

# SBS Showcase – Third year PhD student posters

## 1. Structural Investigation of TRIM proteins in Autophagy

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Members of the TRIM protein family are characterised by an N-terminal TRI-partite Motif, containing a RING domain with E3 ligase activity, up to two B-box binding domains, and a coiled-coil domain. With over 80 members in the human genome, TRIM proteins have a variety of roles, including innate immune surveillance.

TRIM proteins may also have roles in the regulation of autophagy, a cellular process for the recycling of cytosolic components by encapsulating targets within a double membraned vesicle, the phagophore.

The principal mechanism for localising to the phagophore membrane is via binding to a mATG8, such as LC3B, which are anchored to the membrane by C-terminal lipid modification. It is proposed that TRIM proteins also act as a selective autophagy receptor, targeting substrates to the membrane via an LC3-interacting region (LIR) that binds LC3B. Consequently, TRIM proteins may act as receptors and regulators of precision autophagy for specific targets. We have recently determined the co-crystal structure of TRIM5 $\alpha$  and LC3B, that reveals a cryptic binding motif within the coiled-coil domain of TRIM5 $\alpha$ , making this the first example of an LIR with  $\alpha$  helical secondary structure.

To characterise this atypical LIR and probe the mode of binding to the other mATG8s, we have developed fusion constructs of mATG8 proteins with a C-terminal peptide derived from the helical LIR region of TRIM5 $\alpha$ . We have so far determined two crystallographic structures for GABARAPL1 fusion constructs, however their biological interpretation is compromised due to the loss of secondary, tertiary, and quaternary elements in the motif.

## 2. The bronchiolitis lower airways respiratory tract microbiome

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Bronchiolitis is an acute inflammatory condition of the lower airways. It represents one of the most common lower respiratory infections in children, with one-third of children developing bronchiolitis within the first 2 years of life. While the majority of cases are self-resolving, approx. 3% of infants with bronchiolitis will be hospitalised and some will require costly intensive care, with estimates of US\$550 million p.a. in the USA alone. Furthermore, longitudinal studies have associated severe bronchiolitis with an increased incidence of chronic, non-curable respiratory conditions such as asthma and bronchiectasis.

A possible mechanism by which this acute illness could lead to development of chronic disease is the manner in which the respiratory tract microbiome is affected. In the upper respiratory tract of infants with bronchiolitis, individuals in which *Haemophilus* bacteria dominate the respiratory tract microbiome tend to have higher rates of intensive care use, while *Moraxella*-dominated individuals are associated with lower rates of intensive care use. However, the lower respiratory tract microbiota of bronchiolitis has not been studied to date; this is a critical knowledge gap as the lower airways are where most of the disease pathology occurs.

We have performed 16S rRNA gene-based analysis of paired samples from both the lower and upper respiratory tract in children with severe bronchiolitis. Upper respiratory tract samples were collected by use of an anterior nares swab while lower respiratory tract samples were collected through a non-bronchoscopic lavage. These data are, to our knowledge, the first of their kind, and will provide valuable insights into disease pathophysiology potentially opening up the way for future investigations into new therapeutic approaches such as targeted antibiotics or probiotic treatments.

### **3. GWAS of novel protein coding variants discovered through whole genome sequencing.**

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Genome wide association studies using sequence-resolution data potentially allow mapping of causal variants directly, though causal variants may still be difficult to discern from non-functional, equivalently associated variants due to linkage disequilibrium. To attempt to reduce the causative variant search space, we used an annotation-based filtering approach to identify high-priority protein-coding variants in New Zealand dairy cattle, and estimated their effect on milk production traits. We annotated whole genome sequence data with RefSeq and Ensembl gene models, and then identified all candidate protein altering and splicing variants. We then verified the validity of transcript annotations for these variants by examining RNA-seq data from various bovine tissues, resulting in a set of 55,935 high-impact variants. BayesB association mapping was performed with priors determined in BayesCPI for 8 milk production traits. Quantitative trait loci (QTL) were detected for known causal variants (BTA 6, 14 and 20), as well as a number of uncharacterized loci (BTA 3, 12, 14, 15, 29). The top 10 QTL for milk protein percent are presented alongside coinciding lactation effects.

#### 4. A Yeast for Mead Making

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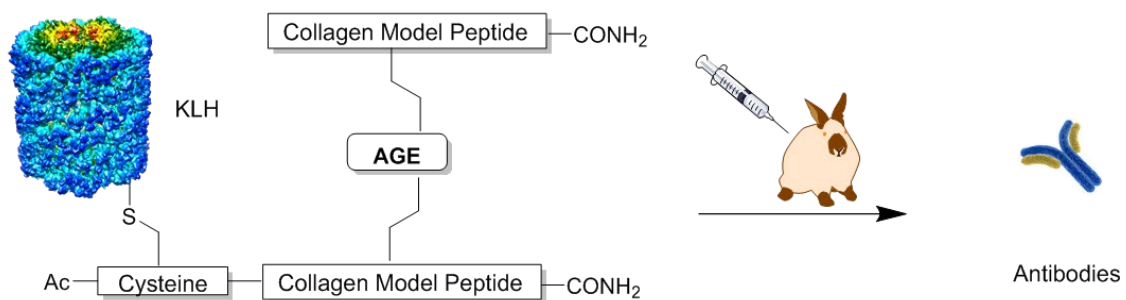
When making mead, problems with fermentation often occur, such as off-tastes, stalling, and slowed fermentation. This is because honey-must (the substrate for mead) is very low in nitrogen, which is a poor environment for *Saccharomyces cerevisiae*. Currently, there is no strain of *S. cerevisiae* specialised to mead fermentation. This project is focussed on directed evolution with *S. cerevisiae* to find a yeast strain that ferments mead consistently. Seven yeast strains were used for directed evolution. Directed evolution occurs as a selection pressure is placed on the population, so that only yeast with useful adaptations are successful. The cycles of directed evolution began with high nitrogen supplementation, which was decreased over the course of the study to place selection pressure for efficient nitrogen utilization on the yeast. In the beginning 12 cycles, the samples lost around 0.9 g of weight from fermentation each cycle. When the nitrogen supplementation decreased to 0 g/L (DAP), weight loss decreased noticeably, indicating that even a low level of supplementation enables higher levels of growth. To further analyse these data, more cycles could be completed, to observe weight loss, as well as genomic sequencing and alcohol quantification.

## 5. Antibodies from Selectively Advanced Glycation Endproduct (AGE) modified Collagen Model Peptides (CMPs)

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Advanced Glycation Endproducts (AGEs) are naturally occurring protein modifications found in humans that are derived from the non-enzymatic reaction of sugars with proteins, which alter the structure and biological properties of modified peptides. AGEs accumulate over time in long-lived peptides, particularly in diabetes patients due to persistent high blood-sugar levels. A positive relationship between increased AGE formation and pathological disease state has been established for diabetes, cancer, cardiovascular and Alzheimer's diseases, making AGEs a group of highly interesting biomarkers. Therefore, a reliable and accessible method to measure and investigate AGEs in complex biological samples is needed. Selective antibodies, generated to recognize AGE modifications in peptides, offer a versatile method. Antibodies currently available are primarily generated from a direct but unselective AGE modification of carrier proteins such as keyhole limpet hemocyanin (KLH), raising the question of their epitope specificity. The aim of this project is the design of a biomimetic immunogen, which will be utilised for the generation of highly specific antibodies that are capable of recognising an AGE on a particular human protein.



**Figure 1.** Design of a biomimetic immunogen and the generation of the antibodies.

The AGE-specific antibodies obtained will be affinity purified and tested in AGE-containing biological samples to determine their ability to selectively detect AGEs in complex systems.

## 6. Analysis of spatial and temporal variations in bacterial community dynamics within stream biofilms

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Stream biofilms play an important ecological role in the bottom up supply of energy and organic matter to aquatic food webs. Their composition is shaped by biotic interactions as well as physicochemical conditions and spatial factors that dictate the extent of microbial dispersal. However, a detailed understanding of the relative importance of spatial and temporal variation in microbial community composition remains poorly understood. Here, we applied 16S rRNA gene sequencing to characterise the biofilm bacterial community composition of 732 stream biofilm samples, collected within six streams located a maximum of 118 km apart and draining different catchment types (forest, urban, rural), at monthly intervals from 2013-2015. We found that monthly variation in bacterial community composition within streams was far more important (PERMANOVA;  $p < 0.01$ ) than the differences observed among different streams and catchment types (PERMANOVA;  $p=0.44$ ). Bacterial community composition over months and years followed a discernible cyclical seasonal pattern, as quantified using periodic regression analysis. Temperature, total nitrogen, light and deficit (DistLM analysis;  $p$  all  $< 0.01$ ) were identified as being the factors most significantly correlated with seasonal variation in bacterial community composition. Overall, these findings suggest that temporal changes are more important than spatial changes across the scales investigated in this study, increasing our understanding of stream microbial community diversity and what governs the distribution and successional changes of microbial taxa.

## 7. Musical Beer; the impact of audible sound on fermentations.

**Harris, A.,** Ganley, A., Jeffs, A.

Sound is ubiquitous in nature and industry, however, its impact on microbes is largely unknown. Recently, the biological effects of audible sound, with a frequency between 20 hertz (Hz) to 20 kHz, have been investigated in yeast. It was found that audible sound positively influences the growth of both *Saccharomyces cerevisiae* and *Candida albicans*. Furthermore, different frequencies of sound altered secondary metabolism of *S. cerevisiae*, producing distinct metabolic profiles. In the current study we are investigating the effects that the two key components of audible sound, frequency and intensity, have on the fermentation characteristics and metabolism of a commercial *Saccharomyces cerevisiae* strain US-05, with the ultimate aim of producing a novel sonic beer. To limit background noise we utilized the University of Auckland's anechoic chamber, which significantly reduces interference from external sounds. Initially, the audible spectrum was divided into four bands, with a silent control. Fermentations were conducted in triplicate in minimal media with maltose, at industry concentration, as the sole carbon source. The general fermentation characteristics, growth rate, sugar consumption and ethanol production were compared to identify an 'active' frequency band. Subsequently, this active frequency band was exposed to three different intensities, to compare the intracellular and extracellular metabolism during exponential growth. Overall, sound was shown to increase the specific growth rate when compared to silence by up to 40% in lower frequencies whereas higher frequencies showed very little change. Applying different sound intensities to fermentations resulted in distinct intra- and extracellular metabolite profiles, from which we could identify fermentations with up to 87% accuracy. Together these results suggest sound, both frequency and intensity, could be used to influence *S. cerevisiae* fermentations in the brewing industry. Furthermore, combining metabolomics with gene expression could provide insight into the mechanism through which sound acts.

## 8. Exploring expression of migraine-associated CGRP receptors in rat brain

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Migraine is an intensely painful neurological disorder affecting one in ten people worldwide, with debilitating consequences. While the exact pathophysiology of migraine remains difficult to untangle, it is clear that the peptide hormone calcitonin gene-related peptide (CGRP) is a key player. Blocking CGRP action has therapeutic benefit in migraine, as shown by the multiple CGRP-inhibiting treatments in development, with three antibody-based therapies recently approved by the FDA.

CGRP is present in the nervous system, brain and vasculature, where it acts at specific cellular receptors. The canonical CGRP receptor is a dimer, comprising a G protein-coupled receptor interacting with an additional protein called RAMP1. Interestingly, an additional dimeric receptor (AMY<sub>1</sub>) is also potentially activated by CGRP, but its role in CGRP activity *in vivo* is not well understood. In particular, there is currently limited information about the localization of AMY<sub>1</sub> receptor in cells and tissues relevant for CGRP biology, such as the brain.

To address this, I have validated antibodies for AMY<sub>1</sub> receptor components and used these to probe areas of the rat brain for AMY<sub>1</sub> receptor expression. The focus is the brainstem, where many pain-sensing pathways are present. The aim of this research is to identify the spatial relationships between CGRP and its receptors in physiologically relevant tissues. This is important for understanding the potential role of the AMY<sub>1</sub> receptor in CGRP biology, which could maximise the therapeutic potential of CGRP for the treatment of migraines.



## 9. Mutational analysis of a kiwifruit *CEN*-like gene

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Members of the phosphatidylethanolamine-binding protein (PEBP) gene family have key roles in regulation of flowering and architecture in various plant species. *FT*- and *TFL1/CEN*-like genes are well-described activators and repressors of flowering, respectively. Recently, it was demonstrated that mutagenesis of two kiwifruit *CEN* genes promoted maturity and flowering. Here we study another kiwifruit *TFL1/CEN*-like gene, which shows homology to a gene implicated in regulation of inflorescence development in *Arabidopsis*, but has not been studied extensively in other species. Here we use CRISPR/Cas9-mediated mutagenesis to be added, removed or altered to specifically target this *CEN*-like gene and produce mutant lines for functional characterization in kiwifruit. *Agrobacterium* mediated transformation was performed to generate the transgenic lines and plants were grown in the containment glasshouse. Multiple independent transgenic lines were genotyped, which confirmed gene editing events in lines 01, 32 and 39 and no evidence of editing in line 05. Identified mutations were mostly small deletions in positions targeted by Guide 3 and Guide 4, giving rise to frameshift mutations or removing up to 10 amino acids. A larger deletion was identified in line 01, which removed 776bp from Guide 3 to 4 in allele II. Modelling of the predicted mutated proteins identified major differences in the structure. For a preliminary study, these plants were defoliated and subjected to 30 days of cold treatment. Axillary buds were excised and expression was tested at the end of the cold treatment. All transgenic lines showed reduced gene expression and earlier bud break compared to the control plant. CRISPR/Cas9-mediated mutagenesis is a promising approach to determine the role of PEBP genes in kiwifruit development, architecture and flowering, and might provide means to engineer *Actinidia* with desired architectural traits.

## 10. A unilateral rabbit model for studying the microbiome in chronic rhinosinusitis

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Chronic rhinosinusitis (CRS) is a morbid condition of the paranasal sinuses which severely impairs patients' quality of life. Symptoms of this inflammatory disease include nasal obstruction or discharge, facial pain or pressure, loss of smell and fatigue. In standard practice, CRS is initially treated with a combination of systemic or topical corticosteroids and antibiotics, which may go on for years. The underlying causes of this chronic condition remain poorly understood and evidence for the efficacy of antibiotics in its treatment is scarce. While various microorganisms such as *Staphylococcus aureus* and members of the genus *Corynebacterium* have been implicated in CRS, there is increasing evidence that bacterial dysbiosis is a feature of the disease. However, due to the highly heterogeneous nature of CRS, determining its aetiology is challenging. Additional factors such as preceding medication and co-morbidities make it difficult to obtain meaningful data from human studies.

The main objective of this study is to establish a unilateral inflammatory sinus model in rabbits that accurately reflects the chronic nature of CRS. Rabbits show a closer anatomical similarity to human sinuses than other model animals, which makes them particularly suitable for such studies. Mechanical obstruction of one side of the animal's sinuses is employed to induce physiologically accurate CRS, enabling study of the microbiome in chronic sinonasal inflammation. Establishing a unilateral model further allows us to use each animal as its own control, which can help mitigate the high inter-individual variation which represents a major challenge in human CRS microbiome research.

We are employing this animal model to investigate the effectiveness of current medical treatments for CRS by determining the effects of antibiotics and corticosteroids on the sinus microbiome, host inflammation and disease progression. Ultimately, the findings of this work will help in developing a better understanding of the medical management of CRS, with the ultimate of improving treatment strategies for human CRS patients.

## 11. Do the gastrointestinal 'satiety' peptides really cause satiety? Testing the hypothesis with whey protein.

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Background: Although a high-protein diet stimulates the secretion of gastrointestinal (GI) peptides and lowers postprandial glycaemia, the hypothesis that protein-induced satiety is regulated by GI peptides, in particular glucagon-like peptide-1 (GLP-1) and Peptide YY (PYY) remain debatable. This study investigates the dose-response relationship between protein intake, postprandial change in GI peptides, glycaemia and eating behaviour.

Design: 24 overweight women received a 380mL whey protein beverage containing either 0g protein (control; 0 kJ), 12.5g protein (low protein, LP; 210 kJ) or 50.0g protein (high protein, HP; 846 kJ) as a preload breakfast on 3 occasions in a randomised, crossover design. Subjective feelings of appetite were measured using Visual Analogue Scales (VAS) and blood samples were collected over 4 hours post-preload. Subsequently, participants received an outcome lunch meal and were instructed to eat ad libitum (freely) until comfortably full. Energy intake (EI) was recorded.

Results: HP significantly increased postprandial circulating insulin, glucagon and GLP-1, and lowered glycaemia (all, preload \* time,  $p < 0.01$ ). Postprandial PYY was constantly higher after HP when compared to control (preload,  $p < 0.01$ ), but with no significant increase throughout the postprandial period (preload \* time,  $p > 0.05$ ). HP did not significantly change the ratings of hunger, fullness, thoughts of food and satisfaction over 4 hours relative to LP and control (all, preload \* time,  $p > 0.05$ ). Unexpectedly, despite high protein and energy content of the HP preload, there was no difference in ad lib EI between the 3 beverages (mean  $\pm$  SEM kJ, control:  $3047 \pm 314$ ; LP:  $2945 \pm 225$ ; HP:  $2961 \pm 259$ ;  $p > 0.05$ ).

Conclusion: Despite the significant increase in circulating GI peptides following HP beverage, there was no evidence that this in turn resulted in a change in eating behaviour.

## **12. What's brown, sticky, and looks like poop? The fine art of masquerade in the North Island lichen moth**

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Camouflage provides some of the most striking examples of defensive colouration in the animal kingdom, with the strategies employed both diverse and extraordinary. While most of these strategies help reduce the probability of being detected by predators, others function to prevent correct recognition. Masquerade is a form of camouflage that acts to deceive predators into misidentifying prey as an unprofitable object that it resembles, such as a leaf or twig. Thus, rather than blending into the background, masquerading prey conceal themselves in plain sight. The endemic North Island lichen moth, *Declana atronivea*, is a fascinating system to investigate masquerade as they appear to employ multiple strategies across the different life stages. The caterpillar's mottled brown and white colouration, lumpy texture, and tendency to curl up on or stand erect from the substrate causes them to resemble bird-droppings and twigs. The moths, on the other hand, with their intricate black and white forewing patterns, look remarkably like the patches of lichen they rest on. However, demonstrating that an organism is using masquerade can prove challenging as it is difficult to disentangle whether predators are detecting and misclassifying the prey, or simply not detecting them at all. In order to be fooled by masquerading prey, predators would first need to have previous experience of the objects their prey are mimicking. To control for this, we will be running a series of 'experience manipulation trials' whereby we manipulate the exposure of naïve baby chickens to the putative masquerade models (twig, droppings and lichen) while keeping their exposure to the masquerading prey (the caterpillars or moths) the same. This will allow us to objectively quantify the purpose of the colouration, shape and behaviour of the caterpillars and moths, and further understand how selection pressures may have shaped these interesting antipredator adaptations.

### **13. Gut Microbiome and Metabolic Health in Pre-diabetic Asian Communities**

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Changes in human diet and lifestyle have led to an increasing incidence of metabolic disorders such as obesity and type 2 diabetes mellitus (T2D) globally. With >1.5 billion adults currently overweight and struggling with poor metabolic health, these disorders represent a significant public health crisis. In mainland China alone, T2D affects roughly 300 million people.

Asian populations are especially susceptible to T2D, often characterised by a 'TOFI' profile (Thin on the Outside, Fat on the Inside). TOFI individuals can be slim but have distinctive visceral distribution of body fat, associated with vital organs such as the liver and pancreas. A recent double-blinded, randomised clinical trial was conducted at the University of Auckland as part of the High-Value Nutrition National Science Challenge. Focusing primarily on identifying nutritional solutions for T2D prevention in Asian consumers, the trial studied 86 pre-diabetic Asian participants, with the tester group incorporating a high-protein nut bar and the placebo group a high-carbohydrate cereal bar into their daily diet for three months.

As the gut microbiota has been linked with metabolic health in humans, we sequenced PCR-amplified 16S ribosomal RNA genes from stool samples collected from the trial participants. We present here the preliminary results from this analysis, which suggest that bacterial diversity and community structure is not greatly impacted by the dietary intervention. Shotgun metagenomic analyses will now be used to study the functional potential of these microorganisms and how these may relate to metabolic homeostasis.

## 14. Robust Digital Archival in a DNA Medium

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It is easy to imagine living in a world where medical records are misplaced, tax and legal documents vanish, engineering techniques are passed on by oral tradition, and scientific data is unreadable, because this is the current state of digital technology. In 2015 the Australian Tax Office lost a petabyte of data due to hardware failure and lack of recovery procedures [1] [2]. Similarly, the 1976 NASA Viking experimental data literally disintegrated [3]. Although data recovery was painstakingly reconstructed from analogue and locked down caches, these examples demonstrate the need for robust, readily decipherable data storage techniques [4] [5]. *Digital obsolescence* compounds this challenge. This is the tendency for software and hardware required for digital data reading to become unsupported or out of production. For example, the open standard for magnetic tape is guaranteed to be backwards compatible for only two generations [6]. In other words, upgrading could break compatibility for media “introduced just six years ago” [7]. As a consequence, there has been less emphasis on digital artefact preservation in library research [8]. For instance, word processing documents slowly lose original formatting through migration due to software deprecation and incompatibilities [5]. Fortunately, these problems can be mitigated via DNA storage technology. First, DNA-based technology are unlikely to become obsolete. As the basis for life on Earth, DNA will continue to be the focus of research and emerging technology. There are many different approaches for interacting with DNA, meaning corporate failure or hardware obsolescence can’t prevent DNA reading or writing. Second, DNA is known to be stable for thousands of years, while magnetic storage is typically replaced every 3-5 years [9] [10] [11]. Finally, the standard genetic code is known to be error resistant. Thus, biologically-inspired encoding techniques can be designed for corruption resilience. Technical details will be explored in the poster session.

## 15. Unravelling the effects of land management on vineyard biodiversity

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Biodiversity affects the productivity, resilience and longevity of natural systems but there is lack of understanding of how it is affected by agricultural management practices. An exciting branch of New Zealand's Vineyard Ecosystems Programme supported by the Ministry of Business, Innovation and Employment and the New Zealand Winegrowers Bragato Research Institute, aims to examine whether management approaches used in vineyards affects their biodiversity. Broadly, *future* managed vineyards use limited synthetic inputs and no herbicides, while *contemporary* managed vineyards do. To understand how these management practices effect biodiversity, total DNA was extracted from soil, one of the land's greatest reservoirs of life, and using next-generation sequencing approaches the bacterial (16S), fungal (ITS2) and metazoan (COI) communities were quantified in 24 vineyards across Hawke's Bay and Marlborough over four years at three growing seasons per year. Biodiversity metrics including the numbers, types and abundances of species were calculated and statistically evaluated to determine the effects of management practice on vineyard biodiversity. Findings suggest the effect of management on biodiversity is statistically significant but small compared with other factors including the year, growing season and geographic location. In fact, the year of sampling explains over 10 times more of the variation compared to management and region explains about 3 times more. The effect of management is also region-dependent with smaller effects seen in Hawke's Bay compared to Marlborough. This suggests biological kingdoms respond differently under different circumstances, and space and time influence the degree to which management affects life.

## 16. Towards creation of a U-DNA genome using synthetic biology tools

**Rickerby, AM.**<sup>1,2</sup>, Poole, AM.<sup>1,3</sup>

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The precursors of DNA are produced from RNA precursors through ribonucleotide reduction, suggesting that DNA evolved after RNA. Furthermore, because deoxythymidine (dT) production from deoxyuridine (dU) requires four additional enzymatic steps, modern DNA appears to have evolved via a uracil-containing intermediate. We have previously<sup>1</sup> noted that the utility of T as fourth base in DNA is indirect; it enables identification of U:G pairs arising from cytosine deamination (C→U) events, only if a repair mechanism already exists to correct such mutations. We therefore argued that the U→T transition was likely driven by the deleterious side-effects of removing uracil resulting from C→U deaminations in a U-DNA genome, where it is not straightforward to distinguish between legitimate U:A pairs and U:G pairs resulting from cytosine deamination. To test our model, we are first using synthetic biology and experimental evolution tools to generate an *E. coli* lineage with a genome that carries uracil instead of thymine, i.e. a U-DNA genome. We will report on progress towards this goal. We will also present results on the detection of uracil directly in DNA using nanopore sequencing. Finally, we will outline our plans to use our resulting line to test our model for the U→T transition.

1. Poole, A., Penny, D. & Sjöberg, B.-M. (2001) *Nature Reviews Molecular Cell Biology*, 2, 147–151.



## 17. Differential internalisation of the CGRP and AMY<sub>1</sub> receptor

**Rees, TA.**<sup>1,2</sup>, Gingell, JJ.<sup>1,2</sup>, Siow, A.<sup>1,3</sup>, Harris, PWR.<sup>1,2,3</sup>, Brimble, MA.<sup>2,3</sup>, Hay, DL<sup>1,2</sup>, Walker, CS<sup>1,2</sup>

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The neuropeptide calcitonin-gene related peptide (CGRP) plays a key role in migraine pathogenesis and is known to potently activate two receptors, the CGRP and the AMY<sub>1</sub> receptor. These receptors are expressed throughout the trigeminal ganglia, which is an important site for migraine and pain transmission. These receptors display different pharmacology, the CGRP receptor responds to CGRP while the AMY<sub>1</sub> receptor responds equally to CGRP, amylin and pramlintide, an amylin mimetic. The CGRP receptor is known to internalise when stimulated, however little is known about the internalisation of the AMY<sub>1</sub> receptor. In this study we aimed to characterise whether the AMY<sub>1</sub> receptor internalises and how this compares to the CGRP receptor.

To study these receptors novel Cy5-labelled [Cy5<sup>3</sup>]-hαCGRP and [Cy5<sup>21</sup>]-pramlintide were synthesised then pharmacologically characterised in transiently transfected Cos-7 cells. Receptor internalisation was studied using high content imaging with the Cy5-labelled peptides and using cell-surface ELISA in transiently transfected HEK293S and Cos-7 cells.

Pharmacological characterisation of [Cy5<sup>3</sup>]-hαCGRP and [Cy5<sup>21</sup>]-pramlintide suggested they had similar cAMP signalling to the unmodified peptides. Internalisation was then quantified using [Cy5<sup>3</sup>]-hαCGRP and [Cy5<sup>21</sup>]-pramlintide. The [Cy5<sup>3</sup>]-hαCGRP had significant internalisation at the CGRP receptor; this was mirrored by the loss of cell surface expression of the receptor components over time in response to unmodified hαCGRP. Interestingly, at the AMY<sub>1</sub> receptor [Cy5<sup>3</sup>]-hαCGRP and [Cy5<sup>21</sup>]-pramlintide did not induce internalisation. This was mirrored by the retention of cell surface expression of the AMY<sub>1</sub> receptor components in response to unmodified hαCGRP or hAmy.

The receptors displayed different internalisation profiles. The AMY<sub>1</sub> receptor does not appear to internalise in response to hαCGRP and pramlintide while the CGRP receptor internalises robustly, as expected. These data suggest that CGRP activity could be regulated by distinct internalisation profiles, with implications for physiology and drug treatment.

## 18. The 'weight' is over: The molecular regulation of kiwifruit (*Actinidia chinensis*) size

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Kiwifruit are an increasingly important crop for the New Zealand (NZ) and world economy, with NZ aiming to reach a return of 4.5B NZD by 2025. An important factor in determining market value is fruit size, with small fruit being rejected from the supply chain. Additionally, when breeding new varieties, a minimum fruit size is needed for the new cultivar to be considered. At present little is known about the molecular regulation of fruit size in kiwifruit. In model fruit species such as tomato it has been shown that final cell number within the mature ovary (pre-fertilisation) and young fruit (post-fertilisation), are key determinants of fruit size. This suggests that the rapid cell division phase during early fruit development is crucial for fruit growth. Plant hormones regulate these phases in an as yet, unknown mechanism. We aim to better understand the regulation of fruit size in kiwifruit.

Firstly, a mapping population that segregates for fruit size was used to identify genetic regions controlling fruit size. There are a growing number of genes that have been implicated in controlling organ size in other plant species. Candidate genes based on homology and assumed function were identified in the newly re-annotated *Actinidia chinensis* (genotype Red5) genome<sup>1</sup>. Combining genetic control locations with candidate genes will help identify potential regulators of fruit size. Candidate genes were tested for differential expression during early fruit development. Functional analyses of these candidate genes will infer if they have a molecular role in the regulation of fruit size.

Understanding the mechanisms controlling fruit size in kiwifruit can help develop new cultivars and molecular tools to increase breeding efficiency, and give tools for growers to ensure that a larger proportion of the crop can be marketed.

1. Pilkington SM, Crowhurst R, Hilario E, et al., (2018). *A manually annotated Actinidia chinensis var. chinensis (kiwifruit) genome highlights the challenges associated with draft genomes and gene prediction in plants*. BMC Genomics. 1-19.

## 19. Investigating dynamics of rDNA copy number at intra-species level

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In most eukaryotes ribosomal DNA (rDNA) genes exist as a tandem array(s) of repeats with high variability in copy number within and between species. The rDNA is central to many critical cellular activities for which its multi-copy nature is believed to be important. However, while the mechanism of rDNA copy number variation is understood, little is known about rDNA dynamics at the population level, partly because of difficulties in measuring copy number. In particular, while different species maintain different “set” rDNA copy numbers, it is not known if this holds true for different populations within the same species. To address this question, we developed a novel approach to measure rDNA copy number from whole genome sequence data using the most frequent (modal) coverage. We validated this method with *Saccharomyces cerevisiae* strains having known, different rDNA copy numbers, and then applied it to calculate the copy numbers of 789 *S. cerevisiae* strains using sequence data from the 1002 Yeast genome project. Interestingly, we found no correlation between phylogeny and rDNA copy number, unlike what is seen above the species level in fungi. We rule out ploidy differences driving this lack of correlation. Another explanation, consistent with different mean copy number across these strains (~92 copies) compared to laboratory *S. cerevisiae* (150-200 copies), is that environmental differences drive rDNA copy number differences. However, our analyses do not provide strong support for this explanation, either. Therefore, our results are consistent with all *S. cerevisiae* populations having a single ‘set’ rDNA copy number, suggesting that the genetic differences that drive copy number differences only manifest above the species levels, at least in *S. cerevisiae*. This further suggests that variation in rDNA copy number in other systems, such as tissue-specific differences in humans, may simply be the result of stochastic copy number variation.

## 20. Managing *Vespula* Wasp Invasion on New Zealand's Islands

Schmack, J., Beggs, J., Ward, D.

Social *Vespula* wasps have successfully invaded New Zealand's native ecosystems including unique offshore islands. The common wasp *V. vulgaris* and the German wasp *V. germanica* cause severe ecological and economic damage to native taxa, apiculture and tourism in New Zealand. In South island beech forest (*Nothofagus* spp.), they significantly reduce the standing crop of carbohydrate resources and thereby, outcompete native taxa. Furthermore, *Vespidae* larvae need invertebrate prey as their protein resource and thereby drastically reduce the survival rate of vulnerable native spiders and caterpillars. Though invasive wasps are widespread throughout New Zealand, little is known about their abundance and impact on offshore islands. We assessed the abundance of *Vespula* wasps on 36 offshore islands off the east coast of New Zealand's North Island and investigated the biogeographic, biotic and anthropogenic drivers of wasp invasion. The abundance of *Vespula* was associated with island isolation and size, canopy cover and human settlement such that smaller, more isolated islands that had dense canopy cover and no human settlement showed lower abundance of *Vespula* than large, close by islands that had little canopy cover and were settled by humans. Biogeographic factors are fixed, but in order to mitigate wasp invasion on New Zealand's islands, vegetation cover could be increased by planting trees and human settlement including farming could be reduced. We hope our findings inform decision makers in conservation planning and help develop sustainable strategies for managing invasive wasps.

## 21. Genetic and phenotypic variation of *Onithochiton neglectus* across a New Zealand latitudinal gradient

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Marine species have varying levels of population connectivity, and understanding their patterns of genetic structure is a way of detecting possible geographical barriers and selective processes. The New Zealand endemic chiton *Onithochiton neglectus* is distributed throughout an environmental gradient, and its brooding behaviour and high morphological variability suggest low gene flow among populations and potential for local adaptation, despite its widespread distribution in the New Zealand East Coast and the Sub-Antarctic Islands. However, *O. neglectus* from the Sub-Antarctic Islands can raft on the holdfast of bull kelp (*Durvillaea antarctica*), which facilitates gene flow among geographically separated populations. In this study, we assessed *O. neglectus* genetic structure by comparing two types of molecular markers: the mitochondrial cytochrome oxidase I gene (COI) and a large panel of Single Nucleotide Polymorphisms (SNP) generated by Genotyping by Sequencing. In addition, we assessed shell shape variation using a Fourier Elliptical analysis, and compared morphological and genetic variation. The results for COI are concordant with the results for SNP, showing three separate *O. neglectus* groups: Northern North Island, Central New Zealand and Southern New Zealand (including the New Zealand South Island and the Sub-Antarctic Islands). Furthermore, the SNP revealed a high level of similarity within populations and high level of divergence between populations, which is exacerbated among groups. However, the shell shape analysis only partially separated two groups, corresponding to a Northern group and a Southern group including the Sub-Antarctic Islands. This scenario suggests that *O. neglectus* have genetically isolated populations where a mechanism to drive connectivity among distant locations is absent. Further analyses with more populations will investigate local adaptation in *O. neglectus*, the potential of sea surface temperature to act as a selective force driving divergence among populations, and the possibility that *O. neglectus* shell shape is an adaptive trait.

## 22. Benzimidazolium-derived *N*-Heterocyclic Carbenes Bridging the Gap between Peptides and Metal Complexes

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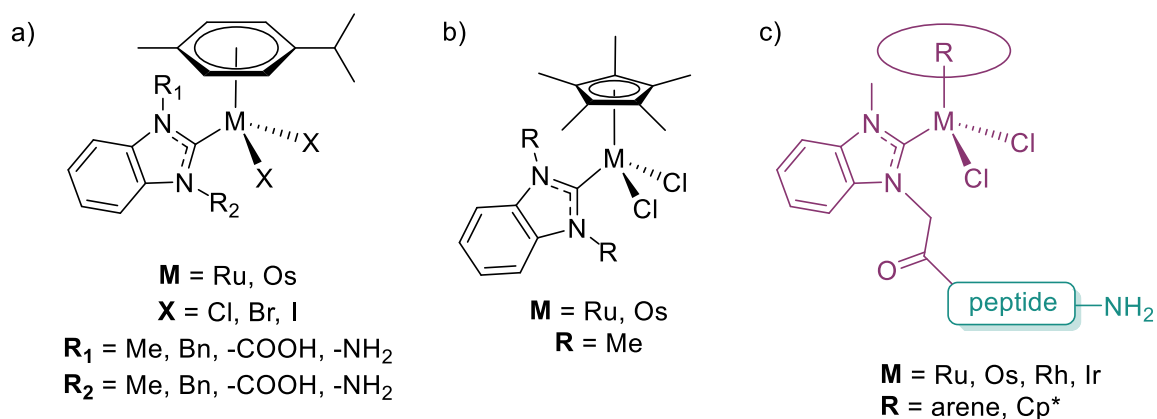
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Cisplatin has long been renowned in the treatment of cancer. Intrinsic and acquired resistance to cisplatin as well as severe systemic toxicity<sup>1</sup> of the drug have spurred the search for new transition metal-based anticancer compounds.<sup>2</sup> In the search for new anticancer drugs beyond platinum, organoruthenium and -osmium pharmacophores have been developed based on the piano stool scaffold and with the general formula [M(arene)(X)(Y)(Z)]. Depending on the structure, they can be tuned to be active against primary tumours or metastases.<sup>3</sup>

Previously developed *N*-heterocyclic carbene (NHC) metal complexes derived from an imidazolium scaffold have shown anticancer activity by targeting thioredoxin reductase (TrxR).<sup>4</sup> Biological evaluation has demonstrated the cytotoxicity of selected symmetrical NHC organo-Ru<sup>II</sup> and -Os<sup>II</sup> complexes in the low to mid micromolar concentration range. These results indicate the promising potential of these complexes as novel anticancer agents with NHC-Ru<sup>II</sup> derivatives recently having been shown to specifically bind to a model protein with a unique binding modality.<sup>5</sup>

In this project, we aim to improve the selectivity of organometallic NHC anticancer agents by synthesising NHC ligands with a peptide component to target specific cell compartments. The small molecules were successfully conjugated onto a selection of peptides known to target mitochondria.<sup>6</sup> A fluorophore will be incorporated to highlight any mitochondrial accumulation that may occur.



**Figure 1.** – a) General structure of benzimidazolium-derived *N*-heterocyclic carbene Ru<sup>II</sup> and Os<sup>II</sup> complexes, b) General structure of benzimidazolium-derived *N*-heterocyclic carbene Rh<sup>III</sup> and Ir<sup>III</sup> complexes, c) General structure of metal-NHC-peptide conjugates.

### **23. Pharmacological Characterisation of PACAP-responsive Receptors Reveals Signalling Bias and Agonist-dependent Antagonism**

**Tasma, Z.**, Hay, DL. and Walker, CS.

Pituitary adenylate cyclase-activating peptide (PACAP) is a neuropeptide widely expressed in the brain and periphery, including sensory and sympathetic nerves. PACAP is known to be involved in nociceptive behaviours and migraine-associated pain. Three different G protein-coupled receptors (GPCRs) are potently activated by PACAP; the PAC<sub>1</sub> receptor, the VPAC<sub>1</sub> receptor and the VPAC<sub>2</sub> receptor. The PAC<sub>1</sub> receptor has several known splice variants in the N-terminal domain, which is important for peptide recognition. These variants include the PAC<sub>1n</sub> receptor (full N-terminus) and the PAC<sub>1s</sub> receptor (21 amino acid N-terminal deletion). To develop new pain treatments targeting PAC<sub>1</sub>, it is essential to understand how PACAP signals and how this signalling is inhibited. Here we pharmacologically characterised the signalling capabilities of PACAP-responsive receptors and how these responses were blocked by antagonists.

The signalling profiles of the human PAC<sub>1</sub>, VPAC<sub>1</sub> and VPAC<sub>2</sub> receptors were examined in transfected Cos7 cells. cAMP, IP<sub>1</sub>, pAkt, pERK and pCREB were measured. The ability of antagonists to block multiple agonists at PACAP-responsive receptors were studied using cAMP assays.

PACAP-responsive receptors coupled to all signalling pathways measured. Limited signalling bias was observed at this receptor family. The PAC<sub>1s</sub> receptor displayed a differential agonist profile where VIP was 20-fold more potent than at the PAC<sub>1n</sub> receptor. Agonist dependent antagonism was observed at the PAC<sub>1n</sub> and PAC<sub>1s</sub> receptors. VIP and PACAP-27 signalling were both more potently blocked by PACAP<sub>6-38</sub> and M65 compared to that of PACAP-38.

The PAC<sub>1s</sub> receptor displayed a distinct pharmacological profile to that of the PAC<sub>1n</sub> receptor, which may play a unique role in pain responses. Differences in antagonist pharmacology at PACAP-responsive receptors suggests the effectiveness of blocking a signalling response can be influenced by which endogenous agonist is present. This agonist dependent effect has potential implications for the development and effectiveness of anti-PAC<sub>1</sub> receptor drugs for pain therapy.

## 24. Gut microbiome of the threatened takahē: Biogeographic patterns and conservation implications

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The New Zealand takahē (*Porphyrio hochstetteri*), once thought to be extinct, is a threatened, flightless rail currently under intense conservation management to boost population numbers. While there has been some previous research into disease-related microbes in takahē, little is known about the community of microbes present in the gastrointestinal tract. Given the importance of gut-associated microbes to herbivore nutrition and immunity, knowledge of these communities is likely to be of great conservation value to the grass-eating takahē. Here we examined the gut microbiomes of 57 takahē at eight separate locations across New Zealand. Faecal samples, taken as a proxy for the hindgut microbial community, were subjected to 16S rRNA gene amplicon sequencing using Illumina MiSeq. Phylogenetic analysis of 2158 ASVs (amplicon sequence variants of 100% sequence identity) revealed six core bacterial phyla (*Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Fusobacteria*, *Proteobacteria* and *Tenericutes*) that dominated the takahē gut microbiome. Location was a significant effect ( $p < 0.001$ , 999 permutations) that accounted for 32% of total variation between microbiome samples. Only one ASV was present in all takahē gut samples, *Lactobacillus* sp, at an average relative abundance of 17%. There was significant variation in *Lactobacillus* abundance between locations sampled. A previously described common commensal bacterium, *Campylobacter* spp., was also present in most takahē samples. These data present a first glimpse of the previously unexplored takahē gut microbiome, and provide a baseline for future microbiological studies and conservation efforts.



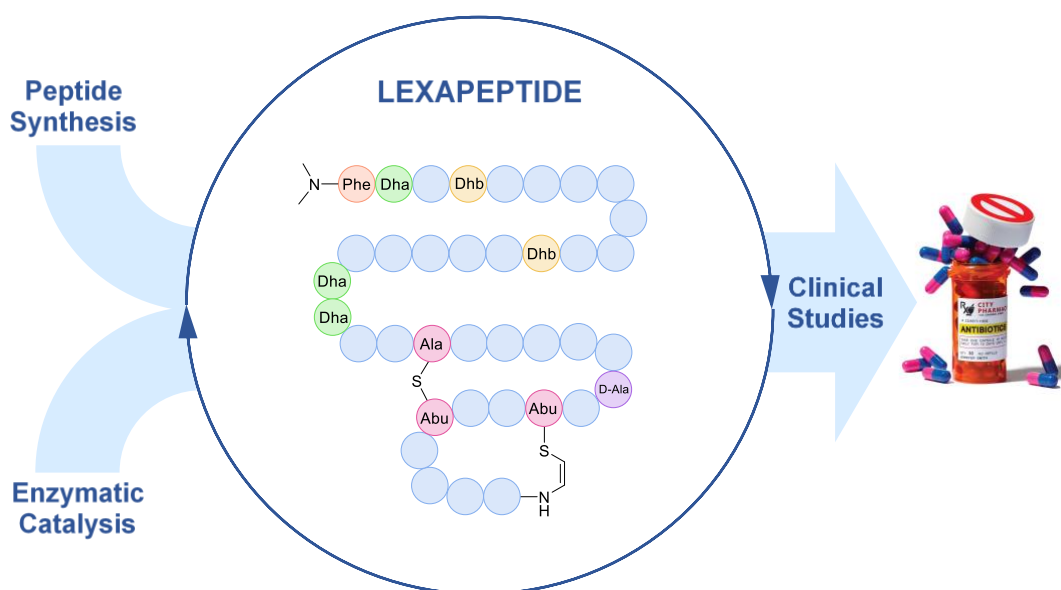
## 25. Chemo-Enzymatic Strategies: Total Synthesis of Lexapeptide

**Williams, TE.**, Harris, P., Bashiri, G., Brimble, MA.

Antimicrobial resistance (AMR) is projected to cause over 10 million deaths per year by 2050, if action is not taken.<sup>1</sup> Despite this, the antibiotic pipeline has only produced seven new and approved antibiotics (2003-2012), perhaps due to a lack of commercial interest. Therefore, it is crucial for new antibacterial compounds to be discovered and synthetic methods to be devised.

Lexapeptide, isolated from a soil bacterium (*Streptomyces rochei*) in 2016 exhibits potent activity against a wide range of multi-drug resistant (MDR) bacteria.<sup>2</sup> Furthermore, its complex structure gives rise to desirable “drug-like” properties such as high stability and target specificity, making it an ideal candidate.<sup>2</sup> To this date there are no known synthetic methods to access this peptide.

Our aim is to devise methods using peptide chemistry in conjunction with enzymatic catalysis to complete the total synthesis of lexapeptide. Following this, the use of lexapeptide as an antibiotic therapy can be evaluated.



<sup>1</sup> C. D. Mathers & D. Loncar, *PLoS Medicine* **3**, 20 (2006).

<sup>2</sup> T. Meifeng, T., X. Min, W. Yimin, & D. Zixin, CN106188253 A (2016).

## **26. Using genomics and transcriptomics to assess the impact of estuarine macro-algae on microbial communities and their roles in biogeochemical cycling**

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Estuaries are considered highly productive ecosystems and important interfaces between freshwater and marine systems. With intense urbanisation and agriculture, estuaries receive high inputs of nitrogen compounds leading to eutrophication and causing overgrowth of macroalgae. This dramatically increases the availability of organic carbon. Excess organic carbon has been linked to the development of anoxic and sulfidic conditions which has the potential to disrupt biogeochemical cycling within estuarine sediment. In this study, analysis of sediment nutrients and community response (environmental genomics and transcriptomics) was undertaken to determine the impact of macroalgae on biogeochemical cycling. Results show that pore water nutrients differ between sites. Higher ammonia and sulfide were associated with sites that have experienced algae cover, and higher nitrate and nitrite was found at sites that had not experienced algae cover. There were subtle changes in the microbial community structure between sites, but the strongest microbial response to algae was functional. Denitrification and ammonia oxidation peak after short term cover, likely fuelled by a small increase in carbon supplied by algae. However both of these processes reduced dramatically in long-term cover sites, which is likely due to a higher input of carbon and increased anoxia. Instead, dissimilatory nitrate reduction to ammonia steadily increased with increasing duration of algal cover, leading to a build-up of ammonium in sediments underlying macroalgal mats

## **27. The effect of acute protein consumption on glycaemic control and insulin response in prediabetic Asian Chinese and Caucasian adults <sup>†</sup>**

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<sup>2</sup>Department of Medicine, University of Auckland, Auckland, NZ

Dietary protein consumption has been shown to decrease postprandial hyperglycaemia. Whey protein in particular stimulates insulin secretion. The aim of this study was to assess the effect of acute whey protein consumption on established and novel markers of type 2 diabetes (T2D) risk; and to investigate whether greater ectopic lipid deposition in the pancreas, key site for insulin secretion, may alter the response to dietary whey protein intervention.

This was a randomised, 3 treatment cross-over study consisting of 24 female Asian Chinese (n=12) and Caucasian (n=12), aged 18-65 years and with BMI 23-40kg/m<sup>2</sup>. At screening, all participants were prediabetic (raised fasting plasma glucose (FPG), 5.6-6.9mmol/L) and were confirmed with >4% or <4% pancreas fat assessed by magnetic resonance imaging (MRI). Each participant attended the Human Nutrition Unit (HNU), University of Auckland for 3 study visits over 3 weeks, with a minimum 7 day wash-out period. At each visit, anthropometry was measured and a fasted baseline (T=0min) blood sample was collected. Following consumption of the test breakfast drink; either water, low-dose protein or high-dose protein, blood samples were collected over a 4 hour period (T=15, 30, 60, 90, 120, 180 and 240 min).

There was no difference in glycaemic control between all 3 groups. Whey protein consumption significantly increased insulin secretion (p<0.05) after both low and high protein drinks, with high protein drink demonstrating a greater effect (P<0.05). Peak plasma insulin was higher in women with low pancreas fat in both protein groups, and was statistically significant (P<0.05) following consumption of the high protein drink. The difference in plasma insulin concentration was significantly (P<0.05) higher 15 minutes after the high protein drink in individuals with low pancreas fat.

The high protein drink significantly increased circulating insulin concentrations, whilst high pancreas fat decreased insulin probably due to pancreatic b-cell dysfunction and decreased insulin secretion.

## 28. Structural insights into product release domains of non-ribosomal peptide synthetases

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Non-ribosomal peptide synthetases (NRPSs) are multi-modular enzymes which function as molecular assembly lines to synthesize a wide range of structurally and functionally diverse peptides. A minimum module of an NRPS consists of an adenylation, peptidyl carrier protein (PCP) and a condensation domain. The adenylation domain 'activates' a specific amino acid by adenylating and transferring it to the 4' -phosphopantetheine arm of the downstream PCP domain. The condensation domain catalyzes the formation of a peptide bond between the activated amino acids in two adjacent modules. The resulting peptide is then transferred to the PCP domain of the downstream module. The peptide product is finally released from the terminal PCP domain by a termination domain usually present in the C-terminus of the last module.

X-ray crystallography has been a valuable tool in elucidating the mechanism of NRPSs, which are characterized by significant domain movements resulting in several intra and inter-domain interactions during different steps of peptide synthesis. One such unexplored interaction is of the termination domain with its upstream PCP domain occurring during the product release step of peptide synthesis. Reductase domains are termination domains known to catalyse NAD(P)H dependent reductive release of their peptide products. In this work, we present the first crystal structure of an NRPS reductase domain along with its upstream PCP domain of an NRPS enzyme (mru\_351) from *Methanobrevibacter ruminantium* which will contribute to our understanding of inter-domain interactions of the product release domains.

## 29. A sticky situation: Shifts in microbial composition, diversity and functioning along a sedimentary gradient.

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Estuaries are interfaces between fluvial and tidal processes, making them highly biodiverse, dynamic and heterogeneous ecosystems. They are also sites where large fractions of riverine nitrogen are removed from the environment as dinitrogen gas. This is driven by microbial communities, especially the nitrifying and denitrifying fractions. Consequently, estuaries are susceptible to a variety of disturbances, such as sedimentation and nutrient enrichment. Land use changes brought by urban and rural development has markedly increased sedimentation rates of terrestrial mud in coastal margins, leading to smothering of native sediment and nutrient enrichment, creating eutrophic and anoxic conditions that are detrimental to biodiversity and ecosystem functioning. To better understand how sedimentation affects estuarine microbial communities, we investigated the influence of mud (< 63 µm) content on the composition and diversity of prokaryotic communities and nitrogen cycling members using amplicon sequencing of 16S rRNA (prokaryotic communities), *amoA* (nitrifiers) and *nosZ* (denitrifiers) genes. The nitrifying and denitrifying potential and expression in sediments was quantified with droplet digital PCR. Concurrently, we determined mud content via dry sieving and assessed sediment and pore water nutrient contents. Chemical analyses suggest that muddier sediments were more nutrient enriched and possibly hypoxic. We found that mud content is a major factor in the structuring of microbial communities and nitrogen cycling fractions. However, nitrifiers exhibited a higher tolerance to mud encroachment. Muddier sediments generally harboured more diverse prokaryotic communities and denitrifying fractions. In addition, we found denitrifiers were enriched and more active in muddier sediments, whereas nitrifiers were less diverse and more active. Our study shows that even small amounts of terrestrial mud can have a large impact on prokaryotic communities and the nitrogen cycling fractions, implying a shift in coastal nitrogen budgets with increasing sedimentation rates.