks.

Herein, we will present work focussed at creating arrays of magnetic material using protein and polymer self-assembly. Specifically, work focused on using protein-protein interactions to build up higher order protein structures, and our efforts to organize and functionalise these structures, will be presented. Proteins like Lsmα and peroxiredoxin self-assemble into robust doughnuts whose pore size can be tuned specifically to encapsulate metal complexes or nanoparticles and then assemble further into stacks to create magnetic, electrical or optical nanorods. We are harnessing this potential to create functional arrays of these self-assembling protein rings. We have explored ways of arranging these protein rings through templating using a self-assembling block copolymer. Challenges in co-assembly of proteins and polymers will be highlighted, as well as fabrication solutions using polymers or proteins alone.

Specifically, the protein core of peroxiredoxin was used to template the synthesis of small (approx.. 4 nm) iron oxide nanoparticles. After the synthesis of the nanoparticles inside the protein ring, the stacking ability of the protein was used to create hybrid protein-iron nanowires. Throughout all of this work, imaging is an important characterisation tool and we will show how we use AFM and other techniques to understand our systems.

1. Manuguri, S., Webster, K., Yewdall, N.A., An, Y. Venugopal, H., Bhugra, V., Turner, A., Domigan, L.J., Gerrard, J.A., Williams, D.E., Malmström, J., *Nano Letters,* **2018**, 18, 5138-5145
2. Malmström, J., Wason, A., Roache, F., Yewdall, N. A., Radjainia, M., Wei, Higgins, M. J., Williams, D. E., Gerrard, J. A., Travas-Sejdic, J. Nanoscale, 47, 19940-19948

K2 - Evolvability and its basis: adaptation of experimentally evolved bacteria

Wünsche, A.[1], Dinh, D.[1], Khan, A.[1], Cooper, T.F.[1,2]

[1]Department of Biology and Biochemistry, University of Houston, Texas USA, [2]School of Natural and Computational Sciences, Massey University, Auckland 0632, New Zealand.

The mutations that underlie adaptation interact so that their combined effect is often differs from an expectation based on their individual effects. Theoretical models predict that these deviations, known as epistasis, can have a major impact on evolutionary outcomes, effecting the rate and repeatability of adaptation. To examine these predictions, we constructed a set of bacterial strains that comprise the complete set of steps in the early part of an evolutionary trajectory. We found that mutation interactions, while unpredictable at an individual mutation level, nevertheless follow global patterns that predict a regular decelerating pattern of fitness increase. These interactions depend on the fitness effect of individual mutations, not on their specific physiological activity, a pattern that promotes evolutionary repeatability by making the effect of late mutations less dependent on the specific identity of earlier mutations. By introducing the same mutations into a set of natural isolate bacterial strains, we extend our finding of global mutation interactions to divergent strains and find that they act in a way to promote the spread of mutations between strains.

K3 - Lens protein biomaterials for use in ocular surgery

Manmeet Kaur[1,2], Judith Glasson[1,2,3], Kishara Semarasekara[2], Trevor Sherwin[3] and Laura J. Domigan[1,2,4,5]

[1]School of Biological Sciences, University of Auckland, [2]Department of Chemical and Materials Engineering, University of Auckland, [3]Department of Ophthalmology, University of Auckland, [4]Biomolecular Interaction Centre, New Zealand, [5]The MacDiarmid Institute for Advanced Materials and Nanotechnology, New Zealand.

Crystallins are the dominant structural protein in the eye lens, where they are responsible for maintaining optical transparency. The structure and packing of crystallin proteins enables this function, and *in vivo*, some classes of crystallin proteins also have therapeutic properties. Our research focuses on the creation of biomaterials from crystallins for use in ocular health applications. Although crystallin proteins may be produced recombinantly, there are multiple classes of crystallins, and gaining significant yield is an issue. As such, our source material is extracted from Hoki fish (*Macruronus novaezelandiae*) eyes[1], a low value by-product of the New Zealand fisheries industry.

We have recently used crystallin proteins to formulate transparent biomaterials – namely, thin films, gels and adhesives[2]. Crystallin thin films are preparing via casting, and have a range of mechanical properties and degradation rates dependant on the concentration of crosslinker and plasticizer. One application that is currently being explored for these films is as stem cell carriers for the treatment of limbal stem cell deficiency, an ocular disease that results in significant pain and loss of visual acuity. We have shown crystallin thin films to be cytocompatible and support *ex vivo* expansion from limbal explants. Proof-of-concept experiments demonstrate that cell transfer takes place from films to a corneal surface, validating transfer capacity in an *in vitro* model of clinical treatment.

In ophthalmology there is also a need for new specialised adhesives for use in ocular surgery. Current adhesives are limited by a lack of transparency, scarring issues, and toxicity. This has led to the formulation of crystallin proteins into a bioadhesive that is biocompatible due to its protein-based formulation, and transparent due to the source material. Current work is focused on the tuning of degradation rate to match host cell infiltration, whilst maintaining adequate adhesive strength and curing time for surgical use.

1. Healy, J., Wong, K., Domigan, L.J., Roux, C., Gerrard, J. and Vasudevamurthy, M. (2012) *Polymorphism and Higher Ordered Structures of Protein Nanofibres*, Biopolymers, 97 (8): 595-606.

2. Domigan, L.J., Sherwin, T., Kaur, M., Glasson, J., Semarasekara, K. (2019) Provisional Patent Application:750438

I2 - Cryo-EM of the malaria parasite PA28/20S proteasome complex reveals an unusual activation mechanism with implications for artemisinin sensitivity

Stanley C. Xie[1], Riley D. Metcalfe[1], Eric Hanssen[1,2], Tuo Yang[1], David L. Gillett[1], Andrew P. Leis[2], Craig J. Morton[1], Michael J. Kuiper[3], Michael W. Parker[1,4], Natalie J. Spillman[1], Wilson Wong[5], Christopher Tsu[6], Lawrence R. Dick[6], Leann Tilley[1] and Michael D.W. Griffin[1].

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The proteasome is a multi-subunit enzyme complex that is responsible for most of the non-lysosomal proteolysis in eukaryotic cells. The malaria parasite, *Plasmodium falciparum* (*Pf*) is highly reliant on its protein turnover machinery, thus the proteasome is a drug target in the treatment of malaria. The activity of the 20S proteasome is regulated by protein complexes, such as the 19S complex. The PA28 regulator (also 11S or REG) has been shown to stimulate 20S proteasome peptidase activity *in vitro*, but its role *in vivo* remains unclear.

We show that genetic deletion of *Pf*PA28 renders parasites more sensitive to anti-malarial drugs, consistent with a role for PA28 in responding to proteotoxic stress. The crystal structure of *Pf*PA28 reveals a bell-shaped structure with a highly charged central channel, and large dynamic loops bordering the apical pore. We solved the structure of *Pf*20S in complex with one and two *Pf*PA28 caps using single-particle cryo-EM, revealing the binding and activation mechanism of *Pf*PA28 and providing evidence that *Pf*PA28 employs a mechanism of 20S activation distinct from other 11S activators and the 19S complex. Cryo-EM data also showed that *Pf*PA28 and *Pf*20S form a dynamic complex, with *Pf*PA28 undergoing large rigid-body motions on *Pf*20S. We propose that the large loops of *Pf*PA28 control entry of substrate to the *Pf*20S lumen and that the dynamic motions of the activator permit lateral transfer of proteasome products through the *Pf*PA28/*Pf*20S interface as an alternative mechanism of substrate egress, avoiding the need for products to traverse the *Pf*PA28 pore.

I3 - Advanced Biotech NZ – Bovine collagen for wound care

Green, BJ

ABNZ was established in 2016 to assess and ultimately develop collagen products from NZ for the US wound care market and beyond. Te Puru means 'the bull'.

Collagen has many uses that are common to the general public that includes protein drink supplements to cosmetics. For the wound care sector Collagen serves as a scaffold for tissue re-growth that supports the healing of chronic wounds. In the US alone, over 5 million patients suffer from chronic wounds with 50% of the collagen products being derived from bovine. With an ageing population and rising statistics around diabetes, the market for collagen products in wound care is growing.

NZ is well regarded internationally as a safe source of bovine biomaterials due to the biosecurity practices employed in NZ as well as a history of being BSE (mad cow disease) free.

ABNZ has worked with biotech companies in the US and has developed and trialled a process to make Type I from cow hides. Trials were undertaken with AgResearch & were successful with collagen yields & quality exceeding expectations.

Further work is underway towards identifying strategic partners to support investment into a bovine collagen operation in NZ to serve offshore markets.

The opportunity for NZ is to take a hide that is worth around $100 on a good day and turn it into pharmaceutical grade products worth greater than 100 times the value (as a wholesale material). Operating costs estimated to be less than 50% of wholesale prices.

ABNZ acknowledges the support over recent years by AgMardt & Callaghan, who respectively providing funding that contributed to costs for trials and market visits, & to Sprout Agritech for their mentoring where ABNZ was a member of the 2018 cohort.

S1 - Artificial leaf surfaces and fluorescently labelled bacteria to investigate phyllosphere microbiology

Mitja Remus-Emsermann[1,2], Michal Bernach[1,2,3], Rudolf Schlechter[1,2], Rebecca Soffe[2,3], Daniela Remus[4] and Volker Nock[2,3]

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[3]Electrical and Computer Engineering, University of Canterbury, Christchurch, New Zealand

[4]Callaghan Innovation, University of Canterbury, Christchurch, New Zealand

Plant leaves are colonised by up to 10^7 bacteria per cm^2. On every plant species, specific bacterial communities can be found. To date, the reasons the recurrence of the bacterial communities is unclear. To better understand the underlying factors that are shaping the communities, we follow a reductionist approach that allows the study of synthetic bacterial communities that are representative for leaf surface communities. To that end, we developed 1) fluorescent protein markers that allow us the identification of several bacterial strains at the single cell resolution and 2) a PDMS-based leaf replica. The fluorescent protein toolbox allows us to tag different environmental bacterial strains with one of eight different fluorescent proteins and by using soft lithography, we are able to replicate the exact surface topography of the delicate *Arabidopsis thaliana* leaf in polydimethylsiloxane (PDMS). By adding excipients to the PDMS, we are able to modulate the permeability of the leaf replica to sustain bacteria that are colonising this surface. By releasing fluorescently labelled bacteria onto leaf replica and by following bacterial colonisation using fluorescent microscopy, we are able to track the bacterial colonisers at the single cell resolution. The observation of fluorescently tagged bacteria at the single cell resolution on leaf replica surfaces enables us to understand the bacterial traits that drive leaf surface colonisation. Understanding the drivers of leaf colonisation is important for future biological plant protection applications.

K4 - Viral proteins that mimic host protein interactions to undermine antimicrobial defenses

Sunde, M.[1], Pham, C.L.L.[1], Shanmugam, N.[1], Strange, M.[1], Baker, M.O.D.G. [1], Steain, M.[2], O' Carroll, A.[3], Brown, J.W.[3], Sierecki, E.[3], Gambin, Y.[3]

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Viruses, including herpes simplex virus, murine cytomegalovirus and varicella zoster virus, are able to evade host defense pathways. Specifically, they are able to evade host cell death by the programmed cell death pathway necroptosis. The host receptor interacting protein kinases 1 and 3 (RIPK1 and RIPK3), and the cytosolic nucleic acid sensor Z-DNA binding protein 1 (ZBP1) contain RIP homotypic interaction motifs (RHIMs). These proteins respond to inflammation and/or the presence of viral nucleic acid by assembling into a functional amyloid structure known as a necrosome, which signals for cell death to restrict the viral infection. Viral proteins that contain RHIMs have been shown to be responsible for inhibition of necroptosis.

We have shown that two viral RHIM proteins, the protein M45 from murine cytomegalovirus and the ICP6 protein expressed by herpes simplex virus-1, are able to form amyloid fibrils and that it is the RHIM within these proteins that renders them amyloidogenic. Additionally, we have characterized a previously unrecognised RHIM in the ORF20 protein from Varicella zoster virus, and we show that it forms amyloid structures with host proteins. We have used a wide range of biophysical techniques, including single molecule fluorescence studies, to demonstrate that these viral RHIM-containing proteins form heteromeric amyloid fibrils through co-assembly with host proteins. We find that the incorporation of a viral protein into the host amyloid fibrils alters the structure and stability of the amyloid complexes. Our results demonstrate that the ability of these viral proteins to form decoy amyloid fibrils with host RHIM-containing proteins likely contributes to the suppression of programmed cell death by members of the herpes family of viruses[1]. The activity of the RHIMs is an example of functional amyloid structures co-opted for competing biological roles by mammalian and viral proteins.

1. Pham, C.L.L., Shanmugam, N., Strange, M., O' Carroll, A., Brown, J.W., Sierecki, E., Gambin, Y., Steain, M., and Sunde, M. (2019). *Viral M45 and necroptosis-associated proteins form heteromeric amyloid assemblies.* EMBO Reports 20(2). pii e46518.

I5 - Cysteine oxidation triggers amyloid fibril formation by the tumour suppressor p16

Christoph Göbl*[1,2,6], Vanessa K Morris*[1,2,7], Loes van Dam[3], Marieke Visscher[3], Paulien E. Polderman[3], Christoph Hartlmüller[1,2], Hesther de Ruiter[3], Manuel Hora[1], Laura Liesinger[4,5], Ruth Birner-Gruenberger[4,5], Harmjan R. Vos[3], Bernd Reif[1], Tobias Madl[4,5], Tobias B. Dansen[3]

[1]Technische Universität München, Garching, 85747, Germany; [2]Institute of Structural Biology, Helmholtz Zentrum München, Neuherberg, 85764, Germany; [3]Center for Molecular Medicine, University Medical Center Utrecht, Utrecht, 3584CG, The Netherlands; [4]BioTechMed-Graz, Graz, 8010, Austria; [5]Medical University of Graz, Graz, 8010, Austria; [6]Princess Margaret Hospital, Toronto, Ontario, Canada; [7]University of Canterbury, Christchurch, 8140, New Zealand

p16INK4A (p16) is an important cell-cycle regulator that is found to be mutated in many cancers. Accumulation of p16 leads to cell cycle arrest though inhibition of the CDK4/6 complex, and can lead to cellular senescence, thus p16 is critically involved in cancer and in aging. We have found that under oxidizing conditions, the single cysteine residue of p16 forms an intermolecular disulfide bond. Formation of the disulfide-linked dimer leads to a dramatic structural rearrangment of the protein, from an all alpha-helical structure, to beta-sheet fibrils. These fibrils have typical features of amyloid fibrils, including binding of diagnostic dyes, presence of cross-beta sheet structure, and typical morphology and dimensions by electron microscopy. Using a multi-disciplinary approach, we find that p16 can be oxidized under relatively mild oxidizing conditions, and show that it forms aggregates both in vitro and in cultured human cells. We furthermore find that p16 amyloid formation abolishes its function as a CDK4/6 inhibitor, and therefore p16 amyloid formation is a novel regulatory mechanism of p16 activity. Collectively, these observations mechanistically link the cellular redox state to the inactivation of p16 through the formation of amyloid fibrils.

S2 - Using Cryo-EM to Understand Seneca Valley Virus Specific Tropism for Cancer Cells

Mihnea Bostina

Department of Microbiology and Immunology and Otago Micro and Nano Imaging, University of Otago, Dunedin, New Zealand

Oncolytic virotherapy is an advancing field of cancer treatment with over 70 clinical trials registered up to date. Oncolytic viruses (OVs) penetrate the tumour cell, replicate and eventually trigger cell lysis, releasing new capsids, which at their turn will attack and kill neighbouring cells (1). A detailed knowledge on OVs receptor expression in cancers as well as their interactions with OVs provides valuable insights into targeting cancers.

Seneca Valley Virus (SVV) is a newly-discovered picornavirus, which has earned a significant reputation as a potent oncolytic agent. Recently, Anthrax toxin receptor 1 (ANTXR1) also known as Tumour Endothelial Marker 8 has been identified as the high-affinity cellular receptor for SVV (2). We will give a structural description of the virus and will discuss the basis of SVV-receptor interaction and the avenues for further development of SVV for cancer therapy (3,4).

1. McCarthy, C, N Jayawardena, LN Burga, M Bostina. **2019**. Developing Picornaviruses for Cancer Therapy. ***Cancers***. 16;11(5). pii: E685.

2. Miles, L, LN Burga, E Gardner, M Bostina, JT Poirier, C Rudin. **2017**. Anthrax toxin receptor 1 (ANTXR1) is the cellular receptor for Seneca Valley Virus in neuroendocrine cancers. ***J Clin Investigation*** 127(8):2957-2967.

3. Strauss, M, N Jayawardena, E Sun, RA Easingwood, LN Burga, M Bostina. **2017**. Cryo-EM Structure of Seneca Valley Virus Procapsid. ***J Virol***. 92(6):e01927-17.

4. Jayawardena, N, LN Burga, RA Easingwood, Y Takizawa, M Wolf, M Bostina. **2018**. Structural basis for Anthrax Toxin Receptor 1 recognition by Seneca Valley Virus. ***PNAS***. 15(46):E10934-E10940.

K5 - The TAFs of TFIID bind and rearrange the topology of the TATA-less RPS5 promoter

Dominika Elmlund

Monash University, Australia

The general transcription factor TFIID is a core promoter selectivity factor that recognizes DNA sequence elements and nucleates the assembly of a pre-initiation complex (PIC). The mechanism by which TFIID recognizes the promoter is poorly understood. The TATA-box binding protein (TBP) is a subunit of the multi-protein TFIID complex believed to be key in this process. We reconstituted transcription from highly purified components on a ribosomal protein gene *(RPS5)* and discovered that TFIIDΔTBP binds and rearranges the promoter DNA topology independent of TBP. TFIIDΔTBP binds ~200 bp of the promoter and changes the DNA topology to a larger extent than the nucleosome core particle. We show that TBP inhibits the DNA binding activities of TFIIDΔTBP and conclude that the complete TFIID complex may represent an auto-inhibited state. Furthermore, we show that the DNA binding activities of TFIIDΔTBP are required for assembly of a PIC poised to select the correct transcription start site (TSS).

I6 - Protein expression is controlled by the accessibility of translation initiation sites

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Recombinant protein production in microbial systems is a widely used technique, yet up to half of these experiments fail at the expression phase. A number of contributing factors to failures have been proposed, e.g. codon-bias, mRNA folding, mRNA:ncRNA avoidance, tRNA abundance and G+C xontent. Determining which, if any, of these features explains experiment failures is an active area of research. We have discovered that an ensemble energy model of RNA folding that captures the accessibility of translation inition sites greatly outperforms other features in predicting the outcomes of 11,430 recombinant protein expression experiments in *Escherichia coli*. We have developed a new computational tool called Tisigner, that optimises the first nine codons of an mRNA to improve (or impair) accessibility. Our evaluations have shown that this approach is generally sufficient to elevate the chances of a successful experiment, with the advantage that straightforward PCR cloning methods can be used to integrate optimised sequences.

I7 - A biometallurgical approach to recovering gold from electronic waste

Dr Will Barker

Mint Innovation, a New Zealand cleantech company, is developing a biometallurgical approach to recovering gold (and other metals) from electronic waste.

This “urban ore”, of which ~45 million tonnes has been recently estimated by the UN-funded International Telecommunications Union to be generated globally per annum, contains up to 500 tonnes of gold. Recovering value from this stream is attractive yet difficult due to the heterogenous nature of e-waste as a feedstock. In addition, existing pyrometallurgical solutions in developed nations require regional to country-sized collection schemes to source enough e-waste to operate profitably. This need for scale is in tension with the fact that the majority of high-metalic value electronic goods, such as computers and smartphones, are ending their lives in developing nations. Regulatory enforcement in these jurisdictions is typically lax, thereby letting small-scale metal recovery methods flourish – at a significant cost to both human and environmental health.

In an effort to provide a process that is both profitable and environmentally sound at the local scale encountered in developing nations, Mint is utilising a biometallurgical approach. Microbes that are selective for specific metals, such as gold, are harnessed for their ability to concentrate metals from parts per million to parts per thousand and higher. Combined with low-cost chemistry and low-CAPEX equipment, Mint’s technology aims to make it viable, both economically and environmentally, to recover gold from e-waste near its point of collection (for both the developed and developing world). This paper will give an update to the mineralogical community on how such an approach is working in practice.

K6 - From Chaperones to the Membrane with a BAM!

Karen G. Fleming

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Bacteria have an amazing ability to directionally sort outer membrane proteins (OMPs) in the absence of an external energy source such as ATP. We gained insight into this process through a holistic computational model. Our OMP Biogenesis Model – termed OmpBioM – integrates parameters from experiments both in vivo and in vitro. It incorporates all major periplasmic chaperones at their cellular concentrations, interaction rate constants and considers biological oligomeric states to predict the periplasmic lifetimes, copy numbers and sorting trajectories for OMPs. Using deterministic and stochastic methods we simulated OMP biogenesis under varying conditions replicating biochemical and genetic findings. OmpBioM stochastic simulations reveal that there are hundreds of binding and unbinding events between periplasmic chaperones and unfolded OMPs within an OMP lifetime. These interactions are thermodynamically favored yet kinetically fast suggesting that the periplasmic conditions are near equilibrium with OMPs being "tossed" from chaperone to chaperone. Following this equilibration in the periplasm, the ultimate, rate-limiting step for OMP incorporation into bacterial outer membranes is folding catalyzed by the essential and conserved BAM complex. OmpBioM provides unique insight into this process by predicting the ranges of rates that are possible for this BAM-stimulated reaction. Overall, a finely tuned balance between thermodynamic and kinetic potentials maximizes OMP folding flux through the periplasm, directs them to the native membrane, and minimizes unnecessary degradation. A kinetic "push" prevents OMPs from incorporation into the wrong membrane; OMP sorting is random in the aqueous periplasm; and – once folded with a BAM! – OMPs are thermodynamically favored and kinetically trapped in their native conformations.

I8 - Insane in the membrane: Biology of bacterial sialic acid metabolism

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Sialic acids comprise a varied group of nine-carbon amino sugars that are widely distributed among mammals and higher metazoans. Commensal and pathogenic bacteria that colonise heavily sialylated niches (e.g. the mammalian respiratory tract and gut) can scavenge sialic acid from their surrounding environment and use it as a carbon, nitrogen and energy source—that is, they eat your glycoconjugates for breakfast. Sequestration and degradation of sialic acid involves specific amino sugar transporters responsible for the import into the bacterial cell and five catabolic enzymes that successively degrade sialic acid. Regulation of this pathway is achieved at the transcription level by specific repressor proteins.

In this talk I will present the first crystal structure of a sialic acid specific sodium solute symporter at 1.95 Å resolution in its outward-open conformation. Happily, this structure was determined in complex with sodium and sialic acid bound, providing insight into how this transporter mediates the movement of sialic acid across the membrane. I will then describe our structural studies on the mechanism by which the pathway is regulated at the gene level.

Overall, the work provides new data that enriches our understanding of the import and degradation of sialic acid in clinically important human bacterial pathogens.

K7 - Pore forming proteins of the immune system: What happens when there are no target-recognition domains?

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The MACPF/CDC family proteins use a common fold to oligomerise into a ring-shaped transmembrane pore capable of either direct cell lysis or passive transport other factors proteins. Members of this family are found in all kingdoms of life with a range of functions including as immune effectors, pathogenicity factors, parasite egress, fungal defense and development.

Decades of structural research on the MACPF/CDC family suggested that oligomer assembly on the target membrane is mediated via a dedicated ancillary domain. This is followed by planar diffusion upon the target membrane into a ring shaped prepore. The prepore undergoes a concerted conformational change to form the final pore. This model of mechanism, however, is only consistent with Cholesterol Dependent Cytolysins (CDCs), pleurotolysin and perforin that all have dedicated membrane binding ancillary domains. It is now emerging that this canonical mechanism does not explain how two important pathogen-targeting systems function: Membrane Attack Complex (MAC) and MPEG-1.

The MAC can target a wide range of eukaryotic and bacterial surfaces. In fact, the MAC can assemble on any surface that triggers the complement pathway and bystander host cells rely on the inhibitor CD59 to prevent unwanted MAC assembly. The MAC is a hetero-oligomer structure that consists of C5b, C6, C7, C8 and 18 units of C9 that assembly and insert in a simultaneous fashion. The MAC components lack any ancillary membrane binding domains; however, the sequential insertion mechanism allows for membrane anchoring once C7 has bound. Our X-ray structure of the soluble C9 component of the MAC in comparison with the near atomic single particle cryo-EM structure of the 22-subunit polyC9 shows how this sequential assembly pathway has evolved and how it is controlled.

Similar to the MAC, MPEG-1 is proposed to target a wide range of bacteria engulfed by macrophage but it is unknown how this broad target recognition is achieved. Moreover, the new SP cryo-EM structures of an MPEG-1 prepore shows an absence of any ancillary domains that can bind specifically to the target surface. Interestingly, the structure of MPEG-1 prepores bound to liposomes alludes to an “upside down” membrane binding orientation which we hypothesise anchors the complex to the vacuole membrane to prevent autolysis. Whilst there is some evidence of a trans-pore mechanism it remains to be discovered how the final MPEG-1 pore is actually formed in the absence of a target specific ancillary domain.

Overall, these new structures of the MAC and MPEG-1 challenge the existing dogma in MACPF/CDC pore assembly. We conclude that MACPF/CDC pores used by the immune system have evolved to be able to target highly variable surfaces by evolving different assembly pathways.

K8 - Protein nanotechnology: towards applications

Professor Juliet A. Gerrard, FRSNZ, HonFRSC

Prime Minister's Chief Science Advisor

Kaitohutohu Mātanga Pūtaiao Matua ki te Pirimia

The enormous potential of proteins as molecular components of nanodevices and smart materials has been heralded for some time, but successful examples of designed protein nanostructures remain scarce. This is in part due to the paucity of generic building blocks that can be manipulated such that they assemble into structures of choice. I will give an overview of our work on doughnut-shaped proteins as new building blocks and present results showing that their supramolecular assembly can be controlled by a combination of site-directed mutagenesis and environmental triggers. I will also present early examples of using these structures as components of materials ordered at the nanoscale and in nanodevices.

I9 - Using Lab-on-a-Chip technology to reduce complexity in plant-fungi interaction studies

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In ecosystems, plants are attacked by a multitude of pathogens, which can cause major crop losses and lead to species extinction. To protect themselves, plants activate a sophisticated immune system and recruit beneficial microbes to their root and leaf systems, which help them grow stronger. Our work aims to unravel the biomolecular machinery driving plant interactions with beneficial and pathogenic microbes. Understanding these mechanisms will provide us with sustainable strategies to protect plants and reduce pesticide use. To achieve these goals, we use micro-engineering and Lab-on-a-Chip (LoC) technologies to better mimic *in-vivo* conditions, while simultaneously reducing experimental complexity through inherent compartmentalization and thus increasing repeatability.

In this paper I will discuss two examples of this approach. Firstly, I will summarize the progress we have made towards the use of LoCs to study biomolecular interactions in the life cycle of pathogenic fungi and oomycetes[1-3]. I will show how our platform is capable of measuring protrusive forces in the microNewton range exerted by pathogenic fungi and oomycetes. In addition, I will demonstrate how this can now be coupled on-chip with trapping, compartmentalization and germination of individual zoospores, with the goal of studying protrusive force generation in pathogenic species and thus provide high-throughput screening of anti-fungal compounds. Secondly, I will provide an update on our efforts to combine these technologies with the study of electrotaxis in zoospores and the integration with Root-on-a-Chip approaches[4] for interaction studies.

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3. Sun, Y., A. Tayagui, A. Garrill and V. Nock (2019). *A monolithic polydimethylsiloxane platform for zoospore capture, germination and single hypha force sensing.* Proceedings of 20th IEEE Transducers/Eurosensors XXXIII.
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S3 - How does a “scorched earth” enzyme work? Experimental and computational studies on the human antiretroviral restriction factor SAMHD1

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The human sterile alpha motif and HD domain-containing protein 1 (SAMHD1) is a retroviral restriction factor in myeloid cells and non-cycling CD4+ T cells, a feature imputed to its phosphohydrolase activity-the enzyme depletes the cellular dNTP levels to the point where reverse transcription of the viral genome is not possible. This “Scorched Earth” approach to retroviral restriction requires a tightly regulated mechanism – which we have explored in this project.

X-Ray Crystallography has shown that the functionally active form of SAMHD1 is an allosterically triggered tetramer which utilizes GTP-Mg+2 -dNTP cross bridges to link and stabilize adjacent monomers. However, very little was known about how this protein assembles into a tetramer and how long the tetramer stays intact. In this joint experimental and computational study, we provide a detailed analysis of the correlation between tetramer assembly and enzymatic activity. We have demonstrated that the EC50 values for SAMHD1 activation by dNTPs correlate with dNTP concentrations in cycling cells. We have also studied the role of phospho-regulation and allosteric site dynamics in SAMHD1 using experiments and simulations. Our computational studies further suggest a reciprocal allosteric “handshake” interaction across adjacent monomers. Finally, we have also uncovered a redox-regulatory role played by surface exposed Cysteine residues. This study has employed a combination of NMR, Analytical Ultracentrifugation, Fluorescence Spectroscopy, Mass Spectrometry and Molecular Dynamics calculations.

While a mechanistic understanding of the complete enzymatic process is still elusive, our results build a compelling model for the operation of this enzyme and its HIV-1 restriction properties in cycling cells. However, the regulatory mechanism of SAMHD1 is a more subtle mechanism than has been previously suspected – allowing this protein to lower substrate dNTP levels far more effectively in non cycling cells than would be suggested by our model. Thus, we have barely scratched the surface in terms of understanding this enigmatic enzyme.

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2: Functionality of Redox-Active Cysteines Is Required for Restriction of Retroviral Replication by SAMHD1. Cell Rep. 2018 Jul 24;24(4):815-823. PMID: 30044979.

3: Allosteric Signal Transduction in HIV-1 Restriction Factor SAMHD1 Proceeds via Reciprocal Handshake across Monomers. J Chem Inf Model. 2017 Oct 23;57(10):2523-2538. PMID: 28956603.

4: Uncovering allostery and regulation in SAMHD1 through molecular dynamics simulations. Proteins. 2017 Oct;85(10):1962. PMID: 28884867.

5: Allosteric Activation of SAMHD1 Protein by Deoxynucleotide Triphosphate (dNTP)-dependent

Tetramerization Requires dNTP Concentrations That Are Similar to dNTP Concentrations Observed in Cycling T Cells. J Biol Chem. 2016 Oct 7;291(41):21407-21413. Epub 2016 Aug 26. PMID: 27566548.

6: Effects of T592 phosphomimetic mutations on tetramer stability and dNTPase activity of SAMHD1 can not explain the retroviral restriction defect. Sci Rep. 2016 Aug 11;6:31353. PMID: 27511536.

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Nov 12;10:131. PMID: 24219908.

S4 - Multifunctional gold nanoparticles targeted against HER2-amplified cells for selective delivery of cytotoxic payloads

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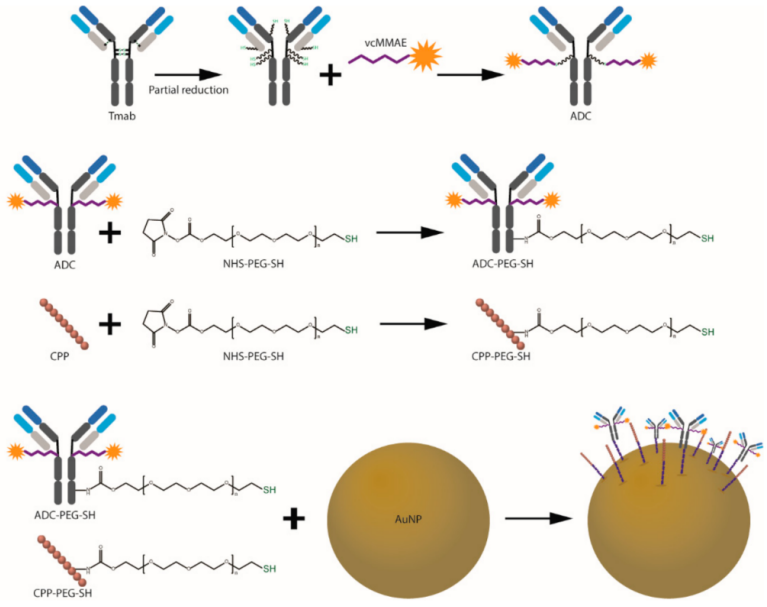
Accelerated growth in solid tumours leads to the formation of abnormal vasculature with increased permeability and reduced lymphatic drainage. This provides an opportunity to employ nanosized materials for selective drug delivery, as the size range of nanoparticles hinders penetration into healthy tissues but allows extravasation into the tumour, leading to preferential accumulation and retention upon systemic delivery. Gold nanoparticles stand out as biocompatible inorganic nanomaterials for such purposes, due to the remarkable ease to tailor their optical properties and chemically modify their surface. Consequently, customized biological properties and enhanced specificity can be granted via surface-attachment of bioactive agents.

Taking advantage of this established surface chemistry, we have synthesized spherical gold nanoparticles functionalized with an anti-HER2 antibody to promote selective interactions with HER2 amplified cancer cells and provide specificity for drug delivery. In the design presented herein, the antibody serves a dual purpose, as it is previously modified with the attachment of MMAE, a potent cytotoxic payload, through a cleavable linker for selective intracellular release. Thus, the synthesized antibody-drug conjugate functions as a drug carrier and as an active-targeting agent on the nanoparticle. The engineered antibody retains high specificity towards its molecular target, as demonstrated by surface-plasmon resonance binding experiments. Evaluation of *in vitro* cellular uptake showed that antibody attachment improves internalization in HER2 overexpressing cell lines, facilitating intracellular drug release. Furthermore, the nanoparticle-bound antibody-drug conjugates retained high antimitotic potency in *in vitro* cytotoxicity assays. Our results underpin the feasibility of employing such nanoparticle engineering strategies to construct formats with enhanced therapeutic potential. In oncology, these are likely to display higher therapeutic indices in tumours with enhanced permeability and retention. Moreover, this approach can readily be adapted to target other types of cancers and maladies.



1. E. Cruz and V. Kayser (2019). *Synthesis and Enhanced Cellular Uptake In Vitro of Anti-HER2 Multifunctional Gold Nanoparticles*. Cancers. 11(6): 870.

K9 - Population Genetics of Allorecognition in the Social Amoeba

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The ability to distinguish self versus nonself— termed allorecognition—is a near universal attribute of multicellular life. However the evolutionary forces acting on allorecognition systems are not well understood. We used molecular evolution and population genomic analyses to elucidate the history of selection acting on allorecognition loci in the social amoeba *Dictyostelium discoideum*. These organisms have an unusual form of multicellularity where cells converge upon starvation and differentiate, forming a fruiting body where some cells altruistically die and produce a stalk that supports the remainder. During this process, allorecognition occurs, causing genetically different cells to segregate into partially distinct fruiting bodies—a trait that is thought to ensure high relatedness and promote cooperation[1]. Allorecognition is carried out by two proteins on the cell surface called *tgrB1* and *tgrC1* [2,3.] Using Illumina and Sanger sequencing, we find that *tgrB1* and *tgrC1* show extraordinary sequence diversity that is orders of magnitude greater than that of other loci in the genome. More important, *tgrB1* and *tgrC1* reside within a larger gene family, consisting of ~59 genes and pseudogenes in clusters near the centromeres. Other members of the gene family are also polymorphic, though less so than *tgrB1* and *tgrC1*, and collectively they show signatures of balancing selection. We also uncover evidence of interlocus gene conversion, suggesting that paralogous genes "donate" novel sequence to *tgrB1* and *tgrC1*, which may explain how these focal recognition genes attain and maintain extraordinary sequence variability. The patterns of molecular evolution of the *tgr* loci is strikingly reminiscent of that of other recognition systems, and we suggest that the unique evolutionary dynamics of large gene families play a critical role in rapidly producing novel sequence variants that allow these loci to function has highly sensitive indicators of relatedness.

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K10 - High concentration protein solutions: insights from analytical ultracentrifugation and analytical electrophoresis

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Methods are needed that incorporate measured quantities into the calculated properties of high-concentration protein solutions. Molecular interactions comprise the sum of the attractive and repulsive potential energies. Repulsive effects are determined by the Stokes radius of and charge on each species- both measurable quantities.

Using analytical electrophoresis (AE), charge measurements at both pH 5.0 and pH 7.4 were made on 12 IgG mAbs and their respective Fc and $F(ab')_2$ fragments. In no case is the calculated charge, based solely on H^+ binding, remotely close to the measured charge. The results illustrate just how little is known about this fundamental property, and how important charge measurement is.[1]

Fluorescence-detected sedimentation (AU-FDS) was used to determine the hydrodynamic nonideality coefficient, k_s. A subset of 7 of the 12 mAbs were fluorescently labelled and tracer quantities sedimented in increasing concentrations of unlabelled IgGs. Weak enthalpically-driven IgG::IgG attractive interactions were observed in all cases. The interactions were found to be variable in strength, affected by both the variable and constant regions, but indiscriminate with respect to IgG subclass. Furthermore, weak attractive interactions were observed for all the mAbs with freshly purified human poly-IgG.[2] We postulate that the generality of the interactions allows for a broader range of epitope spacing for complement activation.[3]

The measurement of the repulsive and attractive terms allows the concentration dependence of high-concentration protein solutions calculated. It is suggested that our approach may be extended to help understand interactions in complex, concentrated systems like serum, cytosol, sputum, urine, CSF, etc.

1 Yang, et al. (2019) "*IgG Charge: Practical and Biological Consequences*" Antibodies, 8:24.

2 Yang, et al. (2018) *"Weak IgG self-and hetero-association characterized by fluorescence analytical ultracentrifugation"* Protein Sci. 27:1334-1348.

3 Yang, et al. (2017) *"IgG cooperativity - Is there allostery? Implications for antibody functions and therapeutic antibody development."* MAbs 9:1231-1252.

S5 - Repair outside the box? Structural and functional diversity of bacterial ATP-dependent DNA ligases

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DNA ligases which catalyse the joining of breaks in the phosphodiester backbone of DNA are essential enzymes for biological DNA replication and repair, and are distinguished by whether their adenylate donor is ATP (eukaryotes, archaea and most viruses) or NAD (all bacteria). In addition to their NAD-dependent replicative enzymes, many species of bacteria encode proteins annotated as ATP-dependent DNA ligases (AD-ligases). Some of these AD-ligases function double-strand break repair and depend on the Ku end-binding protein for activity[1], while others reside in genomes lacking Ku raising questions about whether they possess independent DNA ligase activity and if so, how they engage their substrate.

We have used a combination of bioinformatics, structural biology and *in vitro* assays to investigate bacterial AD-ligases, revealing an unexpected wealth of structural and functional diversity. One example are the minimal 'Lig E' group of AD-ligases which possess only the catalytic core domains of the ligase scaffold and are found in numerous Gram-negative bacteria[2]. Crystal structures capturing both the DNA-free enzyme and the DNA-bound ternary complex revealed that Lig E lacks the dedicated DNA-binding modules found in other DNA ligases, but instead engages its substrate using well-ordered motifs on the core domains[3]. A second example are the three AD-ligases that co-occur in some strains of the major oxygen-producing cyanobacterium *Prochlorococcus marinus.* These isozymes have low sequence similarity, different specific activities and appear to reside in gene clusters with other DNA modifying enzymes indicating the presence of alternate repair pathways. Structures of one of the isozymes capturing pre-ternary and post-ternary complexes in the ligation reaction reveal striking structural homology to the T4 DNA ligase, suggesting a bacteriophage origin for this form[4].

These findings confirm that these enzymes are bona fide DNA ligases, and despite lacking canonical pathway components, many possess high levels of independent ligase activity.

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4 Williamson, A. L., Hanna-Kirsti. *Structural intermediates of a DNA-ligase complex illuminate the role of the catalytic metal ion and mechanism of phosphodiester bond formation*. Nucleic Acids Research, In press.

S6 - Promoters to drive Chimeric Antigen Receptor (CAR) T cell therapy

McLellan A.D.[1], Poudel A.[1], Tan G.M.Y., Halpin J.C., Smith-Bell S.W.J., Dally E.R., Laws G.A., Matheson J.A., He K., Saunderson S.C.[1], Hosseini-Rad S.M.A.[1]

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Chimeric antigen receptor (CAR) T cell cancer immunotherapy combines the specificity of antibodies with the cytolytic activity of T cells to create potent, tumour-killing lymphocytes. CAR T cells are extremely effective in the treatment of B cell lymphomas, even for relapsed patients that have failed chemotherapy. CAR T cell therapy is emerging for the treatment of the more common solid tumours, however this strategy requires further development to address patient safety issues and to improve clinical responses. Our laboratory uses lentiviral and Sleeping Beauty transposon-based gene transfer systems with newly developed bidirectional, or auto-inducible / drug-inducible promoters. These systems maximise the utility of CAR genetic cassettes and 'accessory genes' for improving T cell memory and activity, yet are compact enough to allow efficient gene transfer. Monoclonal antibody technology through both classic hybridoma and M13 phage display approaches are used to develop new tumour-targeting strategies, as well as for development of flow cytometric reagents for CAR T cell quality control for clinical trials. New CAR T cell designs encompass single chain antibody fragment variable (scFv) recognising an antigen present on cancer stem cells, as well as other CAR scFv that target differentiated cancer cells. To mitigate immune responses to self-tissues, scFv engineering and phage display platforms, along with genetic forecast technologies, are currently in development to detect and respond to systemic and tumour-derived signals.

I8 - Learning from viral inhibitory proteins to block the pathway to necroptotic cell death

Petrie, E.J.[1,2], Sandow, J.J.[1,2], Manning, G.[3], Koide, A.[4], Kersten, W.[1], Birkinshaw, R.[1,2], Young, S.[1], Fitzgibbon, C.[1], Lessene, G.[1,2], Lucet, I.S.[1,2], Webb, A.[1,2], Czabotar, P.E.[1,2] Koide, S.[4], Murphy, J.M.[1,2]

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Necroptosis is a lytic programmed cell death mechanism involved in pathogen clearance by promoting an inflammatory response. Although the primary role for necroptosis pathway is associated with innate immune responses, unregulated necroptosis is linked to inflammatory diseases, ischemic-reperfusion injuries and tumour growth. To date no specific inhibitors of necroptosis exist, but would have significant therapeutic value.

The central signalling axis of the necroptosis pathway involves two catalytically-active kinases, RIPK1 and RIPK3, and a catalytically-dead pseudokinase, MLKL. Phosphorylation of MLKL by RIPK3 promotes the formation of killer MLKL assemblies that associate with the inner plasma membrane to cause the damage that ultimately results in an explosive cell death. We recently demonstrated that the key regulatory interaction between RIPK3 and MLKL has evolved to be more tightly controlled in human cells compared to mice[1], suggesting rapid evolution of the pathway.

This talk will describe a class of MLKL orthologs identified in poxvirus (vMLKL) that subvert necroptosis by targeting the regulatory RIPK3/MLKL interaction. Using biophysics, mass-spectrometry and cell biology, we characterised how these vMLKL proteins use species-specific mechanisms to inhibit the activation of endogenous MLKL. Based on observations that vMLKL proteins can block key pathway interactions to inhibit necroptosis, we engineered a library of unique small protein 'monobodies' that specifically bind to different surfaces of human MLKL. Using this library, we identified monobodies that inhibit cell death by blocking the path of MLKL to the plasma membrane. Together the vMLKL proteins have taught us about species specific regulation of necroptosis and created an avenue to develop inhibitors of necroptosis by targeting MLKL interaction interfaces.

1. **Petrie EJ**, Sandow JJ, Jacobsen AV, Smith BJ, Griffin MDW, Lucet IS, et al, Murphy JM *Conformational switching of the pseudokinase domain promotes human MLKL tetramerization and cell death by necroptosis.* Nat Commun. 2018;9(1):2422.

K11 - Elucidation of allosteric mechanism via network analysis of molecular dynamics simulations

Shadfar, Z.[1,2], Razzak, A.[2,3], Ho, T[2,3,4]., Dawes, S[2,3]., Lott, J.S.[2,3], Allison, J.R.[2,3]

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KstR is a TetR-family transcriptional regulator (TFTR) that controls cholesterol catabolism in *Mycobacterium tuberculosis* and is therefore a potential target for the development of new anti-tubercular drugs. Manipulation of KstR repressor function is contingent on gaining an understanding of the allosteric process which converts the protein between the mutually exclusive ligand-bound and DNA-bound states. To date, however, the dynamics of this process have not been studied for any TFTR. Our goal is therefore to determine the allosteric mechanism of action of KstR, and whether this TFTR associates with DNA via conformational selection or induced fit. We have run molecular dynamics (MD) simulations from crystal structures of KstR in its ligand-bound, DNA-bound, and *apo* states to explore the conformational transitions between these three states. We have used a combination of principal component and network analysis to reveal that the transitions involve relative motion of rigid bodies and to characterise these motions. We have also identified the communication pathways that drive these conformational transitions in response to ligand or DNA binding and how these differ between states and transitions.

S7 - iscovery of two inhibitors of ubiquitin chain growth and their future in cells

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The post-translational modification of proteins with ubiquitin has a central role in all eukaryotic cells. As a result, disruption of ubiquitin transfer is associated with diseases including cancer, immunological diseases, and neurological disorders. Proteins can be modified by single ubiquitin moieties or by polyubiquitin chains of eight different linkages, and this spells out a rich code that dictates the fate of the substrate proteins. For example, ubiquitin chains connected by lysine 48 signal for destruction of the substrate by the proteasome, and these chains have a critical role in regulating protein abundance.

The E2 enzyme Ube2k builds only Lys48 chains, and is therefore tightly coupled with degradation of proteins. We have probed how Ube2k can build Lys48 chains by mutating and characterising amino acids around its active site. The results allowed us to model an incoming ubiquitin molecule bound to Ube2k. Using this prediction as a template, we generated protein-based inhibitors of Ube2k, and these are being used as tools for understanding the role of Ube2k in cells.

I9 - Using helices to cope with stress

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Mitogen-activated protein kinase (MAPK) cascades are ubiquitous in eukaryotes as a means of sensing and responding to stressors. In humans, the JNK and P38 stress-activated MAP kinases are activated by upstream MAP kinase kinases (MAP2Ks), which are in-turn activated by a diverse group of MAP3Ks. Although the activation of both MAPKs and MAP2Ks by phosphorylation is well understood, MAP3Ks are less well characterised. Here I will present our recent investigations of how different types of stress-activated MAP3K—the 'ASK' family kinases, and MEKK1—are regulated. Crystallographic structures of previously unidentified domains of ASK3 and MEKK1 reveal all-helical regulatory domains from each. Accompanying functional studies have revealed new mechanisms governing oligomerisation and binding partners of the ASK family of kinases and MEKK1, which are relevant to both pathological signalling and drug sensitivity.

K12 - Protein Self-Assembly and Misassembly

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This talk describes our efforts to elucidate the molecular mechanisms of protein aggregation as well as the kinetics of this process and how these features connect to the biological roles that these structures can have in both health and disease. A particular focus will be on the development and use of physical tools and concepts, including reaction kinetics, to discover molecular assembly pathways in amyloid formation. Moreover we will discuss the development of new microfluidics approaches to study heterogeneous protein self-assembly and their application to explore the molecular determinants of amyloid formation from peptides and proteins.

Summary of Abstracts for the Poster Session

No.	Title	Presenter	Institutions
P1	**Application of Analytical ultracentrifugation on Bio-macromolecular interactions**	Wenqi, L	National Protein Science Facility(Beijing), School of Life Sciences, Tsinghua University, Beijing, CN
P2	**Modulation of M1 and M2 macrophage polarization by engineered nanoparticles**	Syrovets, T	Institute of Pharmacology of Natural Products & Clinical Pharmacology, Ulm University, Ulm, Germany
P3	**Cryo EM Structure of the soluble Formate Dehydrogenase from *Rhodobacter capsulatus***	Mittelstädt, G.	[1]Ferrier Research Institute, Victoria University of Wellington, Wellington, NZ
P4	**Investigating the RNA interactome in colorectal cancer**s	Pinkney, H.R.	[1]Department of Biochemistry, University of Otago, Dunedin, NZ
P5	***N*-Acetylmannosamine-6-phosphate 2-epimerase uses a novel substrate-assisted mechanism to catalyse carbohydrate epimerisation**	Currie, M.J.	[1]School of Biological Sciences, University of Canterbury, Christchurch, NZ
P6	**The Interactions of Oleosins within Complex Food Systems**	Board A.	Biomolecular Interaction Centre, University Of Canterbury, Christchurch, New Zealand.
P7	**Metal ROK rebel: Characterisation of *S. aureus N*-acetylmannosamine kinase**	David Coombes	Biomolecular Interaction Centre and School of Biological Sciences, University of Canterbury, PO Box 4800, Christchurch 8140, New Zealand
P8	**Screening conformationally-selective Nanobodies against TRIB1**	Struwig, J. P. W. V. R.	Department of Biochemistry, University of Otago, Dunedin, NZ.
P9	**Characterisation of the PHD and HARE-HTH Domains of Additional Sex Combs-Like 1–3.**	Reddington, C.J.	Department of Biochemistry, University of Otago, Dunedin, NZ.
P10	**A structural basis for MEKK1's response to microtubule stress**	Filipcik, P.	Department of Biochemistry, University of Otago, Dunedin, NZ

P11	**Structure of the Polycomb Repressive Deubiquitinase complex**	Fellner, M.	Biochemistry Department, University of Otago, Dunedin, NZ
P12	**Protein Scissors for the Killing of Bacteria**	Love, M.J	Biomolecular Interaction Centre and School of Biological Sciences, University of Canterbury, Christchurch 8041, New Zealand
P13	**Substrate specificity in SiaT mutants**	Newton-Vesty, M.C.	[1]School of Biological Sciences, University of Canterbury, Christchurch, New Zealand, [2]Biomolecular Interaction Centre, University of Canterbury, Christchurch, New Zealand
P14	**Characterising conformational change in a TRAP substrate-binding protein**	King-Hudson, T. J.	[1]School of Biological Sciences, University of Canterbury, Christchurch, New Zealand, [2]Biomolecular Interaction Centre, University of Canterbury, Christchurch, New Zealand
P15	**Highly accessible translation initiation sites are predictive of successful heterologous protein expression**	Paul P. Gardner	[1]Department of Biochemistry, School of Biomedical Sciences, University of Otago, Dunedin, NZ [2]Biomolecular Interaction Centre, University of Canterbury, Christchurch, New Zealand
P16	**Defining the DNA binding mechanism of NanR**	Chris Horne	[1]Department of Biochemistry, School of Biomedical Sciences, University of Otago, Dunedin, NZ

P1 - Application of Analytical ultracentrifugation on Bio-macromolecular interactions

Wenqi, L. [1]

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Analytical ultracentrifugation has been utilized to capture the motion of macromolecules in centrifugation field, and is widely applied in assessing protein properties in solution, especially sedimentation coefficient, stokes radius, molecular weight, and Bio-macromolecular interactions. Utilizing analytical ultracentrifugation in our facility, we analyzed some protein-protein interactions, such as: in a sedimentation-velocity, the peak sedimentation coefficients and the calculated molecular weights for the proteins indicated that: PSK induces a monomeric PSK–PSKR1LRR–SERK1LRR complex; in another sedimentation velocity experiment, TBC1D7 and TSC1 (939–992) formed a 2:2 heterotetramer when there was enough or excessiveTBC1D7, and TBC1D7–TSC1 (939–992) complex was an 1:2 heterotrimer when TSC1 (939–992) was in excess were demonstrated.

1. Jizong W,Hongju L., Zhifu H., et.al.(2015), *Allosteric receptor activation by the plant peptide hormone phytosulfokine.Nature. 525: 265–268.*
2. *Gai Z, Chu W, Deng W, Li W, Li H, He A, Nellist M, Wu G(2016). Structure of the TBC1D7-TSC1 complex reveals that TBC1D7 stabilizes dimerization of the TSC1 C-terminal coiled coil region. J Mol Cell Biol. 8 (5): 411-425.*

P2 - Modulation of M1 and M2 macrophage polarization by engineered nanoparticles

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Institute of Pharmacology of Natural Products & Clinical Pharmacology, Ulm University, Ulm, Germany

Macrophages are professional phagocytes and key regulators of innate and adaptive immune responses. Exposure to various cytokines initiates their polarization into either the classical proinflammatory M1 or alternative anti-inflammatory M2 phenotype, which promotes wound healing and supports cancer growth. Thus, the M1/M2 balance critically determines tissue homeostasis. We have investigated effects of the particle surface charge on human M1 and M2 macrophages by using model carboxyl- (PS-COOH) and amino-functionalized (PS-NH_2) polystyrene nanoparticles. M1 macrophages exhibit high expression of CD86 and release proinflammatory TNF-α and IL-1β. By contrast, M2 express CD200R, CD206, efficiently phagocyte *E. coli*, and secrete anti-inflammatory IL-10. PS-COOH led to an increase in protein contents and ATP levels without induction of proliferation, and did not compromise cell viability of both macrophage subsets and phagocytosis of *E. coli* by M2 macrophages. PS-NH_2 significantly decreased release of IL-10 by M2, reduced the ATP contents, impaired *E. coli* phagocytosis, and viability of both macrophage subsets. When nanoparticles were added to macrophages together with polarization stimuli, PS-COOH slightly enhanced release of IL-1β by M1, and significantly inhibited release of IL-10 by both macrophage subsets. Thus, PS-COOH might impair the M2 macrophage polarization without affecting phagocytosis. Given the importance of macrophage subsets and their products in health and disease, functionalized nanoparticles may provide a useful tool to reprogram their activation state for therapeutic benefits.

P3 - Cryo EM Structure of the soluble Formate Dehydrogenase from *Rhodobacter capsulatus*

Mittelstädt, G.[1], Radon, C.[2], Bürger, J.[3], Mielke, T.[3], Leimkühler, S.[2], Wendler, P.[2]

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Essential biological processes such as respiration, anaerobic metabolism and nitrogen fixation depend enzymes that link the two half- reactions, of often complicated, redox reactions through an electron-transfer pathway. One of these oxidoreductases, metal containing formate dehydrogenase (FDH), catalyses the reversible oxidation of formate to carbon dioxide. Enzymatic reduction of carbon dioxide to formate allows for storage of hydrogen as a fuel for industrial applications[1] as well as carbon sequestration from the atmosphere. Prior to this study only limited structural information was available for metal containing FDHs[2].

Here, we present the cryo-electron microscopic structures of molybdenum cofactor-containing *Rhodobacter capsulatus* FDH (*Rcp*FDH) as isolated and in the presence of NADH at 3.26 Å resolution. *Rcp*FDH consists of four subunits, which assemble into a 360 kDa dimer of hetero-tetramers. The structures reveal an intricate electron-transfer pathway formed by 14 Fe/S-clusters between the active site molybdenum, observed in in oxidation states V and VI, and flavin mononucleotide (FMN) at the NAD binding site, which allows for direct and inter-dimer electron transport and shows vibration of the clusters when loaded with electrons.

1. Schuchmann, K. and Müller, V. (2013), *Direct and Reversible Hydrogenation of CO_2 to Formate by a Bacterial Carbon Dioxide Reductase.* Science. 342. pp. 1382-1385.
2. Boyington, J.C. *et al*., (2007). *Crystal Structure of Formate Dehydrogenase H: Catalysis Involving Mo, Molybdopterin, Selenocystein, and an Fe_4S_4 Cluster.* Science. 275 pp. 1305-1308.

P4: Investigating the RNA interactome in colorectal cancer

Pinkney, H.R.[1] , Xiong, H.[1], Gardner, P.P.[1], Diermeier, S.D[1].

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RNA-RNA interactions, such as in ribosomal RNA and spliceosomal RNA, are key contributors to cell functionality. Previous studies have attributed many non-coding RNAs, including MALAT1 and HOTAIR, to cancer pathogenesis. However, investigation into their tumorigenic roles have largely focussed on interactions with DNA and proteins. Due to a lack of high throughput methods, the possibility of investigating global RNA-RNA interactions has remained limited, until recently. New psoralen-based methods have been developed to allow for the crosslinking and pull-down of interacting RNA regions, providing a high-throughput option for investigating novel and potentially disease-relevant RNA interactions.

This discovery-based project uses both *in silico* and *in vitro* techniques to compare RNA-RNA interactions in colorectal cancer to normal colon cells.

Interacting RNA molecules are crosslinked using biotinylated psoralen, allowing for purification on streptavidin beads. The interacting RNA molecules are then ligated together, linearised and sequenced on an Illumina platform.

The resulting chimeric reads are mapped back to the transcriptome, allowing for both identification and quantification of interacting RNA.

This study has identified several potential interactions of interest, including interactions of the long non-coding RNA DANCR, hMaTAR25, and other non-coding RNAs involved in proliferation and metastasis, potentially giving insight into some of the mechanisms underlying tumorigenesis.

Future goals include further characterisation and validation of the interactions found between these RNAs, as well as application of the method to other disease models.

P5: *N*-Acetylmannosamine-6-phosphate 2-epimerase uses a novel substrate-assisted mechanism to catalyse carbohydrate epimerisation

Currie, M.J.[1], North, R.A.[1], Horne, C.R.[1], Muscroft-Taylor, A.C.[1], Dobson, R.C.J.[1]

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N-acetylmannosamine-6-phosphate 2-epimerase (NanE) is a key enzyme in the sialic acid degradation pathway, catalysing the conversion of ManNAc-6P into GlcNAc-6P. The use of this pathway provides a distinct growth advantage and aids in pathogen colonisation and infection in sialic acid rich environments. Furthermore, NanE is required for *Staphylococcus aureus* to grow on sialic acid[1]. These findings highlight the potential of NanE as a target for the design of novel antibiotics that inhibit metabolism. However, prior to inhibitor design, the mechanism of the enzyme must be understood in intricate detail.

A recently published study described the mechanism for NanE from *Clostridium perfringens* for the first time[2]. A close analysis of the crystal structures and enzyme kinetics shows that the currently proposed mechanism is not entirely supported by the published data. We have solved crystal structures of NanE from *S. aureus* and performed kinetic analysis on mutants with active site substitutions. From this, we propose an alternative mechanism for NanE catalysis—a novel proton displacement mechanism mediated by the substrate.

1. Olson, M. E., King, J. M., Yahr, T. L. & Horswill, A. R. (2013). *Sialic acid catabolism in Staphylococcus aureus*. Journal of bacteriology, 195, 1779-1788.
2. Pélissier, M. C., Sebban-Kreuzer, C., Guerlesquin, F., Brannigan, J. A., Bourne, Y. & Vincent, F. (2014). *Structural and Functional Characterization of the Clostridium perfringens N-Acetylmannosamine-6-phosphate 2-Epimerase Essential for the Sialic Acid Salvage Pathway*. Journal of Biological Chemistry, 289, 35215-35224.

P6: The Interactions of Oleosins within Complex Food Systems

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Seeds need fuel to germinate and grow into a plant. Oil bodies are micelles that hold the triacetyl glycerides or “fat” in seeds. They are comprised of a phospholipid monolayer encasing the “fats” inside. Oleosins are proteins that are imbedded in the membrane of the oil body. The terminals of the protein are hydrophilic and sit on the outside of the membrane along with the phospholipid heads. There is a central hydrophobic domain that inserts into the membrane anchoring the protein in the membrane. The hydrophobic d contain a highly preserved proline knot motif that creates a hairpin turn and brings the amino acid chain around to bring the terminal to the top of the membrane. In this work I am working to understand the interaction between Oleosins, protein to protein, any other interactions that may take place with Oleosins.

P7: Metal ROK rebel: Characterisation of *S. aureus N*-acetylmannosamine kinase

David Coombes[1], James S. Davies[1], Michael Newton-Vesty[1], Christopher R. Horne[1], Ramaswamy Subramanian[2], James W. B. Moir[3], Rosmarie Friemann[4,5], Michael D.W. Griffin[6], Santosh Panjikar[7,8], Rachel A. North [1]* & Renwick C.J. Dobson [1,8,]*

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[8]Australian Synchrotron, Clayton, Victoria 3168, Australia

N-Acetylmannosamine kinase (NanK) catalyses the phosphorylation of *N*-acetylmannosamine in the bacterial sialic acid import and degradation pathway. Sequence alignments reveal that Gram-positive *N*-acetylmannosamine kinase enzymes are Repressor, Open-reading frame, Kinase (ROK) family proteins, but many lack the canonical Zn-binding motif, and the sugar-binding motif is altered from ExGH to ExGY.

Here, we study the structure and function of Gram-positive *S. aureus* NanK (*Sa*NanK), which is the first characterisation of a Gram-positive NanK. We report that *Sa*NanK has *bona fide N*-acetylmannosamine kinase activity and the crystal structure of ligand-free, *N*-acetylmannosamine-bound and *N*-acetylglucosamine-bound forms (2.33, 2.20 and 2.20 Å resolution, respectively). These demonstrate, alongside small-angle X-ray scattering data, that *Sa*NanK is a dimer that will switch to a closed conformation upon sugar-substrate binding.

Analysis of the new ExGY motif reveals that ExGY tyrosine is important for function with the pathway substrate, *N*-acetylmannosamine. The residue binds with *N*-acetyl for association of *N*-acetylmannosamine in 'boat' conformation. This is first structure of substrate bound non-Zn-bound ROK kinase and represents a significant departure from binding in Zn-bound NanK.

In place of Zn-binding, *Sa*NanK has an arginine stack, unique to *Staphylococcus species*. Substitution of a stacking arginine residue lowers thermal stability and significantly lowers catalytic rates, which is consistent with the hypothesised role of the canonical Zn-binding motif, in orientating nearby active site residues.

This characterisation of *Sa*NanK provides insight into the structure and function of Gram-positive NanK without the Zn-bound motif and the potential role of Zn-binding that is prevalent throughout the ROK family.

P8: Screening conformationally-selective Nanobodies against TRIB1

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Tribbles pseudokinases (TRIB1–3) play an important role in blood cell and adipocyte differentiation, and act by recruiting developmental transcription factors for ubiquitination. Previous studies have elucidated an allosteric switch that occurs in TRIB1 when binding to its canonical substrate, the transcription factor C/EBP ✓[1]. This mechanism constitutes a shift from an auto-inhibited state to an open, active, state, allowing the E3 ligase-dependent ubiquitination of the bound substrate. Both active and inactive conformations represent exciting targets for the development of specific ligands that could release TRIB1 autoinhibition or block substrate binding. The aim of this project was to develop conformationally-selective nanobodies against TRIB1, which are comprised of isolated variable domains derived from camelid antibodies[2].

From of a library of 96 nanobodies generated against TRIB1, 18 were sequenced, and a final six chosen according to phylogenetic analysis of their amino acid sequences. Further functional experiments to measure the interaction included differential scanning fluorimetry (DSF), isothermal titration calorimetry (ITC), GST-pulldown assays and co-elution assays using liquid chromatography. The results from these experiments identified 3 out of the chosen 6 that bound to and stabilised TRIB1 to a significant degree, and may have potential to modulate oligomeric state of TRIB1. These findings allow the development of an efficient pipeline for the identification of high-affinity, conformationally-selective nanobodies with further potential for therapeutic application.

P9: Characterisation of the PHD and HARE-HTH Domains of Additional Sex Combs-Like 1–3.

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Ubiquitin is added to Lysine at position 119 of the Histone H2A tail by Polycomb Repressive Complex 1, repressing neighbouring gene expression. BRCA1-Associated Protein 1 (BAP1) removes ubiquitin from the Histone H2A tail, in complex with Additional Sex Combs-like 1–3 (ASXL1–3) (Sahtoe *et al.*, 2016). The ASXL proteins are required for BAP1 deubiquitinase activity, forming part of the composite ubiquitin binding site. Recently, BAP1/ASXL were shown to form a double-heterodimer, 2:2 complex *in vitro* (Foglizzo *et al.*, 2018). Double hetero-dimerization would require localised enrichment of BAP1:ASXL at ubH2A substrate. However, targeting of the BAP1:ASXL complex to chromatin is poorly understood.

Notably, a Plant Homeodomain (PHD) exists toward the C-terminus of ASXL1–3. PHD domains conventionally bind either to specific histone modifications or to other proteins — either function could facilitate BAP1:ASXL localisation (Sanchez & Zhou, 2011). Our *in silico* and *in vitro* analyses indicate that the ASXL PHD differs from a canonical PHD. A non-canonical function for the ASXL PHD may convey a unique fashion of BAP1:ASXL localisation. On the other hand, a conserved DNA-binding domain, named the HARE-HTH, exists at the N-terminus of ASXL1–3 (Aravind & Iyer, 2012). Together with the ASXL PHD, the ASXL HARE-HTH domain is thought to mediate the wider localisation of the BAP1:ASXL complex to ubiquitinated nucleosome. This poster discusses the characterisation of the putative chromatin-targeting domains of the ASXL proteins through structural studies, *in vitro* binding assays and proximity-dependent analysis of interactors coupled with Mass Spectrometry.

1. Aravind, L., & Iyer, L. M. (2012). The HARE-HTH and associated domains: Novel modules in the coordination of epigenetic DNA and protein modifications. *Cell Cycle*, *11*(1), 119–131. https://doi.org/10.4161/cc.11.1.18475

2. Foglizzo, M., Middleton, A. J., Burgess, A. E., Crowther, J. M., Dobson, R. C. J., Murphy, J. M., … Mace, P. D. (2018). A bidentate Polycomb Repressive-Deubiquitinase complex is required for efficient activity on nucleosomes. *Nature Communications*, *9*(1). https://doi.org/10.1038/s41467-018-06186-1

3. Sahtoe, D. D., Van Dijk, W. J., Ekkebus, R., Ovaa, H., & Sixma, T. K. (2016). BAP1/ASXL1 recruitment and activation for H2A deubiquitination. *Nature Communications*, *7*, 1–13. https://doi.org/10.1038/ncomms10292

4. Sanchez, R., & Zhou, M. M. (2011). The PHD finger: A versatile epigenome reader. *Trends in Biochemical Sciences*, *36*(7), 364–372. https://doi.org/10.1016/j.tibs.2011.03.005

P10: A structural basis for MEKK1's response to microtubule stress

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Mitogen-activated protein 3-kinase 1 (MEKK1) is the only member of the mitogen-activated protein 3 kinase (MAP3K) family that contains both a kinase domain and a RING E3 ubiquitin ligase domain. These unique characteristics suggest that MEKK1 will have a prominent role in mediating crosstalk between the ubiquitin and kinase signalling systems. MEKK1 is known to be a key upstream regulator in the NF-κB and MAPK functional pathways—major cellular pathways that control stress responses and cell fate. MEKK1 is also required for vinblastine-induced cell death and hyperosmotic stress response.

We present the crystal structure of a central domain of MEKK1 at 2.1Å resolution, which is structurally homologous to tubulin-interacting TOG domains. It consists of six helix-loop-helix motifs with short, conserved loops on the tubulin-interacting side and longer, disordered loops on the opposite side. Our data indicate that MEKK1 TOG shows preference for the curved conformation of tubulin dimers, found in soluble tubulin and at depolymerizing microtubule ends. We propose a model where MEKK1 is activated at the site of microtubule catastrophes via clustering of its signalling elements, the RING and kinase domains. We also observe that mutations in the tubulin-binding domain of MEKK1 are enriched in human tumours and reduce tubulin binding. Accordingly, a rigorous understanding of MEKK1's interaction with tubulin and microtubules could in the future inform choice of cancer chemotherapy, particularly as several first-line cancer treatments include drugs that specifically target tubulin and microtubules.

P11: Structure of the Polycomb Repressive Deubiquitinase complex

Fellner, M.[1], Foglizzoa, M.[1], Middleton A.J.[1], Burgess A.E.[1], Crowther, J.M.[2], Dobson, R.C.J.[2,3], Murphy, J.M.[4], Day, C.L.[1], Mace, P.D.[1]

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DNA packaging is a major determinant of gene expression and cancer development. Histone proteins are able to pack DNA into a compact shape. Their function is governed by epigenetic regulation – posttranslational modification called histone marks. Dysfunction of histone marks are linked to cancer development and progression. Here we investigate how the Polycomb Repressive Deubiquitinase (PR-DUB) complex removes histone marks that silence gene transcription. The goal is to determine the biological relevant oligomeric state of PR-DUB and its functional effect on histone marks. Combining the findings will allow to directly study the complex in the background of disease-causing mutations that confer the highest risk of malignancy. Results can then be used in the prevention and therapy of cancer treatments for patients bearing PR-DUB mutations.**Viral**

P12: Protein Scissors for the Killing of Bacteria

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[2] Institute of Environmental Science and Research, Christchurch 8041, New Zealand

Our extensive use of antibiotics is resulting in the development of multi-drug resistant bacteria. We are at risk of causing a "superbug" resistant to every available antibiotic that would have unprecedented economic and health impacts. There is a global push to decrease antibiotic use in clinical, veterinary and agricultural settings and find novel ways to treat bacteria. The requirement for alternative antimicrobial agents may be fulfilled by bacteriophage endolysins. Endolysins are enzymes encoded by bacteriophage (viruses that infect bacteria) that cleave bacterial peptidoglycan (the tough outer cell wall that protects bacteria) from within the cell at the end of their life cycle allowing for the release of daughter bacteriophage particles. External application of endolysins to susceptible bacteria results in similar lytic activity. This feature underpins the prospect for endolysin-based antimicrobials.

This research characterises the structural and biophysical properties of endolysins for future exploration into the engineering potential of these proteins. Optimizing the functionality will be essential for the future success of endolysins as the basis for antimicrobial drugs.

P13: Substrate specificity in SiaT mutants

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Staphylococcus aureus and *Proteus mirabilis* are pathogenic bacteria that are resistant to many clinical antibiotics and the identification of new inhibitory compounds is a priority for human health. Our approach to this challenge is to investigate the proteins of the sialic acid pathway. Sialic acids are scavenged by *S. aureus* and *P. mirabilis* from mammalian hosts as carbon and nitrogen sources, but also to be used on their cell surfaces in order to evade the host immune system. Our focus here is on the sialic acid transporter SiaT, a membrane protein that can be directly targeted by inhibitory compounds.

N-Acetylneuraminate and *N*-glycolylneuraminate are the most abundant sialic acids, with *S. aureus* SiaT having a higher affinity for *N*-glycolylneuraminate, whereas the homologous SiaT from *P. mirabilis* has a higher affinity for *N*-acetylneuraminate. However, the active sites of these two homologues only differ in three amino acid residues. Examination of the X-ray crystal structure of *P. mirabilis* SiaT, and the subsequently modelled *S. aureus* structure, suggests that these three residues may be entirely responsible for the alternative substrate specificity these two SiaT homologues display.

We are investigating substrate preference by engineering two SiaT triple mutants; the *S. aureus* and *P. mirabilis* SiaT, each with the three active residues substituted to the alternative configuration. The characteristics of these will then be examined by isothermal titration calorimetry, microscale thermophoresis, whole cell growth assays and X-ray crystallography.

P14: Characterising conformational change in a TRAP substrate-binding protein

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TRAPs are transporters found exclusively in prokaryotes such as bacteria, which facilitate the uptake of specific molecules. The sialic acid TRAP transporter, SiaPQM, is required for bacterial uptake of *N*-acetylneuraminic acid (Neu5Ac), an abundant sugar acid on the surface of mammalian cells. The ability to take up and repurpose this molecule offers SiaPQM$^+$ pathogens a competitive advantage when colonising mammalian hosts[1]. Here, we present 2.58 Å resolution structures of the substrate-binding component (SiaP) from the periodontal pathogen *Aggregatibacter actinomycetemcomitans* (AaSiaP), obtained by X-ray crystallography. The crystal structure in the absence of Neu5Ac includes an intermediate between the open and closed conformation. The crystallisation of this intermediate suggests that SiaP is dynamic until Neu5Ac binding stabilises the closed conformation, presumably followed by delivery of Neu5ac to the SiaQM membrane domains for uptake. To better understand the binding and movement this substrate-binding protein (SBP) undergoes in solution, we performed analytical ultracentrifugation (AUC) and small angle-X-ray scattering (SAXS) in the absence and presence of Neu5Ac. The results suggested that SiaP may be present in a semi-closed intermediate in Neu5Ac-free solution, contrary to recent findings[2]. Moreover, a value for the dissociation constant (K_D) of 0.03785 (95% CI: 0.02922 – 0.04891) was estimated for Neu5Ac binding by AaSiaP using differential scanning fluorimetry (DSF). Overall, these findings may be useful for the engineering of SiaP-based biosensors, or for designing SiaP inhibitors to impede the colonisation of sialic acid-utilising pathogens such as *A. actinomycetemcomitans.*

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2. Glaenzer, J., Martin, P. F., Thomas, G. H., Hagelueken, G. (2017). *PELDOR Spectroscopy Reveals Two Defined States of a Sialic Acid TRAP Transporter SBP in solution.* Biophysical Journal. 112(1): 109-120.

P15: Highly accessible translation initiation sites are predictive of successful heterologous protein expression

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Recombinant protein production in microbial systems is well-established, yet half of these experiments have failed in the expression phase. Failures are expected for 'difficult-to-express' proteins, but for others, codon bias, mRNA folding, avoidance, and G+C content have been suggested to explain observed levels of protein expression. However, determining which of these is the strongest predictor is still an active area of research. We used an ensemble average of energy model for RNA to show that the accessibility of translation initiation sites outperforms other features in predicting the outcomes of 11,430 experiments of recombinant protein production in *Escherichia coli*. We developed TIsigner and showed that synonymous codon changes within the first nine codons are sufficient to improve the accessibility of translation initiation sites. Our software produces scores for both input and optimised sequences, so that success/failure can be predicted and prevented by PCR cloning of optimised sequences.

P16: Defining the DNA binding mechanism of NanR

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Bacteria carefully regulate metabolic processes to efficiently colonise and persist within the human host. One mechanism bacteria employ is gene regulation. In *Escherichia coli*, the catabolism of sialic acid is controlled by the GntR-type transcriptional regulator NanR, but the mechanism of gene regulation is unknown. We first demonstrate that NanR binds as a dimer to a total of three direct GGTATA repeats that make up the DNA recognition site, forming a hexameric assembly. Interestingly, we found this binding is cooperative and demonstrate it is mediated by a unique N-terminal extension, likely through protein-protein interactions. To understand how DNA-binding is attenuated by the effector, we solve the co-crystal structure of *E. coli* NanR in the presence of *N*-acetylneuraminic acid to 2.1 Å, supporting the hypothesis that this is the effector molecule, and identify a metal-binding motif that coordinates zinc. The structure is asymmetrical with N-acetylneuraminic acid and zinc only present in one monomer, informing the molecular details of the conformational change that occurs following binding to attenuate DNA-binding activity. Notably, we report the structure of the NanR-DNA complex to 3.9 Å using cryo-electron microscopy, and the first structural evidence of a multimeric assembly process within the GntR superfamily. Together, our results give the first molecular insight into the mechanism of the NanR-DNA interaction in *E. coli*, which enhances our understanding of sialic acid gene regulation in bacteria.